CellReporterXpress

Image Acquisition and Analysis Software

Version 2.7

User Guide

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October 2020

MOLECULAR
DEVICES

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Chapter 1: CellReporterXpress Image Acquisition and Analysis Software

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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.

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

You can contact Molecular Devices Technical Support by submitting a support request through the Molecular Devices Knowledge Base at mdc.custhelp.com.

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

You will need the instrument serial number.

Documentation

Review the product documentation on the Molecular Devices Knowledge Base at mdc.custhelp.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
- 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

Logging In to the Software

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

- Logging In on the Host Computer, see below
- Logging In on a Client Workstation Using a Shortcut, see page 8
- Logging In on a Client Workstation Using Connection Details, see page 8

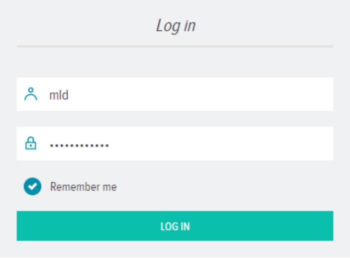
Note: The CellReporterXpress software uses the Windows login credentials of the host computer to authenticate users. If the host computer does not maintain a constant connection to the network, we recommend that user accounts be local accounts (and not roaming or domain accounts). If domain accounts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



mld

....

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**.

Product Documentation

The following guides are available on the Molecular Devices Knowledge Base at mdc.custhelp.com:

- *CellReporterXpress Installation Guide*
- *CellReporterXpress Release Notes*
- *CellReporterXpress User Guide*
- *ImageXpress Pico Pre-Installation Guide*
- *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.

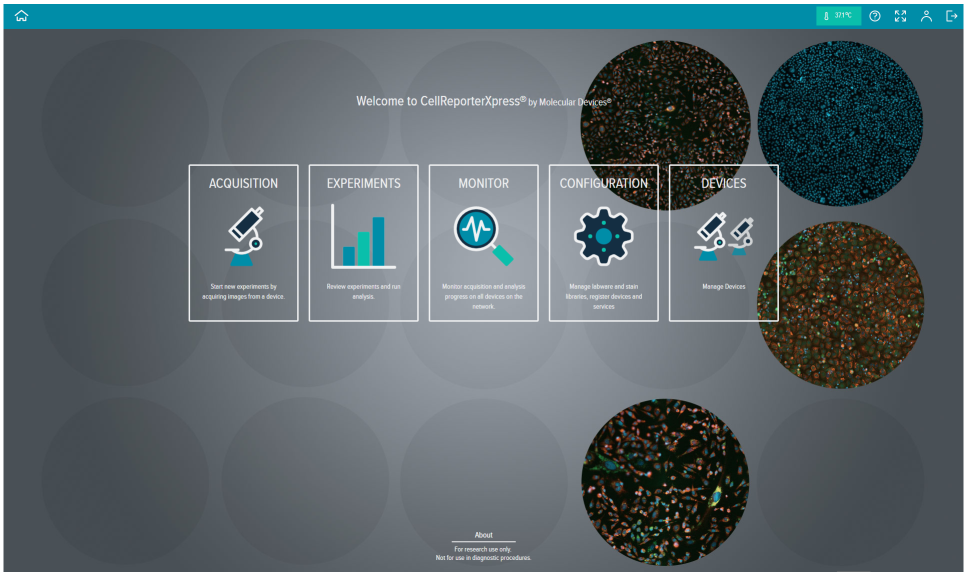
✱ Tip: We recommend that you review the documentation before installing or using the ImageXpress Pico system or the CellReporterXpress software.

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 mdc.custhelp.com for the most up-to-date information.

Chapter 2: Home Page



Welcome to CellReporterXpress® by Molecular Devices®

Start new experiments by acquiring images from a device.

Review experiments and run analysis.

Monitor acquisition and analysis progress on all devices on the network.

Manage labware and stain libraries, register devices and services

Manage Devices

For research use only.
Not for use in diagnostic procedures.

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 113 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, raw images exported in **Experiments** mode, movies downloaded in **Experiments** mode, and maintenance operations run in **Devices** mode. See Monitor Mode on page 209 for details.

Configuration: Click to set the systemwide options that affect all users of the CellReporterXpress software. See Configuration Mode on page 211 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 225 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 253 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 4% **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).

91% **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 1% **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).

37.1°C **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 227 for details.

Chapter 3: Acquisition Mode

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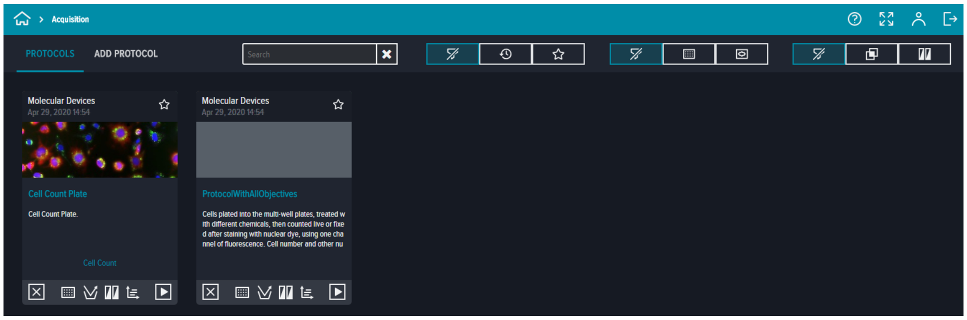
Use **Acquisition** mode to configure experiment settings and run experiments from supported instruments using protocols or templates.

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.



Cell Count Plate.

Cells plated into the multi-well plates, treated w
ith different chemicals, then counted live or fixe
d after staining with nuclear dye, using one cha
nnel of fluorescence. Cell number and other nu

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 66 for details on running a plate protocol or Run Protocol on page 110 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 69 for details.
3. Click **Save Protocol**. See Run Protocol on page 66 for details on running a plate protocol or Run Protocol on page 110 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

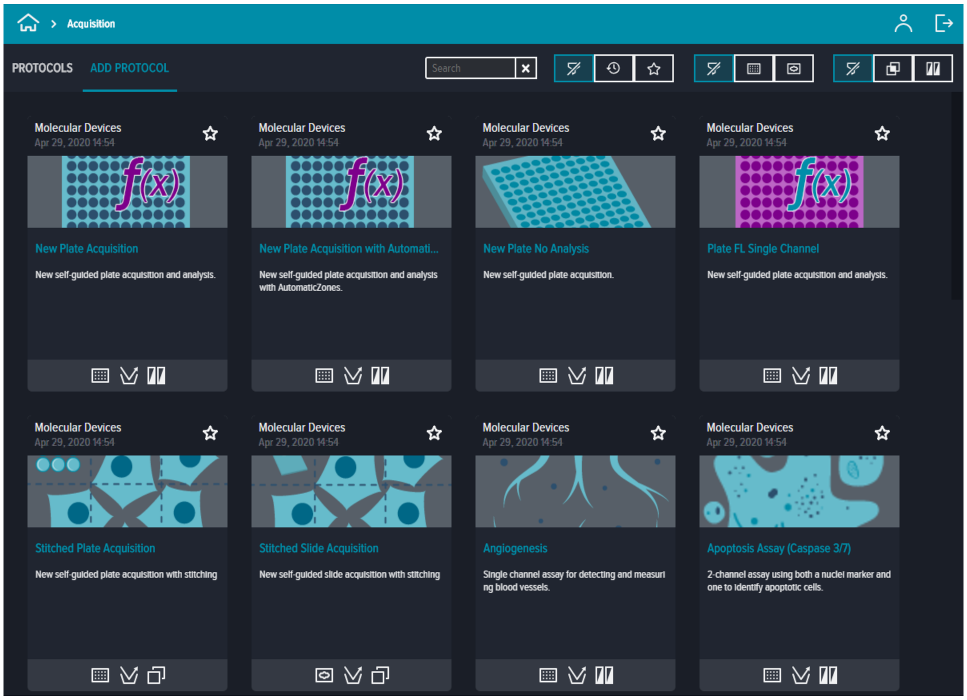
To add a protocol, see Template Library on page 15.

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.



Molecular Devices
Apr 29, 2020 14:54
New Plate Acquisition
New self-guided plate acquisition and analysis.

Molecular Devices
Apr 29, 2020 14:54
New Plate Acquisition with Automati...
New self-guided plate acquisition and analysis with AutomaticZones.

Molecular Devices
Apr 29, 2020 14:54
New Plate No Analysis
New self-guided plate acquisition.

Molecular Devices
Apr 29, 2020 14:54
Plate FL Single Channel
New self-guided plate acquisition and analysis.

Molecular Devices
Apr 29, 2020 14:54
Stitched Plate Acquisition
New self-guided plate acquisition with stitching

Molecular Devices
Apr 29, 2020 14:54
Stitched Slide Acquisition
New self-guided slide acquisition with stitching

Molecular Devices
Apr 29, 2020 14:54
Angiogenesis
Single channel assay for detecting and measuri ng blood vessels.

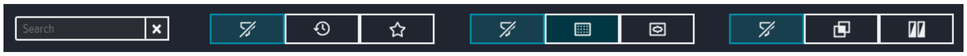
Molecular Devices
Apr 29, 2020 14:54
Apoptosis Assay (Caspase 3/7)
2-channel assay using both a nuclei marker and one to identify apoptotic cells.

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 53 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 54 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 59 for details.

Well Selection is the step where you select the well for the experiment. See Well Selection on page 60 for details.

Device Sensors is the step where you specify the environmental sensors to be monitored for the experiment. The experiment data will indicate if the sensors were within the specified range during the experiment or outside of it. See Device Sensors on page 61 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 63 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 65 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 66 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.

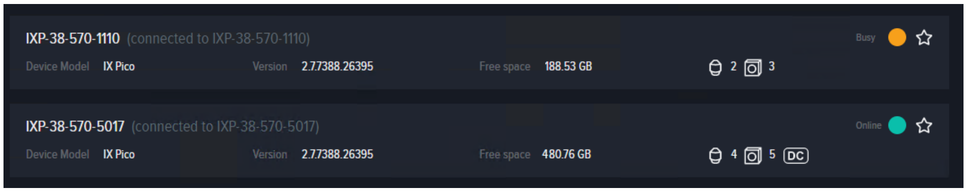
Open Plate Door: Opens the top door on the selected instrument so that you can insert or remove labware.

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 Devices on page 216 for details on adding other instruments in your network.



IXP-38-570-1110 (connected to IXP-38-570-1110) Busy
Device Model IX Pico Version 2.7.7388.26395 Free space 188.53 GB 2 3

IXP-38-570-5017 (connected to IXP-38-570-5017) Online
Device Model IX Pico Version 2.7.7388.26395 Free space 480.76 GB 4 5 DC

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 49 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 28 for details.

Download Plane Images: Exports 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, exports a TIFF image of the current Z stack projection and each individual Z stack plane.

Specifying the Plate Format

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.

To specify the plate format:

1. On the **Acquisition Settings** page, in the **Tools** pane on the right, click 96 **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, you can add a new one. See Labware Library on page 213 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.
- 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.

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 Well, see page 24
- Snapping Well Comparison Previews, see page 28
- Snapping Z Stack Images, see page 32
- Viewing a Live Preview of a Plate, see page 36
- Adjusting an Objective Correction Collar, see page 44
- Understanding How Autofocus Works with a Plate, see page 46
- Troubleshooting Autofocus Issues, see page 49

To continue to the next workflow step, click **Region Selection to Acquire**. See Region Selection to Acquire on page 53 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, wavelength, well, region, focus settings, and exposure settings.

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

Download Plane Images: Exports 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. “Hidden” channels are shown slightly dimmed.

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* Tip: When an image is overexposed, the channel icons indicate the overexposure 🟧. 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.

61.97 µm DIGITAL ZOOM 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.

Note:

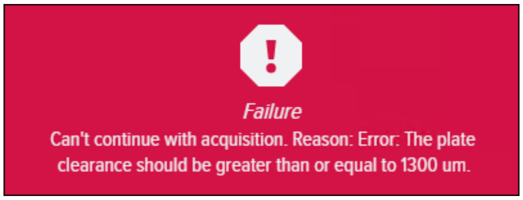
- If the snap fails, the software may display the following message:

Failure

Can't continue with acquisition. Reason: Error: The acquisition was aborted because could not find focus.

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 49 for details.

- If you are using the optional environmental control cassette and the autofocus fails, the software may display the following message:



Failure

Can't continue with acquisition. Reason: Error: The plate clearance should be greater than or equal to 1300 um.

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 213 for details on adding a labware specification.

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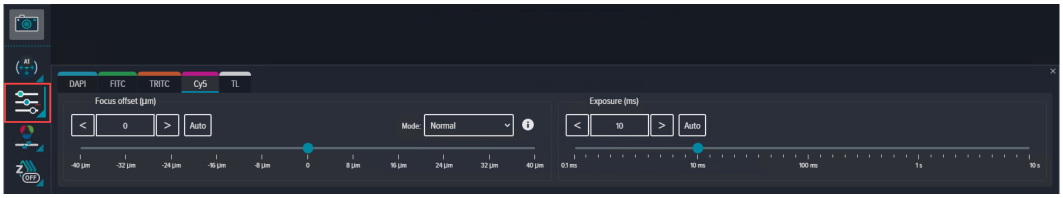
2. At the bottom of the screen on the **Choose Position to Acquire** tab, click **Select Well**.
3. In the well map, select the well for the preview. The instrument snaps a preview image of the newly selected well. The **A1** well is selected by default.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

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**.



7. On the **Focus/Exposure Settings** tab, adjust the settings as needed for each channel:
   a. Use the **Focus offset** controls to adjust the image sharpness. Click the **Mode** drop-down list box to set one of the following autofocus search ranges:
      - **Plate and Well Bottom**: Uses the hardware autofocus and labware thickness value to set the focal plane at the bottom of a well. Works best for flat samples, such as adherent cells.
      - **Normal**: Uses the hardware autofocus, labware specification, and image-based autofocus to find the best focus plane. Recommended for thick or uneven samples in a plate.
      - **Wide**: Similar to Normal mode, but the image-based autofocus has a wider search range.
      - **Superwide**: Similar to Normal mode, but the image-based autofocus searches from the well bottom up.

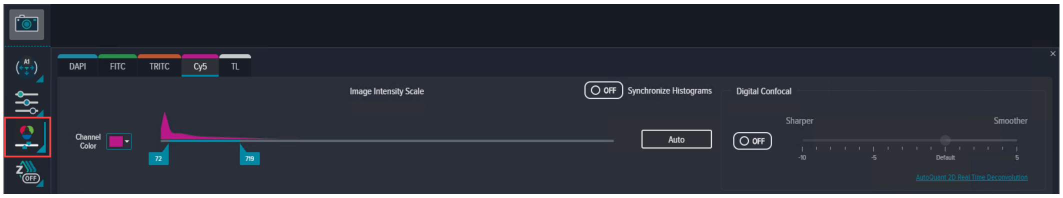
      Click next to the **Mode** drop-down list box or see Understanding How Autofocus Works with a Plate on page 46 for details on getting good autofocus results.
   b. Use the **Exposure** controls to adjust the exposure time for each wavelength, which affects image brightness and results in a broader dynamic range (that is, the difference between the brightest and dimmest object detected).

      When your 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 photodamage your sample.

      > Tip: Try using **Auto** with a known bright sample, such as a positive control.

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

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. Click **Auto** to set the best contrast based on the current preview.

    Note: If you need to adjust the image intensity scale for all channels simultaneously, you can set **Synchronize Histograms** to **On**. In most cases, however, you can leave setting **Off**.

11. To apply the optional digital confocal algorithm to the image, do the following:
    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.

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.

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 32 for details.

To continue to the next workflow step, click **Region Selection to Acquire**. See Region Selection to Acquire on page 53 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, wavelengths, 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: Exports 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. “Hidden” channels are shown slightly dimmed.

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* Tip: When an image is overexposed, the channel icons indicate the overexposure 🟧. 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.

61.97 µm DIGITAL ZOOM 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.
2. In the **Tools** pane on the right, click **Comparison Mode**.

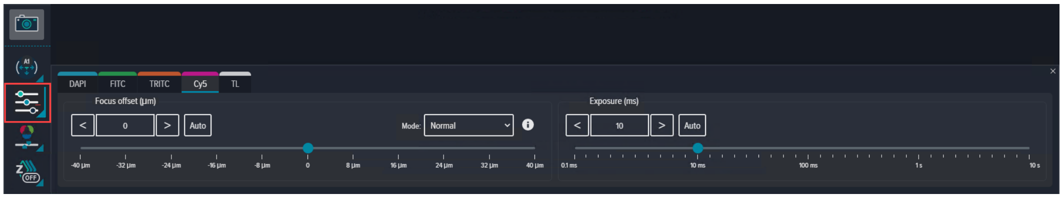
> **Note:** If **Z Stacking** is set to **On**, the Comparison Mode icon is disabled. You must set **Z stacking** to **Off** before you can start Comparison Mode.

3. At the bottom of the screen on the **Choose Position to Acquire** tab, click **Select 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 **A1** well is selected by default.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

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**.



Focus offset (µm): 0

Mode: Normal

Exposure (ms): 10

10. On the **Focus/Exposure Settings** tab, adjust the settings as needed for each channel:
    a. Use the **Focus offset** controls to adjust the image sharpness. Click the **Mode** drop-down list box to set one of the following autofocus search ranges:
        - **Plate and Well Bottom**: Uses the hardware autofocus and labware thickness value to set the focal plane at the bottom of a well. Works best for flat samples, such as adherent cells.
        - **Normal**: Uses the hardware autofocus, labware specification, and image-based autofocus to find the best focus plane. Recommended for thick or uneven samples in a plate.
        - **Wide**: Similar to Normal mode, but the image-based autofocus has a wider search range.
        - **Superwide**: Similar to Normal mode, but the image-based autofocus searches from the well bottom up.

       Click next to the **Mode** drop-down list box or see Understanding How Autofocus Works with a Plate on page 46 for details on getting good autofocus results.

    b. Use the **Exposure** controls to adjust the exposure time for each wavelength, which affects image brightness and results in a broader dynamic range (that is, the difference between the brightest and dimmest object detected).

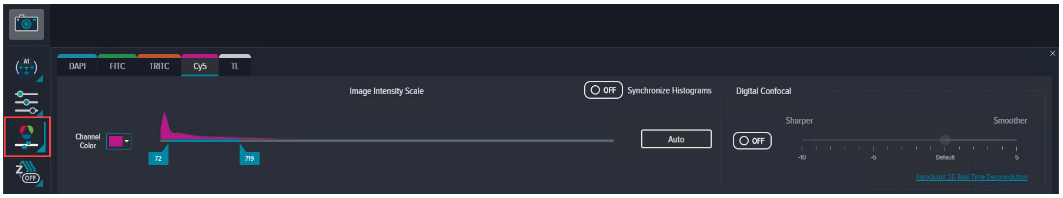
       When your image is in focus, click **Auto** to automatically determine the optimal exposure for the best dynamic range. The longer the exposure, the brighter the experiment image. Dimmer objects get brighter with longer exposure time, as does the background. Increased exposure time slows the experiment because each image takes longer. In addition, it can lead to image saturation and possibly photodamage your sample.

       ✱ Tip: Try using **Auto** with a known bright sample, such as a positive control.

       ---

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. Click **Auto** to set the best contrast based on the current preview.

> **Note:** If you need to adjust the image intensity scale for all channels simultaneously, you can set **Synchronize Histograms** to **On**. In most cases, however, you can leave setting **Off**.

14. To apply the optional digital confocal algorithm to the image, do the following:
    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.

> 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.

> 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.

To continue to the next workflow step, click **Region Selection to Acquire**. See Region Selection to Acquire on page 53 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, wavelength, 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: Exports 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, exports 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. “Hidden” channels are shown slightly dimmed.

Tip: When an image is overexposed, the channel icons indicate the overexposure 🟥. 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.

61.97 µm DIGITAL ZOOM 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.

Z 20µm: 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 20µm 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.

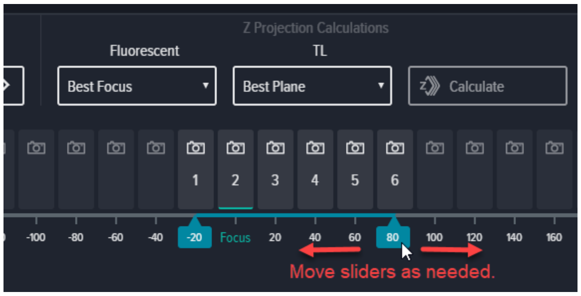
To snap Z stack images:

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
4x	50 µm
10x	20 µm
20x	5 µm
40x	2 µm
63x	1 µm

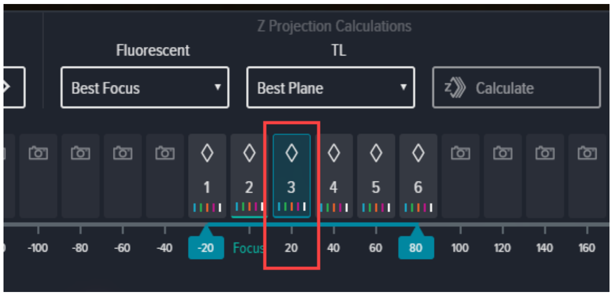
4. As needed, move the sliders to select the Z stack planes to include in the Z stack.



