invitrogen QUICK REFERENCE

Attune™ Cytometric Software

Image Processing Workflow

Pub. No. MAN0028531 Rev. C

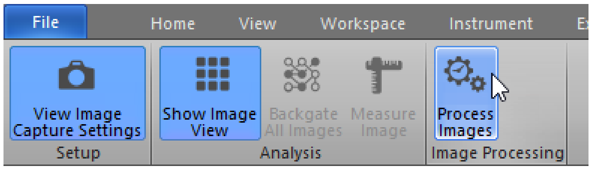
⚠ **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the following product documentation: *Attune™ CytPix™ Flow Cytometer User Guide* (Pub. No. MAN0019440). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

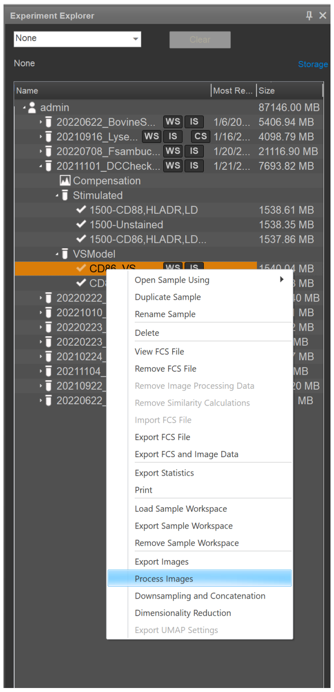
Process images

1. On the **Image Settings** ribbon tab, click **Process Images** to open the **Process Images dialog**.

   Note: The **Image Processing** controls are visible only for Attune™ CytPix™ experiments. You can process images only for samples in the active experiment.



   Alternatively, select **Process Images** in the **Experiment Explorer** context menu.



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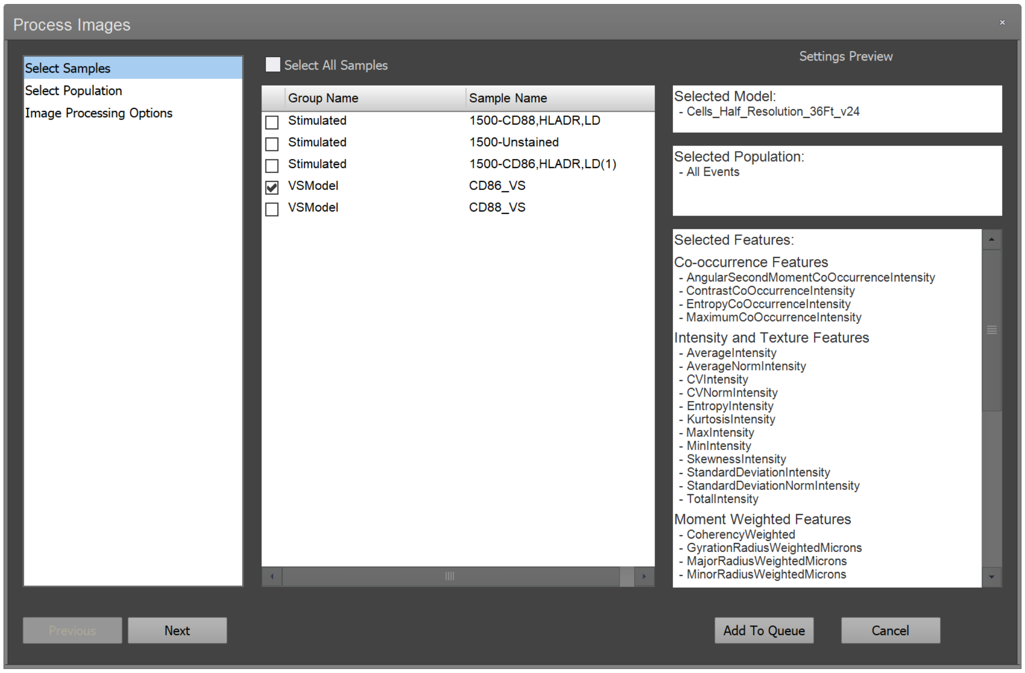
2. On the **Select Samples** screen, select the samples for which to process images. To select or deselect all samples in the experiment, select or deselect the **Select All Samples** option above the sample selection list.



	Group Name	Sample Name
☐	Stimulated	1500-CD88,HLADR,LD
☐	Stimulated	1500-Unstained
☐	Stimulated	1500-CD86,HLADR,LD(1)
☑	VSModel	CD86_VS
☐	VSModel	CD88_VS

Selected Model:
- Cells_Half_Resolution_36Ft_v24

Selected Population:
- All Events

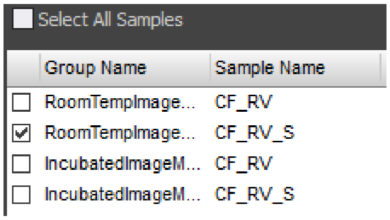
Selected Features:

Co-occurrence Features
- AngularSecondMomentCoOccurrenceIntensity
- ContrastCoOccurrenceIntensity
- EntropyCoOccurrenceIntensity
- MaximumCoOccurrenceIntensity

Intensity and Texture Features
- AverageIntensity
- AverageNormIntensity
- CVIntensity
- CVNormIntensity
- EntropyIntensity
- KurtosisIntensity
- MaxIntensity
- MinIntensity
- SkewnessIntensity
- StandardDeviationIntensity
- StandardDeviationNormIntensity
- TotalIntensity

Moment Weighted Features
- CoherencyWeighted
- GyrationRadiusWeightedMicrons
- MajorRadiusWeightedMicrons
- MinorRadiusWeightedMicrons

Note: If the Process Images dialog is opened from the Experiment Explorer, the samples are automatically selected based on the Sample, Group, or Experiment that was selected in the Experiment Explorer to open the dialog.



	Group Name	Sample Name
☐	RoomTempImage...	CF_RV
☑	RoomTempImage...	CF_RV_S
☐	IncubatedImageM...	CF_RV
☐	IncubatedImageM...	CF_RV_S

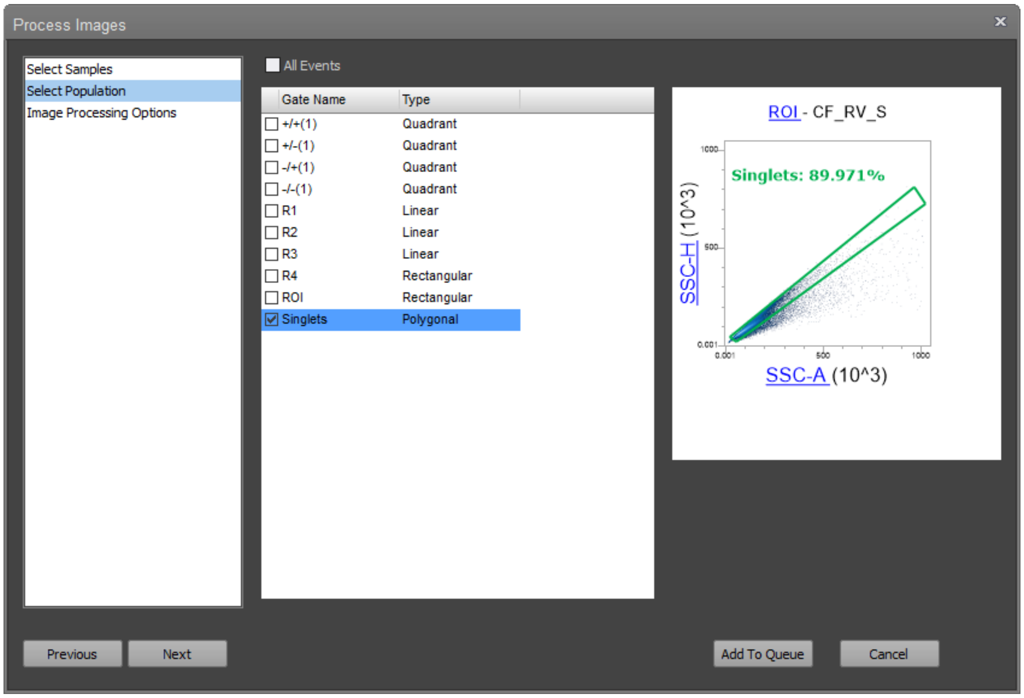
3. Click **Next** to go to the **Select Population** screen to filter the images to be processed based on one or more populations (gates).

   Alternatively, click **Add To Queue** to add the samples to the image processing queue without selecting a population to filter the images.

   Note: When **Add to Queue** is clicked, all selected samples are added to a processing queue and are processed serially in the order they were added. You can manage the sample queue using the Attune™ Image Processing Dashboard (see "Attune™ Image Processing Dashboard" on page 5). Image processing proceeds even if you close the Attune™ Cytometric Software.

4. On the **Select Population** screen, select one or more populations (gates) to process only the events in the selected gates. By default, **All Events** is selected. The preview plot shows the selected population. When no gate is selected, the preview plot does not show any plot.

   Note: The software uses the selected gate to process all samples. If the gate does not exist on the selected workspace, the software tries the next level up in the gating hierarchy (Sample > Group > Experiment). If the software finds no gate, it reverts to **All Events** for that sample.



☐ All Events

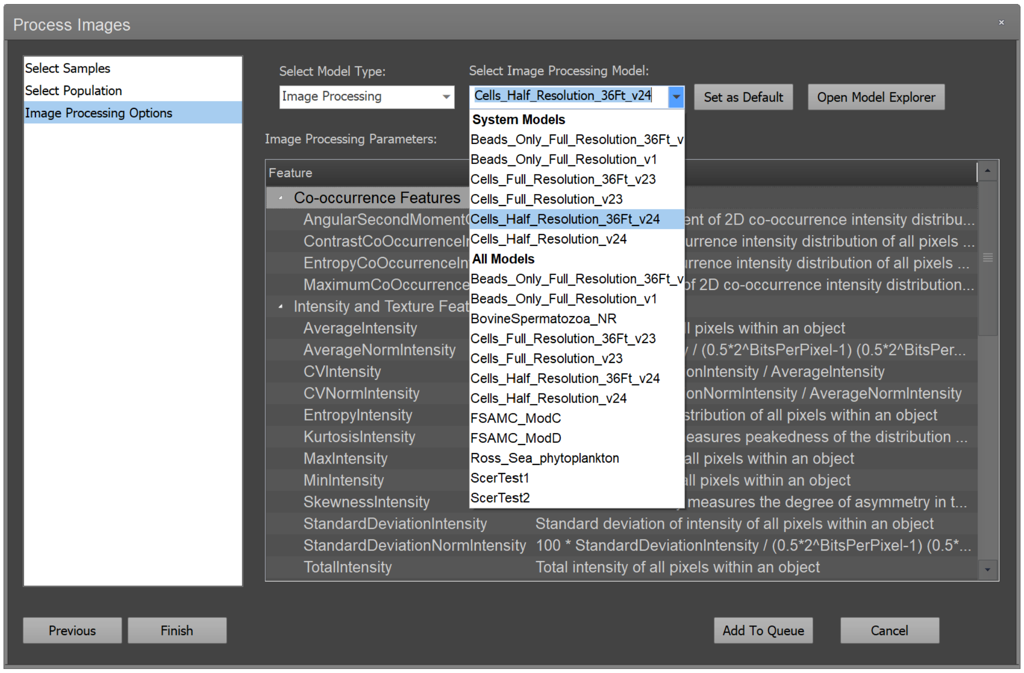
Gate Name	Type
☐ +/+(1)	Quadrant
☐ +/-(1)	Quadrant
☐ -/+(1)	Quadrant
☐ -/-(1)	Quadrant
☐ R1	Linear
☐ R2	Linear
☐ R3	Linear
☐ R4	Rectangular
☐ ROI	Rectangular
☑ Singlets	Polygonal

5. Click **Next** to go to **Image Processing Options** to select an image processing model.

   Alternatively, click **Add to Queue** to add the samples to the image processing queue without selecting an image processing model.

6. On the **Image Processing Options** screen, select an image processing model. For a list and description of image processing models, see “Image processing parameters” on page 9.

IMPORTANT! Regardless of the size of the captured images in the experiment, images are processed at either full resolution (248 × 248 pixels) or at half resolution (124 × 124 pixels). Processing the images at half resolution decreases the data footprint by 4-fold and improves the processing times by >4-fold. However, this comes with a potential trade-off in accuracy.



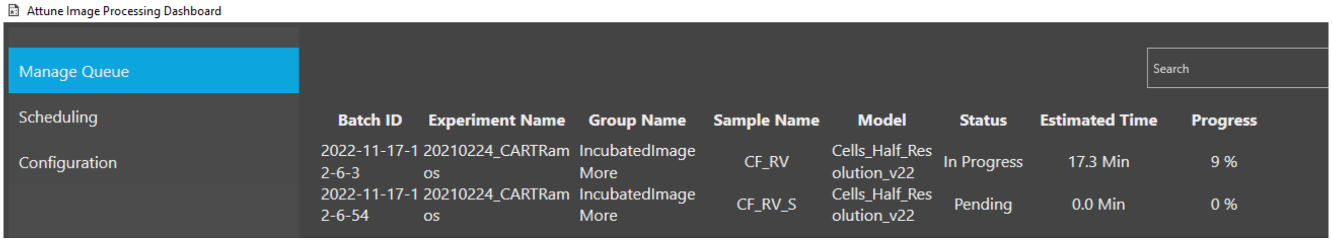
Select Model Type: Image Processing

Select Image Processing Model: Cells_Half_Resolution_36Ft_v24

7. *(Optional)* Click **Set As Default** to set the selected image processing model as the default for subsequent image processing operations.

8. Click **Add To Queue** or **Finish** to start image processing.

   Samples in the processing queue are processed serially in the order they were added. You can view the status of the image processing job in the Attune™ Image Processing Dashboard, which also enables you to manage the sample queue (see “Attune™ Image Processing Dashboard” on page 5).



Batch ID	Experiment Name	Group Name	Sample Name	Model	Status	Estimated Time	Progress
2022-11-17-12-6-3	20210224_CARTRamos	IncubatedImageMore	CF_RV	Cells_Half_Resolution_v22	In Progress	17.3 Min	9 %
2022-11-17-12-6-54	20210224_CARTRamos	IncubatedImageMore	CF_RV_S	Cells_Half_Resolution_v22	Pending	0.0 Min	0 %

Note: If the selected samples already have image processing data, the software displays a warning dialog that enables you to overwrite the existing data, to skip the current sample, or to cancel image processing (see “Reprocess images” on page 8).

Note: Image processing proceeds even if you close the Attune™ Cytometric Software.

