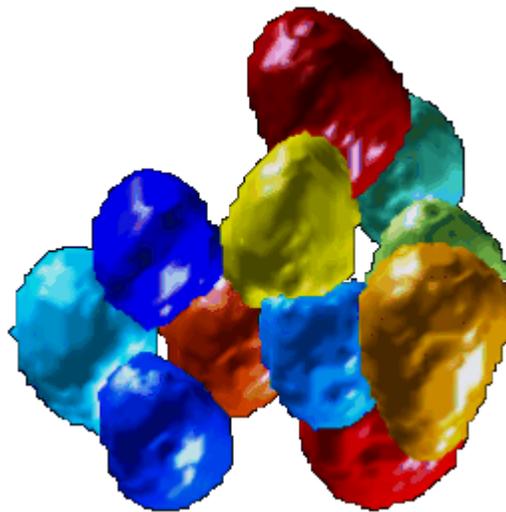


OpenSegSPIM

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OpenSegSPIM

A Quick User Guide

Version 1.1



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***Tips:** A useful hint to the user of OpenSegSPIM when they operate the application.*



***Image Processing Note:** A piece of technical information for readers, who are interested in more details of undertaking image processing operations.*



***Important Note:** An important message required reader's attention during the operation of OpenSegSPIM.*



OpenSegSPIM

Chapter 1: OpenSegSPIM interface

OpenSegSPIM at a quick start : Five main components

The screenshot shows the OpenSegSPIM software interface. On the left, a control panel contains buttons for 'Load image', 'Image enhancement', 'Foreground extraction', 'Detection', 'Segmentation', 'Analysis', 'Save', and 'Batch Process'. The main window displays two side-by-side images: the original grayscale image on the left and the segmented result on the right, where nuclei are highlighted in various colors. Below the images is a 'Nuclei Segmentation' progress bar and a 'Slice' indicator showing 48/116. At the bottom, there are two tables: a configuration table and a statistics table.

Loaded File	Enhancement	Detection & Segmentation	Statistics
Pathname	F:\Research Pr...	Smoothing Gaussian	Mask Threshold coef 1 <Nearest neigh...
Filename	100714_NS6.tif	Contrast adjust... Off	Seeds detection M... Intensity <Nuclei Vol> lu...
xy resolution	0.6500	Window size=	11 Seeds sensitivity 95 <Sphericity>
z_res	1.5000	background re...	17 Number of Seeds 14 Sorted by Volume
reducedsize	1_L_low	0	Watershed based ... Intensity N_manually ad...
Enhanced	L_high	0.9135	Number of nuclei 14

	Volume (um ³)	Sphericity	Nearest neighbour D (pix)	Intensity
1	64.2623	0.9546	69.6941	0.3191
2	66.1846	1.0206	12.6657	0.2157
3	93.9218	1.0206	35.3704	0.1799
4	116.9903	0.9794	11.1209	0.2616
5	148.8468	0.9847	11.8298	0.2486
6	185.6465	1.0047	16.2479	0.2650
7	229.3119	0.9575	11.1209	0.2474



OpenSegSPIM

OpenSegSPIM overview

In the past decades, we have seen a rapid development of microscopy imaging techniques. Recent fast growth of SPIM allowed us to produce large amount of 3D data. However, this is probably the first step. In general, user-friendly, flexible and reliable tools are still a bottleneck of data quantitative analysis in this field.

OpenSegSPIM is a 3-D fully automatic quantitative analysis tool for the nuclei in cell aggregate acquired using SPIM or traditional confocal. The software mainly designs to extract quantitative relevant information from SPIM image stacks, such as the number of nuclei/cells and measure the volume and sphericity of stained nuclei and etc on Light Sheet Microscopy (LSM) images. Typically it is useful quantitative analysis tool to different biological problems such as Neurospheres, Zebrafish embryos, Drosophila embryos, Skin sample, Mouse embryos and Organotypic cell culture.

The screenshot shows the OpenSegSPIM software interface. It features a toolbar at the top left, a main visualization area with two side-by-side image windows, and a data table at the bottom. The interface is annotated with four green circles and numbers:

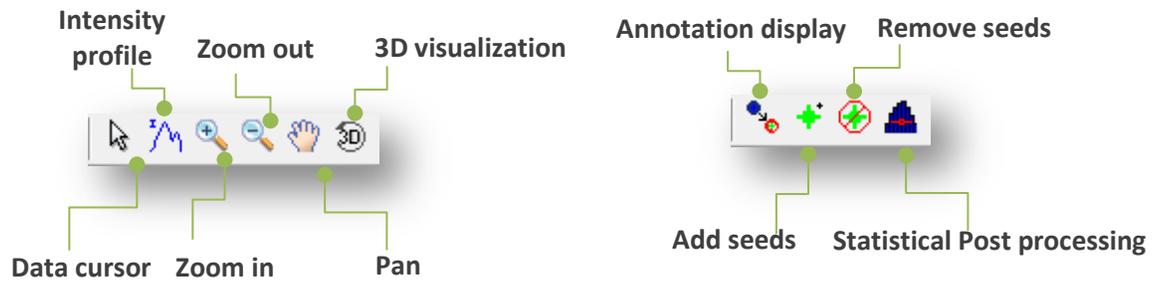
- 1**: Points to the main visualization area, which shows a 3D image of blue-stained nuclei on the left and a segmented view of the same nuclei on the right, each with a coordinate axis.
- 2**: Points to the 'Wizard panel' on the left side of the main window, which contains buttons for 'Load image', 'Image enhancement', 'Foreground extraction', 'Detection', 'Segmentation', 'Analysis', 'Save', and 'Batch Process'.
- 3**: Points to the 'Information panel' at the bottom of the main window, which displays a table of quantitative data for each segmented nucleus.
- 4**: Points to a smaller 'Wizard panel' at the bottom of the interface, which contains buttons for 'Segmentation', 'Analysis', and 'Save'.

Loaded File	Enhancement	Detection & Segmentation	Statistics	Volume (um ³)	Sphericity	Nearest neighbor D (pix)	Intensity	
Pathname F:\Research Pr...	Smoothing Gaussian	Mask Threshold coef	1 <Nearest neigh...	12.0151				
Filename 100714_NIS6.tif	Contrast adjust... Off	Seeds detection M... Intensity	<Nuclei>Vol.> /u...	215.9729				
xy resolution 0.6500	Window size=	11 Seeds sensitivity	95 <Sphericity>	0.9763				
z_res 1.5000	background re...	17 Number of Seeds	14 Sorted by Volume					
reducedsize 1_L_low		0 Watershed based ... Intensity	N_manually ad...	0				
Enhanced L_high		0.9135 Number of nuclei	14					
				1	64.2623	0.9546	69.6941	0.3191
				2	66.1846	1.0206	12.6657	0.2157
				3	93.9218	1.0206	35.3704	0.1799
				4	116.9903	0.9794	11.1209	0.2616
				5	148.8468	0.9847	11.8296	0.2486
				6	185.6465	1.0047	16.2479	0.2650
				7	229.3119	0.9575	11.1209	0.2474



1

Toolbar of OpenSegSPIM



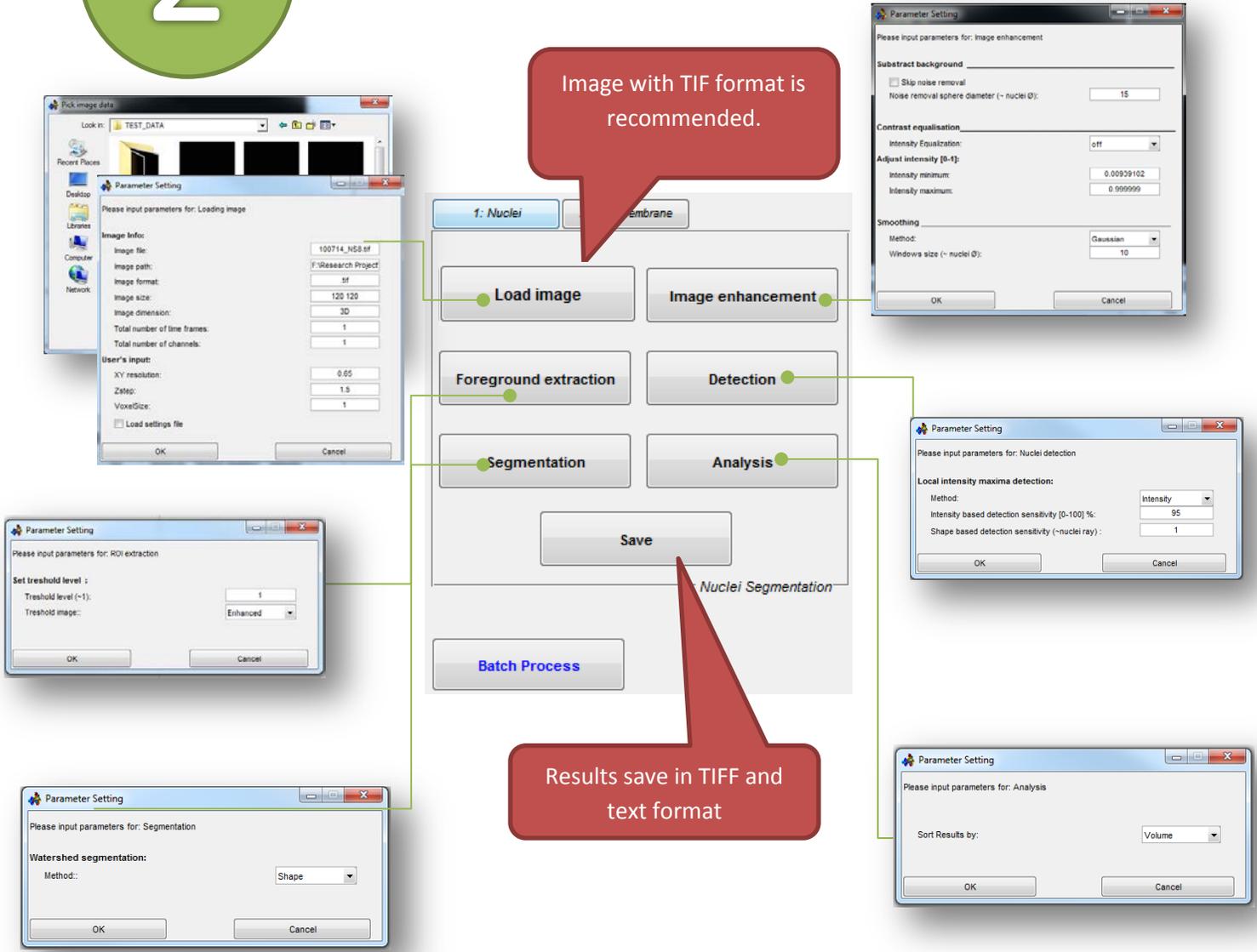
Menu item	Description
Data cursor	Activate ruler to measure nucleus in the viewer and intensity brightness/contrast adjustment.
Zoom in	Enlarge the image by clicking the area of the axes where you want to zoom in, or drag the cursor to draw a box around the area you want to zoom in.  <i>Tips: When in zoom in mode, you can double click to restore the view and use Shift+click to zoom out (i.e., press and hold down the Shift key while clicking the mouse). You can also right-click and zoom out or restore the plot to its original view using the context menu.</i>
Zoom out	Reduce an image by clicking the area of the axes where you want to zoom out, or drag the cursor to the area you want to zoom out.
Pan	When the image is enlarged, click and drag the image and move the current region to another region.
3D visualization	Click to open the 3D viewer to visualize original nucleus and segmented nucleus in 3D space.
Intensity profile	Analytical tool used to show the intensity value in along the line draw by user
Annotation display	Select different mode of viewer to display outline of segmented nucleus.
Add seeds	Insert missing seeds in the visualization viewer at the right hand side.
Remove seeds	Remove an unreliable seed from the visualization viewer at the right hand side.
Statistical Post processing	Open histogram viewer to perform data filtering.