Move sliders as needed.

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.
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.

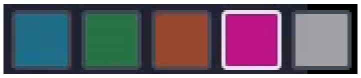
Note: The **Calculate** button becomes enabled when you modify settings that affect the preview image. Click **Calculate** to quickly view the updated preview image.

To continue to the next workflow step, click **Region Selection to Acquire**. See Region Selection to Acquire on page 53 for details.

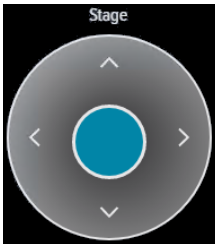
Viewing a Live Preview of a Plate

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, wavelength, 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 focus position offset value increases or decreases accordingly. A value in italics indicates that the current value has not been set as the focus offset position.

Sets the current **Focus Position** value as the focus offset position. The setting is reflected on the **Focus Offset** slider 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.
- 61.97 µm DIGITAL ZOOM 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 will time out and exit after five minutes.

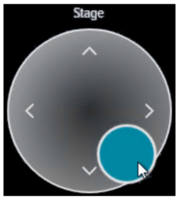
To view a live preview of a plate:

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 **A1** well is selected by default.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

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**.

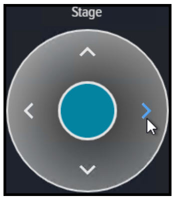
6. Use the stage joystick to move the sample (X-Y) stage to view different areas of the well:
   - For fast stage movements, click and drag the stage joystick in the direction you want to move toward the outermost position.

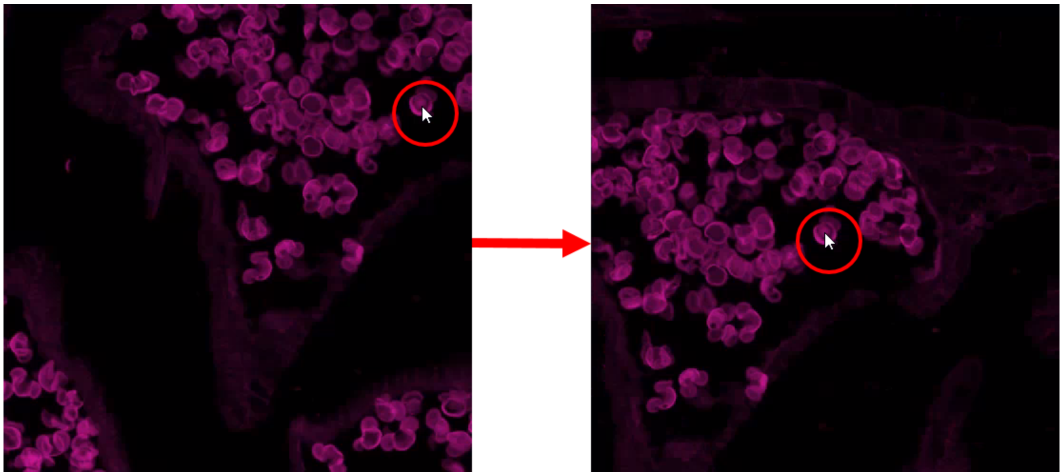
   - For slow stage movements, drag the stage joystick slightly.

   - 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.

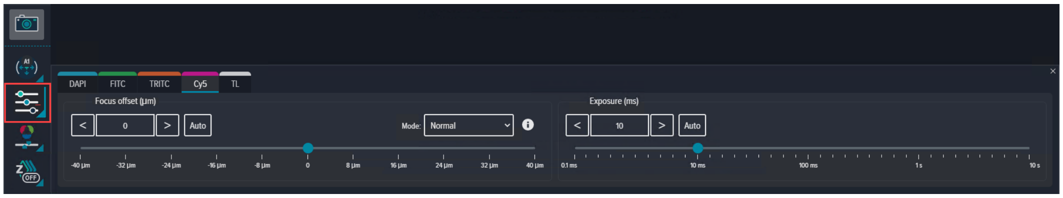
     

   - For fine focus adjustments, click one of the joystick arrows. Each time you click an arrow, the focus adjusts one step. The step size corresponds to half of the depth of field for the selected objective.

10. If needed, snap a preview of the well to confirm your focus offset settings.

11. Click **Focus/Exposure Settings**.



Focus offset (µm): 0

Mode: Normal

Exposure (ms): 10

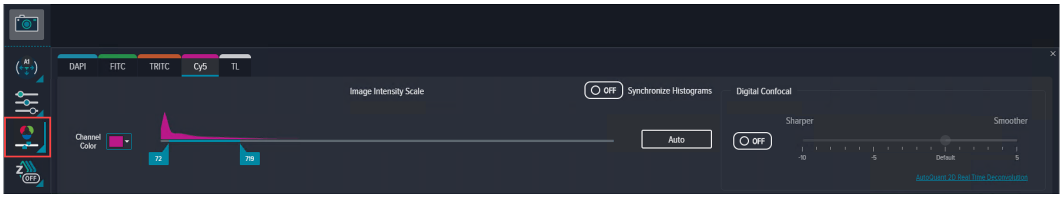
12. Use the **Exposure** controls to adjust the exposure time for each wavelength, which affects image brightness and results in a broader dynamic range (that is, the difference between the brightest and dimmest object detected).

    When your 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 photodamage your sample.

    Tip: Try using **Auto** with a known bright sample, such as a positive control.

    ---

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. Click **Auto** to set the best contrast based on the current preview.

Note: If you need to adjust the image intensity scale for all channels simultaneously, you can set **Synchronize Histograms** to **On**. In most cases, however, you can leave setting **Off**.

15. To apply the optional digital confocal algorithm to the image, do the following:
    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.

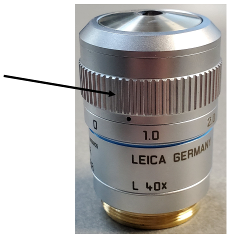
      

19. To save the focus offset for that channel click **Set Offset**. The setting is reflected on the **Focus Offset** slider on the **Focus/Exposure Settings** tab.
20. Repeat these steps as needed to find other regions of interest.
21. Repeat these steps as needed for each channel.

To continue to the next workflow step, click □ **Region Selection to Acquire**. See Region Selection to Acquire on page 53 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!

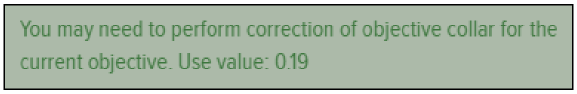
- To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling objectives and filter cubes.
- With the instrument power on, do not manually rotate the objective turret. Manually rotating the objective turret can damage the instrument.

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.

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.


You may need to perform correction of objective collar for the current objective. Use value: 0.19

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.

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 top 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 top door.
12. Click **OK**.

Understanding How Autofocus Works with a Plate

The instrument uses two focus mechanisms:

- **Hardware Autofocus**, which uses an LED beam to find reflective surfaces, is designed for speed. Hardware autofocus works well for adherent samples in plates or chamber slides.
- **Image-based Autofocus** searches a range for the best focus plane based on image contrast. Image-based autofocus (also known as "software autofocus") works well for slides with a coverslip or samples in plates that are not flat, such as suspension cells or spheroids.

Focus Modes

The focus modes differ in their method and search range when defining the Z-position for imaging. The Mode drop-down list box offers the following autofocus search ranges:

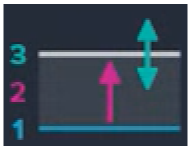
Plate and Well Bottom mode uses the hardware autofocus and labware thickness value to set the focal plane at the bottom of a well. Works best for flat samples, such as adherent cells. The autofocus steps are:

1. The hardware autofocus finds the plate bottom.
2. The hardware autofocus finds the well bottom.

Normal mode uses the hardware autofocus, labware specification, and image-based autofocus to find the best focus plane. Recommended for thick or uneven samples in a plate. The autofocus steps are:

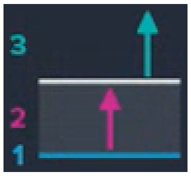
1. The hardware autofocus finds the plate bottom.
2. The software uses the Bottom Thickness value in the labware specification to determine the well bottom.
3. The image-based autofocus searches a range of ±7.5% of the bottom thickness around the well bottom.

Wide mode is similar to Normal mode, but the image-based autofocus has a wider search range. The autofocus steps are:



1. The hardware autofocus finds the plate bottom.
2. The software uses the Bottom Thickness value in the labware specification to determine the well bottom.
3. The image-based autofocus searches a range of ±20% of the bottom thickness around the well bottom.

Superwide mode is similar to Normal mode, but the image-based autofocus searches from the well bottom up. The autofocus steps are:



1. The hardware autofocus finds the plate bottom.
2. The software uses the Bottom Thickness value in the labware specification to determine the well bottom.
3. The image-based autofocus searches a range of 300 µm above the well bottom.

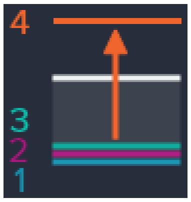
To find the best focus for a plate, start with the default **Plate and Well Bottom** mode. With imaging-quality labware, an accurate labware specification, and a relatively flat sample (for example, most adherent cells), the autofocus should provide good results.

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

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

Mimetas OrganoPlates

Mimetas OrganoPlates have special characteristics. These microfluidic plates have multiple surfaces close to the well bottom, which can make it difficult for the hardware autofocus to differentiate them. To address this situation, the **Bottom Thickness** value in the labware specification for the default Mimetas OrganoPlate is set at the lowest possible setting, which is 0.001 mm. Use **Normal** mode or **Wide** mode; in either case, the search range is very narrow. (The other focus modes will likely not provide good results.) Then, change the **Focus Offset** value on the **Focus/Exposure Settings** tab until you find the desired focus plane. The autofocus steps are:



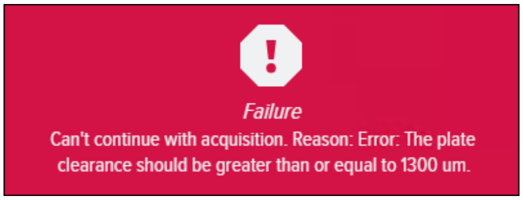
1. The hardware autofocus finds the plate bottom.
2. The software uses the Bottom Thickness value in the labware specification (which is 0.001 mm) to define the well bottom just above of the plate bottom.
3. The image-based autofocus searches a very narrow range around the well bottom (either ±7.5% or ±20% of the 0.001 mm bottom thickness, depending on the selected focus mode).
4. Change the Focus Offset value on the Focus Exposure Settings tab until you find the best focal plane. With the optional Z stacking feature, you may want to acquire a test Z stack to help determine the Focus Offset value.

Using Plates in the Environmental Control Cassette

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

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

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



Failure

Can't continue with acquisition. Reason: Error: The plate clearance should be greater than or equal to 1300 um.

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 213 for details on adding a labware specification.

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 modes. It determines the search range used to detect the plate bottom.
- **Bottom Thickness** is important for the Normal, Wide, and Superwide focus modes. 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 small or too big, 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 these settings. See Labware Library on page 213 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 great. 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.

Troubleshooting Autofocus Issues

The CellReporterXpress software uses hardware and image-based autofocus to determine 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 213 for details.

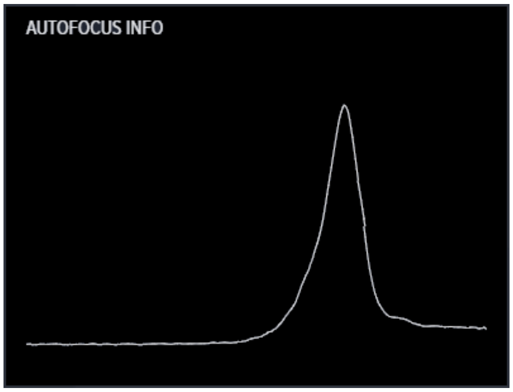
The **Autofocus Info** graph on the **Acquisition Settings** page shows the focus peaks for the most recent autofocus to help you understand how it was determined. This section describes how you can use this information to troubleshoot autofocus issues.

To troubleshoot autofocus issues:

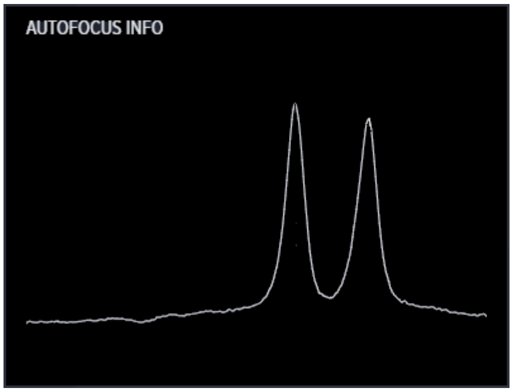
1. On the **Acquisition Settings** page, click **Snap Image** to snap a preview image.
2. On the right side of the screen under **Tools**, click **Autofocus Info** to display a graph showing the focus peaks of the autofocus.
3. Review the **Autofocus Info** graph along the following examples.

Good Autofocus

The following graph shows a good result of a Normal, Wide, or Superwide (single-peak) autofocus. The software typically uses single-peak autofocus for plates with a 4x objective and the **Mode** set to **Normal** on the **Focus/Exposure Settings** tab of the **Acquisition Settings** page.

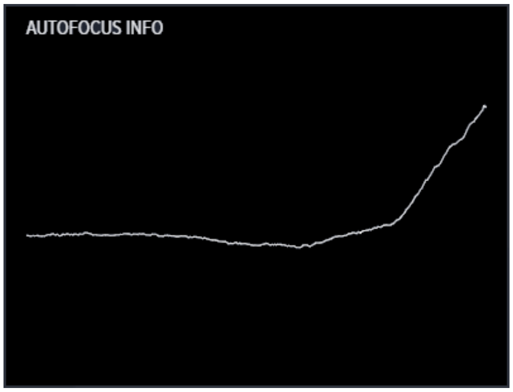


The following graph shows a good result of a Plate and Well Bottom (two-peak) autofocus. The software typically uses two-peak autofocus for plates with the **Mode** set to **Plate and Well Bottom** on the **Focus/Exposure Settings** tab of the **Acquisition Settings** page.

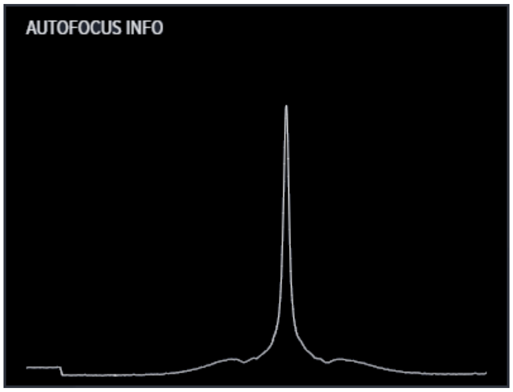


Autofocus Issues

The following graph shows a failed result of a Normal (single-peak) autofocus. The incomplete single peak on the right side of the graph indicates that the value specified the well depth may be too high or the value specified for the skirt height may be too low. See Labware Library on page 213 for details.

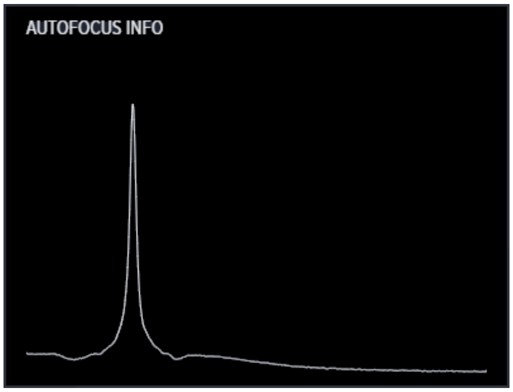


The following graph shows a failed result of a Plate and Well Bottom (two-peak) autofocus, where you would expect to see two peaks on the graph. The single peak in the center indicates that the value specified for the plate thickness may be too small. See Labware Library on page 213 for details.

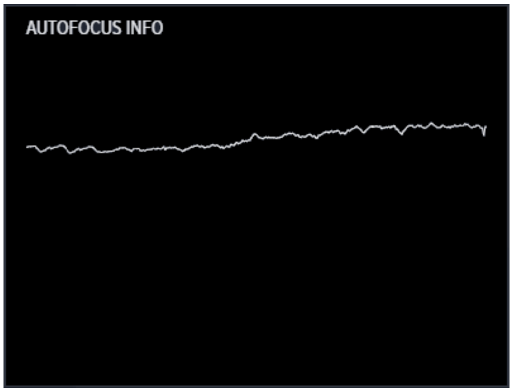


The following graph shows a failed result of a Plate and Well Bottom (two-peak) autofocus. The single peak on the right side of the graph indicates that the value specified the well depth may be too high or the value specified for the skirt height may be too low. See Labware Library on page 213 for details.

The following graph shows a failed result of a Plate and Well Bottom (two-peak) autofocus. The single peak on the left side of the graph indicates that the value specified for the well depth may be too low or the value specified for the skirt height may be too high. See Labware Library on page 213 for details.



The following graph shows a general autofocus failure. No peaks are found, which likely indicates that there is no plate in the labware holder. It is also possible that the wrong labware is selected. If these are not true, remeasure the plate. See Labware Library on page 213 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.

Random: Randomly adds an acquisition region selection overlay. You can control various elements of the acquisition region, including the percentage of the well.

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 54 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.

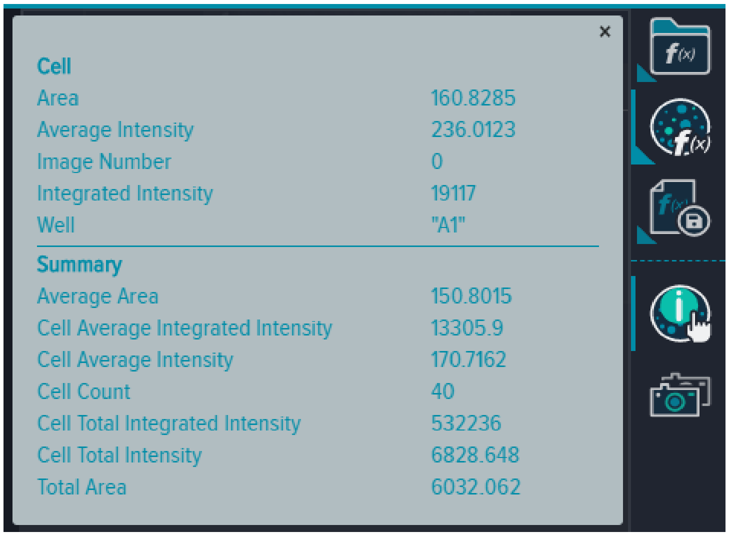
Setting Up an Analysis

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 257 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.



Cell	
Area	160.8285
Average Intensity	236.0123
Image Number	0
Integrated Intensity	19117
Well	"A1"
Summary	
Average Area	150.8015
Cell Average Integrated Intensity	13305.9
Cell Average Intensity	170.7162
Cell Count	40
Cell Total Integrated Intensity	532236
Cell Total Intensity	6828.648
Total Area	6032.062

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

- Testing the Analysis of a Well, see page 56
- Testing the Analysis of Comparison Images, see page 57
- Saving Analysis Settings, see page 58

To continue to the next workflow step, click **Region Selection to Analyze**. See Region Selection to Analyze on page 59 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.

> **Note:** You typically do not need to adjust the image intensity settings, which use the Acquisition settings. If you do, click **Image Intensity Scale** and adjust the settings as needed for each channel. Use the **Channel Color** drop-down list box to change the identification color for the channel. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image. Click **Auto** to set the best contrast based on the current preview.

5. Click **Choose Position to Acquire**.
6. Click **Select Well**.
7. In the plate map, select a different well.
   The software runs a test analysis.
8. 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.
9. Click **Algorithm Input**.
10. Click **Test Analysis** to preview the analysis.
11. 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 58 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.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

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.

Note: You typically do not need to adjust the image intensity settings, which use the Acquisition settings. If you do, click **Image Intensity Scale** and adjust the settings as needed for each channel. Use the **Channel Color** drop-down list box to change the identification color for the channel. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image. Click **Auto** to set the best contrast based on the current preview.

13. 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 page 58 for details.

