CellReporterXpress

Image Acquisition and Analysis Software

Version 2.9

User Guide

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MOLECULAR DEVICES

Product manufactured by Molecular Devices, LLC.
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Chapter 1: CellReporterXpress Image Acquisition and Analysis Software

The CellReporterXpress® Image Acquisition and Analysis Software is the user interface for the Molecular Devices® ImageXpress® Pico Automated Cell Imaging System.

The CellReporterXpress software integrates image acquisition and analysis into a unified workflow. Along with the ImageXpress Pico system, the CellReporterXpress software streamlines automated imaging to offer a simplified solution for scaling up microscopy. Its features include:

- A web-based interface that runs on many browsers, including those found on iPads and Android tablets.
- Over 25 available predefined experimental protocols.
- High-powered analysis tools equivalent to those found in desktop applications.
- Easy-to-manage data with no requirement to configure a database.
- A simplified user interface that is easy to learn and easy to use.

Logging In to the Software

This section describes the various ways to log in to the CellReporterXpress software:

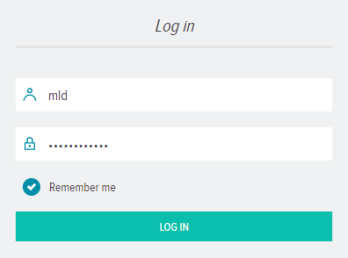
Note: The CellReporterXpress software uses the Windows login credentials of the host computer to authenticate users. If the host computer does not maintain a constant connection to the network, we recommend that user accounts be local accounts (and not roaming or domain accounts). If domain accounts are required, the host computer must remain connected to the domain network at all times.

Logging In on the Host Computer

Note: If you have just powered on or restarted the host computer, wait five minutes before logging in. This allows time for the CellReporterXpress software services to start.

To log in to the CellReporterXpress software on the host computer:

1. On the host computer, do one of the following to display the CellReporterXpress Log In screen:
   - On the desktop, double-click **MD.CellReporterXpress**.
   - Click **Start > Molecular Devices > MD.CellReporterXpress**.



2. In the **Login** field, enter the user name for a Windows account on the host computer.
3. In the **Password** field, enter the required password for the Windows account.
4. Click **LOG IN**.

Logging In on a Client Workstation Using a Shortcut

If you installed a client workstation, you can use the installed shortcut to connect to the CellReporterXpress software on the host computer.

To log in to the CellReporterXpress software from a client workstation with a shortcut:

1. On the client workstation, do one of the following to display the CellReporterXpress Log In screen:
   - On the desktop, double-click  **MD.CellReporterXpress**.
   - Click **Start > Molecular Devices > MD.CellReporterXpress**.
2. In the **Login** field, enter the user name for a Windows account on the host computer.
3. In the **Password** field, enter the required password for the Windows account.
4. Click **LOG IN**.

Logging In on a Client Workstation Using Connection Details

You can enter the connection details to connect a computer or tablet to the CellReporterXpress software on the host computer.

Note: If you are logging in on a client workstation for the first time after an update, we recommend that you restart the computer or tablet and clear the browser cache before connecting to the software on the host computer.

To log in to the CellReporterXpress software from a client workstation using connection details:

1. On the computer or tablet, open a supported browser.
2. In the address bar, enter either the IP address or the host computer name along with the port being used by the remote client (by default, 80) in the following format:

   ```
   http://address:port
   ```

   For example, if the host computer is named CellReporterXpress, enter:

   ```
   http://CellReporterXpress:80
   ```

   Or, if the IP address of the host computer is 192.168.1.1, enter:

   ```
   http://192.168.1.1:80
   ```
3. In the **Login** field, enter the user name for a Windows account on the host computer.
4. In the **Password** field, enter the required password for the Windows account.
5. Click **LOG IN**.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest level of technical service.

Our Support website—www.moleculardevices.com/service-support—describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, you can submit a request to Molecular Devices Technical Support.

Technical Support

To contact Molecular Devices Technical Support, submit a support request through the Molecular Devices Knowledge Base at support.moleculardevices.com.

You can also submit a support request by phone. For regional support contact information, go to www.moleculardevices.com/contact.

To expedite support, be prepared to provide the instrument serial number. The serial number is located on the back panel of the instrument.

Documentation

Review the product documentation on the Molecular Devices Knowledge Base at support.moleculardevices.com, including installation guides and user guides. In addition, online Help is available within the CellReporterXpress software. Press **F1** to access Help for the current page.

Additional Resources

Web-based microscopy courses:

- www.leica-microsystems.com/science-lab/science-lab-home
- www.ibiology.org/ibioeducation/taking-courses/ibiology-microscopy-short-course.html

The *Molecular Probes Handbook* offers advice on fluorescent probes and can help you determine if there are better stains available for your analysis:

- www.thermofisher.com/us/en/home/references/molecular-probes-the-handbook.html

Product Documentation

The following guides are available on the Molecular Devices Knowledge Base at support.moleculardevices.com:

- *CellReporterXpress Installation & IT Guide*
- *CellReporterXpress Firewall & Antivirus Supplement*
- *CellReporterXpress User Guide*
- *CellReporterXpress Release Notes*
- *ImageXpress Pico Pre-Installation Guide*
- *ImageXpress Pico EC Gas Requirements Pre-Installation Guide*
- *ImageXpress Pico Product Safety Sheet*
- *ImageXpress Pico Installation Guide*
- *ImageXpress Pico User Guide*
- *ImageXpress Pico Calibration Kit Guide*

In addition, the CellReporterXpress software includes context-sensitive Help that you can access from within the software. Just press the **F1** key from within the software to view Help for the current page.

Tip: We recommend that you review the documentation before installing or using the ImageXpress Pico system or the CellReporterXpress software.

About This Guide

This guide is intended for the scientist using the CellReporterXpress software. It is a PDF version of the Help that is integrated into the CellReporterXpress software.

The information in this guide is subject to change without notice. We recommend that you review the guide on the Molecular Devices Knowledge Base at support.moleculardevices.com for the most up-to-date information.

Chapter 2: Home Page

The **Home** page is the first page displayed when you log in to the CellReporterXpress software. It contains the following tiles that enable you to access the software modes:

Acquisition: Click to configure experiment settings and run experiments from supported instruments using protocols or templates. See Acquisition Mode on page 13 for details.

Experiments: Click to view images and analysis data collected in **Acquisition** mode and perform additional offline analysis. See Experiments Mode on page 125 for details.

Monitor: Click to view the progress and completion status of various CellReporterXpress software activities, including experiments run in **Acquisition** mode or **Experiments** mode, images exported in **Experiments** mode, movies downloaded in **Experiments** mode, and maintenance operations run in **Devices** mode. See Monitor Mode on page 213 for details.

Configuration: Click to set the systemwide options that affect all users of the CellReporterXpress software. See Configuration Mode on page 215 for details.

Devices: Click to manage and configure instruments for acquisition, including installing and calibrating objectives and filter cubes and controlling the temperature inside the instrument. See Devices Mode on page 229 for details.

Some of the tiles will be used often. Others will be used infrequently after you have set up the system.

The toolbar at the top of the CellReporterXpress window is always available. On the left are page navigation tabs, which are a “breadcrumb trail” indicating the path you used to get to the currently displayed page. To return to a previous page, click on that previously visited page tab.

The right side of the toolbar includes the following icons:

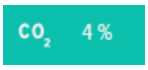
 **Help**: Opens the Help.

 **Full Screen**: Expands the software window to fill the entire screen of your computer or tablet.

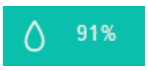
 **User Preferences**: Enables you to specify the way the software looks and, for some functions, to specify the way the functions work. The options you set become your personal preferences and stay set every time you use the software. See Configuration Settings on page 259 for details.

 **Log out**: Logs out of the CellReporterXpress software and returns to the Log In screen.

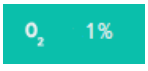
The toolbar may also contain a toolbar notification for an environmental control sensor:



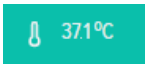
CO_2 Level: Shows the current CO_2 level inside the environmental control cassette. The color of the toolbar notification indicates if the CO_2 level is within the range you set (green) or outside of it (yellow).



Humidity Level: Shows the current humidity level inside the environmental control cassette. The color of the toolbar notification indicates if the humidity level is within the fixed range (green) or outside of it (yellow).



O_2 Level: Shows the current O_2 level inside the environmental control cassette. The color of the toolbar notification indicates if the O_2 level is within the range you set (green) or outside of it (yellow).



Temperature: Shows the current temperature inside the instrument. The color of the toolbar notification indicates if the temperature is within the range you set (green) or outside of it (yellow).

Click a toolbar notification to open the control panel for that environmental control component. See Sensors on page 231 for details.

Chapter 3: Acquisition Mode

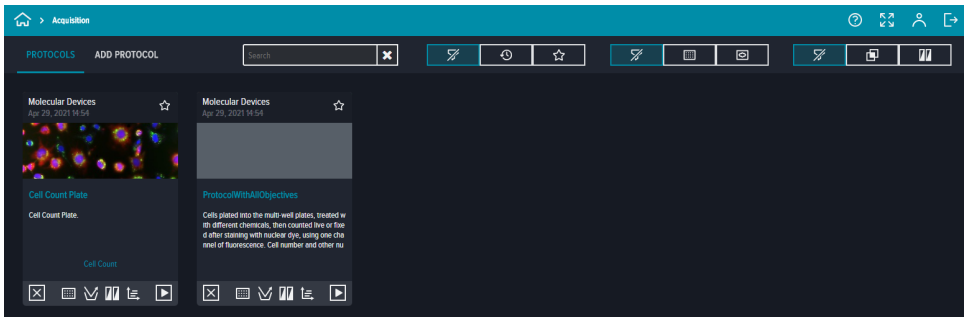
Use **Acquisition** mode to configure experiment settings and run experiments from supported instruments using protocols or templates.

On the **Home** page, click the **Acquisition** tile to enter **Acquisition** mode. The **Protocol** library appears.

Protocol Library

A protocol enables you to reuse a specific configuration for an experiment. It includes no experiment data, only configuration settings. A typical protocol enables you to insert labware into the instrument, select the protocol, and then run the protocol to perform the experiment and collect data.

A protocol is created from a template. When you click **Add Protocol** in the Protocol library, the Template library appears. Each template guides you through the configuration process for a particular experiment. When you save a modified template, it becomes a protocol in the Protocol library. See Template Library on page 15 for details.



Each protocol in the library is displayed as a card. The cards are listed in alphabetical order by protocol name. Along with the protocol name and description, each card indicates the user who created the protocol, the date and time of creation, a **Favorite** icon (that you can use to flag frequently used protocols), a **Delete** icon, and a **Run** icon.

Each card contains icons to indicate protocol properties, including:

 **Locked**

 **Plate** or  **Slide**

 **Fluorescence** or  **Colorimetric**

 **Stitched** or  **Not Stitched**

 **Analysis On** or  **Analysis Off**

From the Protocol library, you can run a protocol, modify a protocol, add a protocol, or delete a protocol.

Running a Protocol

To run a protocol:

1. Click the card you want to run.
2. Click  **Run**. See Run Protocol on page 71 for details on running a plate protocol or Run Protocol on page 121 for details on running a slide protocol.

Modifying a Protocol

To modify a protocol:

1. Click the card you want to modify.
2. Go to each workflow step you want to modify and make changes as needed. See Plate Acquisition Workflow on page 17 or Slide Acquisition Workflow on page 74 for details.
3. Click  **Save Protocol**. See Run Protocol on page 71 for details on running a plate protocol or Run Protocol on page 121 for details on running a slide protocol.

Deleting a Protocol

To delete a protocol:

1. Click the card you want to delete.
2. Click  **Delete**.

Adding a Protocol

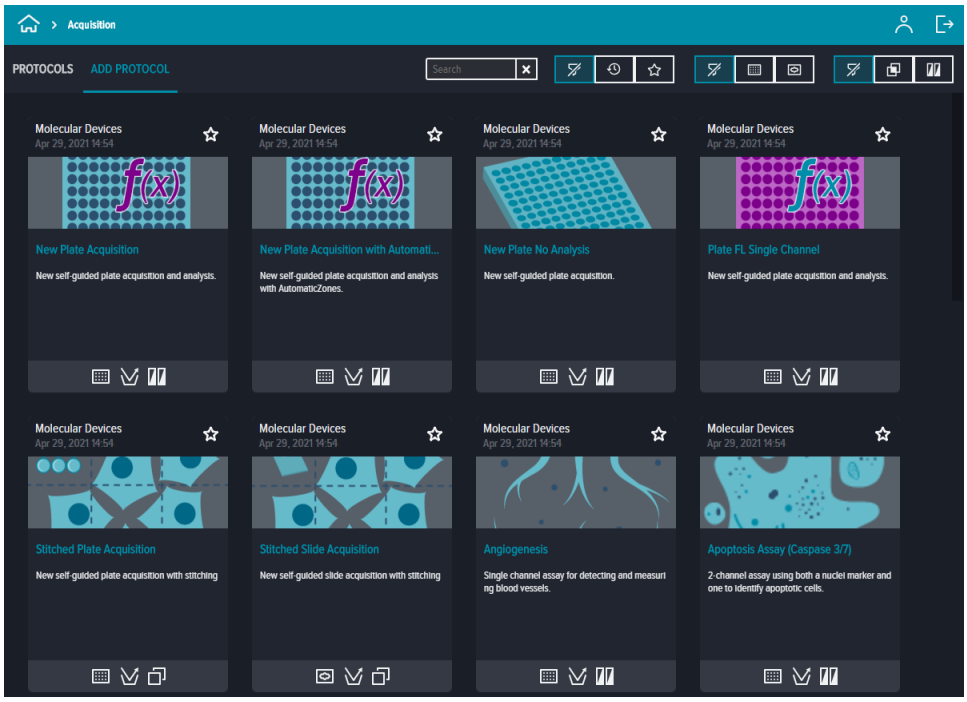
To add a protocol, see Template Library on page 15.

Template Library

When you click **Add Protocol** in the Protocol library, the Template library appears. Each template guides you through the configuration process for a particular experiment.

Most templates are designed for typical experiments and have restricted settings options. The restrictions help you focus on the most appropriate options for that experiment type. Two unrestricted templates (**New Plate Acquisition** and **New Slide Acquisition**) allow you to select from all the available experiment settings options.

Note: You cannot create new templates. When you save a template, it becomes a protocol. See Protocol Library on page 13 for details.



Each template in the library is displayed as a card. The cards are listed in alphabetical order by template name. Along with the template name, each card indicates the template description, a **Favorite** icon (that you can use to flag frequently used protocols). In addition, each card contains summary icons to indicate template settings, including:

- **Plate** or **Slide**
- **Fluorescence** or **Colorimetric**
- **Stitched** or **Not Stitched**

Search and Filters

To limit the number of visible cards, use the **Search** field and filter icons at the top of the Protocol library and the Template library.

Using Search

Use search to find specific words in the titles and descriptions of protocols or templates.

To use search:

1. Click in the **Search** field.
2. Enter the word you want to search, and press **ENTER**.

Using Filters

Use the filter icons to control which template cards or protocol cards are shown. Active filter icons are highlighted. By default, **All** is the active filter. Filter options include:

Recent or **Favorites**

Plates or **Slides**

Stitched or **Not Stitched**

To use filter icons:

Click the filter icon you want to use.

The icon is highlighted and only the cards that match the filter option are shown.

Plate Acquisition Workflow

Select a plate template to begin the plate acquisition workflow. The **New Plate Acquisition** template is an unrestricted template that allows you to select from all the available plate experiment settings options. Other plate templates may offer restricted settings options to help you focus on the most appropriate options for that experiment type.

The icons in the **Steps** pane on the left side of the page guide you through the plate experiment configuration process. The tools and controls in the pane on the right side of the page vary according to the step being configured and the experiment type.

Note: Depending on the selected template, some steps, tools, and options may not appear or may not be available. Use the **New Plate Acquisition** template to access all steps, tools, and options.

The plate acquisition workflow is as follows:


Acquisition Device is the first step for all acquisition workflows. In this step, you select the instrument for the acquisition and insert your experiment-ready labware. See Acquisition Device on page 19 for details.


Acquisition Settings is the step where you set up image acquisition for the experiment, including the stains and objective to be used. You can preview the image capture and adjust channel identification color, histogram, focus, and exposure settings. See Acquisition Settings on page 21 for details.


Region Selection to Acquire is the step where you select the region of the well to be acquired. The page shows a representation of a well with a region selection overlay. You must select at least one region to run an experiment. See Region Selection to Acquire on page 58 for details.


Analysis Settings is the step where you set up image analysis for the experiment. The data generated based on these settings becomes the analysis data for the experiment. See Analysis Settings on page 59 for details.


Region Selection to Analyze is the step where you select the region of interest to be analyzed. You must select at least one region to run an experiment. See Region Selection to Analyze on page 64 for details.


Well Selection is the step where you select the well for the experiment. See Well Selection on page 65 for details.


Device Sensors is the step where you specify the environmental sensors to be monitored for the experiment. The experiment data indicates if the sensors were within the specified range during the experiment. See Device Sensors on page 66 for details.


Time Series is an optional step where you set up a time series for image acquisition. This enables you to acquire images at multiple time points. See Time Series on page 68 for details.


Save Protocol is an optional step where you can save the protocol you have created. You will typically save a protocol only when you intend to run it frequently. After you save a protocol, it appears as a card in the Protocol library. See Save Protocol on page 70 for details.


Run Protocol is the last step in the workflow. In this step, you can review the settings for the experiment and address any issues. See Run Protocol on page 71 for details.

Acquisition Device


Acquisition Device is the first step for all acquisition workflows. In this step, you select the instrument for the acquisition and insert your experiment-ready labware. See the *ImageXpress Pico User Guide* for details on inserting labware into the instrument.

The right side of the page includes the following icons:


Shutdown Device: Prepares the software to power off the selected instrument.


Restart Device: Restarts the selected instrument.


Open Plate Door: Opens the top door on the selected instrument so that you can insert or remove labware.


Close Plate Door: Closes the top door.


Set Up for Adjustment of Objective Collar: Moves the objective turret so that you can adjust the correction collar on the selected objective.


Finish Adjustment of Objective Collar: Moves the objective turret back into position after you adjust a correction collar.

The software displays a tile for each available instrument. See Adding an Instrument to the My Devices List on page 223 for details on adding other instruments in your network.

For each instrument, a status indicator shows one of the following:

Online

Offline or **Incompatible**

Busy

Error

In addition, the following information appears for each instrument:

- Instrument Name
- Instrument Serial Number
- Device Model
- Version
- Free Space
- Number of Installed Objectives
- Number Installed Filter Cubes
- Digital Confocal License

Note: If you click the **Favorite** icon for a device, that device will be selected by default. Otherwise, the last used device is default.

To select an acquisition device:

On the **Acquisition Device** page, select the instrument you want to use.

The selected device is highlighted.

To continue to the next workflow step, click **Acquisition Settings**. See Acquisition Settings on page 21 for details.

Acquisition Settings


Acquisition Settings is the step where you set up image acquisition for the experiment, including the stains and objective to be used. You can preview the image capture and adjust channel identification color, histogram, focus, and exposure settings.

The right side of the page includes the following icons:

Plate Format: Specifies the plate format for the acquisition.


Stains: Specifies the stains for the acquisition and the order in which they are acquired.

Objectives: Specifies the objective for the acquisition.


Autofocus Info: Shows the focus peaks for the most recent autofocus to help you understand how it was calculated and troubleshoot focus issues. See Troubleshooting Autofocus Issues on page 53 for details.


Comparison Mode: Captures two preview images, which enables you to compare the uniformity of the image quality or compare settings on two different phenotypes (such as positive and negative controls). See Snapping Well Comparison Previews on page 30 for details.


Download Plane Images: Downloads TIFF images of the current preview. Without Z stack images, the downloaded image is the current preview. With Z stack images, the downloaded image is determined by the current selection: either the Z stack projection or an individual Z stack plane.


Download Z Stacking Images: With Z stack images, downloads a TIFF image of the current Z stack projection and each individual Z stack plane.

Specifying the Plate Format

To achieve good autofocus results, it is very important to specify an appropriate and accurate plate format for the labware you are using.

Note:

- The only supported dish format (**6 Dish Glass Bottom 35 mm**) is characteristically similar to a plate format, so it is listed as a plate.
- The 2.5x objective has a large field of view and may not provide a useful image with a 384-well plate.
- When using the environment control cassette with the 2.5x objective, autofocus may fail or be inconsistent. See Understanding How Autofocus Works with a Plate on page 49 for details.

To specify the plate format:

1. On the **Acquisition Settings** page, in the **Tools** pane on the right, click 

Plate Format.
2. In the **Plate Format** pane, select the plate format you want to use.

Note:

- If an appropriate plate format does not exist, click **Add New Plate** to define a new labware specification. See Labware Library on page 217 for details.
- If you are using the plate skirt height adapter with the optional environmental control cassette, select a plate format that adds 1.5 mm to the actual **Plate Height** value.

Specifying the Stains

To specify the stains:

1. On the **Acquisition Settings** page, in the **Tools** pane on the right, click 
Stains.
2. In the **Stains** pane, select the stains you want to acquire.
3. Click  **Move Stain Up** and  **Move Stain Down** as needed to select the order in which the stains will be acquired.

Tip:

- For an acquisition with transmitted light, acquire the transmitted light stain first or last. If you plan to perform transmitted light segmentation, you will likely want to set the transmitted light stain to be acquired first.
- Avoid acquiring Cy5 as the first stain as it can interfere with the hardware autofocus.
- DAPI is typically the best choice for first stain, if you are acquiring that stain.

Specifying the Objective

To specify the objective:

1. On the **Acquisition Settings** page, in the **Tools** pane on the right, click 

Objectives.
2. In the **Objectives** pane, select the objective you want to use.

Note: The 2.5x objective has a large field of view and may not provide a useful image with a 384-well plate.

As part of configuring acquisition settings, you may do the following:

- Snapping a Preview of a Well, see page 24
- Snapping Well Comparison Previews, see page 30
- Snapping Z Stack Images, see page 36
- Viewing a Live Preview of a Plate, see page 40
- Adjusting an Objective Correction Collar, see page 46
- Understanding How Autofocus Works with a Plate, see page 49
- Troubleshooting Autofocus Issues, see page 53

To continue to the next workflow step, click  **Region Selection to Acquire**. See Region Selection to Acquire on page 58 for details.

Snapping a Preview of a Well

You can view a preview of the acquisition by snapping an image. The preview uses the selected objective, stain, well, region, focus settings, exposure settings, and histogram.

After snapping the preview, you can use the icon on the right side of the page to do the following:

 **Download Plane Images**: Downloads TIFF images of the current preview. Without Z stack images, the downloaded image is the current preview. With Z stack images, the downloaded image is determined by the current selection: either the Z stack projection or an individual Z stack plane.

Use the image viewer controls as needed to view the preview:

 Toggles the available channels for the preview by clicking on the channel icons. The icons for visible channels are brighter and have a white border. “Hidden” channels are dimmed and do not have a white border.

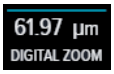
 **Tip:** When a channel is overexposed, the overexposure  indicator appears. Lower the **Exposure** value as needed.

 Zooms in on the image.

 Zooms out on the image.

 Resets the view zoom to the original image size.

 Views the image full screen.



Indicates the current zoom scale. **Digital Zoom** indicates that the image is zoomed in digitally (that is, not optically) and might not represent the actual quality of the image.

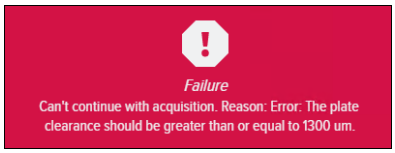
To snap a preview of a well:

1. On the **Acquisition Settings** page, click  **Snap Image** to snap an initial preview image. A thumbnail of the preview image appears in the **History** pane.

Note:

- If autofocus fails, the software may display the following message:

  In this case, you may want to check the Autofocus Info graph to review the focus peaks for the most recent autofocus to understand how the system determined focus. See Troubleshooting Autofocus Issues on page 53 for details.
- If autofocus fails with the optional environmental control cassette, the software may display the following message:



  In this case, you may need to insert the plate skirt height adapter and select a plate format that adds 1.5 mm to the actual **Plate Height** value. See the *ImageXpress Pico User Guide* for details on inserting the plate skirt height adapter. See Labware Library on page 217 for details on adding a labware specification.
- Molecular Devices Technical Support has compiled information that can help you diagnose and resolve autofocus issues. For details, log in to the Molecular Devices Knowledge Base at support.moleculardevices.com, and search for **ImageXpress Pico: Focus Troubleshooting**.

2. At the bottom of the screen on the 

Choose Position to Acquire tab, click 

Select Well.
3. In the well map, select the well for the preview. The instrument snaps a preview image of the newly selected well. The top left well is selected by default.

4. In the single well map, click and drag the  selection tool to select the region of the well for the preview, if needed. The center area of the well is selected by default.
5. When you find a region of interest, click **Save Position** to save the current field of view to the **Region Selection to Acquire** page.

> **Note:** You can click **Save Position** for each region of interest you find. Each time you click, the software adds a region on the **Region Selection to Acquire** page.

6. Click  **Focus/Exposure Settings**.

> **Note:** Click  **Focus Mode Descriptions** or see Understanding How Autofocus Works with a Plate on page 49 for details on getting good autofocus results.

7. On the **Focus/Exposure Settings** tab, do the following:
   a. Under **Detect Surface**, select one of the following hardware autofocus settings:
      - **Plate Bottom**: A single-peak autofocus designed for samples in 3D matrices or clear mounted coverslips, where the second surface appears "invisible" to autofocus. It is also suitable for imaging thin-bottom plates with a low-magnification objective.
      - **Well Bottom**: A two-peak autofocus designed for samples in a liquid medium, such as well plates or chamber slides. It is not recommended for samples in 3D matrices, thin-bottom plates with a low-magnification objective, or dry plates. It is not available with the 2.5x objective.
      - **Well Insert**: A three-peak autofocus designed for well inserts in well plates or any labware design that has a distinct third surface.
   b. If needed, under **Find Best Plane**, select one of the following software autofocus options to improve autofocus:
      - **Normal Search Range**: The Normal Search Range option is centered on the surface selected by the hardware autofocus. It searches a range around that surface of 15% of the Bottom Thickness value from the labware specification (that is, 7.5% above the surface and 7.5% below it).
      - **Wide Search Range**: The Wide Search Range option is centered on the surface selected by the hardware autofocus. It searches a range of 40% of the Bottom Thickness value from the labware specification (that is, 20% above the surface and 20% below it).
      - **Superwide Search Range**: The Superwide Search Range option uses the surface selected by the hardware autofocus and searches a range of 300 µm above that surface (unlike the other options, which search around the surface).

c. If needed, select the **Anchor Focus Position** check box to save current focus position and disable the autofocus controls. The software uses the saved focus position for any ensuing snaps of preview images and for the acquisition. An anchor icon  indicates the snapped image previews in the **History** pane that are using the anchored focus position.

Note:

- Typically, the instrument runs autofocus each time you snap a preview image or acquire an image. When you select the **Anchor Focus Position** check box, the instrument no longer runs autofocus. Instead, the software uses the saved focus position across all sites and wells, which speeds up acquisition.
- Clear the check box to remove the saved anchored focus position setting. The instrument resumes autofocus the next time you snap an image preview or run an acquisition.
- For best results when you anchor the focus position, use the 2.5x objective or 4x objective. In addition, ensure that both your plate and your sample are relatively flat.
- The **Anchor Focus Position** check box is not available if any of the following are true:
  - No preview images have been snapped (that is, the **History** pane is empty).
  - The selected objective has just been changed.
  - The 40x objective or 63x objective is selected.
  - Live preview is on.

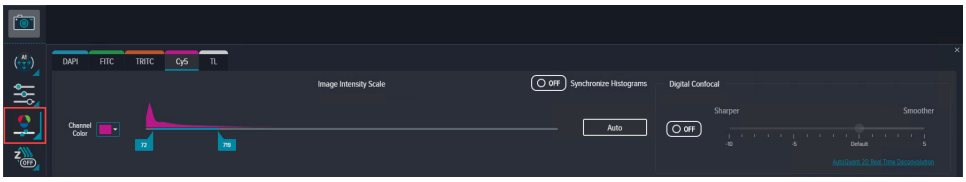
d. If needed, use the **Focus Offset** controls to adjust the image sharpness for each wavelength.

e. If needed, use the **Exposure** controls to adjust the exposure time for each channel. Exposure time affects image brightness and results in a broader dynamic range (that is, the difference between the brightest and dimmest object detected).
When the image is in focus, click **Auto** to automatically determine the optimal exposure for the best dynamic range. The longer the exposure, the brighter the experiment image. Dimmer objects get brighter with longer exposure time, as does the background. Increased exposure time slows the experiment because each image takes longer. In addition, it can lead to image saturation and possibly cause photobleaching or phototoxicity of your sample.

Tip: Try using **Auto** with a known bright sample, such as a positive control. Then, if needed, decrease the exposure about 10% to 20%.

8. Click  **Snap Image** again to refresh the preview.
A thumbnail of the preview image appears in the **History** pane.

9. Click  **Image Intensity Settings**.



10. On the **Image Intensity Settings** tab, adjust the settings as needed for each channel:
    a. Use the **Channel Color** drop-down list box to change the identification color for the channel. The default channel color is based on the emission wavelength of the selected stain.
    b. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image.
    c. Click **Auto** to set the best contrast based on the current preview.
    d. If you need to adjust the image intensity scale for all channels simultaneously, you can set **Synchronize Histograms** to **On**.

    Note: In most cases, however, you can set **Synchronize Histograms** to **Off**.

11. With an optional digital confocal license, do the following to apply the optional digital confocal algorithm to the image:
    a. Set **Digital Confocal** to **On**.
    b. As needed, move the slider to the right to smooth the image.
    c. As needed, move the slider to the left to sharpen the image.

    AutoQuant integrated innovation

    Digital confocal enhances contrast, improves resolution, and sharpens the image using the AutoQuant 2D RealTime Deconvolution algorithm. The algorithm calculates and inverts an ideal point spread function (PSF) for the optics based on image acquisition properties (not the actual image) such as the numerical aperture, refraction index, wavelength, and so on. The algorithm then applies the inverted PSF to the original image in Fourier space, which is a single multiplier (instead of a convolution). A Weiner filter uses a constant K value to correct noise in the image.

12. Repeat these steps as needed until you are satisfied with the quality of the preview image.

    Tip:
    - The **History** pane contains thumbnails of previous preview images. Click a thumbnail to show that preview image and revert the settings to the configuration used to acquire it.
    - An anchor icon  on a thumbnail indicates the snapped image preview is using the anchored focus position.

13. Consider snapping Z stack images if your sample includes any of the following:
    - More than one focus plane within the field of view.
    - Objects of different depths.
    - Objects with varying depths relative to the focus plane.
    - Thick objects.

    See Snapping Z Stack Images on page 36 for details.

To continue to the next workflow step, click  **Region Selection to Acquire**. See Region Selection to Acquire on page 58 for details.

Snapping Well Comparison Previews

You can view previews of the acquisition by snapping comparison images from two wells (or two regions of the same well). This enables you to compare the uniformity of the image quality or compare settings on two different phenotypes (such as positive and negative controls). As with snapping an image of a single well, the comparison images use the selected objective, stain, well, region, focus settings, exposure settings, and histogram.

After snapping the preview, you can use the icon on the right side of the page to do the following:

 **Download Plane Images**: Downloads TIFF images of the current preview. Without Z stack images, the downloaded image is the current preview. With Z stack images, the downloaded image is determined by the current selection: either the Z stack projection or an individual Z stack plane.

Use the image viewer controls as needed to view the preview:

 Toggles the available channels for the preview by clicking on the channel icons. The icons for visible channels are brighter and have a white border. “Hidden” channels are dimmed and do not have a white border.

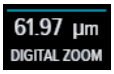
Tip: When a channel is overexposed, the overexposure  indicator appears. Lower the **Exposure** value as needed.

 Zooms in on the image.

 Zooms out on the image.

 Resets the view zoom to the original image size.

 Views the image full screen.



Indicates the current zoom scale. **Digital Zoom** indicates that the image is zoomed in digitally (that is, not optically) and might not represent the actual quality of the image.

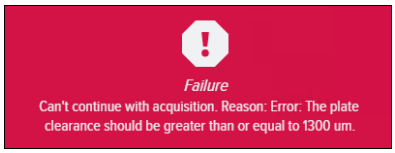
To snap well comparison images:

1. On the **Acquisition Settings** page, click  **Snap Image** to snap an initial preview image. A thumbnail of the preview image appears in the **History** pane.

Note:

- If autofocus fails, the software may display the following message:

  In this case, check the Autofocus Info graph to review the focus peaks for the most recent autofocus to understand how the system determined focus. See Troubleshooting Autofocus Issues on page 53 for details.
- If autofocus fails with the optional environmental control cassette, the software may display the following message:



  In this case, insert the plate skirt height adapter and select a plate format that adds 1.5 mm to the actual **Plate Height** value. See the *ImageXpress Pico User Guide* for details on inserting the plate skirt height adapter. See Labware Library on page 217 for details on adding a labware specification.
- Molecular Devices Technical Support has compiled information that can help you diagnose and resolve autofocus issues. For details, log in to the Molecular Devices Knowledge Base at support.moleculardevices.com, and search for **ImageXpress Pico: Focus Troubleshooting**.

2. In the **Tools** pane on the right, click  **Comparison Mode**.

Note: If **Z Stacking** is set to **On**, the Comparison Mode icon is not available. You must set **Z stacking** to **Off** before you can start Comparison Mode.

3. At the bottom of the screen on the 

Choose Position to Acquire tab, click 

Select Well on the left.
4. In the well map, select the first well for the preview. The instrument snaps a preview image of the newly selected well. The top left well is selected by default.

5. Click 

Select Well on the right.
6. In the well map, select the second well for the preview. The instrument snaps a preview image of the newly selected well. The bottom right well is selected by default.
7. In the single well map on the left, click and drag the  selection tool to select the region of the first well for the preview, if needed. The center area of the well is selected by default.
8. Repeat steps as needed in the single well map on the right to select the region of the second well for the preview, if needed.
9. Click  **Focus/Exposure Settings**.

Note: Click  **Focus Mode Descriptions** or see Understanding How Autofocus Works with a Plate on page 49 for details on getting good autofocus results.

10. On the **Focus/Exposure Settings** tab, do the following:
    a. Under **Detect Surface**, select one of the following hardware autofocus settings:
        - **Plate Bottom**: A single-peak autofocus designed for samples in 3D matrices or clear mounted coverslips, where the second surface appears "invisible" to autofocus. It is also suitable for imaging thin-bottom plates with a low-magnification objective.
        - **Well Bottom**: A two-peak autofocus designed for samples in a liquid medium, such as well plates or chamber slides. It is not recommended for samples in 3D matrices, thin-bottom plates with a low-magnification objective, or dry plates. It is not available with the 2.5x objective.
        - **Well Insert**: A three-peak autofocus designed for well inserts in well plates or any labware design that has a distinct third surface.
    b. If needed, under **Find Best Plane**, select one of the following software autofocus options to improve autofocus:
        - **Normal Search Range**: The Normal Search Range option is centered on the surface selected by the hardware autofocus. It searches a range around that surface of 15% of the Bottom Thickness value from the labware specification (that is, 7.5% above the surface and 7.5% below it).
        - **Wide Search Range**: The Wide Search Range option is centered on the surface selected by the hardware autofocus. It searches a range of 40% of the Bottom Thickness value from the labware specification (that is, 20% above the surface and 20% below it).
        - **Superwide Search Range**: The Superwide Search Range option uses the surface selected by the hardware autofocus and searches a range of 300 µm above that surface (unlike the other options, which search around the surface).

c. If needed, select the **Anchor Focus Position** check box to save current focus position and disable the autofocus controls. The software uses the saved focus position for any ensuing snaps of preview images and for the acquisition. An anchor icon  indicates the snapped image previews in the **History** pane that are using the anchored focus position.

Note:

- Typically, the instrument runs autofocus each time you snap a preview image or acquire an image. When you select the **Anchor Focus Position** check box, the instrument no longer runs autofocus. Instead, the software uses the saved focus position across all sites and wells, which speeds up acquisition.
- Clear the check box to remove the saved anchored focus position setting. The instrument resumes autofocus the next time you snap an image preview or run an acquisition.
- For best results when you anchor the focus position, use the 2.5x objective or 4x objective. In addition, ensure that both your plate and your sample are relatively flat.
- The **Anchor Focus Position** check box is not available if any of the following are true:
  - No preview images have been snapped (that is, the **History** pane is empty).
  - The selected objective has just been changed.
  - The 40x objective or 63x objective is selected.
  - Live preview is on.

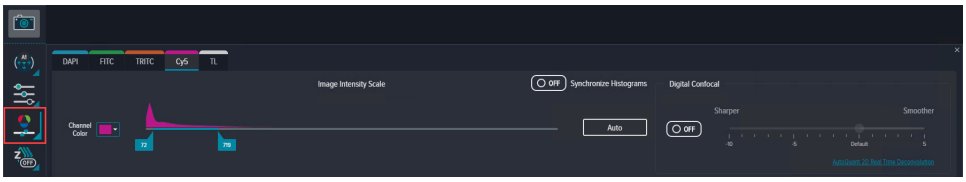
d. If needed, use the **Focus Offset** controls to adjust the image sharpness for each wavelength.

e. If needed, use the **Exposure** controls to adjust the exposure time for each channel. Exposure time affects image brightness and results in a broader dynamic range (that is, the difference between the brightest and dimmest object detected).
When the image is in focus, click **Auto** to automatically determine the optimal exposure for the best dynamic range. The longer the exposure, the brighter the experiment image. Dimmer objects get brighter with longer exposure time, as does the background. Increased exposure time slows the experiment because each image takes longer. In addition, it can lead to image saturation and possibly cause photobleaching or phototoxicity of your sample.

Tip: Try using **Auto** with a known bright sample, such as a positive control. Then, if needed, decrease the exposure about 10% to 20%.

11. Click  **Snap Image** again to refresh the preview.

12. Click  **Image Intensity Settings**.



13. On the **Image Intensity Settings** tab, adjust the settings as needed for each channel:
    a. Use the **Channel Color** drop-down list box to change the identification color for the channel. The default channel color is based on the emission wavelength of the selected stain.
    b. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image.
    c. Click **Auto** to set the best contrast based on the current preview.
    d. If you need to adjust the image intensity scale for all channels simultaneously, you can set **Synchronize Histograms** to **On**.

    Note: In most cases, however, you can set **Synchronize Histograms** to **Off**.

14. With an optional digital confocal license, do the following to apply the optional digital confocal algorithm to the image:
    a. Set **Digital Confocal** to **On**.
    b. As needed, move the slider to the right to smooth the image.
    c. As needed, move the slider to the left to sharpen the image.

AutoQuant integrated innovation

Digital confocal enhances contrast, improves resolution, and sharpens the image using the AutoQuant 2D RealTime Deconvolution algorithm. The algorithm calculates and inverts an ideal point spread function (PSF) for the optics based on image acquisition properties (not the actual image) such as the numerical aperture, refraction index, wavelength, and so on. The algorithm then applies the inverted PSF to the original image in Fourier space, which is a single multiplier (instead of a convolution). A Weiner filter uses a constant K value to correct noise in the image.

15. Repeat these steps as needed until you are satisfied with the quality of the preview image.

Tip:

- The **History** pane contains thumbnails of previous preview images. Click a thumbnail to show that preview image and revert the settings to the configuration used to acquire it.
- An anchor icon  on a thumbnail indicates the snapped image preview is using the anchored focus position.

To continue to the next workflow step, click  **Region Selection to Acquire**. See Region Selection to Acquire on page 58 for details.

Snapping Z Stack Images

With the optional Z stacking feature, you can view previews of the acquisition by snapping Z stack images. A Z stack is comprised of a series of images captured at the specified focus offsets using the selected objective, stain, well, region, focus settings, and exposure settings. Z stack images can be helpful if your sample includes any of the following:

- More than one focus plane within the field of view.
- Objects of different depths.
- Objects with varying depths relative to the focus plane.
- Thick objects.

As you set up the acquisition, you can view and download individual planes of the Z stack or the entire Z stack projection. The Z stack projection is saved with the experiment data when you run the protocol.

Note: Only the Z stack projection is saved with the experiment data. Images for individual planes are not saved.

After snapping the preview, you can use the icons on the right side of the page to do the following:


Download Plane Images: Downloads TIFF images of the current preview. Without Z stack images, the downloaded image is the current preview. With Z stack images, the downloaded image is determined by the current selection: either the Z stack projection or an individual Z stack plane.


Download Z Stacking Images: With Z stack images, downloads a TIFF image of the current Z stack projection and each individual Z stack plane.

Use the image viewer controls as needed to view the preview:

 Toggles the available channels for the preview by clicking on the channel icons. The icons for visible channels are brighter and have a white border. “Hidden” channels are dimmed and do not have a white border.

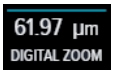
Tip: When a channel is overexposed, the overexposure  indicator appears. Lower the **Exposure** value as needed.

 Zooms in on the image.

 Zooms out on the image.

 Resets the view zoom to the original image size.

 Views the image full screen.



Indicates the current zoom scale. **Digital Zoom** indicates that the image is zoomed in digitally (that is, not optically) and might not represent the actual quality of the image.

: With Z stack images, indicates the type of image. **ZP** indicates a Z stack projection. For an individual Z stack plane, displays the focus offset amount. **Z 20µm** indicates 20 µm above focus.

Note: Before snapping Z stack images, you may first want to snap a preview of a well. See Snapping a Preview of a Well on page 24 for details.

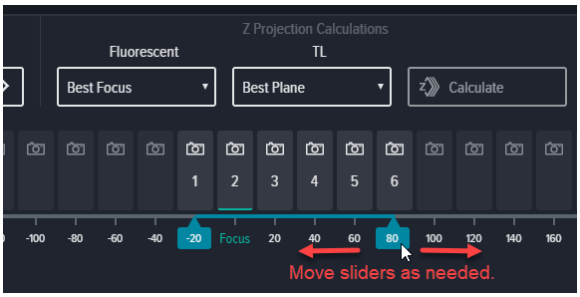
To snap Z stack images:

1. On the **Acquisition Settings** page, click  **Z Stacking**.
2. In the bottom pane, set **Z Stacking** to **On**.

3. In the **Focus Step (µm)** field, modify the distance between the Z stack planes, as needed. The default focus step distance varies based on the objective.

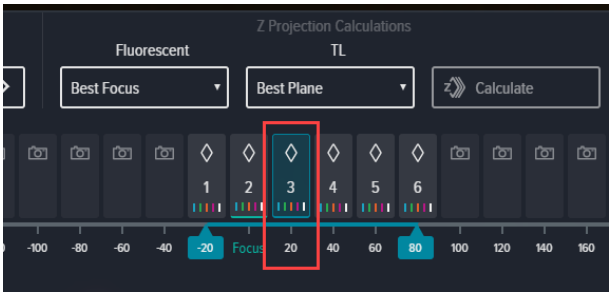
Objective	Default Focus Step Distance
2.5x	50 µm
4x	50 µm
10x	20 µm
20x	5 µm
40x	2 µm
63x	1 µm

4. As needed, move the sliders to select the Z stack planes to include in the Z stack.



5. Click  **Snap Z Stacking Images** to snap the Z stack.
The Z stack projection appears, and the Z stack projection button highlights to indicate that you are viewing the projection.

6. Click a Z stack plane button to view the preview for that plane.



7. If you are performing a fluorescent light acquisition, click the **Fluorescent** drop-down list box, and select one of the following to improve the Z stack images:
   - **Maximum**: For each corresponding pixel position in the images, finds the pixel that has the highest intensity value out of all the values in all the planes and outputs this value to the projection image.
   - **Best Focus**: Calculates the regions of best focus in an image stack on a pixel-by-pixel basis. Similar to the **Maximum** setting, this setting uses the best focused pixels from the Z stack to create the projection image.
   - **Best Focus Smaller Objects**: Breaks up images into very small zones to find the best focus across all Z planes for each zone, which brings fine details and smaller objects into focus. This option is very computationally demanding and may run slower than the Best Focus option or Best Focus Larger Objects option.
   - **Best Focus Larger Objects**: Breaks up images into small zones to find the best focus across all Z planes for each zone, which brings greater detail of larger objects into focus. This option is computationally demanding and may run slower than to the Best Focus option.
8. If you are performing a transmitted light acquisition, click the **TL** drop-down list box, and select one of the following to improve the Z stack images:
   - **Average**: For each corresponding pixel position in the images, averages the gray scale values of the pixels in all the planes, and outputs this value to the projection image.
   - **Best Plane**: Scores the images of best focus in an image stack and uses the plane with the best focus score to create the projection image.
   - **Best Focus Smaller Objects**: Breaks up images into very small zones to find the best focus across all Z planes for each zone, which brings fine details and smaller objects into focus. This option is very computationally demanding and may run slower than the Best Focus option or Best Focus Larger Objects option.
   - **Best Focus Larger Objects**: Breaks up images into small zones to find the best focus across all Z planes for each zone, which brings greater detail of larger objects into focus. This option is computationally demanding and may run slower than to the Best Focus option.

Note: The **Calculate** button becomes enabled when you modify settings that affect the preview image. Click **Calculate** to quickly view the updated preview image.

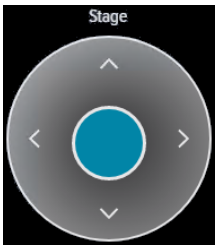
To continue to the next workflow step, click **Region Selection to Acquire**. See Region Selection to Acquire on page 58 for details.

Viewing a Live Preview of a Plate

Live preview enables you to move the sample (X-Y) stage to explore a continually updated, dynamic image of the sample. Using the two virtual joysticks (one for stage movement and one for focus control), you can quickly find a region of interest. The live preview uses the selected objective, stain, well, focus settings, and exposure settings.

Use the following controls as needed to view the live preview:

 Selects the active channel for live preview. Only one channel at a time can be active.



Moves the sample (X-Y) stage. Click and drag the stage joystick in the direction you want to move the stage. Drag it a small distance from the center to slowly move the stage. Larger movements will move the stage more quickly. Release the stage joystick to stop stage movement. You can also click the arrows inside the stage joystick to move the stage in small steps.

Adjusts the focus offset of the image. Click and drag the focus joystick to adjust the focus. As you adjust the offset, the focus position value updates. Drag the joystick a small distance from the center to fine-tune the focus. Larger movements will change the focus more quickly. Release the focus joystick to stop changing the focus. You can also click the arrows inside the focus joystick to adjust the focus in small steps. The step size corresponds to half of the depth of field for the selected objective.

Indicates the current focus offset value, which is the difference between the current focus position and the autofocus position. As you drag the focus joystick, the **Offset from Autofocus** value increases or decreases accordingly. A value in italics indicates that the current value has not been set as the focus offset.

Sets the current **Offset from Autofocus** value as the focus offset. This setting is reflected by the value in the **Focus Offset** field on the **Focus/Exposure Settings** tab.

Click to Center: Moves the stage to center the image on the spot that you click.

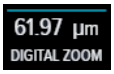
In addition, use the image viewer controls as needed to view the live preview:

 Zooms in on the image.

 Zooms out on the image.

 Resets the view zoom to the original image size.

 Views the image full screen.



Indicates the current zoom scale. **Digital Zoom** indicates that the image is zoomed in digitally (that is, not optically) and might not represent the actual quality of the image.

Tip: Several factors (for example, the labware is not completely flat or a thick sample is in multiple focus planes) may cause unexpected results when you click **Save Offset** after using the stage and focus joysticks. This can affect the focus of the acquisition when you run the experiment. For best results, you may want to snap a preview for your region of interest after turning off live preview.

Note: To avoid overexposing your sample, live preview automatically turns off after five minutes.

To view a live preview of a plate:

1. On the **Acquisition Settings** page, click 

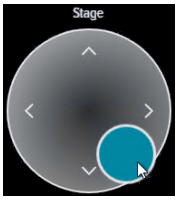
Choose Position to Acquire.
2. Click 

Select Well.
3. In the well map, select the well for the preview. The instrument snaps a preview image of the newly selected well. The top left well is selected by default.

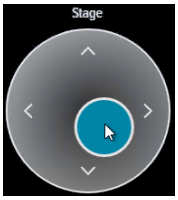
4. In the single well map, click and drag the  selection tool to select the region of the well for the preview, if needed. The center area of the well is selected by default.

5. In the bottom pane, set **Live Preview** to **On**.

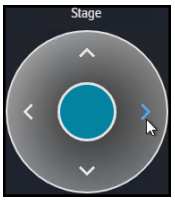
6. Use the stage joystick to move the sample (X-Y) stage to view different areas of the well:
   - For fast stage movements, click and drag the stage joystick in the direction you want to move toward the outermost position.



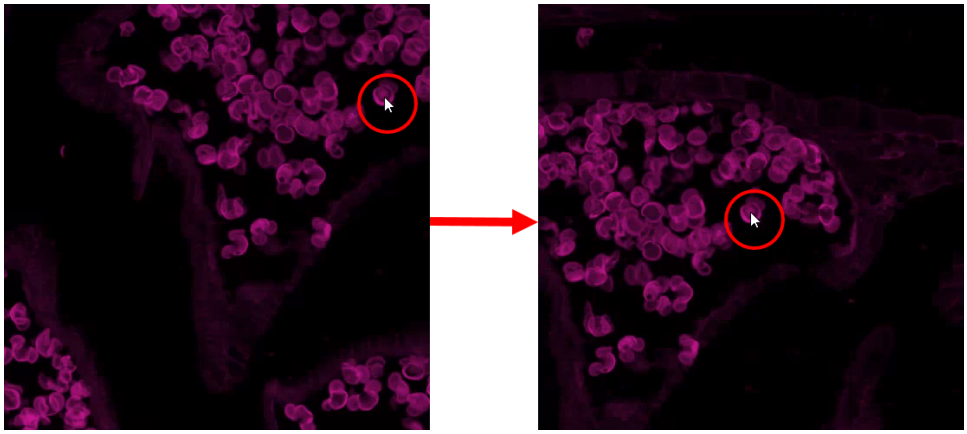
   - For slow stage movements, drag the stage joystick slightly.



   - For very small stage movements to fine-tune the stage position, click one of the joystick arrows. Each time you click an arrow, the stage moves one step.



7. Click on the image as needed to center the image on the clicked spot.



8. When you find a region of interest, click **Save Position** to save the current field of view to the **Region Selection to Acquire** page.

> **Note:** You can click **Save Position** for each region of interest you find. Each time you click, the software adds a region on the **Region Selection to Acquire** page.

9. Use the focus joystick to adjust the focus offset of the live preview. As you adjust the offset, the focus position value updates.
   - For large focus adjustments, click and drag the focus joystick toward the outermost position.

   - For smaller focus adjustments, drag the focus joystick slightly.

   - For fine focus adjustments, click one of the joystick arrows. Each time you click an arrow, the focus adjusts one step. The step size corresponds to half of the depth of field for the selected objective.

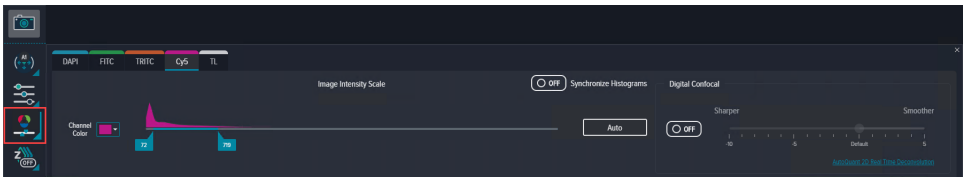
10. If needed, click  **Snap Image** to confirm your focus offset settings.
11. Click  **Focus/Exposure Settings**.

Note: Click  **Focus Mode Descriptions** or see Understanding How Autofocus Works with a Plate on page 49 for details on getting good autofocus results.

12. If needed, use the **Exposure** controls to adjust the exposure time for each channel. Exposure time affects image brightness and results in a broader dynamic range (that is, the difference between the brightest and dimmest object detected).
    When the image is in focus, click **Auto** to automatically determine the optimal exposure for the best dynamic range. The longer the exposure, the brighter the experiment image. Dimmer objects get brighter with longer exposure time, as does the background. Increased exposure time slows the experiment because each image takes longer. In addition, it can lead to image saturation and possibly cause photobleaching or phototoxicity of your sample.

    Tip: Try using **Auto** with a known bright sample, such as a positive control. Then, if needed, decrease the exposure about 10% to 20%.

13. Click  **Image Intensity Settings**.



14. On the **Image Intensity Settings** tab, adjust the settings as needed for each channel:
    a. Use the **Channel Color** drop-down list box to change the identification color for the channel. The default channel color is based on the emission wavelength of the selected stain.
    b. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image.
    c. Click **Auto** to set the best contrast based on the current preview.
    d. If you need to adjust the image intensity scale for all channels simultaneously, you can set **Synchronize Histograms** to **On**.

    Note: In most cases, however, you can set **Synchronize Histograms** to **Off**.

15. With an optional digital confocal license, do the following to apply the optional digital confocal algorithm to the image:
    a. Set **Digital Confocal** to **On**.
    b. As needed, move the slider to the right to smooth the image.
    c. As needed, move the slider to the left to sharpen the image.

    AutoQuant integrated innovation Digital confocal enhances contrast, improves resolution, and sharpens the image using the AutoQuant 2D RealTime Deconvolution algorithm. The algorithm calculates and inverts an ideal point spread function (PSF) for the optics based on image acquisition properties (not the actual image) such as the numerical aperture, refraction index, wavelength, and so on. The algorithm then applies the inverted PSF to the original image in Fourier space, which is a single multiplier (instead of a convolution). A Weiner filter uses a constant K value to correct noise in the image.

