Gulbenkian Institute for Molecular Medicine

Flow Cytometry Data

Cell View Lens Plugin

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June 2026

Guide Information

This guide was developed and validated using:

- **FlowJo v10.10.1**
- **CellView Lens Plugin v2.6**

Support data was generated using a BD FACSDiscover S8 Cell Sorter.

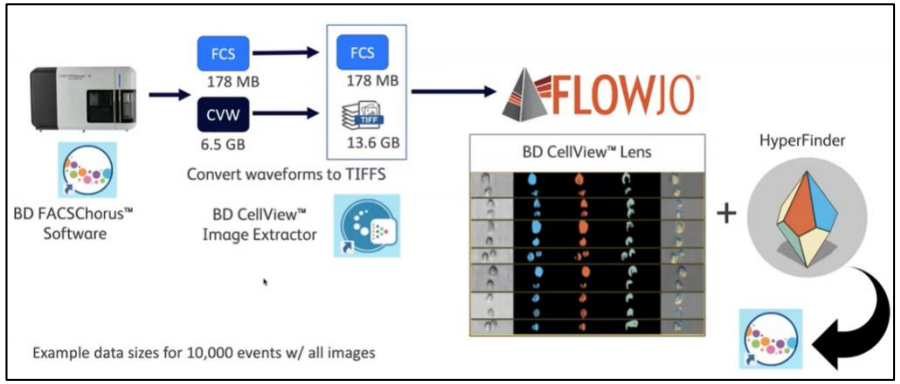
When analyzing S8/A8 data in FlowJo V10, please follow the recommended **scaling guidelines**:
https://www.flowjo.com/docs/flowjo10/advanced-features/bd-facs-discover-s8-data/s8-preference-recomendations

For complete **CellView Lens documentation**, please refer to:
https://www.flowjo.com/docs/flowjo10/advanced-features/cellview-lens

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Using Cell View Lens Plugin

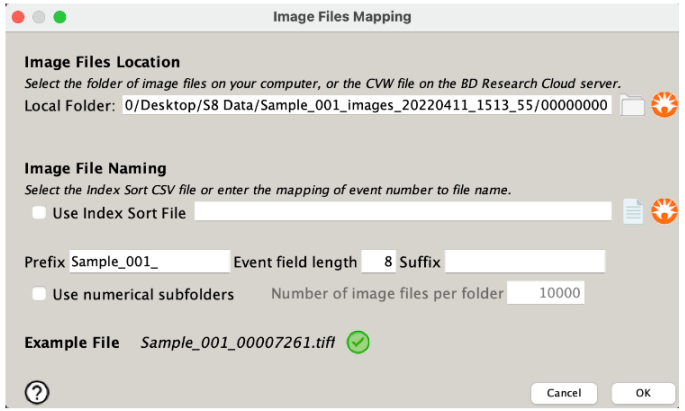
The BD CellView™ Lens plugin for FlowJo™ v10 provides the complete set of tools for visually exploring the image data captured and exported from the BD FACSDiscover™ S8 cell sorter and A8 analyzer.



Start by Extracting your images from the CVW file using the BD CellView Image Extractor.

Then, load your FCS file into a workspace and analyze the data as you normally would to identify populations of interest.

Select a **population**, then choose the BD CellView™ Lens plugin from the Workspace – Plugins menu to create a plugin node for that population. The first use of the plugin in a workspace pops open the Event to Image File Mapping dialog box.



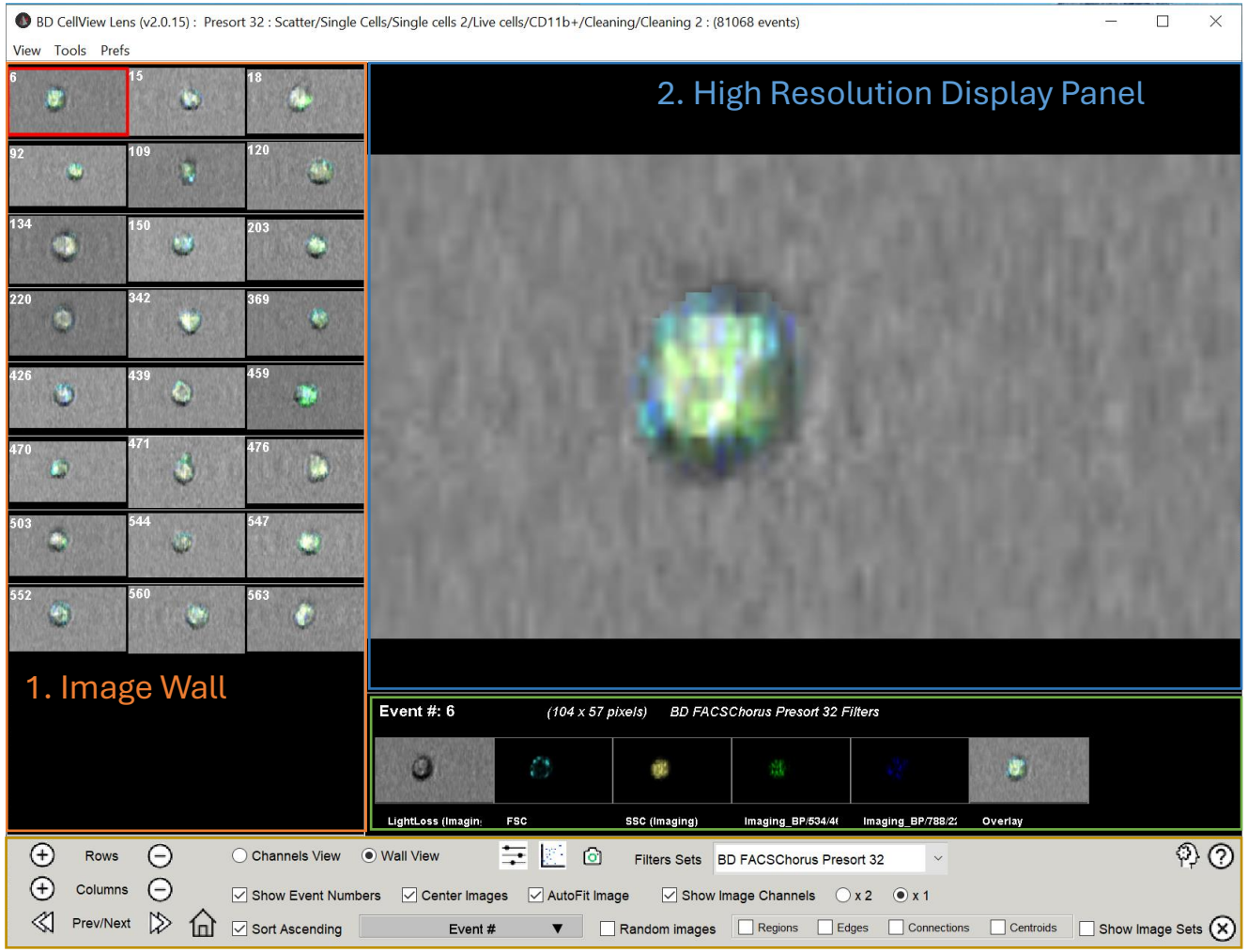
Once images have been linked you will notice that a new node with a camera lens icon displays below the selected population.