Saving Analysis Settings

When you are satisfied with the quality of the preview in Testing the Analysis of a Well on page 56 or Testing the Analysis of Comparison Images on page 57, 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 59 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 60 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 61 for details.

Device Sensors

Device Sensors is the step where you specify the environmental sensors to be monitored for the experiment. The experiment data will indicate if the sensors were within the specified range during the experiment or outside of it.

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 227 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 63 for details.

Time Series

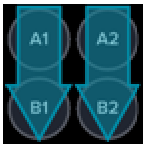
Time Series is an optional step where you set up a time series for image acquisition. This enables you to acquire images at multiple time points.

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 227 for details.

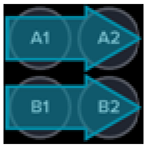
To set up a time series:

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:

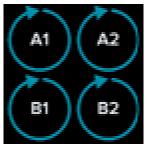
All Wells: Performs a complete time series on all selected wells. This option requires the least amount of time because all selected wells are included in each time series. This means that the fewest number of time series are performed.



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.



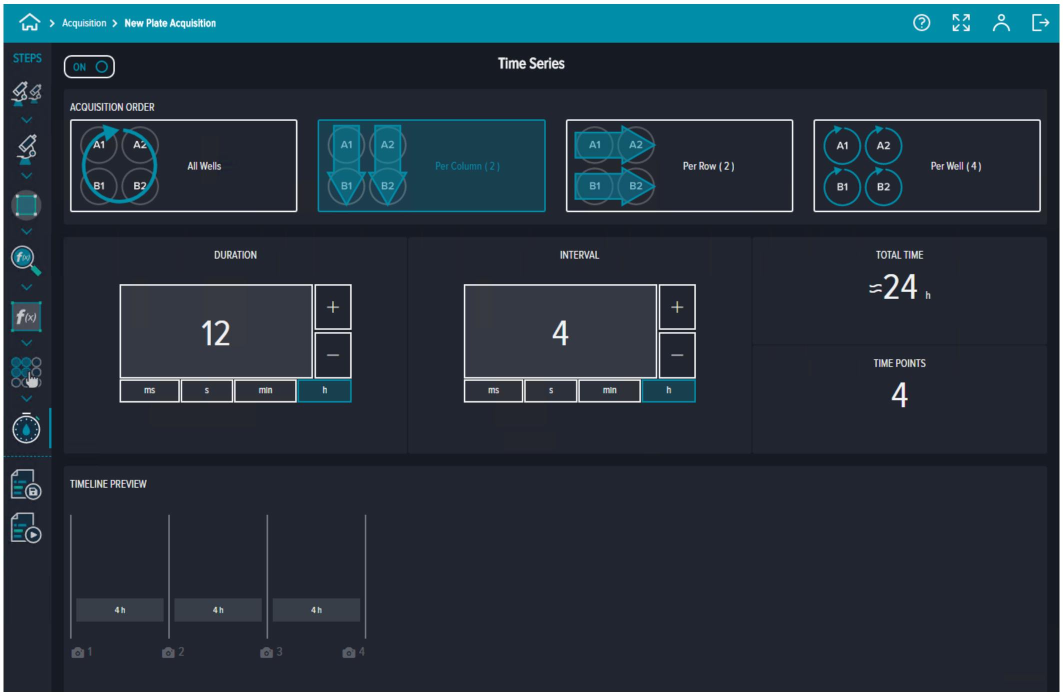
Per Well: Performs a complete time series on each selected well, then moves on to the next well. This option requires the most time because the time series is repeated for each selected well.

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
   - Wavelength
   - 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.



DURATION 12 h

INTERVAL 4 h

TOTAL TIME ≈24 h

TIME POINTS 4

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 65 or Run Protocol on page 66 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** screen, 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 255 for details.
4. Click **Save Protocol**.

To continue to the next workflow step, click **Run Protocol**. See Run Protocol on page 66 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, in the **Experiment Details** pane, 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 experiment labware.
   c. If needed, in the **Experiment Description** field, enter a description of the experiment.
   d. To save TIFF images of the acquisition, select the **Save Raw Images** check box.
   e. 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 68 for details.
   f. On the **Acquisition Parameters** tab, review the settings.
   g. 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 computer 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 255 for details.

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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 209 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.

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.

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 71 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 73 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 101 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 102 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 107 for details.

Device Sensors is the step where you specify the environmental sensors to be monitored for the experiment. The experiment data will indicate if the sensors were within the specified range during the experiment or outside of it. See Device Sensors on page 108 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 109 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 110 for details.

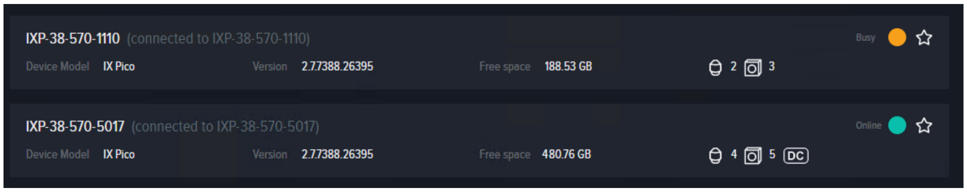
Acquisition Device

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 Devices on page 216 for details on adding other instruments in your network.



IXP-38-570-1110 (connected to IXP-38-570-1110) Busy
Device Model IX Pico Version 2.7.7388.26395 Free space 188.53 GB 2 3

IXP-38-570-5017 (connected to IXP-38-570-5017) Online
Device Model IX Pico Version 2.7.7388.26395 Free space 480.76 GB 4 5 DC

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 73.

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 99 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 79 for details.

Download Plane Images: Exports 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, exports a TIFF image of the current Z stack projection and each individual Z stack plane.

Specifying the Labware Specification

To specify the labware specification:

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, you can add a new one. See Labware Library on page 213 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 75
- Snapping Slide Comparison Previews, see page 79
- Snapping Z Stack Images, see page 83
- Viewing a Live Preview of a Slide, see page 87
- Adjusting an Objective Correction Collar, see page 95
- Understanding How Autofocus Works with a Slide, see page 97
- Troubleshooting Autofocus Issues, see page 99

To continue to the next workflow step, click **Region Selection to Acquire**. See Region Selection to Acquire on page 101 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, wavelength, slide, region, focus settings, and exposure settings.

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

Download Plane Images: Exports 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. "Hidden" channels are shown slightly dimmed.

Tip: When an image is overexposed, the channel icons indicate the overexposure 🟥. 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.

61.97 µm DIGITAL ZOOM 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.

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

Failure

Can't continue with acquisition. Reason: Error: The acquisition was aborted because could not find focus.

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 99 for details.

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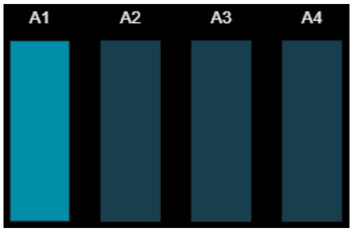
2. At the bottom of the screen on the **Choose Position to Acquire** tab, click **Select Slide**.
3. In the slide holder map, select the slide for the preview. The instrument snaps a preview image of the newly selected slide. The **A1** slide is selected by default.



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.

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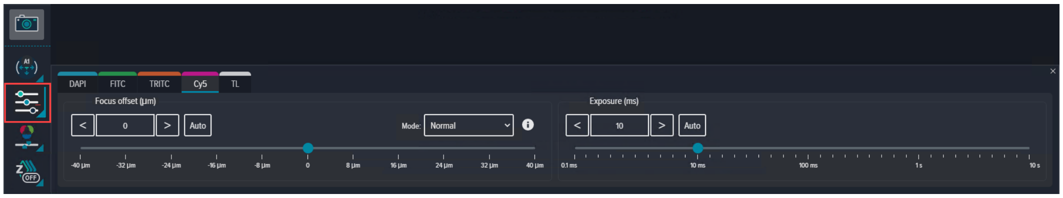
7. Click **Focus/Exposure Settings**.



Focus offset (µm) 0

Mode: Normal

Exposure (ms) 10

8. On the **Focus/Exposure Settings** tab, adjust the settings as needed for each channel:
   a. Use the **Focus offset** controls to adjust the image sharpness. Click the **Mode** drop-down list box to set one of the following autofocus search ranges:
      - **Plate and Well Bottom**: Uses the hardware autofocus and labware thickness value to set the focal plane at the bottom of a chamber on a chamber slide. Works best for flat samples, such as adherent cells. Not intended for use with slides with a coverslip.
      - **Normal**: Uses the hardware autofocus, labware specification, and image-based autofocus to find the best focus plane. Works well for slides with a coverslip.
      - **Wide**: Similar to Normal mode, but the image-based autofocus has a wider search range.
      - **Superwide**: Similar to Normal mode, but the image-based autofocus searches from the slide/coverslip top up.

      Click ⓘ next to the **Mode** drop-down list box or see Understanding How Autofocus Works with a Slide on page 97 for details on getting good autofocus results.

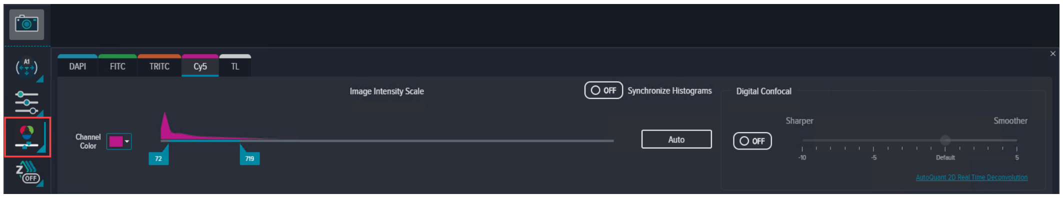
   b. Use the **Exposure** controls to adjust the exposure time for each wavelength, which affects image brightness and results in a broader dynamic range (that is, the difference between the brightest and dimmest object detected).

      When your 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 photodamage your sample.

      Tip: Try using **Auto** with a known bright sample, such as a positive control.

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

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. Click **Auto** to set the best contrast based on the current preview.

    Note: If you need to adjust the image intensity scale for all channels simultaneously, you can set **Synchronize Histograms** to **On**. In most cases, however, you can leave setting **Off**.

12. To apply the optional digital confocal algorithm to the image, do the following:
    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.

    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.

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 83 for details.

To continue to the next workflow step, click **Region Selection to Acquire**. See Region Selection to Acquire on page 101 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, wavelengths, 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**: Exports 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. “Hidden” channels are shown slightly dimmed.

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* Tip: When an image is overexposed, the channel icons indicate the overexposure 🟥. 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.

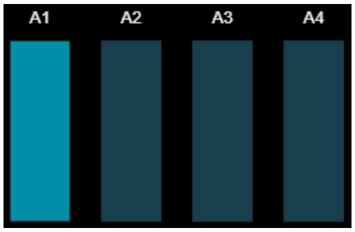
61.97 µm DIGITAL ZOOM Indicates the current zoom scale. **Digital Zoom** indicates that the image is zoomed in digitally (that is, not optically) and might not represent the actual quality of the image.

To snap slide comparison images:

1. On the **Acquisition Settings** page, click **Snap Image** to snap an initial preview image.
2. In the **Tools** pane on the right, click **Comparison Mode**.

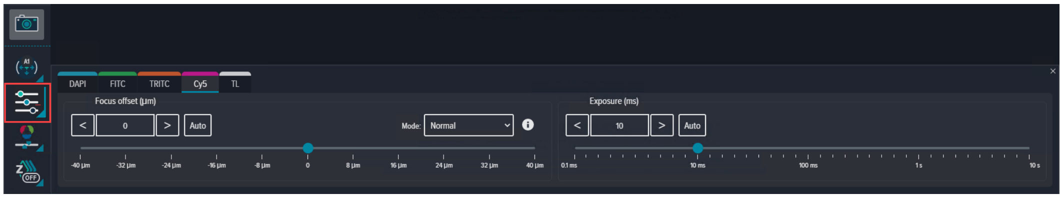
> **Note:** If **Z Stacking** is set to **On**, the Comparison Mode icon is disabled. You must set **Z stacking** to **Off** before you can start Comparison Mode.

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**.



11. On the **Focus/Exposure Settings** tab, adjust the settings as needed for each channel:
    a. Use the **Focus offset** controls to adjust the image sharpness. Click the **Mode** drop-down list box to set one of the following autofocus search ranges:
        - **Plate and Well Bottom**: Uses the hardware autofocus and labware thickness value to set the focal plane at the bottom of a chamber on a chamber slide. Works best for flat samples, such as adherent cells. Not intended for use with slides with a coverslip.
        - **Normal**: Uses the hardware autofocus, labware specification, and image-based autofocus to find the best focus plane. Works well for slides with a coverslip.
        - **Wide**: Similar to Normal mode, but the image-based autofocus has a wider search range.
        - **Superwide**: Similar to Normal mode, but the image-based autofocus searches from the slide/coverslip top up.

       Click ⓘ next to the **Mode** drop-down list box or see Understanding How Autofocus Works with a Slide on page 97 for details on getting good autofocus results.

    b. Use the **Exposure** controls to adjust the exposure time for each wavelength, which affects image brightness and results in a broader dynamic range (that is, the difference between the brightest and dimmest object detected).

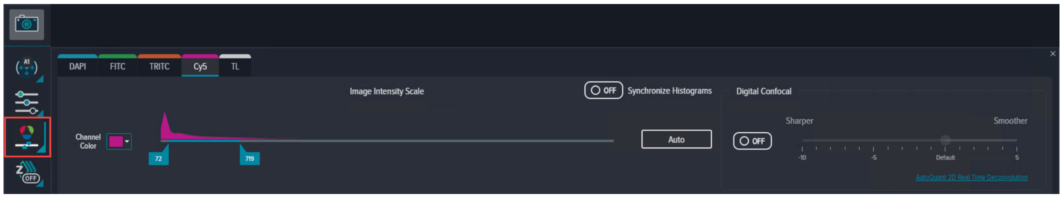
       When your image is in focus, click **Auto** to automatically determine the optimal exposure for the best dynamic range. The longer the exposure, the brighter the experiment image. Dimmer objects get brighter with longer exposure time, as does the background. Increased exposure time slows the experiment because each image takes longer. In addition, it can lead to image saturation and possibly photodamage your sample.

       Tip: Try using **Auto** with a known bright sample, such as a positive control.

       ---

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. Click **Auto** to set the best contrast based on the current preview.

Note: If you need to adjust the image intensity scale for all channels simultaneously, you can set **Synchronize Histograms** to **On**. In most cases, however, you can leave setting **Off**.

15. To apply the optional digital confocal algorithm to the image, do the following:
    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.

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.

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.

To continue to the next workflow step, click **Region Selection to Acquire**. See Region Selection to Acquire on page 101 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, wavelength, 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: Exports 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, exports 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. “Hidden” channels are shown slightly dimmed.

Tip: When an image is overexposed, the channel icons indicate the overexposure 🟥. 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.

61.97 µm DIGITAL ZOOM 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.

Z 20µm: 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 20µm 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 75 for details.

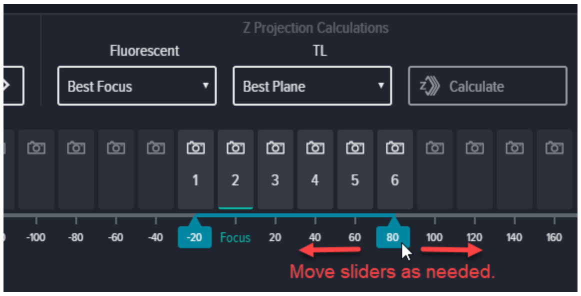
To snap Z stack images:

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.

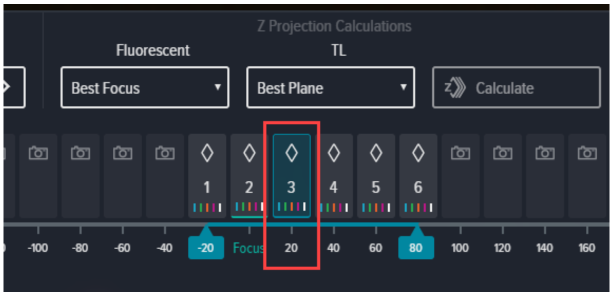
Objective	Default Focus Step Distance
4x	50 µm
10x	20 µm
20x	5 µm
40x	2 µm
63x	1 µm

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.
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.
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.

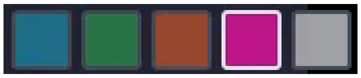
Note: The **Calculate** button becomes enabled when you modify settings that affect the preview image. Click **Calculate** to quickly view the updated Z stack images.

To continue to the next workflow step, click **Region Selection to Acquire**. See Region Selection to Acquire on page 101 for details.

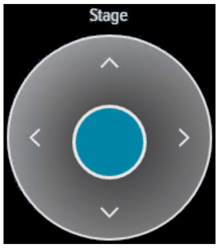
Viewing a Live Preview of a Slide

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, wavelength, 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 focus position offset value increases or decreases accordingly. A value in italics indicates that the current value has not been set as the focus offset position.

Sets the current **Focus Position** value as the focus offset position. The setting is reflected on the **Focus Offset** slider 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.

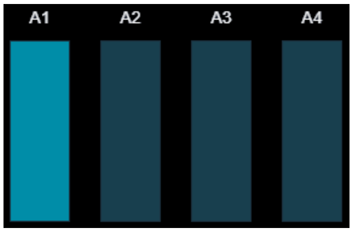
61.97 µm DIGITAL ZOOM Indicates the current zoom scale. **Digital Zoom** indicates that the image is zoomed in digitally (that is, not optically) and might not represent the actual quality of the image.

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 will time out and exit after five minutes.

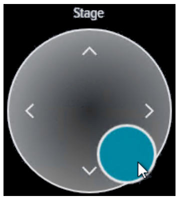
To view a live preview of a slide:

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**.

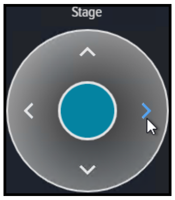
7. Use the stage joystick to move the sample (X-Y) stage to view different areas of the slide:
   - For fast stage movements, click and drag the stage joystick in the direction you want to move toward the outermost position.

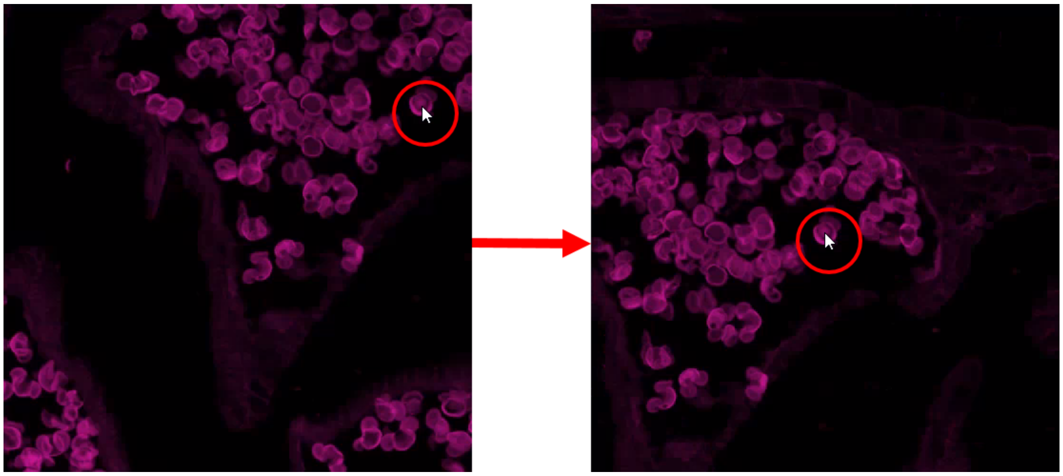
   - For slow stage movements, drag the stage joystick slightly.

   - 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.

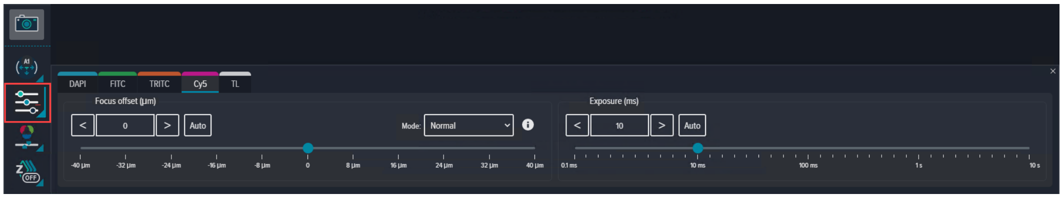
      

    - For fine focus adjustments, click one of the joystick arrows. Each time you click an arrow, the focus adjusts one step. The step size corresponds to half of the depth of field for the selected objective.

