

Spectral Imaging Toolbox 0.1

User manual

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Contents

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This non-technical user manual includes the following:

1. Getting started
2. Input GUI
3. Object separator
4. Membrane segmentation
5. Spherical Object mode
6. Output

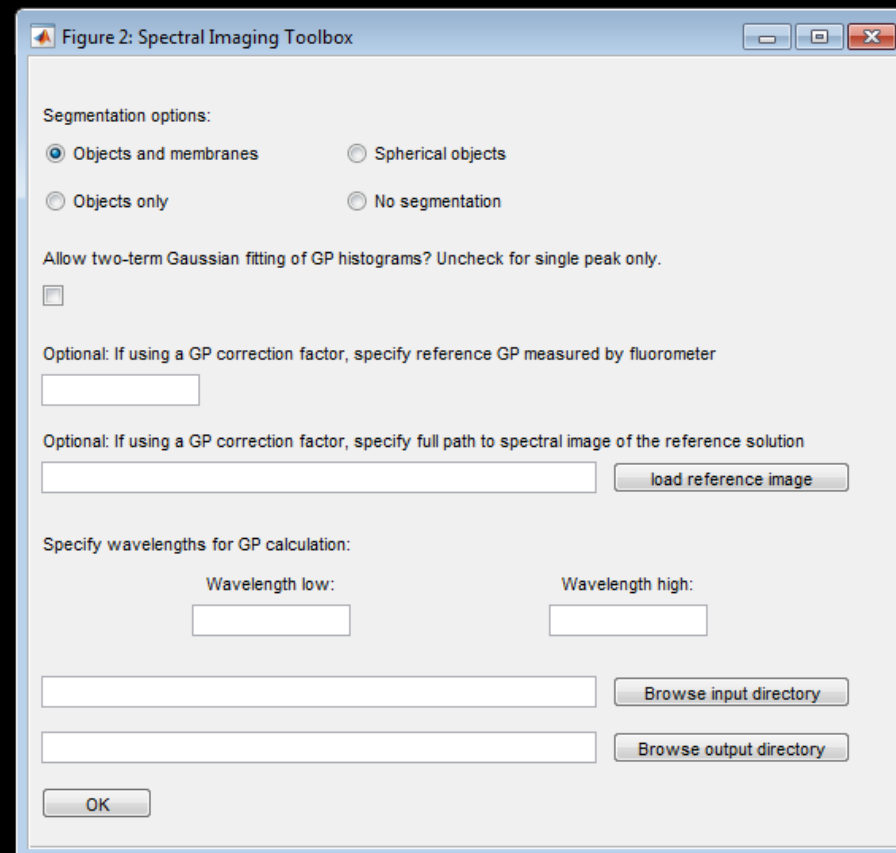
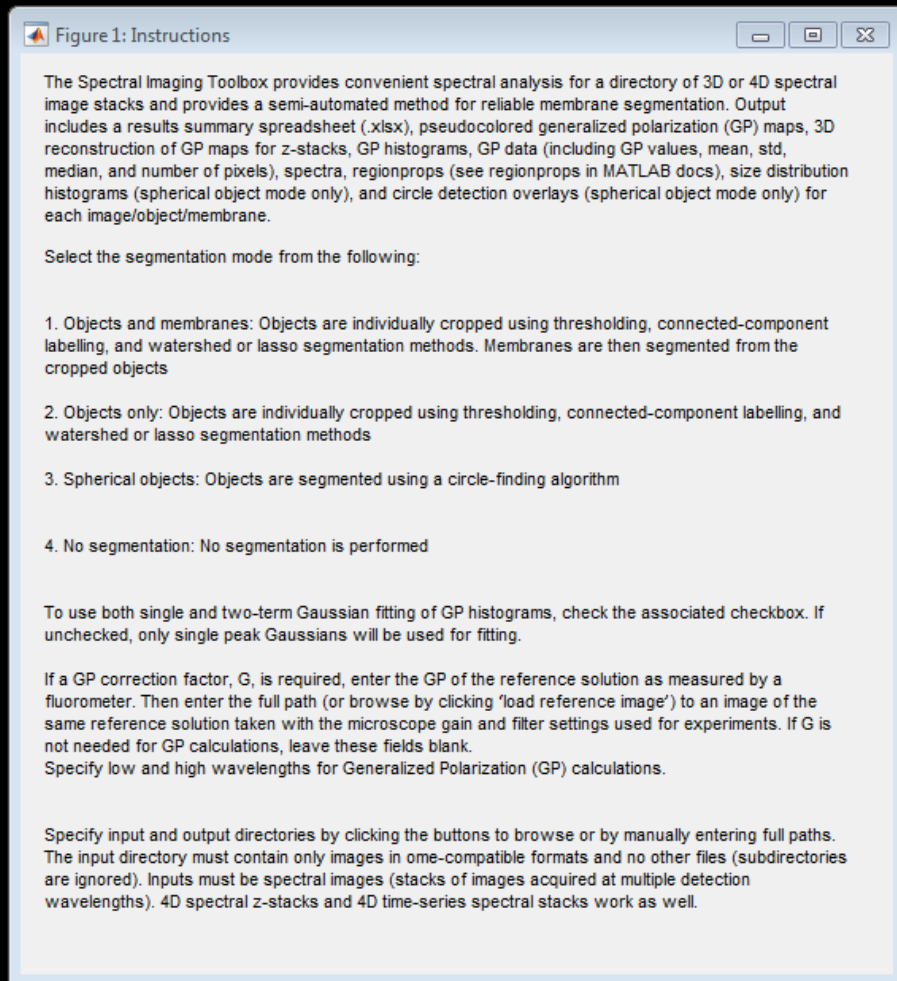
A few important points

- The Spectral Imaging Toolbox is designed for batch processing. Simply select an input folder with multiple spectral images of a single fluorescent probe and all the images will be processed.
- Many users we spoke to are interested in spectral analysis of an ROI in the image (ie: for intracellular vesicles). This can be achieved by lasso-segmentation. Choose 'objects' or 'objects and membranes' to use lasso-segmentation.
- The 'spherical objects' segmentation mode is often less effective than the 'objects and membranes' segmentation mode for spherical vesicles. We recommend trying both modes on a few representative spectral images before processing a larger data set.

Getting started

1. Download the Spectral Imaging Toolbox .zip folder from the MATLAB file exchange
2. Add it and its subfolders to your MATLAB path
3. Run `spectrallmagingToolbox.m`
 - This launches the Input Graphical User Interface (GUI)

Input GUI

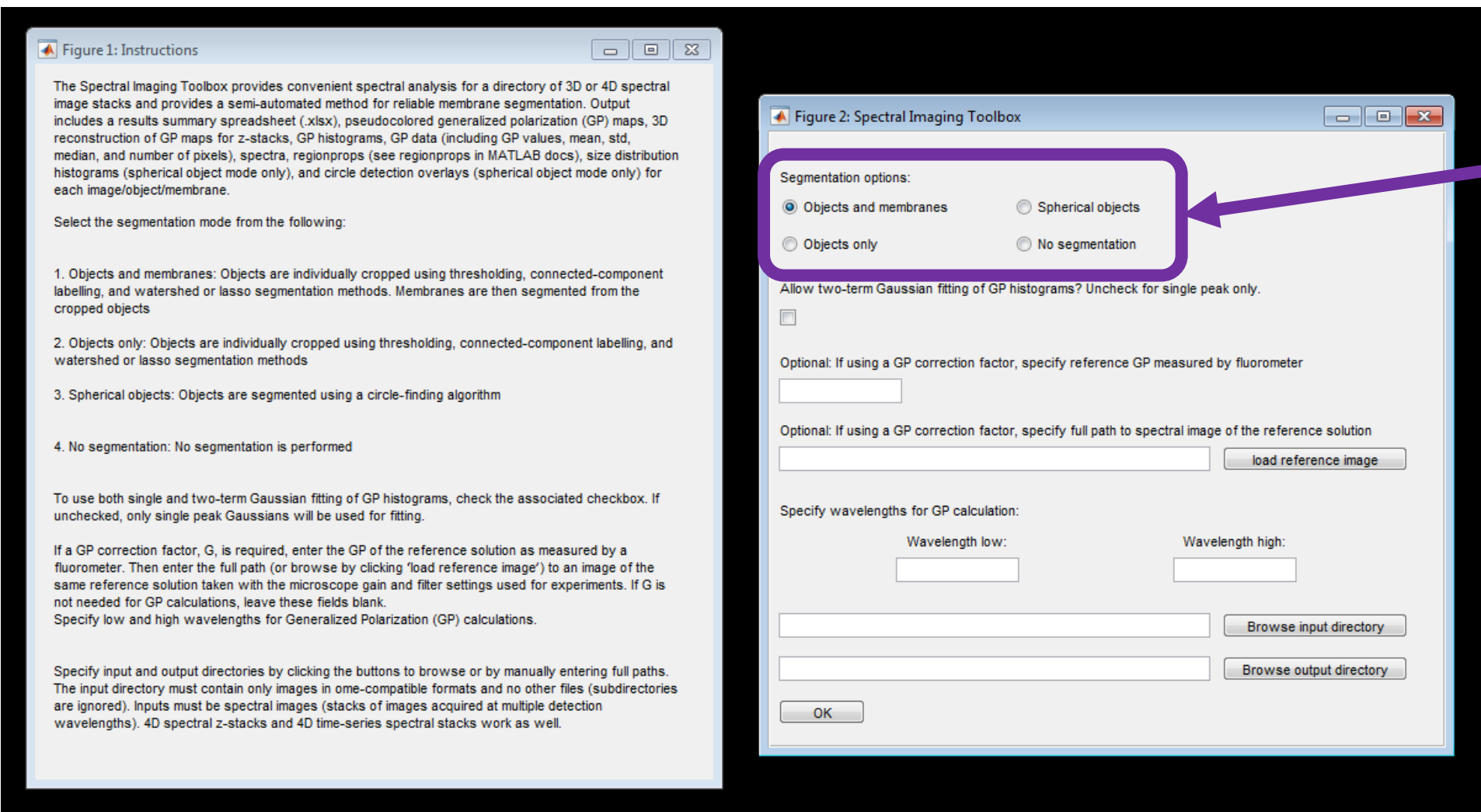


Input GUI: Segmentation mode

Select the segmentation mode from the following:

1. Objects and membranes
 - Objects are individually cropped using thresholding, connected-component labelling, and watershed or lasso segmentation methods
 - Membranes are then segmented from the cropped objects
2. Objects only
 - Objects are individually cropped using thresholding, connected-component labelling, and watershed or lasso segmentation methods
3. Spherical objects
 - Objects are segmented using a circle-finding algorithm
4. No segmentation
 - No segmentation is performed

