

# CellReporterXpress<sup>®</sup>

Image Acquisition and Analysis Software

Version 2.5

## User Guide

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## Chapter 1: CellReporterXpress Image Acquisition and Analysis Software

The Molecular Devices® 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](http://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 our Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance after consulting the Knowledge Base, 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 Knowledge Base or by phone. To find regional support contact information, visit [www.moleculardevices.com/contact](http://www.moleculardevices.com/contact).

You need the instrument serial number.

### Documentation

Review the product documentation on the Knowledge Base, 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](http://www.leica-microsystems.com/science-lab)
- [www.ibiology.org/ibioeducation/taking-courses/ibiology-microscopy-short-course.html](http://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.lifetechnologies.com/us/en/home/references/molecular-probes-the-handbook.html](http://www.lifetechnologies.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 page 8](#)
- [Logging In on a Client Workstation Using a Shortcut, see page 9](#)
- [Logging In on a Client Workstation Using Connection Details, see page 10](#)



**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, Molecular Devices recommends that user accounts be Local accounts (and not Roaming or Domain accounts). If Domain accounts are required, the Host computer should remain connected to the domain network at all times.

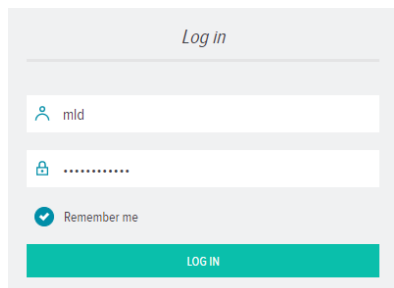
---



## Logging In on the Host Computer

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

1. On the host computer, do one of the following to display the CellReporterXpress Log In screen:

- On the desktop, double-click  **MD.CellReporterXpress**.
- Click **Start > Molecular Devices > MD.CellReporterXpress**.



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



---

**Note:** A password is required to log in to the CellReporterXpress software. If the Windows account has no password, you cannot log in to the software.

---

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.



**Note:** A password is required to log in to the CellReporterXpress software. If the Windows account has no password, you cannot log in to the software.

---

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.

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.



**Note:** A password is required to log in to the CellReporterXpress software. If the Windows account has no password, you cannot log in to the software.

---

5. Click **LOG IN**.

## Product Documentation

The following guides are available on the Molecular Devices Knowledge Base at [mdc.custhelp.com](http://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.



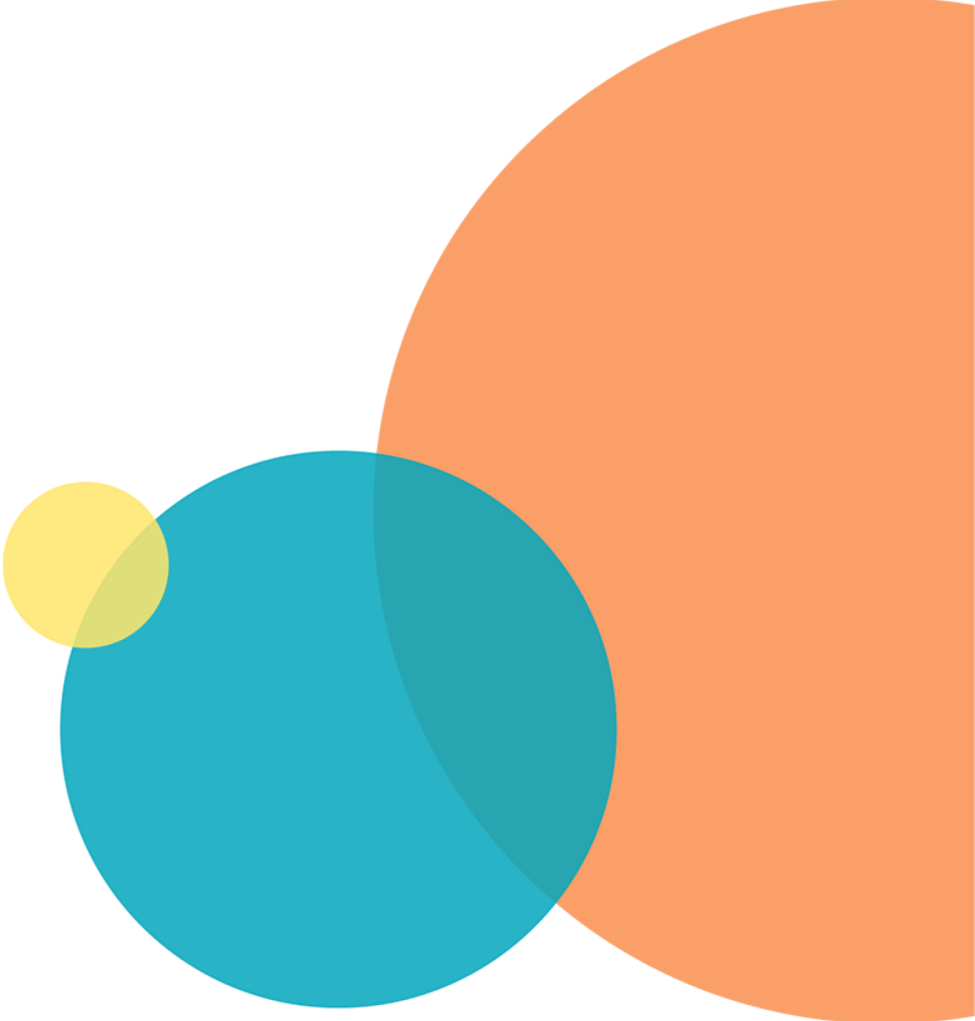
**Tip:** Molecular Devices recommends that you review the documentation before installing or using the ImageXpress Pico system or the CellReporterXpress software.

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## 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. Molecular Devices recommends that you review the guide on the Knowledge Base for the most up-to-date information.





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 17](#) for details.



**Experiments:** Click to view images and analysis data collected in **Acquisition** mode and perform additional offline analysis. See [Experiments Mode on page 111](#) 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, and maintenance operations run in **Devices** mode. See [Monitor Mode on page 199](#) for details.



**Configuration:** Click to set the systemwide options that affect all users of the CellReporterXpress software. See [Configuration Mode on page 201](#) 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 215](#) 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.

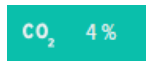


**User Preferences:** Enables you to specify the way the software looks and, for some functions, to specify the way the functions work. The options you set become your personal preferences and stay set every time you use the software. See [Configuration Settings on page 245](#) for details.



**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<sub>2</sub> Level:** Shows the current CO<sub>2</sub> level inside the environmental control cassette. The color of the toolbar notification indicates if the CO<sub>2</sub> level is within the range you set (green) or outside of it (yellow).



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

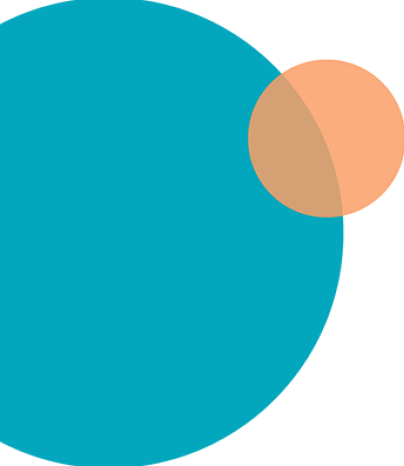


**O<sub>2</sub> Level:** Shows the current O<sub>2</sub> level inside the environmental control cassette. The color of the toolbar notification indicates if the O<sub>2</sub> level is within the range you set (green) or outside of it (yellow).



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

Click a toolbar notification to open the control panel for that environmental control component. See [Sensors on page 218](#) for details.






## Chapter 3: Acquisition Mode

# 3

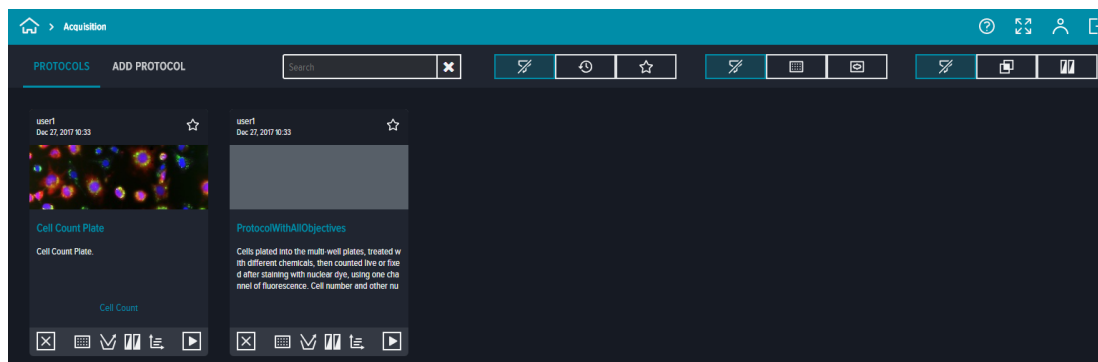
Use **Acquisition** mode to configure experiment settings and run experiments from supported instruments using protocols or templates.




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

### Protocol Library

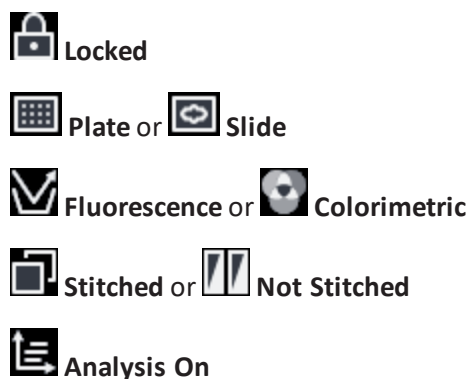
A protocol enables you to reuse a specific configuration for an experiment. It includes no experiment data, only configuration settings. A typical protocol enables you to insert labware into the instrument, select the protocol, and then run the protocol to perform the experiment and collect data.

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 19](#) for details.



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


Each card contains icons to indicate protocol properties, including:



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 67](#) for details on running a plate protocol or [Run Protocol on page 107](#) 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 21](#) or [Slide Acquisition Workflow on page 70](#) for details.
3. Click  **Save Protocol**. See [Run Protocol on page 67](#) for details on running a plate protocol or [Run Protocol on page 107](#) 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 19](#).

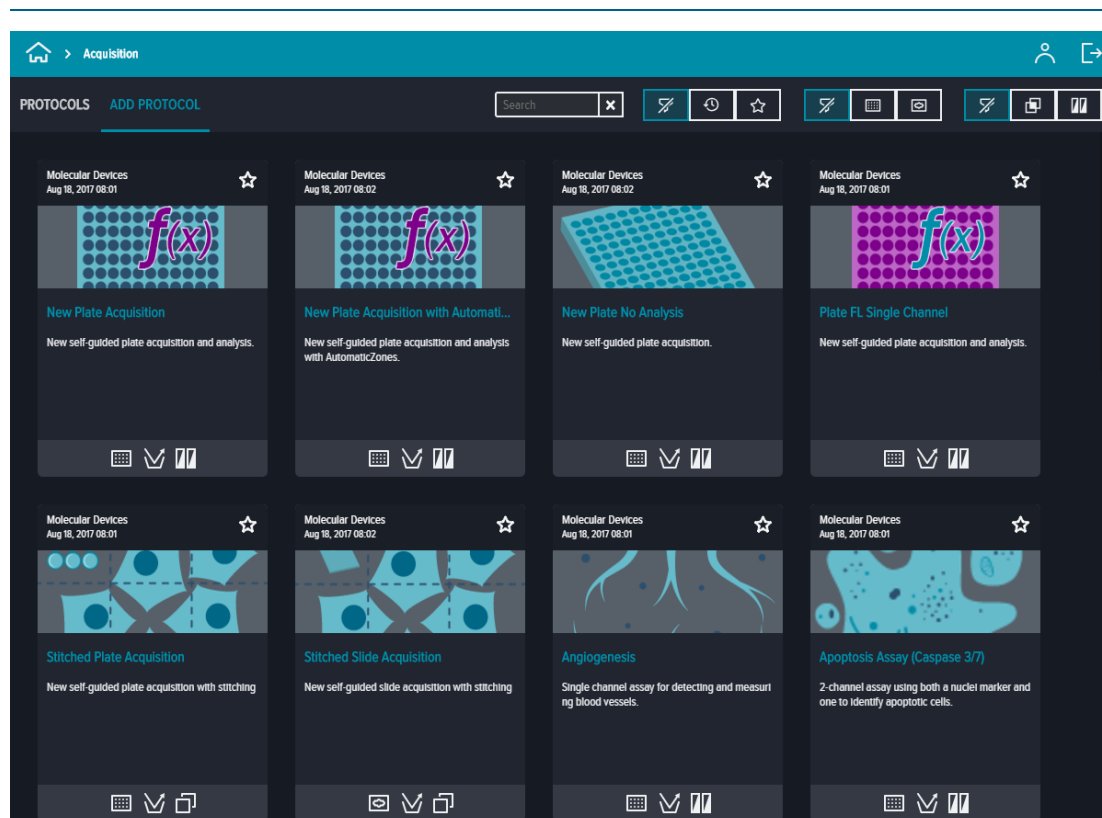
## Template Library


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.

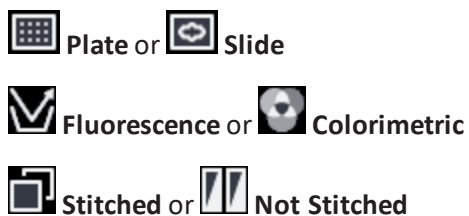
Most templates are designed for typical experiments and have restricted settings options. The restrictions help you focus on the most appropriate options for that experiment type. Two unrestricted templates (**New Plate Acquisition** and **New Slide Acquisition**) allow you to select from all the available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



**Note:** You cannot create new templates. When you save a template, it becomes a protocol. See [Protocol Library on page 17](#) for details.



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



## 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  **No Stitching**

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 23](#) 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 24](#) 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 66](#) 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 67](#) 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 can control multiple instruments, which can be configured differently. If your software is configured to control multiple instruments, you may need to select the instrument that is appropriate for your experiment. Different instruments may contain different objectives or filter cubes or may include different features, such as digital confocal or environmental control.



**Note:** If you click the  **Favorite** icon for a device, that device will be selected by default. Otherwise, the last used device will be selected by 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 24](#) 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.



**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 32](#) 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 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  **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 203](#) 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 use.
3. Click  **Move Stain Up** and  **Move Stain Down** as needed to select the order in which the stains will be collected.



**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 collected first.

## 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 27](#)
- [Snapping Well Comparison Previews, see page 32](#)
- [Snapping Z Stack Images, see page 36](#)
- [Viewing a Live Preview of a Plate, see page 40](#)
- [Adjusting an Objective Correction Collar, see page 47](#)
- [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.



**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.



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.



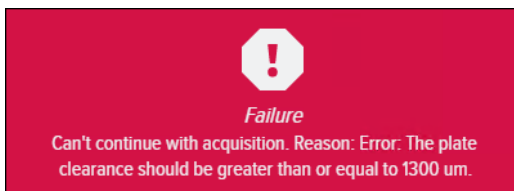
**Note:**

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



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.

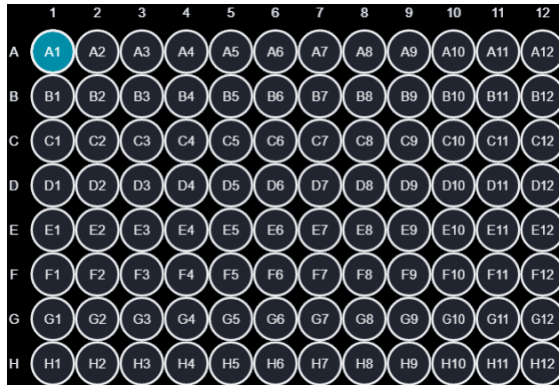
- If you are using the optional environmental control cassette and the autofocus fails, the software may display the following message:




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 203](#) for details on adding a labware format.

2. At the bottom of the screen on the  **Choose Position to Acquire** tab, click  **Select Well**.

- 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.



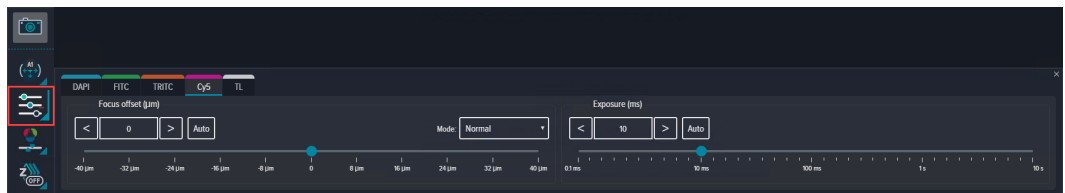
- In the single well map, click and drag the  selection tool to select the region of the well for the preview, if needed. The center area of the well is selected by default.
- When you find a region of interest, click **Save Position** to save the current field of view to the **Region Selection to Acquire**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



- Click **Focus/Exposure Settings**.




7. On the **Focus/Exposure Settings** tab, adjust the settings as needed for each channel:
  - Use the **Focus offset** controls to adjust the image sharpness. Click the **Mode** drop-down list box to set one of the following autofocus ranges:
    - **Normal**: Hardware and image autofocus mode using a short search range.
    - **Wide**: Hardware and image autofocus mode using a wide range.
    - **Super Wide**: Hardware and image autofocus mode designed for samples that do not sit directly on the well bottom.
    - **Plate and Well Bottom**: Hardware-only autofocus mode designed for speed.

After you set the focus mode, click **Auto** next to the **Focus Offset** controls to determine the focus offset between stains. This is particularly useful when using the **Plate and Well Bottom** focus mode.



- 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).

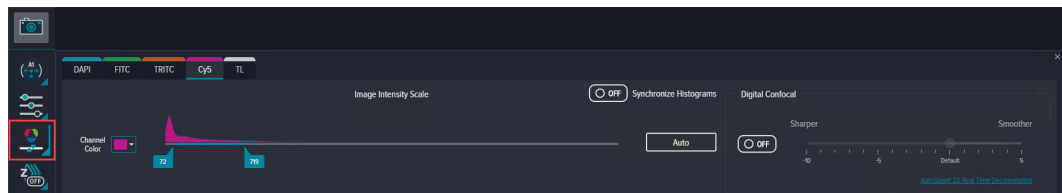
When your image is in focus, click **Auto** to automatically determine the optimal exposure for the best dynamic range. The longer the exposure, the brighter the experiment image. Dimmer objects get brighter with longer exposure time, as does the background. Increased exposure time slows the experiment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

---

 **Tip:** You may want to use **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:
  - 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.
  - 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.



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 36](#) 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.



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



. Lower the **Exposure** value as needed.



Zooms in on the image.



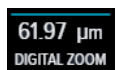
Zooms out on the image.



Resets the view zoom to the original image size.



Views the image full screen.



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**.



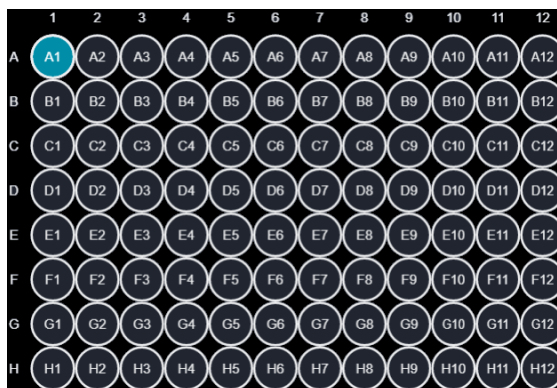
**Note:** If **Z Stacking** is set to **On**, the Comparison Mode icon is disabled. You must set **Z stacking** to **Off** before you can start Comparison Mode.


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.

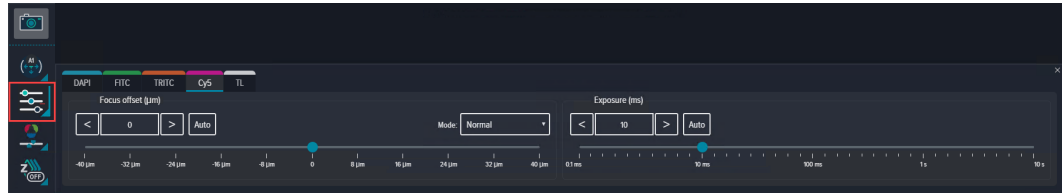


5. Click  **Select Well** on the right.
6. In the well map, select the second well for the preview. The instrument snaps a preview image of the newly selected well. The bottom right well is selected by default.

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**.



10. On the **Focus/Exposure Settings** tab, adjust the settings as needed for each channel:
- Use the **Focus offset** controls to adjust the image sharpness. Click the **Mode** drop-down list box to set one of the following autofocus ranges:
    - **Normal**: Hardware and image autofocus mode using a short search range.
    - **Wide**: Hardware and image autofocus mode using a wide range.
    - **Super Wide**: Hardware and image autofocus mode designed for samples that do not sit directly on the well bottom.
    - **Plate and Well Bottom**: Hardware-only autofocus mode designed for speed.

After you set the focus mode, click **Auto** next to the **Focus Offset** controls to determine the focus offset between stains. This is particularly useful when using the **Plate and Well Bottom** focus mode.

- 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.

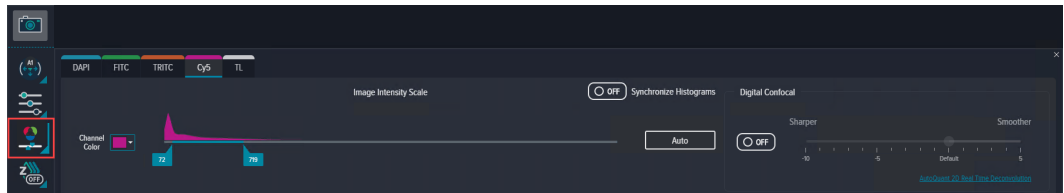


**Tip:** You may want to use **Auto** with a known bright sample, such as a positive control.



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:
- 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.
  - 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:
- Set **Digital Confocal** to **On**.
  - As needed, move the slider to the right to smooth the image.
  - 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 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.



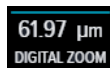
Zooms out on the image.



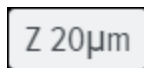
Resets the view zoom to the original image size.



Views the image full screen.



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




: With Z stack images, indicates the type of image. **ZP** indicates a Z stack projection. For an individual Z stack plane, displays the focus offset amount. **Z 20μm** indicates 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 27](#) 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**.

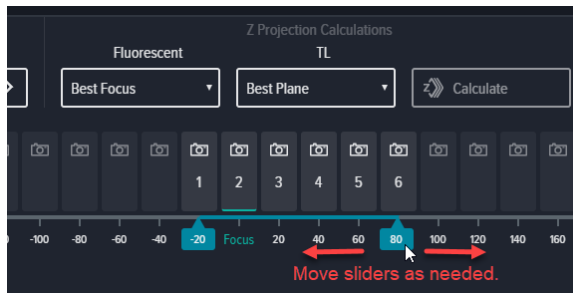


3. In the **Focus Step ( $\mu\text{m}$ )** field, modify the distance between the Z stack planes, as needed.

The default focus step distance varies based on the objective.

Objective	Default Focus Step Distance
4x	50 $\mu\text{m}$
10x	20 $\mu\text{m}$
20x	5 $\mu\text{m}$
40x	2 $\mu\text{m}$
63x	1 $\mu\text{m}$

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

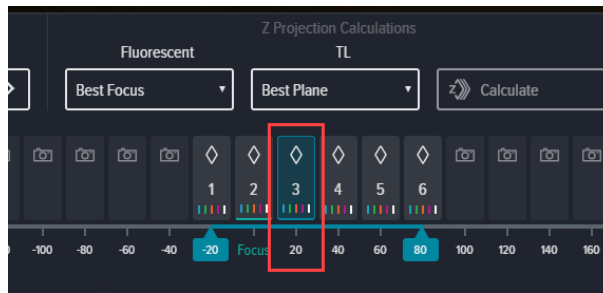


5. Click  **Snap Z Stacking Images** to snap the Z stack.

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.



**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.

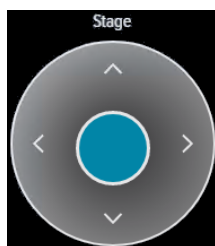
## Viewing a Live Preview of a Plate

Live preview enables you to view 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 to explore the live preview to find a region of interest. 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.



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.



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.



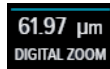
Zooms out on the image.



Resets the view zoom to the original image size.



Views the image full screen.



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.

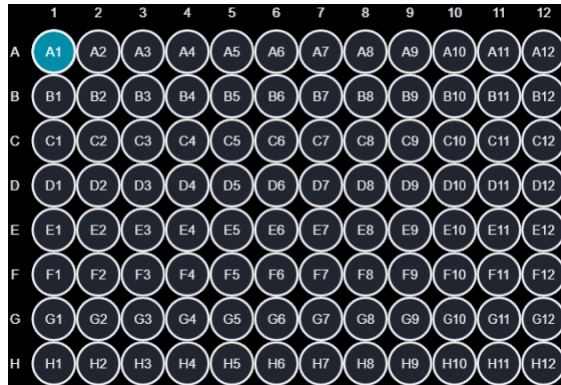


**Note:** To avoid overexposing your sample, live preview will time out and exit after five minutes.

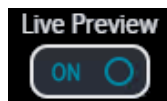
To view a live preview of a plate:

1. On the **Acquisition Settings** page, click  **Choose Position to Acquire**.
2. Click  **Select Well**.

- 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.



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

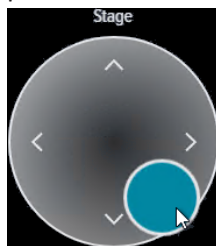


- Click and drag the stage joystick in the direction you want move the sample (X-Y) stage to view different areas of the well.

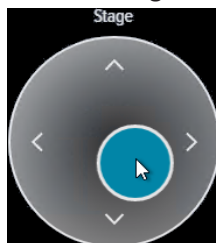


**Note:**

- For fast stage movements, drag the stage joystick toward the outermost position.