11. If needed, snap a preview of the slide to confirm your focus offset settings.

12. Click **Focus/Exposure Settings**.



Focus offset (µm): 0

Mode: Normal

Exposure (ms): 10

13. Use the **Exposure** controls to adjust the exposure time for each wavelength, which affects image brightness and results in a broader dynamic range (that is, the difference between the brightest and dimmest object detected).

    When your 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 photodamage your sample.

Tip: Try using **Auto** with a known bright sample, such as a positive control.

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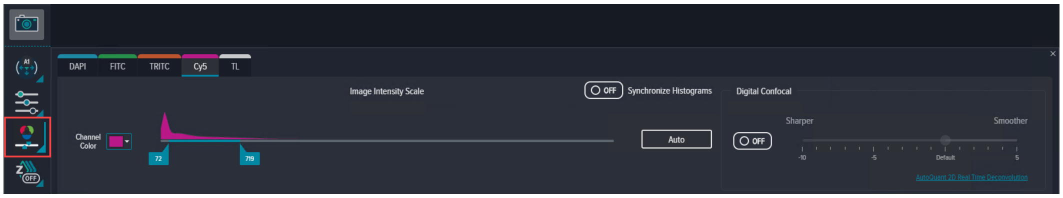
14. Click **Image Intensity Settings**.



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. Click **Auto** to set the best contrast based on the current preview.

> **Note:** If you need to adjust the image intensity scale for all channels simultaneously, you can set **Synchronize Histograms** to **On**. In most cases, however, you can leave setting **Off**.

16. To apply the optional digital confocal algorithm to the image, do the following:
    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.

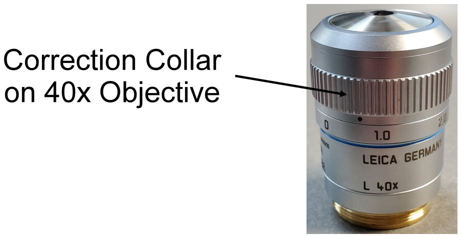
    - For fine focus adjustments, click one of the joystick arrows. Each time you click an arrow, the focus adjusts one step. The step size corresponds to half of the depth of field for the selected objective.

20. To save the focus offset for that channel click **Set Offset**. The setting is reflected on the **Focus Offset** slider 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 101 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!

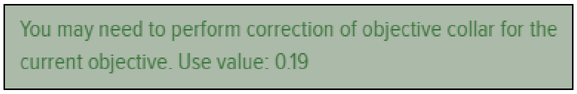
- To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling objectives and filter cubes.
- With the instrument power on, do not manually rotate the objective turret. Manually rotating the objective turret can damage the instrument.

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.

   You may need to perform correction of objective collar for the current objective. Use value: 0.19

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 top 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 top door.
12. Click **OK**.

Understanding How Autofocus Works with a Slide

The instrument uses two focus mechanisms:

- **Hardware Autofocus**, which uses an LED beam to find reflective surfaces, is designed for speed. Hardware autofocus works well for adherent samples in plates or chamber slides.
- **Image-based Autofocus** searches a range for the best focus plane based on image contrast. Image-based autofocus (also known as "software autofocus") works well for slides with a coverslip or samples in plates that are not flat, such as suspension cells or spheroids.

Focus Modes

The focus modes differ in their method and search range when defining the Z-position for imaging. The Mode drop-down list box offers the following autofocus search ranges:

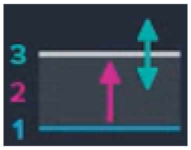
Plate and Well Bottom mode uses the hardware autofocus and labware thickness value to set the focal plane at the bottom of a chamber on a chamber slide. Works best for flat samples, such as adherent cells. Not intended for use with slides with a coverslip. The autofocus steps are:

1. The hardware autofocus finds the chamber slide bottom.
2. The hardware autofocus finds the well bottom.

Normal mode uses the hardware autofocus, labware specification, and image-based autofocus to find the best focus plane. Works well for slides with a coverslip. The autofocus steps are:

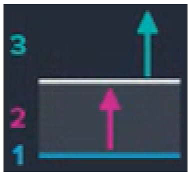
1. The hardware autofocus finds the slide/coverslip bottom.
2. The software uses the Surface Thickness value in the labware specification to determine the slide/coverslip surface.
3. The image-based autofocus searches a range of ±7.5% of the surface thickness or 25 µm around the slide/coverslip top (whichever is greater).

Wide mode is similar to Normal mode, but the image-based autofocus has a wider search range. Recommended for thick and uneven samples on slides with a coverslip. The autofocus steps are:



1. The hardware autofocus finds the slide/coverslip bottom.
2. The software uses the Surface Thickness value in the labware specification to determine the slide/coverslip surface.
3. The image-based autofocus searches a range of ±20% of the surface thickness around the slide/coverslip top.

Superwide mode is similar to Normal mode, but the image-based autofocus searches from the slide/coverslip top up. The autofocus steps are:



1. The hardware autofocus finds the slide/coverslip bottom.
2. The software uses the Surface Thickness value in the labware specification to determine the slide/coverslip surface.
3. The image-based autofocus searches a range of 300 µm above the slide/coverslip top.

Slides with a Coverslip

To find the best focus for a slide with a coverslip, first try **Normal** mode. If you are unsatisfied with the focus and your sample is thick or uneven, try **Wide** mode and **Superwide** mode to improve the result.

After you set the focus mode, click **Auto** next to the **Focus Offset** controls to determine the focus offset between stains. Do this for each stain in the acquisition. The image-based autofocus bases its search on the first stain in the acquisition, so consider the order in which the stains are acquired. See Acquisition Settings on page 73 for details.

Most Chamber Slides

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

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

After you set the focus mode, click **Auto** next to the **Focus Offset** controls to determine the focus offset between stains. Do this for each stain in the acquisition. The image-based autofocus bases its search on the first stain in the acquisition, so consider the order in which the stains are acquired. See Acquisition Settings on page 73 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 for the Normal, Wide, and Superwide focus modes. It determines the base focus search range around the coverslip surface where the sample is located. If the Surface Thickness value is too small or too big, 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 213 for details.

Troubleshooting Autofocus Issues

The CellReporterXpress software uses hardware and image-based autofocus to determine 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 213 for details.

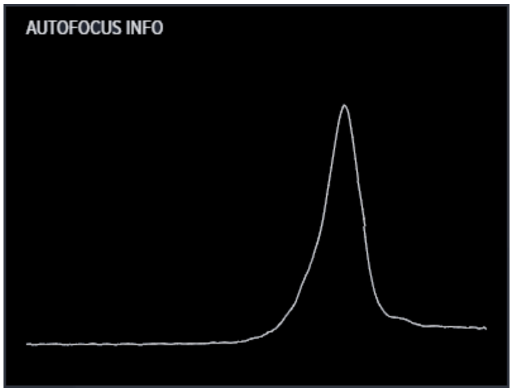
The **Autofocus Info** graph on the **Acquisition Settings** page shows the focus peaks for the most recent autofocus to help you understand how it was determined. This section describes how you can use this information to troubleshoot autofocus issues.

To troubleshoot autofocus issues:

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.

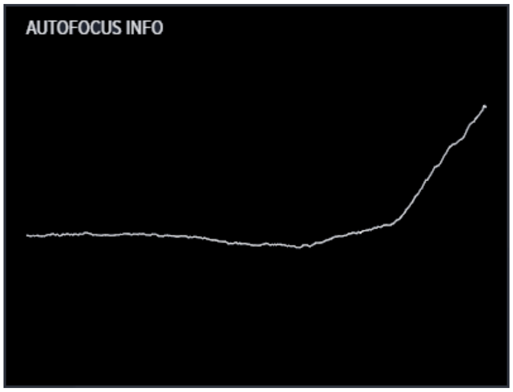
Good Autofocus

The following graph shows a good result of a Normal, Wide, or Superwide (single-peak) autofocus. The software typically uses single-peak autofocus for slides.

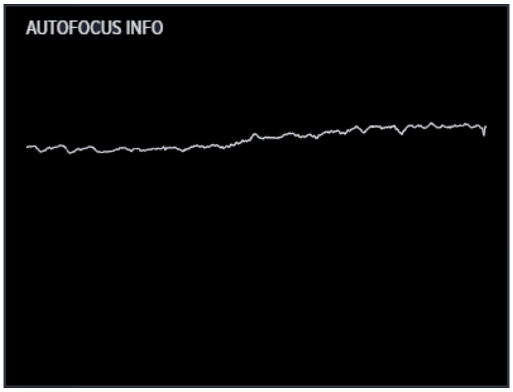


Autofocus Issues

The following graph shows a failed result of a Normal, Wide, or Superwide (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 213 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. It is also possible that the wrong labware is selected. See Labware Library on page 213 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 109 or Run Protocol on page 110 for details.

To continue to the next workflow step for all other acquisitions, click **Analysis Settings**. See Analysis Settings on page 102 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.

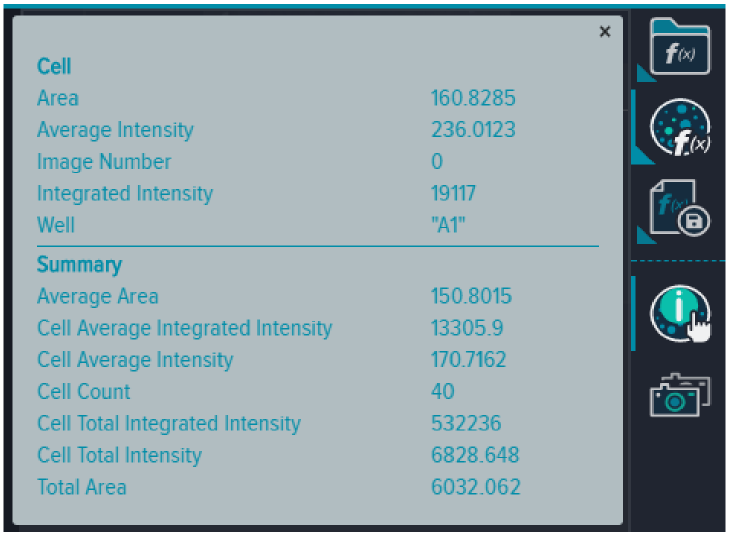
Setting Up an Analysis

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 257 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.



Cell	
Area	160.8285
Average Intensity	236.0123
Image Number	0
Integrated Intensity	19117
Well	"A1"
Summary	
Average Area	150.8015
Cell Average Integrated Intensity	13305.9
Cell Average Intensity	170.7162
Cell Count	40
Cell Total Integrated Intensity	532236
Cell Total Intensity	6828.648
Total Area	6032.062

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

- Testing the Analysis of a Region, see page 104
- Testing the Analysis of Comparison Images, see page 105
- Saving Analysis Settings, see page 106

To continue to the next workflow step, click **Region Selection to Analyze**. See Region Selection to Analyze on page 107 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.

   Note: You typically do not need to adjust the image intensity settings, which use the Acquisition settings. If you do, click **Image Intensity Scale** and adjust the settings as needed for each channel. Use the **Channel Color** drop-down list box to change the identification color for the channel. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image. Click **Auto** to set the best contrast based on the current preview.

5. Click **Choose Position to Acquire**.
6. Click **Select 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, 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.
9. Click **Algorithm Input**.
10. Click **Test Analysis** to preview the analysis.
11. 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 106 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 A1 **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 H12 **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.

> **Note:** You typically do not need to adjust the image intensity settings, which use the Acquisition settings. If you do, click **Image Intensity Scale** and adjust the settings as needed for each channel. Use the **Channel Color** drop-down list box to change the identification color for the channel. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image. 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 page 106 for details.

Saving Analysis Settings

When you are satisfied with the quality of the preview in Testing the Analysis of a Region on page 104 or Testing the Analysis of Comparison Images on page 105, 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 107 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 108 for details.

Device Sensors

Device Sensors is the step where you specify the environmental sensors to be monitored for the experiment. The experiment data will indicate if the sensors were within the specified range during the experiment or outside of it.

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

See Sensors on page 227 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 109 or Run Protocol on page 110 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** screen, 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 255 for details.
4. Click **Save Protocol**.

To continue to the next workflow step, click **Run Protocol**. See Run Protocol on page 110 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, in the **Experiment Details** pane, 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 experiment labware.
   c. If needed, in the **Experiment Description** field, enter a description of the experiment.
   d. To save TIFF images of the acquisition, select the **Save Raw Images** check box.
   e. 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 112 for details.
   f. On the **Acquisition Parameters** tab, review the settings.
   g. 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 computer 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 255 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 209 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.

> 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.

3. Click **Run Protocol** to return to **Run Protocol** page.

Chapter 4: Experiments Mode

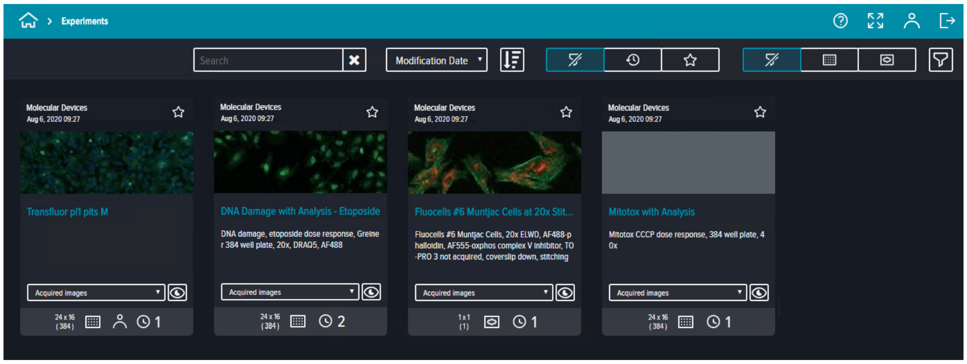
4

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.



Molecular Devices
Aug 6, 2020 09:27
Transfluor pl1 pits M
24 x 16 (384) ▦ 👤 🕓 1

Molecular Devices
Aug 6, 2020 09:27
DNA Damage with Analysis - Etoposide
DNA damage, etoposide dose response, Greiner 384 well plate, 20x, DRAQ5, AF488
24 x 16 (384) ▦ 🕓 2

Molecular Devices
Aug 6, 2020 09:27
Fluocells #6 Muntjac Cells at 20x Stit...
Fluocells #6 Muntjac Cells, 20x ELWD, AF488-phalloidin, AF555-oxphos complex V inhibitor, TO-PRO 3 not acquired, coverslip down, stitching
1 x 1 (1) ▭ 🕓 1

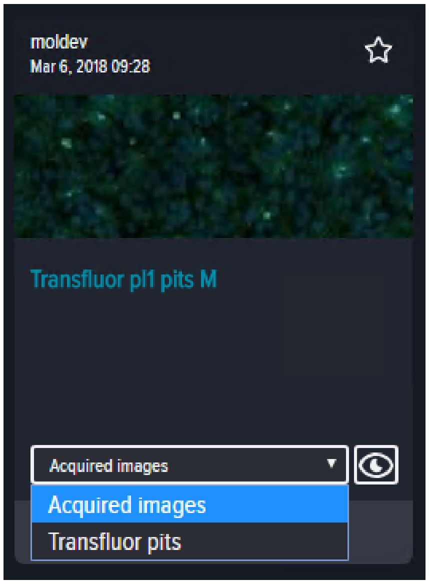
Molecular Devices
Aug 6, 2020 09:27
Mitotox with Analysis
Mitotox CCCP dose response, 384 well plate, 40x
24 x 16 (384) ▦ 🕓 1

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:

- 12 x 8 (96) **Geometry**
- ▦ **Plate** or ▭ **Slide**
- 👤 **Shared**
- 🕓 4 **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**

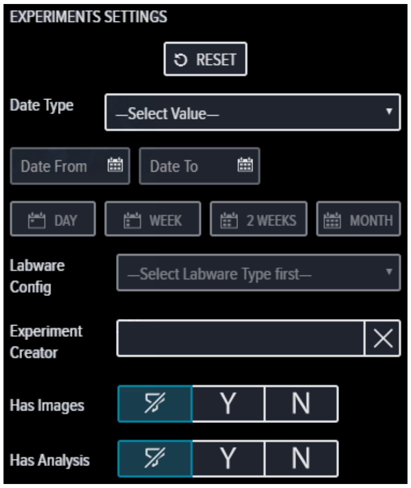
To use filter icons:

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

Complex Filters

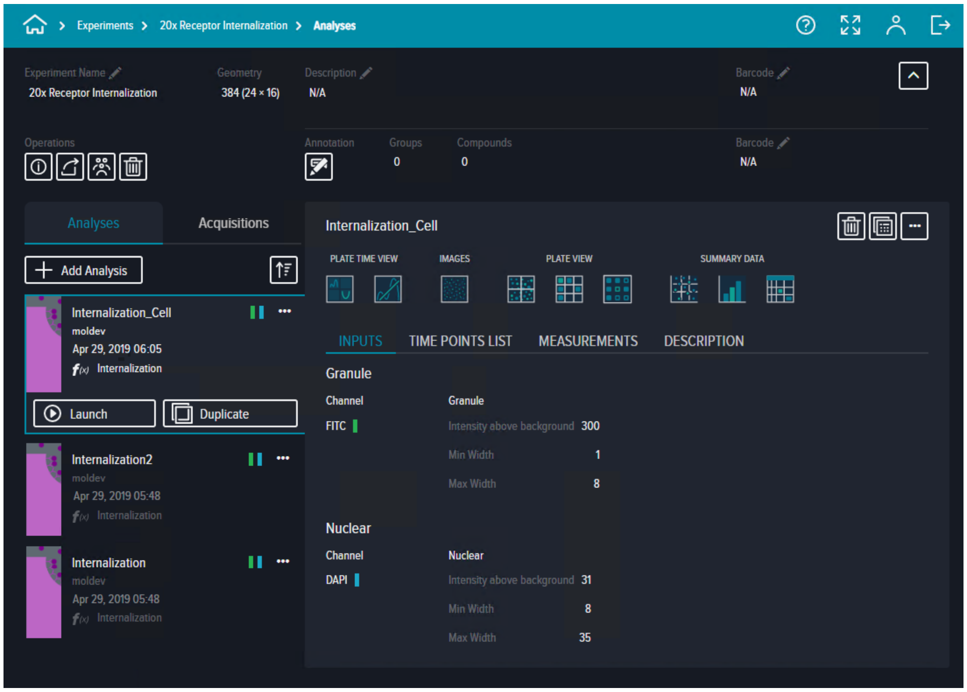
Click **Filter** to create more complex filters. For example, you can filter for experiments created within a specific date range or filter for experiments created by one of three specified users.

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.



Experiment Name: 20x Receptor Internalization
Geometry: 384 (24 × 16)
Description: N/A
Barcode: N/A
Groups: 0
Compounds: 0
Barcode: N/A

Internalization_Cell
moldev
Apr 29, 2019 06:05
f(x) Internalization

Internalization2
moldev
Apr 29, 2019 05:48
f(x) Internalization

Internalization
moldev
Apr 29, 2019 05:48
f(x) Internalization

Internalization_Cell

Granule
Channel: FITC
Granule
Intensity above background 300
Min Width 1
Max Width 8

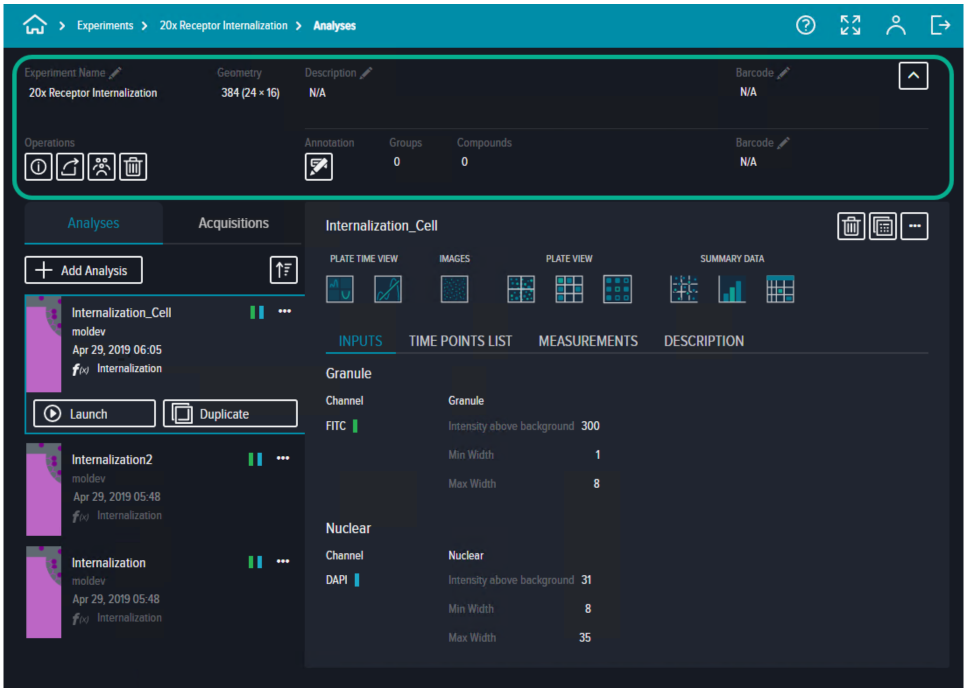
Nuclear
Channel: DAPI
Nuclear
Intensity above background 31
Min Width 8
Max Width 35

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.