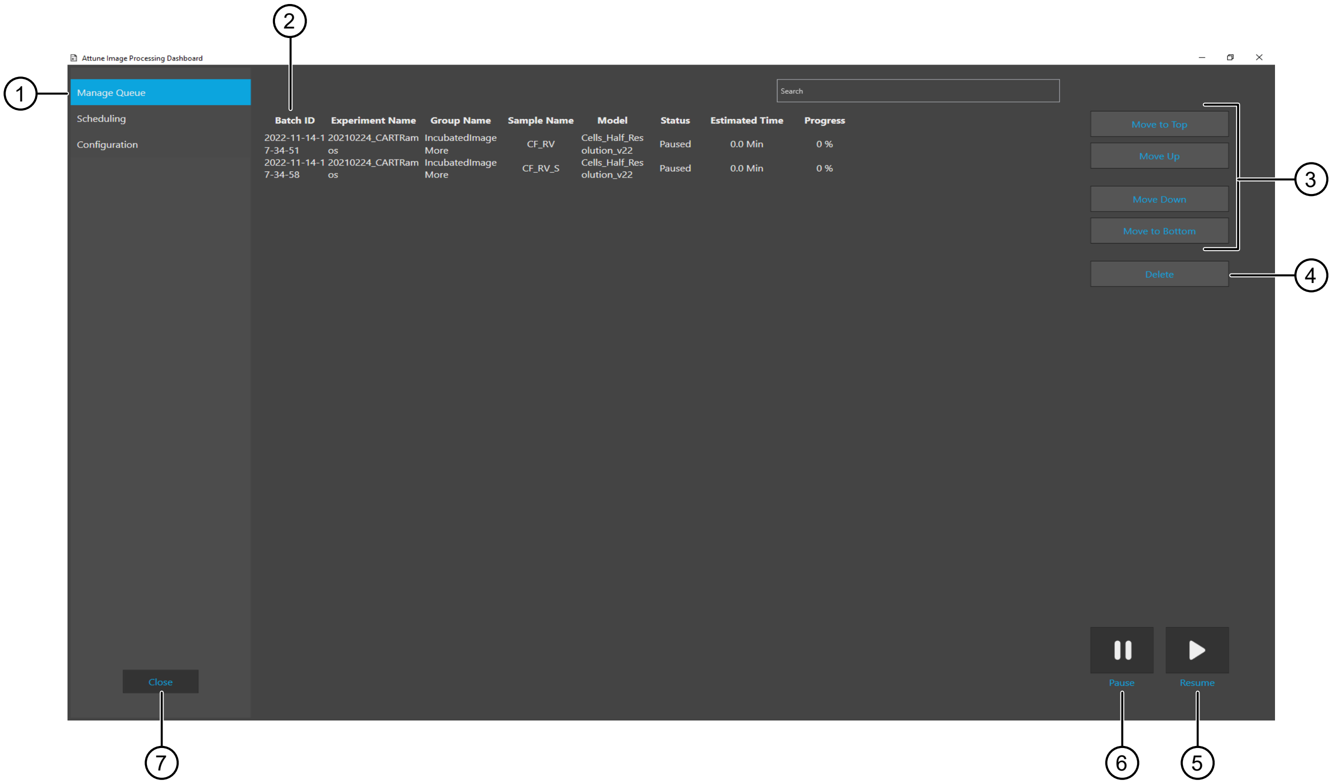
Note: Image processing is paused during sample acquisition.

Attune™ Image Processing Dashboard

The Attune™ Image Processing Dashboard is automatically opened when you power on the computer that runs the Attune™ Cytometric Software and it functions independently of the Attune™ Software.

The Attune™ Image Processing Dashboard enables you to:

- View processing queue, status, and progress (page 6)
- Reprioritize the processing queue (page 6)
- Cancel processing requests (page 6)
- Pause or resume processing (page 6)
- Schedule processing



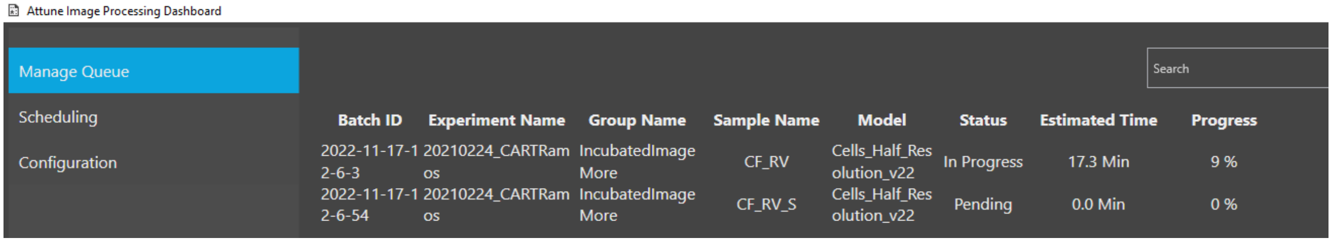
Batch ID	Experiment Name	Group Name	Sample Name	Model	Status	Estimated Time	Progress
2022-11-14-17-34-51	20210224_CARTRamos	IncubatedImage More	CF_RV	Cells_Half_Resolution_v22	Paused	0.0 Min	0 %
2022-11-14-17-34-58	20210224_CARTRamos	IncubatedImage More	CF_RV_S	Cells_Half_Resolution_v22	Paused	0.0 Min	0 %

Figure 1 **Attune™ Image Processing Dashboard**

① Manage Queue tab
② Processing queue
③ Move request up or down buttons
④ Delete button
⑤ Resume processing button
⑥ Pause processing button
⑦ Close dashboard button

View processing queue, status, and progress

You can view the processing queue, status, and progress in the **Manage Queue** tab of the Attune™ Image Processing Dashboard:



Batch ID	Experiment Name	Group Name	Sample Name	Model	Status	Estimated Time	Progress
2022-11-17-12-6-3	20210224_CARTRamos	IncubatedImageMore	CF_RV	Cells_Half_Resolution_v22	In Progress	17.3 Min	9 %
2022-11-17-12-6-54	20210224_CARTRamos	IncubatedImageMore	CF_RV_S	Cells_Half_Resolution_v22	Pending	0.0 Min	0 %

Figure 2 Processing queue

- **Batch ID:** Unique identification assigned to the processing job based on the date and time the sample was added to the processing queue.
- **Experiment Name:** Name of the experiment in Experiment Explorer from which the sample was selected for processing.
- **Group Name:** Group name in Experiment Explorer from which the sample was selected for processing.
- **Sample Name:** Name of the sample in Experiment Explorer that was selected for processing.
- **Model:** Processing model selected in Process Images wizard.
- **Status:** Processing status for the sample (In Progress, Pending, or Paused).
- **Estimated Time:** Estimated time for the completion of processing for the sample.
- **Progress:** Percentage of the processing job that is completed.

Note: Processing is paused during sample acquisition.

Reprioritize the image processing queue

To move a processing request up or down in the image processing queue, select the request, then use the **Move to Top**, **Move Up**, **Move Down**, and **Move to Bottom** buttons to move it to the desired position in the queue.

Cancel image processing requests

To cancel an image processing request, select the request in the image processing queue, then click **Delete**.

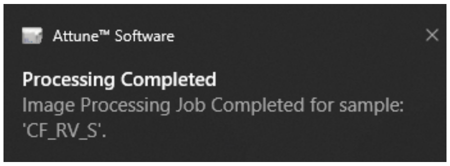
Pause or resume image processing

1. To pause an ongoing image processing of a sample, select the sample, then click **Pause**.
2. To resume image processing, click **Resume**.

Completion of image processing

When the Attune™ Cytometric Software is running and image processing is completed for any sample in the processing queue that is specific to the currently logged-in user, the software provides the following notifications:

- System tray displays the **Processing Complete** notification.



Attune™ Software

Processing Completed

Image Processing Job Completed for sample: 'CF_RV_S'.

Note: Windows notifications must be enabled to see system tray notifications.

- Message bar displays the **Image Processing Job Complete** notification with an option to reload the sample and see the results.

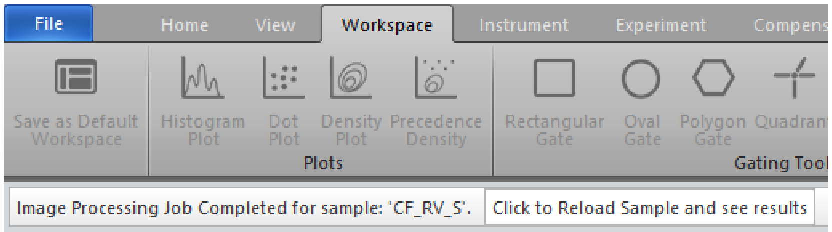


Image Processing Job Completed for sample: 'CF_RV_S'.

Note: Clearing the message by clicking **X** or **Reload Sample and see results** button hides the message bar.

- If the Attune™ Software is running in the SAE ("Security, Auditing, and Electronic Signature") mode and the experiment was signed, the message bar displays a warning that indicates that changes to the experiment will invalidate any existing signatures.

  Note: Attune™ Software version 7.1 is not 21 CFR Part 11 compliant.

  Note: The features including **Train Model**, **Image Similarity**, and **Dimensionality Reduction** are not supported when using SAE mode.

When image processing is completed for a sample, the icon next to the sample in the Experiment Explorer is updated to indicate that the sample has both FCS and image processing data.

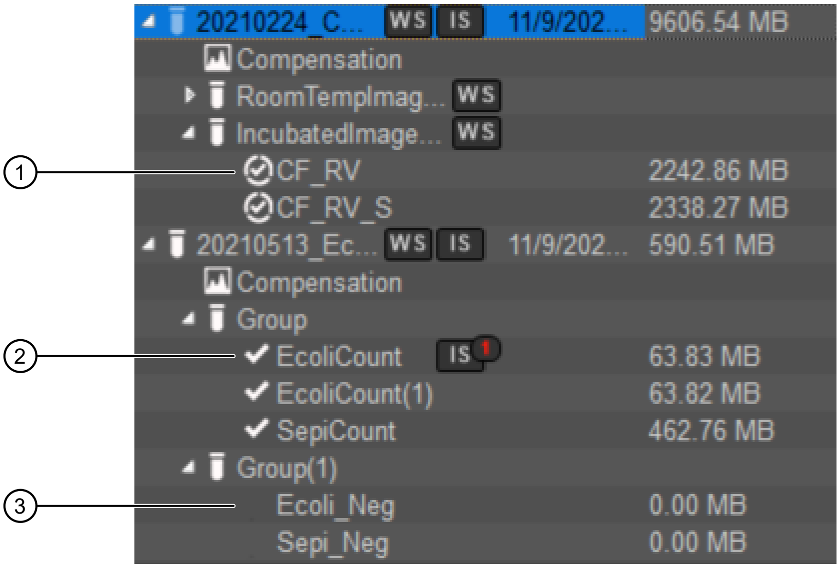


Figure 3 Experiment Explorer containing samples with and without image processing data

① Sample with FCS and Image Processing data

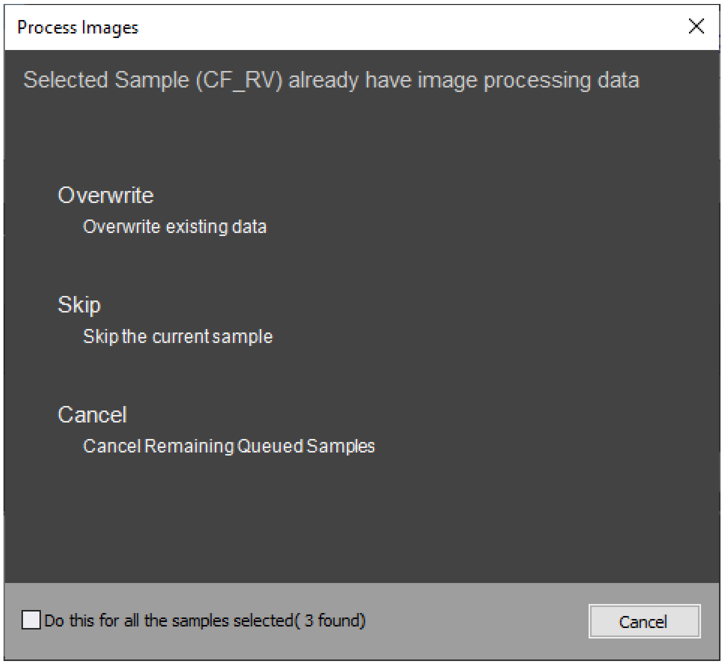
② Sample with FCS data only

③ Sample with no data

Reprocess images

You can reprocess images in a sample to allow a different processing model or algorithm, or to use other populations or features to extract cell image information. When samples are reprocessed, any ongoing processing is canceled and the new processing request is added to the queue.

1. Select the samples, populations, and image processing options as described in Process images. When you start processing a request for a sample that has already been processed, the software displays a dialog that states that the selected sample already has image processing data. The software displays the dialog for each sample that meets this condition.



Selected Sample (CF_RV) already have image processing data

2. To process the selected samples and overwrite the existing image processing data, select **Overwrite**.

   Note: If you have selected multiple samples with existing image processing data, you can apply the **Overwrite** option to all samples that meet this condition by selecting **Do this for all the samples selected** checkbox. When the last sample that meets this condition is confirmed, the image processing dialog is closed.

   The selected samples that already have image processing data are added to the queue and processed along with any samples that do not have existing data.

   IMPORTANT! When image processing completes for each sample, any existing results are overwritten.

3. To skip the samples with existing image processing data and process only the samples that do not have existing data, select **Skip**.

   Note: If you have selected multiple samples with existing image processing data, you can apply the **Skip** option to all samples that meet this condition by selecting **Do this for all the samples selected** checkbox. When the last sample that meets this condition is confirmed, the image processing dialog is closed.

   The selected samples that already have image processing data are not added to the queue and only those samples that do not have existing data are processed.

4. To cancel the processing request without adding the selected samples to the image processing queue, select **Cancel**.

   The image processing dialog remains open, allowing you to update the sample selection, if desired.