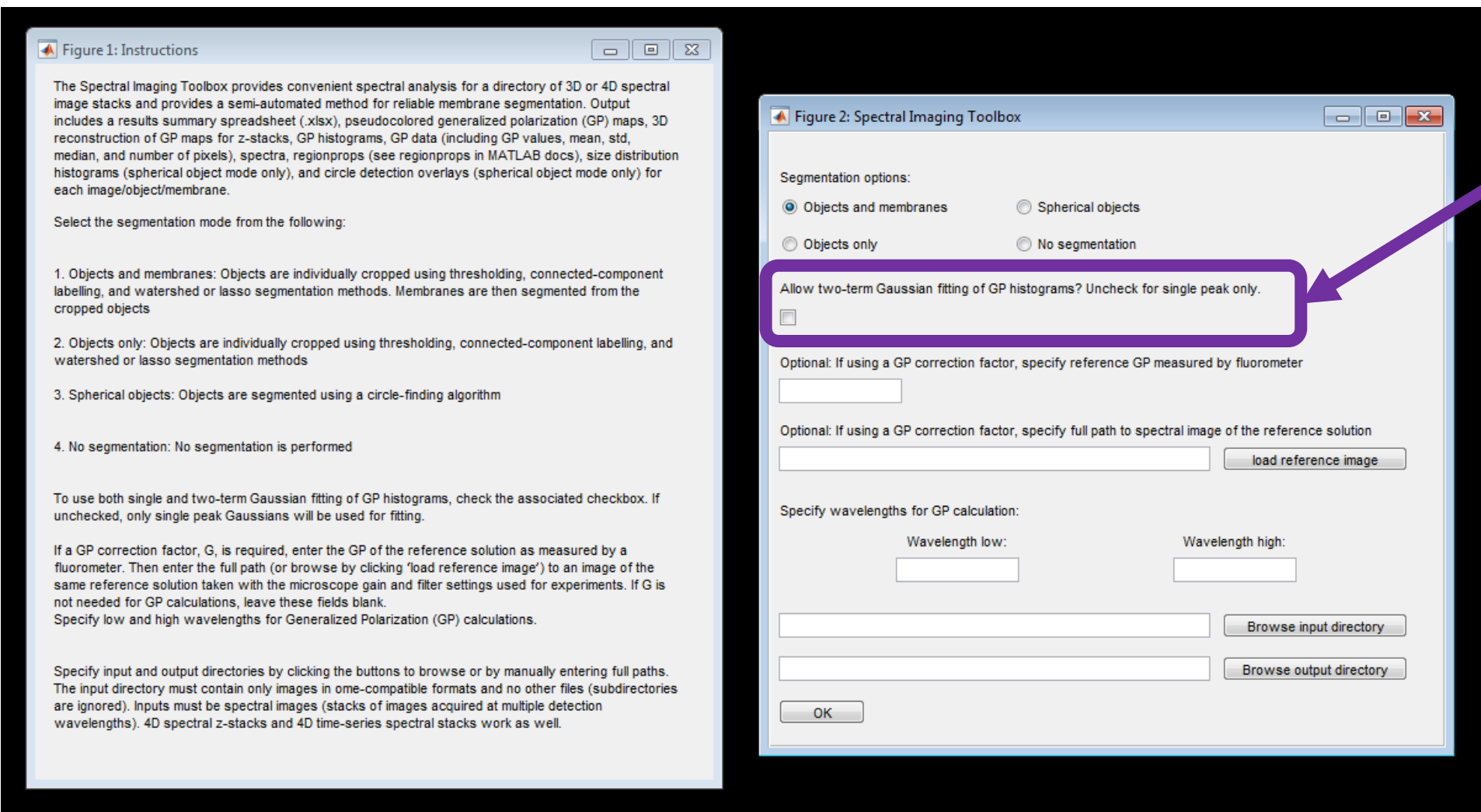
Input GUI: Segmentation mode



Input GUI: GP histogram fitting

To enable fitting of GP histograms with two-term Gaussians in addition to single peak Gaussians check the associated checkbox. If checked, the better fit will be determined on the basis of the lowest root-mean squared error (RMSE). If unchecked, only single peak Gaussians will be used for fitting GP histograms.

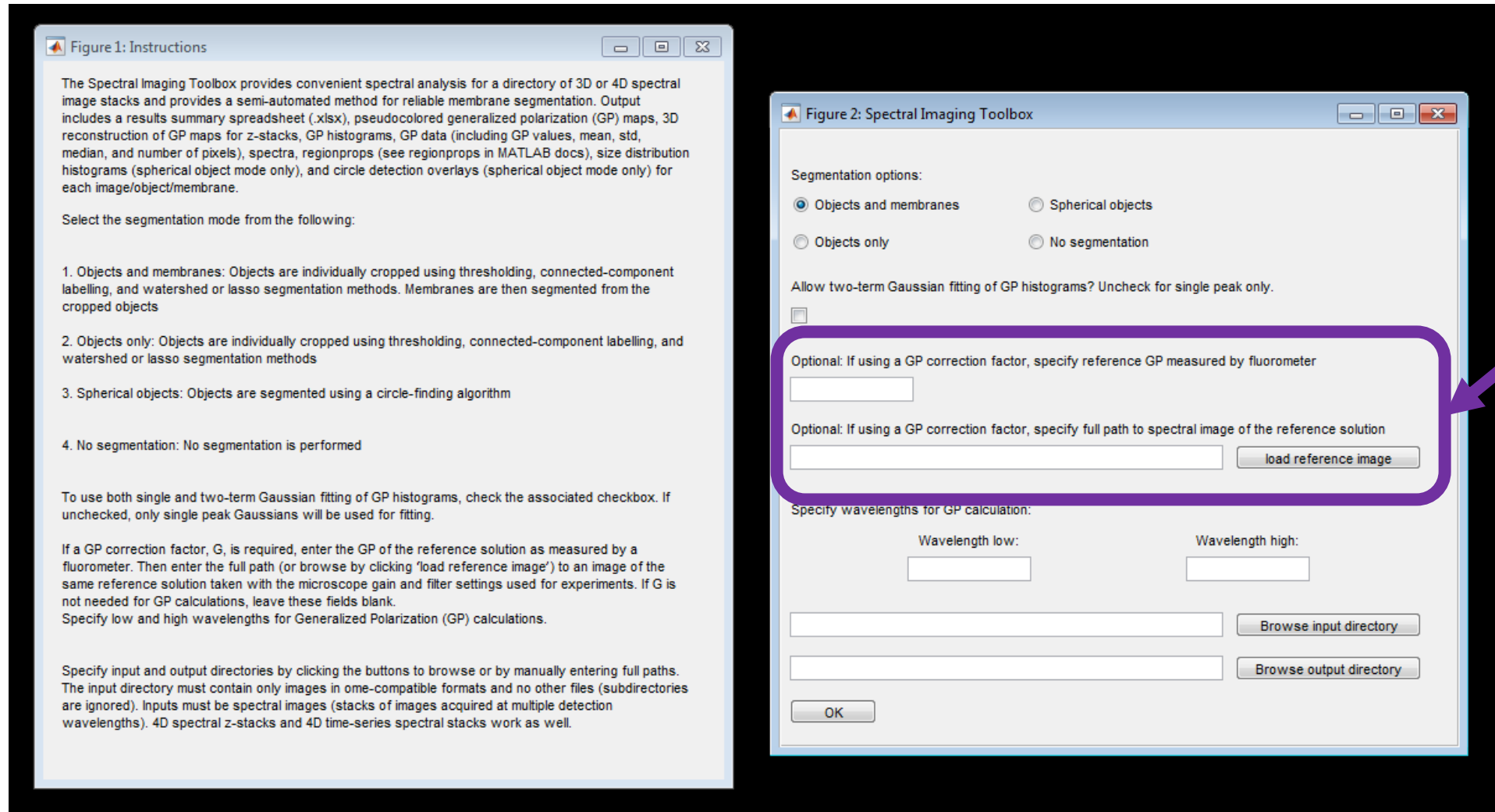
Input GUI: GP histogram fitting



Input GUI: GP correction factor, G

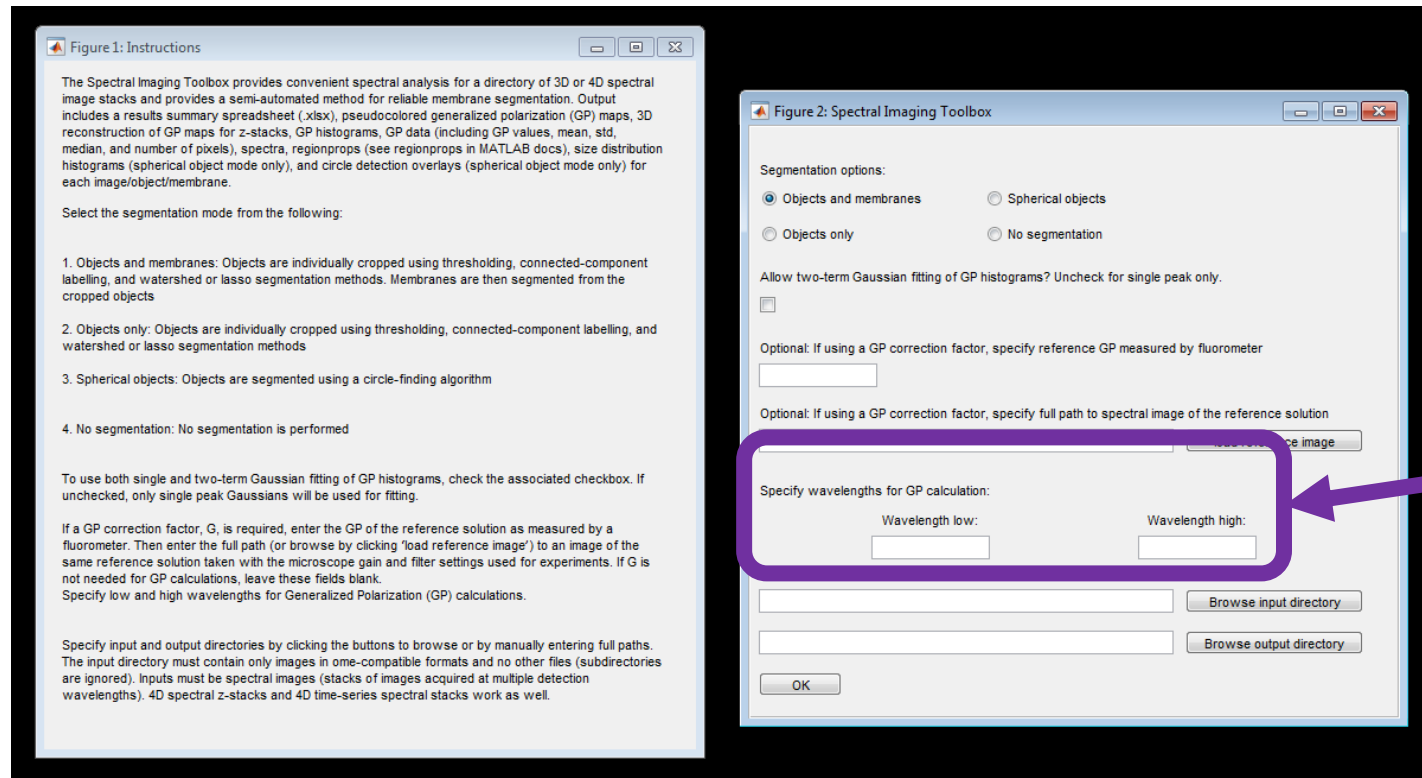
If a GP correction factor, G, is required, enter the GP of the reference solution as measured by a fluorometer. Then enter the full path (or browse by clicking 'load reference image') to an image of the same reference solution taken with the microscope gain and filter settings used for experiments. If G is not needed for GP calculations, leave these fields blank.