Experiment Name: 20x Receptor Internalization
Geometry: 384 (24 × 16)
Description: N/A
Barcode: N/A
Annotation
Groups: 0
Compounds: 0
Barcode: N/A

Internalization_Cell
moldev
Apr 29, 2019 06:05
f(x) Internalization

Internalization2
moldev
Apr 29, 2019 05:48
f(x) Internalization

Internalization
moldev
Apr 29, 2019 05:48
f(x) Internalization

Internalization_Cell

Granule
Channel: FITC
Granule
Intensity above background: 300
Min Width: 1
Max Width: 8

Nuclear
Channel: DAPI
Nuclear
Intensity above background: 31
Min Width: 8
Max Width: 35

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 119 for details.
- **Annotation**: Click **Edit Annotations** to open the **Annotations** page to upload or edit annotations. See Creating Annotations on page 122 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. See Exporting Experiment Images on page 120 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 121 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. You can export an image as tiles or at full size. With a full-size image, you can include a scale bar and a time stamp.

Any image larger than 2 GB is scaled down to a 2 GB file size, which is the file size limit for a TIFF file. If your image is over 2 GB and you want to export it at full resolution, export the image as tiles. You can then use a TIFF image processing program to join the tiles together for a full-resolution image, if needed.

In addition, if you plan to import the image into Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis Software for further processing and analysis, the image can be scaled to meet the 32K x 32K pixel size required by the MetaXpress software.

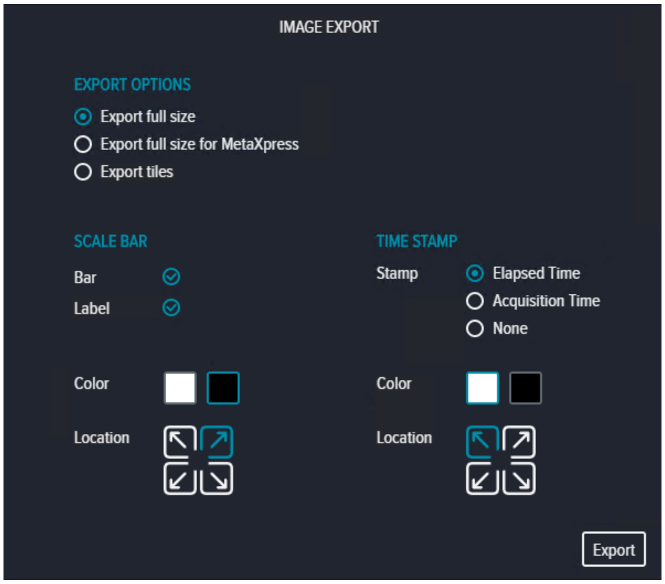


IMAGE EXPORT

EXPORT OPTIONS
● Export full size
○ Export full size for MetaXpress
○ Export tiles

SCALE BAR
Bar ✓
Label ✓

TIME STAMP
Stamp ● Elapsed Time ○ Acquisition Time ○ None

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 209 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 Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis 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 209 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 255 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.

	1	2	3	4	5	6	7	8
A	A1 0.01 positive good	A2 0.06 negative neutral	A3 0.11 negative good	A4 0.16 test bad	A5 0.21 test bad	A6 0.26 positive good	A7 0.31 positive good	A8 0.36 negative neutral
B	B1 0.03 positive bad	B2 0.12 negative neutral	B3 0.33 negative neutral	B4 0.31 test good	B5 0.36 test bad	B6 0.41 positive bad	B7 0.46 positive good	B8 0.51 negative neutral
C	C1 0.05 positive neutral	C2 0.24 negative good	C3 0.99 negative neutral	C4 0.46 test good	C5 0.51 test good	C6 0.56 positive neutral	C7 0.61 positive bad	C8 0.66 negative bad
D	D1 0.07 positive neutral	D2 0.48 negative bad	D3 2.97 negative neutral	D4 0.61 test good	D5 0.66 test bad	D6 0.71 positive neutral	D7 0.76 positive neutral	D8 0.81 negative good
E	E1 0.09 positive good	E2 0.96 negative bad	E3 8.91 negative good	E4 0.76 test neutral	E5 0.81 test bad	E6 0.86 positive bad	E7 0.91 positive bad	E8 0.96 negative good
F	F1 0.11 positive neutral	F2 1.92 negative neutral	F3 26.73 negative good	F4 0.91 test neutral	F5 0.96 test neutral	F6 1.01 positive bad	F7 1.06 positive bad	F8 1.11 negative good

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 this page 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 125 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.

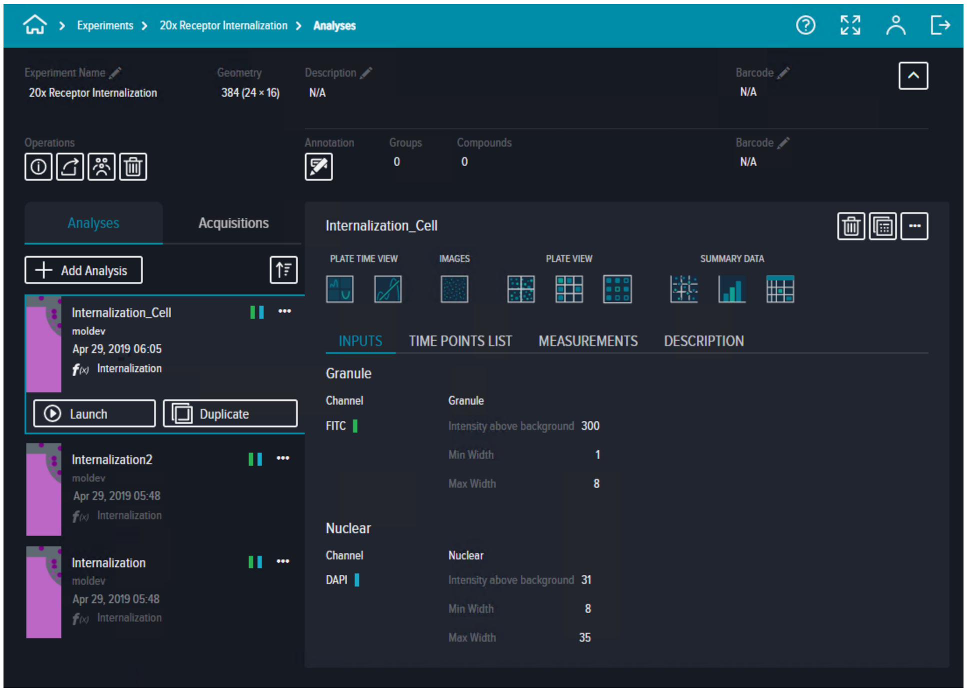
   : Assigns the series from bottom to top, then left to right.

   : 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.



Experiment Name: 20x Receptor Internalization
Geometry: 384 (24 × 16)
Description: N/A
Barcode: N/A
Groups: 0
Compounds: 0
Barcode: N/A

Internalization_Cell
moldev
Apr 29, 2019 06:05
f(x) Internalization

Internalization2
moldev
Apr 29, 2019 05:48
f(x) Internalization

Internalization
moldev
Apr 29, 2019 05:48
f(x) Internalization

Internalization_Cell

Granule
Channel: FITC
Granule
Intensity above background: 300
Min Width: 1
Max Width: 8

Nuclear
Channel: DAPI
Nuclear
Intensity above background: 31
Min Width: 8
Max Width: 35

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 127 or Add Slide Analysis Workflow on page 138 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 151 and Slide Views on page 185 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 128 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 128 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 133 for details.

Well Selection for Analysis is the step where you select the wells for the experiment. See Well Selection for Analysis on page 134 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 135 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, see below 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.

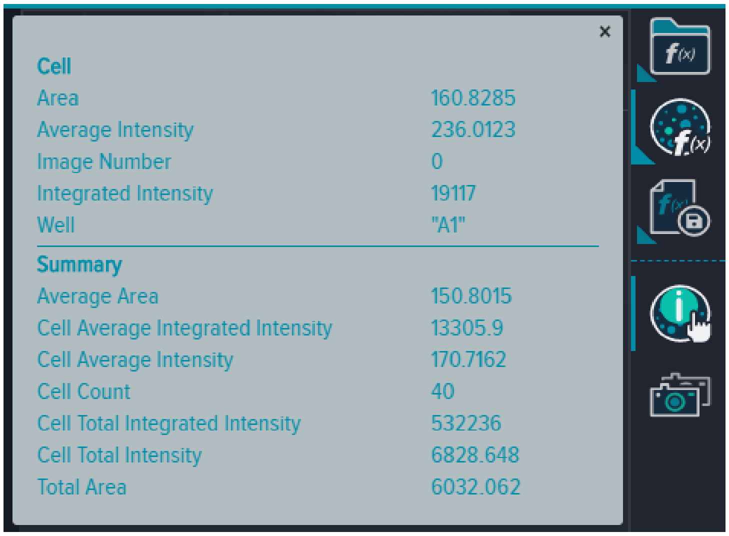
Setting Up an Analysis

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 257 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.



Cell	
Area	160.8285
Average Intensity	236.0123
Image Number	0
Integrated Intensity	19117
Well	"A1"
Summary	
Average Area	150.8015
Cell Average Integrated Intensity	13305.9
Cell Average Intensity	170.7162
Cell Count	40
Cell Total Integrated Intensity	532236
Cell Total Intensity	6828.648
Total Area	6032.062

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

- Testing the Analysis of a Well, see page 130
- Testing the Analysis of Comparison Images, see page 131
- Saving Analysis Settings, see page 132

To continue to the next workflow step, click **Region Selection to Analyze**. See Region Selection to Analyze on page 133 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.

   Note: You typically do not need to adjust the image intensity settings, which use the Acquisition settings. If you do, click **Image Intensity Scale** and adjust the settings as needed for each channel. Use the **Channel Color** drop-down list box to change the identification color for the channel. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image. Click **Auto** to set the best contrast based on the current preview.

5. Click **Choose Position to Acquire**.
6. Click **Select Well**.
7. In the plate map, select a different well and time point.
   The software runs a test analysis.
8. 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.
9. Click **Algorithm Input**.
10. Click **Test Analysis** to preview the analysis.
11. 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 132 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 T1: A1 **Select Well** on the left.
7. In the well map, select the first well and time point for the preview.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

8. Click T1: H12 **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.

Note: You typically do not need to adjust the image intensity settings, which use the Acquisition settings. If you do, click **Image Intensity Scale** and adjust the settings as needed for each channel. Use the **Channel Color** drop-down list box to change the identification color for the channel. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image. Click **Auto** to set the best contrast based on the current preview.

13. 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, see below for details.

Saving Analysis Settings

When you are satisfied with the quality of the preview in Testing the Analysis of a Well on page 130 or Testing the Analysis of Comparison Images on page 131, 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 133 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 134 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 135 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, in the **Experiment Details** pane, 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 experiment labware.
    c. If needed, in the **Experiment Description** field, enter a description of the experiment.
    d. To save TIFF images of the acquisition, select the **Save Raw Images** check box.
    e. 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 137 for details.
    f. On the **Acquisition Parameters** tab, review the settings.
    g. 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 computer 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 255 for details.

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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 209 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 139 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 139 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 144 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 145 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 page 139 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.

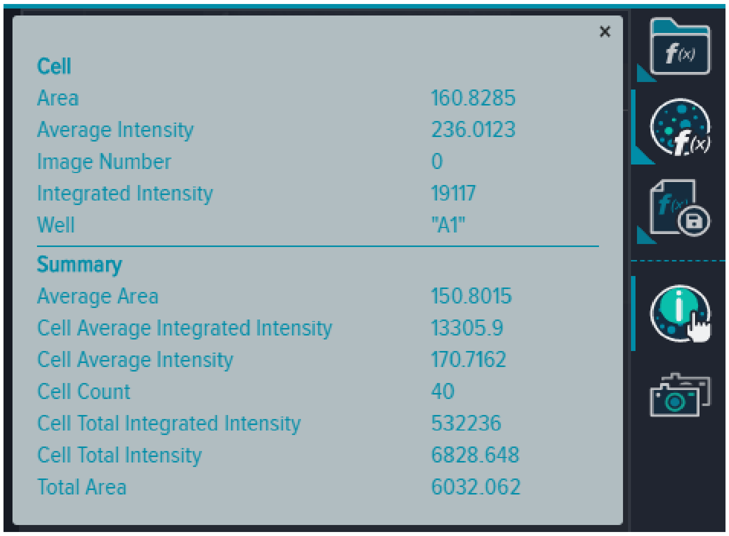
Setting Up an Analysis

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 257 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.



Cell	
Area	160.8285
Average Intensity	236.0123
Image Number	0
Integrated Intensity	19117
Well	"A1"
Summary	
Average Area	150.8015
Cell Average Integrated Intensity	13305.9
Cell Average Intensity	170.7162
Cell Count	40
Cell Total Integrated Intensity	532236
Cell Total Intensity	6828.648
Total Area	6032.062

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

- Testing the Analysis of a Region on page 141
- Testing the Analysis of Comparison Images, see page 142
- Saving Analysis Settings, see page 143

To continue to the next workflow step, click **Region Selection to Analyze**. See Region Selection to Analyze on page 144 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.

   Note: You typically do not need to adjust the image intensity settings, which use the Acquisition settings. If you do, click **Image Intensity Scale** and adjust the settings as needed for each channel. Use the **Channel Color** drop-down list box to change the identification color for the channel. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image. Click **Auto** to set the best contrast based on the current preview.

5. Click **Choose Position to Acquire**.
6. Click T1: A1 **Select 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, 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.
9. Click **Algorithm Input**.
10. Click **Test Analysis** to preview the analysis.
11. 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 143 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 T1: A1 **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 T1: H12 **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.

Note: You typically do not need to adjust the image intensity settings, which use the Acquisition settings. If you do, click **Image Intensity Scale** and adjust the settings as needed for each channel. Use the **Channel Color** drop-down list box to change the identification color for the channel. Use the **Image Intensity Scale** controls to adjust the contrast between the dark and bright pixels in the image. 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, see below for details.

Saving Analysis Settings

When you are satisfied with the quality of the preview in Testing the Analysis of a Region on page 141 or Testing the Analysis of Comparison Images on page 142, 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 144 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 145 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, in the **Experiment Details** pane, 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 experiment labware.
   c. If needed, in the **Experiment Description** field, enter a description of the experiment.
   d. To save TIFF images of the acquisition, select the **Save Raw Images** check box.
   e. 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 147 for details.
   f. On the **Acquisition Parameters** tab, review the settings.
   g. 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 computer 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 255 for details.

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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 209 for details.

Fixing Invalid Parameters

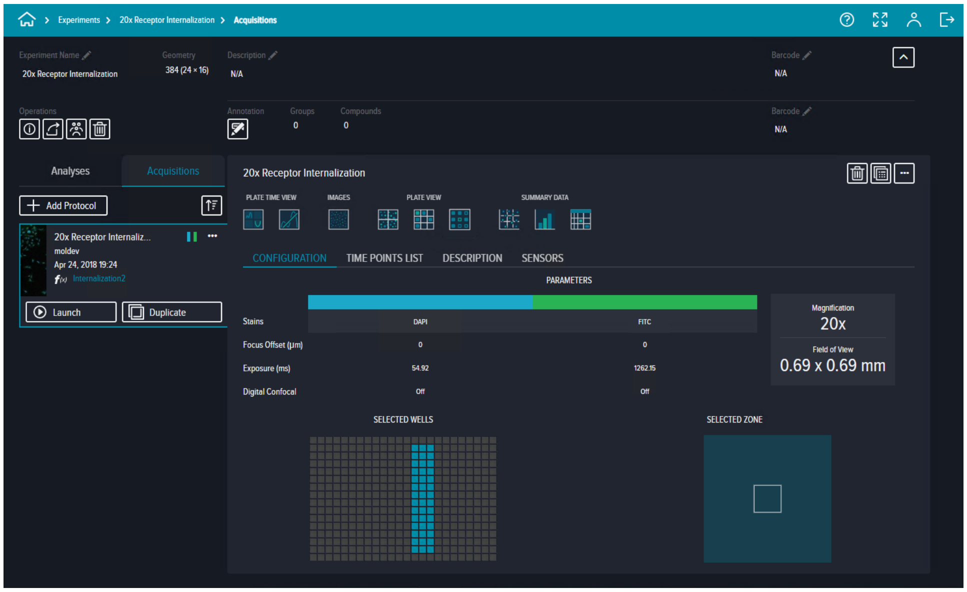
Invalid parameter settings are indicated by a ⊗ icon. Click the icon to display the reason for the invalid parameter.

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.



Experiment Name: 20x Receptor Internalization
Geometry: 384 (24 × 16)
Description: N/A
Barcode: N/A
Groups: 0
Compounds: 0
Barcode: N/A

20x Receptor Internaliz...
moldev
Apr 24, 2018 19:24
f(x) Internalization2

20x Receptor Internalization

PARAMETERS

Stains	DAPI	FITC
Focus Offset (µm)	0	0
Exposure (ms)	54.92	1262.15
Digital Confocal	Off	Off

Magnification: 20x
Field of View: 0.69 x 0.69 mm

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 149 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 151 and Slide Views on page 185 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. You can export an image as tiles or at full size. With a full-size image, you can include a scale bar and a time stamp.

Any image larger than 2 GB is scaled down to a 2 GB file size, which is the file size limit for a TIFF file. If your image is over 2 GB and you want to export it at full resolution, export the image as tiles. You can then use a TIFF image processing program to join the tiles together for a full-resolution image, if needed.

In addition, if you plan to import the image into Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis Software for further processing and analysis, the image can be scaled to meet the 32K x 32K pixel size required by the MetaXpress software.

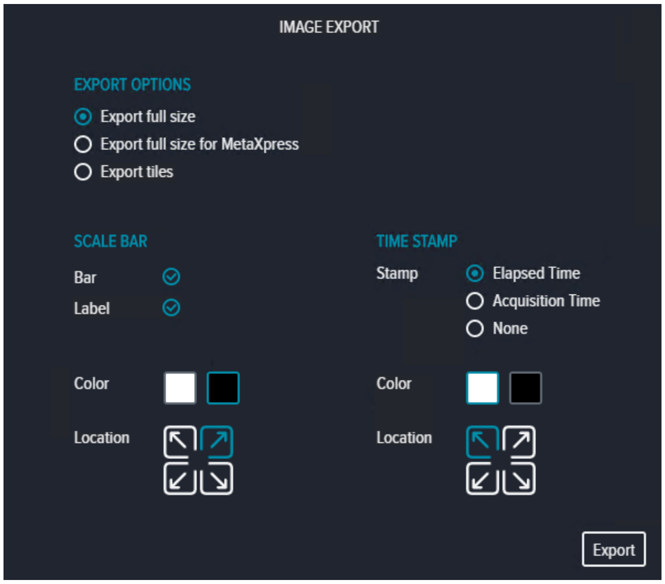


IMAGE EXPORT

EXPORT OPTIONS

● Export full size
○ Export full size for MetaXpress
○ Export tiles

SCALE BAR

Bar ✓
Label ✓

TIME STAMP

Stamp ● Elapsed Time
○ Acquisition Time
○ None

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 209 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 Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis 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 209 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 153 for details.

Time Graph: Shows measurements over time for selected wells. See Plate Time Graph on page 154 for details.

Thumbnail View: Shows an overview of the well images in low resolution. See Plate Thumbnail View on page 155 for details.

Data View: Shows a heatmap with up to four measurements displayed in the wells. See Plate Data View on page 158 for details.

Heatmap: Shows a heatmap of one measurement. See Plate Heatmap on page 160 for details.

Images: Shows high-resolution images for deep zoom viewing. See Plate Images on page 162 for details.

Summary Views

Scatter Plot: Shows a scatter plot of two summary measurements. See Summary Scatter Plot on page 167 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 169 for details.

Table: Shows a table with summary well-level measurements. See Summary Table on page 172 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 175 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 177 for details.

Cell Zoom Views

Note: You must select at least one bin in a Cellular view to enable the Cell Zoom views.