Image processing parameters

Feature	Description	PnR[1]
Co-occurrence features		
AngularSecondMomentCoOccurrenceIntensity	Angular second moment of co-occurrence intensity distribution of pixels within the mask[2]	2^1
ContrastCoOccurrenceIntensity	Contrast of co-occurrence intensity distribution of pixels within the mask[3]	2^{10}
EntropyCoOccurrenceIntensity	Entropy of co-occurrence intensity distribution of pixels within the mask[4]	2^{10}
MaximumCoOccurrenceIntensity	Maximum probability of co-occurrence intensity distribution of all pixels within the mask[2]	2^1
Intensity and Texture features		
AverageIntensity	Average intensity of all pixels within an object	2^{10}
AverageNormIntensity	100 × AverageIntensity / (0.5 × $2^{(\text{BitsPerPixel} - 1)}$)	—
CVIntensity	100 × StandardDeviationIntensity / AverageIntensity	—
CVNormIntensity	100 × StandardDeviationNormIntensity / AverageNormIntensity	—
EntropyIntensity	Entropy of intensity distribution of all pixels within an object	—
KurtosisIntensity	Kurtosis of intensity distribution of all pixels within an object[5]	—
MaxIntensity	Maximum intensity of all pixels within an object	2^{10}
MinIntensity	Minimum intensity of all pixels within an object	2^{10}
SkewnessIntensity	Skewness of intensity distribution of all pixels within an object[6]	—
StandardDeviationIntensity	Standard deviation of intensity of all pixels within an object	2^{10}
StandardDeviationNormIntensity	100 × StandardDeviationIntensity / (0.5 × $2^{(\text{BitsPerPixel} - 1)}$)	—
TotalIntensity	Total intensity of all pixels within an object	—
Moment-weighted features		
CoherencyWeighted	Measure of the alignment of the substructures within an object	2^4
GyrationRadiusWeightedMicrons	Gyration radius (in microns) along the Z axis	2^7
MajorRadiusWeightedMicrons	Half the distance (in microns) across an ellipse along its long axis	2^7
MinorRadiusWeightedMicrons	Half the distance (in microns) across an ellipse along its short axis	2^7
Object features		
ParticleCount	Number of cells within the identified object	2^3
Particle interaction features		
ClumpIndexMax	Maximum number of particles among clumps	2^6
ObjectCount	Number of objects, where each object is encompassed by an outer mask	2^6
Pixel features		
NumPixels	Number of pixels contained within identified objects	2^{16}

Feature	Description	PnR[1]
Shape features		
AreaSquareMicrons	Area of the object measured within mask based on pixel count = NumPixels × (PixelSize.MicronsX × PixelSize.MicronsY)	2^{13}
CircularityPercent	Percent circularity of an object = 100 / PerimeterToArea (100 for a circular object)	2^{9}
EccentricityPercent	Eccentricity of an ellipse = 100 × Sqrt(1 – ShortAxisMicrons^2 / LongAxisMicrons^2)	0 to 100
MajorDiameterMicrons	Distance across an ellipse along its long axis = MajorRadius × 2 × PixelSize.MicronsX	2^{7}
MinorDiameterMicrons	Distance across an ellipse along its short axis = MajorRadius × 2 × PixelSize.MicronsX	2^{7}
PerimeterMicrons	Perimeter of an object	2^{15}
PseudoDiameterMicrons	Diameter of a circle with an area equal to the area of the object = 2 × Sqrt(Object.Area in µm^2/ pi)	2^{15}
MinorMajorRatioPercent	Short to long axes ratio of an object as a percentage = 100 × ShortAxisMicrons / LongAxisMicrons	0 to 100
System features		
ConfidenceScore	Indicates that one or more objects intersects with the FOV of the image	—
IsOnBorder	Indicates that one or more objects intersects with the FOV of the image	—
IsProcessable	Indicates that the image is processable	—
IsProcessed	Indicates that the image was processed. This is generated when the image processing FCS file is loaded and merged with the raw FCS file data	—

[1] $PnR is the range for the selected parameter n.

[2] May be most valuable as a dimensionality reduction parameter as meaningful influence may be difficult to detect in a 1D/2D space.

[3] Contrast co-occurrence intensity is used to quantify the intensity contrast between a pixel and its neighbor.

[4] Entropy co-occurrence intensity is used to quantify the complexity of pixel intensity distributions in an image.

[5] Kurtosis of intensity measures the peakedness of the distribution of all pixels within an object. See “Kurtosis” on page 11.

[6] Skewness of intensity measures the degree of asymmetry in the pixel data of an object. See “Skewness” on page 11.

Kurtosis

Kurtosis is measures how peaked a histogram is and it is based on the size of a distribution's tails. The kurtosis of a normal distribution is 0. Distributions with short tails compared to a normal distribution have negative kurtosis (platykurtic) and distributions with relatively long tails have positive kurtosis (leptokurtic).

In the context of image processing, kurtosis describes whether distribution of gray tones is more spread-out (flat) or more concentrated around the mean (peaked).

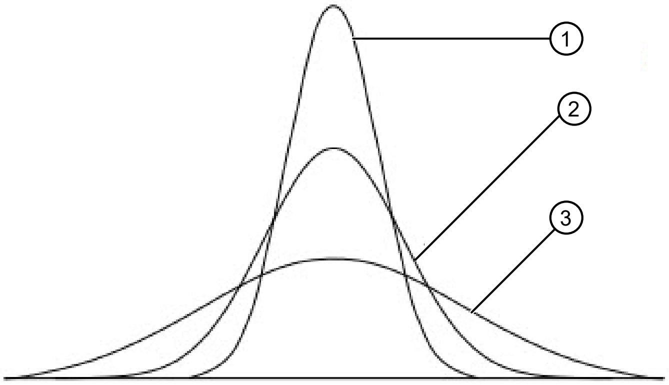


Figure 4 Kurtosis

① Positive kurtosis (leptokurtic)
② Normal distribution (mesokurtic)
③ Negative kurtosis (platykurtic)

Skewness

Skewness measures the degree of asymmetry exhibited by the data. If skewness equals zero, the histogramis symmetric about the mean.

In the context of image processing, skewness indicates the imbalance between the number of pixels that are darker or brighter than the mean.

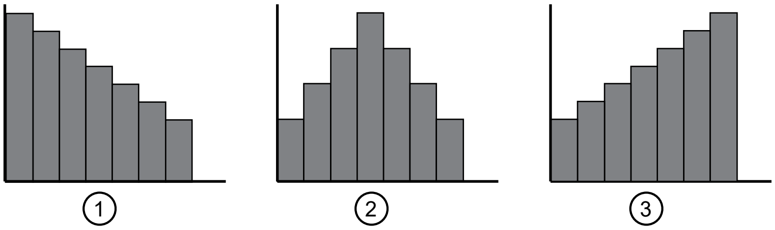


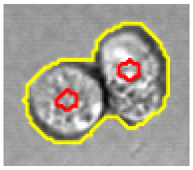
Figure 5 Skewness

① Positively skewed histogram
② Symmetric distribution histogram
③ Negatively skewed histogram

Customize Images

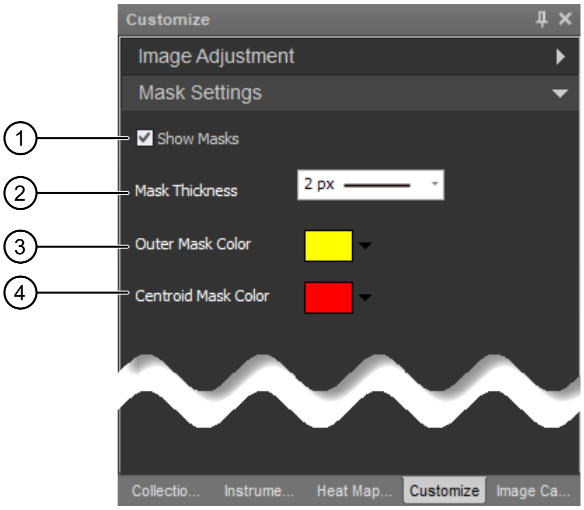
Change mask settings

When image processing is completed, the software can also return the image masks that were generated by the image processing.



- Image masks are binary representations of an image where an object is identified by the presence of a signal versus its absence. They provide a visual confirmation as to how the image processing “saw” the cell or the particle.
- The pixel coordinates of the mask contour are returned as outputs in the image processing results.
- The results include both the outer masks and the centroid masks (number of spots or cells detected in the Field of View). In the example above, the outer masks are depicted in yellow and the centroid masks in red.

1. In **Image View**, select an image, then click the **Customize** tab in the left panel to show the **Mask Settings** controls.



☑ Show Masks

Mask Thickness 2 px

Outer Mask Color 🟨

Centroid Mask Color 🟥

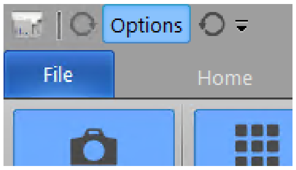
① Show Masks option
② Mask Thickness dropdown
③ Outer Mask Color dropdown
④ Centroid Mask Color dropdown

Figure 6 Customize panel Mask Settings controls

2. To show or hide the image masks, select or deselect **Show Masks**.
3. To change the line thickness of the mask contour, select the desired thickness in pixels from the **Mask Thickness** dropdown.
4. To change the color of the outer or the centroid mask, select the desired color from the **Outer Mask Color** or the **Centroid Mask Color** dropdown.

Change default mask colors

1. On the **Quick Access** toolbar, click **Options** to open the **Options** dialog.



2. In the **Options** dialog, click the **Image Options** tab, then under **Mask Options** select the desired **Mask Colors** for the **Outer** and **Centroid** masks.

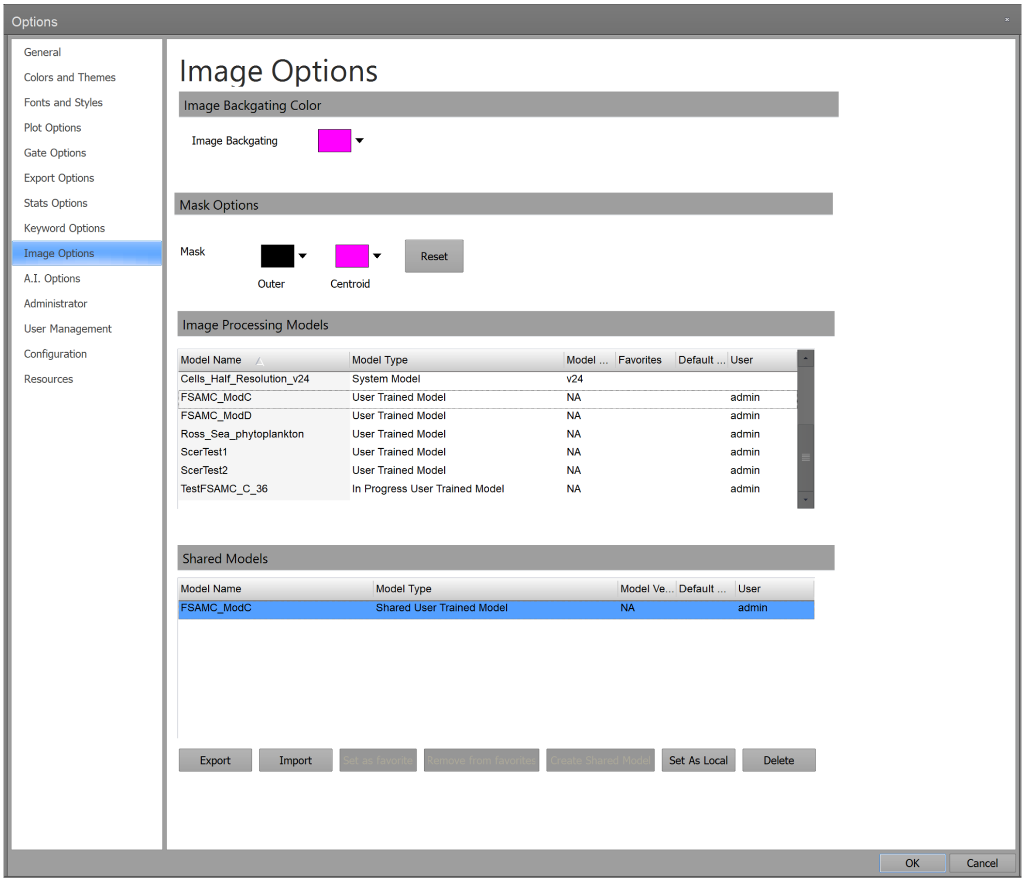


Image Processing Models

Model Name	Model Type	Model ...	Favorites	Default ...	User
Cells_Half_Resolution_v24	System Model	v24			
FSAMC_ModC	User Trained Model	NA			admin
FSAMC_ModD	User Trained Model	NA			admin
Ross_Sea_phytoplankton	User Trained Model	NA			admin
ScerTest1	User Trained Model	NA			admin
ScerTest2	User Trained Model	NA			admin
TestFSAMC_C_36	In Progress User Trained Model	NA			admin

Shared Models

Model Name	Model Type	Model Ve...	Default ...	User
FSAMC_ModC	Shared User Trained Model	NA		admin

3. To reset the default mask colors, click **Reset**.

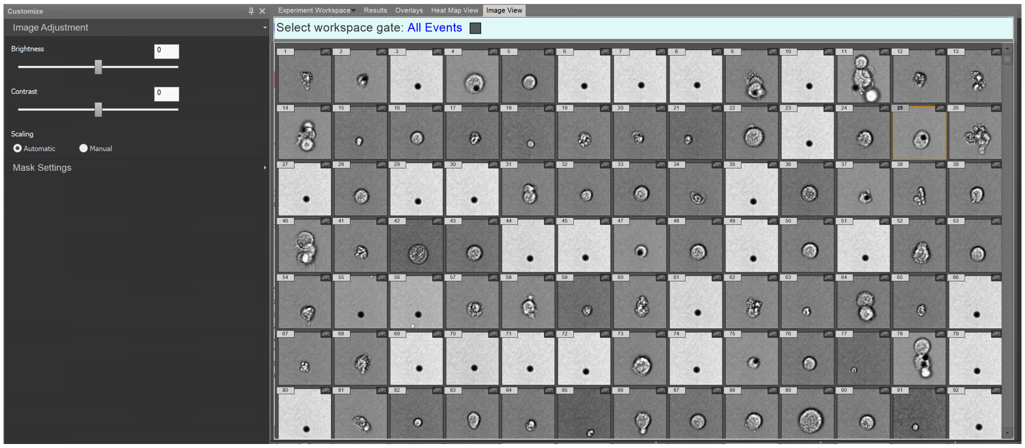
4. Click **OK** to close the **Options** dialog.

Image scaling

The Attune™ CytPix™ Flow Cytometer captures images with 10 bits of dynamic range (0 to 1023) and stores them as 16-bit greyscale TIFF image files (maximum intensity of 65536). Because computer monitors can only display RGB between 0 to 255 per channel, the range that a single 8-bit byte can offer (which enables 16,777,216 colors – 256^3 colors and another 256 levels of opacity), images greater than 8 bits have to be scaled down to 8 bits per channel.

By default, the Attune™ Cytometric Software scales down the images such that the minimum intensity in each image becomes 0 and the maximum intensity becomes 255. This automatic scaling can result in visual artifacts, such as black images or images in which the background appears differently in each image.

