FLUIDIGM®

FLDM-00075[01]

CyTOF Software v7.0 for Imaging Mass Cytometry

User Guide

For Research Use Only. Not for use in diagnostic procedures.

FLDM-00075[01]

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Introduction

About This Guide

This guide is intended for use with the Hyperion™ Imaging System and CyTOF® Software v7.0.

NOTE An HTML version of this user guide is available within CyTOF Software v7.0. To open the user guide in your web browser, click the Help button in the upper-right corner of the CyTOF Software screen.

About CyTOF Software v7.0

CyTOF Software v7.0 is 64-bit dual-interface software designed for Imaging Mass Cytometry™ and cell suspension-based applications for Hyperion Imaging System and Helios™ system users. The software has the built-in capability to switch sample introduction modes, providing users the flexibility to configure hardware as required.

CyTOF Software v7.0 is intended for operation on Windows® 7 Pro and Windows 10 Pro 64-bit computer systems (PN 104042, 104043, or 104050) with Windows Administrator privileges.

Supported Systems

- Helios system, including the AS-5 Autosampler
- CyTOF 2-to-Helios Upgrade instruments
- Hyperion Imaging System: Hyperion Tissue Imager coupled with a Helios system

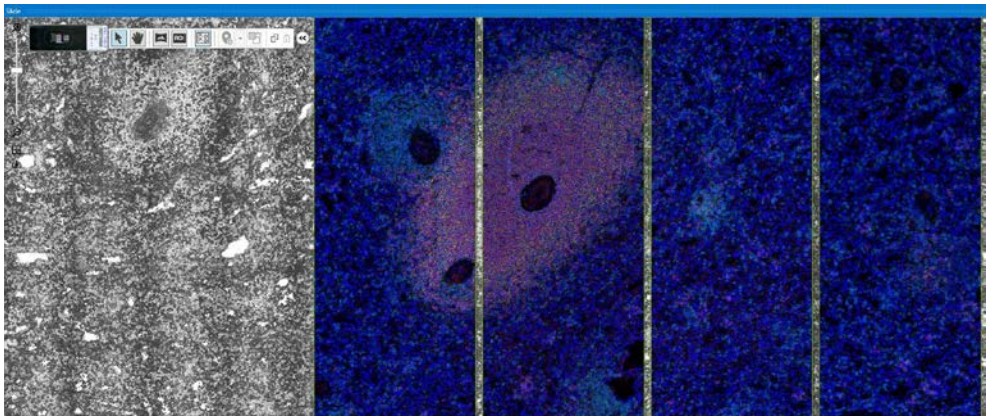
IMPORTANT CyTOF Software v7.0 is not compatible with CyTOF, CyTOF 2, or CyTOF C5 upgrade instruments.

What's New in v7.0

Features

- **New user interface.** A new user interface with docking windows provides a highly configurable workspace. Explore the new workspace.
- **Offline batch creation.** Install CyTOF Software v7.0 on a personal computer to prepare batches offline. This is especially convenient for tissue microarray (TMA) experiments that may require the selection of several regions of interest (ROIs), working with annotated images, and supporting off-site users.
- **Improved workflow to define ROIs and panoramas.** New tools enable you to precisely define ROIs and panoramas and adjust the positions and dimensions.
    - Copy and paste functionality provides easier and faster selection of multiple ROIs directly on the sample image.
    - Import a list of ROI coordinates and dimensions from a CSV file.
- **Image coregistration.** For improved accuracy of ablation when using sample images, use the instrument camera to coregister a sample image with the physical sample inserted in the Hyperion Tissue Imager.
- **ROIs and panoramas are no longer linked.** Select ROIs anywhere within the slide boundaries. Panoramas are not required. The MCD file structure has changed to support this improvement.
- **Fiducial creation.** Burn fiducials on a sample slide before starting your run or before removing the slide from the Hyperion Tissue Imager. Coregister the fiducials to calibrate the slide position before resuming acquisition of ROIs at a later time.
- **Improved detector voltage optimization routine.** The new detector voltage algorithm supports 10x higher ^{131}Xe signal.
- **High-redundancy auto focus.** Auto focusing routine uses a 9-point pattern for redundancy resulting in more reliable and effective focusing.
- **Support for larger ROIs.** CyTOF Software v7.0 adjusts the stage height during ablation to compensate for slide topography and periodically optimizes the detector voltage. These improvements result in uniform ablation of ROIs >1 mm^2.
- **Faster panorama creation.** A 1 cm^2 panorama is completed 40% faster than with earlier versions of CyTOF Software, and >40% for smaller panoramas.
- **Automated calibration of reference energy.** Calibration of reference energy to 0 dB for ablation of tuning film is now automated.
- **Preventive maintenance reminder.** A reminder is displayed when it is time to schedule preventive maintenance. You can choose to automatically notify the Fluidigm Service Department by email or snooze the reminder.

- **Live ion image in color.** View multi-color ion images to assess sample and data quality in real-time or post-acquisition.



Data Storage Requirements

IMPORTANT To reduce the risk of acquisition interruption due to insufficient disk space and to prevent the effect of network latency on performance, save data to the E: drive. Do not save data to the C: drive, an external hard drive, or a network drive.

Estimate Storage Requirements

When acquisition is started, data storage requirements are estimated based on the following requirements. If there is insufficient space on the hard drive, acquisition is canceled and a message is displayed in Log Manager.

- A 1000 x 1000 µm ROI with 10 channels requires 50 MB of disk space.
- A text file for a 1000 x 1000 µm ROI requires 8MB per channel.
- Optical images, such as panoramas, imported images, and before and after ablation images, increase the size of the MCD file. The size increase varies depending on the dimensions and resolution of the optical images.

IMPORTANT For best software responsiveness, the combined area of all panoramas and imported images (with a resolution of 1 µm/pixel) should be less than 20,000 x 10,000 µm.

Computer Resources

- **Preserve as much free space on the C: drive as possible.** Do not install software that is not required for the instrument.
- **Allow system resources for data acquisition.** Do not run additional software or process data during acquisition. These actions reduce the system resources available for acquisition.

- **Disable or reschedule automatic processes.** Windows Automatic Update, disk defragmentation, and virus scanning, require computer resources. If a process is scheduled to run during acquisition, acquisition may fail due to insufficient computer resources.

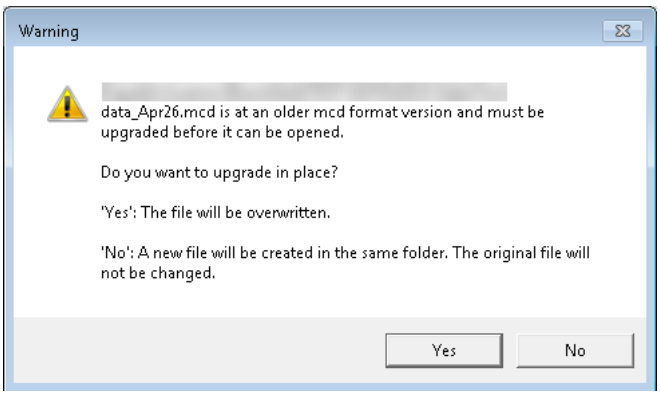
Additional Recommendations

- Do not defragment the drives on the acquisition computer. Frequent defragmentation decreases the lifespan of the drives.
- Do not map the E: drive to a network. This may compromise the read and write operations of the acquisition software.

MCD File Compatibility

MCD files acquired with an earlier version of CyTOF Software must be updated for compatibility with CyTOF Software v7.0. The changes are to the file schema and do not impact data integrity.

When an MCD file created with an earlier version of CyTOF Software is opened, a prompt is displayed.



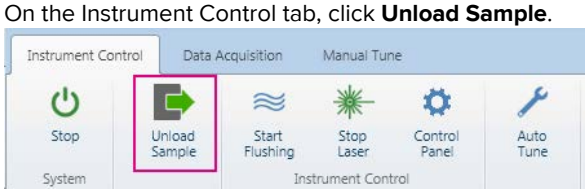
Click **Yes** to upgrade and replace the original file. Click **No** to save the original data file and create a v7.0 compatible copy.

IMPORTANT v7.0 MCD files are not compatible with earlier versions of CyTOF Software.

Acquisition Quick Reference

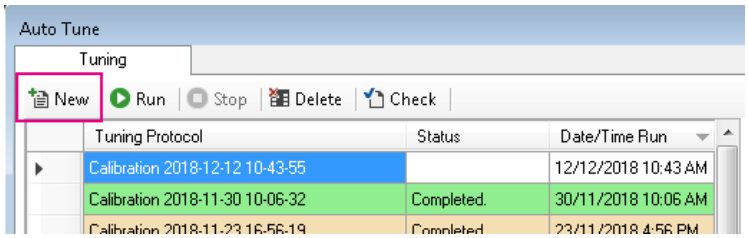
This topic provides a brief overview of key operational features of CyTOF® Software v7.0.

All users are encouraged to read the topics in the Acquire Data section of the Help guide for more detailed instructions on how to use the new features in v7.0.

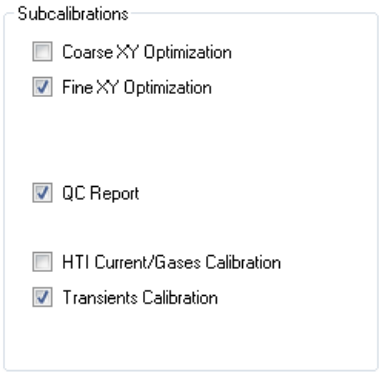
How To	Steps
Start the system	On the ribbon, click the **Instrument Control** tab, and then click **Start**.  The startup sequence takes approximately 7 minutes. Allow the system 30 minutes to warm up before running Auto Tune. **NOTE** If the system has been off for >1 day, plasma may be extinguished. For more information, see Start the System.
Unload or load a slide	**1** On the Instrument Control tab, click **Unload Sample**.  **2** After the stage on the Hyperion™ Tissue Imager fully extends, open the sample window and, if necessary, remove the previously loaded slide. **3** Load the slide onto the stage and close the sample window. **4** After the sample is loaded, click **Load Sample**  .

How To	Steps
Run Auto Tune	**1** On the ribbon, click the **Instrument Control** tab, and then click **Auto Tune**.

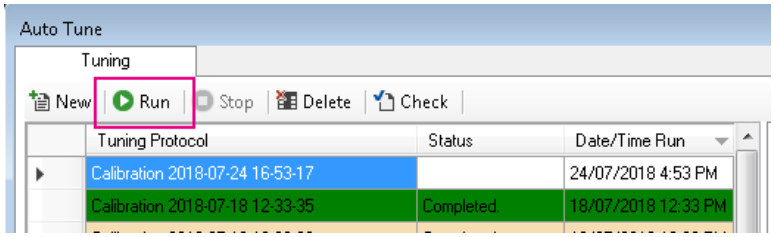
2 On the Auto Tune window, click **New** to create a new entry in the Tuning Protocol table.



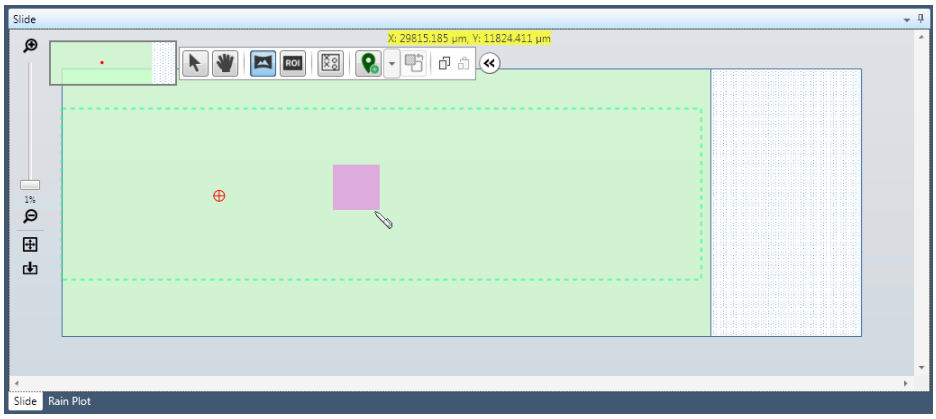
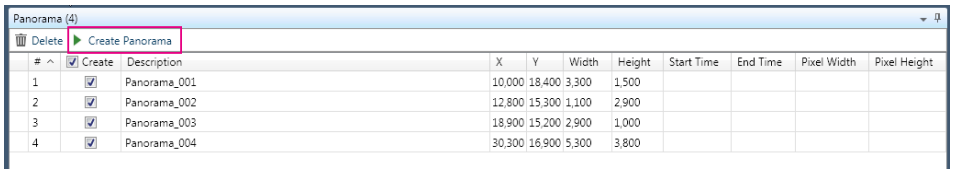
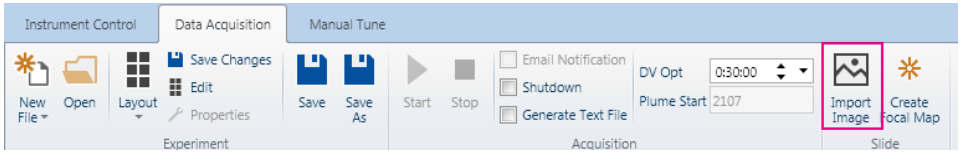
3 Under Subcalibrations, check any combination of subcalibrations to run. For example:

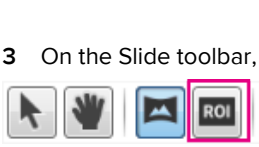
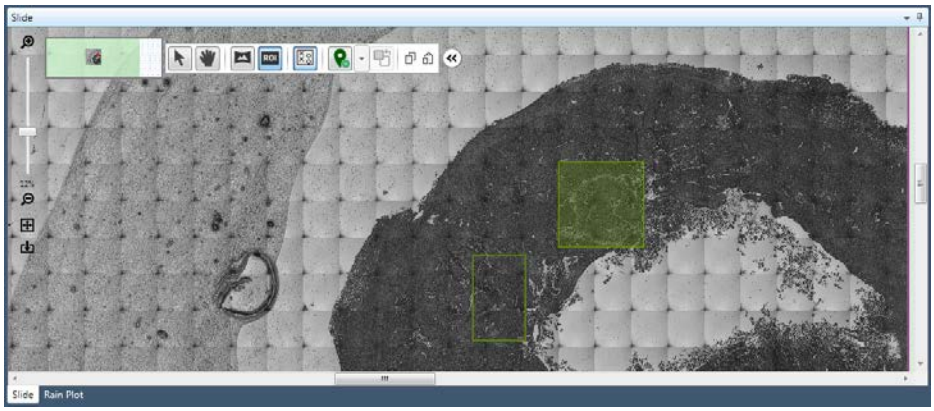


4 Click **Run** to start Auto Tune.



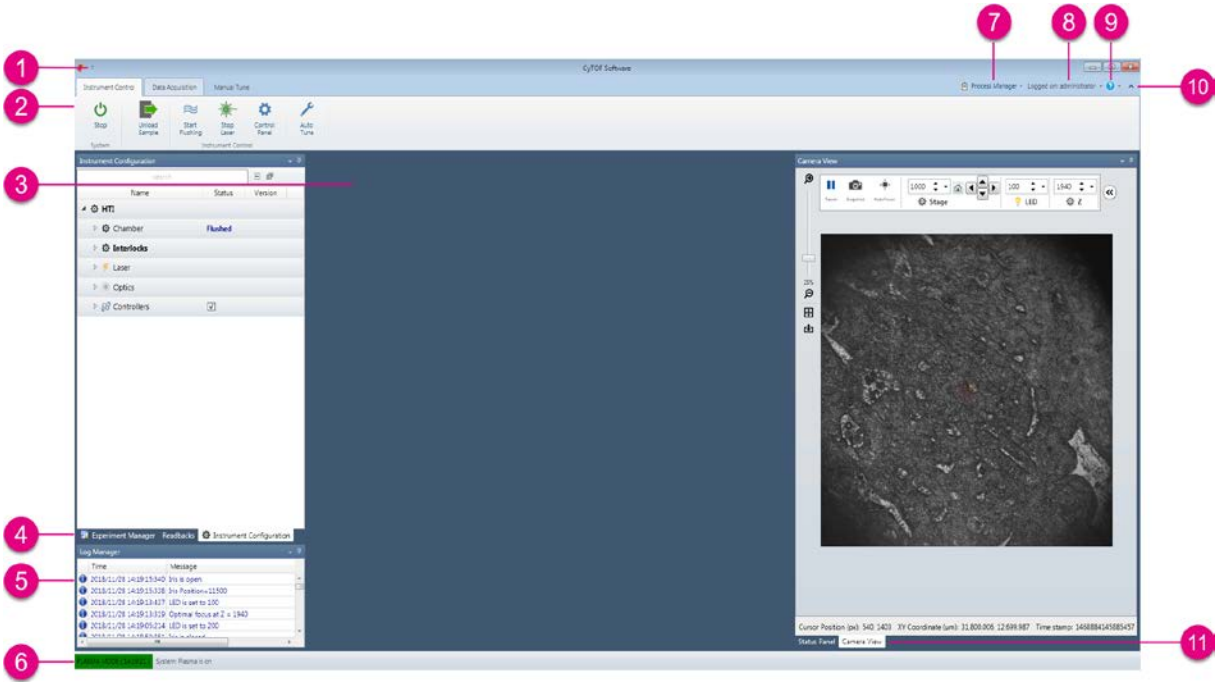
For more information, see About Auto Tune and Run Auto Tune.

How To	Steps
Create a panorama	**1** If an MCD file is not opened, click the **Data Acquisition** tab, and then click **New File**. **2** On the Slide toolbar, click the **Panorama** tool  .  **3** Click and drag to select an area on the Slide Layout.  **4** Click the Panorama tab to display the Panorama table. **5** Verify that the Create checkbox is checked and then click **Create Panorama**.  For more information, see Create a Panorama and Import an Image.
Import an image	**1** If an MCD file is not open, click the **Data Acquisition** tab, and then click **New File**. **2** On the Data Acquisition tab, click **Import Image**.  **3** Browse to locate an image of the sample loaded in the Hyperion Tissue Imager. Click to choose it, and then click **Open**. **4** In the Import Slide Image window, crop, rotate, or rescale the image, if necessary, and then click **Apply** to import the image into the MCD file. It is displayed on the Slide Layout and a row is added to the Panorama table. **5** Coregister the image with the sample before selecting ROIs for acquisition. For more information, see Import an Image.

How To	Steps
Draw and acquire a region of interest (ROI)	**1** If an MCD file is not open, click the **Data Acquisition** tab, and then click **New File**. **2** Create a panorama or import and coregister an image. **3** On the Slide toolbar, click the **ROI** tool  **4** Click and drag to select an area on the panorama.  **5** Click the **ROI** tab to display the ROI table. **6** Apply an acquisition template. **a** On the ROI toolbar, click **Template**.  **b** Double-click a template in the list to apply the acquisition settings. **7** To acquire the ROI, verify that the **Acquire** checkbox is checked and then click **Start**. For more information, see Draw or Import ROIs, Create, Modify, or Delete Acquisition Templates, Apply Acquisition Settings, and Start Data Acquisition.

Explore the User Interface

User Interface Overview



Name	Description
Quick Access Toolbar	A customizable toolbar that provides access to user-specified tools at all times. Click the down arrow to customize the toolbar.
Ribbon	Contains tabbed toolbars—Instrument Control, Data Acquisition, and Manual Tune (CyTOF® Administrators only)—that group buttons by task.
Workspace	Data acquisition-related tasks are performed in the Workspace after an MCD file is opened.
Instrument Configuration, Experiment Manager and Readbacks tabs	The Instrument Configuration tab displays the status of components within the Hyperion™ Tissue Imager. The Experiment Manager tab displays the content structure of all opened MCD files. Readbacks displays readbacks from the multifunction board (MFB), the radio frequency generator (RFG), and XY.
Log Manager	The Log Manager displays messages related to instrument status.

Name	Description
Instrument Status	The Instrument Status bar Indicates the instrument state: On is green. System start up or shut down is yellow. Idle or error is red.
Process Manager	The Process Manager displays progress of individual processes and subprocesses performed by the Hyperion Tissue Imager. The active process is displayed. Click to expand and see all recent processes.
User Management	User Management displays the account that currently logged on to the software. Click the down arrow to access User Management settings.
Help	Click the Help icon to access Help and to view the About box.
Show/Hide the Ribbon	Hide or show the ribbon. When the ribbon is hidden, the toolbar tabs are displayed, but the toolbar is hidden when you click elsewhere.
Camera View and Status Panel tabs	Camera View provides navigation controls that move the stage and bring different parts of the sample into view. The Status Panel is a visual display of the status of Hyperion Tissue Imager components.

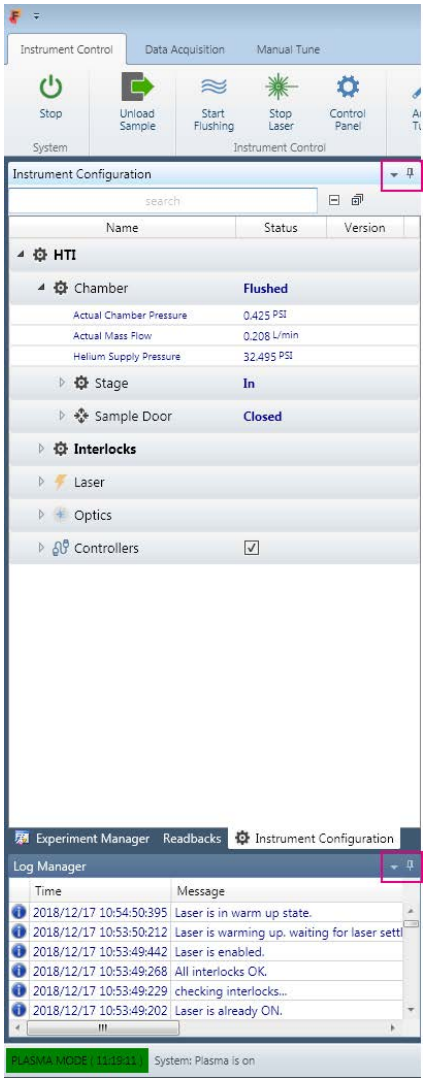
Configure the Workspace

To configure or auto-hide a tab

In the top right corner of each pane within the workspace is an arrow and pushpin. Click the arrow to display the configuration menu or click the pushpin to auto-hide the active tab.

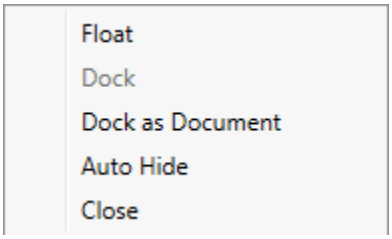
To view or re-dock a hidden tab

When auto-hide is applied, the active tab is collapsed on the side of the workspace. Click the button to expand the tab, and then click the pushpin to re-dock the tab.



The Window Configuration Menu

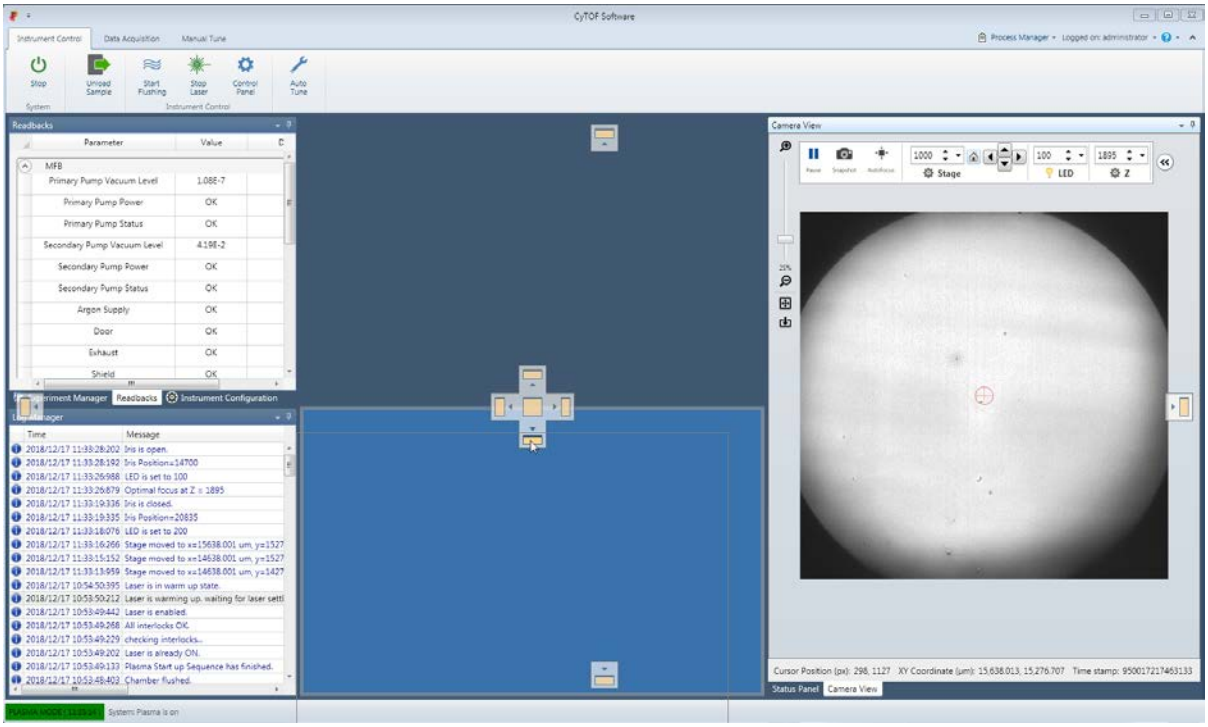
Right-click a window title bar or click the arrow in the upper right corner to access the following window configuration menu:



Menu Item	Action
Float	Allows you to position the window anywhere within the workspace.
Dock	Snaps a floating window back to its previous docked location.
Dock as Document	Displays the window as a tabbed document page. To access window-positioning controls on a tabbed document, right-click the tab.
Auto Hide	Hides the window. A tab is displayed in place of the window. Click the tab to restore the window to view.
Close	Close the window.

Drag and Drop Windows

Windows can be easily repositioned by dragging them into the preferred section of the workspace. Click the window title bar and drag the window toward the center of the workspace. A yellow positioning diagram is displayed. Hover the mouse pointer over the area where you would like to drop the window and release the mouse button.



Ribbon

The new ribbon design contains tabbed toolbars that organize the controls by task.

- Instrument Control
- Data Acquisition
- Manual Tune (CyTOF® Administrators only)

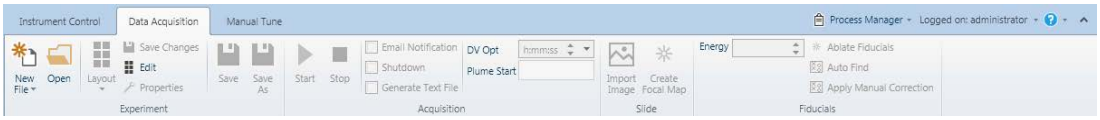
Tabs and Groups

The controls on each tab are sub-categorized into groups.

Instrument Control Tab

Group	Function
System	Start or stop the Hyperion Imaging System.
Instrument Control	• Load or unload a slide. • Flush the chamber. • Start or stop the laser. • Open the Control Panel. • Tune the instrument with Auto Tune.

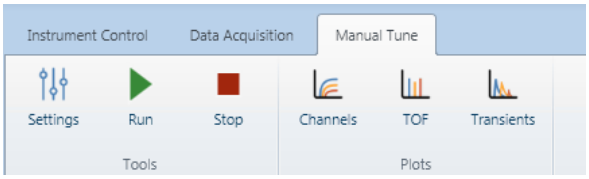
Data Acquisition Tab



Group	Function
Experiment	• Create, open, and save MCD files. • Change the Slide Layout. • Edit Slide Layouts (CyTOF Administrators only).
Acquisition	• Start and stop data acquisition • Enable email notifications, shut down the instrument, and generate text files. • Configure DV optimization intervals. • Observe the Plume Start value.
Slide	• Import and edit an image. • Create a focal map.
Fiducials	• Specify an ablation energy to use when creating fiducials. • Ablate fiducials. • Find fiducials.

Manual Tune Tab

The Manual Tune tab is available to CyTOF Administrators only.

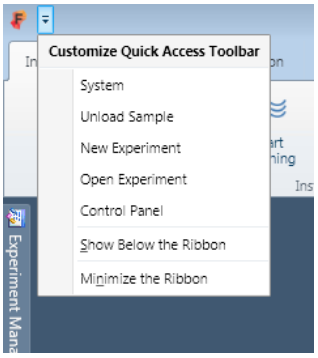


Group	Function
Tools	• Modify Manual Tune settings, such as: • The analytes to monitor while tuning • The parameter to ramp and the settings to use during parameter ramping, such as start/stop values and step size • Ablation parameters, such as ablation energy, ablation frequency, ablation coordinates and dimensions • Start and stop manual tune.
Plots	• Channels displays the Mass(es) Per Reading plot. • TOF displays the Time of Flight Per Reading (Mass Peak) plot. • Transients displays the Transients Per Reading plot.

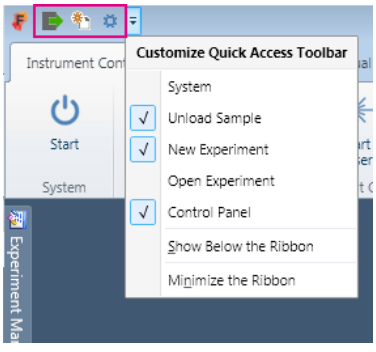
Quick Access Toolbar

Add shortcuts to common controls for quick access at all times.

1 Click the down arrow to access the available controls.

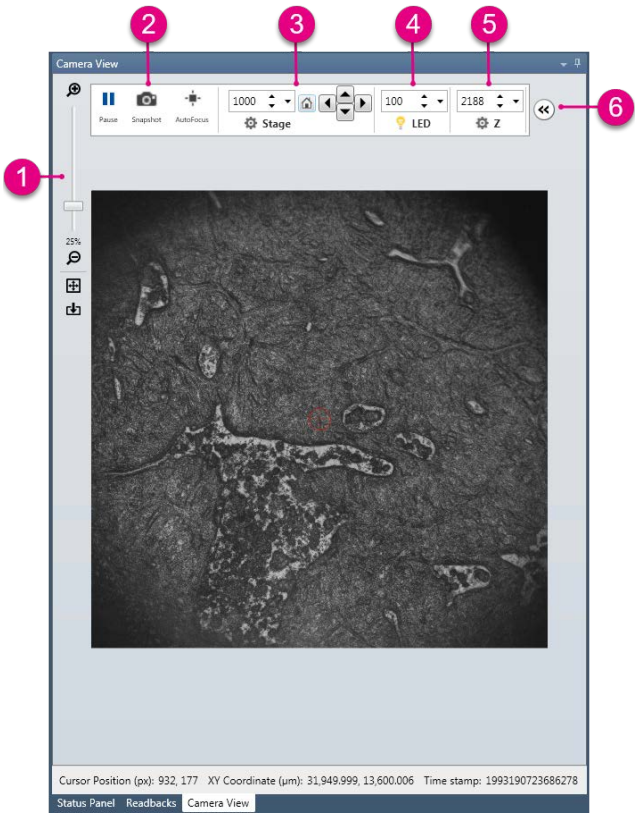


2 Click controls in the list to add them to the Quick Access Toolbar.



Camera View

The Camera View toolbar provides controls to navigate the slide to view the sample. The camera starts automatically when the software is opened.



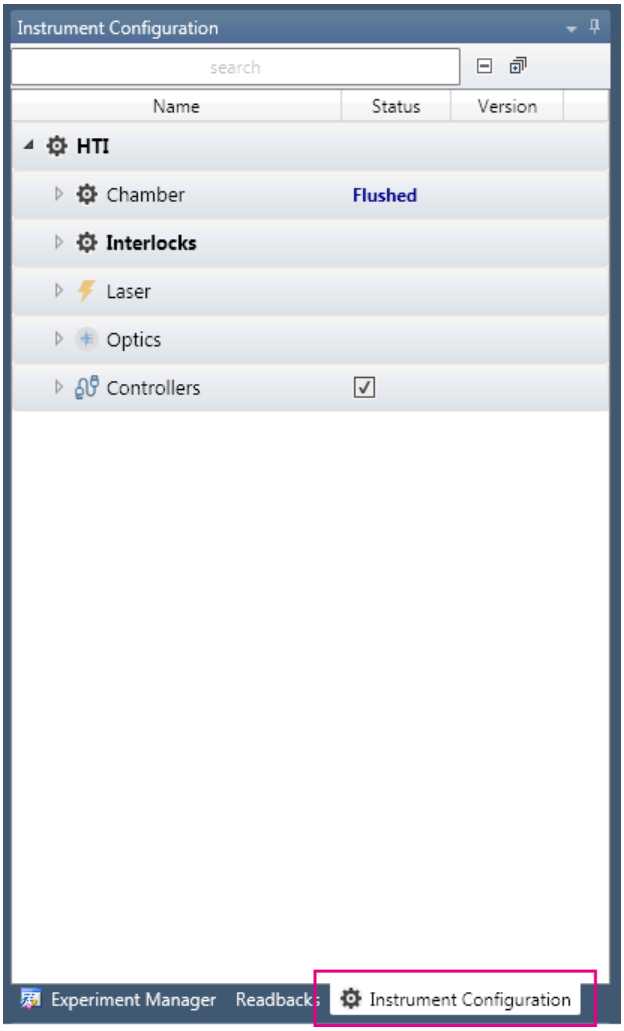
Control Name	Description
1 Zoom	• Use the slider to zoom in and out (or use the mouse wheel). • Click  to fit the visible camera area into the Camera window. • Click  to reset zoom to 25%.
2 Camera	• Click **Pause** to pause the camera. The last view remains displayed. • Click **Snapshot** to save the current camera view to a PNG (.png) file. • Click **AutoFocus** to automatically bring the image into focus. AutoFocus iteratively adjusts the LED strength and the stage height (Z) until optimal focus is achieved.
3 Stage	• Enter a step size (µm) into the text box to set the distance the stage will travel when moved in any direction. The default step size is 1000 µm. • Click an arrow button to move the stage up, down, left, or right. • Click Home to position the stage at the home coordinates.

Control Name	Description
4 LED	• This is an optional setting. LED brightness is optimized by AutoFocus. • Enter a value between 0 and 4095 to adjust the LED brightness. The greater the value, the brighter the LED. The default value is 100.
5 Z	• This is an optional setting. The stage height is optimized by AutoFocus. • Enter a value between 0 and 4095 to adjust the stage height. The greater the value, the higher the stage.
6 Collapse	• Click  to hide the toolbar.