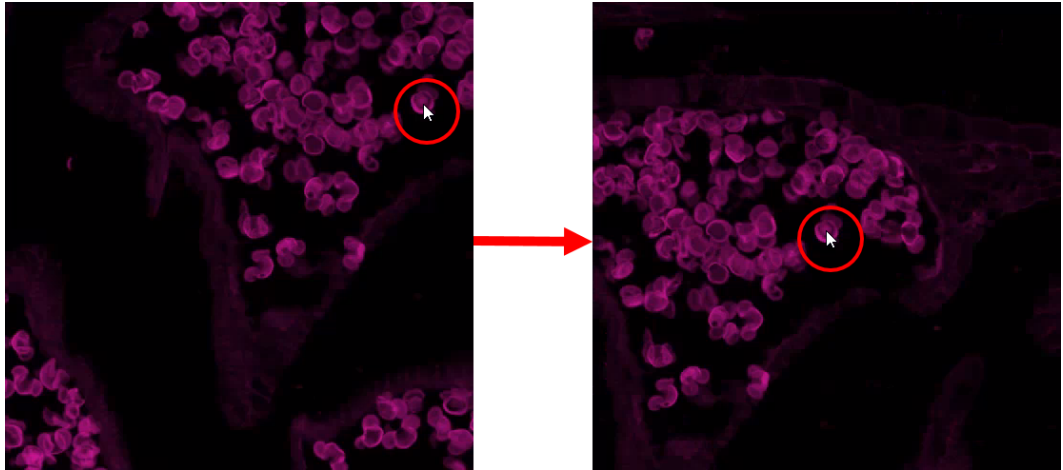


- For slow stage movements, drag the stage joystick slightly.



- When you find an appropriate location, release the stage joystick.

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. Click and drag the focus joystick to adjust the focus offset of the live preview. As you adjust the offset, the focus position value updates.



**Note:**

- For large focus adjustments, drag the focus joystick toward the outermost position.




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

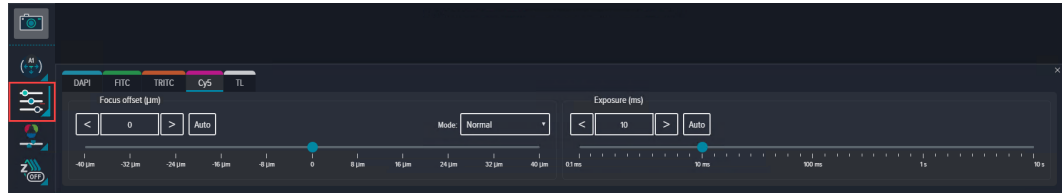


11. When you find the best possible focus, release the focus joystick.




**Tip:** You may want to snap a preview of the well to confirm your focus offset settings.

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

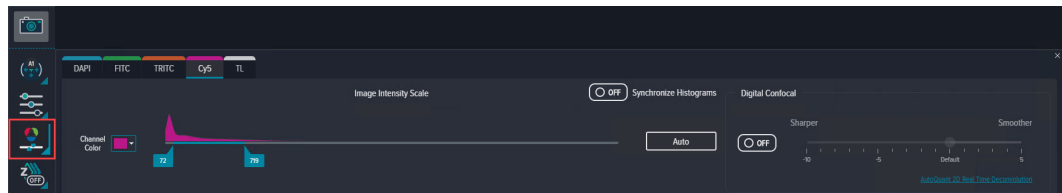


13. Use the **Exposure** controls to adjust the exposure time for each wavelength, which affects image brightness and results in a broader dynamic range (that is, the difference between the brightest and dimmest object detected).


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:** You may want to use **Auto** with a known bright sample, such as a positive control.

14. Click  **Image Intensity Settings**.



15. On the **Image Intensity Settings** tab, adjust the settings as needed for each channel:
- 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.
  - 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.



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. Click and drag the focus joystick to find the best focus position for the current channel.



**Note:**

- For large focus adjustments, drag the focus joystick toward the outermost position.



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



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 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.

Correction Collar  
on 40x Objective



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, Molecular Devices recommends 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 objective 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 objective door.
12. Click **OK**.





## Troubleshooting Autofocus Issues

The CellReporterXpress software uses hardware and software autofocus to determine the best focus for the sample. Autofocus issues 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 203](#) for details.

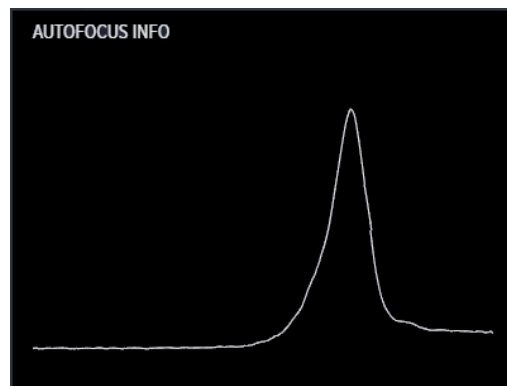
The **Autofocus Info** graph on the **Acquisition Settings** page shows the focus peaks for the most recent autofocus to help you understand how it was determined. This section describes how you can use this information to troubleshoot autofocus issues.

To troubleshoot autofocus issues:

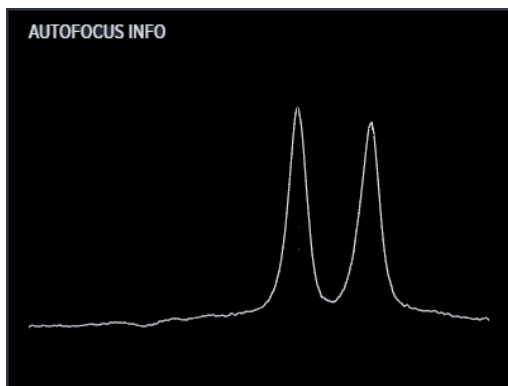
1. On the **Acquisition Settings** page, click  **Snap Image** to snap a preview image.
2. On the right side of the screen under **Tools**, click  **Autofocus Info** to display a graph showing the focus peaks of the autofocus.
3. Review the **Autofocus Info** graph along the following examples.

### 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.

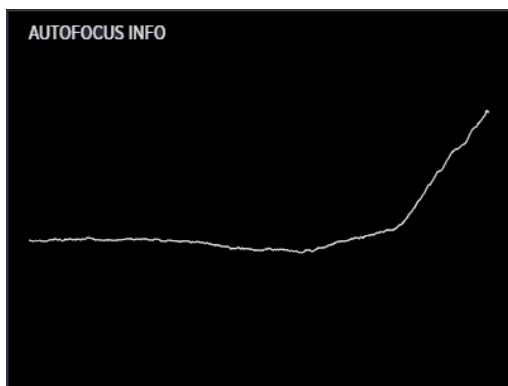


The following graph shows a good result of a Plate and Well Bottom (two-peak) autofocus. The software typically uses two-peak autofocus for plates with the **Mode** set to **Plate and Well Bottom** on the **Focus/Exposure Settings** tab of the **Acquisition Settings** page.

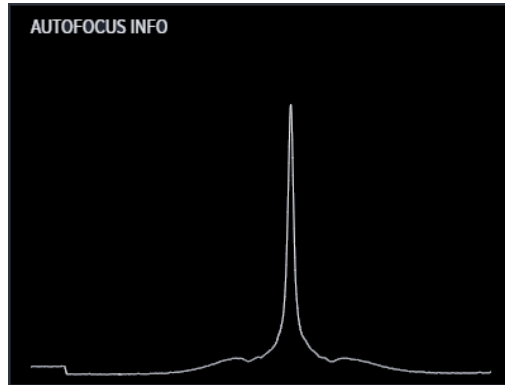


### Autofocus Issues

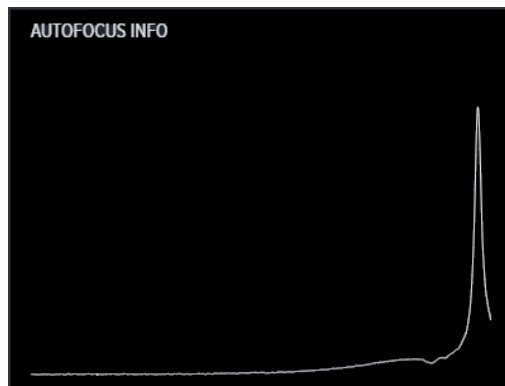
The following graph shows a failed result of a Normal (single-peak) autofocus. The incomplete single peak on the right side of the graph indicates that the value specified the well depth may be too high or the value specified for the skirt height may be too low. See [Labware Library on page 203](#) for details.



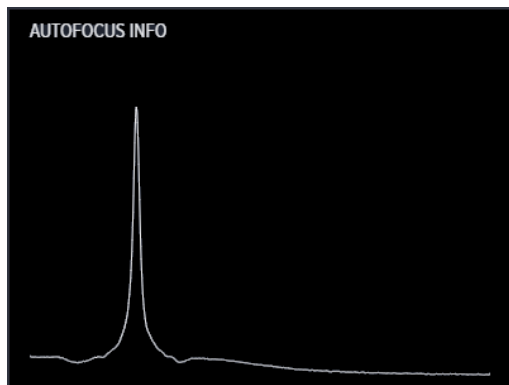
The following graph shows a failed result of a Plate and Well Bottom (two-peak) autofocus, where you would expect to see two peaks on the graph. The single peak in the center indicates that the value specified for the plate thickness may be too small. See [Labware Library on page 203](#) for details.



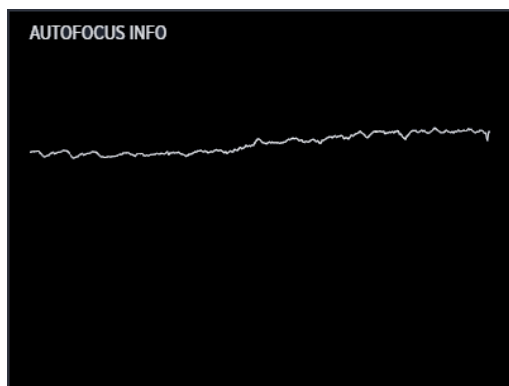
The following graph shows a failed result of a Plate and Well Bottom (two-peak) autofocus. The single peak on the right side of the graph indicates that the value specified the well depth may be too high or the value specified for the skirt height may be too low. See [Labware Library on page 203](#) 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 203](#) 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 203](#) 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 251](#) 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.

<b>Cell</b>	
Area	160.8285
Average Intensity	236.0123
Image Number	0
Integrated Intensity	19117
Well	"A1"
<b>Summary</b>	
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 Well

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

### Selecting Individuals Well

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

### Deselecting Individuals Well

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<sub>2</sub> level, and O<sub>2</sub> 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).

### Monitoring the CO<sub>2</sub> Level Sensor

To monitor the CO<sub>2</sub> level sensor:

1. On the **Device Sensors** page, in the **CO<sub>2</sub> Level** row, in the **Min** field, enter the lower limit for the CO<sub>2</sub> level range as a percentage.
2. In the **Max** field, enter the upper limit for the CO<sub>2</sub> 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<sub>2</sub> Level Sensor

To monitor the O<sub>2</sub> level sensor:

1. On the **Device Sensors** page, in the **O<sub>2</sub> Level** row, in the **Min** field, enter the lower limit for the O<sub>2</sub> level range as a percentage.
2. In the **Max** field, enter the upper limit for the O<sub>2</sub> 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 component 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<sub>2</sub> level, O<sub>2</sub> level, or temperature as needed, and then acquiring the next set of time points. See [Sensors on page 218](#) for details.

To set up a time series:

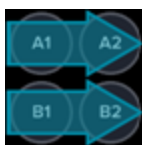
1. On the upper left of the **Time Series** page, set the toggle switch to **On**.
2. In the **Acquisition Order** section, click one of the following icons to indicate the order in which wells will be acquired for your time series:



**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

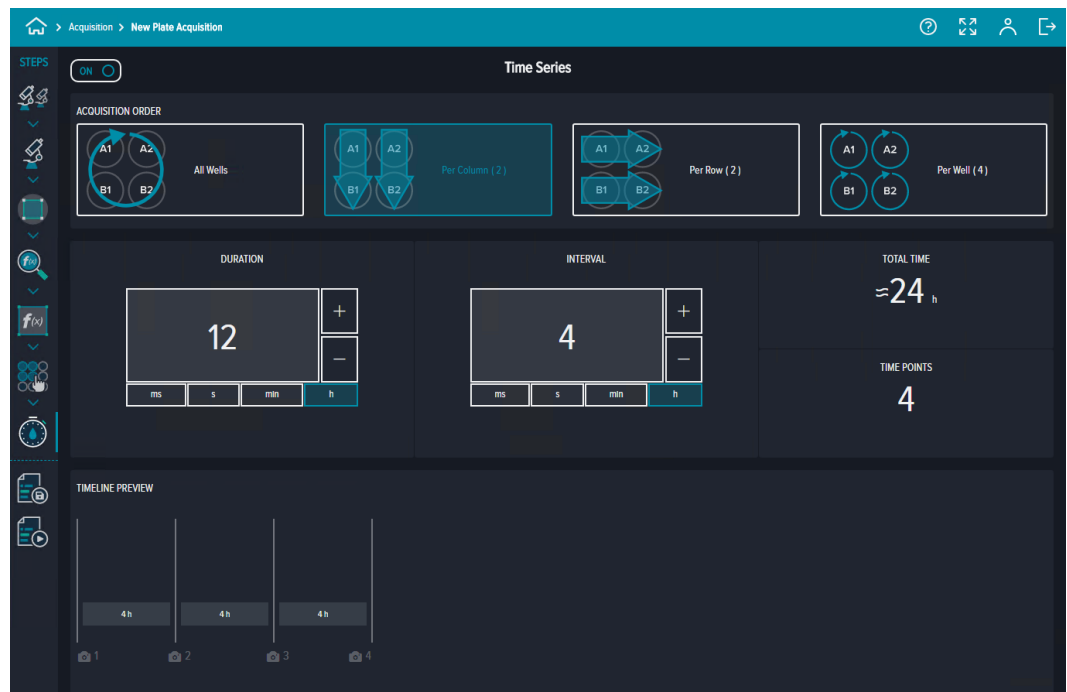
---

 **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.



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 66](#) or [Run Protocol on page 67](#) 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:** Molecular Devices recommends 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** checkbox 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** checkbox 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** checkbox to prevent other users from modifying the settings on the **Well Selection** page.




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

---

- 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 248](#) for details.

---

4. Click **Save Protocol**.



To continue to the next workflow step, click **Run Protocol**. See [Run Protocol on page 67](#) 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. (Optional) In the **Barcode** field, enter the barcode for the experiment labware.
  - c. (Optional) In the **Experiment Description** field, enter a description of the experiment.
  - d. 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 69](#) for details.
2. If you want to review the settings for image storage during and after acquisition, click  **Storage** and do the following:
  - a. 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.
  - b. In **Data Storage Settings** field, select a mapped folder for image storage after acquisition. See [Data Storage on page 212](#) for details on registering external computers and mapping folders for image storage. Select the **Preserve Raw Images** checkbox to save TIFF images of the acquisition.
3. If you want to manage the shared status of the experiment to restrict other users from viewing it, click  **Public** and do the following:
  - a. Select the **Private** checkbox.
  - b. 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 248](#) 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 199](#) 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 72](#) 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 98](#) 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 99](#) 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 104](#) 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 105](#) 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 106](#) 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 107](#) 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 can control multiple instruments, which can be configured differently. If your software is configured to control multiple instruments, you may need to select the instrument that is appropriate for your experiment. Different instruments may contain different objectives or filter cubes or may include different features, such as digital confocal or environmental control.



**Note:** If you click the  **Favorite** icon for a device, that device will be selected by default. Otherwise, the last used device will be selected by 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 format 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 96](#) 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 each individual Z stack plane.

## Specifying the Labware Format

To specify the labware format:

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






**Note:** If an appropriate slide format does not exist, you can add a new one. See [Labware Library on page 203](#) 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 use.
3. Click  **Move Stain Up** and  **Move Stain Down** as needed to select the order in which the stains will be collected.



**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 collected first.

## 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 94](#)
- [Troubleshooting Autofocus Issues, see page 96](#)

To continue to the next workflow step, click  **Region Selection to Acquire**. See [Region Selection to Acquire on page 98](#) 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.



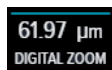
Zooms out on the image.



Resets the view zoom to the original image size.



Views the image full screen.



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.



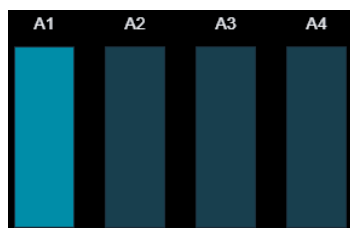
**Note:** If the snap fails, the software may display the following message:





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 96](#) for details.

2. At the bottom of the screen on the  **Choose Position to Acquire** tab, click  **Select Slide**.

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



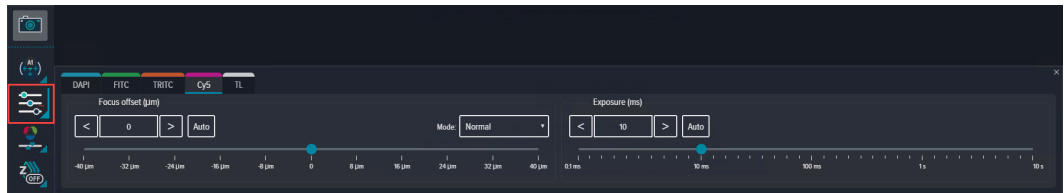
4. Click  **Snap Overview** to snap a low-resolution overview image of the entire slide in the single slide map. This can help you identify a region of interest for your experiment. The overview image is created by the overview camera, which uses no objective.

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



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

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




8. On the **Focus/Exposure Settings** tab, adjust the settings as needed for each channel:
- Use the **Focus offset** controls to adjust the image sharpness. Click the **Mode** drop-down list box to set one of the following autofocus ranges:
    - **Normal**: Hardware and image autofocus mode using a short search range.
    - **Wide**: Hardware and image autofocus mode using a wide range.
    - **Super Wide**: Hardware and image autofocus mode designed for samples that do not sit directly on the well bottom.
    - **Plate and Well Bottom**: Hardware-only autofocus mode designed for speed.

After you set the focus mode, click **Auto** next to the **Focus Offset** controls to determine the focus offset between stains. This is particularly useful when using the **Plate and Well Bottom** focus mode.

- 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.

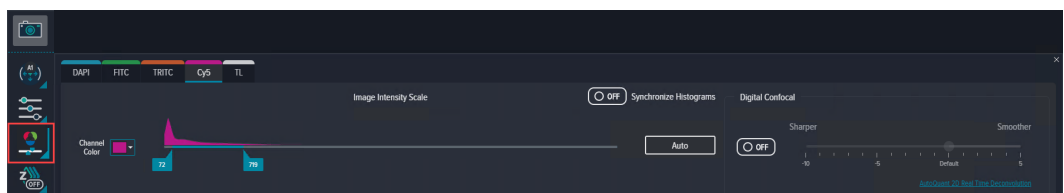
---

 **Tip:** You may want to use **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:
  - 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.
  - 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.



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 98](#) 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.



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



. Lower the **Exposure** value as needed.



Zooms in on the image.



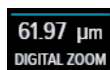
Zooms out on the image.



Resets the view zoom to the original image size.



Views the image full screen.





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:

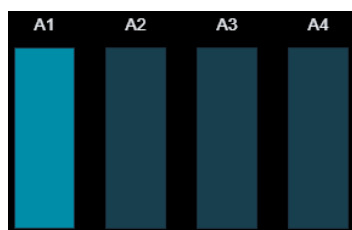
1. On the **Acquisition Settings** page, click  **Snap Image** to snap an initial preview image.
2. In the **Tools** pane on the right, click  **Comparison Mode**.






**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.

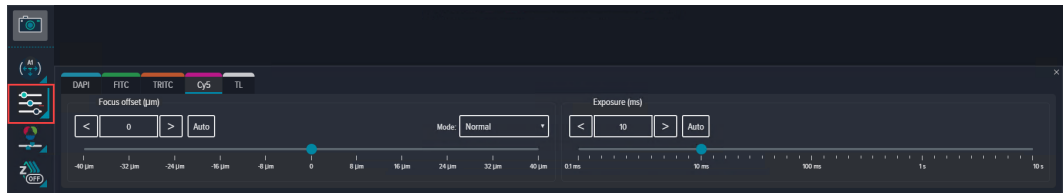


5. Click  **Select Slide** on the right.
6. In the slide holder map, select the second slide for the preview. The instrument snaps a preview image of the newly selected slide.
7. Click  **Snap Overview** to snap a low-resolution overview image of the entire slide in the single slide map. This can help 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:
- Use the **Focus offset** controls to adjust the image sharpness. Click the **Mode** drop-down list box to set one of the following autofocus ranges:
    - **Normal**: Hardware and image autofocus mode using a short search range.
    - **Wide**: Hardware and image autofocus mode using a wide range.
    - **Super Wide**: Hardware and image autofocus mode designed for samples that do not sit directly on the well bottom.
    - **Plate and Well Bottom**: Hardware-only autofocus mode designed for speed.

After you set the focus mode, click **Auto** next to the **Focus Offset** controls to determine the focus offset between stains. This is particularly useful when using the **Plate and Well Bottom** focus mode.

- 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).

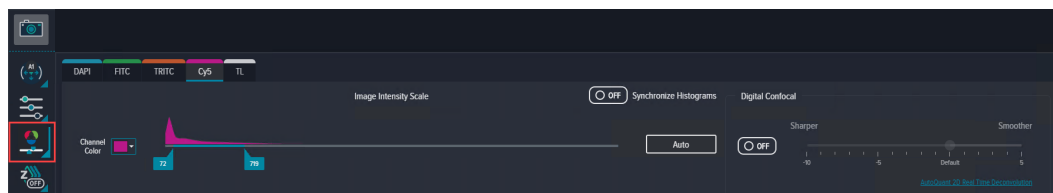
When your image is in focus, click **Auto** to automatically determine the optimal exposure for the best dynamic range. The longer the exposure, the brighter the experiment image. Dimmer objects get brighter with longer exposure time, as does the background. Increased exposure time slows the experiment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



**Tip:** You may want to use **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:
- 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.
  - 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:
- Set **Digital Confocal** to **On**.
  - As needed, move the slider to the right to smooth the image.
  - 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 98](#) 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 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.



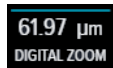
Zooms out on the image.



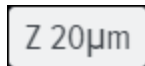
Resets the view zoom to the original image size.



Views the image full screen.



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




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

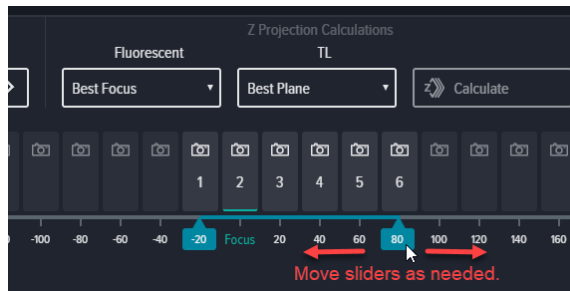


3. In the **Focus Step ( $\mu\text{m}$ )** field, modify the distance between the Z stack planes, as needed.

The default focus step distance varies based on the objective.

Objective	Default Focus Step Distance
4x	50 $\mu\text{m}$
10x	20 $\mu\text{m}$
20x	5 $\mu\text{m}$
40x	2 $\mu\text{m}$
63x	1 $\mu\text{m}$

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

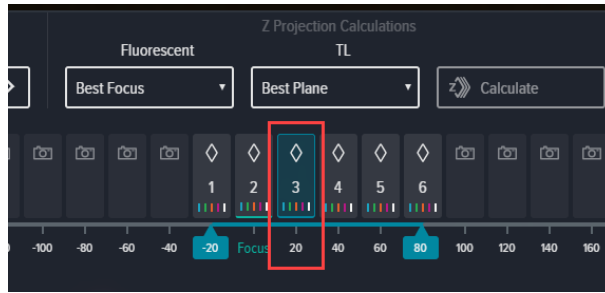


5. Click  **Snap Z Stacking Images** to snap the Z stack.

The Z stack projection appears, and the Z stack projection button highlights to indicate that you are viewing the projection.



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



- 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.
- 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.
- 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 98](#) for details.

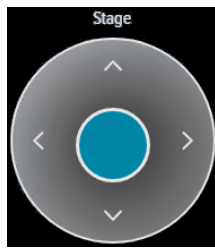
## Viewing a Live Preview of a Slide

Live preview enables you to view 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 to explore the live preview to find a region of interest. 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.



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.



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.



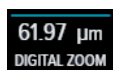
Zooms out on the image.



Resets the view zoom to the original image size.



Views the image full screen.



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.



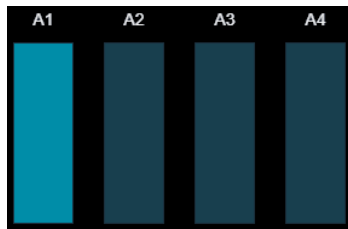
**Note:** To avoid overexposing your sample, live preview will time out and exit after five minutes.


To view a live preview of a slide:


1. On the **Acquisition Settings** page, click  **Choose Position to Acquire**.
2. Click  **Select Slide**.

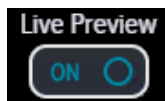


- In the slide holder map, select the slide for the preview. The instrument snaps a preview image of the newly selected slide. The **A1** slide is selected by default.



- 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.

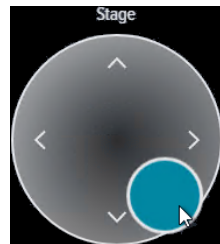
- In the single slide map, click and drag the  selection tool to select the region of the slide for the preview, if needed. The center area of the slide is selected by default.
- In the bottom pane, set **Live Preview** to **On**.



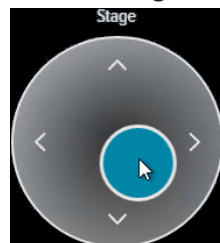
- Click and drag the stage joystick in the direction you want move the sample (X-Y) stage to view different areas of the slide.

**Note:**

- For fast stage movements, drag the stage joystick toward the outermost position.