The Attune™ Cytometric Software enables you to manually scale the captured images, so that all images have the same minimum and maximum value (that is, they are normalized to a fixed intensity range). Setting a fixed-range normalization value enables images to be compared, where image scaling is identical.



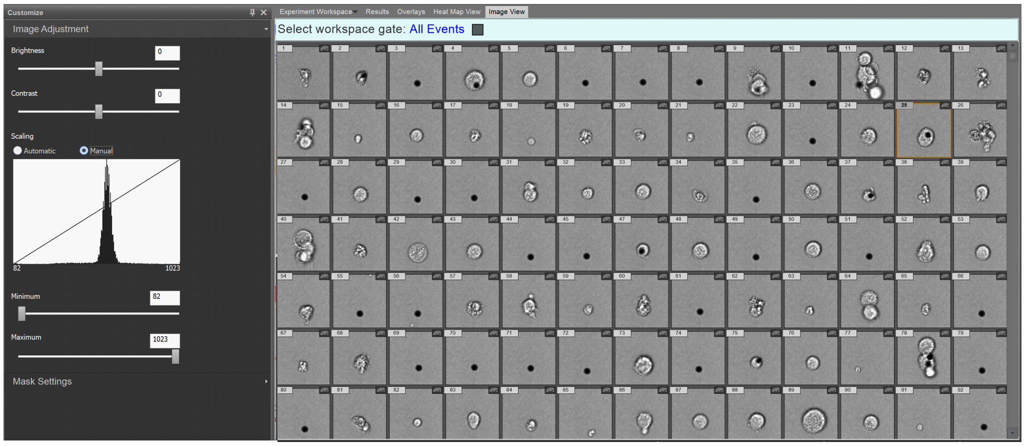
Brightness 0

Contrast 0

Scaling ● Automatic ○ Manual

Select workspace gate: All Events

Figure 7 Automatic scaling Automatic scaling (default) sets the minimum and maximum intensity within each image to 0 and 255, respectively.



Brightness 0

Contrast 0

Scaling ○ Automatic ● Manual

Minimum 82

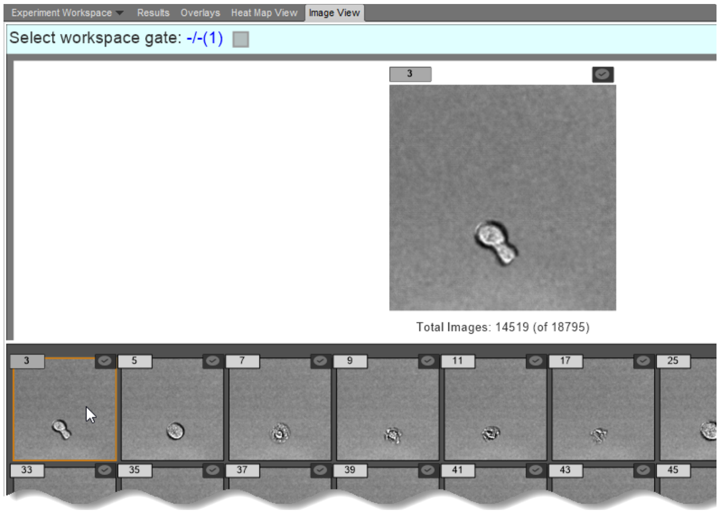
Maximum 1023

Select workspace gate: All Events

Figure 8 Manual scaling Manual scaling enables you to set the minimum and maximum intensity for each image such that the specified minimum and maximum values are scaled to 0 and 255, respectively, which normalizes the images to a fixed intensity range.

Manually adjust image scaling

1. In **Image View**, select the image to use for manual scaling.

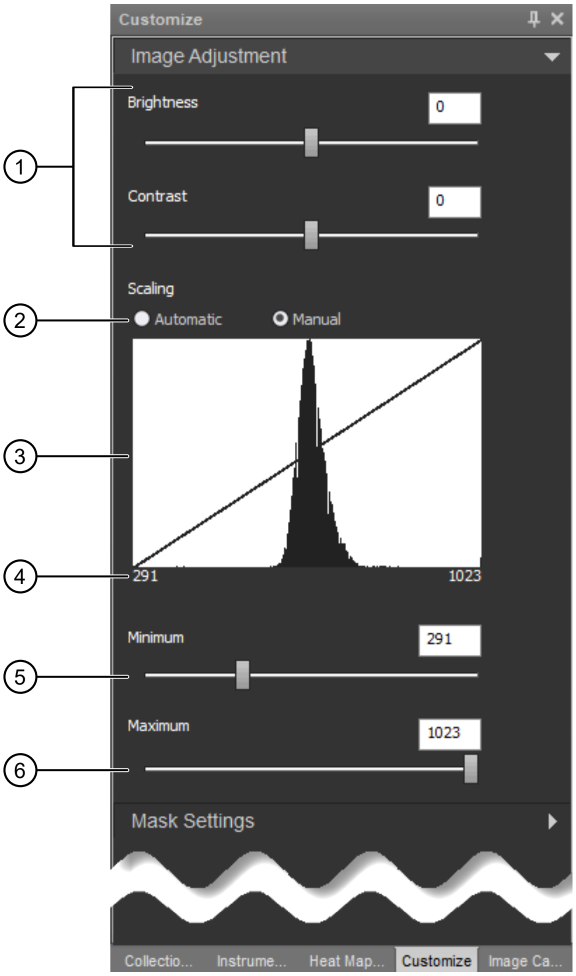


Select workspace gate: -/-(1)

Total Images: 14519 (of 18795)

2. In the **Customize** panel, select **Manual** for **Scaling** under **Image Adjustment**.

   The software displays the histogram for the active image that was selected in **Image View**, and the **Minimum** and **Maximum** values reflect the raw intensity values for the selected image.



Brightness 0

Contrast 0

Scaling ○ Automatic ● Manual

Minimum 291

Maximum 1023

① Brightness and Contrast sliders
② Scaling selection (Automatic or Manual)
③ Image histogram
④ Minimum and maximum raw intensity values
⑤ Minimum intensity slider
⑥ Maximum intensity slider

Figure 9 Customize panel Image Adjustment controls

3. To adjust image scaling, move the **Minimum** and **Maximum** sliders until the you are satisfied with the images displayed in **Image View**. Alternatively, type in the desired **Minimum** and **Maximum** intensity value.

   Note: Image scaling is applied to **all** images in the Image View and gallery, as well as the cell images in the Experiment Workspace. During acquisition, the image scaling is only applied to newly acquired images; the existing preview images remain unchanged until the acquisition completes.

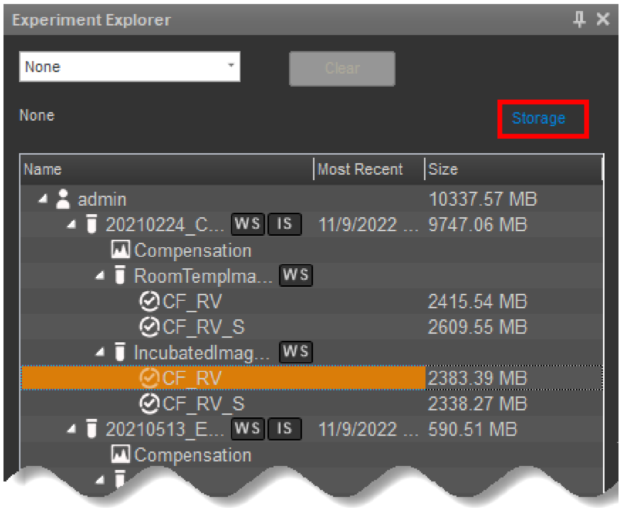
Data management

There are many types of data associated with the Attune™ instrument and software, including tube and plate experiment data, as well as instrument and user management data. Addition of imaging to the flow cytometry workflow also increases data storage requirements greatly. To ensure that there is sufficient disk space to acquire experiment data, export and clean up experiment data.

For more information about how to manage and back up data in the Attune™ Cytometric Software, see "Appendix B: Data Management in Attune™ Cytometric Software" in the *Attune™ Cytometric Software User Guide*.

Disk usage

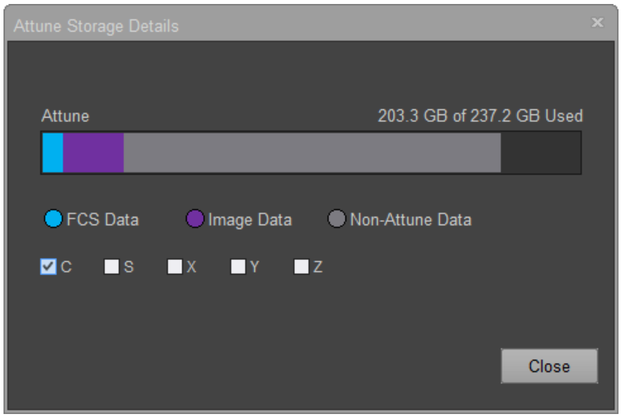
To view the data storage details on the instrument, click the **Storage** hyperlink at the top of the **Experiment Explorer**, which opens the **Attune Storage Details** window.



Name	Most Recent	Size
admin		10337.57 MB
20210224_C... WS IS	11/9/2022 ...	9747.06 MB
Compensation		
RoomTemplma... WS		
CF_RV		2415.54 MB
CF_RV_S		2609.55 MB
IncubatedImag... WS		
CF_RV		2383.39 MB
CF_RV_S		2338.27 MB
20210513_E... WS IS	11/9/2022 ...	590.51 MB
Compensation		

The **Attune Storage Details** displays the total used space and the total combined space broken into:

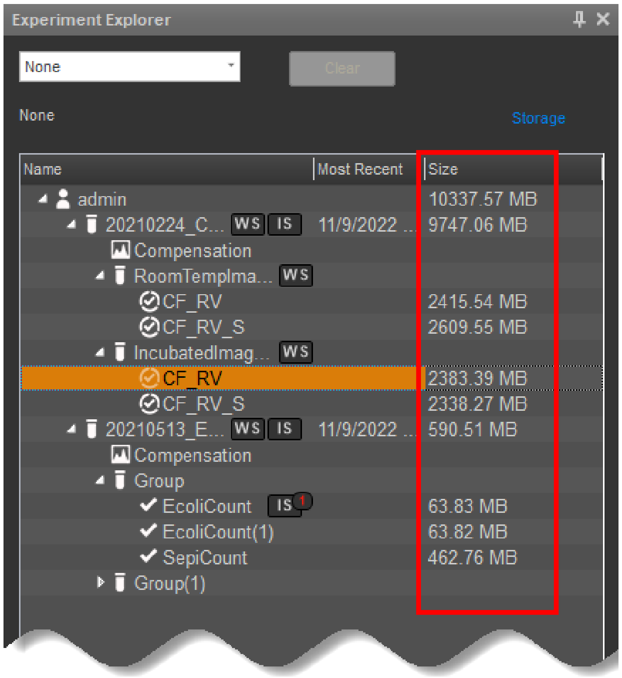
- FCS Data
- Image Data
- Non-Attune Data



Attune 203.3 GB of 237.2 GB Used

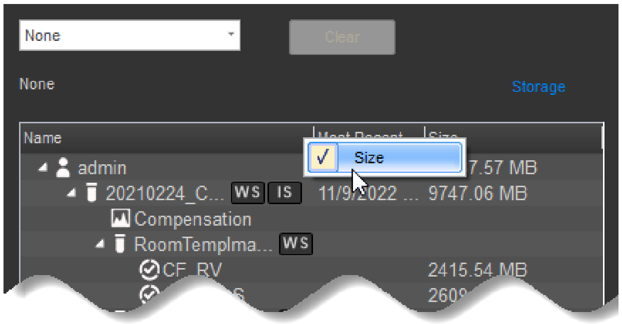
☑ C ☐ S ☐ X ☐ Y ☐ Z

The size details for Experiments, Groups, and Samples are shown in the **Size** column in **Experiment Explorer**, which are calculated on sign in and when any changes are made to an experiment (data added or removed).



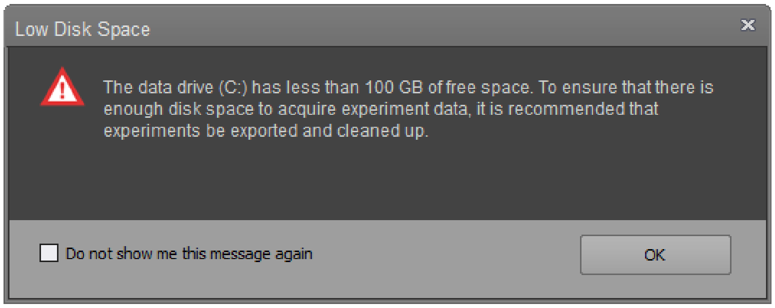
Name	Most Recent	Size
admin		10337.57 MB
20210224_C... WS IS	11/9/2022 ...	9747.06 MB
Compensation		
RoomTemplma... WS		
CF_RV		2415.54 MB
CF_RV_S		2609.55 MB
IncubatedImag... WS		
CF_RV		2383.39 MB
CF_RV_S		2338.27 MB
20210513_E... WS IS	11/9/2022 ...	590.51 MB
Compensation		
Group		
EcoliCount IS		63.83 MB
EcoliCount(1)		63.82 MB
SepiCount		462.76 MB
Group(1)		

- The size data are displayed for all samples that contain data and include the size of the FCS file, the image data, and all metadata for the sample.
- The size data are displayed for the Experiment and include the summed size of all Sample data and any Experiment metadata.
- To sort the size data in ascending or descending order, click the **Size column header**.
- To hide or show the size column, right-click the **Experiment Explorer heading row**, then deselect or select **Size**.



Data storage warnings

By default, when activating experiments, the Attune™ Cytometric Software displays the **Low Disk Space** warning if the available disk space is less than 100 GB on the primary data (image data and experiment data) drives.



The data drive (C:) has less than 100 GB of free space. To ensure that there is enough disk space to acquire experiment data, it is recommended that experiments be exported and cleaned up.