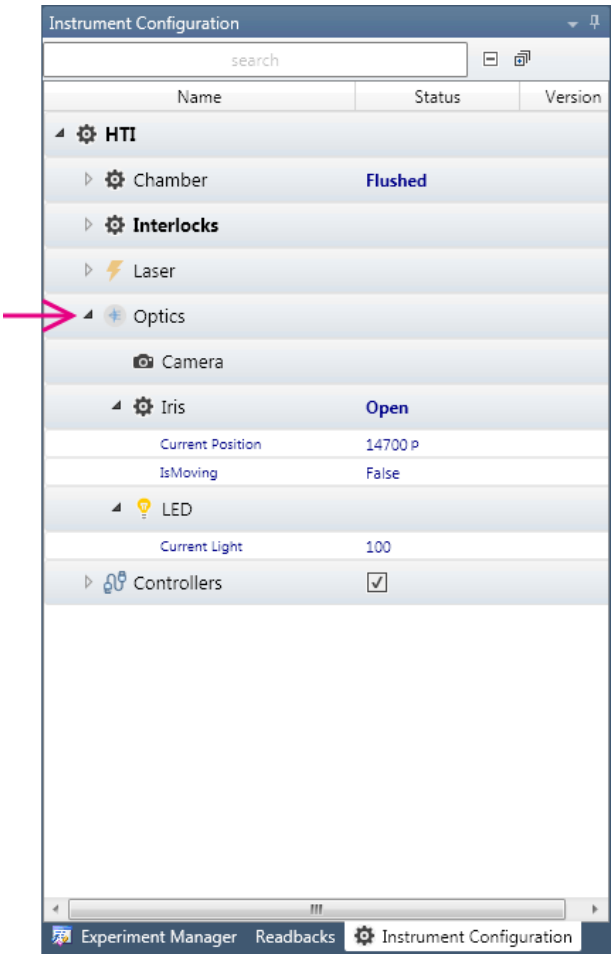
Instrument Configuration

When the software starts, the Instrument Configuration tab is displayed on the left side of the workspace. It displays the read-only status of each hardware component within the Hyperion™ Tissue Imager.

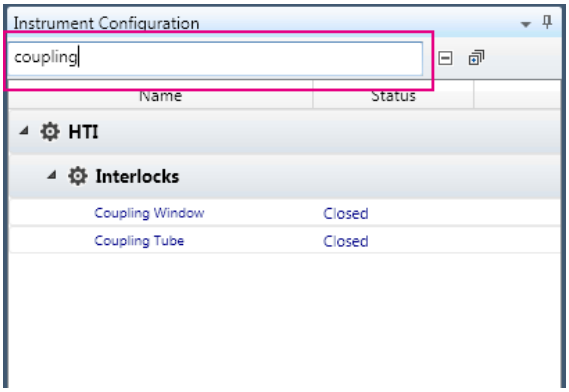
NOTE When Instrument Configuration is not displayed, click the Instrument Configuration tab to display it.



Click the arrow to expand and view components within each section.



To quickly locate a component by name, type the name into the search bar at the top of the pane.

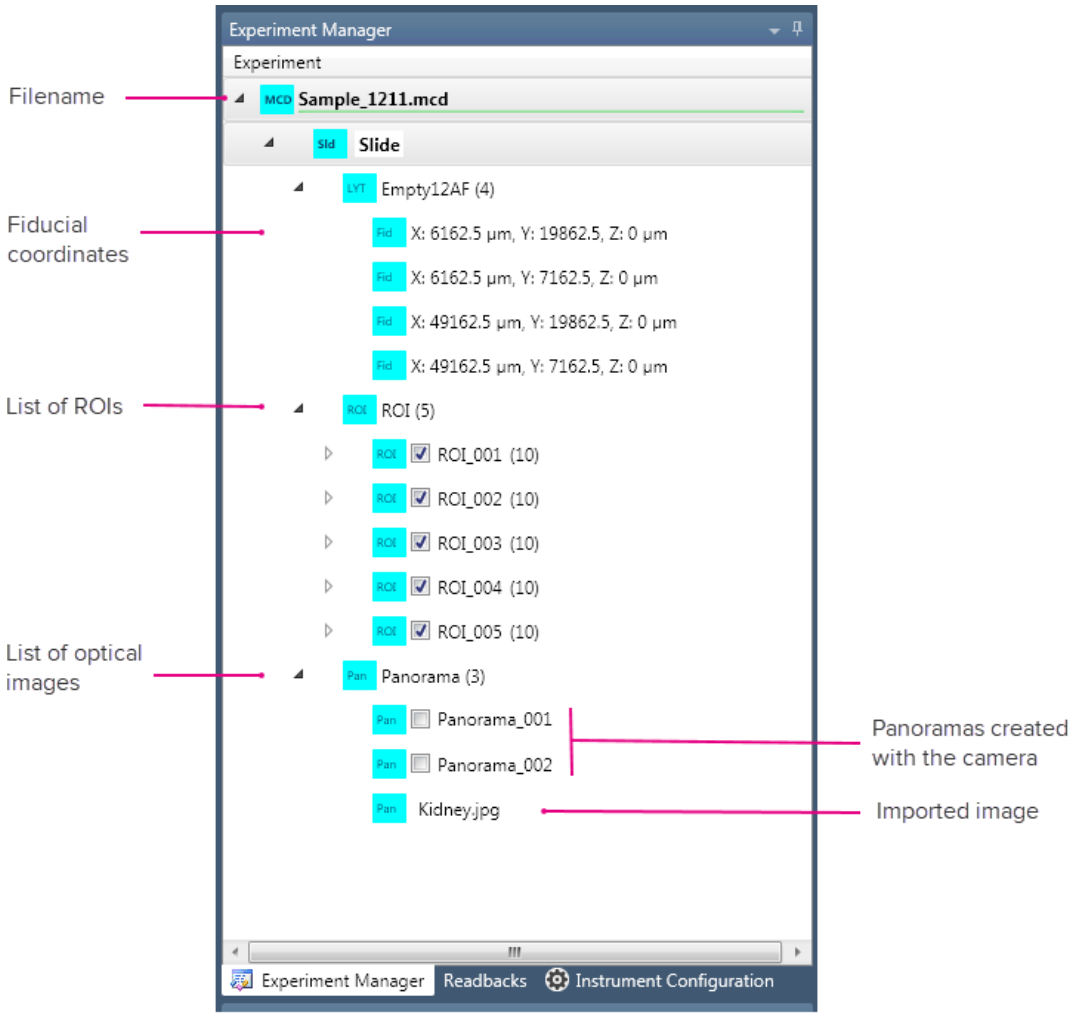


Experiment Manager

Experiment Manager is automatically displayed on the left side of the workspace when an MCD file is created or opened. Experiment Manager displays the file contents—such as Slide Layout, fiducial coordinates, panoramas, and ROIs—of all opened MCD files. Use tools within Experiment Manager to easily create, locate, and view panoramas and ROIs.

Experiment Manager Overview

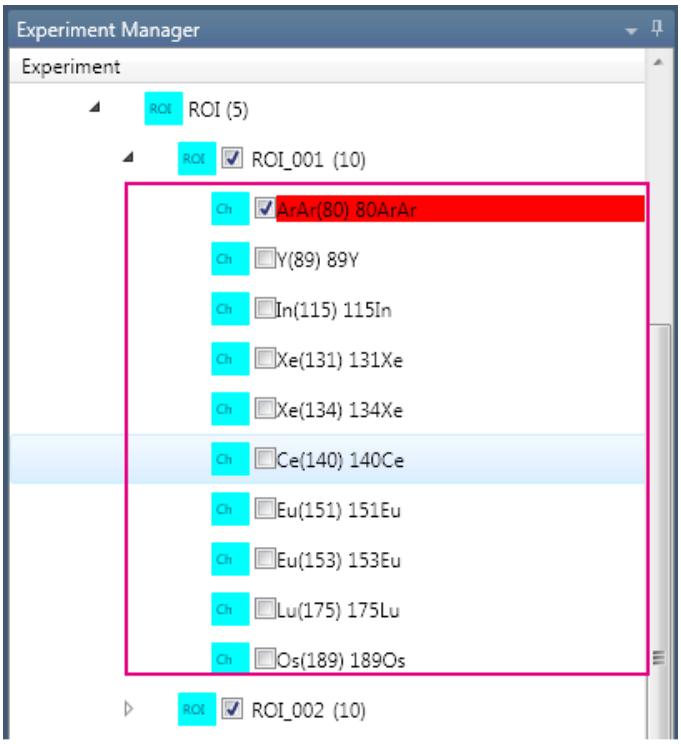
In v7.0, ROIs are no longer grouped by panorama, as they were in earlier versions of CyTOF® Software. As a result, ROIs and panoramas are equivalent on a hierarchal level. With v7.0 the slide is the parent container for both panoramas and ROIs.



Display and Select Channels

Expand individual ROIs to display the channels. Selected channels are displayed in the Live Ion Image during and after acquisition. By default, the first channel is selected and is displayed in red.

NOTE Channels are defined in the acquisition template and cannot be edited in Experiment Manager.

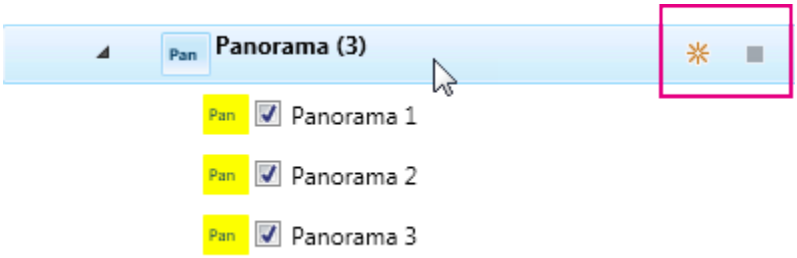


Toolbars

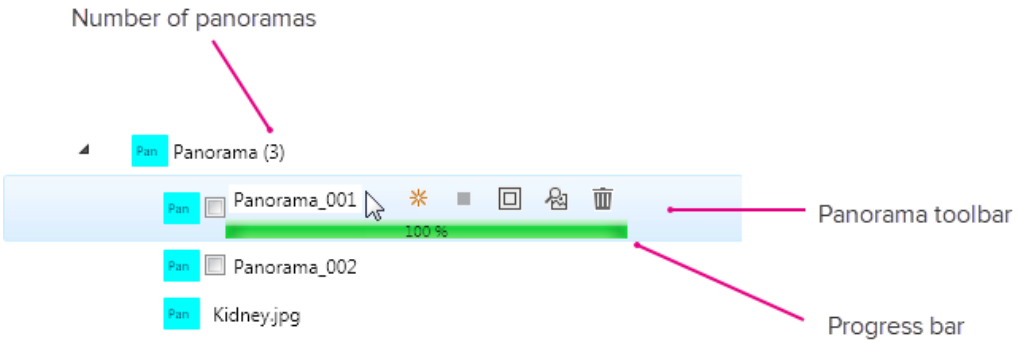
Each component within the Experiment Manager has a toolbar. Move the mouse pointer over an object to display the toolbar.

Panorama Toolbar

The top-level panorama toolbar has two buttons—Create All ( ) and Stop ( ). Click to create every panorama. Click to stop creating panoramas.



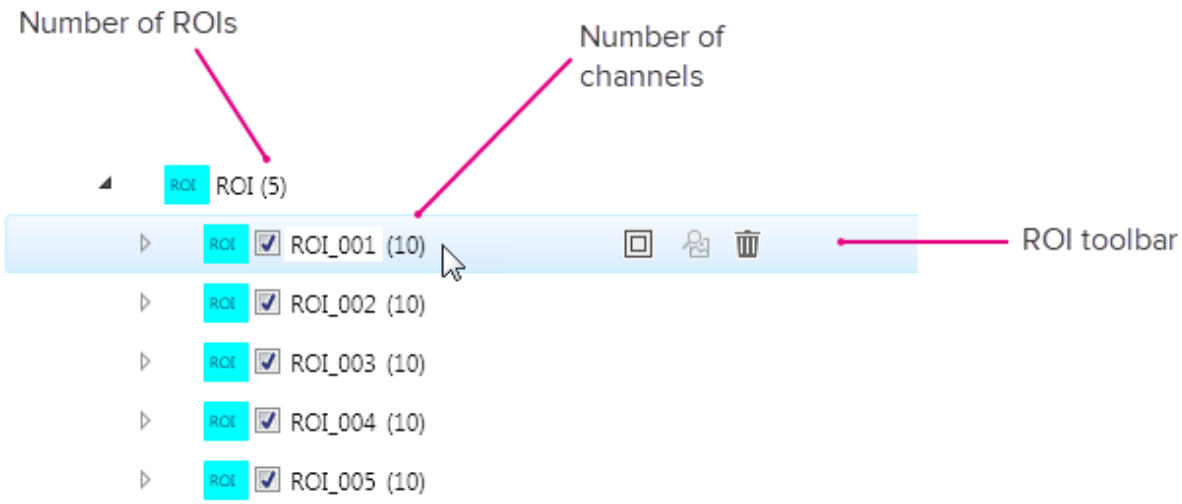
Move the pointer over an individual panorama to display a toolbar specific to that panorama.



The Panorama toolbar has five tools:

Tool	Description
	Click to create the current panorama. A progress bar is displayed after panorama creation starts.
	Click to stop creating the panorama.
	Click to view the panorama.
	Click to locate the panorama on the slide.
	Click to delete the panorama.

ROI Toolbar



The ROI toolbar has three tools:

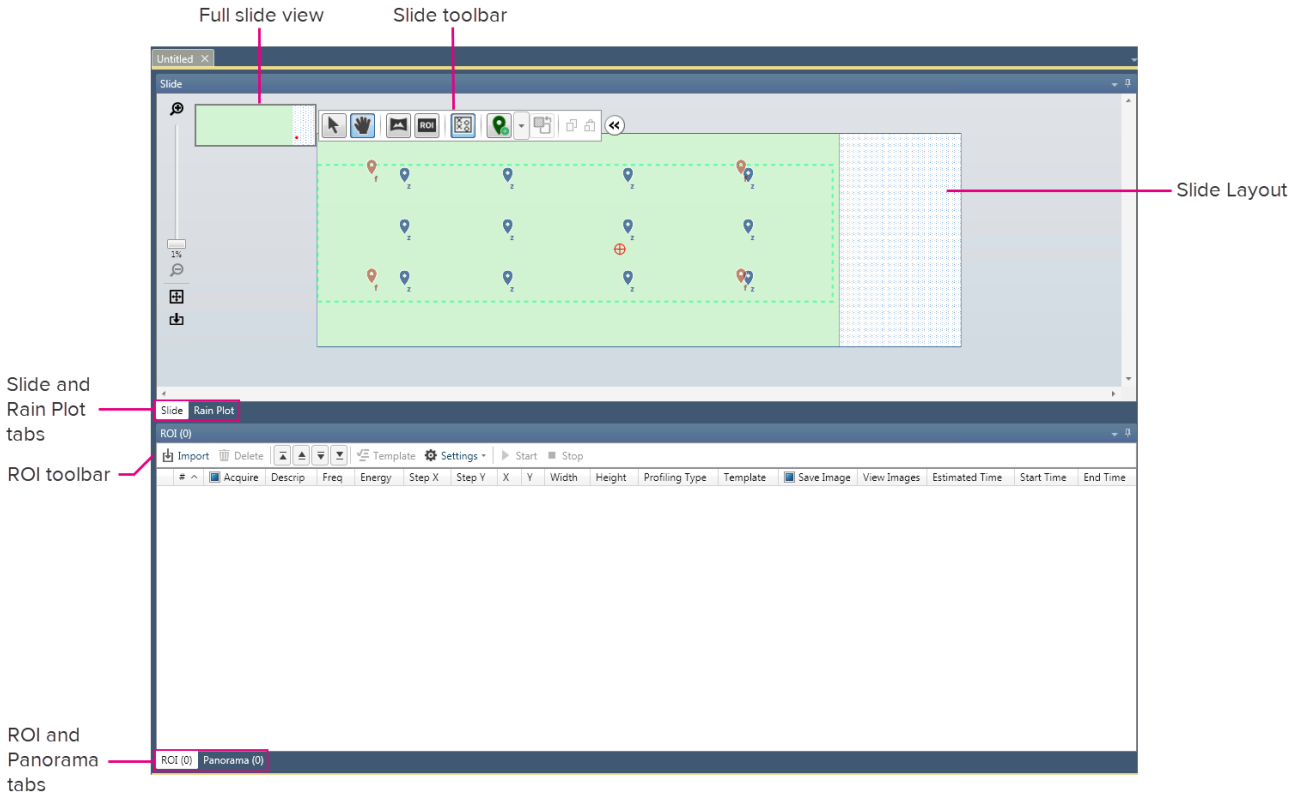
Tool	Description
	Click to view the ROI.
	Click to locate the ROI on the slide.
	Click to delete the ROI that has not been ablated.

Acquisition Interface

After an MCD file is created or opened, the file contents and the tools required to import or create optical images, identify regions of interest (ROI), set acquisition parameters, and start acquisition are displayed on two panes in the workspace.

The upper pane consists of two tabs—the Slide tab, which displays the Slide Layout, and the Rain Plot tab, which displays the counts per push per channel so you can identify events as they occur during acquisition

The lower pane consists of two tabs—the ROI tab, which displays the list of selected ROIs



Name	Description
Full slide view	Intended to assist with slide navigation while zoomed in on the Slide Layout. The red dot indicates the position of the mouse cursor relative to the entire slide.
Slide toolbar	Contains the tools required to navigate the Slide Layout, create panoramas, create ROIs, reposition fiducial pins, and coregister imported images.
Slide Layout	Visually represents the slide loaded in the Hyperion™ Tissue Imager. Displays the locations of the focal and fiducial pins, panoramas, and ROIs.

Name	Description
Slide and Rain Plot tabs	Click to display the Slide Layout or the Rain Plot.
ROI toolbar	Contains ROI table tools to import ROIs from CSV file, delete unacquired ROIs, reorder ROIs, apply an acquisition template, modify acquisition settings, and start and stop acquisition.
ROI and Panorama tabs	Click to display the ROI table or Panorama table.

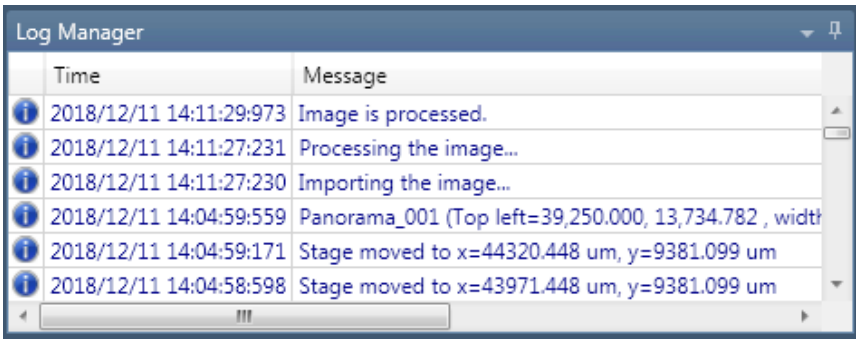
Slide Toolbar

Tool Name	Icon	Description
Select		Click an object to select it or select multiple objects by clicking and dragging over a collection of objects or holding the Ctrl key and clicking each object.
Pan		Move the Slide Layout view horizontally, diagonally, or vertically.
Draw Panorama		Click and drag to select an area on the Slide Layout for panorama creation.
Draw ROI		Click and drag to select an area on the slide layout for ROI acquisition.
Show Fiducials		Focal and fiducial pins are displayed by default. Click once to hide the pins. Click again to show the pins.
Add Coregistration Landmark		Click to add a coregistration pin to the optical image on the Slide Layout. Coregistration pin placement must match the position of the camera cross hair. See Coregister an Image for more information.
Apply Coregistration		Apply Coregistration is tool is dimmed when fewer than three coregistration landmarks are pinned or when coregistration is already applied.
		After three coregistration landmarks have been pinned, the Apply Coregistration tool turns green to indicate that coregistration can be applied. Click to apply coregistration.
Copy		Copies the selected object(s) to the clipboard.

Tool Name	Icon	Description
Paste		Pastes copied objects onto the Slide Layout.
Minimize		Hides the Slide toolbar.

Log Manager

Log Manager is located in the bottom left corner of the workspace. It displays a list of time-stamped messages that describe the real-time operational status of the Hyperion Imaging System.

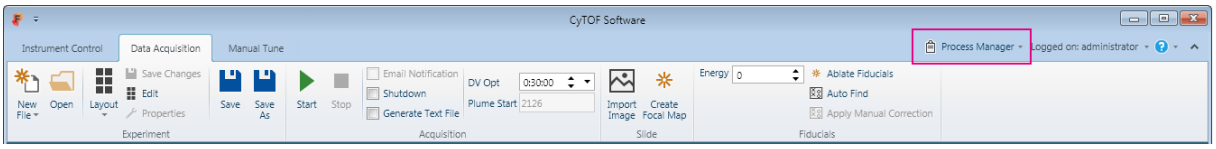


Message Categories

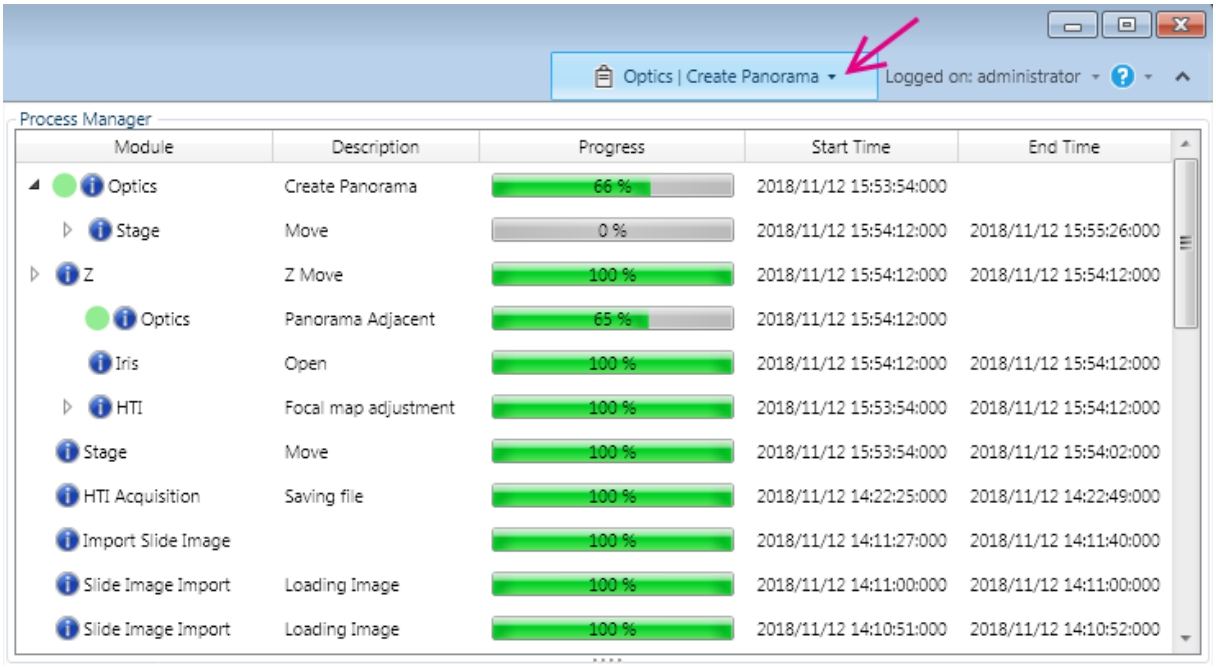
Symbol	Category	Description
	General Information	Describes normal operational functions of the instrument.
	Warning	Describes an unexpected occurrence. Operation is not interrupted.
	Error	Describes an unexpected occurrence. Operation is interrupted.

Process Manager

Process Manager displays the progress of processes and sub-processes as they are performed by software and hardware components within the Hyperion Imaging System.



The Process Manager button updates to display the active process. Click the button to see the progress of multiple subprocesses.



Configure User Management

About User Management

NOTE Accounts created with an earlier version of CyTOF® Software are preserved. If you upgraded from an earlier version of CyTOF Software, you do not need to create new accounts.

When CyTOF Software is installed for the first time, a default administrator account is created. The user name is **Administrator** and there is no password. To create a password for this account, see Change the account password.

Use the Administrator account to create additional accounts.

Authorization Levels

Every account must be assigned an authorization level, either User or Administrator.

User authorization allows the user to:

- Tune the instrument using existing tuning settings
- Create new acquisition templates
- Acquire data
- Update personal CyTOF account information

Administrator authorization grants access to all User functionality, and allows the Administrator to:

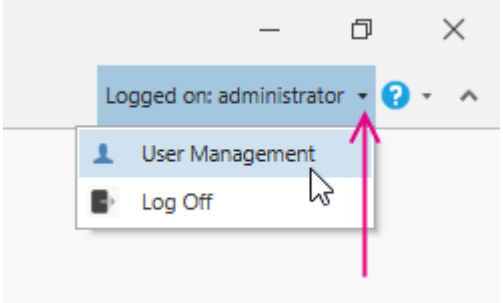
- Modify Auto Tune parameters.
- Access and run Manual Tune.
- Create new Slide Layouts.
- Create new acquisition templates.
- Manage advanced instrument parameters.
- Create, modify, or delete CyTOF accounts.

Create an Account (CyTOF Administrators only)

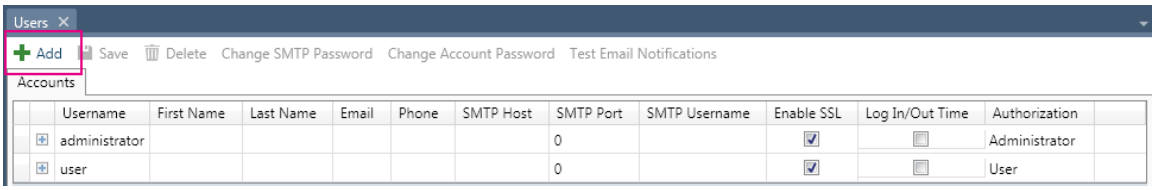
CyTOF Administrators can create new user accounts. The minimum information required to create a new account is username, password, and authorization level (User or Administrator). The default authorization level is User.

To create a new account

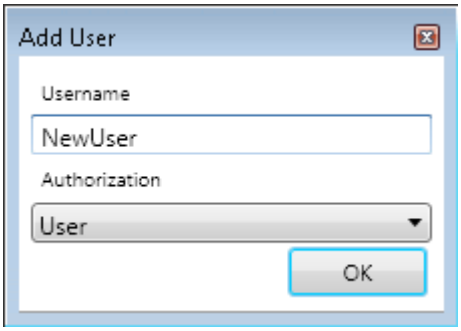
1 On the right side of the ribbon, next to the active user account, click the down arrow, and then click **User Management**.



2 On the User Management tab, click **Add** to display the Add User dialog box.

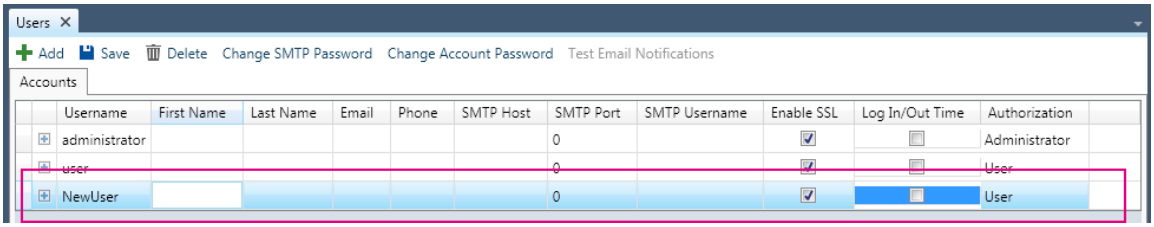


3 In the Add User dialog box, enter a username, choose an Authorization level, and then click **OK**.



4 (Optional) On the User Management tab, double-click the following cells to enter information:

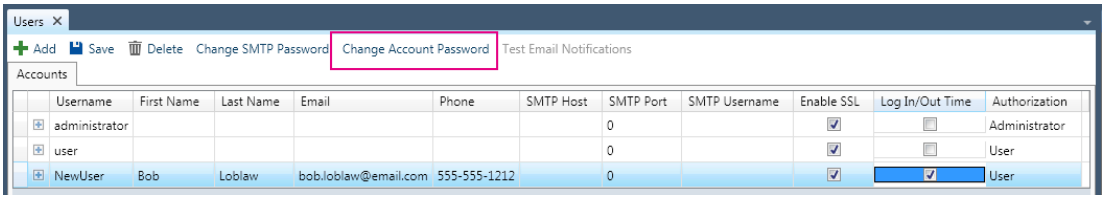
- First Name
- Last Name
- Email
- Phone
- Log In/Out Time: Check this box to maintain a record of the login and logout activity for this account. For more information, see View Log In/Out History.
- Authorization: Choose an authorization level appropriate for the account. For more information, see Authorization Levels.



5 After the account information is entered, create a password for the account.

a Click the row to select it.

b Click **Change Account Password**.



c Enter a new password and click **OK**. When prompted, enter the password again to confirm, and click **OK**.

6 Click **Save** to save the account.

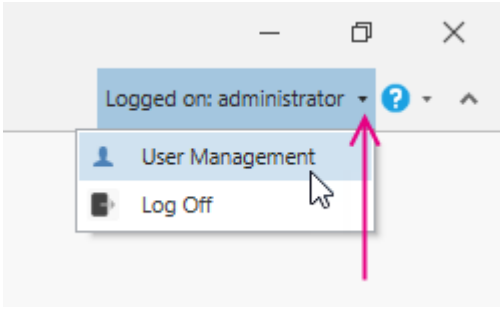
7 Repeat steps 1–6 to create another account or close the User Management tab to exit.

Modify an Account

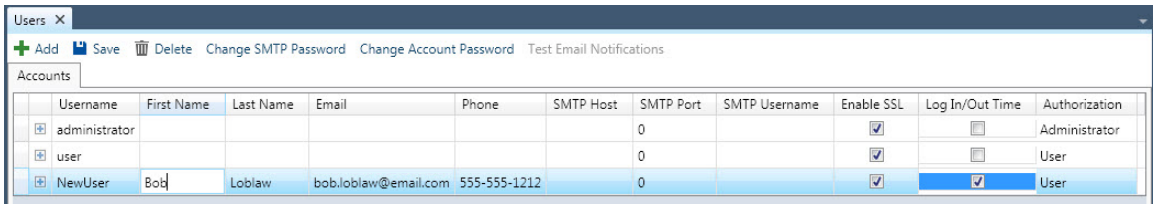
CyTOF Administrators can modify the credentials and permissions of all accounts. CyTOF Software Users can only modify their own account.

To modify an account

1 On the right side of the ribbon, next to the active user account, click the down arrow, and then click **User Management**.



2 On the User Management tab, double-click any cell to add or edit content.



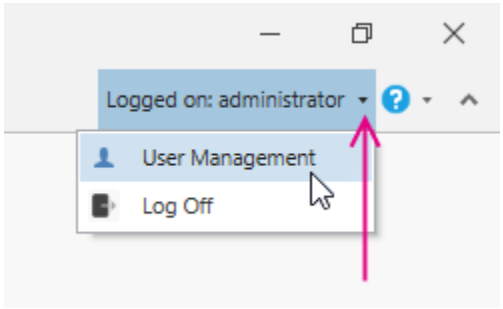
NOTE Users with User authorization can only modify the account they are logged into.

3 After all changes are made to the account, click **Save** to apply.

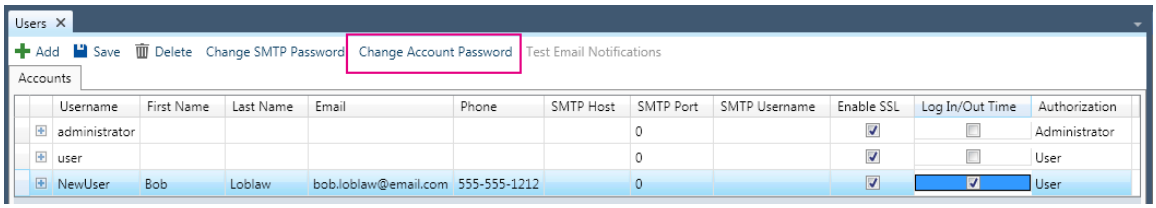
Change an Account Password

To change an account password

1 On the right side of the ribbon, next to the active user account, click the down arrow, and then click **User Management**.



2 On the User Management tab, click **Change Account Password**.



3 When prompted, enter a new password and click **OK**.

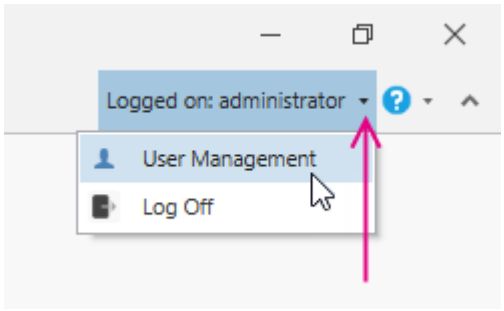
4 Re-enter the password to confirm, and click **OK**. The password is now updated.

Delete an Account (CyTOF Administrators only)

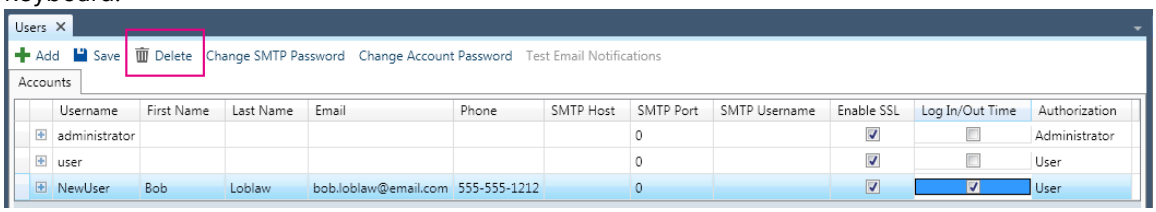
Only CyTOF Administrators can delete accounts.

To delete an account

1 On the right side of the ribbon, next to the active user account, click the down arrow, and then click **User Management**.



2 In the Accounts table, click the account to delete and then press **Delete** on your keyboard.

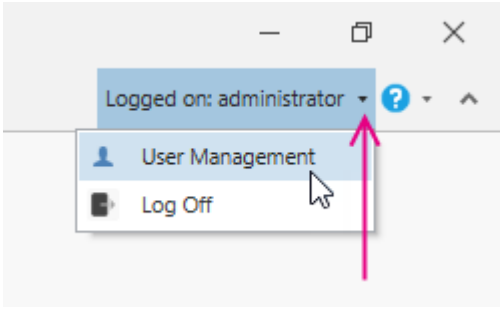


View Log In/Out History (CyTOF Administrators only)

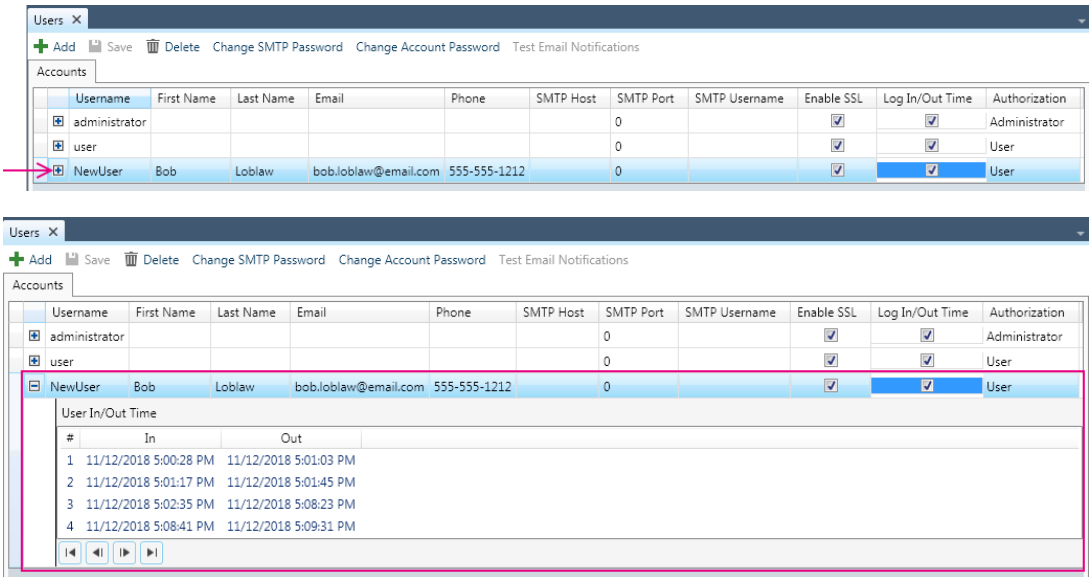
If Log In/Out Time is enabled for an account in CyTOF Software, the time stamp is recorded each time the account is used to log on to and off of the software.

