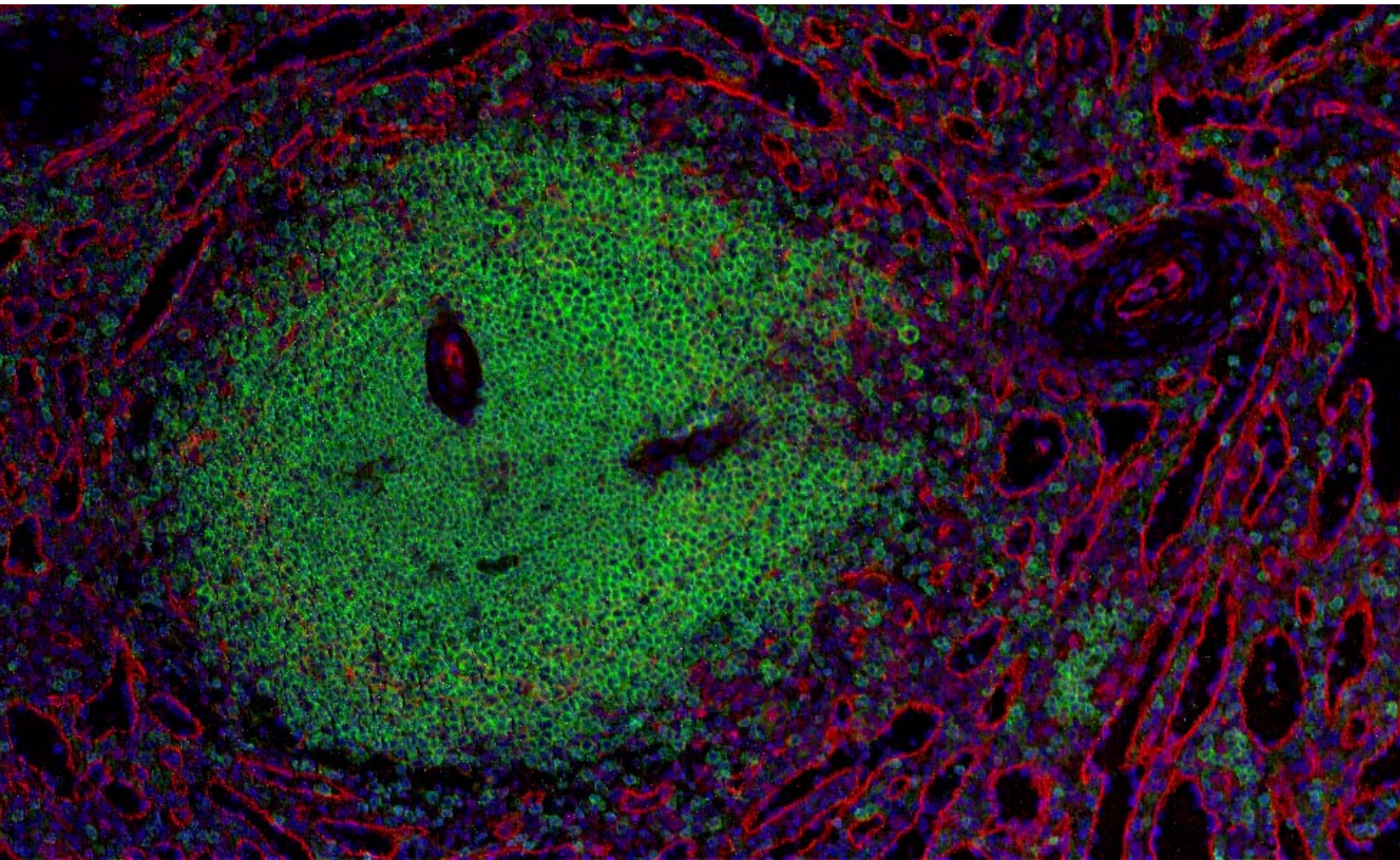


MCD Viewer v1.0.560.6

USER GUIDE



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Contents

About This Guide	4
Revision History	4
Introduction	5
About MCD Viewer	5
What's New in v1.0.560.6	5
Workspace	6
File Menu	6
Channel List	8
Channel Settings	9
Tabs	9
Chapter 1: Data Visualization	10
Create a Merged Image	10
Open Imaging Data for a Region of Interest	10
Select Channels	12
Adjust Channel Settings	13
Add a Scale Bar	14
Overlay Optical Images (Optional)	16
Save a Merged Image	19
Selected Region Summary	20
Select a Region	20
Delete a Selected Region	21
Save the Selected Region Summary	22
View Thumbnails	22
View Optical Images	22
View Total Ion Current	23
Chapter 2: Export Imaging Data	24
Export to OME-TIFF	24
Export to Text	27
Appendix A: Exported TIFF File Structure	29
Important Tags	29
OME-TIFF Metadata	31
Resources	31

About This Guide

This guide is intended for MCD™ Viewer v1.0.560.6 users who are processing Imaging Mass Cytometry™ data (MCD and text file format) acquired with the Hyperion™ Imaging System and CyTOF® Software 6.7 and later.

Revision History

Revision	Date	Description of change
1	May 29, 2017	Initial version.
2	Dec 03, 2018	Describes changes made to support data generated by CyTOF Software v7.0. Regions of interest are no longer grouped by panorama.
3	Dec 20, 2019	Adds information for Windows non-administrative users and the new default setting for gamma.

Introduction

About MCD Viewer

MCD Viewer is post-acquisition data processing software that allows users to visualize, review, and export Imaging Mass Cytometry data acquired with the Hyperion Imaging System and CyTOF Software 6.7 and later.

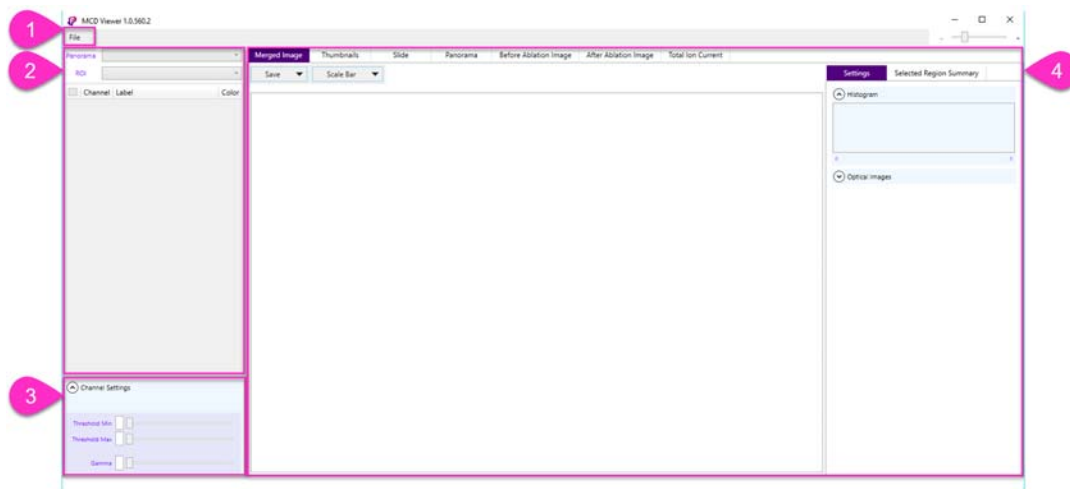
- Create and save merged ion images to 8- or 16-bit color TIFF files for presentation and publication purposes.
- Export raw data to the following formats for additional processing and analysis with third-party software:
 - Single-page or multipage 16-bit grayscale OME-TIFF (.ome.tiff)
 - Single-page or multipage 32-bit grayscale OME-TIFF (.ome.tiff)
 - Text (.txt)

What's New in v1.0.560.6

- The Gamma default setting for merged images and total ion current images (see [Advanced Channel Settings](#)) is changed from 0.5 to 1.
- MCD Viewer can now be run from non-administrative Windows® accounts.