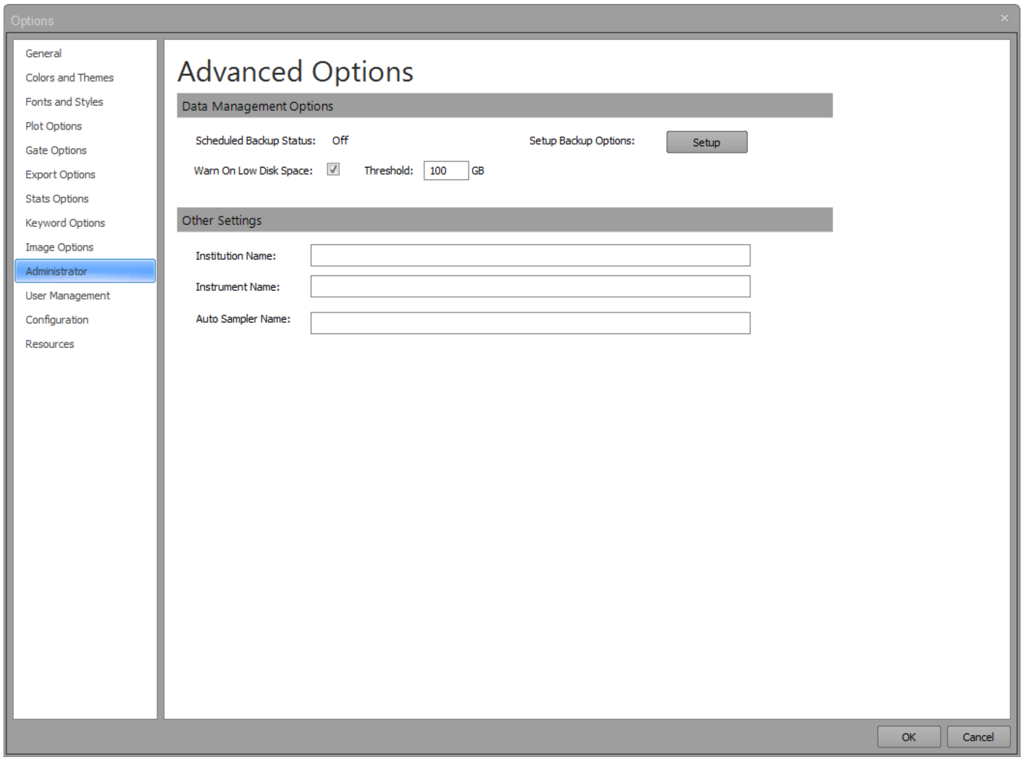
☐ Do not show me this message again

- To ignore and suppress the warning for the length of the session, select **Do not show me this message again**, then click **OK**.
- To disable the warning or to change the low disk threshold, see "Change low disk space warning options" on page 18.

Change low disk space warning options

Users with Administrator or System Administrator accounts can change backup and low disk space warning options in the **Administrator** tab of the **Options** dialog.

1. On the **Quick Access** toolbar, click **Options** to open the Options dialog, then select the **Administrator** tab.



Scheduled Backup Status: Off

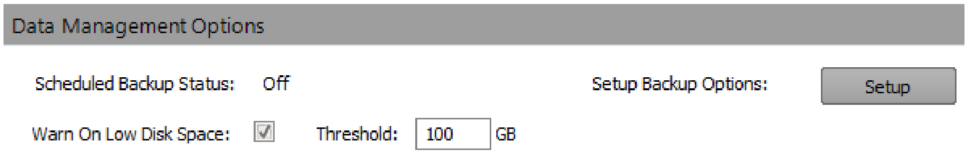
Warn On Low Disk Space: ☑ Threshold: 100 GB

Institution Name:

Instrument Name:

Auto Sampler Name:

2. To disable the Low Disk Space warning, deselect **Warn On Low Disk Space**.



Scheduled Backup Status: Off

Warn On Low Disk Space: ☑ Threshold: 100 GB

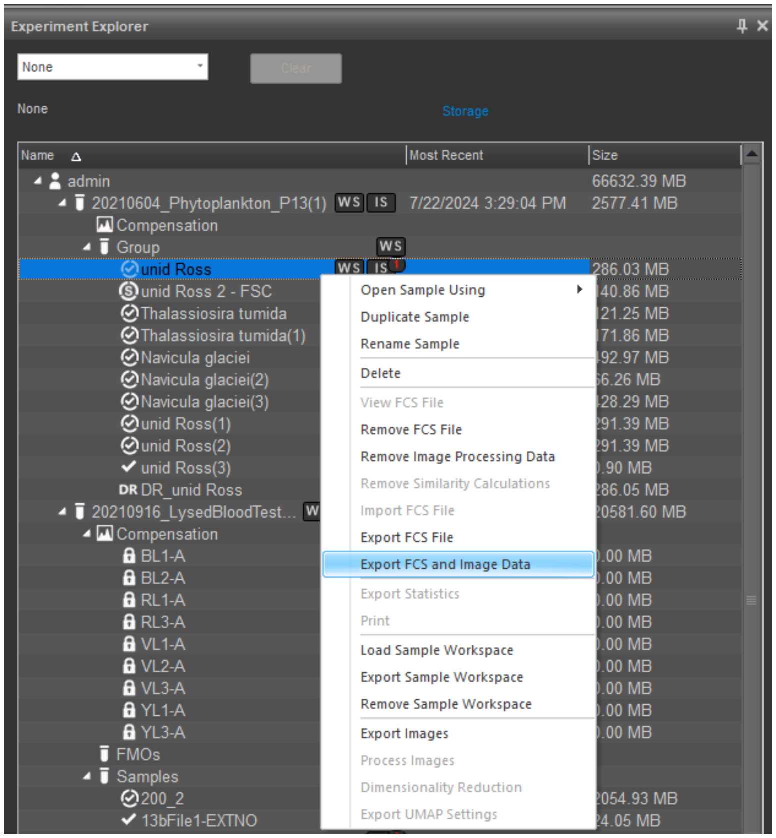
3. To change the threshold of available disk space on the primary disk drives below which the Low Disk Space warning is displayed, enter the desired value (in GB) in the **Threshold** field.

   Note: By default, the threshold is set to 100 GB. You can enter a threshold value of between 1 GB and 1000 GB.

Data management - Export data

Export FCS and Image Data

To export FCS files and imaging data together for a Sample, right-click the Sample in Experiment Explorer, then select **Export FCS and Image Data**.



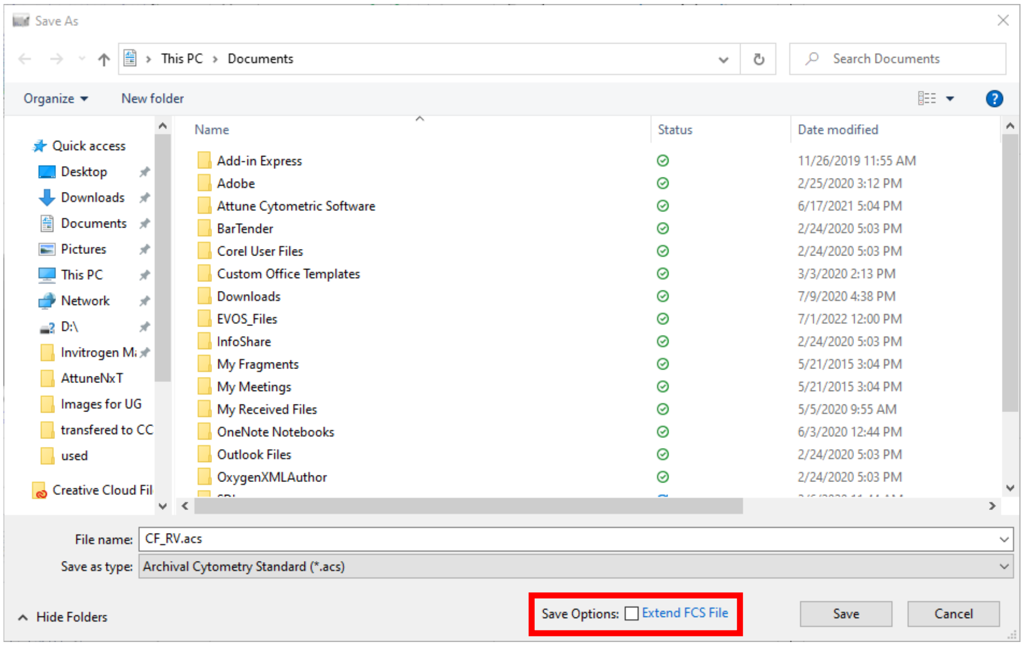
- By default, images, extended parameters (image processing data), and image mask are exported into a single zip file based on the ACS file format.
- Exported ACS files can be imported into a sample, which imports the FCS data and all imaging data.
- Exported ACS files can be opened in third party software that supports the ACS standard (such as FlowJo™ Software and FCS Express™ Software).

Export FCS files with extended data

When exporting sample data that has image processing data, the image processing results can be appended to the original FCS file, which creates a single FCS file that includes the image processing parameters.

1. To export FCS files or FCS files and imaging data together, right-click the sample, then select **Export FCS Files** or **Export FCS and Image Data**.

2. In the **Save As/Folder Selection** dialog, select **Extend FCS File** for **Save Options** to add the new data to the original FCS file.



File name: CF_RV.acs

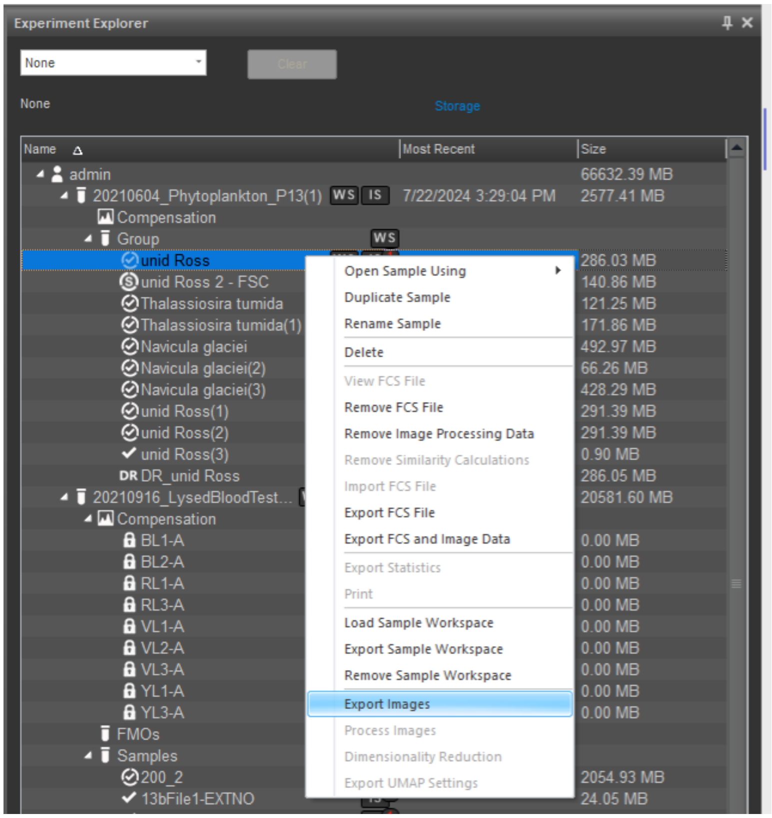
Save as type: Archival Cytometry Standard (*.acs)

Save Options: ☐ Extend FCS File

When **Extend FCS File** option is selected, the original FCS file is appended with the image processing parameter data, and the $ORIGINALITY, $LAST_MODIFIER, and $LAST_MODIFIED keywords in the FCS file are updated to indicate that the data have been appended and modified.

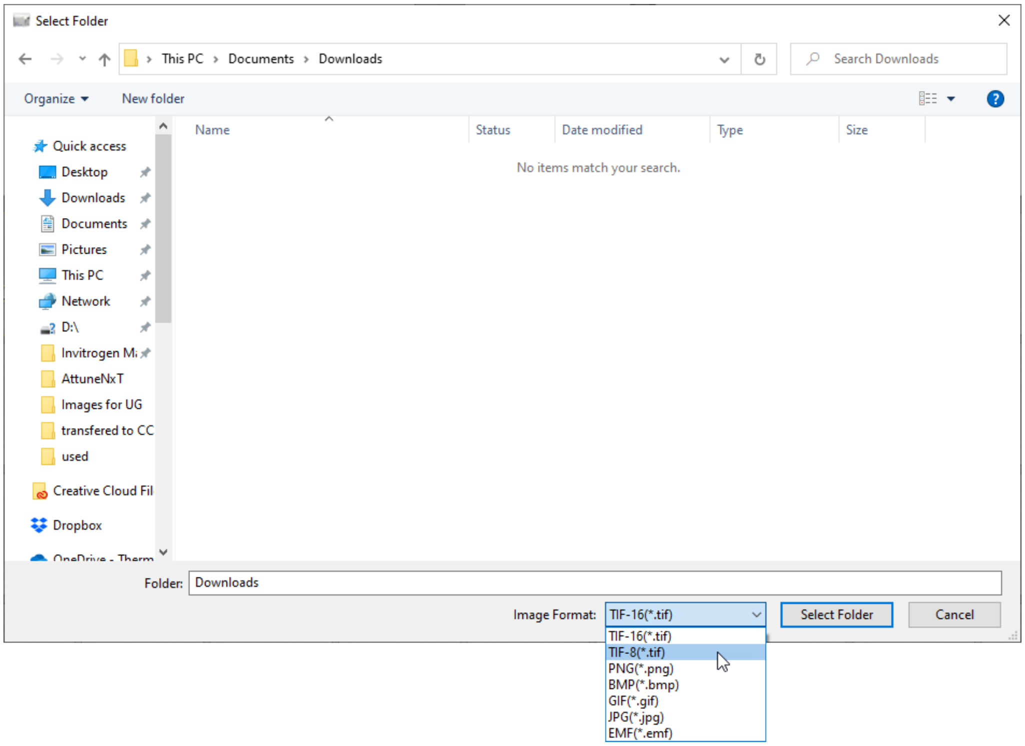
Export Images (from Experiment Explorer)

To export images for an Experiment, Group, or Sample, right-click the Experiment, Group, or Sample in Experiment Explorer, then select **Export Images**.



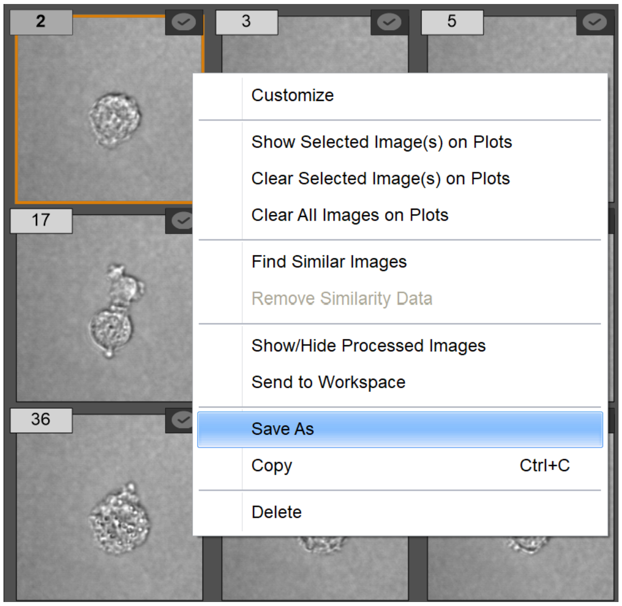
- When images are exported, all images for the selected sample(s) are exported into a single zip file.
- By default, images are exported as TIF-16 files, which is the native file format collected from the Attune™ CytPix™ Flow Cytometer.
- You can select to save the images as TIF-16, TIF-8, PNG, BMP, GIF, EMF, or JPG files in the **Save As/Folder Selection** dialog.