Name	Statistic	#Cells
Sample_001.fcs		11000
Cells	87.6	9636
BD_CellView_Lens of Cells		Ready

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Exploring Images

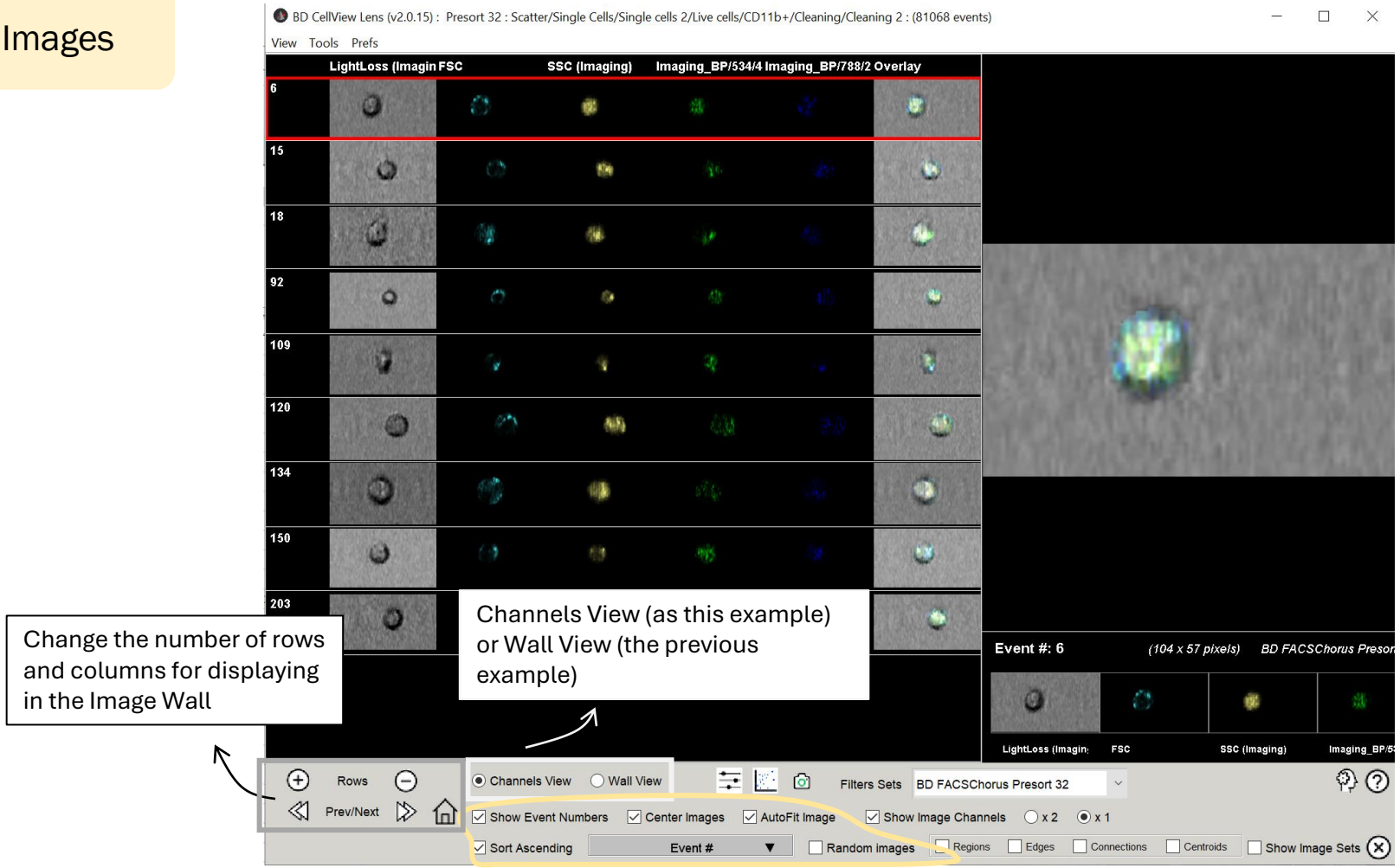


3. Channels of selected image

4. Image Display Controls

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Exploring Images



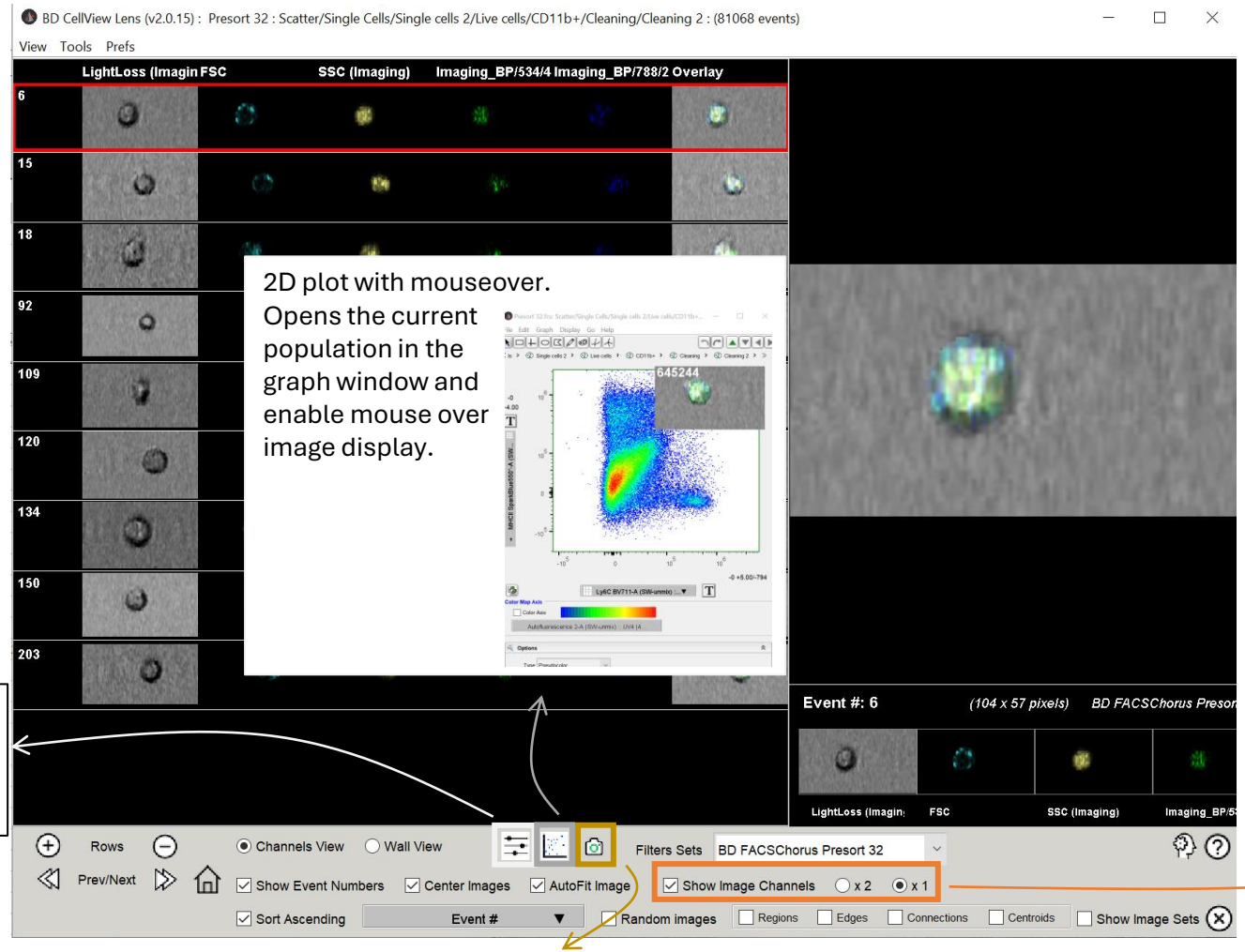
Change the number of rows and columns for displaying in the Image Wall

Channels View (as this example) or Wall View (the previous example)