16. Do the following to reset the autofocus:
    a. In the bottom pane, set **Live Preview** to **Off**.
    b. In the bottom pane, set **Live Preview** to **On**.

    Note: This will autoscale the histogram. You may need to re-adjust the histogram scaling.

17. Click 

Choose Position to Acquire.
18. Use the focus joystick to find the best focus position for the current channel. As you adjust the offset, the focus position value updates.
    - For large focus adjustments, click and drag the focus joystick toward the outermost position.

    - For smaller focus adjustments, drag the focus joystick slightly.

    - For fine focus adjustments, click one of the joystick arrows. Each time you click an arrow, the focus adjusts one step. The step size corresponds to half of the depth of field for the selected objective.

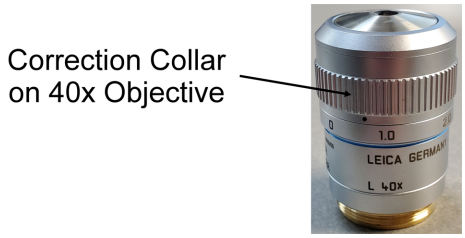
      

19. To save the focus offset for that channel click **Set Offset**. This setting is reflected by the value in the **Focus Offset** field on the **Focus/Exposure Settings** tab.
20. Repeat these steps as needed to find other regions of interest.
21. Repeat these steps as needed for each channel.

To continue to the next workflow step, click  **Region Selection to Acquire**. See Region Selection to Acquire on page 58 for details.

Adjusting an Objective Correction Collar

The 40x objective and 63x objective have application-optimized correction collars to compensate for well bottom thickness or coverslip thickness. The collars have a range of 0 mm to 2 mm correction. Changing this setting adjusts the distances between components inside the objective barrel. Image quality and resolution are very dependent on properly setting these collars.



The settings to be used depend on the well bottom thickness of the plate or the coverslip thickness on the slide on which the specimen is mounted. In general, set the correction collar for the physical thickness of the plate or slide that you are imaging. The physical thickness can be determined by the plate specifications from the plate manufacturer.

Note: Do not use a plate, slide, or coverslip with a thickness that is out of the range of the correction collar for the selected objective.

Observe the following when handling an objective:

CAUTION!

- To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling objectives and filter cubes.
- With the instrument power on, do not manually rotate the objective turret. Manually rotating the objective turret can damage the instrument.

You would typically adjust a correction collar as part of setting up an acquisition.

Note:

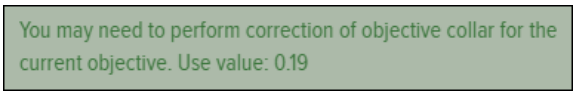
- When using the environmental control cassette with a 40x objective, you must adjust the objective correction collar an additional 0.7 mm above the recommended setting to account for the thickness of the cassette glass bottom. If the bottom thickness of the plate plus 0.7 mm is greater than the range of the 40x objective correction collar, then this plate is not compatible with this configuration. Try a different plate type, use a lower magnification objective, or image without the environmental control cassette.
- The 63x objective is not compatible with the environmental control cassette. Do not install the 63x objective when the environmental control cassette is loaded.

To adjust a correction collar for a plate:

1. On the **Acquisition Settings** page, on the right side of the screen under **Tools**, click 

Plate Format.
2. In the **Plate Format** list, select the plate format.
3. On the right side of the screen under **Tools**, click 

Objectives.
4. In the **Objectives** list, select the objective.
If a correction collar adjustment is required, the software displays the recommended setting.



Note: When using the environmental control cassette with a 40x objective, you must adjust the objective correction collar an additional 0.7 mm above the recommended setting to account for the thickness of the cassette glass bottom. If the bottom thickness of the plate plus 0.7 mm is greater than the range of the 40x objective correction collar, then this plate is not compatible with this configuration. Try a different plate type, use a lower magnification objective, or image without the environmental control cassette.

5. On the left side of the screen under **Steps**, click  **Acquisition Device**.
6. On the right side of the screen, click  **Set Up for Adjustment of Objective Collar**.
7. Click **OK**.
   The maintenance door opens.
8. If needed, loosen the objective from the instrument by gently turning it counterclockwise.
9. Rotate the correction collar to its new setting.

   Tip: You might need a flashlight to see the markings for the graduated scale on the barrel and its current setting.

10. If you loosened the objective, tighten it by gently turning it clockwise.

    Note: When tightening the objective, take care to avoid changing the correction collar setting.

11. Close the maintenance door.
12. On the right side of the screen under **Tools**, click  **Finish Adjustment of Objective Collar** to exit maintenance mode.
13. Click **OK**.

Understanding How Autofocus Works with a Plate

The instrument uses two autofocus mechanisms:

- **Detect Surface**, which is hardware autofocus. Hardware autofocus uses an LED beam to find reflective surfaces and is designed for speed. It works well for adherent samples in plates or chamber slides.
- **Find Best Plane**, which is image-based autofocus (also known as software autofocus). When enabled, image-based autofocus searches a range for the best focus plane based on image contrast. It works well for slides with a coverslip or for samples in a plate that are not flat, such as suspension cells or spheroids.

Detect Surface

The Detect Surface options use hardware autofocus to detect surfaces. The following autofocus options are available for plates:

Plate Bottom

Hardware autofocus detects the surface closest to the objective (that is, the plate bottom). It then applies the bottom thickness from the labware configuration as an offset to place the focus plane at the well bottom.

The Plate Bottom option is designed for samples in 3D matrices or clear mounted coverslips, where the second surface appears "invisible" to autofocus. It is also suitable for imaging thin-bottom plates with a low-magnification objective.

Well Bottom

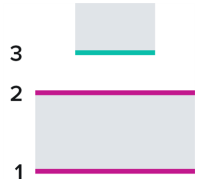
Hardware autofocus detects the two surfaces closest to the objective (that is, the plate bottom and well bottom). It sets the focus plane at the well bottom.

The Well Bottom option is designed for samples in a liquid medium, such as well plates or chamber slides. It is not recommended for samples in 3D matrices, thin-bottom plates with a low-magnification objective, or dry plates. It is not available with the 2.5x objective.

Well Insert

Hardware autofocus detects the three surfaces closest to the objective (that is, the plate bottom, well bottom, and well insert). It then sets the focus plane at the bottom of the well insert membrane.

The Well Insert option is designed for well inserts in well plates or any labware design that has a distinct third surface.

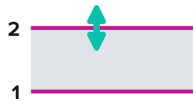


Note: To detect the plate bottom with the environmental control cassette loaded, the software adjusts the autofocus range to ignore the cassette glass bottom.

Find Best Plane

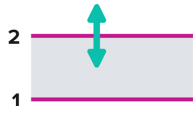
If the Detect Surface options do not provide satisfactory focus, select the **Find Best Plane** option to add image-based autofocus for the first channel within the selected search range. Adding image-based autofocus is useful for thicker samples, samples with variable best focus planes, or labware with variable thickness.

Normal Search Range



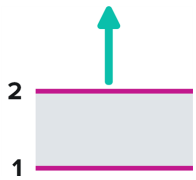
The Normal Search Range option is centered on the surface selected by the hardware autofocus. It searches a range around that surface of 15% of the Bottom Thickness value from the labware specification (that is, 7.5% above the surface and 7.5% below it).

Wide Search Range



The Wide Search Range option is centered on the surface selected by the hardware autofocus. It searches a range of 40% of the Bottom Thickness value from the labware specification (that is, 20% above the surface and 20% below it).

Superwide Search Range



The Superwide Search Range option uses the surface selected by the hardware autofocus and searches a range of 300 µm above that surface (unlike the other options, which search around the surface).

Autofocus Strategies for Plates

To find the best focus for a plate, start with the default **Well Bottom** option. With imaging-quality labware, an accurate labware specification, and a relatively flat sample (for example, most adherent cells), autofocus should provide good results.

If you are unsatisfied with the focus and your sample is thick or uneven, turn on image-based autofocus (by selecting the **Find Best Plane** check box) and select the **Normal Search Range** option. If this does not improve the result, use the **Wide Search Range** option or **Superwide Search Range** option to increase the search range.

After you select the focus option, click **Auto** next to the **Focus Offset** controls to determine the focus offset between stains. Do this for each stain in the acquisition. Image-based autofocus bases its search on the first stain in the acquisition, so consider the order in which stains are acquired. See Acquisition Settings on page 21 for details.

Autofocus Strategies for Mimetas OrganoPlates

Mimetas OrganoPlates have special characteristics. These microfluidic plates have multiple surfaces close to the well bottom, which can make them difficult for hardware autofocus to differentiate. To address this situation, make sure that the **Bottom Thickness** value in the labware specification for the default Mimetas OrganoPlate matches the manufacturer's specification.

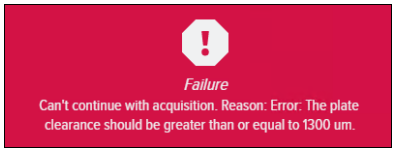
To find the best focus for a Mimetas OrganoPlate, select the **Plate Bottom** option (with image-based autofocus off). Then, change the **Focus Offset** value on the **Focus/Exposure Settings** tab until you find the desired focus plane.

Using the Environmental Control Cassette with a Plate

To find the best focus for a plate in the optional environmental control cassette, start with the default **Well Bottom** mode. With imaging-quality labware, an accurate labware specification, and a relatively flat sample (for example, most adherent cells), autofocus should provide good results.

The environmental control cassette increases the distance between the sample and the objective. It is possible that a plate with a high skirt height or thick well bottom may elevate the sample beyond the working distance of higher magnification objectives. In this case, try a different plate type. See the *ImageXpress Pico User Guide* for details on the working distance of each objective.

Conversely, the skirt height of some plates may be too low. If you are using the environmental control cassette and autofocus fails, the software may display the following message:



In this case, you may need to insert the plate skirt height adapter and select a plate format that adds 1.5 mm to the actual **Plate Height** value. See the *ImageXpress Pico User Guide* for details on inserting the plate skirt height adapter. See Labware Library on page 217 for details on adding a labware specification.

Using the Environmental Control Cassette with the 2.5x Objective

When using the environment control cassette with the 2.5x objective, autofocus may fail or be inconsistent. We recommend using a plate with a high clearance or using the plate skirt height adapter. See the *ImageXpress Pico User Guide* for details on inserting the plate skirt height adapter.

If you are still unable to achieve good focus, try using the 4x objective.

Labware Specification Settings

While all settings in the labware specification are important, the following settings play a large role in achieving good autofocus:

- **Clearance** is important for all focus options. It determines the search range used to detect the plate bottom.
- **Bottom Thickness** is important when using image-based autofocus. It determines the base focus search range around the well or chamber bottom where the sample is located. If the Bottom Thickness value is too low or too high, the search range will not include the sample, and the instrument will not be able to find a focus plane.
- **Well Diameter** is important when imaging whole wells. An accurate setting ensures that the search range includes sites on the edge or outside of the well.

If autofocus issues occur, you may need to adjust the **Bottom Thickness** and **Well Diameter** settings. (The **Clearance** setting is calculated and cannot be adjusted.) See Labware Library on page 217 for details.

Labware Quality

The ideal plate for imaging features black well sides and a single-piece, thin, glass bottom, which provides optimal autofocus performance and image quality. A plastic-bottom plate will also work well as long as the variability in the bottom thickness is not too high. If you experience autofocus issues with a plastic-bottom plate, try using a glass-bottom plate or a different plastic-bottom plate.

See the *ImageXpress Pico User Guide* for details on selecting the best plate for your assay.

Anchoring the Focus Position

When you are satisfied with the quality of the image preview, you can select the **Anchor Focus Position** check box on the **Focus/Exposure Settings** tab of the **Acquisition Settings** page to save current focus position and disable the autofocus controls. The instrument will no longer run autofocus each time you snap a preview image or acquire an image. Instead, the software will use the saved focus position across all sites, which speeds up acquisition. For best results, use the 2.5x objective or 4x objective. In addition, ensure that both your plate and your sample are relatively flat.

See Snapping a Preview of a Well on page 24 for details.

Troubleshooting Autofocus Issues

The CellReporterXpress software uses hardware and image-based autofocus to find the best focus for the sample. When autofocus issues occur, they are typically caused by an incorrect plate holder configuration in the Labware Library. In many cases, the issue can be addressed by measuring plate dimensions and adjusting the plate holder configuration. See Labware Library on page 217 for details.

The **Autofocus Info** graph on the **Acquisition Settings** page shows the focus peaks for the most recent autofocus to help you understand how it was determined.

Note: With the environmental control cassette loaded, the software adjusts the autofocus range so that no peak appears for the cassette glass bottom.

This section describes how you can use this information to troubleshoot autofocus issues.

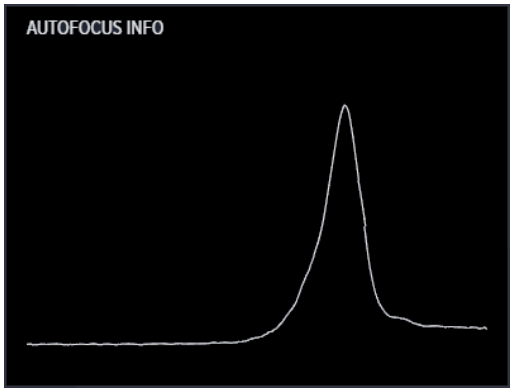
Tip: Molecular Devices Technical Support has compiled information that can help you diagnose and resolve autofocus issues. For details, log in to the Molecular Devices Knowledge Base at support.moleculardevices.com, and search for **ImageXpress Pico: Focus Troubleshooting**.

To troubleshoot autofocus issues using the Autofocus Info graph:

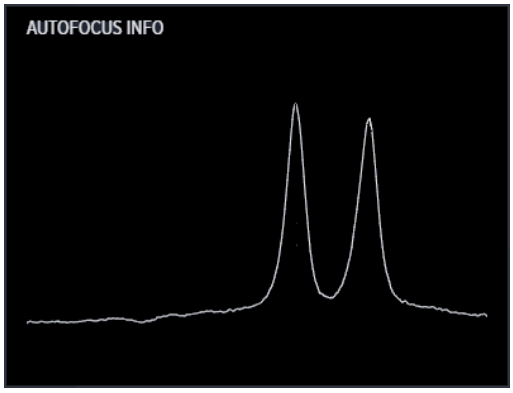
1. On the **Acquisition Settings** page, click **Snap Image** to snap a preview image.
2. On the right side of the screen under **Tools**, click **Autofocus Info** to display a graph showing the focus peaks of the autofocus.
3. Review the **Autofocus Info** graph along the following examples.

Good Autofocus

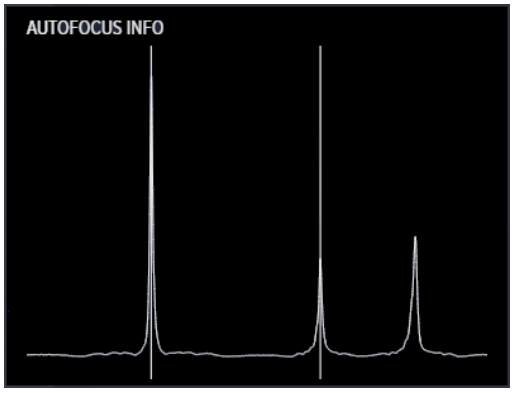
The following graph shows a good result of a single-peak autofocus (Plate Bottom option selected). Single-peak autofocus is typically used for thin bottom plates with a 2x objective or 4x objective.



The following graph shows a good result of a two-peak autofocus (Well Bottom option selected).

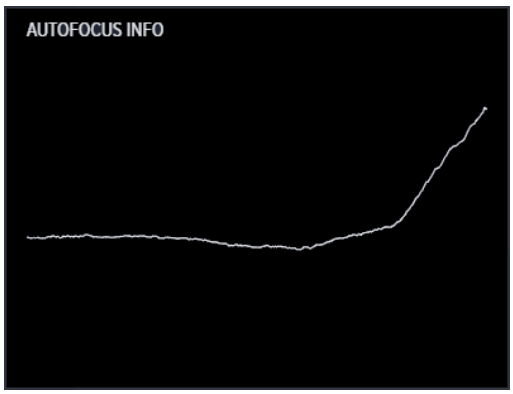


The following graph shows a good result of a three-peak autofocus (Well Insert option selected).

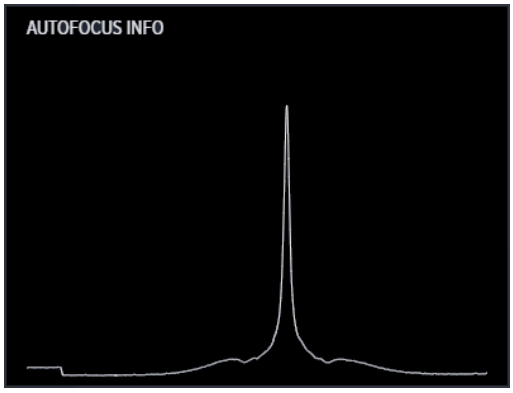


Autofocus Issues

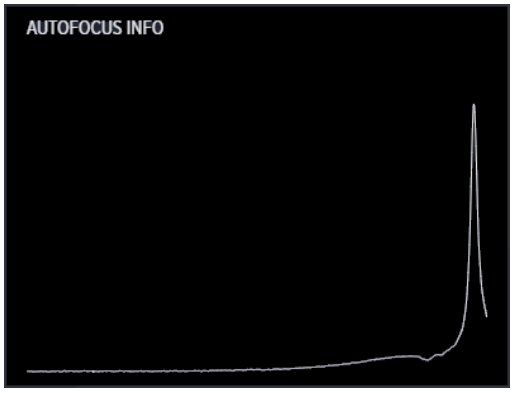
The following graph shows a failed result of a single-peak autofocus (Plate Bottom option selected). The incomplete single peak on the right side of the graph indicates that the value specified for the Well Depth value may be too high or the Clearance value (which measures skirt height) may be too low. See Labware Library on page 217 for details.



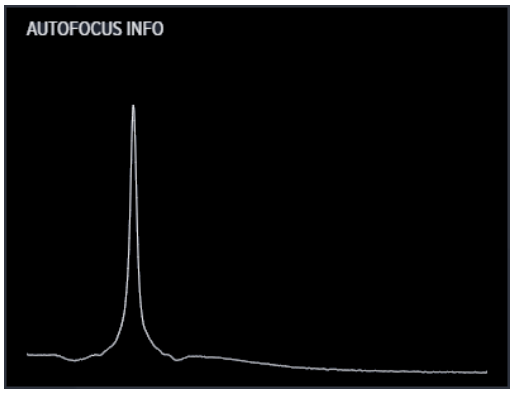
The following graph shows a failed result of a two-peak autofocus (Well Bottom option selected), where you would expect to see two peaks on the graph. The single peak in the center indicates that the Bottom Thickness value may be too small or too high. See Labware Library on page 217 for details.



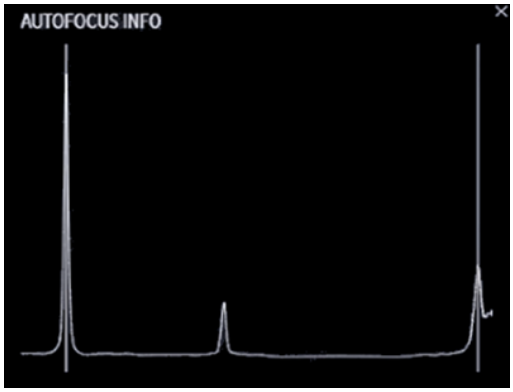
The following graph shows a failed result of a two-peak autofocus (Well Bottom option selected). The single peak on the right side of the graph indicates that the value specified the Well Depth value may be too high or the Clearance value (which measures skirt height) may be too low. See Labware Library on page 217 for details.



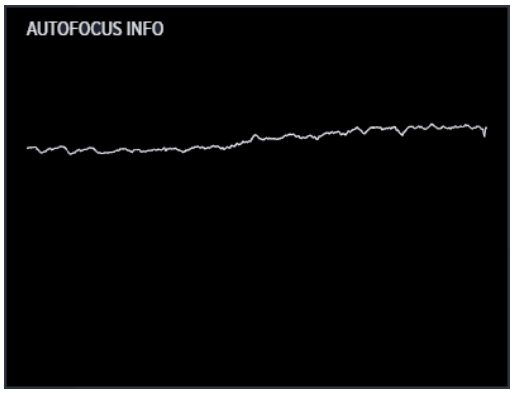
The following graph shows a failed result of a two-peak autofocus (Well Bottom option selected). The single peak on the left side of the graph indicates that the value specified the Well Depth value may be too low or the Clearance value (which measures skirt height) may be too high. See Labware Library on page 217 for details.



The following graph shows a failed result of a two-peak autofocus (Well Bottom option selected). The third peak on the right indicates that the meniscus from the low liquid levels is interfering with the autofocus. Confirm that the wells are at least halfway filled with media.



The following graph shows a general autofocus failure. No peaks are found, which likely indicates that there is no plate in the labware holder or the labware holder is not inserted correctly. It is also possible that the wrong labware is selected. If these are not true, remeasure the plate with no sample in it. See Labware Library on page 217 for details.



Region Selection to Acquire

Region Selection to Acquire is the step where you select the region of the well to be acquired. The page shows a representation of a well with a region selection overlay. You must select at least one region to run an experiment.

You can change the region selection area by resizing and moving the overlay. If needed, you can set multiple region selection overlays.

Note: Several factors (including the number of wells, the magnification of the objective, and the use of the environmental control cassette) may prevent you from selecting some regions within certain wells.

The right side of the page includes the following icons:

From Center: Adds an acquisition region selection overlay in the center of the well. You can control various elements of the acquisition region, including the percentage of the well and the shape of the selection overlay.

Activate Edit Mode: Activates the selection handles on the acquisition region selection overlay, enabling you to manually move and size it.

Add Acquisition Region: Adds a new acquisition region selection overlay that you can size and move into position.

Delete Selected Region: Removes the selected region overlay.

Clear All Regions: Removes all the visible region overlays.

Toggle Actual Area to Capture: Shows what the selected camera objective will snap based on the field of view for the lens. You may need to adjust the region selection or the objective selection based on this area.

To continue to the next workflow step, click  **Analysis Settings**. See Analysis Settings on page 59 for details.

Analysis Settings


Analysis Settings is the step where you set up image analysis for the experiment. The data generated based on these settings becomes the analysis data for the experiment.

The right side of the page includes the following icons:


Choose Analysis: Toggles analysis on or off and selects the analysis for the experiment.


Measurements: Specifies the cell measurements included in the analysis.


Save Analysis: Saves the analysis for use in future experiments.


Cell Info Mode: Displays information on a selected cell.


Comparison Mode: Captures two preview images, which enables you to compare the uniformity of the image quality.

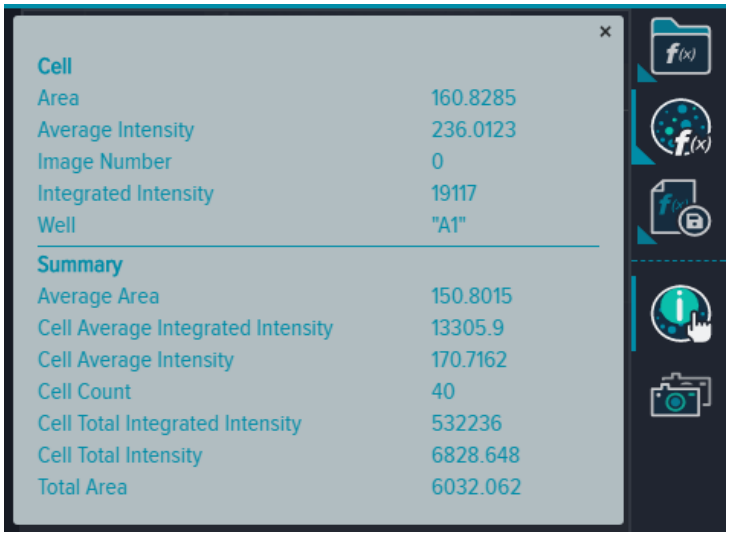
Setting Up an Analysis

To set up an analysis:

1. On the **Analysis Settings** page, in the **Tools** pane on the right, click **Choose Analysis**.
2. Set **Analysis** to **On**.
3. Select a fluorescence or transmitted light analysis. See Analysis Descriptions on page 263 for details on the available analyses.
4. Click **Measurements**.
5. In the **Measurements** pane, select the measurements for the analysis.

   Note: The recommended measurements for the analysis are selected by default.

6. In the bottom pane, click **Test Analysis** to calculate the summary measurements using the preview image.
7. To view cell information, click **Cell Info Mode** and select a detected cell in the image preview.



As part of configuring analysis settings, you may do the following:

- Testing the Analysis of a Well, see page 61
- Testing the Analysis of Comparison Images, see page 62
- Saving Analysis Settings, see page 63

To continue to the next workflow step, click **Region Selection to Analyze**. See Region Selection to Analyze on page 64 for details.

Testing the Analysis of a Well

The preview represents the image quality to expect when you run your experiment.

To test the analysis of a well:

1. On the **Acquisition Analysis** page, in the bottom pane, click  **Test Analysis**.
2. Click  **Algorithm Input**.
3. On the **Algorithm Input** tab, adjust the settings as needed to optimize object detection. Settings vary depending on the selected analysis type. Setting options typically control intensity, minimum width, and maximum width for each stain used in the acquisition.
4. Click  **Test Analysis** to preview the analysis.
5. If needed, click  **Image Intensity Settings** and do the following to adjust the settings as needed for each channel:

   Note: You typically do not need to adjust the image intensity settings for the analysis, which are based on the acquisition settings. If you adjust these settings, it affects only the image display; it has no effect on any analysis measurements.

   a. Use the **Channel Color** drop-down list box to change the identification color for the channel.
   b. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image.
   c. Click **Auto** to set the best contrast based on the current preview.
6. Click  **Choose Position to Acquire**.
7. Click  **Select Well**.
8. In the plate map, select a different well.
   The software runs a test analysis.
9. In the single well map, click and drag the  selection tool to select the region of the well for the preview, if needed. The center area of the well is selected by default.
   The software runs a test analysis.
10. Click  **Algorithm Input**.
11. Click  **Test Analysis** to preview the analysis.
12. Repeat these steps as needed until you are satisfied with the quality of the preview.

After you are satisfied with the quality of the preview, you may want to save the analysis settings for later reuse. See Saving Analysis Settings on page 63 for details.

Testing the Analysis of Comparison Images

You can preview two images from different wells to compare the uniformity of the image quality.

To test the analysis of comparison images:

1. On the **Acquisition Analysis** page, in the **Tools** pane on the right, click  **Comparison Mode**.
2. In the bottom pane, click  **Test Analysis**.
3. Click  **Algorithm Input**.
4. On the **Algorithm Input** tab, adjust the settings as needed to optimize object detection. The settings affect both previews. Settings vary depending on the selected analysis type. Setting options typically control intensity, minimum width, and maximum width for each stain used in the acquisition.
5. On the left side of the pane, click the  **Choose Well and Area to Acquire** tab.
6. Click  **Select Well** on the left.
7. In the well map, select the first well for the preview.

8. Click  **Select Well** on the right.
9. In the well map, select the second well for the preview.
10. In the single well map on the left, click and drag the  selection tool to select the region of the first well for the preview, if needed. The center area of the well is selected by default.
11. Repeat the previous step in the single well map on the right to select the region of the second well for the preview, if needed.
12. Click  **Test Analysis** to preview the analysis.

13. If needed, click  **Image Intensity Settings** and do the following to adjust the settings as needed for each channel:

    Note: You typically do not need to adjust the image intensity settings for the analysis, which are based on the acquisition settings. If you adjust these settings, it affects only the image display; it has no effect on any analysis measurements.

    a. Use the **Channel Color** drop-down list box to change the identification color for the channel.
    b. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image.
    c. Click **Auto** to set the best contrast based on the current preview.
14. Repeat these steps as needed until you are satisfied with the quality of the previews.

After you are satisfied with the quality of the preview, you may want to save the analysis settings for later reuse. See Saving Analysis Settings on this page for details.

Saving Analysis Settings

When you are satisfied with the quality of the preview in Testing the Analysis of a Well on page 61 or Testing the Analysis of Comparison Images on page 62, you may want to save the analysis settings for later reuse.

To save analysis settings:

1. On the **Acquisition Analysis** page, in the **Tools** pane on the right, click  **Save Analysis**.
2. In the **Save Analysis** pane, in the **Analysis Settings** field, enter a descriptive name.
3. If needed, add an avatar image by doing one of the following:
   - Click **Use Captured Picture**.
   - Click **Click to upload**, select an image file, and click **Open**.
4. Click **Save**.

To continue to the next workflow step, click  **Region Selection to Analyze**. See Region Selection to Analyze on page 64 for details.

Region Selection to Analyze

Region Selection to Analyze is the step where you select the region of interest to be analyzed. You must select at least one region to run an experiment.

Note: Several factors (including the number of wells, the magnification of the objective, and the use of the environmental control cassette) may prevent you from selecting some regions within certain wells.

The right side of the page includes the following icons:

From Center: Adds an analysis region selection overlay in the center of the well or slide. You can control various elements of the analysis region, including the percentage of the well and the shape of the selection overlay.

Activate Edit Mode: Activates the selection handles on the acquisition region selection overlay, enabling you to manually move and size it.

Add Analysis Region: Adds a new analysis region selection overlay that you can size and move into position.

Delete Selected Region: Removes the selected region overlay.

Clear All Regions: Removes all the visible region overlays.

To continue to the next workflow step, click  **Well Selection**. See Well Selection on page 65 for details.

Well Selection


Well Selection is the step where you select the well for the experiment.

The **Well Selection** page shows a map for the labware selected in the **Acquisition Settings** step. By default, no wells are selected. Select and deselect wells as needed. You must select at least one well to run an experiment.

The right side of the page includes the following icons:


Select All: Selects all wells.


Clear All Regions: Removes all well selections.


Note: Several factors (including the number of wells, the magnification of the objective, and the use of the environmental control cassette) may prevent you from selecting some regions within certain wells.

Selecting a Group of Wells

On the **Well Selection** page, in the well map, click and drag to select a series of well.

Selecting Individual Wells

On the **Well Selection** page, in the well map, click a well to select it.

Deselecting Individual Wells

On the **Well Selection** page, in the well map, click a selected well to deselect it.

To continue to the next workflow step, click 
Device Sensors. See Device Sensors on page 66 for details.

Device Sensors

Device Sensors is the step where you specify the environmental sensors to be monitored for the experiment. The experiment data indicates if the sensors were within the specified range during the experiment.

With the optional environmental control system and the environmental control cassette in the instrument, you can monitor the temperature inside the environmental control cassette up to 40°C (104°F). You can also monitor the humidity level, CO_2 level, and O_2 level within the environmental control cassette.

Without the environmental control cassette in the instrument, you can monitor only the temperature inside the instrument up to 40°C (104°F).

See Sensors on page 231 for details on regulating temperature, humidity level, CO_2 level, and O_2 level.

Monitoring the CO_2 Level Sensor

To monitor the CO_2 level sensor:

1. On the **Device Sensors** page, in the **CO_2 Level** row, in the **Min** field, enter the lower limit for the CO_2 level range as a percentage.
2. In the **Max** field, enter the upper limit for the CO_2 level range as a percentage.

   Note: The lower limit and upper limit values must be within the range of 1% to 15%.

3. Set **Monitor** to **On**.

Monitoring the Humidity Level Sensor

The humidity level monitor range is fixed at 75% to 95%.

To monitor the humidity level sensor:

On the **Device Sensors** page, in the **Humidity Level** row, set **Monitor** to **On**.

Monitoring the O_2 Level Sensor

To monitor the O_2 level sensor:

1. On the **Device Sensors** page, in the **O_2 Level** row, in the **Min** field, enter the lower limit for the O_2 level range as a percentage.
2. In the **Max** field, enter the upper limit for the O_2 level range as a percentage.

   Note: The lower limit and upper limit values must be within the range of 1% to 15%.

3. Set **Monitor** to **On**.

Monitoring the Temperature Sensor

To monitor the temperature sensor:

1. On the **Device Sensors** page, in the **Temperature** row, in the **Min** field, enter the lower limit value for the temperature range in degrees Celsius.
2. In the **Max** field, enter the upper limit value for the temperature range in degrees Celsius.

   Note: The lower limit and upper limit values must be within the range of 25°C to 40°C (77°F to 104°F).

3. Set **Monitor** to **On**.

To continue to the next workflow step, click  **Time Series**. See Time Series on page 68 for details.

Time Series

Time Series is an optional step where you set up a time series for image acquisition. This enables you to acquire images at multiple time points.

Note: The software does not support adjusting an environmental control setting during a time series acquisition. If your experiment requires this, perform a discontinuous time series by acquiring the first set of time points, adjusting the humidity level, CO_2 level, O_2 level, or temperature as needed, and then acquiring the next set of time points. See Sensors on page 231 for details.

To set up a time series:

1. On the upper left of the **Time Series** page, set the toggle switch to **On**.
2. In the **Acquisition Order** section, click one of the following icons to indicate the order in which wells will be acquired for your time series:

All Wells: Performs a complete time series on all selected wells. This option requires the least amount of time because all selected wells are included in each time series. This means that the fewest number of time series are performed.

Per Column: Performs a complete time series on all selected wells in a column, then moves on to the next column. The leftmost column is acquired first. This option requires more time because the time series is repeated for each selected column.

Per Row: Performs a complete time series on all selected wells in a row, then moves on to the next row. The topmost row is acquired first. This option requires more time because the time series is repeated for each selected row.

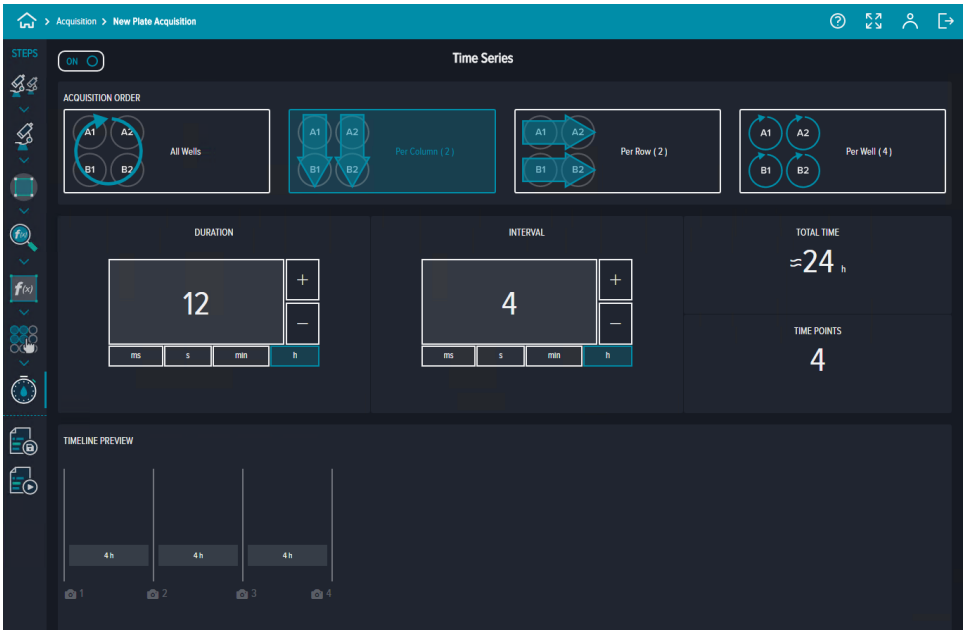
Per Well: Performs a complete time series on each selected well, then moves on to the next well. This option requires the most time because the time series is repeated for each selected well.

3. In the **Duration** section, do the following to specify the duration of your time series:
   a. Click in the value field and enter a value.
   b. Click the appropriate unit.
4. In the **Interval** section, do the following to specify the interval between the time points in your time series:
   a. Click in the value field and enter a value.
   b. Click the appropriate unit.

   The software displays the recommended interval, which is based on several factors, including the following:
   - Number of wells and the number of regions per well
   - Stain
   - Exposure time
   - Acquisition order

   Tip: For best results, specify at least the recommended interval. If you specify a lower interval, the software performs back-to-back acquisitions using the specified acquisition order. The actual interval will likely be greater than the specified interval.

   The software displays the total time required for the time series and the number of time points to be acquired for each selected well, along with a visual representation of the time series.



5. Review the time series settings.
6. If needed, repeat these steps to adjust the time series settings.

To continue to the next workflow step, click either **Save Protocol** or **Run Protocol**. See Save Protocol on page 70 or Run Protocol on page 71 for details.

Save Protocol

 **Save Protocol** is an optional step where you can save the protocol you have created. You will typically save a protocol only when you intend to run it frequently. After you save a protocol, it appears as a card in the Protocol library.

Tip: We recommend creating protocols sparingly to avoid unnecessarily filling your Protocol library, which can make it difficult to find a protocol.

The right side of the page includes the following icon:

 **Lock Protocol**: Manages the ability of other users to modify the protocol.

Note: Other users are not prevented from viewing or running a locked protocol.

To save a protocol:

1. On the **Save Protocol** page, in the **Protocol Name** field, enter a name for the protocol.
2. (Optional) In the **Protocol Description** field, enter a description of the protocol.
3. Do the following to restrict other users from modifying the protocol:
   a. On the right side of the screen, click  **Lock Protocol**.
   b. Select the **Private** check box to prevent other users from modifying the settings on the **Acquisition Device**, **Acquisition Settings**, **Analysis Settings**, and **Time Series** pages for the protocol.
   c. Select the **Lock region selection** check box to prevent other users from modifying the settings on the **Region Selection to Acquire** and **Region Selection to Analyze** pages.
   d. Select the **Lock well selection** check box to prevent other users from modifying the settings on the **Well Selection** page.

      Note: The **Lock region selection** check box and **Lock well selection** check box are not enabled until you select the **Private** check box.

   e. To specify other users who can modify a locked protocol, click the **Share With** field and select users from the list.

      Tip: You can set default sharing permissions in  **Configuration Settings**. See Sharing Permissions on page 261 for details.

4. Click **Save Protocol**.

To continue to the next workflow step, click  **Run Protocol**. See Run Protocol on page 71 for details.

Run Protocol


Run Protocol is the last step in the workflow. In this step, you can review the settings for the experiment and address any issues.

When you run the experiment, the software acquires images and analyzes the data according to your settings. When the experiment completes, the software saves all acquired data as specified. Acquired data is then available for viewing and analysis in **Experiments** mode.

The right side of the page includes the following icons:


Experiment Details: Displays acquisition and analysis settings and enables you to validate settings before running the experiment.


Storage: Specifies image storage location during and after acquisition.


Public and 
Private: Manages the shared status of the experiment.


Open Plate Door: Opens the top door on the selected instrument so that you can insert or remove labware.


Close Plate Door: Closes the top door.


Run Experiment: Runs the experiment using the specified acquisition and analysis settings. This icon becomes enabled when all the settings on the **Validation** tab in the **Experiment Details** pane are valid and an **Experiment Name** has been entered.

To run a protocol:

1. On the **Run Protocol** page, do the following:
   a. In the **Experiment Name** field, enter a name to identify the experiment in the Experiments library.
   b. If needed, in the **Barcode** field, enter the barcode for the labware.
   c. If needed, in the **Experiment Description** field, enter a description of the experiment.
   d. To save TIFF files of the acquisition images, select the **Save Raw Images** check box.
   e. To export TIFF files of the acquisition images for use with the Molecular Devices IN Carta® Image Analysis Software, select the **Export to IN Carta** check box.

   Note: If you select the **Export to IN Carta** check box, the images of the acquisition will be cropped as required by the IN Carta software. With colorimetric images, each image will be separated into three, 16-bit component images (that is, one red, one green, and one blue). In addition, the export will include the required XDCE file.

   f. On the **Validation** tab, verify that all the required settings are valid. A  icon indicates a valid setting and a  icon indicates an invalid or missing setting. All acquisition settings must be valid to run the experiment. See Fixing Invalid Parameters on page 73 for details.
   g. On the **Acquisition Parameters** tab, review the settings.
   h. On the **Analysis Parameters** tab, review the settings.
2. If you want to review the settings for image storage during and after acquisition, do the following:
   a. Click  **Storage.**
   b. In the **Available Temporary Storage on Device** field, specify the drive for temporary image storage during acquisition. See the *ImageXpress Pico User Guide* for details on adding external temporary storage.
3. If you want to manage the shared status of the experiment to restrict other users from viewing it, do the following:
   a. Click  **Public**.
   b. Select the **Private** check box.
   c. If you want to specify other users who can view a private experiment, click the **Share With** field and select users from the list.

   Tip: You can set default sharing permissions in  **Configuration Settings**. See Sharing Permissions on page 261 for details.

4. If you have not already done so, do the following to insert your experiment-ready labware into the instrument:
   a. Click  **Open Plate Door** to open the top door on the instrument.
   b. Insert your experiment-ready labware into the instrument. See the *ImageXpress Pico User Guide* for details.
   c. Click  **Close Plate Door** to close the top door on the instrument.
5. Click  **Run Experiment** to run the experiment.

The **Monitor** page opens to display the progress of the running experiment. See Monitor Mode on page 213 for details.

Fixing Invalid Parameters

Invalid parameter settings are indicated by a  icon. Click the icon to display the reason for the invalid parameter.

To fix an invalid parameter:

Tip:
- If Data Temp Storage is insufficient, consider adding external temporary storage. See the *ImageXpress Pico User Guide* for details.
- If Data Storage is insufficient, consider adding more storage to the host computer or (in a server configuration) adding a remote storage computer. See the *CellReporterXpress Installation Guide* for details on setting up a server configuration.

1. Click the link next to the  icon to open the workflow step for the invalid parameter.
2. Address the issue.
3. Click  **Run Protocol** to return to **Run Protocol** page.

Slide Acquisition Workflow

Select a slide template to begin the slide acquisition workflow. The **New Slide Acquisition** template is an unrestricted template that allows you to select from all slide experiment settings options. Other slide templates may offer fewer or restricted options.

The icons in the **Steps** pane on the left side of the page guide you through the slide experiment configuration process. The tools and controls in the pane on the right side of the page vary according to the step being configured and the experiment type.

Note: Depending on the selected template, some steps, tools, and options may not appear or may not be available. Use the **New Slide Acquisition** template to access all steps, tools, and options.

The slide acquisition workflow is as follows:


Acquisition Device is the first step for all acquisition workflows. In this step, you select the instrument for the acquisition and insert your experiment-ready labware. See Acquisition Device on page 76 for details.


Acquisition Settings is the step where you set up image acquisition for the experiment, including the stains and objective to be used. You can preview the image capture and adjust channel identification color, histogram, focus, and exposure settings. See Acquisition Settings on page 78 for details.


Region Selection to Acquire is the step where you select the region of the slide to be acquired. The page shows a representation of a slide. You must select at least one region to run an experiment. See Region Selection to Acquire on page 112 for details.


Analysis Settings is the step where you set up image analysis for the experiment. The data generated based on these settings becomes the analysis data for the experiment. This step is not included in the slide workflow for a colorimetric or stitched acquisition. See Analysis Settings on page 113 for details.

 **Region Selection to Analyze** is the step where you select the region of interest to be analyzed. You must select at least one region to run an experiment. This step is not included in the slide workflow for a colorimetric or stitched acquisition. See Region Selection to Analyze on page 118 for details.

 **Device Sensors** is the step where you specify the environmental sensors to be monitored for the experiment. The experiment data indicates if the sensors were within the specified range during the experiment. See Device Sensors on page 119 for details.

 **Save Protocol** is an optional step where you can save the protocol you have created. You will typically save a protocol only when you intend to run it frequently. After you save a protocol, it appears as a card in the Protocol library. See Save Protocol on page 120 for details.

 **Run Protocol** is the last step in the workflow. In this step, you can review the settings for the experiment and address any issues. See Run Protocol on page 121 for details.

Acquisition Device


Acquisition Device is the first step for all acquisition workflows. In this step, you select the instrument for the acquisition and insert your experiment-ready labware. See the *ImageXpress Pico User Guide* for details on inserting labware into the instrument.

The right side of the page includes the following icons:


Shutdown Device: Prepares the software to power off the selected instrument.


Restart Device: Restarts the selected instrument.


Open Plate Door: Opens the top door on the selected instrument so that you can insert or remove labware.


Close Plate Door: Closes the top door.


Set Up for Adjustment of Objective Collar: Moves the objective turret so that you can adjust the correction collar on the selected objective.


Finish Adjustment of Objective Collar: Moves the objective turret back into position after you adjust a correction collar.

The software displays a tile for each available instrument. See Adding an Instrument to the My Devices List on page 223 for details on adding other instruments in your network.

For each instrument, a status indicator shows one of the following:

Online

Offline or **Incompatible**

Busy

Error

In addition, the following information appears for each instrument:

- Instrument Name
- Instrument Serial Number
- Device Model
- Version
- Free Space
- Number of Installed Objectives
- Number Installed Filter Cubes
- Digital Confocal License

Note: If you click the **Favorite** icon for a device, that device will be selected by default. Otherwise, the last used device is default.

To select an acquisition device:

On the **Acquisition Device** page, select the instrument you want to use.

The selected device is highlighted.

To continue to the next workflow step, click **Acquisition Settings**. See Acquisition Settings on page 78.

Acquisition Settings

Acquisition Settings is the step where you set up image acquisition for the experiment, including the stains and objective to be used. You can preview the image capture and adjust channel identification color, histogram, focus, and exposure settings.

The right side of the page includes the following icons:

Slide Format: Specifies the labware specification for the acquisition.

Stains: Specifies the stains for the acquisition.

Objectives: Specifies the objective for the acquisition.

Autofocus Info: Shows the focus peaks for the most recent autofocus to help you understand how it was calculated and troubleshoot focus issues. See Troubleshooting Autofocus Issues on page 110 for details.

Comparison Mode: Captures two preview images, which enables you to compare the uniformity of the image quality or compare settings on two different phenotypes (such as positive and negative controls). See Snapping Slide Comparison Previews on page 87 for details.

Download Plane Images: Downloads TIFF images of the current preview. Without Z stack images, the downloaded image is the current preview. With Z stack images, the downloaded image is determined by the current selection: either the Z stack projection or an individual Z stack plane.

Download Z Stacking Images: With Z stack images, downloads a TIFF image of the current Z stack projection and each individual Z stack plane.

Specifying the Slide Format

To achieve good autofocus results, it is very important to specify an appropriate and accurate slide format for the labware you are using.

Note: Autofocus may not provide good results when using the 2.5x objective with the default **4 Slide Holder Coverslip Up** format. You may need to define a new slide format specification with an increased **Support Ledge Height** value. See Labware Library on page 217 for details.

To specify the slide format:

1. On the **Acquisition Settings** page, in the **Tools** pane on the right, click  **Slide Format**.
2. In the **Slide Format** pane, select the slide format you want to use.

   Note: If an appropriate slide format does not exist, click **Add New Slide** to define a new labware specification. See Labware Library on page 217 for details.

3. If your slide has a frosted area at one end, in the **Slide Frost Area** section, enter details on the size and position of the frosted area.

Specifying the Stains

To specify the stains:

1. On the **Acquisition Settings** page, in the **Tools** pane on the right, click  **Stains**.
2. In the **Stains** pane, select the stains you want to acquire.
3. Click  **Move Stain Up** and  **Move Stain Down** as needed to select the order in which the stains will be acquired.

Tip:

- For an acquisition with transmitted light, acquire the transmitted light stain first or last. If you plan to perform transmitted light segmentation, you will likely want to set the transmitted light stain to be acquired first.
- Avoid acquiring Cy5 as the first stain as it can interfere with the hardware autofocus.
- DAPI is typically the best choice for first stain, if you are acquiring that stain.

Specifying the Objective

To specify the objective:

1. On the **Acquisition Settings** page, in the **Tools** pane on the right, click 

Objectives.
2. In the **Objectives** pane, select the objective you want to use.

As part of configuring acquisition settings, you may do the following:

- Snapping a Preview of a Slide, see page 81
- Snapping Slide Comparison Previews, see page 87
- Snapping Z Stack Images, see page 93
- Viewing a Live Preview of a Slide, see page 98
- Adjusting an Objective Correction Collar, see page 104
- Understanding How Autofocus Works with a Slide, see page 106
- Troubleshooting Autofocus Issues, see page 110

To continue to the next workflow step, click  **Region Selection to Acquire**. See Region Selection to Acquire on page 112 for details.

Snapping a Preview of a Slide

You can view a preview of the acquisition by snapping an image. The preview uses the selected objective, stain, slide, region, focus settings, exposure settings, and histogram.

After snapping the preview, you can use the icon on the right side of the page to do the following:

 **Download Plane Images**: Downloads TIFF images of the current preview. Without Z stack images, the downloaded image is the current preview. With Z stack images, the downloaded image is determined by the current selection: either the Z stack projection or an individual Z stack plane.

Use the image viewer controls as needed to view the preview:

 Toggles the available channels for the preview by clicking on the channel icons. The icons for visible channels are brighter and have a white border. “Hidden” channels are dimmed and do not have a white border.

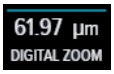
Tip: When a channel is overexposed, the overexposure indicator appears. Lower the **Exposure** value as needed.

 Zooms in on the image.

 Zooms out on the image.

 Resets the view zoom to the original image size.

 Views the image full screen.



Indicates the current zoom scale. **Digital Zoom** indicates that the image is zoomed in digitally (that is, not optically) and might not represent the actual quality of the image.

To snap a preview of a slide:

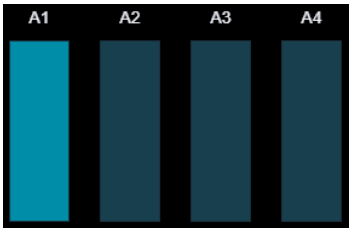
1. On the **Acquisition Settings** page, click  **Snap Image** to snap an initial preview image. A thumbnail of the preview image appears in the **History** pane.

Note:

- If autofocus fails, the software may display the following message:

  In this case, check the Autofocus Info graph to review the focus peaks for the most recent autofocus to understand how the system determined focus. See Troubleshooting Autofocus Issues on page 110 for details.
- Molecular Devices Technical Support has compiled information that can help you diagnose and resolve autofocus issues. For details, log in to the Molecular Devices Knowledge Base at support.moleculardevices.com, and search for **ImageXpress Pico: Focus Troubleshooting**.

2. At the bottom of the screen on the  **Choose Position to Acquire** tab, click  **Select Slide**.
3. In the slide holder map, select the slide for the preview. The instrument snaps a preview image of the newly selected slide. The **A1** slide is selected by default.



4. Click  **Snap Overview** to snap a low-resolution overview image of the entire slide in the single slide map. This can help you identify a region of interest for your experiment. The overview image is created by the overview camera, which uses no objective.
5. In the single slide map, click and drag the  selection tool to select the region of the slide for the preview, if needed. The center area of the slide is selected by default.
6. When you find a region of interest, click **Save Position** to save the current field of view to the **Region Selection to Acquire** page.

Note: You can click **Save Position** for each region of interest you find. Each time you click, the software adds a region on the **Region Selection to Acquire** page.

7. Click  **Focus/Exposure Settings**.

Note: Click **Focus Mode Descriptions** or see Understanding How Autofocus Works with a Slide on page 106 for details on getting good autofocus results.

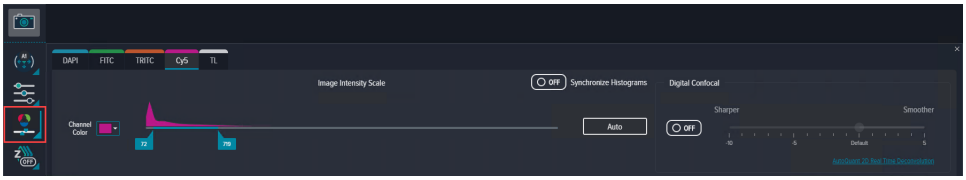
8. On the **Focus/Exposure Settings** tab, do the following:
   a. Under **Detect Surface**, select one of the following hardware autofocus settings:
      - **Plate Bottom**: A single-peak autofocus designed for samples in 3D matrices or clear mounted coverslips, where the second surface appears "invisible" to autofocus.
      - **Well Bottom**: A two-peak autofocus designed for samples in a liquid medium, such as well plates or chamber slides. It is not recommended for samples in 3D matrices or clear mounted coverslips. It is not available with the 2.5x objective.
   b. If needed, under **Find Best Plane**, select one of the following software autofocus options to improve autofocus:
      - **Normal Search Range**: The Normal Search Range option is centered on the surface selected by the hardware autofocus. It searches a range around that surface of 15% of the Bottom Thickness value from the labware specification (that is, 7.5% above the surface and 7.5% below it) of the Bottom Thickness value from the labware specification or 50 µm (that is, 25 µm above the surface and 25 µm below it), whichever is greater.
      - **Wide Search Range**: The Wide Search Range option is centered on the surface selected by the hardware autofocus. It searches a range of 40% of the Bottom Thickness value from the labware specification (that is, 20% above the surface and 20% below it).
      - **Superwide Search Range**: The Superwide Search Range option uses the surface selected by the hardware autofocus and searches a range of 300 µm above that surface (unlike the other options, which search around the surface).
   c. If needed, select the **Anchor Focus Position** check box to save current focus position and disable the autofocus controls. The software uses the saved focus position for any ensuing snaps of preview images and for the acquisition. An anchor icon  indicates the snapped image previews in the **History** pane that are using the anchored focus position.