Input GUI: GP correction factor, G



Input GUI: Wavelengths

Specify low and high wavelengths for Generalized Polarization (GP) calculations

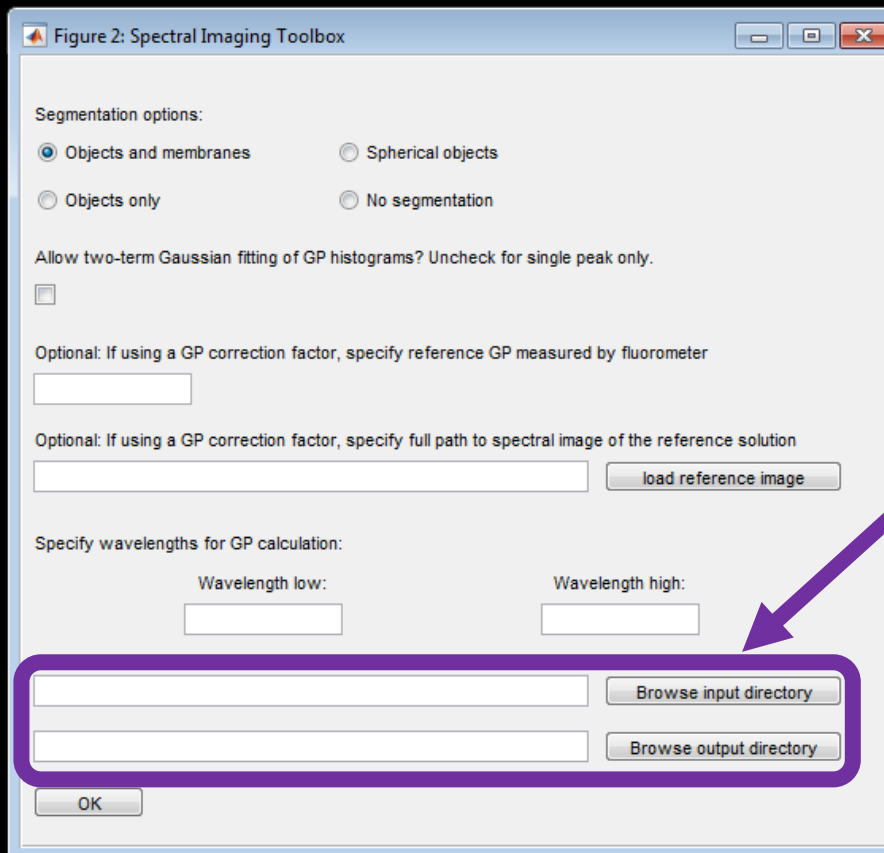
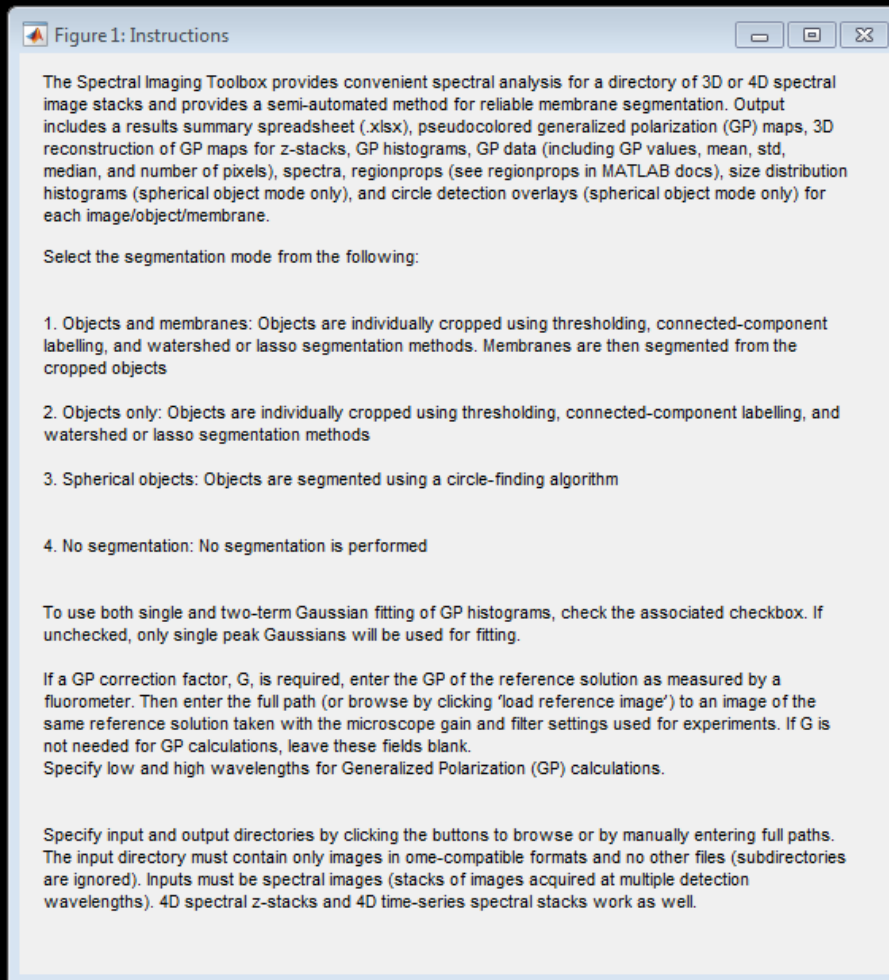


Input GUI: Input and output directories

Specify input and output directories by clicking the buttons to browse your computer or by manually entering full paths

- The input directory must contain only images in ome-compatible formats and no other files (subdirectories are ignored)
- Inputs must be spectral images (stacks of images acquired at multiple detection wavelengths)
- 4D spectral z-stacks and 4D time-series spectral stacks work as well

Input GUI: Input and output directories



Object separator

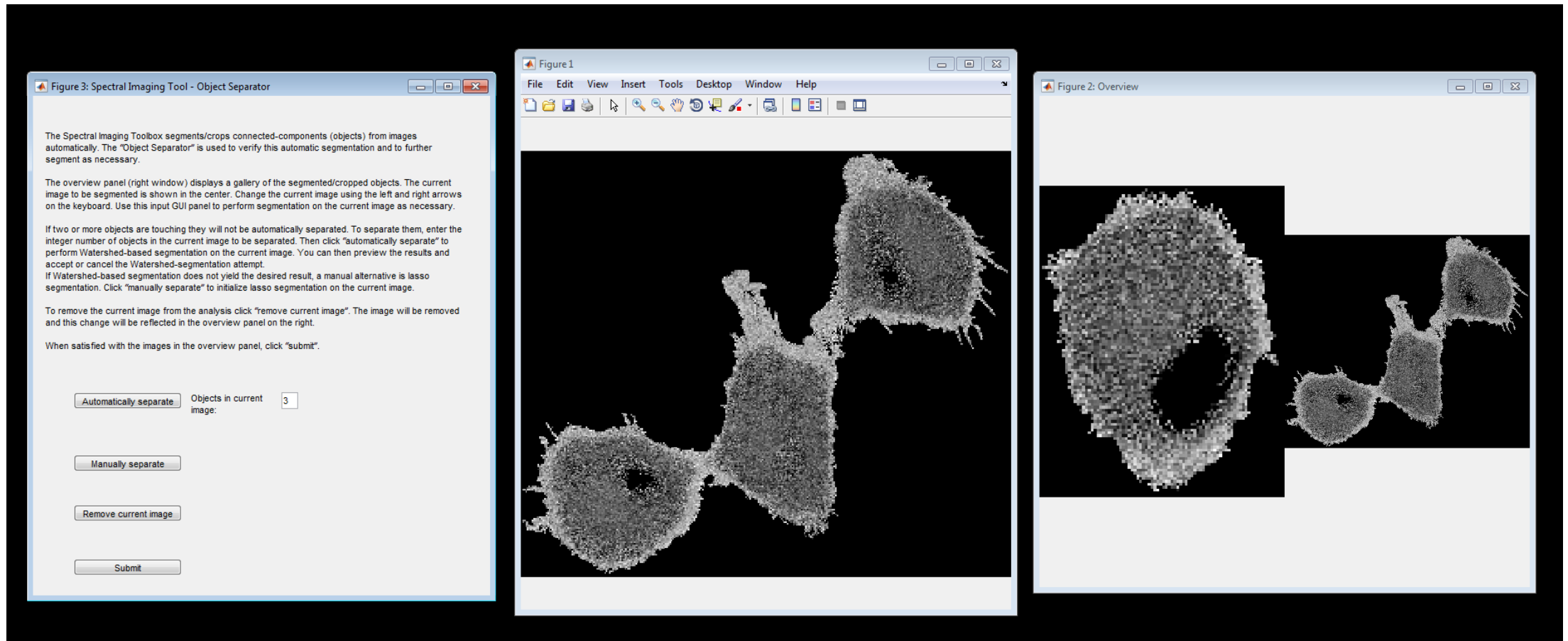
- In “objects and membrane” and “objects only” segmentation modes the “Object Separator” is called
- The Spectral Imaging Toolbox segments/crops connected-components (objects) from the images in the input folder automatically
- The “Object Separator” is used to verify this automatic segmentation and to further segment as necessary
- There are three panels to be aware of:
 1. Input panel (left window)
 2. Current image panel (middle window)
 - Navigate through the images using the left and right arrows on the keyboard
 3. Overview panel (right window)
 - This panel displays a gallery of the segmented/cropped objects
- When satisfied with the images in the overview panel, click “submit” in the input panel