To view Log In/Out History

1 On the right side of the ribbon, next to the active user account, click the down arrow, and then click **User Management**.



2 Click ⊞ next to the account to expand the User In/Out Time history.



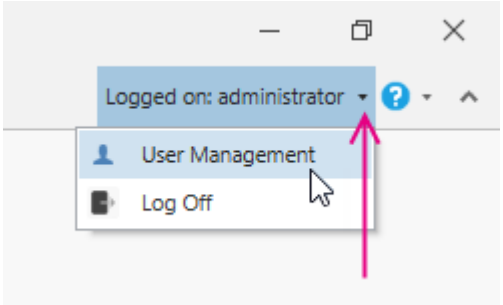
Configure Email Notifications

When the email notifications setting is enabled, the software sends an email when acquisition successfully completes. An email notification is also sent if acquisition unexpectedly stops.

NOTE You must be familiar with the SMTP settings for your mail server. Contact your IT department for assistance.

To configure email notifications

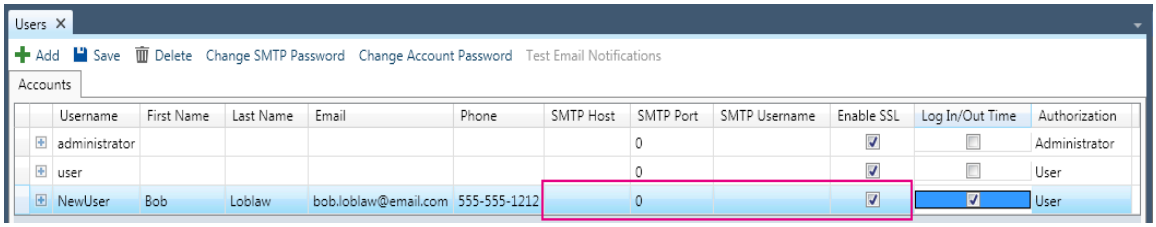
1 On the right side of the ribbon, next to the active user account, click the down arrow, and then click **User Management**.



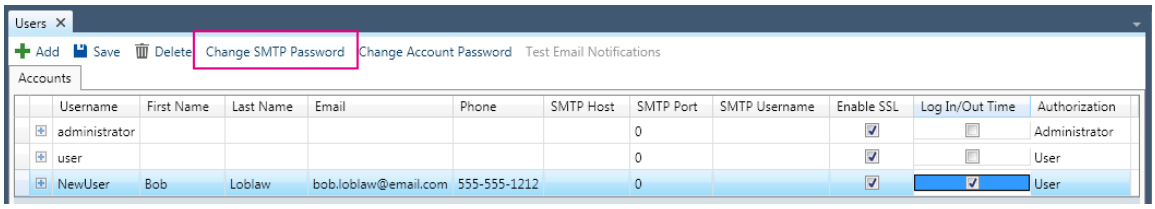
2 In the Accounts table, locate the account to configure email notifications for, and then enter the following information in the appropriate cells:

- SMTP Host
- SMTP Port
- SMTP Username
- Enable SSL

NOTE CyTOF Administrators will see all accounts and CyTOF Users will see only their accounts.



3 Click **Change SMTP Password**.



4 Enter the network password. Click **OK**.

About Instrument Control

Start or Stop the System

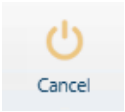
It takes approximately 7 minutes for the system startup sequence to complete. During this time the ablation chamber is flushed with helium, plasma is ignited, and hardware components are initialized.

NOTE If the system has been off for one or more days, additional flushing is required to rid the chamber of oxygen before the system is started. To flush the chamber, click the **Instrument Control** tab, and then click **Start Flushing**.

Start the system

After flushing is complete, click **Start**.

The button now displays Cancel. To cancel system startup, click **Cancel**.



After the system startup sequence is complete the button becomes a stop button.

IMPORTANT Allow 30 minutes for the system to fully warm up before you start tuning.

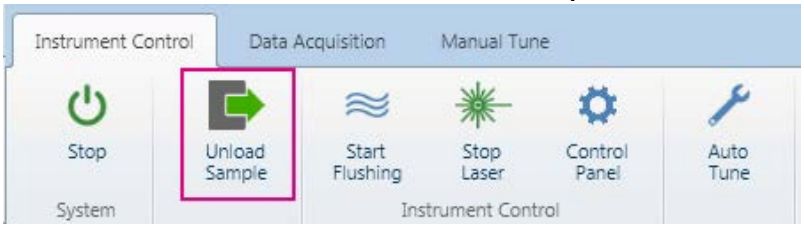
Stop the system

When you are finished acquiring data and want to stop the system, click **Stop**.

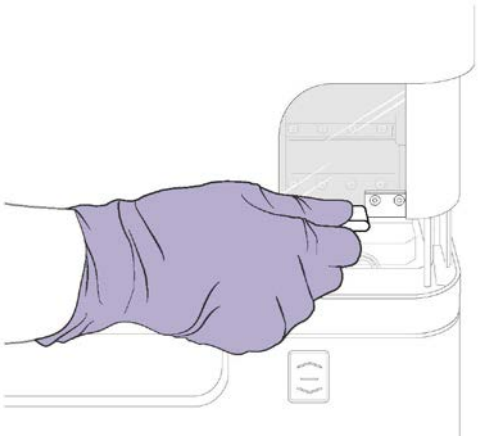
NOTE Stopping the system stops plasma. To stop the laser between acquisitions, click **Stop Laser**.

Unload and Load a Sample

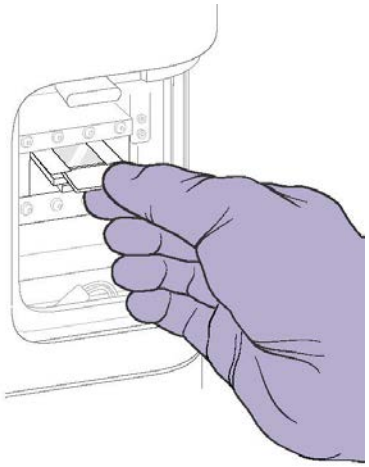
1 On the Instrument Control tab, click **Unload Sample**.



2 After the stage on the Hyperion™ Tissue Imager fully extends, open the sample window and, if necessary, remove the previously loaded slide.



3 Load the slide onto the stage and close the sample window.



4 After the sample is loaded, click **Load Sample**. 

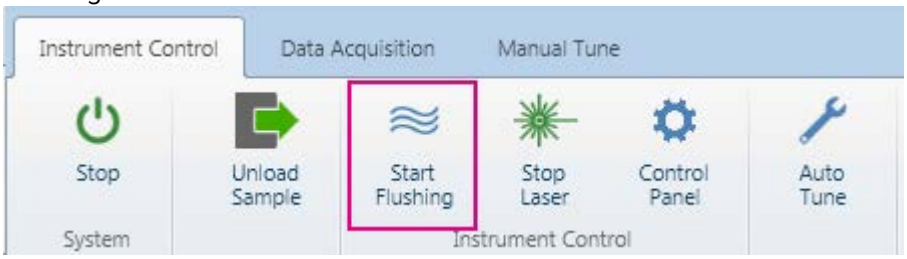
NOTE For more information about loading and unloading slides, see the Hyperion Imaging System User Guide (400311).

Flush the Ablation Chamber

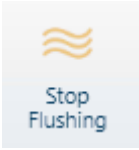
The ablation chamber is automatically flushed with helium when the system is started. This rids the chamber of oxygen, which can extinguish plasma.

To flush the ablation chamber

1 Click **Start Flushing**. After the chamber begins flushing the button toggles to Stop Flushing.



2 Click **Stop Flushing** to stop flushing the ablation chamber.

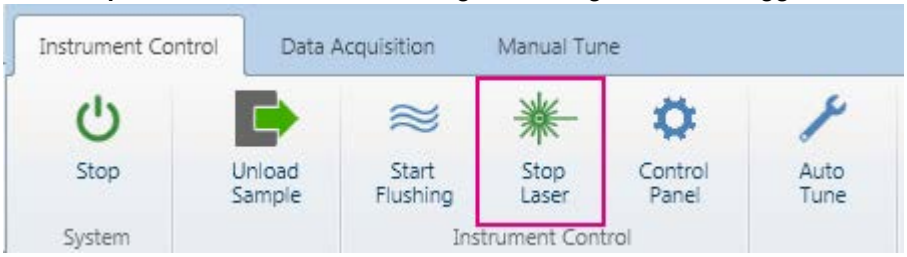


Stop or Start the Laser

The laser is automatically started when the system is started. To stop the laser from firing between acquisitions without stopping plasma, click **Stop Laser**.

To stop or start the laser

1 Click **Stop Laser**. After the chamber begins flushing the button toggles to Start Laser.



2 Click **Start Laser** to start the laser at any time.

NOTE The laser will not start if a safety switch is tripped.

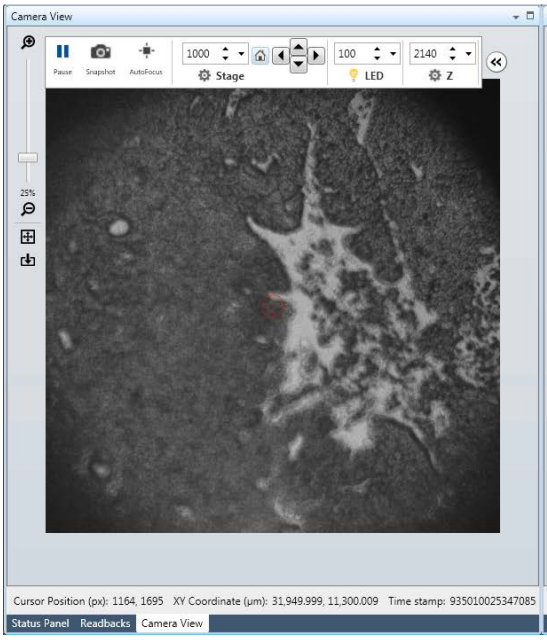
Use the Camera

Camera View provides the tools necessary to navigate and view the sample in the Hyperion Tissue Imager.

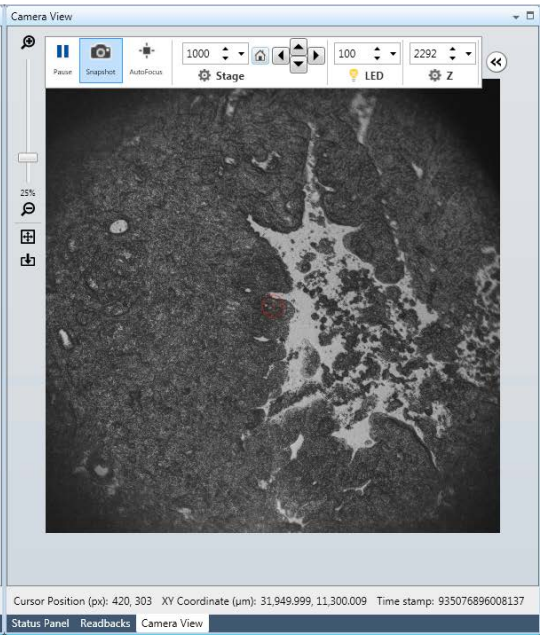
AutoFocus

If the image in Camera View is blurry, click **AutoFocus** to optimize the stage height and field brightness to bring the image into focus.

Before AutoFocus



After AutoFocus



Slide Navigation

Use the Stage controls to horizontally and vertically move the stage in increments to view the sample.

To move the stage

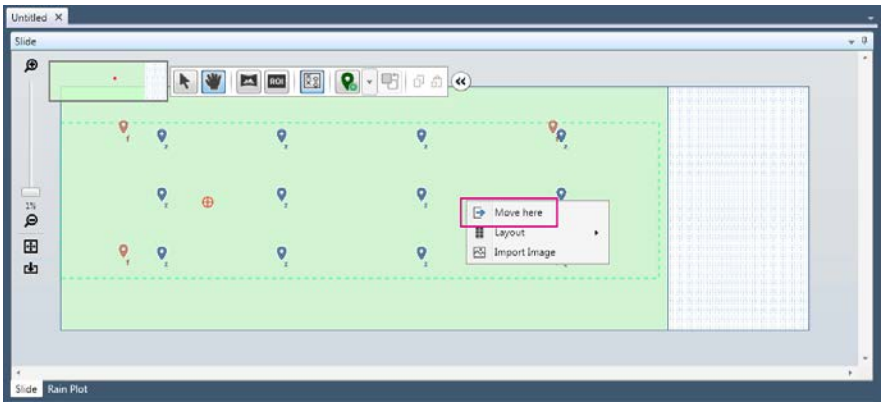
1. Specify the distance the stage will move with each click by entering a value in the Stage textbox. The default value is 1000 µm..
2. Click the directional controls to move the stage horizontally or vertically.

To move the stage to the Home position

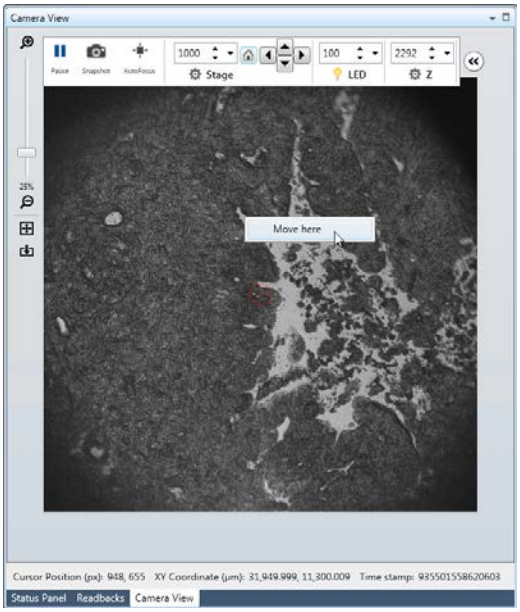
Click the Home ( ) button.

To move the camera to a specific location

1 Right-click on the **Slide Layout** and click **Move here.**



2 In the Camera View window, right-click a location, and then click **Move here**.



NOTE If the image becomes blurry, click **AutoFocus**.

Control Panel Overview (CyTOF Administrators only)

The Control Panel is available to CyTOF® Administrators. It contains five tabs:

- Switch Box
- Analog Controls
- Plasma
- General
- Devices

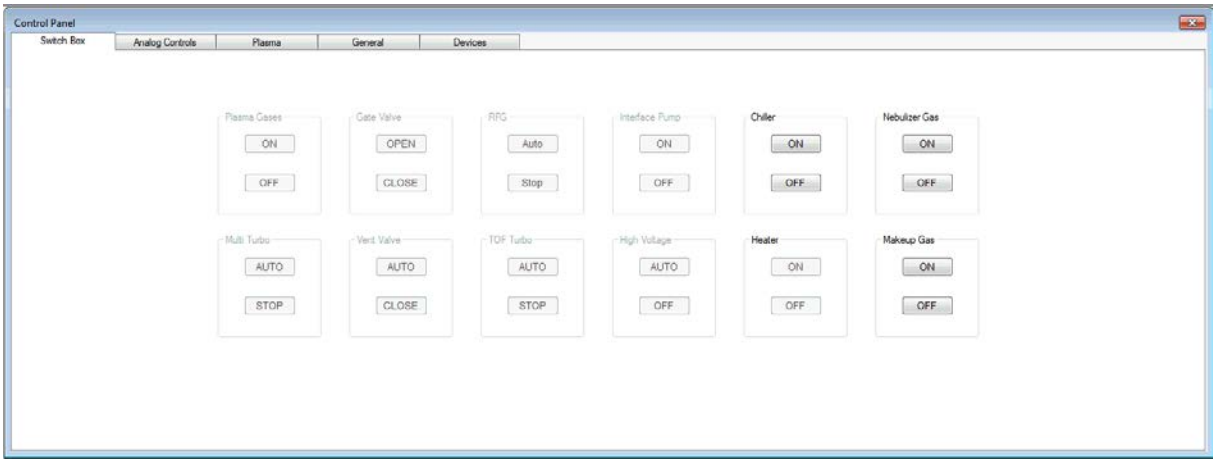
To open the Control Panel

On the ribbon, click the Instrument Control tab, and then click **Control Panel**.

Switch Box

On the Switch Box tab you can turn the following hardware components off or on:

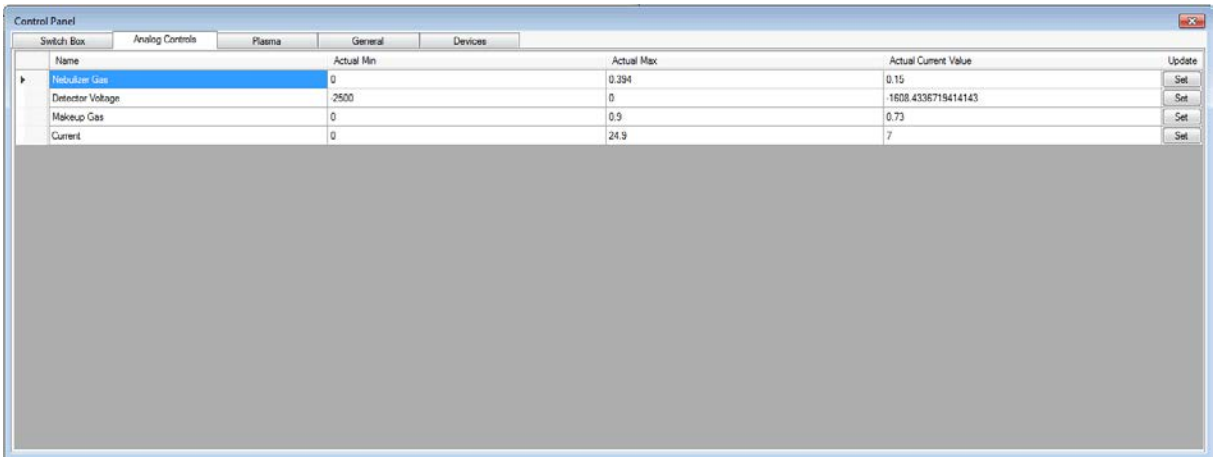
- Chiller
- Nebulizer Gas
- Makeup Gas



Analog Controls

On the Analog Controls tab you can see the operating range and update the default setting for the following parameters:

- Nebulizer Gas
- Detector Voltage
- Makeup Gas
- Current



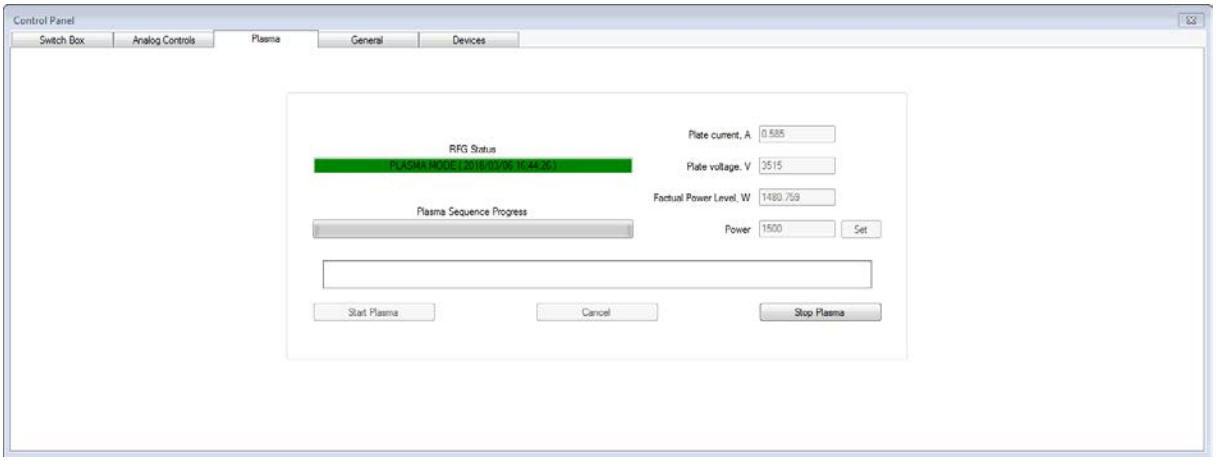
Actual Min and Actual Max define the valid operating range for each parameter. These values are fixed and cannot be changed. The Actual Current Value is the value presently applied for each parameter. The Actual Current Value can be modified if the Detector Voltage Optimization subcalibration or Gases/Current subcalibration fails. See If Detector Voltage Optimization Fails for more information.

To change the Actual Current Value

1. Under Actual Current Value, double-click the cell.
2. Enter a value within the acceptable range for the parameter (Actual Min–Actual Max).
3. Click **Set**.

Plasma

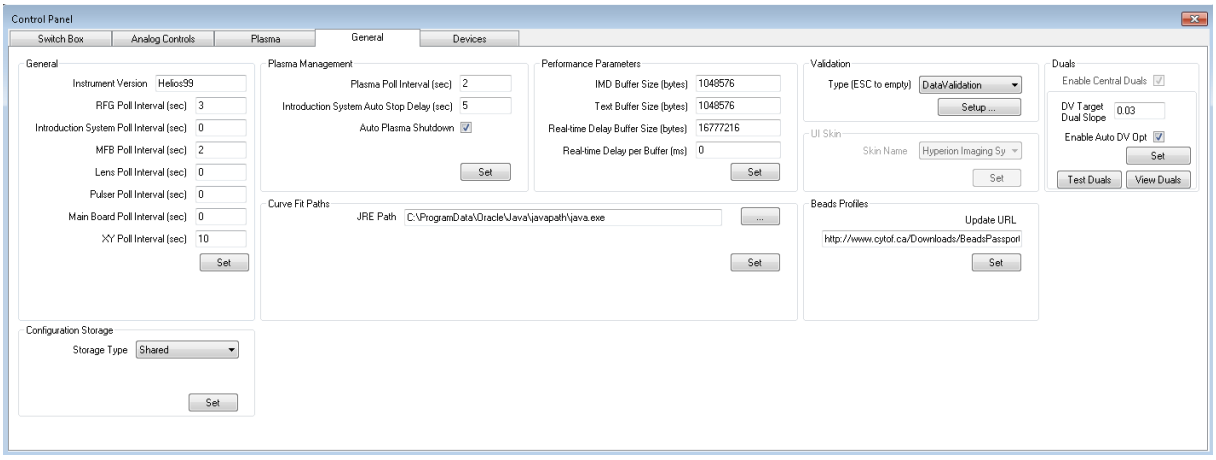
On the Plasma tab you can manually start plasma, stop plasma, and cancel the plasma start-up sequence.



General

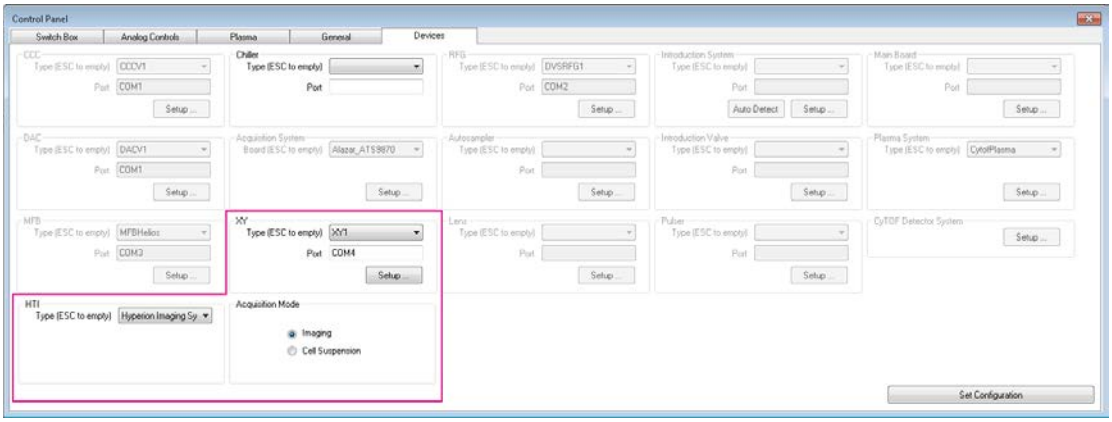
The settings on the General tab are not intended for typical workflow instrument usage. These settings are intended for troubleshooting and they should only be adjusted with instruction from Fluidigm technical support, a field application scientist, or a field service engineer.

IMPORTANT Some settings below may be instrument-dependent and may not reflect the settings on your instrument.



Devices

The Devices tab allows you to switch between cell suspension and imaging acquisition modes and configure your hardware accordingly. This section only describes settings that can be modified by CyTOF Administrators.



HTI

Displays **Hyperion Imaging System** to indicate the Hyperion Tissue Imager is connected and recognized.

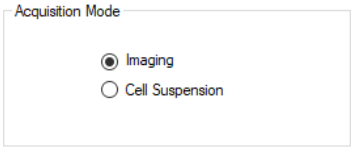
XY

The XY settings allow you to manually adjust the Helios™ torch alignment with the vacuum interface.

IMPORTANT These settings are not required for daily operation and should only be used when instructed by Fluidigm Service or Support for troubleshooting. To adjust XY alignment, run a tuning protocol that includes the XY subcalibration. For more information, see Create a Custom Tuning Protocol and Run a Tuning Protocol.

Acquisition Mode

Switch between imaging and cell suspension acquisition modes.

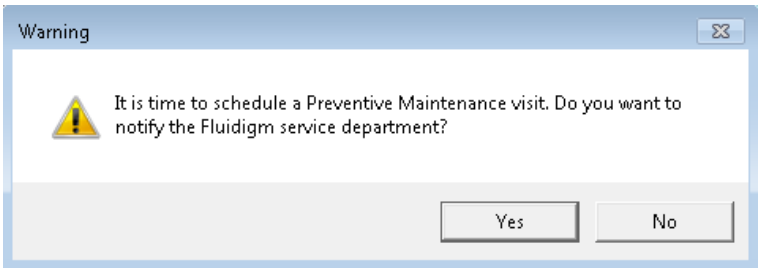


- If you are using Helios as a stand-alone instrument, click **Cell Suspension**, and then click **Set Configuration**.
- If you are using the Hyperion™ Imaging System, click **Imaging**, and then click **Set Configuration**.

NOTE See Switch Acquisition Modes for more information.

Preventive Maintenance Reminder

CyTOF Software monitors instrument usage and reminds you when it is time to schedule a Preventive Maintenance (PM) service call.



If your computer has internet access, click **Yes** to automatically send an email to Fluidigm Support to schedule a PM service call.

If your computer does not have internet access or the email was blocked by a firewall, click **Yes** to acknowledge the message and then visit techsupport.fluidigm.com to schedule a PM service call.

If you do not want to immediately schedule a service call, click **No** to set the notification to snooze. The notification is displayed again after the snooze interval has passed.

Switch Acquisition Modes (CyTOF Administrators only)

CyTOF Software supports two acquisition modes:

- Cell suspension, for data acquisition with Helios
- Imaging, for data acquisition with the Hyperion Imaging System

Each acquisition mode has a unique user interface.

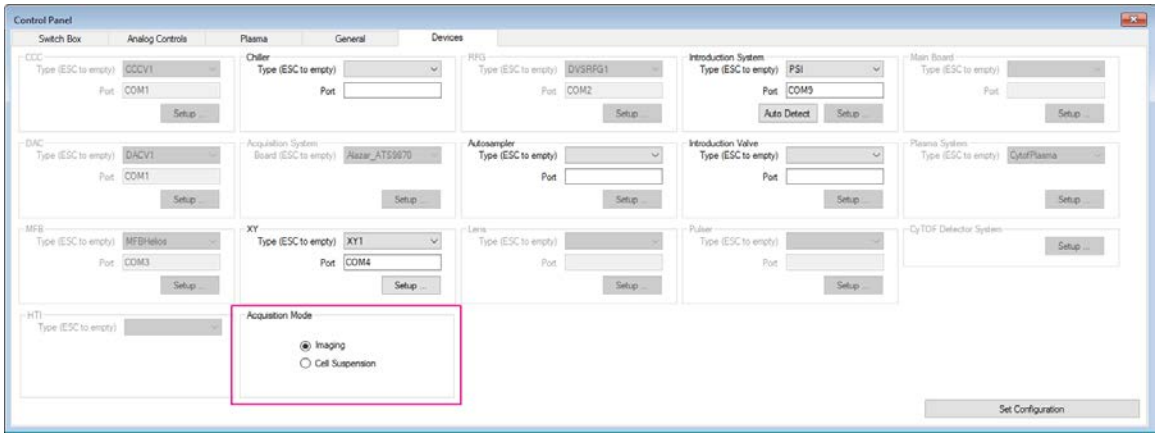
Switch from Cell Suspension to Imaging Mode

To switch from cell suspension to imaging mode

NOTE To acquire data in imaging mode, the Hyperion Imaging System must be configured. Connect the hardware according to the instructions in the **Hyperion Imaging System User Guide**.

1 On the Toolbar, under System, click **Control Panel**.

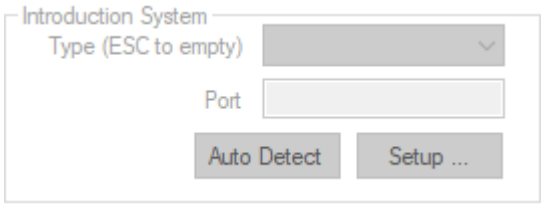
2 Click the **Devices** tab, and then under Acquisition Mode, click **Imaging**



3 Click **Set Configuration**. CyTOF Software restarts.

4 When prompted, log on to the software as an administrator. The Hyperion Imaging System user interface is displayed.

NOTE After the software restarts in Imaging mode, Introduction System (Control Panel > Devices) is empty and grayed out.



5 If the software does not automatically detect the Hyperion Imaging System:

a Close CyTOF Software.

b Check all cable connections.

c Confirm that the hardware is on.

d Open CyTOF software again. CyTOF Software should recognize the hardware.

Switch from Imaging to Cell Suspension Mode

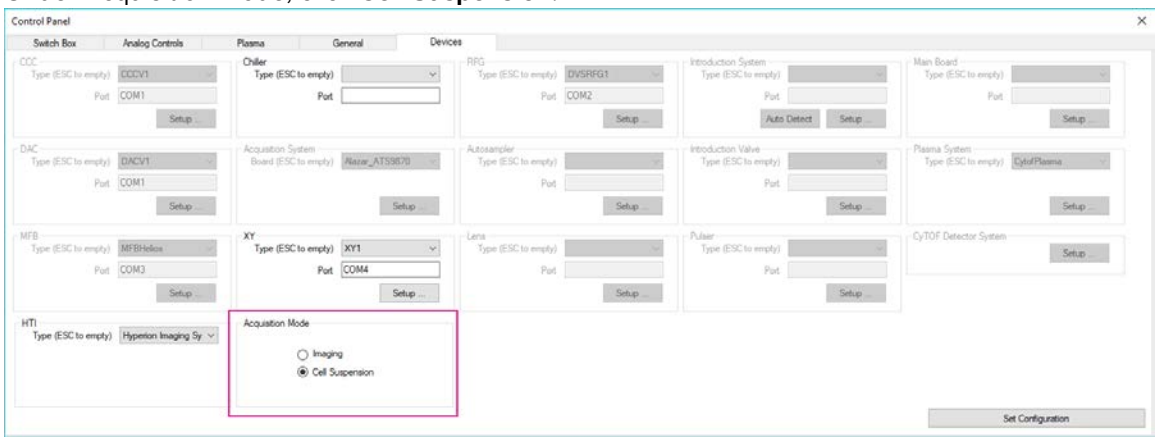
To switch from imaging mode to cell suspension mode

NOTE After the hardware is connected according the instructions in the Helios User Guide, configure the system in the software.

1 On the Instrument Control tab, click **Control Panel**.

2 On the Control Panel, click the **Devices** tab.

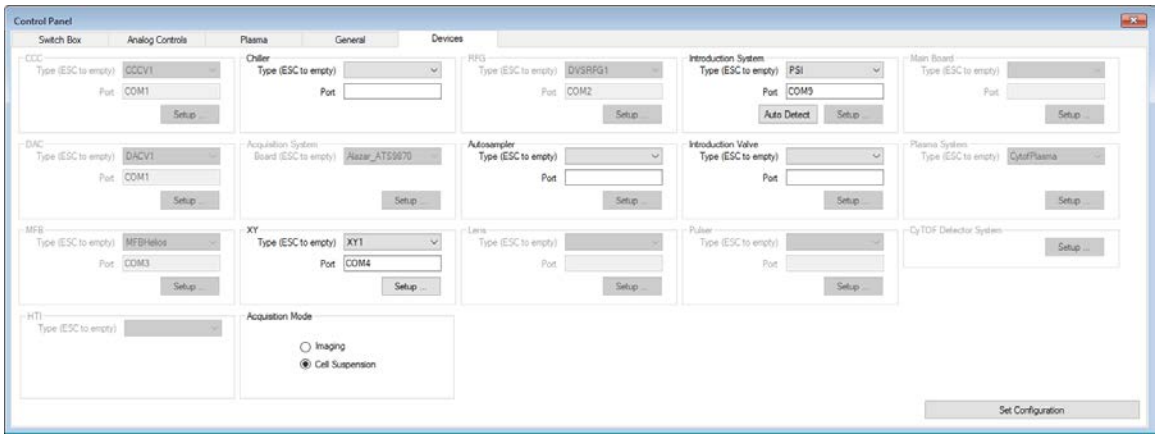
3 Under Acquisition Mode, click **Cell Suspension**.



4 Click **Set Configuration**. CyTOF Software restarts.

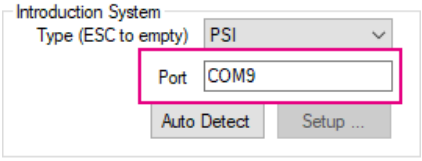
5 When prompted, log on to the software as an administrator. The Helios user interface is displayed.

6 On the Toolbar, under System, click **Control Panel**, and then click the **Devices** tab.



7 Under Acquisition Mode, confirm that Cell Suspension is selected.

8 Under Introduction System, click **Auto Detect**. If the COM port is not automatically set to COM9, manually enter it.



9 If the software does not automatically detect Helios and PSI (Sample Loader):

a Close CyTOF Software.

b Check all cable connections.

c Confirm that the hardware is on.

d Open CyTOF Software again.

e Repeat Step 8. The software should recognize the Sample Loader.