2

Wizard panel - Nuclei segmentation

Image with TIF format is recommended.



Results save in TIFF and text format

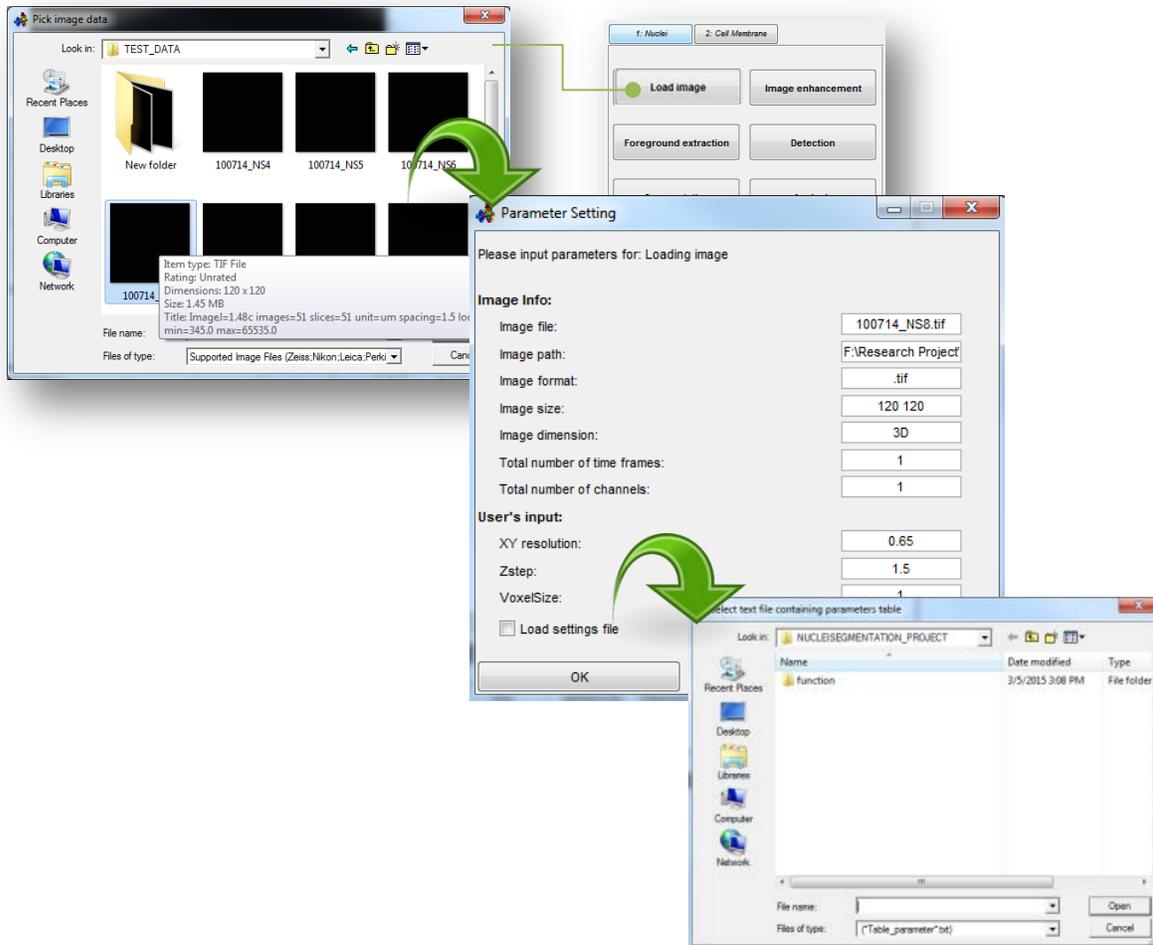
Nuclei segmentation wizard panel



Components	Description
LoadImage	Load image stacks to start the quantitative analysis
Image enhancement	Enhance the loaded image stacks by using the following approaches: <ul style="list-style-type: none">• Artefact and noise removal• Brightness and contrast adjustment
Foreground extraction	Extract the outline of targeted nucleus.
Detection	Identify each nucleus with seed detection.
Segmentation	Segment the nucleus aggregate (separate each of them)
Analysis	Count nucleus and compute the quantitative information such as volume and sphericity of each nucleus.
Save	Click to save the configured parameter value and segmented image stacks.



Load image



Load image parameter setting	Description
Pick image data	Load image stacks to start the quantitative analysis
Image info	The information is automatic collected via image metadata and display in the parameter setting dialog.
User' input	<p>XY resolution and Z-step are extracted from image metadata. However, users are allowed to input according to their need.</p> <p> Tips: User can load back the parameters that have been saved previously. Hence, tick on "Load setting file" and click ok button will prompt a configuration pickup dialog to select required setting file.</p>