Customize the Image Wall by centering or auto-fitting images. By default, images are sorted by event number. You can choose how they are displayed: sorted in ascending/descending order by a selected parameter/morphological feature or shown randomly

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Exploring Images



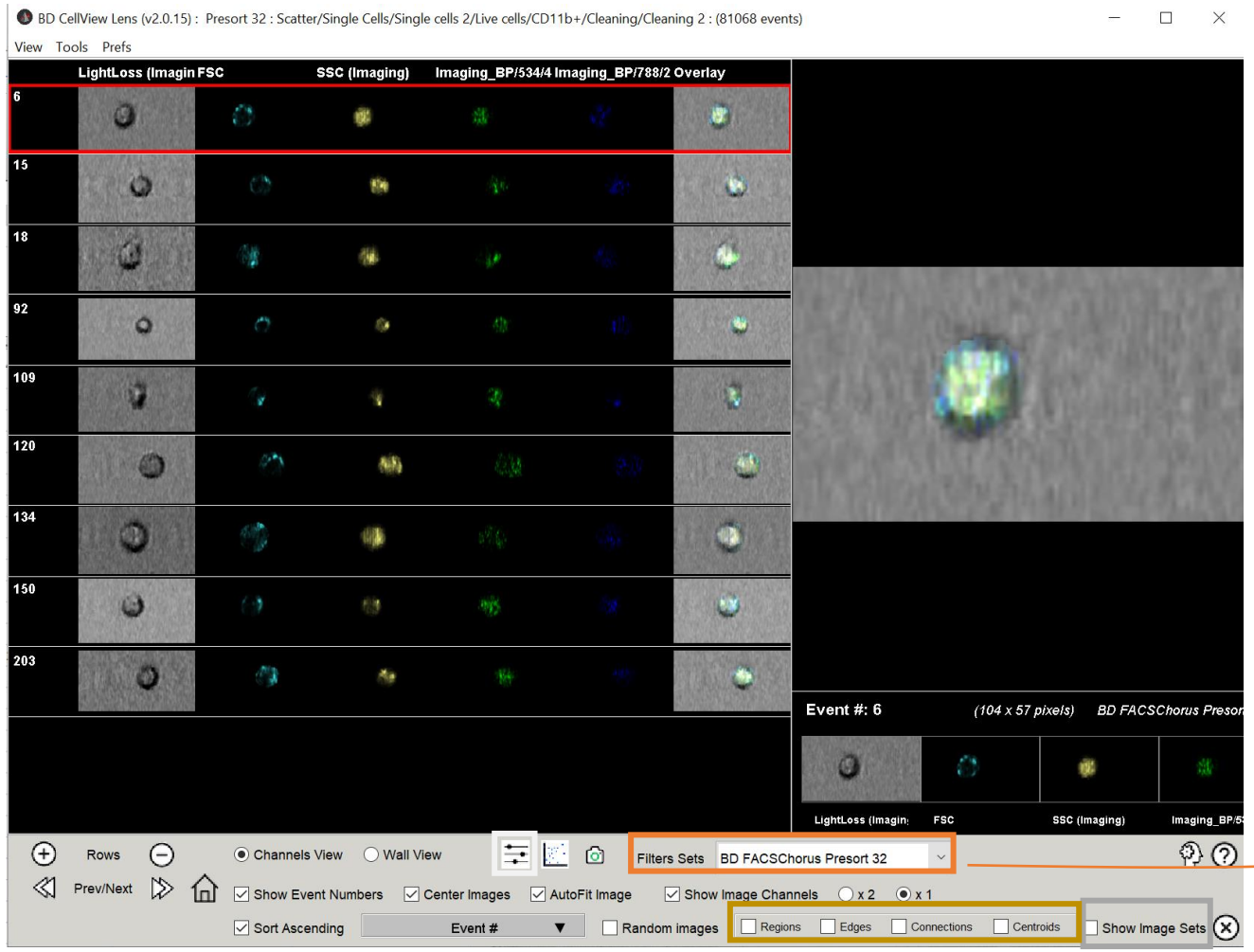
Open the controls for modifying the appearance of the images (Filter sets) (**Page 7**)