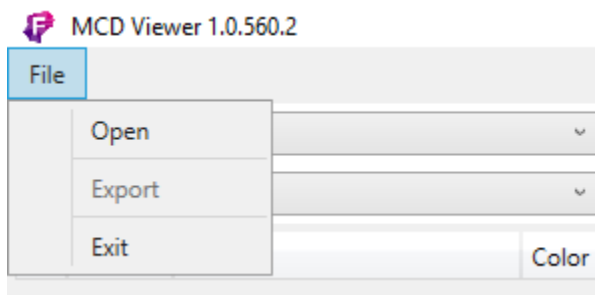
NOTE Windows administrative privileges are still required to install the software.

Workspace



- 1 File menu
- 2 Channel list
- 3 Channel settings
- 4 Tabs

File Menu



Open: Open MCD or text file types.

Export: Display the Export dialog box.

Exit: Exit and close MCD Viewer.

Supported File Types

MCD

MCD (.mcd) files created by CyTOF Software 6.7 and later. MCD files contain imaging data for one or more regions of interest.

Text

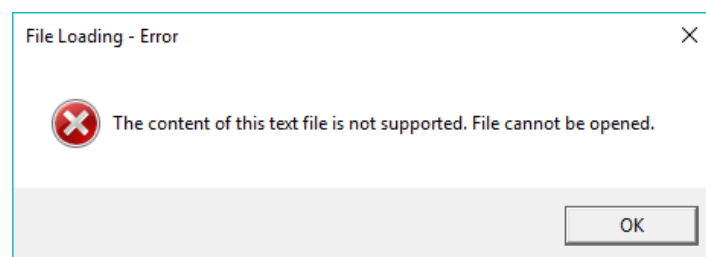
Tab-delimited text (.txt) files created by CyTOF Software 6.7 and later. Text files contain imaging data for individual regions of interest (ROIs). The header row is formatted Start_push, End_push, Pushes_duration, X, Y, Z, channel(label):

header row

	A	B	C	D	E	F	G	H	I	J	K
	Start_push	End_push	Pushes_duration	X	Y	Z	As755(As7	Se766(Se7	Se777(Se7	Se788(Se7	Br799(Br7)
2	2000	2191	192	0	0	0	200.335	200.181	213.075	199.039	180.007
3	2192	2383	192	1	0	1	191.017	200.164	206.277	189.537	173.151
4	2384	2575	192	2	0	2	183.372	189.886	200.751	182.686	165.81
5	2576	2767	192	3	0	3	173.586	178.744	190.62	175.639	162.841
6	2768	2959	192	4	0	4	163.689	171.917	179.456	165.32	153.258
7	2960	3151	192	5	0	5	163.467	162.279	169.404	155.673	143.62
8	3152	3343	192	6	0	6	154.682	157.547	151.784	142.328	133.87
9	3344	3535	192	7	0	7	143.145	148.355	140.323	130.562	125.773
10	3536	3727	192	8	0	8	133.526	143.452	132.731	119.432	114.594
11	3728	3919	192	9	0	9	129.745	137.742	123.387	110.847	111.456
12	3920	4111	192	10	0	10	118.319	123.508	112.195	103.829	102.49
13	4112	4303	192	11	0	11	104.113	109.051	98.598	91.916	92.347
14	4304	4495	192	12	0	12	95.773	100.528	93.496	87.973	86.043
15	4496	4687	192	13	0	13	96.023	96.152	86.318	76.424	77.414
16	4688	4879	192	14	0	14	90.407	87.509	79.927	71.139	71.082
17	4880	5071	192	15	0	15	73.254	64.902	62.568	63.554	63.657
18	5072	5263	192	16	0	16	66.832	56.852	57.325	57.213	63.741
19	5264	5455	192	17	0	17	57.575	49.136	49.176	50.698	55.111
20	5456	5647	192	18	0	18	44.981	46.211	41.585	39.373	43.26
21	5648	5839	192	19	0	19	38.893	35.153	34.384	31.151	31.184

Pano 02_2_ROI 01_1

IMPORTANT Do not modify text files containing imaging data. The following error is displayed if a modified or unsupported text file is opened:



Channel List

Panorama: 20170317_Eric_spleen_0007000A
ROI: 251_10min_1000x1000_4dB_200Hz

<input type="checkbox"/>	Channel	Label	Color
<input type="checkbox"/>	ArAr(80)	80ArAr	<input type="text"/>
<input type="checkbox"/>	In(115)	115In-lipid	<input type="text"/>
<input type="checkbox"/>	Sn(120)	120Sn	<input type="text"/>
<input type="checkbox"/>	I(127)	127I	<input type="text"/>
<input type="checkbox"/>	Cs(133)	133Cs	<input type="text"/>
<input type="checkbox"/>	Xe(134)	134Xe	<input type="text"/>
<input type="checkbox"/>	Ba(138)	138Ba	<input type="text"/>
<input checked="" type="checkbox"/>	Nd(143)	143Nd-VIM	<input type="text"/>
<input type="checkbox"/>	Sm(149)	149Sm-CD45RO	<input type="text"/>
<input type="checkbox"/>	Gd(155)	155Gd-CD45RA	<input type="text"/>
<input type="checkbox"/>	Gd(156)	156Gd-CD4	<input type="text"/>
<input type="checkbox"/>	Dy(161)	161Dy-CD20	<input type="text"/>
<input type="checkbox"/>	Dy(162)	162Dy-CD8a	<input type="text"/>
<input type="checkbox"/>	Dy(163)	163Dy-CD235	<input type="text"/>
<input type="checkbox"/>	Dy(164)	164DyCD15	<input type="text"/>
<input type="checkbox"/>	Er(166)	166Er-CD74	<input type="text"/>
<input type="checkbox"/>	Tm(169)	169Tm-collagen	<input type="text"/>

Channel Settings

Nd(143) 143Nd-VIM

Threshold Min: 0
Threshold Max: 90.98
(Dual count) 0.00 1,082.00

Advanced

The channel list displays all channels acquired for the selected ROI. The channel list populates after a data file is opened and a panorama and ROI are selected.

NOTE Text files contain data for individual ROIs only, so the panorama name and ROI name are automatically loaded when a text file is opened.

channel name
and label

Channel Settings

Channel settings are displayed at the bottom of the channel list. Channel settings apply to individual channels. Changes are reflected in the merged image.

Tabs

For ease of use, the workspace is divided into tabs. Click a tab to bring it forward.

- **Merged Image:** Merge and display individual ion images.
- **Thumbnails:** View thumbnails of individual channel ion images.
- **Slide:** View the optical image of the slide (if acquired).
- **Panorama:** View the panorama image (if acquired).
- **Before Ablation Image:** View the sample before ablation (if acquired).
- **After Ablation Image:** View the sample after ablation (if acquired).
- **Total Ion Current:** View an ion image representative of the sum of all ions across the full signal range for the selected channels.

Chapter 1: Data Visualization

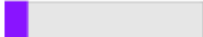
Create a Merged Image

Select one or more channels to create a merged image for evaluation of experimental results. Save images as 8- or 16-bit color TIFF files.

Open Imaging Data for a Region of Interest

The channel list populates after an MCD or text file is opened and a panorama and ROI are selected from the lists. Text files containing data from individual ROIs begin to load immediately.

The time required to load the channel data varies depending on the ROI dimensions and the number of channels acquired. While the data is loading, a progress bar is displayed in the lower left corner of the MCD™ Viewer user interface.

