Sorting Surfaces with the Sortomato

The Sortomato is a utility for sorting Surpass objects into new objects based on their statistical properties. The Sortomato works with Spots and Surfaces objects. If the objects have custom statistical properties, they can also be used to sort the objects. In the following example, objects are sorted based on their mean intensity in two channels to remove Surfaces that were created from bleedthrough of the CFSE channel into the CMTMR channel.
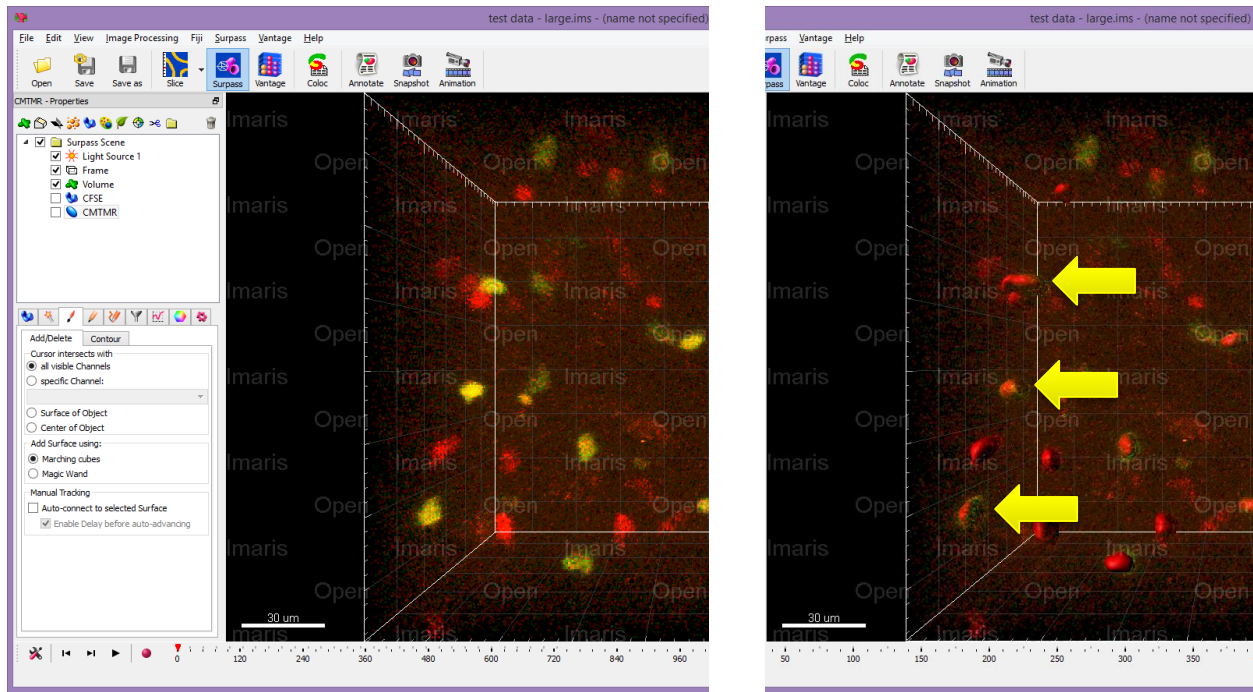

Figure 1. Surfaces have been created from the CMTMR channel, but CFSE bleeds through into the CMTMR channel, resulting in unwanted Surfaces in the CMTMR Surfaces.

1. To start, click the Sortomato menu option in the Tools tab of the Surfaces object (*Figure 2*). The Sortomato can also be found in the Image Processing menu under the Spots and Surfaces submenus.

2. In the Sortomato, click the Objects dropdown menu and choose the CMTMR objects (*Figure 2*). The Sortomato will gather the statistics, including any custom properties for the CMTMR surfaces and present them as options for creating plots. Additionally, if the objects have been tracked, they can be plotted based on their track properties, e.g., Track Speed Mean, Min, etc. However, see *Note 1* below.

3. Click the 2D graph button to create a figure for plotting two properties against each other. 3D plots can also be created for visualizing three properties, but cannot be used for sorting.