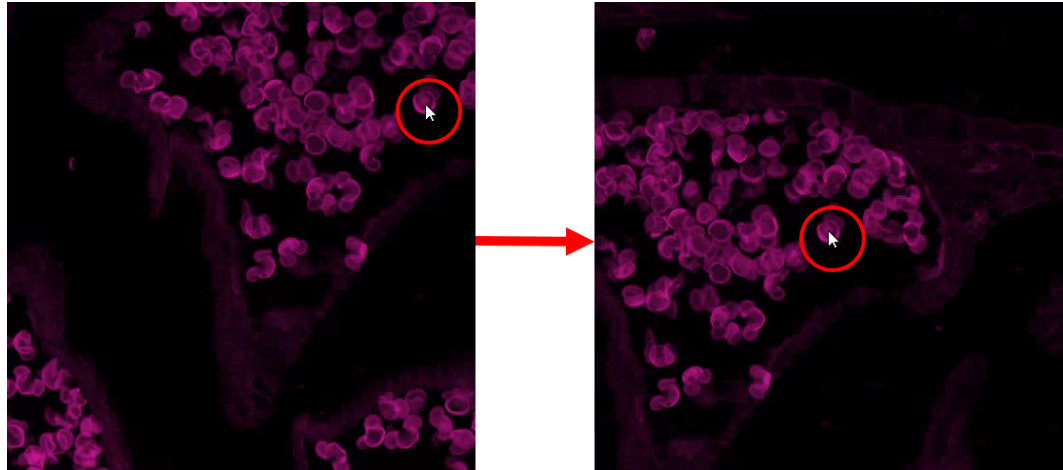


- For slow stage movements, drag the stage joystick slightly.



- When you find an appropriate location, release the stage joystick.

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



10. 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.

11. Click and drag the focus joystick to adjust the focus offset of the live preview. As you adjust the offset, the focus position value updates.



**Note:**

- For large focus adjustments, drag the focus joystick toward the outermost position.



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

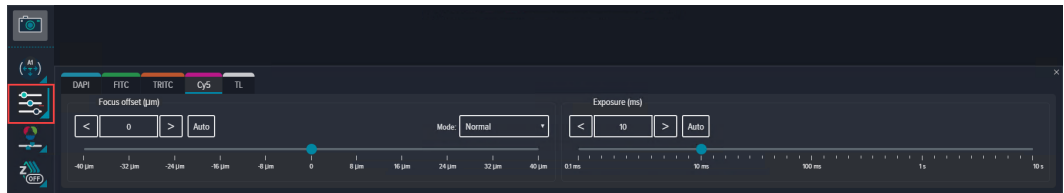


12. When you find the best possible focus, release the focus joystick.



**Tip:** You may want to snap a preview of the slide to confirm your focus offset settings.

13. Click  **Focus/Exposure Settings**.



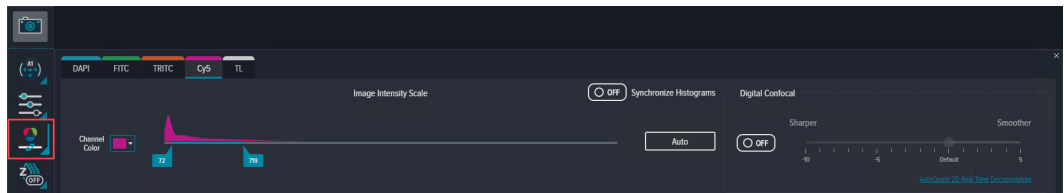
14. Use the **Exposure** controls to adjust the exposure time for each wavelength, which affects image brightness and results in a broader dynamic range (that is, the difference between the brightest and dimmest object detected).

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:** You may want to use **Auto** with a known bright sample, such as a positive control.

15. Click  **Image Intensity Settings**.



16. On the **Image Intensity Settings** tab, adjust the settings as needed for each channel:
- 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.
  - 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**.

17. 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.

---

18. 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.

---



19. Click **Choose Position to Acquire**.

20. Click and drag the focus joystick to find the best focus position for the current channel.



**Note:**

- For large focus adjustments, drag the focus joystick toward the outermost position.



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



21. 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.
22. Repeat these steps as needed to find other regions of interest.
23. Repeat these steps as needed for each channel.

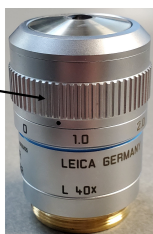


To continue to the next workflow step, click **Region Selection to Acquire**. See [Region Selection to Acquire on page 98](#) for details.

## 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.

Correction Collar  
on 40x Objective



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, Molecular Devices recommends 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 objective 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 objective door.
12. Click **OK**.

## Troubleshooting Autofocus Issues

The CellReporterXpress software uses hardware and software autofocus to determine the best focus for the sample. Autofocus issues 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 203](#) for details.

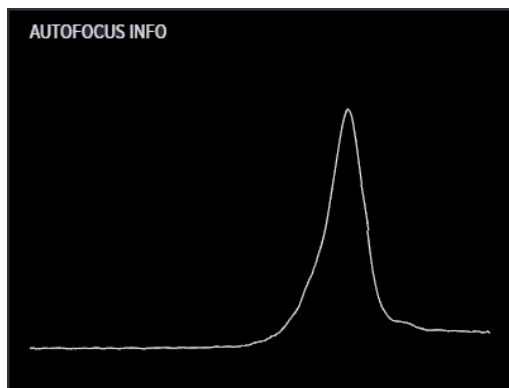
The **Autofocus Info** graph on the **Acquisition Settings** page shows the focus peaks for the most recent autofocus to help you understand how it was determined. This section describes how you can use this information to troubleshoot autofocus issues.

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

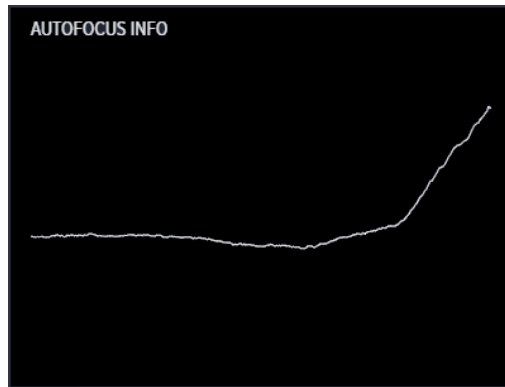
The following graph shows a good result of a Normal, Wide, or Superwide (single-peak) autofocus. The software typically uses single-peak autofocus for slides.



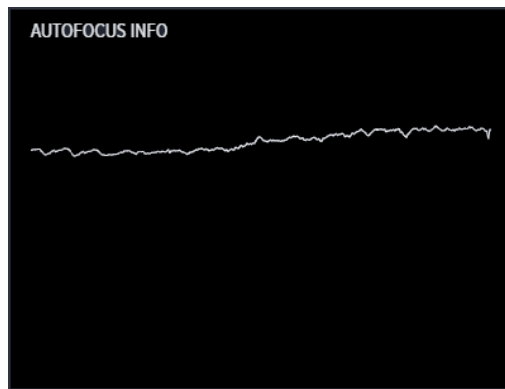


### Autofocus Issues

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 203](#) 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 203](#) 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 106](#) or [Run Protocol on page 107](#) for details.



To continue to the next workflow step for all other acquisitions, click **Analysis Settings.** See [Analysis Settings on page 99](#) 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 251](#) 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.

<b>Cell</b>	
Area	160.8285
Average Intensity	236.0123
Image Number	0
Integrated Intensity	19117
Well	"A1"
<b>Summary</b>	
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 101](#)
- [Testing the Analysis of Comparison Images, see page 102](#)
- [Saving Analysis Settings, see page 103](#)

To continue to the next workflow step, click  **Region Selection to Analyze**. See [Region Selection to Analyze on page 104](#) 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 103](#) for details.

## Testing the Analysis of Comparison Images

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

To test the analysis of comparison images:

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

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



**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 103](#) for details.

### Saving Analysis Settings

When you are satisfied with the quality of the preview in [Testing the Analysis of a Region on page 101](#) or [Testing the Analysis of Comparison Images on page 102](#), 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 104](#) 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 105](#) 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).

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**.



**Save Protocol** or



**Run**

To continue to the next workflow step, click either **Save Protocol** or **Run Protocol**. See [Save Protocol on page 106](#) or [Run Protocol on page 107](#) 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:** Molecular Devices recommends 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** checkbox 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** checkbox 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** checkbox is not enabled until you select the **Private** checkbox.

---

- 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 248](#) for details.

---

4. Click **Save Protocol**.



To continue to the next workflow step, click **Run Protocol**. See [Run Protocol on page 107](#) 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. (Optional) In the **Barcode** field, enter the barcode for the experiment labware.
  - c. (Optional) In the **Experiment Description** field, enter a description of the experiment.
  - d. 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 109](#) for details.
2. If you want to review the settings for image storage during and after acquisition, click  **Storage** and do the following:
  - a. 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.
  - b. In **Data Storage Settings** field, select a mapped folder for image storage after acquisition. See [Data Storage on page 212](#) for details on registering external computers and mapping folders for image storage. Select the **Preserve Raw Images** checkbox to save TIFF images of the acquisition.
3. If you want to manage the shared status of the experiment to restrict other users from viewing it, click  **Public** and do the following:
  - a. Select the **Private** checkbox.
  - b. 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 248](#) 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 199](#) 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:

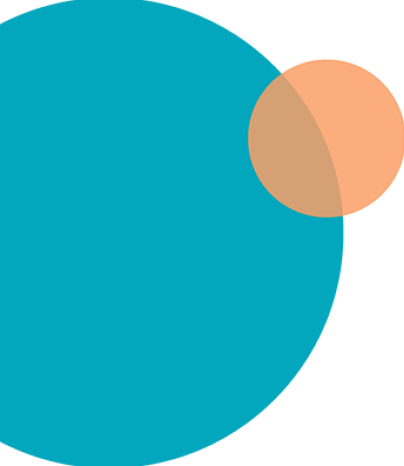
1. Click the link next to the  icon to open the workflow step for the invalid parameter.
2. Address the issue.



#### 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

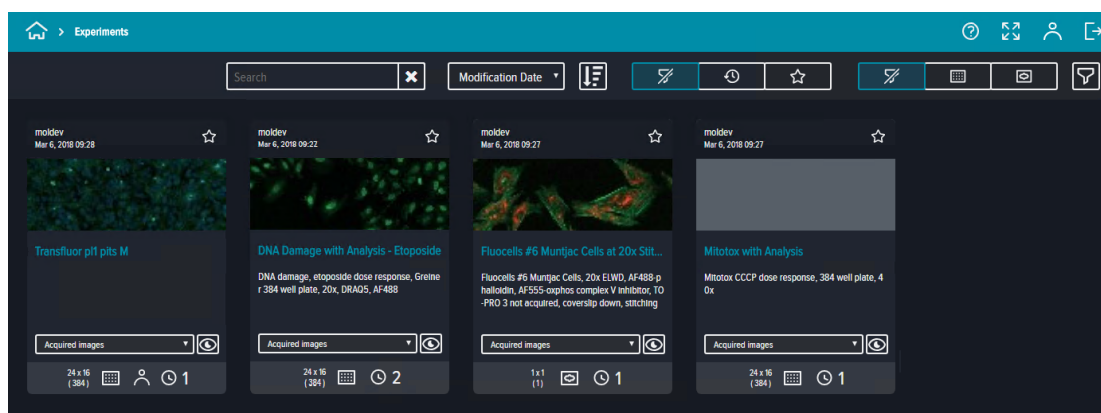
Use **Experiments** mode to view images and analysis data collected in **Acquisition** mode and perform additional offline analysis.



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

### Experiments Library

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



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


**12 x 8**  
**(96)** Geometry

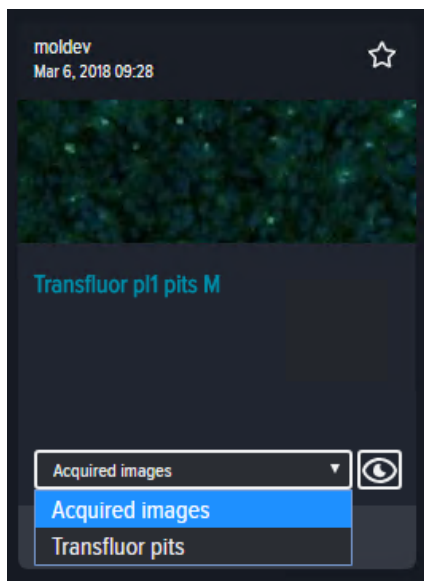
**Plate or Slide**

**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:

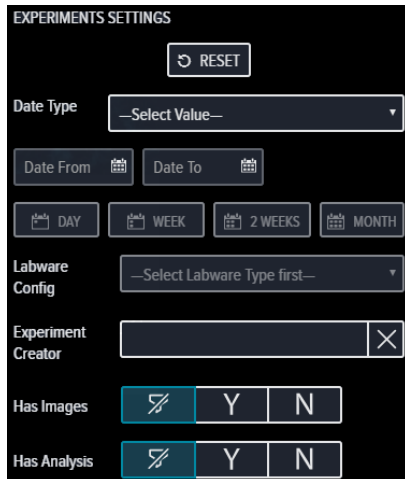
Select the filter icon you want to use. The icon is highlighted and only the cards matching the filter option are shown.

## Complex Filters

Click the  **Filter** icon 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.



The screenshot shows the 'EXPERIMENTS SETTINGS' dialog box with the following fields and controls:

- RESET** button
- Date Type** dropdown menu with "--Select Value--"
- Date From** and **Date To** date pickers
- Radio buttons for **DAY**, **WEEK**, **2 WEEKS**, and **MONTH**
- Labware Config** dropdown menu with "--Select Labware Type first--"
- Experiment Creator** text input field with a clear (X) button
- Has Images** filter with a toggle switch and **Y** / **N** buttons
- Has Analysis** filter with a toggle switch and **Y** / **N** buttons

## Experiments Page

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

The screenshot displays the 'Experiments' page for '20x Receptor Internalization'. The top navigation bar shows the breadcrumb: Experiments > 20x Receptor Internalization > Analyses. The main header area contains experiment details: Experiment Name (20x Receptor Internalization), Geometry (384 (24 x 16)), Description (N/A), and Barcode (N/A). Below this, there are sections for Operations, Annotation, Groups (0), Compounds (0), and Barcode (N/A). The main content area is divided into three sections:

- Analyses (Left):** A list of analyses with tabs for 'Analyses' and 'Acquisitions'. The list includes:
  - Internalization\_Cell (moldev, Apr 29, 2019 06:05, Internalization)
  - Internalization2 (moldev, Apr 29, 2019 05:48, Internalization)
  - Internalization (moldev, Apr 29, 2019 05:48, Internalization)
- Internalization\_Cell Detail View (Right):** A detailed view of the selected analysis, showing various views (PLATE TIME VIEW, IMAGES, PLATE VIEW, SUMMARY DATA) and tabs (INPUTS, TIME POINTS LIST, MEASUREMENTS, DESCRIPTION). The 'INPUTS' tab is active, showing:
 

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

Nuclear	
Channel	Nuclear
DAPI	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.

The screenshot displays the 'Experiment Details' pane in the software. At the top, the breadcrumb navigation shows 'Experiments > 20x Receptor Internalization > Analyses'. The main details section includes:

- Experiment Name:** 20x Receptor Internalization (with an edit icon)
- Geometry:** 384 (24 x 16)
- Description:** N/A (with an edit icon)
- Barcode:** N/A (with an edit icon)

Below this, there are sections for 'Operations' (with icons for stop, refresh, share, and delete), 'Annotation' (with an edit icon), 'Groups' (0), 'Compounds' (0), and another 'Barcode' field (N/A, with an edit icon).

The main content area is divided into two tabs: 'Analyses' and 'Acquisitions'. Under 'Analyses', there is a list of analyses:

- Internalization\_Cell:** Created by 'moldev' on 'Apr 29, 2019 06:05'. It has a 'Launch' button and a 'Duplicate' button.
- Internalization2:** Created by 'moldev' on 'Apr 29, 2019 05:48'.
- Internalization:** Created by 'moldev' on 'Apr 29, 2019 05:48'.

The 'Internalization\_Cell' analysis is selected, showing a detailed view with tabs for 'PLATE TIME VIEW', 'IMAGES', 'PLATE VIEW', and 'SUMMARY DATA'. The 'INPUTS' tab is active, showing a table of parameters:

Channel	Granule
FITC	Intensity above background: 300
	Min Width: 1
	Max Width: 8
Channel	Nuclear
DAPI	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 changing sharing permissions. See [Experiment Operations on page 117](#) for details.
- **Annotation:** Click **Edit Annotations** to open the **Annotations** page to upload or edit annotations. See [Annotations on page 119](#) 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 (one image per stain per well 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 tiled or stitched images. Images larger than 2 GB are scaled down to a 2 GB file size. In addition, if you plan to import the images into Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis Software for further processing and analysis, you can scale the image accordingly to meet the 32K x 32K pixel size required by the MetaXpress software.

 **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 Others from Viewing an Experiment on this page](#) for details.

 **Delete Experiment:** Permanently deletes an experiment and all experiment data, including 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 well 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 tiled or stitched images. Images larger than 2 GB are scaled down to a 2 GB file size. In addition, if you plan to import the images into Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis Software for further processing and analysis, you can scale the image accordingly to meet the 32K x 32K pixel size required by the MetaXpress software.

To export experiment images:

1. On the **Experiments** page, under **Operations**, click  **Export Experiment Images**.
2. Do one of the following:
  - a. Click **Export** to export the image as a TIFF file. If the resulting file size is larger than 2 GB, the file will be scaled down to 2 GB.
  - b. Click **Export for MetaXpress** to export the image as a TIFF file that can be imported into Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis Software.

The **Monitor** page opens to display the progress of the export. See [Monitor Mode on page 199](#) for details.


### Restricting Others from Viewing an Experiment

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 248](#) for details.


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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.

## 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 28.73 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 software 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 this page](#) 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, then 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.

The screenshot displays the 'Analyses' tab for the experiment '20x Receptor Internalization'. The top navigation bar shows the breadcrumb: Experiments > 20x Receptor Internalization > Analyses. Below the navigation, experiment details are shown: Experiment Name (20x Receptor Internalization), Geometry (384 (24 x 16)), Description (N/A), and Barcode (N/A). The main content area is divided into two tabs: 'Analyses' (selected) and 'Acquisitions'. Under 'Analyses', there is an 'Add Analysis' button and a list of three analyses: 'Internalization\_Cell', 'Internalization2', and 'Internalization'. Each analysis entry includes a thumbnail, name, user (moldev), timestamp, and a 'Launch' button. The 'Internalization\_Cell' analysis is selected, and its details are shown on the right. The details include a 'Granule' section with 'Channel' (FITC) and 'Intensity above background' (300), and a 'Nuclear' section with 'Channel' (DAPI) and 'Intensity above background' (31). Both sections also show 'Min Width' and 'Max Width' values.

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 124](#) or [Add Slide Analysis Workflow on page 135](#) 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 146](#) and [Slide Views on page 178](#) 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 125](#) 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 125](#) 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 130](#) for details.



**Well Selection for Analysis** is the step where you select the wells for the experiment. See [Well Selection for Analysis on page 131](#) 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 132](#) 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 125](#) 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 251](#) 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.

<b>Cell</b>	
Area	160.8285
Average Intensity	236.0123
Image Number	0
Integrated Intensity	19117
Well	"A1"
<b>Summary</b>	
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 127
- [Testing the Analysis of Comparison Images](#), see page 128
- [Saving Analysis Settings](#), see page 129

To continue to the next workflow step, click  **Region Selection to Analyze**. See [Region Selection to Analyze on page 130](#) 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 129](#) 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**.

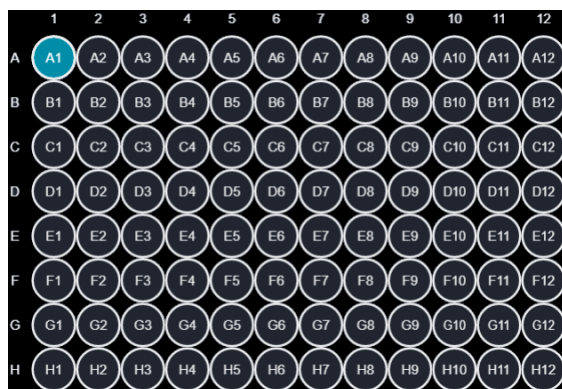
3. Click  **Algorithm Input**.

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.



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 on page 129](#) for details.

### Saving Analysis Settings

When you are satisfied with the quality of the preview in [Testing the Analysis of a Well on page 127](#) or [Testing the Analysis of Comparison Images on page 128](#), 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 130](#) 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 131](#) 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 Individuals Wells

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

### Deselecting Individuals 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 132](#) 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. (Optional) In the **Barcode** field, enter the barcode for the experiment labware.
  - c. (Optional) In the **Experiment Description** field, enter a description of the experiment.
  - d. 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 134](#) for details.
2. If you want to review the settings for image storage during and after acquisition, click  **Storage** and do the following:
  - a. 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.
  - b. In **Data Storage Settings** field, select a mapped folder for image storage after acquisition. See [Data Storage on page 212](#) for details on registering external computers and mapping folders for image storage. Select the **Preserve Raw Images** checkbox to save TIFF images of the acquisition.
3. If you want to manage the shared status of the experiment to restrict other users from viewing it, click  **Public** and do the following:
  - a. Select the **Private** checkbox.
  - b. 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 248](#) 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 199](#) 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 135](#) 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 136](#) 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 141](#) 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 142](#) 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 136](#) 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 251](#) 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.

<b>Cell</b>	
Area	160.8285
Average Intensity	236.0123
Image Number	0
Integrated Intensity	19117
Well	"A1"
<b>Summary</b>	
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 138](#)
- [Testing the Analysis of Comparison Images, see page 139](#)
- [Saving Analysis Settings, see page 140](#)

To continue to the next workflow step, click  **Region Selection to Analyze**. See [Region Selection to Analyze on page 141](#) 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.





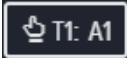
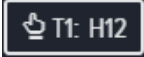


5. Click  **Choose Position to Acquire**.
6. Click  **Select Slide**.
7. Click  **Snap Overview** to snap a low-resolution overview image of the entire slide in the single slide map. This can help you identify a region of interest for your experiment. The overview image is created by the overview camera, 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 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 140](#) for details.

### Testing the Analysis of Comparison Images

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

To test the analysis of comparison images:

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

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



**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 140](#) for details.

### Saving Analysis Settings

When you are satisfied with the quality of the preview in [Testing the Analysis of a Region on page 138](#) or [Testing the Analysis of Comparison Images on page 139](#), 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 141](#) 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 142](#) 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. (Optional) In the **Barcode** field, enter the barcode for the experiment labware.
  - c. (Optional) In the **Experiment Description** field, enter a description of the experiment.
  - d. 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 144](#) for details.
2. If you want to review the settings for image storage during and after acquisition, click  **Storage** and do the following:
  - a. 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.
  - b. In **Data Storage Settings** field, select a mapped folder for image storage after acquisition. See [Data Storage on page 212](#) for details on registering external computers and mapping folders for image storage. Select the **Preserve Raw Images** checkbox to save TIFF images of the acquisition.
3. If you want to manage the shared status of the experiment to restrict other users from viewing it, click  **Public** and do the following:
  - a. Select the **Private** checkbox.
  - b. 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 248](#) 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 199](#) for details.

### Fixing Invalid Parameters

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

To fix an invalid parameter:

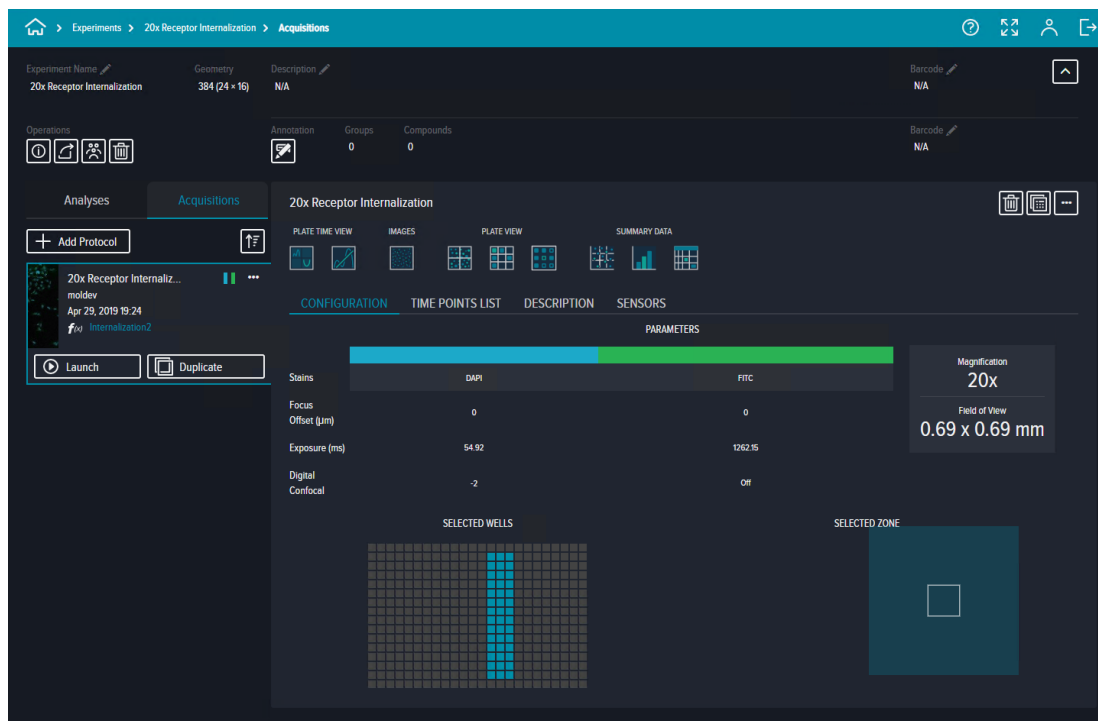
1. Click the link next to the  icon to open the workflow step for the invalid parameter.
2. Address the issue.

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




## Experiment Acquisition Details

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



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

- **Launch**: Opens the acquisition for the experiment and enables you to perform additional acquisitions (using the same protocol).
- **Duplicate**: Opens the acquisition for the experiment and enables you to modify the protocol and perform additional acquisitions.

In addition, you can click  to save the protocol as a template or delete the acquisition.

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

- **Inputs**: Displays the parameters for the acquisition set in **Acquisition** mode.
- **Time Points List**: Displays the timepoints for the acquisition and allows you to get details on specific timepoints.
- **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 146](#) and [Slide Views on page 178](#) 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 148](#) for details.



**Time Graph:** Shows measurements over time for selected wells. See [Plate Time Graph on page 150](#) for details.



**Thumbnail View:** Shows an overview of the well images in low resolution. See [Plate Thumbnail View on page 151](#) for details.



**Data View:** Shows a heatmap with up to four measurements displayed in the wells. See [Plate Data View on page 153](#) for details.



**Heatmap:** Shows a heatmap of one measurement. See [Plate Heatmap on page 155](#) for details.



**Images:** Shows high-resolution images for deep zoom viewing. See [Plate Images on page 157](#) for details.

### Summary Views



**Scatter Plot:** Shows a scatter plot of two summary measurements. See [Summary Scatter Plot on page 160](#) 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 162](#) for details.