Width: 1000, Height: 999  108Pd(Pd108Di)

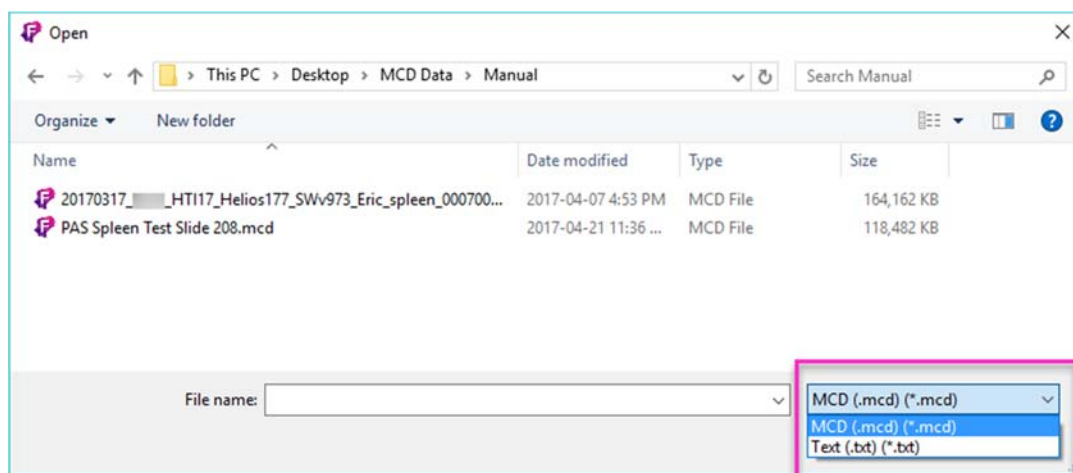
To Open Imaging Data

- 1 Click **File > Open**.

IMPORTANT Data must be stored on a local hard drive.

- 2 Browse to locate an MCD (.mcd) or text (.txt) file acquired with CyTOF® Software.

The default file type is MCD (.mcd). To browse for text files, change the file type to Text (.txt).



NOTE After a file is opened, a dialog box prompts you to choose a panorama and ROI to load.

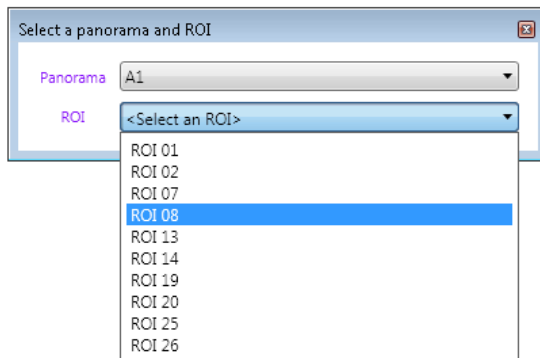
3 Choose an ROI to load.

IMPORTANT ROIs load differently depending on the CyTOF Software version used to acquire the data.

- Load an ROI from Data Acquired with v6.7
- Load an ROI from Data Acquired with v7.0

Load an ROI from Data Acquired with v6.7

ROIs are grouped by panorama. When prompted, choose a panorama, and then choose from a list of ROIs within that panorama.



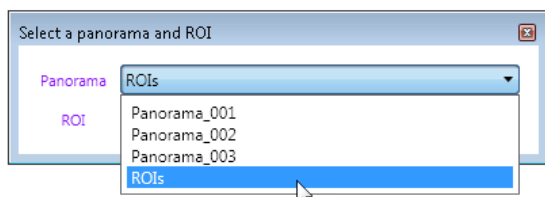
- From the Panorama drop-down list, click the panorama that contains the ROI you want to load. The ROI drop-down list is populated with the ROIs within the selected panorama.
- From the ROI drop-down list, choose the ROI you want to load.

NOTE The **Panorama** and **ROI** boxes (above the channel list) update according to the selections made. Use these controls to change the panorama and ROI selections after the initial ROI has loaded.

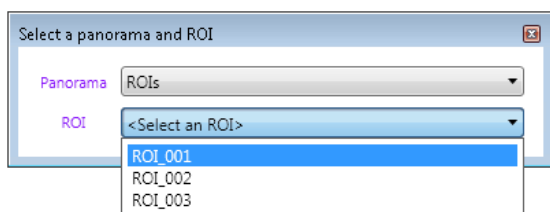
Load an ROI from Data Acquired with v7.0

Unlike how they are organized in v6.7, ROIs are not grouped by panorama in MCD files acquired with v7.0. Instead, panoramas and ROIs are separate objects.

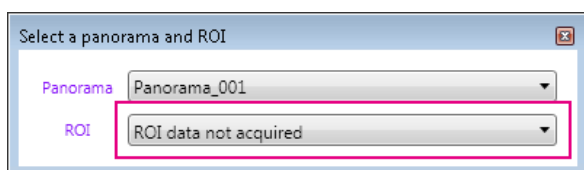
- a From the Panorama drop-down list, click **ROIs**. The ROI drop-down is populated with all ROIs acquired to the MCD file.



- b From the ROI drop-down list, click the ROI you want to load.



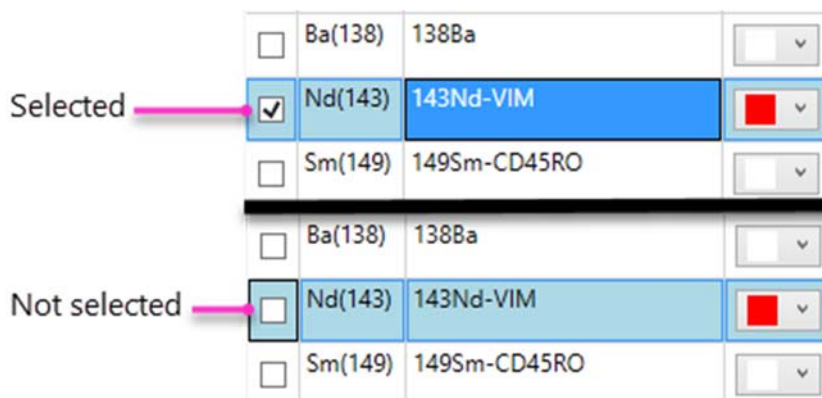
NOTE Choose a panorama other than “ROIs” to view the panoramic image. The following message is displayed indicating there is no ROI data associated with the chosen panorama. Close the dialog box and then click the Panorama tab to see the image.



Select Channels

Selected channels are merged and displayed in the Merged Image tab.

- 1 After the channel list is populated, click the box next to a channel to select it and enable channel settings. (Highlighting a row does not select the channel.)



2 Click the **Merged Image** tab if it is not active.

OR

If required, to aid channel selection, preview ion images in the Thumbnails tab.

a Click the **Thumbnails** tab.

- Click a thumbnail to highlight the corresponding channel in the channel list.
- Click a channel in the channel list to highlight the corresponding thumbnail.

b Click the **Merged Image** tab to view the merged ion image of the selected channels.

3 Click the color list next to a selected channel to assign a color.

NOTE The default color is white.

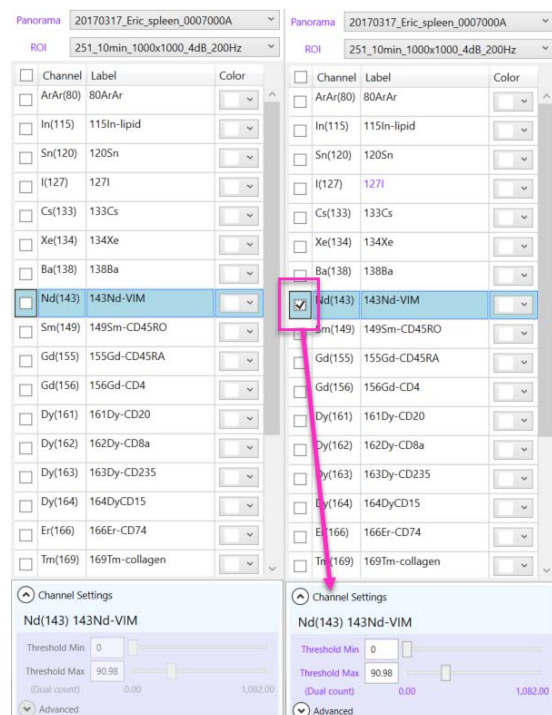
Adjust Channel Settings

To optimize the appearance of the merged image, adjust the Channel Settings.