Customize the display in the High-Resolution Display Panel and respective channels

Take Snapshot for Layout Editor

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Exploring Images



Adjustments made to the image filtering can be saved as filter sets, selected here, and applied

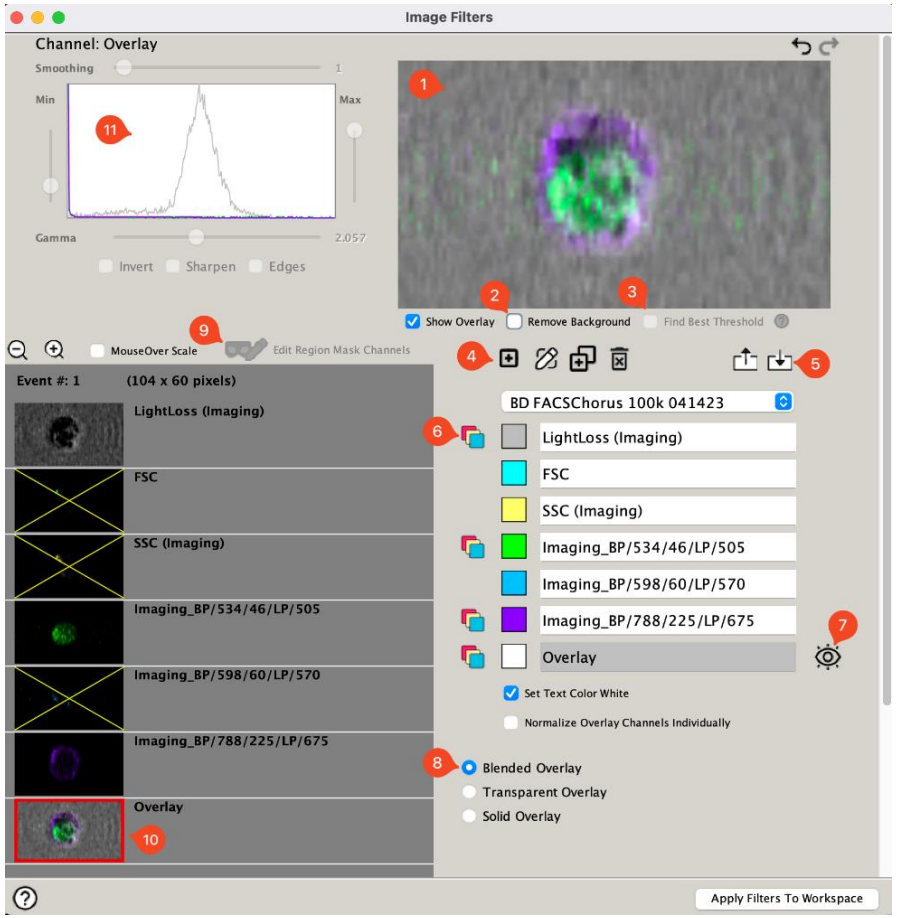
Manually curate a set of your favorite images (**Page 8**)

Visualize Region Masks **(Page 12)**

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Adjusting Image Filters

Refine the display of images in each channel by adding different color options, image processing filters, and by controlling the composition of the Overlay channel.



No.	Tool Description
1	**Image preview**. Displays either the selected channel or all active channels if Show Overlay is selected
2	**Remove Background**. Using the Region Mask Channel(s) (see No. 9), contiguous edges will be identified and any signal outside that region will be removed as background.
3	**Find Best Threshold**. If Remove Background is selected checking this box will use an algorithm to identify the optimal boundary.
4	Create, rename, duplicate, or delete filter set. Organizational tools for filter sets
5	Save or load filter sets. Particularly well-crafted filter sets can be saved as an external file and imported into other workspaces.
6	**Channel controls**. Clicking to the left of the color box will make a channel active and turn on the blue/yellow/box icon. Active channels will be used to create the overlay image and can be viewed in the image wall. Clicking on the colored box will change the highlight color of the channel.
7	**Displayed channel indicator**. The eye icon next to a channel displays which channel will be displayed in the image wall (if you are in channel view, all active channels will be displayed). Clicking to the right of any channel will move the eye to that channel. Only one channel can be the displayed channel at a time.
8	**Overlay options**. Select Blended, Transparent, or Solid to control how the colors are mixed from multiple channels.
9	**Edit Region Mask Channels** - If Remove Background is checked on clicking this button will show a mask icon for each channel used to determine the image mask, meaning the edge of the identified cell(s) that will be kept after background removal. Control-clicking on any channel will toggle it on/off. If edit region is selected, the Show Overlay button becomes the Show Region Mask button, which allows you to display the area identified as 'cell' vs. noise that will be removed. See further information on Region Masks in page 12.
10	**Channel controls**. The red box indicates which channel is being edited currently. Double clicking on a channel toggles it on/off.
11	**Channel manual editing tools**. These tools can be used to adjust smoothing, and display thresholds for the selected channel.