Tune the Instrument

About Instrument Tuning

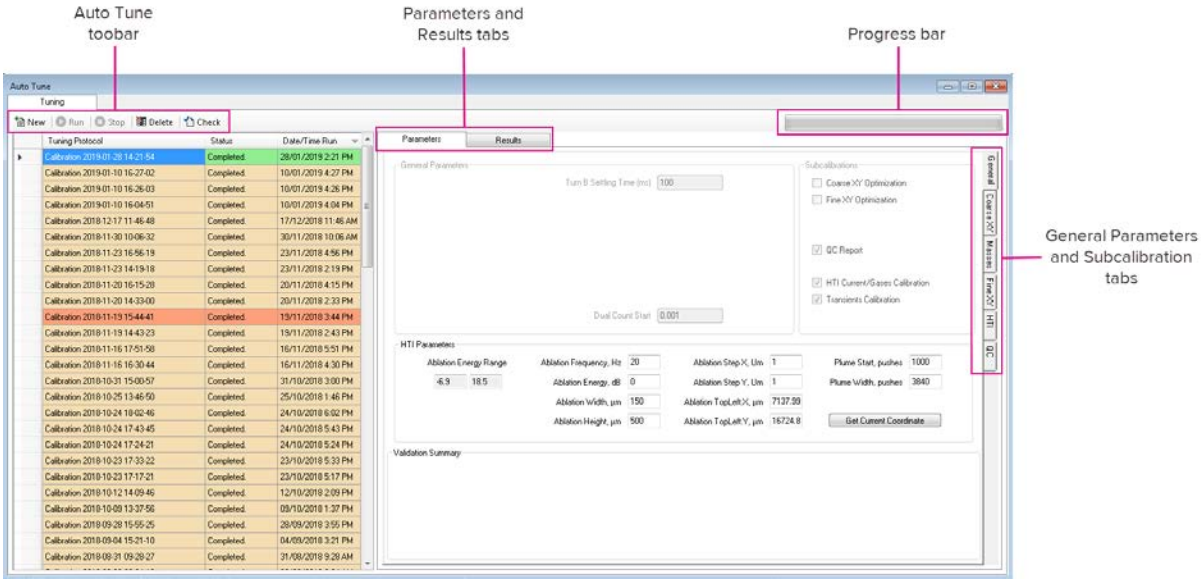
CyTOF® Software offers fully automated instrument tuning and calibration for the Hyperion™ Imaging System. Run Auto Tune daily to ensure optimal system performance.

For troubleshooting or to optimize an individual parameter, CyTOF Administrators can use Manual Tune.

Required Materials

- 3-Element Full Coverage Tuning Slide (PN 201088)

About Auto Tune



Subcalibrations

Auto Tune automatically tunes and calibrates the instrument by performing the subcalibrations chosen by the user. General parameters and parameters specific to each subcalibration are divided into tabs on the right side of the Auto Tune window.

Subcalibration	Description
Coarse XY Optimization	Optimizes the coarse alignment of the torch with the vacuum interface. **IMPORTANT** This subcalibration is only required when hardware maintenance is performed. It should not be run on a regular basis.
Fine XY Optimization	Optimizes the fine alignment of the torch with the vacuum interface to provide maximum signal during calibration. Optimal alignment is important for maximum transmission of ions into the vacuum interface.
QC Report	Generates a QC report, displayed on the Results tab, after the tuning protocol is completed.
HTI Current/Gases Calibration	Optimizes current and makeup gas flow for optimal ion transmission.
Transients Calibration	Minimizes crosstalk by optimizing the helium flow rate.

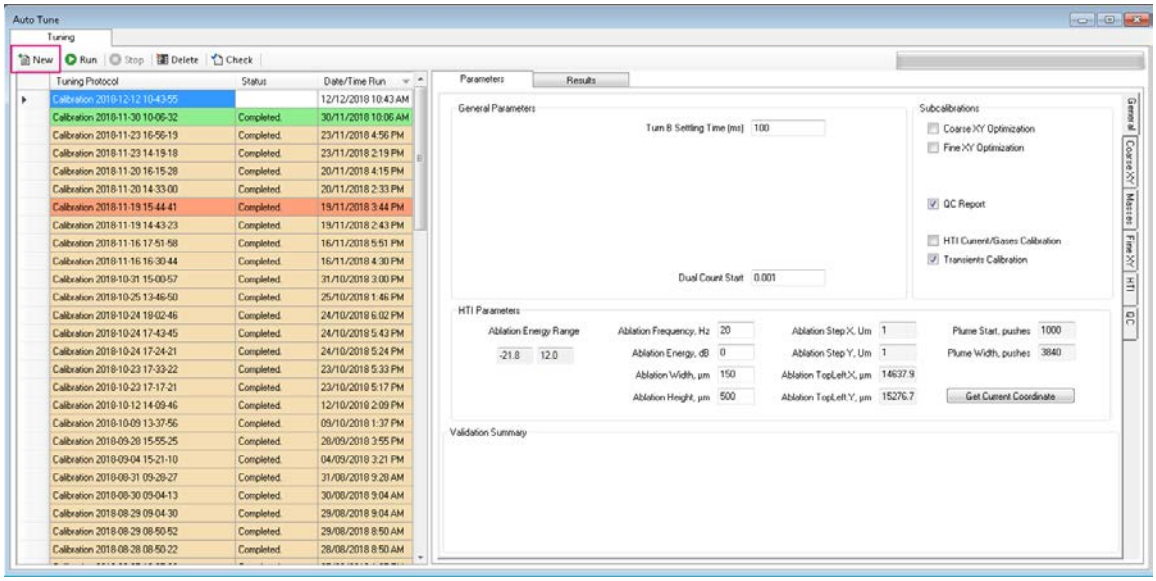
Run Auto Tune

After the system is started and warmed up, run Auto Tune to optimize system performance.

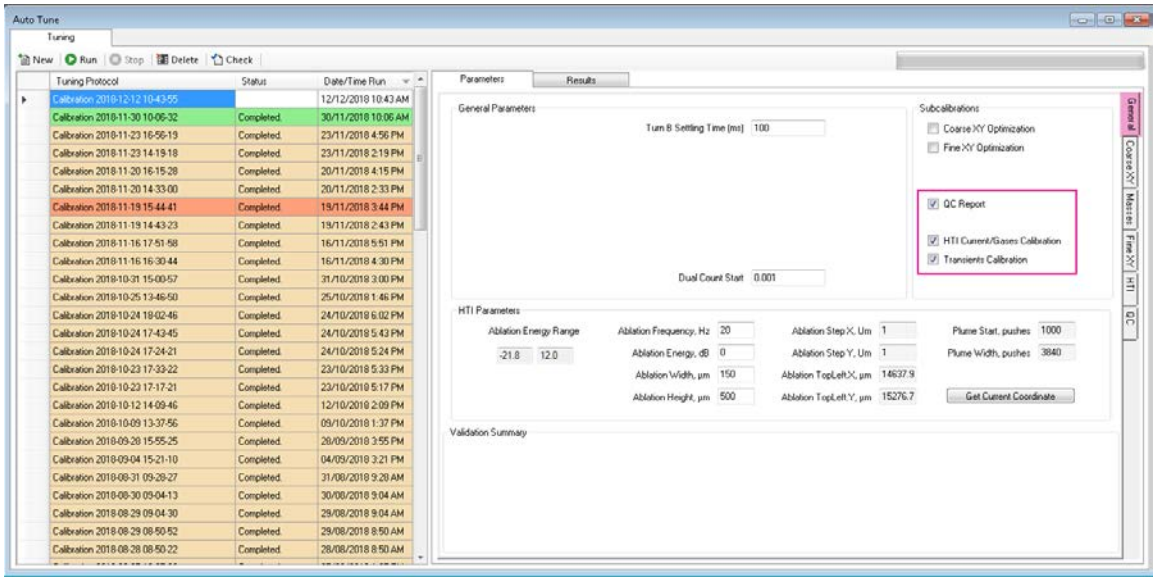
NOTE Load a 3-Element Full Coverage Tuning Slide (PN 201088) before you begin. See Unload and Load a Sample.

1 On the Instrument Control tab, click **Auto Tune**.

2 Click **New**. A new row with a date and time stamp is added to the top of the Tuning Protocol table.



3 On the General tab, under Subcalibrations, check **QC Report**, **Enable HTI Current/Gases Calibration,** and **Transients Calibration**.

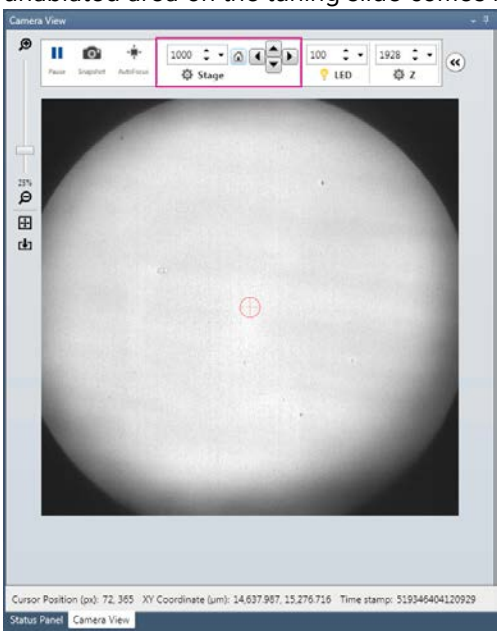


NOTE Include Fine XY Optimization if low sensitivity is consistently observed. Include Coarse XY Optimization and Fine XY Optimization if:

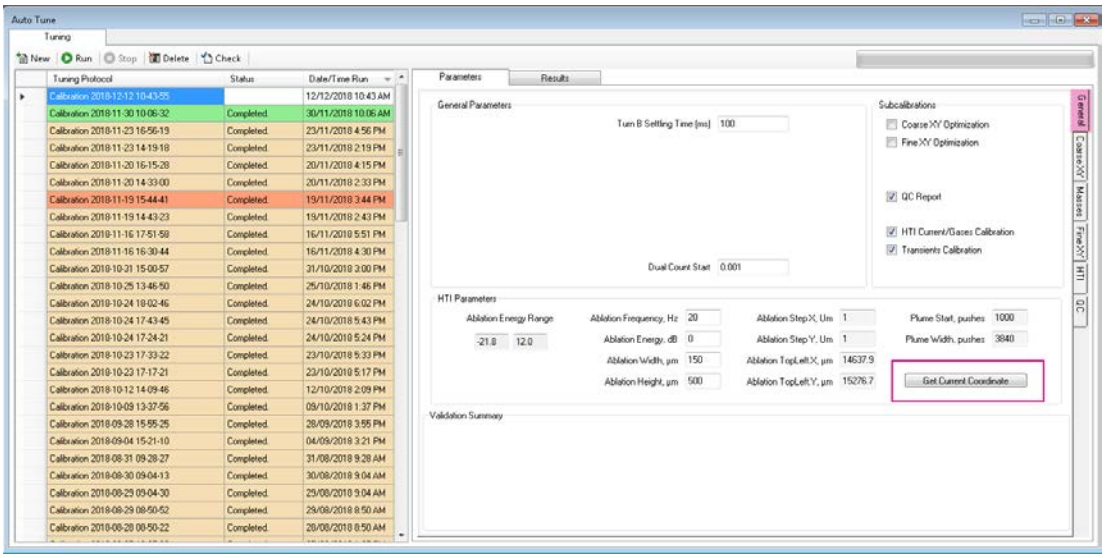
- the acquisition mode is switched from Cell Suspension to Imaging
- the injector and coupling have been removed and replaced
- consistently low signal that may be improved by Helios XY realignment

4 Find an unablated area of tuning film to ablate while tuning.

a In the Camera View window, use the Stage controls to move the stage until a clean, unablated area on the tuning slide comes into view.



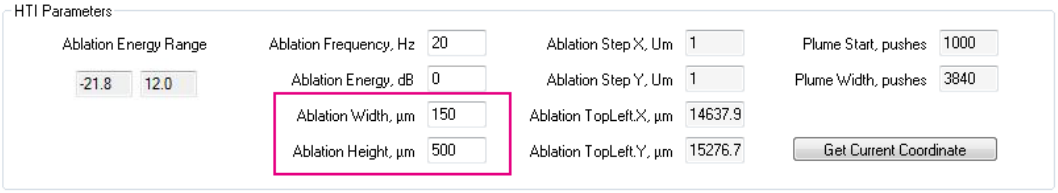
b In the Auto Tune window, under HTI Parameters, click **Get Current Coordinates**.



c Specify the ROI dimensions:

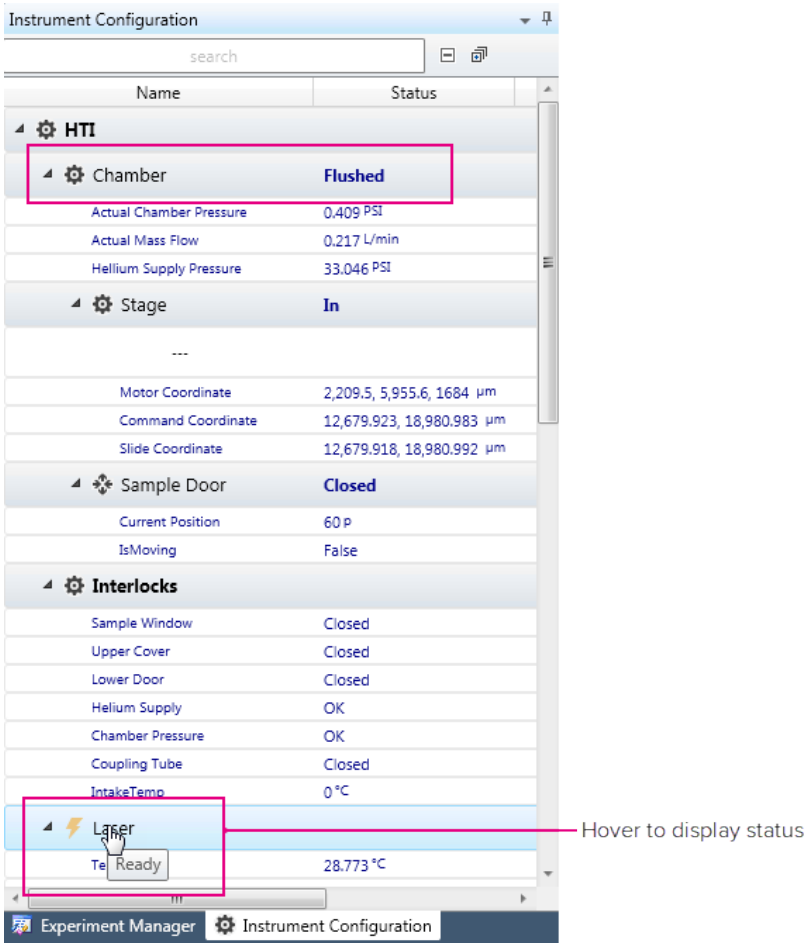
- In the Ablation Width, µm text box, enter 150.
- In the Ablation Height, µm text box, enter 500.

The dimensions for ablation should be at least 150 µm x 500 µm to ensure that the area is large enough to accommodate all of the subcalibrations in the tuning protocol.

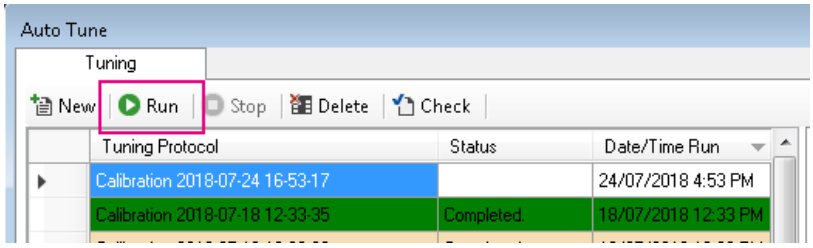


NOTE Use the default Ablation Frequency value of 20 Hz for the best resolution of single-element transients. The Ablation Energy required to effectively ablate tuning film is 0 dB. If 0 dB does not effectively ablate tuning film, optimize the reference energy offset (CyTOF Administrators only). See Recalibrate the Reference Energy Offset.

5 In the Instrument Configuration tab, verify that the Chamber status is Flushed and the Laser status is Ready.

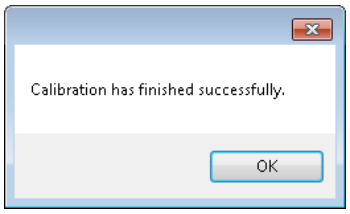


6 In the Auto Tune window, click **Run**.

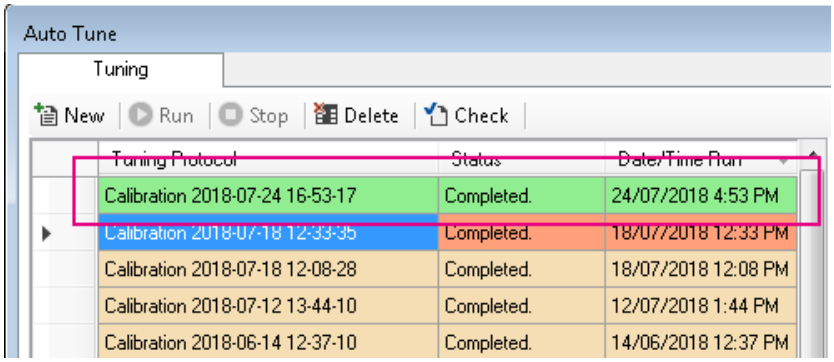


NOTE The entire Auto Tuning (with the two selected Subcalibrations) takes approximately 10 minutes. A progress bar provides a visual indication of the time remaining until completion.

7 Click **OK** in the message box that displays after calibration has finished successfully.



The Tuning Protocol status changes to Completed and the row turns green.



8 Click the **Results** tab to view the results of the tuning.

The pass criteria for Auto Tune are:

- Resolution (Mass 1): >400
- Transients Cross Talk 1: <0.15
- Transients Cross Talk 2: <0.05
- Mean Duals for ^{175}Lu, per laser shot: >500

NOTE If the instrument was switched from Cell Suspension mode to Imaging mode, the mean dual count for 75Lu may be 80% of what they were before to switching to Cell Suspension mode.

IMPORTANT If Auto Tune fails, refer to the Hyperion Imaging System User Guide (400311) for troubleshooting tips.

About Manual Tune (CyTOF Administrators only)

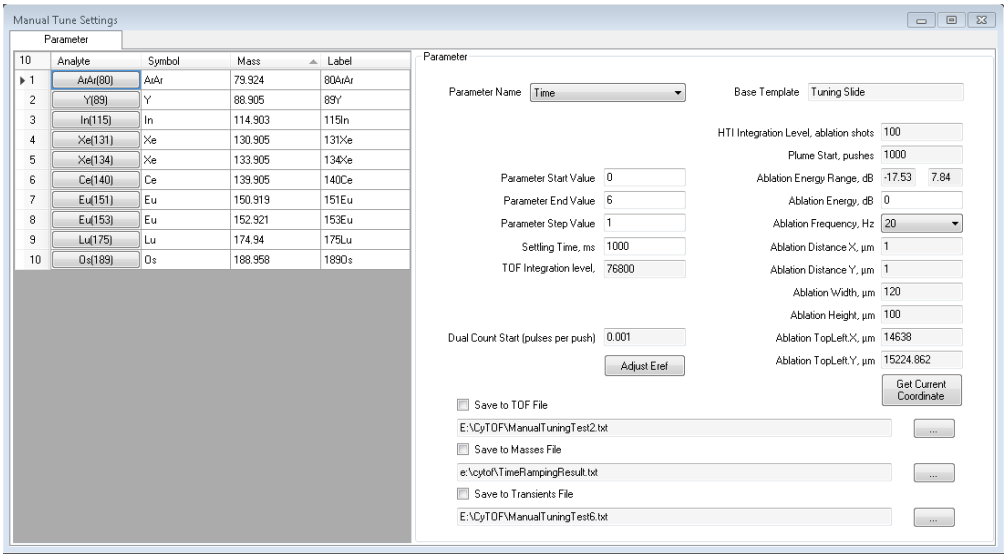
CyTOF Administrators can optimize individual parameters using Manual Tune. Procedures to optimize the following parameters are provided:

- Ablation Energy
- XY (Torch Alignment)
- Helium Mass Flow
- Current
- Makeup Gas

Additional parameters should only be modified with the guidance of Fluidigm technical support,

Manual Tune Settings

The Manual Tune Settings window allows you to manually ramp individual parameters over a specified range in order to determine the value that produces the best signal.



Choose from the following recommended parameters in the Parameter Name list:

- Hyperion Tissue Imager parameters
  - Helium Mass Flow
  - Ablation Energy
  - Z
- DAC parameters
  - Current

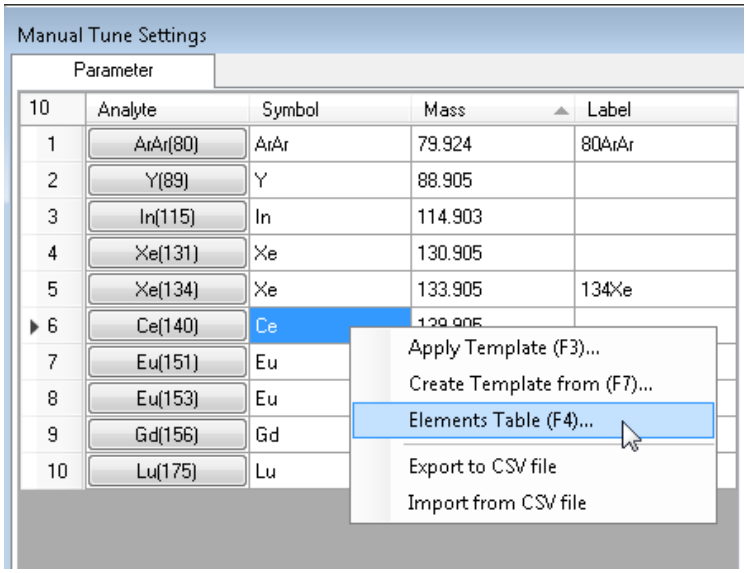
  - Detector Voltage
  - Makeup Gas
  - Nebulizer Gas
- XY parameters
  - X
  - Y

Specify Tuning Elements

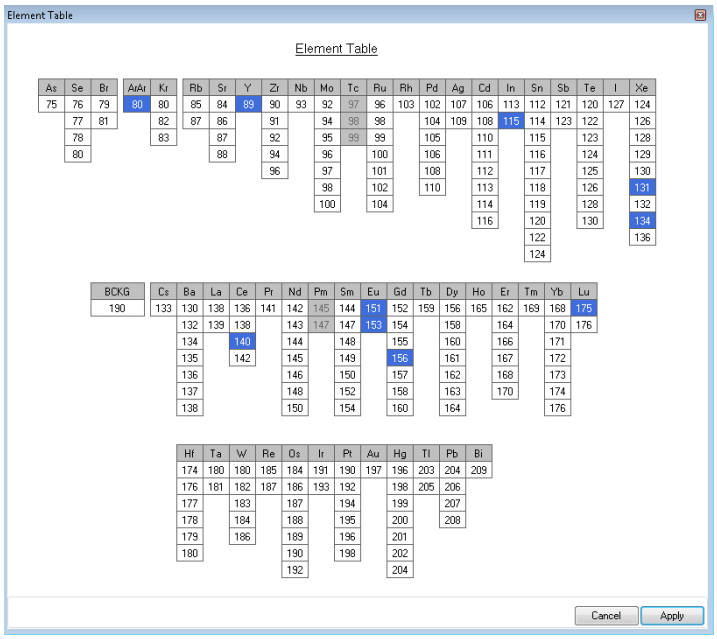
Elements in the Analyte table are scanned at each step within the scanned range for the chosen parameter.

To include or exclude any element in the Elements Table

1 Right-click on the table and click **Elements Table (F4)...**



2 Click an element to select (blue) or deselect (white) and then click **Apply**.



Optimize Parameters

Optimize Helium Mass Flow (CyTOF Administrators only)

Optimize helium flow by ramping the Helium Mass Flow parameter across a range of values. Dual count measurements for ^{175}Lu are taken across the range at intervals defined by the Parameter Step Value. At each step, a corresponding transient curve is generated and displayed. Each curve represents the dual count signal of ^{175}Lu at a particular flow rate. The optimal Helium Mass Flow value is the value that produces ^{175}Lu with the highest intensity and with minimal cross talk.

NOTE Load a 3-Element Full Coverage Tuning Slide (PN 201088) before you begin. See Unload and Load a Sample.

1 On the ribbon, click the **Manual Tune** tab, and then click **Settings**.

2 In the Manual Tune Settings window, in the Parameter Name list, click **HTI → Helium Mass Flow**.

3 Enter the following values:

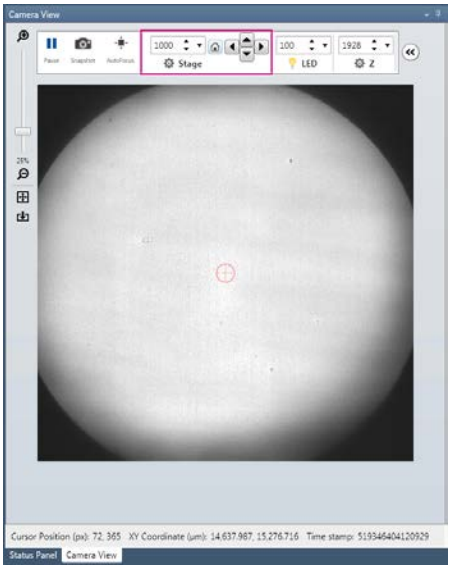
Setting	Value
Parameter Start	0.1
Parameter Stop	0.25

Setting	Value
Step Value	0.01
Settling Time, ms	10000
Ablation Energy, dB	0 **NOTE** If 0 dB is insufficient, see Recalibrate the Reference Energy Offset
Ablation Frequency, Hz	20
Ablation Distance X, µm	1
Ablation Distance, Y µm	1

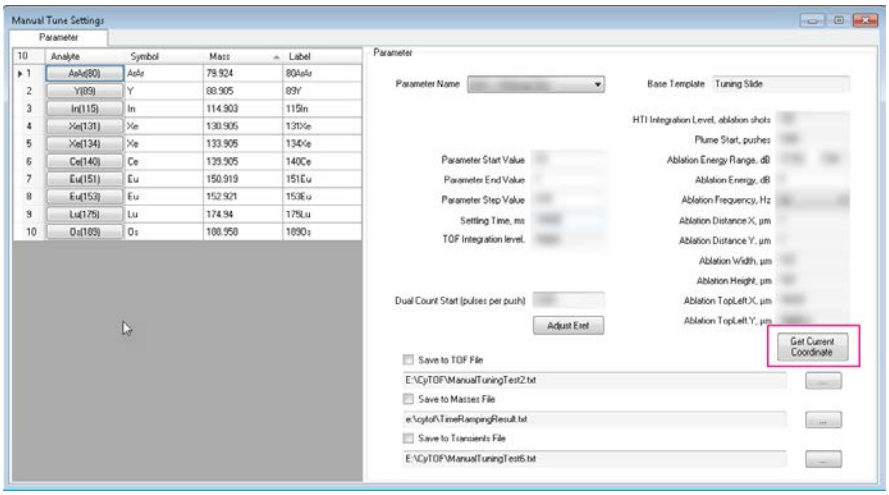
4 Verify that the following elements are in the Analyte table: Y(89), Ce(140), Lu(175). If they are not, right-click the Analyte table and then click **Elements Table (F4)...** to add them.

5 Set the ablation area.

a In the Camera View window, use the Stage controls to move to a clean, unablated area on the tuning slide.



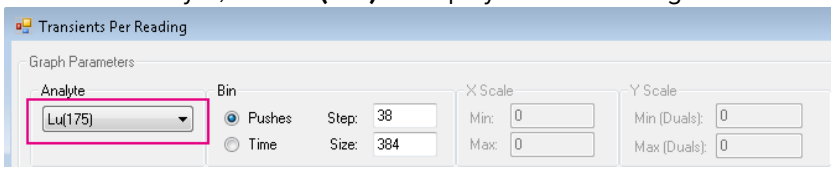
b On the Manual Tune Settings window, click **Get Current Coordinates**.



c Enter Ablation Width and Ablation Height values large enough to accommodate the number of measurements. For the range and step size used in this procedure, 200 x 500 µm is sufficient.

NOTE A wider range or a smaller step size increases the number of measurements taken. A large area may be required to complete all of the measurements.

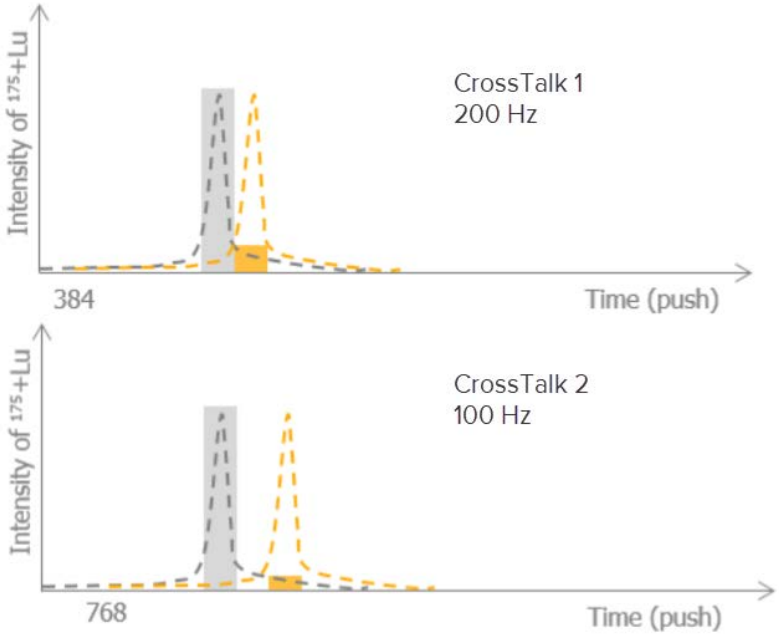
6 On the Manual Tune toolbar, click **Transient** to open the Transients Per Reading window and under Analyte, click **Lu(175)** to display the lutetium signal measured at each step.



7 Click **Run**.

8 Identify the helium flow value that corresponds to the maximum lutetium signal observed with low values for CrossTalk 1 (<15%) and CrossTalk 2 (<5%). Right-click to select the row and click **Select Value**.

NOTE CrossTalk 1 and CrossTalk 2 values represent the estimated overlap from one plume to the next at ablation frequencies of 200 Hz and 100 Hz, respectively.



9 Click **OK** in the message that is displayed to indicate Helium Mass Flow is successfully set to the selected value.

NOTE If transient intensities are low, check that the coupling tubing is correctly connected to the ablation chamber and aligned. See the Hyperion Imaging System User Guide for more information.

Optimize Makeup Gas (CyTOF Administrators only)

Optimize makeup gas flow by ramping the Makeup Gas parameter across a range of values. Dual count measurements for ^{175}Lu are taken across the range at intervals defined by the Parameter Step Value. At each step, a corresponding transient curve is generated and displayed. Each curve represents the dual count signal of ^{175}Lu at a particular flow rate. The optimal Helium Mass Flow value is the value that produces ^{175}Lu with the highest intensity and with minimal cross talk.

NOTE Load a 3-Element Full Coverage Tuning Slide (PN 201088) before you begin. See Unload and Load a Sample.

1 On the ribbon, click the **Manual Tune** tab, and then click **Settings**.

2 In the Manual Tune Settings window, in the Parameter Name list, click **HTI → Makeup Gas**.

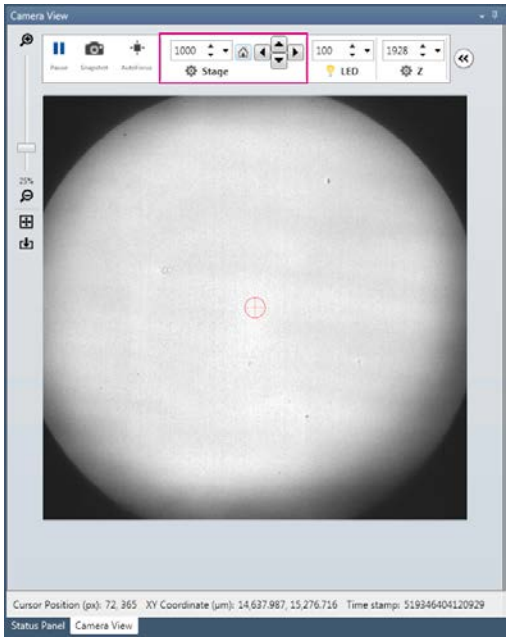
3 Enter the following values:

Setting	Value
Parameter Start	0.5
Parameter Stop	1.0
Step Value	0.05
Settling Time, ms	10000
Ablation Energy, dB	0 NOTE If 0 dB is insufficient, see Recalibrate the Reference Energy Offset
Ablation Frequency, Hz	20
Ablation Distance X, µm	1
Ablation Distance, Y µm	1

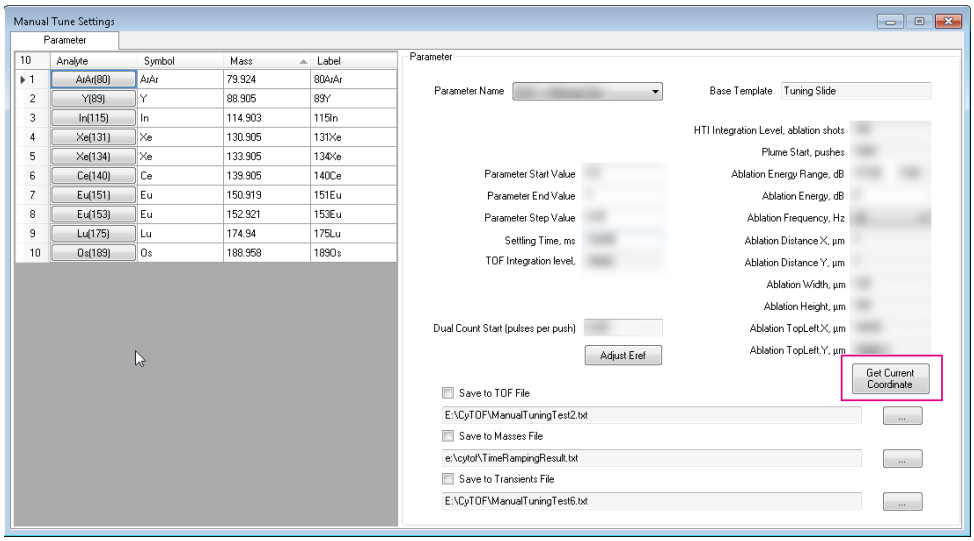
4 Verify that the following elements are in the Analyte table: Y(89), Ce(140), Lu(175). If they are not, right-click on the Analyte table and then click **Elements Table (F4)...** to add them.

5 Set the ablation area.

a In the Camera View window, use the Stage controls to move to a clean, unablated area on the tuning slide.



b On the Manual Tune Settings window, click **Get Current Coordinates**.



c Enter Ablation Width and Ablation Height values large enough to accommodate the number of measurements. For the range and step size used in this procedure, 200 x 500 µm is sufficient.

NOTE A wider range or a smaller step size increases the number of measurements taken. A large area may be required to complete all of the measurements.

6 Display the Transient and Channels plots.

On the Manual Tune toolbar, in the Plots group, click:

- **Transient** to open the Transients Per Reading window. In the Transients Per Reading window, under Analyte, click **Lu(175)**.
- **Channels** to open Masses Per Reading window.

7 Click **Run**.

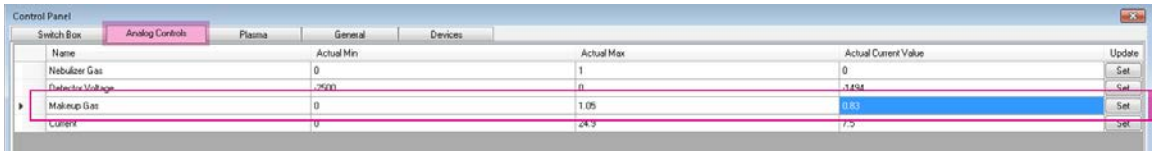
8 For Makeup Gas, record the value that corresponds to the highest Lu(175) response in the Masses Per Reading window.