IMPORTANT! When saving in any format other than TIF-16, images are converted to 8-bit images, which results in loss of image pixel data.



Save Images (from Image View)

To save images from the **Image View** and **Image Gallery**, right-click the image, then select **Save As**.

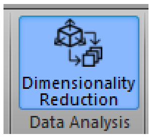


- By default, the selected image is saved as a TIF-8 file.
- You can select to save the images as TIF-16, TIF-8, PNG, BMP, GIF, EMF, or JPG files in the **Save As/Folder Selection** dialog.

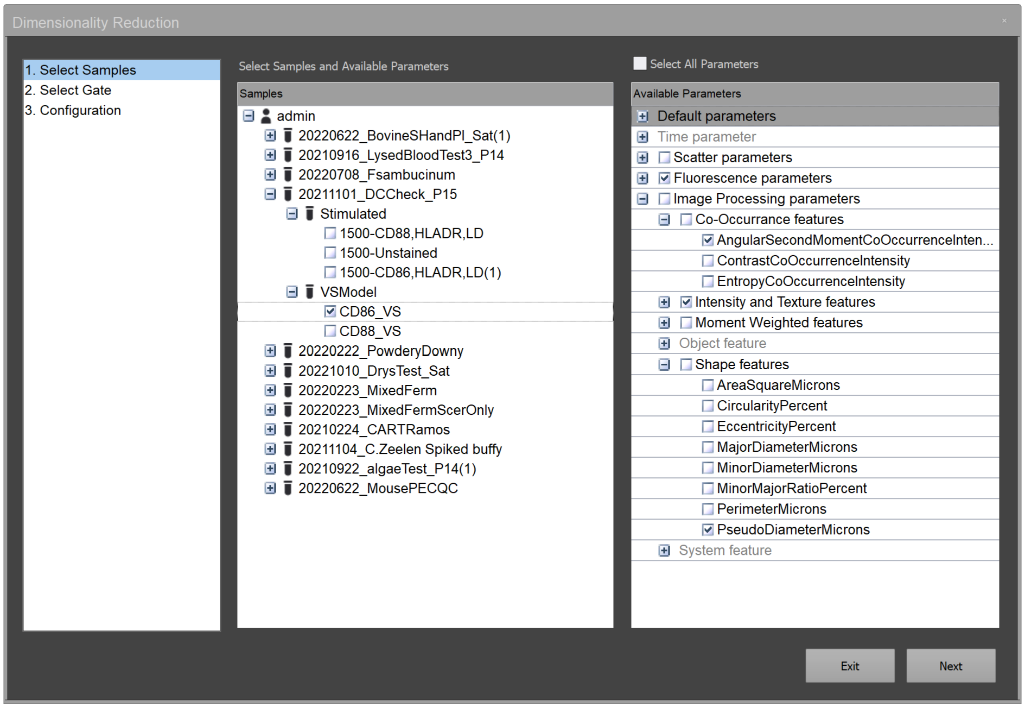
  Note: TIF-16 is the native file format collected from the Attune™ CytPix™ Flow Cytometer. Saving images in any other format results in loss of image pixel data.
- If images have been processed and mask overlays are turned on, images saved from the gallery or image view have the mask data overlayed on the images.
- If multiple images are selected, selected images are saved as individual image files.

Dimensionality Reduction

1. On the Quick Access toolbar, click the **Workspace** tab, then click **Dimensionality Reduction**.



2. Select the samples for analysis. Click **Next**.



Select Samples and Available Parameters

Samples

- admin
  - 20220622_BovineSHandPI_Sat(1)
  - 20210916_LysedBloodTest3_P14
  - 20220708_Fsambucinum
  - 20211101_DCCheck_P15
    - Stimulated
      - ☐ 1500-CD88,HLADR,LD
      - ☐ 1500-Unstained
      - ☐ 1500-CD86,HLADR,LD(1)
    - VSModel
      - ☑ CD86_VS
      - ☐ CD88_VS
  - 20220222_PowderyDowny
  - 20221010_DrysTest_Sat
  - 20220223_MixedFerm
  - 20220223_MixedFermScerOnly
  - 20210224_CARTRamos
  - 20211104_C.Zeelen Spiked buffy
  - 20210922_algaeTest_P14(1)
  - 20220622_MousePECQC

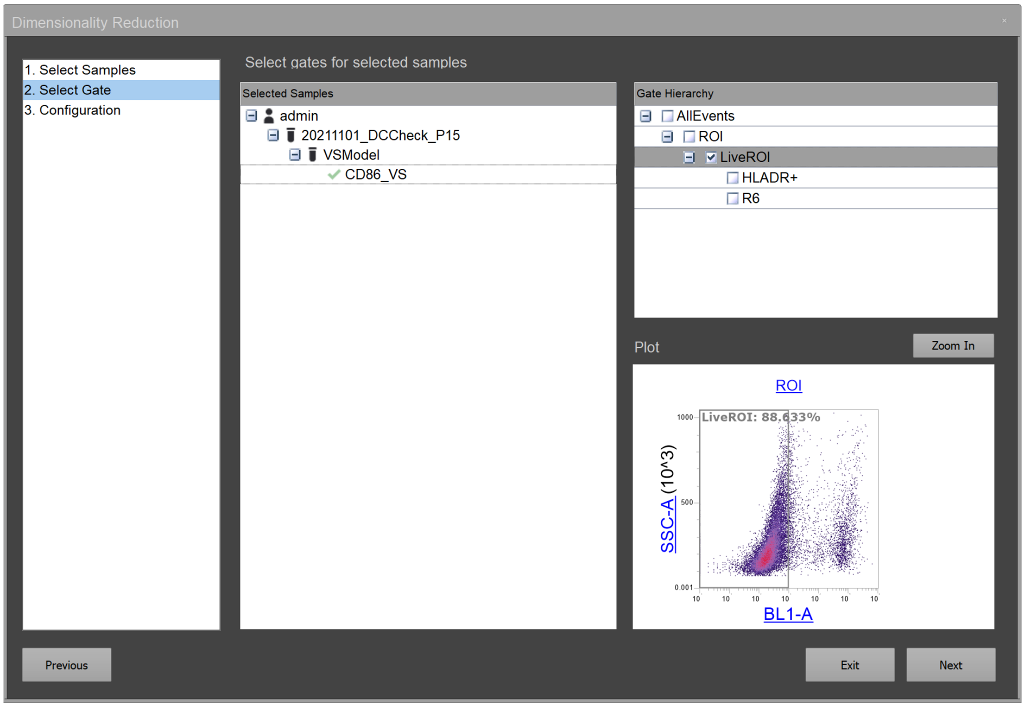
☐ Select All Parameters

Available Parameters

- Default parameters
- Time parameter
- ☐ Scatter parameters
- ☑ Fluorescence parameters
- ☐ Image Processing parameters
  - ☐ Co-Occurance features
    - ☑ AngularSecondMomentCoOccurrenceInten...
    - ☐ ContrastCoOccurrenceIntensity
    - ☐ EntropyCoOccurrenceIntensity
  - ☑ Intensity and Texture features
  - ☐ Moment Weighted features
  - Object feature
  - ☐ Shape features
    - ☐ AreaSquareMicrons
    - ☐ CircularityPercent
    - ☐ EccentricityPercent
    - ☐ MajorDiameterMicrons
    - ☐ MinorDiameterMicrons
    - ☐ MinorMajorRatioPercent
    - ☐ PerimeterMicrons
    - ☑ PseudoDiameterMicrons
  - System feature

Note: One sample or multiple samples can be selected for analysis. Certain parameters are selected by default, while many are optional.

3. In the **Gate Hierarchy** window, select the appropriate gate. Click **AllEvents** to select all collected events.



Select gates for selected samples

Selected Samples

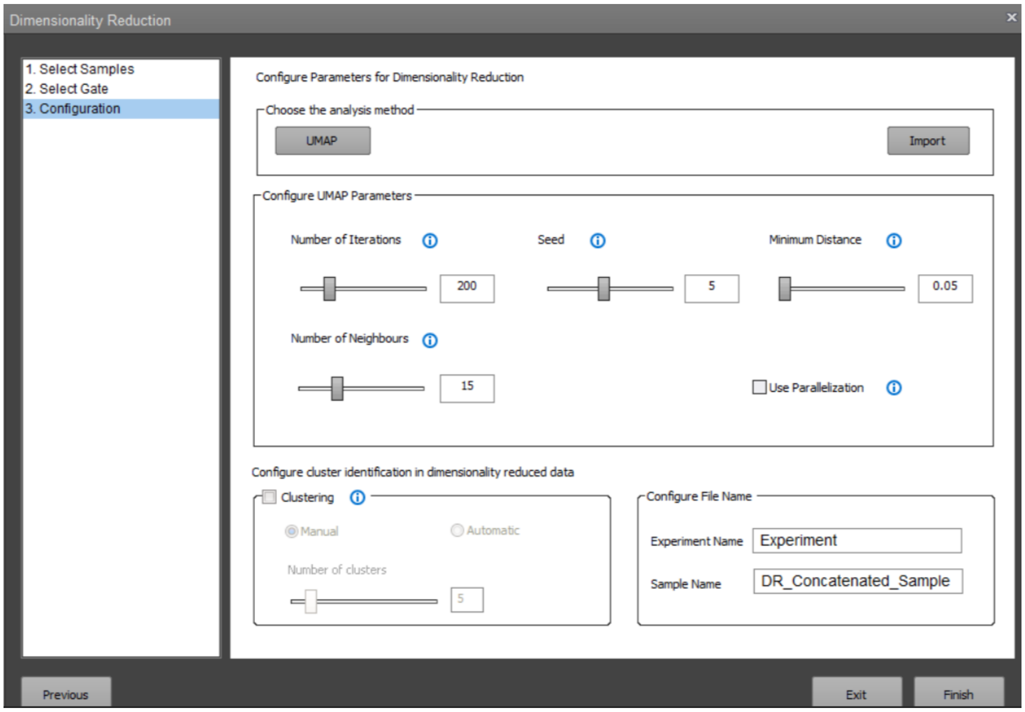
- admin
  - 20211101_DCCheck_P15
    - VSModel
      - ✓ CD86_VS

Gate Hierarchy

- ☐ AllEvents
  - ☐ ROI
    - ☑ LiveROI
      - ☐ HLADR+
      - ☐ R6

4. Click **Next**.

5. Configure the parameters for dimensionality reduction.



Number of Iterations: 200
Seed: 5
Minimum Distance: 0.05
Number of Neighbours: 15
☐ Use Parallelization
☐ Clustering
● Manual ○ Automatic
Number of clusters: 5
Experiment Name: Experiment
Sample Name: DR_Concatenated_Sample

   a. Choose the analysis method by clicking **UMAP**.
   b. Configure the UMAP parameters.

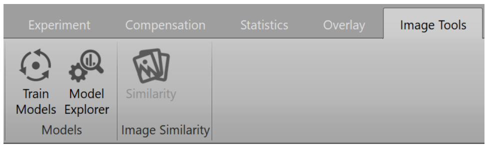
Number of Iterations (1–1,000)	Iteration refers to one complete pass of the entire training dataset through the learning algorithm. The number of iterations determines how many times the learning algorithm will work through the entire dataset, updating the model's parameters each time. A higher number of iterations typically results in a more compressed and separated cluster representation by may require more processing time.
Number of Neighbours (1–50)	Specifies how many neighbouring points each data point should consider when constructing the initial weighted graph. More neighbors results in tighter and sharper clusters.
Seed (1–10)	Seed value, or random seed parameter, controls the random initialization during dimensionality reduction. By setting a same seed value, this ensures that running UMAP on the same dataset produces the same visualizatio, which enables consistent interpretation.
Minimum Distance (0.05–1)	The minimum distance parameter signifies the closeness or how tightly the data points are packed while being embedded in low dimensions. Minimum distance influences how the cluster of cells appear. A low minimum distance will allow data points to be packed very close together, leading to denser and tighter clusters, whereas a high distance will result in loose clusters with more spacing.
Use Parallelization	In flow cytometry data analysis, parallelization plays a crucial role in optimizing the computational efficiency of the algorithm when dealing with the large, high-dimensional datasets. Parallelization allows UMAP to distribute computations across multiple CPU cores or processing units, significantly reducing the time required to perform these operations. Without parallelization, these calculations could take a very long time for large datasets. Note that parallelization often results in variance between runs.

   c. *(Optional)* Click **Clustering**. Select **Manual** or **Automatic** and a value of 2 to 30 to determine the number of clusters present in DR output data.
   d. *(Optional)* Configure the file name by entering the **Experiment** name if 2 or more samples. A single experiment can be given a **Sample** name.

6. Click **Finish**.

Train Models

1. On the **Quick Access** toolbar, click the **Image Tools** tab, then click **Train Models**.



The screen displays the user-driven training workflow.


User Driven Training

1 Model Information

Choose the model that you want to use as a base for new model training.

2 Choose your training samples

Select your samples for training.

3 Train your model

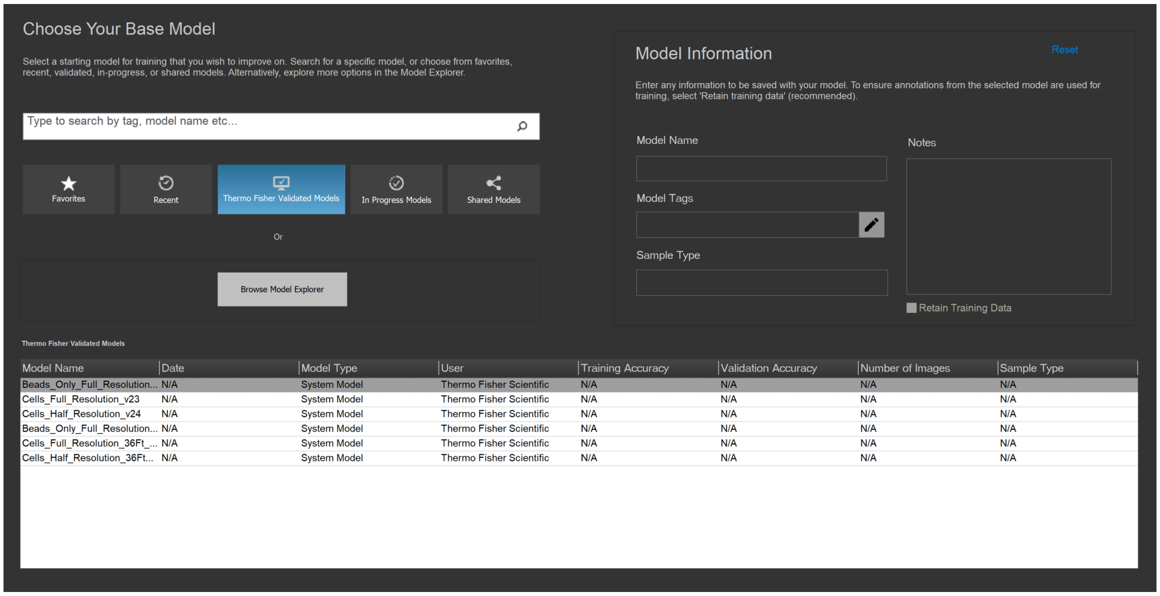
Annotate your images and train the model.