Important note: The actual metadata might be lost or inaccurate when the original microscopy image stacks are transfer to TIF. Users need to pay extra attention.

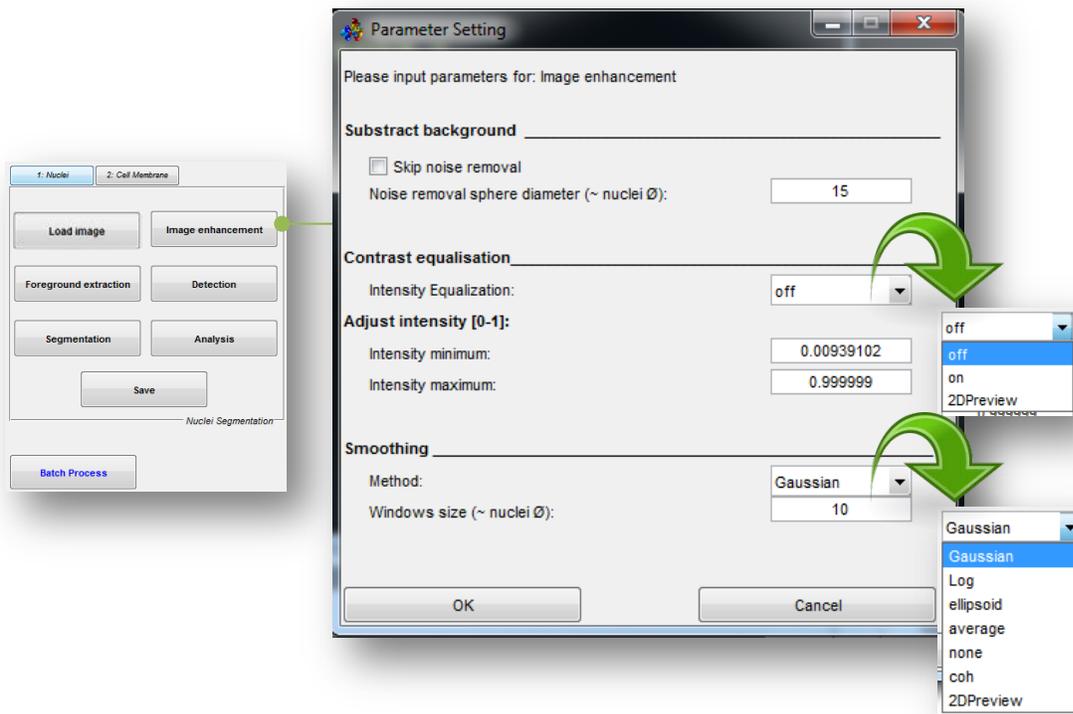


Image enhancement parameter setting	Description
Subtract background	Remove big artifacts, such as non-uniform background by knowing the approximate diameter of nucleus.
Contrast equalization	Increases the global contrast of images, especially when the usable data of the image is represented by close contrast values. Default value is <i>off</i>
Adjust intensity	Increase the visibility of nuclei\cells by adjusting the brightness (min and max intensity value).  <i>Tips: min and max intensity value can be obtained automatically during adjusting the brightness contract at cell viewer (Please refer to visualization panel)</i>
Smoothing	Remove noise and periodic components from data sets while preserving underlying patterns.
2DPreview	Select 2DPreview option from combo box to visualize the original image and processed images based on the configuration value setting accordingly. (Please refer to image processing note)