9 Subtract 0.1 from this value and enter the new value in Control Panel > Analog Controls.

a On the ribbon, click the **Instrument Control** tab. Click **Control Panel**, and then click **Analog Controls**.

b In the Makeup Gas row, enter the new value in the Actual Current Value column. Click **Set**.

c Close Control Panel. When prompted to save the changes, click **Yes**.



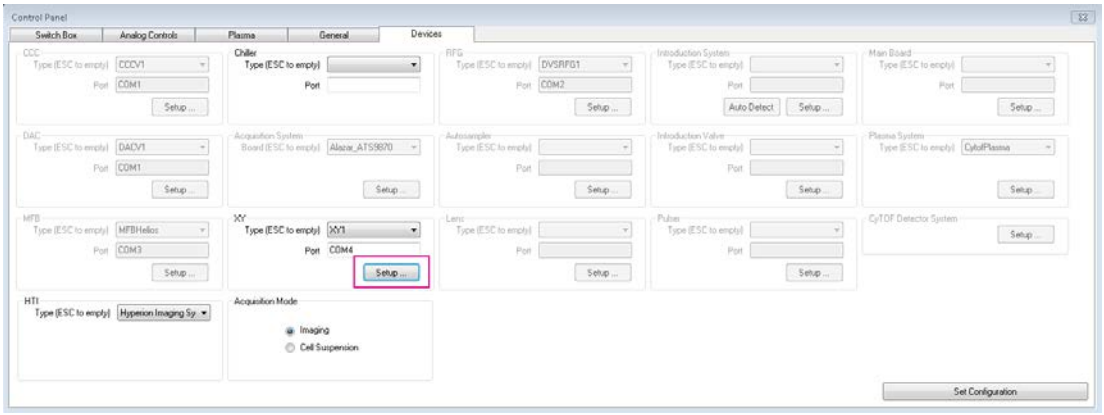
Optimize Torch Alignment—XY (CyTOF Administrators only)

NOTE Load a 3-Element Full Coverage Tuning Slide (PN 201088) before you begin. See Unload and Load a Sample.

1 Open the Control Panel and record the X and Y values to determine an appropriate scan range.

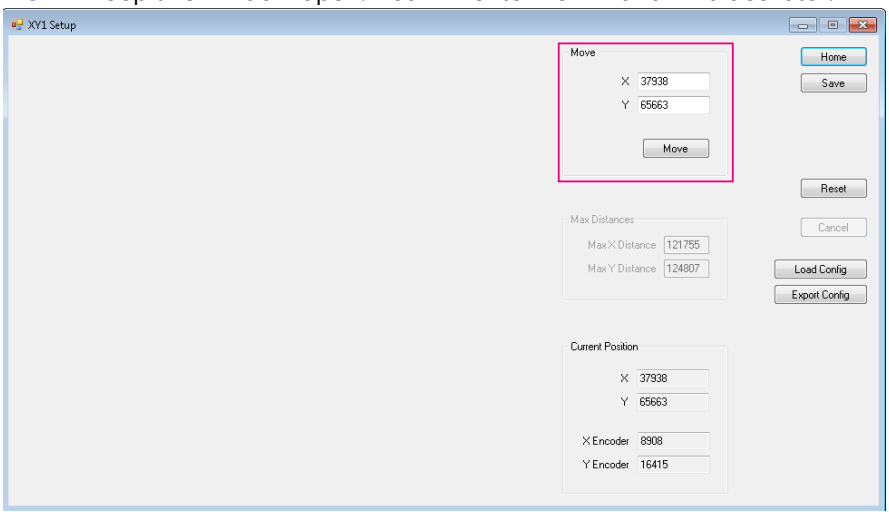
   a On the ribbon, click the **Instrument Control** tab, and then click **Control Panel**.

   b In Control Panel, click the **Devices** tab, and under XY, click **Setup**.



   c On the XY1 Setup window, under Move, record the X and Y values.

   NOTE Keep this window open. You will enter new X and Y values later.



2 On the ribbon, click the **Manual Tune** tab, and then click **Settings**.

3 In the Manual Tune Settings window, Under Parameter Name, click **XY → Y** .

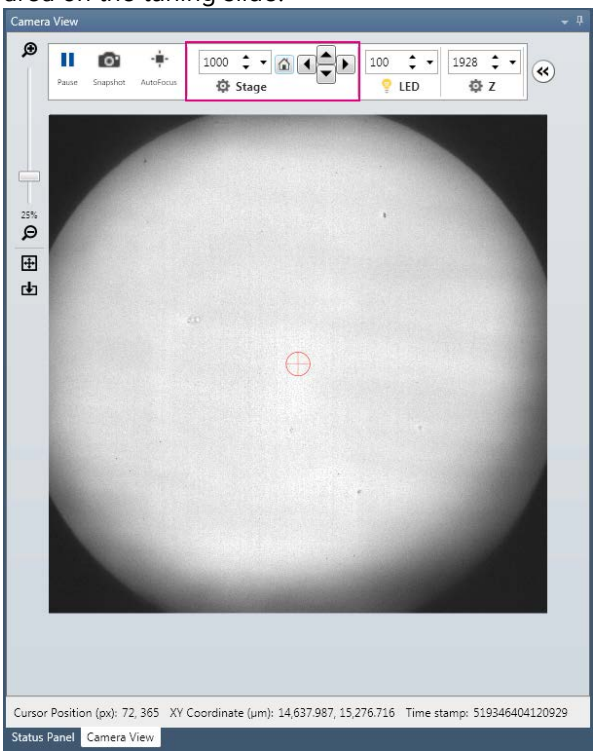
4 Enter the following values. Use the Y value recorded in Step 1 to determine Parameter Start and Parameter Stop values.

Setting	Value
Parameter Start	Y value – 20,000
Parameter Stop	Y value + 20,000
Step Value	5000
Settling Time, ms	5000
Ablation Energy, dB	0 NOTE If 0 dB is insufficient, see Recalibrate the Reference Energy Offset
Ablation Frequency, Hz	20
Ablation Distance X, µm	1
Ablation Distance, Y µm	1

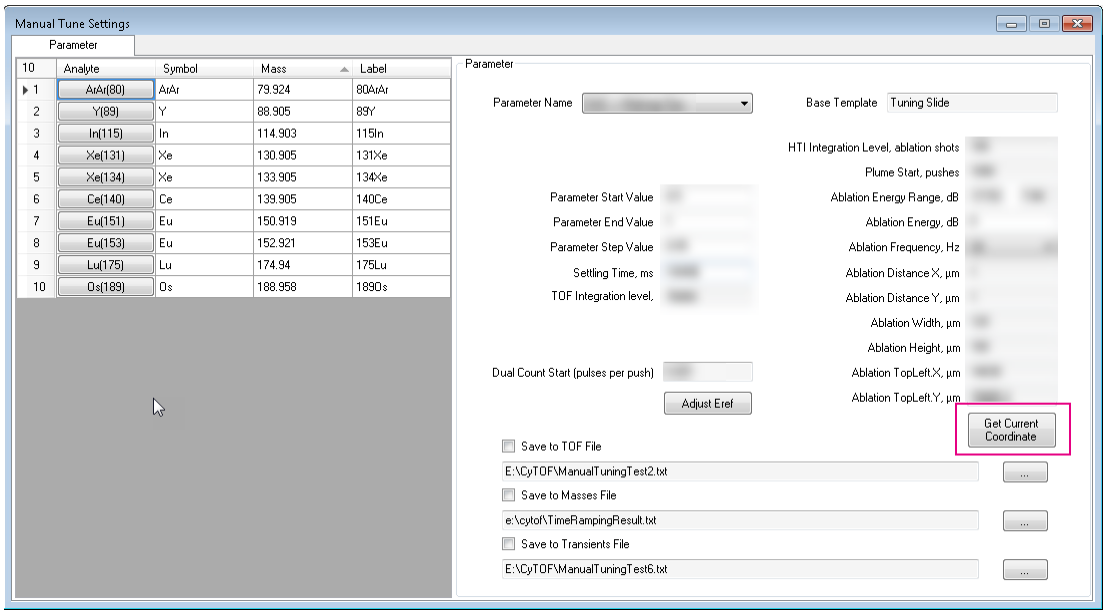
5 Verify that the following elements are in the Analyte table: Y(89), Ce(140), Lu(175). If they are not, right-click the Analyte table and then click **Elements Table (F4)...** to add them.

6 Set the ablation area.

a In the Camera View window, use the Stage controls to move to a clean, unablated area on the tuning slide.



b On the Manual Tune Settings window, click **Get Current Coordinates**.



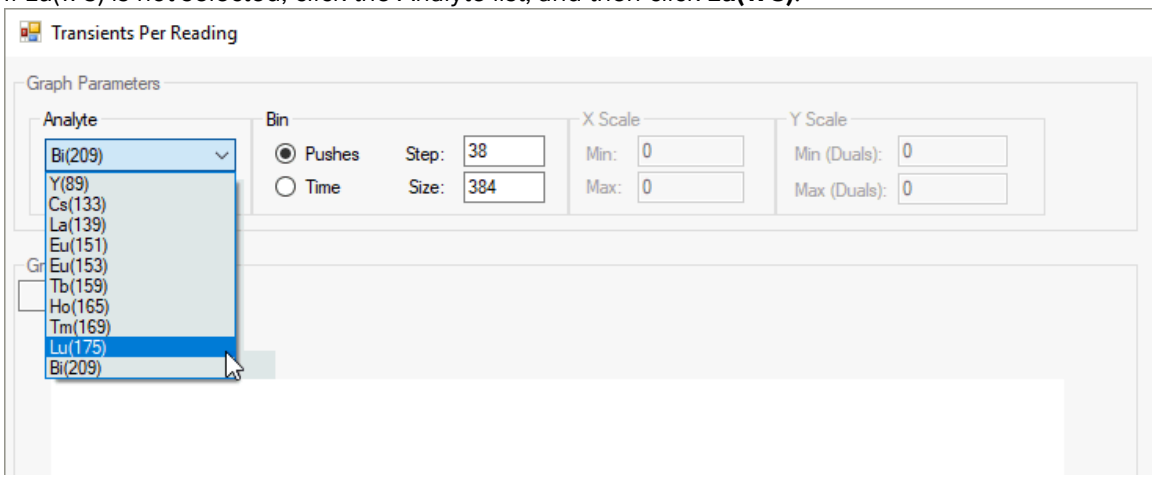
c Enter Ablation Width and Ablation Height values large enough to accommodate the number of measurements. For the range and step size used in this procedure, 200 x 500 µm is sufficient.

NOTE A wider range or a smaller step size increases the number of measurements taken. A large area may be required to complete all of the measurements.

7 On the Manual Tune toolbar, click **Run**.

8 Click **Transients** to view real-time transient signals.

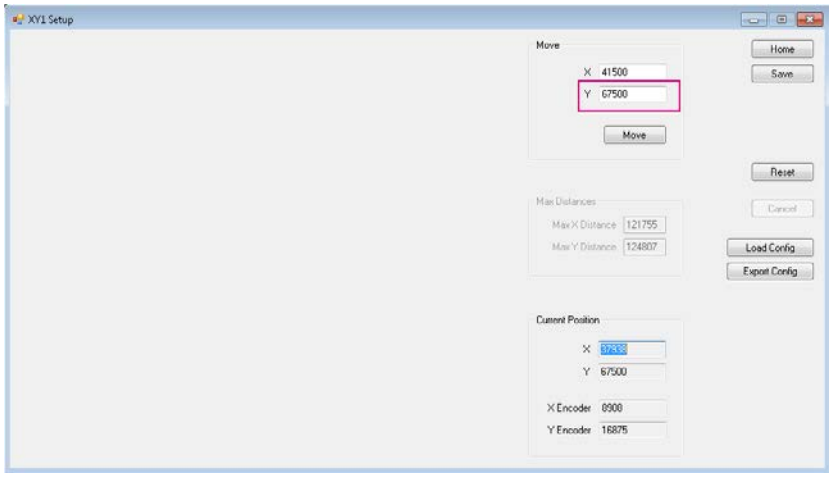
9 If Lu(175) is not selected, click the Analyte list, and then click **Lu(175)**.



NOTE Transient peaks are displayed in the Transients Per Reading window after ions reach the detector.

10 Ablation stops after all measurements are taken. On the Transients Per Reading window, identify the value that provides the most intense Lu(175) peak.

11 Narrow the scan range using this value ±5,000 (enter new start and stop values). Decrease the step size to 500 and start scanning. Open the Transients Per Reading window. After scanning is complete, identify the Y value that produces the most intense Lu(175) signal and enter it into the Y text box in the XY1 Setup window. Click **Move** to reposition the torch.



12 Repeat Steps 1–11 for the X position. In the Manual Tune Settings window, under Parameter Name click **XY → X** from the drop-down. Verify that the most intense signal for Lu is >100,000. Enter the X value that produces the most intense signal into the X text box on the XY1 Setup window and click **Move**.

IMPORTANT After the X position is optimized, repeat this procedure for optimal alignment.

Optimize Current (CyTOF Administrators only)

IMPORTANT Perform this procedure only when a 175Lu signal >100,000 cps cannot be achieved. This procedure optimizes the system sensitivity.

NOTE Load a 3-Element Full Coverage Tuning Slide (PN 201088) before you begin. See Unload and Load a Sample.

1 On the ribbon, click the **Manual Tune** tab, and then click **Settings**.

2 In the Manual Tune Settings window, in the Parameter Name list, click **DAC → Current**.

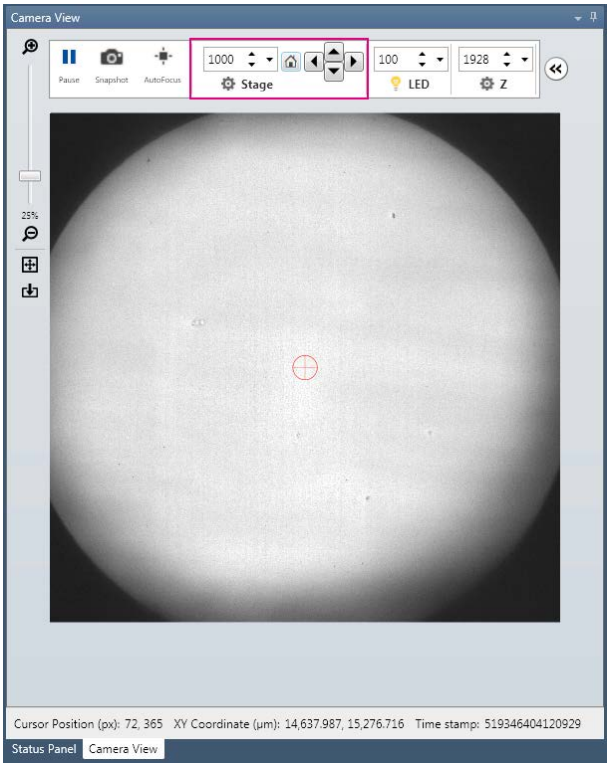
3 Enter the following values:

Setting	Value
Parameter Start	0
Parameter Stop	10
Step Value	0.5
Settling Time, ms	20
Ablation Energy, dB	0 NOTE If 0 dB is insufficient, see Recalibrate the Reference Energy Offset
Ablation Frequency, Hz	20
Ablation Distance X, µm	1
Ablation Distance, Y µm	1

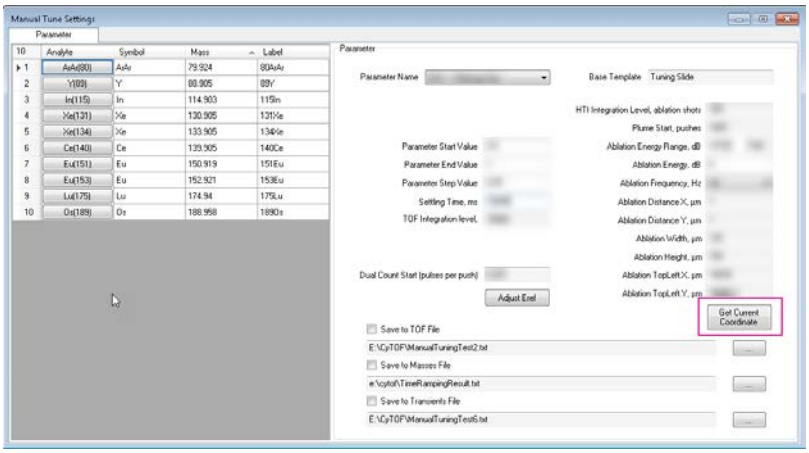
4 Verify that the following elements are in the Analyte table: Y(89), Ce(140), Lu(175). If they are not, right-click the Analyte table and then click **Elements Table (F4)...** to add them.

5 Set the ablation area.

a In the Camera View window, use the Stage controls to move to a clean, unablated area on the tuning slide.



b On the Manual Tune Settings window, click **Get Current Coordinates**.



c Enter Ablation Width and Ablation Height values large enough to accommodate the number of measurements. For the range and step size used in this procedure, 200 x 500 µm is sufficient.

NOTE A wider range or a smaller step size increases the number of measurements taken. A large area may be required to complete all of the measurements.

6 Display the Transient and Channels plots.

On the Manual Tune toolbar, in the Plots group, click:

- **Transient** to open the Transients Per Reading window. In the Transients Per Reading window, under Analyte, click **Lu(175)**.
- **Channels** to open Masses Per Reading window.

7 Click **Run**. After the data for each step within the range is acquired the transient curve appears in the Transients Per Reading window.

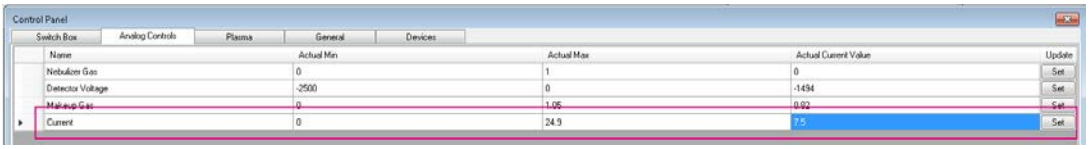
8 Record the value for Current that corresponds to the highest Lu(175) response in the Masses Per Reading window.

9 Enter the new value in Control Panel > Analog Controls.

a On the ribbon, click the **Instrument Control** tab. Click **Control Panel**, and then click **Analog Controls**.

b In the Current row, enter the value recorded in Step 8 in the Actual Current Value column. Click **Set**.

c Close Control Panel. When prompted to save the changes, click **Yes**.



NOTE If the Lu(175) Maximum is <50,000 then perform the Optimize XY procedure.

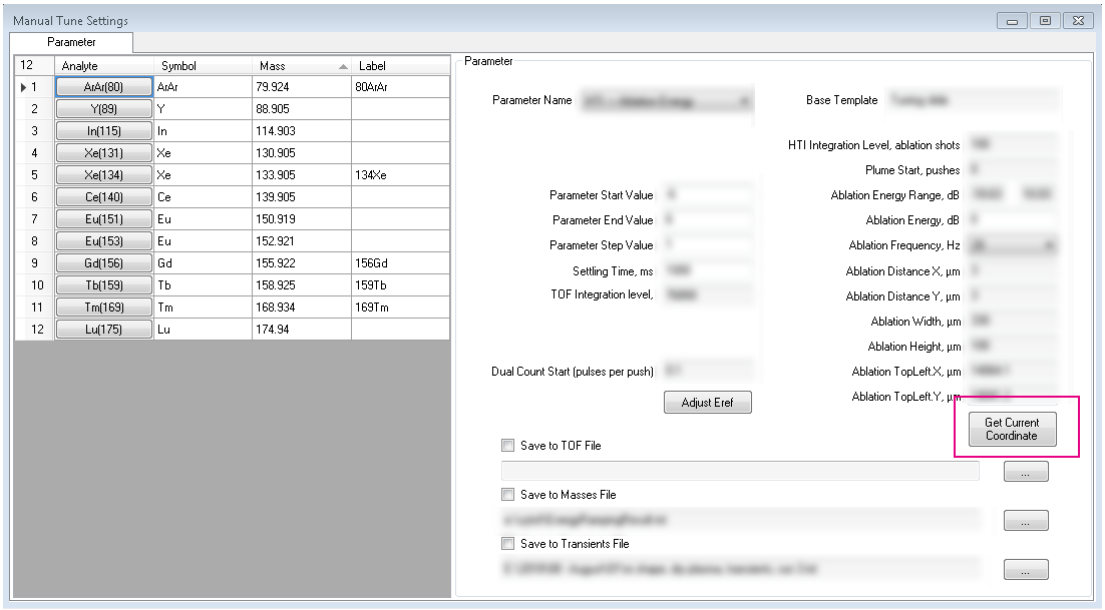
Recalibrate the Reference Energy Offset

The ablation energy required to effectively ablate tuning film with a new laser is 0 dB. Over time, and at a rate dependent on laser usage, the amount of energy required to ablate tuning film (and samples) increases. Recalibrate the reference energy offset to restore 0 dB as the energy required to ablate tuning film and eliminate the need to update existing acquisition methods.

NOTE Load a 3-Element Full Coverage Tuning Slide (PN 201088) before you begin. See Unload and Load a Sample.

1. On the ribbon, click the **Manual Tune** tab, and then click **Settings**.
2. Use Camera View to locate an unablated section of tuning film on the slide. The area required to complete this calibration is 120 x 100 µm.
3. Set the camera coordinates as the location to start ablating:

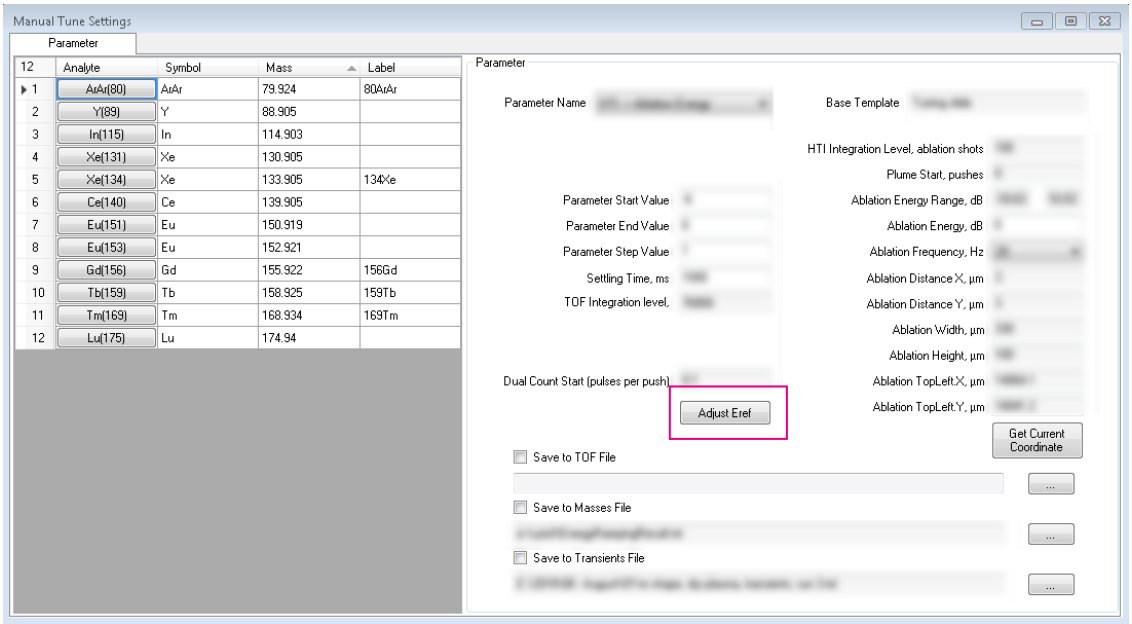
   In Manual Tune Settings, under Parameter, click **Get Current Coordinates**. The values for Ablation TopLeftX, µm and Ablation TopLeftY, µm are updated to match the camera coordinates.



4. Apply the Tuning Slide acquisition template. Right-click the **Analyte** table and click **Apply Template**. Double-click **Tuning Slide**.

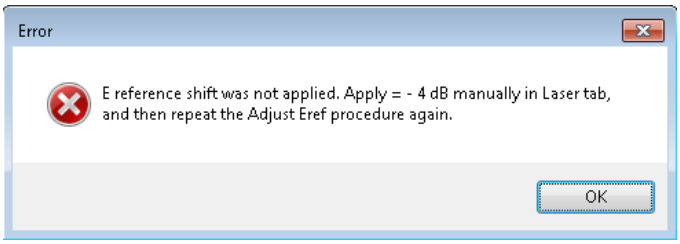
5 To start re-calibrating the reference energy, click **Adjust Eref**.

NOTE Recalibration takes approximately 5 minutes to complete. The instrument cannot be used during this time.



After re-calibration completes, a message that the Eref adjustment completed successfully is displayed in Log Manager.

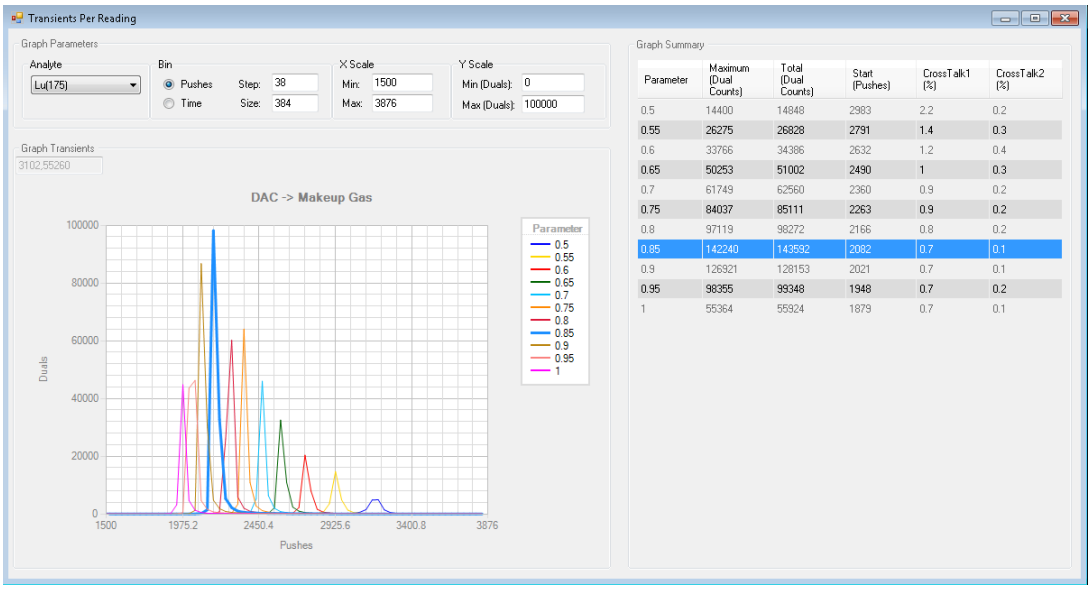
NOTE If the following message is displayed, contact Fluidigm Technical Support for assistance.



Plots

Transients Per Reading Plot

The Transients Per Reading plot displays the transient signal detected for a specific analyte at each measurement taken during manual tuning.



Graph Parameters

- Specify an analyte to monitor.
- Specify whether to bin data by push or by time.
- Set the X and Y axis scale.

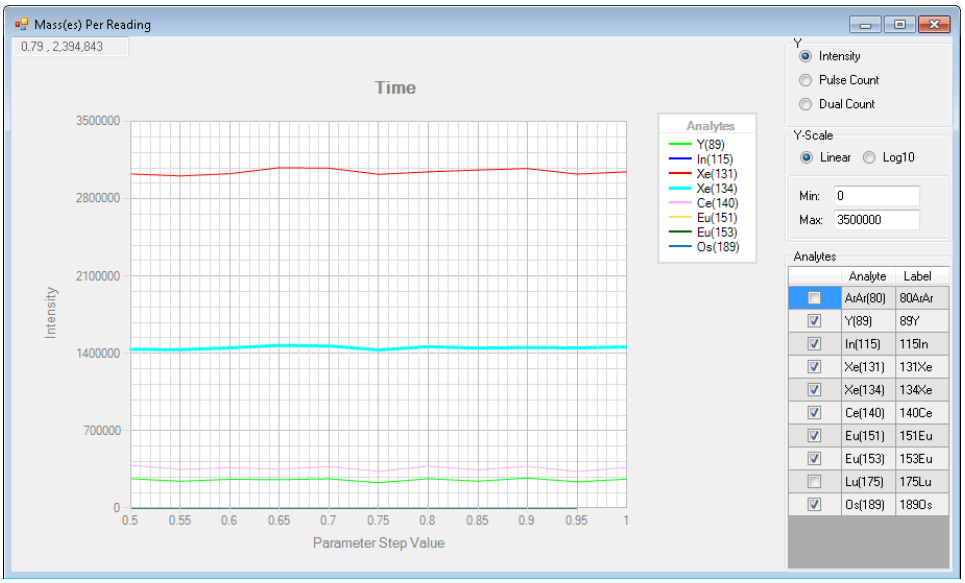
Graph Summary

The Graph Summary displays the following values for each measurement:

- Maximum (Dual Counts): The most intense measurement in the peak
- Total (Dual Counts): The peak area
- Start (Pushes): The number of pushes at which the peak start was measured
- CrossTalk1: The estimated percent overlap of ions from one transient to the next at 200 Hz ablation frequency
- CrossTalk2: The estimated percent overlap of ions from one transient to the next at 100 Hz frequency

Masses Per Reading Plot

On the Manual Tune window, click Channels to display the Masses Per Reading (MPR) plot. The MPR plot allows users to view the integrated intensity, pulse count, or dual count per reading for specified channels. The reading duration is defined in the Manual Tune Settings window, in the HTI Integration Level, ablation shots text box. The default value is 100 shots.



MPR Plot Settings

Setting	Description
Y	Choose to display integrated intensity, pulse count, or dual count for all pushes within the reading duration. • Intensity: The analog signal intensity. • Pulse Count: The number of pulses counted within a given mass channel. When the ion flux within the mass channel is low, the ion flux is most precisely determined by counting the number of pulses. • Dual Count: The effective ion flux for a given mass channel. At sufficiently low ion flux, the dual count is taken from the Pulse Count value. As the ion flux increases, ion pulses begin to arrive at the detector at the same time. In this situation, pulse count underestimates the true ion count, and integrated intensity becomes a more accurate measurement. Integrated intensity is then multiplied by the dual coefficient to calculate the amount of incoming ions.
Y-scale	Choose to display data on a linear or logarithmic scale.
Min/Max	Adjust the Y-axis range to zoom the view in or out.

Select Channels to Display

The channels shown in the Analyte table are the channels specified in the tuning protocol or acquisition protocol, depending on whether data is being acquired in Acquire mode or Tune mode.

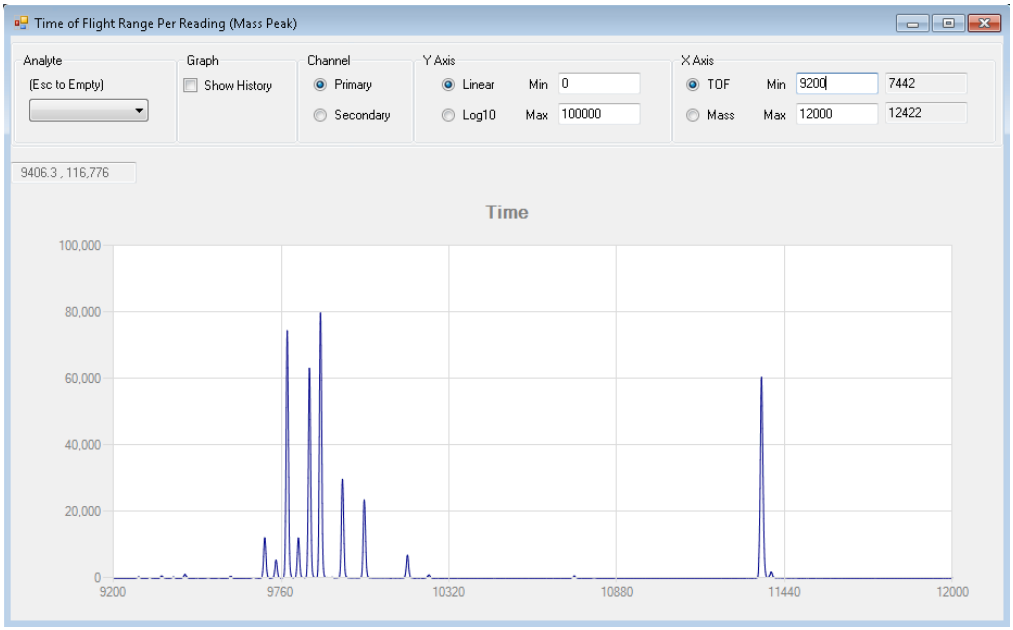
By default all channels in the Analyte table in the protocol are selected and displayed.

To specify channels to display

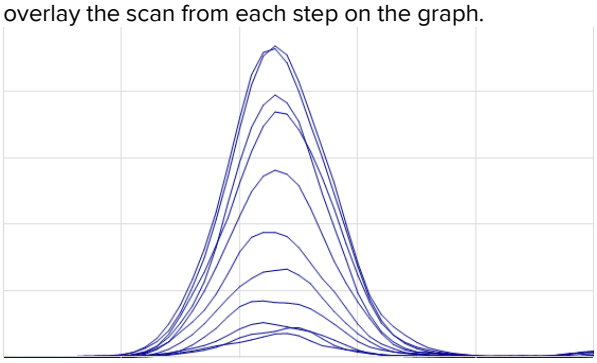
In the Analytes table, uncheck the channels to exclude from view.

Time of Flight (TOF) Plot

The TOF plot displays the intensity of ions from a single push against the time-of-flight (ns) scale or mass scale (amu).



TOF Plot Settings

Setting	Description
Analyte	The list of metal isotopes present in the tuning film or sample being acquired. To view a specific isotope, select it from the drop-down list, or press the **Esc** key to view all isotopes.
Graph	When ramping a parameter in **Manual Tune**, check the **Show History** box to overlay the scan from each step on the graph.  By default, **Show History** is unchecked.
Channel	The signal from the detector is split between two channels. The signal to the Primary channel (Channel A) is amplified. The signal to the Secondary channel (Channel B) is dampened. The difference between the two signals is a factor of 50. • Click **Primary** to display the amplified signal. • Click **Secondary** to display the dampened signal. **NOTE** If the Secondary channel signal is higher than the Primary channel signal, the cables from the computer to the instrument have become reversed and must be swapped.
Y axis	Choose the range and scale to display (linear or logarithmic). The default range is 0–10,000,000.
X axis	Display either time-of-flight (ns) or mass number (amu).

Acquire Data

About Data Acquisition

Imaging Mass Cytometry™ data are saved to an MCD (.mcd) file. In addition to channel data, each MCD file contains additional sample-specific information:

- Imported images
- Panoramic images created with the Hyperion™ Tissue Imager
- Regions of interest (ROIs)
- Optical images of ROIs taken before and after ablation
- Focal map data for optimal laser and camera focus
- Fiducial coordinates

Data Acquisition Enhancements in v7.0

Prepare MCD Files on a Non-Acquisition Computer

To support flexible and collaborative workflows and to maximize instrument availability, install CyTOF® Software v7.0 on a non-acquisition computer. You can perform the following tasks:

- Create new MCD files
- Create or edit Slide Layouts
- Import images of the sample
- Draw panoramas and ROIs
- Create, edit, and apply acquisition templates

Later, transfer the MCD file with the corresponding sample to the Hyperion Imaging System. Load the slide into the Hyperion Tissue Imager, coregister the sample image and ROIs to the physical sample, and then acquire data.