Note:
- Typically, the instrument runs autofocus each time you snap a preview image or acquire an image. When you select the **Anchor Focus Position** check box, the instrument no longer runs autofocus. Instead, the software uses the saved focus position across all sites and regions, which speeds up acquisition.
- Clear the check box to remove the saved anchored focus position setting. The instrument resumes autofocus the next time you snap an image preview or run an acquisition.
- For best results when you anchor the focus position, use the 2.5x objective or 4x objective. In addition, ensure that both your slide and your sample are relatively flat.
- The **Anchor Focus Position** check box is not available if any of the following are true:
  - No preview images have been snapped (that is, the **History** pane is empty).
  - The selected objective has just been changed.
  - The 40x objective or 63x objective is selected.
  - Live preview is on.

d. If needed, use the **Focus Offset** controls to adjust the image sharpness for each wavelength.
e. If needed, use the **Exposure** controls to adjust the exposure time for each channel. Exposure time affects image brightness and results in a broader dynamic range (that is, the difference between the brightest and dimmest object detected).
When the image is in focus, click **Auto** to automatically determine the optimal exposure for the best dynamic range. The longer the exposure, the brighter the experiment image. Dimmer objects get brighter with longer exposure time, as does the background. Increased exposure time slows the experiment because each image takes longer. In addition, it can lead to image saturation and possibly cause photobleaching or phototoxicity of your sample.

> **Tip:** Try using **Auto** with a known bright sample, such as a positive control. Then, if needed, decrease the exposure about 10% to 20%.

9. Click  **Snap Image** again to refresh the preview.
A thumbnail of the preview image appears in the **History** pane.
10. Click  **Image Intensity Settings**.



11. On the **Image Intensity Settings** tab, adjust the settings as needed for each channel:
a. Use the **Channel Color** drop-down list box to change the identification color for the channel. The default channel color is based on the emission wavelength of the selected stain.
b. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image.
c. Click **Auto** to set the best contrast based on the current preview.
d. If you need to adjust the image intensity scale for all channels simultaneously, you can set **Synchronize Histograms** to **On**.

> **Note:** In most cases, however, you can set **Synchronize Histograms** to **Off**.

12. With an optional digital confocal license, do the following to apply the optional digital confocal algorithm to the image:
    a. Set **Digital Confocal** to **On**.
    b. As needed, move the slider to the right to smooth the image.
    c. As needed, move the slider to the left to sharpen the image.

    AutoQuant integrated innovation Digital confocal enhances contrast, improves resolution, and sharpens the image using the AutoQuant 2D RealTime Deconvolution algorithm. The algorithm calculates and inverts an ideal point spread function (PSF) for the optics based on image acquisition properties (not the actual image) such as the numerical aperture, refraction index, wavelength, and so on. The algorithm then applies the inverted PSF to the original image in Fourier space, which is a single multiplier (instead of a convolution). A Weiner filter uses a constant K value to correct noise in the image.

13. Repeat these steps as needed until you are satisfied with the quality of the preview image.

    Tip:
    - The **History** pane contains thumbnails of previous preview images. Click a thumbnail to show that preview image and revert the settings to the configuration used to acquire it.
    - An anchor icon on a thumbnail indicates the snapped image preview is using the anchored focus position.

14. Consider snapping Z stack images if your sample includes any of the following:
    - More than one focus plane within the field of view.
    - Objects of different depths.
    - Objects with varying depths relative to the focus plane.
    - Thick objects.

    See Snapping Z Stack Images on page 93 for details.

To continue to the next workflow step, click **Region Selection to Acquire**. See Region Selection to Acquire on page 112 for details.

Snapping Slide Comparison Previews

You can view previews of the acquisition by snapping comparison images from two slides (or two regions of the same slide) or compare settings on two different phenotypes (such as positive and negative controls). This enables you to compare the uniformity of the image quality. As with snapping an image of a single slide, the comparison images use the selected objective, stain, slide, region, focus settings, exposure settings, and histogram.

After snapping the preview, you can use the icon on the right side of the page to do the following:

 **Download Plane Images**: Downloads TIFF images of the current preview. Without Z stack images, the downloaded image is the current preview. With Z stack images, the downloaded image is determined by the current selection: either the Z stack projection or an individual Z stack plane.

Use the image viewer controls as needed to view the preview:

 Toggles the available channels for the preview by clicking on the channel icons. The icons for visible channels are brighter and have a white border. “Hidden” channels are dimmed and do not have a white border.

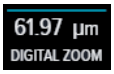
Tip: When a channel is overexposed, the overexposure  indicator appears. Lower the **Exposure** value as needed.

 Zooms in on the image.

 Zooms out on the image.

 Resets the view zoom to the original image size.

 Views the image full screen.



Indicates the current zoom scale. **Digital Zoom** indicates that the image is zoomed in digitally (that is, not optically) and might not represent the actual quality of the image.

To snap slide comparison images:

1. On the **Acquisition Settings** page, click  **Snap Image** to snap an initial preview image. A thumbnail of the preview image appears in the **History** pane.

Note: If autofocus fails, the software may display the following message:

In this case, check the Autofocus Info graph to review the focus peaks for the most recent autofocus to understand how the system determined focus. See Troubleshooting Autofocus Issues on page 110 for details.

Note:

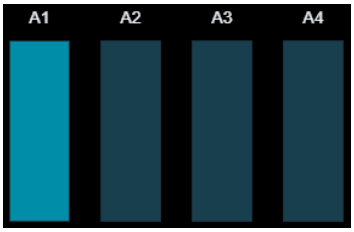
- If autofocus fails, the software may display the following message:

  In this case, check the Autofocus Info graph to review the focus peaks for the most recent autofocus to understand how the system determined focus. See Troubleshooting Autofocus Issues on page 110 for details.
- Molecular Devices Technical Support has compiled information that can help you diagnose and resolve autofocus issues. For details, log in to the Molecular Devices Knowledge Base at support.moleculardevices.com, and search for **ImageXpress Pico: Focus Troubleshooting**.

2. In the **Tools** pane on the right, click  **Comparison Mode**.

Note: If **Z Stacking** is set to **On**, the Comparison Mode icon is disabled. You must set **Z stacking** to **Off** before you can start Comparison Mode.

3. At the bottom of the screen on the  **Choose Position to Acquire** tab, click  **Select Slide** on the left.
4. In the slide holder map, select the first slide for the preview. The instrument snaps a preview image of the newly selected slide. The **A1** slide is selected by default.



5. Click  **Select Slide** on the right.

6. In the slide holder map, select the second slide for the preview. The instrument snaps a preview image of the newly selected slide.
7. Click  **Snap Overview** to snap a low-resolution overview image of the entire slide in the single slide map. This can help you identify a region of interest for your experiment. The overview image is created by the overview camera, which uses no objective.
8. In the single slide map on the left, click and drag the  selection tool to select the region of the first slide for the preview, if needed. The center area of the slide is selected by default.
9. Repeat steps 7 and 8 in the single slide map on the right to select the region of the second slide for the preview, if needed.
10. Click  **Focus/Exposure Settings**.

Note: Click  **Focus Mode Descriptions** or see Understanding How Autofocus Works with a Slide on page 106 for details on getting good autofocus results.

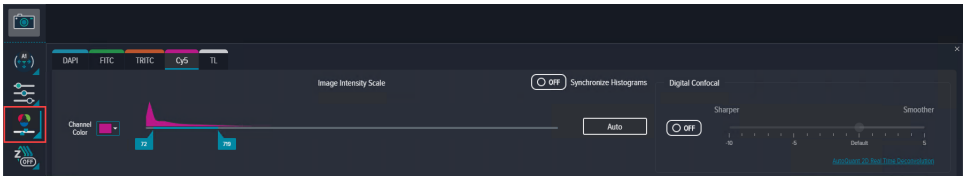
11. On the **Focus/Exposure Settings** tab, do the following:
    a. Under **Detect Surface**, select one of the following hardware autofocus settings:
        - **Plate Bottom**: A single-peak autofocus designed for samples in 3D matrices or clear mounted coverslips, where the second surface appears "invisible" to autofocus.
        - **Well Bottom**: A two-peak autofocus designed for samples in a liquid medium, such as well plates or chamber slides. It is not recommended for samples in 3D matrices or clear mounted coverslips. It is not available with the 2.5x objective.
    b. If needed, under **Find Best Plane**, select one of the following software autofocus options to improve autofocus:
        - **Normal Search Range**: The Normal Search Range option is centered on the surface selected by the hardware autofocus. It searches a range around that surface of 15% of the Bottom Thickness value from the labware specification (that is, 7.5% above the surface and 7.5% below it) of the Bottom Thickness value from the labware specification or 50 µm (that is, 25 µm above the surface and 25 µm below it), whichever is greater.
        - **Wide Search Range**: The Wide Search Range option is centered on the surface selected by the hardware autofocus. It searches a range of 40% of the Bottom Thickness value from the labware specification (that is, 20% above the surface and 20% below it).
        - **Superwide Search Range**: The Superwide Search Range option uses the surface selected by the hardware autofocus and searches a range of 300 µm above that surface (unlike the other options, which search around the surface).
    c. If needed, select the **Anchor Focus Position** check box to save current focus position and disable the autofocus controls. The software uses the saved focus position for any ensuing snaps of preview images and for the acquisition. An anchor icon  indicates the snapped image previews in the **History** pane that are using the anchored focus position.

Note:
- Typically, the instrument runs autofocus each time you snap a preview image or acquire an image. When you select the **Anchor Focus Position** check box, the instrument no longer runs autofocus. Instead, the software uses the saved focus position across all sites and regions, which speeds up acquisition.
- Clear the check box to remove the saved anchored focus position setting. The instrument resumes autofocus the next time you snap an image preview or run an acquisition.
- For best results when you anchor the focus position, use the 2.5x objective or 4x objective. In addition, ensure that both your slide and your sample are relatively flat.
- The **Anchor Focus Position** check box is not available if any of the following are true:
    - No preview images have been snapped (that is, the **History** pane is empty).
    - The selected objective has just been changed.
    - The 40x objective or 63x objective is selected.
    - Live preview is on.

d. If needed, use the **Focus Offset** controls to adjust the image sharpness for each wavelength.
e. If needed, use the **Exposure** controls to adjust the exposure time for each channel. Exposure time affects image brightness and results in a broader dynamic range (that is, the difference between the brightest and dimmest object detected).
When the image is in focus, click **Auto** to automatically determine the optimal exposure for the best dynamic range. The longer the exposure, the brighter the experiment image. Dimmer objects get brighter with longer exposure time, as does the background. Increased exposure time slows the experiment because each image takes longer. In addition, it can lead to image saturation and possibly cause photobleaching or phototoxicity of your sample.

> **Tip:** Try using **Auto** with a known bright sample, such as a positive control. Then, if needed, decrease the exposure about 10% to 20%.

12. Click  **Snap Image** again to refresh the preview.
13. Click  **Image Intensity Settings**.



14. On the **Image Intensity Settings** tab, adjust the settings as needed for each channel:
a. Use the **Channel Color** drop-down list box to change the identification color for the channel. The default channel color is based on the emission wavelength of the selected stain.
b. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image.
c. Click **Auto** to set the best contrast based on the current preview.
d. If you need to adjust the image intensity scale for all channels simultaneously, you can set **Synchronize Histograms** to **On**.

> **Note:** In most cases, however, you can set **Synchronize Histograms** to **Off**.

15. With an optional digital confocal license, do the following to apply the optional digital confocal algorithm to the image:
    a. Set **Digital Confocal** to **On**.
    b. As needed, move the slider to the right to smooth the image.
    c. As needed, move the slider to the left to sharpen the image.

AutoQuant integrated innovation Digital confocal enhances contrast, improves resolution, and sharpens the image using the AutoQuant 2D RealTime Deconvolution algorithm. The algorithm calculates and inverts an ideal point spread function (PSF) for the optics based on image acquisition properties (not the actual image) such as the numerical aperture, refraction index, wavelength, and so on. The algorithm then applies the inverted PSF to the original image in Fourier space, which is a single multiplier (instead of a convolution). A Weiner filter uses a constant K value to correct noise in the image.

16. Repeat these steps as needed until you are satisfied with the quality of the preview image.

Tip:

- The **History** pane contains thumbnails of previous preview images. Click a thumbnail to show that preview image and revert the settings to the configuration used to acquire it.
- An anchor icon on a thumbnail indicates the snapped image preview is using the anchored focus position.

To continue to the next workflow step, click **Region Selection to Acquire**. See Region Selection to Acquire on page 112 for details.

Snapping Z Stack Images

With the optional Z stacking feature, you can view previews of the acquisition by snapping Z stack images. A Z stack is comprised of a series of images captured at the specified focus offsets using the selected objective, stain, slide, region, focus settings, and exposure settings. Z stack images can be helpful if your sample includes any of the following:

- More than one focus plane within the field of view.
- Objects of different depths.
- Objects with varying depths relative to the focus plane.
- Thick objects.

As you set up the acquisition, you can view and download individual planes of the Z stack or the entire Z stack projection. The Z stack projection is saved with the experiment data when you run the protocol.

Note: Only the Z stack projection is saved with the experiment data. Images for individual planes are not saved.

After snapping the preview, you can use the icons on the right side of the page to do the following:


Download Plane Images: Downloads TIFF images of the current preview. Without Z stack images, the downloaded image is the current preview. With Z stack images, the downloaded image is determined by the current selection: either the Z stack projection or an individual Z stack plane.


Download Z Stacking Images: With Z stack images, downloads a TIFF image of the current Z stack projection and each individual Z stack plane.

Use the image viewer controls as needed to view the preview:

 Toggles the available channels for the preview by clicking on the channel icons. The icons for visible channels are brighter and have a white border. "Hidden" channels are dimmed and do not have a white border.

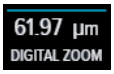
Tip: When a channel is overexposed, the overexposure  indicator appears. Lower the **Exposure** value as needed.

 Zooms in on the image.

 Zooms out on the image.

 Resets the view zoom to the original image size.

 Views the image full screen.



Indicates the current zoom scale. **Digital Zoom** indicates that the image is zoomed in digitally (that is, not optically) and might not represent the actual quality of the image.

: With Z stack images, indicates the type of image. **ZP** indicates a Z stack projection. For an individual Z stack plane, displays the focus offset amount. **Z 20µm** indicates 20 µm above focus.

Note: Before snapping Z stack images, you may first want to snap a preview of the slide. See Snapping a Preview of a Slide on page 81 for details.

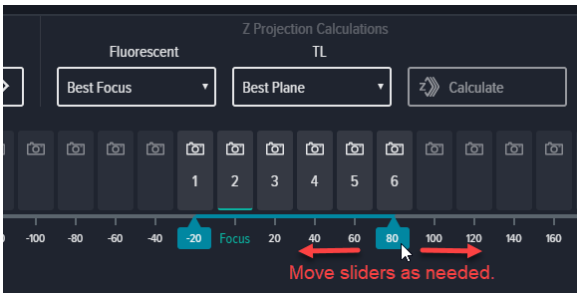
To snap Z stack images:

1. On the **Acquisition Settings** page, click  **Z Stacking**.
2. In the bottom pane, set **Z Stacking** to **On**.

3. In the **Focus Step (µm)** field, modify the distance between the Z stack planes, as needed. The default focus step distance varies based on the objective.

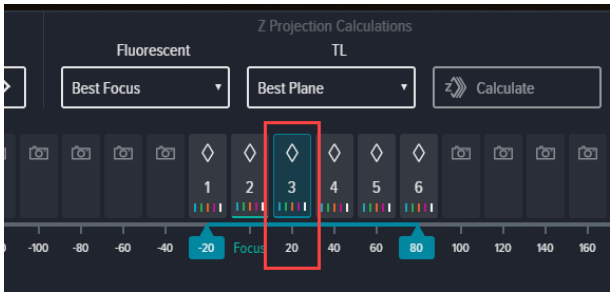
Objective	Default Focus Step Distance
2.5x	50 µm
4x	50 µm
10x	20 µm
20x	5 µm
40x	2 µm
63x	1 µm

4. As needed, move the sliders to select the Z stack planes to include in the Z stack.



5. Click  **Snap Z Stacking Images** to snap the Z stack.
The Z stack projection appears, and the Z stack projection button highlights to indicate that you are viewing the projection.

6. Click a Z stack plane button to view the preview for that plane.



7. If you are performing a fluorescent light acquisition, click the **Fluorescent** drop-down list box, and select one of the following to improve the Z stack images:
   - **Maximum**: For each corresponding pixel position in the images, finds the pixel that has the highest intensity value out of all the values in all the planes and outputs this value to the projection image.
   - **Best Focus**: Calculates the regions of best focus in an image stack on a pixel-by-pixel basis. Similar to the **Maximum** setting, this setting uses the best focused pixels from the Z stack to create the projection image.
   - **Best Focus Smaller Objects**: Breaks up images into very small zones to find the best focus across all Z planes for each zone, which brings fine details and smaller objects into focus. This option is very computationally demanding and may run slower than the Best Focus option or Best Focus Larger Objects option.
   - **Best Focus Larger Objects**: Breaks up images into small zones to find the best focus across all Z planes for each zone, which brings greater detail of larger objects into focus. This option is computationally demanding and may run slower than to the Best Focus option.
8. If you are performing a transmitted light acquisition, click the **TL** drop-down list box, and select one of the following to improve the Z stack images:
   - **Average**: For each corresponding pixel position in the images, averages the gray scale values of the pixels in all the planes, and outputs this value to the projection image.
   - **Best Plane**: Scores the images of best focus in an image stack and uses the plane with the best focus score to create the projection image.
   - **Best Focus Smaller Objects**: Breaks up images into very small zones to find the best focus across all Z planes for each zone, which brings fine details and smaller objects into focus. This option is very computationally demanding and may run slower than the Best Focus option or Best Focus Larger Objects option.
   - **Best Focus Larger Objects**: Breaks up images into small zones to find the best focus across all Z planes for each zone, which brings greater detail of larger objects into focus. This option is computationally demanding and may run slower than to the Best Focus option.

9. If you are performing a colorimetric acquisition, click the **Colorimetric** drop-down list box, and select one of the following to improve the Z stack images:
   - **Minimum**: For each corresponding pixel position in the images, finds the pixel that has the lowest intensity value out of all the values in all the planes and outputs this value to the new image.
   - **Best Focus**: Calculates the regions of best focus in an image stack on a pixel-by-pixel basis. Similar to the **Maximum** setting, this setting uses the best focused pixels from the Z stack to create the projection image.
   - **Best Focus Smaller Objects**: Breaks up images into very small zones to find the best focus across all Z planes for each zone, which brings fine details and smaller objects into focus. This option is very computationally demanding and may run slower than the Best Focus option or Best Focus Larger Objects option.
   - **Best Focus Larger Objects**: Breaks up images into small zones to find the best focus across all Z planes for each zone, which brings greater detail of larger objects into focus. This option is computationally demanding and may run slower than to the Best Focus option.

Note: The **Calculate** button becomes enabled when you modify settings that affect the preview image. Click **Calculate** to quickly view the updated Z stack images.

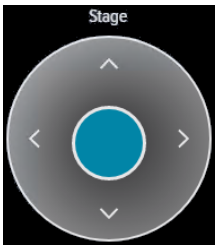
To continue to the next workflow step, click  **Region Selection to Acquire**. See Region Selection to Acquire on page 112 for details.

Viewing a Live Preview of a Slide

Live preview enables you to move the sample (X-Y) stage to explore a continually updated, dynamic image of the sample. Using the two virtual joysticks (one for stage movement and one for focus control), you can quickly find a region of interest. The live preview uses the selected objective, stain, slide, focus settings, and exposure settings.

Use the following controls as needed to view the live preview:

 Selects the active channel for live preview. Only one channel at a time can be active.



Moves the sample (X-Y) stage. Click and drag the stage joystick in the direction you want to move the stage. Drag it a small distance from the center to slowly move the stage. Larger movements will move the stage more quickly. Release the stage joystick to stop stage movement. You can also click the arrows inside the stage joystick to move the stage in small steps.

Adjusts the focus offset of the image. Click and drag the focus joystick to adjust the focus. As you adjust the offset, the focus position value updates. Drag the joystick a small distance from the center to fine-tune the focus. Larger movements will change the focus more quickly. Release the focus joystick to stop changing the focus. You can also click the arrows inside the focus joystick to adjust the focus in small steps. The step size corresponds to half of the depth of field for the selected objective.

Indicates the current focus offset value, which is the difference between the current focus position and the autofocus position. As you drag the focus joystick, the **Offset from Autofocus** value increases or decreases accordingly. A value in italics indicates that the current value has not been set as the focus offset.

Sets the current **Offset from Autofocus** value as the focus offset. This setting is reflected by the value in the **Focus Offset** field on the **Focus/Exposure Settings** tab.

Click to Center: Moves the stage to center the image on the spot that you click.

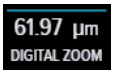
In addition, use the image viewer controls as needed to view the live preview:

 Zooms in on the image.

 Zooms out on the image.

 Resets the view zoom to the original image size.

 Views the image full screen.



Indicates the current zoom scale. **Digital Zoom** indicates that the image is zoomed in digitally (that is, not optically) and might not represent the actual quality of the image.

Tip: Several factors (for example, the labware is not completely flat or a thick sample is in multiple focus planes) may cause unexpected results when you click **Save Offset** after using the stage and focus joysticks. This can affect the focus of the acquisition when you run the experiment. For best results, you may want to snap a preview for your region of interest after turning off live preview.

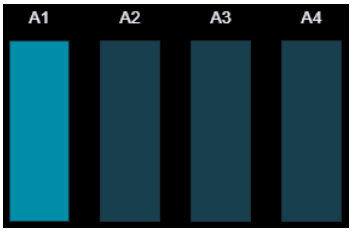
Note: To avoid overexposing your sample, live preview automatically turns off after five minutes.

To view a live preview of a slide:

1. On the **Acquisition Settings** page, click 

Choose Position to Acquire.
2. Click 

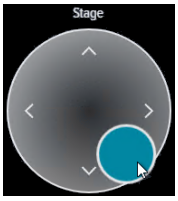
Select Slide.
3. In the slide holder map, select the slide for the preview. The instrument snaps a preview image of the newly selected slide. The **A1** slide is selected by default.



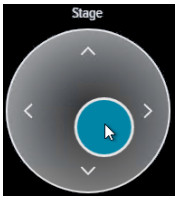
4. Click  **Snap Overview** to snap a low-resolution overview image of the entire slide in the single slide map. This can help you identify a region of interest for your experiment. The overview image is created by the overview camera, which uses no objective.
5. In the single slide map, click and drag the  selection tool to select the region of the slide for the preview, if needed. The center area of the slide is selected by default.

6. In the bottom pane, set **Live Preview** to **On**.

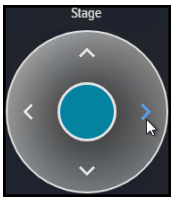
7. Use the stage joystick to move the sample (X-Y) stage to view different areas of the slide:
   - For fast stage movements, click and drag the stage joystick in the direction you want to move toward the outermost position.



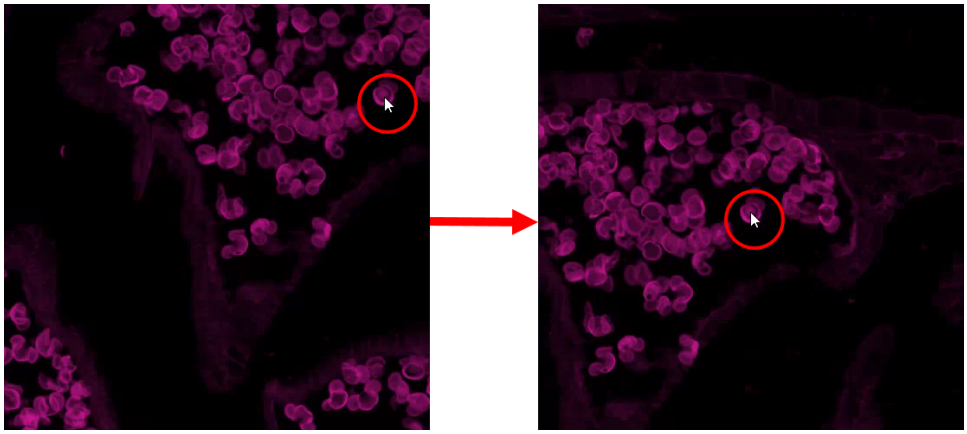
   - For slow stage movements, drag the stage joystick slightly.



   - For very small stage movements to fine-tune the stage position, click one of the joystick arrows. Each time you click an arrow, the stage moves one step.



8. Click on the image as needed to center the image on the clicked spot.



9. When you find a region of interest, click **Save Position** to save the current field of view to the **Region Selection to Acquire** page.

> **Note:** You can click **Save Position** for each region of interest you find. Each time you click, the software adds a region on the **Region Selection to Acquire** page.

10. Use the focus joystick to adjust the focus offset of the live preview. As you adjust the offset, the focus position value updates.
    - For large focus adjustments, click and drag the focus joystick toward the outermost position.

    - For smaller focus adjustments, drag the focus joystick slightly.

    - For fine focus adjustments, click one of the joystick arrows. Each time you click an arrow, the focus adjusts one step. The step size corresponds to half of the depth of field for the selected objective.

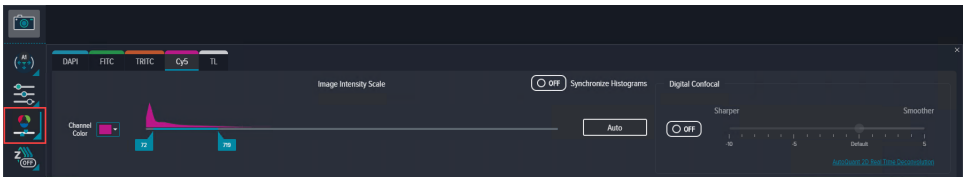
11. If needed, click  **Snap Image** to confirm your focus offset settings.
12. Click  **Focus/Exposure Settings**.

Note: Click  **Focus Mode Descriptions** or see Understanding How Autofocus Works with a Slide on page 106 for details on getting good autofocus results.

13. If needed, use the **Exposure** controls to adjust the exposure time for each channel. Exposure time affects image brightness and results in a broader dynamic range (that is, the difference between the brightest and dimmest object detected).
    When the image is in focus, click **Auto** to automatically determine the optimal exposure for the best dynamic range. The longer the exposure, the brighter the experiment image. Dimmer objects get brighter with longer exposure time, as does the background. Increased exposure time slows the experiment because each image takes longer. In addition, it can lead to image saturation and possibly cause photobleaching or phototoxicity of your sample.

    Tip: Try using **Auto** with a known bright sample, such as a positive control. Then, if needed, decrease the exposure about 10% to 20%.

14. Click  **Image Intensity Settings**.



15. On the **Image Intensity Settings** tab, adjust the settings as needed for each channel:
    a. Use the **Channel Color** drop-down list box to change the identification color for the channel. The default channel color is based on the emission wavelength of the selected stain.
    b. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image.
    c. Click **Auto** to set the best contrast based on the current preview.
    d. If you need to adjust the image intensity scale for all channels simultaneously, you can set **Synchronize Histograms** to **On**.

    Note: In most cases, however, you can set **Synchronize Histograms** to **Off**.

16. With an optional digital confocal license, do the following to apply the optional digital confocal algorithm to the image:
    a. Set **Digital Confocal** to **On**.
    b. As needed, move the slider to the right to smooth the image.
    c. As needed, move the slider to the left to sharpen the image.

    AutoQuant integrated innovation Digital confocal enhances contrast, improves resolution, and sharpens the image using the AutoQuant 2D RealTime Deconvolution algorithm. The algorithm calculates and inverts an ideal point spread function (PSF) for the optics based on image acquisition properties (not the actual image) such as the numerical aperture, refraction index, wavelength, and so on. The algorithm then applies the inverted PSF to the original image in Fourier space, which is a single multiplier (instead of a convolution). A Weiner filter uses a constant K value to correct noise in the image.

17. Do the following to reset the autofocus:
    a. In the bottom pane, set **Live Preview** to **Off**.
    b. In the bottom pane, set **Live Preview** to **On**.

    Note: This will autoscale the histogram. You may need to re-adjust the histogram scaling.

18. Click 

Choose Position to Acquire.
19. Use the focus joystick to find the best focus position for the current channel. As you adjust the offset, the focus position value updates.
    - For large focus adjustments, click and drag the focus joystick toward the outermost position.

    - For smaller focus adjustments, drag the focus joystick slightly.

    - For fine focus adjustments, click one of the joystick arrows. Each time you click an arrow, the focus adjusts one step. The step size corresponds to half of the depth of field for the selected objective.

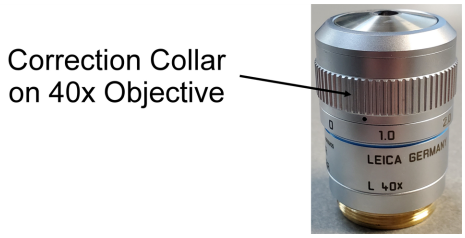
      

20. To save the focus offset for that channel click **Set Offset**. This setting is reflected by the value in the **Focus Offset** field on the **Focus/Exposure Settings** tab.
21. Repeat these steps as needed to find other regions of interest.
22. Repeat these steps as needed for each channel.

To continue to the next workflow step, click  **Region Selection to Acquire**. See Region Selection to Acquire on page 112 for details.

Adjusting an Objective Correction Collar

The 40x objective and 63x objective have application-optimized correction collars to compensate for well bottom thickness or coverslip thickness. The collars have a range of 0 mm to 2 mm correction. Changing this setting adjusts the distances between components inside the objective barrel. Image quality and resolution are very dependent on properly setting these collars.



The settings to be used depend on the well bottom thickness of the plate or the coverslip thickness on the slide on which the specimen is mounted. In general, set the correction collar for the physical thickness of the plate or slide that you are imaging. The physical thickness can be determined by the plate specifications from the plate manufacturer.

Note: Do not use a plate, slide, or coverslip with a thickness that is out of the range of the correction collar for the selected objective.

Observe the following when handling an objective:

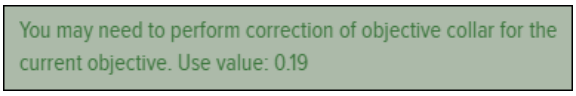
CAUTION!

- To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling objectives and filter cubes.
- With the instrument power on, do not manually rotate the objective turret. Manually rotating the objective turret can damage the instrument.

You would typically adjust a correction collar as part of setting up an acquisition.

To adjust an objective correction collar for a slide:

1. On the **Acquisition Settings** page, on the right side of the screen under **Tools**, click  **Slide Format**.
2. In the **Slide Format** list, select the slide format.
3. On the right side of the screen under **Tools**, click  **Objectives**.
4. In the **Objectives** list, select the objective.
   If a correction collar adjustment is required, the software displays the recommended setting.

5. On the left side of the screen under **Steps**, click  **Acquisition Device**.
6. On the right side of the screen, click  **Set Up for Adjustment of Objective Collar**.
7. Click **OK**.
   The maintenance door opens.
8. If needed, loosen the objective from the instrument by gently turning it counterclockwise.
9. Rotate the correction collar to its new setting.

   Tip: You might need a flashlight to see the markings for the graduated scale on the barrel and its current setting.

10. If you loosened the objective, tighten it by gently turning it clockwise.

    Note: When tightening the objective, take care to avoid changing the correction collar setting.

11. Close the maintenance door.
12. On the right side of the screen under **Tools**, click  **Finish Adjustment of Objective Collar** to exit maintenance mode.
13. Click **OK**.

Understanding How Autofocus Works with a Slide

The instrument uses two autofocus mechanisms:

- **Detect Surface**, which is hardware autofocus. Hardware autofocus uses an LED beam to find reflective surfaces and is designed for speed. It works well for adherent samples in plates or chamber slides.
- **Find Best Plane**, which is image-based autofocus (also known as software autofocus). When enabled, image-based autofocus searches a range for the best focus plane based on image contrast. It works well for slides with a coverslip or for samples in a plate that are not flat, such as suspension cells or spheroids.

Detect Surface

The Detect Surface options use hardware autofocus to detect surfaces. The following autofocus options are available for slides:

Plate Bottom

Hardware autofocus detects the surface closest to the objective, which is the coverslip surface (when loaded with coverslip down). It then applies the bottom thickness from the labware configuration as an offset to place the focus plane at the top of the slide.

The Plate Bottom option is designed for samples in 3D matrices or clear mounted coverslips, where the second surface appears "invisible" to autofocus.

Well Bottom

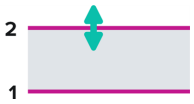
Hardware autofocus detects the two surfaces closest to the objective (that is, the slide top and bottom). It sets the focus plane at the top of the slide.

The Well Bottom option is designed for samples in a liquid medium, such as well plates or chamber slides. It is not recommended for samples in 3D matrices or clear mounted coverslips. It is not available with the 2.5x objective.

Find Best Plane

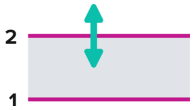
If the Detect Surface options do not provide satisfactory focus, select the **Find Best Plane** option to add image-based autofocus for the first channel within the selected search range. Adding image-based autofocus is useful for thicker samples, samples with variable best focus planes, or labware with variable thickness.

Normal Search Range



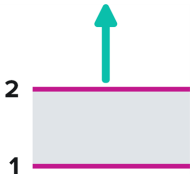
The Normal Search Range option is centered on the surface selected by the hardware autofocus. It searches a range around that surface of 15% of the Bottom Thickness value from the labware specification (that is, 7.5% above the surface and 7.5% below it) of the Bottom Thickness value from the labware specification or 50 µm (that is, 25 µm above the surface and 25 µm below it), whichever is greater.

Wide Search Range



The Wide Search Range option is centered on the surface selected by the hardware autofocus. It searches a range of 40% of the Bottom Thickness value from the labware specification (that is, 20% above the surface and 20% below it).

Superwide Search Range



The Superwide Search Range option uses the surface selected by the hardware autofocus and searches a range of 300 µm above that surface (unlike the other options, which search around the surface).

Autofocus Strategies for Slides with a Coverslip

To find the best focus for a slide with a coverslip, first try the default **Plate Bottom** option.

Tip:

- If suitable for your sample, we recommend that you load slides with the coverslip down.
- When using the 4-slide holder, slide variations (thickness, material, sealing, and so on) may affect autofocus quality. We recommend using the same type of slide in each position.

If you are unsatisfied with the focus and your sample is thick or uneven, try the **Wide** option or **Superwide** option to improve the result.

After you select the focus option, click **Auto** next to the **Focus Offset** controls to determine the focus offset between stains. Do this for each stain in the acquisition. Image-based autofocus bases its search on the first stain in the acquisition, so consider the order in which stains are acquired. See Acquisition Settings on page 78 for details.

Autofocus Strategies for Most Chamber Slides

To find the best focus for most chamber slides, start with the **Well Bottom** option. With imaging-quality labware, an accurate labware specification, and a relatively flat sample (for example, most adherent cells), autofocus should provide good results.

If you are unsatisfied with the focus and your sample is thick or uneven, select the **Normal Search Range** option. If this does not improve the result, use the **Wide Search Range** option or **Superwide Search Range** option to increase the search range.

After you select the focus option, click **Auto** next to the **Focus Offset** controls to determine the focus offset between stains. Do this for each stain in the acquisition. Image-based autofocus bases its search on the first stain in the acquisition, so consider the order in which stains are acquired. See Acquisition Settings on page 78 for details.

Labware Specification Setting

While all settings in the labware specification are important, the following setting plays a large role in achieving good autofocus:

- **Surface Thickness** is important when using image-based autofocus. It determines the base focus search range around the coverslip surface (with the coverslip down) where the sample is located. If the Surface Thickness value is too low or too high, the search range will not include the sample, and the instrument will not be able to find a focus plane.

If autofocus issues occur, you may need to adjust this setting. See Labware Library on page 217 for details.

Anchoring the Focus Position

When you are satisfied with the quality of the image preview, you can select the **Anchor Focus Position** check box on the **Focus/Exposure Settings** tab of the **Acquisition Settings** page to save current focus position and disable the autofocus controls. The instrument will no longer run autofocus each time you snap a preview image or acquire an image. Instead, the software will use the saved focus position across all sites, which speeds up acquisition. For best results, use the 2.5x objective or 4x objective. In addition, ensure that both your slide and your sample are relatively flat.

See Snapping a Preview of a Slide on page 81 for details.

Troubleshooting Autofocus Issues

The CellReporterXpress software uses hardware and image-based autofocus to find the best focus for the sample. When autofocus issues occur, they are typically caused by an incorrect slide holder configuration in the Labware Library. In many cases, the issue can be addressed by adjusting the slide holder configuration. See Labware Library on page 217 for details.

The **Autofocus Info** graph on the **Acquisition Settings** page shows the focus peaks for the most recent autofocus to help you understand how it was determined.

This section describes how you can use this information to troubleshoot autofocus issues.

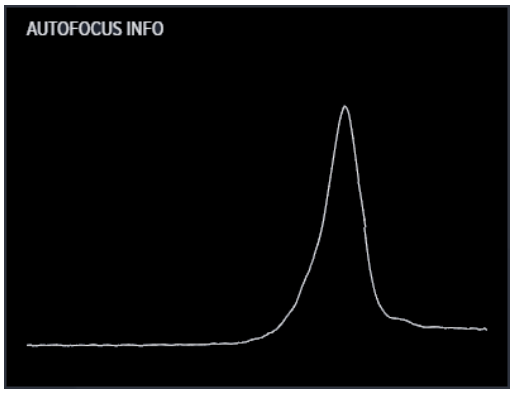
Tip: Molecular Devices Technical Support has compiled information that can help you diagnose and resolve autofocus issues. For details, log in to the Molecular Devices Knowledge Base at support.moleculardevices.com, and search for **ImageXpress Pico: Focus Troubleshooting**.

To troubleshoot autofocus issues using the Autofocus Info graph:

1. On the **Acquisition Settings** page, click **Snap Image** to snap a preview image.
2. On the right side of the screen under **Tools**, click **Autofocus Info** to display a graph showing the focus peaks of the autofocus.
3. Review the **Autofocus Info** graph along the following examples.

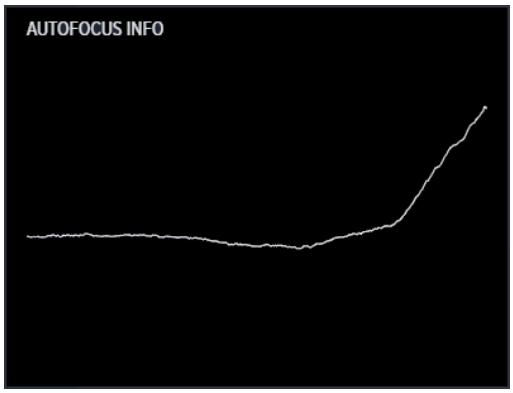
Good Autofocus

The following graph shows a good result of a single-peak autofocus (Plate Bottom option selected). Single-peak autofocus is typically used for slides with coverslips.

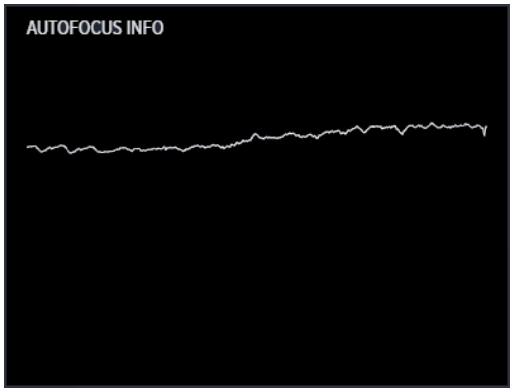


Autofocus Issues

The following graph shows a failed result of a single-peak autofocus of a slide with the coverslip down. It indicates that coverslip may be too high. You may want to increase the value specified for the support ledge height. See Labware Library on page 217 for details.



The following graph shows a general autofocus failure. No peaks are found, which likely indicates that there is no slide in the selected position or the labware holder is not inserted correctly. It is also possible that the wrong labware is selected. See Labware Library on page 217 for details.



Region Selection to Acquire


Region Selection to Acquire is the step where you select the region of the slide to be acquired. The page shows a representation of a slide. You must select at least one region to run an experiment.

By default, there are no slide region selections. You can add, resize, move, and delete region selection overlays. If needed, you can set multiple region selection overlays.


Note: Several factors (including the slide holder format and the magnification of the objective) may prevent you from selecting some regions near the edges a slide.

Click 
Snap Overview to snap a low-resolution overview image of the entire slide in the single slide map. This can help you identify a region of interest for your experiment. The overview image is created by the overview camera, which uses no objective.

The right side of the page includes the following icons:


Add Acquisition Region: Adds a new acquisition region selection overlay that you can size and move into position.


Delete Selected Region: Removes the selected region overlay.


Clear All Regions: Removes all the visible region overlays.


Toggle Actual Area to Capture: Shows what the selected camera objective will snap based on the field of view for the lens. You may need to adjust the region selection or the objective selection based on this area.

To continue to the next workflow step for a colorimetric or stitched acquisition, click either 
Save Protocol or 
Run Protocol. See Save Protocol on page 120 or Run Protocol on page 121 for details.

To continue to the next workflow step for all other acquisitions, click 
Analysis Settings. See Analysis Settings on page 113 for details.

Analysis Settings


Analysis Settings is the step where you set up image analysis for the experiment. The data generated based on these settings becomes the analysis data for the experiment.

The right side of the page includes the following icons:


Choose Analysis: Toggles analysis on or off and selects the analysis for the experiment.


Measurements: Specifies the cell measurements included in the analysis.


Save Analysis: Saves the analysis for use in future experiments.


Cell Info Mode: Displays information on a selected cell.


Comparison Mode: Captures two preview images, which enables you to compare the uniformity of the image quality.

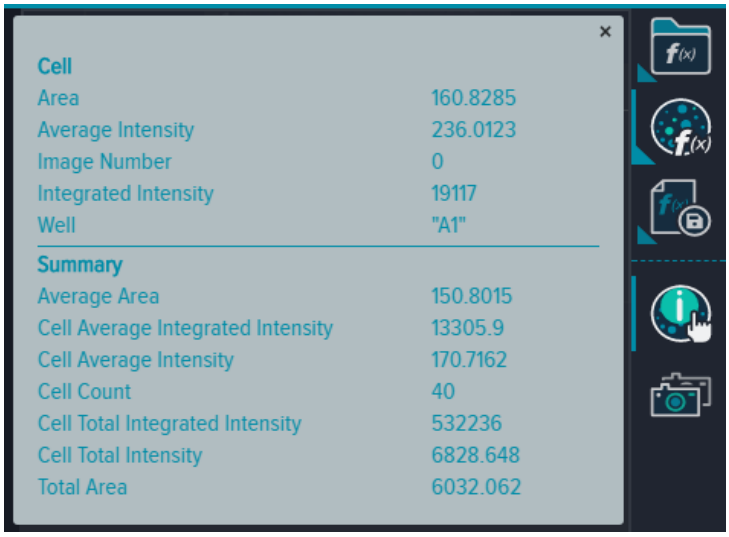
Setting Up an Analysis

To set up an analysis:

1. On the **Analysis Settings** page, in the **Tools** pane on the right, click **Choose Analysis**.
2. Set **Analysis** to **On**.
3. Select a fluorescence or transmitted light analysis. See Analysis Descriptions on page 263 for details on the available analyses.
4. Click **Measurements**.
5. In the **Measurements** pane, select the measurements for the analysis.

   Note: The recommended measurements for the analysis are selected by default.

6. In the bottom pane, click **Test Analysis** to calculate the summary measurements using the preview image.
7. To view cell information, click **Cell Info Mode** and select a detected cell in the image preview.



As part of configuring analysis settings, you may do the following:

- Testing the Analysis of a Region, see page 115
- Testing the Analysis of Comparison Images, see page 116
- Saving Analysis Settings, see page 117

To continue to the next workflow step, click **Region Selection to Analyze**. See Region Selection to Analyze on page 118 for details.

Testing the Analysis of a Region

The preview represents the image quality to expect when you run your experiment.

To test the analysis of a region:

1. On the **Acquisition Analysis** page, in the bottom pane, click  **Test Analysis**.
2. Click  **Algorithm Input**.
3. On the **Algorithm Input** tab, adjust the settings as needed to optimize object detection. Settings vary depending on the selected analysis type. Setting options typically control intensity, minimum width, and maximum width for each stain used in the acquisition.
4. Click  **Test Analysis** to preview the analysis.
5. If needed, click  **Image Intensity Settings** and do the following to adjust the settings as needed for each channel:

   Note: You typically do not need to adjust the image intensity settings for the analysis, which are based on the acquisition settings. If you adjust these settings, it affects only the image display; it has no effect on any analysis measurements.

   a. Use the **Channel Color** drop-down list box to change the identification color for the channel.
   b. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image.
   c. Click **Auto** to set the best contrast based on the current preview.
6. Click  **Choose Position to Acquire**.
7. Click  **Select Slide**.
8. Click  **Snap Overview** to snap a low-resolution overview image of the entire slide in the single slide map. This can help you identify a region of interest for your experiment. The overview image is created by the overview camera, which uses no objective.
9. In the single slide map, click and drag the  selection tool to select the region of the slide for the preview, if needed. The center area of the slide is selected by default.
   The software runs a test analysis.
10. Click  **Algorithm Input**.
11. Click  **Test Analysis** to preview the analysis.
12. Repeat these steps as needed until you are satisfied with the quality of the preview.

After you are satisfied with the quality of the preview, you may want to save the analysis settings for later reuse. See Saving Analysis Settings on page 117 for details.

Testing the Analysis of Comparison Images

You can preview two images from different regions to compare the uniformity of the image quality.

To test the analysis of comparison images:

1. On the **Acquisition Analysis** page, in the **Tools** pane on the right, click  **Comparison Mode**.
2. In the bottom pane, click  **Test Analysis**.
3. Click  **Algorithm Input**.
4. On the **Algorithm Input** tab, adjust the settings as needed to optimize object detection. The settings affect both previews. Settings vary depending on the selected analysis type. Setting options typically control intensity, minimum width, and maximum width for each stain used in the acquisition.
5. On the left side of the pane, click the  **Choose Position to Acquire** tab.
6. Click  **Select Slide** on the left.
7. In the slide map, select the first slide for the preview. The A1 slide is selected by default.
8. Click  **Select Slide** on the right.
9. In the slide map, select the second slide for the preview.
10. Click  **Snap Overview** to snap a low-resolution overview image of the entire slide in the single slide map. This can help you identify a region of interest for your experiment. The overview image is created by the overview camera, which uses no objective.
11. In the single slide map on the left, click and drag the  selection tool to select the region of the first slide for the preview, if needed. The center area of the slide is selected by default.
12. Repeat the previous step in the single slide map on the right to select the region of the second slide for the preview, if needed.

13. Click  **Test Analysis** to preview the analysis.

1. If needed, click  **Image Intensity Settings** and do the following to adjust the settings as needed for each channel:

Note: You typically do not need to adjust the image intensity settings for the analysis, which are based on the acquisition settings. If you adjust these settings, it affects only the image display; it has no effect on any analysis measurements.

   a. Use the **Channel Color** drop-down list box to change the identification color for the channel.
   b. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image.
   c. Click **Auto** to set the best contrast based on the current preview.

14. Repeat these steps as needed until you are satisfied with the quality of the previews.

After you are satisfied with the quality of the preview, you may want to save the analysis settings for later reuse. See Saving Analysis Settings on this page for details.

Saving Analysis Settings

When you are satisfied with the quality of the preview in Testing the Analysis of a Region on page 115 or Testing the Analysis of Comparison Images on page 116, you may want to save the analysis settings for later reuse.

To save analysis settings:

1. On the **Acquisition Analysis** page, in the **Tools** pane on the right, click  **Save Analysis**.
2. In the **Save Analysis** pane, in the **Analysis Settings** field, enter a descriptive name.
3. If needed, add an avatar image by doing one of the following:
   - Click **Use Captured Picture**.
   - Click **Click to upload**, select an image file, and click **Open**.
4. Click **Save**.

To continue to the next workflow step, click  **Region Selection to Analyze**. See Region Selection to Analyze on page 118 for details.

Region Selection to Analyze

Region Selection to Analyze is the step where you select the region of interest to be analyzed. You must select at least one region to run an experiment.

Note: Several factors (including the slide holder format and the magnification of the objective) may prevent you from selecting some regions near the edges a slide.

Click  **Snap Overview** to snap a low-resolution overview image of the entire slide in the single slide map. This can help you identify a region of interest for your experiment. The overview image is created by the overview camera, which uses no objective.

The right side of the page includes the following icons:

 **From Center**: Adds an analysis region selection overlay in the center of the well or slide. You can control various elements of the analysis region, including the percentage of the slide and the shape of the selection overlay.

 **Activate Edit Mode**: Activates the selection handles on the acquisition region selection overlay, enabling you to manually move and size it.

 **Add Analysis Region**: Adds a new analysis region selection overlay that you can size and move into position.

 **Delete Selected Region**: Removes the selected region overlay.

 **Clear All Regions**: Removes all the visible region overlays.

To continue to the next workflow step, click  **Device Sensors**. See Device Sensors on page 119 for details.

Device Sensors


Device Sensors is the step where you specify the environmental sensors to be monitored for the experiment. The experiment data indicates if the sensors were within the specified range during the experiment.

You can monitor the temperature inside the instrument up to 40°C (104°F).

See Sensors on page 231 for details on regulating temperature.

To monitor the temperature sensor:

1. In the **Temperature** row, under **Monitoring Range**, in the **Min** field, enter the lower limit value for the temperature range in degrees Celsius.
2. In the **Max** field, enter the upper limit value for the temperature range in degrees Celsius.

   Note: The lower limit and upper limit values must be within the range of 25°C to 40°C (77°F to 104°F).

3. Set the notification to **On**.

To continue to the next workflow step, click either  **Save Protocol** or  **Run Protocol**. See Save Protocol on page 120 or Run Protocol on page 121 for details.

Save Protocol

 **Save Protocol** is an optional step where you can save the protocol you have created. You will typically save a protocol only when you intend to run it frequently. After you save a protocol, it appears as a card in the Protocol library.

Tip: We recommend creating protocols sparingly to avoid unnecessarily filling your Protocol library, which can make it difficult to find a protocol.

The right side of the page includes the following icon:

 **Lock Protocol**: Manages the ability of other users to modify the protocol.

Note: Other users are not prevented from viewing or running a locked protocol.

To save a protocol:

1. On the **Save Protocol** page, in the **Protocol Name** field, enter a name for the protocol.
2. (Optional) In the **Protocol Description** field, enter a description of the protocol.
3. Do the following to restrict other users from modifying the protocol:
   a. On the right side of the screen, click  **Lock Protocol**.
   b. Select the **Private** check box to prevent other users from modifying the settings on the **Acquisition Device**, **Acquisition Settings**, and **Analysis Settings** pages for the protocol.
   c. Select the **Lock region selection** check box to prevent other users from modifying the settings on the **Region Selection to Acquire** and **Region Selection to Analyze** pages.

      Note: The **Lock region selection** check box is not enabled until you select the **Private** check box.

   d. To specify other users who can modify a locked protocol, click the **Share With** field and select users from the list.

   Tip: You can set default sharing permissions in  **Configuration Settings**. See Sharing Permissions on page 261 for details.
4. Click **Save Protocol**.

To continue to the next workflow step, click  **Run Protocol**. See Run Protocol on page 121 for details.

Run Protocol

 **Run Protocol** is the last step in the workflow. In this step, you can review the settings for the experiment and address any issues.

When you run the experiment, the software acquires images and analyzes the data according to your settings. When the experiment completes, the software saves all acquired data as specified. Acquired data is then available for viewing and analysis in **Experiments** mode.

The right side of the page includes the following icons:

 **Experiment Details**: Displays acquisition and analysis settings and enables you to validate settings before running the experiment.

 **Storage**: Specifies image storage location during and after acquisition.

 **Public** and  **Private**: Manages the shared status of the experiment.

 **Open Plate Door**: Opens the top door on the selected instrument so that you can insert or remove labware.

 **Close Plate Door**: Closes the top door.

 **Run Experiment**: Runs the experiment using the specified acquisition and analysis settings. This icon becomes enabled when all the settings on the **Validation** tab in the **Experiment Details** pane are valid and an **Experiment Name** has been entered.

To run a protocol:

1. On the **Run Protocol** page, do the following:
   a. In the **Experiment Name** field, enter a name to identify the experiment in the Experiments library.
   b. If needed, in the **Barcode** field, enter the barcode for the labware.
   c. If needed, in the **Experiment Description** field, enter a description of the experiment.
   d. To save TIFF files of the acquisition images, select the **Save Raw Images** check box.
   e. To export TIFF files of the acquisition images for use with the Molecular Devices IN Carta Image Analysis Software, select the **Export to IN Carta** check box.

   Note: If you select the **Export to IN Carta** check box, the images of the acquisition will be cropped as required by the IN Carta software. With colorimetric images, each image will be separated into three, 16-bit component images (that is, one red, one green, and one blue). In addition, the export will include the required XDCE file.

   f. On the **Validation** tab, verify that all the required settings are valid. A  icon indicates a valid setting and a  icon indicates an invalid or missing setting. All acquisition settings must be valid to run the experiment. See Fixing Invalid Parameters on page 123 for details.
   g. On the **Acquisition Parameters** tab, review the settings.
   h. On the **Analysis Parameters** tab, review the settings.
2. If you want to review the settings for image storage during and after acquisition, do the following:
   a. Click  **Storage.**
   b. In the **Available Temporary Storage on Device** field, specify the drive for temporary image storage during acquisition. See the *ImageXpress Pico User Guide* for details on adding external temporary storage.
3. If you want to manage the shared status of the experiment to restrict other users from viewing it, do the following:
   a. Click  **Public**.
   b. Select the **Private** check box.
   c. If you want to specify other users who can view a private experiment, click the **Share With** field and select users from the list.

   Tip: You can set default sharing permissions in  **Configuration Settings**. See Sharing Permissions on page 261 for details.