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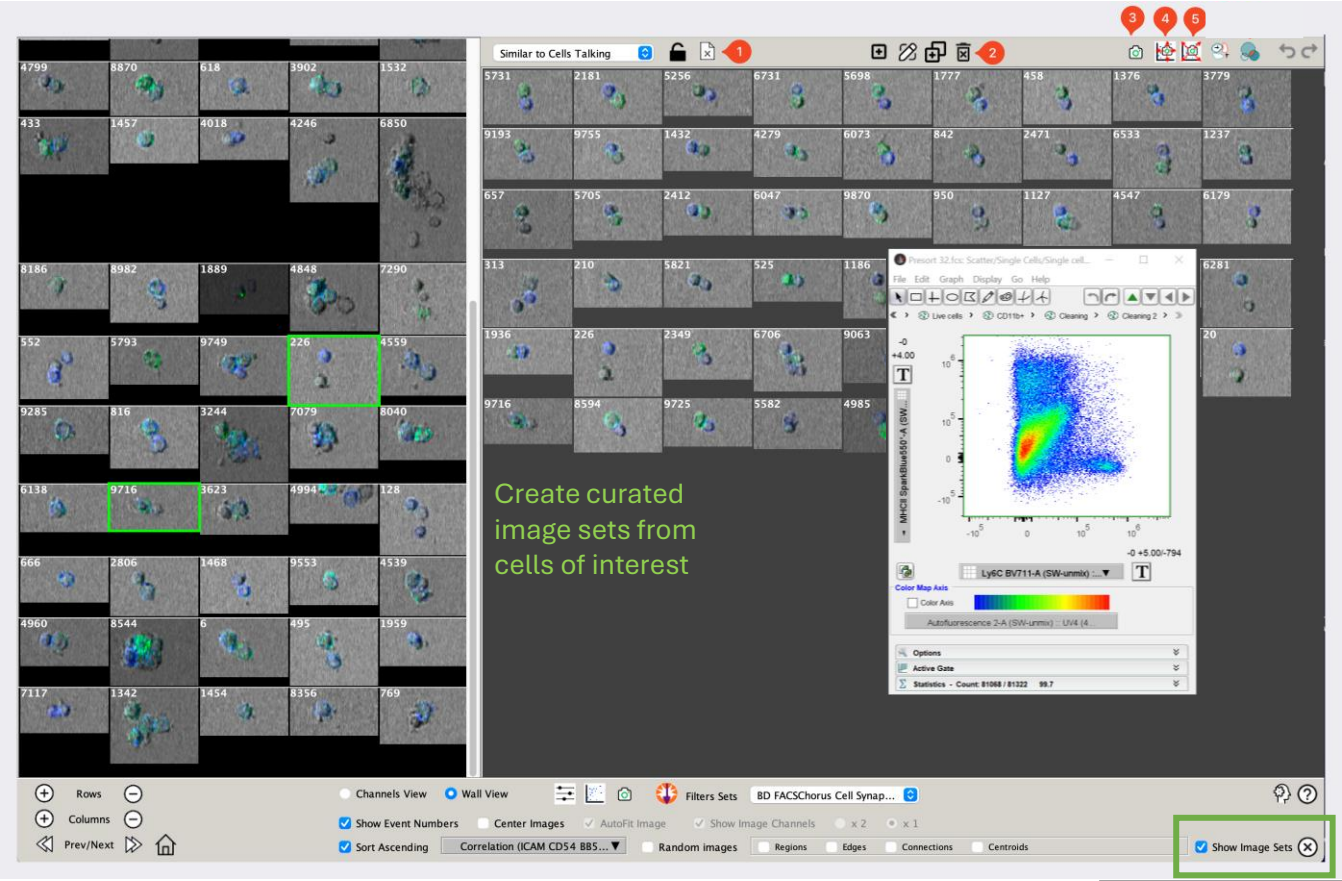
Creating Image Sets

1. Image set name, unlock, and remove all images from the set

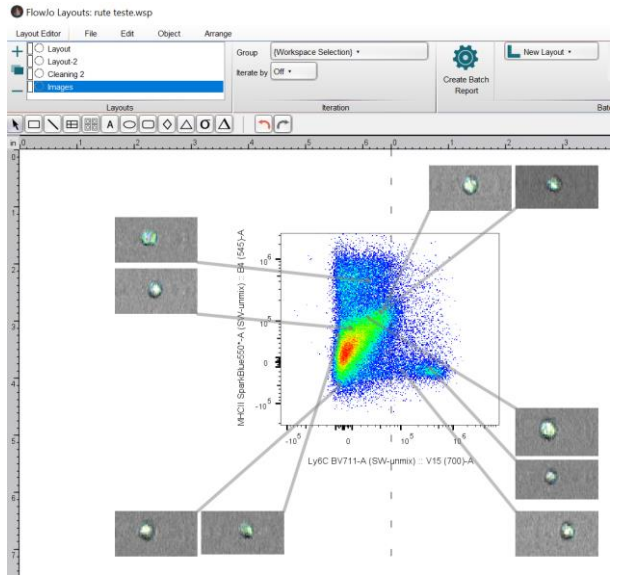
2. Make a new image set, edit the name, duplicate a set, and delete the entire set

3. Add the images in the current image set to the layout editor
4. Create a 2D plot with image callouts, aligned around edges of the plot
5. Create a 2D plot with image callouts, aligned in quadrants

Call out 3 will create a graphic of the wall of images in the image set. If a 2D plot has been created for these images (edges turn green), call out 4 will create a graph in the layout editor of the selected 2D plot with the images in the set's position indicated, and the images placed along the border of the plot. Call out 5 will do the same but arrange the images in quadrants.