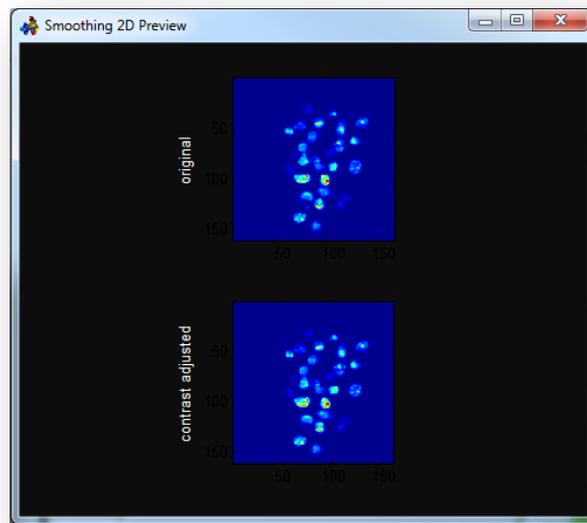
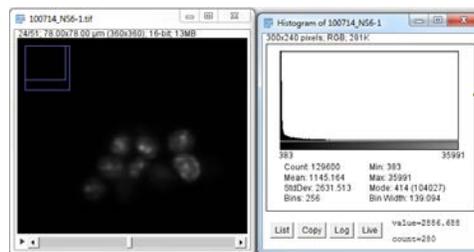
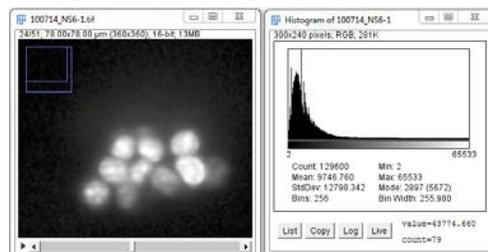


Image processing note: **Contrast equalization** is based on **histogram equalization algorithm** for adjusting image intensities to enhance the contrast by stretch out the intensity range.



Original image with intensity histogram

For instance, from the image above, you can see that the pixels seem clustered around the middle and right of the available range of intensities. Histogram Equalization stretch out intensity range. Take a look at the image below: After applying the equalization, we get an histogram like the figure in the below. The resulting image is shown in the picture at left.



Processed image with stretched intensity histogram

Implementation of this algorithm, readers is advised to follow the tutorial at following:

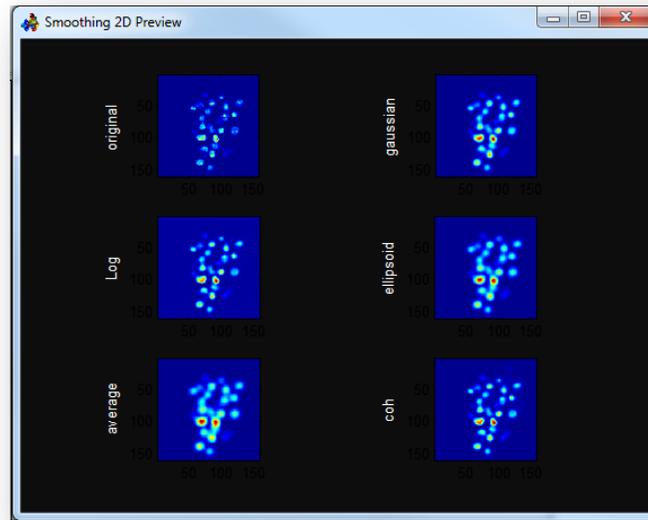


Image processing note:

Smoothing also known as blurring, it is a simple and often used for image processing operation.

Gaussian Filter: The filter compute with a $m \times n$ mask that computed based on Gaussian function.

$$G(x, y, z) = Ae^{-\frac{(x-\mu_x)^2}{2\sigma^2_x} - \frac{(y-\mu_y)^2}{2\sigma^2_y} - \frac{(z-\mu_z)^2}{2\sigma^2_z}}$$

Where μ is the mean and σ represents the variance.