Object separator

Input panel

Current image panel

Overview panel

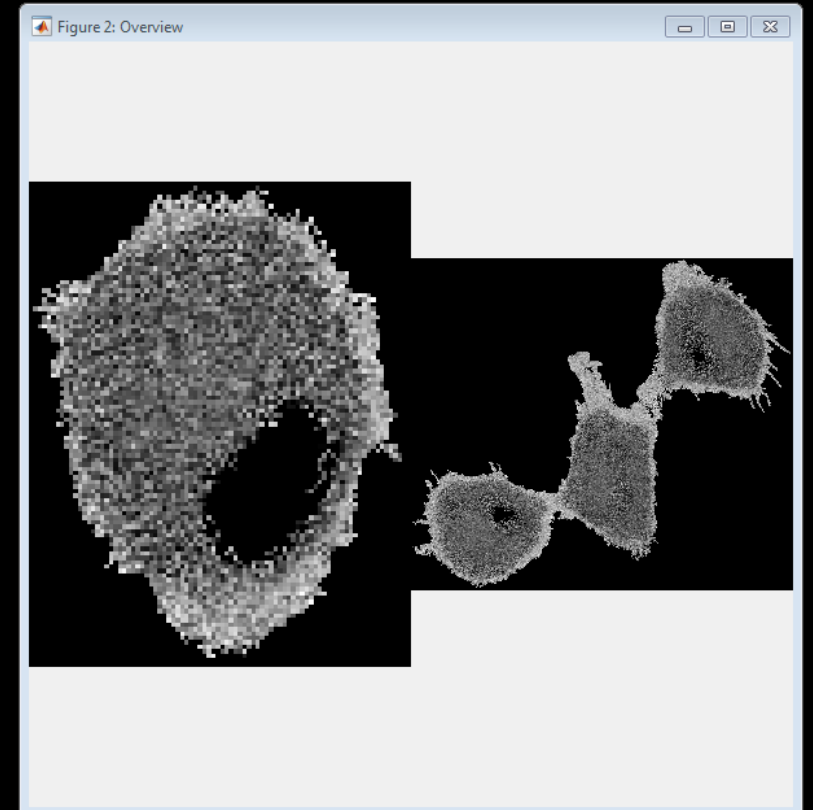
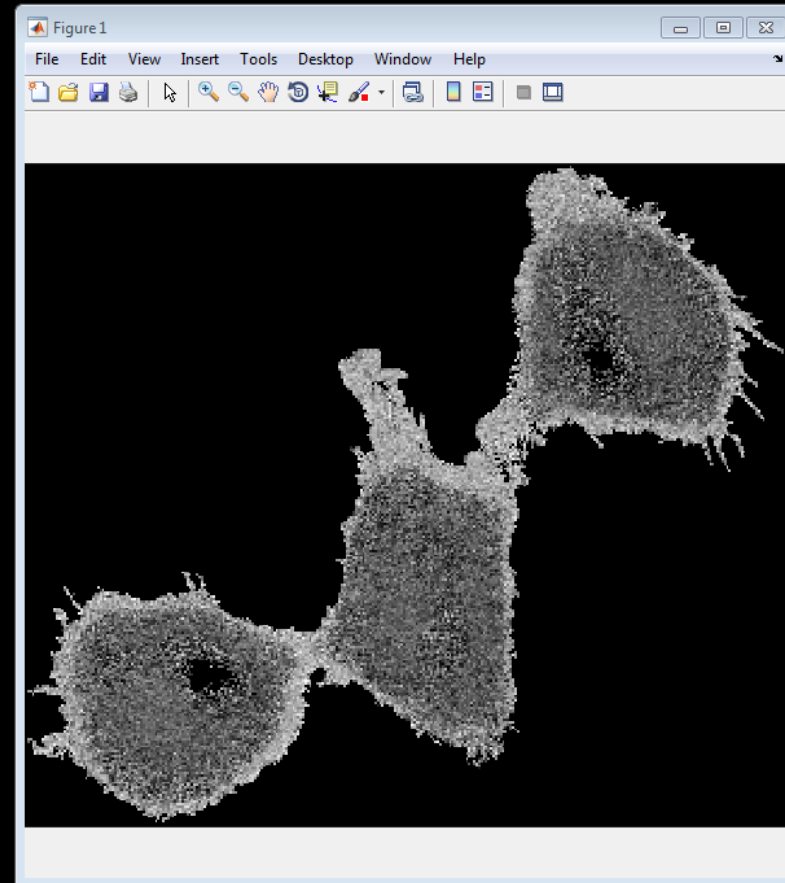
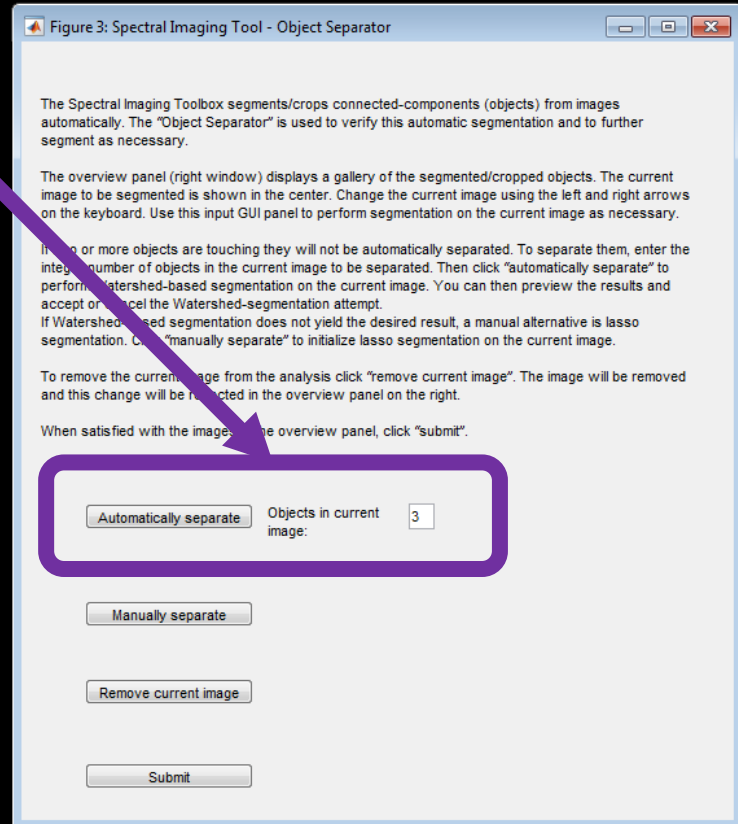


Object separator: Watershed segmentation

- If two or more objects are touching they will not be automatically separated. The first option is based on the Watershed transform.
- First enter an integer number of objects to be separated
- Then click “automatically separate” to perform Watershed-based segmentation on the current image
- This will launch a preview panel where you can accept or reject the Watershed-based segmentation attempt

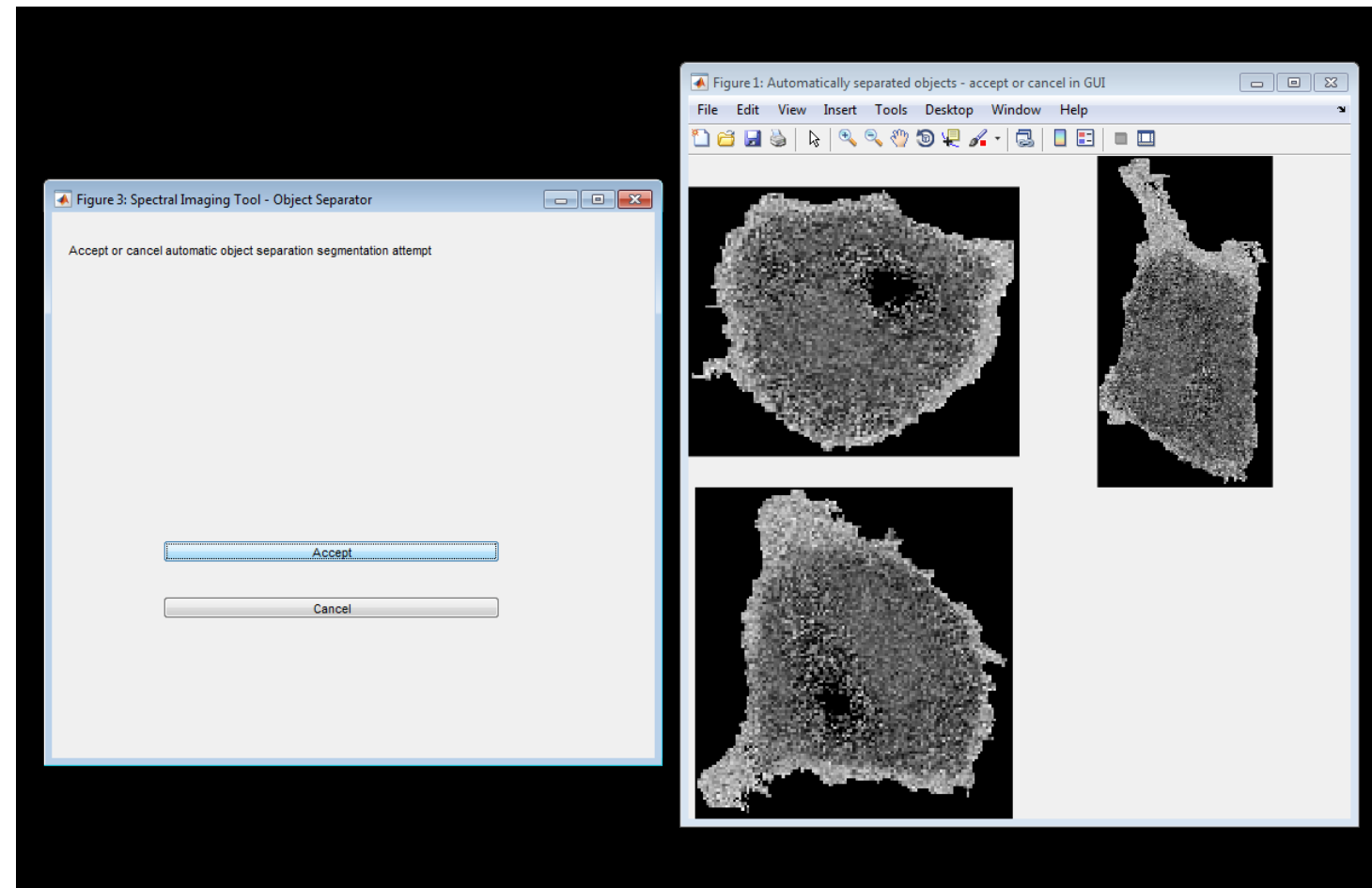
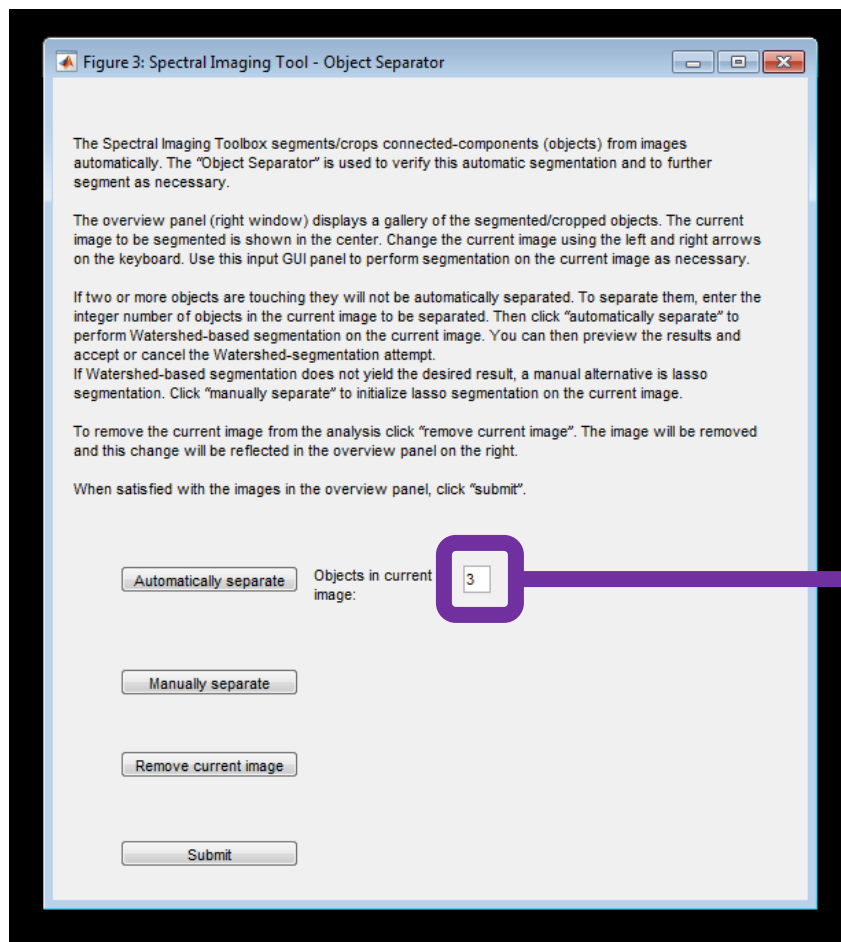
Object separator: Watershed segmentation