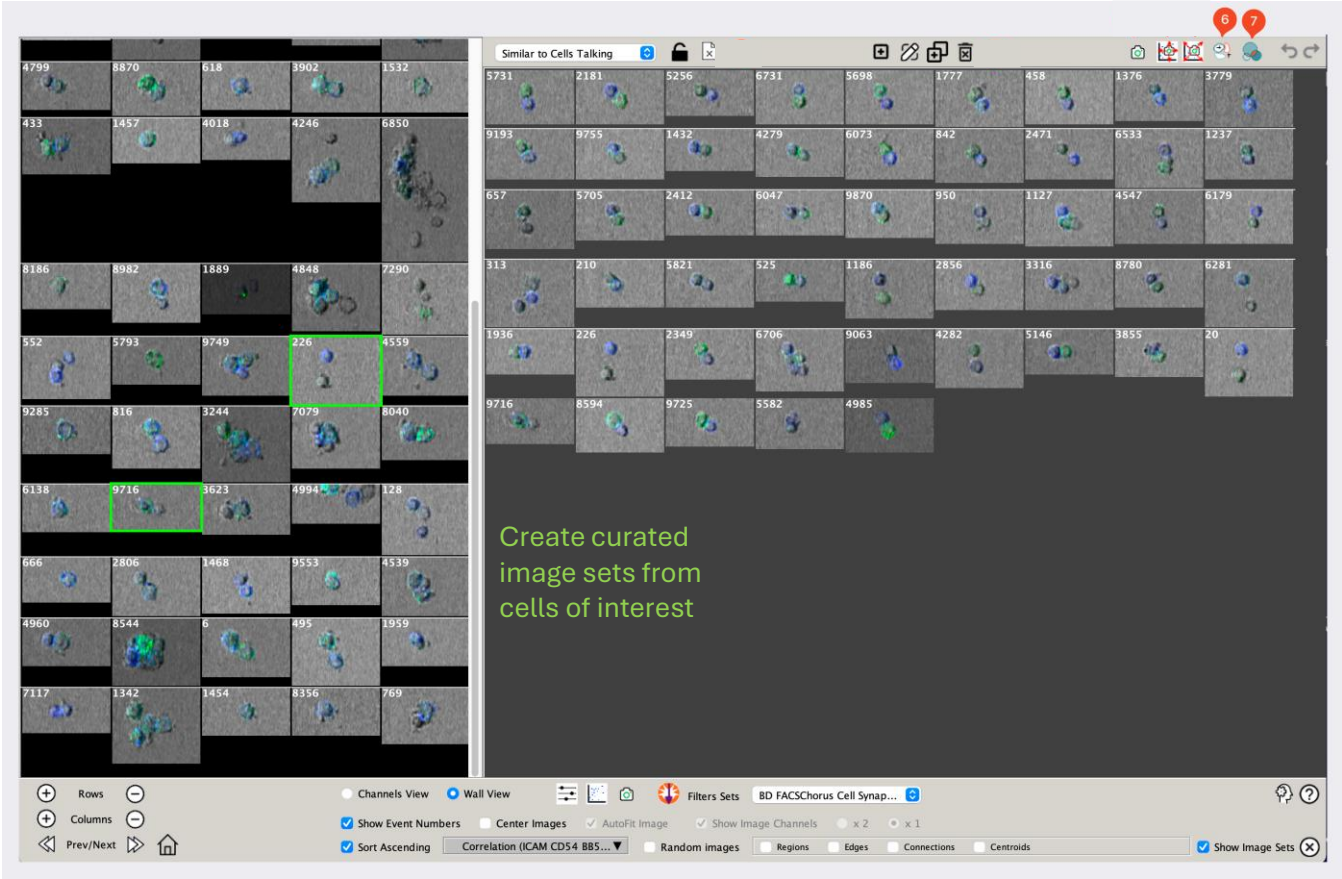


0. Click to activate the Image Set window

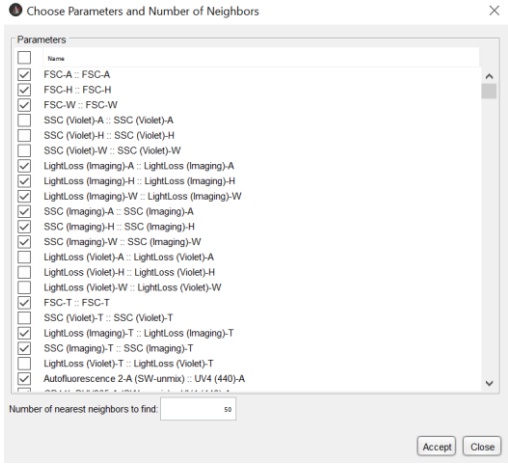


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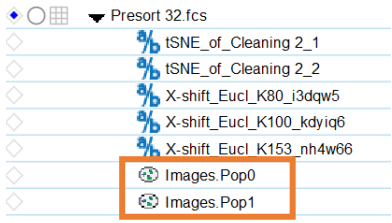
Creating Image Sets



6. Find similar images to the current ImageSet using KNN algorithm.
Select the parameters (features) to use for similarity matching and specify the number of cells to search

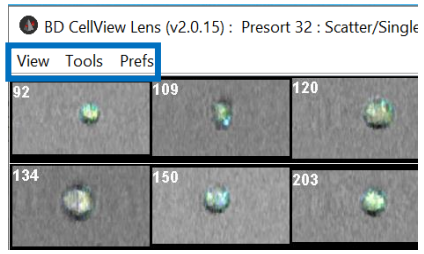


7. Create a population of these cells in the FlowJo hierarchy for downstream analysis, visualization, or HyperFinder-based gate optimization.
Two new populations will be created in the workspace: Images.Pop1 containing the selected images and Images.Pop0 containing all remaining events



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Exploring Cell View Lens Taskbar



View: Recapitulates the Image View Controls in menu form with associated keyword shortcuts listed.

Prefs: Contains a pair of settings allowing the user to show placeholders and/or warnings for missing images.