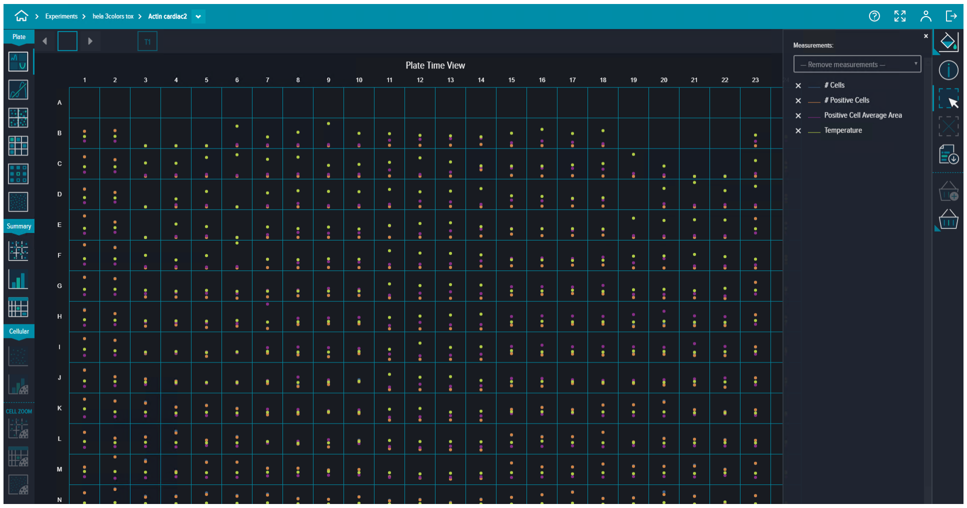
Scatter Mode: Shows a scatter plot graph of two measurements. See Cell Zoom Level Scatter Plot on page 179 for details.

Cell Level Table: Shows a table with cellular measurements. See Cell Zoom Level Table on page 181 for details.

Cell Level Images: Shows high-resolution images for deep zoom viewing of individual cells. See Cell Zoom Level Images on page 183 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.

Add to Quick List: Saves selections to the Quick List for easy access.

Quick List: Opens the Quick List where you can access saved selections.

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:

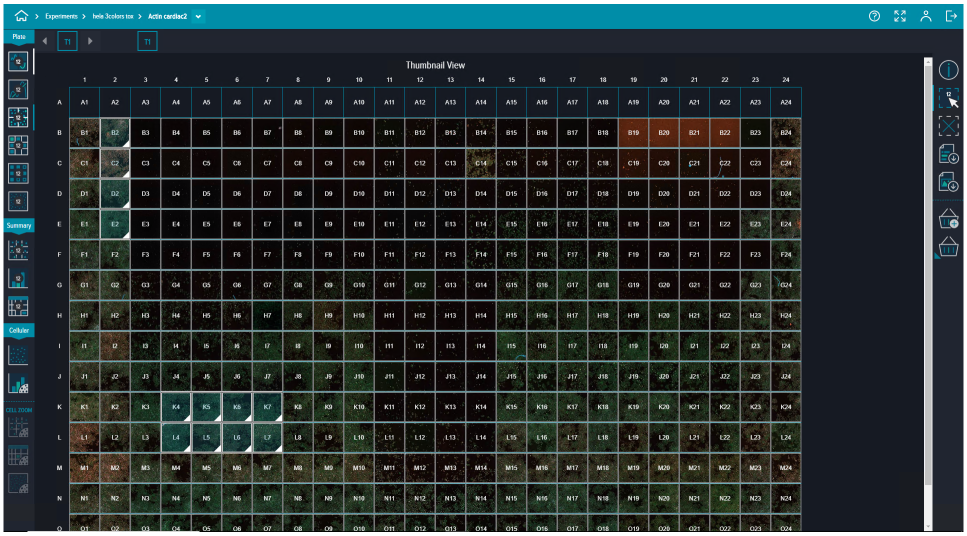
Measurements: Selects the measurements to show.

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 162 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 Raw Images**: Exports the selected thumbnail images as TIFF files. See Exporting Thumbnail Images on page 156 for details.
- **Add to Quick List**: Saves selections to the Quick List for easy access.
- **Quick List**: Opens the Quick List where you can access saved selections.

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. You can export an image as tiles or at full size. With a full-size image, you can include a scale bar and a time stamp.

Any image larger than 2 GB is scaled down to a 2 GB file size, which is the file size limit for a TIFF file. If your image is over 2 GB and you want to export it at full resolution, export the image as tiles. You can then use a TIFF image processing program to join the tiles together for a full-resolution image, if needed.

In addition, if you plan to import the image into Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis Software for further processing and analysis, the image can be scaled to meet the 32K x 32K pixel size required by the MetaXpress software.

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 Raw 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 209 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 Raw 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 Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis 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.

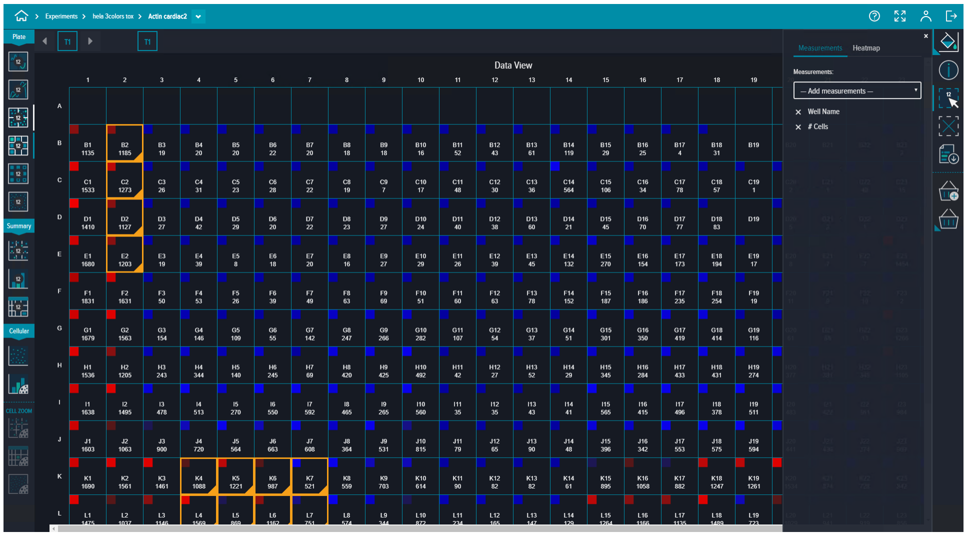
   b. In the **Color** field, select the color for the time stamp (either white or black).
   c. In the **Location** field, select the location on the image for the time stamp (top-left, top-right, bottom-left, or bottom-right).
6. Click **Export**.

The **Monitor** page opens to display the progress of the export. See Monitor Mode on page 209 for details.

Plate Data View

The **Data View** shows a heatmap with up to four measurements displayed in the wells.

Tip: The heatmap color scheme can be changed in **Configuration Settings**. See Color Scheme on page 254 for details.



Data View

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A																			
B	B1 1135	B2 1185	B3 19	B4 20	B5 20	B6 22	B7 20	B8 18	B9 18	B10 16	B11 52	B12 43	B13 61	B14 119	B15 29	B16 25	B17 4	B18 31	B19
C	C1 1533	C2 1273	C3 26	C4 31	C5 23	C6 28	C7 22	C8 19	C9 7	C10 17	C11 48	C12 30	C13 36	C14 564	C15 106	C16 34	C17 78	C18 57	C19 1
D	D1 1410	D2 1127	D3 27	D4 42	D5 29	D6 20	D7 22	D8 23	D9 27	D10 24	D11 40	D12 38	D13 60	D14 21	D15 45	D16 70	D17 77	D18 83	D19
E	E1 1680	E2 1203	E3 19	E4 39	E5 8	E6 18	E7 20	E8 16	E9 27	E10 29	E11 26	E12 39	E13 45	E14 132	E15 270	E16 154	E17 173	E18 194	E19 17
F	F1 1831	F2 1631	F3 50	F4 53	F5 26	F6 39	F7 49	F8 63	F9 69	F10 51	F11 60	F12 63	F13 78	F14 152	F15 187	F16 186	F17 235	F18 254	F19 19
G	G1 1679	G2 1563	G3 154	G4 146	G5 109	G6 55	G7 142	G8 247	G9 266	G10 282	G11 107	G12 54	G13 37	G14 51	G15 301	G16 350	G17 419	G18 414	G19 116
H	H1 1536	H2 1205	H3 243	H4 344	H5 140	H6 245	H7 69	H8 420	H9 425	H10 492	H11 42	H12 27	H13 52	H14 29	H15 345	H16 284	H17 433	H18 431	H19 274
I	I1 1638	I2 1495	I3 478	I4 513	I5 270	I6 550	I7 592	I8 465	I9 265	I10 560	I11 35	I12 35	I13 43	I14 41	I15 565	I16 415	I17 496	I18 378	I19 511
J	J1 1603	J2 1063	J3 900	J4 720	J5 564	J6 663	J7 608	J8 364	J9 531	J10 815	J11 79	J12 65	J13 90	J14 48	J15 396	J16 342	J17 553	J18 575	J19 594
K	K1 1690	K2 1561	K3 1461	K4 1088	K5 1221	K6 987	K7 521	K8 559	K9 703	K10 614	K11 90	K12 82	K13 82	K14 61	K15 895	K16 1058	K17 882	K18 1247	K19 1261
L	L1 1475	L2 1037	L3 1146	L4 1569	L5 869	L6 1162	L7 751	L8 574	L9 344	L10 872	L11 234	L12 165	L13 147	L14 129	L15 1264	L16 1166	L17 1135	L18 1489	L19 723

Measurements:

- Well Name
- # Cells

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.

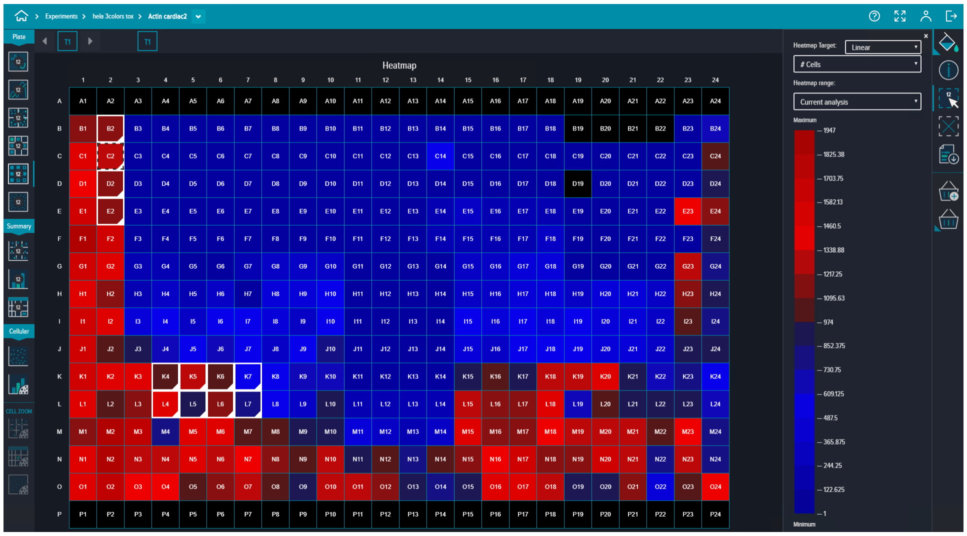
Add to Quick List: Saves selections to the Quick List for easy access.

Quick List: Opens the Quick List where you can access saved selections.

Plate Heatmap

The **Heatmap** shows a heatmap of one measurement.

* Tip: The heatmap color scheme can be changed in **Configuration Settings**. See Color Scheme on page 254 for details.



Heatmap Target: Linear

Cells

Heatmap range: Current analysis

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.

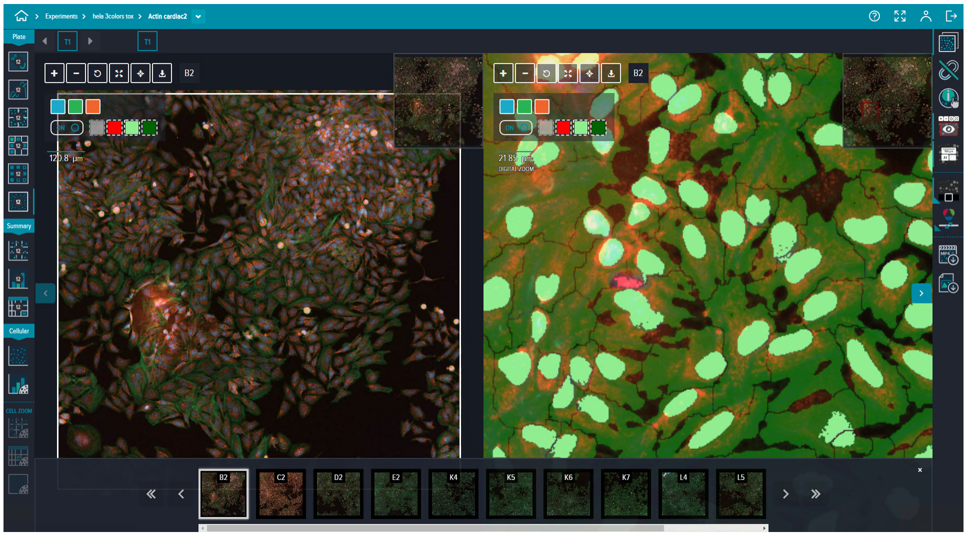
Add to Quick List: Saves selections to the Quick List for easy access.

Quick List: Opens the Quick List where you can access saved selections.

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 164 for details. This function is not available with a tablet.

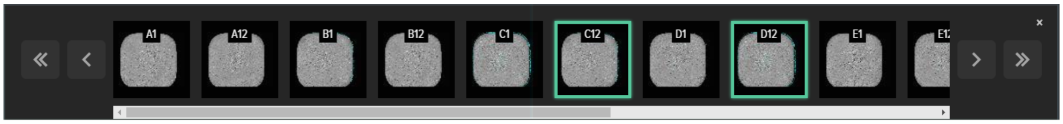
Export Raw Images: Exports the current plate image as a TIFF file. See Exporting Images on page 165 for details.

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 209 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. You can export an image as tiles or at full size. With a full-size image, you can include a scale bar and a time stamp.

Any image larger than 2 GB is scaled down to a 2 GB file size, which is the file size limit for a TIFF file. If your image is over 2 GB and you want to export it at full resolution, export the image as tiles. You can then use a TIFF image processing program to join the tiles together for a full-resolution image, if needed.

In addition, if you plan to import the image into Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis Software for further processing and analysis, the image can be scaled to meet the 32K x 32K pixel size required by the MetaXpress software.

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 Raw 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 209 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 Raw 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 Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis 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 209 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: The heatmap color scheme can be changed in **Configuration Settings**. See Color Scheme on page 254 for details.

X Axis: Linear

\# Negative Cells

Y Axis: Linear

\# Cells

Aggregate Function: —None—

Min-Max Options: Current T

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.

Add to Quick List: Saves selections to the Quick List for easy access.

Quick List: Opens the Quick List where you can access saved selections.

Axes/Color Pane

Use the **Axes/Color** pane to specify the data that appears in the graph.

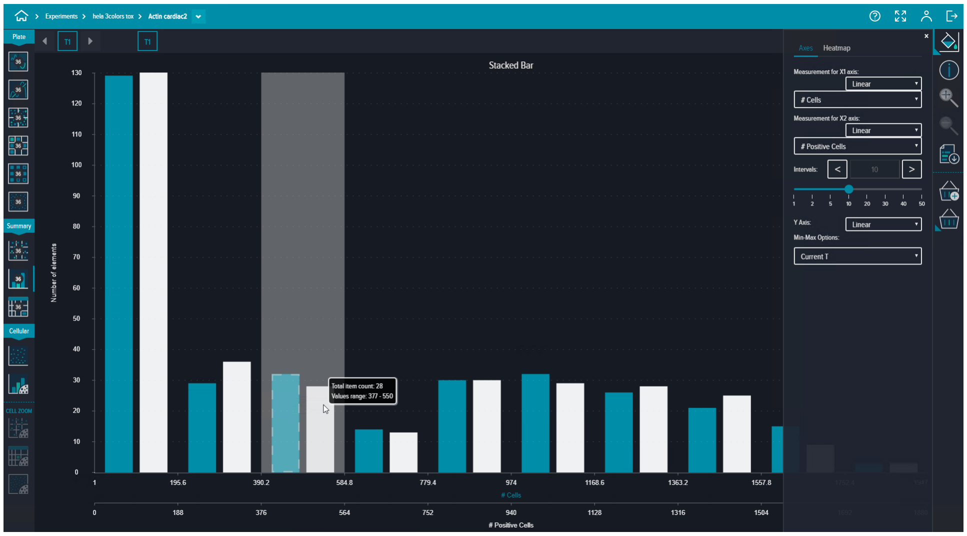
The following tabs are available:

- **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: The heatmap color scheme can be changed in **Configuration Settings**. See Color Scheme on page 254 for details.



Total item count: 28
Values range: 377 - 550

Measurement for X1 axis: Linear
Cells

Measurement for X2 axis: Linear
Positive Cells

Intervals: 10

Y Axis: Linear

Min-Max Options: Current T

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.

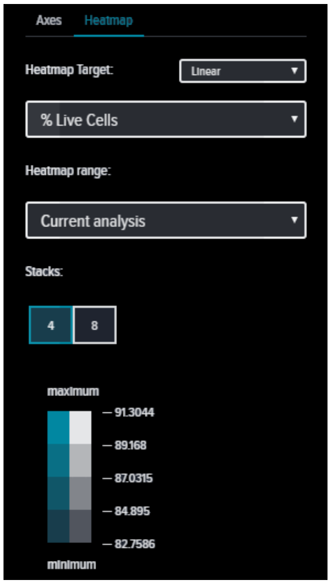
Export Current View to an Image: Downloads a JPEG image of the page view, including selections. This function is not available with a tablet.

Add to Quick List: Saves selections to the Quick List for easy access.

Quick List: Opens the Quick List where you can access saved selections.

Axes/Color Pane

Use the **Axes/Color** pane to specify the data that appears in the graph.



Heatmap Target: Linear

% Live Cells

Heatmap range: Current analysis

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.

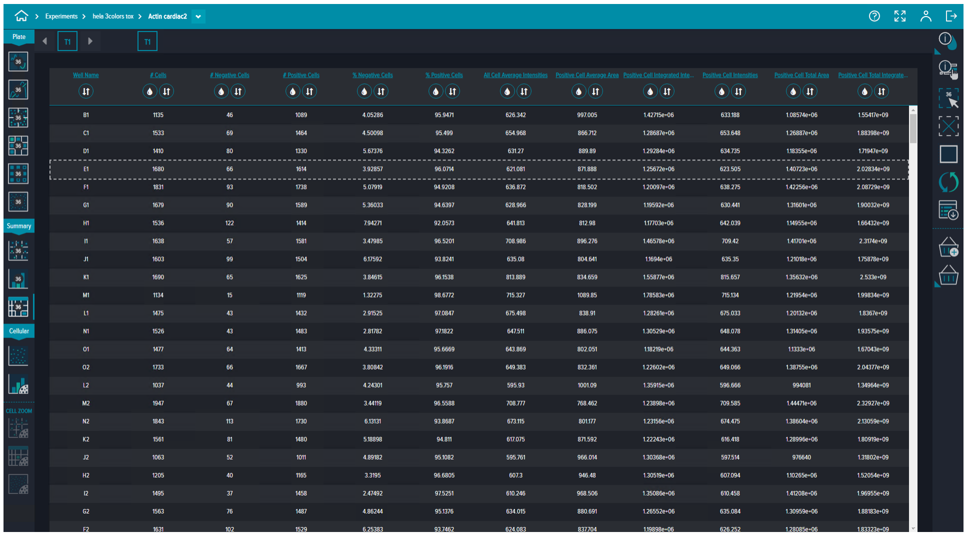
The **Stacks** field, shows the range of values for each shade in the heatmap. You can show either **4** or **8** stacks.

Summary Table

The **Table** shows a table view of all well-level measurements.

*

Tip: The heatmap color scheme can be changed in **Configuration Settings**. See Color Scheme on page 254 for details.