4. In the figure window that opens, select the Intensity Mean - Channel 2 property from the Y dropdown menu in the upper-left of the figure area. In the X dropdown menu at the lower-right of the figure area, select the Intensity Mean - Channel 1 property.
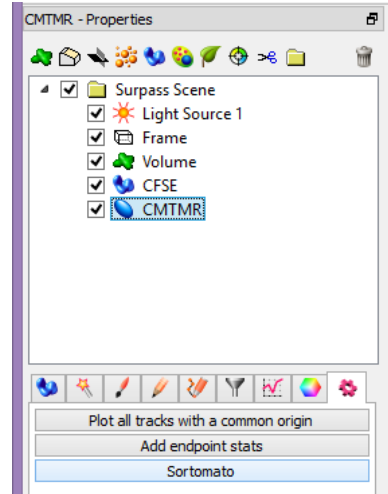

Figure 2. The Sortomato can be started from Surpass Tools menu option, as well as the Image Processing Spots and Surfaces submenus.
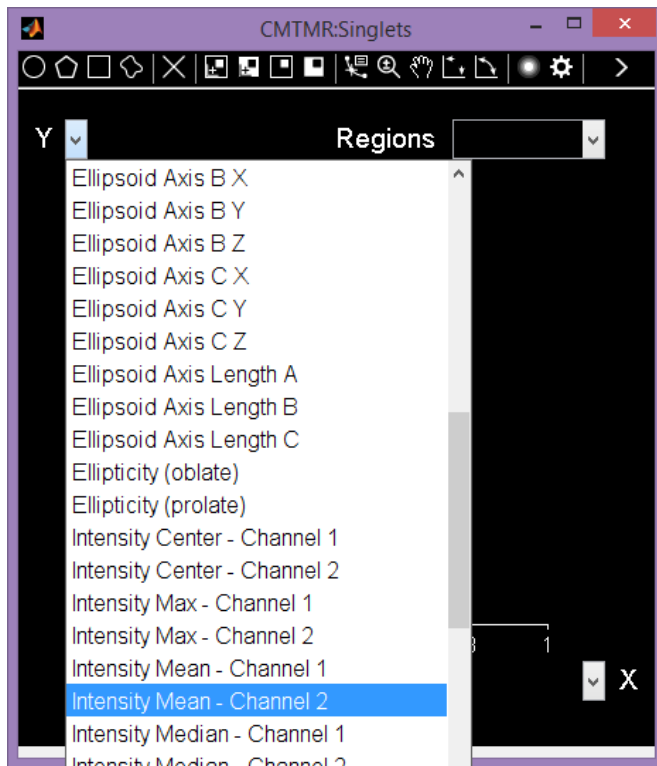

Figure 3. Select the object from the menu, then create a new graph for the objects.

Figure 4. Create a plot of the Surfaces' mean intensity in channel 2 (Y dropdown) against their mean intensity in channel 1 (X dropdown).
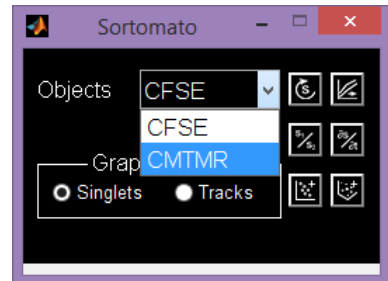
5. In the toolbar at the top of the window, click the Rectangle icon to draw a rectangular region of interest by dragging the cursor over the group of objects on the right side of the graph. This represents the surfaces with a high mean intensity in Channel 1 (*Figure 5*).

6. We can now sort these objects into a new Surpass object. On the toolbar, select the icon to sort the objects outside the selected region, or right click within the rectangle and choose Sort objects -> Outside region from the context menu (*Figure 5*).

7. In Imaris, a new Surfaces object will be created with Surfaces from Rectangle 1 (*Figure 6*).

8. Alternatively, the objects within Rectangle 1 can be removed from the CMTMR Surfaces object. First, select those objects using the Select Imaris objects choice in the context menu. The objects within Rectangle 1 in the graph will be highlighted in Imaris when you go to the Surfaces' Surpass Edit tab. Press Delete to remove these objects (Figure 6).
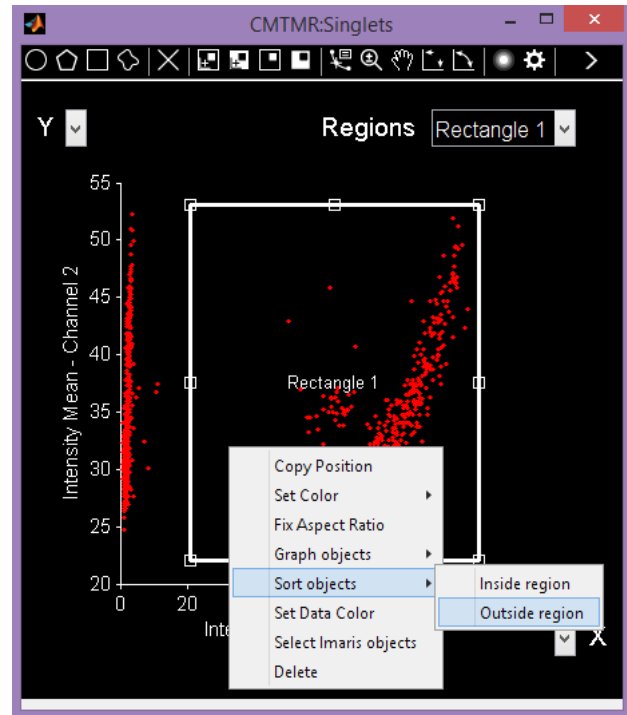