Channel settings apply to the selected channel. Settings for each channel must be optimized individually.

The default Threshold Min (minimum signal threshold) setting is 0 and the default Threshold Max (maximum signal threshold) setting is 98% of the cumulative signal.

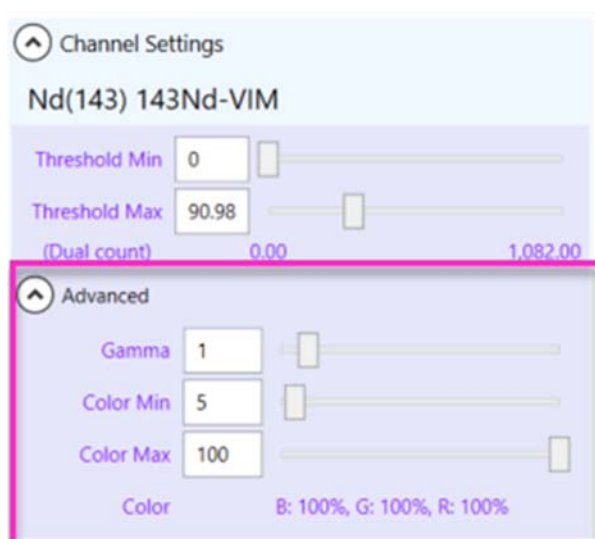
NOTE Channel Settings are enabled when the channel check box is checked.



To Adjust Channel Settings

- 1 Select a channel in the channel list.
- 2 To increase or decrease the signal range displayed in the merged image, adjust the Threshold Min and Threshold Max by entering a value in the text box or adjusting the slider.

Advanced Channel Settings



The Advanced Channel Settings include:

- Gamma correction
- Color Min/Max

To optimize the tonal levels of the merged image, adjust Gamma. The default Gamma setting is 1.

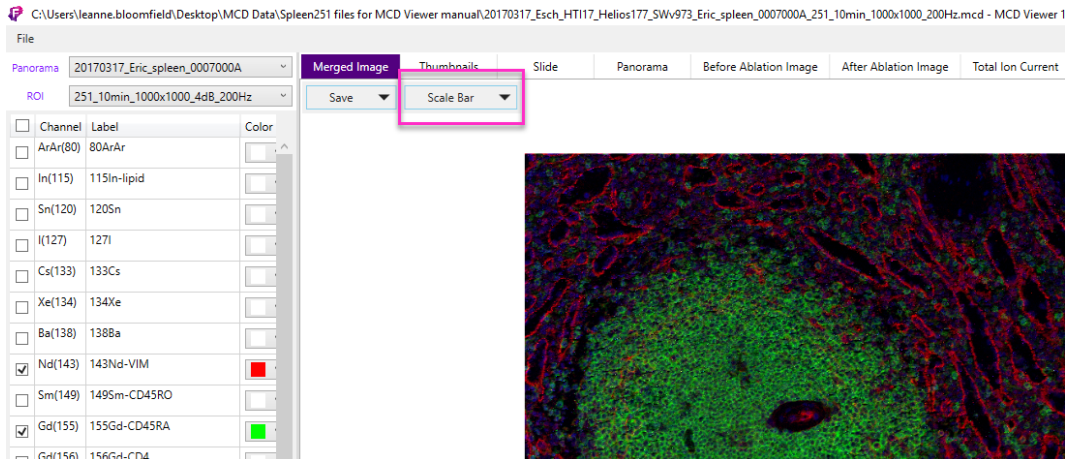
To set the minimum and maximum color intensity applied across the signal range, adjust the Color Min and Color Max settings. By default, Color Min is set to 5% to help visually discern low-intensity pixels from the image background.

Add a Scale Bar

Add a customized scale bar to the merged image.

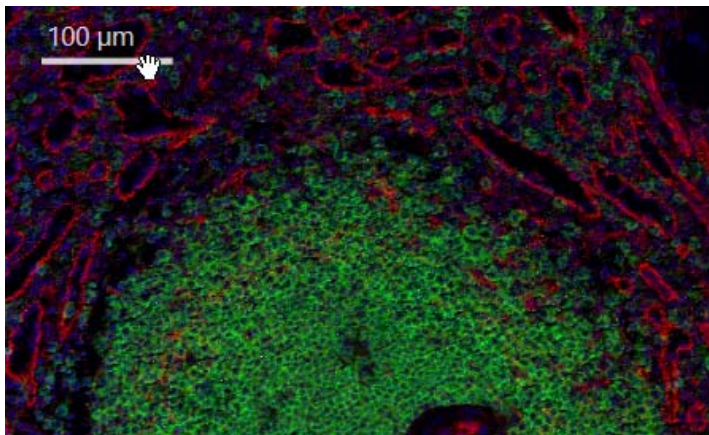
To Add a Scale Bar

- 1 In the Merged Image tab, above the merged image, click **Scale Bar > Show Scale Bar**.

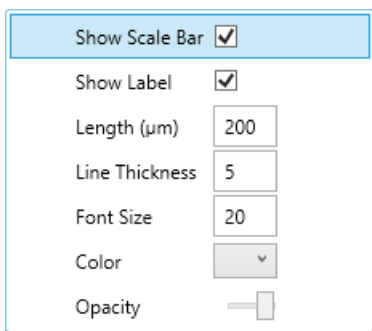


- 2 To see the scale bar as changes are made, click and drag it to the lower half of the merged image.

- a Move the mouse over the scale bar until the mouse cursor changes to a hand.



- b Click and drag the scale bar to another location on the image.
 - c Click **Scale Bar** to return to the scale bar menu.



- 3 To display the length and units above the scale bar, click **Show Label**.

- 4 Modify the scale bar settings to achieve the desired appearance:

- a Length (μm): Enter the length of the scale bar in microns.

- b** Line Thickness: Enter the line thickness in pixels (px).
 - c** Font Size: Applies to the label. Enter the font size in points (pt).
 - d** Color: Click to select a scale bar color.
 - e** Opacity: Adjust the slider to increase or decrease the scale bar opacity.
- 5** To save the scale bar with the merged image, click **Save > With Burn-in**.

Overlay Optical Images (Optional)

If images of the sample were taken before and after ablation and saved to the MCD file at the time of acquisition, they can be overlaid on the merged image.

The overlay feature allows easy comparison of the merged image to the original sample, for example, to show localization of a particular marker to a specific region of the tissue.