Tools (Part 1): Contain some additional tools that can be applied to images (others are shortcuts for previously mentioned tools)

Image Sets: Manually curate a set of your favorite images

Take Snapshot for Layout Editor: Click to add the current images to the layout editor.

Take a Snapshot of Image Set: Add a picture of the current image set to the layout editor.

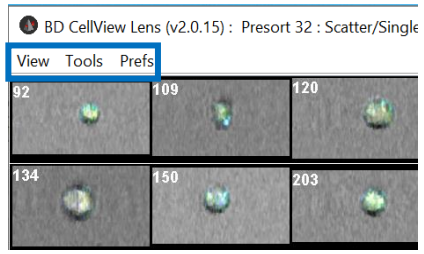
Take a 2D Snapshot with Images on Sides: Option if you have the 2D mapping tool open. It will create a 2D plot + map images to events in the plot and place it in the layout editor with the images on the side of the plot.

Take a 2D Snapshot with Images in Quadrants: Option if you have the 2D mapping tool open. It will create a 2D plot + map images to events in the plot and place it in the layout editor with the images in quadrants around the plot.

Run Slideshow: Cycles through each cell in the selection, promoting it to the high-resolution plot.

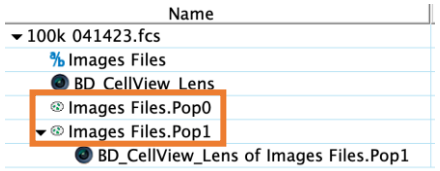
Export Selected CellView Population: If you want to isolate a population or populations but preserve the image relationships. This tool exports the current selection as an FCS file. From a single sample, select the population(s) of interest. Add a CellView Lens node to this population, open it, and export. To concatenate populations from multiple samples, multi-select the CellView Lens nodes of all desired populations, open all of them in CellView Lens image browser windows and choose the export option via the Tools menu. Concatenation will be done automatically.

Exploring Cell View Lens Taskbar



Tools (part 2): Contain some additional tools that can be applied to images (others are shortcuts for previously mentioned tools)

Make Image File Gates: Creates a gate that separates cells that have an associated image from those that do not. Useful for index sorted data, that contains all of the non-indexed data. For large files the process will take a couple minutes then return a derived parameter called Images Files, and two populations, Pop0 and Pop1. Pop1 will be the events with images associated.



Make Image Parameters: Will create a set of derived parameters on the root file in the hierarchy. Most useful when Regions have been used.

See further information on Region Masks in page 12.

Create PNG files: It creates a folder in the location of your choosing with a PNG file for each event with an associated image.

Generate Image Files Report: Creates a text file listing all of the TIFF files used in the platform

Create Bisector Gate: Divides current population into 2 populations based on selection of a specific image to bisect the data on.

Open Image filter tools: Click this to open the controls for modifying the appearance of the images.

Open Index Sort Plate View: Opens a dialogue to connect index sort plate information with the selected cells and then opens a plate view of the data.

Open Event to Image Mapping Tool: Reopens the dialogue for connecting images to cells.

Open 2D plot with mouseover: Click this to open the current population in the graph window and enable mouseover image display.

Open Machine Learning Tools: Opens the toolbox for using machine learning to classify events based on images.

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Creating Region Masks

Region Masks segments each cell image, allowing calculation of region-based statistics such as the **number of sub-regions, area, circumference, and distances between sub-regions**. These measurements can be used to identify and sort biologically interesting populations.

1. Open the Image Filter Tool and **create a new image filter.**

2. Disable all channels except the relevant ones.

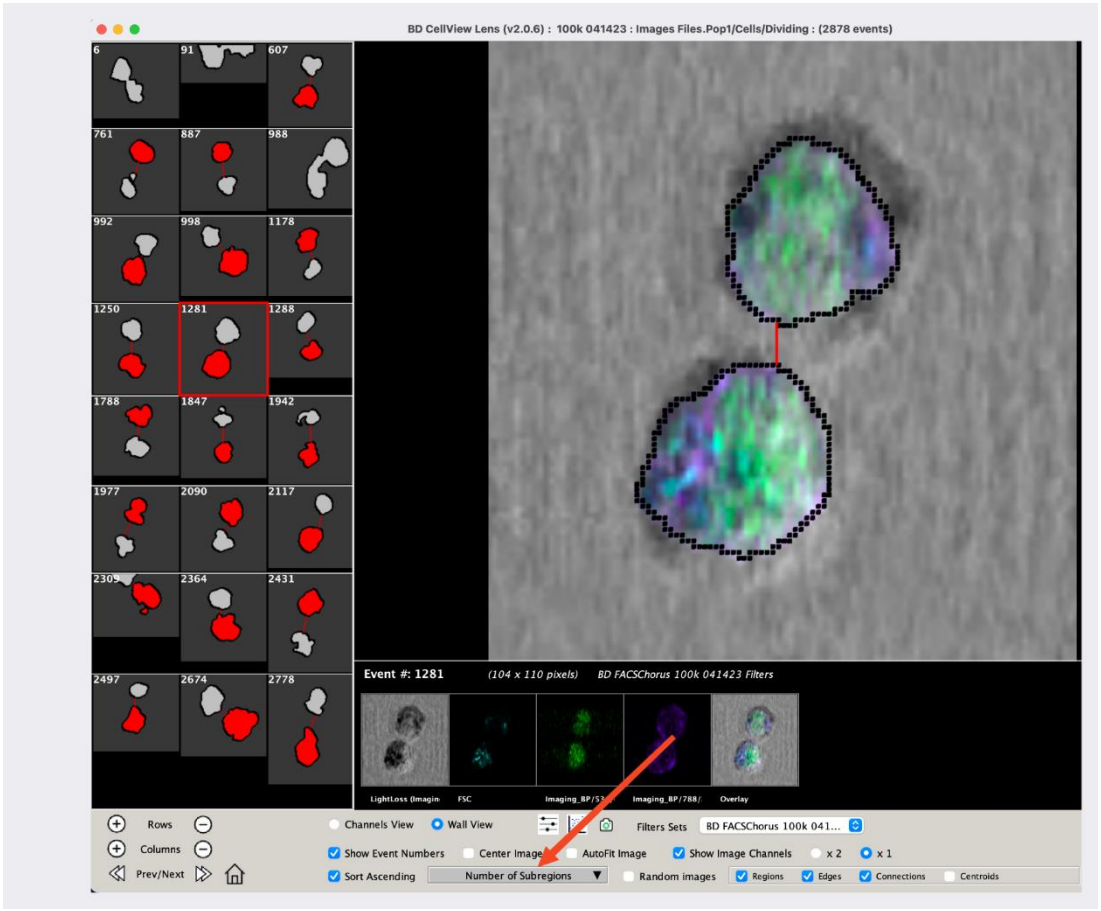
3. Enable **Remove Background** to generate a **Region Mask.**