4. If you have not already done so, do the following to insert your experiment-ready labware into the instrument:
   a. Click  **Open Plate Door** to open the top door on the instrument.
   b. Insert your experiment-ready labware into the instrument. See the *ImageXpress Pico User Guide* for details.
   c. Click  **Close Plate Door** to close the top door on the instrument.
5. Click  **Run Experiment** to run the experiment.

The **Monitor** page opens to display the progress of the running experiment. See Monitor Mode on page 213 for details.

Fixing Invalid Parameters

Invalid parameter settings are indicated by a  icon. Click the icon to display the reason for the invalid parameter.

To fix an invalid parameter:

Tip:
- If Data Temp Storage is insufficient, consider adding external temporary storage. See the *ImageXpress Pico User Guide* for details.
- If Data Storage is insufficient, consider adding more storage to the host computer or (in a server configuration) adding a remote storage computer. See the *CellReporterXpress Installation Guide* for details on setting up a server configuration.

1. Click the link next to the  icon to open the workflow step for the invalid parameter.
2. Address the issue.
3. Click  **Run Protocol** to return to **Run Protocol** page.

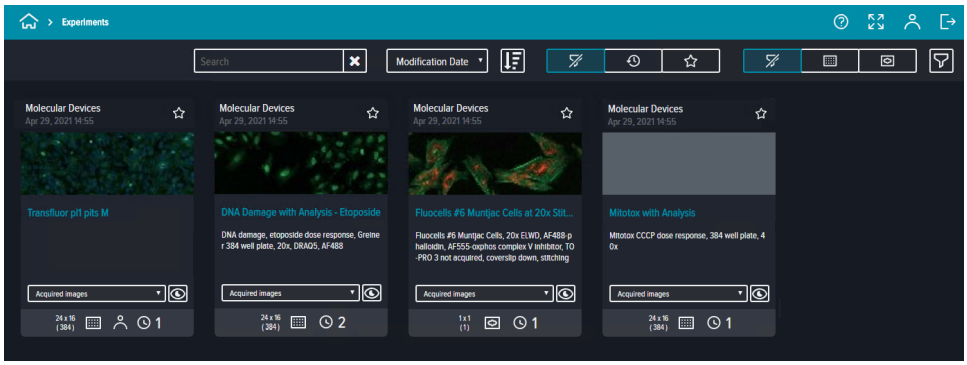
Chapter 4: Experiments Mode

Use **Experiments** mode to view images and analysis data collected in **Acquisition** mode and perform additional offline analysis.

On the **Home** page, click the  **Experiments** tile to enter **Experiments** mode. The **Experiments** library opens.

Experiments Library

When you run a protocol in **Acquisition** mode, a card is created in the **Experiments** library. The card links all the processed images and any analysis data associated with the experiment run.



The experiment cards contain the experiment name and description, along with the name of the user who ran the experiment, the date and time of the experiment run, a  **Favorite** icon (that you can use to flag certain experiments), and a  **View** icon. In addition, each card contains icons to indicate experiment properties, including:

 **Geometry**

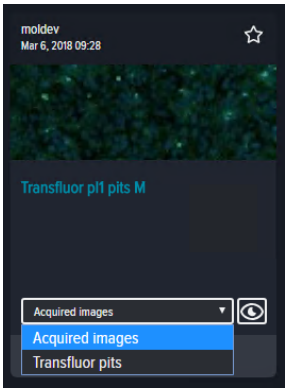
 **Plate** or  **Slide**

 **Shared**

 **Time Points**

Each experiment card includes the following functionality:

- Click a card to display the **Experiments** page, which shows the details for that experiment. From here, you can review analyses and acquisitions details.
- Click **View** (with the default value of **Acquired Images** selected) to display the **Thumbnail View** page for that experiment.
- Click the drop-down list box on the card to select acquired images or analysis data. The options vary depending on what was acquired during the experiment.



Search and Filters

To limit the number of visible cards, use the **Search** field, the sort field, and the filter controls at the top of the Experiments library.

Search

Use search to find specific words in the titles and descriptions of experiments.

To use search:

1. Click in the **Search** field.
2. Enter the word you want to find then press **ENTER**.

Sort

Use sort to arrange the experiment tiles. You can sort in ascending or descending order based on the experiment name, creation date, or modification date.

To use sort:

1. Click the drop-down list box, and select one of the following sort types:
   - **Creation Date**
   - **Modification Date**
   - **Name**
2. Click the sort order icon to specify one of the following sort orders:
   - Sorts from A to Z or from earliest to latest.
   - Sorts from Z to A or from latest to earliest.

Basic Filters

Use the filter icons to control which experiment cards are shown. Active filter icons are highlighted. Filter options include:

- **Recent** or **Favorites**
- **Plates** or **Slides**

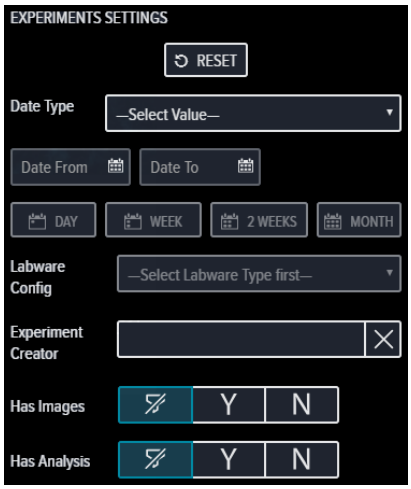
To use filter icons:

Select the filter icon you want to use. The icon is highlighted and only the cards matching the filter option are shown.

Complex Filters

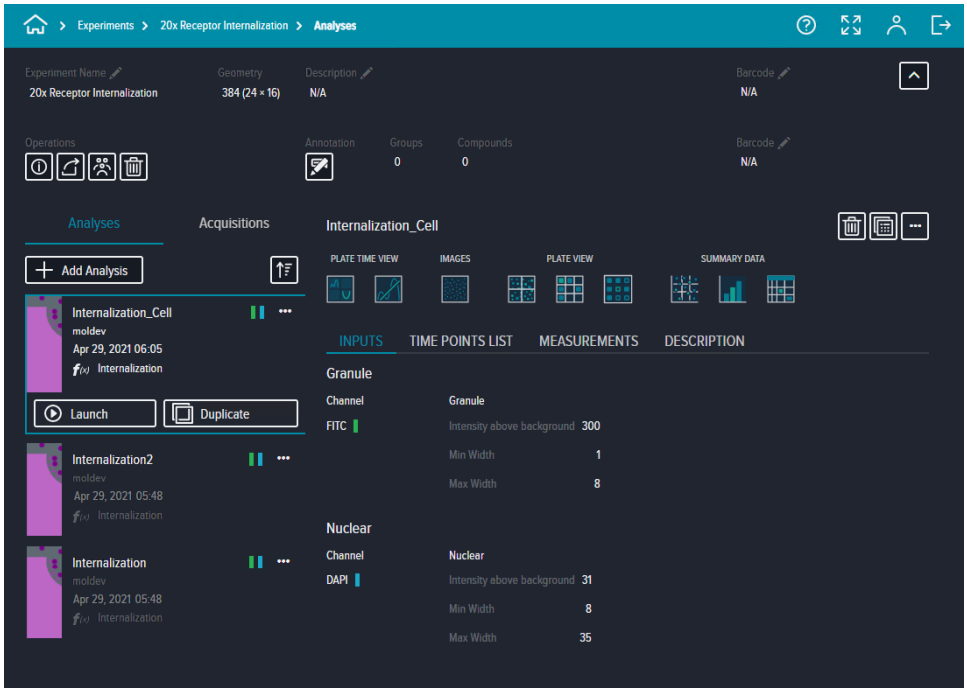
Click **Filter** to create more complex filters. For example, you can filter for experiments created within a specific date range or filter for experiments created by one of three specified users.

Note: In order to use the **Labware Config** field, you must first select either **Plates** or **Slides** from the basic filter icons at the top of the page.



Experiments Page

The **Experiments** page shows the data for an experiment. From here, you can review general experiment details and access analyses and acquisitions details.

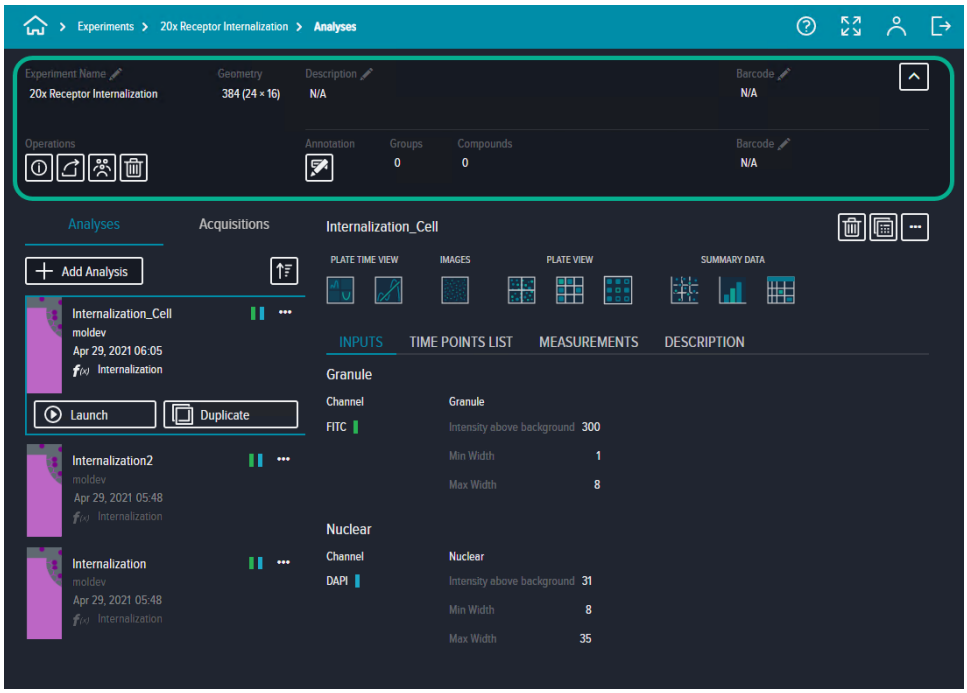


The screen is divided into three sections:

- The top section shows experiment details.
- The bottom left section contains tabs that show analyses and acquisitions for the experiment.
- The bottom right section contains details for the selected analysis or acquisition.

Experiment Details

The top section of the **Experiments** page shows details for the experiment.



The experiment details pane includes the following:

- **Experiment Name**: Indicates the name of the experiment. This field is editable.
- **Geometry**: Indicates the dimensions of the labware used for the experiment.
- **Description**: Indicates the description of the experiment. This field is editable.
- **Barcode**: Indicates the barcode of the plate for the experiment. This field is editable.
- **Operations**: Provides tools to manage the experiment, including exporting experiment images, importing times points, and managing the shared status of an experiment. See Experiment Operations on page 131 for details.
- **Annotation**: Click **Edit Annotations** to open the **Annotations** page to upload or edit annotations. See Creating Annotations on page 135 for details.
- **Groups**: Indicates the number of annotation groups currently in use.
- **Compounds**: Indicates the number of annotation compounds currently in use.
- **Barcode**: Indicates the barcode for the experiment annotations. This field is editable.

Note: The **Edit** icon indicates that a value can be edited. Click the value to edit it.

Experiment Operations

The **Experiments** page includes the following tools to help you manage the experiment:

 **Properties**: Displays properties including storage information and creation and modification details. You can upload an image avatar for the experiment.

 **Export Experiment Images**: Exports images from the experiment as TIFF files for use the Molecular Devices MetaXpress® High-Content Image Acquisition and Analysis Software, the Molecular Devices IN Carta Image Analysis Software, or an image viewer. See Exporting Experiment Images on page 132 for details.

 **Public** or  **Private**: Indicates the shared status of the experiment. You can manage this status to restrict other users from viewing the experiment. See Restricting Experiments on page 134 for details.

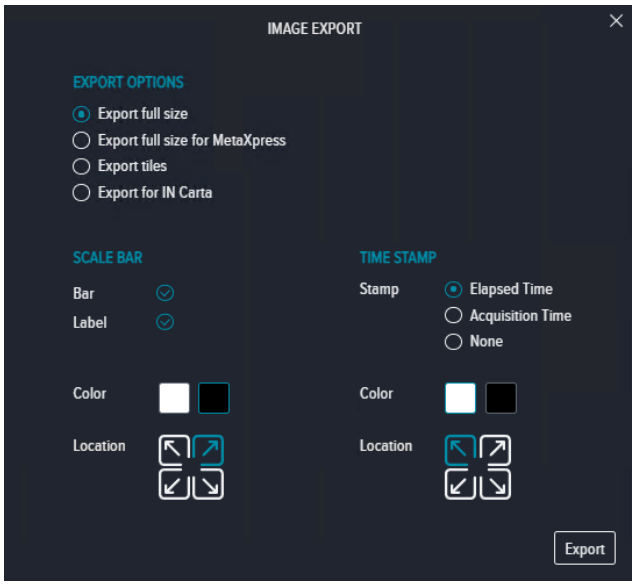
 **Delete Experiment**: Permanently deletes an experiment and all experiment data, including all acquisition and analysis details.

 **CAUTION!** After you delete an experiment, it cannot be recovered.

Exporting Experiment Images

You can export images—one image per stain per region per time point—from the experiment as TIFF files. Exported images are stored in the C:\crx-export folder on the host computer. The software provides several options for the export:

- You can export TIFF file images as tiles or at full size.
- With a full-size image, you can include a scale bar and a time stamp.
- Images larger than 2 GB are scaled down to a 2 GB file size, which is the file size limit for a TIFF file. If an image is larger than 2 GB and you want to export it at full resolution, you can export image tiles. You can then use a TIFF image processing program to join the tiles together for a full-resolution image, if needed.
- If you plan to import the images into the MetaXpress High-Content Image Acquisition and Analysis Software, the images will be scaled to meet the required 32K × 32K pixel size.
- If you plan to import the images into the IN Carta Image Analysis Software, the images will be cropped as required by the IN Carta software. With colorimetric images, each image will be separated into three, 16-bit component images (that is, one red, one green, and one blue). In addition, the export will include the required XDCE file.



Exporting Experiment Images as Tiles

To export experiment images as tiles:

1. On the **Experiments** page, under **Operations**, click **Export Experiment Images**.
2. In the **Image Export** dialog, in the **Export Options** section, select the **Export Tiles** option to export each image tile as a separate TIFF file.
3. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Exporting Full-Size Experiment Images

To export full-sized experiment images:

1. On the **Experiments** page, under **Operations**, click  **Export Experiment Images**.
2. In the **Image Export** dialog, do one of the following in the **Export Options** section:
   - Select the **Export Full Size** option to export a full-size image as a single TIFF file. If the resulting file size is larger than 2 GB, the file will be scaled down to 2 GB.
   - Select the **Export Full Size for MetaXpress** option to export a full-size image as a single TIFF file that can be imported into MetaXpress software.
3. If you want to include a scale bar in the exported image, do the following in the **Scale Bar** section:
   a. Select the **Bar** check box.
   b. If you want to include a label to the scale bar, select the **Label** check box.
   c. In the **Color** field, select the color for the scale bar (either white or black).
   d. In the **Location** field, select the location on the image for the scale bar (top-left, top-right, bottom-left, or bottom-right).
4. If you want to include a time stamp in the exported image, do the following in the **Time Stamp** section:
   a. In the **Stamp** field, do one of the following:
      - Select the **Elapsed Time** option to include a time stamp in the exported image that shows the time elapsed from the earliest time point selected for the export.
      - Select the **Acquisition Time** option to include a time stamp in the exported image that shows the date and time of the acquisition.
      - Select **None** to not include a time stamp in the exported image.
   b. In the **Color** field, select the color for the time stamp (either white or black).
   c. In the **Location** field, select the location on the image for the time stamp (top-left, top-right, bottom-left, or bottom-right).
5. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Exporting Images for the IN Carta Software

To export images for use with the IN Carta software:

1. On the **Experiments** page, under **Operations**, click **Export Experiment Images**.
2. In the **Image Export** dialog, select the **Export for IN Carta** option.

   Note: A message appears explaining how the exported images may be cropped to meet the requirements of the IN Carta software.

3. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Restricting Experiments

You can manage the shared status of an experiment to restrict other users from viewing it. The shared status icon under **Operations** indicates the current shared status, either **Public** or **Private**.

Tip: You can set default sharing permissions in **Configuration Settings**. See Sharing Permissions on page 261 for details.

To restrict other users from viewing the experiment:

1. On the **Experiments** page, under **Operations**, click **Public**.
2. Select the **Private** check box.
3. If you want to specify other users who can view a private experiment, click the **Share With** field and select users from the list.

Creating Annotations

Click **Annotation** on the **Experiments** page to open the **Annotations** page. The **Annotations** page enables you to attach annotations to the experiment. You can manually enter annotations or import them from a CSV file. The annotation data is available as Measurements that can be used in heatmaps, bar graphs, scatter plots, and tables.

Tip: Positive Group and Negative Group values are recognized by the software and can be used later in relevant calculations, like Z Prime.

The right side of the page includes the following icons:

Assign Values: Assigns annotation values to selected wells or slides.


Edit Annotation Names: Adds annotation field names that you can assign to the default field names. Default field names are **group**, **compound**, and **concentration**.


Configure Display Annotations: Specifies which annotation data to show and the heatmap measurements to use.

Import/Export Annotations: Imports annotations from a CSV file and exports annotations to a CSV file. See Importing Annotations on page 137 for details.


Map Annotations: Assigns annotation field names to the default field names.

Selection Mode: Activates selection mode, which enables you to select wells or regions. Click and drag to select multiple wells. Click individual wells or regions to select and deselect them. The number on the icon indicates the number of selected wells or regions.


Deselect All: Deselect all selected wells.

Importing Annotations

You can import annotations from a CSV file. For best results, use one of the sample templates available in the *CellReporterXpress Help* and customize it to your needs.

To import annotations:

1. Click  **Upload Annotations**.
2. In the **Import/Export Annotations** pane, click **Choose File**.
3. Browse to select the CSV file with your annotations.
4. In the **Import Mode** field, select one of the following options:
   - **Replace**: Overwrites current annotations.
   - **Keep Existing**: Adds to existing annotations.
5. In the **File Format** field, select either **Plate** or **Column**.
6. Click **Upload**.

Assigning Annotation Values

To assign values to selected wells or slides:

1. On the **Annotations** page, click  **Assign Values**.
2. Select the wells or slides you want to annotate.
   Click and drag to select a series of wells or slides. Click individual wells or slides to select and deselect them.
3. If needed, do one of the following:
   - Click  **Clear All Values** to clear all values in selected wells or slides.
   - Click  **Clear Values for Selected Wells** for a field to clear values for that field in selected wells or slides.
4. Enter annotation values as needed.
5. For a numeric value field, click  **Assign Series** if you want to assign a series of values. See Assigning a Series of Annotation Values to a Numeric Field on page 138 for details.
6. Do one of the following:
   - Click  **Apply All Values** to apply all values to selected wells or slides.
   - Click  **Apply Value to Selection** for a field to apply values for that field in selected wells or slides.

Assigning a Series of Annotation Values to a Numeric Field

For numeric value fields, you can assign a series of annotation values.

To assign a series:

1. On the **Annotations** page, click  **Assign Values**.
2. For a numeric value field, click  **Assign Series**.
3. In the **X-direction** field, enter the number of well or slides to repeat values in a horizontal direction.
4. In the **Y-direction** field, enter the number of well or slides to repeat values in a vertical direction.
5. In the **Start From** section, select one of the following icons to indicate the order of the series:

    : Assigns the series from top to bottom, then left to right.

    : Assigns the series from top to bottom, then right to left.

    : Assigns the series from right to left, then bottom to top.

    : Assigns the series from right to left, then top to bottom.

    : Assigns the series from bottom to top, then right to left.

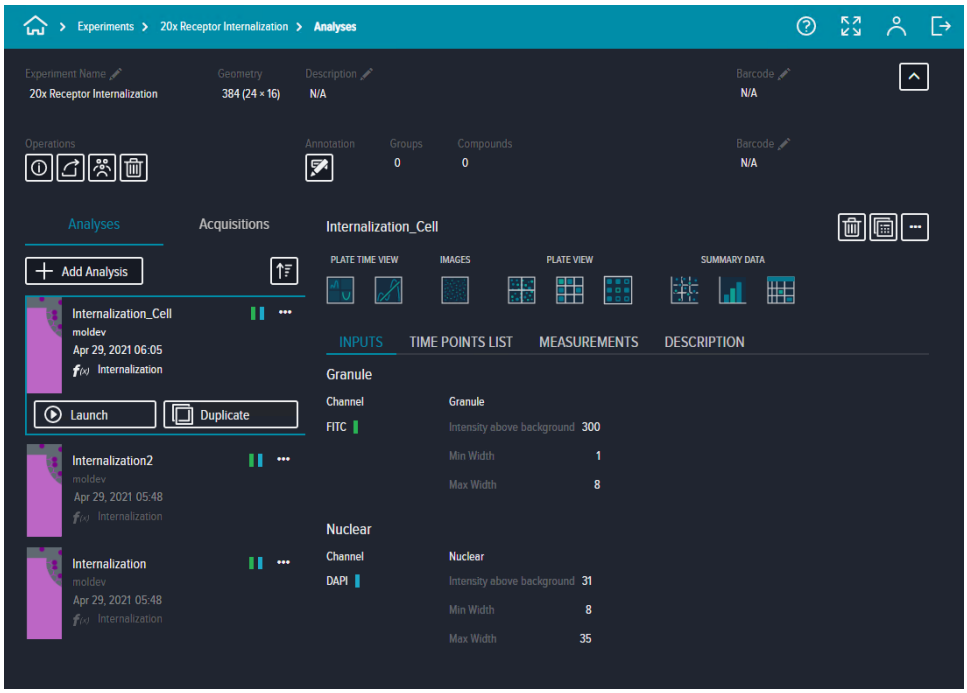
    : Assigns the series from bottom to top, then left to right.

    : Assigns the series from left to right, then bottom to top.

    : Assigns the series from left to right, the top to bottom.
6. In the **Starting Value** field, enter the starting value for the series.
7. Click the **Step By** drop-down list box, and select the operator for the series.
8. In the **Step By** field, enter the step value for the series.
9. Click **Save**.

Experiment Analysis Details

The bottom section of the **Experiments** page shows analysis and acquisition details for the experiment. The left pane in the bottom section contains two tabs: **Analyses** and **Acquisitions**. Click the **Analyses** tab to show analysis details.



On the **Analyses** tab, each analysis for the experiment is listed. The following functions are available:

- **Launch**: Opens the analysis settings and enables you to select a time point to rerun the analysis.
- **Duplicate**: Opens a copy of the analysis settings and enables you to select a time point and modify the analysis settings. Then you can run save and run the new analysis.
- **Add Analysis**: Opens the analysis settings and enables to select a time point and set up new analysis settings.

All three functions use the add analysis workflow. See Add Plate Analysis Workflow on page 140 or Add Slide Analysis Workflow on page 149 for details.

In addition, you can click  to save an analysis as a template or delete an analysis.

The section on the right shows analysis data on the following tabs:

- **Inputs**: Displays the parameters for the analysis set in **Acquisition** mode.
- **Time Points List**: Displays the time points for the acquisition and allows you to select specific time points to analyze.
- **Measurements**: Remove or export selected cell measurements.
- **Description**: View and edit the analysis name, description, and avatar.

You can also access various plate views or slide views. See Plate Views on page 160 and Slide Views on page 191 for details.

Add Plate Analysis Workflow

The icons in the **Steps** pane on the left side of the page guide you through the plate experiment configuration process. The tools and controls in the pane on the right side of the page vary according to the step being configured and the experiment type.

The add plate analysis workflow is as follows:


Time Points is the step where you select the time points for the experiment. See Time Points on page 141 for details.


Analysis Settings is the step where you set up image analysis for the experiment. The data generated based on these settings becomes the analysis data for the experiment. See Analysis Settings on page 141 for details.


Region Selection to Analyze is the step where you select the region of interest to be analyzed. You must select at least one region to run an experiment. See Region Selection to Analyze on page 146 for details.


Well Selection for Analysis is the step where you select the wells for the experiment. See Well Selection for Analysis on page 147 for details.


Run Protocol is the last step in the workflow. In this step, you can review the settings for the experiment and address any issues. See Run Protocol on page 148 for details.

Time Points

 **Time Points** is the step where you select the time points for the experiment.

By default, all time points from the acquisition are selected. Click the time points as needed to select the time points for the experiment.

To continue to the next workflow step, click  **Analysis Settings**. See Analysis Settings on this page for details.

Analysis Settings

 **Analysis Settings** is the step where you set up image analysis for the experiment. The data generated based on these settings becomes the analysis data for the experiment.

The right side of the page includes the following icons:

 **Choose Analysis**: Toggles analysis on or off and selects the analysis for the experiment.

 **Measurements**: Specifies the cell measurements included in the analysis.

 **Save Analysis**: Saves the analysis for use in future experiments.

 **Cell Info Mode**: Displays information on a selected cell.

 **Comparison Mode**: Captures two preview images, which enables you to compare the uniformity of the image quality.

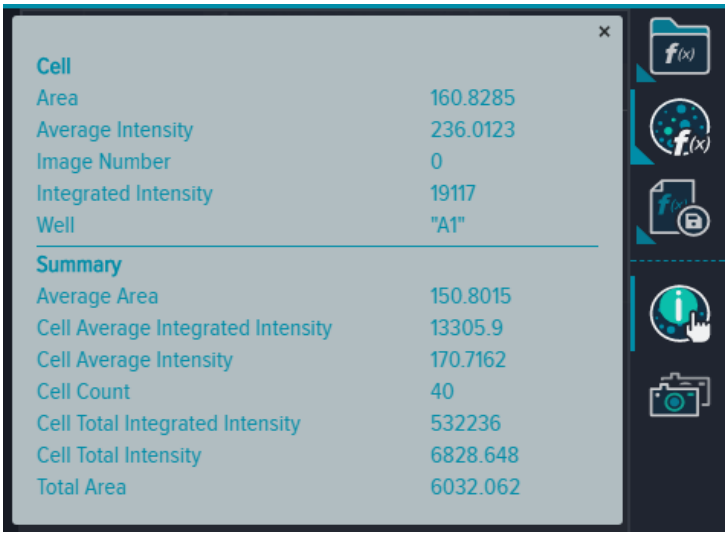
Setting Up an Analysis

To set up an analysis:

1. On the **Analysis Settings** page, in the **Tools** pane on the right, click **Choose Analysis**.
2. Set **Analysis** to **On**.
3. Select a fluorescence or transmitted light analysis. See Analysis Descriptions on page 263 for details on the available analyses.
4. Click **Measurements**.
5. In the **Measurements** pane, select the measurements for the analysis.

   Note: The recommended measurements for the analysis are selected by default.

6. In the bottom pane, click **Test Analysis** to calculate the summary measurements using the preview image.
7. To view cell information, click **Cell Info Mode** and select a detected cell in the image preview.



As part of configuring analysis settings, you may do the following:

- Testing the Analysis of a Well, see page 143
- Testing the Analysis of Comparison Images, see page 144
- Saving Analysis Settings, see page 145

To continue to the next workflow step, click **Region Selection to Analyze**. See Region Selection to Analyze on page 146 for details.

Testing the Analysis of a Well

The preview represents the image quality to expect when you run your experiment.

To test the analysis of a well:

1. On the **Acquisition Analysis** page, in the bottom pane, click  **Test Analysis**.
2. Click  **Algorithm Input**.
3. On the **Algorithm Input** tab, adjust the settings as needed to optimize object detection. Settings vary depending on the selected analysis type. Setting options typically control intensity, minimum width, and maximum width for each stain used in the acquisition.
4. Click  **Test Analysis** to preview the analysis.
5. If needed, click  **Image Intensity Settings** and do the following to adjust the settings as needed for each channel:

   Note: You typically do not need to adjust the image intensity settings for the analysis, which are based on the acquisition settings. If you adjust these settings, it affects only the image display; it has no effect on any analysis measurements.

   a. Use the **Channel Color** drop-down list box to change the identification color for the channel.
   b. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image.
   c. Click **Auto** to set the best contrast based on the current preview.
6. Click  **Choose Position to Acquire**.
7. Click  **Select Well**.
8. In the plate map, select a different well and time point.
   The software runs a test analysis.
9. In the single well map, click and drag the  selection tool to select the region of the well for the preview, if needed. The center area of the well is selected by default.
   The software runs a test analysis.
10. Click  **Algorithm Input**.
11. Click  **Test Analysis** to preview the analysis.
12. Repeat these steps as needed until you are satisfied with the quality of the preview.

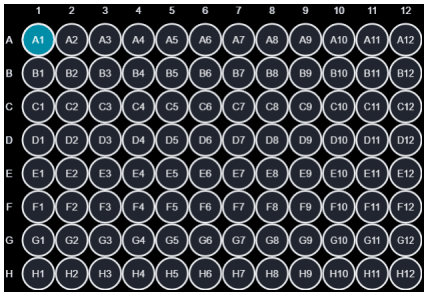
When you are satisfied with the quality of the preview, you may want to save the analysis settings for later reuse. See Saving Analysis Settings on page 145 for details.

Testing the Analysis of Comparison Images

You can preview two images from different wells to compare the uniformity of the image quality.

To test the analysis of comparison images:

1. On the **Acquisition Analysis** page, in the **Tools** pane on the right, click  **Comparison Mode**.
2. In the bottom pane, click  **Test Analysis**.
3. Click  **Algorithm Input**.
4. On the **Algorithm Input** tab, adjust the settings as needed to optimize object detection. The settings affect both previews. Settings vary depending on the selected analysis type. Setting options typically control intensity, minimum width, and maximum width for each stain used in the acquisition.
5. On the left side of the pane, click the  **Choose Well and Area to Acquire** tab.
6. Click  **Select Well** on the left.
7. In the well map, select the first well and time point for the preview.



8. Click  **Select Well** on the right.
9. In the well map, select the second well and time point for the preview.
10. In the single well map on the left, click and drag the  selection tool to select the region of the first well for the preview, if needed. The center area of the well is selected by default.
11. Repeat the previous step in the single well map on the right to select the region of the second well for the preview, if needed.
12. Click  **Test Analysis** to preview the analysis.

13. If needed, click  **Image Intensity Settings** and do the following to adjust the settings as needed for each channel:

    Note: You typically do not need to adjust the image intensity settings for the analysis, which are based on the acquisition settings. If you adjust these settings, it affects only the image display; it has no effect on any analysis measurements.

    a. Use the **Channel Color** drop-down list box to change the identification color for the channel.
    b. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image.
    c. Click **Auto** to set the best contrast based on the current preview.
14. Repeat these steps as needed until you are satisfied with the quality of the previews.

After you are satisfied with the quality of the preview, you may want to save the analysis settings for later reuse. See Saving Analysis Settings on this page for details.

Saving Analysis Settings

When you are satisfied with the quality of the preview in Testing the Analysis of a Well on page 143 or Testing the Analysis of Comparison Images on page 144, you may want to save the analysis settings for later reuse.

To save analysis settings:

1. On the **Acquisition Analysis** page, in the **Tools** pane on the right, click  **Save Analysis**.
2. In the **Save Analysis** pane, in the **Analysis Settings** field, enter a descriptive name.
3. If needed, add an avatar image by doing one of the following:
   - Click **Use Captured Picture**.
   - Click **Click to upload**, select an image file, and click **Open**.
4. Click **Save**.

To continue to the next workflow step, click  **Region Selection to Analyze**. See Region Selection to Analyze on page 146 for details.

Region Selection to Analyze

Region Selection to Analyze is the step where you select the region of interest to be analyzed. You must select at least one region to run an experiment.

Note: Several factors (including the number of wells, the magnification of the objective, and the use of the environmental control cassette) may prevent you from selecting some regions within certain wells.

The right side of the page includes the following icons:

From Center: Adds an analysis region selection overlay in the center of the well or slide. You can control various elements of the analysis region, including the percentage of the well and the shape of the selection overlay.

Activate Edit Mode: Activates the selection handles on the acquisition region selection overlay, enabling you to manually move and size it.

Add Analysis Region: Adds a new analysis region selection overlay that you can size and move into position.

Delete Selected Region: Removes the selected region overlay.

Clear All Regions: Removes all the visible region overlays.

To continue to the next workflow step, click  **Well Selection for Analysis**. See Well Selection for Analysis on page 147 for details.

Well Selection for Analysis

 **Well Selection for Analysis** is the step where you select the wells for the experiment.

The Well Selection for Analysis page shows a plate map from the acquisition. All acquired wells are selected by default. Select and deselect wells as needed. You must select at least one well to run an experiment.

The right side of the page includes the following icons:

 **Select All**: Selects all wells from the acquisition.

 **Clear All Regions**: Removes all well selections.

Selecting a Group of Wells

On the **Well Selection** page, in the plate map, click and drag to select a series of wells.

Selecting Individual Wells

On the **Well Selection** page, in the plate map, click a well to select it.

Deselecting Individual Wells

On the **Well Selection** page, in the plate map, click a selected well to deselect it.

To continue to the next workflow step, click  **Run Protocol**. See Run Protocol on page 148 for details.

Run Protocol


Run Protocol is the last step in the workflow. In this step, you can review the settings for the experiment and address any issues.

When you run the experiment, the software analyzes the data according to your settings. When the experiment completes, the software saves all acquired data as specified. Acquired data is then available for viewing and analysis in **Experiments** mode.

The right side of the page includes the following icons:


Experiment Details: Displays acquisition and analysis settings and enables you to validate settings before running the experiment.


Run Experiment: Runs the experiment using the specified analysis settings. This icon becomes enabled when all the settings on the **Validation** tab are valid and an **Analysis Name** has been entered.

To run a protocol:

1. On the **Run Protocol** page, in the **Analysis Name** field, enter a name to identify the analysis.
2. If needed, in the **Analysis Description** field, enter a description of the analysis.
3. On the **Validation** tab, verify that all the required settings are valid. A  icon indicates a valid setting and a  icon indicates an invalid or missing setting. All acquisition settings must be valid to add the analysis. See Fixing Invalid Parameters on this page for details.
4. Click  **Run Experiment** to run the experiment.

The **Monitor** page opens to display the progress of the running experiment. See Monitor Mode on page 213 for details.

Fixing Invalid Parameters

Invalid parameter settings are indicated by a  icon. Click the icon to display the reason for the invalid parameter.

To fix an invalid parameter:

1. Click the link next to the  icon to open the workflow step for the invalid parameter.
2. Address the issue.
3. Click  **Run Protocol** to return to **Run Protocol** page.

Add Slide Analysis Workflow

The icons in the **Steps** pane on the left side of the page guide you through the slide experiment configuration process. The tools and controls in the pane on the right side of the page vary according to the step being configured and the experiment type.

The add slide analysis workflow is as follows:

 **Time Points** is the step where you select the time points for the experiment. See Time Points on page 150 for details.

 **Analysis Settings** is the step where you set up image analysis for the experiment. The data generated based on these settings becomes the analysis data for the experiment. See Analysis Settings on page 150 for details.

 **Region Selection to Analyze** is the step where you select the region of interest to be analyzed. You must select at least one region to run an experiment. See Region Selection to Analyze on page 155 for details.

 **Run Protocol** is the last step in the workflow. In this step, you can review the settings for the experiment and address any issues. See Run Protocol on page 156 for details.

Time Points

 **Time Points** is the step where you select the time points for the experiment.

By default, all time points from the acquisition are selected. Click the time points as needed to select the time points for the experiment.

To continue to the next workflow step, click  **Analysis Settings**. See Analysis Settings on this page for details.

Analysis Settings

 **Analysis Settings** is the step where you set up image analysis for the experiment. The data generated based on these settings becomes the analysis data for the experiment.

The right side of the page includes the following icons:

 **Choose Analysis**: Toggles analysis on or off and selects the analysis for the experiment.

 **Measurements**: Specifies the cell measurements included in the analysis.

 **Save Analysis**: Saves the analysis for use in future experiments.

 **Cell Info Mode**: Displays information on a selected cell.

 **Comparison Mode**: Captures two preview images, which enables you to compare the uniformity of the image quality.

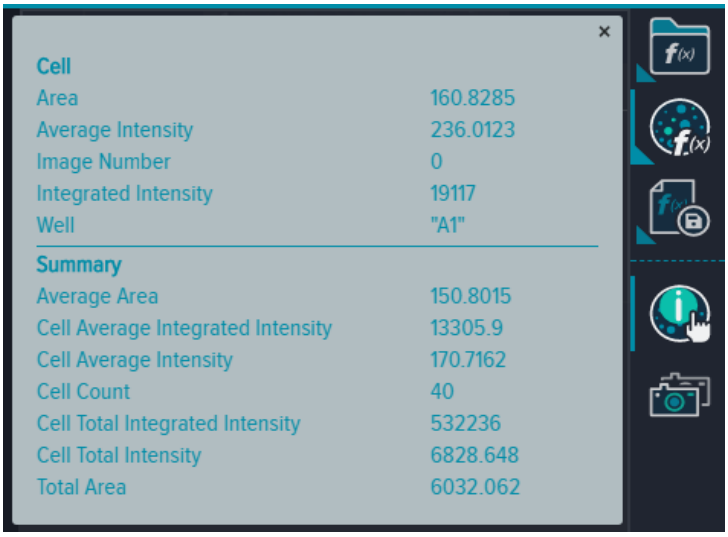
Setting Up an Analysis

To set up an analysis:

1. On the **Analysis Settings** page, in the **Tools** pane on the right, click **Choose Analysis**.
2. Set **Analysis** to **On**.
3. Select a fluorescence or transmitted light analysis. See Analysis Descriptions on page 263 for details on the available analyses.
4. Click **Measurements**.
5. In the **Measurements** pane, select the measurements for the analysis.

   Note: The recommended measurements for the analysis are selected by default.

6. In the bottom pane, click **Test Analysis** to calculate the summary measurements using the preview image.
7. To view cell information, click **Cell Info Mode** and select a detected cell in the image preview.



As part of configuring analysis settings, you may do the following:

- Testing the Analysis of a Region, see page 152
- Testing the Analysis of Comparison Images, see page 153
- Saving Analysis Settings, see page 154

To continue to the next workflow step, click **Region Selection to Analyze**. See Region Selection to Analyze on page 155 for details.

Testing the Analysis of a Region

The preview represents the image quality to expect when you run your experiment.

To test the analysis of a region:

1. On the **Acquisition Analysis** page, in the bottom pane, click  **Test Analysis**.
2. Click  **Algorithm Input**.
3. On the **Algorithm Input** tab, adjust the settings as needed to optimize object detection. Settings vary depending on the selected analysis type. Setting options typically control intensity, minimum width, and maximum width for each stain used in the acquisition.
4. Click  **Test Analysis** to preview the analysis.
5. If needed, click  **Image Intensity Settings** and do the following to adjust the settings as needed for each channel:

   Note: You typically do not need to adjust the image intensity settings for the analysis, which are based on the acquisition settings. If you adjust these settings, it affects only the image display; it has no effect on any analysis measurements.

   a. Use the **Channel Color** drop-down list box to change the identification color for the channel.
   b. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image.
   c. Click **Auto** to set the best contrast based on the current preview.
6. Click  **Choose Position to Acquire**.
7. Click  **Select Slide**.
8. Click  **Snap Overview** to snap a low-resolution overview image of the entire slide in the single slide map. This can help you identify a region of interest for your experiment. The overview image is created by the overview camera, which uses no objective.
9. In the single slide map, click and drag the  selection tool to select the region of the slide for the preview, if needed. The center area of the slide is selected by default.
   The CellReporterXpress software runs a test analysis.
10. Click  **Algorithm Input**.
11. Click  **Test Analysis** to preview the analysis.
12. Repeat these steps as needed until you are satisfied with the quality of the preview.

After you are satisfied with the quality of the preview, you may want to save the analysis settings for later reuse. See Saving Analysis Settings on page 154 for details.

Testing the Analysis of Comparison Images

You can preview two images from different regions to compare the uniformity of the image quality.

To test the analysis of comparison images:

1. On the **Acquisition Analysis** page, in the **Tools** pane on the right, click  **Comparison Mode**.
2. In the bottom pane, click  **Test Analysis**.
3. Click  **Algorithm Input**.
4. On the **Algorithm Input** tab, adjust the settings as needed to optimize object detection. The settings affect both previews. Settings vary depending on the selected analysis type. Setting options typically control intensity, minimum width, and maximum width for each stain used in the acquisition.
5. On the left side of the pane, click the 

Choose Position to Acquire tab.
6. Click 

Select Slide on the left.
7. In the slide map, select the first slide and time point for the preview. The A1 slide is selected by default.
8. Click 

Select Slide on the right.
9. In the slide map, select the second slide and time point for the preview.
10. Click  **Snap Overview** to snap a low-resolution overview image of the entire slide in the single slide map. This can help you identify a region of interest for your experiment. The overview image is created by the overview camera, which uses no objective.
11. In the single slide map on the left, click and drag the  selection tool to select the region of the first slide for the preview, if needed. The center area of the slide is selected by default.
12. Repeat the previous step in the single slide map on the right to select the region of the second slide for the preview, if needed.
13. Click  **Test Analysis** to preview the analysis.

14. If needed, click  **Image Intensity Settings** and do the following to adjust the settings as needed for each channel:

    Note: You typically do not need to adjust the image intensity settings for the analysis, which are based on the acquisition settings. If you adjust these settings, it affects only the image display; it has no effect on any analysis measurements.

    a. Use the **Channel Color** drop-down list box to change the identification color for the channel.
    b. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image.
    c. Click **Auto** to set the best contrast based on the current preview.
15. Repeat these steps as needed until you are satisfied with the quality of the previews.

After you are satisfied with the quality of the preview, you may want to save the analysis settings for later reuse. See Saving Analysis Settings on this page for details.

Saving Analysis Settings

When you are satisfied with the quality of the preview in Testing the Analysis of a Region on page 152 or Testing the Analysis of Comparison Images on page 153, you may want to save the analysis settings for later reuse.

To save analysis settings:

1. On the **Acquisition Analysis** page, in the **Tools** pane on the right, click  **Save Analysis**.
2. In the **Save Analysis** pane, in the **Analysis Settings** field, enter a descriptive name.
3. If needed, add an avatar image by doing one of the following:
   - Click **Use Captured Picture**.
   - Click **Click to upload**, select an image file, and click **Open**.
4. Click **Save**.

To continue to the next workflow step, click  **Region Selection to Analyze**. See Region Selection to Analyze on page 155 for details.

Region Selection to Analyze


Region Selection to Analyze is the step where you select the region of interest to be analyzed. You must select at least one region to run an experiment.


Note: Several factors (including the slide holder format and the magnification of the objective) may prevent you from selecting some regions near the edges a slide.

Click  **Snap Overview** to snap a low-resolution overview image of the entire slide in the single slide map. This can help you identify a region of interest for your experiment. The overview image is created by the overview camera, which uses no objective.

The right side of the page includes the following icons:

 **From Center**: Adds an analysis region selection overlay in the center of the well or slide. You can control various elements of the analysis region, including the percentage of the slide and the shape of the selection overlay.

 **Activate Edit Mode**: Activates the selection handles on the acquisition region selection overlay, enabling you to manually move and size it.

 **Add Analysis Region**: Adds a new analysis region selection overlay that you can size and move into position.

 **Delete Selected Region**: Removes the selected region overlay.

 **Clear All Regions**: Removes all the visible region overlays.

To continue to the next workflow step, click  **Run Protocol**. See Run Protocol on page 156 for details.

Run Protocol

 **Run Protocol** is the last step in the workflow. In this step, you can review the settings for the experiment and address any issues.

When you run the experiment, the software analyzes the data according to your settings. When the experiment completes, the software saves all acquired data as specified. Acquired data is then available for viewing and analysis in **Experiments** mode.

The right side of the page includes the following icons:

 **Experiment Details**: Displays acquisition and analysis settings and enables you to validate settings before running the experiment.

 **Run Experiment**: Runs the experiment using the specified analysis settings. This icon becomes enabled when all the settings on the **Validation** tab are valid and an **Experiment Name** has been entered.

To run a protocol:

1. On the **Run Protocol** page, in the **Analysis Name** field, enter a name to identify the analysis.
2. If needed, in the **Analysis Description** field, enter a description of the analysis.
3. On the **Validation** tab, verify that all the required settings are valid. A  icon indicates a valid setting and a  icon indicates an invalid or missing setting. All acquisition settings must be valid to run the experiment. See Fixing Invalid Parameters on this page for details.
4. Click  **Run Experiment** to run the experiment.

The **Monitor** page opens to display the progress of the running experiment. See Monitor Mode on page 213 for details.

Fixing Invalid Parameters

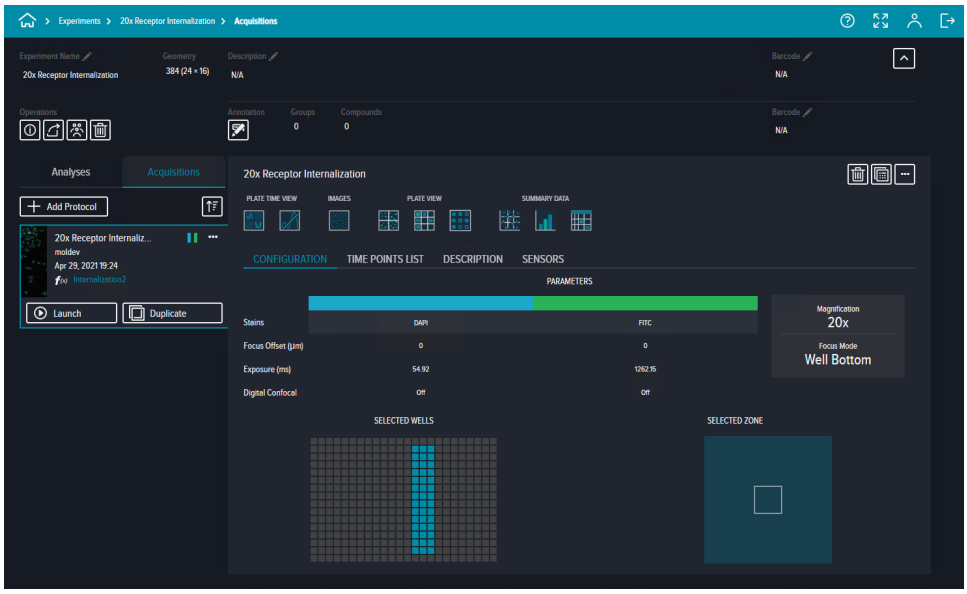
Invalid parameter settings are indicated by a  icon. Click the icon to display the reason for the invalid parameter.

To fix an invalid parameter:

1. Click the link next to the  icon to open the workflow step for the invalid parameter.
2. Address the issue.
3. Click  **Run Protocol** to return to **Run Protocol** page.

Experiment Acquisition Details

The bottom section of the **Experiments** page shows analysis and acquisition details for the experiment. The left pane in the bottom section contains two tabs: **Analyses** and **Acquisitions**. Click the **Acquisitions** tab to show acquisition details.



On the **Acquisitions** tab, each acquisition for the experiment is listed. The following functions are available:

- **Launch**: Opens the acquisition for the experiment and enables you to perform additional acquisitions (using the same protocol).
- **Duplicate**: Opens the acquisition for the experiment and enables you to modify the protocol and perform additional acquisitions.

In addition, you can click  to save the protocol as a template or delete the acquisition.

The section on the right shows acquisition data on the following tabs:

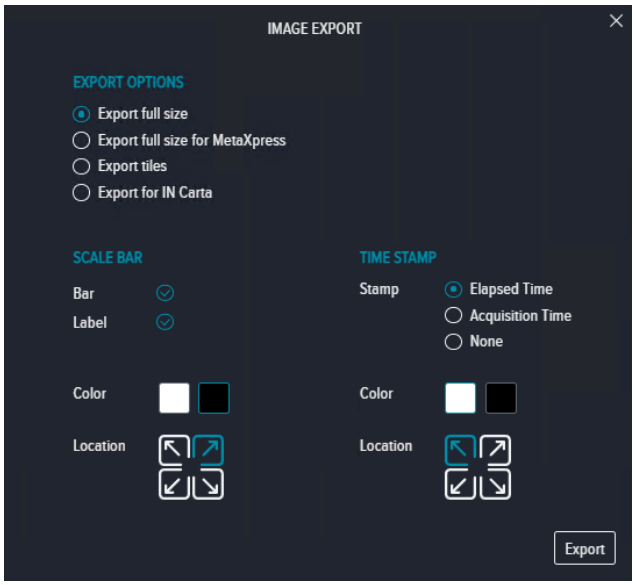
- **Inputs**: Displays the parameters for the acquisition set in **Acquisition** mode.
- **Time Points List**: Displays the time points for the acquisition. You can get details on specific time points, delete time points, and export time point images. See Exporting Time Point Images on page 158 for details.
- **Description**: View and edit the analysis name, description, and avatar.
- **Sensors**: Displays details on the environmental control settings for the acquisition.

You can also access various plate views or slide views. See Plate Views on page 160 and Slide Views on page 191 for details.

Exporting Time Point Images

You can export images—one image per stain per region per time point—from the experiment as TIFF files. Exported images are stored in the C:\crx-export folder on the host computer. The software provides several options for the export:

- You can export TIFF file images as tiles or at full size.
- With a full-size image, you can include a scale bar and a time stamp.
- Images larger than 2 GB are scaled down to a 2 GB file size, which is the file size limit for a TIFF file. If an image is larger than 2 GB and you want to export it at full resolution, you can export image tiles. You can then use a TIFF image processing program to join the tiles together for a full-resolution image, if needed.
- If you plan to import the images into the MetaXpress High-Content Image Acquisition and Analysis Software, the images will be scaled to meet the required 32K × 32K pixel size.
- If you plan to import the images into the IN Carta Image Analysis Software, the images will be cropped as required by the IN Carta software. With colorimetric images, each image will be separated into three, 16-bit component images (that is, one red, one green, and one blue). In addition, the export will include the required XDCE file.



Exporting Time Point Images as Tiles

To export time point images as tiles:

1. On the **Experiments** page, in the acquisition details, click the **Time Points List** tab.
2. Select the time points you want to export.
3. Click **Other Actions** and select **Export Time Point(s) Images**.
4. In the **Image Export** dialog, in the **Export Options** section, select the **Export Tiles** option to export each image tile as a separate TIFF file.
5. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Exporting Full-Size Time Point Images

To export full-size time point images:

1. On the **Experiments** page, in the acquisition details, click the **Time Points List** tab.
2. Select the time points you want to export.
3. Click **Other Actions** and select **Export Time Point(s) Images**.
4. In the **Image Export** dialog, do one of the following in the **Export Options** section:
   - Select the **Export Full Size** option to export a full-size image as a single TIFF file. If the resulting file size is larger than 2 GB, the file will be scaled down to 2 GB.
   - Select the **Export Full Size for MetaXpress** option to export a full-size image as a single TIFF file that can be imported into MetaXpress software.
5. If you want to include a scale bar in the exported image, do the following in the **Scale Bar** section:
   a. Select the **Bar** check box.
   b. If you want to include a label to the scale bar, select the **Label** check box.
   c. In the **Color** field, select the color for the scale bar (either white or black).
   d. In the **Location** field, select the location on the image for the scale bar (top-left, top-right, bottom-left, or bottom-right).
6. If you want to include a time stamp in the exported image, do the following in the **Time Stamp** section:
   a. In the **Stamp** field, do one of the following:
      - Select the **Elapsed Time** option to include a time stamp in the exported image that shows the time elapsed from the earliest time point selected for the export.
      - Select the **Acquisition Time** option to include a time stamp in the exported image that shows the date and time of the acquisition.
      - Select **None** to not include a time stamp in the exported image.
   b. In the **Color** field, select the color for the time stamp (either white or black).
   c. In the **Location** field, select the location on the image for the time stamp (top-left, top-right, bottom-left, or bottom-right).
7. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Exporting Time Point Images for the IN Carta Software

To export time point images for use with the IN Carta Image Analysis Software:

1. On the **Experiments** page, in the acquisition details, click the **Time Points List** tab.
2. Select the time points you want to export.
3. Click **Other Actions** and select **Export Time Point(s) Images**.
4. In the **Image Export** dialog, select the **Export for IN Carta** option.

   Note: A message appears explaining how the exported images may be cropped to meet the requirements of the IN Carta software.

5. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Plate Views

The following plate views are available:

Plate Views


Plate Time View: Shows measurements over time for each well. See Plate Time View on page 162 for details.


Time Graph: Shows measurements over time for selected wells. See Plate Time Graph on page 163 for details.


Thumbnail View: Shows an overview of the well images in low resolution. See Plate Thumbnail View on page 164 for details.


Data View: Shows a heatmap with up to four measurements displayed in the wells. See Plate Data View on page 167 for details.


Heatmap: Shows a heatmap of one measurement. See Plate Heatmap on page 168 for details.


Images: Shows high-resolution images for deep zoom viewing. See Plate Images on page 169 for details.

Summary Views


Scatter Plot: Shows a scatter plot of two summary measurements. See Summary Scatter Plot on page 174 for details.


Stacked Bar: Shows a histogram-style bar graph with up to two different measurements side by side. You can add a third measurement to subdivide each bar. See Summary Stacked Bar on page 176 for details.


Table: Shows a table with summary well-level measurements. See Summary Table on page 178 for details.

Cellular Views

Note: You must select at least one well in a Plate view or a Summary view to enable the Cellular views.

 **Cell Level Density Heatmap**: Shows a scatter plot-style graph of two measurements. See Cell Level Density Heatmap on page 181 for details.

 **Cell Level Stacked Bar**: Shows a histogram-style bar graph with up to two different measurements side by side. You can add a third measurement to subdivide each bar. See Cell Level Stacked Bar on page 183 for details.