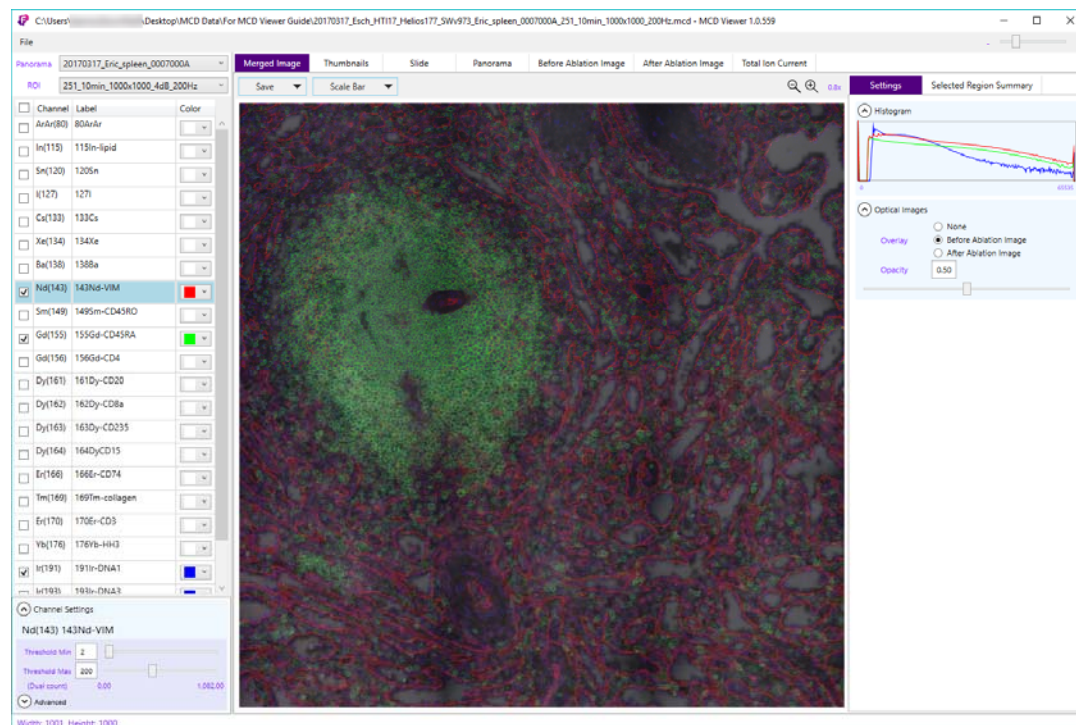
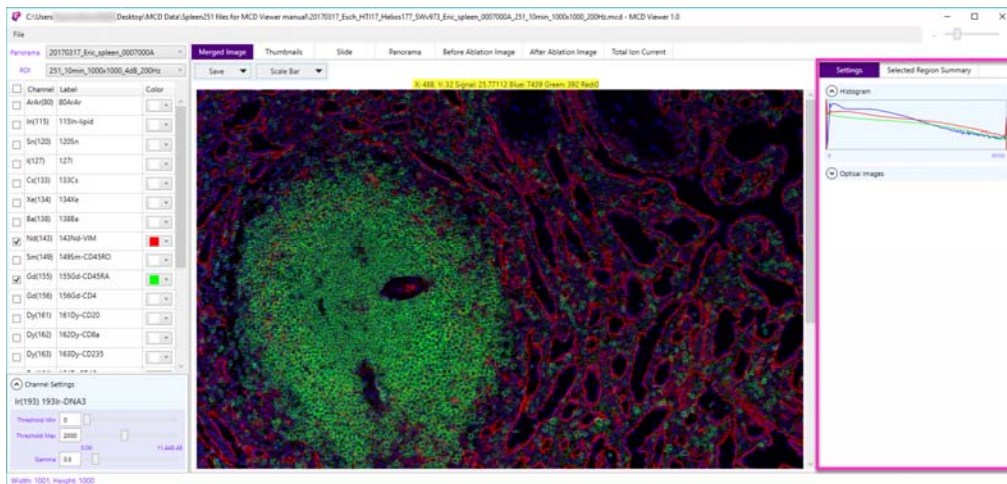


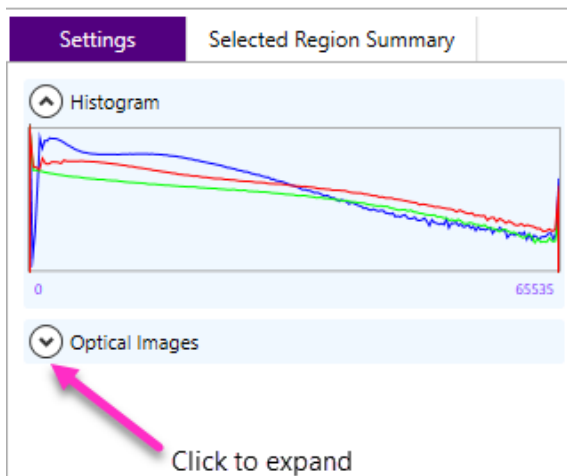
Figure 1. Image before ablation overlaid on merged image

To Overlay an Optical Image

- 1 On the Merged Image tab, click the **Settings** tab.

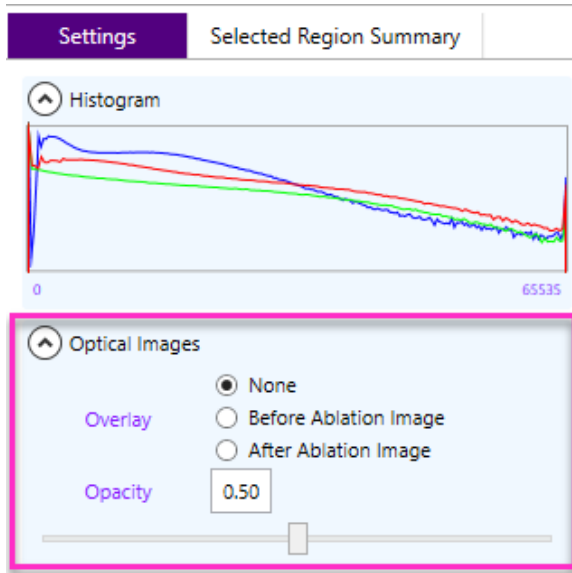


- 2 Click **Optical Images** to expand the controls.



3 Click one of the following Overlay options:

- **None** (default)
- **Before Ablation Image**
- **After Ablation Image**



4 To increase or decrease the opacity of the optical image, adjust the **Opacity** slider or enter a value in the text box.

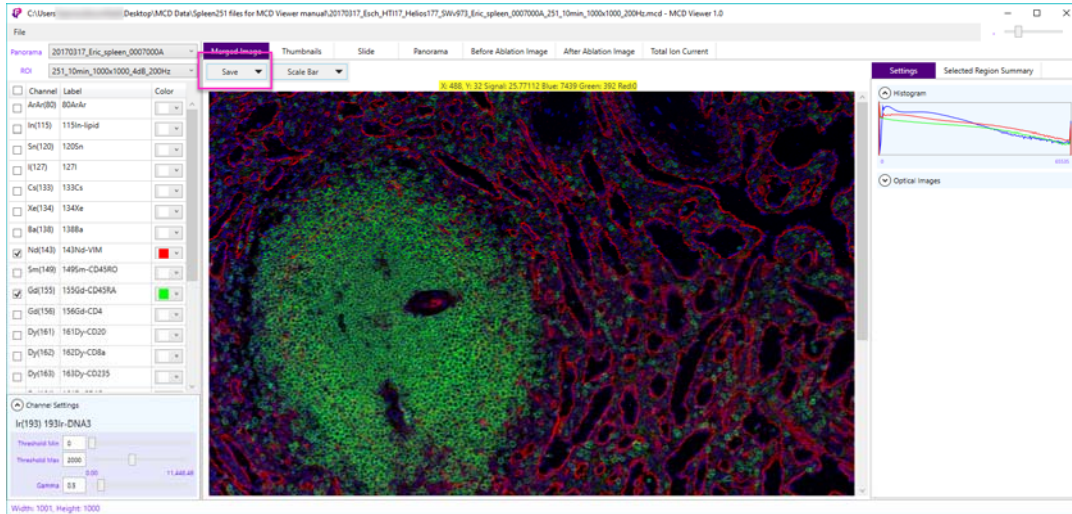
5 To save the image, click **Save > With Burn-in**.

Save a Merged Image

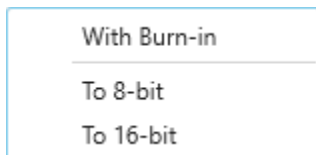
Merged images are saved as 8- or 16-bit color TIFF images.

To Save a Merged Image

- 1 Click **Save** to expand the menu.



Click to choose one of the following options from the menu.



- **With Burn-in:** To save to an 8-bit color TIFF. This option is available when an optical image or object, such as a scale bar, is overlaid on the merged image. Choose this option to save all overlaid layers with the merged image.
- **To 8-bit:** To save the merged image to an 8-bit color TIFF.
IMPORTANT Overlaid optical images or objects are not saved.
- **To 16-bit:** To save the merged image to a 16-bit color TIFF (increased color depth compared to 8-bit).
IMPORTANT Overlaid optical images or objects are not saved.