4. Open **Edit Region Mask Channels** and, while holding Ctrl, enable only the relevant channel (mask icon). Use Ctrl-click again to disable channels if needed.

5. Select the **Region Mask channel** and adjust:

- **Min** (vertical slider) for the region mask pixel threshold.
- **R. Smooth** (horizontal slider) for smoothing.
- Optionally use the **LightLoss** image as a visual guide (it cannot be used to create the mask).
- Use **Show Region Mask** to preview sub-regions in different colors while adjusting settings.

6. Click **'Edit Region Mask Channels'** to disable editing and then click **'Apply Filters to Workspace'**. Any open Image Browser windows will now use your new image filter.



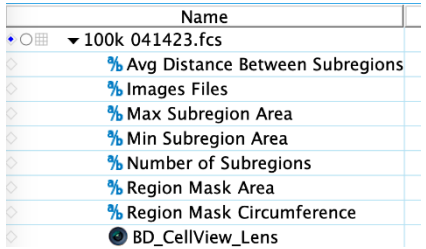
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Generating derived parameters

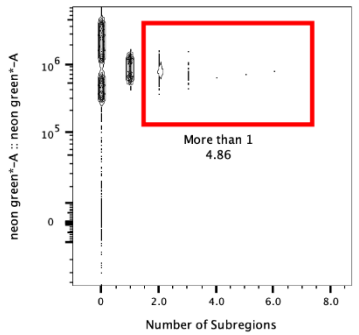
To create **new imaging derived parameters** using the Region Mask:

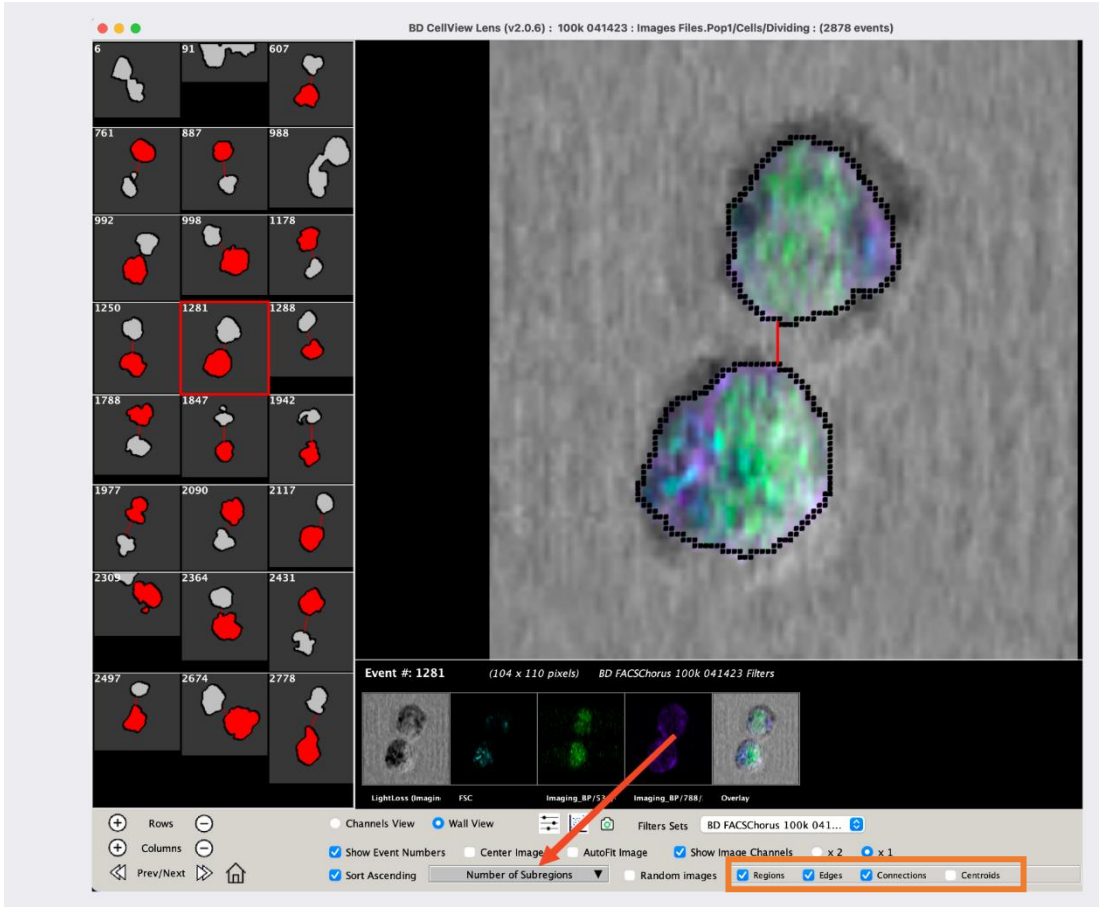
7. Open an image browser window on the root sample population. Only from this window you can create new imaging derived parameters.

8. From the menu bar 'Tools' menu, select the operation to **'Make Image Parameters'**. The calculation will take a little time depending on how many cells with images are your experiment, but on completion a set of **derived parameters** will be created



They can be displayed on an axis and gated to separate events with the most sub-regions or the greatest distance between sub-regions.





You can use the toggle buttons at the bottom to help you visualize some of these.

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