Well Name	# Cells	# Negative Cells	# Positive Cells	% Negative Cells	% Positive Cells	All Cell Average Intensities	Positive Cell Average Area	Positive Cell Integrated Inte...	Positive Cell Intensities	Positive Cell Total Area	Positive Cell Total Integrate...
B1	1135	46	1089	4.05286	95.9471	626.342	997.005	1.42715e+06	633.188	1.08574e+06	1.55417e+09
C1	1533	69	1464	4.50098	95.499	654.968	866.712	1.28687e+06	653.648	1.26887e+06	1.88398e+09
D1	1410	80	1330	5.67376	94.3262	631.27	889.89	1.29284e+06	634.735	1.18355e+06	1.71947e+09
E1	1680	66	1614	3.92857	96.0714	621.081	871.888	1.25672e+06	623.505	1.40723e+06	2.02834e+09
F1	1831	93	1738	5.07919	94.9208	636.872	818.502	1.20097e+06	638.275	1.42256e+06	2.08729e+09
G1	1679	90	1589	5.36033	94.6397	628.966	828.199	1.19592e+06	630.441	1.31601e+06	1.90032e+09
H1	1536	122	1414	7.94271	92.0573	641.813	812.98	1.17703e+06	642.039	1.14955e+06	1.66432e+09
I1	1638	57	1581	3.47985	96.5201	708.986	896.276	1.46578e+06	709.42	1.41701e+06	2.3174e+09
J1	1603	99	1504	6.17592	93.8241	635.08	804.641	1.1694e+06	635.35	1.21018e+06	1.75878e+09
K1	1690	65	1625	3.84615	96.1538	813.889	834.659	1.55877e+06	815.657	1.35632e+06	2.533e+09
M1	1134	15	1119	1.32275	98.6772	715.327	1089.85	1.78583e+06	715.134	1.21954e+06	1.99834e+09
L1	1475	43	1432	2.91525	97.0847	675.498	838.91	1.28261e+06	675.033	1.20132e+06	1.8367e+09
N1	1526	43	1483	2.81782	97.1822	647.511	886.075	1.30529e+06	648.078	1.31405e+06	1.93575e+09
O1	1477	64	1413	4.33311	95.6669	643.869	802.051	1.18219e+06	644.363	1.1333e+06	1.67043e+09
O2	1733	66	1667	3.80842	96.1916	649.383	832.361	1.22602e+06	649.066	1.38755e+06	2.04377e+09
L2	1037	44	993	4.24301	95.757	595.93	1001.09	1.35915e+06	596.666	994081	1.34964e+09
M2	1947	67	1880	3.44119	96.5588	708.777	768.462	1.23898e+06	709.585	1.44471e+06	2.32927e+09
N2	1843	113	1730	6.13131	93.8687	673.115	801.177	1.23156e+06	674.475	1.38604e+06	2.13059e+09
K2	1561	81	1480	5.18898	94.811	617.075	871.592	1.22243e+06	616.418	1.28996e+06	1.80919e+09
J2	1063	52	1011	4.89182	95.1082	595.761	966.014	1.30368e+06	597.514	976640	1.31802e+09
H2	1205	40	1165	3.3195	96.6805	607.3	946.48	1.30519e+06	607.094	1.10265e+06	1.52054e+09
I2	1495	37	1458	2.47492	97.5251	610.246	968.506	1.35086e+06	610.458	1.41208e+06	1.96955e+09
G2	1563	76	1487	4.86244	95.1376	634.015	880.691	1.26552e+06	635.084	1.30959e+06	1.88183e+09
F2	1631	102	1529	6.25383	93.7462	624.083	837.704	1.19898e+06	626.252	1.28085e+06	1.83323e+09

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.

Add to Quick List: Saves selections to the Quick List for easy access.

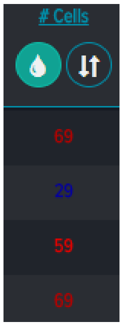
Quick List: Opens the Quick List where you can access saved selections.

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 💧.



Cells
69
29
59
69

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.

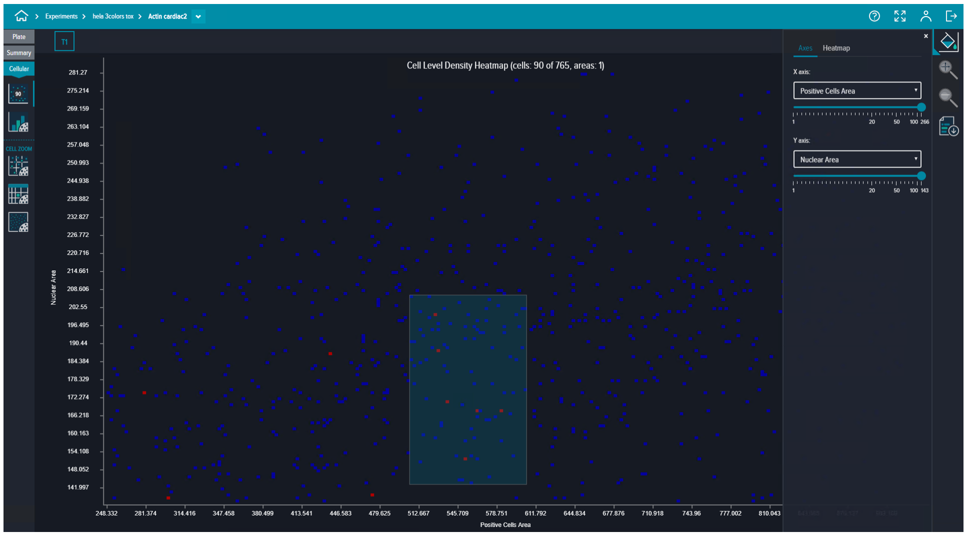
Cell Level Density Heatmap

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: The heatmap color scheme can be changed in **Configuration Settings**. See Color Scheme on page 254 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.



Cell Level Density Heatmap (cells: 90 of 765, areas: 1)

X axis: Positive Cells Area

Y axis: Nuclear Area

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:

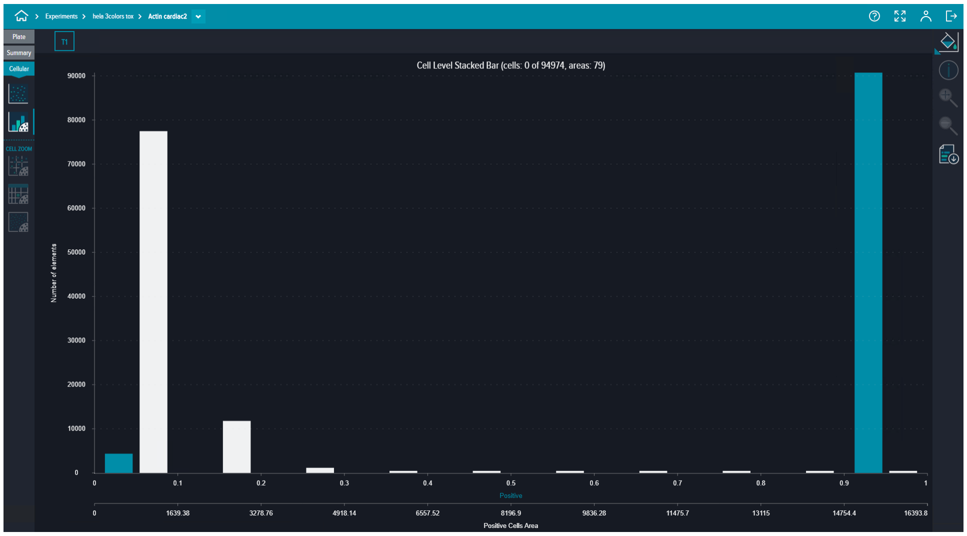
- **Axes**: Specifies the measurements for two scatter plot axes and intervals.
- **Heatmap**: Specifies the heatmap coloring to the graph data.

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.

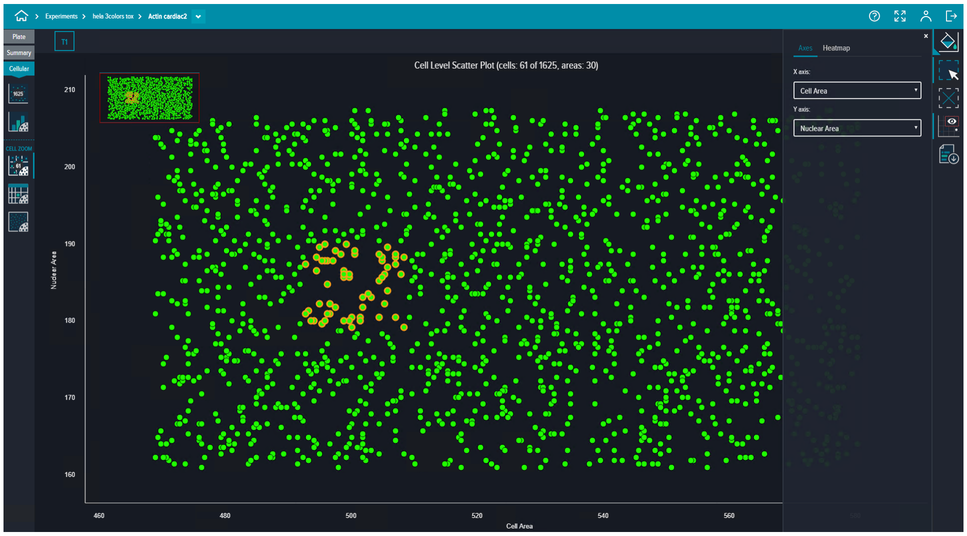
Cell Zoom Level Scatter Plot

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: The heatmap color scheme can be changed in **Configuration Settings**. See Color Scheme on page 254 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.



Cell Level Scatter Plot (cells: 61 of 1625, areas: 30)

X axis: Cell Area

Y axis: Nuclear Area

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: The heatmap color scheme can be changed in **Configuration Settings**. See Color Scheme on page 254 for details.

Well Name	Cell Area	Nuclear Area	Positive	Positive Cells Area	Positive Cells Average Inten...	Positive Cells Integrated Int...	Wavelength 1 Average Nucl...	Wavelength 1 Integrated Nu...	Wavelength 2 Average Cell ...	Wavelength 2 Average Nucl...	Wavelength 2 Integrated C...
L1	506.632	187.224	1	506.632	551.415	644604	322.988	139531	551.415	587.002	644604
L1	494.064	181.59	1	494.064	531.239	605612	335.117	140414	531.239	570.578	605612
N1	506.632	189.824	1	506.632	584.189	682917	311.091	136258	584.189	558.594	682917
N1	502.731	183.324	1	502.731	595.112	690330	304.671	128876	595.112	592.232	690330
N1	509.232	179.423	1	509.232	593.317	697148	323.623	133980	593.317	571.604	697148
N1	504.032	183.324	1	504.032	663.942	772164	325.799	137813	663.942	711.884	772164
O1	499.698	185.924	1	499.698	607.012	699885	478.189	205143	607.012	670.054	699885
O1	496.664	190.258	1	496.664	597.06	684231	278.415	122224	597.06	611.74	684231
O1	500.564	185.924	1	500.564	528.981	610973	291.448	125031	528.981	517.084	610973
O1	498.398	189.391	1	498.398	532.931	612871	297.297	129919	532.931	543.927	612871
O1	497.964	188.091	1	497.964	535.992	615855	301.136	130693	535.992	555.009	615855
O1	497.097	185.057	1	497.097	621.488	712847	302.131	129010	621.488	641.452	712847
H2	499.264	188.958	1	499.264	579.957	668110	267.743	116736	579.957	596.592	668110
G2	500.998	180.723	1	500.998	637.849	737353	251.18	104742	637.849	747.125	737353
B2	500.131	186.357	1	500.131	564.922	651920	305.737	131467	564.922	600.686	651920
K5	506.199	186.357	1	506.199	605.437	707150	278.942	119945	605.437	567.379	707150
K5	497.531	181.157	1	497.531	539.807	619698	305.036	127505	539.807	553.684	619698
M6	505.332	185.491	1	505.332	630.736	735438	296.792	127027	630.736	633.979	735438
M6	500.131	190.258	1	500.131	653.991	754706	336.866	147884	653.991	636.622	754706
M6	502.298	180.723	1	502.298	638.412	739920	343.192	143111	638.412	532.022	739920
O10	495.364	186.791	1	495.364	557.653	637397	232.884	100373	557.653	579.313	637397
O10	505.765	185.924	1	505.765	661.393	771846	342.1	146761	661.393	756.958	771846
N10	495.797	189.824	1	495.797	590.184	675171	257.356	112722	590.184	579.413	675171
N10	500.998	179.423	1	500.998	642.694	742954	301.118	124663	642.694	652.63	742954
M15	494.497	180.29	1	494.497	558.324	637048	319.538	132928	558.324	566.959	637048

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.

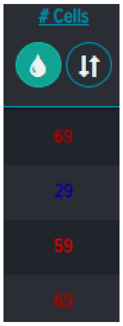
Export: Downloads the currently configured table as a CSV file. This function is not available with a tablet.

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 .



Cells
69
29
59
69

Sorting Data

To sort rows by the values in the column, click .

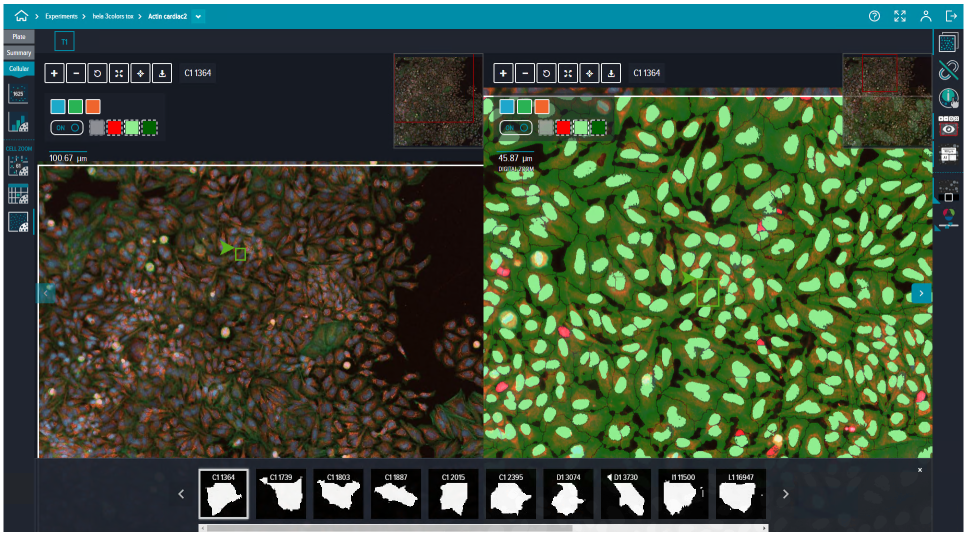
- Click once to sort lowest to highest.
- Click again to sort highest to lowest.
- Click a third time to deactivate sorting for the column.

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 187 for details.

Images: Shows high-resolution images for deep zoom viewing. See Slide Images on page 191 for details.

Summary Views

Table: Shows a table with summary slide region-level measurements. See Summary Table on page 196 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 199 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 201 for details.

Cell Zoom Views

Note: You must select at least one bin in a Cellular view to enable the Cell Zoom views.

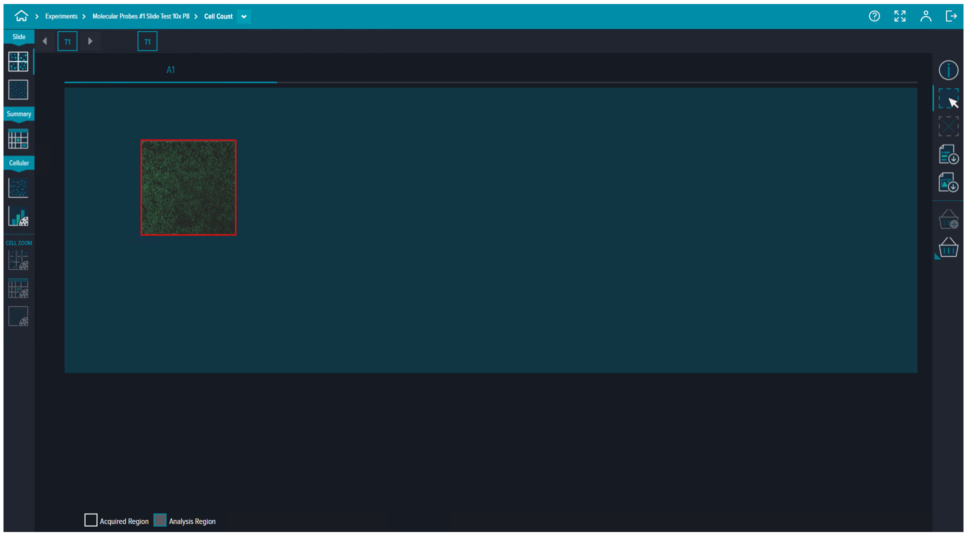
Scatter Mode: Shows a scatter plot graph of two measurements. See Cell Zoom Level Scatter Plot on page 203 for details.

Cell Level Table: Shows a table with cellular measurements. See Cell Zoom Level Table on page 205 for details.

Cell Level Images: Shows high-resolution images for deep zoom viewing of individual cells. See Cell Zoom Level Images on page 207 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 191 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 Raw Images**: Exports the selected thumbnail images as TIFF files. See Exporting Thumbnail Images on page 189 for details.
- **Add to Quick List**: Saves selections to the Quick List for easy access.
- **Quick List**: Opens the Quick List where you can access saved selections.

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. You can export an image as tiles or at full size. With a full-size image, you can include a scale bar and a time stamp.

Any image larger than 2 GB is scaled down to a 2 GB file size, which is the file size limit for a TIFF file. If your image is over 2 GB and you want to export it at full resolution, export the image as tiles. You can then use a TIFF image processing program to join the tiles together for a full-resolution image, if needed.

In addition, if you plan to import the image into Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis Software for further processing and analysis, the image can be scaled to meet the 32K x 32K pixel size required by the MetaXpress software.

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 Raw 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 209 for details.

Exporting Full-Size Thumbnail Images

To export full-sized images:

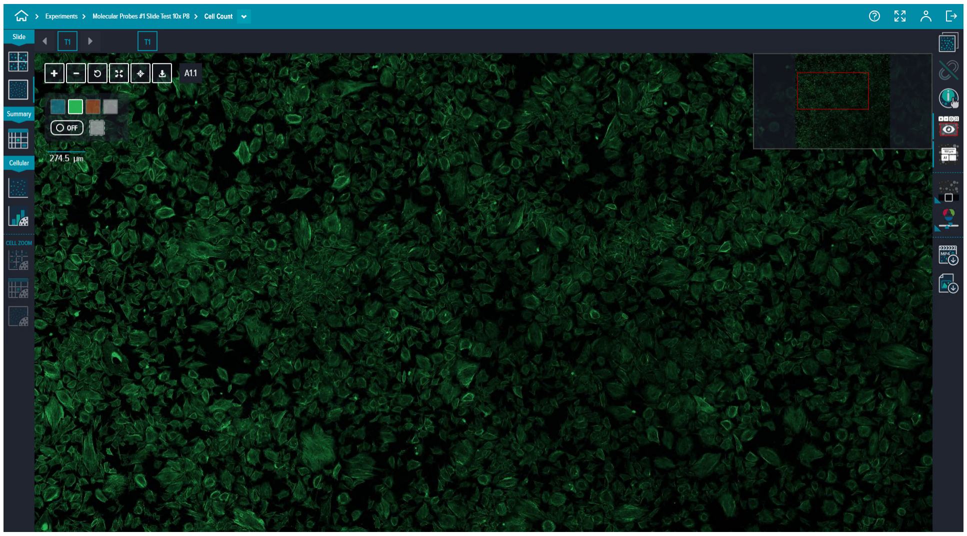
1. On the **Thumbnail View** page, select the regions you want to export.
2. On the right side of the page, click **Export Raw Images**.
3. In the Image Export dialog, do one of the following in the **Export Options** section:
   - Select the **Export Full Size** option to export a full-size image as 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 Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis 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 209 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 193 for details. This function is not available with a tablet.

Export Raw Images: Exports the current slide image as a TIFF file. See Exporting Images on page 194 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 209 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. You can export an image as tiles or at full size. With a full-size image, you can include a scale bar and a time stamp.

Any image larger than 2 GB is scaled down to a 2 GB file size, which is the file size limit for a TIFF file. If your image is over 2 GB and you want to export it at full resolution, export the image as tiles. You can then use a TIFF image processing program to join the tiles together for a full-resolution image, if needed.

In addition, if you plan to import the image into Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis Software for further processing and analysis, the image can be scaled to meet the 32K x 32K pixel size required by the MetaXpress software.

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 Raw 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 209 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 Raw 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 Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis 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 209 for details.

Summary Table

The ▦ **Table** shows a table view of all region-level measurements.