Cell Zoom Views

Note: You must select at least one bin in a Cellular view to enable the Cell Zoom views.

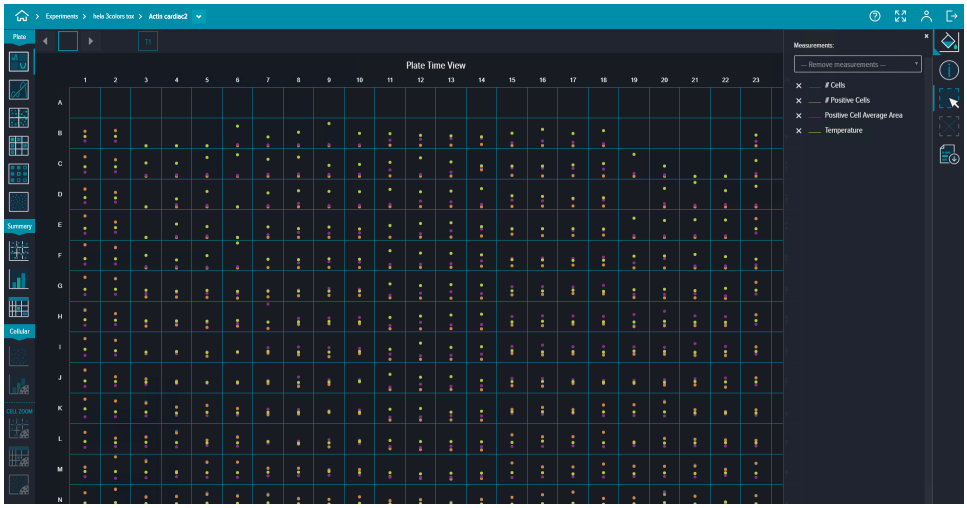
 **Scatter Mode**: Shows a scatter plot graph of two measurements. See Cell Zoom Level Scatter Plot on page 185 for details.

 **Cell Level Table**: Shows a table with cellular measurements. See Cell Zoom Level Table on page 187 for details.

 **Cell Level Images**: Shows high-resolution images for deep zoom viewing of individual cells. See Cell Zoom Level Images on page 189 for details.

Plate Time View

The  **Plate Time View** shows measurements over time for each well.



The right side of the page includes the following icons:

 **Measurements**: Selects the measurements to show.

 **Well Info Mode**: Shows summary measurements for the selected well.

 **Selection Mode**: Activates selection mode, which enables you to select wells. Click and drag to select multiple wells. The number on the icon indicates the number of selected wells.

 **Deselect All**: Deselects all wells.

 **Export Current View to an Image**: Downloads a JPEG image of the page view, including selections. This function is not available with a tablet.

Plate Time Graph

The **Time Graph** shows measurements over time for selected wells.

Double-click on a well image to navigate to the **Images View** page for that well.

The right side of the page includes the following icons:

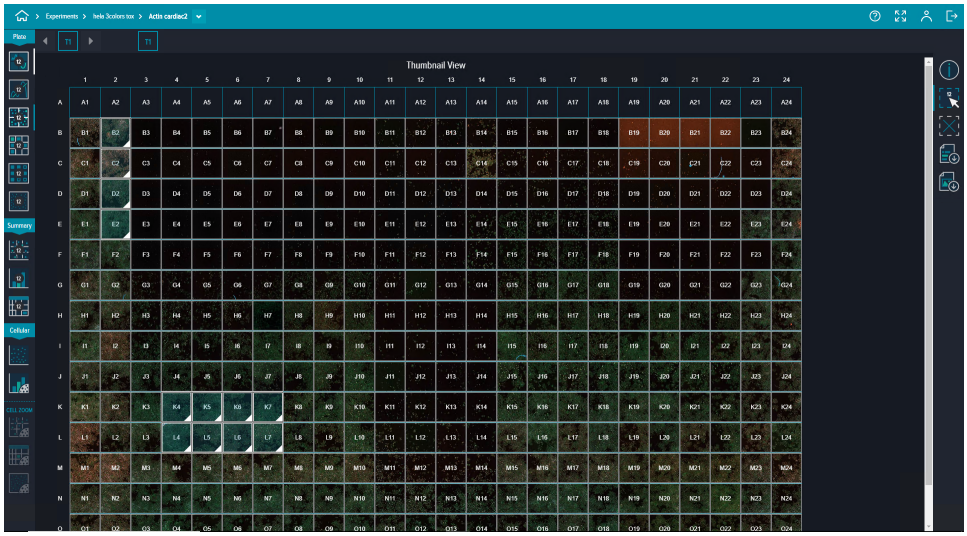
Measurements: Selects the measurements to show.

Toggle Grid Lines: Toggles the display of grid lines on the graph.

Toggle Value Labels: Displays the numeric value for the selected measurement at each time point.

Plate Thumbnail View

The **Thumbnail View** shows an overview of the well images in low resolution. Double-click on a well image to view the image for that well. See Plate Images on page 169 for details.



The right side of the page includes the following icons:

Well Info Mode: Shows summary measurements for the selected well.

Selection Mode: Activates selection mode, which enables you to select wells. Click and drag to select multiple wells. The number on the icon indicates the number of selected wells.

Deselect All: Deselects all wells.

Export Current View to an Image: Downloads a JPEG image of the page view, including selections. This function is not available with a tablet.

Export Images: Exports the selected thumbnail images as TIFF files for use the MetaXpress High-Content Image Acquisition and Analysis Software, the IN Carta Image Analysis Software, or an image viewer. See Exporting Thumbnail Images on page 165 for details.

Exporting Thumbnail Images

You can export images—one image per stain per region per time point—from the experiment as TIFF files. Exported images are stored in the C:\crx-export folder on the host computer. The software provides several options for the export:

- You can export TIFF file images as tiles or at full size.
- With a full-size image, you can include a scale bar and a time stamp.
- Images larger than 2 GB are scaled down to a 2 GB file size, which is the file size limit for a TIFF file. If an image is larger than 2 GB and you want to export it at full resolution, you can export image tiles. You can then use a TIFF image processing program to join the tiles together for a full-resolution image, if needed.
- If you plan to import the images into the MetaXpress High-Content Image Acquisition and Analysis Software, the images will be scaled to meet the required 32K × 32K pixel size.
- If you plan to import the images into the IN Carta Image Analysis Software, the images will be cropped as required by the IN Carta software. With colorimetric images, each image will be separated into three, 16-bit component images (that is, one red, one green, and one blue). In addition, the export will include the required XDCE file.

Exporting Thumbnail Images as Tiles

To export thumbnail images as tiles:

1. On the **Thumbnail View** page, select the wells you want to export.
2. On the right side of the page, click  **Export Images**.
3. In the **Image Export** dialog, in the **Export Options** section, select the **Export Tiles** option to export each image tile as a separate TIFF file.
4. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Exporting Full-Size Thumbnail Images

To export full-sized thumbnail images:

1. On the **Thumbnail View** page, select the wells you want to export.
2. On the right side of the page, click  **Export Images**.
3. In the **Image Export** dialog, do one of the following in the **Export Options** section:
   - Select the **Export Full Size** option to export a full-size image as a single TIFF file. If the resulting file size is larger than 2 GB, the file will be scaled down to 2 GB.
   - Select the **Export Full Size for MetaXpress** option to export a full-size image as a single TIFF file that can be imported into MetaXpress software.
4. If you want to include a scale bar in the exported image, do the following in the **Scale Bar** section:
   a. Select the **Bar** check box.
   b. If you want to include a label to the scale bar, select the **Label** check box.
   c. In the **Color** field, select the color for the scale bar (either white or black).
   d. In the **Location** field, select the location on the image for the scale bar (top-left, top-right, bottom-left, or bottom-right).
5. If you want to include a time stamp in the exported image, do the following in the **Time Stamp** section:
   a. In the **Stamp** field, do one of the following:
      - Select the **Elapsed Time** option to include a time stamp in the exported image that shows the time elapsed from the earliest time point selected for the export.
      - Select the **Acquisition Time** option to include a time stamp in the exported image that shows the date and time of the acquisition.
      - Select **None** to not include a time stamp in the exported image.
   b. In the **Color** field, select the color for the time stamp (either white or black).
   c. In the **Location** field, select the location on the image for the time stamp (top-left, top-right, bottom-left, or bottom-right).
6. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Exporting Images for the IN Carta Software

To export images for use with the IN Carta software:

1. On the **Thumbnail View** page, select the wells you want to export.
2. On the right side of the page, click  **Export Images**.
3. In the **Image Export** dialog, select the **Export for IN Carta** option.

   Note: A message appears explaining how the exported images may be cropped to meet the requirements of the IN Carta software.

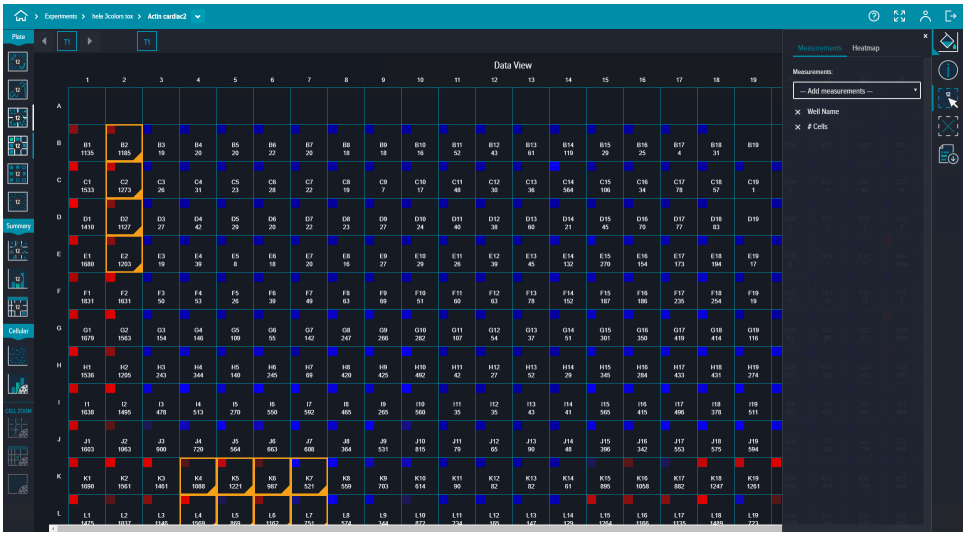
4. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Plate Data View

The  **Data View** shows a heatmap with up to four measurements displayed in the wells.

Tip: You can change the heatmap color scheme in **Configuration Settings**. See Color Scheme on page 260 for details.



The right side of the page includes the following icons:

 **Measurements/Heatmap**: Selects the measurements to show and the measurements to use for the heatmap.

 **Well Info Mode**: Shows summary measurements for the selected well.

 **Selection Mode**: Activates selection mode, which enables you to select wells. Click and drag to select multiple wells. The number on the icon indicates the number of selected wells.

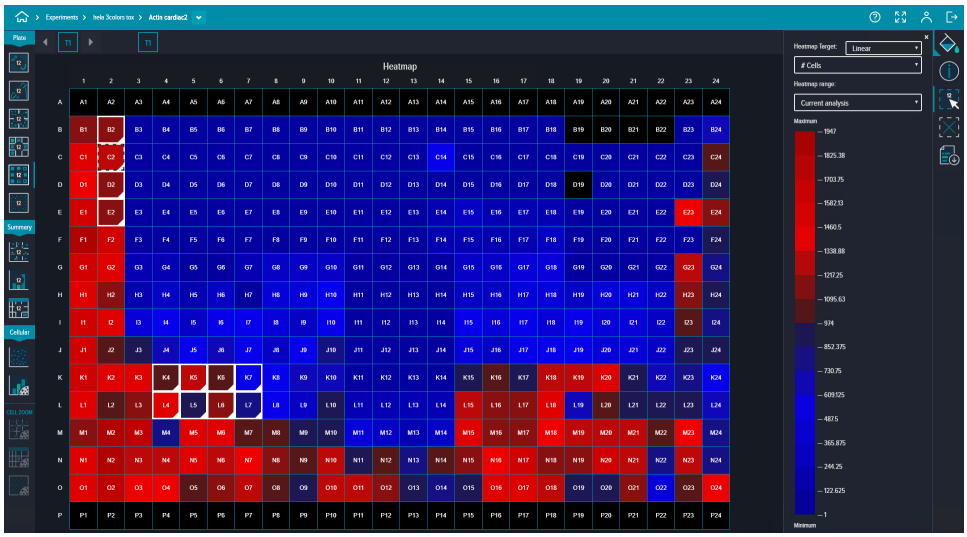
 **Deselect All**: Deselects all wells.

 **Export Current View to an Image**: Downloads a JPEG image of the page view, including selections. This function is not available with a tablet.

Plate Heatmap

The  **Heatmap** shows a heatmap of one measurement.

Tip: You can change the heatmap color scheme in  **Configuration Settings**. See Color Scheme on page 260 for details.



The right side of the page includes the following icons:

 **Color**: Selects the measurement to use as the heatmap and the heatmap color scale to display.

 **Well Info Mode**: Shows summary measurements for the selected well.

 **Selection Mode**: Activates selection mode, which enables you to select wells. Click and drag to select multiple wells. The number on the icon indicates the number of selected wells.

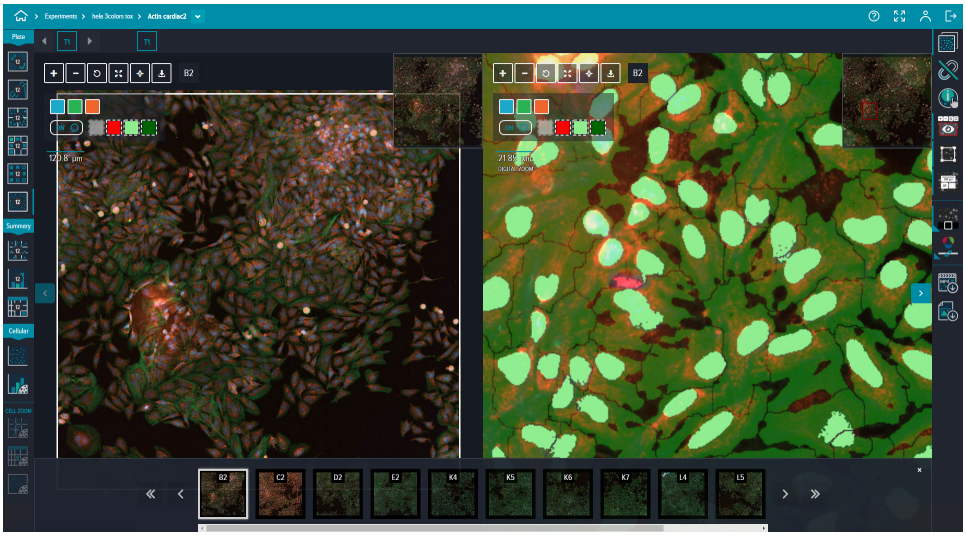
 **Deselect All**: Deselects all wells.

 **Export Current View to an Image**: Downloads a JPEG image of the page view, including selections. This function is not available with a tablet.

Plate Images

The **Images** show high-resolution images for deep zoom viewing.

Click on the image to zoom in on objects of interest. You can also use your mouse wheel to zoom in and out.



The right side of the page includes the following icons:

 **Show One Image / Show Two Images**: Toggles between a single image and two side-by-side images.

 **Link Images** or  **Unlink Images**: When showing two images, toggles between controlling images together or independently.

 **Cell Info Mode**: Shows cellular and summary measurements.

 **Show Navigation Maps**: Toggles the visualization tools in the top left corner and the mini-map in the upper right corner.

 **Show / Hide Analysis Zone**: Toggles the display of the selected analysis region.

 **Show Scale and Zone**: Toggles the measurement scale and the well number.

 **Show Image Gallery**: Toggles the image gallery at the bottom of the screen.

 **Show Channel Settings**: Toggles the display scaling tools at the bottom of the screen.

 **Download MP4 Movie**: Downloads a movie of the current plate image over time as an MP4 file. See Downloading Movies on page 171 for details. This function is not available with a tablet.

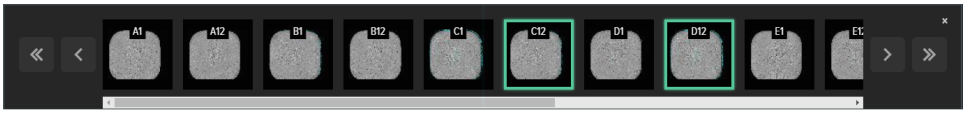
 **Export Images**: Exports the current plate image as a TIFF file for use the MetaXpress High-Content Image Acquisition and Analysis Software, the IN Carta Image Analysis Software, or an image viewer. See Exporting Images on page 172 for details.

Comparing Images

By default, a single image appears. You can display two views of the same image, which enables you to compare images.

To compare images:

1. Click  **Show Two Images**.
2. At the bottom of the page, in the image gallery, drag and drop image thumbnails to the comparison panes as needed.



3. To synchronize image zooming and changing positions in both panes, click  **Unlink Images**. The icon toggles to show the current state, which is  **Link Images**.

Downloading Movies

You can download a movie of the current plate image over time as an MP4 file. Downloaded movies are stored in the C:\crx-export folder on the host computer. You can include a time stamp in the movie.

Note: This function is not available with a tablet.

To download a movie:

1. On the **Image** page, select the well/slide you want to download as a movie.
2. On the right side of the page, click  **Download MP4 Movie**.
3. If you want to include a time stamp in the downloaded movie, do the following in the **Time Stamp** section:
   a. In the **Stamp** field, do one of the following:
      - Select the **Elapsed Time** option to include a time stamp in the downloaded movie that shows the time elapsed from the earliest selected time point.
      - Select **the Acquisition Time** option to include a time stamp in the downloaded movie that shows the date and time of the acquisition.
      - Select **None** to not include a time stamp in the downloaded movie.
   b. In the **Color** field, select the color for the time stamp (either white or black).
   c. In the **Location** field, select the location in the movie for the time stamp (top-left, top-right, bottom-left, or bottom-right).
4. Click **Export**.

The **Monitor** page opens to display the progress of the download. See Monitor Mode on page 213 for details.

Exporting Images

You can export images—one image per stain per region per time point—from the experiment as TIFF files. Exported images are stored in the C:\crx-export folder on the host computer. The software provides several options for the export:

- You can export TIFF file images as tiles or at full size.
- With a full-size image, you can include a scale bar and a time stamp.
- Images larger than 2 GB are scaled down to a 2 GB file size, which is the file size limit for a TIFF file. If an image is larger than 2 GB and you want to export it at full resolution, you can export image tiles. You can then use a TIFF image processing program to join the tiles together for a full-resolution image, if needed.
- If you plan to import the images into the MetaXpress High-Content Image Acquisition and Analysis Software, the images will be scaled to meet the required 32K × 32K pixel size.
- If you plan to import the images into the IN Carta Image Analysis Software, the images will be cropped as required by the IN Carta software. With colorimetric images, each image will be separated into three, 16-bit component images (that is, one red, one green, and one blue). In addition, the export will include the required XDCE file.

Exporting Images as Tiles

To export images as tiles:

1. On the **Image** page, select the well you want to export.
2. On the right side of the page, click  **Export Images**.
3. In the **Image Export** dialog, in the **Export Options** section, select the **Export Tiles** option to export each image tile as a separate TIFF file.
4. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Exporting Full-Size Images

To export full-sized images:

1. On the **Image** page, select the well you want to export.
2. On the right side of the page, click  **Export Images**.
3. In the **Image Export** dialog, do one of the following in the **Export Options** section:
   - Select the **Export Full Size** option to export a full-size image as a single TIFF file. If the resulting file size is larger than 2 GB, the file will be scaled down to 2 GB.
   - Select the **Export Full Size for MetaXpress** option to export a full-size image as a single TIFF file that can be imported into MetaXpress software.
4. If you want to include a scale bar in the exported image, do the following in the **Scale Bar** section:
   a. Select the **Bar** check box.
   b. If you want to include a label to the scale bar, select the **Label** check box.
   c. In the **Color** field, select the color for the scale bar (either white or black).
   d. In the **Location** field, select the location on the image for the scale bar (top-left, top-right, bottom-left, or bottom-right).
5. If you want to include a time stamp in the exported image, do the following in the **Time Stamp** section:
   a. In the **Stamp** field, do one of the following:
      - Select the **Elapsed Time** option to include a time stamp in the exported image that shows the time elapsed from the earliest time point selected for the export.
      - Select the **Acquisition Time** option to include a time stamp in the exported image that shows the date and time of the acquisition.
      - Select **None** to not include a time stamp in the exported image.
   b. In the **Color** field, select the color for the time stamp (either white or black).
   c. In the **Location** field, select the location on the image for the time stamp (top-left, top-right, bottom-left, or bottom-right).
6. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Exporting Images for the IN Carta Software

To export images for use with the IN Carta software:

1. On the **Image** page, select the well you want to export.
2. On the right side of the page, click  **Export Images**.
3. In the **Image Export** dialog, select the **Export for IN Carta** option.

   Note: A message appears explaining how the exported images may be cropped to meet the requirements of the IN Carta software.

4. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Summary Scatter Plot

The **Scatter Plot** shows a scatter plot of two summary measurements. Use the mini map at the upper left as a guide while moving through data in the graph.

Tip: You can change the heatmap color scheme in **Configuration Settings**. See Color Scheme on page 260 for details.

The right side of the page includes the following icons:

 **Axes/Color**: Selects the measurements to show and the measurements to use for the heatmap.

 **Selection Mode**: Activates selection mode, which enables you to select wells. Click and drag to select multiple wells. The number on the icon indicates the number of selected wells.

 **Deselect All**: Deselects all wells.

 **Show Selected Only**: Shows or hides the selected data or all the data.

 **Toggle Mini Map**: Shows or hides the small overview of the graph at the top left.

 **Toggle Grid Lines**: Toggles the display of grid lines on the graph.

 **Aggregation Function Line**: Not used in this version of the CellReporterXpress software.

 **Export Current View to an Image**: Downloads a JPEG image of the page view, including selections. This function is not available with a tablet.

Axes/Color Pane

Use the  **Axes/Color** pane to specify the data that appears in the graph.

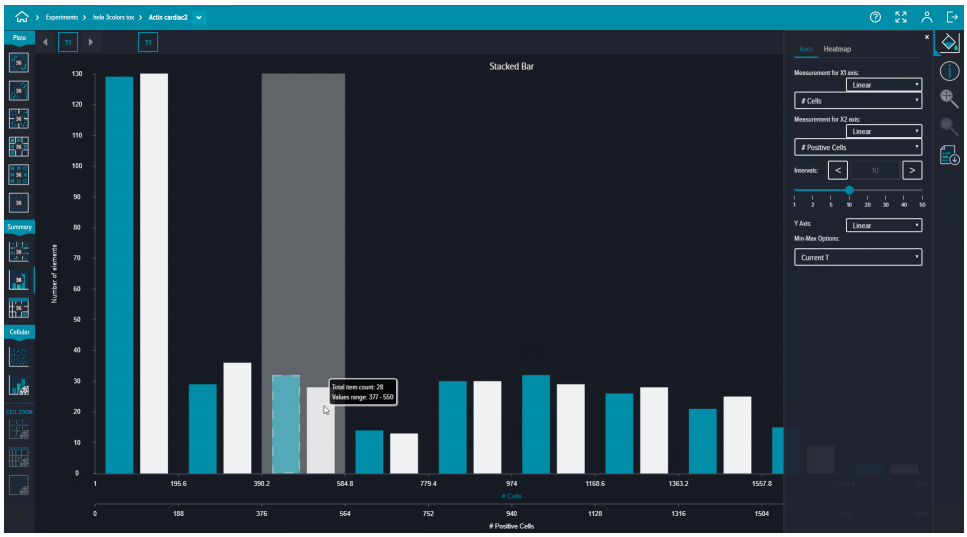
The following tabs are available:

- **Axes**: Specifies the measurements for two scatter plot axes.
- **Heatmap**: Specifies the heatmap coloring to the graph data.
- **Labels**: Specifies the label text next to the data in the graph.

Summary Stacked Bar

The **Stacked Bar** shows a histogram-style bar graph with up to two different measurements side by side. You can add a third measurement to subdivide each bar.

Tip: You can change the heatmap color scheme in **Configuration Settings**. See Color Scheme on page 260 for details.



The right side of the page includes the following icons:

 **Axes/Color**: Selects the measurements to show and the measurements to use for the heatmap.

 **Well Info**: Shows summary measurements for the selected well.

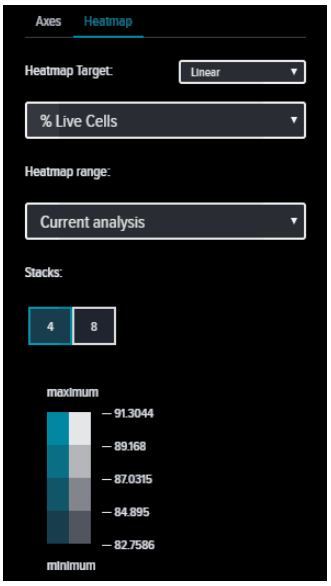
 **Zoom In**: When bins are selected, replots the selected bars only for a more granular view.

 **Zoom Out**: When bins are selected, replots the selected bars only for a more general view.

 **Export Current View to an Image**: Downloads a JPEG image of the page view, including selections. This function is not available with a tablet.

Axes/Color Pane

Use the  **Axes/Color** pane to specify the data that appears in the graph.



The following tabs are available:

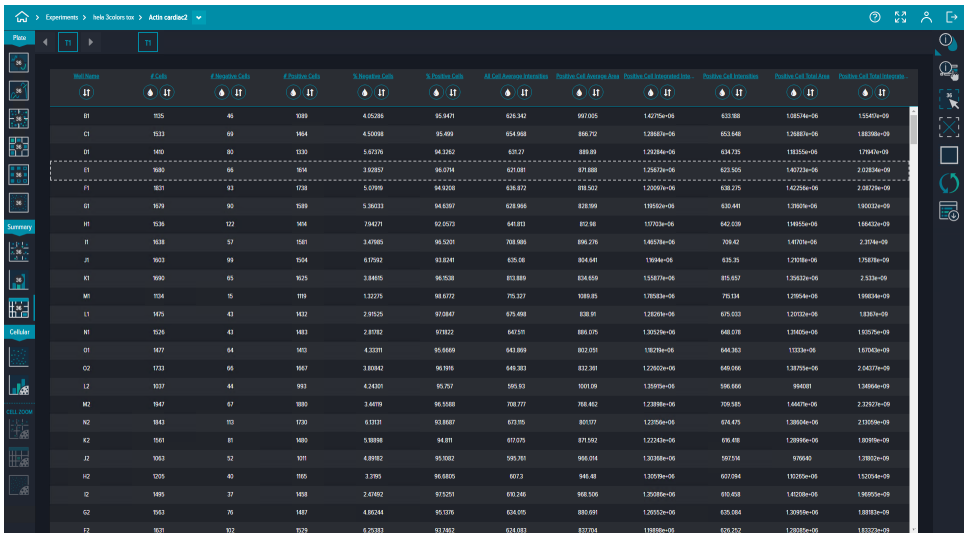
- **Axes**: Specifies the measurements for two scatter plot axes and intervals.
- **Heatmap**: Specifies the heatmap coloring to the graph data.

The **Stacks** field shows the range of values for each shade in the heatmap. You can show either **4** or **8** stacks.

Summary Table

The  **Table** shows a table view of all well-level measurements.

Tip: You can change the heatmap color scheme in **Configuration Settings**. See Color Scheme on page 260 for details.



The right side of the page includes the following icons:

 **Legend**: Enables you to select a measurement and show the mapping between color and values. Provides information used when column heatmaps are active.

 **Well Info Mode**: Shows summary measurements for the selected well.

 **Selection Mode**: Activates selection mode, which enables you to select wells. Click and drag to select multiple wells. The number on the icon indicates the number of selected wells.

 **Deselect All**: Deselects all wells.

 **Show Selected Only**: Shows or hides the selected data or all the data.

 **Reset**: Reverts the table to the default configuration.

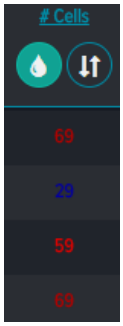
 **Export**: Downloads the currently configured table as a CSV file. This function is not available with a tablet.

Changing Columns

To change the column headings and associated data, click the heading name and then select from the menu options.

Adding Heatmap Coloring

To add heatmap coloring to a column of data, click  .



Sorting Data

To sort rows by the values in the column, click  .

- Click once to sort lowest to highest.
- Click again to sort highest to lowest.
- Click a third time to deactivate sorting for the column.

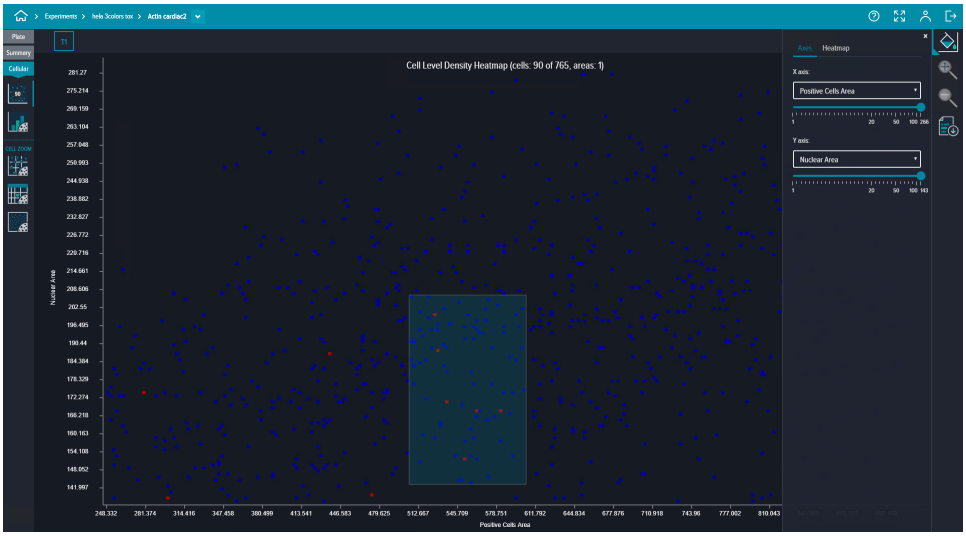
Cell Level Density Heatmap

The **Cell Level Density Heatmap** shows a scatter plot-style graph of two measurements. Each spot represents all the cells with similar measurements. The heatmap color of the spot is based on the cell count for the spot.

Note: You must select at least one well in a Plate view or a Summary view to enable the Cellular views.

Tip: You can change the heatmap color scheme in **Configuration Settings**. See Color Scheme on page 260 for details.

The page heading indicates the number of selected cells (out of the total number of cells plotted on the graph) and the number of selected wells.



The right side of the page includes the following icons:

 **Axes/Color**: Selects the measurements to show and the measurements to use for the heatmap.

 **Zoom In**: When bins are selected, replots the selected bars only for a more granular view.

 **Zoom Out**: When bins are selected, replots the selected bars only for a more general view.

 **Export Current View to an Image**: Downloads a JPEG image of the page view, including selections. This function is not available with a tablet.

Axes/Color Pane

Use the  **Axes/Color** pane to specify the data that appears in the graph.

The following tabs are available:

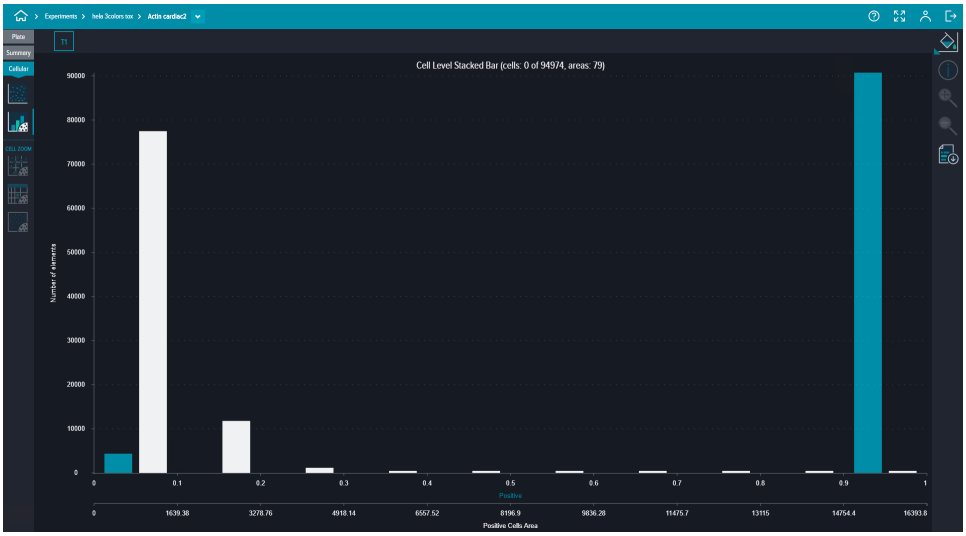
- **Axes**: Specifies the measurements for two scatter plot axes and intervals.
- **Heatmap**: Specifies the heatmap coloring to the graph data.

Cell Level Stacked Bar

The **Cell Level Stacked Bar** shows a histogram-style bar graph with up to two different measurements side by side. You can add a third measurement to subdivide each bar.

Note: You must select at least one well in a Plate view or a Summary view to enable the Cellular views.

The page heading indicates the number of selected cells (out of the total number of cells plotted on the graph) and the number of selected wells.



The right side of the page includes the following icons:

 **Axes/Color**: Selects the measurements to show and the measurements to use for the heatmap.

 **Cell Info**: Shows summary measurements for the selected well.

 **Zoom In**: When bins are selected, replots the selected bars only for a more granular view.

 **Zoom Out**: When bins are selected, replots the selected bars only for a more general view.

 **Export Current View to an Image**: Downloads a JPEG image of the page view, including selections. This function is not available with a tablet.

Axes/Color Pane

Use the  **Axes/Color** pane to specify the data that appears in the graph.

The following tabs are available:

- **Axes**: Specifies the measurements for two scatter plot axes and intervals.
- **Heatmap**: Specifies the heatmap coloring to the graph data.

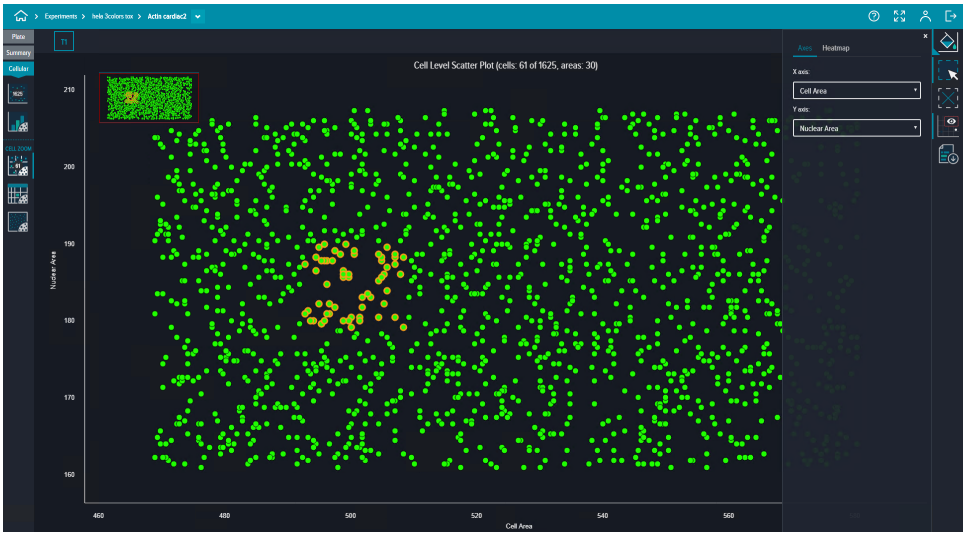
Cell Zoom Level Scatter Plot

The **Scatter Mode** shows a scatter plot of two measurements.

Note: You must select at least one bin in a Cellular view to enable the Cell Zoom views.

Tip: You can change the heatmap color scheme in **Configuration Settings**. See Color Scheme on page 260 for details.

The page heading indicates the number of selected cells (out of the total number of cells plotted on the graph) and the number of selected wells.



The right side of the page includes the following icons:

 **Axes/Color**: Selects the measurements to show and the measurements to use for the heatmap.

 **Selection Mode**: Activates selection mode, which enables you to select wells. Click and drag to select multiple wells. The number on the icon indicates the number of selected wells.

 **Deselect All**: Deselects all wells.

 **Toggle Mini Map**: Shows or hides the small overview of the graph at the top left.

 **Export Current View to an Image**: Downloads a JPEG image of the page view, including selections. This function is not available with a tablet.

Axes/Color Pane

Use the  **Axes/Color** pane to specify the data that appears in the graph.

The following tabs are available:

- **Axes**: Specifies the measurements for two scatter plot axes.
- **Heatmap**: Specifies the heatmap coloring to the graph data.

Cell Zoom Level Table

The  **Cell Level Table** shows a table with cell-level measurements.

Note: You must select at least one bin in a Cellular view to enable the Cell Zoom views.

Tip: You can change the heatmap color scheme in **Configuration Settings**. See Color Scheme on page 260 for details.

The right side of the page includes the following icons:


Legend: Enables you to select a measurement and show the mapping between color and values. Provides information used when column heatmaps are active.


Reset: Reverts the table to the default configuration.


Well Info Mode: Shows summary measurements for the selected well.

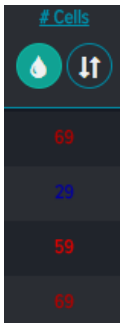

Export: Downloads the currently configured table as a CSV file. This function is not available with a tablet.

Changing Columns

To change the column headings and associated data, click the heading name and then select from the menu options.

Adding Heatmap Coloring

To add heatmap coloring to a column of data, click  .



Sorting Data

To sort rows by the values in the column, click  .

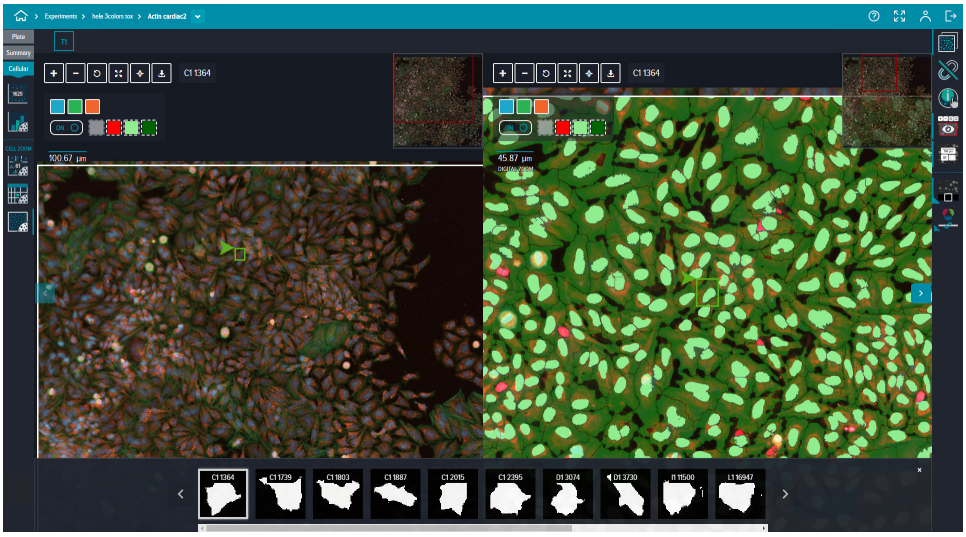
- Click once to sort lowest to highest.
- Click again to sort highest to lowest.
- Click a third time to deactivate sorting for the column.

Cell Zoom Level Images

The **Cell Level Images** page shows high-resolution images for deep zoom viewing. At the bottom of the page, zoom-level segments appear.

Click on the image to zoom in on objects of interest. You can also use your mouse wheel to zoom in and out.

Note: You must select at least one bin in a Cellular view to enable the Cell Zoom views.



The right side of the page includes the following icons:

 **Show One Image / Show Two Images**: Toggles between a single image and two side-by-side images.

 **Link Images** or  **Unlink Images**: When showing two images, toggles between controlling images together or independently.

 **Cell Info Mode**: Shows cellular and summary measurements.

 **Show Navigation Maps**: Toggles the visualization tools in the top left corner and the mini-map in the upper right corner.

 **Show Scale and Zone**: Toggles the measurement scale and the well number.

 **Show Image Gallery**: Toggles the image gallery at the bottom of the screen.

 **Show Channel Settings**: Toggles the display scaling tools at the bottom of the screen.

Comparing Images

By default, a single image appears. You can display two views of the same image, which enables you to compare images.

To compare images:

1. Click  **Show Two Images**.
2. At the bottom of the page, in the image gallery, drag and drop image thumbnails to the comparison panes as needed.

3. To synchronize image zooming and changing positions in both panes, click  **Unlink Images**. The icon toggles to  **Link Images**.

Slide Views

The following slide data views are available:

Slide Views


Thumbnail View: Shows an overview of the slide images in low resolution. See Slide Thumbnail View on page 192 for details.


Images: Shows high-resolution images for deep zoom viewing. See Slide Images on page 195 for details.

Summary Views


Table: Shows a table with summary slide region-level measurements. See Summary Table on page 200 for details.

Cellular Views

Note: You must select at least one region in a Slide view or a Summary view to enable the Cellular views.


Cell Level Density Heatmap: Shows a scatter plot-style graph of two measurements. See Cell Level Density Heatmap on page 202 for details.


Cell Level Stacked Bar: Shows a histogram-style bar graph with up to two different measurements side by side. You can add a third measurement to subdivide each bar. See Cell Level Stacked Bar on page 204 for details.

Cell Zoom Views

Note: You must select at least one bin in a Cellular view to enable the Cell Zoom views.

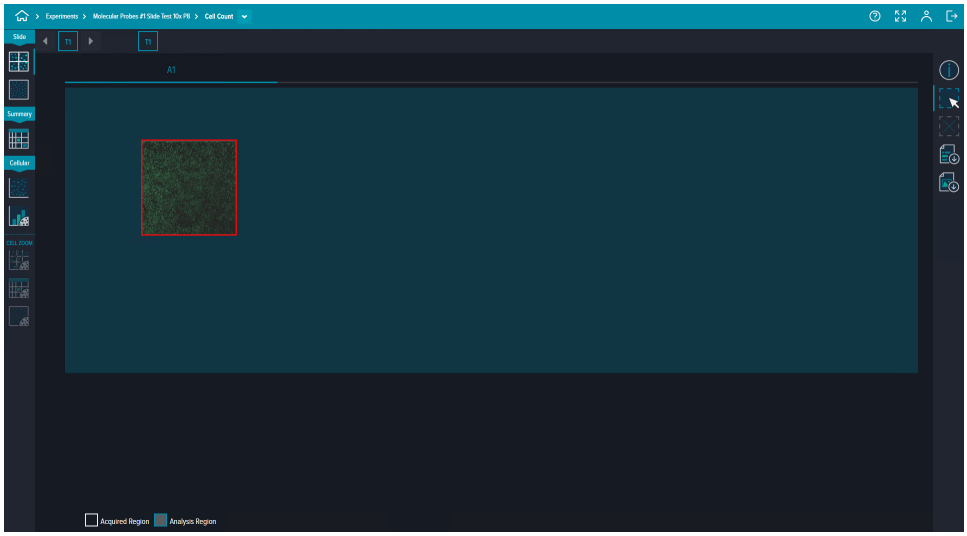

Scatter Mode: Shows a scatter plot graph of two measurements. See Cell Zoom Level Scatter Plot on page 206 for details.


Cell Level Table: Shows a table with cellular measurements. See Cell Zoom Level Table on page 208 for details.


Cell Level Images: Shows high-resolution images for deep zoom viewing of individual cells. See Cell Zoom Level Images on page 210 for details.

Slide Thumbnail View

The **Thumbnail View** shows an overview of the slide regions in low resolution. You can double-click on a region to view the image for that region. See Slide Images on page 195 for details.



The right side of the page includes the following icons:

Well Info: Shows summary measurements for the selected region.

Selection Mode: Activates selection mode, which enables you to select regions. Press **SHIFT** and click to select multiple regions. The number on the icon indicates the number of selected regions.

Deselect All: Deselects all regions.

Export Current View to an Image: Downloads a JPEG image of the page view, including selections. This function is not available with a tablet.

Export Images: Exports the selected thumbnail images as TIFF files for use the MetaXpress High-Content Image Acquisition and Analysis Software, the IN Carta Image Analysis Software, or an image viewer. See Exporting Thumbnail Images on page 193 for details.

Exporting Thumbnail Images

You can export images—one image per stain per region per time point—from the experiment as TIFF files. Exported images are stored in the C:\crx-export folder on the host computer. The software provides several options for the export:

- You can export TIFF file images as tiles or at full size.
- With a full-size image, you can include a scale bar and a time stamp.
- Images larger than 2 GB are scaled down to a 2 GB file size, which is the file size limit for a TIFF file. If an image is larger than 2 GB and you want to export it at full resolution, you can export image tiles. You can then use a TIFF image processing program to join the tiles together for a full-resolution image, if needed.
- If you plan to import the images into the MetaXpress High-Content Image Acquisition and Analysis Software, the images will be scaled to meet the required 32K × 32K pixel size.
- If you plan to import the images into the IN Carta Image Analysis Software, the images will be cropped as required by the IN Carta software. With colorimetric images, each image will be separated into three, 16-bit component images (that is, one red, one green, and one blue). In addition, the export will include the required XDCE file.

Exporting Thumbnail Images as Tiles

To export thumbnail images as tiles:

1. On the **Thumbnail View** page, select the regions you want to export.
2. On the right side of the page, click  **Export Images**.
3. In the **Image Export** dialog, in the **Export Options** section, select the **Export Tiles** option to export each image tile as a separate TIFF file.
4. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Exporting Full-Size Thumbnail Images

To export full-sized images:

1. On the **Thumbnail View** page, select the regions you want to export.
2. On the right side of the page, click  **Export Images**.
3. In the **Image Export** dialog, do one of the following in the **Export Options** section:
   - Select the **Export Full Size** option to export a full-size image as a single TIFF file. If the resulting file size is larger than 2 GB, the file will be scaled down to 2 GB.
   - Select the **Export Full Size for MetaXpress** option to export a full-size image as a single TIFF file that can be imported into MetaXpress software.
4. If you want to include a scale bar in the exported image, do the following in the **Scale Bar** section:
   a. Select the **Bar** check box.
   b. If you want to include a label to the scale bar, select the **Label** check box.
   c. In the **Color** field, select the color for the scale bar (either white or black).
   d. In the **Location** field, select the location on the image for the scale bar (top-left, top-right, bottom-left, or bottom-right).
5. If you want to include a time stamp in the exported image, do the following in the **Time Stamp** section:
   a. In the **Stamp** field, do one of the following:
      - Select the **Elapsed Time** option to include a time stamp in the exported image that shows the time elapsed from the earliest time point selected for the export.
      - Select the **Acquisition Time** option to include a time stamp in the exported image that shows the date and time of the acquisition.
      - Select **None** to not include a time stamp in the exported image.
   b. In the **Color** field, select the color for the time stamp (either white or black).
   c. In the **Location** field, select the location on the image for the time stamp (top-left, top-right, bottom-left, or bottom-right).
6. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Exporting Images for the IN Carta Software

To export images for use with the IN Carta software:

1. On the **Thumbnail View** page, select the regions you want to export.
2. On the right side of the page, click  **Export Images**.
3. In the **Image Export** dialog, select the **Export for IN Carta** option.

   Note: A message appears explaining how the exported images may be cropped to meet the requirements of the IN Carta software.

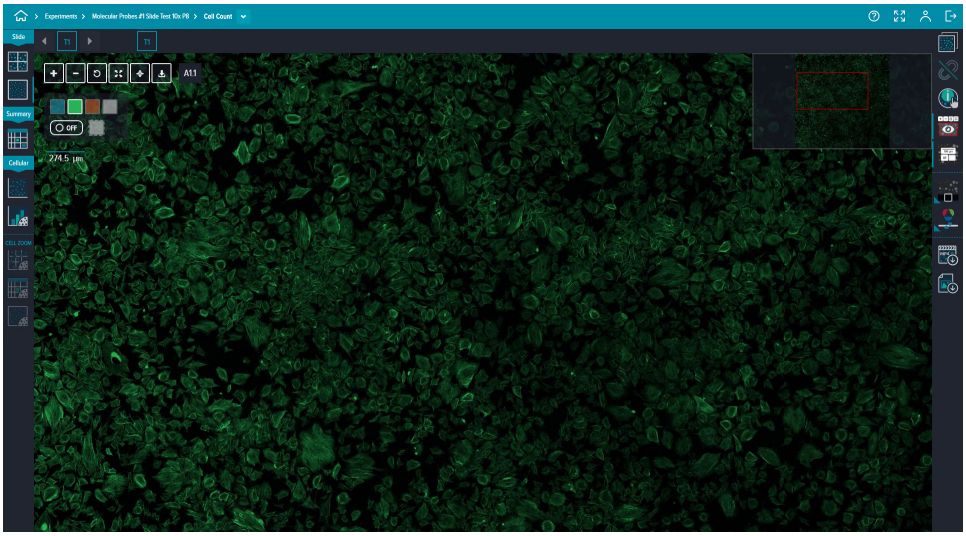
4. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Slide Images

The  **Images** show high-resolution images for deep zoom viewing.

Click on the image to zoom in on objects of interest. You can also use your mouse wheel to zoom in and out.



The right side of the page includes the following icons:

 **Show One Image / Show Two Images**: Toggles between a single image and two side-by-side images.

 **Link Images** or  **Unlink Images**: When showing two images, toggles between controlling images together or independently.

 **Cell Info Mode**: Shows cellular and summary measurements.

 **Show Navigation Maps**: Toggles the visualization tools in the top left corner and the mini-map in the upper right corner.

 **Show / Hide Analysis Zone**: Toggles the display of the selected analysis region.

 **Show Scale and Region**: Toggles the display of the measurement scale and the slide number.

 **Show Image Gallery**: Toggles the image gallery at the bottom of the screen.

 **Show Channel Settings**: Toggles the display scaling tools at the bottom of the screen.

 **Download MP4 Movie**: Downloads a movie of the selected slide image over time as an MP4 file. See Downloading a Movie on page 197 for details. This function is not available with a tablet.

 **Export Images**: Exports the current slide image as a TIFF file for use the MetaXpress High-Content Image Acquisition and Analysis Software, the IN Carta Image Analysis Software, or an image viewer. See Exporting Images on page 198 for details.

Comparing Images

By default, a single image appears. You can display two views of the same image, which enables you to compare images.

To compare images:

1. Click  **Show Two Images**.
2. At the bottom of the page, click  **Show Image Gallery**.
3. In the image gallery, drag and drop image thumbnails to the comparison panes as needed.
4. To synchronize image zooming and changing positions in both panes, click  **Unlink Images**. The icon toggles to show the current state, which is  **Link Images**.

Downloading a Movie

You can download a movie of the current plate image over time as an MP4 file. Downloaded movies are stored in the C:\crx-export folder on the host computer. You can include a time stamp in the movie.

Note: This function is not available with a tablet.

To download a movie:

1. On the **Image** page, select the well/slide you want to download as a movie.
2. On the right side of the page, click  **Download MP4 Movie**.
3. If you want to include a time stamp in the downloaded movie, do the following in the **Time Stamp** section:
   a. In the **Stamp** field, do one of the following:
      - Select the **Elapsed Time** option to include a time stamp in the downloaded movie that shows the time elapsed from the earliest selected time point.
      - Select **the Acquisition Time** option to include a time stamp in the downloaded movie that shows the date and time of the acquisition.
      - Select **None** to not include a time stamp in the downloaded movie.
   b. In the **Color** field, select the color for the time stamp (either white or black).
   c. In the **Location** field, select the location in the movie for the time stamp (top-left, top-right, bottom-left, or bottom-right).
4. Click **Export**.

The **Monitor** page opens to display the progress of the download. See Monitor Mode on page 213 for details.

Exporting Images

You can export images—one image per stain per region per time point—from the experiment as TIFF files. Exported images are stored in the C:\crx-export folder on the host computer. The software provides several options for the export:

- You can export TIFF file images as tiles or at full size.
- With a full-size image, you can include a scale bar and a time stamp.
- Images larger than 2 GB are scaled down to a 2 GB file size, which is the file size limit for a TIFF file. If an image is larger than 2 GB and you want to export it at full resolution, you can export image tiles. You can then use a TIFF image processing program to join the tiles together for a full-resolution image, if needed.
- If you plan to import the images into the MetaXpress High-Content Image Acquisition and Analysis Software, the images will be scaled to meet the required 32K × 32K pixel size.
- If you plan to import the images into the IN Carta Image Analysis Software, the images will be cropped as required by the IN Carta software. With colorimetric images, each image will be separated into three, 16-bit component images (that is, one red, one green, and one blue). In addition, the export will include the required XDCE file.