2 In the Save As dialog box:

- a** Browse to a folder to save the image.
- b** Enter a filename or accept the default filename.

NOTE The default filename format follows this convention:
ROI_ChannelColorChannelLabel.tiff. For example, 1000x1000_4dB_200Hz_Red143Nd-
VIM_Lime155Gd-CD45RA_Blue191Ir-DNA1_251_10min.tiff

3 Click **Save**.

NOTE A text file containing the channel settings used to create the merged image is saved with the same name as the merged image file.

Selected Region Summary

Generate and export basic statistical data for selected areas within the merged image. The following statistics are calculated by channel based on the raw data in the selected region.

- Min: the minimum channel signal
- Max: the maximum channel signal
- Integral: the sum of all channel signal values within the selected region
- Mean: the sum of all signal values (dual counts) divided by the total number of signal values
- CV(%): percent coefficient of variation, calculated using population standard deviation

To Display the Selected Region Summary

Click the **Merged Image** tab, and then click the **Selected Region Summary** tab.

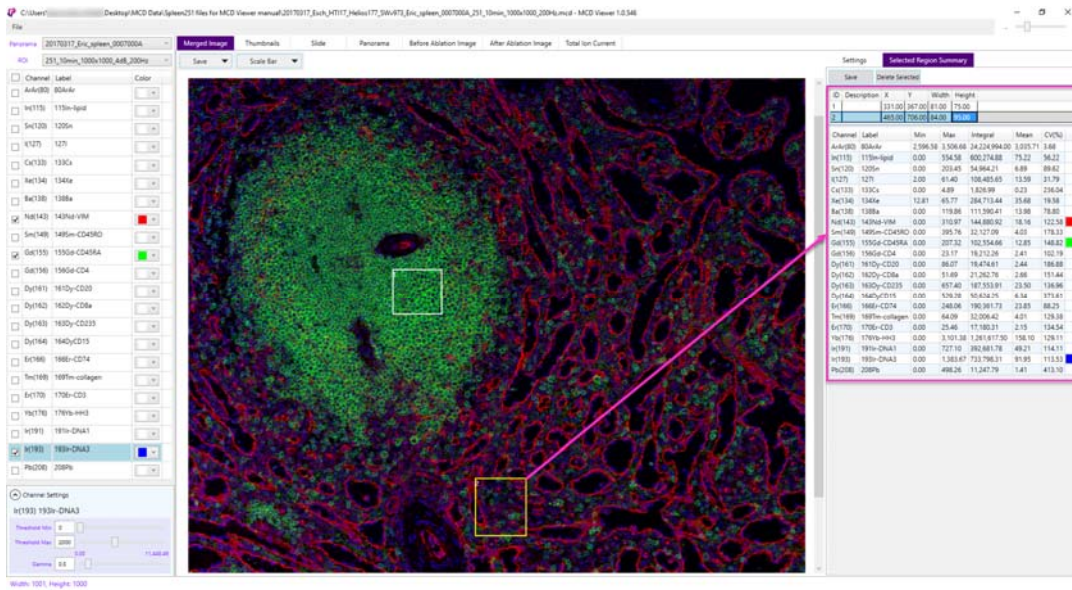
Select a Region

Select one or more regions within a merged image. A number is assigned to each region as it is selected. The ID number is displayed on the merged image and in the ID table (upper right) that is populated as regions are selected.

To Select a Region

- 1** On a merged image, click and diagonally drag to select an area.
- 2** Repeat Step 1 until each required region is selected.

- To review the Selected Region Summary for a specific region, click a row in the ID table.
The statistical data, by channel, for the selected region is displayed below.



NOTE Double-click the **Description** cell in the ID table to enter a description for the selected region.

Delete a Selected Region

Delete a row from the ID table to delete the selected region from the merged image. The remaining selected regions are not renumbered.

To Delete a Selected Region

- Click to select a row in the ID table. Click and drag upward or downward to select multiple rows.
- Click **Delete Selected** or press the **Delete** key.

Settings		Selected Region Summary				
		Save	Delete Selected			
ID	Description	X	Y	Width	Height	
1		331.00	367.00	81.00	75.00	
2		465.00	706.00	84.00	95.00	
Channel	Label	Min	Max	Integral	Mean	CV(%)
ArAr(80)	80ArAr	2,596.58	3,506.68	24,224,994.00	3,035.71	3.68
In(115)	115In-lipid	0.00	554.58	600,274.88	75.22	56.22
Sn(120)	120Sn	0.00	203.45	54,964.21	6.89	89.62
I(127)	127I	2.00	61.40	108,485.65	13.59	31.79
Cs(133)	133Cs	0.00	4.89	1,826.99	0.23	236.04
Xe(134)	134Xe	12.81	65.77	284,713.44	35.68	19.58
Ba(138)	138Ba	0.00	119.86	111,590.41	13.98	78.80
Nd(143)	143Nd-VIM	0.00	310.97	144,880.92	18.16	122.58
Sm(149)	149Sm-CD45RO	0.00	395.76	32,127.09	4.03	178.33

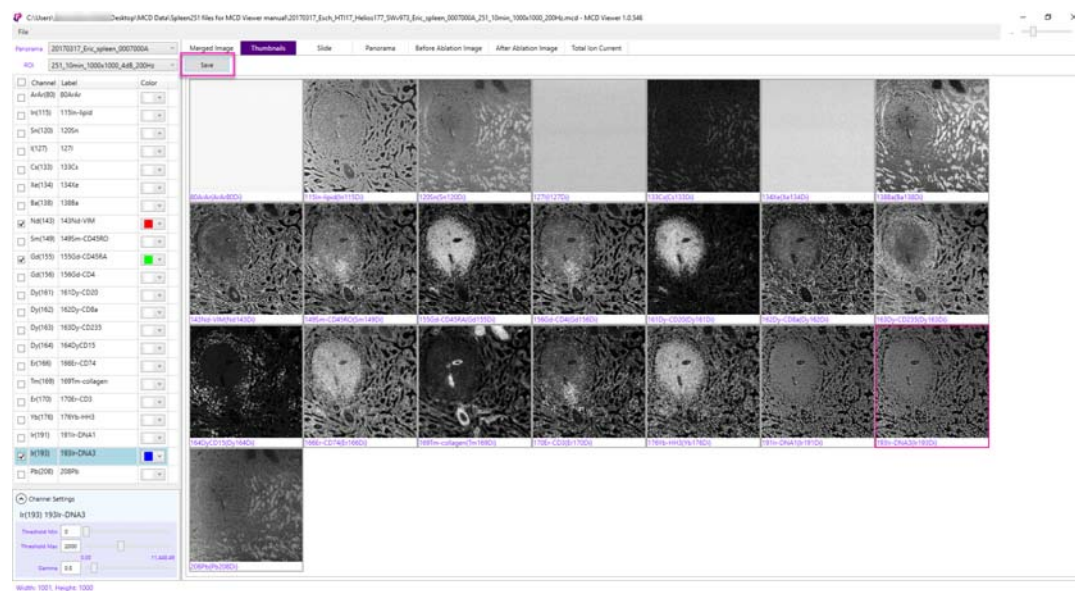
Save the Selected Region Summary

To save the contents of the selected region summary to a tab-delimited text file, choose the **Selected Region Summary** tab, and then click **Save**.

View Thumbnails

The Thumbnails tab displays a collage of channel-specific ion images. Below each thumbnail is the corresponding channel name and label.

To save the thumbnail collage as a 16-bit TIFF, click **Save**.



View Optical Images

You can individually view and save any of the following images that are stored in the MCD file:

- Slide image
- Panorama
- Before ablation image
- After ablation image

NOTE If the MCD file was acquired with CyTOF Software v7.0, the panorama is no longer linked to the ROI, so the Panorama tab is empty. To view a specific panorama, choose it from the Panorama drop-down list, and then click the **Panorama** tab.