**Table:** Shows a table with summary well-level measurements. See [Summary Table on page 165](#) 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 168](#) 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 170](#) 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 172](#) for details.



**Cell Level Table:** Shows a table with cellular measurements. See [Cell Zoom Level Table on page 174](#) for details.

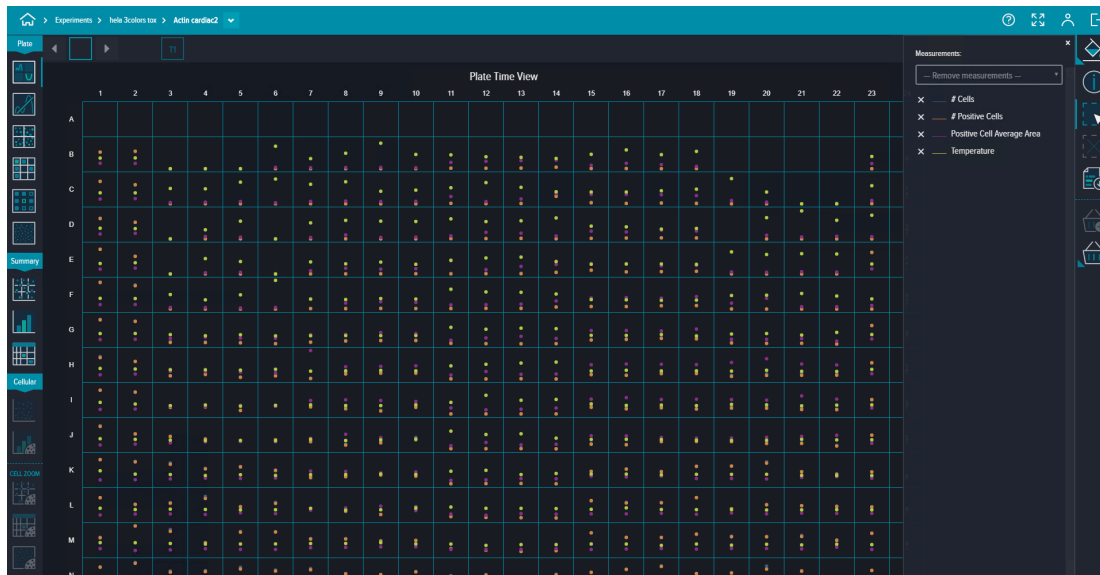


**Cell Level Images:** Shows high-resolution images for deep zoom viewing of individual cells. See [Cell Zoom Level Images on page 176](#) for details.

## 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.



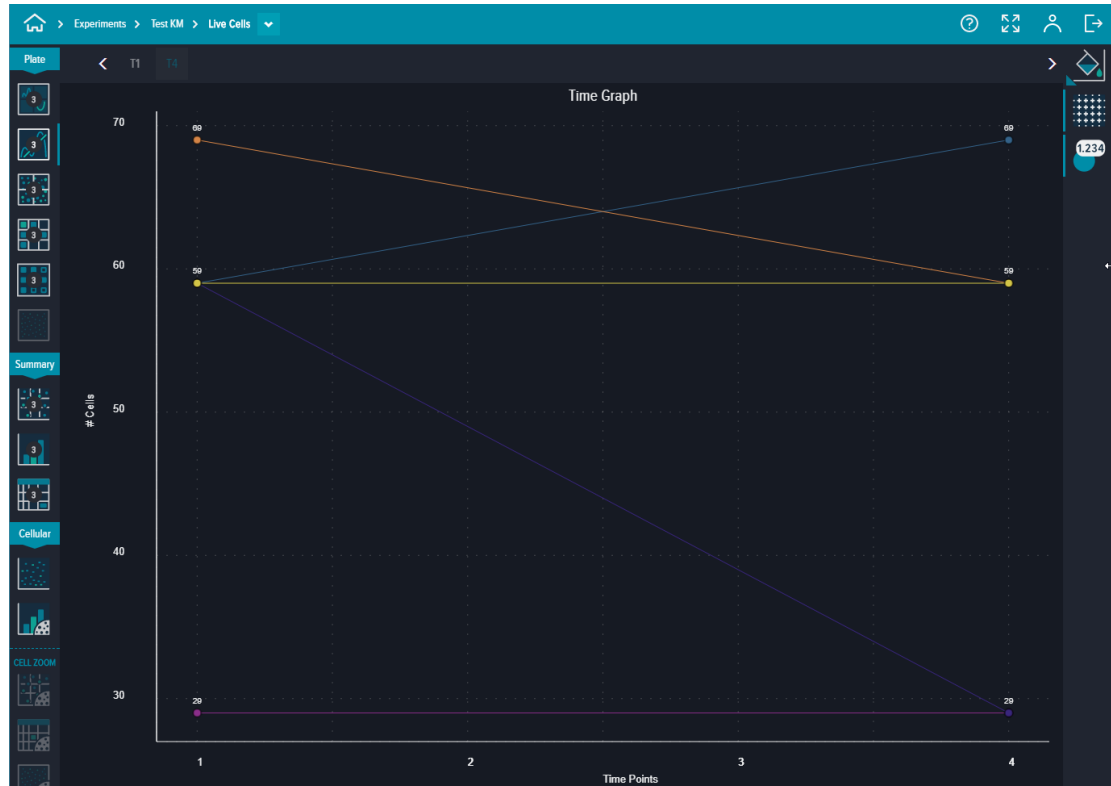
**Quick List:** Opens the Quick List where you can access saved selections.

## 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.

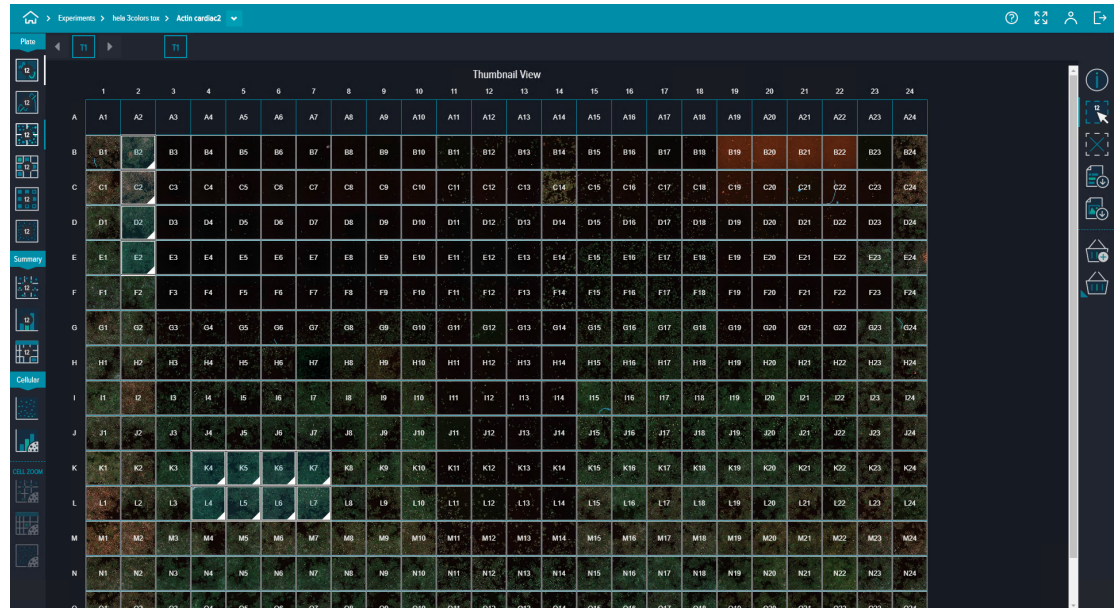


**Toggle Value Labels:** Displays the numeric value for the selected measurement at each timepoint.

## 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 157](#) 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 TIFF images (one image per stain per well per time point) of the selected wells. Exported images are stored in the C:\crx-export folder on the host computer. You can export tiled or stitched images. Images larger than 2 GB are scaled down to a 2 GB file size. In addition, if you plan to import the images into Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis Software for further processing and analysis, you can scale the image accordingly to meet the 32K x 32K pixel size required by the MetaXpress software.



**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.




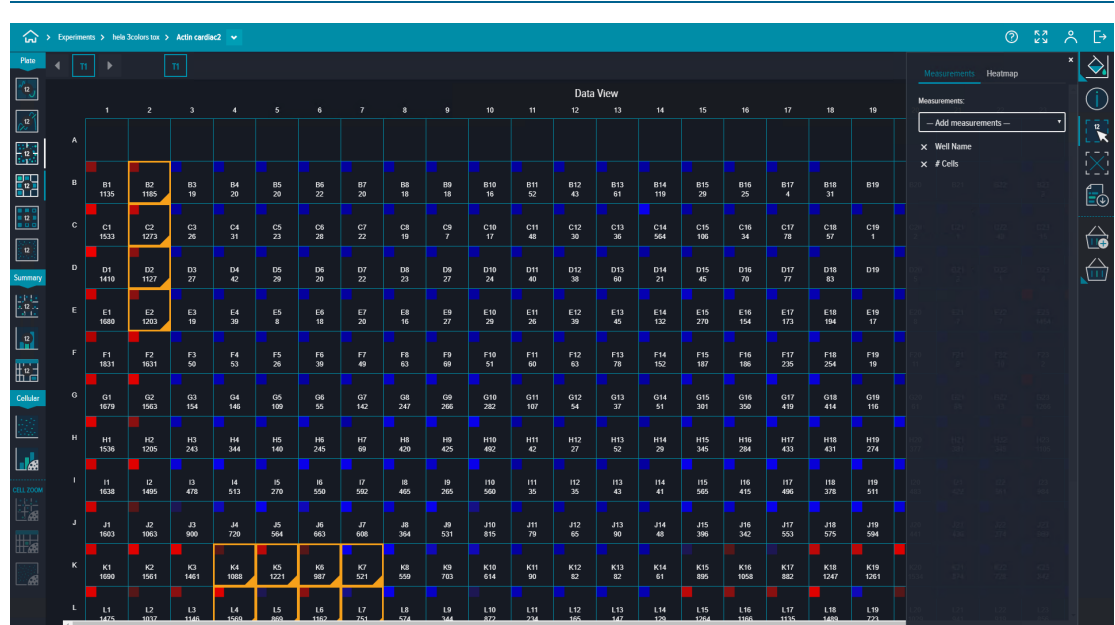
## Plate Data View



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 246 for details.



The right side of the page includes the following icons:



**Measurements/Heatmap:** Selects the measurements to show and the measurements to use for the heatmap.



**Well Info Mode:** Shows summary measurements for the selected well.



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



**Deselect All:** Deselects all wells.



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



**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.

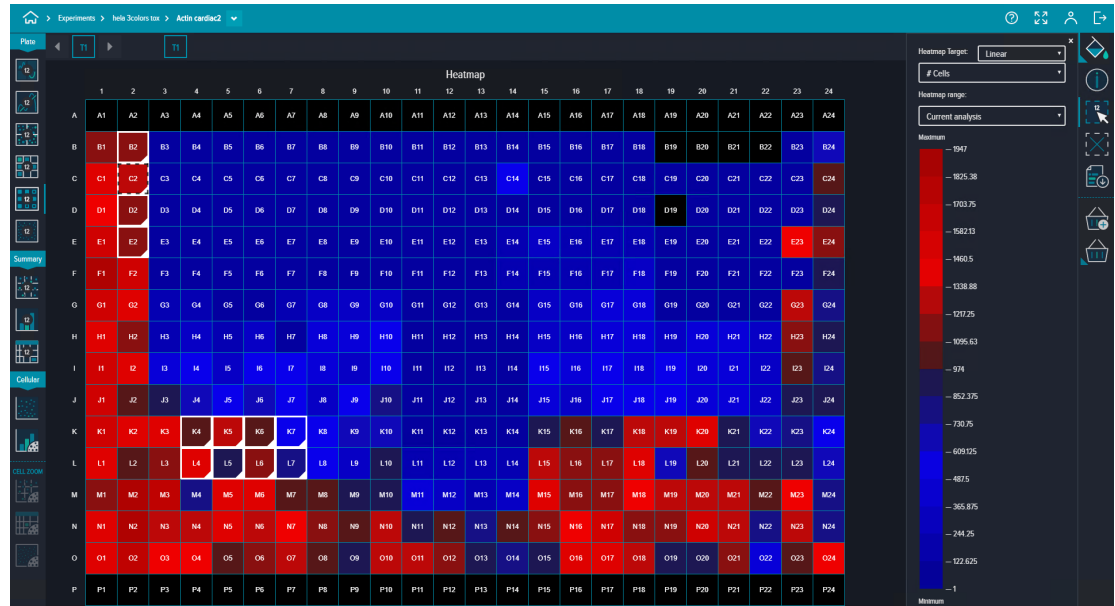
## Plate Heatmap



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



**Tip:** The heatmap color scheme can be changed in  **Configuration Settings**. See [Color Scheme on page 246](#) for details.



The right side of the page includes the following icons:



**Color:** Selects the measurement to use as the heatmap and the heatmap color scale to display.



**Well Info Mode:** Shows summary measurements for the selected well.



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



**Deselect All:** Deselects all wells.



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



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



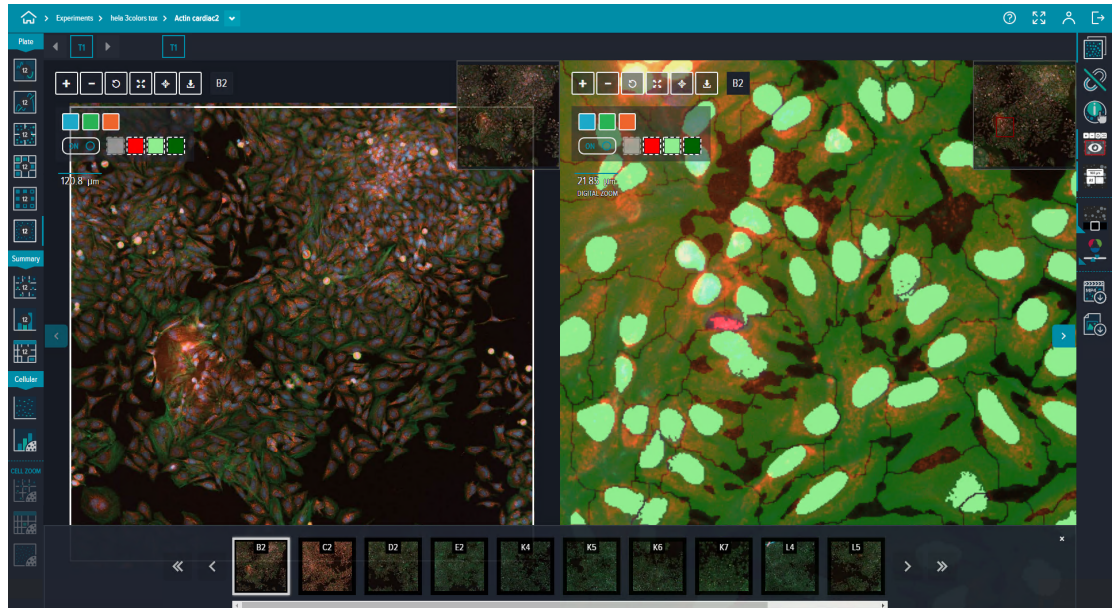
**Quick List:** Opens the Quick List where you can access saved selections.

## 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 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 an MP4 video of the current plate image over time. This function is not available with a tablet.




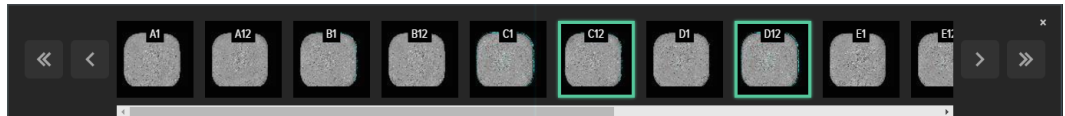
**Export Raw Images:** Exports a TIFF image (one image per stain per well per time point) of the current plate image. Exported images are stored in the C:\crx-export folder on the host computer. You can export tiled or stitched images. Images larger than 2 GB are scaled down to a 2 GB file size. In addition, if you plan to import the images into Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis Software for further processing and analysis, you can scale the image accordingly to meet the 32K x 32K pixel size required by the MetaXpress software.



## 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**.

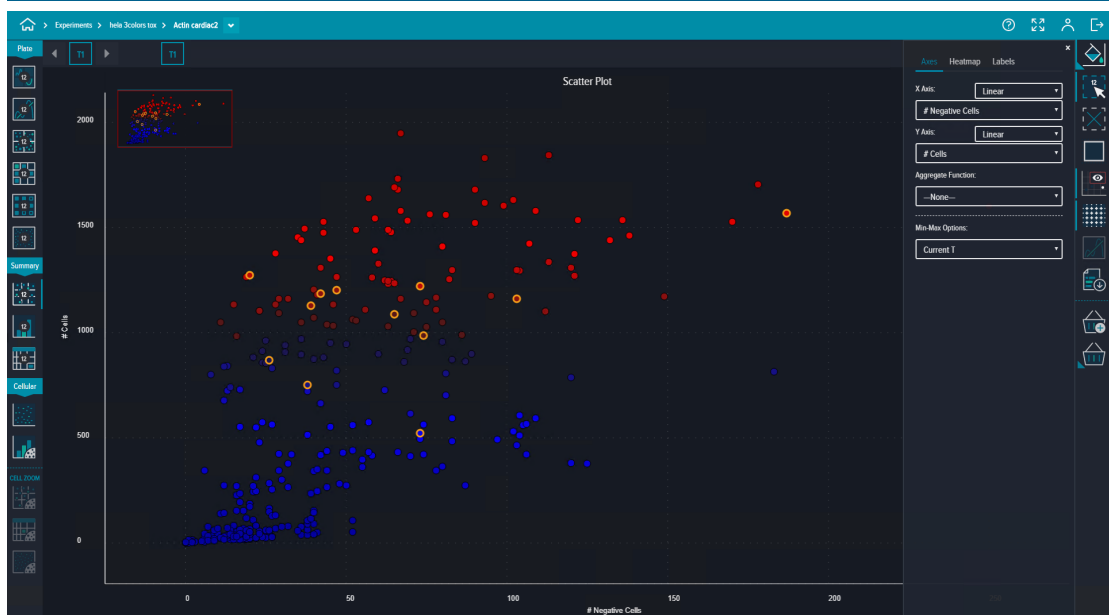
## Summary Scatter Plot



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 246 for details.





The right side of the page includes the following icons:



**Axes/Color:** Selects the measurements to show and the measurements to use for the heatmap.



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



**Deselect All:** Deselects all wells.



**Show Selected Only:** Shows or hides the selected data or all the data.



**Toggle Mini Map:** Shows or hides the small overview of the graph at the top left.



**Toggle Grid Lines:** Toggles the display of grid lines on the graph.



**Aggregation Function Line:** Not used in this version of the CellReporterXpress software.



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



**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 246](#) for details.



The right side of the page includes the following icons:



**Axes/Color:** Selects the measurements to show and the measurements to use for the heatmap.



**Well Info:** Shows summary measurements for the selected well.



**Zoom In:** When bins are selected, replots the selected bars only for a more granular view.



**Zoom Out:** When bins are selected, replots the selected bars only for a more general view.



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



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

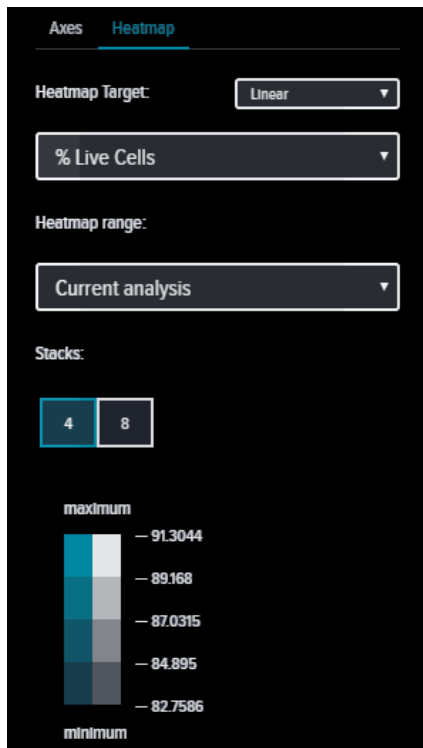


**Quick List:** Opens the Quick List where you can access saved selections.

## 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 and intervals.
- **Heatmap:** Specifies the heatmap coloring to the graph data.

### Stacks


**Stacks** show the range of values for each shade in the heatmap. The number of shades of each color can be adjusted to either **4** or **8**.

## 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 246 for details.

Well Name	# Cells	# Negative Cells	# Positive Cells	% Negative Cells	% Positive Cells	All Cell Averages Intensity	Positive Cell Average Area	Positive Cell Intensity	Positive Cell Total Area	Positive Cell Total Intensity	
B1	1135	46	1089	4.0526	95.9471	626.342	997.005	1.4275e-06	633.188	1.0831e-06	1.5547e-09
C1	1533	69	1464	4.5008	95.499	654.988	866.712	1.28687e-06	653.648	1.26887e-06	1.86398e-09
D1	1480	80	1400	5.3716	94.6282	631.37	889.89	1.2028e-06	634.735	1.0235e-06	1.7947e-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	1631	63	1568	5.0799	94.9208	636.872	818.502	1.20097e-06	638.375	1.4225e-06	2.08729e-09
G1	1679	90	1589	5.36033	94.6397	628.966	828.999	1.09592e-06	630.441	1.3302e-06	1.90032e-09
H1	1536	122	1414	7.9421	92.0573	641.813	812.98	1.0702e-06	642.039	1.4805e-06	1.66432e-09
I1	1638	57	1581	3.47965	96.5201	708.986	896.276	1.46076e-06	709.42	1.4070e-06	2.3174e-09
J1	1603	99	1504	6.17592	93.8241	635.08	804.641	1.0194e-06	635.35	1.2198e-06	1.76878e-09
K1	1690	85	1605	5.02958	94.9704	613.889	834.659	1.55877e-06	615.657	1.35022e-06	2.532e-09
L1	1134	15	1119	1.32275	98.6772	715.327	1089.85	1.78633e-06	715.134	1.21954e-06	1.98034e-09
M1	1475	43	1432	2.91525	97.0847	675.498	838.91	1.2826e-06	675.033	1.20137e-06	1.8367e-09
N1	1526	42	1482	2.75182	97.2482	647.511	866.075	1.30529e-06	648.078	1.21405e-06	1.93575e-09
O1	1477	64	1413	4.3331	95.6669	643.869	802.051	1.18276e-06	644.363	1.1333e-06	1.67043e-09
O2	1733	66	1667	3.80642	96.1936	649.383	832.361	1.27602e-06	649.066	1.38755e-06	2.04377e-09
L2	1037	44	993	4.24301	95.757	595.93	1001.09	1.3395e-06	596.665	944081	1.34946e-09
M2	1947	67	1880	3.4419	96.5588	708.777	788.462	1.23896e-06	709.585	1.44476e-06	2.32927e-09
N2	1643	113	1530	6.8131	93.8987	673.115	801.177	1.23756e-06	674.475	1.38504e-06	2.11059e-09
K2	1561	81	1480	5.18988	94.811	617.075	871.592	1.22243e-06	616.418	1.28996e-06	1.80919e-09
J2	1063	52	1011	4.8982	95.1082	595.761	966.014	1.30368e-06	597.514	93640	1.38102e-09
H2	1205	40	1165	3.3195	96.6805	607.3	946.48	1.3059e-06	607.094	1.0205e-06	1.52054e-09
I2	1495	37	1458	2.47462	97.5251	610.246	968.506	1.35086e-06	610.458	1.42038e-06	1.96955e-09
G2	1563	76	1487	4.86244	95.1376	634.015	880.661	1.26552e-06	635.084	1.30959e-06	1.88183e-09
F2	1031	102	929	9.79633	90.2037	624.013	877.014	1.09091e-06	620.262	1.2005e-06	1.81125e-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.




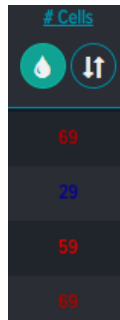
**Quick List:** Opens the Quick List where you can access saved selections.

## 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 .



## 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.

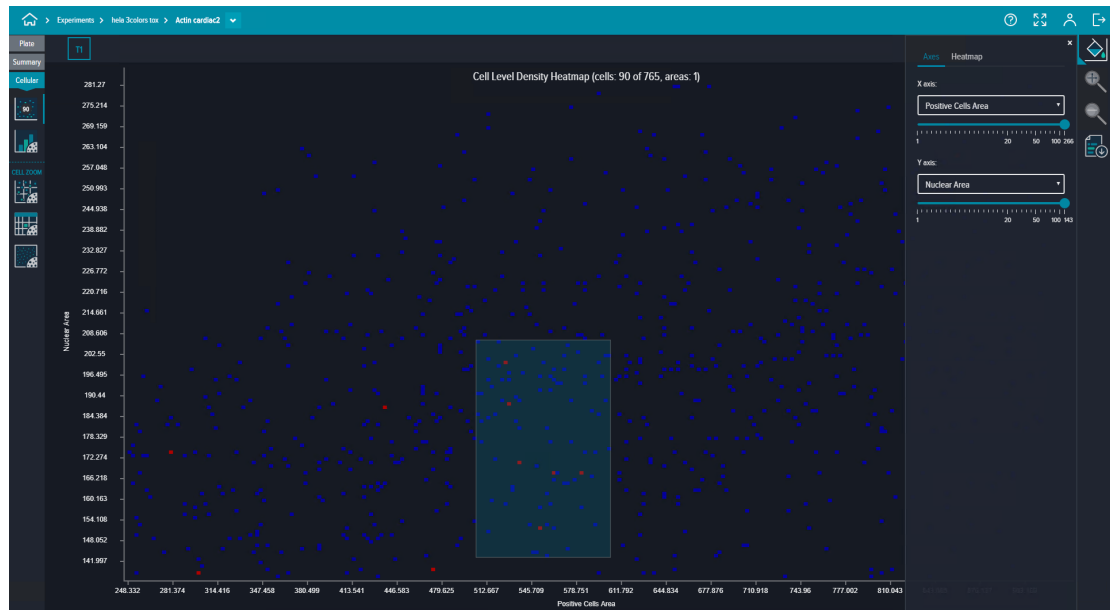


**Note:** You must select at least one well in a Plate view or a Summary view to enable the Cellular views.



**Tip:** The heatmap color scheme can be changed in  **Configuration Settings**. See [Color Scheme on page 246](#) for details.