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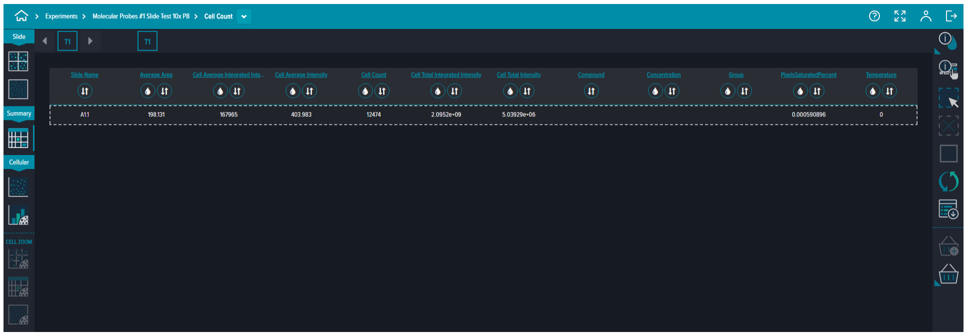
*

Tip: The heatmap color scheme can be changed in 👤 **Configuration Settings**. See Color Scheme on page 254 for details.



Slide Name	Average Area	Cell Average Integrated Inte...	Cell Average Intensity	Cell Count	Cell Total Integrated Intensity	Cell Total Intensity	Compound	Concentration	Group	PixelsSaturatedPercent	Temperature
A1.1	198.131	167965	403.983	12474	2.0952e+09	5.03929e+06				0.000590896	0

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.

Add to Quick List: Saves selections to the Quick List for easy access.

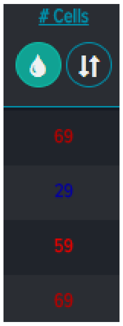
Quick List: Opens the Quick List where you can access saved selections.

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 💧.



Cells
69
29
59
69

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.

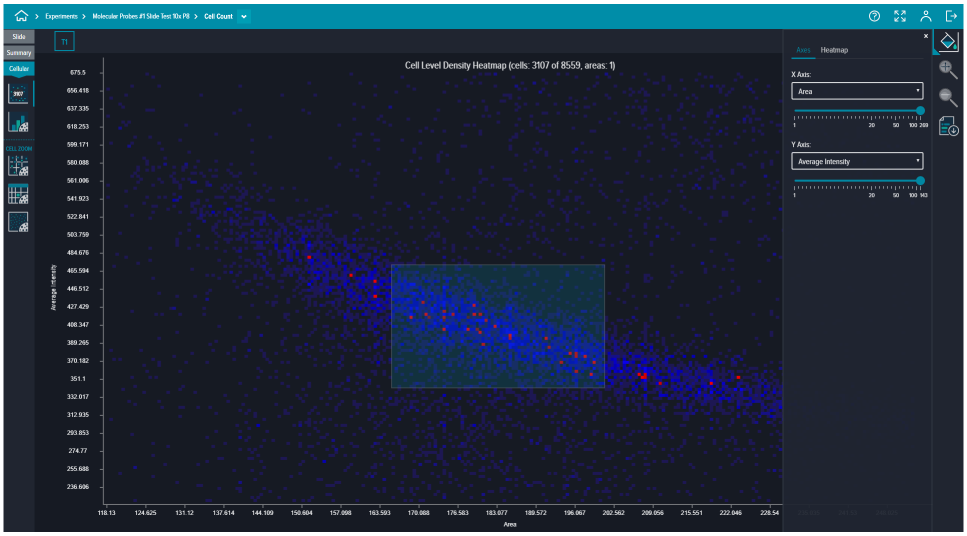
Cell Level Density Heatmap

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: The heatmap color scheme can be changed in **Configuration Settings**. See Color Scheme on page 254 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.



Cell Level Density Heatmap (cells: 3107 of 8559, areas: 1)

X Axis: Area

Y Axis: Average Intensity

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:

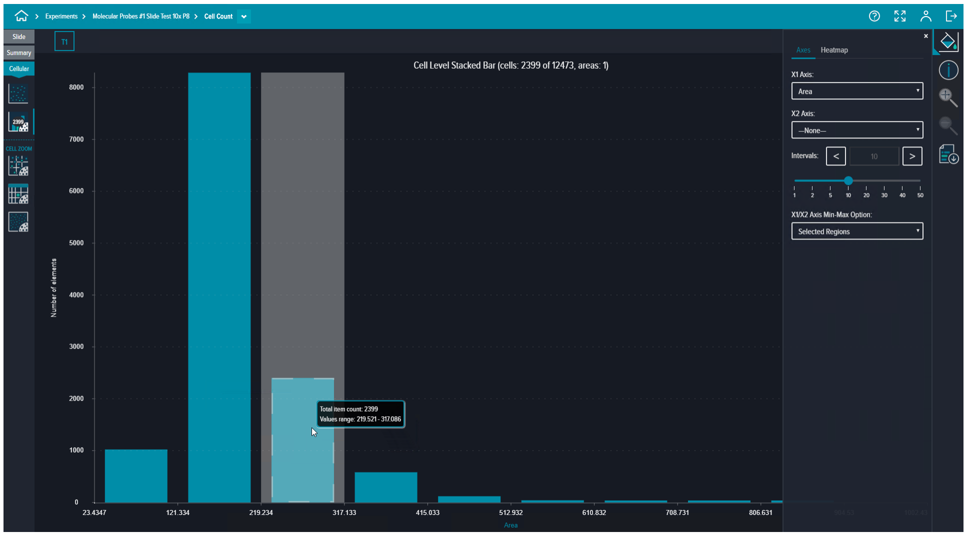
- **Axes**: Specifies the measurements for two scatter plot axes and intervals.
- **Heatmap**: Specifies the heatmap coloring to the graph data.

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.



Cell Level Stacked Bar (cells: 2399 of 12473, areas: 1)

Total item count: 2399
Values range: 219.521 - 317.086

X1 Axis: Area

X2 Axis: —None—

Intervals: 10

X1/X2 Axis Min-Max Option: 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.

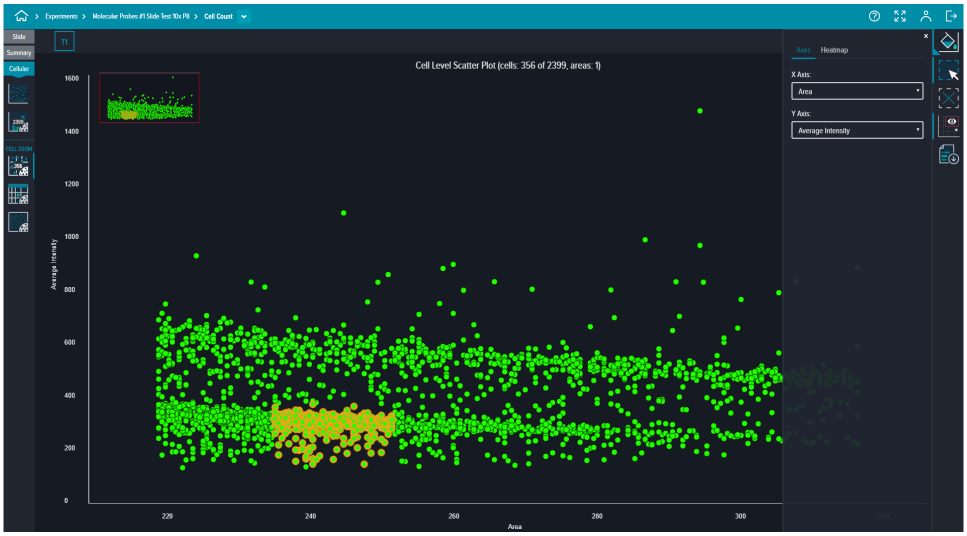
Cell Zoom Level Scatter Plot

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: The heatmap color scheme can be changed in **Configuration Settings**. See Color Scheme on page 254 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.



Cell Level Scatter Plot (cells: 356 of 2399, areas: 1)

X Axis: Area

Y Axis: Average Intensity

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: The heatmap color scheme can be changed in **Configuration Settings**. See Color Scheme on page 254 for details.

Slide Name	Area	Average Intensity	Integrated Intensity	PixelsSaturatedPercent	Temperature	Average Area	Cell Average Integrated Int...	Cell Average Intensity
A1.1	246.782	265.934	137222	0.000590896	0	198.131	167965	403.983
A1.1	237.695	276.099	137221	0.000590896	0	198.131	167965	403.983
A1.1	236.738	293.139	145104	0.000590896	0	198.131	167965	403.983
A1.1	248.695	284.992	148196	0.000590896	0	198.131	167965	403.983
A1.1	237.217	316.772	157119	0.000590896	0	198.131	167965	403.983
A1.1	241.043	289.879	146099	0.000590896	0	198.131	167965	403.983
A1.1	244.391	296.157	151336	0.000590896	0	198.131	167965	403.983
A1.1	237.217	291.877	144771	0.000590896	0	198.131	167965	403.983
A1.1	239.608	292.691	146638	0.000590896	0	198.131	167965	403.983
A1.1	249.651	308.797	161192	0.000590896	0	198.131	167965	403.983
A1.1	244.869	278.695	142692	0.000590896	0	198.131	167965	403.983
A1.1	239.13	279.26	139630	0.000590896	0	198.131	167965	403.983
A1.1	242.478	294.067	149092	0.000590896	0	198.131	167965	403.983
A1.1	242.478	311.369	157864	0.000590896	0	198.131	167965	403.983
A1.1	245.825	290.233	149180	0.000590896	0	198.131	167965	403.983
A1.1	235.782	297.349	146593	0.000590896	0	198.131	167965	403.983
A1.1	247.26	289.617	149732	0.000590896	0	198.131	167965	403.983
A1.1	241.521	286.376	144620	0.000590896	0	198.131	167965	403.983
A1.1	250.608	285.239	149465	0.000590896	0	198.131	167965	403.983
A1.1	239.608	315.375	158003	0.000590896	0	198.131	167965	403.983
A1.1	235.782	319.308	157419	0.000590896	0	198.131	167965	403.983
A1.1	246.782	291.196	150257	0.000590896	0	198.131	167965	403.983
A1.1	249.173	272.47	141957	0.000590896	0	198.131	167965	403.983
A1.1	236.26	304.3	150324	0.000590896	0	198.131	167965	403.983

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.

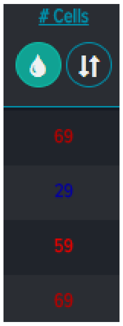
Export: Downloads the currently configured table as a CSV file. This function is not available with a tablet.

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 ⇅.

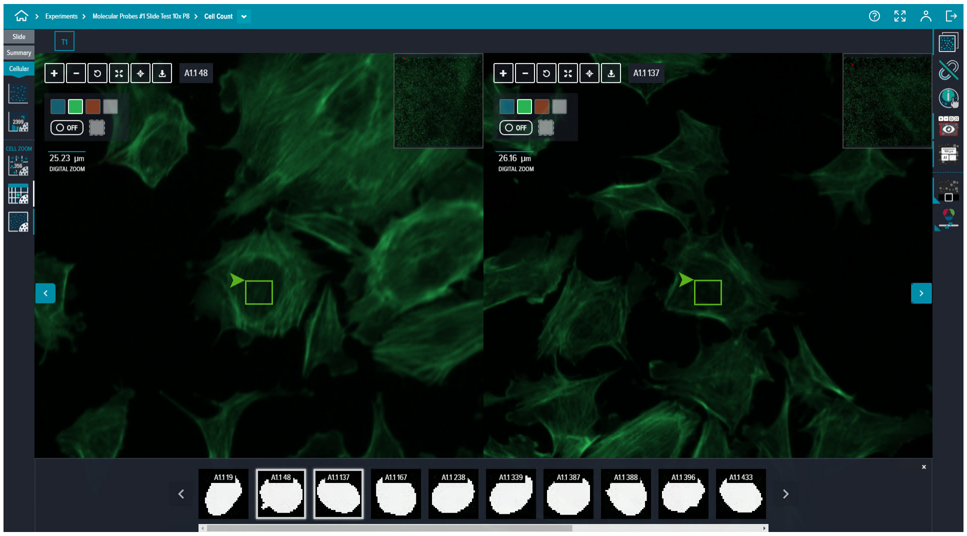
- Click once to sort lowest to highest.
- Click again to sort highest to lowest.
- Click a third time to deactivate sorting for the column.

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.

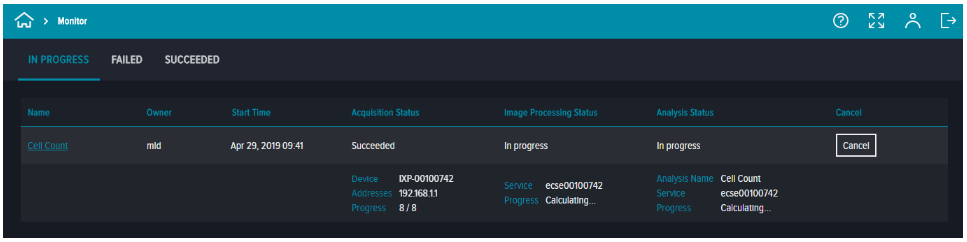
3. To synchronize image zooming and changing positions in both panes, click **Unlink Images**. The icon toggles to **Link Images**.

Chapter 5: Monitor Mode

5

Use **Monitor** mode to view the progress and completion status of various CellReporterXpress software activities, including experiments run in **Acquisition** mode or **Experiments** mode, raw 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.



Name	Owner	Start Time	Acquisition Status	Image Processing Status	Analysis Status	Cancel
Cell Count	mld	Apr 29, 2019 09:41	Succeeded	In progress	In progress	Cancel
			Device IXP-00100742 Addresses 192.168.1.1 Progress 8 / 8	Service ecse00100742 Progress Calculating...	Analysis Name Cell Count Service ecse00100742 Progress Calculating...	

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 119 for details on permanently deleting an experiment.

Chapter 6: Configuration Mode

6

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 212 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 213 for details.
- **Devices**: Specifies the instruments available for acquisition and maintenance. You can add instruments as needed. See Devices on page 216 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 220 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 222 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.

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

To add a plate format to the library:

1. Insert the new plate in the instrument.

Note: For best results, we recommend that you 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 **Measure Plate Dimensions**.
5. Click the **Select the instrument** drop-down list box, and select the instrument you are using.
6. Click **Open Door**.
7. Insert the plate.
8. Click **Close Door**.
9. Click **Measure Plate Dimensions**.
   The instrument measures the well depth and bottom thickness.
10. Click **Finish**.
11. Click **Edit**.

Name: * 96 Corning 3603 [1]
Left Edge to Left Center: 14.3 mm
Top Edge to Top Center: 11.2 mm
Well Depth: 10.67 mm
Horizontal Center to Center: 9 mm
Vertical Center to Center: 9 mm
Bottom Thickness: 0.5 mm
✓ Clear Well Bottom
Plate Length: 127.8 mm
Plate Width: 85.5 mm
Plate Height: 14.2 mm
Well Diameter: 6.35 mm
○ Well Shape Square
Material: Plastic
Clearance: 3.03 mm

12. In the **Name** field, enter a name for the new plate format.
13. Edit the specifications for the plate format as needed.
14. Click **Save**.

Adding a Slide Format

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**.

Name: * 4 Slide Holder [1]

Left Edge to Left Center: 20 mm

Top Edge to Top Center: 53 mm

Opening Depth: 8.33 mm

Holder Length: 148 mm

Holder Width: 97 mm

Holder Height: 9 mm

Opening Length: 27 mm

Opening Width: 70 mm

Surface Thickness: 0.17 mm

Horizontal Center to Center: 29 mm

Material: Glass

Support Ledge Height: 0.5 mm

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 mdc.custhelp.com and search for article number **20356**.

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 Devices on page 216 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 PC 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 PC 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 for remote storage, 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 computer registered for remote storage, 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.

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.

   Note: 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`).

2. Click + **Map Existing Folder on Storage Computer**.

The mapped folder appears in the **List of Mapped Folders**.

Note: To increase security for remote storage, 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 216 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 226 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 227 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 240 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 245 for details.
- **Maintenance**: Displays a tile for each maintenance activity that can be performed on the selected instrument. See Maintenance on page 250 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 230 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 a compressed gas supply 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 your safety officer defines 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 63 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. With a plate, see Adjusting an Objective Correction Collar on page 44 for details. With slides, see Adjusting an Objective Correction Collar on page 95 for details.
- 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 233 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 three or four days. 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 63 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 250 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. With a plate, see Adjusting an Objective Correction Collar on page 44 for details. With slides, see Adjusting an Objective Correction Collar on page 95 for details.
- 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 236 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 a compressed gas supply 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 your safety officer defines 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 63 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. With a plate, see Adjusting an Objective Correction Collar on page 44 for details. With slides, see Adjusting an Objective Correction Collar on page 95 for details.
- 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 239 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). So, 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 63 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. With a plate, see Adjusting an Objective Correction Collar on page 44 for details. With slides, see Adjusting an Objective Correction Collar on page 95 for details.

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
1	4x
2	10x
3	20x
4	empty
5	40x or 63x
6	empty

Note:

- Depending on how your ImageXpress Pico system is configured, you may not have all the objectives.
- The 40x objective and the 63x objective cannot be installed in the instrument simultaneously.
- The 63x objective cannot be installed when the environmental control cassette is loaded.

Installing an Objective in an Empty Slot

You must install objectives in specific slots in the turret. See Installing an Objective on page 240 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 240 for details. Because of this, the only reason to replace an objective with another objective of a different magnification is when you are a 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 **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 244 for details.

Replacing an Objective (Same Magnification)

You must install objectives in specific slots in the turret. See Installing an Objective on page 240 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 244 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.

HC PL FLUOTAR 10x/0.32 objective

Calibrated

- 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
- FITC
- TRITC
- Cy5
- Texas Red
- CFP

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 249 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 249 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:

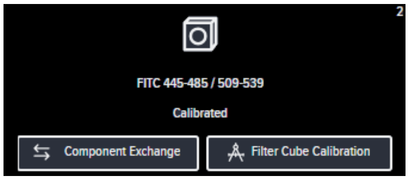
- Fluorescent Bead Slide (9100-0099)
- Red Plastic Slide (1-GP-7)
- Green Plastic Slide (1-GP-8)
- Orange Plastic Slide (1-GP-11)

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

If your instrument includes the optional environmental control system, you can run air dry to clear any condensation that builds up inside the system. Condensation in the environmental control system can prevent the instrument from consistently achieving a humidity level of between 75% and 95%.

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 209 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

8

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 254 for details.

Vocabulary: Specifies the language for the CellReporterXpress user interface. See Vocabulary on page 254 for details.

Color Scheme: Specifies the color scheme used for heatmaps in **Experiments** mode. See Color Scheme on page 254 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 254 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 255 for details.

Miscellaneous: Specifies various CellReporterXpress software preferences, including image preferences and timeouts. See Miscellaneous on page 256 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 212 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 wavelength can have an offset to image the part of the sample to be analyzed. The default setting is 40µm.
- **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.
- **Reached Max. Timepoints**: Specifies the maximum number of time points in a single acquisition. The default setting is 100 time points.

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 262: 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 264: 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 266: Two-channel assay for detecting autophagic granules. Use the Autophagy analysis to detect the infection of viral particles into cells.

Cell Count, see page 268: 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 270: 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 273: 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 277: 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 282: 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 289: Two-channel assay for detecting endosomes. Use the Endocytosis analysis to detect the internalization of endosomes into cells.

Internalization, see page 291: 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 293: 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 295: 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 297: 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 299: 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 301: 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 304: 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 306: 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 309: 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 312: 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 318: 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 315: Single-channel, transmitted light assay to find beads.

Transmitted Light Cell Count, see page 315: Single-channel, transmitted light assay to find a range of cells.

Transmitted Light Cell Count, see page 315: Single-channel, transmitted light assay to find larger cells.

Transmitted Light Cell Count, see page 315: 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 316: Two-channel, transmitted light assay to find beads, then scoring for an additional fluorescence channel.

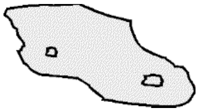
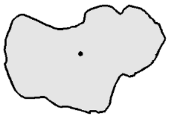
Transmitted Light Cell Scoring, see page 316: Two-channel, transmitted light assay to find a range of cells, then scoring for an additional fluorescence channel.

Transmitted Light Cell Scoring, see page 316: 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 316: 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.

Measurement	Description
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.

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.

Measurement	Description
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.
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.

Measurement	Description
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.
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.

Measurement	Description
Wavelength 4 Total Integrated Cell Intensity	Total integrated intensity of cells positive for Marker 3.
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.

Measurement	Description
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.
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. You can use a nuclear wavelength (for example, DAPI, Hoechst, or DRAQ5) with transmitted light or fluorescent images to help identify the cell body.

Tip:

- Exposure time should be optimized for outgrowth intensity, not cell body intensity. The autoexpose function may give images that are too dim in the outgrowths.
- If cell bodies are saturating and this is interfering with cell body identification, you can acquire the same channel a second time at a lower exposure time. Use the dimmer image as the “nuclear stain” image.
- 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.

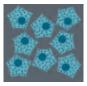
Summary Measurements

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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