To View and Save an Optical Image

- 1 Click the **Slide**, **Panorama**, **Before Ablation Image**, or **After Ablation Image** tab.
- 2 Click **Save**.

View Total Ion Current

The total ion current (TIC) is an ion image that represents the summed signal of the selected channels. The following default channel settings are applied to the image:

- Signal range of 0% to 98% of the cumulative signal.
- Gamma correction of 1.

To Generate and Save a TIC

- 1 Click the **Total Ion Current** tab.
- 2 Check the boxes next to the channels to select (if not previously selected).
- 3 Click **Generate**.
- 4 Click **Save**.

Chapter 2: Export Imaging Data

Export MCD (.mcd) and text (.txt) files to single-page or multipage 16- or 32-bit grayscale OME-TIFF or text files for analysis with third-party software.

Files are exported to the same Windows® directory as the source file, with a different directory structure for each type of exported file:

- Single-page OME-TIFF files:
File Name\ROI ID_ROI Name\Channel_ChannelLabel.ome.tiff
- Multipage OME-TIFF files:
File Name\ROI ID_ROI name\ROI ID_ROI Name.ome.tiff
- Text files:
File Name\AcquisitionDate_ROI Name.txt

Export to OME-TIFF

Exported OME-TIFF (.ome.tiff) file headers contain tags that define raster image data that is specific to each channel. File headers also contain an OME-XML metadata block compatible with Open Microscopy Environment (OME). For additional information about the file format and the OME-TIFF XML metadata block, see [Appendix A: Exported TIFF File Structure](#).

A tab-delimited text file summarizing the exported content is created and saved to the export directory. This file contains the page number (for multipage OME-TIFFs), channel name, channel label, minimum signal value, and maximum signal value.

Page	Channel	Label	MinValue	MaxValue
0	ArAr(80)	80ArAr	0	3708
1	In(115)	115In-lipid	0	7383
2	Sn(120)	120Sn	0	868
3	I(127)	127I	0	124
4	Cs(133)	133Cs	0	99
5	Xe(134)	134Xe	0	250
6	Ba(138)	138Ba	0	5204
7	Nd(143)	143Nd-VIM	0	1082
8	Sm(149)	149Sm-CD45RO	0	559
9	Gd(155)	155Gd-CD45RA	0	1541

NOTE Depending on file size, file export may take several minutes to complete.

To Export to OME-TIFF

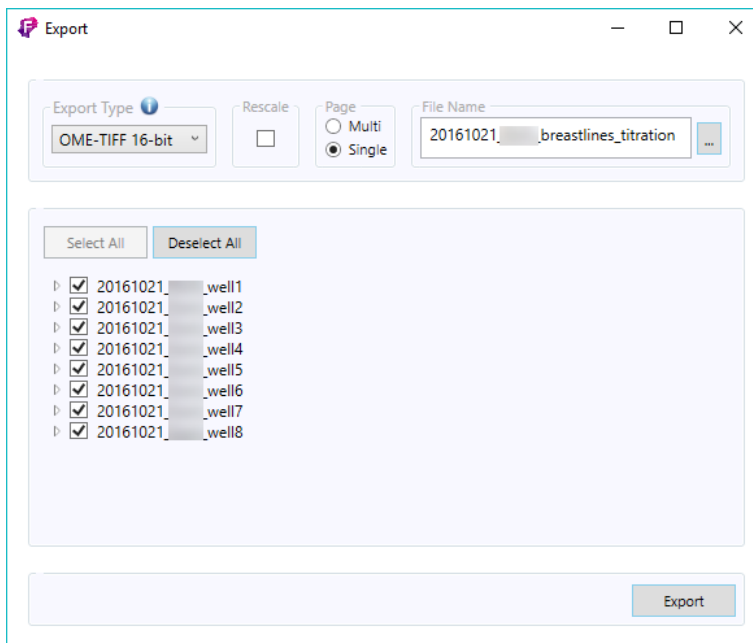
- 1 Click **File > Open**.

IMPORTANT Data must be stored on a local hard drive.

- 2 After the file is opened, click **File > Export**.

- 3 Under Export Type, click **OME-TIFF 16-bit** or **OME-TIFF 32-bit**.


IMPORTANT Select OME-TIFF 16-bit for processing with third-party data processing applications that do not support 32-bit OME-TIFF files.



IMPORTANT To preserve the raw data values for data processing purposes, do not select Rescale. Rescale converts the raw data values to either 0–65535 (for 16-bit TIFF) or 0–1 (for 32-bit TIFF) for viewing in Windows. Rescale is unchecked by default.

- 4 Under Page, click **Multi** or **Single**:

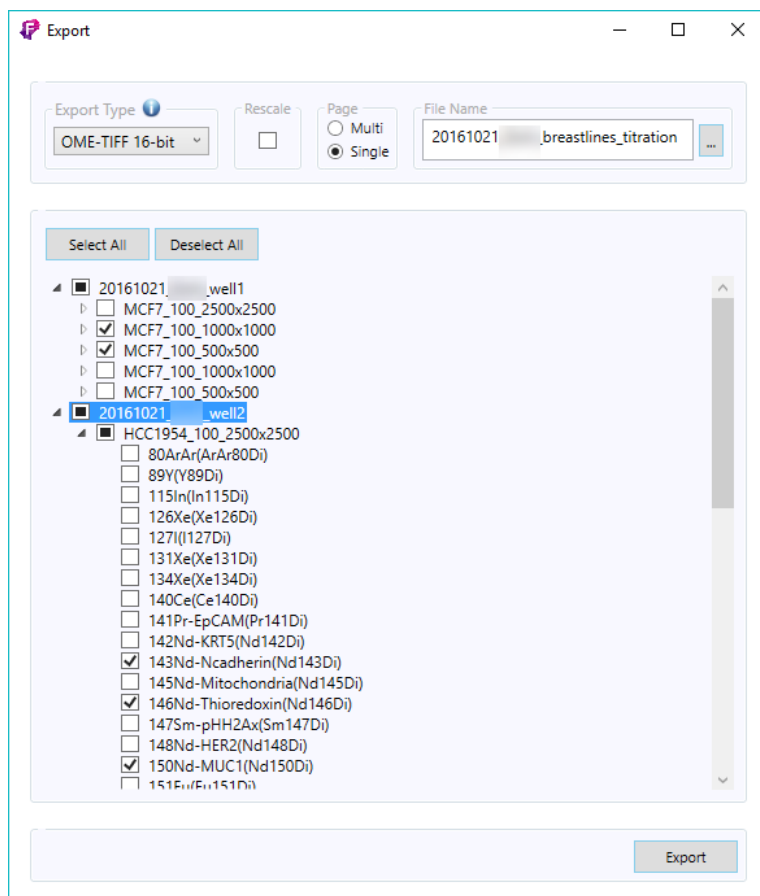
- Choose **Multi** to export data to a multipage TIFF file where an ion image for each channel is displayed on an individual page within the same TIFF. Choose this option if fewer TIFF files are desired and single-page TIFF files are not required for processing with third-party software.
- Choose **Single** to export to single-page TIFF files where each ion image is exported to an individual TIFF file. Choose this option if single-page TIFF files are required for processing with third-party software.

- 5 Under **File Name**, click the browse button . Choose a location and name for the exported file folder. Click **Save**.

- 6 Select all or a subset of ROIs and channels to export. All ROIs and channels are selected by default.