Initializing Watershed-based segmentation



Object separator: Watershed segmentation

Preview panel

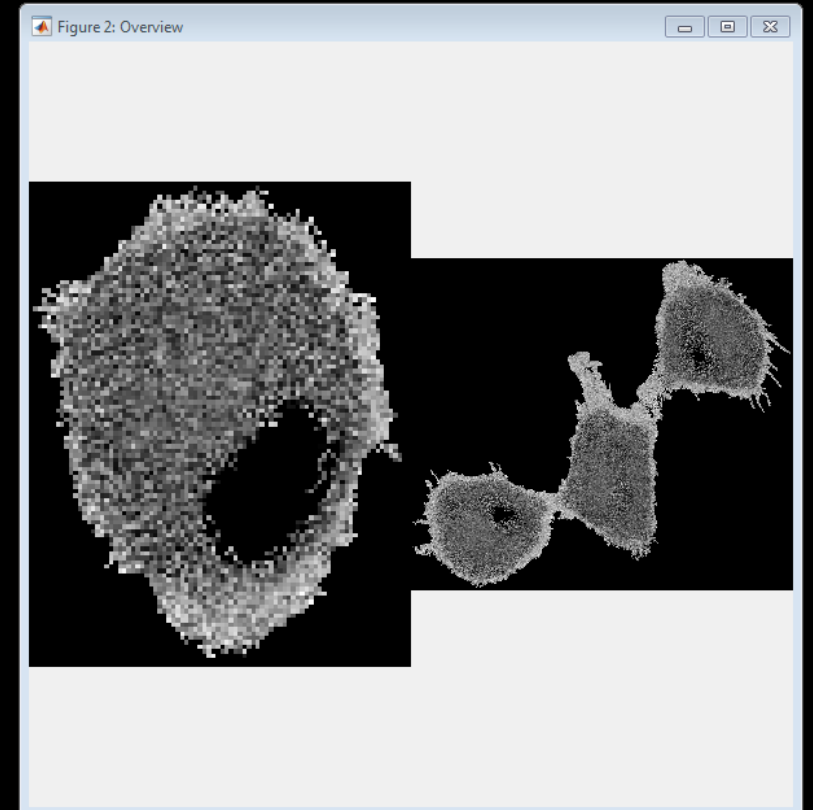
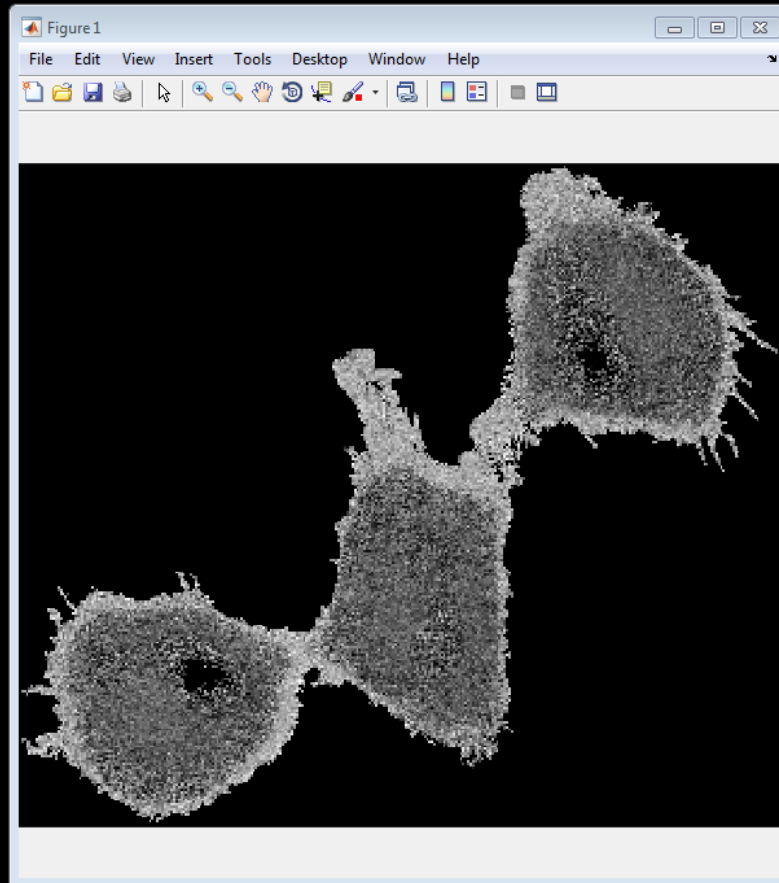
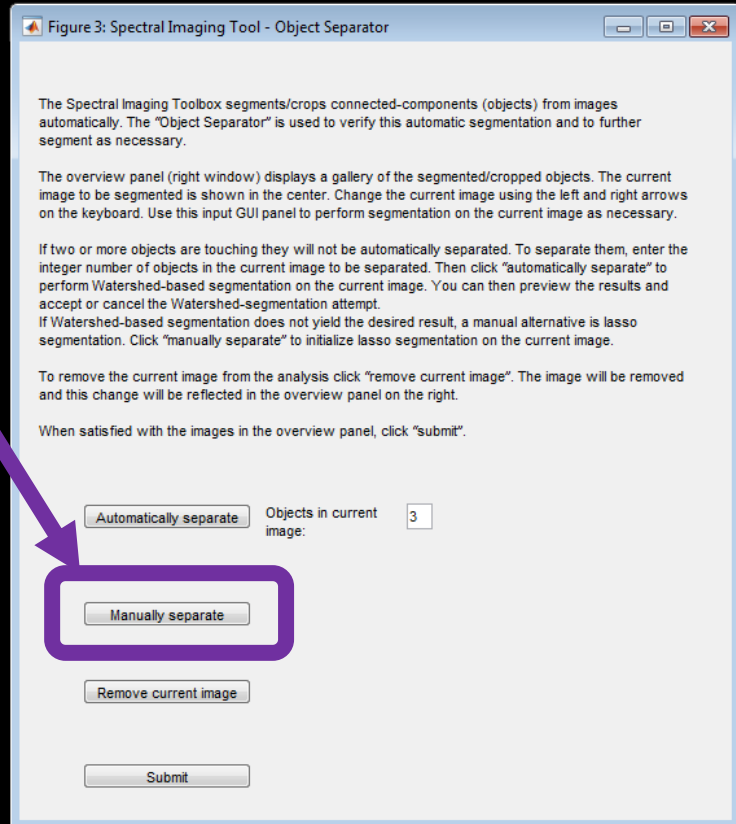


Object separator: Lasso segmentation

- For various reasons the Watershed-based segmentation will not always work for separating objects. The manual alternative is lasso segmentation.
- Lasso segmentation can also be used to specify any ROI for analysis such as intracellular vesicles
- Click “manually separate” to initialize lasso segmentation on the current image

Object separator: Lasso segmentation

Initializing lasso segmentation

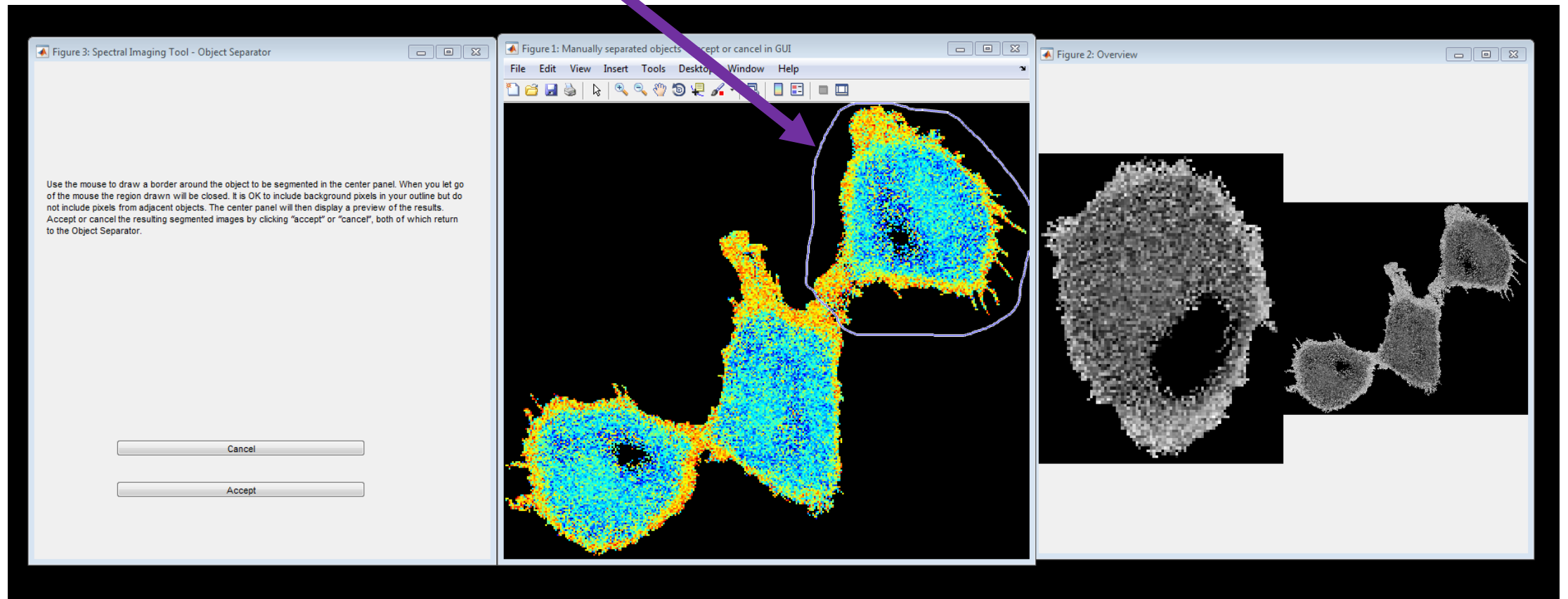


Object separator: Lasso segmentation

- After clicking “manually separate” in the input panel of the Object Separator, two new panels are launched
 1. GUI (left)
 2. Lasso segmentation panel (center)
- Use the mouse to draw a border around the object to be separated in the Lasso segmentation panel (center)
 - When you let go of the mouse the region drawn will be closed
 - The Lasso segmentation panel will then display a preview of the results
- Accept or cancel the resulting segmented images by clicking “accept” or “cancel”, both of which return to the Object Separator