Exporting Images as Tiles

To export images as tiles:

1. On the **Image** page, select the regions you want to export.
2. On the right side of the page, click  **Export Images**.
3. In the **Image Export** dialog, in the **Export Options** section, select the **Export Tiles** option to export each image tile as a separate TIFF file.
4. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Exporting Full-Size Images

To export full-sized images:

1. On the **Image** page, select the regions you want to export.
2. On the right side of the page, click  **Export Images**.
3. In the **Image Export** dialog, do one of the following in the **Export Options** section:
   - Select the **Export Full Size** option to export a full-size image as a single TIFF file. If the resulting file size is larger than 2 GB, the file will be scaled down to 2 GB.
   - Select the **Export Full Size for MetaXpress** option to export a full-size image as a single TIFF file that can be imported into MetaXpress software.
4. If you want to include a scale bar in the exported image, do the following in the **Scale Bar** section:
   a. Select the **Bar** check box.
   b. If you want to include a label to the scale bar, select the **Label** check box.
   c. In the **Color** field, select the color for the scale bar (either white or black).
   d. In the **Location** field, select the location on the image for the scale bar (top-left, top-right, bottom-left, or bottom-right).
5. If you want to include a time stamp in the exported image, do the following in the **Time Stamp** section:
   a. In the **Stamp** field, do one of the following:
      - Select the **Elapsed Time** option to include a time stamp in the exported image that shows the time elapsed from the earliest time point selected for the export.
      - Select the **Acquisition Time** option to include a time stamp in the exported image that shows the date and time of the acquisition.
      - Select **None** to not include a time stamp in the exported image.
   b. In the **Color** field, select the color for the time stamp (either white or black).
   c. In the **Location** field, select the location on the image for the time stamp (top-left, top-right, bottom-left, or bottom-right).
6. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Exporting Images for the IN Carta Software

To export images for use with the IN Carta software:

1. On the **Image** page, select the regions you want to export.
2. On the right side of the page, click  **Export Images**.
3. In the **Image Export** dialog, select the **Export for IN Carta** option.

   Note: A message appears explaining how the exported images may be cropped to meet the requirements of the IN Carta software.

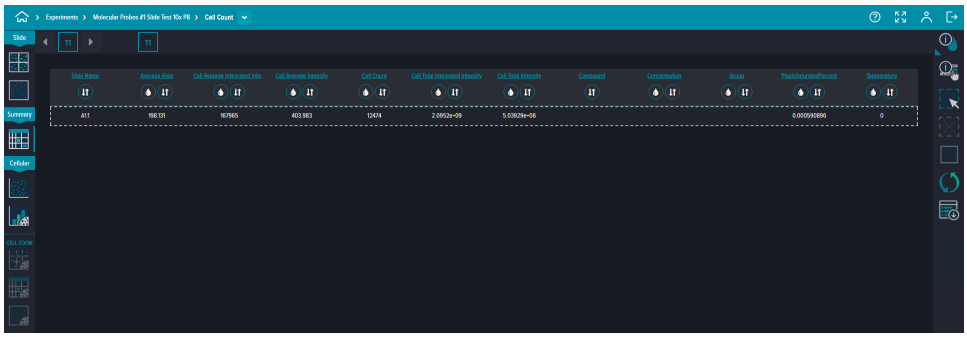
4. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 213 for details.

Summary Table

The  **Table** shows a table view of all region-level measurements.

Tip: You can change the heatmap color scheme in **Configuration Settings**. See Color Scheme on page 260 for details.



The right side of the page includes the following icons:

Legend: Enables you to select a measurement and show the mapping between color and values. Provides information used when column heatmaps are active.

Region Info Mode: Shows the summary measurements for the last row selected.

Selection Mode: Activates selection mode, which enables you to select regions. Press **SHIFT** and click to select multiple regions. The number on the icon indicates the number of selected regions.

Deselect All: Deselects all regions.

Show Selected Only: Shows or hides the selected data or all the data.

Reset: Reverts the table to the default configuration.

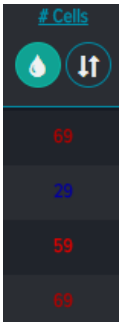
Export: Downloads the currently configured table as a CSV file. This function is not available with a tablet.

Changing Columns

To change the column headings and associated data, click the heading name and then select from the menu options.

Adding Heatmap Coloring

To add heatmap coloring to a column of data, click  .



Sorting Data

To sort rows by the values in the column, click  .

- Click once to sort lowest to highest.
- Click again to sort highest to lowest.
- Click a third time to deactivate sorting for the column.

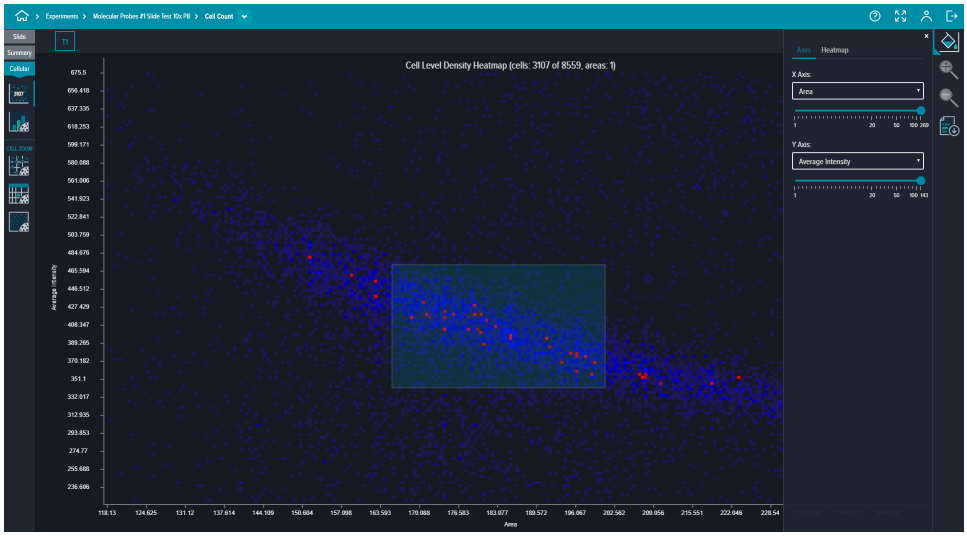
Cell Level Density Heatmap

The **Cell Level Density Heatmap** shows a scatter plot-style graph of two measurements. Each spot represents all the cells with similar measurements. The heatmap color of the spot is based on the cell count for the spot.

Note: You must select at least one region in a Slide view or a Summary view to enable the Cellular views.

Tip: You can change the heatmap color scheme in **Configuration Settings**. See Color Scheme on page 260 for details.

The page heading indicates the number of selected cells (out of the total number of cells plotted on the graph) and the number of selected regions.



The right side of the page includes the following icons:

 **Axes/Color**: Selects the measurements to show and the measurements to use for the heatmap.

 **Zoom In**: When bins are selected, replots the selected bars only for a more granular view.

 **Zoom Out**: When bins are selected, replots the selected bars only for a more general view.

 **Export Current View to an Image**: Downloads a JPEG image of the page view, including selections. This function is not available with a tablet.

Axes/Color Pane

Use the  **Axes/Color** pane to specify the data that appears in the graph.

The following tabs are available:

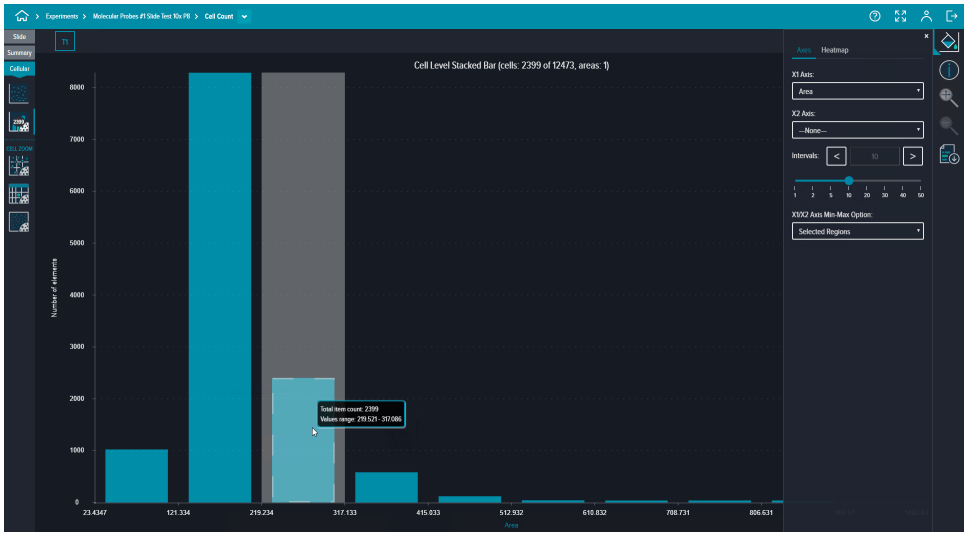
- **Axes**: Specifies the measurements for two scatter plot axes and intervals.
- **Heatmap**: Specifies the heatmap coloring to the graph data.

Cell Level Stacked Bar

The **Cell Level Stacked Bar** shows a histogram-style bar graph with up to two different measurements side by side. You can add a third measurement to subdivide each bar.

Note: You must select at least one region in a Slide view or a Summary view to enable the Cellular views.

The page heading indicates the number of selected cells (out of the total number of cells plotted on the graph) and the number of selected regions.



The right side of the page includes the following icons:

 **Axes/Color**: Selects the measurements to show and the measurements to use for the heatmap.

 **Cell Info**: Shows summary measurements for the selected region.

 **Zoom In**: When bins are selected, replots the selected bars only for a more granular view.

 **Zoom Out**: When bins are selected, replots the selected bars only for a more general view.

 **Export Current View to an Image**: Downloads a JPEG image of the page view, including selections. This function is not available with a tablet.

Axes/Color Pane

Use the  **Axes/Color** pane to specify the data that appears in the graph.

The following tabs are available:

- **Axes**: Specifies the measurements for two scatter plot axes and intervals.
- **Heatmap**: Specifies the heatmap coloring to the graph data.

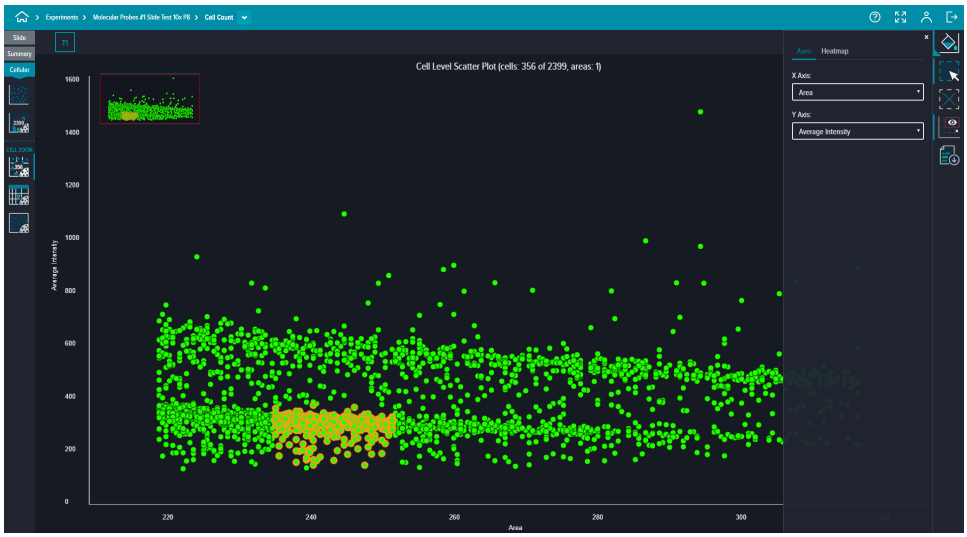
Cell Zoom Level Scatter Plot

The  **Scatter Mode** shows a scatter plot of two measurements.

Note: You must select at least one bin in a Cellular view to enable the Cell Zoom views.

Tip: You can change the heatmap color scheme in **Configuration Settings**. See Color Scheme on page 260 for details.

The page heading indicates the number of selected cells (out of the total number of cells plotted on the graph) and the number of selected regions.



The right side of the page includes the following icons:

 **Axes/Color**: Selects the measurements to show and the measurements to use for the heatmap.

 **Selection Mode**: Activates selection mode, which enables you to select regions. Press **SHIFT** and click to select multiple regions. The number on the icon indicates the number of selected regions.

 **Deselect All**: Deselects all regions.

 **Toggle Mini Map**: Shows or hides the small overview of the graph at the top left.

 **Export Current View to an Image**: Downloads a JPEG image of the page view, including selections. This function is not available with a tablet.

Axes/Color Pane

Use the  **Axes/Color** pane to specify the data that appears in the graph.

The following tabs are available:

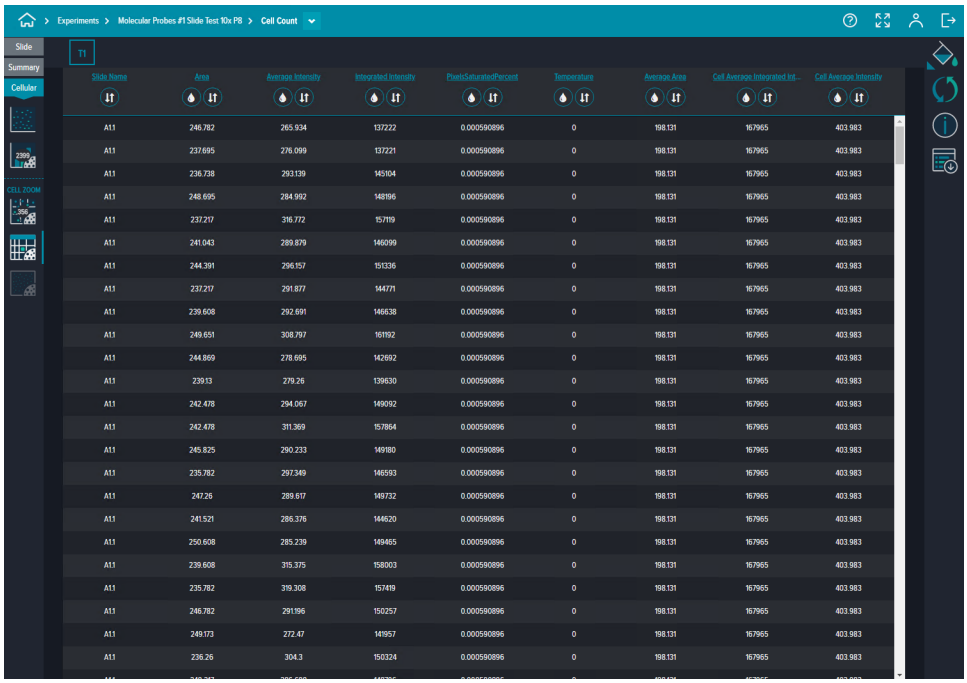
- **Axes**: Specifies the measurements for two scatter plot axes.
- **Heatmap**: Specifies the heatmap coloring to the graph data.

Cell Zoom Level Table

The **Cell Level Table** shows a table with cell-level measurements.

Note: You must select at least one bin in a Cellular view to enable the Cell Zoom views.

Tip: You can change the heatmap color scheme in **Configuration Settings**. See Color Scheme on page 260 for details.



The right side of the page includes the following icons:


Legend: Enables you to select a measurement and show the mapping between color and values. Provides information used when column heatmaps are active.


Reset: Reverts the table to the default configuration.


Well Info: Shows the summary measurements for the last row selected.

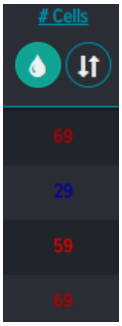

Export: Downloads the currently configured table as a CSV file. This function is not available with a tablet.

Changing Columns

To change the column headings and associated data, click the heading name and then select from the menu options.

Adding Heatmap Coloring

To add heatmap coloring to a column of data, click  .



Sorting Data

To sort rows by the values in the column, click  .

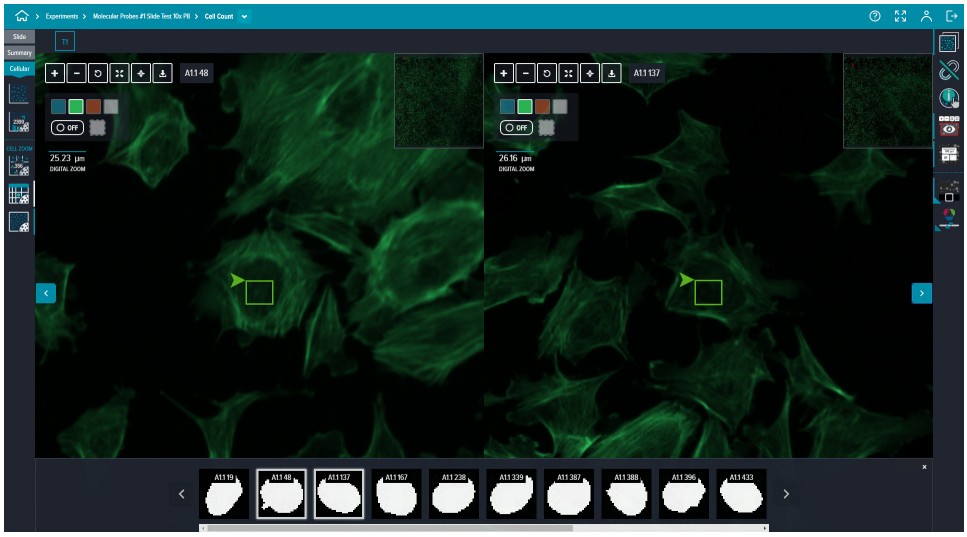
- Click once to sort lowest to highest.
- Click again to sort highest to lowest.
- Click a third time to deactivate sorting for the column.

Cell Zoom Level Images

The **Cell Level Images** page shows high-resolution images for deep zoom viewing. At the bottom of the page, zoom-level segments appear.

Click on the image to zoom in on objects of interest. You can also use your mouse wheel to zoom in and out.

Note: You must select at least one bin in a Cellular view to enable the Cell Zoom views.



The right side of the page includes the following icons:

 **Show One Image / Show Two Images**: Toggles between a single image and two side-by-side images.

 **Link Images** or  **Unlink Images**: When showing two images, toggles between controlling images together or independently.

 **Cell Info Mode**: Shows cellular and summary measurements.

 **Show Navigation Maps**: Toggles the visualization tools in the top left corner and the mini-map in the upper right corner.

 **Show Scale and Zone**: Toggles the measurement scale and the well number.

 **Show Image Gallery**: Toggles the image gallery at the bottom of the screen.

 **Show Channel Settings**: Toggles the display scaling tools at the bottom of the screen.

Comparing Images

By default, a single image appears. You can display two views of the same image, which enables you to compare images.

To compare images:

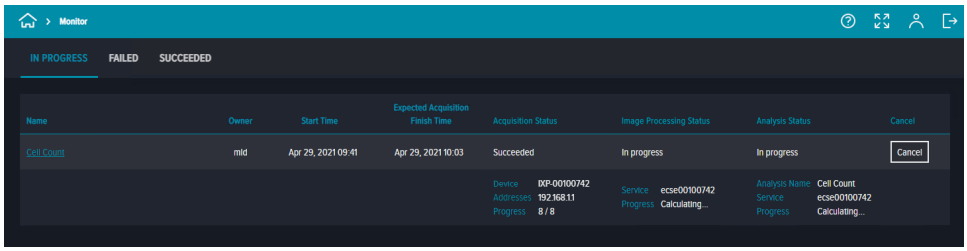
1. Click  **Show Two Images**.
2. At the bottom of the page, in the image gallery, drag and drop image thumbnails to the comparison panes as needed.

3. To synchronize image zooming and changing positions in both panes, click  **Unlink Images**. The icon toggles to  **Link Images**.

Chapter 5: Monitor Mode

Use **Monitor** mode to view the progress and completion status of various CellReporterXpress software activities, including experiments run in **Acquisition** mode or **Experiments** mode, images exported in **Experiments** mode, movies downloaded in **Experiments** mode, and maintenance operations run in **Devices** mode.

On the **Home** page, click the  **Monitor** tile to enter **Monitor** mode.



The following tabs are available:

- **In Progress**: Displays details on currently running experiments, exports, and maintenance operations.
- **Failed**: Displays details on failed experiments, exports, and maintenance operations. An error message may appear to describe the reason for the failure. Details remain listed on the **Failed** tab until you delete them.
- **Succeeded**: Displays details on successful experiments, exports, and maintenance operations. Details remain listed on the **Succeeded** tab until you delete them.

Viewing the Analysis for an Experiment

To view the analysis for an experiment:

On the **Monitor** page, on any tab, click an experiment name in the **Name** field to view images and analysis data on the **Experiments** page.

Canceling a Running Experiment

To cancel a running experiment:

On the **Monitor** page, on the **In Progress** tab, click **Cancel**.

The experiment details move to the **Succeeded** tab and a card for the canceled experiment is created on the **Experiments** page.

Responding to a Failed Experiment

There can be many reasons that an experiment fails. If the reason is caused by an issue with the CellReporterXpress software or your network, the instrument may continue performing acquisition. In this case, the status light remains yellow.

To respond to a failed experiment:

1. On the **Monitor** page, on the **Failed** tab, review the error message.
2. Restart the instrument. See the *ImageXpress Pico User Guide* for details on restarting your instrument.
3. Check all network connections.
4. Confirm that you have enough temporary storage for the experiment. See the *ImageXpress Pico User Guide* for details on adding external temporary storage.
5. Retry running the experiment.

Deleting Details from the Monitor Page

To delete the details of an experiment, export, download, or maintenance operation:

1. Do one of the following:
   - On the **Monitor** page, on the **Succeeded** tab, select the **Delete** check box for each item you want to delete.
   - On the **Monitor** page, on the **Failed** tab, select the **Delete** check box for each item you want to delete.
2. Click **Delete**.
3. Click **OK**.

Note: Only the details of the status of the experiment are deleted. The card for the experiment remains in **Experiments** mode. See Experiment Operations on page 131 for details on permanently deleting an experiment.

Chapter 6: Configuration Mode

Use **Configuration** mode to set the systemwide options that affect all users of the CellReporterXpress software.

On the **Home** page, click the  **Configuration** tile to enter **Configuration** mode.

The left side of the page includes the following icons:

 **Stain Library**: Specifies the stain definitions available to all users of the CellReporterXpress software. You can add, edit, and delete stain definitions as needed. See Stain Library on page 216 for details.

 **Labware Library**: Specifies the labware specifications available to all users. A default library of formats for plates, dishes, and slides is provided. You can add more plate and slide formats as needed. See Labware Library on page 217 for details.

 **Devices**: Specifies the instruments available for acquisition and maintenance. You can add instruments as needed. See Devices on page 220 for details.

 **Image Analysis Computers**: Specifies the registered computers and mapped folders that are available for image storage. In all configurations, you can perform image analysis on the host computer. In a server configuration, you can also perform image analysis on any networked computer that has been registered for external image analysis. See Image Analysis Computers on page 224 for details.

 **Data Storage**: Specifies the registered computers and mapped folders that are available for storage. In all configurations, you can store image and experiment data on the host computer. In a server configuration, you can also store data on any networked computer running the Location Service (MD.LocationService) that has been registered for external image storage. See Data Storage on page 226 for details.

Stain Library

The  **Stain Library** page specifies the stain definitions available to all users of the CellReporterXpress software. You can add, edit, and delete stain definitions as needed.

Adding a Stain Definition

To add a stain definition to the library:

1. On the **Stain Library** page, click **Add Stain**.
2. In the **Group Name** field, enter the stain-equivalent filter name.
3. In the **Stain Name** field, enter the dye name.
4. Click the **Color** drop-down list box, and select a representative display color.
5. In the **Excitation** field, enter the center excitation wavelength for the new stain. This value determines the LEDs used to illuminate the sample.
6. In the **Emission** field, enter the center emission wavelength for the new stain. This value determines which filter cube is used for detection.
7. In the **Edit** field, click  **OK**.

Editing a Stain Definition

To edit a stain definition in the library:

1. On the **Stain Library** page, in the row for the stain you want to edit, click  **Edit**.
2. Make the changes as needed.
3. In the **Edit** field, click  **OK**.

Deleting a Stain Definition

To delete a stain definition from the library:

1. On the **Stain Library** page, in the row of the stain you want to delete, click  **Delete**.
2. Click **OK**.

Labware Library

The  **Labware Library** page specifies the labware specifications available to all users. A default library of formats for plates, dishes, and slides is provided. You can add more plate and slide formats as needed.

Tip: To achieve the best autofocus results during an acquisition, it is very important to specify a plate or slide format that is accurate for your labware.

Note: The software provides a default format for the optional dish holder, which holds up to six 35 mm dishes. This is the only supported dish format. The dish format is characteristically similar to a plate, so it is listed as a plate format.

Viewing a Labware Specification

To view a labware specification:

1. On the **Labware Library** page, click the tab for the type of labware specification you want to view (either **Plates** or **Slides**).
2. In the row of a labware specification you want to view, click **View**.
3. When you are finished viewing the labware specification, click **Close**.

Adding a Plate Format

You can define a new plate format specification and add it the library.

Tip: One reason to add a plate format is when you need to use the plate skirt height adapter after autofocus failure with the environmental control cassette. In this case, you must define a new plate format specification that adds 1.5 mm to the actual **Plate Height** value.

To add a plate format to the library:

1. Insert the new plate in the instrument.

   Note: For best results, use a dry plate.

2. On the **Labware Library** page, click the **Plates** tab.
3. In the row of a plate format that is similar to the one you want to add, click **Duplicate**.
4. In the new row, click **Edit**.

5. In the **Name** field, enter a name for the new plate format.
6. Confirm that the value in the **Material** field is correct.
7. Confirm that the **Well Shape Square** check box is set correctly.
8. Click **Save**.
9. Click **Measure Plate Dimensions**.
10. Click the **Select the instrument** drop-down list box, and select the instrument you are using.
11. Click **Open Door** to open the top door.
12. Insert the plate.

    Note: Always measure just the plate; do not measure the plate inside the environmental control cassette. The software automatically compensates for the thickness of the cassette.

13. Click **Close Door**.
14. Click **Measure Plate Dimensions**.
    The instrument measures the well depth and bottom thickness.
15. Click **Finish**.

Adding a Slide Format

You can define a new slide format specification and add it the library.

Tip: One reason to add a slide format is when autofocus does not provide good results with the 2.5x objective and the default **4 Slide Holder Coverslip Up** format. In this case, define a slide format specification with an increased **Support Ledge Height** value. For details, log in to the Molecular Devices Knowledge Base at support.moleculardevices.com, and search for **ImageXpress Pico: Focus Troubleshooting**.

To add a slide format to the library:

1. On the **Labware Library** page, click the **Slides** tab.
2. In the row of a slide format that is similar to the one that you want to add, click **Duplicate**.
3. In the new row, click **Edit**.

4. In the **Name** field, enter a name for the new slide format.
5. Edit the specifications for the slide format as needed.
6. Click **Save**.

Deleting a Labware Specification

You can delete a labware specification that you have added to the library.

Note: You cannot delete a default format.

To delete a format from the library:

1. On the **Labware Library** page, click the tab for the type of labware specification you want to delete (either **Plates** or **Slides**).
2. In the row of the labware specification you want to delete, click **Delete**.
3. Click **OK**.

Devices

The  **Devices** page specifies the instruments available for acquisition and maintenance. You can add instruments as needed using one of the following connections:

- **Direct Connection**: A direct Ethernet connection between the instrument and the host computer running the CellReporterXpress software in a standalone configuration.
- **Remote Connection**: A network Ethernet connection between the instrument and the host computer running the CellReporterXpress software in a network configuration or a server configuration.

The **Devices** page contains two tabs:

- **My Devices**: Lists all registered instruments available to you for acquisition and maintenance.
- **Add Device**: Lists all registered instruments connected to the host computer. From here, you can add instruments to the list on the **My Devices** tab, which makes them available for acquisition and maintenance.

You must add at least one instrument to the list on the **My Devices** tab to acquire an image.

For each instrument, a status indicator shows one of the following:

 **Online**

 **Offline** or **Incompatible**

 **Busy**

 **Error**

Note: If the status indicator for an instrument shows  **Incompatible**, the instrument firmware version may not be compatible with the software version. For details on updating the firmware, log in to the Molecular Devices Knowledge Base at support.moleculardevices.com, and search for **ImageXpress Pico: Firmware Update**.

Registering an Instrument

Registering a Directly Connected Instrument

To register a directly connected instrument:

1. Confirm that the instrument is connected to the host computer running the CellReporterXpress software using the **LAN1** port on the back of the device.
2. On the **Devices** page, on the **Add Device** tab, click **Connect LAN1**.
   The instrument appears in the list on the **Add Device** tab.
3. If needed, in the tile for that instrument, click + **Add to My Devices**.
   The instrument appears in the list on the **My Devices** tab.

Tip: We recommend that you do not directly connect an instrument to the host computer using the LAN2 port unless advised to do so by Molecular Devices Technical Support.

Registering a Remotely Connected Instrument Using Autodiscovery

To register a remotely connected instrument using autodiscovery:

1. Confirm that the instrument is connected to the network using the **LAN2** port on the back of the device.
2. Confirm that the host computer running the CellReporterXpress software is connected to the network.
   Within five minutes, the instrument appears in the list on the **Add Device** tab. It may initially indicate offline status, but it should change to online status shortly after it appears.
   If the instrument does not appear in the list on the **Add Device** tab, your computer or your network may be set up to block autodiscovery. In this case, do one of the following:
   - Enable network discovery on the computer where the CellReporterXpress software is installed and try again.
   - Register the instrument using manual discovery. See Registering a Remotely Connected Instrument Using Manual Discovery on page 222 for details.
3. If needed, in the tile for that instrument, click + **Add to My Devices**.
   The instrument appears in the list on the **My Devices** tab.

Registering a Remotely Connected Instrument Using Manual Discovery

The host computer or your network may be set up to block autodiscovery. In that case, you can register a remotely connected instrument using manual discovery.

To register a remotely connected instrument using manual discovery:

1. Confirm that the instrument is connected to the network using the **LAN2** port on the back of the device.
2. Confirm that the host computer running the CellReporterXpress software is connected to the network.
3. On the **Devices** page, on the **Add Device** tab, in the **Remote Connection** field, enter the name or the IP address of the instrument you want to add.

   Note: The instrument name begins with "IXP-" followed by the serial number, which is on the back of the instrument. See the *ImageXpress Pico User Guide* for details on locating the serial number.

   The device IP address is determined by your network. Contact your network administrator for details.

4. Click **Register Device**.
   The remotely connected instrument appears in the list on the **Add Device** tab.
   If the instrument does not appear in the list on the **Add Device** tab, contact your network administrator.
5. If needed, in the tile for that instrument, click **Add to My Devices**.
   The instrument appears in the list on the **My Devices** tab.

Unregistering an Instrument

To unregister a registered instrument:

1. On the **Devices** page, on the **Add Device** tab, locate the instrument you want to unregister.
2. Click **Unregister**.
3. Click **OK**.

Using the My Devices List

Adding an Instrument to the My Devices List

Add a registered instrument to the list on the **My Devices** tab to make the device available for acquisition and maintenance. Your setting affects your login only. You must add at least one instrument to the list on the **My Devices** tab to acquire an image.

To add an instrument to the My Devices list:

1. On the **Devices** page, on the **Add Device** tab, locate the instrument you want to make available.
2. Click **Add to My Devices**.
   The instrument appears in the list on the **My Devices** tab.

Removing an Instrument from the My Devices List

Remove an instrument from to the list on the **My Devices** tab to make the device no longer available for acquisition and maintenance. Your setting affects your login only.

Note: After you remove an instrument from the My Devices list, it remains available in the software and can be added again.

To remove an instrument from the My Devices list:

1. On the **Devices** page, on the **My Devices** tab, locate the instrument you want to make available.
2. Click **Remove from My Devices**.
   The instrument is removed from the list on the **My Devices** tab.

Flagging a Frequently Used Instrument

The tile for each instrument on the **My Devices** tab contains a **Favorite** icon that you can use to flag a frequently used instrument. Flagged instruments will appear at the top of the list on the **My Devices** tab. Your setting affects your login only.

To flag a frequently used instrument:

Click **Favorite** to flag a frequently used instrument.

Image Analysis Computers

The **Image Analysis Computers** page specifies the registered computers and mapped folders that are available for image storage. In all configurations, you can perform image analysis on the host computer. In a server configuration, you can also perform image analysis on any networked computer that has been registered for external image analysis. The CellReporterXpress software determines which registered computer will be used for each analysis.

In a server configuration, any registered computer running the Analysis Service (MD.AnalysisService) can perform image analysis. See the *CellReporterXpress Installation Guide* for details on setting up a server configuration.

All registered computers appear in the Registered Image Analysis Computers list with one of the following status indicators:

 **Online**

 **Offline**

A registered computer may indicate offline status due to an issue with the network, the firewall, or the Analysis Service (that is, it is not present or not started).

Registering a Remote Analysis Computer

To register a computer for image analysis:

1. On the **Image Analysis Computers** page, in the **Add Image Analysis Computer** field, enter the computer name or the IP address of the computer you want to register.
2. Click  **Add Image Analysis Computer**.

The computer appears in the **Registered Image Analysis Computers** list.

Removing a Registered Computer

You can remove a computer from the **Registered Image Analysis Computers** list, which prevents it from being used to perform image analysis. After you remove a registered computer, the MD.AnalysisService remains on that computer.

To remove a registered computer:

1. On the **Image Analysis Computers** page, in row for the registered computer you want to remove, click  **Unregister**.
2. Click **OK**.

Restarting the Analysis Service

If a registered computer indicates offline status or an error occurs when testing analysis, you may need to restart the MD.AnalysisService on that computer.

To restart the analysis service on a registered computer:

1. On the **Image Analysis Computers** page, in row for the registered computer with the analysis service you want to restart, click  **Restart**.
2. Click **OK**.

Data Storage

The  **Data Storage** page specifies the registered computers and mapped folders that are available for storage. In all configurations, you can store image and experiment data on the host computer. In a server configuration, you can also store data on any networked computer running the Location Service (MD.LocationService) that has been registered for external image storage. There is no limit to the number of remote storage computers in a configuration. You can select the registered computer and mapped folder to be used for storage when you run a protocol. See the *CellReporterXpress Installation Guide* for details on setting up a server configuration.

All registered computers appear in the List of Registered Storage Computers and all mapped folders appear in the List of Mapped Folders with one of the following status indicators:

 **Online**

 **Offline**

A registered computer or mapped folder may indicate offline status due to an issue with the network, the firewall, or the Location Service (that is, it is not present or not started).

Registering a Remote Storage Computer

To register a computer for image storage:

1. On the **Data Storage** page, in the **Add Data Storage Computer** field, enter the computer name or the IP address of the computer you want to register.
2. Click  **Add Data Storage**.

The computer appears in the **List of Registered Storage Computers**.

Note: To increase security on remote computers registered for storage operations, you may want to set the Location Service (MD.LocationService) as a local service on each remote storage computer. Once you do this, you must set each folder mapped for storage to allow write access to the software. See the "Secure Setup for Remote Storage" section in the *CellReporterXpress Installation Guide* for details.

Mapping a Folder for Storage

On the host computer and on any remote computer registered for storage operations, the following folder is created and mapped for storage by default:

```
C:\ProgramData\Molecular Devices\MD.LocationService\Data
```

You can map other folders for image storage as needed.

Note:

- Before you can map a folder on a network drive for storage, you must set up the Location Service accordingly. See the *CellReporterXpress Installation & IT Guide* for details.
- When specifying a folder on a network drive for storage, you must use the full, UNC path of the folder (for example, `\\servername\folder`). The software does not support using a virtual path that uses a drive letter (for example, `J:\folder`).
- The software does not have write access to folders under the `C:\Users` folder. This means you cannot map some common folders—such as the **Desktop** folder, **Documents** folder, or **Downloads** folder—for storage.

To map a folder for image storage:

1. On the **Data Storage** page, in the **Map Folder on Storage Computer** field, enter the full path of the folder you want to map.
2. Click + **Map Existing Folder on Storage Computer**.

The mapped folder appears in the **List of Mapped Folders**.

Note: To increase security on remote computers registered for storage operations, you may want to set the Location Service (MD.LocationService) as a local service on each remote storage computer. Once you do this, you must set each folder mapped for storage to allow write access to the software. See the "Secure Setup for Remote Storage" section in the *CellReporterXpress Installation Guide* for details.

Removing a Registered Computer

You can remove a computer from the **List of Registered Storage Computers**, which prevents it from being used for image storage. After you remove a registered computer, the MD.LocationService remains on that computer.

To remove a registered computer:

1. On the **Data Storage** page, in row for the registered computer you want to remove, click **Unregister**.
2. Click **OK**.

The computer no longer appears in the **List of Registered Storage Computers**.

Removing a Mapped Folder

You can remove a mapped folder from the **List of Mapped Folders**, which prevents it from being used for image storage. After you remove a mapped folder, the folder and the images it contains remain on the computer.

To remove a mapped folder:

1. On the **Data Storage** page, in row for the mapped you want to remove, click **Remove from List**.
2. Click **OK**.

The mapped folder no longer appears in the **List of Mapped Folders**.

Chapter 7: Devices Mode

Use **Devices** mode to manage and configure instruments for acquisition, including installing and calibrating objectives and filter cubes and controlling the temperature inside the instrument.

On the **Home** page, click the  **Devices** tile to enter **Devices** mode.

The right side of the page includes the following icons:

 **Shutdown Device**: Prepares the software to power off the selected instrument.

 **Restart Device**: Restarts the selected instrument.

 **Open Plate Door**: Opens the top door on the selected instrument so that you can insert or remove labware.

 **Close Plate Door**: Closes the top door.

The **Available Acquisition Devices** list on the **Devices** page shows the instruments available to you. See Devices on page 220 for details on adding an instrument to the **Available Acquisition Devices** list.

Click  **Show Device Options** for a device to view device details on the following tabs:

- **Info**: Displays details about the selected instrument, including serial number, version, and free space. See Info on page 230 for details.
- **Sensors**: Displays the current environmental details for selected instrument, including temperature, humidity level, CO_2 level, and O_2 level. You can set a target value for each environmental detail and subscribe to a toolbar notification to help you monitor environmental conditions. See Sensors on page 231 for details.
- **Objectives**: Displays a tile for each objective slot in the selected instrument. Each tile shows the registered objective for that slot and the calibration state of the objective. From here, you can install and calibrate objectives. See Objectives on page 246 for details.
- **Filters**: Displays a tile for each filter cube slot in the selected instrument. Each tile shows the registered filter cube for that slot and the calibration state of the filter cube. From here, you can install and calibrate filter cubes. See Filters on page 252 for details.
- **Maintenance**: Displays a tile for each maintenance activity that can be performed on the selected instrument. See Maintenance on page 257 for details.

Info

The **Info** tab displays details about the selected instrument, including the following:

- Instrument Name
- Instrument Serial Number
- Device Model
- Connected on
- Version
- Free Space
- IP
- MAC
- Number of Installed Objectives
- Number Installed Filter Cubes
- Digital Confocal License

Sensors

Without the optional environmental control system, the **Sensors** tab displays the current temperature inside the selected instrument.

With the optional environmental control system, the **Sensors** tab displays the current temperature, humidity level, CO_2 level, and O_2 level inside the environmental control cassette.

You can set a target value to regulate each component:

Component	Set Range	Notes
CO_2	1% to 15%	
Humidity	85%	Humidity level is fixed.
O_2	1% to 15% and ambient	
Temperature	25°C to 40°C (77°F to 104°F)	Environmental control cassette is not required. Minimum achievable temperature setting is: • 6°C (11°F) above ambient without cassette. • 8°C (14°F) above ambient with cassette.

For each component, you can subscribe to a toolbar notification to help you monitor environmental conditions.

Note:

- If the environmental control cassette is not inserted in the instrument, the **Sensors** tab and the toolbar notification (if enabled) show no value for the humidity level. Values may be displayed for CO_2 level and O_2 level, but these values are not meaningful for an experiment because they are measured within the gas mixer and not near the labware.
- Environmental control is fully supported for plate and dish acquisitions only. For slide acquisitions, only temperature control is available; humidity, CO_2, and O_2 control is not available.

Regulating CO_2 Level

With the optional environmental control system, you can regulate the CO_2 level inside the environmental control cassette within the range of 1% to 15%. When you set a target level and start CO_2 level control, the instrument supplies CO_2 to the environmental control cassette to achieve the target.

The current CO_2 level inside the environmental control cassette is shown on the **Sensors** tab and on the CO_2 level toolbar notification (if enabled). See Enabling a CO2 Level Toolbar Notification on page 235 for details.

Before you can start CO_2 level control, you must set up the environmental control system and insert the environmental control cassette. See the *ImageXpress Pico User Guide* for details.

WARNING! Use compressed gas supplies in a well-ventilated area. The instrument is not air-tight. Gas can escape into the atmosphere surrounding the instrument. When you use potentially toxic gas, observe the cautionary procedures defined by your safety officer to maintain a safe work environment, such as the use of an automatic warning system.

Note:

- Before and during an experiment with CO_2 level control, check the gas supply.
- The software does not support adjusting an environmental control setting during a time series acquisition. If your experiment requires this, perform a discontinuous time series by acquiring the first set of time points, adjusting the humidity level, CO_2 level, O_2 level, or temperature as needed, and then acquiring the next set of time points. See Time Series on page 68 for details.
- When using the environment control cassette with the 2.5x objective, autofocus may fail or be inconsistent. See Understanding How Autofocus Works with a Plate on page 49 for details.
- When using the environmental control cassette with a 40x objective, you must adjust the objective correction collar an additional 0.7 mm above the recommended setting to account for the thickness of the cassette glass bottom. If the bottom thickness of the plate plus 0.7 mm is greater than the range of the 40x objective correction collar, then this plate is not compatible with this configuration. Try a different plate type, use a lower magnification objective, or image without the environmental control cassette. With a plate, see Adjusting an Objective Correction Collar on page 46 for details. With slides, see Adjusting an Objective Correction Collar on page 104 for details.
- The 63x objective is not compatible with the environmental control cassette. Do not install the 63x objective when the environmental control cassette is loaded.
- If you open or remove the environmental control cassette while gas is flowing, gas flow stops.
- If you experience an issue with the environmental control system, review the troubleshooting tips in the *ImageXpress Pico User Guide* before contacting Molecular Devices Technical Support.
- In any mixture of CO_2 and air, the maximum O_2 concentration drops as the CO_2 level increases, as shown in the following table:

CO_2 Level	Maximum O_2 Concentration
0%	21%
5%	20%
10%	19%

For best results, if you are regulating the CO_2 level, set the O_2 level accordingly.

Starting CO_2 Level Control

To start CO_2 level control:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the instrument where you want to start CO_2 level control.
2. Click the **Sensors** tab.
3. In the **CO_2 Level** row, under **Component State**, in the **Target** field, enter a target CO_2 level as a percentage.

    **Note:** The target CO_2 level must be within the range of 1% to 15%.

4. Click  **Start Regulation**.
   The indicator in the **State** field enables to show that CO_2 level control is on.

Modifying CO_2 Level Control

When CO_2 level control is on, you can set a new target CO_2 level.

To start temperature control:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the instrument where you want to set a new CO_2 level.
2. Click the **Sensors** tab.
3. In the **CO_2 Level** row, under **Component State**, in the **Target** field, enter a new target CO_2 level as a percentage.

    **Note:** The target CO_2 level must be within the range of 1% to 15%.

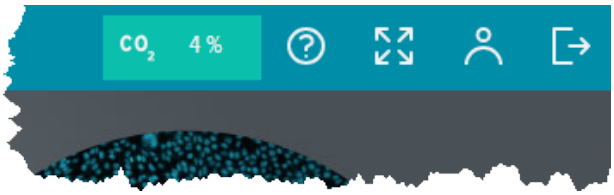
4. Click  **Start Regulation**.
   The indicator in the **State** field remains enabled.

Stopping CO_2 Level Control

To stop CO_2 level control:

1. On the **Devices** page, click **Show Device Options** to expand the details for the instrument where you want to stop CO_2 level control.
2. Click the **Sensors** tab.
3. In the **CO_2 Level** row, under **Component State**, click **Stop Regulation**.

Enabling a CO_2 Level Toolbar Notification



You can enable a toolbar notification to monitor the CO_2 level in the environmental control cassette. The toolbar notification appears at the top of the CellReporterXpress window. The color of the toolbar notification indicates if the CO_2 level is within the specified range (green) or outside of it (yellow).

Click on a toolbar notification to open an environmental control panel.

Note: If the environmental control cassette is not inserted in the instrument, the **Sensors** tab and the toolbar notification (if enabled) show no value for the humidity level. Values may be displayed for CO_2 level and O_2 level, but these values are not meaningful for an experiment because they are measured within the gas mixer and not near the labware.

To enable a CO_2 level toolbar notification:

1. On the **Devices** page, click **Show Device Options** to expand the details for the instrument where you want to subscribe to a CO_2 level toolbar notification.
2. Click the **Sensors** tab.
3. In the **CO_2 Level** row, under **Notification Settings**, in the **Min** field, enter the lower limit value for the CO_2 level range as a percentage.
4. In the **Max** field, enter the upper limit value for the CO_2 level range as a percentage.

   Note: The lower limit and upper limit values must be within the range of 1% to 15%.

5. Click **Start Notification**.

Regulating Humidity Level

With the optional environmental control system, you can regulate the humidity level inside the environmental control cassette. When you start humidity level control, the instrument introduces 85% humidity into the environmental control cassette.

The current humidity level inside the environmental control cassette is shown on the **Sensors** tab and on the humidity level toolbar notification (if enabled). See Enabling a Humidity Level Toolbar Notification on page 238 for details.

Before you can start humidity level control, you must set up the environmental control system and insert the environmental control cassette. See the *ImageXpress Pico User Guide* for details.

CAUTION!

- Do not operate humidity level control if the water level is below the minimum indicator. Operating without enough water can damage the instrument and the humidifying column.
- Before and during an experiment with humidity level control, check the water level in the humidifying column and refill as needed. (Be aware that refilling the humidifying column during an experiment can reduce the humidity level for several minutes.)
- Only use 18 Mohm•cm ultrapure water to fill the humidifying column.
- Confirm that the stopper is firmly seated in the humidifying column. A loose stopper can allow gas leakage and other environmental control system issues.

Note:

- The humidifying column holds 130 ml (4.4 oz) of ultrapure water, which is enough to continuously provide humidity for up to 72 hours. When the water level approaches the minimum indicator (approximately one-third full), add 18 Mohm•cm ultrapure water to reach the maximum level.
- If the humidifying column does run dry, restart the instrument after refilling the column.
- To prevent condensation, humidity may not start immediately after you insert the sample.
- Fill all unused wells with media to reduce overall evaporation.
- The software does not support adjusting an environmental control setting during a time series acquisition. If your experiment requires this, perform a discontinuous time series by acquiring the first set of time points, adjusting the humidity level, CO_2 level, O_2 level, or temperature as needed, and then acquiring the next set of time points. See Time Series on page 68 for details.
- If the instrument is unable to consistently achieve a humidity level of between 75% and 95%, you may need to run air dry to clear condensation from the environmental control system. See Running Air Dry on page 257 for details.
- When using the environmental control cassette with a 40x objective, you must adjust the objective correction collar an additional 0.7 mm above the recommended setting to account for the thickness of the cassette glass bottom. If the bottom thickness of the plate plus 0.7 mm is greater than the range of the 40x objective correction collar, then this plate is not compatible with this configuration. Try a different plate type, use a lower magnification objective, or image without the environmental control cassette. With a plate, see Adjusting an Objective Correction Collar on page 46 for details. With slides, see Adjusting an Objective Correction Collar on page 104 for details.
- The 63x objective is not compatible with the environmental control cassette. Do not install the 63x objective when the environmental control cassette is loaded.
- If you experience an issue with the environmental control system, review the troubleshooting tips in the *ImageXpress Pico User Guide* before contacting Molecular Devices Technical Support.

Starting Humidity Level Control

To start humidity level control:

1. On the **Devices** page, click **Show Device Options** to expand the details for the instrument where you want to start humidity level control.
2. Click the **Sensors** tab.
3. In the **Humidity** row, under **Component State**, click **Start Regulation**.
   The indicator in the **State** field enables to show that humidity control is on.

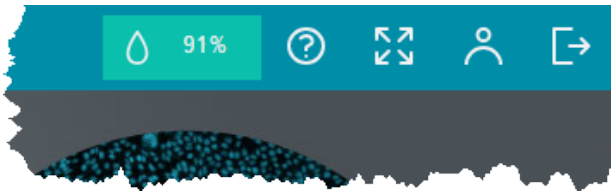
CAUTION! When humidity level control is on, the base of the humidifying column can heat up to 50°C (122°F). Avoid touching the temperature-controlled parts of the system.

Stopping Humidity Level Control

To stop humidity level control:

1. On the **Devices** page, click **Show Device Options** to expand the details for the instrument where you want to stop humidity level control.
2. Click the **Sensors** tab.
3. In the **Humidity** row, under **Component State**, click **Stop Regulation**.

Enabling a Humidity Level Toolbar Notification



You can enable a toolbar notification to monitor the humidity level in the environmental control cassette. The toolbar notification appears at the top of the CellReporterXpress window. The color of the toolbar notification indicates if the temperature is within the fixed range (green) or outside of it (yellow).

Click on a toolbar notification to open an environmental control panel.

Note: If the environmental control cassette is not inserted in the instrument, the **Sensors** tab and the toolbar notification (if enabled) show no value for the humidity level. Values may be displayed for CO_2 level and O_2 level, but these values are not meaningful for an experiment because they are measured within the gas mixer and not near the labware.

To enable a toolbar notification for humidity:

1. On the **Devices** page, click **Show Device Options** to expand the details for the instrument where you want to subscribe to a humidity level toolbar notification.
2. Click the **Sensors** tab.
3. Click **Start Notification**.

Regulating O_2 Level

With the optional environmental control system, you can regulate the O_2 level inside the environmental control cassette within the range of 1% to 15%. When you set a target level and start O_2 level control, the instrument supplies O_2 to the environmental control cassette to achieve the target O_2 level.

The current O_2 level inside the environmental control cassette is shown on the **Sensors** tab and on the O_2 level toolbar notification (if enabled). See Enabling an O2 Level Toolbar Notification on page 242 for details.

Before you can start O_2 level control, you must set up the environmental control system and insert the environmental control cassette. See the *ImageXpress Pico User Guide* for details.

WARNING! Use compressed gas supplies in a well-ventilated area. The instrument is not air-tight. Gas can escape into the atmosphere surrounding the instrument. When you use potentially toxic gas, observe the cautionary procedures defined by your safety officer to maintain a safe work environment, such as the use of an automatic warning system.

Note:

- Before and during an experiment with O_2 level control, check the gas supply.
- The software does not support adjusting an environmental control setting during a time series acquisition. If your experiment requires this, perform a discontinuous time series by acquiring the first set of time points, adjusting the humidity level, CO_2 level, O_2 level, or temperature as needed, and then acquiring the next set of time points. See Time Series on page 68 for details.
- When using the environmental control cassette with a 40x objective, you must adjust the objective correction collar an additional 0.7 mm above the recommended setting to account for the thickness of the cassette glass bottom. If the bottom thickness of the plate plus 0.7 mm is greater than the range of the 40x objective correction collar, then this plate is not compatible with this configuration. Try a different plate type, use a lower magnification objective, or image without the environmental control cassette. With a plate, see Adjusting an Objective Correction Collar on page 46 for details. With slides, see Adjusting an Objective Correction Collar on page 104 for details.
- The 63x objective is not compatible with the environmental control cassette. Do not install the 63x objective when the environmental control cassette is loaded.
- If you open or remove the environmental control cassette while gas is flowing, gas flow stops.
- If you experience an issue with the environmental control system, review the troubleshooting tips in the *ImageXpress Pico User Guide* before contacting Molecular Devices Technical Support.
- In any mixture of CO_2 and air, the maximum O_2 concentration drops as the CO_2 level increases, as shown in the following table:

CO_2 Level	Maximum O_2 Concentration
0%	21%
5%	20%
10%	19%

For best results, if you are regulating the CO_2 level, set the O_2 level accordingly.

Starting O_2 Level Control

To start O_2 level control:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the device where you want to start O_2 level control.
2. Click the **Sensors** tab.
3. In the **O_2 Level** row, under **Component State**, in the **Target** field, enter a target O_2 level as a percentage.

    **Note:** The target O_2 level must be within the range of 1% to 15%.

4. Click  **Start Regulation**.
   The indicator in the **State** field enables to show that O_2 level control is on.

Modifying O_2 Level Control

When O_2 level control is on, you can set a new target O_2 level.

To start temperature control:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the device where you want to set a new O_2 level.
2. Click the **Sensors** tab.
3. In the **O_2 Level** row, under **Component State**, in the **Target** field, enter a new target O_2 level as a percentage.

    **Note:** The target O_2 level must be within the range of 1% to 15%.

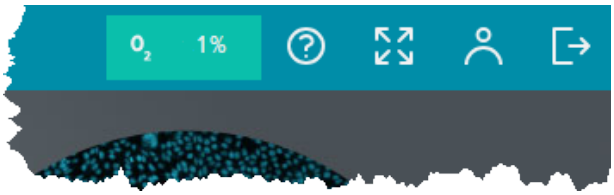
4. Click  **Start Regulation**.
   The indicator in the **State** field enables to show that O_2 level control is on.

Stopping O_2 Level Control

To stop O_2 level control:

1. On the **Devices** page, click **Show Device Options** to expand the details for the device where you want to stop O_2 level control.
2. Click the **Sensors** tab.
3. In the **O_2 Level** row, under **Component State**, click **Stop Regulation**.

Enabling an O_2 Level Toolbar Notification



You can enable a toolbar notification to monitor the O_2 level in the environmental control cassette. The toolbar notification appears at the top of the CellReporterXpress window. The color of the toolbar notification indicates if the O_2 level is within the specified range (green) or outside of it (yellow).

Click on a toolbar notification to open the temperature control panel.

Note: If the environmental control cassette is not inserted in the instrument, the **Sensors** tab and the toolbar notification (if enabled) show no value for the humidity level. Values may be displayed for CO_2 level and O_2 level, but these values are not meaningful for an experiment because they are measured within the gas mixer and not near the labware.