LoG filter: is Laplacian of Gaussian (LoG) where Gaussian filter required to be applying before Laplacian. It is derivative filters used to find areas of rapid change (edges) in images.

$$LoG(x, y, z) = -\frac{1}{\pi\sigma^4} \left[1 - \frac{x^2 + y^2 + z^2}{2\sigma^2} \right] e^{-\frac{x^2+y^2+z^2}{2\sigma^2}}$$

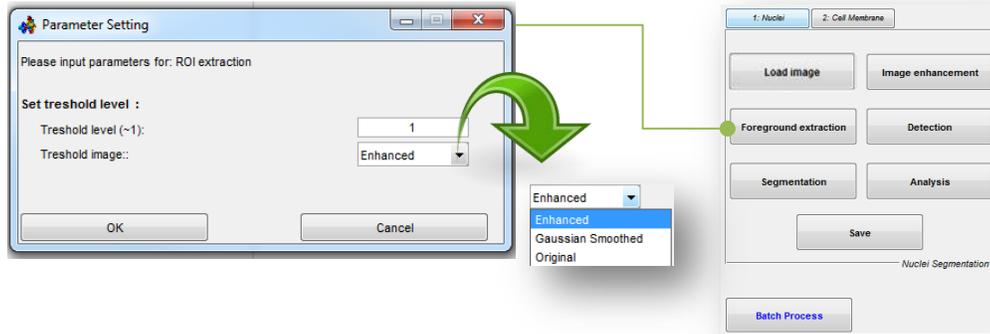
Ellipsoid filter: The filter computes with a ellipsoid as a mask that generates the data using the following equation:

$$E(x, y, z) = \frac{(x - xc)^2}{xr^2} + \frac{(y - yc)^2}{yr^2} + \frac{(z - zc)^2}{zr^2}$$



OpenSegSPIM

Foreground Extraction



Foreground Extraction parameter setting	Description
Threshold level	Set the threshold floating value from 0 to 1 to extract the foreground.  <i>Tips: We recommended user to set the threshold value approximate to 1 in order to obtain a good ROI.</i>
Threshold image	Optional to let user processed the ROI extraction based on original image, Gaussian smooth image or image enhanced since beginning.

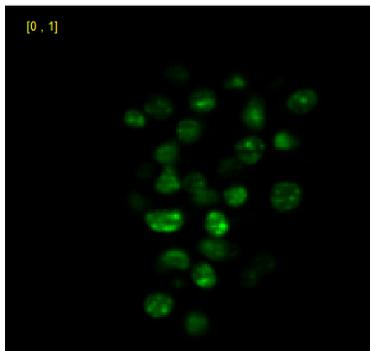


Image processing note:

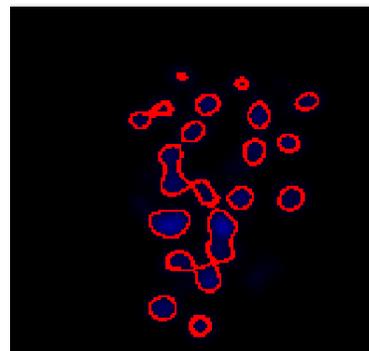
Thresholding approach: ROI extraction using Thresholding approach is in-expensive computation and strait forward approach. Thresholding operation define as

$$f = \begin{cases} 1, & t > f(x,y) \\ 0, & \text{otherwise} \end{cases}$$

Where t denotes the threshold value, f is the grayscale image, and $f(x,y)$ is intensity value of coordinate x and y . For example:



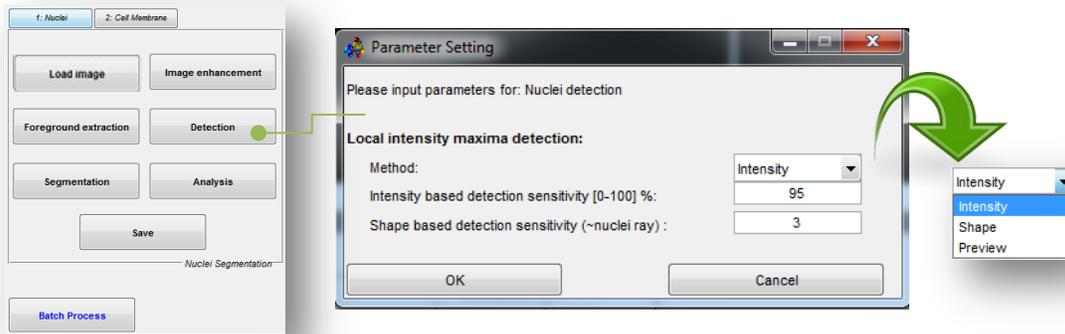
Grayscale Image (cell)