To select a subset of ROIs and channels:

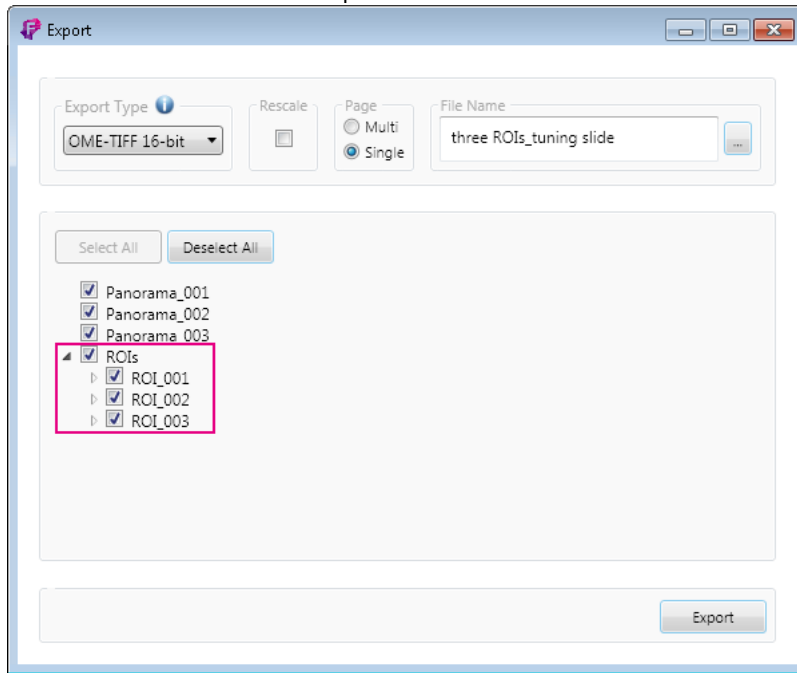
- a Expand the tree view.
- b Check the boxes of ROIs and channels to export. Uncheck all other checkboxes.



- 7 After ROIs and channels are selected, click **Export**. A progress bar indicates export progress, and the Export button becomes a Cancel button.
- 8 To cancel file export, click **Cancel**. It may take a few moments to complete cancellation because the file in progress is exported first. All files exported up to this time are saved.

NOTE For MCD files acquired with CyTOF® Software v7.0, all ROIs are not linked to panoramas. To choose from all ROIs within the MCD file, expand the ROIs section and check

the ROIs and channels for export.

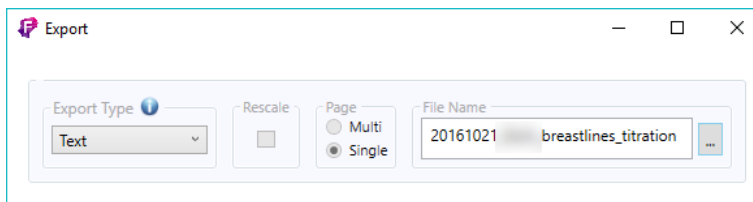



Export to Text

Exported text files are tab-delimited files that contain the original signal values and associated X/Y coordinates for selected channels.

NOTE Depending on file size, file export may take several minutes to complete.

- 1 Click **File > Open**.
- 2 After the file is opened, click **File > Export**.
- 3 Under Export Type, click **Text**.



- 4 Under File Name, click the browse button . Choose a location and name for the exported file folder. Click **Save**.
- 5 Select all or a subset of ROIs and channels to export. By default, all ROIs and channels are selected.

- 6** To select a subset of ROIs and channels:
 - a** Expand the tree view containing the list of ROIs and channels.
 - b** Either click individual checkboxes or click **Deselect All** to clear selections from the list.
 - c** Click to select the ROIs and channels for export.
- 7** After ROIs and channels are selected, click **Export**. A progress bar indicates export progress, and the Export button becomes a Cancel button.
- 8** To cancel file export, click **Cancel**. It may take a few moments to complete cancellation because the file in progress is exported first. All files exported up to this time are saved.

Appendix A: Exported TIFF File Structure

Each TIFF and OME-TIFF file header contains tags that define the raster image data that is specific to each channel.

Each pixel in the TIFF file has 4 bytes. Since the data was written in IEEE floating-point format, the pixel values must be read as floats (not as 32-bit unsigned or signed integers).

During conversion from MCD to TIFF, the dual count signals are converted to floats and, when rescaling is applied, mapped to a 0–1 float value range to accommodate the 32-bit register of the TIFF file. The `SMinSampleValue` and `SMaxSampleValue` tags store the minimum and maximum dual count values per channel. These values are required to back-calculate the original dual count signal values.

Important Tags

The following tags are defined within each image file directory and should be extracted in order to determine pixel values and reconstruct the original dual count signals.

Table 1. Tags for extraction to assist with data processing

Tag	Specification
SamplesPerPixel	Tag = 277 (115.H) Type = SHORT The number of components per pixel. This number is 3 for RGB images unless extra samples are present.
BitsPerSample	BitsPerSample Tag = 258 (102.H) Type = SHORT The number of bits per component. Allowable values for Baseline TIFF grayscale images are 4 and 8, allowing either 16 or 256 distinct shades of gray.

Tag	Specification
RowsPerStrip	<p>The number of rows per strip</p> <p>Tag = 278 (116.H)</p> <p>Type = SHORT or LONG</p> <p>N = 1</p> <p>TIFF image data is organized into strips for faster random access and efficient I/O buffering. RowsPerStrip and ImageLength together tell us the number of strips in the entire image. The equation is:</p> $\text{StripsPerImage} = \text{floor}((\text{ImageLength} + \text{RowsPerStrip} - 1) / \text{RowsPerStrip})$ <p>StripsPerImage is not a field. It is a value that a TIFF reader will compute, because it specifies the number of StripOffsets and StripByteCounts for the image.</p> <p>Note that either SHORT or LONG values can be used to specify RowsPerStrip. SHORT values may be used for small TIFF files. It should be noted, however, that earlier TIFF specification revisions required LONG values and that some software may not accept SHORT values.</p> <p>The default is $2^{32} - 1$, which is effectively infinity. That is, the entire image is one strip. Use of a single strip is not recommended. Choose RowsPerStrip such that each strip is about 8K bytes, even if the data is not compressed, to make buffering simpler for readers. The 8K value is fairly arbitrary but seems to work well.</p>
XResolution	<p>The number of pixels per ResolutionUnit in the ImageWidth direction</p> <p>Tag = 282 (11A.H)</p> <p>Type = RATIONAL</p> <p>N = 1</p> <p>It is not mandatory that the image be actually displayed or printed at the size implied by this parameter. It is up to the application to use this information as it wishes.</p> <p>No default</p>
YResolution	<p>YResolution</p> <p>The number of pixels per ResolutionUnit in the ImageLength direction</p> <p>Tag = 283 (11B.H)</p> <p>Type = RATIONAL</p> <p>N = 1</p> <p>No default</p>
SMinSampleValue	<p>Tag = 340 (154.H)</p> <p>Type = the field type that best matches the sample data</p> <p>N = SamplesPerPixel</p> <p>This field specifies the minimum sample value. Note that a value should be given for each data sample. That is, if the image has 3 SamplesPerPixel, 3 values must be specified.</p> <p>The default for SMinSampleValue and SMaxSampleValue is the full range of the data type.</p>

Tag	Specification
SMaxSampleValue	Tag = 341 (155.H) Type = the field type that best matches the sample data N = SamplesPerPixel This new field specifies the maximum sample value.
PageName	The name of the page from which this image was scanned Tag = 285 (11D.H) Type = ASCII No default

Source: Adobe TIFF 6.0 Specification Final, June 3, 1992

OME-TIFF Metadata

An XML metadata block is included in the OME-TIFF file header. It includes the following content:

- Instrument ID (Hyperion™ Tissue Imager)
- Image ID (channel ID) and Name (channel name)
- Pixels DimensionOrder (PixelsDimensionOrder.XYZTC), Type (float), SizeX (ROI width), and SizeY (ROI height)
- Channel ID (channel ID) and Name (channel name)
- AcquiredDate = current date
- SamplesPerPixel = 1

Resources

TIFF 6.0 specifications: itu.int/itudoc/itu-t/com16/tiff-fx/docs/tiff6.pdf

OME-TIFF specifications: docs.openmicroscopy.org/ome-model/5.6.4/ome-tiff/specification.html



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