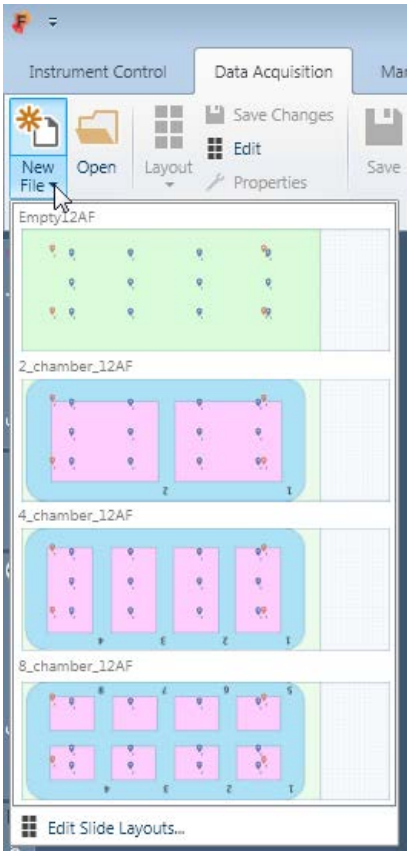
NOTE See CyTOF Software v7.0 Release Notes (FLDM-400338) for minimum system requirements and installation instructions for offline acquisition preparation.

Image Coregistration

For best accuracy, coregister images imported into an MCD file to the physical sample in the Hyperion Tissue Imager. Use the camera within the Hyperion Tissue Imager to identify landmarks that correspond with the imported image. A minimum of three landmarks must be identified to successfully coregister an image.

Slide Layouts

When a new MCD file is created, a Slide Layout is applied. A Slide Layout contains focal pins and fiducial pins. Focal pins indicate where pre-acquisition measurements are taken to compensate for slide curvature during panorama creation and sample ablation. Fiducial pins indicate where fiducials will be created. Slide Layouts provided with CyTOF Software have 12 focal pins and 4 fiducial pins.



CyTOF Users can choose from existing Slide Layouts. CyTOF Administrators can modify existing Slide Layouts and create new ones. New Slide Layouts are available to all users from Data Acquisition > New File.

Four Slide Layouts are provided with the software:

Plain slide (Empty12AF)
2-well chamber slide (2_chamber_12AF)
4-well chamber slide (4_chamber_12AF)
8-well chamber slide (8_chamber_12AF)

NOTE The default Slide Layout is applied to MCD files acquired with earlier versions of CyTOF Software when opened with CyTOF Software v7.0. This does not affect or change the data acquired.

Focal Pins

A focal map (or profile) is created from a series of measurements taken at slide coordinates marked by focal pins on the Slide Layout. The focal map determines the optimal stage height at various slide coordinates during acquisition to compensate for slide curvature resulting in improved camera focus and more uniform ablation of larger ROIs.

A focal map is automatically created when panorama creation or ROI acquisition is started. It takes approximately 2 minutes to create the focal map.

Fiducial Coordinates

Fiducial pins indicate where to create fiducial marks. Create fiducial marks to use for slide registration if you want to remove a sample and resume acquisition to the same MCD file another time.

For more information, see Create Fiducials and Find Fiducials.

NOTE The Slide Layout can be changed at any time unless fiducials have been created.

Data Acquisition Workflow

If CyTOF Software v7.0 is installed on a non-acquisition computer, you can perform preliminary work on that computer and then transfer the MCD file with the corresponding sample to a Hyperion Imaging System for acquisition. You can also perform all of your work directly on the acquisition computer connected to the Hyperion™ Imaging System.

The following workflows provide a general overview of the data acquisition workflow. For more detailed information about specific functionality, see the topics in the Acquire Data > How To section of this Help guide.

Starting on a Non-Acquisition Computer

1. Create a new MCD file.
2. Import an image of the sample.
3. Select regions of interest for ablation.
4. Apply acquisition settings.
5. Save the MCD file and transfer it with the sample to a tuned Hyperion Imaging System.
6. Coregister the image and ROIs to the sample in the Hyperion Tissue Imager.
7. (Optional) Create panoramic images of the sample.
8. Acquire data.

Starting on the Hyperion Imaging System

1. Create a new MCD file.
2. Import and coregister an image or create a panoramic image of your sample.
3. Select regions of interest for ablation.
4. Apply acquisition settings.
5. Start acquisition.

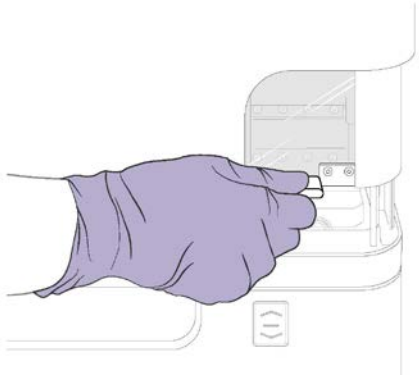
(Optional) Create Fiducials

To remove the slide and acquire data later, create fiducials on the slide at any time before or after acquisition. Fiducial coordinates are saved to the MCD file. When the sample is reloaded for acquisition, open the same MCD file and register to the fiducials to realign the Slide Layout to the physical sample in the Hyperion Tissue Imager. For more about fiducials, see Create Fiducials and Find Fiducials.

Unload and Load a Sample

1 On the Instrument Control tab, click **Unload Sample**.

2 After the stage on the Hyperion™ Tissue Imager fully extends, open the sample window and, if necessary, remove the previously loaded slide.



3 Load the slide onto the stage and close the sample window.

4 After the sample is loaded, click **Load Sample**.

NOTE For more information about loading and unloading slides, see the Hyperion Imaging System User Guide (400311).

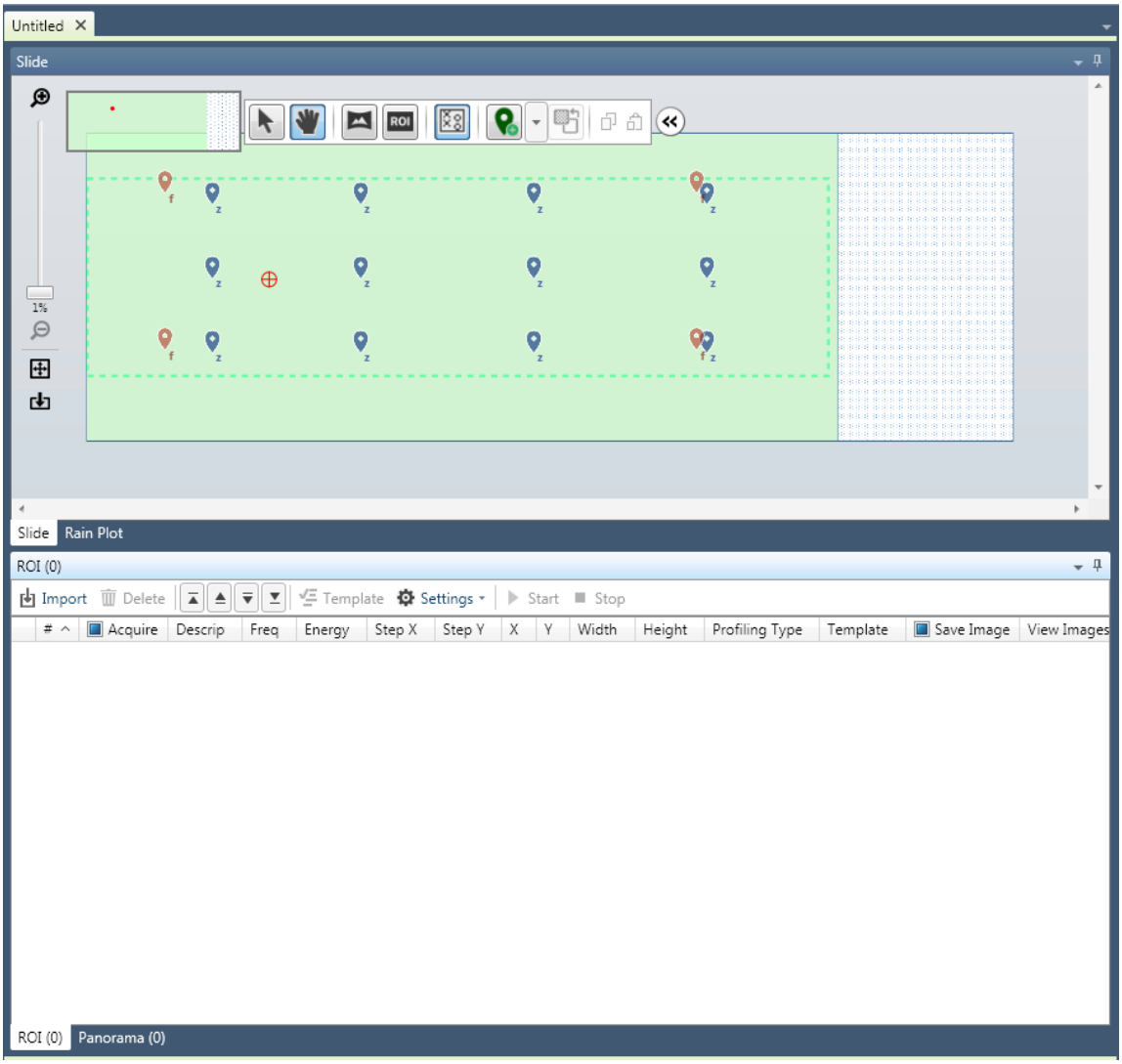
Create or Open an MCD File

After you create or open an MCD file, the Slide and Rain Plot tabs are displayed in the top pane of the workspace and the ROI and Panorama tables are displayed on tabs in the bottom pane.

The Slide Layout is shown on the Slide tab. If a Slide Layout is not selected when the MCD file is created, the default Slide Layout—which is the Slide Layout that was last used—is applied.

The Slide Layout shows the locations of focal pins (blue) and fiducial pins (red). For more information, see Slide Layouts, Move Pins on the Slide Layout, and Create or Modify a Slide Layout.

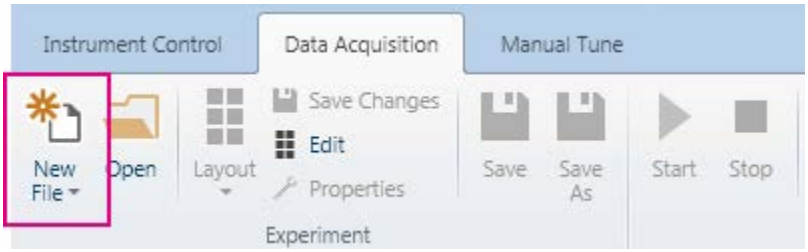
NOTE Do not acquire data from more than one slide to an MCD file. Create a new MCD file for each slide.



Create a New MCD File

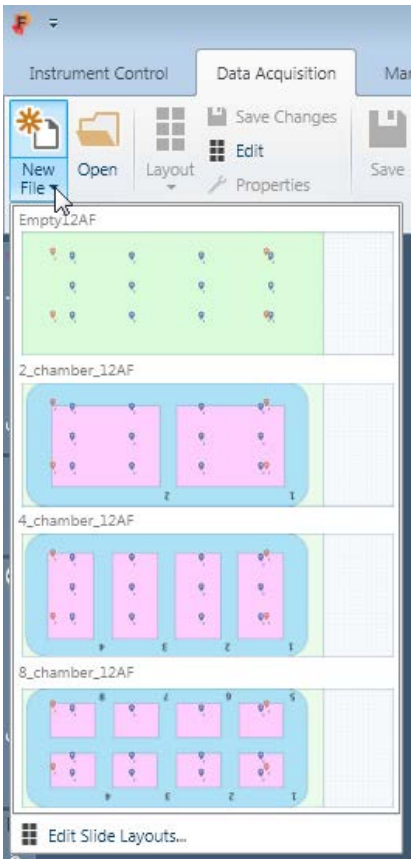
To create a new MCD file with the default Slide Layout

On the ribbon, click the **Data Acquisition** tab, and then click **New File**.



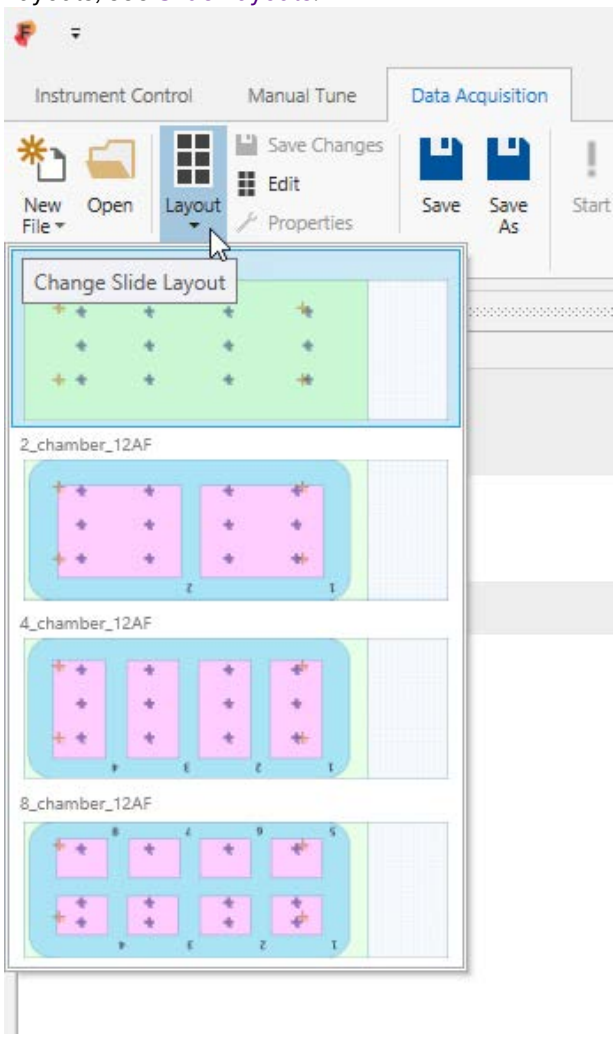
To create a new MCD file with a specific Slide Layout

On the ribbon, click the Data Acquisition tab, and then click the New File down arrow to display the Slide Layout list. Click the Slide Layout to apply it.



Change the Slide Layout

1 (Optional) To change the Slide Layout, on the Data Acquisition toolbar, click **Layout**, and then click the Slide Layout that you want to use. For more information about Slide Layouts, see Slide Layouts.

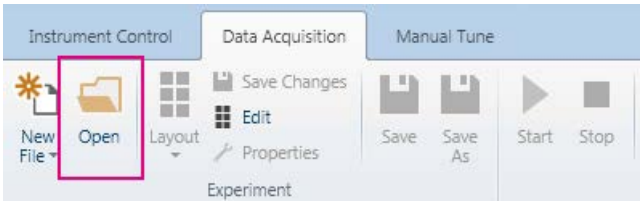


NOTE The last Slide Layout used becomes the new default Slide Layout. CyTOF® Administrators can create or modify Slide Layouts.

2 Focal pins and fiducial pins are displayed on the Slide Layout by default. Click the Show Fiducials tool to hide all pins from view. Click again to display the pins.

Open an MCD File

1 On the ribbon, click **Data Acquisition**, and then click **Open**.



NOTE An MCD file created with an earlier version of CyTOF Software must be upgraded for compatibility with v7.0. For more information, see MCD File Compatibility.

2 Browse to locate the MCD file and double-click the file to open it. The file contents are displayed in Experiment Manager, the Slide Layout, and the ROI and Panorama tables.

3 Focal pins and fiducial pins are displayed on the Slide Layout by default. Click **Show Fiducials** to hide all pins from view. Click again to display the pins.

Move Pins on the Slide Layout

A Slide Layout contains two types of pins:

- Focal pins to mark the coordinates where focal measurements are taken
- Fiducial pins to mark the coordinates where fiducials (optional) are created

Move a Fiducial Pin

Four fiducial pins are included in each Slide Layout. Fiducial pins indicate where fiducials are created. If a fiducial pin is placed over the sample or hydrophobic barrier, move the fiducial pin before creating fiducials.

For more information, see Create Fiducials and Find Fiducials.

To move fiducial pins

1 In the Slide window, click  .

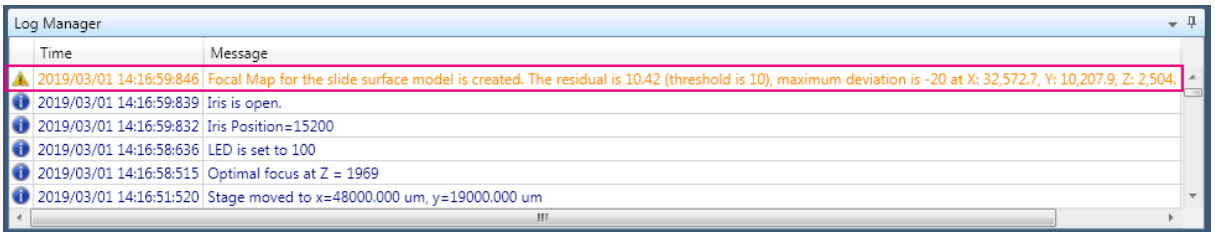
2 Click and drag the coordinate mark you want to move.

Move a Focal Pin

If a focal measurement is taken in an area where light is not properly reflected, such as over thick tissue, a warning is displayed in the Log Manager. If the ROIs queued for acquisition are >1,000 µm2:

- Switch to Local Profiling: In the ROI table, set Profiling Type to Local
- Move the focal pin and create the Focal Map.

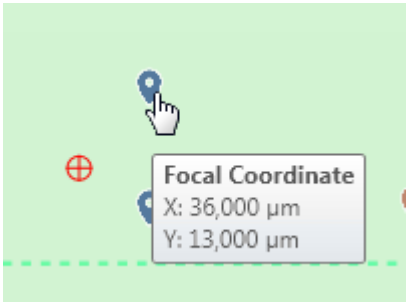
Otherwise, acquisition begins after the Focal Map is created.



To move a focal pin

1 On the Slide toolbar, click  .

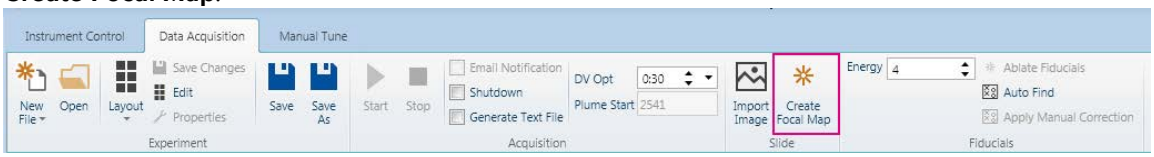
2 Locate the pin with coordinates that match the warning message displayed in Log Manager. Hover over a focal pin to display its coordinates.



3 Use Camera View to identify a location that is adequately lit and contains a thinner section of the tissue. The red cross hair on the Slide Layout updates to reflect the camera position.

4 On the Slide Layout, click and drag the focal pin to red cross hair.

5 After the focal pin is moved, re-create the Focal Map. On the Data Acquisition tab, click **Create Focal Map**.



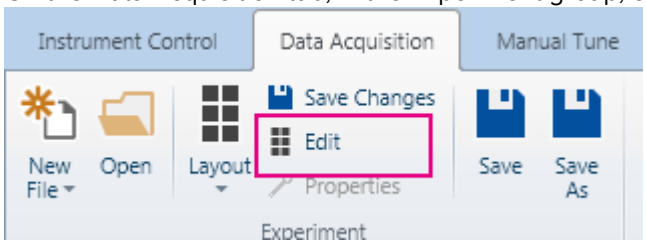
Create or Modify a Slide Layout (CyTOF Administrators only)

The Slide Layouts provided with CyTOF® Software contain 12 focal pins and 4 fiducial pins and are designed to work with standard slide types. The pins can be repositioned as needed in the MCD file to accommodate the sample location or slide type. These changes are saved to the MCD file, not to the Slide Layout.

To add or remove focal or fiducial pins or create a Slide Layout intended for a non-standard slide type, open the Slide Layout editor. CyTOF Administrators can create or modify existing Slide Layouts, including Slide Layouts provided with the software.

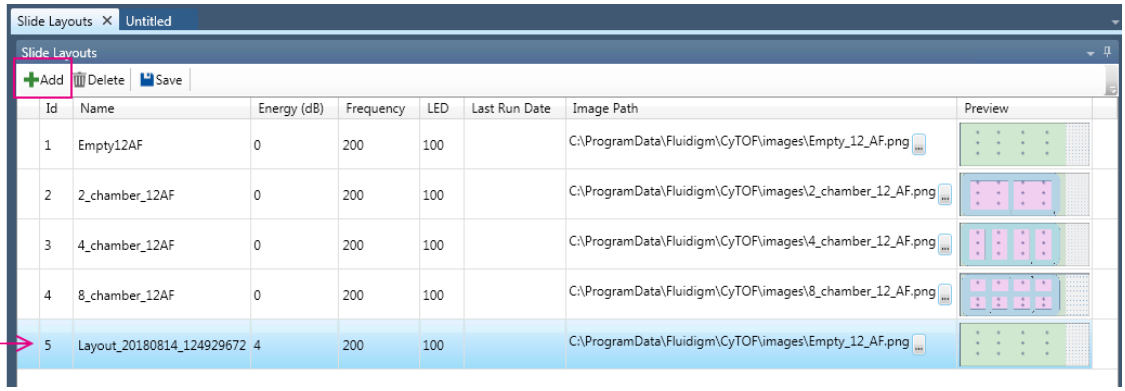
To create or modify a Slide Layout

1 On the Data Acquisition tab, in the Experiment group, click **Edit**.



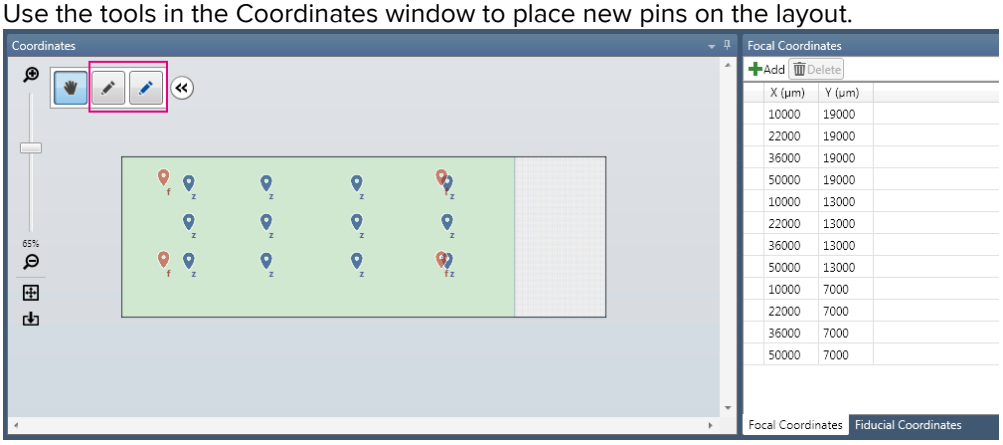
2 In the Slide Layouts window choose a Slide Layout to modify or create a new Slide Layout.

- To choose a Slide Layout to modify, click a Slide Layout in the list.
- To create a new Slide Layout, click **Add**.

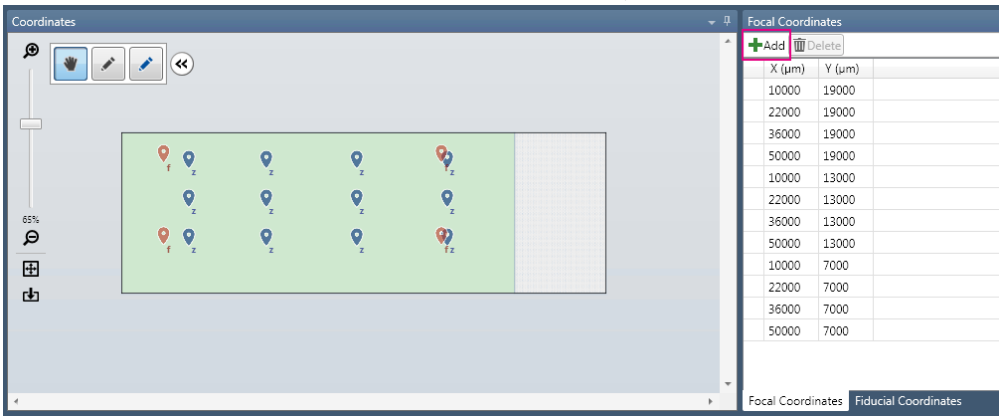


3 Add new focal or fiducial pins in one of these ways:

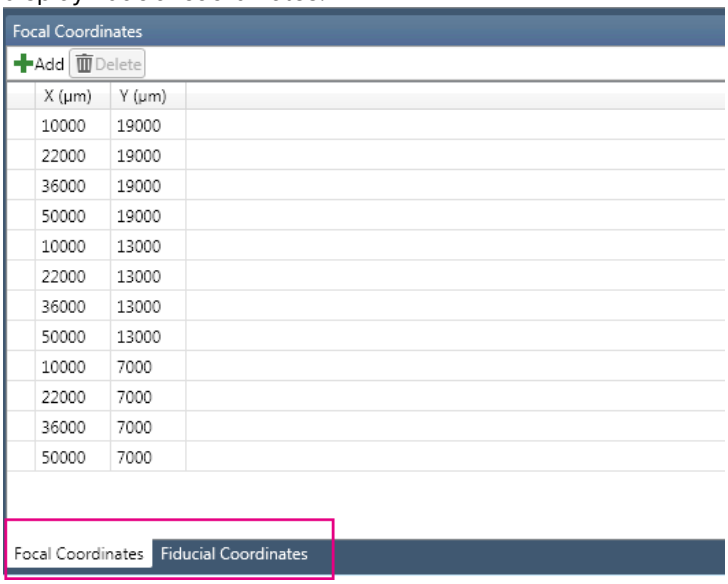
- Use the tools in the Coordinates window to place new pins on the layout.



- On the Focal Coordinates or Fiducial Coordinates tab, click **Add.**



Focal Coordinates are displayed by default. Click the Fiducial Coordinates tab to display fiducial coordinates.

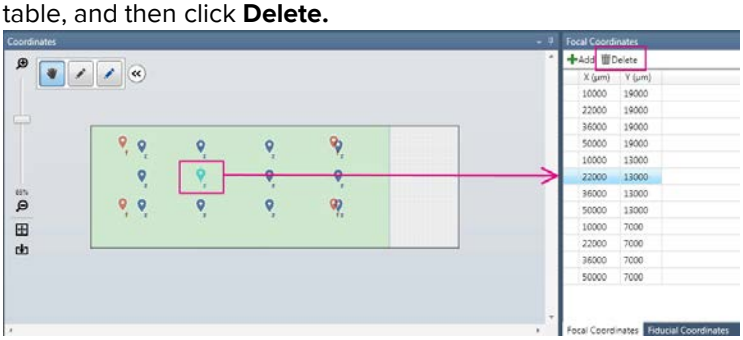


4 Move the pins by clicking the Hand tool and then clicking and dragging a coordinate marker.

NOTE If a pin is placed out of bounds, a red square is displayed and the pin coordinates are highlighted in the table. A Slide Layout cannot be saved if a pin is in an invalid location.

5 To delete focal or fiducial pins in one of these ways:

- In the Coordinates window, click a focal pin to highlight the row containing the coordinates in the Focal Coordinates table, and then click **Delete**.
- Click the Focal Coordinates or Fiducial Coordinates tab, click the coordinate in the table, and then click **Delete.**



Import an Image

To help navigate the sample and identify regions of interest (ROIs), import an image of the sample. Before acquiring data, coregister the image and objects, such as unacquired ROIs, to the sample loaded in the Hyperion™ Tissue Imager by identifying common physical landmarks.

NOTE For most accurate coregistration, import an exact image of the sample loaded in the Hyperion Tissue Imager. Images of sequential tissue sections can be used for qualitative analysis only.

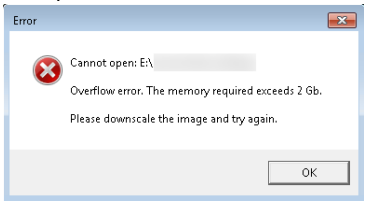
Image Recommendations

- Import full slide images with a resolution between 20 µm/pixel (or approximately 1,200 dpi) and 5 µm/pixel for best accuracy.
- Downscale partial slide Images with dimensions >10,000 x 10,000 pixels or an equivalent number of pixels. Otherwise, the software may become slow to respond.
- The following file types are supported: bitmap (.bmp), uncompressed TIFF (.tif/.tiff), JPG (.jpg/.jpeg) and PNG (.png). Compressed file types, such as JPG and PNG, are saved to the MCD file as bitmap images. Choose uncompressed file types to best estimate the impact of the file size on the MCD file size.

NOTE The JPEG2000 compression format is not supported.

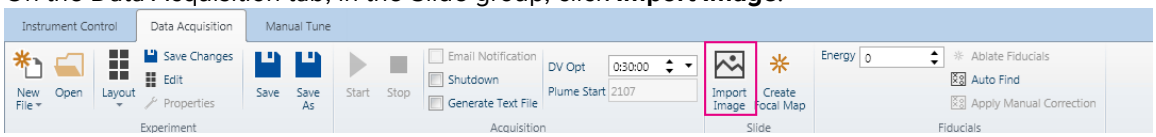
- To improve software speed and performance, crop images so that only the relevant portion of the image is imported.

  IMPORTANT Image files that require >2 GB of memory are not supported and cannot be opened. Downscale the image using external software to import it.



To import an image

1 On the Data Acquisition tab, in the Slide group, click **Import Image**.

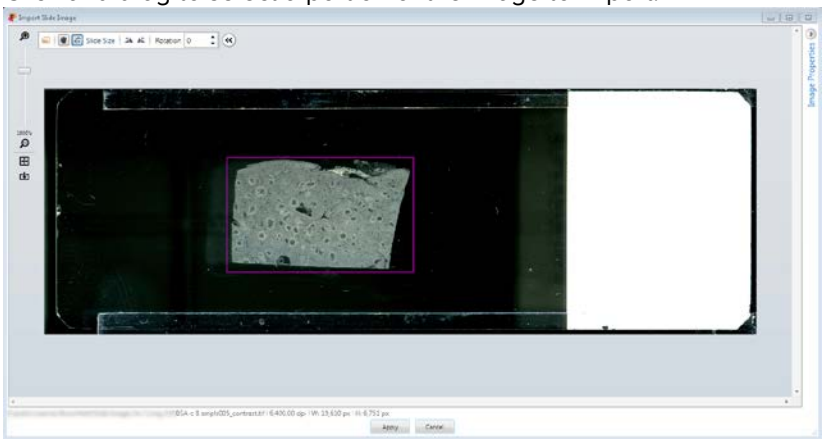


2 Navigate to the image file and double-click to open the file. The file is displayed in the Import Slide Image window.

3 (Optional) Crop the image.

a Click the Crop tool .

b Click and drag to select a portion of the image to import.



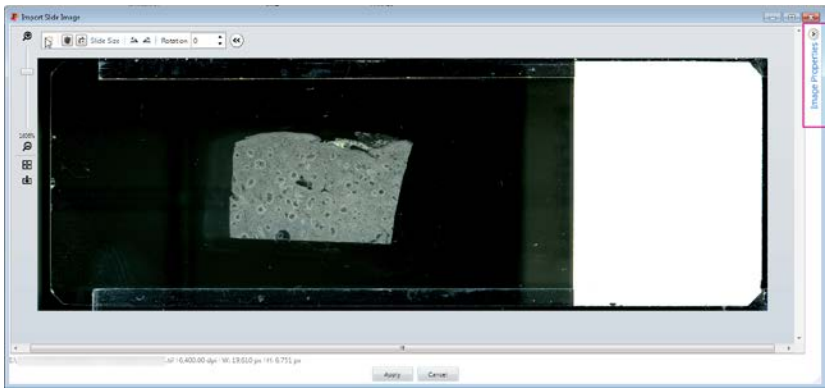
4 If necessary, rotate the image to orient it relative to the sample in the Hyperion Tissue Imager. The frosted end of the slide must be on the right.

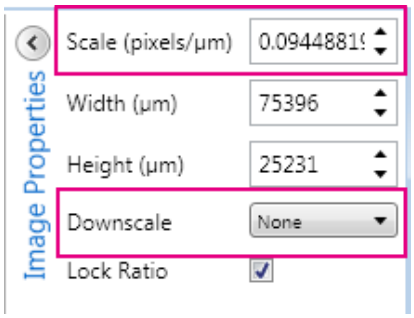
Rotate the image in one of these ways:

- Click or to rotate the image 90° in either direction.
- Enter a value in the Rotation text box and press Enter to rotate the image a specific number of degrees.

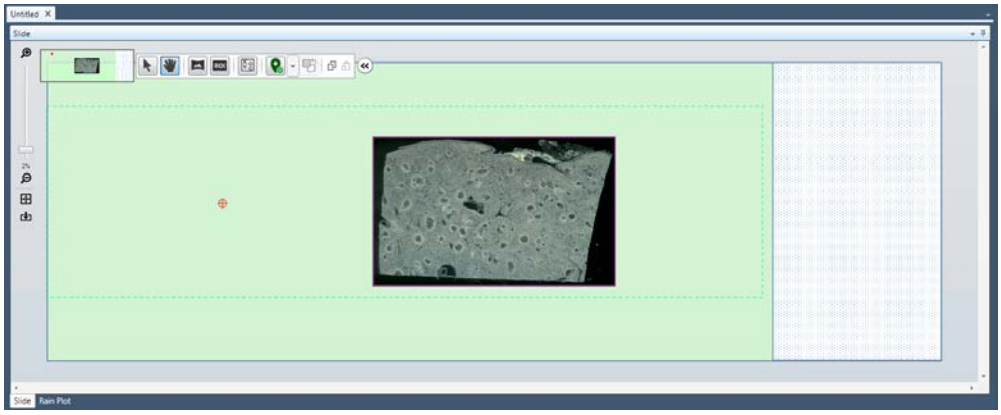
NOTE The image cannot be rotated after it is imported.

5 If the image resolution is higher than recommended, click **Image Properties**. In the Scale text box, enter the image scale, and next to Downscale, click **1 µm/pixel** or **5 µm/pixel** to reduce the resolution. For more information, see Image Properties.





6 Click **Apply** to apply the changes to the image file. The Slide Image Window closes and the image is displayed on the Slide Layout of the active MCD file.



NOTE To avoid accidentally moving or resizing an imported image, lock the image in place. Click the lock icon in the upper right corner of the Slide Layout. When the lock icon is red, the image is locked. Click the icon again to unlock the image.