2. Click **Next**.

3. Select a base model for training. Use the search bar to find a specific model or select a model from the 6 choices:

Favorites	Previously created models with up to 10 favorited models.
Recent	The 10 most recently created models.
Thermo Fisher Validated Models	Thermo Fisher system models: • Cells_Half_Resolution_v24 • Cells_Full_Resolution_v23 • Beads_Only_Full_Resolution_v1 • Cells_Half_Resolution_36Ft_v24 • Cells_Full_Resolution_36Ft_v23 • Beads_Only_Full_Resolution_36Ft_v1
In Progress Models	Models started in the building process and saved.
Shared Models	Models shared in the same system.
Browse Model Explorer	Opens **Model Explorer** for further searching of Annotation, System, and User Models with model information for each selection.

The screen below shows **Thermo Fisher Validated Models** selected with the list view of available models.



Choose Your Base Model

Select a starting model for training that you wish to improve on. Search for a specific model, or choose from favorites, recent, validated, in-progress, or shared models. Alternatively, explore more options in the Model Explorer.

Type to search by tag, model name etc...

Model Information

Enter any information to be saved with your model. To ensure annotations from the selected model are used for training, select 'Retain training data' (recommended).

Model Name:

Model Tags:

Sample Type:

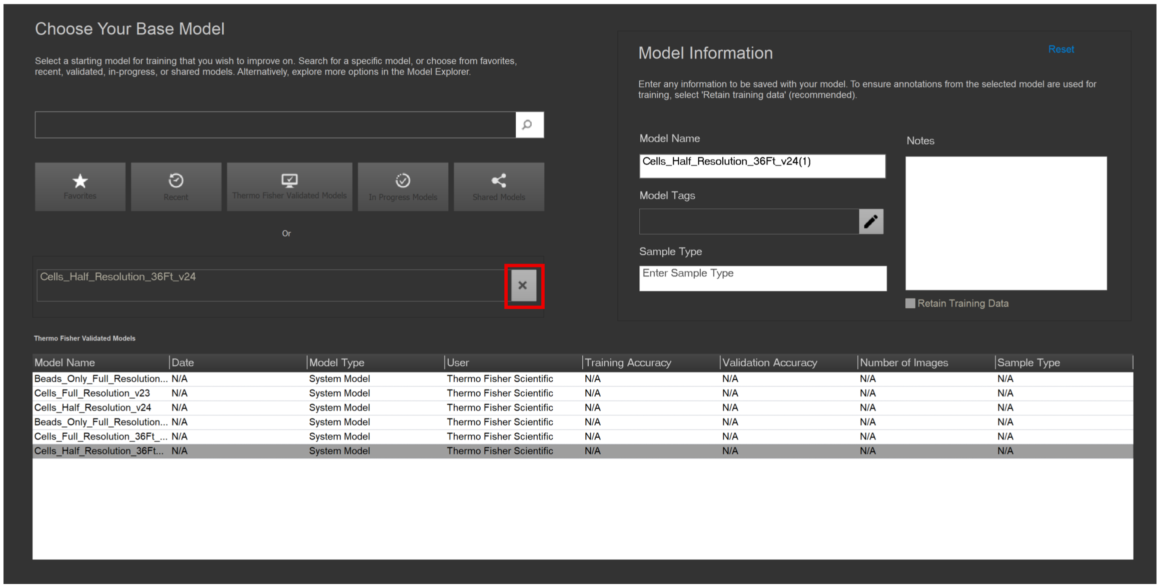
Notes:

☐ Retain Training Data

Thermo Fisher Validated Models

Model Name	Date	Model Type	User	Training Accuracy	Validation Accuracy	Number of Images	Sample Type
Beads_Only_Full_Resolution...	N/A	System Model	Thermo Fisher Scientific	N/A	N/A	N/A	N/A
Cells_Full_Resolution_v23	N/A	System Model	Thermo Fisher Scientific	N/A	N/A	N/A	N/A
Cells_Half_Resolution_v24	N/A	System Model	Thermo Fisher Scientific	N/A	N/A	N/A	N/A
Beads_Only_Full_Resolution...	N/A	System Model	Thermo Fisher Scientific	N/A	N/A	N/A	N/A
Cells_Full_Resolution_36Ft_...	N/A	System Model	Thermo Fisher Scientific	N/A	N/A	N/A	N/A
Cells_Half_Resolution_36Ft...	N/A	System Model	Thermo Fisher Scientific	N/A	N/A	N/A	N/A

The screen below shows the model selected (gray row) with the model name shown above. Clicking **X** next to the name field closes the model selection to allow for another selection.



Choose Your Base Model

Select a starting model for training that you wish to improve on. Search for a specific model, or choose from favorites, recent, validated, in-progress, or shared models. Alternatively, explore more options in the Model Explorer.

Cells_Half_Resolution_36Ft_v24

Model Information

Enter any information to be saved with your model. To ensure annotations from the selected model are used for training, select 'Retain training data' (recommended).

Model Name: Cells_Half_Resolution_36Ft_v24(1)

Model Tags:

Sample Type: Enter Sample Type

Notes:

☐ Retain Training Data

Thermo Fisher Validated Models

Model Name	Date	Model Type	User	Training Accuracy	Validation Accuracy	Number of Images	Sample Type
Beads_Only_Full_Resolution...	N/A	System Model	Thermo Fisher Scientific	N/A	N/A	N/A	N/A
Cells_Full_Resolution_v23	N/A	System Model	Thermo Fisher Scientific	N/A	N/A	N/A	N/A
Cells_Half_Resolution_v24	N/A	System Model	Thermo Fisher Scientific	N/A	N/A	N/A	N/A
Beads_Only_Full_Resolution...	N/A	System Model	Thermo Fisher Scientific	N/A	N/A	N/A	N/A
Cells_Full_Resolution_36Ft_...	N/A	System Model	Thermo Fisher Scientific	N/A	N/A	N/A	N/A
Cells_Half_Resolution_36Ft...	N/A	System Model	Thermo Fisher Scientific	N/A	N/A	N/A	N/A

The **Save & Exit** and **Next** buttons are active once a model selection is made.

4. In the **Model Information** panel, edit the **Model Name** and **Sample Type** and click ✎ **(Model Tags)** to access **Tag Management**. Enter notes as needed in the **Notes** field.

   Note: The **Retain Training Data** checkbox is automatically checked when using a user-trained model as a base model. This ensures that data used to create the base model are retained accurately. This option is not available for vendor-supplied models.

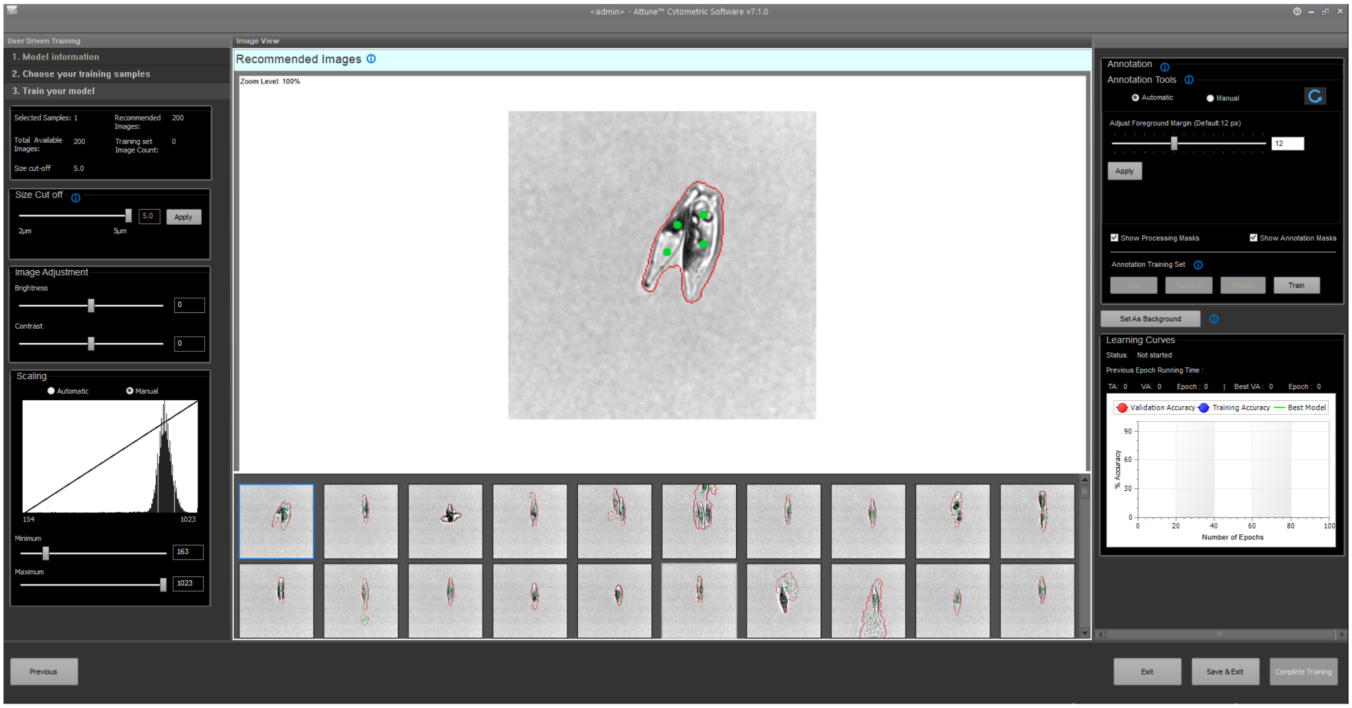
5. Click **Save & Exit** to save your model building progress and exit the model training. You may return to this model at a later time to complete training. Click **Next** to choose samples for creating an image library.

6. Select your training library by clicking through the various fields and entering appropriate values.

   Note: Click ⓘ **(Information)** throughout the screen to view details of each function.

Note: Base model masks from initial steps can be seen outlining the cells and marking the centroids.

Selected Samples	Number of samples used to create the image library
Total Available Images	Number of images in the library (recommended images plus retained images)
Size cut-off	Enter a number in the field or use the slider bar to select from 2–5 µm in 0.5-µm increments (system models default to 5 µm). Click **Apply**.
Recommended Images	Size of the selected training library (200–1,000 images)
Training set Image Count	Number of images in the training set (annotated images a user adds plus retained images)
Image Adjustment	Use the slider bars to adjust the **Brightness** and **Contrast** when needed.


<admin> - Attune™ Cytometric Software v7.1.0

User Driven Training

1. Model information
2. Choose your training samples
3. Train your model

Selected Samples: 1
Recommended Images: 200
Total Available Images: 200
Training set Image Count: 0
Size cut-off 5.0

Size Cut off: 5.0

Brightness: 0
Contrast: 0

Scaling: ○ Automatic ● Manual

Minimum: 163
Maximum: 1023

Zoom Level: 100%

Annotation Tools: ● Automatic ○ Manual

Adjust Foreground Margin (Default:12 px): 12

☑ Show Processing Masks ☑ Show Annotation Masks

Learning Curves

Status: Not started

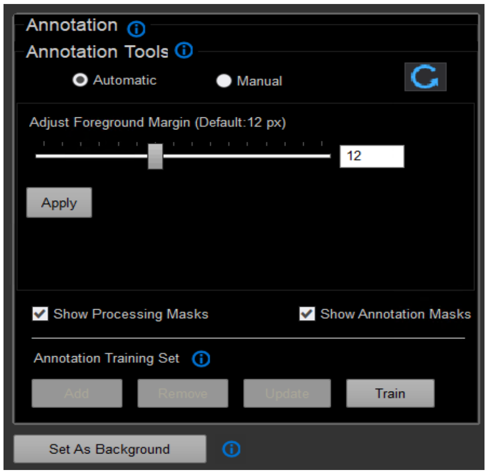
Previous Epoch Running Time :

TA: 0 VA: 0 Epoch : 0 | Best VA : 0 Epoch : 0

7. Annotate the images using the **Annotation Tools**.

   a. Click **Automatic** or **Manual**.

   Note: Automatic suggests annotation masks and centroids. **Manual** allows the user to draw the foreground (pink), background (blue), and centroid (yellow) annotations. To clear all annotations on the selected image, click ↻ **(Clear)**.

Automatic	Manual
 - Use the slider bar to **Adjust the Foreground Margin** (default value is 12 px). The margin helps define with certainty where the objects begin and end. - *(Optional)* Check **Show Processing Masks**. Check **Show Annotation Masks**. - Click **Apply**. **Note:** Automatically annotated images can be manually edited after initial annotation.	 - *(Optional)* Click **Foreground**, **Background**, or **Centroid** to manually annotate features. Use the brush tools and **Brush Size** slider bar to change the brushing tool size for the **Foreground** and **Background** features. - *(Optional)* Click **Show Processing Masks**. Click **Show Annotation Masks**.

   b. Centroids can be added using the software recommended position using the **Suggest Center** button or **Centroid** tool. Centroid positions can be edited by clicking and moving the centroids on each image. To remove all added centroids on an image, click **Remove**.

   c. Under **Annotation Training Set**, click **Add**, **Remove**, or **Update** to affect images in the training set, or click **Train** to continue the training after a minimum of 10 images are added to the training set.

   Note: To add multiple images, use the mouse to drag and click across images. **Ctrl + right click** can be used to select images that are not sequential.

   d. If the image lacks noticeable objects, click **Set As Background**. This applies a background mask to the image, which helps to train the algorithm to recognize background areas.

8. Watch the processing masks update in the training library as the model trains. If desired, add additional annotations during live training or click **Pause** to pause the training to add more images.

9. Click **Complete Training**.

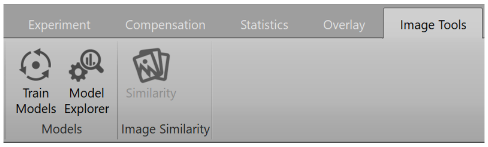
   Note: The **Complete Training** button is enabled after completion of 20 epochs.