After thresholding process, cells are automatically identify



Detection

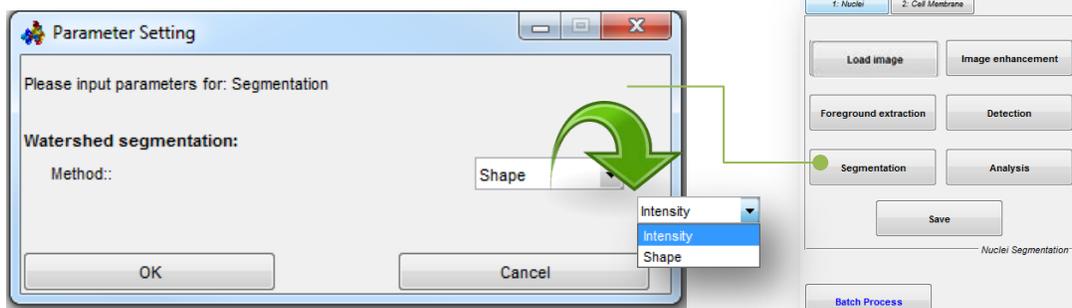


Detection parameter setting	Description
Local intensity maxima detection	Set the local intensity maxima method to detect seed of cells. The features used to seed detection are included: <ul style="list-style-type: none"> • Intensity • Shape
Intensity based detection sensitivity	Percentage of threshold level used to finding peak and valleys of intensity value.
Shape based detection sensitivity	Percentage of threshold level used to finding peak and valleys of distance value of shape.
	 Important note: <i>If the seeds are not detected correctly, users are advised to add annotation using the seeds editing tool as described at toolbar section and page. 32.</i>



OpenSegSPIM

Segmentation

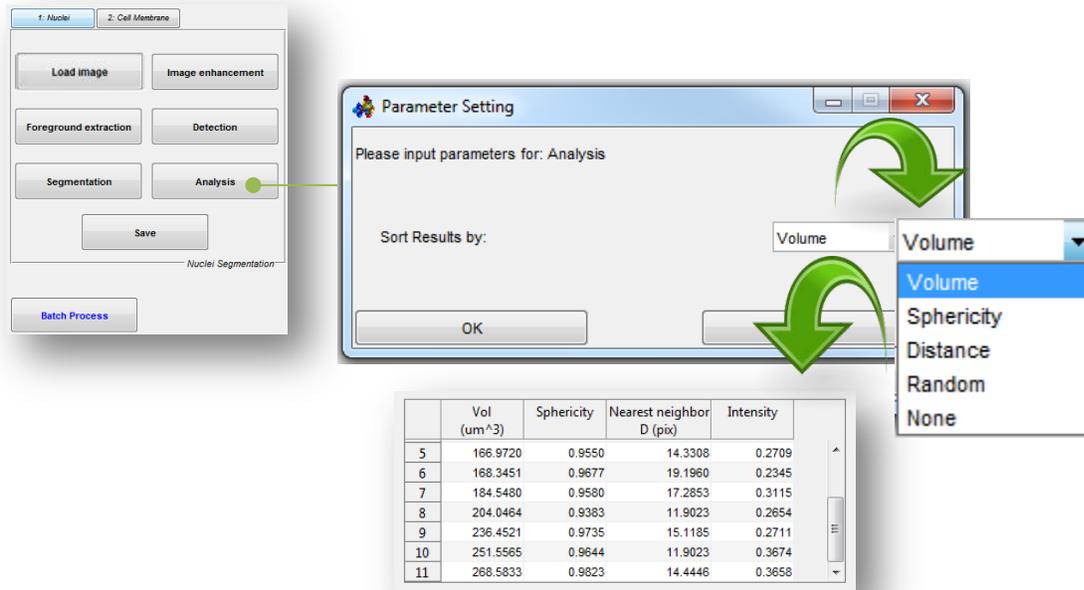


Segmentation parameter setting	Description
Segmentation method	Set the feature of watershed segmentation to segment cells. The features used to segmentation are included: <ul style="list-style-type: none">• Intensity• Shape
Intensity	Apply watershed algorithm based on intensity value.
Shape	Apply watershed algorithm based on distance (shape) value.