Figure 6. Click the Rectangle icon and drag a rectangle region over the objects in the right size of the graph. Once you have create the region, sort the objects whose properties fall within the rectangle by choosing the Sort icon in the toolbar, or right-clicking within Rectangle 1 and selecting Sort objects -> Inside region.
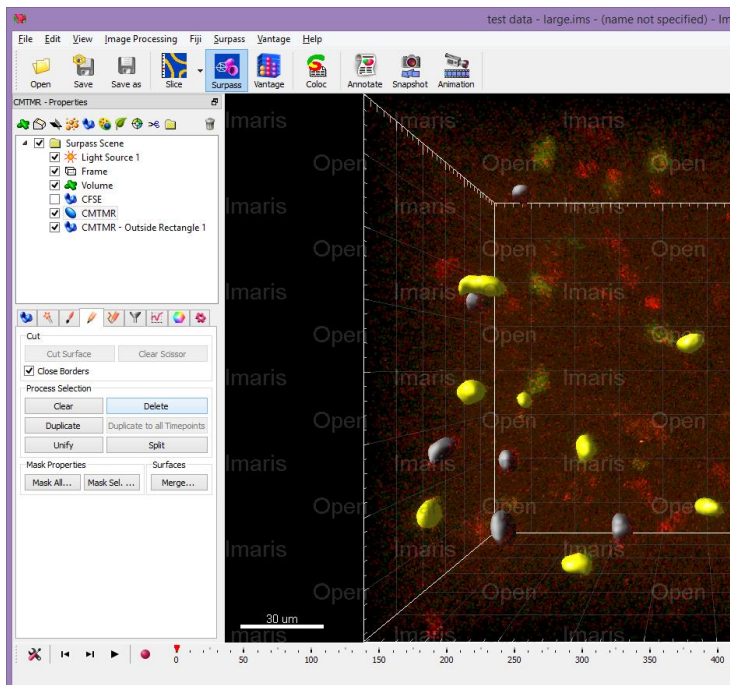

Figure 5. After pressing sort, a new Surfaces object is created from the objects outside Rectangle 1. This represents the Channel 1 (CFSE) low group of objects (CMTMR – Outside Rectangle 1, shown in gray). Alternatively, select the objects corresponding to the Rectangle 1 group using the context menu option and delete them from the CFSE object.

Notes

1. After tracking, Spots and Surfaces objects can be sorted based on the properties of the individual Spots or Surfaces. However, the track edges will not be returned to Imaris. This is to avoid breaking up tracks if some objects in the track do not meet the sorting criteria. As a result, it is best to sort objects first, then track, or only sort based on Track statistical properties.
2. The example used here can be sorted using the built-in 1D Filtering provided by Imaris. However, more complicated sorting based on a series of property comparisons can be done within the Sortomato by first creating graphs of sub-groups of objects, then sorting.
3. The data can be represented as contour plots if a large number of Spots or Surfaces are present.
4. The graphs and raw data to create the plots can be exported using the export options on the toolbar.
5. In the Sortomato menu, there are buttons to export the data organized by track (one track per column, with data for each time point placed into the row entries). If the data is untracked, it can more easily exported using the built-in Export tools in Surpass on the Statistics tab.
6. The Sortomato can also calculate new statistics based on the derivatives of statistical properties, or by performing binary arithmetic on two statistical properties, e.g., the ratio of two channels.
7. To run the non-compiled version under MATLAB, the Image Processing Toolbox is required.
8. If there is a large number of Spots or Surfaces and a large number of statistical properties, the maximum Java heap size will need to be increased. In MATLAB 2009a, this requires you to create and edit a java.opts file and add it to the MATLAB $ARCH folder. To increase the max heap size, follow the instructions here: http://www.mathworks.com/matlabcentral/answers/92813-how-do-i-increase-the-heap-space-for-the-java-vm-in-matlab-6-0-r12-and-later-versions.