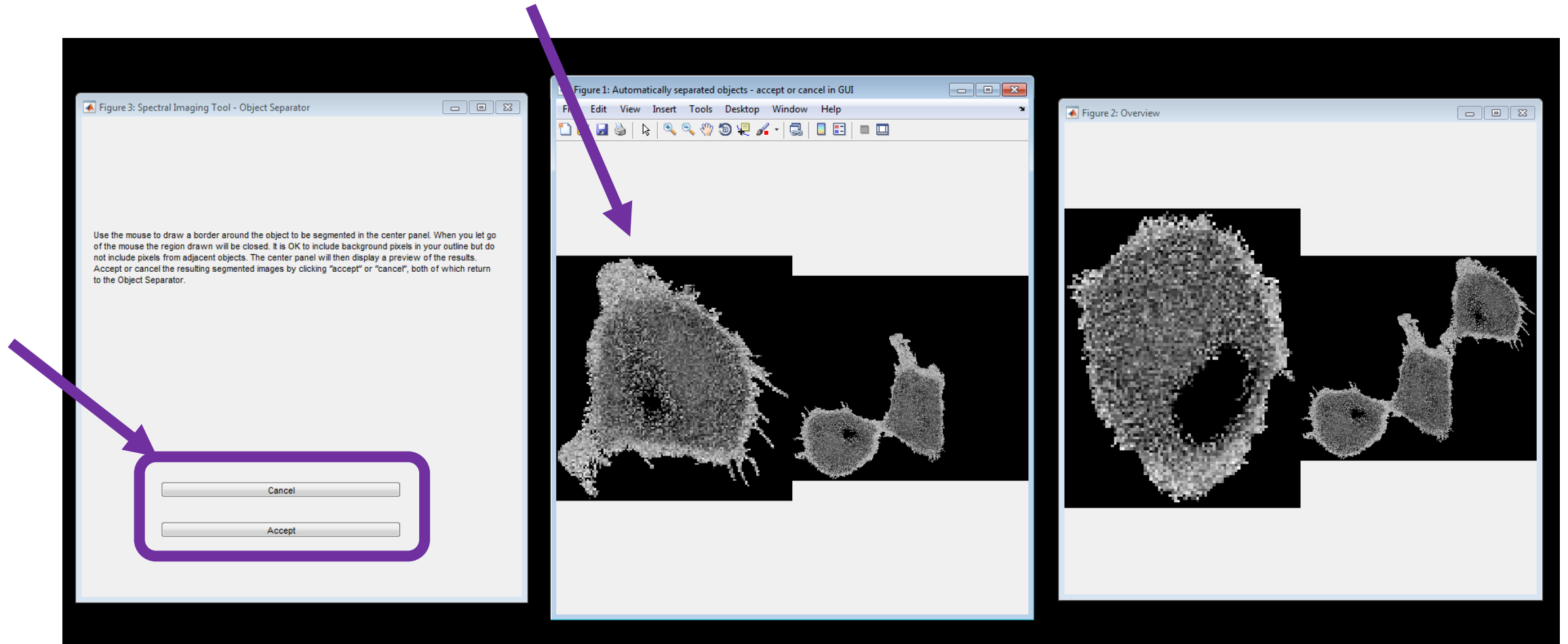
Object separator: Lasso segmentation

Outline the region to segment



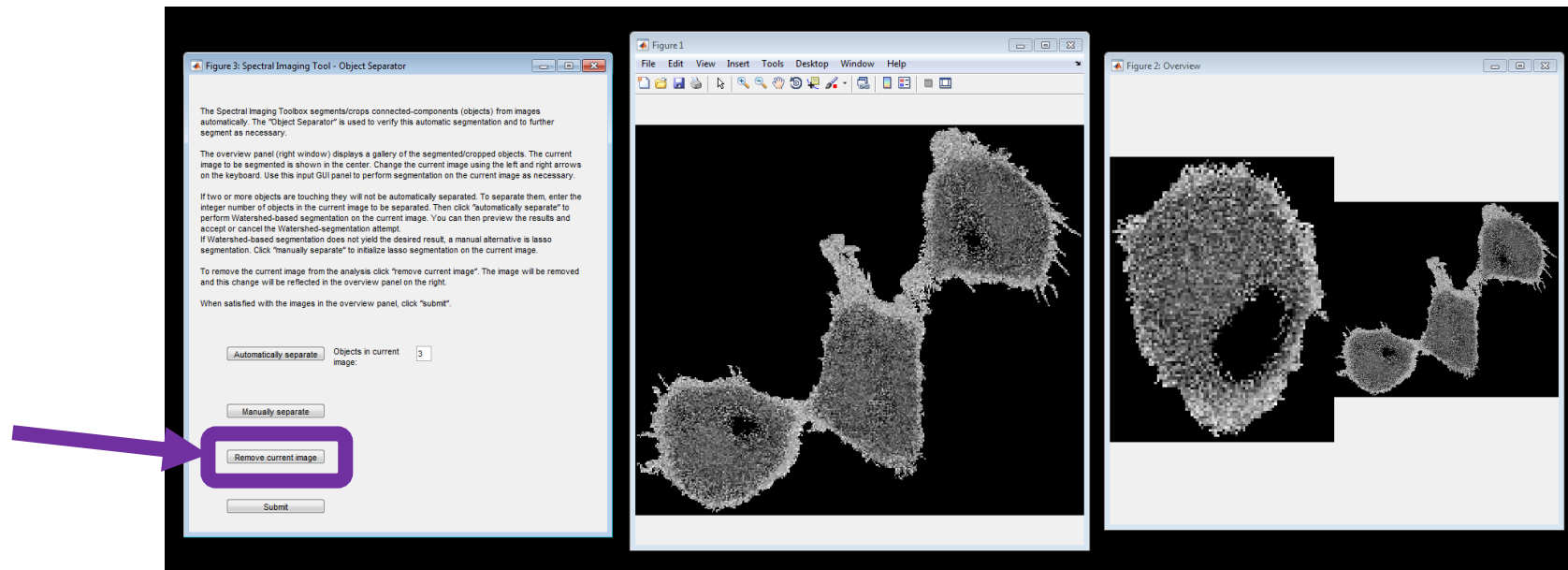
Object separator: Lasso segmentation

Preview and accept or cancel the segmentation



Object separator: Removing images

- To remove the current image from the analysis click “remove current image”
- The image will be removed and this change will be reflected in the overview panel



Membrane segmentation: Review

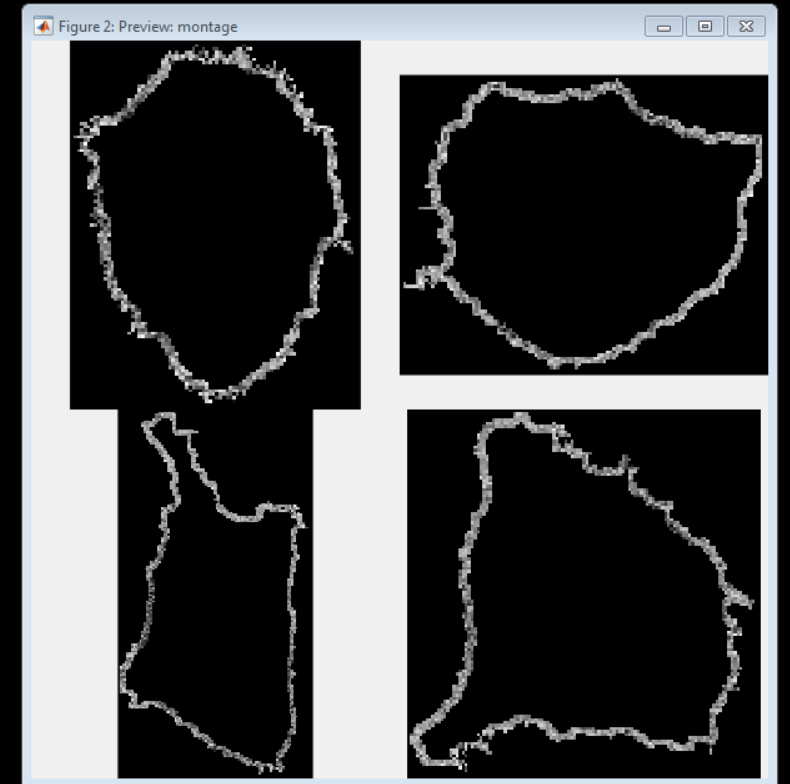
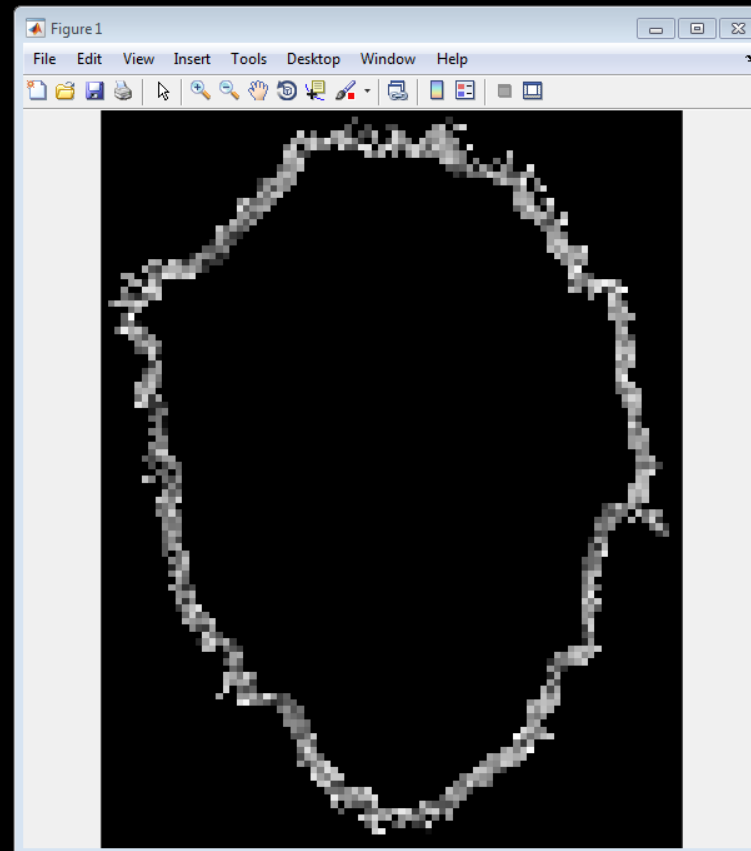
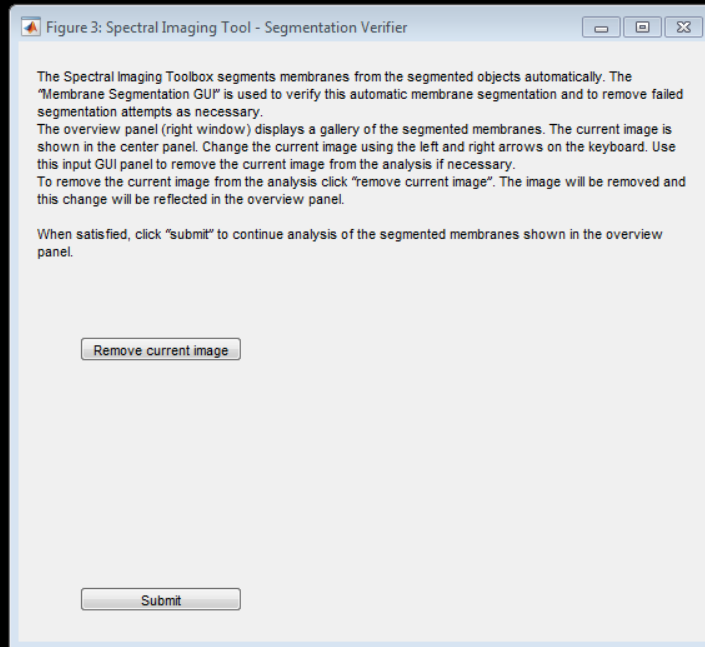
- If in “objects and membranes” segmentation mode, the membranes are auto-segmented from the cropped images submitted in the Object Separator
- The segmented membranes are then displayed for review in a similar 3 panel GUI as follows:
 1. Input panel (left window)
 2. Current image panel (middle window)
 - Navigate through the segmented membranes using the left and right arrows on the keyboard
 3. Overview panel (right window)
 - This panel displays a gallery of the segmented membranes
- When satisfied, click “submit” to continue analysis of the segmented membranes shown in the overview panel