The page heading indicates the number of selected cells (out of the total number of cells plotted on the graph) and the number of selected wells.





The right side of the page includes the following icons:



**Axes/Color:** Selects the measurements to show and the measurements to use for the heatmap.



**Zoom In:** When bins are selected, replots the selected bars only for a more granular view.



**Zoom Out:** When bins are selected, replots the selected bars only for a more general view.



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

### Axes/Color Pane



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

The following tabs are available:

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

## Cell Level Stacked Bar

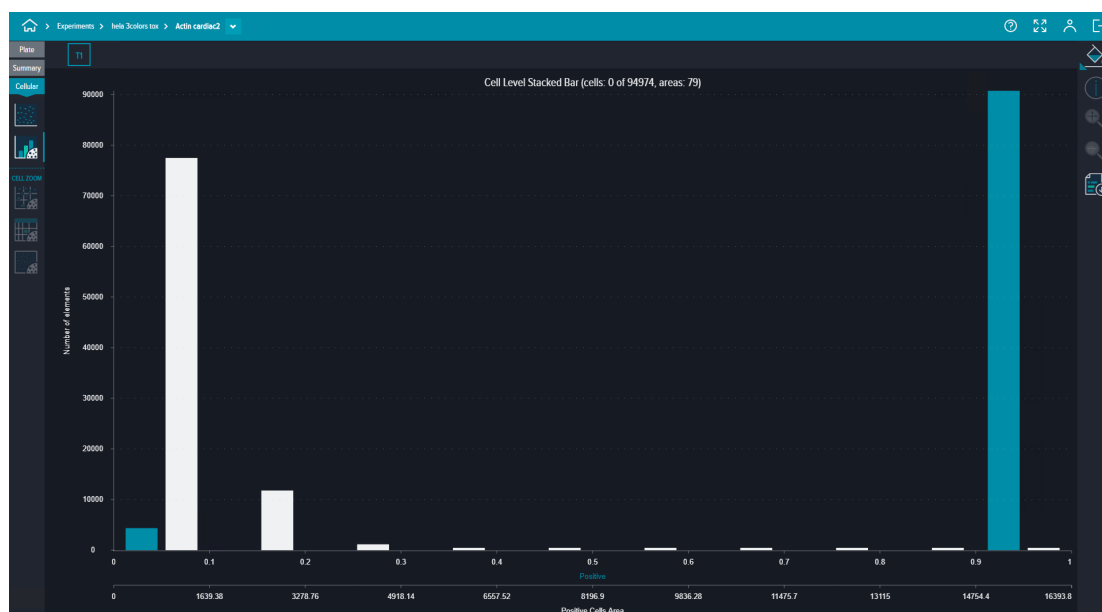


The **Cell Level Stacked Bar** shows a histogram-style bar graph with up to two different measurements side by side. You can add a third measurement to subdivide each bar.



**Note:** You must select at least one well in a Plate view or a Summary view to enable the Cellular views.

The page heading indicates the number of selected cells (out of the total number of cells plotted on the graph) and the number of selected wells.



The right side of the page includes the following icons:



**Axes/Color:** Selects the measurements to show and the measurements to use for the heatmap.



**Cell Info:** Shows summary measurements for the selected well.



**Zoom In:** When bins are selected, replots the selected bars only for a more granular view.



**Zoom Out:** When bins are selected, replots the selected bars only for a more general view.



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

### Axes/Color Pane



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

The following tabs are available:

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

## Cell Zoom Level Scatter Plot




The **Scatter Mode** shows a scatter plot of two measurements.

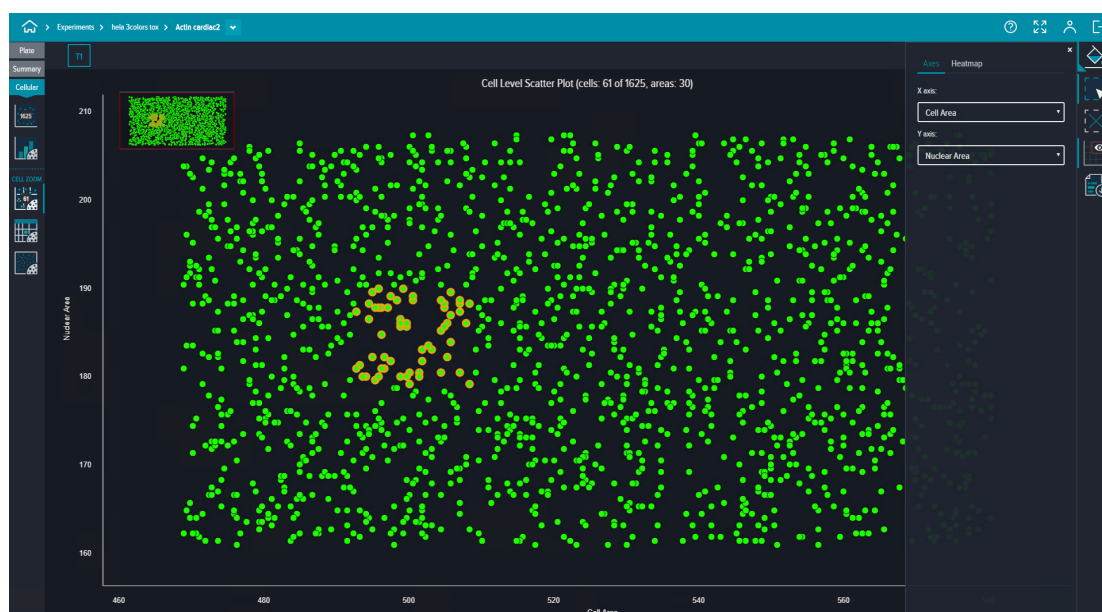


**Note:** You must select at least one bin in a Cellular view to enable the Cell Zoom views.



**Tip:** The heatmap color scheme can be changed in  **Configuration Settings**. See [Color Scheme on page 246](#) for details.

The page heading indicates the number of selected cells (out of the total number of cells plotted on the graph) and the number of selected wells.



The right side of the page includes the following icons:



**Axes/Color:** Selects the measurements to show and the measurements to use for the heatmap.



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



**Deselect All:** Deselects all wells.



**Toggle Mini Map:** Shows or hides the small overview of the graph at the top left.



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

### Axes/Color Pane



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

The following tabs are available:

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

## Cell Zoom Level Table




The **Cell Level Table** shows a table with cell-level measurements.



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



**Tip:** The heatmap color scheme can be changed in  **Configuration Settings**. See [Color Scheme](#) on page 246 for details.

The screenshot shows the software interface with a 'Cellular' view selected. The 'Cell Level Table' displays a grid of data for various cell bins. The table has columns for 'Well Name', 'Cell Area', 'Nucleus Area', 'Positives', and multiple 'Positives Cells' categories (e.g., 'Positives Cells Average Image', 'Positives Cells Unweighted Bin', etc.). Each row represents a cell bin, and the cells contain numerical values representing measurements.

Well Name	Cell Area	Nucleus Area	Positives	Positives Cells Area	Positives Cells Average Image	Positives Cells Unweighted Bin	Positives Cells Unweighted Bin	Positives Cells Unweighted Bin	Positives Cells Unweighted Bin	Positives Cells Unweighted Bin	Positives Cells Unweighted Bin	Positives Cells Unweighted Bin
L1	506.632	192224	1	506.632	551.455	644604	322.988	139531	551.455	587002	644604	
L1	484.064	181.55	1	484.064	531.230	605602	335.017	140414	531.230	530.578	605602	
N1	506.632	189.824	1	506.632	584.189	682907	311.091	138258	584.189	558.584	682907	
N1	502.728	183.324	1	502.728	595.712	690330	304.671	128876	595.712	592.232	690330	
N1	509.232	179.423	1	509.232	593.317	697488	323.623	133980	593.317	571.604	697488	
N1	504.032	183.324	1	504.032	663.842	772764	325.799	137813	663.842	711.884	772764	
O1	499.688	185.924	1	499.688	607012	699885	478.189	205143	607012	670.054	699885	
O1	496.664	190.258	1	496.664	59706	684231	278.415	122224	59706	611.74	684231	
O1	500.564	183.924	1	500.564	528.281	610273	291.448	125021	528.281	571.084	610273	
O1	498.388	189.391	1	498.388	532.831	612871	297297	125999	532.831	543.927	612871	
O1	497.964	188.091	1	497.964	535.992	615855	301136	130693	535.992	555.009	615855	
O1	497097	188.052	1	497097	621488	702847	302131	129010	621488	641.452	702847	
H2	499.284	188.958	1	499.284	579.957	668710	267743	116736	579.957	596.582	668710	
G2	500.998	180.723	1	500.998	637849	737253	251.18	104742	637849	747253	737253	
E2	500131	186.357	1	500131	584.922	659200	305.737	133467	584.922	600.686	659200	
K3	508.199	186.337	1	508.199	605.437	707190	278.942	119945	605.437	587378	707190	
K5	497531	18157	1	497531	538.807	619698	305.036	127505	538.807	553.684	619698	
M6	505.332	185.491	1	505.332	630.736	735438	296.792	127027	630.736	633.979	735438	
M6	500131	190.258	1	500131	603.991	754206	338.866	143884	603.991	636.622	754206	
M6	502.298	180.723	1	502.298	638.412	739900	343.192	143111	638.412	532.022	739900	
O10	495.364	186.791	1	495.364	557653	637297	232.884	100373	557653	579.313	637297	
O10	505.765	183.924	1	505.765	601383	77846	3421	146761	601383	756.958	77846	
N10	495.797	189.824	1	495.797	590184	675071	257356	112722	590184	579.411	675071	
N10	500.998	179.423	1	500.998	642.694	742954	301118	124663	642.694	652.63	742954	
L10	454.813	130.25	1	454.813	508.321	637081	305.018	132228	508.321	556.653	637081	

The right side of the page includes the following icons:



**Legend:** Enables you to select a measurement and show the mapping between color and values. Provides information used when column heatmaps are active.



**Reset:** Reverts the table to the default configuration.



**Well Info Mode:** Shows summary measurements for the selected well.




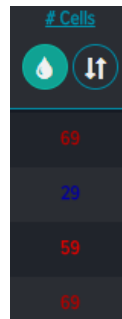
**Export:** Downloads the currently configured table as a CSV file. This function is not available with a tablet.

### Changing Columns

To change the column headings and associated data, click the heading name and then select from the menu options.

### Adding Heatmap Coloring

To add heatmap coloring to a column of data, click .



### Sorting Data

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

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

## Cell Zoom Level Images

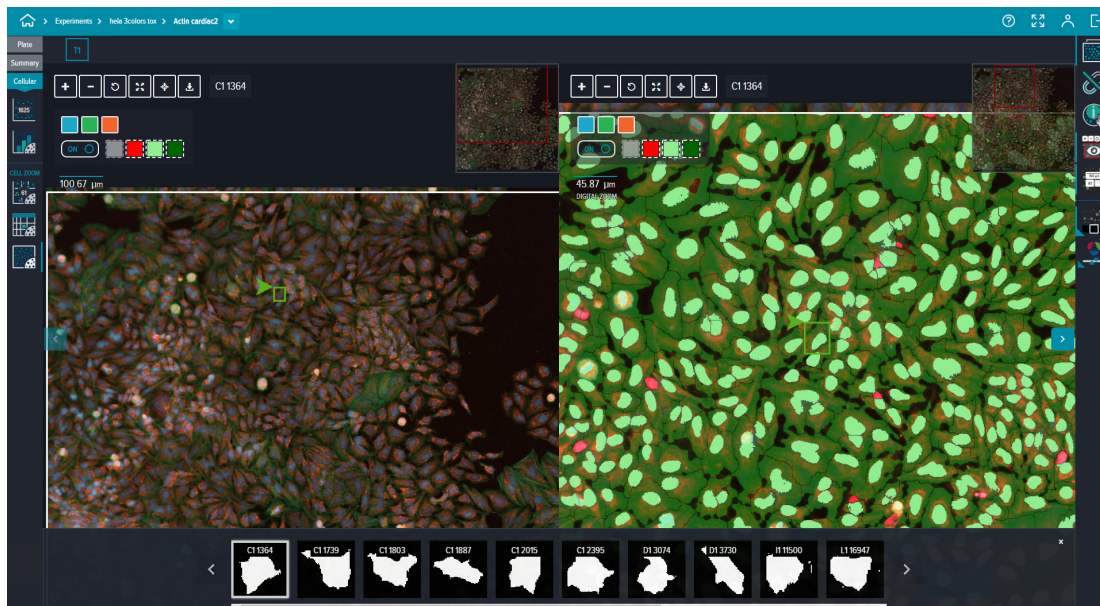


The **Cell Level Images** page shows high-resolution images for deep zoom viewing. At the bottom of the page, zoom-level segments appear.

Click on the image to zoom in on objects of interest. You can also use your mouse wheel to zoom in and out.



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





The right side of the page includes the following icons:



**Show One Image / Show Two Images:** Toggles between a single image and two side-by-side images.



**Link Images** or **Unlink Images:** When showing two images, toggles between controlling images together or independently.



**Cell Info Mode:** Shows cellular and summary measurements.



**Show Navigation Maps:** Toggles the visualization tools in the top left corner and the mini-map in the upper right corner.



**Show Scale and Zone:** Toggles the measurement scale and the well number.



**Show Image Gallery:** Toggles the image gallery at the bottom of the screen.



**Show Channel Settings:** Toggles the display scaling tools at the bottom of the screen.

### Comparing Images

By default, a single image appears. You can display two views of the same image, which enables you to compare images.

To compare images:



1. Click **Show Two Images**.
2. At the bottom of the page, in the image gallery, drag and drop image thumbnails to the comparison panes as needed.



3. To synchronize image zooming and changing positions in both panes, click  **Unlink**

**Images.** The icon toggles to  **Link Images**.

## Slide Views

The following slide data views are available:

### Slide Views



**Thumbnail View:** Shows an overview of the slide images in low resolution. See [Slide Thumbnail View on page 180](#) for details.



**Images:** Shows high-resolution images for deep zoom viewing. See [Slide Images on page 182](#) for details.

### Summary Views



**Table:** Shows a table with summary slide region-level measurements. See [Summary Table on page 185](#) for details.

### Cellular Views



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**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 188](#) 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 190](#) 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 192](#) for details.



**Cell Level Table:** Shows a table with cellular measurements. See [Cell Zoom Level Table on page 194](#) for details.

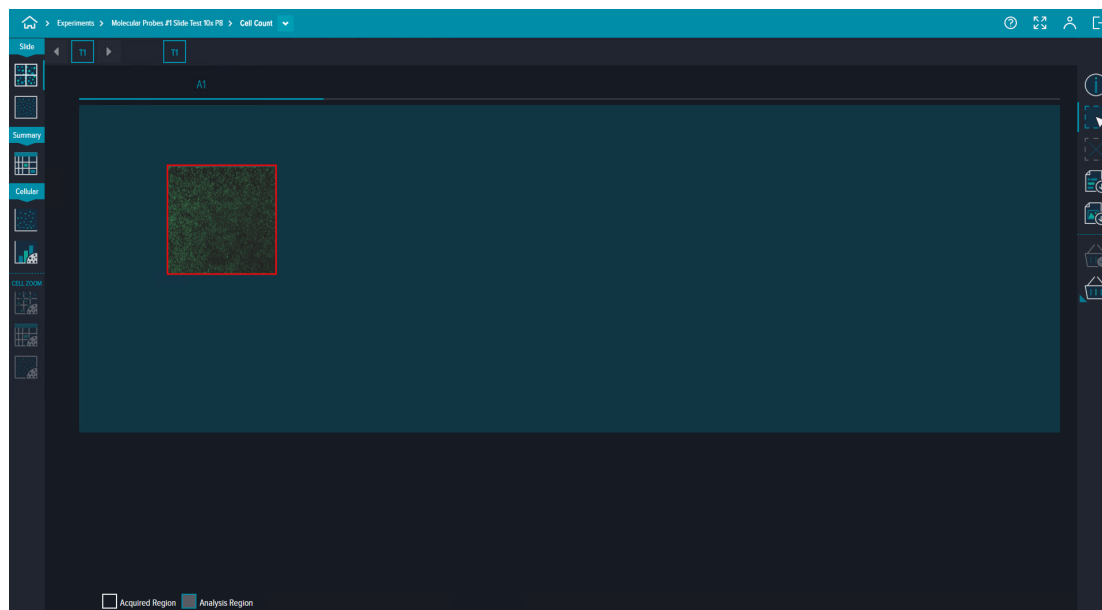


**Cell Level Images:** Shows high-resolution images for deep zoom viewing of individual cells. See [Cell Zoom Level Images on page 196](#) for details.

## Slide Thumbnail View



The **Thumbnail View** shows an overview of the slide regions in low resolution. Double-click on a region to view the **Image** for that region. See [Slide Images](#) on [page 182](#) 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 TIFF images (one image per stain per region) of the selected regions. Exported images are stored in the C:\crx-export folder on the host computer. You can export tiled or stitched images. Images larger than 2 GB are scaled down to a 2 GB file size. In addition, if you plan to import the images into Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis Software for further processing and analysis, you can scale the image accordingly to meet the 32K x 32K pixel size required by the MetaXpress software.



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



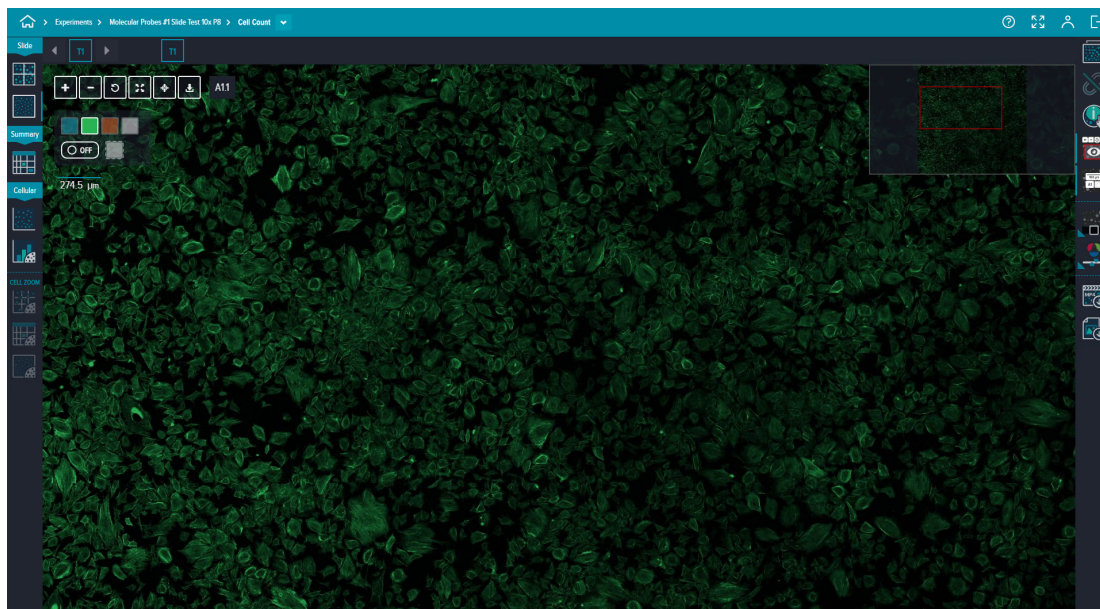
**Quick List:** Opens the Quick List where you can access saved selections.

## Slide Images



The **Slide 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 Scale and Zone:** Toggles 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 an MP4 video of the selected slide image over time. This function is not available with a tablet.






**Export Raw Images:** Exports TIFF images (one image per stain per slide) of the current slide image. Exported images are stored in the C:\crx-export folder on the host computer. You can export tiled or stitched images. Images larger than 2 GB are scaled down to a 2 GB file size. In addition, if you plan to import the images into Molecular Devices® MetaXpress® High-Content Image Acquisition and Analysis Software for further processing and analysis, you can scale the image accordingly to meet the 32K x 32K pixel size required by the MetaXpress software.

## 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**.




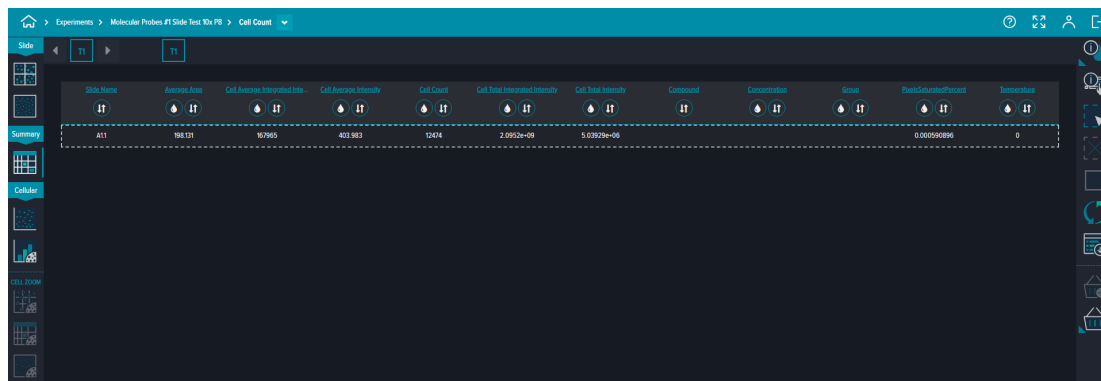
## Summary Table



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



**Tip:** The heatmap color scheme can be changed in  **Configuration Settings**. See [Color Scheme on page 246](#) for details.



The screenshot shows a software interface with a summary table. The table has the following columns and data:

Slide Name	Average Area	Cell Average Integrated Int.	Cell Average Intensity	Cell Count	Cell Total Integrated Intensity	Cell Total Intensity	Compound	Concentration	Group	PixelSubstratePercent	Temperature
A11	198.131	107965	431.983	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.




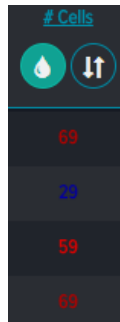
**Quick List:** Opens the Quick List where you can access saved selections.

### 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 .



### 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.

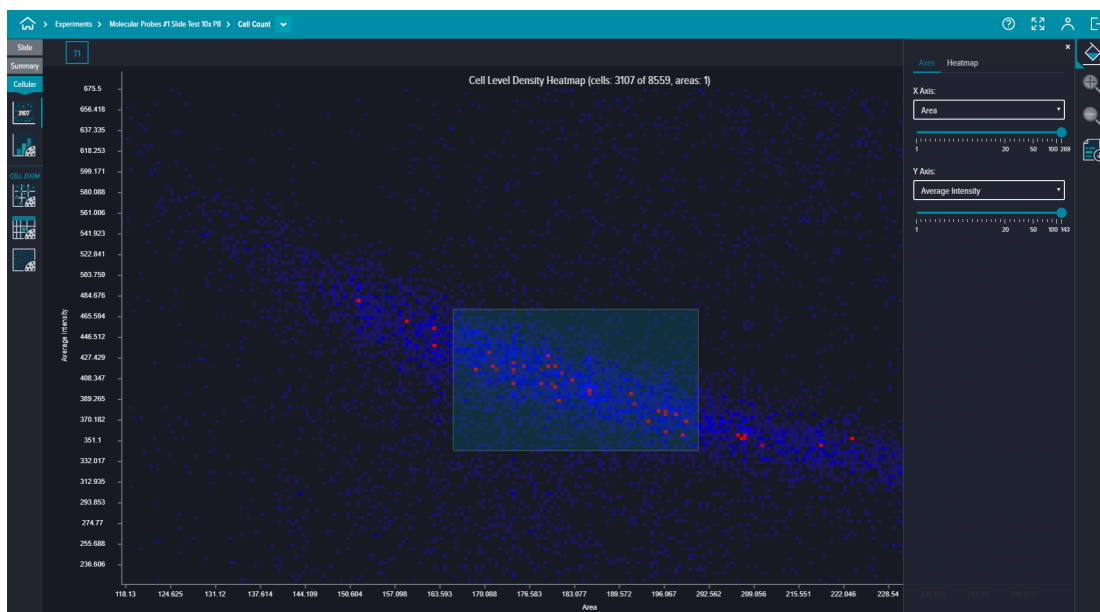


**Note:** You must select at least one region in a Slide view or a Summary view to enable the Cellular views.



**Tip:** The heatmap color scheme can be changed in  **Configuration Settings**. See [Color Scheme on page 246](#) for details.

The page heading indicates the number of selected cells (out of the total number of cells plotted on the graph) and the number of selected regions.



The right side of the page includes the following icons:



**Axes/Color:** Selects the measurements to show and the measurements to use for the heatmap.



**Zoom In:** When bins are selected, replots the selected bars only for a more granular view.



**Zoom Out:** When bins are selected, replots the selected bars only for a more general view.



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

### Axes/Color Pane



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

The following tabs are available:

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

## Cell Level Stacked Bar

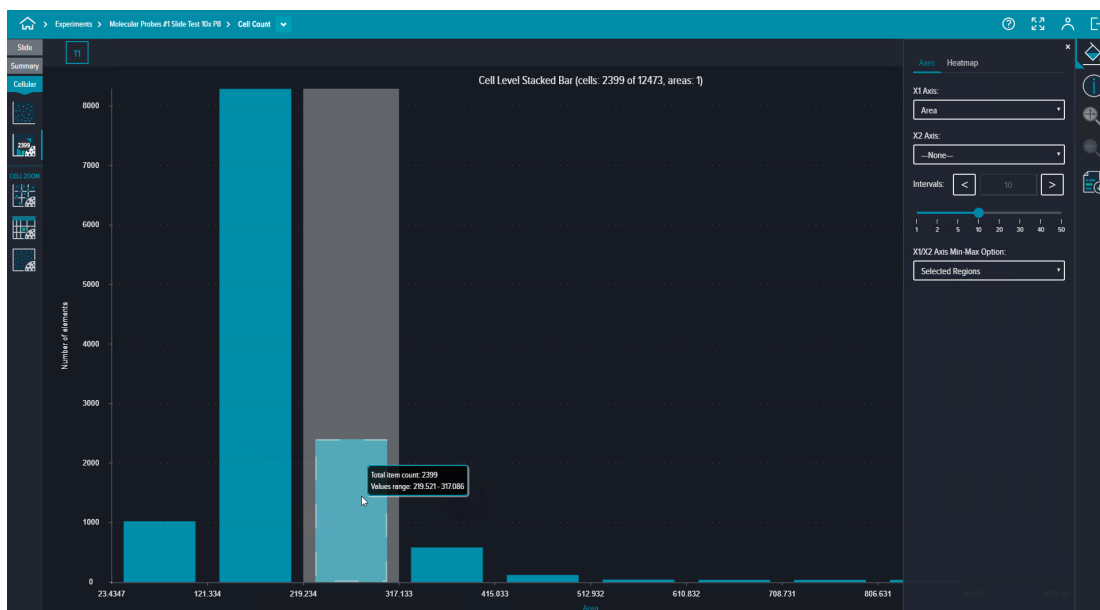


The **Cell Level Stacked Bar** shows a histogram-style bar graph with up to two different measurements side by side. You can add a third measurement to subdivide each bar.