   A **Model Training Complete** window displays upon completion of the training. The new model is automatically optimized in the **Image Processing Dashboard**.

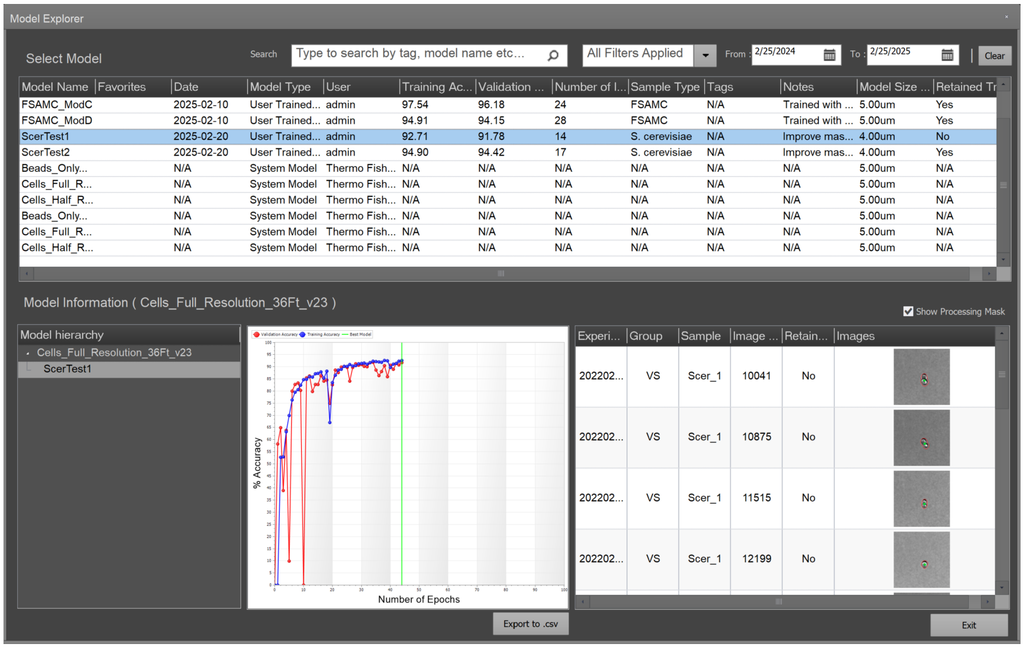
Model Explorer

The **Model Explorer** feature provides details and data for models present in the system. This model metadata assists in choosing a base model for training or processing data. The screen is divided into the **Select Model** and **Model Information** panes.

1. On the **Quick Access** toolbar, click the **Image Tools** tab, then click **Model Explorer**.



The screen displays the **Model Explorer** window.



Model Name	Favorites	Date	Model Type	User	Training Ac...	Validation ...	Number of I...	Sample Type	Tags	Notes	Model Size ...	Retained Tr
FSAMC_ModC		2025-02-10	User Trained...	admin	97.54	96.18	24	FSAMC	N/A	Trained with ...	5.00um	Yes
FSAMC_ModD		2025-02-10	User Trained...	admin	94.91	94.15	28	FSAMC	N/A	Trained with ...	5.00um	Yes
ScerTest1		2025-02-20	User Trained...	admin	92.71	91.78	14	S. cerevisiae	N/A	Improve mas...	4.00um	No
ScerTest2		2025-02-20	User Trained...	admin	94.90	94.42	17	S. cerevisiae	N/A	Improve mas...	4.00um	Yes
Beads_Only...		N/A	System Model	Thermo Fish...	N/A	N/A	N/A	N/A	N/A	N/A	5.00um	N/A
Cells_Full_R...		N/A	System Model	Thermo Fish...	N/A	N/A	N/A	N/A	N/A	N/A	5.00um	N/A
Cells_Half_R...		N/A	System Model	Thermo Fish...	N/A	N/A	N/A	N/A	N/A	N/A	5.00um	N/A
Beads_Only...		N/A	System Model	Thermo Fish...	N/A	N/A	N/A	N/A	N/A	N/A	5.00um	N/A
Cells_Full_R...		N/A	System Model	Thermo Fish...	N/A	N/A	N/A	N/A	N/A	N/A	5.00um	N/A
Cells_Half_R...		N/A	System Model	Thermo Fish...	N/A	N/A	N/A	N/A	N/A	N/A	5.00um	N/A

Experi...	Group	Sample	Image ...	Retain...	Images
202202...	VS	Scer_1	10041	No	
202202...	VS	Scer_1	10875	No	
202202...	VS	Scer_1	11515	No	
202202...	VS	Scer_1	12199	No	

Model Information is further divided into a **Model hierarchy** pane and a **Samples, Images, and Processing Masks** pane.

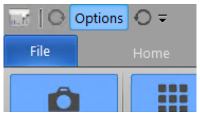
- **Model hierarchy** is presented as a clickable hierarchical table where users can browse through model history, especially if successive model generations have been trained on top of each other. If a model is deleted, it will still be represented in the hierarchical table but will show no data. A warning triangle will indicate the deletion and notify users of the status.
- **Samples, Images, and Processing Masks** is a table showing all images in the training set that were used to train the model. This can be used to see how well that model performed on the training data and to determine its appropriateness for use or need for further training. The Experiment, Group, Sample, and Image Number are present to enable traceability. Further, information about whether this is a Retained Image is also provided. Each image is shown with the option to see the processing masks of the model on top of the image. Processing masks can be clicked on or off as needed.
  The training curves at the time of model saving are shown with an option to export data to a .csv file to plot in another software.
- **Export to .csv** allows for exporting training curves.

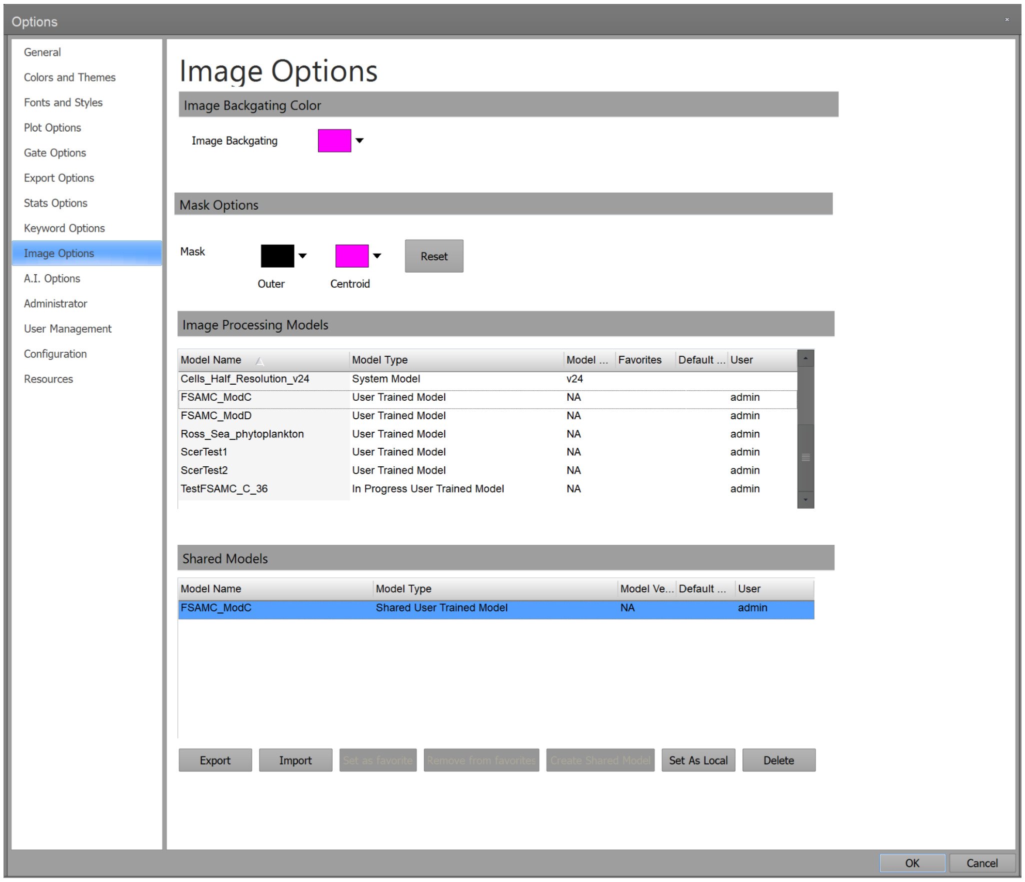
2. Select a model by clicking on the desired model row or using the search field to type in the desired details (for example, tag or model name). Models can also be searched using the date fields. Click **Clear** to return to empty search fields.

3. Click **Confirm** to select the appropriate model.

Model sharing and importing

1. Click **Options ▸ Image Options**.





Model Name	Model Type	Model ...	Favorites	Default ...	User
Cells_Half_Resolution_v24	System Model	v24			
FSAMC_ModC	User Trained Model	NA			admin
FSAMC_ModD	User Trained Model	NA			admin
Ross_Sea_phytoplankton	User Trained Model	NA			admin
ScerTest1	User Trained Model	NA			admin
ScerTest2	User Trained Model	NA			admin
TestFSAMC_C_36	In Progress User Trained Model	NA			admin

Model Name	Model Type	Model Ve...	Default ...	User
FSAMC_ModC	Shared User Trained Model	NA		admin

2. To create a shared model, select a **User Trained Model** from the list of **Image Processing Models**.

3. Click **Create Shared Model**, following software prompts as provided. The new shared model will now appear in the **Shared Models** list.

4. To export a shared model, select a model from the **Shared Models** list and click **Export**. This will open a file explorer window to save the model's .utm file.

5. To import a shared model, click **Import** to navigate to a model's .utm file location.

   The imported shared model appears in blue in the **Shared Models** list.

6. Click **Delete** to remove a model. This is the only place to perform this function.

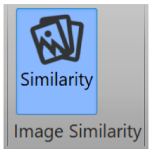
Similarity

Note: Process samples with an image processing model before performing the **Similarity** function.

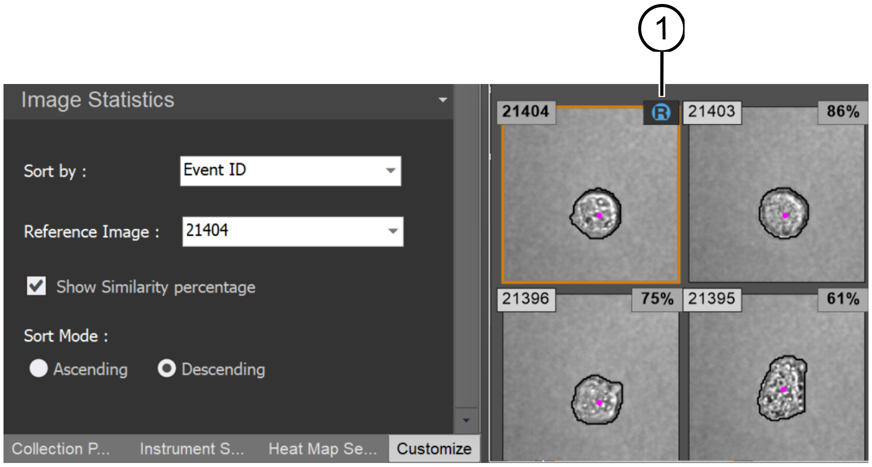
Note: Similarity data are calculated for all images within the selected sample, not for subsets of data (gates).

Note: Any objects with a centroid count different than the selected reference image will result in 0% similarity (for example, if a singlet reference image is selected, all doublets will appear as 0% similar).

1. Select an image, then click **Similarity** in the Process Images ribbon. Alternatively, select an image, then right-click and select **Find Similar Images**.



2. *(Optional)* In the **Customize** panel, scroll down to the **Image Statistics** section to adjust the **Similarity** settings.

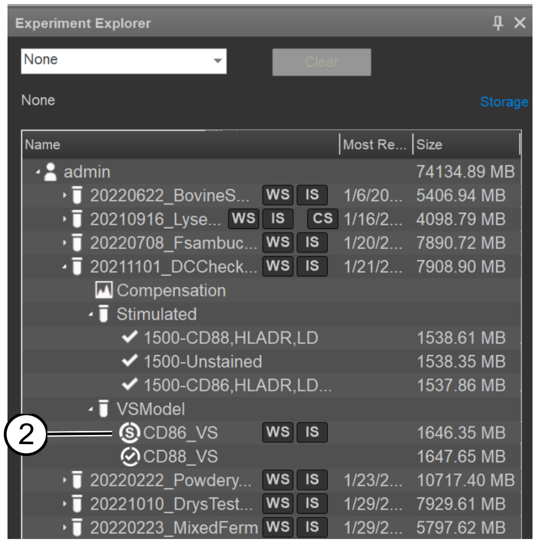


Sort by : Event ID

Reference Image : 21404

☑ Show Similarity percentage

Sort Mode :
○ Ascending ● Descending



Name	Most Re...	Size
admin		74134.89 MB
20220622_BovineS... WS IS	1/6/20...	5406.94 MB
20210916_Lyse... WS IS CS	1/16/2...	4098.79 MB
20220708_Fsambuc... WS IS	1/20/2...	7890.72 MB
20211101_DCCheck... WS IS	1/21/2...	7908.90 MB
Compensation		
Stimulated		
✓ 1500-CD88,HLADR,LD		1538.61 MB
✓ 1500-Unstained		1538.35 MB
✓ 1500-CD86,HLADR,LD...		1537.86 MB
VSModel		
CD86_VS WS IS		1646.35 MB
CD88_VS		1647.65 MB
20220222_Powdery... WS IS	1/23/2...	10717.40 MB
20221010_DrysTest... WS IS	1/29/2...	7929.61 MB
20220223_MixedFerm WS IS	1/29/2...	5797.62 MB

① Icon indicates reference image

② Icon next to **Experiment Explorer** sample listing indicating **Similarity** has been applied

- **Sort by**: Select **Event ID** or **Similarity Percentage**
- **Reference Image**: Displays image selections
- **Show Similarity percentage**: Turns on or off the percentage shown in the corner of each image
- **Sort Mode**: Select **Ascending** or **Descending**

Limited product warranty

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Revision history: Pub. No. MAN0028531 C

Revision	Date	Description
C	29 April 2025	Removed Downsampling and Concatenation topic for versioning of software.
B	18 March 2025	Added new v7.1 software features including Model Explorer, Similarity, Dimensionality Reduction, and Downsampling and Concatenation for Data Analysis.
A.0	17 March 2023	Initial release.