Membrane segmentation: Review

Input panel

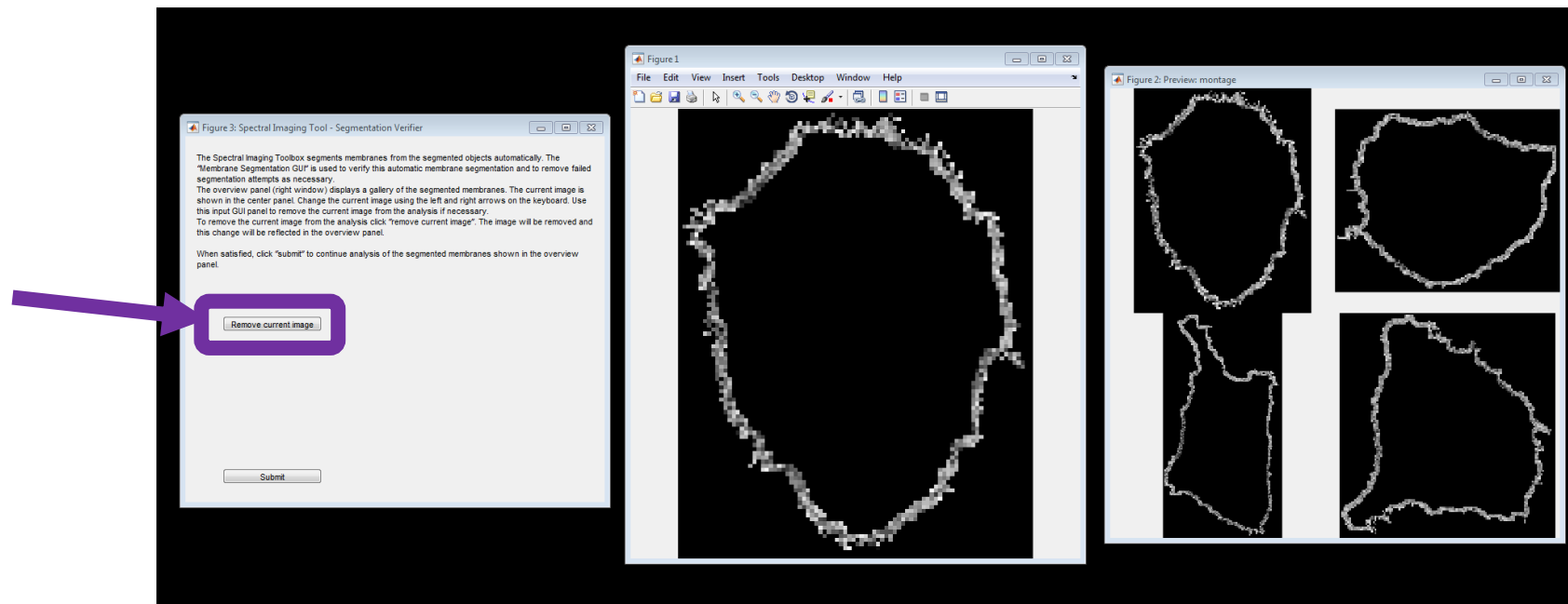
Current image panel

Overview panel



Membrane segmentation: Removing images

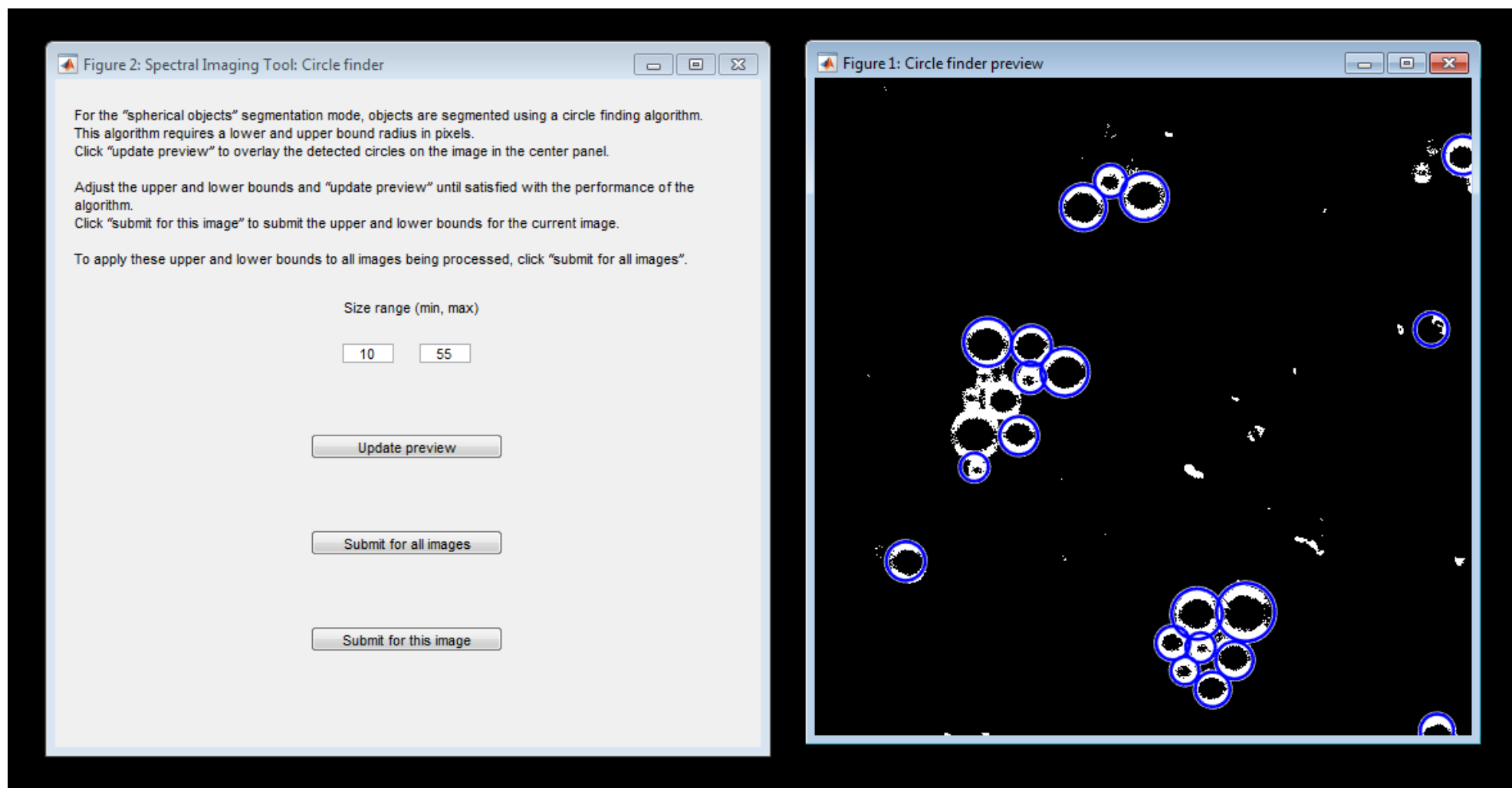
- As in the Object Separator, to remove the current image from the analysis click “remove current image”
- The image will be removed and this change will be reflected in the overview panel



Spherical object mode

- If “spherical objects” is chosen for the segmentation mode, objects are segmented using a circle finding algorithm
- This algorithm requires a lower and upper bound radius in pixels
- For each image the image is shown in the center panel and a GUI for setting these upper and lower bounds in the left panel
- Click “update preview” to overlay the detected circles on the image in the center panel
- Adjust the upper and lower bounds and “update preview” until satisfied with the performance of the algorithm
- Click “submit for this image” to submit the upper and lower bounds for the current image
- To apply these upper and lower bounds to all images being processed, click “submit for all images”

Spherical object mode



Output: File trees

The Spectral Imaging Toolbox outputs a results folder containing a results summary .xlsx spreadsheet and the following subfolders for each segmentation mode:

<u>No segmentation mode</u>	<u>Objects mode</u>	<u>Objects and membranes mode</u>	<u>Spherical objects mode</u>
1. No segmentation	1. No segmentation 2. Objects	1. No segmentation 2. Objects 3. Membranes	1. No segmentation 2. Spherical objects

Contents of each subfolder:

<u>No segmentation</u>	<u>Objects</u>	<u>Membranes</u>	<u>Spherical objects</u>
1. GP <ul style="list-style-type: none">GP data* (.mat)GP maps (.fig)3D GP maps** (.fig)	1. GP <ul style="list-style-type: none">GP data* (.mat)GP maps (.fig)	1. GP <ul style="list-style-type: none">GP data* (.mat)GP maps (.fig)	1. GP <ul style="list-style-type: none">GP data* (.mat)GP maps (.fig)
2. GP Histograms <ul style="list-style-type: none">GP histogram data (.mat)GP histograms (.fig)	2. GP Histograms <ul style="list-style-type: none">GP histogram data (.mat)GP histograms (.fig)	2. GP Histograms <ul style="list-style-type: none">GP histogram data (.mat)GP histograms (.fig)	2. GP Histograms <ul style="list-style-type: none">GP histogram data (.mat)GP histograms (.fig)
3. Spectra <ul style="list-style-type: none">Spectra data (.mat)Spectra plots (.fig)	3. Spectra <ul style="list-style-type: none">Spectra data (.mat)Spectra plots (.fig)	3. Spectra <ul style="list-style-type: none">Spectra data (.mat)Spectra plots (.fig)	3. Spectra <ul style="list-style-type: none">Spectra data (.mat)Spectra plots (.fig)
	4. Object properties <ul style="list-style-type: none">Region properties*** (.mat)		4. Size distribution <ul style="list-style-type: none">Diameters (.mat)Size histogram data (.mat)Size histogram (.fig)
			5. Circle detection <ul style="list-style-type: none">Detected circles**** (.mat)

* "GP data" includes Generalized Polarization (GP) map values, mean, standard deviation, median, and pixel count
** 3D reconstruction of GP data is performed automatically for z-stacks
*** See regionprops in MATLAB
**** See viscircles in MATLAB

Output: Summary spreadsheets

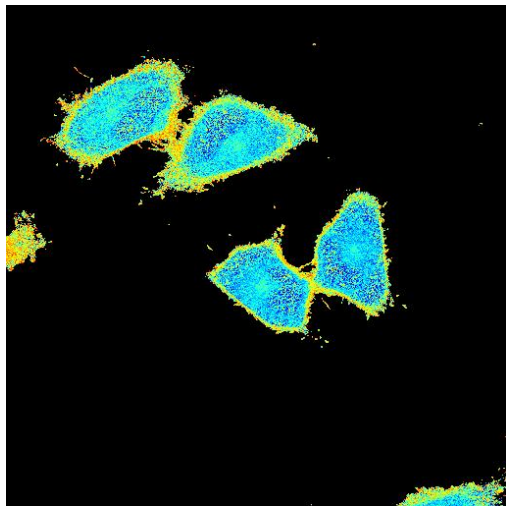
- Results are summarized in .xlsx spreadsheets for convenient access in OpenOffice, Excel, or Google Docs spreadsheets

	A	B	C	D	E	F
1	Filename - Object ID	Mean GP	Standard deviation GP	Median GP	Number of Pixels	Diameter (microns)
2	Sample 2 - Image 1.czi object1	0.52951925	0.341336208	0.58290547	1156	24.73901574
3	Sample 2 - Image 1.czi object2	0.54124818	0.311463689	0.59090909	1521	29.88465651
4	Sample 2 - Image 1.czi object3	0.51417003	0.332519005	0.58333333	1332	27.52721323
5	Sample 2 - Image 1.czi object4	0.53359636	0.342065133	0.5890411	2025	35.5516253
6	Sample 2 - Image 1.czi object5	0.49933015	0.342390308	0.57002801	1444	28.95812729
7	Sample 2 - Image 1.czi object6	0.53003362	0.313051595	0.59183673	2116	37.20117107
8	Sample 2 - Image 1.czi object7	0.50595873	0.344516211	0.57894737	2116	36.21857338
9	Sample 2 - Image 1.czi object8	0.53605329	0.315825626	0.58817134	735	25.8731937
10	Sample 2 - Image 1.czi object9	0.55396829	0.32654343	0.6354616	1560	30.20603234
11	Sample 2 - Image 1.czi object10	0.49917694	0.318772367	0.56521739	1225	26.20399072
12	Sample 2 - Image 1.czi object11	0.5233253	0.31616234	0.58823529	2304	38.97263598
13	Sample 2 - Image 1.czi object12	0.55203238	0.292202071	0.6056338	1156	24.14719615
14	Sample 2 - Image 1.czi object13	0.5348318	0.328080587	0.6	2116	36.75554893
15	Sample 2 - Image 1.czi object14	0.57592446	0.300772714	0.6287683	988	29.49781057
16	Sample 2 - Image 1.czi object15	0.51125402	0.331434649	0.58208955	1024	22.99847338
17	Sample 2 - Image 1.czi object16	0.51904627	0.334971252	0.57575758	930	21.63119101
18	Sample 2 - Image 1.czi object17	0.56648846	0.305009083	0.63636364	1521	30.05335688
19	Sample 2 - Image 1.czi object18	0.51293162	0.338895332	0.59710145	3306	48.77327688
20	Sample 2 - Image 1.czi object19	0.50162262	0.34287531	0.56442688	961	22.11339557
21	Sample 2 - Image 1.czi object20	0.58888503	0.23607257	0.60824742	1190	25.64855693