**Note:** You must select at least one region in a Slide view or a Summary view to enable the Cellular views.

The page heading indicates the number of selected cells (out of the total number of cells plotted on the graph) and the number of selected regions.



The right side of the page includes the following icons:



**Axes/Color:** Selects the measurements to show and the measurements to use for the heatmap.



**Cell Info:** Shows summary measurements for the selected region.



**Zoom In:** When bins are selected, replots the selected bars only for a more granular view.



**Zoom Out:** When bins are selected, replots the selected bars only for a more general view.



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

### Axes/Color Pane



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

The following tabs are available:

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

## Cell Zoom Level Scatter Plot




The **Scatter Mode** shows a scatter plot of two measurements.

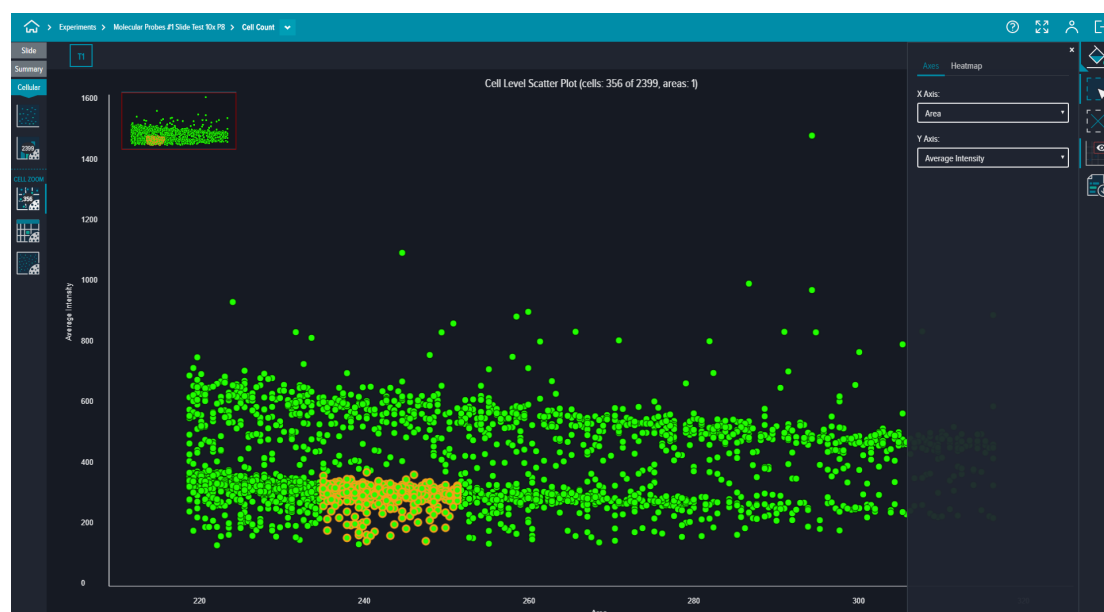


**Note:** You must select at least one bin in a Cellular view to enable the Cell Zoom views.



**Tip:** The heatmap color scheme can be changed in  **Configuration Settings**. See [Color Scheme on page 246](#) for details.

The page heading indicates the number of selected cells (out of the total number of cells plotted on the graph) and the number of selected regions.





The right side of the page includes the following icons:



**Axes/Color:** Selects the measurements to show and the measurements to use for the heatmap.



**Selection Mode:** Activates selection mode, which enables you to select regions. Press **SHIFT** and click to select multiple regions. The number on the icon indicates the number of selected regions.



**Deselect All:** Deselects all regions.



**Toggle Mini Map:** Shows or hides the small overview of the graph at the top left.



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

### Axes/Color Pane



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

The following tabs are available:

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

## Cell Zoom Level Table




The **Cell Level Table** shows a table with cell-level measurements.



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



**Tip:** The heatmap color scheme can be changed in  **Configuration Settings**. See **Color Scheme** on page 246 for details.

Slide Name	Area	Average Intensity	Integrated Intensity	Fluorescence Percent	Temperature	Average Area	Cell Average Integrated Intensity	Cell Average Intensity
A11	246.782	265.934	137222	0.000590896	0	198.131	167965	403.983
A11	237.695	276.099	137221	0.000590896	0	198.131	167965	403.983
A11	236.738	293.139	145104	0.000590896	0	198.131	167965	403.983
A11	248.695	284.992	148196	0.000590896	0	198.131	167965	403.983
A11	237.217	316.772	157119	0.000590896	0	198.131	167965	403.983
A11	241.043	289.879	146099	0.000590896	0	198.131	167965	403.983
A11	244.391	296.157	151336	0.000590896	0	198.131	167965	403.983
A11	237.217	291.877	144771	0.000590896	0	198.131	167965	403.983
A11	238.608	292.691	146638	0.000590896	0	198.131	167965	403.983
A11	249.651	308.797	161192	0.000590896	0	198.131	167965	403.983
A11	244.869	278.695	142692	0.000590896	0	198.131	167965	403.983
A11	239.13	279.26	139630	0.000590896	0	198.131	167965	403.983
A11	242.478	294.067	149092	0.000590896	0	198.131	167965	403.983
A11	242.478	311.369	157864	0.000590896	0	198.131	167965	403.983
A11	245.826	290.233	149180	0.000590896	0	198.131	167965	403.983
A11	235.782	297.349	146593	0.000590896	0	198.131	167965	403.983
A11	247.26	289.617	149732	0.000590896	0	198.131	167965	403.983
A11	241.521	288.376	144620	0.000590896	0	198.131	167965	403.983
A11	250.608	285.239	149465	0.000590896	0	198.131	167965	403.983
A11	239.608	315.375	158003	0.000590896	0	198.131	167965	403.983
A11	235.782	319.308	157419	0.000590896	0	198.131	167965	403.983
A11	246.782	291.196	150257	0.000590896	0	198.131	167965	403.983
A11	249.173	272.47	141957	0.000590896	0	198.131	167965	403.983
A11	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.




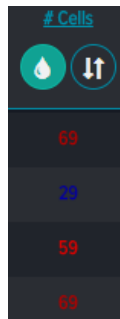
**Export:** Downloads the currently configured table as a CSV file. This function is not available with a tablet.

### 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

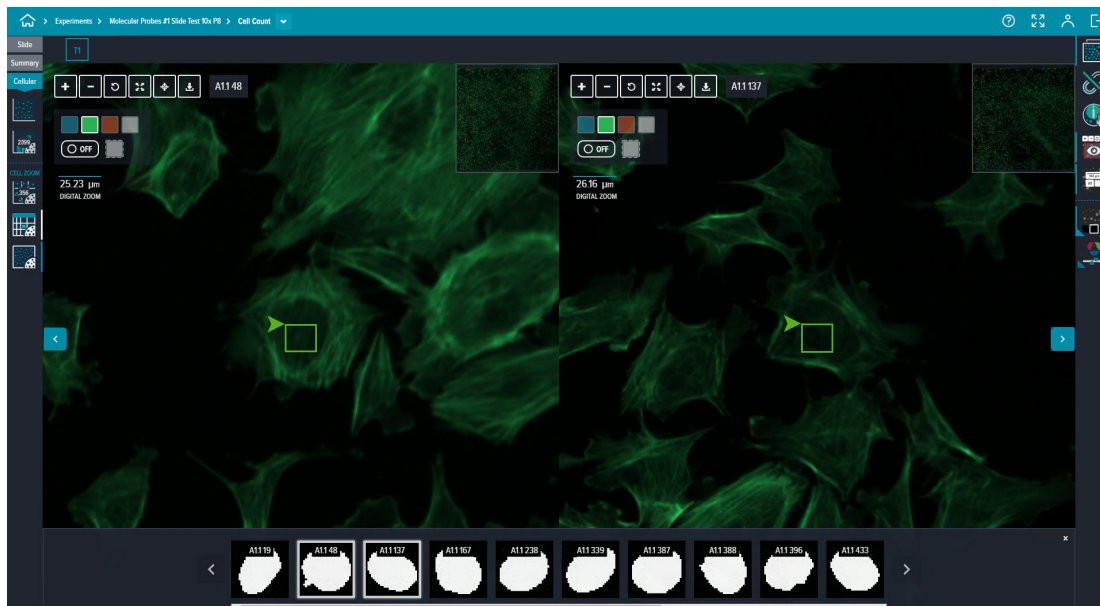


The **Cell Level Images** page shows high-resolution images for deep zoom viewing. At the bottom of the page, zoom-level segments appear.

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:

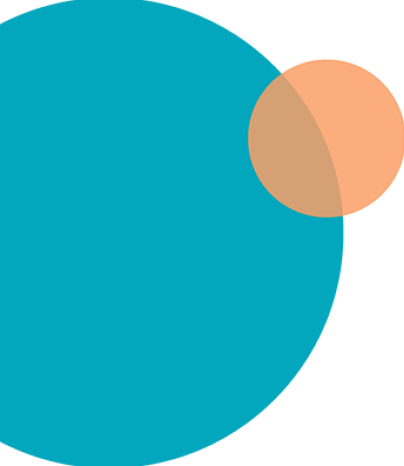


1. Click **Show Two Images**.
2. At the bottom of the page, in the image gallery, drag and drop image thumbnails to the comparison panes as needed.



3. To synchronize image zooming and changing positions in both panes, click  **Unlink**

**Images.** The icon toggles to  **Link Images**.



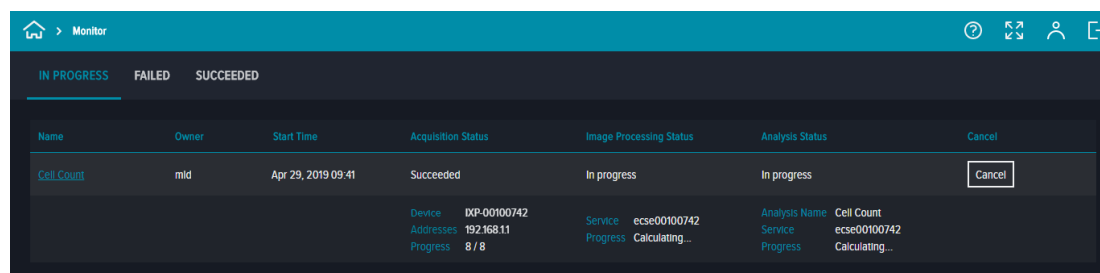
## Chapter 5: Monitor Mode

# 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, and maintenance operations run in **Devices** mode.



On the **Home** page, click the **Monitor** tile to enter **Monitor** mode.



The following tabs are available:

- **In Progress:** Displays details on currently running experiments, exports, and maintenance operations.
- **Failed:** Displays details on failed experiments, exports, and maintenance operations. An error message may appear to describe the reason for the failure. Details remain listed on the **Failed** tab until you delete them.
- **Succeeded:** Displays details on successful experiments, exports, and maintenance operations. Details remain listed on the **Succeeded** tab until you delete them.

### Viewing the Analysis for an Experiment

To view the analysis for an experiment:

On the **Monitor** page, on any tab, click an experiment name in the **Name** field to view images and analysis data on the **Experiments** page.

### Canceling a Running Experiment

To cancel a running experiment:

On the **Monitor** page, on the **In Progress** tab, click **Cancel**.

The experiment details move to the **Succeeded** tab and a card for the canceled experiment is created on the **Experiments** page.

## Responding to a Failed Experiment

There can be many reasons that an experiment fails. If the reason is caused by an issue with the CellReporterXpress software or your network, the instrument may continue performing acquisition. In this case, the status light remains yellow.

To respond to a failed experiment:

1. On the **Monitor** page, on the **Failed** tab, review the error message.
2. Restart the instrument. See the *ImageXpress Pico User Guide* for details on restarting your instrument.
3. Check all network connections.
4. Confirm that you have enough temporary storage for the experiment. See the *ImageXpress Pico User Guide* for details on adding external temporary storage.
5. Retry running the experiment.

## Deleting the Details of a Successful Experiment or Maintenance Operation

To delete the details of a successful experiment or maintenance operation:

1. On the **Monitor** page, on the **Succeeded** tab, select the **Delete** check box for each successful experiment you want to delete.
2. Click **Delete**.
3. Click **OK**.



**Note:** Only the details of the status of the successful experiment are deleted. The card for the successful experiment remains in **Experiments** mode.

---

## Deleting the Details of a Failed Experiment or Maintenance Operation

To delete the details of a failed experiment or maintenance operation:

1. On the **Monitor** page, on the **Failed** tab, select the **Delete** check box for each failed experiment you want to delete.
2. Click **Delete**.
3. Click **OK**.



**Note:** Only the details of the status of the failed experiment are deleted. The card for the failed experiment remains in **Experiments** mode.

---



Use **Configuration** mode to set the systemwide options that affect all users of the CellReporterXpress software.

On the **Home** page, click the  **Configuration** tile to enter **Configuration** mode.

The left side of the page includes the following icons:



**Stain Library:** Specifies the stain definitions available to all users of the CellReporterXpress software. You can add, edit, and delete stain definitions as needed. See [Stain Library on page 202](#) for details.



**Labware Library:** Specifies the labware formats 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 203](#) for details.



**Devices:** Specifies the instruments available for acquisition and maintenance. You can add instruments as needed. See [Devices on page 206](#) 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 210](#) 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 212](#) 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 formats 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 Format

To view a labware format:

1. On the **Labware Library** page, click the tab for the type of labware format you want to view.
2. In the row of a labware format you want to view, click  **View**.
3. When you are finished viewing the labware format, 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, Molecular Devices recommends 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**.

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**.



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 Format

You can delete a labware format 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 (either **Plates** or **Slides**) for the format you want to delete.
2. In the row of the format 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.

See the *CellReporterXpress Installation Guide* for details on setting up the various configurations.

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 available to the software. From here, you can add devices to the list on the **My Devices** tab, which makes them available to you 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. See the Molecular Devices Knowledge Base for details on updating the firmware.

---

The tile for each instrument on the **My Devices** tab contains a  **Favorite** icon. Click  **Favorite** to indicate a frequently used device. Note that the favorite setting affects your login only.

## 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.



**Tip:** Molecular Devices recommends that you do not directly connect an instrument to the host computer using the LAN2 port unless advised to do so by Molecular Devices Technical Support.

---

## Registering a Remotely Connected Instrument Using Autodiscovery

To register a remotely connected instrument using autodiscovery:

1. Confirm that the instrument is connected to the network using the **LAN2** port on the back of the device.
2. Confirm that the host computer running the CellReporterXpress software is connected to the network.

Within five minutes, the instrument appears in the list on the **Add Device** tab. It may initially indicate offline status, but it should change to online status shortly after it appears.

If the instrument does not appear in the list on the **Add Device** tab, your computer or your network may be set up to block autodiscovery. In this case, do one of the following:

- Enable network discovery on the computer where the CellReporterXpress software is installed and try again.
- Register the instrument using manual discovery. See [Registering a Remotely Connected Instrument Using Manual Discovery on page 208](#) for details.

## Registering a Remotely Connected Instrument Using Manual Discovery

Your 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.

## 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 Device**.
3. Click **OK**.



### 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 Device**.

The instrument appears in the list on the My Devices tab.

### Removing an Instrument from the My Devices List


Remove an instrument from 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 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  **Remove Device**.

The instrument appears in the list on the My Devices tab.

## 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  **Delete**.
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 Increasing Network Security for Image Storage 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 *Increasing Network Security for Image Storage* 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:

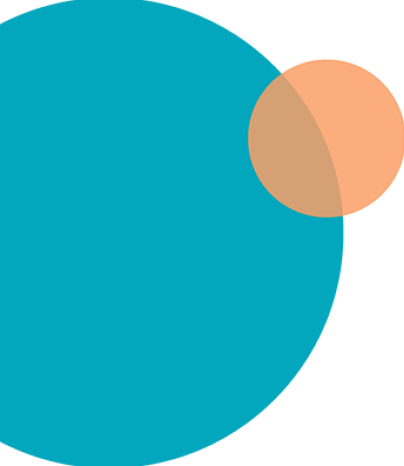
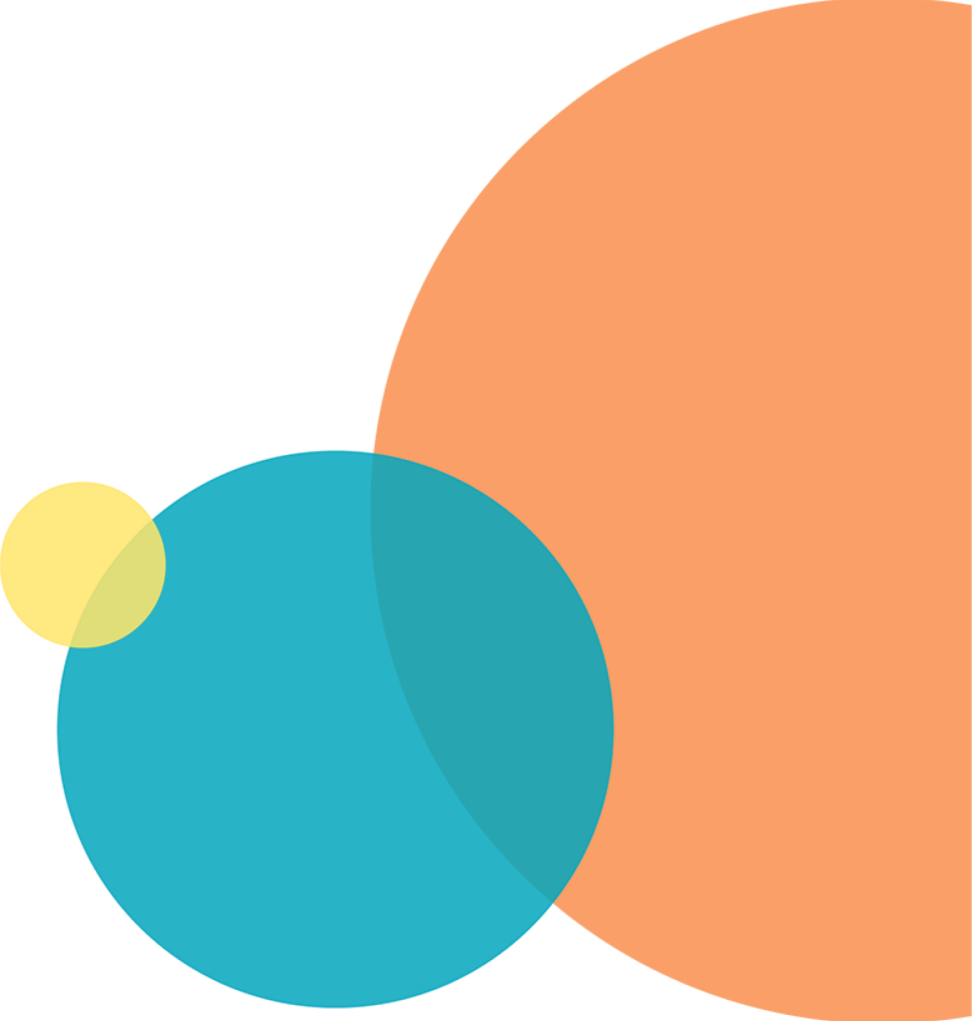
1. On the **Data Storage** page, in row for the registered computer you want to remove, click  **Delete**.
2. Click **OK**.

## 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  **Delete**.
2. Click **OK**.



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 206](#) 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 217](#) for details.
- **Sensors:** Displays the current environmental details for selected instrument, including temperature, humidity level, CO<sub>2</sub> level, and O<sub>2</sub> 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 218](#) 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 231](#) 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 237](#) for details.
- **Maintenance:** Displays a tile for each maintenance activity that can be performed on the selected instrument. See [Maintenance on page 243](#) for details.



## Info

The **Info** tab displays details about the selected instrument, including the following:

- Device Name
- 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<sub>2</sub> level, and O<sub>2</sub> level inside the environmental control cassette.

You can set a target value to regulate each component:

Component	Set Range	Notes
CO <sub>2</sub>	1% to 15%	
Humidity	85%	Humidity level is fixed.
O <sub>2</sub>	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 all sensors, 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<sub>2</sub> level and O<sub>2</sub> level, but these values are not meaningful for an experiment because they are measured within the gas mixer and not near the sample (X-Y) plate.
- Environmental control is fully supported for plate and dish acquisitions only. For slide acquisitions, only temperature control is available; humidity, CO<sub>2</sub>, and O<sub>2</sub> control is not available.

## Regulating CO<sub>2</sub> Level

With the optional environmental control system, you can regulate the CO<sub>2</sub> level inside the environmental control cassette within the range of 1% to 15%. When you set a target level and start CO<sub>2</sub> level control, the instrument supplies CO<sub>2</sub> to the environmental control cassette to achieve the target.

The current CO<sub>2</sub> level inside the environmental control cassette is shown on the **Sensors** tab and on the CO<sub>2</sub> level toolbar notification (if enabled). See [Enabling a CO<sub>2</sub> Level Toolbar Notification on page 221](#) for details.

Before you can start CO<sub>2</sub> 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!** 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<sub>2</sub> level control, check the gas supply.
  - The software does not support adjusting an environmental control component 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<sub>2</sub> level, O<sub>2</sub> 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 47](#) for details. With slides, see [Adjusting an Objective Correction Collar on page 94](#) for details.
  - If you open or remove the environmental control cassette while gas is flowing, gas flow stops.
-

## Starting CO<sub>2</sub> Level Control


To start CO<sub>2</sub> level control:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the instrument where you want to start CO<sub>2</sub> level control.
2. Click the **Sensors** tab.
3. In the **CO<sub>2</sub> Level** row, under **Component State**, in the **Target** field, enter a target CO<sub>2</sub> level as a percentage.



**Note:** The target CO<sub>2</sub> 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<sub>2</sub> level control is on.

## Modifying CO<sub>2</sub> Level Control

When CO<sub>2</sub> level control is on, you can set a new target CO<sub>2</sub> 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<sub>2</sub> level.
2. Click the **Sensors** tab.
3. In the **CO<sub>2</sub> Level** row, under **Component State**, in the **Target** field, enter a new target CO<sub>2</sub> level as a percentage.





**Note:** The target CO<sub>2</sub> level must be within the range of 1% to 15%.

---

4. Click  **Start Regulation**.  
The indicator in the **State** field remains enabled.

## Stopping CO<sub>2</sub> Level Control

To stop CO<sub>2</sub> level control:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the instrument where you want to stop CO<sub>2</sub> level control.
2. Click the **Sensors** tab.
3. In the **CO<sub>2</sub> Level** row, under **Component State**, click  **Stop Regulation**.


## Enabling a CO<sub>2</sub> Level Toolbar Notification

You can enable a toolbar notification to monitor the CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> level and O<sub>2</sub> level, but these values are not meaningful for an experiment because they are measured within the gas mixer and not near the sample (X-Y) plate.

To enable a CO<sub>2</sub> 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<sub>2</sub> level toolbar notification.
2. Click the **Sensors** tab.
3. In the **CO<sub>2</sub> Level** row, under **Notification Settings**, in the **Min** field, enter the lower limit value for the CO<sub>2</sub> level range as a percentage.
4. In the **Max** field, enter the upper limit value for the CO<sub>2</sub> 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 224](#) 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 component 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<sub>2</sub> level, O<sub>2</sub> 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 243](#) 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 47](#) for details. With slides, see [Adjusting an Objective Correction Collar on page 94](#) for details.

**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<sub>2</sub> level and O<sub>2</sub> level, but these values are not meaningful for an experiment because they are measured within the gas mixer and not near the sample (X-Y) plate.

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<sub>2</sub> Level

With the optional environmental control system, you can regulate the O<sub>2</sub> level inside the environmental control cassette within the range of 1% to 15%. When you set a target level and start O<sub>2</sub> level control, the instrument supplies O<sub>2</sub> to the environmental control cassette to achieve the target O<sub>2</sub> level.

The current O<sub>2</sub> level inside the environmental control cassette is shown on the **Sensors** tab and on the O<sub>2</sub> level toolbar notification (if enabled). See [Enabling an O<sub>2</sub> Level Toolbar Notification on page 227](#) for details.

Before you can start O<sub>2</sub> 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!** 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<sub>2</sub> level control, check the gas supply.
  - The software does not support adjusting an environmental control component 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<sub>2</sub> level, O<sub>2</sub> 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 47](#) for details. With slides, see [Adjusting an Objective Correction Collar on page 94](#) for details.
  - If you open or remove the environmental control cassette while gas is flowing, gas flow stops.
-

## Starting O<sub>2</sub> Level Control

To start O<sub>2</sub> level control:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the device where you want to start O<sub>2</sub> level control.
2. Click the **Sensors** tab.
3. In the **O<sub>2</sub> Level** row, under **Component State**, in the **Target** field, enter a target O<sub>2</sub> level as a percentage.



**Note:** The target O<sub>2</sub> 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<sub>2</sub> level control is on.

## Modifying O<sub>2</sub> Level Control

When O<sub>2</sub> level control is on, you can set a new target O<sub>2</sub> 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<sub>2</sub> level.
2. Click the **Sensors** tab.
3. In the **O<sub>2</sub> Level** row, under **Component State**, in the **Target** field, enter a new target O<sub>2</sub> level as a percentage.





**Note:** The target O<sub>2</sub> 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<sub>2</sub> level control is on.

## Stopping O<sub>2</sub> Level Control

To stop O<sub>2</sub> level control:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the device where you want to stop O<sub>2</sub> level control.
2. Click the **Sensors** tab.
3. In the **O<sub>2</sub> Level** row, under **Component State**, click  **Stop Regulation**.


## Enabling an O<sub>2</sub> Level Toolbar Notification

You can enable a toolbar notification to monitor the O<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> level and O<sub>2</sub> level, but these values are not meaningful for an experiment because they are measured within the gas mixer and not near the sample (X-Y) plate.