To enable an O_2 level toolbar notification:

1. On the **Devices** page, click **Show Device Options** to expand the details for the instrument where you want to subscribe to a O_2 level toolbar notification.
2. Click the **Sensors** tab.
3. In the **O_2 Level** row, under **Notification Settings**, in the **Min** field, enter the lower limit value for the O_2 level range as a percentage.
4. In the **Max** field, enter the upper limit value for the O_2 level range as a percentage.

   Note: The lower limit and upper limit values must be within the range of 1% to 15%.

5. Click **Start Notification**.

Regulating Temperature

You can regulate the temperature inside the instrument or (with the optional environmental control system) inside the environmental control cassette within a range of 25°C to 40°C (77°F to 104°F). When you set a target temperature and start temperature control, the inside of the instrument or the inside of the environmental control cassette is warmed to the target temperature.

The current temperature inside the instrument or the environmental control cassette is shown on the **Sensors** tab and on the temperature toolbar notification (if enabled). See Enabling a Temperature Toolbar Notification on page 245 for details.

If you are using temperature control with the environmental control cassette, you must set up the environmental control system and insert the cassette. See the *ImageXpress Pico User Guide* for details.

Note:

- The ambient room temperature must be at least 6°C (10.8°F) below the set temperature (without the environmental control cassette) or at least 8°C (14.5°F) below the set temperature (with the environmental control cassette). To achieve the minimum temperature setting of 25°C (77°F) without the environmental control cassette, the room temperature can be no higher than 19°C (66.2°F). To achieve the minimum temperature setting with the environmental control cassette, the room temperature can be no higher than 17°C (62.5°F).
- After starting or modifying temperature control, allow the temperature to reach the target value before inserting the sample.
- Without the environmental control cassette, the temperature sensor detects the temperature inside the instrument, not the temperature of the samples in the plate. With the environmental control cassette, the temperature sensor detects the temperature inside the cassette.
- If you are not using the environmental control cassette, you may want to use a seal or lid on the sample to prevent evaporation.
- Once warmed, it may take longer for the temperature inside the instrument to cool than it took to warm it.
- The software does not support adjusting an environmental control setting during a time series acquisition. If your experiment requires this, perform a discontinuous time series by acquiring the first set of time points, adjusting the humidity level, CO_2 level, O_2 level, or temperature as needed, and then acquiring the next set of time points. See Time Series on page 68 for details.
- When using the environmental control cassette with a 40x objective, you must adjust the objective correction collar an additional 0.7 mm above the recommended setting to account for the thickness of the cassette glass bottom. If the bottom thickness of the plate plus 0.7 mm is greater than the range of the 40x objective correction collar, then this plate is not compatible with this configuration. Try a different plate type, use a lower magnification objective, or image without the environmental control cassette. With a plate, see Adjusting an Objective Correction Collar on page 46 for details. With slides, see Adjusting an Objective Correction Collar on page 104 for details.
- The 63x objective is not compatible with the environmental control cassette. Do not install the 63x objective when the environmental control cassette is loaded.

Starting Temperature Control

To start temperature control:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the device where you want to start temperature control.
2. Click the **Sensors** tab.
3. In the **Temperature** row, under **Component State**, in the **Target** field, enter a target temperature value in degrees Celsius.

    **Note:** The target temperature value must be within the range of 25°C to 40°C (77°F to 104°F).

4. Click  **Start Regulation**.
   The indicator in the **State** field enables to show that temperature control is on.
5. After the temperature reaches the target level, insert the sample.

Modifying Temperature Control

When temperature control is on, you can set a new temperature.

To modify temperature control:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the device where you want to set a new temperature.
2. Click the **Sensors** tab.
3. In the **Temperature** row, under **Component State**, in the **Target** field, enter a new target temperature value in degrees Celsius.

    **Note:** The target temperature level must be within the range of 25°C to 40°C (77°F to 104°F).

4. Click  **Start Regulation**.
   The indicator in the **State** field remains enabled.
5. After the temperature reaches the target level, insert the sample.

Stopping Temperature Control

To stop temperature control:

1. On the **Devices** page, click **Show Device Options** to expand the details for the device where you want to stop temperature control.
2. Click the **Sensors** tab.
3. In the **Temperature** row, under **Component State**, click **Stop Regulation**.

Enabling a Temperature Toolbar Notification

You can enable a toolbar notification to monitor temperature conditions inside the instrument or inside the environmental control cassette. The toolbar notification appears at the top of the CellReporterXpress window. The color of the toolbar notification indicates if the temperature is within the specified range (green) or outside of it (yellow).

Click on a toolbar notification to open an environmental control panel.

To enable a temperature toolbar notification:

1. On the **Devices** page, click **Show Device Options** to expand the details for the instrument where you want to subscribe to a temperature toolbar notification.
2. Click the **Sensors** tab.
3. In the **Temperature** row, under **Notification Settings**, in the **Min** field, enter the lower limit value for the temperature range in degrees Celsius.
4. In the **Max** field, enter the upper limit value for the temperature range in degrees Celsius.

   Note: The lower limit and upper limit values must be within the range of 25°C to 40°C (77°F to 104°F).

5. Click **Start Notification**.

Objectives

The **Objectives** tab displays a tile for each objective slot in the selected instrument. Each tile shows the registered objective for that slot and the calibration state of the objective. From here, you can install and calibrate objectives.

Installing an Objective

Before installing an objective, review the following:

- Access only the user-serviceable components inside the instrument as described in this guide. Avoid contact with other components as they can be damaged or knocked out of alignment.
- To prevent dust from collecting inside the instrument, keep the top door and the maintenance door closed (unless you are inserting labware or performing maintenance tasks).
- Ensure that the top door and the maintenance door are closed before starting the instrument.

CAUTION!

- To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling objectives and filter cubes.
- With the instrument power on, do not manually rotate the objective turret. Manually rotating the objective turret can damage the instrument.

Molecular Devices precalibrates the objectives to specific slots in the turret. You must install the objectives as follows:

Slot	Objective Magnification	Notes
1	4x	The 4x objective is required for autofocus and must be installed in slot 1.
2	10x	The 10x objective is required for autofocus and must be installed in slot 2.
3	2.5x	Do not install the 2.5x objective and the 20x objective in the instrument simultaneously.
	20x	
4	empty	Slot 4 must be empty.
5	40x	Do not install the 40x objective and the 63x objective in the instrument simultaneously.
	63x	The 63x objective is not compatible with the environmental control cassette. Do not install the 63x objective when the environmental control cassette is loaded.
6	empty	Slot 6 must be empty.

Note:

- Depending on how your ImageXpress Pico system is configured, you may not have all the objectives.
- If no filter cubes are installed, an installed objective will appear to be not installed. We recommend that at least one filter cube be installed before installing an objective. See Installing a Filter Cube on page 252 for details.

Installing an Objective in an Empty Slot

You must install objectives in specific slots in the turret. See Installing an Objective on page 246 for details.

To install an objective in an empty slot:

1. On the **Devices** page, click **Show Device Options** to expand the details for the instrument where you want to install an objective.
2. Click the **Objectives** tab.
3. In the tile for the objective slot where you want to install, click **Component Exchange**.
4. Click the **Choose Objective** drop-down list box, and select the objective you want to install.
5. Click **Open Maintenance Door**.
6. Install the objective in the slot by gently turning it clockwise.

CAUTION! Retain the objective case. When not installed in the instrument, an objective should always be stored in its case.

7. Do both of the following:
   a. Manually close the maintenance door.
   b. In the CellReporterXpress software, click **Close Maintenance Door**.
8. Click **Apply**.

Replacing an Objective (Different Magnification)

You must install objectives in specific slots in the turret. See Installing an Objective on page 246 for details. Because of this, the only reasons to replace an objective with another objective of a different magnification are the following:

- When you are swapping the 2.5x objective and the 20x objective in slot 3.
- When you are swapping the 40x objective and the 63x objective in slot 5.

To replace an objective with an objective of a different magnification:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the instrument where you want to replace an objective.
2. Click the **Objectives** tab.
3. In the tile for objective slot **3** or slot **5**, click  **Component Exchange**.
4. Click the **Choose Objective** drop-down list box, and select the objective you want to install.
5. Click **Open Maintenance Door**.
6. Remove the objective to be replaced from the instrument by gently turning it counterclockwise.

   CAUTION! When not installed in the instrument, an objective should always be stored in its case.

7. Install the replacement objective in the slot by gently turning it clockwise.

   CAUTION! Retain the objective case for future storage needs. When not installed in the instrument, an objective should always be stored in its case.

8. Do both of the following:
   a. Manually close the maintenance door.
   b. In the CellReporterXpress software, click **Close Maintenance Door**.
9. Click **Apply**.

After you replace an objective, you may need to calibrate it. See Calibrating an Objective on page 251 for details.

Replacing an Objective (Same Magnification)

You must install objectives in specific slots in the turret. See Installing an Objective on page 246 for details. The typical reason to replace an objective with another objective of the same magnification is when you are replacing a damaged objective.

To replace an objective with an objective of the same magnification:

1. On the **Devices** page, click **Show Device Options** to expand the details for the instrument where you want to replace an objective.
2. Click the **Objectives** tab.
3. In the tile for the objective slot where you want to install, click **Component Exchange**.
4. Click the **Choose Objective** drop-down list box, and select **None**.
5. Click **Open Maintenance Door**.
6. Remove the objective to be replaced from the instrument by gently turning it counterclockwise.

   CAUTION! When not installed in the instrument, an objective should always be stored in its case.

7. Install the replacement objective in the slot by gently turning it clockwise.

   CAUTION! Retain the objective case for future storage needs. When not installed in the instrument, an objective should always be stored in its case.

8. Do both of the following:
   a. Manually close the maintenance door.
   b. In the CellReporterXpress software, click **Close Maintenance Door**.
9. Click **Close**.

After you replace an objective with another objective, you may need to calibrate it. See Calibrating an Objective on page 251 for details.

Note: If you re-install the same objective (after cleaning it, for example), you typically do not need to calibrate it.

Calibrating an Objective

After you install a new objective, you may need to calibrate it. **Molecular Devices precalibrates the objectives included with the initial purchase of the instrument. You must calibrate any objectives purchased after that time.** A calibration kit, which is included with any after-sales objective purchase, includes the following:

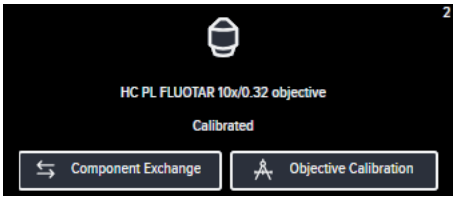
- Fluorescent Bead Slide (9100-0099)
- Red Plastic Slide (1-GP-7)
- Green Plastic Slide (1-GP-8)
- Orange Plastic Slide (1-GP-11)

You will need to provide the following item for the calibration process:

- 4-Slide Holder (5068503)

To calibrate an objective:

1. On the **Devices** page, click → **Show Device Options** to expand the details for the device where you want to calibrate an objective.
2. Click the **Objectives** tab.
3. Note the status of the objective, which is indicated in the tile.



   - If the tile indicates **Not Calibrated**, continue to the next step to perform the calibration procedure.
   - If the tile indicates **Calibrated**, you typically do not need to perform the calibration procedure and you can skip this procedure.

> **Note:** An exception to this is when you replace an objective with another objective of the same magnification. In this case, you should continue to the next step and perform the calibration procedure regardless of the label in the tile.

4. In the tile for the objective you want to calibrate, click **Objective Calibration**.
5. Follow the on-screen instructions to complete the calibration.

> **Tip:**
> - In the **Choose Slide Holder** drop-down list box, select **4 Slide Holder**.
> - Insert the slides face down in the slide holder (that is, printed side down).
> - When each step completes, click **Next** to continue to the next step.

Filters

The **Filters** tab displays a tile for each filter cube slot in the selected instrument. Each tile shows the registered filter cube for that slot and the calibration state of the filter cube. From here, you can install and calibrate filter cubes.

Installing a Filter Cube

Before installing a filter cube, review the following:

- Access only the user-serviceable components inside the instrument as described in this guide. Avoid contact with other components as they can be damaged or knocked out of alignment.
- To prevent dust from collecting inside the instrument, keep the top door and the maintenance door closed (unless you are inserting labware or performing maintenance tasks).
- Ensure that the top door and the maintenance door are closed before starting the instrument.

CAUTION! To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling objectives and filter cubes.

The following filter cubes are available:

- DAPI
- CFP
- FITC
- TRITC
- Texas Red
- Cy5

Note:

- Depending on how you have configured your ImageXpress Pico system, you may not have received all the filter cubes.
- Do not install a filter cube in slot 6 of the turret. Slot 6 must be empty (in both the instrument and the software).

Installing a Filter Cube in an Empty Slot

To install a filter cube in an empty slot:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the device where you want to install a filter cube.
2. Click the **Filters** tab.
3. In the tile for the filter cube slot where you want to install, click  **Component Exchange**.

   Note: Do not install a filter cube in slot 6 of the turret. Slot 6 must be empty (in both the instrument and the software).

4. Click the **Choose Filter** drop-down list box, and select the filter cube you want to install.
5. Click **Open Maintenance Door**.
6. If needed, slightly rotate the filter cube turret by hand to get direct access to the filter cube slot.
7. Install the filter cube in the slot by gently pushing it into the slot until it “snaps” into place.

   CAUTION! Retain the filter cube packaging. When not installed in the instrument, a filter cube should always be stored in its original packaging.

8. Do both of the following:
   a. Manually close the maintenance door.
   b. In the CellReporterXpress software, click **Close Maintenance Door**.
9. Click **Apply**.

Replacing a Filter Cube (Different Wavelength)

To replace a filter cube with a filter cube of a different wavelength:

1. On the **Devices** page, click **Show Device Options** to expand the details for the device where you want to replace a filter cube.
2. Click the **Filters** tab.
3. In the tile for the filter cube slot where you want to install, click **Component Exchange**.
4. Click the **Choose Filter** drop-down list box, and select the filter cube you want to install.
5. Click **Open Maintenance Door**.
6. If needed, slightly rotate the filter cube turret by hand to get direct access to the filter cube slot.
7. Remove the filter cube to be replaced from the instrument by gently pulling it toward you.

   CAUTION! When not installed in the instrument, a filter cube should always be stored in its original packaging.

8. Install the filter cube in the slot by gently pushing it into the slot until it “snaps” into place.

   CAUTION! Retain the filter cube packaging for future storage needs. When not installed in the instrument, a filter cube should always be stored in its original packaging.

9. Do both of the following:
   a. Manually close the maintenance door.
   b. In the CellReporterXpress software, click **Close Maintenance Door**.
10. Click **Apply**.

After you replace a filter cube, you may need to calibrate it. See Calibrating a Filter Cube on page 256 for details.

Note: If you re-install the same filter cube (after cleaning it, for example), you do not need to calibrate it.

Replacing a Filter Cube (Same Wavelength)

The typical reason to replace a filter cube with another filter cube of the same wavelength is when you are replacing a damaged filter cube.

To replace a filter cube with a filter cube of the same wavelength:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the device where you want to replace a filter cube.
2. Click the **Filters** tab.
3. In the tile for the filter cube slot where you want to replace, click  **Component Exchange**.
4. Click the **Choose Filter** drop-down list box, and select **None**.
5. Click **Open Maintenance Door**.
6. If needed, slightly rotate the filter cube turret by hand to get direct access to the filter cube slot.
7. Remove the filter cube to be replaced from the instrument by gently pulling it toward you.

   CAUTION! When not installed in the instrument, a filter cube should always be stored in its original packaging.

8. Install the filter cube in the slot by gently pushing it into the slot until it “snaps” into place.

   CAUTION! Retain the filter cube packaging for future storage needs. When not installed in the instrument, a filter cube should always be stored in its original packaging.

9. Do both of the following:
   a. Manually close the maintenance door.
   b. In the CellReporterXpress software, click **Close Maintenance Door**.
10. Click **Close**.

After you replace a filter cube, you may need to calibrate it. See Calibrating a Filter Cube on page 256 for details.

Note: If you re-install the same filter cube (after cleaning it, for example), you do not need to calibrate it.

Calibrating a Filter Cube

After you install a new filter cube, you may need to calibrate it. **Molecular Devices precalibrates the filter cubes included with the initial purchase of the instrument. You must calibrate any filter cubes purchased after that time.** A calibration kit, which is included with any after-sales filter cube purchase, includes the following:

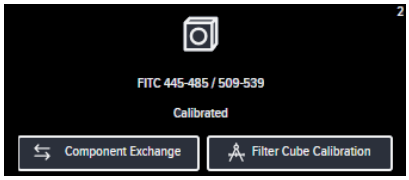
- Fluorescent Bead Slide (9100-0099)
- Red Plastic Slide (1-GP-7)
- Green Plastic Slide (1-GP-8)
- Orange Plastic Slide (1-GP-11)

You will need to provide the following item for the calibration process:

- 4-Slide Holder (5068503)

To calibrate a filter cube:

1. On the **Devices** page, click → **Show Device Options** to expand the details for the device where you want to calibrate a filter cube.
2. Click the **Filters** tab.
3. Note the status of the filter cube, which is indicated in the tile.



   - If the tile indicates **Not Calibrated**, continue to the next step to perform the calibration procedure.
   - If the tile indicates **Calibrated**, you typically do not need to perform the calibration procedure and you can skip this procedure.

   Note: An exception to this is when you replace a filter cube with the same type of filter cube. In this case, you should continue to the next step and perform the calibration procedure regardless of the label in the tile.

4. In the tile for the filter cube you want to calibrate, click **Filter Cube Calibration**.
5. Follow the on-screen instructions to complete the calibration.

Tip:

- In the **Choose Slide Holder** drop-down list box, select **4 Slide Holder**.
- Insert the slides face down in the slide holder (that is, printed side down).
- When each step completes, click **Next** to continue to the next step.

Maintenance

The **Maintenance** tab displays a tile for each maintenance activity that can be performed on the selected instrument.

Running Air Dry

With the optional environmental control system, condensation can build up inside the internal and external tubing. Condensation can prevent the instrument from consistently achieving a humidity level of between 75% and 95%.

Use the air dry maintenance to circulate hot, dry air throughout the environmental control system tubing to clear any condensation.

Note:

- Air dry can take 60 minutes or more. During that time, the instrument will be busy.
- Humidity control is not used during air dry, so the process runs successfully both with and without water in the humidifying column. If you already have water in the column, we recommend that you leave it there while running air dry.
- Before packing the instrument for transport, run air dry.
- Before running air dry, load the environmental control cassette in the instrument. Confirm that there is no sample in the cassette.

While air dry is in progress, you can monitor the status on the Monitor page. See Monitor Mode on page 213 for details.

To run air dry:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the instrument where you want to run air dry.
2. Click the **Maintenance** tab.
3. Click **Air Dry**.
4. Click **Start Air Dry**.
5. Click **Finish**.

The wizard closes and air dry runs.

Restoring Instrument Configuration

Run restore instrument configuration to revert the instrument to the factory settings or to a recent snapshot. This maintenance is for use in cases of unexpected instrument failure.

CAUTION! This maintenance reverts all instrument configuration settings, including calibration settings. Do not run this maintenance unless specifically instructed to do so by Molecular Devices.

Note: When you restore the instrument configuration, only the instrument settings are reverted. All CellReporterXpress data, including saved protocols and experiment data, remains intact.

Restoring the instrument configuration can take one to two minutes. During that time, the instrument will be busy. After the maintenance completes, the instrument automatically reboots. When the status light on the instrument is green, the instrument is ready to use.

Before restoring the instrument configuration, you may want to try performing a hard shutdown of the instrument and restarting the CellReporterXpress host computer. See the *ImageXpress Pico User Guide* for details on performing a hard shutdown.

Restoring to a Recent Snapshot

Restoring to a recent snapshot may remove the issue that caused the unexpected instrument failure.

To restore the instrument to a recent snapshot:

1. On the **Devices** page, click **Show Device Options** to expand the details for the instrument where you want to revert the settings.
2. Click the **Maintenance** tab.
3. Click **Restore Instrument Configuration**.
4. Click **Restore to Recent Snapshot**.
5. Click **Finish**.

Restoring to Factory Settings

To restore the instrument to factory settings:

1. On the **Devices** page, click **Show Device Options** to expand the details for the instrument where you want to revert the settings.
2. Click the **Maintenance** tab.
3. Click **Restore Instrument Configuration**.
4. Click **Restore to Factory Settings**.
5. Click **Finish**.

Chapter 8: Configuration Settings

Use **Configuration Settings** to customize the CellReporterXpress software interface for your login only.

In the toolbar, click  **View Preferences** to display the **Configuration Settings** page.

The left side of the page includes the following icons:

 **Themes**: Specifies the color scheme for the CellReporterXpress user interface. See Themes on page 260 for details.

 **Vocabulary**: Specifies the language for the CellReporterXpress user interface. See Vocabulary on page 260 for details.

 **Color Scheme**: Specifies the color scheme used for heatmaps in **Experiments** mode. See Color Scheme on page 260 for details.

 **Stains**: Specifies the stain definitions available in the CellReporterXpress software. You can edit certain details of the stain definitions. See Stains on page 260 for details.

 **Sharing Permissions**: Specifies the default sharing permissions for protocols and experiments. You can modify this default setting for each protocol and experiment to set permissions individually. See Sharing Permissions on page 261 for details.

 **Miscellaneous**: Specifies various CellReporterXpress software preferences, including image preferences and timeouts. See Miscellaneous on page 262 for details.

Themes

The  **Themes** settings specify the color scheme for the CellReporterXpress user interface. Your setting affects your login only.

Vocabulary

The  **Vocabulary** settings specify the language for the CellReporterXpress user interface. Your setting affects your login only.

English Technical is the only vocabulary setting available with the current version of the CellReporterXpress software.

Color Scheme

The  **Color Scheme** settings specify the color scheme used for heatmaps in **Experiments** mode. Your setting affects your login only.

Stains

The  **Stains** settings specify the stain definitions available in the CellReporterXpress software. You can edit certain details of the stain definitions. Your edits affect your login only.

All edited stain definitions display a  **Restore Original** icon in the leftmost column.

Note: To add stain definitions to the library or edit the stain definition details of the stains available to all users, go to the **Stain Library** page in **Configuration** mode. See Stain Library on page 216 for details.

Editing a Stain Definition for Your Login

To edit a stain for your login:

1. On the **Stains** page, in the row for the stain you want to edit, click  **Edit**.
2. Make the changes as needed.
3. In the **Edit** field, click  **Apply**.

Restoring a Stain Definition to Its Systemwide Setting

To restore a stain definition to its systemwide setting:

On the **Stains** page, in the row for the stain you want to restore, click  **Restore Original**.

Sharing Permissions

The **Sharing Permissions** settings specify the default sharing permissions for protocols and experiments. You can modify this default setting for each protocol and experiment to set permissions individually. Your settings affect your login only. By default, sharing permissions for protocols and experiments are unlocked and unrestricted.

Sharing permissions function differently for protocols and experiments:

- A locked protocol can be viewed and run by all other users, but only specified users can modify it.
- A private experiment can only be viewed by specified users.

Setting Default Protocol Sharing Permissions

The default protocol sharing permission is unlocked.

To set your default protocol sharing permission to share protocols only with specific users:

1. On the **Sharing Permissions** page, under **Default Protocol Sharing Permissions**, click  **Unlocked**.
2. Click the **Share with** drop-down list box, and select user names as needed to assign permissions.

Setting Default Experiment Sharing Permissions

The default experiment sharing permission is unlocked.

To set your default experiment sharing permission to share experiments only with specific users:

1. On the **Sharing Permissions** page, under **Default Experiment Sharing Permissions**, click  **Unlocked**.
2. Click the **Share with** drop-down list box, and select user names as needed to assign permissions.

Miscellaneous

The **Miscellaneous** settings specify various CellReporterXpress software preferences, including image preferences and timeouts. Your settings affect your login only.

The following settings are available:

- **Deep Zoom Images Preferences**: Specifies the image type (either **PNG** or **JPG**) for acquired images. If you select **JPG**, set the level of quality to be used. Higher quality means less compression and larger files, which affects the time required to open, redraw, and transfer acquired images.
- **Snap Image Preferences**: Specifies the image type (either **PNG** or **JPG**) and image resolution for preview images. If you select **JPG**, set the level of quality to be used. Higher quality means less compression and larger files, which affects the time required to open preview images.
- **Minimum Range for Focus Offset**: Specifies the minimum range for focus offset after focusing. Each channel can have an offset to image the part of the sample to be analyzed. The default setting is 40 µm.
- **Session Timeout**: Specifies the amount of time of inactivity before a session times out and logs off. Note that acquisitions (including time series acquisitions) continue after the session logs off. The default setting is 30 minutes.
- **Numeric Data Significant Figures**: Specifies the number of significant figures shown for measurements when measured analysis values appear. This setting also affects the number of significant digits saved when exporting measurements. The default setting is 6 figures.
- **Storage Unit (byte) Decimal Places**: Specifies how many decimal digits appear in a data storage value. The default setting is 2 decimal digits.
- **Length Unit (mm) Decimal Places**: Specifies how many decimal digits appear in a data storage value. The default setting is 2 decimal digits.

Appendix A: Analysis Descriptions

Note: Depending on your CellReporterXpress software license, some analyses may not be available.

Fluorescence Analyses

The software includes the following fluorescence analyses:

 Angiogenesis Skeletonization, see page 268: Single channel analysis for detecting and measuring blood vessels. Use the Angiogenesis Skeletonization analysis to identify and measure tubes (long thin objects) and nodes (connecting points between tubes) in a single wavelength. In addition to tube formation assays, you can also measure neurite outgrowth in assays where the cell bodies are indistinct or outside the field of view.

 Apoptosis, see page 270: Two-channel analysis using both a nuclei marker and a marker to identify apoptotic cells. Use the Apoptosis analysis to identify and measure cells entering programmed cell death using an apoptotic stain.

 Autophagy, see page 272: Two-channel assay for detecting autophagic granules. Use the Autophagy analysis to detect the infection of viral particles into cells.

 Cell Count, see page 274: Single-channel assay for counting cells based on a nuclei stain. Use the Cell Count analysis to segment images in order to identify and differentiate cell nuclei. The segmentation labels each isolated and identified cell to let you see a visual separation between cells that are close or touching.

 Cell Differentiation, see page 276: Two-channel assay using both a nuclei marker and a marker to identify differentiated cells. Use the Cell Differentiation analysis to identify two subpopulations of cells based on a fluorescent marker for all nuclei (for example, DAPI, Hoechst, or DRAQ5) and an additional marker for differentiated cells of interest. The additional marker can label cytoplasm or entire cell.

 Cell Scoring, see page 279: Two-channel assay for scoring cells based on a marker. Use the Cell Scoring analysis to identify two subpopulations of cells based on a fluorescent marker for all nuclei (for example, DAPI, Hoechst, or DRAQ5) and an additional marker (Marker 1) for cells of interest. The additional marker can label nuclei, cytoplasm or both.

 Cell Scoring: 3 Channels, see page 283: Three-channel assay for scoring cells based on a nuclear stain and two markers. Use the Cell Scoring: 3 Channels analysis to identify subpopulations of cells based on a fluorescent marker for all nuclei (for example, DAPI, Hoechst, or DRAQ5) and two additional markers (Marker 1 and Marker 2) for cells of interest. The additional markers can label nuclei, cytoplasm or both. You can use a fluorescent channel as more than one marker.

 Cell Scoring: 4 Channels, see page 288: Four-channel assay for scoring cells based on a nuclear stain and three markers. Use the Cell Scoring: 4 Channels analysis to identify subpopulations of cells based on a fluorescent marker for all nuclei (for example, DAPI, Hoechst, or DRAQ5) and three additional markers (Marker 1, Marker 2, and Marker 3) for cells of interest. The additional markers can label nuclei, cytoplasm or both. You can use a fluorescent channel as more than one marker.

 Endocytosis, see page 295: Two-channel assay for detecting endosomes. Use the Endocytosis analysis to detect the internalization of endosomes into cells.

 Internalization, see page 297: Two-channel assay for detecting internalizing granules. Use the Internalization analysis to identify and measure receptor internalization through granule measurements inside cells.

 Live Cells, see page 299: Two-channel assay using a nuclei marker for all cells and a second marker to identify live cells. Use the Live Cells analysis to determine the count of live and dead cells in appropriately prepared live/dead assays. This analysis lets you use two separate wavelengths and two separate stains. One stain identifies all cells, and the other stain identifies live cells. The dead cell count is determined by subtracting the live cell count from the total cell count.

 Lysosomal Degradation, see page 301: Two-channel assay for detecting lysosomes. Use the Lysosomal Degradation analysis to identify lysosome granules. A nuclear wavelength (for example, DAPI, Hoechst, or DRAQ5) is used to determine the number of granules per cell.

 Mitochondria, see page 303: Two-channel assay for detecting mitochondria. The Mitochondria analysis uses two stains to detect and measure mitochondria in cells. One stain typically detects the nuclei and the other detects the mitochondria.

 Mitotic Index, see page 305: Two-channel assay using a nuclei marker and a second marker to identify mitotic cells. Use the Mitotic Index analysis to differentiate between mitotic cells and interphase cells in the normal cell cycle and quantify the various data extracted during image analysis.

 Neurite Tracing, see page 307: Single-channel assay for measuring neurite outgrowth. Use the Neurite Tracing analysis to measure multiple biologies. Neurites are any extension off a cell body. For example, cilia, blood vessels, nanotubes, and so on.

 Phagocytosis, see page 310: Two-channel assay for detecting phagocytic vacuoles. The Phagocytosis analysis detects and measures phagosomes or the particles ingested or engulfed during the phagocytosis process in cells.

 Pits and Vesicles, see page 312: Two-channel assay for detecting GPCR pits and vesicles. Use the Pits and Vesicles analysis to detect the internalization of a fluorescent marker to small, coated pits and/or larger internalized vesicles.

 Protein Expression Index, see page 315: Two-channel assay using both a nuclei marker and one to measure protein expression. Use the Protein Expression Index analysis to measure the expression level of a protein of interest though differences in intensity levels.

 Translocation, see page 318: Two-channel assay for the quantification of cellular signaling events and intracellular trafficking. Use the Translocation analysis to measure intensity movement from one compartment to another (for instance, the nucleus to the cytoplasm).

 Viral Infectivity, see page 324: Two-channel assay using both a nuclei marker and a marker to detect cells infected with a virus. Use the Viral Infectivity analysis to detect and measure cells infected with viruses. It can quantify the number of cells in a field of view infected as well as the level of infection through differences of intensities using a marker for the virus.

Transmitted Light Analyses

The software includes transmitted light analyses for Cell Count and Cell Scoring.

Use the Transmitted Light Cell Count analyses to segment brightfield images in order to identify and differentiate cells. The segmentation labels each isolated and identified cell to let you see a visual separation between cells that are close or touching.


Transmitted Light Cell Count, see page 321: Single-channel, transmitted light assay to find beads.


Transmitted Light Cell Count, see page 321: Single-channel, transmitted light assay to find a range of cells.


Transmitted Light Cell Count, see page 321: Single-channel, transmitted light assay to find larger cells.


Transmitted Light Cell Count, see page 321: Single-channel, transmitted light assay to find smaller cells.

Use the Transmitted Light Cell Scoring analyses to identify two subpopulations of cells based on a brightfield image for all cells and a fluorescent marker for cells of interest. The fluorescent marker can label nuclei, cytoplasm, or both.


Transmitted Light Cell Scoring, see page 322: Two-channel, transmitted light assay to find beads, then scoring for an additional fluorescence channel.

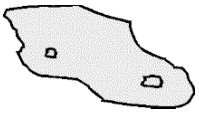
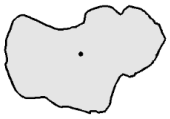

Transmitted Light Cell Scoring, see page 322: Two-channel, transmitted light assay to find a range of cells, then scoring for an additional fluorescence channel.


Transmitted Light Cell Scoring, see page 322: Two-channel, transmitted light assay to find larger cells, such as HeLa cells, then scoring for an additional fluorescence channel.


Transmitted Light Cell Scoring, see page 322: Two-channel, transmitted light assay to find smaller cells, such as CHO cells, then scoring for an additional fluorescence channel.

See the referenced page for details on an analysis.

Analysis Measurement Definitions

Measurement	Definition	Example
Total Area	The area of the entire object or feature.	
Width	The horizontal dimension of the object or feature.	
Height	The vertical dimension of the object or feature.	
Centroid X and Centroid Y	The X and Y coordinates of the centroid of the object or feature in calibrated units, where the centroid is the center of mass of the object or feature. Note that, depending on the shape of the object (for example, if the object is concave), the centroid might not be inside the object.	
Average Intensity	The average of all the intensity values for the pixels in the object or feature.	N/A
Integrated Intensity	The sum of all the intensity values for the pixels in the object or feature.	N/A
Intensity Standard Deviation	The standard deviation around the mean of the average intensity value in the object or feature.	N/A
Maximum Intensity	The maximum pixel intensity value in the object or feature.	N/A
Minimum Intensity	The minimum pixel intensity value in the object or feature.	N/A

Angiogenesis Skeletonization

Use the Angiogenesis Skeletonization analysis to identify and measure tubes (long thin objects) and nodes (connecting points between tubes) in a single wavelength. In addition to tube formation assays, you can also measure neurite outgrowth in assays where the cell bodies are indistinct or outside the field of view.

Algorithm Input Parameters

Cell tab

Channel: Select the fluorescent channel used for the tubes and nodes.

Cell

- **Intensity**: The minimum intensity above local background that is used for finding the tubes and nodes. This value is a minimum and should be set slightly lower than the difference in intensity between the dimmest part of the tube (typically the thinnest part) and its local background.
- **Min Width**: The minimum value for the smallest tubes that you want to detect. The width refers to the distance across in µm. Tubes smaller than the minimum width will be ignored.
- **Max Width**: The maximum value for the largest tubes that you want to detect. The width refers to the short axis of a tube in µm. Tubes larger than the maximum width will be identified as nodes.

Summary Measurements

Measurement	Description
# Cells	Number of cells
Total Tubule Length	Total length of the tubes in µm.
Total Tubule Area	Total tube area (excluding nodes) in μm^2.
Segments	Number of tube segments connecting branch points and/or ends.
Branch Points	Number of junctions connecting segments (excluding nodes, which are not considered branches).
Nodes	Number of connected blobs with a thickness exceeding the maximum width, excluded from length and area measures.
Total Node Area	Total node area in μm^2.
Connected Sets	Number of distinct objects detected in the image not connected to one another (no path of connected pixels of tubes or nodes connects the objects). Measures the overall connectivity of the growth network (a completely connected network would have just one connected set of pixels).
Mean Tubule Length	Total tube length divided by the number of segments.
Mean Tubule Area	Total tube area divided by the number of segments.
Tube Length Per Set	Total tube length in microns divided by the number of connected sets.
Mean Node Area	Total node area in μm^2 divided by the number of nodes.

Cell Measurements

Cell measurements are not used with this analysis.

Apoptosis


Use the Apoptosis analysis to identify and measure cells entering programmed cell death using an apoptotic stain.

Note:

- The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.
- You may want to use a particular fluorescent channel as more than one marker. For example, you might use a DAPI channel set to a higher cutoff to indicate cells about to undergo mitosis.

Algorithm Input Parameters

Nuclei tab

Channel: Select the fluorescent channel used to image nuclei.

Nuclei

- **Intensity**: The minimum intensity above local background that is used for finding the nuclei.
- **Min Width**: The minimum value for the smallest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei smaller than the minimum width will be ignored.
- **Max Width**: The maximum value for the largest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei larger than the maximum width will be split.

Marker tab

Channel: Select the fluorescent channel for the apoptosis marker.

Marker

- **Intensity**: The minimum intensity above local background that is used for detecting the apoptosis marker. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: If the apoptosis marker stains the whole cell or only the cytoplasm, the minimum value for the smallest cell that you want to detect. If the apoptosis marker stains only the nucleus, use the same value from the Nuclei tab.
- **Max Width**: If the apoptosis marker stains the whole cell or only the cytoplasm, the maximum value for the largest cell that you want to detect. If the apoptosis marker stains only the nucleus, use the same value from the Nuclei tab.

Summary Measurements

Measurement	Description
# Cells	Number of cells identified by the nuclear stain.
# Positive Cells	Number of apoptic cells.
% Positive Cells	Percentage of apoptic cells to the number of cells.
# Negative Cells	Number of non-apoptic cells.
% Negative Cells	Percentage of non-apoptic cells to the number of cells.
Positive Cell Average Intensities	Average pixel intensity of cells positive for the marker over the positive cell area.
All Cell Avg Intensities	Average pixel intensity of the marker over all the cell areas.
Average Nuclear Area	Sum of nuclear area for all nuclei divided by the number of cells.
Average Nuclear Integrated Intensity	Sum of the integrated nuclear intensity values in all nuclei divided by the number of cells.
Average Nuclear Intensity	Average of apoptic cells average intensity values in the nuclear area.

Cell Measurements

Measurement	Description
Positive	Returns 1 if cell is scored as positive for the marker; otherwise returns 0.
Cell Area	Area of the cell (white segmentation mask).
Nuclear Area	Area of the nucleus (green segmentation mask).
Wavelength 1 Integrated Nuclear Intensity	Total pixel intensity of the nuclear stain over the nuclear area in an individual cell.
Wavelength 1 Average Nuclear Intensity	Average pixel intensity of the nuclear stain over all the nuclear areas in an individual cell (intensity per pixel).
Wavelength 2 Integrated Nuclear Intensity	Total pixel intensity of the marker stain in the nucleus of an individual cell.
Wavelength 2 Average Nuclear Intensity	Average pixel intensity of the marker stain in the nucleus of an individual cell.
Wavelength 2 Integrated Cell Intensity	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Wavelength 2 Average Cell Intensity	Average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Positive Cells Average Intensity	The average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Positive Cells Integrated Intensity	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Autophagy

 Use the Autophagy analysis to detect the infection of viral particles into cells.

Note: The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.

Algorithm Input Parameters

Granule tab

Channel: Select the fluorescent channel used to image granules.

Granule

- **Intensity above background**: The minimum intensity above local background that is used for finding the granules.
- **Min Width**: The minimum value for the smallest granule you want to detect. The width refers to the short axis of a granule in µm. Granules smaller than the minimum width are ignored.
- **Max Width**: The maximum value for the largest granule you want to detect. The width refers to the short axis of a granule in µm. Granules larger than the maximum width are split.

Nuclear tab

Channel: Select the fluorescent channel for nuclei.

Nuclear

- **Intensity above background**: The minimum intensity above local background that is used for detecting nuclei. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: The minimum value for the smallest nuclei you want to detect.
- **Max Width**: The maximum value for the largest nuclei you want to detect.

Summary Measurements

Measurement	Description
Total Object Count	Number of objects.
Total Granule Count	Number of granules.
Avg Granule Count	Average number of granules per object.
Total Granule Area	Total area in μm^2 of granules.
Avg Granule Intensity	Average pixel intensity calculated over all granules in the image.
Avg Granule Integrated Intensity	Average of the integrated pixel intensity values for all granules in the image.

Cell Measurements

Measurement	Description
Granule Count	Number of granules detected for a specific cell. (Note that a granule is assigned to its nearest nucleus).
Granule Total Area	Total area in μm^2 covered by all the granules assigned to a specific cell.
Granule Integrated Intensity	Total pixel intensity of the granules assigned to a specific cell.
Granule Intensity	Average pixel intensity of the granules assigned to a specific cell.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Cell Count

Use the Cell Count analysis to segment images in order to identify and differentiate cell nuclei. The segmentation labels each isolated and identified cell to let you see a visual separation between cells that are close or touching.

Any nuclei-like shape in the image with a width that is less than the specified minimum width is considered to be noise and is excluded from the segmentation. The value that you specify for the maximum width can be used to exclude any blobs larger than the specified size and to control the locality of background intensity estimates near each nucleus.

Note: The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.

Algorithm Input Parameters

Nuclei tab

Channel: Select the fluorescent channel used to image nuclei.

Nuclei

- **Intensity**: The minimum intensity above local background that is used for finding the nuclei.
- **Min Width**: The minimum value for the smallest nuclei you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei smaller than the minimum width will be ignored.
- **Max Width**: The maximum value for the largest nuclei you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei larger than the maximum width will be split.

Summary Measurements

Measurement	Description
Cell Count	Total number of cells.
Cell Total Intensity	Sum of Cells Average Intensity
Cell Average Intensity	Cell Total Intensity / Cell Count
Cell Total Integrated Intensity	Sum of Cells Integrated Intensity
Cell Average Integrated Intensity	Cell Total Integrated Intensity / Cell Count
Total Area	Sum of Cells Area
Average Area	Total Area / Cell Count

Cell Measurements

Measurement	Description
Area	Area in μm^2 of the cell (white segmentation mask).
Integrated Intensity	The total pixel intensity of the nuclei stain of an individual cell.
Average Intensity	The average pixel intensity of the nuclear stain.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Cell Differentiation

Use the Cell Differentiation analysis to identify two subpopulations of cells based on a fluorescent marker for all nuclei (for example, DAPI, Hoechst, or DRAQ5) and an additional marker for differentiated cells of interest. The additional marker can label cytoplasm or entire cell.

The output of the analysis includes the number of cells scored positive as detected by the marker.

In the segmentation mask, all identified cells are indicated.

- Cells scored positive for the marker are shown in light and dark green.
- Cells scored negative for the marker are shown in red.

Note:

- The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.
- You may want to use a particular fluorescent channel as more than one marker. For example, you might use a DAPI channel set to a higher cutoff to indicate cells about to undergo mitosis.

Algorithm Input Parameters

Nuclei tab

Channel: Select the fluorescent channel used to image nuclei.

Nuclei

- **Intensity**: The minimum intensity above local background that is used for finding the nuclei.
- **Min Width**: The minimum value for the smallest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei smaller than the minimum width will be ignored.
- **Max Width**: The maximum value for the largest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei larger than the maximum width will be split.

Marker tab

Channel: Select the fluorescent channel for the marker.

Marker

- **Intensity**: The minimum intensity above local background that is used for detecting the marker. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: If the marker stains the whole cell or only the cytoplasm, the minimum value for the smallest cell that you want to detect. If the marker stains only the nucleus, use the same value from the Nuclei tab.
- **Max Width**: If the marker stains the whole cell or only the cytoplasm, the maximum value for the largest cell that you want to detect. If the marker stains only the nucleus, use the same value from the Nuclei tab.

Summary Measurements

Measurement	Description
# Cells	Number of cells identified by the nuclear stain.
# Positive Cells	Number of cells positive for the marker.
% Positive Cells	Percentage of cells positive for the marker to the number of cells.
# Positive Cells	Number of cells negative for the marker.
% Positive Cells	Percentage of cells negative for the marker to the number of cells.
Positive Cell Average Intensities	Average pixel intensity of cells positive for the marker over the positive cell area.
All Cell Average Intensities	Average pixel intensity of the marker over all the cell areas.
Positive Cell Integrated Intensities	Total pixel intensity of the marker stain over the cell area in all cells positive for the marker
Positive Cell Total Integrated Intensity	Sum of Positive Cells Integrated Intensity
Positive Cell Total Intensity	Sum of Positive Cells Average Intensity
Positive Cell Total Area	Sum of Positive Cells Area
Positive Cell Average Area	Sum of Positive Cells Area / # Positive Cells (or 0 if there are no positive cells).

Cell Measurements

Measurement	Description
Positive	Returns 1 if cell is scored as positive for the marker; otherwise returns 0.
Cell Area	Area of the cell (white segmentation mask).
Nuclear Area	Area of the nucleus (green segmentation mask).
Wavelength 1 Integrated Nuclear Intensity	Total pixel intensity of the nuclear stain over the nuclear area in an individual cell.
Wavelength 1 Average Nuclear Intensity	Average pixel intensity of the nuclear stain over all the nuclear areas in an individual cell (intensity per pixel).
Wavelength 2 Integrated Nuclear Intensity	Total pixel intensity of the marker stain in the nucleus of an individual cell.
Wavelength 2 Average Nuclear Intensity	Average pixel intensity of the marker stain in the nucleus of an individual cell.
Wavelength 2 Integrated Cell Intensity	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Wavelength 2 Average Cell Intensity	Average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Positive Cells Area	Area of the cell if scored as positive.
Positive Cells Average Intensity	The average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Positive Cells Integrated Intensity	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Cell Scoring

Use the Cell Scoring analysis to identify two subpopulations of cells based on a fluorescent marker for all nuclei (for example, DAPI, Hoechst, or DRAQ5) and an additional marker (Marker 1) for cells of interest. The additional marker can label nuclei, cytoplasm or both.

The output of the analysis includes the number of cells scored positive as detected by Marker 1.

In the segmentation mask, all identified cells are indicated.

- Cells scored positive for Marker 1 are shown in green.
- Cells scored negative for Marker 1 are shown in red.

Note:

- The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.
- You may want to use a particular fluorescent channel as more than one marker. For example, you might use a DAPI channel set to a higher cutoff to indicate cells about to undergo mitosis.

Algorithm Input Parameters

Nuclei tab

Channel: Select the fluorescent channel used to image nuclei.

Segmentation Parameters

- **Intensity**: The minimum intensity above local background that is used for finding the nuclei.
- **Min Width**: The minimum value for the smallest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei smaller than the minimum width will be ignored.
- **Max Width**: The maximum value for the largest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei larger than the maximum width will be split.

Marker 1 tab

Channel: Select the fluorescent channel for Marker 1.

Segmentation Parameters

- **Intensity**: The minimum intensity above local background that is used for detecting Marker 1. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: If Marker 1 stains the whole cell or only the cytoplasm, the minimum value for the smallest cell that you want to detect. If Marker 1 stains only the nucleus, use the same value from the Nuclei tab.
- **Max Width**: If Marker 1 stains the whole cell or only the cytoplasm, the maximum value for the largest cell that you want to detect. If Marker 1 stains only the nucleus, use the same value from the Nuclei tab.

Scoring Parameters

- **Stained Area**: Select the area (Nucleus, Cytoplasm, or Both) that must be stained with Marker 1 for the cell to be scored as positive.

Summary Measurements

Measurement	Description
# Cells	Number of cells identified by the nuclear stain.
# Positive Cells	Number of cells positive for Marker 1.
% Positive Cells	Percentage of cells positive for Marker 1 to the number of cells.
# Negative Cells	Number of cells negative for Marker 1.
% Negative Cells	Percentage of cells negative for Marker 1 to the number of cells.
Positive Cell Average Intensities	Average pixel intensity of cells positive for Marker 1 over the positive cell area.
All Cell Average Intensities	Average pixel intensity of Marker 1 over all the cell areas.
Positive Cell Integrated Intensities	Total pixel intensity of the marker stain over the cell area in all cells positive for Marker 1.
Positive Cell Total Integrated Intensity	Sum of Positive Cells Integrated Intensity
Positive Cell Total Intensity	Sum of Positive Cells Average Intensity
Positive Cell Total Area	Sum of Positive Cells Area
Positive Cell Average Area	Sum of Positive Cells Area / # Positive Cells (or 0 if there are no positive cells).

Cell Measurements

Measurement	Description
Positive	Returns 1 if cell is scored as positive for Marker 1; otherwise returns 0.
Cell Area	Area of the cell (white segmentation mask).
Nuclear Area	Area of the nucleus (green segmentation mask).
Wavelength 1 Integrated Nuclear Intensity	Total pixel intensity of the nuclear stain over the nuclear area in an individual cell.
Wavelength 1 Average Nuclear Intensity	Average pixel intensity of the nuclear stain over all the nuclear areas in an individual cell (intensity per pixel).
Wavelength 2 Integrated Nuclear Intensity	Total pixel intensity of Marker 1 stain in the nucleus of an individual cell.
Wavelength 2 Average Nuclear Intensity	Average pixel intensity of Marker 1 stain in the nucleus of an individual cell.
Wavelength 2 Integrated Cell Intensity	Total pixel intensity of Marker 1 stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Wavelength 2 Average Cell Intensity	Average pixel intensity of Marker 1 stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Positive Cells Area	Area of the cell if scored as positive.
Positive Cells Average Intensity	The average pixel intensity of Marker 1 stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Positive Cells Integrated Intensity	Total pixel intensity of Marker 1 stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Cell Scoring: 3 Channels

Use the Cell Scoring: 3 Channels analysis to identify subpopulations of cells based on a fluorescent marker for all nuclei (for example, DAPI, Hoechst, or DRAQ5) and two additional markers (Marker 1 and Marker 2) for cells of interest. The additional markers can label nuclei, cytoplasm or both. You can use a fluorescent channel as more than one marker.

In the segmentation mask, all identified cells are indicated as follows:

- Cells scored positive for Marker 1 are shown in red.
- Cells scored positive for Marker 2 are shown in blue.
- Cells scored positive for Marker 1 and Marker 2 are shown in magenta.
- Cell scored negative for both markers are shown in dark gray.

Note:

- The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.
- You may want to use a particular fluorescent channel as more than one marker. For example, you might use a DAPI channel set to a higher cutoff to indicate cells about to undergo mitosis.

Algorithm Input Parameters

Nuclei tab

Channel: Select the fluorescent channel used to image nuclei.

Segmentation Parameters

- **Intensity**: The minimum intensity above local background that is used for finding the nuclei.
- **Min Width**: The minimum value for the smallest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei smaller than the minimum width will be ignored.
- **Max Width**: The maximum value for the largest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei larger than the maximum width will be split.

Marker 1 tab

Channel: Select the fluorescent channel for Marker 1.

Segmentation Parameters

- **Intensity**: The minimum intensity above local background that is used for detecting Marker 1. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: If Marker 1 stains the whole cell or only the cytoplasm, the minimum value for the smallest cell that you want to detect. If Marker 1 stains only the nucleus, use the same value from the Nuclei tab.
- **Max Width**: If Marker 1 stains the whole cell or only the cytoplasm, the maximum value for the largest cell that you want to detect. If Marker 1 stains only the nucleus, use the same value from the Nuclei tab.

Scoring Parameters

- **Stained Area**: Select the area (Nucleus, Cytoplasm, or Both) that must be stained with Marker 1 for the cell to be scored as positive.
- **Min Stained Area**: The minimum area in μm^2 that must be stained with Marker 1 for the cell to be scored as positive. This helps eliminate false positives such as cells with a small but bright speck.

Marker 2 tab

Channel: Select the fluorescent channel for the second marker, Marker 2.

Segmentation Parameters

- **Intensity**: Enter the minimum intensity above local background that is used for detecting Marker 2. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: If Marker 2 stains the whole cell or only the cytoplasm, enter the minimum value for the smallest cell that you want to detect. If Marker 2 stains only the nucleus, use the same value from the Nuclei tab.
- **Max Width**: If Marker 2 stains the whole cell or only the cytoplasm, enter the maximum value for the largest cell that you want to detect. If Marker 2 stains only the nucleus, use the same value from the Nuclei tab.

Scoring Parameters

- **Stained Area**: Select the area (Nucleus, Cytoplasm, or Both) that must be stained with Marker 2 for the cell to be scored as positive.
- **Min Stained Area**: Set the minimum area in μm^2 that must be stained with Marker 2 for the cell to be scored as positive. This helps eliminate false positives such as cells with a small but bright speck.

Summary Measurements

Measurement	Description
# Cells	Number of cells identified by the nuclei stain.
# Wavelength 2&3 Negative Cells	Number of cells negative for Marker 1 and Marker 2.
# Wavelength 2 Positive Cells	Number of cells positive for Marker 1.
# Wavelength 3 Positive Cells	Number of cells positive for Marker 2.
# Wavelength 2&3 Positive Cells	Number of cells positive for Marker 1 and Marker 2.
% Wavelength 2&3 Negative Cells	Percentage of cells negative for Marker 1 and Marker 2 to the number of cells.
% Wavelength 2 Positive Cells	Percentage of cells positive for Marker 1 to the number of cells.
% Wavelength 3 Positive Cells	Percentage of cells positive for Marker 2 to the number of cells.

Measurement	Description
% Wavelength 2&3 Positive Cells	Percentage of cells positive for Marker 1 and Marker 2 to the number of cells.
Wavelength 2&3 Negative Average Cell Area	Average area of cells negative for Marker 1 and Marker 2 per number of cells negative for Marker 1 and Marker 2.
Wavelength 2&3 Negative Total Cell Area	Total area of cells negative for Marker 1 and Marker 2.
Wavelength 2 Positive Average Cell Area	Average area of cells positive for Marker 1 per number of cells positive for Marker 1.
Wavelength 2 Positive Total Cell Area	Total area of cells positive for Marker 1.
Wavelength 3 Positive Average Cell Area	Average area of cells positive for Marker 2 per number of cells positive for Marker 2.
Wavelength 3 Positive Total Cell Area	Total area of cells positive for Marker 2.
Wavelength 2&3 Positive Average Cell Area	Average area of cells positive for Marker 1 and Marker 2 per number of cells positive for Marker 1 and Marker 2.
Wavelength 2&3 Positive Total Cell Area	Total area of cells positive for Marker 1 and Marker 2.
Wavelength 2 Average Integrated Cell Intensities	Integrated Intensities in cell area (cytoplasm area + nucleus area) of cells positive for Marker 1 divided by the number of cells positive for Marker 1.
Wavelength 2 Average Integrated Cytoplasm Intensities	Integrated intensities of cells positive for Marker 1 in cytoplasm area divided by the number of cells positive for Marker 1.
Wavelength 2 Average Integrated Nuclear Intensities	Integrated intensities of cells positive for Marker 1 in nucleus area divided by the number of cells positive for Marker 1.
Wavelength 2 Total Integrated Cell Intensity	Total integrated intensity of cells positive for Marker 1.
Wavelength 2 Average Cell Intensities	Average of all cells positive for Marker 1 average intensity values in the cell area (cytoplasm + nucleus area).
Wavelength 2 Average Nuclear Intensities	Average of cells positive for Marker 1 average intensity values in the nuclear area.
Wavelength 2 Average Cytoplasm Intensities	Average of cells positive for Marker 1 average intensity values in the cytoplasm area.
Wavelength 3 Average Integrated Cell Intensities	Integrated Intensities in cell area (cytoplasm area + nucleus area) of cells positive for Marker 2 divided by the number of cells positive for Marker 2.