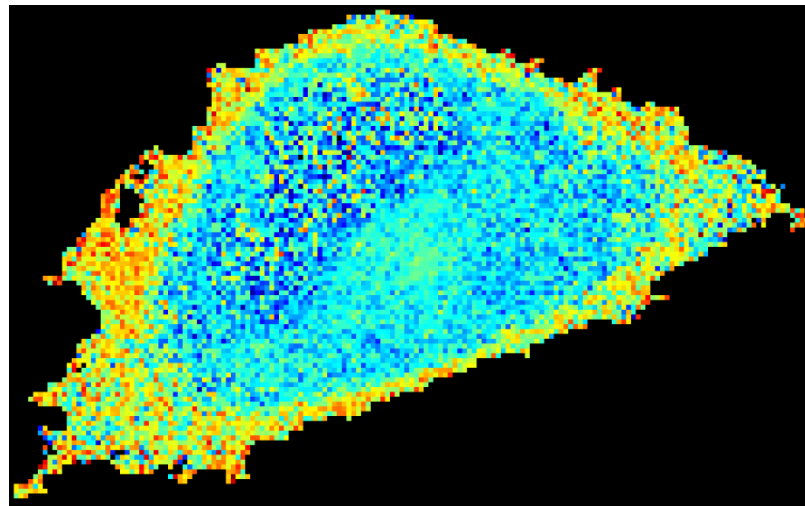
Output: GP maps

- Pseudo-coloured GP maps are generated using the 'jet' colormap with the range of colors from GP values of -1 to 1
- NaN-valued pixels are black

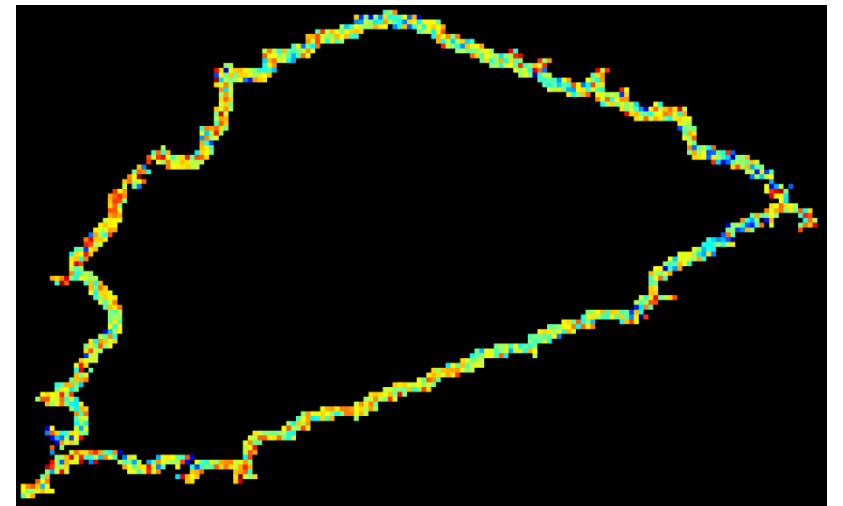
No segmentation



Object segmentation

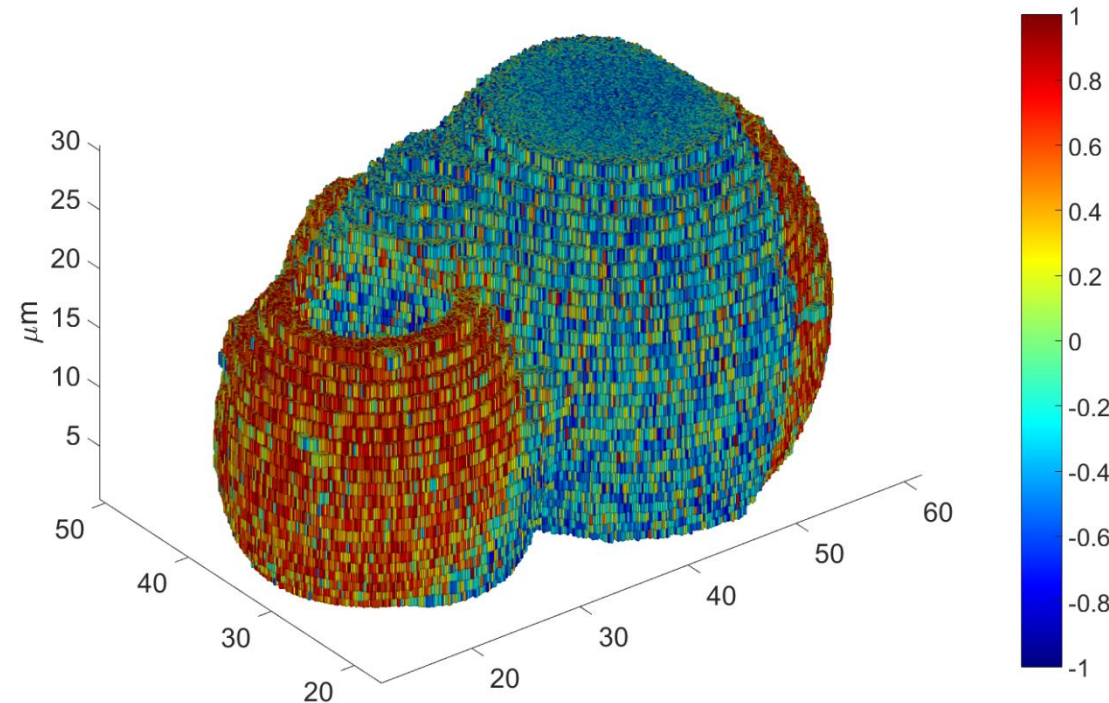


Membrane segmentation



Output: 3D GP maps

3D GP maps are automatically reconstructed from GP data calculated for each slice of a z-stack and using pixel-to-micron conversions in the meta-data



Output: GP data

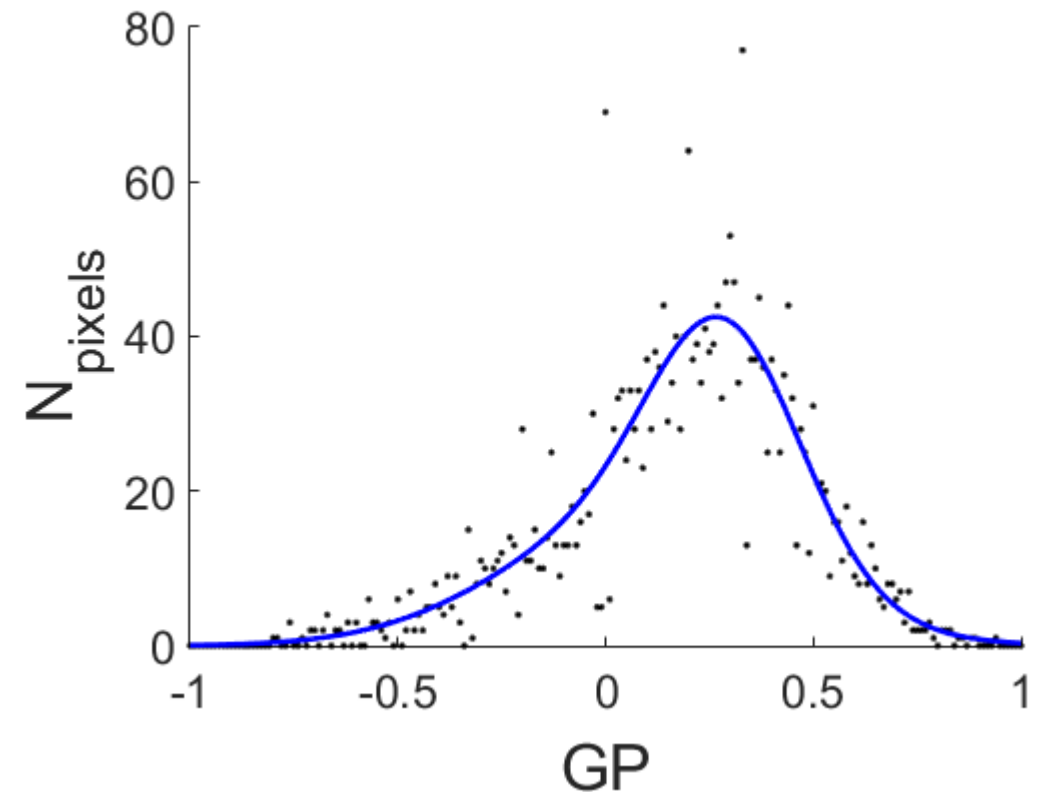
GP data includes .mat files containing:

- Matrices of GP values in the same form as the GP maps for each for each image/object/membrane
- The mean of the GP values for each image/object/membrane
- The median of the GP values for each image/object/membrane
- The standard deviation of the GP values for each image/object/membrane
- The number of pixels used to calculate GP values in each image/object/membrane

These .mat files can be readily loaded in MATLAB and used to generate summary figures, plots, or comparisons etc.

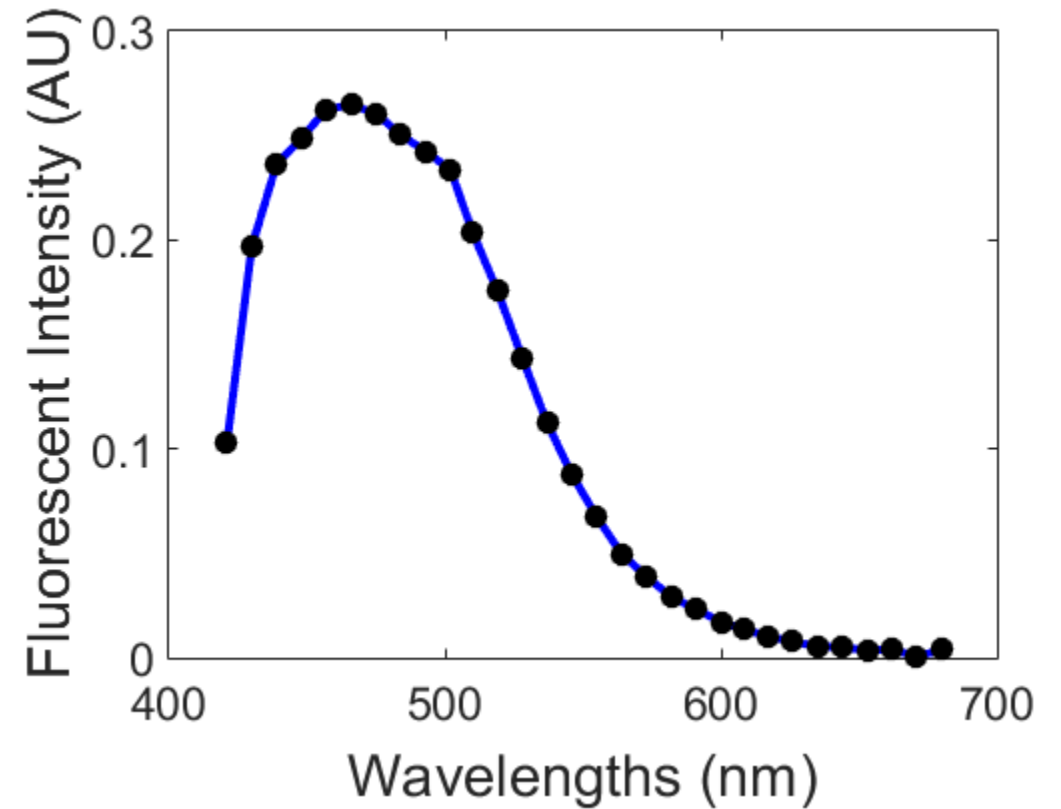
Output: GP histograms

- GP distributions are plotted as shown to the right
- They are fitted with a one or two term Gaussian chosen by the lowest RMSE
- Information about the fit, bins, counts etc. are included in the associated “*histogramData.mat” file



Output: Spectra

- Each point is the mean of the signal-pixel intensities for each wavelength image in the spectral image
- Spectra for segmented objects or membranes are calculated using the segmented signal-pixel intensities



Output: Size distributions and circle detection

- In spherical object mode the output includes a size distribution of the detected objects used in the analysis
- The circle detection performance can be easily visualized in the circle detection images where the circles are overlaid on the GP map of the pre-segmentation image