To enable an O<sub>2</sub> 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<sub>2</sub> level toolbar notification.
2. Click the **Sensors** tab.
3. In the **O<sub>2</sub> Level** row, under **Notification Settings**, in the **Min** field, enter the lower limit value for the O<sub>2</sub> level range as a percentage.
4. In the **Max** field, enter the upper limit value for the O<sub>2</sub> 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 230](#) 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 degrees below the set temperature (without the environmental control cassette) or at least 8 degrees 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 component 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<sub>2</sub> level, O<sub>2</sub> 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 47](#) for details. With slides, see [Adjusting an Objective Correction Collar on page 94](#) 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 enclosure as described in the procedure. Avoid contact with other components as they can be damaged or knocked out of alignment.
- To prevent dust from collecting inside the instrument, keep all access doors closed unless you are performing maintenance tasks.
- Ensure that all components and access doors are closed before starting the instrument.



#### CAUTION!

- To prevent skin oils from damaging the optical coatings, Molecular Devices recommends 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 231](#) 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 for future storage needs. 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 231](#) for details. Because of this, the only reason to replace an objective with another objective of a different magnification is when you are swapping the 40x objective and the 63x objective in slot 5.

To replace an objective with an objective of a different magnification:

1. On the **Devices** page, click  **Show Device Options** to expand the details for the instrument where you want to replace an objective.
2. Click the **Objectives** tab.
3. In the tile for objective slot **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 235](#) for details.

## Replacing an Objective (Same Magnification)

You must install objectives in specific slots in the turret. See [Installing an Objective on page 231](#) 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 235](#) 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)

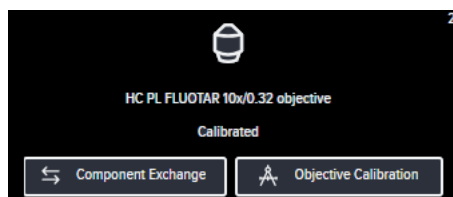
You will need to provide the following item for the calibration process:

- 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.


- Note the status of the objective, which is indicated in the tile.



- If the tile indicates **Not Calibrated**, continue to the next step to perform the calibration procedure.
- If the tile indicates **Calibrated**, you typically do not need to perform the calibration procedure and you can skip this procedure.



**Note:** An exception to this is when you replace an objective with another objective of the same magnification. In this case, you should continue to the next step and perform the calibration procedure regardless of the label in the tile.

- In the tile for the objective you want to calibrate, click  **Objective Calibration**.
- 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 enclosure as described in the procedure. Avoid contact with other components as they can be damaged or knocked out of alignment.
- To prevent dust from collecting inside the instrument, keep all access doors closed unless you are performing maintenance tasks.
- Ensure that all components and access doors are closed before starting the instrument.



**CAUTION!** To prevent skin oils from damaging the optical coatings, Molecular Devices recommends 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 for future storage needs. 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 241](#) 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 241](#) 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)

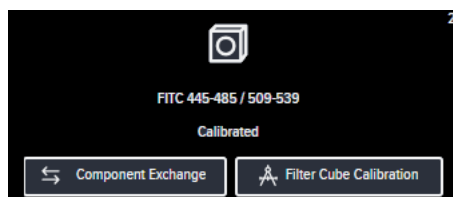
You will need to provide the following item for the calibration process:

- 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 condensation builds up within the environmental control system, you can run air dry to clear it. 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, Molecular Devices recommends that you leave it there while running 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 199](#) 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**.

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 246](#) for details.



**Vocabulary:** Specifies the language for the CellReporterXpress user interface. See [Vocabulary on page 246](#) for details.



**Color Scheme:** Specifies the color scheme used for heatmaps in **Experiments** mode. See [Color Scheme on page 246](#) 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 247](#) 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 248](#) for details.



**Miscellaneous:** Specifies various CellReporterXpress software preferences, including image preferences and timeouts. See [Miscellaneous on page 249](#) 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 202](#) 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.



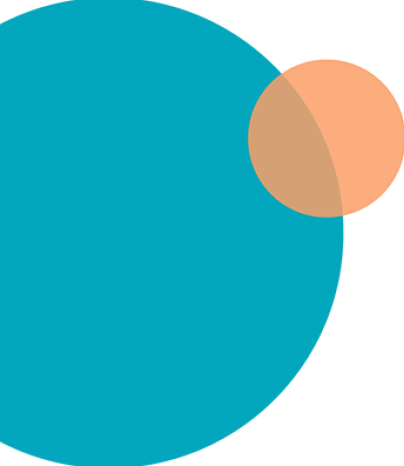
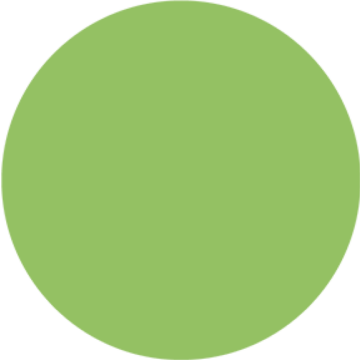
## Miscellaneous



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 40 $\mu$ m.
- **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.





**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 257:** 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 259:** 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 262:** Two-channel assay for detecting autophagic granules. Use the Autophagy analysis to detect the infection of viral particles into cells.



**Cell Count, see page 264:** 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 266:** 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 270:** 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 274:** 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 279:** 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 287:** Two-channel assay for detecting endosomes. Use the Endocytosis analysis to detect the internalization of endosomes into cells.



**Internalization, see page 289:** 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 291:** 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 294: 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 296: 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 298: 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 302: 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 306: 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 308: 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 311: 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 through differences in intensity levels.



**Translocation**, see page 314: 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 321:** 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 317:** Single-channel, transmitted light assay to find beads.



**Transmitted Light Cell Count, see page 317:** Single-channel, transmitted light assay to find a range of cells.



**Transmitted Light Cell Count, see page 317:** Single-channel, transmitted light assay to find larger cells.



**Transmitted Light Cell Count, see page 317:** 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 319:** Two-channel, transmitted light assay to find beads, then scoring for an additional fluorescence channel.



**Transmitted Light Cell Scoring, see page 319:** Two-channel, transmitted light assay to find a range of cells, then scoring for an additional fluorescence channel.



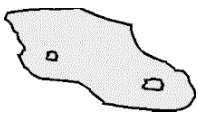



[Transmitted Light Cell Scoring, see page 319](#): Two-channel, transmitted light assay to find larger cells, such as HeLa cells, then scoring for an additional fluorescence channel.



[Transmitted Light Cell Scoring, see page 319](#): 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	Image Example/Calculation
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  $\mu\text{m}$ . 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  $\mu\text{m}$ . Tubes larger than the maximum width will be identified as nodes.

## Summary Measurements

Measurement	Description
<b># Cells</b>	Number of cells
<b>Total Tubule Length</b>	Total length of the tubes in $\mu\text{m}$ .
<b>Total Tubule Area</b>	Total tube area (excluding nodes) in $\mu\text{m}^2$ .
<b>Segments</b>	Number of tube segments connecting branch points and/or ends.
<b>Branch Points</b>	Number of junctions connecting segments (excluding nodes, which are not considered branches).
<b>Nodes</b>	Number of connected blobs with a thickness exceeding the maximum width, excluded from length and area measures.
<b>Total Node Area</b>	Total node area in $\mu\text{m}^2$ .
<b>Connected Sets</b>	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).
<b>Mean Tubule Length</b>	Total tube length divided by the number of segments.
<b>Mean Tubule Area</b>	Total tube area divided by the number of segments.
<b>Tube Length Per Set</b>	Total tube length in microns divided by the number of connected sets.
<b>Mean Node Area</b>	Total node area in $\mu\text{m}^2$ 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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
<b># Cells</b>	Number of cells identified by the nuclear stain.
<b># Positive Cells</b>	Number of apoptic cells.
<b>% Positive Cells</b>	Percentage of apoptic cells to the number of cells.
<b># Negative Cells</b>	Number of non-apoptic cells.
<b>% Negative Cells</b>	Percentage of non-apoptic cells to the number of cells.
<b>Positive Cell Average Intensities</b>	Average pixel intensity of cells positive for the marker over the positive cell area.
<b>All Cell Avg Intensities</b>	Average pixel intensity of the marker over all the cell areas.
<b>Average Nuclear Area</b>	Sum of nuclear area for all nuclei divided by the number of cells.
<b>Average Nuclear Integrated Intensity</b>	Sum of the integrated nuclear intensity values in all nuclei divided by the number of cells.
<b>Average Nuclear Intensity</b>	Average of apoptic cells average intensity values in the nuclear area.

## Cell Measurements

Measurement	Description
<b>Positive</b>	Returns 1 if cell is scored as positive for the marker; otherwise returns 0.
<b>Cell Area</b>	Area of the cell (white segmentation mask).
<b>Nuclear Area</b>	Area of the nucleus (green segmentation mask).
<b>Wavelength 1 Integrated Nuclear Intensity</b>	Total pixel intensity of the nuclear stain over the nuclear area in an individual cell.
<b>Wavelength 1 Average Nuclear Intensity</b>	Average pixel intensity of the nuclear stain over all the nuclear areas in an individual cell (intensity per pixel).
<b>Wavelength 2 Integrated Nuclear Intensity</b>	Total pixel intensity of the marker stain in the nucleus of an individual cell.
<b>Wavelength 2 Average Nuclear Intensity</b>	Average pixel intensity of the marker stain in the nucleus of an individual cell.
<b>Wavelength 2 Integrated Cell Intensity</b>	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Wavelength 2 Average Cell Intensity</b>	Average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Positive Cells Average Intensity</b>	The average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Positive Cells Integrated Intensity</b>	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Cell Segmentation</b>	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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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
<b>Total Object Count</b>	Number of objects.
<b>Total Granule Count</b>	Number of granules.
<b>Avg Granule Count</b>	Average number of granules per object.
<b>Total Granule Area</b>	Total area in $\mu\text{m}^2$ of granules.
<b>Avg Granule Intensity</b>	Average pixel intensity calculated over all granules in the image.
<b>Avg Granule Integrated Intensity</b>	Average of the integrated pixel intensity values for all granules in the image.

## Cell Measurements

Measurement	Description
<b>Granule Count</b>	Number of granules detected for a specific cell. (Note that a granule is assigned to its nearest nucleus).
<b>Granule Total Area</b>	Total area in $\mu\text{m}^2$ covered by all the granules assigned to a specific cell.
<b>Granule Integrated Intensity</b>	Total pixel intensity of the granules assigned to a specific cell.
<b>Granule Intensity</b>	Average pixel intensity of the granules assigned to a specific cell.
<b>Cell Segmentation</b>	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  $\mu\text{m}$ . 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  $\mu\text{m}$ . Nuclei larger than the maximum width will be split.



## Summary Measurements

Measurement	Description
<b>Cell Count</b>	Total number of cells.
<b>Cell Total Intensity</b>	Sum of Cells Average Intensity
<b>Cell Average Intensity</b>	Cell Total Intensity / Cell Count
<b>Cell Total Integrated Intensity</b>	Sum of Cells Integrated Intensity
<b>Cell Average Integrated Intensity</b>	Cell Total Integrated Intensity / Cell Count
<b>Total Area</b>	Sum of Cells Area
<b>Average Area</b>	Total Area / Cell Count

## Cell Measurements

Measurement	Description
<b>Area</b>	Area in $\mu\text{m}^2$ of the cell (white segmentation mask).
<b>Integrated Intensity</b>	The total pixel intensity of the nuclei stain of an individual cell.
<b>Average Intensity</b>	The average pixel intensity of the nuclear stain.
<b>Cell Segmentation</b>	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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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
<b># Cells</b>	Number of cells identified by the nuclear stain.
<b># Positive Cells</b>	Number of cells positive for the marker.
<b>% Positive Cells</b>	Percentage of cells positive for the marker to the number of cells.
<b># Positive Cells</b>	Number of cells negative for the marker.
<b>% Positive Cells</b>	Percentage of cells negative for the marker to the number of cells.
<b>Positive Cell Average Intensities</b>	Average pixel intensity of cells positive for the marker over the positive cell area.
<b>All Cell Average Intensities</b>	Average pixel intensity of the marker over all the cell areas.
<b>Positive Cell Integrated Intensities</b>	Total pixel intensity of the marker stain over the cell area in all cells positive for the marker
<b>Positive Cell Total Integrated Intensity</b>	Sum of Positive Cells Integrated Intensity
<b>Positive Cell Total Intensity</b>	Sum of Positive Cells Average Intensity
<b>Positive Cell Total Area</b>	Sum of Positive Cells Area
<b>Positive Cell Average Area</b>	Sum of Positive Cells Area / # Positive Cells (or 0 if there are no positive cells).

## Cell Measurements

Measurement	Description
<b>Positive</b>	Returns 1 if cell is scored as positive for the marker; otherwise returns 0.
<b>Cell Area</b>	Area of the cell (white segmentation mask).
<b>Nuclear Area</b>	Area of the nucleus (green segmentation mask).
<b>Wavelength 1 Integrated Nuclear Intensity</b>	Total pixel intensity of the nuclear stain over the nuclear area in an individual cell.
<b>Wavelength 1 Average Nuclear Intensity</b>	Average pixel intensity of the nuclear stain over all the nuclear areas in an individual cell (intensity per pixel).
<b>Wavelength 2 Integrated Nuclear Intensity</b>	Total pixel intensity of the marker stain in the nucleus of an individual cell.
<b>Wavelength 2 Average Nuclear Intensity</b>	Average pixel intensity of the marker stain in the nucleus of an individual cell.
<b>Wavelength 2 Integrated Cell Intensity</b>	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Wavelength 2 Average Cell Intensity</b>	Average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Positive Cells Area</b>	Area of the cell if scored as positive.
<b>Positive Cells Average Intensity</b>	The average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Positive Cells Integrated Intensity</b>	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Cell Segmentation</b>	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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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 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.

## Summary Measurements

Measurement	Description
<b># Cells</b>	Number of cells identified by the nuclear stain.
<b># Positive Cells</b>	Number of cells positive for Marker 1.
<b>% Positive Cells</b>	Percentage of cells positive for Marker 1 to the number of cells.
<b># Negative Cells</b>	Number of cells negative for Marker 1.
<b>% Negative Cells</b>	Percentage of cells negative for Marker 1 to the number of cells.
<b>Positive Cell Average Intensities</b>	Average pixel intensity of cells positive for Marker 1 over the positive cell area.
<b>All Cell Average Intensities</b>	Average pixel intensity of Marker 1 over all the cell areas.
<b>Positive Cell Integrated Intensities</b>	Total pixel intensity of the marker stain over the cell area in all cells positive for Marker 1.
<b>Positive Cell Total Integrated Intensity</b>	Sum of Positive Cells Integrated Intensity
<b>Positive Cell Total Intensity</b>	Sum of Positive Cells Average Intensity
<b>Positive Cell Total Area</b>	Sum of Positive Cells Area
<b>Positive Cell Average Area</b>	Sum of Positive Cells Area / # Positive Cells (or 0 if there are no positive cells).



## Cell Measurements

Measurement	Description
<b>Positive</b>	Returns 1 if cell is scored as positive for Marker 1; otherwise returns 0.
<b>Cell Area</b>	Area of the cell (white segmentation mask).
<b>Nuclear Area</b>	Area of the nucleus (green segmentation mask).
<b>Wavelength 1 Integrated Nuclear Intensity</b>	Total pixel intensity of the nuclear stain over the nuclear area in an individual cell.
<b>Wavelength 1 Average Nuclear Intensity</b>	Average pixel intensity of the nuclear stain over all the nuclear areas in an individual cell (intensity per pixel).
<b>Wavelength 2 Integrated Nuclear Intensity</b>	Total pixel intensity of Marker 1 stain in the nucleus of an individual cell.
<b>Wavelength 2 Average Nuclear Intensity</b>	Average pixel intensity of Marker 1 stain in the nucleus of an individual cell.
<b>Wavelength 2 Integrated Cell Intensity</b>	Total pixel intensity of Marker 1 stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Wavelength 2 Average Cell Intensity</b>	Average pixel intensity of Marker 1 stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Positive Cells Area</b>	Area of the cell if scored as positive.
<b>Positive Cells Average Intensity</b>	The average pixel intensity of Marker 1 stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Positive Cells Integrated Intensity</b>	Total pixel intensity of Marker 1 stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Cell Segmentation</b>	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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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 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 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.

Measurement	Description
<b>% Wavelength 2&amp;3 Negative Cells</b>	Percentage of cells negative for Marker 1 and Marker 2 to the number of cells.
<b>% Wavelength 2 Positive Cells</b>	Percentage of cells positive for Marker 1 to the number of cells.
<b>% Wavelength 3 Positive Cells</b>	Percentage of cells positive for Marker 2 to the number of cells.
<b>% Wavelength 2&amp;3 Positive Cells</b>	Percentage of cells positive for Marker 1 and Marker 2 to the number of cells.
<b>Wavelength 2&amp;3 Negative Average Cell Area</b>	Average area of cells negative for Marker 1 and Marker 2 per number of cells negative for Marker 1 and Marker 2.
<b>Wavelength 2&amp;3 Negative Total Cell Area</b>	Total area of cells negative for Marker 1 and Marker 2.
<b>Wavelength 2 Positive Average Cell Area</b>	Average area of cells positive for Marker 1 per number of cells positive for Marker 1.
<b>Wavelength 2 Positive Total Cell Area</b>	Total area of cells positive for Marker 1.
<b>Wavelength 3 Positive Average Cell Area</b>	Average area of cells positive for Marker 2 per number of cells positive for Marker 2.
<b>Wavelength 3 Positive Total Cell Area</b>	Total area of cells positive for Marker 2.
<b>Wavelength 2&amp;3 Positive Average Cell Area</b>	Average area of cells positive for Marker 1 and Marker 2 per number of cells positive for Marker 1 and Marker 2.
<b>Wavelength 2&amp;3 Positive Total Cell Area</b>	Total area of cells positive for Marker 1 and Marker 2.
<b>Wavelength 2 Average Integrated Cell Intensities</b>	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.
<b>Wavelength 2 Average Integrated Cytoplasm Intensities</b>	Integrated intensities of cells positive for Marker 1 in cytoplasm area divided by the number of cells positive for Marker 1.
<b>Wavelength 2 Average Integrated Nuclear Intensities</b>	Integrated intensities of cells positive for Marker 1 in nucleus area divided by the number of cells positive for Marker 1.
<b>Wavelength 2 Total Integrated Cell Intensity</b>	Total integrated intensity of cells positive for Marker 1.

Measurement	Description
<b>Wavelength 2 Average Cell Intensities</b>	Average of all cells positive for Marker 1 average intensity values in the cell area (cytoplasm + nucleus area).
<b>Wavelength 2 Average Nuclear Intensities</b>	Average of cells positive for Marker 1 average intensity values in the nuclear area.
<b>Wavelength 2 Average Cytoplasm Intensities</b>	Average of cells positive for Marker 1 average intensity values in the cytoplasm area.
<b>Wavelength 3 Average Integrated Cell Intensities</b>	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.
<b>Wavelength 3 Average Integrated Cytoplasm Intensities</b>	Integrated intensities of cells positive for Marker 2 in cytoplasm area divided by the number of cells positive for Marker 2.
<b>Wavelength 3 Average Integrated Nuclear Intensities</b>	Integrated intensities of cells positive for Marker 2 in nucleus area divided by the number of cells positive for Marker 2.
<b>Wavelength 3 Total Integrated Cell Intensity</b>	Total integrated intensity of cells positive for Marker 2.
<b>Wavelength 3 Average Cell Intensities</b>	Average of all cells positive for Marker 2 average intensity values in the cell area (cytoplasm + nucleus area).
<b>Wavelength 3 Average Nuclear Intensities</b>	Average of cells positive for Marker 2 average intensity values in the nuclear area.
<b>Wavelength 3 Average Cytoplasm Intensities</b>	Average of cells positive for Marker 2 average intensity values in the cytoplasm area.

## Cell Measurements

Measurement	Description
<b>Cell Area</b>	The total area in $\mu\text{m}^2$ 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.
<b>Nuclear Area</b>	The total area in $\mu\text{m}^2$ of the nucleus.
<b>Average Nuclear Intensity</b>	Average pixel intensity of the nuclear stain in the nuclear area.
<b>Wavelength 2&amp;3 Negative</b>	Returns 1 if cell is scored as negative for Marker 1 and Marker 2; otherwise returns 0.
<b>Wavelength 2 Positive</b>	Returns 1 if cell is scored as positive for Marker 1; otherwise returns 0.
<b>Wavelength 3 Positive</b>	Returns 1 if cell is scored as positive for Marker 2; otherwise returns 0.

Measurement	Description
<b>Wavelength 2&amp;3 Positive</b>	Returns 1 if cell is scored as positive for Marker 1 and Marker 2; otherwise 0.
<b>Wavelength 2 Average Cell Intensity</b>	Average pixel intensity for Marker 1 in cell area (cytoplasm area + nucleus area).
<b>Wavelength 2 Integrated Cell Intensity</b>	Total pixel intensities for Marker 1 in cell area (cytoplasm area + nucleus area).
<b>Wavelength 2 Average Nuclear Intensity</b>	Average pixel intensity for Marker 1 in nuclear area.
<b>Wavelength 2 Integrated Nuclear Intensity</b>	Total of pixel intensities for Marker 1 in nuclear area.
<b>Wavelength 2 Average Cytoplasm Intensity</b>	Average pixel intensity for Marker 1 in cytoplasm area.
<b>Wavelength 2 Integrated Cytoplasm Intensity</b>	Total of pixel intensities for Marker 1 in cytoplasm area.
<b>Wavelength 3 Average Cell Intensity</b>	Average pixel intensity for Marker 2 in cell area (cytoplasm area + nucleus area).
<b>Wavelength 3 Integrated Cell Intensity</b>	Total pixel intensities for Marker 2 in cell area (cytoplasm area + nucleus area).
<b>Wavelength 3 Average Nuclear Intensity</b>	Average pixel intensity for Marker 2 in nuclear area.
<b>Wavelength 3 Integrated Nuclear Intensity</b>	Total of pixel intensities for Marker 2 in nuclear area.
<b>Wavelength 3 Average Cytoplasm Intensity</b>	Average pixel intensity for Marker 2 in cytoplasm area.
<b>Wavelength 3 Integrated Cytoplasm Intensity</b>	Total of pixel intensities for Marker 2 in cytoplasm area.
<b>Cell Segmentation</b>	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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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 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 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 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.

Measurement	Description
<b>% Wavelength 2&amp;3 Positive Cells</b>	Percentage of cells positive for Marker 1 and Marker 2 to the number of cells.
<b>% Wavelength 2&amp;4 Positive Cells</b>	Percentage of cells positive for Marker 1 and Marker 3 to the number of cells.
<b>% Wavelength 3&amp;4 Positive Cells</b>	Percentage of cells positive for Marker 2 and Marker 3 to the number of cells.
<b>% Wavelength 2&amp;3&amp;4 Positive Cells</b>	Percentage of cells positive for Marker 1 and Marker 2 to the number of cells.
<b>Wavelength 2&amp;3&amp;4 Negative Average Cell Area</b>	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.
<b>Wavelength 2 Positive Average Cell Area</b>	Average area of cells positive for Marker 1 per number of cells positive for Marker 1.
<b>Wavelength 3 Positive Average Cell Area</b>	Average area of cells positive for Marker 2 per number of cells positive for Marker 2.
<b>Wavelength 4 Positive Average Cell Area</b>	Average area of cells positive for Marker 3 per number of cells positive for Marker 3.
<b>Wavelength 2&amp;3 Positive Average Cell Area</b>	Average area of cells positive for Marker 1 and Marker 2 per number of cells positive for Marker 1 and Marker 2.
<b>Wavelength 2&amp;4 Positive Average Cell Area</b>	Average area of cells positive for Marker 1 and Marker 3 per number of cells positive for Marker 1 and Marker 3.
<b>Wavelength 3&amp;4 Positive Average Cell Area</b>	Average area of cells positive for Marker 2 and Marker 3 per number of cells positive for Marker 2 and Marker 3.
<b>Wavelength 2&amp;3&amp;4 Positive Average Cell Area</b>	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.
<b>Wavelength 2&amp;3&amp;4 Negative Total Cell Area</b>	Total area of cells negative for Marker 1, Marker 2, and Marker 3.
<b>Wavelength 2 Positive Total Cell Area</b>	Total area of cells positive for Marker 1.
<b>Wavelength 3 Positive Total Cell Area</b>	Total area of cells positive for Marker 2.
<b>Wavelength 4 Positive Total Cell Area</b>	Total area of cells positive for Marker 3.

Measurement	Description
<b>Wavelength 2&amp;3 Positive Total Cell Area</b>	Total area of cells positive for Marker 1 and Marker 2.
<b>Wavelength 2&amp;4 Positive Total Cell Area</b>	Total area of cells positive for Marker 1 and Marker 3.
<b>Wavelength 3&amp;4 Positive Total Cell Area</b>	Total area of cells positive for Marker 2 and Marker 3.
<b>Wavelength 2&amp;3&amp;4 Positive Average Cell Area</b>	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.
<b>Wavelength 2 Average Integrated Cell Intensities</b>	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.
<b>Wavelength 2 Average Integrated Cytoplasm Intensities</b>	Integrated intensities of cells positive for Marker 1 in cytoplasm area divided by the number of cells positive for Marker 1.
<b>Wavelength 2 Average Integrated Nuclear Intensities</b>	Integrated intensities of cells positive for Marker 1 in nucleus area divided by the number of cells positive for Marker 1.
<b>Wavelength 2 Total Integrated Cell Intensity</b>	Total integrated intensity of cells positive for Marker 1.
<b>Wavelength 2 Average Cell Intensities</b>	Average of all cells positive for Marker 1 average intensity values in the cell area (cytoplasm + nucleus area).
<b>Wavelength 2 Average Nuclear Intensities</b>	Average of cells positive for Marker 1 average intensity values in the nuclear area.
<b>Wavelength 2 Average Cytoplasm Intensities</b>	Average of cells positive for Marker 1 average intensity values in the cytoplasm area.
<b>Wavelength 3 Average Integrated Cell Intensities</b>	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.
<b>Wavelength 3 Average Integrated Cytoplasm Intensities</b>	Integrated intensities of cells positive for Marker 2 in cytoplasm area divided by the number of cells positive for Marker 2.
<b>Wavelength 3 Average Integrated Nuclear Intensities</b>	Integrated intensities of cells positive for Marker 2 in nucleus area divided by the number of cells positive for Marker 2.
<b>Wavelength 3 Total Integrated Cell Intensity</b>	Total integrated intensity of cells positive for Marker 2.