Measurement	Description
Wavelength 3 Average Integrated Cytoplasm Intensities	Integrated intensities of cells positive for Marker 2 in cytoplasm area divided by the number of cells positive for Marker 2.
Wavelength 3 Average Integrated Nuclear Intensities	Integrated intensities of cells positive for Marker 2 in nucleus area divided by the number of cells positive for Marker 2.
Wavelength 3 Total Integrated Cell Intensity	Total integrated intensity of cells positive for Marker 2.
Wavelength 3 Average Cell Intensities	Average of all cells positive for Marker 2 average intensity values in the cell area (cytoplasm + nucleus area).
Wavelength 3 Average Nuclear Intensities	Average of cells positive for Marker 2 average intensity values in the nuclear area.
Wavelength 3 Average Cytoplasm Intensities	Average of cells positive for Marker 2 average intensity values in the cytoplasm area.

Cell Measurements

Measurement	Description
Cell Area	The total area in μm^2 of the cell's stained area (nucleus, cytoplasm, or both) as determined by the combination of all wavelengths, particularly the wavelengths that use of cytoplasm or both nucleus and cytoplasm.
Nuclear Area	The total area in μm^2 of the nucleus.
Average Nuclear Intensity	Average pixel intensity of the nuclear stain in the nuclear area.
Wavelength 2&3 Negative	Returns 1 if cell is scored as negative for Marker 1 and Marker 2; otherwise returns 0.
Wavelength 2 Positive	Returns 1 if cell is scored as positive for Marker 1; otherwise returns 0.
Wavelength 3 Positive	Returns 1 if cell is scored as positive for Marker 2; otherwise returns 0.
Wavelength 2&3 Positive	Returns 1 if cell is scored as positive for Marker 1 and Marker 2; otherwise 0.
Wavelength 2 Average Cell Intensity	Average pixel intensity for Marker 1 in cell area (cytoplasm area + nucleus area).
Wavelength 2 Integrated Cell Intensity	Total pixel intensities for Marker 1 in cell area (cytoplasm area + nucleus area).
Wavelength 2 Average Nuclear Intensity	Average pixel intensity for Marker 1 in nuclear area.
Wavelength 2 Integrated Nuclear Intensity	Total of pixel intensities for Marker 1 in nuclear area.
Wavelength 2 Average Cytoplasm Intensity	Average pixel intensity for Marker 1 in cytoplasm area.

Measurement	Description
Wavelength 2 Integrated Cytoplasm Intensity	Total of pixel intensities for Marker 1 in cytoplasm area.
Wavelength 3 Average Cell Intensity	Average pixel intensity for Marker 2 in cell area (cytoplasm area + nucleus area).
Wavelength 3 Integrated Cell Intensity	Total pixel intensities for Marker 2 in cell area (cytoplasm area + nucleus area).
Wavelength 3 Average Nuclear Intensity	Average pixel intensity for Marker 2 in nuclear area.
Wavelength 3 Integrated Nuclear Intensity	Total of pixel intensities for Marker 2 in nuclear area.
Wavelength 3 Average Cytoplasm Intensity	Average pixel intensity for Marker 2 in cytoplasm area.
Wavelength 3 Integrated Cytoplasm Intensity	Total of pixel intensities for Marker 2 in cytoplasm area.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Cell Scoring: 4 Channels

Use the Cell Scoring: 4 Channels analysis to identify subpopulations of cells based on a fluorescent marker for all nuclei (for example, DAPI, Hoechst, or DRAQ5) and three additional markers (Marker 1, Marker 2, and Marker 3) for cells of interest. The additional markers can label nuclei, cytoplasm or both. You can use a fluorescent channel as more than one marker.

Note:

- The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.
- You may want to use a particular fluorescent channel as more than one marker. For example, you might use a DAPI channel set to a higher cutoff to indicate cells about to undergo mitosis.

In the segmentation mask, all identified cells are indicated as follows:

- Cells scored positive for Marker 1 are shown in red.
- Cells scored positive for Marker 2 are shown in blue.
- Cells scored positive for Marker 3 are shown in yellow.
- Cells scored positive for Marker 1 and Marker 2 are shown in magenta.
- Cells scored positive for Marker 1 and Marker 3 are shown in orange.
- Cells scored positive for Marker 2 and Marker 3 are shown in dark green.
- Cells scored positive for all markers are shown in brown.
- Cells scored negative for all markers are shown in dark gray.

Algorithm Input Parameters

Nuclei tab

Channel: Select the fluorescent channel used to image nuclei.

Segmentation Parameters

- **Intensity**: The minimum intensity above local background that is used for finding the nuclei.
- **Min Width**: The minimum value for the smallest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei smaller than the minimum width will be ignored.
- **Max Width**: The maximum value for the largest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei larger than the maximum width will be split.

Marker 1 tab

Channel: Select the fluorescent channel for Marker 1.

Segmentation Parameters

- **Intensity**: The minimum intensity above local background that is used for detecting Marker 1. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: If Marker 1 stains the whole cell or only the cytoplasm, the minimum value for the smallest cell that you want to detect. If Marker 1 stains only the nucleus, use the same value from the Nuclei tab.

- **Max Width**: If Marker 1 stains the whole cell or only the cytoplasm, the maximum value for the largest cell that you want to detect. If Marker 1 stains only the nucleus, use the same value from the Nuclei tab.

Scoring Parameters

- **Stained Area**: Select the area (Nucleus, Cytoplasm, or Both) that must be stained with Marker 1 for the cell to be scored as positive.
- **Min Stained Area**: The minimum area in μm^2 that must be stained with Marker 1 for the cell to be scored as positive. This helps eliminate false positives such as cells with a small but bright speck.

Marker 2 tab

Channel: Select the fluorescent channel for the second marker, Marker 2.

Segmentation Parameters

- **Intensity**: Enter the minimum intensity above local background that is used for detecting Marker 2. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: If Marker 2 stains the whole cell or only the cytoplasm, enter the minimum value for the smallest cell that you want to detect. If Marker 2 stains only the nucleus, use the same value from the Nuclei tab.
- **Max Width**: If Marker 2 stains the whole cell or only the cytoplasm, enter the maximum value for the largest cell that you want to detect. If Marker 2 stains only the nucleus, use the same value from the Nuclei tab.

Scoring Parameters

- **Stained Area**: Select the area (Nucleus, Cytoplasm, or Both) that must be stained with Marker 2 for the cell to be scored as positive.
- **Min Stained Area**: Set the minimum area in μm^2 that must be stained with Marker 2 for the cell to be scored as positive. This helps eliminate false positives such as cells with a small but bright speck.

Marker 3 tab

Channel: Select the fluorescent channel for the second marker, Marker 3.

Segmentation Parameters

- **Intensity**: Enter the minimum intensity above local background that is used for detecting Marker 3. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: If Marker 3 stains the whole cell or only the cytoplasm, enter the minimum value for the smallest cell that you want to detect. If maker 3 stains only the nucleus, use the same value from the Nuclei tab.
- **Max Width**: If Marker 3 stains the whole cell or only the cytoplasm, enter the maximum value for the largest cell that you want to detect. If maker 3 stains only the nucleus, use the same value from the Nuclei tab.

Scoring Parameters

- **Stained Area**: Select the area (Nucleus, Cytoplasm, or Both) that must be stained with Marker 3 for the cell to be scored as positive.
- **Min Stained Area**: Set the minimum area in μm^2 that must be stained with Marker 3 for the cell to be scored as positive. This helps eliminate false positives such as cells with a small but bright speck.

Summary Measurements

Measurement	Description
# Cells	Number of cells identified by the nuclei stain.
# Wavelength 2&3&4 Negative Cells	Number of cells negative for Marker 1, Marker 2, and Marker 3.
# Wavelength 2 Positive Cells	Number of cells positive for Marker 1.
# Wavelength 3 Positive Cells	Number of cells positive for Marker 2.
# Wavelength 4 Positive Cells	Number of cells positive for Marker 3.
# Wavelength 2&3 Positive Cells	Number of cells positive for Marker 1 and Marker 2.
# Wavelength 2&4 Positive Cells	Number of cells positive for Marker 1 and Marker 3.
# Wavelength 3&4 Positive Cells	Number of cells positive for Marker 2 and Marker 3.
# Wavelength 2&3&4 Positive Cells	Number of cells positive for Marker 1, Marker 2, and Marker 3.
% Wavelength 2&3&4 Positive Cells	Percentage of cells positive for Marker 1, Marker 2, and Marker 3 to the number of cells.
% Wavelength 2 Positive Cells	Percentage of cells positive for Marker 1 to the number of cells.
% Wavelength 3 Positive Cells	Percentage of cells positive for Marker 2 to the number of cells.
% Wavelength 4 Positive Cells	Percentage of cells positive for Marker 3 to the number of cells.
% Wavelength 2&3 Positive Cells	Percentage of cells positive for Marker 1 and Marker 2 to the number of cells.
% Wavelength 2&4 Positive Cells	Percentage of cells positive for Marker 1 and Marker 3 to the number of cells.
% Wavelength 3&4 Positive Cells	Percentage of cells positive for Marker 2 and Marker 3 to the number of cells.
% Wavelength 2&3&4 Positive Cells	Percentage of cells positive for Marker 1 and Marker 2 to the number of cells.
Wavelength 2&3&4 Negative Average Cell Area	Average area of cells negative for Marker 1, Marker 2, and Marker 3 per number of cells negative for Marker 1, Marker 2, Marker 3.

Measurement	Description
Wavelength 2 Positive Average Cell Area	Average area of cells positive for Marker 1 per number of cells positive for Marker 1.
Wavelength 3 Positive Average Cell Area	Average area of cells positive for Marker 2 per number of cells positive for Marker 2.
Wavelength 4 Positive Average Cell Area	Average area of cells positive for Marker 3 per number of cells positive for Marker 3.
Wavelength 2&3 Positive Average Cell Area	Average area of cells positive for Marker 1 and Marker 2 per number of cells positive for Marker 1 and Marker 2.
Wavelength 2&4 Positive Average Cell Area	Average area of cells positive for Marker 1 and Marker 3 per number of cells positive for Marker 1 and Marker 3.
Wavelength 3&4 Positive Average Cell Area	Average area of cells positive for Marker 2 and Marker 3 per number of cells positive for Marker 2 and Marker 3.
Wavelength 2&3&4 Positive Average Cell Area	Average area of cells positive for Marker 1, Marker 2, and Marker 3 per number of cells positive for Marker 1, Marker 2, and Marker 3.
Wavelength 2&3&4 Negative Total Cell Area	Total area of cells negative for Marker 1, Marker 2, and Marker 3.
Wavelength 2 Positive Total Cell Area	Total area of cells positive for Marker 1.
Wavelength 3 Positive Total Cell Area	Total area of cells positive for Marker 2.
Wavelength 4 Positive Total Cell Area	Total area of cells positive for Marker 3.
Wavelength 2&3 Positive Total Cell Area	Total area of cells positive for Marker 1 and Marker 2.
Wavelength 2&4 Positive Total Cell Area	Total area of cells positive for Marker 1 and Marker 3.
Wavelength 3&4 Positive Total Cell Area	Total area of cells positive for Marker 2 and Marker 3.
Wavelength 2&3&4 Positive Average Cell Area	Average area of cells positive for Marker 1, Marker 2, and Marker 3 per number of cells positive for Marker 1, Marker 2, and Marker 3.
Wavelength 2 Average Integrated Cell Intensities	Integrated Intensities in cell area (cytoplasm area + nucleus area) of cells positive for Marker 1 divided by the number of cells positive for Marker 1.
Wavelength 2 Average Integrated Cytoplasm Intensities	Integrated intensities of cells positive for Marker 1 in cytoplasm area divided by the number of cells positive for Marker 1.

Measurement	Description
Wavelength 2 Average Integrated Nuclear Intensities	Integrated intensities of cells positive for Marker 1 in nucleus area divided by the number of cells positive for Marker 1.
Wavelength 2 Total Integrated Cell Intensity	Total integrated intensity of cells positive for Marker 1.
Wavelength 2 Average Cell Intensities	Average of all cells positive for Marker 1 average intensity values in the cell area (cytoplasm + nucleus area).
Wavelength 2 Average Nuclear Intensities	Average of cells positive for Marker 1 average intensity values in the nuclear area.
Wavelength 2 Average Cytoplasm Intensities	Average of cells positive for Marker 1 average intensity values in the cytoplasm area.
Wavelength 3 Average Integrated Cell Intensities	Integrated Intensities in cell area (cytoplasm area + nucleus area) of cells positive for Marker 2 divided by the number of cells positive for Marker 2.
Wavelength 3 Average Integrated Cytoplasm Intensities	Integrated intensities of cells positive for Marker 2 in cytoplasm area divided by the number of cells positive for Marker 2.
Wavelength 3 Average Integrated Nuclear Intensities	Integrated intensities of cells positive for Marker 2 in nucleus area divided by the number of cells positive for Marker 2.
Wavelength 3 Total Integrated Cell Intensity	Total integrated intensity of cells positive for Marker 2.
Wavelength 3 Average Cell Intensities	Average of all cells positive for Marker 2 average intensity values in the cell area (cytoplasm + nucleus area).
Wavelength 3 Average Nuclear Intensities	Average of cells positive for Marker 2 average intensity values in the nuclear area.
Wavelength 3 Average Cytoplasm Intensities	Average of cells positive for Marker 2 average intensity values in the cytoplasm area.
Wavelength 4 Average Integrated Cell Intensities	Integrated Intensities in cell area (cytoplasm area + nucleus area) of cells positive for Marker 3 divided by the number of cells positive for Marker 3.
Wavelength 4 Average Integrated Cytoplasm Intensities	Integrated intensities of cells positive for Marker 3 in cytoplasm area divided by the number of cells positive for Marker 3.
Wavelength 4 Average Integrated Nuclear Intensities	Integrated intensities of cells positive for Marker 3 in nucleus area divided by the number of cells positive for Marker 3.
Wavelength 4 Total Integrated Cell Intensity	Total integrated intensity of cells positive for Marker 3.

Measurement	Description
Wavelength 4 Average Cell Intensities	Average of all cells positive for Marker 3 average intensity values in the cell area (cytoplasm + nucleus area).
Wavelength 4 Average Nuclear Intensities	Average of cells positive for Marker 3 average intensity values in the nuclear area.
Wavelength 4 Average Cytoplasm Intensities	Average of cells positive for Marker 3 average intensity values in the cytoplasm area.

Cell Measurements

Measurement	Description
Cell Area	The total area in μm^2 of the cell's stained area (nucleus, cytoplasm, or both) as determined by the combination of all wavelengths, particularly the wavelengths that use of cytoplasm or both nucleus and cytoplasm.
Nuclear Area	The total area in μm^2 of the nucleus.
Average Nuclear Intensity	Average pixel intensity of the nuclear stain in the nuclear area.
Wavelength 2&3&4 Negative	Returns 1 if cell is scored as negative for Marker 1, Marker 2, and Marker 3; otherwise returns 0.
Wavelength 2 Positive	Returns 1 if cell is scored as positive for Marker 1; otherwise returns 0.
Wavelength 3 Positive	Returns 1 if cell is scored as positive for Marker 2; otherwise returns 0.
Wavelength 4 Positive	Returns 1 if cell is scored as positive for Marker 3; otherwise returns 0.
Wavelength 2&3 Positive	Returns 1 if cell is scored as positive for Marker 1 and Marker 2; otherwise 0.
Wavelength 2&3 Positive	Returns 1 if cell is scored as positive for Marker 1 and Marker 3; otherwise 0.
Wavelength 3&4 Positive	Returns 1 if cell is scored as positive for Marker 2 and Marker 3; otherwise 0.
Wavelength 2&3&4 Positive	Returns 1 if cell is scored as positive for Marker 1, Marker 2, and Marker 3; otherwise 0.
Wavelength 2 Average Cell Intensity	Average pixel intensity for Marker 1 in cell area (cytoplasm area + nucleus area).
Wavelength 2 Integrated Cell Intensity	Total pixel intensities for Marker 1 in cell area (cytoplasm area + nucleus area).
Wavelength 2 Average Nuclear Intensity	Average pixel intensity for Marker 1 in nuclear area.
Wavelength 2 Integrated Nuclear Intensity	Total of pixel intensities for Marker 1 in nuclear area.
Wavelength 2 Average Cytoplasm Intensity	Average pixel intensity for Marker 1 in cytoplasm area.
Wavelength 2 Integrated Cytoplasm Intensity	Total of pixel intensities for Marker 1 in cytoplasm area.

Measurement	Description
Wavelength 3 Average Cell Intensity	Average pixel intensity for Marker 2 in cell area (cytoplasm area + nucleus area).
Wavelength 3 Integrated Cell Intensity	Total pixel intensities for Marker 2 in cell area (cytoplasm area + nucleus area).
Wavelength 3 Average Nuclear Intensity	Average pixel intensity for Marker 2 in nuclear area.
Wavelength 3 Integrated Nuclear Intensity	Total of pixel intensities for Marker 2 in nuclear area.
Wavelength 3 Average Cytoplasm Intensity	Average pixel intensity for Marker 2 in cytoplasm area.
Wavelength 3 Integrated Cytoplasm Intensity	Total of pixel intensities for Marker 2 in cytoplasm area.
Wavelength 4 Average Cell Intensity	Average pixel intensity for Marker 3 in cell area (cytoplasm area + nucleus area).
Wavelength 4 Integrated Cell Intensity	Total pixel intensities for Marker 3 in cell area (cytoplasm area + nucleus area).
Wavelength 4 Average Nuclear Intensity	Average pixel intensity for Marker 3 in nuclear area.
Wavelength 4 Integrated Nuclear Intensity	Total of pixel intensities for Marker 3 in nuclear area.
Wavelength 4 Average Cytoplasm Intensity	Average pixel intensity for Marker 3 in cytoplasm area.
Wavelength 4 Integrated Cytoplasm Intensity	Total of pixel intensities for Marker 3 in cytoplasm area.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Endocytosis


Use the Endocytosis analysis to detect the internalization of endosomes into cells.

Note: The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.

Algorithm Input Parameters

Granule tab

Channel: Select the fluorescent channel used to image granules.

Granule

- **Intensity above background**: The minimum intensity above local background that is used for finding the granules.
- **Min Width**: The minimum value for the smallest granule you want to detect. The width refers to the short axis of a granule in µm. Granules smaller than the minimum width are ignored.
- **Max Width**: The maximum value for the largest granule you want to detect. The width refers to the short axis of a granule in µm. Granules larger than the maximum width are split.

Nuclear tab

Channel: Select the fluorescent channel for nuclei.

Nuclear

- **Intensity above background**: The minimum intensity above local background that is used for detecting nuclei. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: The minimum value for the smallest nuclei you want to detect.
- **Max Width**: The maximum value for the largest nuclei you want to detect.

Summary Measurements

Measurement	Description
Total Object Count	Number of objects.
Total Granule Count	Number of granules.
Avg Granule Count	Average number of granules per object.
Total Granule Area	Total area in μm^2 of granules.
Avg Granule Intensity	Average pixel intensity calculated over all granules in the image.
Avg Granule Integrated Intensity	Average of the integrated pixel intensity values for all granules in the image.

Cell Measurements

Measurement	Description
Granule Count	Number of granules detected for a specific cell. (Note that a granule is assigned to its nearest nucleus).
Granule Total Area	Total area in μm^2 covered by all the granules assigned to a specific cell.
Granule Integrated Intensity	Total pixel intensity of the granules assigned to a specific cell.
Granule Intensity	Average pixel intensity of the granules assigned to a specific cell.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Internalization

Use the Internalization analysis to identify and measure receptor internalization through granule measurements inside cells.

Note: The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.

Algorithm Input Parameters

Granule tab

Channel: Select the fluorescent channel used to image granules.

Granule

- **Intensity above background**: The minimum intensity above local background that is used for finding the granules.
- **Min Width**: The minimum value for the smallest granule you want to detect. The width refers to the short axis of a granule in µm. Granules smaller than the minimum width are ignored.
- **Max Width**: The maximum value for the largest granule you want to detect. The width refers to the short axis of a granule in µm. Granules larger than the maximum width are split.

Nuclear tab

Channel: Select the fluorescent channel for nuclei.

Nuclear

- **Intensity above background**: The minimum intensity above local background that is used for detecting nuclei. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: The minimum value for the smallest nuclei you want to detect.
- **Max Width**: The maximum value for the largest nuclei you want to detect.

Summary Measurements

Measurement	Description
Total Object Count	Number of objects.
Total Granule Count	Number of granules.
Avg Granule Count	Average number of granules per object.
Total Granule Area	Total area in μm^2 of granules.
Avg Granule Intensity	Average pixel intensity calculated over all granules in the image.
Avg Granule Integrated Intensity	Average of the integrated pixel intensity values for all granules in the image.

Cell Measurements

Measurement	Description
Granule Count	Number of granules detected for a specific cell. (Note that a granule is assigned to its nearest nucleus).
Granule Total Area	Total area in μm^2 covered by all the granules assigned to a specific cell.
Granule Integrated Intensity	Total pixel intensity of the granules assigned to a specific cell.
Granule Intensity	Average pixel intensity of the granules assigned to a specific cell.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Live Cells

Use the Live Cells analysis to determine the count of live and dead cells in appropriately prepared live/dead assays. This analysis lets you use two separate wavelengths and two separate stains. One stain identifies all cells, and the other stain identifies live cells. The dead cell count is determined by subtracting the live cell count from the total cell count.

Note: The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.

Algorithm Input Parameters

Nuclei tab

Channel: Select the fluorescent channel used to image nuclei.

Nuclei

- **Intensity**: The minimum intensity above local background that is used for finding the nuclei.
- **Min Width**: The minimum value for the smallest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei smaller than the minimum width will be ignored.
- **Max Width**: The maximum value for the largest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei larger than the maximum width will be split.

Marker tab

Channel: Select the fluorescent channel used to image live cells.

Marker

- **Intensity**: The minimum intensity above local background that is used for detecting the marker. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: If the marker stains the whole cell or only the cytoplasm, the minimum value for the smallest cell that you want to detect. If the marker stains only the nucleus, use the same value from the Nuclei tab.
- **Max Width**: If the marker stains the whole cell or only the cytoplasm, the maximum value for the largest cell that you want to detect. If the marker stains only the nucleus, use the same value from the Nuclei tab.

Summary Measurements

Measurement	Description
# Cells	Number of cells.
# Live Cells	Number of cells positive for the marker.
% Live Cells	Percentage of positive cells to total cells.
# Dead Cells	Number of cells negative for the marker in the image as defined in the segmentation parameters.
% Dead Cells	Percentage of positive cells to total cells.
All Cell Intensities	Total pixel intensity of all cells in the image.
Live Cell Intensities	Total pixel intensity of cells positive for the marker.
Live Cell Average Area	Average area in μm^2 of cells positive for the marker.
Live Cell Total Area	Total area in μm^2 of cells positive for the marker.

Cell Measurements

Measurement	Description
Positive	Returns 1 if cell is scored as positive, otherwise returns 0.
Cell Area	Area of the cell (white segmentation mask).
Nuclear Area	Area of the nucleus (green segmentation mask).
Wavelength 1 Integrated Nuclear Intensity	The total pixel intensity of the nuclear stain over the nuclear area.
Wavelength 1 Average Nuclear Intensity	The average pixel intensity of the nuclear stain (intensity per pixel).
Wavelength 2 Integrated Nuclear Intensity	The total pixel intensity of the marker stain in the nucleus of an individual cell.
Wavelength 2 Average Nuclear Intensity	The average pixel intensity of the marker stain in the nucleus of an individual cell.
Wavelength 2 Integrated Cell Intensity	The total pixel intensity of the marker stain in the cell area (cytoplasm + nucleus or cytoplasm if selected in the settings) of the individual cell.
Wavelength 2 Average Cell Intensity	Average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Live Cells Average Intensity	The average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Live Cells Integrated Intensity	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Live Cells Area	Total area in μm^2 of the cell if positive for the marker.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Lysosomal Degradation

Use the Lysosomal Degradation analysis to identify lysosome granules. A nuclear wavelength (for example, DAPI, Hoechst, or DRAQ5) is used to determine the number of granules per cell.

Note: The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.

Algorithm Input Parameters

Granule tab

Channel: Select the fluorescent channel used to image granules.

Granule

- **Intensity above background**: The minimum intensity above local background that is used for finding the granules.
- **Min Width**: The minimum value for the smallest granule you want to detect. The width refers to the short axis of a granule in µm. Granules smaller than the minimum width are ignored.
- **Max Width**: The maximum value for the largest granule you want to detect. The width refers to the short axis of a granule in µm. Granules larger than the maximum width are split.

Nuclear tab

Channel: Select the fluorescent channel for nuclei.

Nuclear

- **Intensity above background**: The minimum intensity above local background that is used for detecting nuclei. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: The minimum value for the smallest nuclei you want to detect.
- **Max Width**: The maximum value for the largest nuclei you want to detect.

Summary Measurements

Measurement	Description
Total Object Count	Number of objects.
Total Granule Count	Number of granules.
Avg Granule Count	Average number of granules per object.
Total Granule Area	Total area in μm^2 of granules.
Avg Granule Intensity	Average pixel intensity calculated over all granules in the image.
Avg Granule Integrated Intensity	Average of the integrated pixel intensity values for all granules in the image.

Cell Measurements

Measurement	Description
Granule Count	Number of granules detected for a specific cell. (Note that a granule is assigned to its nearest nucleus).
Granule Total Area	Total area in μm^2 covered by all the granules assigned to a specific cell.
Granule Integrated Intensity	Total pixel intensity of the granules assigned to a specific cell.
Granule Intensity	Average pixel intensity of the granules assigned to a specific cell.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Mitochondria

The Mitochondria analysis uses two stains to detect and measure mitochondria in cells. One stain typically detects the nuclei and the other detects the mitochondria.

Note: The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.

Algorithm Input Parameters

Granule tab

Channel: Select the fluorescent channel used to image granules.

Granule

- **Intensity above background**: The minimum intensity above local background that is used for finding the granules.
- **Min Width**: The minimum value for the smallest granule you want to detect. The width refers to the short axis of a granule in µm. Granules smaller than the minimum width are ignored.
- **Max Width**: The maximum value for the largest granule you want to detect. The width refers to the short axis of a granule in µm. Granules larger than the maximum width are split.

Nuclear tab

Channel: Select the fluorescent channel for nuclei.

Nuclear

- **Intensity above background**: The minimum intensity above local background that is used for detecting nuclei. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: The minimum value for the smallest nuclei you want to detect.
- **Max Width**: The maximum value for the largest nuclei you want to detect.

Summary Measurements

Measurement	Description
Total Object Count	Number of objects.
Total Granule Count	Number of granules.
Avg Granule Count	Average number of granules per object.
Total Granule Area	Total area in μm^2 of granules.
Avg Granule Intensity	Average pixel intensity calculated over all granules in the image.
Avg Granule Integrated Intensity	Average of the integrated pixel intensity values for all granules in the image.

Cell Measurements

Measurement	Description
Granule Count	Number of granules detected for a specific cell. (Note that a granule is assigned to its nearest nucleus).
Granule Total Area	Total area in μm^2 covered by all the granules assigned to a specific cell.
Granule Integrated Intensity	Total pixel intensity of the granules assigned to a specific cell.
Granule Intensity	Average pixel intensity of the granules assigned to a specific cell.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Mitotic Index

Use the Mitotic Index analysis to differentiate between mitotic cells and interphase cells in the normal cell cycle and quantify the various data extracted during image analysis.

Label the cells with a DNA stain and a mitosis-specific stain such as Histone 3 S10 phosphorylation. The DNA stain labels all the cells and only the mitotic cells are labeled with the second stain. The DNA stain and the associated wavelength are used to differentiate all cells in the image from non-cell material and the background. The mitotic stain differentiates mitotic cells from the background and interphase cells. When properly stained, the mitotic cells typically appear to be significantly brighter than the interphase cells in the mitotic staining image.

Note:

- The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.
- You may want to use a particular fluorescent channel as more than one marker. For example, you might use a DAPI channel set to a higher cutoff to indicate cells about to undergo mitosis.

Algorithm Input Parameters

Nuclei tab

Channel: Select the fluorescent channel used to image nuclei.

Nuclei

- **Intensity**: The minimum intensity above local background that is used for finding the nuclei.
- **Min Width**: The minimum value for the smallest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei smaller than the minimum width will be ignored.
- **Max Width**: The maximum value for the largest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei larger than the maximum width will be split.

Marker tab

Channel: Select the fluorescent channel for the marker.

Marker

- **Intensity**: The minimum intensity above local background that is used for detecting the marker. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: If the marker stains the whole cell or only the cytoplasm, the minimum value for the smallest cell that you want to detect. If the marker stains only the nucleus, use the same value from the Nuclei tab.
- **Max Width**: If the marker stains the whole cell or only the cytoplasm, the maximum value for the largest cell that you want to detect. If the marker stains only the nucleus, use the same value from the Nuclei tab.

Summary Measurements

Measurement	Description
# Cells	Number of cells identified by the nuclear stain.
# Cells	Number of cells positive for the marker.
% Mitotic Cells	Percentage of cells positive for the marker to the number of cells.
# Non-Mitotic Cells	Number of cells negative for the marker.
% Non-Mitotic Cells	Percentage of cells negative for the marker to the number of cells.
All Cell Intensities	Average pixel intensity of over all the cell areas.
Mitotic Cell Intensity	Sum of Mitotic Cells Average Intensity

Cell Measurements

Measurement	Description
Positive	Returns 1 if cell is scored as positive for the marker; otherwise returns 0.
Cell Area	Area of the cell (white segmentation mask).
Nuclear Area	Area of the nucleus (green segmentation mask).
Wavelength 1 Integrated Nuclear Intensity	Total pixel intensity of the nuclear stain over the nuclear area in an individual cell.
Wavelength 1 Average Nuclear Intensity	Average pixel intensity of the nuclear stain over all the nuclear areas in an individual cell (intensity per pixel).
Wavelength 2 Integrated Nuclear Intensity	Total pixel intensity of stain in the nucleus of an individual cell.
Wavelength 2 Average Nuclear Intensity	Average pixel intensity of stain in the nucleus of an individual cell.
Wavelength 2 Integrated Cell Intensity	Total pixel intensity of stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Wavelength 2 Average Cell Intensity	Average pixel intensity of stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Mitotic Cells Average Intensity	The average pixel intensity of stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Mitotic Cells Integrated Intensity	Total pixel intensity of stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Neurite Tracing

Use the Neurite Tracing analysis to measure multiple biologies. Neurites are any extension off a cell body. For example, cilia, blood vessels, nanotubes, and so on.

The Neurite Tracing analysis uses a single-channel assay for measuring neurite outgrowth to identify and measure cell bodies and processes (neurites) attached to cell bodies. Neurites are extensions attached to a cell body and are identified using width, length, and intensity.

Tip:

- Exposure time should be optimized for outgrowth intensity, not cell body intensity. The autoexposure function may give images that are too dim in the outgrowths.
- If the outgrowths are going in and out of the plane of focus, interrupting the connectivity of the outgrowth, it may lead to inaccurate neurite detection. In this case, you may want to collect a Z-stack of the neurite image using the Best Focus setting.
- If neurites are sparse and/or you are imaging at high magnification, you may want to use a stitched protocol to collect multiple sites. Neurites not connected to a cell body are ignored.

Algorithm Input Parameters

Neurite Channel tab

Channel: Select the fluorescent channel for the analysis.

Cell Bodies

- **Intensity above background**: The estimated intensity threshold of cell bodies compared to neighboring background values. This setting determines the gray-level sensitivity of cell body detection.
- **Min Width**: The approximate minimum cell body width in µm. The width fields aid in estimating what intensity fluctuations are potential cell bodies compared to background fluctuations.
- **Max Width**: The approximate maximum cell body width in µm.

Outgrowths

- **Intensity above background**: The estimated intensity threshold of neurites as compared to neighboring background values. This setting determines the gray-level sensitivity of outgrowth detection.
- **Min Width**: The approximate minimum outgrowth width. The width fields aid in the differentiation between cell body deformations and actual outgrowths. It also helps to estimate the range of outgrowth widths that can be distinguished from random noise patterns and background. This value is entered in integer pixel units.
- **Max Width**: The approximate maximum outgrowth width.

Summary Measurements

Measurement	Description
Cell Count	Number of cell bodies in the image.
Total Outgrowth	Total length of skeletonized outgrowth in µm (corrected for diagonal lengths).
Avg Outgrowth	Average skeletonized outgrowth in um corrected for diagonal lengths divided by the number of cells.
Total Branches	Total number of branching junctions in the image.
Avg Branches	Total branches divided by number of cells.
Total Processes	Number of outgrowths in the image that are connected to cell bodies.
Avg Processes	Total processes divided by number of cells.
Avg Cell Body Area	Total cell body area divided by the number of cells.
Avg Cell Body Intensity	Average pixel intensity of the neurite stain over all the outgrowths detected in the image.

Cell Measurements

Measurement	Description
Outgrowth	Total amount of skeletonized outgrowth in µm (corrected for diagonal lengths) associated with the cell.
# Processes	Number of outgrowths that connect to the cell body.
# Branches	Number of branching junctions of all the processes connected to the cell.
Avg Process Growth	Total outgrowth in µm divided by number of processes of the cell.
Max Process Growth	Maximum value of the outgrowth lengths (in um) associated with the cell's various processes.
Median Primary Process Length	Median value of the outgrowth lengths (in µm) associated with the cell's various processes.
Overall Straightness	Ratio varying between 0 (not straight) and 1 (perfectly straight) defined as end-to-end Euclidean distance between the cell's segment junctions divided by corresponding actual neurite curve length (the sum of end-to-end lengths divided by the sum of curve lengths).
Average Outgrowth Intensity	Average pixel intensity of the neurite stain over all the outgrowths for this cell.
Mean Width of All Processes	Total area of all outgrowths divided by the total length of all outgrowths.
Mean Width of All Primary Processes	Total area of primary processes (from cell body to first branch point) divided by the total length of the primary processes.

Measurement	Description
Mean Width of All Secondary Processes	Not used in this version of the CellReporterXpress software.
Number of Secondary Processes	Number of outgrowths that are connected with primary process branches.
Number of Segments	Total number of segments. Segments connect two branches, a cell body to a branch, or a branch point to end point.
Number of End Points	Total number of outgrowth terminal points.
Mean Angular Vector	Each segment is a vector. All vectors that belong to the cell are summed together; the total is the mean vector. The mean vector indicates the general magnitude and direction of growth of the cell. The mean angular vector is the direction of the mean vector. Angles are measured from the “three o’clock” (east) position and will range from 0 to 180 degrees. Angles toward the “twelve o’clock” (north) positions are expressed as positive numbers, and angles toward the “six o’clock” (south) position are expressed as negative numbers.
Mean Vector Magnitude	Magnitude of the mean vector.
Average Cell Body Intensity	Total intensity of all cell bodies divided by number of cell bodies.
Cell Body Area	Total area of the cell body in μm^2 (excluding outgrowths).
Cell Body CentroidX	The X coordinates of the centroid of the object. The centroid is the center of mass of the object, ignoring pixel intensities. Depending on the shape of the object, the centroid may not be inside the object (for example, if the object is concave).
Cell Body CentroidY	The Y coordinates of the centroid of the object. The centroid is the center of mass of the object, ignoring pixel intensities. Depending on the shape of the object, the centroid may not be inside the object (for example, if the object is concave).
Number of Spines	Not used in this version of the CellReporterXpress software.
Mean Spine Area	Not used in this version of the CellReporterXpress software.
Average Spine Intensity	Not used in this version of the CellReporterXpress software.

Phagocytosis

 The Phagocytosis analysis detects and measures phagosomes or the particles ingested or engulfed during the phagocytosis process in cells.

 **Note:** The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.

Algorithm Input Parameters

Granule tab

Channel: Select the fluorescent channel used to image granules.

Granule

- **Intensity above background**: The minimum intensity above local background that is used for finding the granules.
- **Min Width**: The minimum value for the smallest granule you want to detect. The width refers to the short axis of a granule in µm. Granules smaller than the minimum width are ignored.
- **Max Width**: The maximum value for the largest granule you want to detect. The width refers to the short axis of a granule in µm. Granules larger than the maximum width are split.

Nuclear tab

Channel: Select the fluorescent channel for nuclei.

Nuclear

- **Intensity above background**: The minimum intensity above local background that is used for detecting nuclei. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: The minimum value for the smallest nuclei you want to detect.
- **Max Width**: The maximum value for the largest nuclei you want to detect.

Summary Measurements

Measurement	Description
Total Object Count	Number of objects.
Total Granule Count	Number of granules.
Avg Granule Count	Average number of granules per object.
Total Granule Area	Total area in μm^2 of granules.
Avg Granule Intensity	Average pixel intensity calculated over all granules in the image.
Avg Granule Integrated Intensity	Average of the integrated pixel intensity values for all granules in the image.

Cell Measurements

Measurement	Description
Granule Count	Number of granules detected for a specific cell. (Note that a granule is assigned to its nearest nucleus).
Granule Total Area	Total area in μm^2 covered by all the granules assigned to a specific cell.
Granule Integrated Intensity	Total pixel intensity of the granules assigned to a specific cell.
Granule Intensity	Average pixel intensity of the granules assigned to a specific cell.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Pits and Vesicles


Use the Pits and Vesicles analysis to detect the internalization of a fluorescent marker to small, coated pits and/or larger internalized vesicles.

Note: The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.

Algorithm Input Parameters

Pits and Vesicles tab

Channel: Select the fluorescent channel for the analysis.

Pits

- **Intensity above background**: The estimated intensity threshold of cell bodies compared to neighboring background values. This setting determines the gray-level sensitivity of pit detection.
- **Min Width**: The minimum value for the smallest pit you want to detect. The width refers to the short axis of a pit in µm. Pits smaller than the minimum width are ignored.
- **Max Width**: The maximum value for the largest pit you want to detect. The width refers to the short axis of a pit in µm. Pits larger than the maximum width are split.

Vesicles

- **Intensity above background**: The estimated intensity threshold of vesicles as compared to neighboring background values. This setting determines the gray-level sensitivity of vesicle detection.
- **Min Width**: The minimum value for the smallest vesicle you want to detect. The width refers to the short axis of a vesicle in µm. Vesicles smaller than the minimum width are ignored.
- **Max Width**: The maximum value for the largest vesicle you want to detect. The width refers to the short axis of a vesicle in µm. Vesicles larger than the maximum width are split.

Nuclear tab

Channel: Select the fluorescent channel for the analysis.

Nuclear

- **Intensity above background**: The minimum intensity above local background that is used for detecting nuclei. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: The minimum width in µm for the smallest nuclei you want to detect.
- **Max Width**: The maximum width in µm for the largest nuclei you want to detect.

Summary Measurements

Measurement	Description
Cell Count	Number of cells identified.
Pit Count	Number of pits.
Avg Pit Count	Number of pits to the total number of nuclei.
Total Pit Area	Total area in μm^2 of pits for all cells.
Avg Pit Intensity	Average pixel intensity over all the pit areas.
Avg Pit Integrated Intensity	Average pixel intensity over all the pit areas.
Vesicle Count	Number of vesicles.
Avg Vesicle Count	Number of vesicles divided by the number of nuclei.
Total Vesicle Area	Total area in μm^2 of vesicles for all cells.
Avg Vesicle Intensity	Average pixel intensity over all the vesicle areas.
Avg Vesicle Integrated Intensity	Average pixel intensity over all the vesicle areas.
Total Nuclear Area	Total area in μm^2 of the nuclei for all cells.
Avg Nuclear Intensity	Average pixel intensity of the nuclear stain over all the nuclei.
Avg Nuclear Integrated Intensity	Average pixel intensity over all the nuclei.

Cell Measurements

Measurement	Description
Pit Count	Number of pits detected for a specific cell. Note that a pit is assigned to its nearest nucleus.
Pit Total Area	Total area in μm^2 covered by all the pits assigned to a specific cell.
Pit Integrated Intensity	Total pixel intensity of the pits assigned to a specific cell.
Pit Average Intensity	Average pixel intensity of the pits assigned to a specific cell.
Vesicle Count	Number of vesicles detected for a specific cell. Note that a vesicle is assigned to its nearest nucleus.
Vesicle Total Area	Total area in μm^2 covered by all the vesicles assigned to a specific cell.
Vesicle Integrated Intensity	Total pixel intensity of the vesicles assigned to a specific cell.
Vesicle Average Intensity	Average pixel intensity of the vesicles assigned to a specific cell.
Nuclear Total Area	Total square microns of a specific nucleus.
Nuclear Integrated Intensity	Total pixel intensity of the nuclear stain in a specific nucleus.
Nuclear Average Intensity	Average pixel intensity of the nuclear stain in a specific nucleus.
Texture Index	Standard deviation of intensity values of a specific cell.
Gradient Index	Texture-dependent measurement that reflects the amount of local intensity contrast. Measures the difference between the maximum and minimum intensity within a local neighborhood of a specific cell.
Laplacian Index	Similar to the morphological gradient, also reflects fluctuations in the gradient of a specific cell.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Protein Expression Index

 Use the Protein Expression Index analysis to measure the expression level of a protein of interest though differences in intensity levels.

Algorithm Input Parameters

Nuclei tab

Channel: Select the fluorescent channel used to image nuclei.

Nuclei

- **Intensity**: The minimum intensity above local background that is used for finding the nuclei.
- **Min Width**: The minimum value for the smallest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei smaller than the minimum width will be ignored.
- **Max Width**: The maximum value for the largest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei larger than the maximum width will be split.

Marker tab

Channel: Select the fluorescent channel for the marker.

Marker

- **Intensity**: The minimum intensity above local background that is used for detecting the marker. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: The minimum value for the smallest area expressing the protein of interest in the cells that you want to detect.
- **Max Width**: The maximum value for the largest area expressing the protein of interest in the cells that you want to detect.

Summary Measurements

Measurement	Description
# Cells	Number of cells identified by the nuclear stain.
# Positive Cells	Number of cells positive for the marker.
% Positive Cells	Percentage of cells positive for the marker to the number of cells.
# Positive Cells	Number of cells negative for the marker.
% Positive Cells	Percentage of cells negative for the marker to the number of cells.
All Cell Average Intensities	Average pixel intensity of the marker over all the cell areas.
Positive Cell Average Intensities	Average pixel intensity of cells positive for the marker over the positive cell area.
Positive Cell Integrated Intensity	Total pixel intensity of the marker stain over the cell area in all cells positive for the marker
Positive Cell Total Intensity	Sum of Positive Cells Average Intensity
Positive Cell Total Integrated Intensity	Sum of Positive Cells Integrated Intensity

Cell Measurements

Measurement	Description
Positive	Returns 1 if cell is scored as positive for the marker; otherwise returns 0.
Cell Area	Area of the cell (white segmentation mask).
Nuclear Area	Area of the nucleus (green segmentation mask).
Wavelength 1 Integrated Nuclear Intensity	Total pixel intensity of the nuclear stain over the nuclear area in an individual cell.
Wavelength 1 Average Nuclear Intensity	Average pixel intensity of the nuclear stain over all the nuclear areas in an individual cell (intensity per pixel).
Wavelength 2 Integrated Nuclear Intensity	Total pixel intensity of the marker stain in the nucleus of an individual cell.
Wavelength 2 Average Nuclear Intensity	Average pixel intensity of the marker stain in the nucleus of an individual cell.
Wavelength 2 Integrated Cell Intensity	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Wavelength 2 Average Cell Intensity	Average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Positive Cells Average Intensity	The average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Positive Cells Integrated Intensity	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Translocation


Use the Translocation analysis to measure intensity movement from one compartment to another (for instance, the nucleus to the cytoplasm).

Note: The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.

Algorithm Input Parameters

Compartment tab

Channel: Select the fluorescent channel for the nuclear stain (for a nuclear translocation assay).

Compartment

- **Intensity above background**: The estimated intensity threshold of the compartment stain compared to neighboring background values.
- **Max Width**: The approximate maximum compartment width in µm.

Probe tab

Channel: Select the fluorescent channel for the marker that is moving in or out of the compartment.

Probe

- **Classify Positive if Coefficient >=**: The threshold for the Pearson's correlation coefficient. The Pearson's correlation coefficient of the pixel intensity of the two stains in the entire cell region (nucleus + gap + cytoplasm) is a method of identifying a cell as positive for translocation. A value of 1 indicates a perfect correlation (that is, the two stains perfectly overlap). A value of 0 indicates that the stains are independent. A value of -1 indicates the stain is excluded from the compartment.

Summary Measurements

Measurement	Description
Cell Count	Number of cells in the image.
Total Inner Intensity	Total pixel intensity of the probe in all inner regions for the site after background subtraction (note this correlates with cell count).
Total Outer Intensity	Total pixel intensity of the probe in all outer regions for the site after background subtraction (note that this correlates with cell count).
Avg Inner Area	Average area in μm^2 of all inner regions for the site after background subtraction.
Avg Outer Area	Average area in μm^2 of all outer regions for the site after background subtraction.
Avg Inner Intensity	Average pixel intensity of the probe in all inner regions for the site after background subtraction (independent of cell count).
Avg Outer Intensity	Average pixel intensity of the probe in all outer regions for the site after background subtraction (independent of cell count)
# Translocated Cells	Total number of cells classified as positive for translocation.

Cell Measurements

Measurement	Description
Inner Area	Average area in μm^2 of the inner region after background subtraction.
Outer Area	Average area in μm^2 of the outer region after background subtraction.
Total Inner Intensity	Total pixel intensity of the probe in the inner region after background subtraction.
Total Outer Intensity	Total pixel intensity of the probe in the outer region after background subtraction.
Avg Inner Intensity	Average pixel intensity of the probe in the inner region after background subtraction.
Median Inner Intensity	Median (middle) pixel intensity value of the probe in the inner region after background subtraction.
Avg Outer Intensity	Average pixel intensity of the probe in the outer region after background subtraction.
Median Outer Intensity	Median (middle) pixel intensity value of the probe in the outer region after background subtraction.
Correlation Coefficient	The Pearson's correlation coefficient of the pixel intensity of the two stains in the entire cell region (nucleus + gap + cytoplasm). A value of 1 indicates a perfect correlation (that is, the two stains perfectly overlap). A value of 0 indicates that the stains are independent. A value of -1 indicates the stain is excluded from the compartment.
Classification	Returns 1 for positive translocation classification (nuclear staining) and 0 for negative translocation classification (cytoplasmic staining).
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Transmitted Light Cell Count

Use the Transmitted Light Cell Count analyses to segment brightfield images in order to identify and differentiate cells. The segmentation labels each isolated and identified cell to let you see a visual separation between cells that are close or touching.

Tip: The accuracy of the transmitted light counting depends on the focal plane used for the transmitted light channel. Use the analysis algorithm to evaluate the selected focal plane, rather than by picking what looks best by eye.

Four analyses are available:


Transmitted Light Cell Count, Beads: Single-channel, transmitted light assay to find beads.


Transmitted Light Cell Count, General: Single-channel, transmitted light assay to find a range of cells.


Transmitted Light Cell Count, Large Cells: Single-channel, transmitted light assay to find larger cells.


Transmitted Light Cell Count, Small Cells: Single-channel, transmitted light assay to find smaller cells.

Algorithm Input Parameters

Cell tab

Channel: Select the channel used to image cells.

Summary Measurements

Measurement	Description
Cell Count	Total number of cells.
Total Area	Sum of Cells Area
Avg Area	Total Area / Cell Count

Cell Measurements

Measurement	Description
Cell Area	Area in μm^2 of the cell (white segmentation mask).
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Transmitted Light Cell Scoring

Use the Transmitted Light Cell Scoring analyses to identify two subpopulations of cells based on a brightfield image for all cells and a fluorescent marker for cells of interest. The fluorescent marker can label nuclei, cytoplasm, or both.

The output of the analysis includes the number of cells scored positive as detected by the marker.

In the segmentation mask, all identified cells are indicated.

- Cells scored positive for Marker 1 are shown in green.
- Cells scored negative for Marker 1 are shown in red.

Four analyses are available:


Transmitted Light Cell Scoring, Beads: Two-channel, transmitted light assay to find beads, then scoring for an additional fluorescence channel.


Transmitted Light Cell Scoring, General: Two-channel, transmitted light assay to find a range of cells, then scoring for an additional fluorescence channel.


Transmitted Light Cell Scoring, Large Cells: Two-channel, transmitted light assay to find larger cells, such as HeLa cells, then scoring for an additional fluorescence channel.


Transmitted Light Cell Scoring, Small Cells: Two-channel, transmitted light assay to find smaller cells, such as CHO cells, then scoring for an additional fluorescence channel.

Algorithm Input Parameters

Cell tab

Channel: Select the brightfield channel used to image cells.

Marker tab

Channel: Select the fluorescent channel used for the marker.

Marker

- **Intensity**: The minimum intensity above local background that is used for detecting the marker. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: The minimum value for the smallest cell that you want to detect.
- **Max Width**: The maximum value for the largest cell that you want to detect.

Summary Measurements

Measurement	Description
# Cells	Total number of cells.
# Positive Cells	Total number of cells in the image that were positive for the marker.
% Positive Cells	Percentage of cells positive for the marker to the total number of cells in the image.
# Negative Cells	Total number of cells in the image that were negative for the marker.
% Negative Cells	Percentage of cells positive for the marker to the total number of cells in the image.
All Cell Intensities	Average pixel intensity of the marker over all the cell areas.
Positive Cell Intensities	Average pixel intensity of cells positive for the marker over the positive cell area.
Positive Avg Cell Area	Sum of Positive Cells Area / # Positive Cells (or 0 if there are no positive cells).
Positive Total Cell Area	Sum of Positive Cells Area

Cell Measurements

Measurement	Description
Cell Area	Area of the cell (white segmentation mask).
Intensity Standard Deviation	Per cell standard deviation measurement for transmitted light.
Positive	Returns 1 if cell is scored as positive for the marker; otherwise returns 0.
Marker Integrated Intensity	If the cell is positive, the integrated intensity for fluorescence using marker segmentation (may be partial of the cell).
Marker Average Intensity	If the cell is positive, the average intensity for fluorescence using marker segmentation (may be partial of the cell).
Cell Integrated Intensity	Integrated intensity for fluorescence using transmitted light segmentation (entire cell).
Cell Average Intensity	Average intensity for fluorescence using transmitted light segmentation (entire cell).
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

Viral Infectivity

Use the Viral Infectivity analysis to detect and measure cells infected with viruses. It can quantify the number of cells in a field of view infected as well as the level of infection through differences of intensities using a marker for the virus.

Note:

- The total number of cells is determined by the nuclear channel and not by the markers. It is possible that a cell can be positive for a marker, but does not have a stained nucleus associated with it. In this case, that cell will not be counted.
- You may want to use a particular fluorescent channel as more than one marker. For example, you might use a DAPI channel set to a higher cutoff to indicate cells about to undergo mitosis.

Algorithm Input Parameters

Nuclei tab

Channel: Select the fluorescent channel used to image nuclei.

Nuclei

- **Intensity**: The minimum intensity above local background that is used for finding the nuclei.
- **Min Width**: The minimum value for the smallest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei smaller than the minimum width will be ignored.
- **Max Width**: The maximum value for the largest nuclei that you want to detect. The width refers to the short axis of a nucleus in µm. Nuclei larger than the maximum width will be split.

Marker tab

Channel: Select the fluorescent channel for the marker.

Marker

- **Intensity**: The minimum intensity above local background that is used for detecting the marker. You may want to use both positive and negative control images to optimize this value.
- **Min Width**: The minimum value for the smallest virus area in the cells that you want to detect.
- **Max Width**: The maximum value for the largest virus area in the cells that you want to detect.

Summary Measurements

Measurement	Description
# Cells	Number of cells identified by the nuclear stain.
# Positive Cells	Number of cells positive for the marker.
% Positive Cells	Percentage of cells positive for the marker to the number of cells.
# Positive Cells	Number of cells negative for the marker.
% Positive Cells	Percentage of cells negative for the marker to the number of cells.
Positive Cell Average Intensities	Average pixel intensity of cells positive for the marker over the positive cell area.
Positive Cell Integrated Intensity	Total pixel intensity of the marker stain over the cell area in all cells positive for the marker
Positive Cell Total Intensity	Sum of Positive Cells Average Intensity
Positive Cell Total Integrated Intensity	Sum of Positive Cells Integrated Intensity

Cell Measurements

Measurement	Description
Positive	Returns 1 if cell is scored as positive for the marker; otherwise returns 0.
Cell Area	Area of the cell (white segmentation mask).
Nuclear Area	Area of the nucleus (green segmentation mask).
Wavelength 1 Integrated Nuclear Intensity	Total pixel intensity of the nuclear stain over the nuclear area in an individual cell.
Wavelength 1 Average Nuclear Intensity	Average pixel intensity of the nuclear stain over all the nuclear areas in an individual cell (intensity per pixel).
Wavelength 2 Integrated Nuclear Intensity	Total pixel intensity of the marker stain in the nucleus of an individual cell.
Wavelength 2 Average Nuclear Intensity	Average pixel intensity of the marker stain in the nucleus of an individual cell.
Wavelength 2 Integrated Cell Intensity	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Wavelength 2 Average Cell Intensity	Average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Positive Cells Average Intensity	The average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Positive Cells Integrated Intensity	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
Cell Segmentation	Saves the cell segmentation overlay along with the experiment data.

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