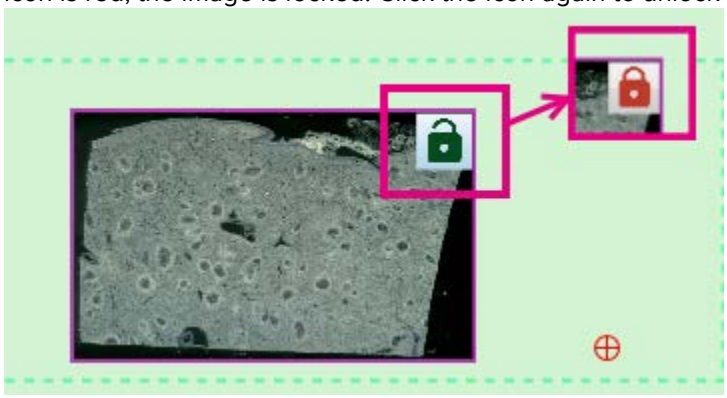
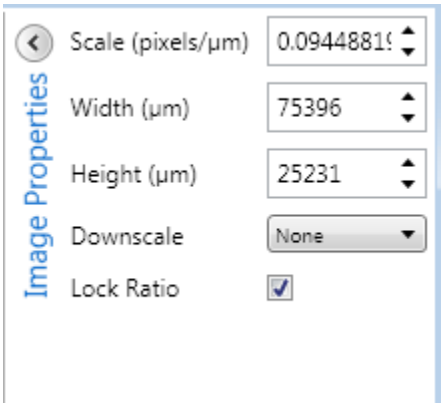


Image Properties

Use Image Properties to modify the scale of an image.



Property	Description
Scale (pixels/µm)	Displays the scale of an image in pixels/µm. The initial value is calculated from the horizontal resolution of the image. Enter a new value to change the scale. If you do not know the scale, divide the image resolution in dpi by 25,400 µm to convert from dpi to pixels/µm. The image resolution must be at least 1,200 dpi.
Width (µm)	Displays the image width in µm. Enter a new value to change the width. When Lock Ratio is checked, the height is automatically adjusted to keep the aspect ratio.
Height (µm)	Displays the image height in µm. Enter a new value to change the width. When Lock Ratio is checked, the width is automatically adjusted to keep the aspect ratio.
Downscale	The default setting is None. To downscale an image, choose **1 µm/pixel** or **5 µm/pixel**.
Lock Ratio	When checked, the aspect ratio of the image is maintained when it is resized.

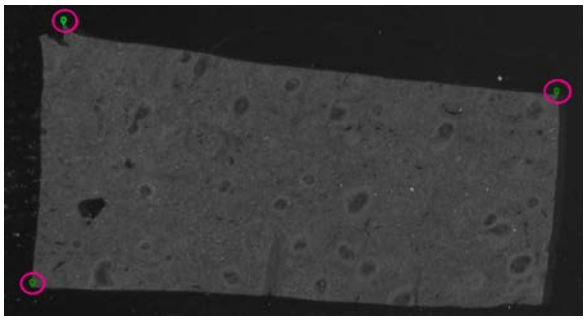
Coregister an Image

For most accurate ablation, coregister an imported image to the physical sample. Use Camera View to identify distinct sample features to use as coregistration landmarks and locate the corresponding features in the sample image. At least three, well-spaced landmarks are required for coregistration.

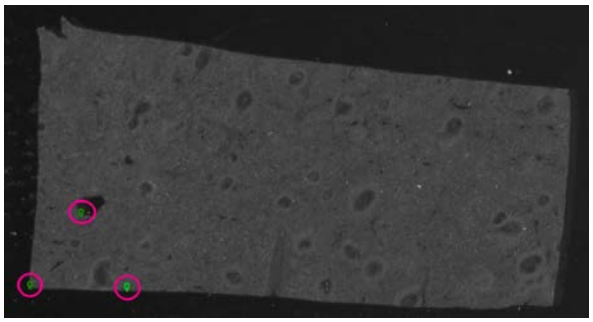
Landmark Positioning

Coregistration landmarks must be placed as far apart from each other as possible for the most accurate results.

Coregistration pins are correctly spaced.

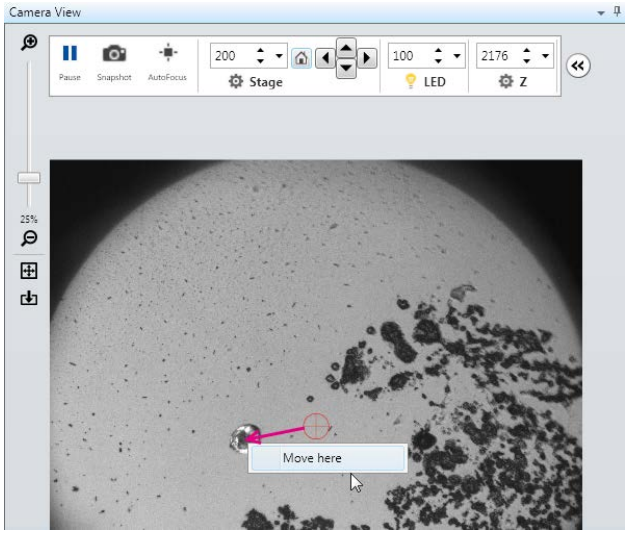


Coregistration pins are too close together.



To coregister a sample image

1. Identify a distinct feature on the imported image, then use Camera View directional controls to navigate the sample to bring the corresponding feature into view.
2. In the Camera View window, right-click directly over the feature and click **Move here** to position the camera cross hair over the feature.

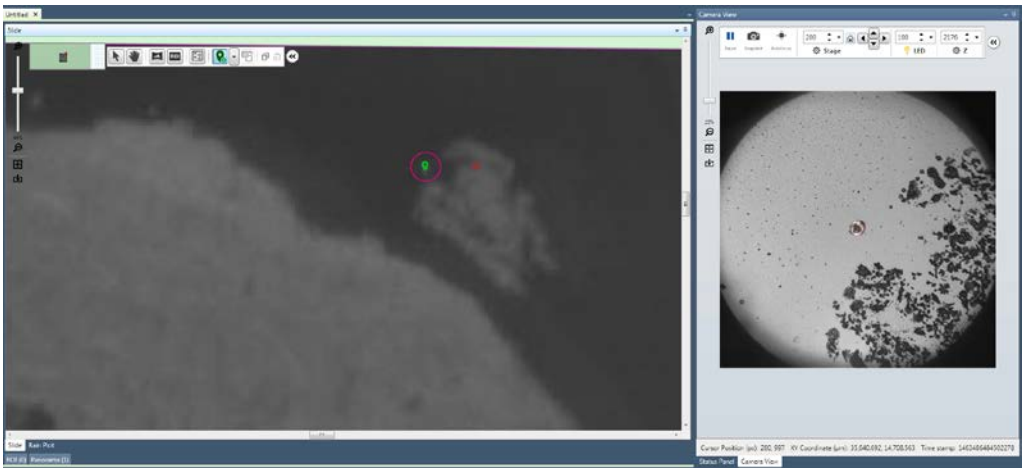


3 On the Slide Layout, place a coregistration pin over the same feature on the imported image.

IMPORTANT Be sure the camera cross hair is positioned over the landmark before placing a coregistration pin on the imported image.

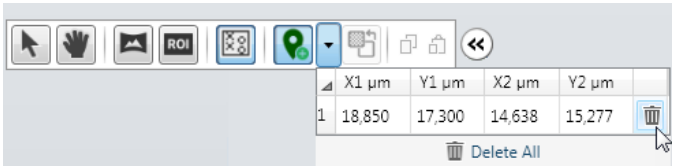
a On the Slide toolbar, click the **Add co-registration landmark** tool  .

b On the imported image, click the image to place the pin.



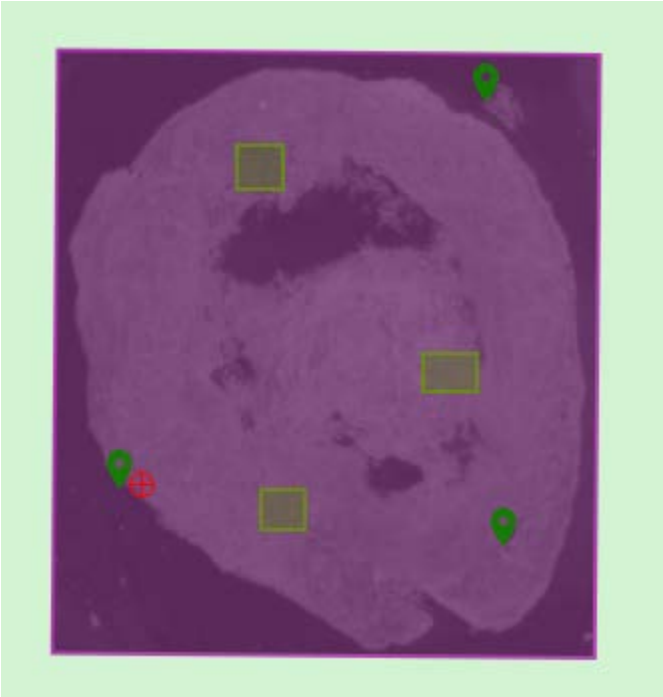
IMPORTANT A coregistration pin placed on the imported image should mirror the cross hair position in Camera View as closely as possible for best accuracy.

c If the pin is not correctly placed, click the down-arrow next to the Add coregistration landmark tool  , and then click the **Delete** icon for the fiducial pin to delete.

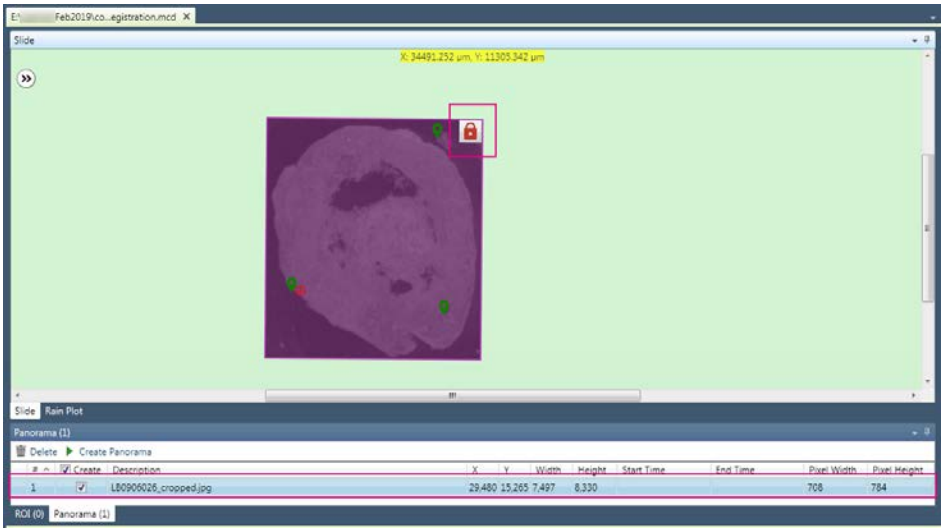


4 Repeat Steps 1 to 3 at least two more times, choosing landmarks that are well-spaced. A minimum of three landmarks is required for coregistration.

5 After three landmarks are identified and coregistration pins are placed, Apply Coregistration  remains dimmed until the image is selected for coregistration. To coregister additional objects such as regions of interest (ROIs), press and hold Ctrl and click the additional objects. Highlighted objects are selected.



NOTE If an image is locked, it must be selected on the Panorama table in the pane below the image. Click the **Panorama** tab and then click the row that corresponds to the image.



6 After the image and other objects are selected for coregistration, click Apply Coregistration  .

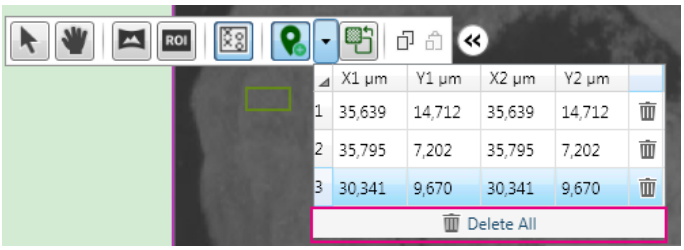
A message is displayed indicating that coregistration has been applied. The imported image may be rescaled. The image and selected objects will slightly shift and then the imported image is locked in place.

IMPORTANT If the dimensions of an imported image change as a result of coregistration, the dimensions of unacquired ROIs and panoramas also change relative to the imported image.

7 To verify coregistration accuracy, right-click a coregistration pin on the Slide Layout and click **Move here**. The position of the cross hair in Camera View and the cross hair on the image on the Slide Layout should match. Repeat at each coregistration pin and other areas on the image.

8 If you are not satisfied with the accuracy of coregistration, delete the coregistration landmarks and repeat Steps 1 to 7. To delete the coregistration landmarks:

On the Slide toolbar, click the down-arrow next to the Coregistration tool, and then click **Delete All**.



9 After the image is successfully coregistered, you can use it to determine where to select ROIs.

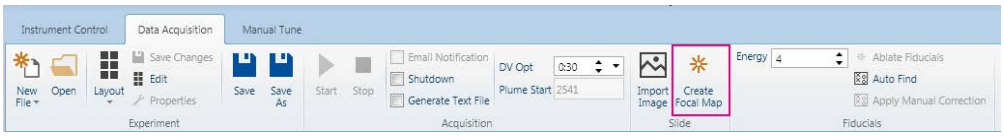
Create a Focal Map

A focal map (or profile) is created from a series of measurements taken at slide coordinates marked by focal pins on the Slide Layout. The focal map determines the optimal stage height at various slide coordinates during acquisition to compensate for slide curvature resulting in improved camera focus and more uniform ablation of larger ROIs.

If a focal map is not already been created, it is automatically created when panorama creation is started or when acquisition of ROIs is started. It takes approximately 2 minutes to create a focal map.

To create a focal map

After an MCD file is opened and the sample slide is loaded into the Hyperion™ Tissue Imager, click the **Data Acquisition** tab, and then click **Create Focal Map**.



After focal map creation starts, Create Focal Map becomes Cancel Focal Map. 

To cancel focal map creation

Click **Cancel Focal Map**.

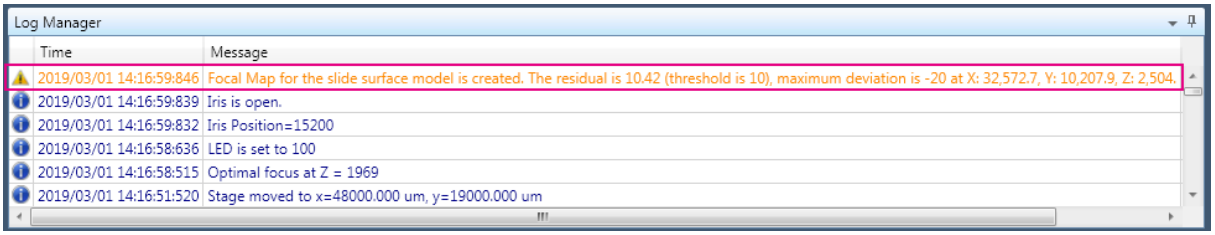
If the Focal Map was Created with Warnings

If a focal measurement is taken in an area where light is not properly reflected, such as over a thick tissue, a warning is displayed in the Log Manager. For ROIs >1,000 µm2:

NOTE Switch to Local Profiling: In the ROI table, set Profiling Type to Local to create a local focal map for each ROI at the time of acquisition.

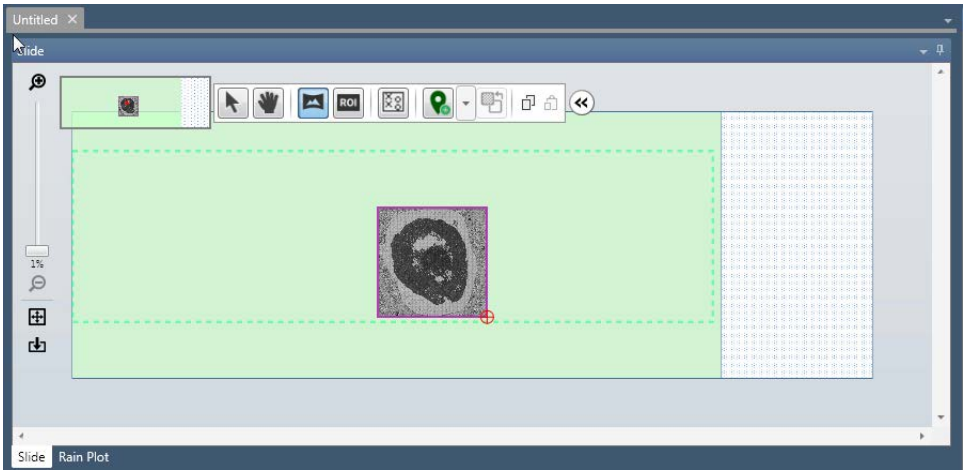
Move the focal pin and re-create the Focal Map. For more information, see Move a Focal Pin.

NOTE If the Focal Map was created automatically after starting acquisition, acquisition proceeds unless stopped by the user.



Create a Panorama

A panorama is a stitched image of the sample created using the camera in the Hyperion Tissue Imager. Use the Panorama tool to select one or more areas on the Slide Layout to indicate where to create panoramas.



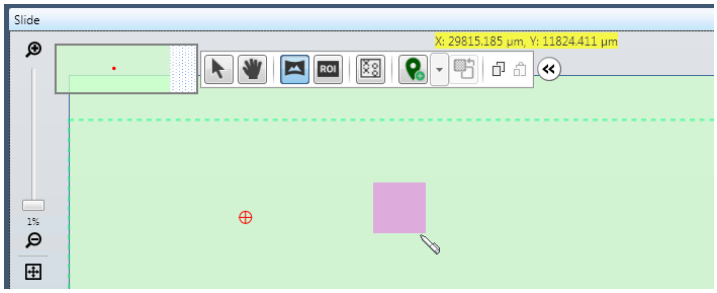
NOTE Panoramas are saved in the MCD file as bitmap images. For best software responsiveness:

- Limit total panorama area to a maximum of 200,000,000 µm2 (or an equivalent area).
- Do not recreate existing panoramas. Original image data is not deleted from the MCD file so the file size increases each time a panorama is recreated. Instead, delete the panorama from the Panorama table and then create a new one.

To create a panorama

1 On the Slide toolbar, click the **Draw Panorama** tool.

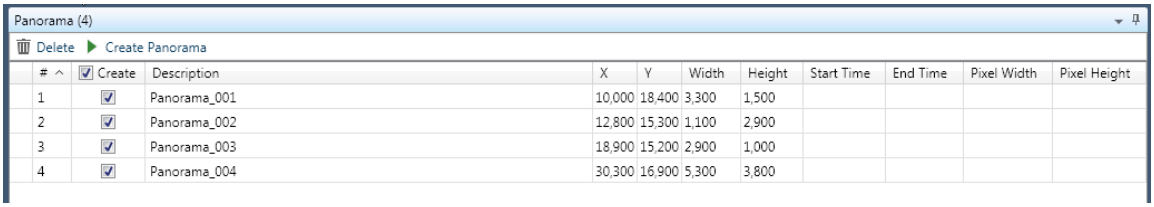
2 Within the boundaries, but at least 500 µm from the left edge, click and diagonally drag the cursor to highlight the area. Repeat, if necessary.



3 After a panorama is selected on the Slide Layout, it appears in the Panorama table in the pane below. Double-click any of the following cells to enter a different value:

- **Description**: A descriptive name for the panorama
- **X**: Top-left horizontal coordinate
- **Y**: Top-left vertical coordinate
- **Width**: Panorama width in µm
- **Height**: Panorama height in µm

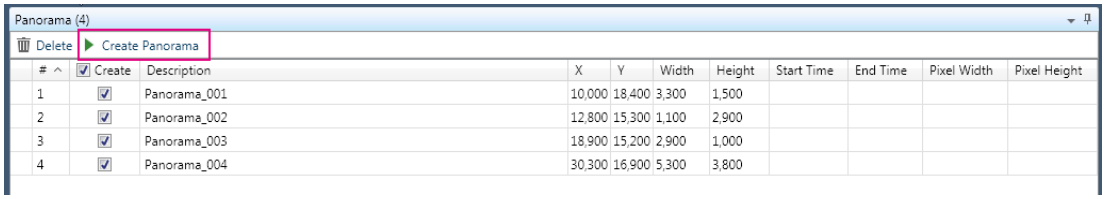
NOTE Imported images are also displayed in the Panorama table, but the contents cannot be edited.



#	Create	Description	X	Y	Width	Height	Start Time	End Time	Pixel Width	Pixel Height
1	☑	Panorama_001	10,000	18,400	3,300	1,500				
2	☑	Panorama_002	12,800	15,300	1,100	2,900				
3	☑	Panorama_003	18,900	15,200	2,900	1,000				
4	☑	Panorama_004	30,300	16,900	5,300	3,800				

4 Create the panoramas from the Panorama table or Experiment Manager.

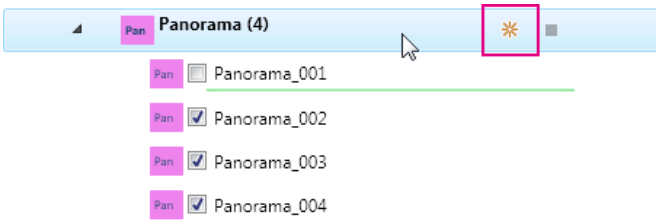
- To create panoramas from the Panorama table, check all of the panoramas you want to create, and then click **Create Panorama**.



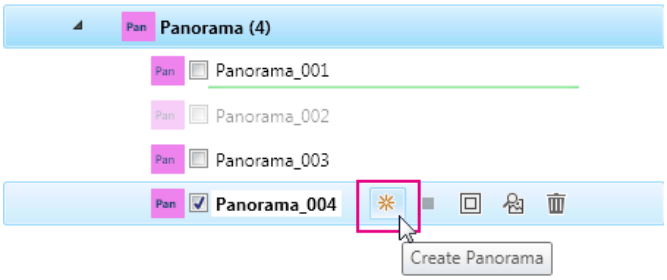
#	Create	Description	X	Y	Width	Height	Start Time	End Time	Pixel Width	Pixel Height
1	☑	Panorama_001	10,000	18,400	3,300	1,500				
2	☑	Panorama_002	12,800	15,300	1,100	2,900				
3	☑	Panorama_003	18,900	15,200	2,900	1,000				
4	☑	Panorama_004	30,300	16,900	5,300	3,800				

- To create panoramas from Experiment Manager

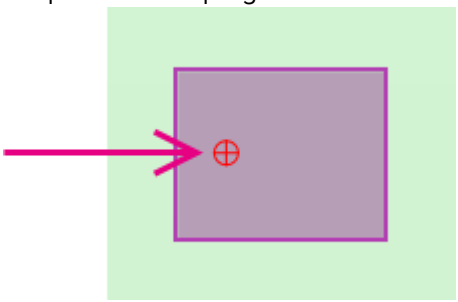
  a Create multiple panoramas: Check all of the panoramas you want to create, hover over the top level panorama to display the toolbar, and then click the Create tool  .



b Create an individual panorama: Check the panorama you want to create, hover over the panorama to display the toolbar, and then click .

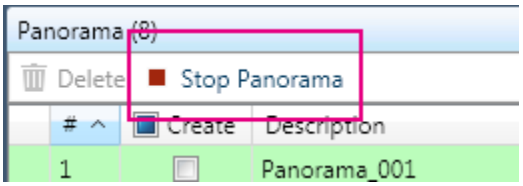


NOTE If a Focal Map is not already created, it will be created before the camera begins moving and taking snapshots. This takes approximately 2 minutes. After the camera begins to move, a red cursor shows the current camera position relative to the panorama. A progress bar is also displayed in Experiment Manager.



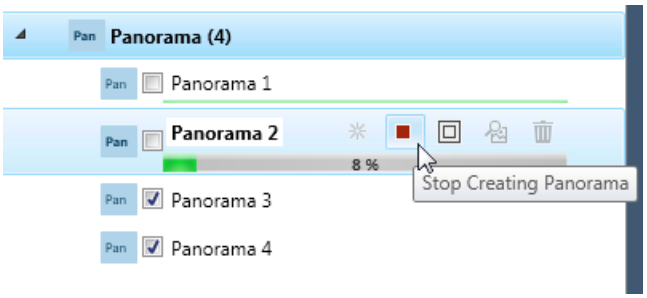
To stop creating a panorama from the Panorama table

Click **Stop Panorama**.



To stop creating a panorama from Experiment Manager

Click the Stop (■) button.

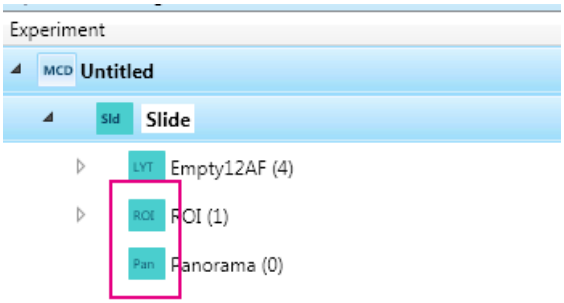


Resize Panorama Selections

Panorama selections can be resized by adjusting the handles on the panorama on the Slide Layout before you create it or by entering exact dimensions in the Width and Height cells in the Panorama table. Double-click each cell to edit the contents. Panoramas cannot be resized after they are created.

Toggle View

Click the icon next to an ROI to hide or show it on the Slide Layout.



Draw or Import ROIs

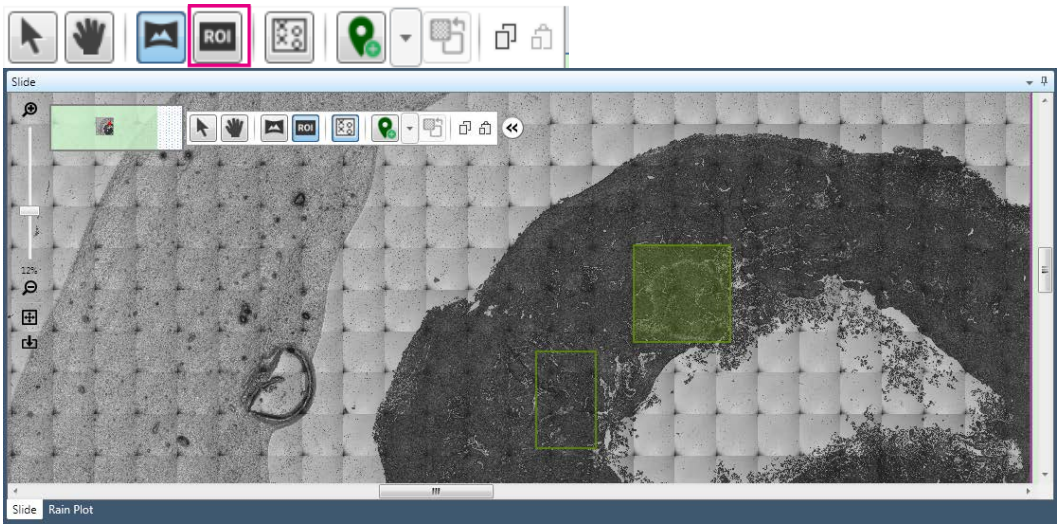
On an optical image of the sample—an imported image or panorama—the ROI tool is used to identify a section of that sample (region of interest, or ROI) for analysis. After an ROI is selected, it is added to the Experiment Manager and the ROI table.

ROIs can be selected by using the Draw ROI tool or by importing ROI coordinates and dimensions. You can duplicate, modify, and delete ROIs after you have drawn or imported them into the ROI table.

Draw ROIs with the Draw ROI Tool

To draw an ROI using the Draw ROI tool

Click the Draw ROI tool, and within the slide boundaries, click and diagonally drag the cursor to highlight the area.



Import ROIs from a CSV File

To create a compatible CSV file, download the CSV template from the Fluidigm website, modify the template in Notepad or Excel, and save as a new CSV file or follow the instructions below.

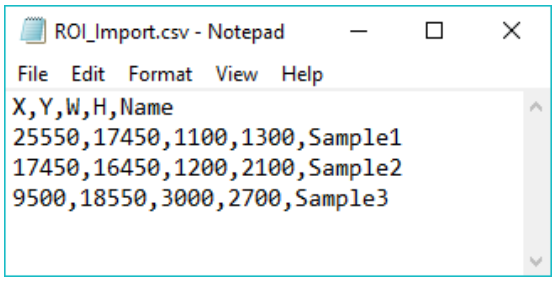
To import ROI coordinates and dimensions from a CSV file

1 Create a comma-separated value (CSV) file that contains the following parameters:

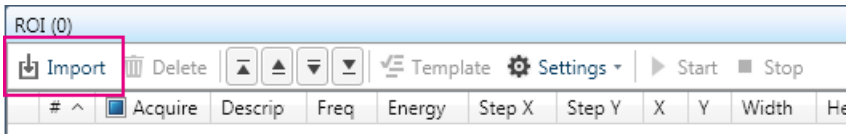
- X and Y coordinates that correspond to the upper left corner of the ROI
- Width and height of the ROI in µm
- (Optional) Sample name

The following column headers must be included: X, Y, H, W.

Name is optional. If ROI names are not included, default ROI names are assigned after the file is imported.



2 On the ROI table, click **Import**. Browse for the CSV file you want to import and double-click the file to open it. ROIs are displayed on the Slide Layout and added to the ROI table.



Copy and Paste ROIs

Use copy and paste to replicate existing ROIs. Click and drag the new ROIs to reposition them.

NOTE Ctrl + C (copy) and Ctrl + V (paste) hotkeys are supported.

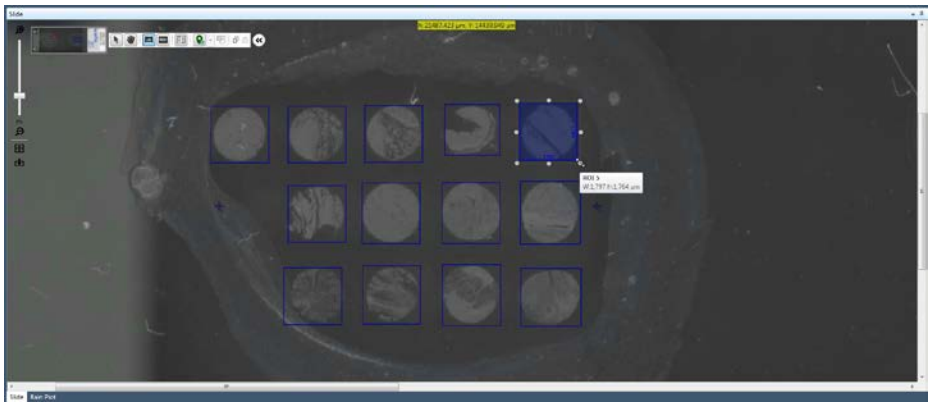
1 On the Slide toolbar, click **Select** tool  .

2 Click an ROI to select it (Ctrl + click for multiple selections, or click and drag around a group to select all).

3 Click the **Copy** tool (or Ctrl+C) and then click the **Paste** tool (or Ctrl+V).

4 Drag the new ROIs to move them.

5 If necessary, click and drag the handles to resize.



Modify ROIs in the ROI Table

After an ROI is selected on the Slide Layout, it appears in the ROI table in the pane below the image. Double-click any of the following cells to enter a different value:

- **Description**: A descriptive name for the ROI
- **X**: Top-left horizontal coordinate
- **Y**: Top-left vertical coordinate
- **Width**: ROI width in µm
- **Height**: ROI height in µm

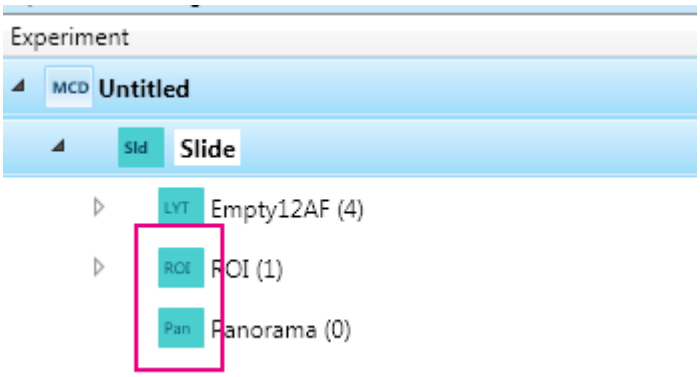
NOTE The remaining columns are related to acquisition settings and data acquisition. For more information, see Apply Acquisition Settings.

Resize ROIs

Resize ROIs by adjusting the handles on the ROI on the Slide Layout or by entering exact dimensions in the Width and Height cells in the ROI table. Double-click each cell to edit the contents.

Toggle View

Click the icon next to an ROI to hide or show it on the Slide Layout.



Delete an ROI

Delete an ROI in one of these ways:

From the Slide Layout

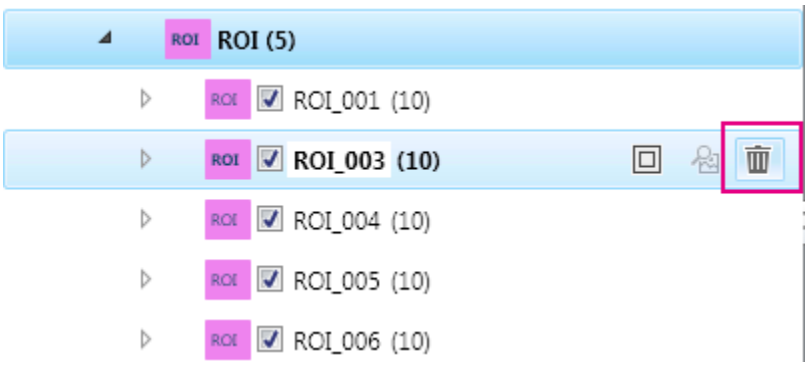
1 On the Slide Layout, click an ROI to select it (Ctrl + click for multiple selections).

2 Press the Delete key.

From Experiment Manager

1 Move the cursor over the ROI you want to delete to display the toolbar.

2 Click **Delete** icon.



From the ROI Table

1 Click the row to select the appropriate ROI.

2 Click **Delete.**

Create, Modify, or Delete Acquisition Templates

Acquisition templates contain acquisition parameters, such as the channel list and channel labels, ablation energy, ablation frequency, and step size.

Open the Acquisition Templates window to create new acquisition templates, modify existing acquisition templates, or delete acquisition templates.

Open the Acquisition Templates Window

The Acquisition Templates window can be opened from Data Acquisition (CyTOF® Administrators and Users) or Manual Tune (CyTOF Administrators only).

From Data Acquisition

1 On the ribbon, click the **Data Acquisition** tab.

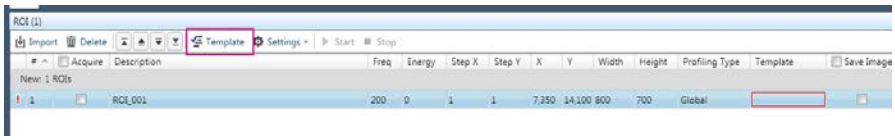
2 Click **Open** to open an existing MCD file with at least one region of interest (ROI) or create a new file. To create a new file:

   a Click **New File**.

   b On the Slide toolbar, click the ROI tool, and then draw an ROI on the Slide Layout.

3 In the ROI table, click an ROI to enable the Template button.

4 Click **Template**.



From Manual Tune (CyTOF Administrators only)

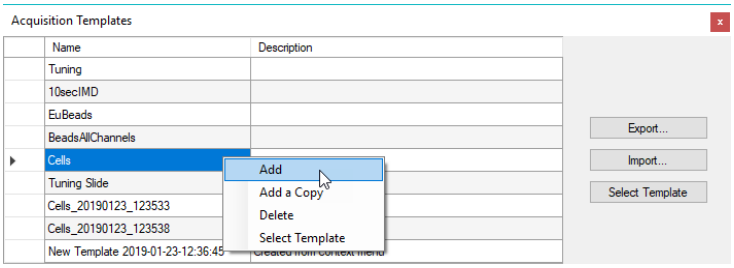
1 On the ribbon, click the **Manual Tune** tab.