Measurement	Description
<b>Wavelength 3 Average Cell Intensities</b>	Average of all cells positive for Marker 2 average intensity values in the cell area (cytoplasm + nucleus area).
<b>Wavelength 3 Average Nuclear Intensities</b>	Average of cells positive for Marker 2 average intensity values in the nuclear area.
<b>Wavelength 3 Average Cytoplasm Intensities</b>	Average of cells positive for Marker 2 average intensity values in the cytoplasm area.
<b>Wavelength 4 Average Integrated Cell Intensities</b>	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.
<b>Wavelength 4 Average Integrated Cytoplasm Intensities</b>	Integrated intensities of cells positive for Marker 3 in cytoplasm area divided by the number of cells positive for Marker 3.
<b>Wavelength 4 Average Integrated Nuclear Intensities</b>	Integrated intensities of cells positive for Marker 3 in nucleus area divided by the number of cells positive for Marker 3.
<b>Wavelength 4 Total Integrated Cell Intensity</b>	Total integrated intensity of cells positive for Marker 3.
<b>Wavelength 4 Average Cell Intensities</b>	Average of all cells positive for Marker 3 average intensity values in the cell area (cytoplasm + nucleus area).
<b>Wavelength 4 Average Nuclear Intensities</b>	Average of cells positive for Marker 3 average intensity values in the nuclear area.
<b>Wavelength 4 Average Cytoplasm Intensities</b>	Average of cells positive for Marker 3 average intensity values in the cytoplasm area.

## Cell Measurements

Measurement	Description
<b>Cell Area</b>	The total area in $\mu\text{m}^2$ 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.
<b>Nuclear Area</b>	The total area in $\mu\text{m}^2$ of the nucleus.
<b>Average Nuclear Intensity</b>	Average pixel intensity of the nuclear stain in the nuclear area.
<b>Wavelength 2&amp;3&amp;4 Negative</b>	Returns 1 if cell is scored as negative for Marker 1, Marker 2, and Marker 3; otherwise returns 0.
<b>Wavelength 2 Positive</b>	Returns 1 if cell is scored as positive for Marker 1; otherwise returns 0.
<b>Wavelength 3 Positive</b>	Returns 1 if cell is scored as positive for Marker 2; otherwise returns 0.
<b>Wavelength 4 Positive</b>	Returns 1 if cell is scored as positive for Marker 3; otherwise returns 0.

Measurement	Description
<b>Wavelength 2&amp;3 Positive</b>	Returns 1 if cell is scored as positive for Marker 1 and Marker 2; otherwise 0.
<b>Wavelength 2&amp;3 Positive</b>	Returns 1 if cell is scored as positive for Marker 1 and Marker 3; otherwise 0.
<b>Wavelength 3&amp;4 Positive</b>	Returns 1 if cell is scored as positive for Marker 2 and Marker 3; otherwise 0.
<b>Wavelength 2&amp;3&amp;4 Positive</b>	Returns 1 if cell is scored as positive for Marker 1, Marker 2, and Marker 3; otherwise 0.
<b>Wavelength 2 Average Cell Intensity</b>	Average pixel intensity for Marker 1 in cell area (cytoplasm area + nucleus area).
<b>Wavelength 2 Integrated Cell Intensity</b>	Total pixel intensities for Marker 1 in cell area (cytoplasm area + nucleus area).
<b>Wavelength 2 Average Nuclear Intensity</b>	Average pixel intensity for Marker 1 in nuclear area.
<b>Wavelength 2 Integrated Nuclear Intensity</b>	Total of pixel intensities for Marker 1 in nuclear area.
<b>Wavelength 2 Average Cytoplasm Intensity</b>	Average pixel intensity for Marker 1 in cytoplasm area.
<b>Wavelength 2 Integrated Cytoplasm Intensity</b>	Total of pixel intensities for Marker 1 in cytoplasm area.
<b>Wavelength 3 Average Cell Intensity</b>	Average pixel intensity for Marker 2 in cell area (cytoplasm area + nucleus area).
<b>Wavelength 3 Integrated Cell Intensity</b>	Total pixel intensities for Marker 2 in cell area (cytoplasm area + nucleus area).
<b>Wavelength 3 Average Nuclear Intensity</b>	Average pixel intensity for Marker 2 in nuclear area.
<b>Wavelength 3 Integrated Nuclear Intensity</b>	Total of pixel intensities for Marker 2 in nuclear area.
<b>Wavelength 3 Average Cytoplasm Intensity</b>	Average pixel intensity for Marker 2 in cytoplasm area.
<b>Wavelength 3 Integrated Cytoplasm Intensity</b>	Total of pixel intensities for Marker 2 in cytoplasm area.
<b>Wavelength 4 Average Cell Intensity</b>	Average pixel intensity for Marker 3 in cell area (cytoplasm area + nucleus area).

Measurement	Description
<b>Wavelength 4 Integrated Cell Intensity</b>	Total pixel intensities for Marker 3 in cell area (cytoplasm area + nucleus area).
<b>Wavelength 4 Average Nuclear Intensity</b>	Average pixel intensity for Marker 3 in nuclear area.
<b>Wavelength 4 Integrated Nuclear Intensity</b>	Total of pixel intensities for Marker 3 in nuclear area.
<b>Wavelength 4 Average Cytoplasm Intensity</b>	Average pixel intensity for Marker 3 in cytoplasm area.
<b>Wavelength 4 Integrated Cytoplasm Intensity</b>	Total of pixel intensities for Marker 3 in cytoplasm area.
<b>Cell Segmentation</b>	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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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
<b>Total Object Count</b>	Number of objects.
<b>Total Granule Count</b>	Number of granules.
<b>Avg Granule Count</b>	Average number of granules per object.
<b>Total Granule Area</b>	Total area in $\mu\text{m}^2$ of granules.
<b>Avg Granule Intensity</b>	Average pixel intensity calculated over all granules in the image.
<b>Avg Granule Integrated Intensity</b>	Average of the integrated pixel intensity values for all granules in the image.

## Cell Measurements

Measurement	Description
<b>Granule Count</b>	Number of granules detected for a specific cell. (Note that a granule is assigned to its nearest nucleus).
<b>Granule Total Area</b>	Total area in $\mu\text{m}^2$ covered by all the granules assigned to a specific cell.
<b>Granule Integrated Intensity</b>	Total pixel intensity of the granules assigned to a specific cell.
<b>Granule Intensity</b>	Average pixel intensity of the granules assigned to a specific cell.
<b>Cell Segmentation</b>	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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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
<b>Total Object Count</b>	Number of objects.
<b>Total Granule Count</b>	Number of granules.
<b>Avg Granule Count</b>	Average number of granules per object.
<b>Total Granule Area</b>	Total area in $\mu\text{m}^2$ of granules.
<b>Avg Granule Intensity</b>	Average pixel intensity calculated over all granules in the image.
<b>Avg Granule Integrated Intensity</b>	Average of the integrated pixel intensity values for all granules in the image.

## Cell Measurements

Measurement	Description
<b>Granule Count</b>	Number of granules detected for a specific cell. (Note that a granule is assigned to its nearest nucleus).
<b>Granule Total Area</b>	Total area in $\mu\text{m}^2$ covered by all the granules assigned to a specific cell.
<b>Granule Integrated Intensity</b>	Total pixel intensity of the granules assigned to a specific cell.
<b>Granule Intensity</b>	Average pixel intensity of the granules assigned to a specific cell.
<b>Cell Segmentation</b>	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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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
<b># Cells</b>	Number of cells.
<b># Live Cells</b>	Number of cells positive for the marker.
<b>% Live Cells</b>	Percentage of positive cells to total cells.
<b># Dead Cells</b>	Number of cells negative for the marker in the image as defined in the segmentation parameters.
<b>% Dead Cells</b>	Percentage of positive cells to total cells.
<b>All Cell Intensities</b>	Total pixel intensity of all cells in the image.
<b>Live Cell Intensities</b>	Total pixel intensity of cells positive for the marker.
<b>Live Cell Average Area</b>	Average area in $\mu\text{m}^2$ of cells positive for the marker.
<b>Live Cell Total Area</b>	Total area in $\mu\text{m}^2$ of cells positive for the marker.

## Cell Measurements

Measurement	Description
<b>Positive</b>	Returns 1 if cell is scored as positive, otherwise returns 0.
<b>Cell Area</b>	Area of the cell (white segmentation mask).
<b>Nuclear Area</b>	Area of the nucleus (green segmentation mask).
<b>Wavelength 1 Integrated Nuclear Intensity</b>	The total pixel intensity of the nuclear stain over the nuclear area.
<b>Wavelength 1 Average Nuclear Intensity</b>	The average pixel intensity of the nuclear stain (intensity per pixel).
<b>Wavelength 2 Integrated Nuclear Intensity</b>	The total pixel intensity of the marker stain in the nucleus of an individual cell.
<b>Wavelength 2 Average Nuclear Intensity</b>	The average pixel intensity of the marker stain in the nucleus of an individual cell.
<b>Wavelength 2 Integrated Cell Intensity</b>	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.
<b>Wavelength 2 Average Cell Intensity</b>	Average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Live Cells Average Intensity</b>	The average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Live Cells Integrated Intensity</b>	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Live Cells Area</b>	Total area in $\mu\text{m}^2$ of the cell if positive for the marker.
<b>Cell Segmentation</b>	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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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
<b>Total Object Count</b>	Number of objects.
<b>Total Granule Count</b>	Number of granules.
<b>Avg Granule Count</b>	Average number of granules per object.
<b>Total Granule Area</b>	Total area in $\mu\text{m}^2$ of granules.
<b>Avg Granule Intensity</b>	Average pixel intensity calculated over all granules in the image.
<b>Avg Granule Integrated Intensity</b>	Average of the integrated pixel intensity values for all granules in the image.

## Cell Measurements

Measurement	Description
<b>Granule Count</b>	Number of granules detected for a specific cell. (Note that a granule is assigned to its nearest nucleus).
<b>Granule Total Area</b>	Total area in $\mu\text{m}^2$ covered by all the granules assigned to a specific cell.
<b>Granule Integrated Intensity</b>	Total pixel intensity of the granules assigned to a specific cell.
<b>Granule Intensity</b>	Average pixel intensity of the granules assigned to a specific cell.
<b>Cell Segmentation</b>	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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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
<b>Total Object Count</b>	Number of objects.
<b>Total Granule Count</b>	Number of granules.
<b>Avg Granule Count</b>	Average number of granules per object.
<b>Total Granule Area</b>	Total area in $\mu\text{m}^2$ of granules.
<b>Avg Granule Intensity</b>	Average pixel intensity calculated over all granules in the image.
<b>Avg Granule Integrated Intensity</b>	Average of the integrated pixel intensity values for all granules in the image.

## Cell Measurements

Measurement	Description
<b>Granule Count</b>	Number of granules detected for a specific cell. (Note that a granule is assigned to its nearest nucleus).
<b>Granule Total Area</b>	Total area in $\mu\text{m}^2$ covered by all the granules assigned to a specific cell.
<b>Granule Integrated Intensity</b>	Total pixel intensity of the granules assigned to a specific cell.
<b>Granule Intensity</b>	Average pixel intensity of the granules assigned to a specific cell.
<b>Cell Segmentation</b>	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.

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### 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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
<b># Cells</b>	Number of cells identified by the nuclear stain.
<b># Cells</b>	Number of cells positive for the marker.
<b>% Mitotic Cells</b>	Percentage of cells positive for the marker to the number of cells.
<b># Non-Mitotic Cells</b>	Number of cells negative for the marker.
<b>% Non-Mitotic Cells</b>	Percentage of cells negative for the marker to the number of cells.
<b>All Cell Intensities</b>	Average pixel intensity of over all the cell areas.
<b>Mitotic Cell Intensity</b>	Sum of Mitotic Cells Average Intensity

## Cell Measurements

Measurement	Description
<b>Positive</b>	Returns 1 if cell is scored as positive for the marker; otherwise returns 0.
<b>Cell Area</b>	Area of the cell (white segmentation mask).
<b>Nuclear Area</b>	Area of the nucleus (green segmentation mask).
<b>Wavelength 1 Integrated Nuclear Intensity</b>	Total pixel intensity of the nuclear stain over the nuclear area in an individual cell.
<b>Wavelength 1 Average Nuclear Intensity</b>	Average pixel intensity of the nuclear stain over all the nuclear areas in an individual cell (intensity per pixel).
<b>Wavelength 2 Integrated Nuclear Intensity</b>	Total pixel intensity of stain in the nucleus of an individual cell.
<b>Wavelength 2 Average Nuclear Intensity</b>	Average pixel intensity of stain in the nucleus of an individual cell.
<b>Wavelength 2 Integrated Cell Intensity</b>	Total pixel intensity of stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Wavelength 2 Average Cell Intensity</b>	Average pixel intensity of stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Mitotic Cells Average Intensity</b>	The average pixel intensity of stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Mitotic Cells Integrated Intensity</b>	Total pixel intensity of stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Cell Segmentation</b>	Saves the cell segmentation overlay along with the experiment data.

## Neurite Tracing



Use the Neurite Tracing analysis to measure multiple biologicals. 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.

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**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  $\mu\text{m}$ . 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  $\mu\text{m}$ .

### 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
<b>Cell Count</b>	Number of cell bodies in the image.
<b>Total Outgrowth</b>	Total length of skeletonized outgrowth in $\mu\text{m}$ (corrected for diagonal lengths).
<b>Avg Outgrowth</b>	Average skeletonized outgrowth in $\mu\text{m}$ corrected for diagonal lengths divided by the number of cells.
<b>Total Branches</b>	Total number of branching junctions in the image.
<b>Avg Branches</b>	Total branches divided by number of cells.
<b>Total Processes</b>	Number of outgrowths in the image that are connected to cell bodies.
<b>Avg Processes</b>	Total processes divided by number of cells.
<b>Avg Cell Body Area</b>	Total cell body area divided by the number of cells.
<b>Avg Cell Body Intensity</b>	Average pixel intensity of the neurite stain over all the outgrowths detected in the image.

## Cell Measurements

Measurement	Description
<b>Outgrowth</b>	Total amount of skeletonized outgrowth in $\mu\text{m}$ (corrected for diagonal lengths) associated with the cell.
<b># Processes</b>	Number of outgrowths that connect to the cell body.
<b># Branches</b>	Number of branching junctions of all the processes connected to the cell.
<b>Avg Process Growth</b>	Total outgrowth in $\mu\text{m}$ divided by number of processes of the cell.
<b>Max Process Growth</b>	Maximum value of the outgrowth lengths (in $\mu\text{m}$ ) associated with the cell's various processes.
<b>Median Primary Process Length</b>	Median value of the outgrowth lengths (in $\mu\text{m}$ ) associated with the cell's various processes.
<b>Overall Straightness</b>	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).
<b>Average Outgrowth Intensity</b>	Average pixel intensity of the neurite stain over all the outgrowths for this cell.
<b>Mean Width of All Processes</b>	Total area of all outgrowths divided by the total length of all outgrowths.



Measurement	Description
<b>Mean Width of All Primary Processes</b>	Total area of primary processes (from cell body to first branch point) divided by the total length of the primary processes.
<b>Mean Width of All Secondary Processes</b>	Not used in this version of the CellReporterXpress software.
<b>Number of Secondary Processes</b>	Number of outgrowths that are connected with primary process branches.
<b>Number of Segments</b>	Total number of segments. Segments connect two branches, a cell body to a branch, or a branch point to end point.
<b>Number of End Points</b>	Total number of outgrowth terminal points.
<b>Mean Angular Vector</b>	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.
<b>Mean Vector Magnitude</b>	Magnitude of the mean vector.
<b>Average Cell Body Intensity</b>	Total intensity of all cell bodies divided by number of cell bodies.
<b>Cell Body Area</b>	Total area of the cell body in $\mu\text{m}^2$ (excluding outgrowths).
<b>Cell Body CentroidX</b>	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).
<b>Cell Body CentroidY</b>	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).
<b>Number of Spines</b>	Not used in this version of the CellReporterXpress software.
<b>Mean Spine Area</b>	Not used in this version of the CellReporterXpress software.
<b>Average Spine Intensity</b>	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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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
<b>Total Object Count</b>	Number of objects.
<b>Total Granule Count</b>	Number of granules.
<b>Avg Granule Count</b>	Average number of granules per object.
<b>Total Granule Area</b>	Total area in $\mu\text{m}^2$ of granules.
<b>Avg Granule Intensity</b>	Average pixel intensity calculated over all granules in the image.
<b>Avg Granule Integrated Intensity</b>	Average of the integrated pixel intensity values for all granules in the image.

## Cell Measurements

Measurement	Description
<b>Granule Count</b>	Number of granules detected for a specific cell. (Note that a granule is assigned to its nearest nucleus).
<b>Granule Total Area</b>	Total area in $\mu\text{m}^2$ covered by all the granules assigned to a specific cell.
<b>Granule Integrated Intensity</b>	Total pixel intensity of the granules assigned to a specific cell.
<b>Granule Intensity</b>	Average pixel intensity of the granules assigned to a specific cell.
<b>Cell Segmentation</b>	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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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  $\mu\text{m}$  for the smallest nuclei you want to detect.
- **Max Width:** The maximum width in  $\mu\text{m}$  for the largest nuclei you want to detect.

## Summary Measurements

Measurement	Description
<b>Cell Count</b>	Number of cells identified.
<b>Pit Count</b>	Number of pits.
<b>Avg Pit Count</b>	Number of pits to the total number of nuclei.
<b>Total Pit Area</b>	Total area in $\mu\text{m}^2$ of pits for all cells.
<b>Avg Pit Intensity</b>	Average pixel intensity over all the pit areas.
<b>Avg Pit Integrated Intensity</b>	Average pixel intensity over all the pit areas.
<b>Vesicle Count</b>	Number of vesicles.
<b>Avg Vesicle Count</b>	Number of vesicles divided by the number of nuclei.
<b>Total Vesicle Area</b>	Total area in $\mu\text{m}^2$ of vesicles for all cells.
<b>Avg Vesicle Intensity</b>	Average pixel intensity over all the vesicle areas.
<b>Avg Vesicle Integrated Intensity</b>	Average pixel intensity over all the vesicle areas.
<b>Total Nuclear Area</b>	Total area in $\mu\text{m}^2$ of the nuclei for all cells.
<b>Avg Nuclear Intensity</b>	Average pixel intensity of the nuclear stain over all the nuclei.
<b>Avg Nuclear Integrated Intensity</b>	Average pixel intensity over all the nuclei.

## Cell Measurements

Measurement	Description
<b>Pit Count</b>	Number of pits detected for a specific cell. Note that a pit is assigned to its nearest nucleus.
<b>Pit Total Area</b>	Total area in $\mu\text{m}^2$ covered by all the pits assigned to a specific cell.
<b>Pit Integrated Intensity</b>	Total pixel intensity of the pits assigned to a specific cell.
<b>Pit Average Intensity</b>	Average pixel intensity of the pits assigned to a specific cell.
<b>Vesicle Count</b>	Number of vesicles detected for a specific cell. Note that a vesicle is assigned to its nearest nucleus.
<b>Vesicle Total Area</b>	Total area in $\mu\text{m}^2$ covered by all the vesicles assigned to a specific cell.
<b>Vesicle Integrated Intensity</b>	Total pixel intensity of the vesicles assigned to a specific cell.
<b>Vesicle Average Intensity</b>	Average pixel intensity of the vesicles assigned to a specific cell.
<b>Nuclear Total Area</b>	Total square microns of a specific nucleus.
<b>Nuclear Integrated Intensity</b>	Total pixel intensity of the nuclear stain in a specific nucleus.
<b>Nuclear Average Intensity</b>	Average pixel intensity of the nuclear stain in a specific nucleus.
<b>Texture Index</b>	Standard deviation of intensity values of a specific cell.
<b>Gradient Index</b>	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.
<b>Laplacian Index</b>	Similar to the morphological gradient, also reflects fluctuations in the gradient of a specific cell.
<b>Cell Segmentation</b>	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 through 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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
<b># Cells</b>	Number of cells identified by the nuclear stain.
<b># Positive Cells</b>	Number of cells positive for the marker.
<b>% Positive Cells</b>	Percentage of cells positive for the marker to the number of cells.
<b># Positive Cells</b>	Number of cells negative for the marker.
<b>% Positive Cells</b>	Percentage of cells negative for the marker to the number of cells.
<b>All Cell Average Intensities</b>	Average pixel intensity of the marker over all the cell areas.
<b>Positive Cell Average Intensities</b>	Average pixel intensity of cells positive for the marker over the positive cell area.
<b>Positive Cell Integrated Intensity</b>	Total pixel intensity of the marker stain over the cell area in all cells positive for the marker
<b>Positive Cell Total Intensity</b>	Sum of Positive Cells Average Intensity
<b>Positive Cell Total Integrated Intensity</b>	Sum of Positive Cells Integrated Intensity



## Cell Measurements

Measurement	Description
<b>Positive</b>	Returns 1 if cell is scored as positive for the marker; otherwise returns 0.
<b>Cell Area</b>	Area of the cell (white segmentation mask).
<b>Nuclear Area</b>	Area of the nucleus (green segmentation mask).
<b>Wavelength 1 Integrated Nuclear Intensity</b>	Total pixel intensity of the nuclear stain over the nuclear area in an individual cell.
<b>Wavelength 1 Average Nuclear Intensity</b>	Average pixel intensity of the nuclear stain over all the nuclear areas in an individual cell (intensity per pixel).
<b>Wavelength 2 Integrated Nuclear Intensity</b>	Total pixel intensity of the marker stain in the nucleus of an individual cell.
<b>Wavelength 2 Average Nuclear Intensity</b>	Average pixel intensity of the marker stain in the nucleus of an individual cell.
<b>Wavelength 2 Integrated Cell Intensity</b>	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Wavelength 2 Average Cell Intensity</b>	Average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Positive Cells Average Intensity</b>	The average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Positive Cells Integrated Intensity</b>	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Cell Segmentation</b>	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  $\mu\text{m}$ .

#### Probe tab

**Channel:** Select the fluorescent channel for the marker that is moving in or out of the compartment.

#### Probe

- **Classify Positive if Coefficient  $\geq$ :** 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
<b>Cell Count</b>	Number of cells in the image.
<b>Total Inner Intensity</b>	Total pixel intensity of the probe in all inner regions for the site after background subtraction (note this correlates with cell count).
<b>Total Outer Intensity</b>	Total pixel intensity of the probe in all outer regions for the site after background subtraction (note that this correlates with cell count).
<b>Avg Inner Area</b>	Average area in $\mu\text{m}^2$ of all inner regions for the site after background subtraction.
<b>Avg Outer Area</b>	Average area in $\mu\text{m}^2$ of all outer regions for the site after background subtraction.
<b>Avg Inner Intensity</b>	Average pixel intensity of the probe in all inner regions for the site after background subtraction (independent of cell count).
<b>Avg Outer Intensity</b>	Average pixel intensity of the probe in all outer regions for the site after background subtraction (independent of cell count)
<b># Translocated Cells</b>	Total number of cells classified as positive for translocation.

## Cell Measurements

Measurement	Description
<b>Inner Area</b>	Average area in $\mu\text{m}^2$ of the inner region after background subtraction.
<b>Outer Area</b>	Average area in $\mu\text{m}^2$ of the outer region after background subtraction.
<b>Total Inner Intensity</b>	Total pixel intensity of the probe in the inner region after background subtraction.
<b>Total Outer Intensity</b>	Total pixel intensity of the probe in the outer region after background subtraction.
<b>Avg Inner Intensity</b>	Average pixel intensity of the probe in the inner region after background subtraction.
<b>Median Inner Intensity</b>	Median (middle) pixel intensity value of the probe in the inner region after background subtraction.
<b>Avg Outer Intensity</b>	Average pixel intensity of the probe in the outer region after background subtraction.
<b>Median Outer Intensity</b>	Median (middle) pixel intensity value of the probe in the outer region after background subtraction.
<b>Correlation Coefficient</b>	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.
<b>Classification</b>	Returns 1 for positive translocation classification (nuclear staining) and 0 for negative translocation classification (cytoplasmic staining).
<b>Cell Segmentation</b>	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.

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**\* 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.

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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 $\mu\text{m}^2$ 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  $\mu\text{m}$ . 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  $\mu\text{m}$ . 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
<b># Cells</b>	Number of cells identified by the nuclear stain.
<b># Positive Cells</b>	Number of cells positive for the marker.
<b>% Positive Cells</b>	Percentage of cells positive for the marker to the number of cells.
<b># Positive Cells</b>	Number of cells negative for the marker.
<b>% Positive Cells</b>	Percentage of cells negative for the marker to the number of cells.
<b>Positive Cell Average Intensities</b>	Average pixel intensity of cells positive for the marker over the positive cell area.
<b>Positive Cell Integrated Intensity</b>	Total pixel intensity of the marker stain over the cell area in all cells positive for the marker
<b>Positive Cell Total Intensity</b>	Sum of Positive Cells Average Intensity
<b>Positive Cell Total Integrated Intensity</b>	Sum of Positive Cells Integrated Intensity

## Cell Measurements

Measurement	Description
<b>Positive</b>	Returns 1 if cell is scored as positive for the marker; otherwise returns 0.
<b>Cell Area</b>	Area of the cell (white segmentation mask).
<b>Nuclear Area</b>	Area of the nucleus (green segmentation mask).
<b>Wavelength 1 Integrated Nuclear Intensity</b>	Total pixel intensity of the nuclear stain over the nuclear area in an individual cell.
<b>Wavelength 1 Average Nuclear Intensity</b>	Average pixel intensity of the nuclear stain over all the nuclear areas in an individual cell (intensity per pixel).
<b>Wavelength 2 Integrated Nuclear Intensity</b>	Total pixel intensity of the marker stain in the nucleus of an individual cell.
<b>Wavelength 2 Average Nuclear Intensity</b>	Average pixel intensity of the marker stain in the nucleus of an individual cell.
<b>Wavelength 2 Integrated Cell Intensity</b>	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Wavelength 2 Average Cell Intensity</b>	Average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Positive Cells Average Intensity</b>	The average pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Positive Cells Integrated Intensity</b>	Total pixel intensity of the marker stain in the cell (nucleus + cytoplasm or cytoplasm if selected in the settings) of an individual cell.
<b>Cell Segmentation</b>	Saves the cell segmentation overlay along with the experiment data.

## Contact Us

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