2 Click **Settings** to display Manual Tune Settings.

3 Right-click the Analyte table and click **Apply Template**.

Create an Acquisition Template

In the Acquisition Templates window, right-click anywhere on the template list and click **Add** to create a new, empty template. A row is added at the end of the table.



NOTE To create a new template from an existing template, right-click a specific template and click **Add a Copy**.

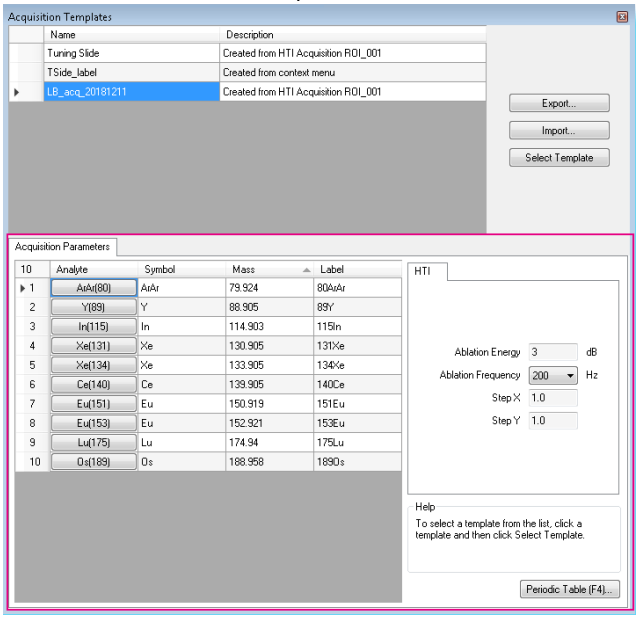
Modify an Acquisition Template

You can add or remove channels, add or modify channel labels, and modify HTI™ acquisition parameters, such as ablation energy, ablation frequency, step X and step Y.

NOTE Do not modify the template name. If the case of the characters in the template is changed—for example, if TemplateName is changed to templatename—the analyte list and acquisition settings are lost. To restore the analyte list and acquisition settings, restore the original template name.

To modify a template

1 Click a template in the Acquisition Templates window to display Acquisition Parameters on the tab below the template list.

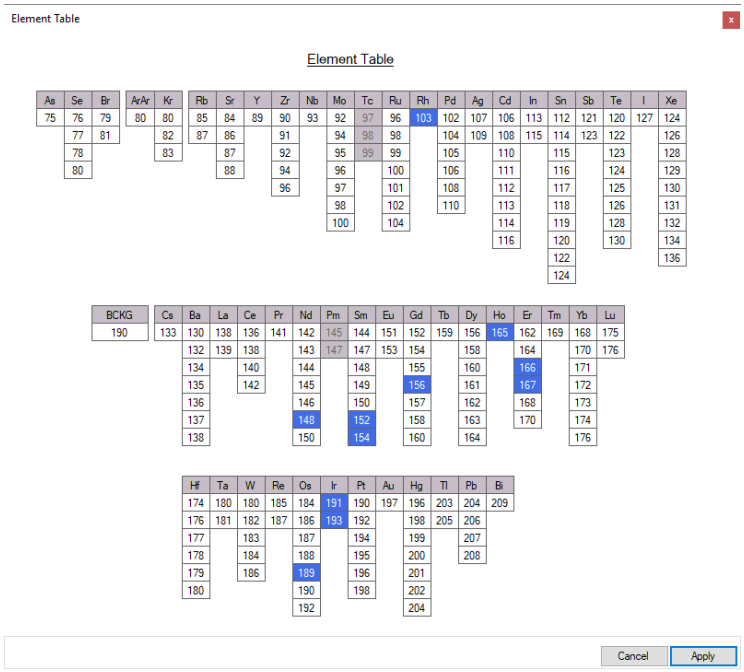


2 Add or remove channels.

a On the Acquisition Parameters tab, right-click the Analyte table, and then click **Periodic Table (F4)...**

b On the Element Table, click to select or deselect individual elements. Selected isotopes appear blue.

NOTE To select all isotopes an element, click the column header.



c After the elements are selected, click **Apply**.

NOTE You can also import a list of elements from a CSV file. See Import a Channel List.

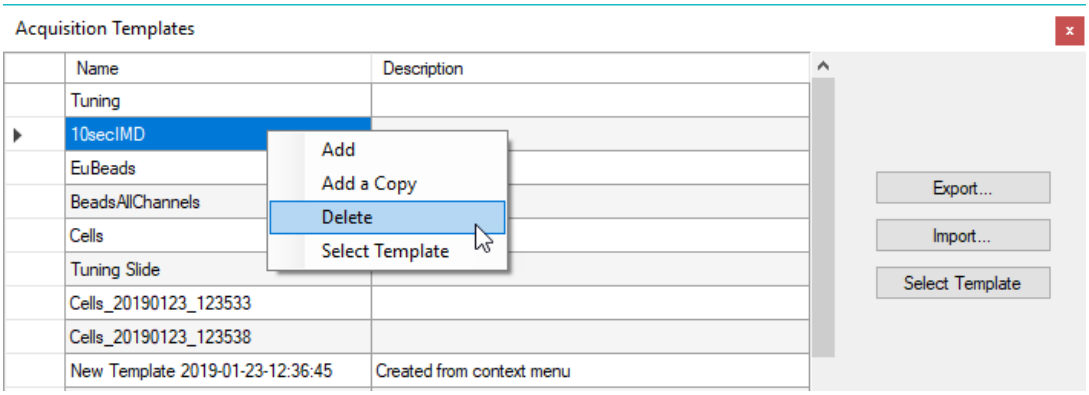
3 Modify the HTI parameters. Enter preferred values into the following text boxes:

- Ablation Energy (dB)
- Ablation Frequency (Hz)
- Step X
- Step Y

NOTE Changes to acquisition templates are automatically saved when the Acquisition Templates window is closed.

Delete an Acquisition Template

In the Acquisition Template window, in the template list, click to select the template you want to delete, right-click the row, and click **Delete**.



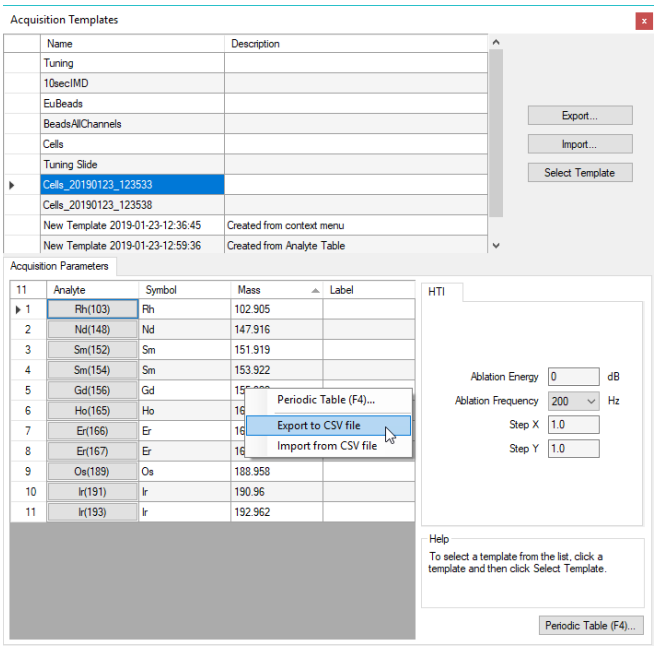
Export or Import a Channel List

Channel lists can be exported from an acquisition template to a comma separated value (CSV) file. Channel lists in CSV format can also be imported into an acquisition template.

Export a Channel List

1 Open the Acquisition Templates window and click the template that contains the channel list you want to export.

2 Right-click the Analyte table displayed below the template list and click **Export to CSV file**.



3 When prompted, browse to the preferred directory, enter a filename, and click **Save**.

Modify an Exported Channel List

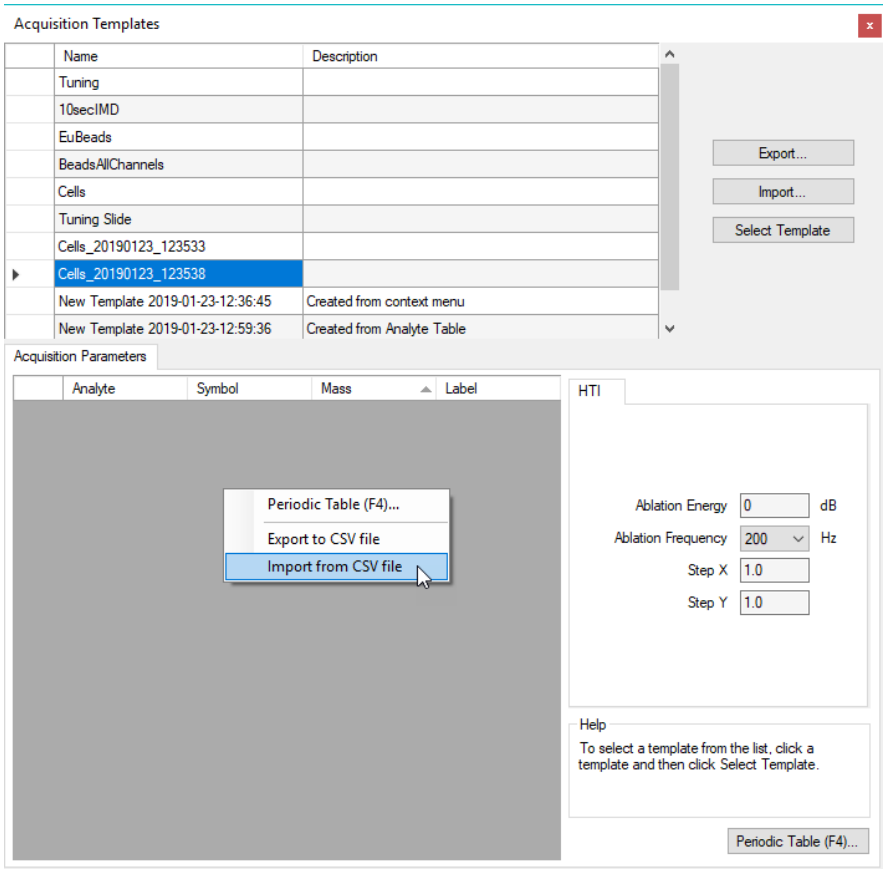
Channels can be added or removed from an exported CSV file and imported back into CyTOF Software. It is important to maintain the integrity of the file format. Otherwise the content is not imported correctly.

The file must be modified in Notepad or Excel® and saved with the .csv file extension. After the file is opened, enter the elements following the format within the file. Do not change the headings and do not leave blank lines.

Import a Channel List

1 Open the Acquisition Templates window and click a template that you want to import channels to.

2 Right-click the Analyte table displayed below the template list and click **Import from CSV file**.



3 Browse to locate the file and click **Open**. After the file loads, the Analyte table is populated with the channel list.

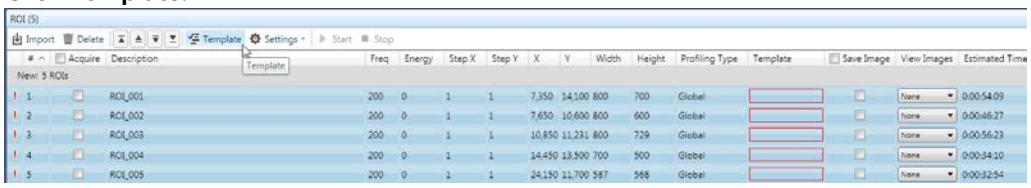
Apply Acquisition Settings

Using an Acquisition Template

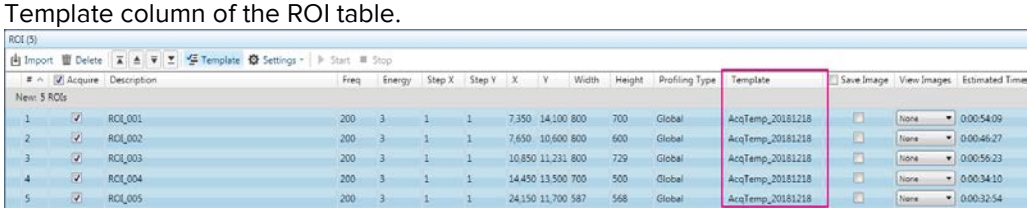
Before acquiring an ROI, an acquisition template must be selected for each ROI. An acquisition template contains the list of channels and acquisition parameters, such as ablation energy and ablation frequency.

1 On the ROI table, select one or more ROIs. Click an individual row to select one ROI. Ctrl + click (or click and drag) to select multiple ROIs.

2 Click **Template**.



3 In the Acquisition Templates window, double-click the template you want to apply. The Acquisition Template window closes and the template name is displayed in the Template column of the ROI table.

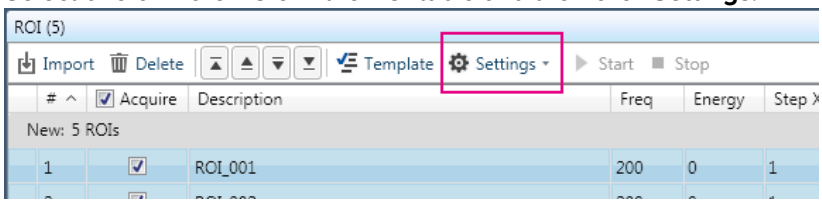


(Optional) Modify Acquisition Parameters with Quick Settings

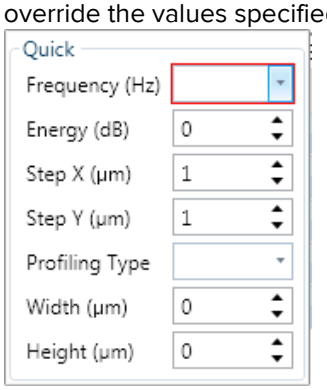
After an acquisition template is applied, you can modify acquisition parameters for individual ROIs by manually entering values in the ROI table or for multiple ROIs by selecting them in the table and applying Quick Settings.

To apply Quick Settings

1 Select one or more ROIs in the ROI table and then click **Settings**.



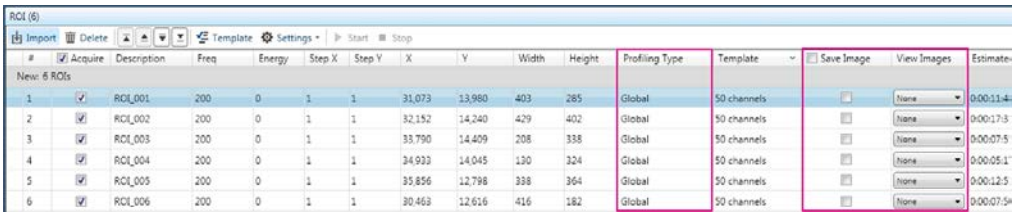
2 In the Quick Settings box, modify the values you want to apply. These values will override the values specified in the acquisition template.



3 Click anywhere to exit Quick Settings. Quick Settings will be applied to the selected ROIs.

NOTE If an acquisition template is applied after Quick Settings are applied, the acquisition template settings are applied.

Modify Additional ROI Table Settings



Profiling Type

With CyTOF® Software v7.0, before the first panorama is created or the first ROI is ablated, a global focal map is created to map the curvature of the slide. The default Profiling Type setting, Global, refers to the Global focal map and is recommended for ROIs with dimensions >500 µm. For ROIs <500 µm2, set Profiling Type to Local to create a local nine-point focal map for the ROI.

Double-click the cell to choose a different Profiling Type.

NOTE Set Profiling Type to None to deactivate profiling. This is not recommended for routine acquisition and should be used for troubleshooting purposes only.

Save Image

Check the **Save Image** box to save optical images of the ROI taken before and after ablation.

View Images

Choose to display an optical image or ion image of a specific ROI on the Slide Layout.

NOTE To display optical images, **Save Images** must be checked.

- **After**: Displays an optical image of the ROI taken after ablation completes.
- **Before**: Displays an optical image of the ROI taken before ablation starts.
- **Ion**: Displays a multicolor ion image in real-time or post-acquisition based on the channels selected for display.

To display specific channels as an ion image

1. In the View Images column, click the cell and then click **Ion**.
2. In Experiment Manager, click the corresponding ROI to display the channel list.
3. Check the channels to view and assign display colors.

When an ROI with live ion image selected is acquired, the live ion image is automatically displayed when acquisition begins. By default, the first channel in the list is selected and displayed in red.

For more information, see View the Live Ion Image.

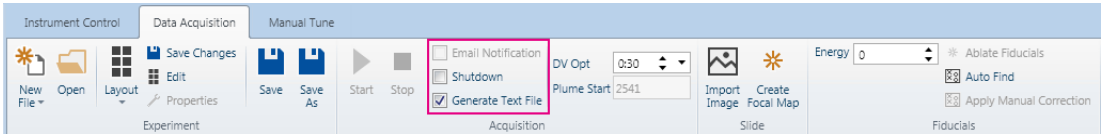
Start Data Acquisition

Start acquisition after an acquisition template is applied and all acquisition settings are configured in the ROI table.

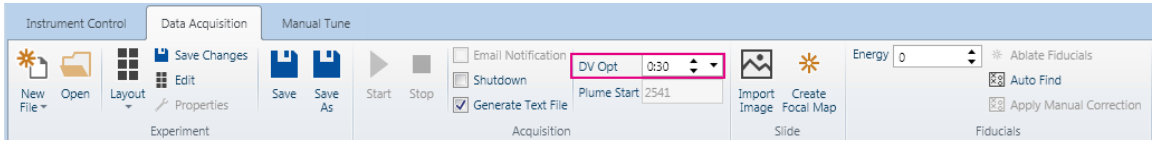
1 On the Data Acquisition toolbar, check any of the following options:

- **Email Notification**: Sends an email notification when acquisition is complete. This option is only enabled when email notifications are configured for the active account. To enable email notifications, contact your CyTOF administrator.
- **Shutdown**: Turns the instrument off after acquisition is complete.
- **Generate Text File**: Creates a text file containing signal values and associated X/Y coordinates of selected channels for each ROI. Text files are named in the following format: *MCDFileName_ROIDescription_ROI ID*. By default, Generate Text File is enabled.

IMPORTANT A text file for a 1000 x 1000 µm ROI requires 8MB per channel. Remove acquired text files from the acquisition computer regularly to ensure adequate disk space for acquisition.

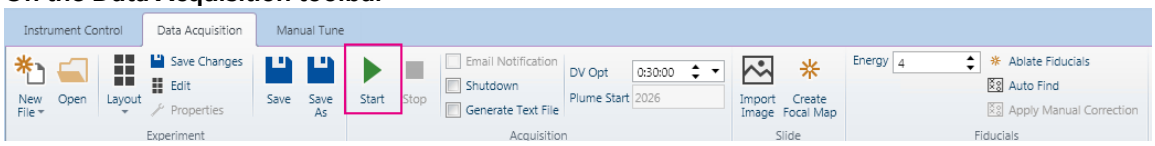


2 (Optional) Change the detector voltage optimization time interval.
By default, the detector voltage is optimized every 30 mins during acquisition. To change frequency of detector voltage optimization, in the DV Opt text box, enter a value in hours and minutes by either typing in the numbers or using the arrows. The format is HH:MM.

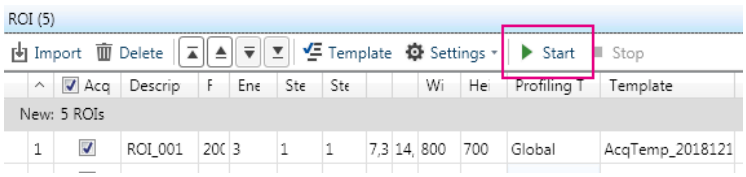


3 Click **Start** on the Data Acquisition toolbar or on the ROI table toolbar.

On the Data Acquisition toolbar

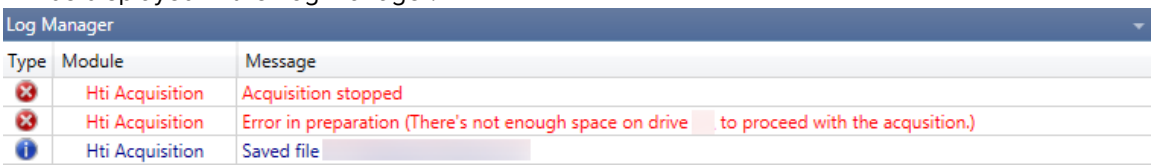


On the ROI table



NOTE

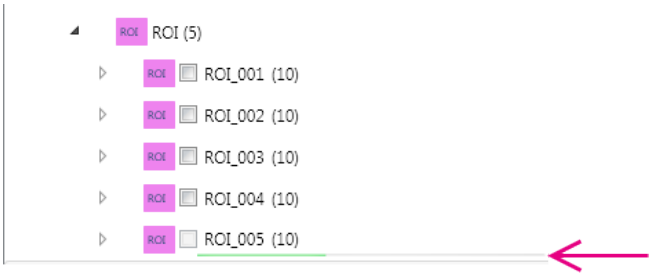
- If the Start button is unavailable because criteria for data acquisition have not been met, hover over the Start button to display a tooltip describing the issue.
- If there is not enough space on the hard drive to meet the estimated storage requirements for the acquisition, acquisition will not start and the following message will be displayed in the Log Manager:



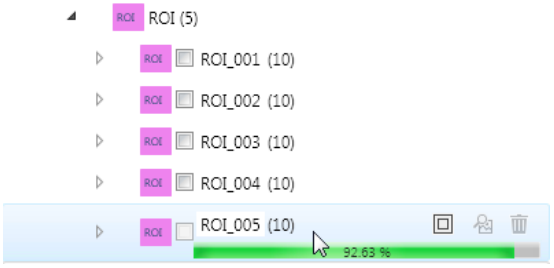
For more information, see Data Storage Requirements.

- If a Focal Map is not already created, it will be created before ablation of the first ROI begins. This takes approximately 2 minutes.

  After acquisition starts, a progress bar is displayed in Experiment Manager below the ROI name.



  Hover over the ROI name in Experiment Manager to display the full progress bar.



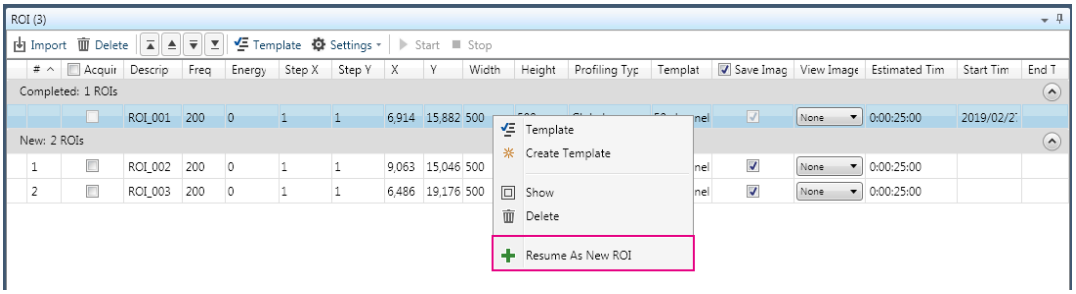
Resume Acquisition of a Partially Acquired ROI

If a region of interest (ROI) is partially acquired, acquisition of the remaining ROI can be resumed as a new ROI.

To resume acquisition of an ROI

1 In the ROI table, right-click the ROI you want to resume.

2 Click **Resume as New ROI**. A new ROI of the unacquired area is added in a row at the end of the ROI table. The same acquisition parameters are applied automatically.



3 Click **Start** to acquire the new ROI.

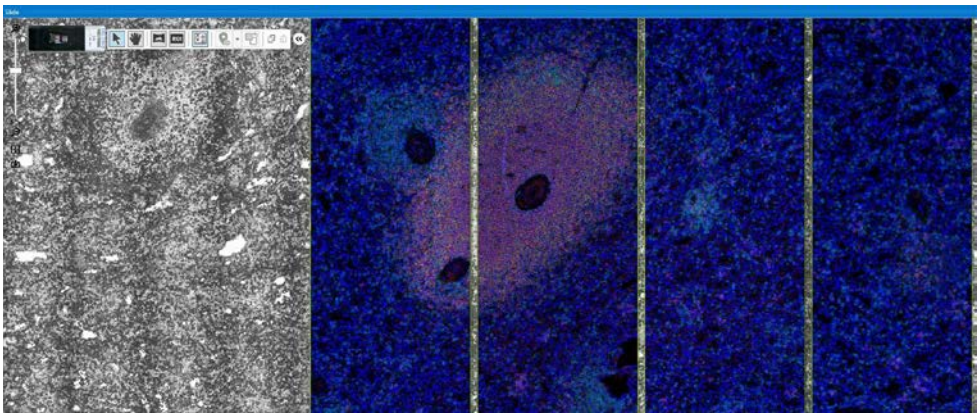
View the Rain Plot

Rain Plot displays the counts per push per channel so you can identify events as they occur. By default, 384 pushes are shown and the display is refreshed in 1-second intervals. These settings can be changed on the Rain Plot tab.

To view the Rain Plot, click the Rain Plot tab in the Slide pane.

View the Live Ion Image

Live Ion Image allows you to view data on the Slide Layout as an ROI is being ablated. Configure the channels you want to see before or during acquisition.

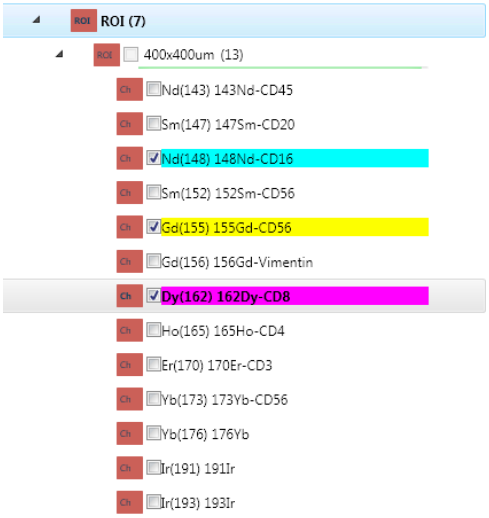


1 Specify the channels to display

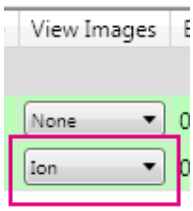
   a In Experiment Manager, click the ROI to view the channel list.

   b Check the channels you want to see.

   c Choose a color for each channel.



2 In the ROI table, in the View Images column, choose **Ion** to display the ion image on the Slide Layout.



Create Fiducials

The position of a slide relative to the camera differs slightly each time it is loaded into the Hyperion Tissue Imager. If you must unload a slide and want to resume acquisition to the same MCD file later, create fiducials as a point of reference for registration to ensure that the contents of the Slide Layout are aligned with the actual slide. After you reload the slide and open the MCD file, use the Auto Find algorithm to automatically locate the fiducials on the slide based on the coordinates saved to the MCD file. For more information, see Find Fiducials.

Slide Layouts provided with CyTOF® Software contain four red fiducial pins. Fiducial pins can be moved to accommodate sample position. It takes approximately 8 minutes to create 4 fiducials.

To create fiducials

1 If you cannot see the fiducial pins on the Slide Layout, click the Show Fiducials tool.

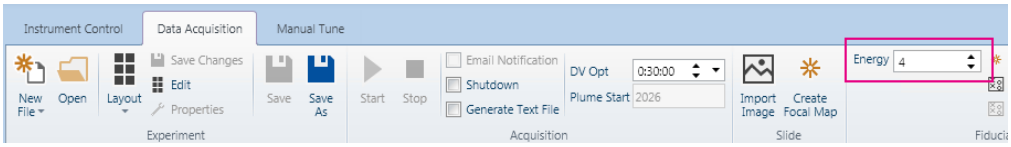
2 Inspect the area around each fiducial pin. If necessary, reposition the pin to an area free of sample and, if possible, free of hydrophobic barrier. To move a fiducial pin:

   a On the Slide toolbar, click the Select tool  , and then right-click a fiducial pin. Click **Move To Fiducial**. Camera View updates to display the area.

   b If the area is adequate for fiducial creation, then repeat for the remaining fiducial pins.

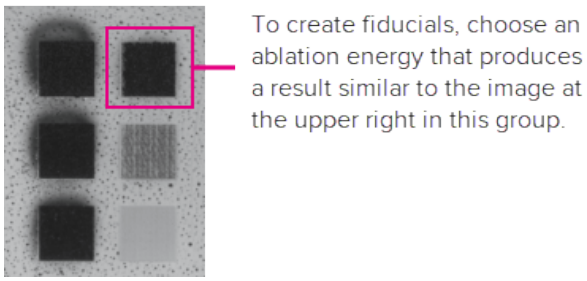
c If the area is not adequate, use the camera to find a suitable area within the green, dashed slide boundary. After a suitable area is located, click  , and then click and drag the fiducial pin to reposition it over the red cursor (indicating camera position) on the Slide Layout.

3 On the Data Acquisition toolbar, in the Fiducials group, enter an ablation energy to use for fiducials.



NOTE **About optimal laser energy for fiducials and Auto Find.** When you reload the slide to resume acquisition, coregister the Slide Layout to the fiducials using Auto Find. Auto Find automatically detects the fiducials and coregisters the Slide Layout to the actual slide. For Auto Find to work, fiducials must be dark, with clean, straight edges (no feathering). Depending on the age of the instrument laser, the optimal ablation energy can be approximately 60—80% of the energy range maximum. Hover over the Energy text box to see the energy range for your instrument.

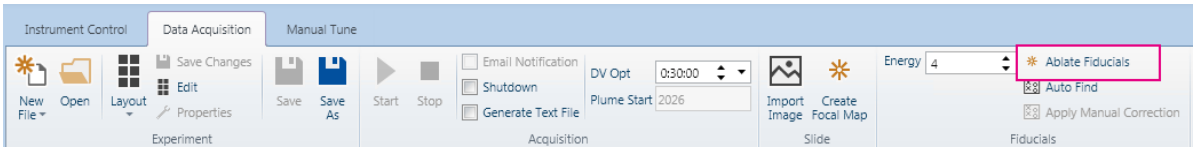
To more accurately determine optimal laser energy, create small ROIs away from the sample. Set the ablation energy for the first ROI to the maximum energy value for your system, and then step the energy down by 1 dB for the subsequent ROIs. For example, if the maximum ablation energy for your instrument is 12.43, use 12 dB, 11 dB, 10 dB, 9 dB, 8 dB. Choose the ablation energy that produces a result similar to the image below.



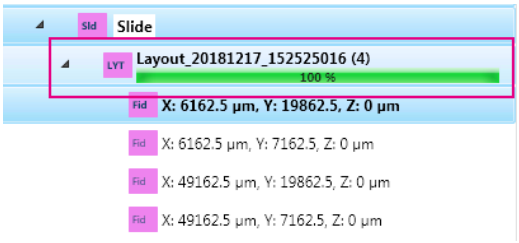
If Auto Find does not work, you can manually locate the fiducial marks and click **Apply Manual Correction** to coregister to the fiducials. For more information, see Find Fiducials.

4 Click **Ablate Fiducials**.

NOTE Fiducials can only be created once. After fiducials are created, the Ablate Fiducials button becomes unavailable.



After fiducials are created, the fiducial coordinates are saved to the MCD file and the progress bar in Experiment Manager reaches 100%



Find Fiducials

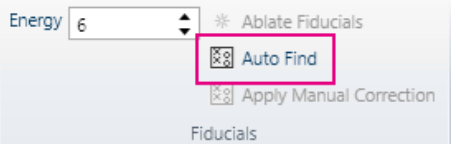
To coregister a Slide Layout to fiducials, open the MCD file that corresponds to the slide. Use the Auto Find feature to locate fiducials and align the Slide Layout to the slide loaded in the Hyperion™ Tissue Imager.

Automatically Find Fiducials

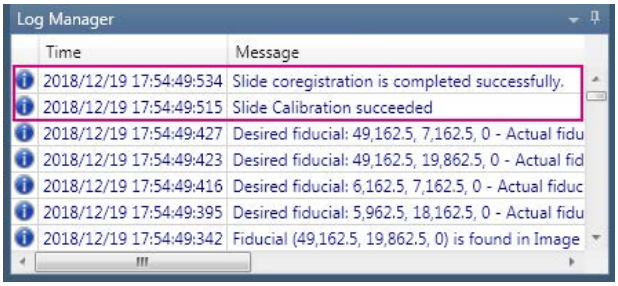
1 Load your sample into the Hyperion Tissue Imager.

2 Open the associated MCD file.

3 On the Data Acquisition tab, in the Fiducials group, click **Auto Find**. The stage moves to each set of coordinates to bring the fiducial into view of the camera. The camera creates a small panoramic image of the area.



If fiducials are found and coregistration is successful, the following message is displayed in Log Manager.



NOTE If the fiducials are not found, a message is displayed in Log Manager. Manually locate fiducials to coregister the Slide Layout contents to the fiducials.

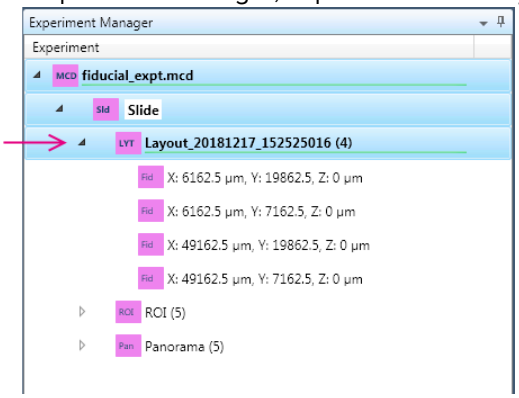
Manually Find Fiducials

If fiducials are not dark enough or were burned onto a hydrophobic barrier, Auto Find may fail. If this occurs, manual correction must be applied to coregister the Slide Layout contents to the fiducials.

NOTE Auto Find must be run before manual correction is performed. This procedure requires the panoramic images created by Auto Find.

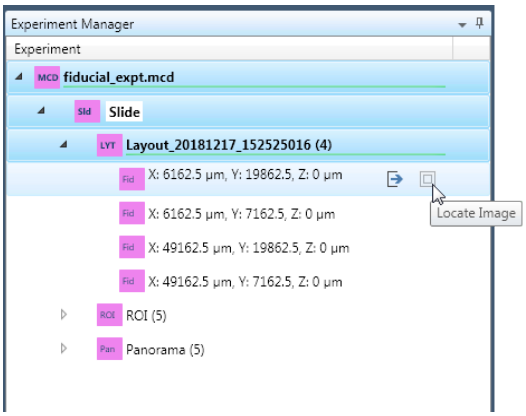
To apply manual correction

1 In Experiment Manager, expand the Slide Layout section to see the fiducial coordinates.

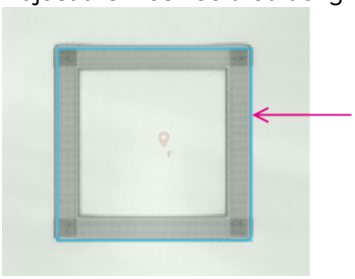


2 Hover over the first set of coordinates and click **Locate Image** to display the panorama created by Auto Find.

NOTE If the Locate Image button is not available, the panoramas are not available. Run Auto Find.



3 Adjust the mask so that it aligns with the fiducial.



4 Repeat Steps 2 and 3 for the remaining coordinates.

5 Click **Apply Manual Correction** to register the fiducials. Log Manager displays the following message: Slide coregistration is complete.

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