OpenSegSPIM

Analysis

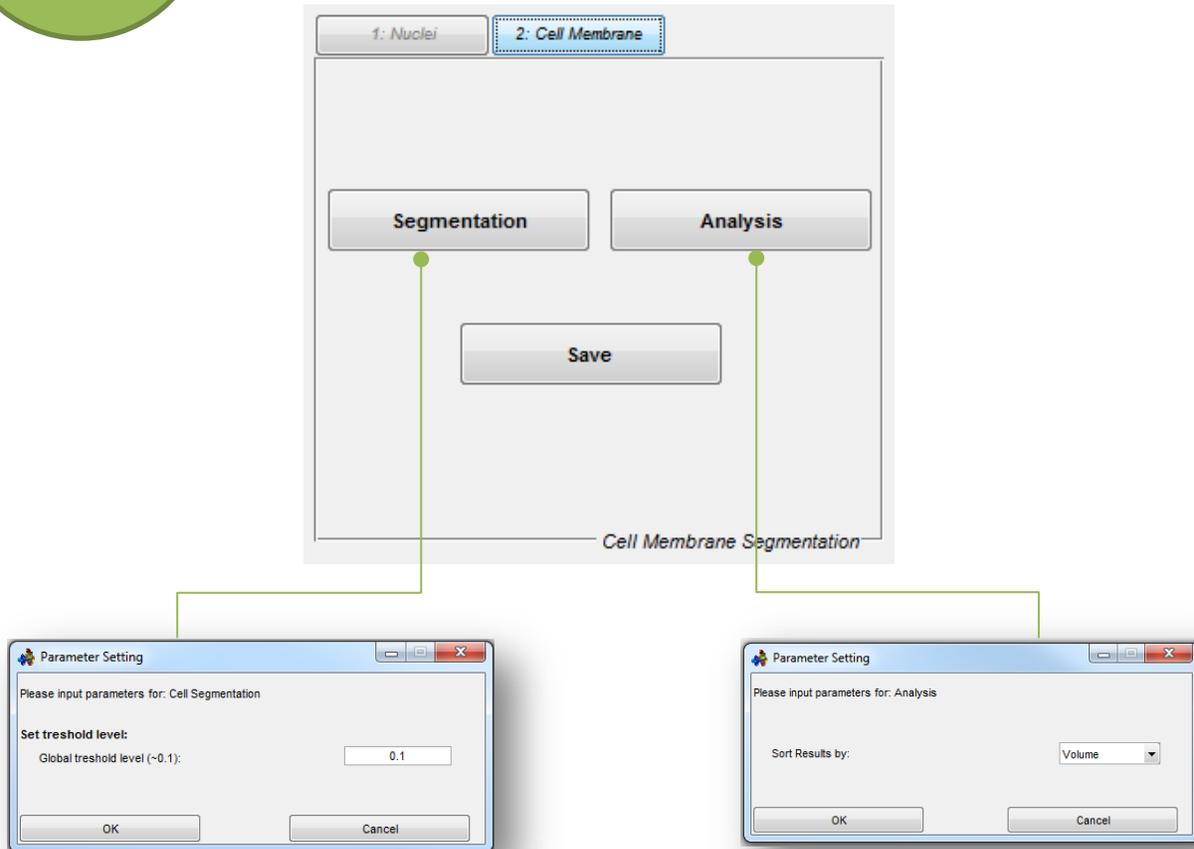


Analysis parameter setting	Description
Sort results by	Select the preferred quantitative measurements of nuclei for quick analysis.



2

Wizard panel – Cell membrane segmentation



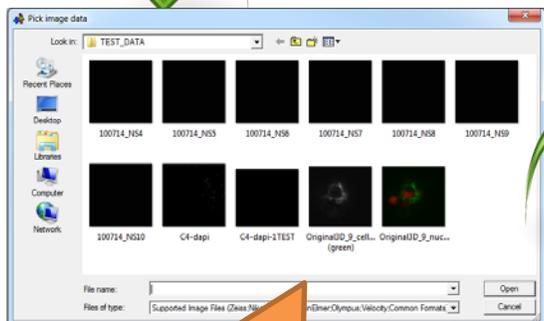
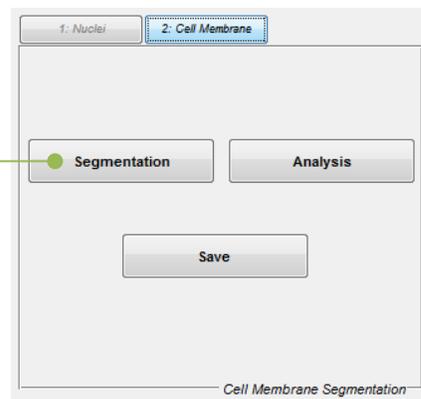
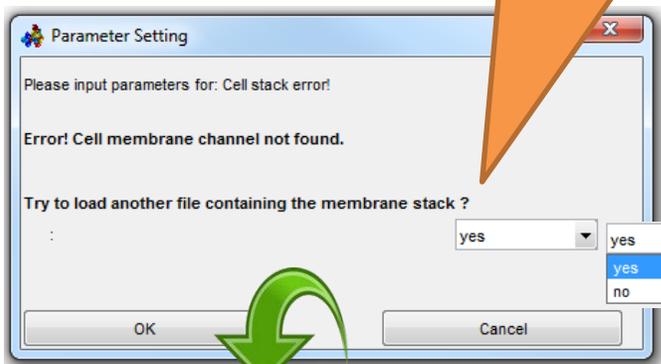
Components	Description
Segmentation	Segment the cell membrane.
Analysis	Count cell and compute the quantitative information such as volume and sphericity of each cell.
Save	Click to save the configured parameter value and segmented image stacks.



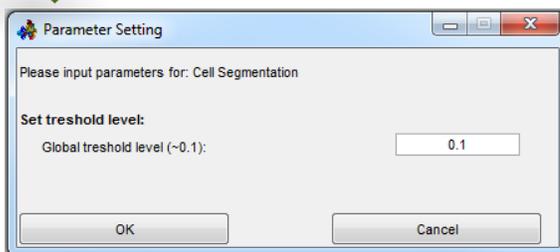
OpenSegSPIM

Segmentation

To process the cell membrane segmentation, the cell membrane stack image is required. Select YES to find the membrane channel stack image file.



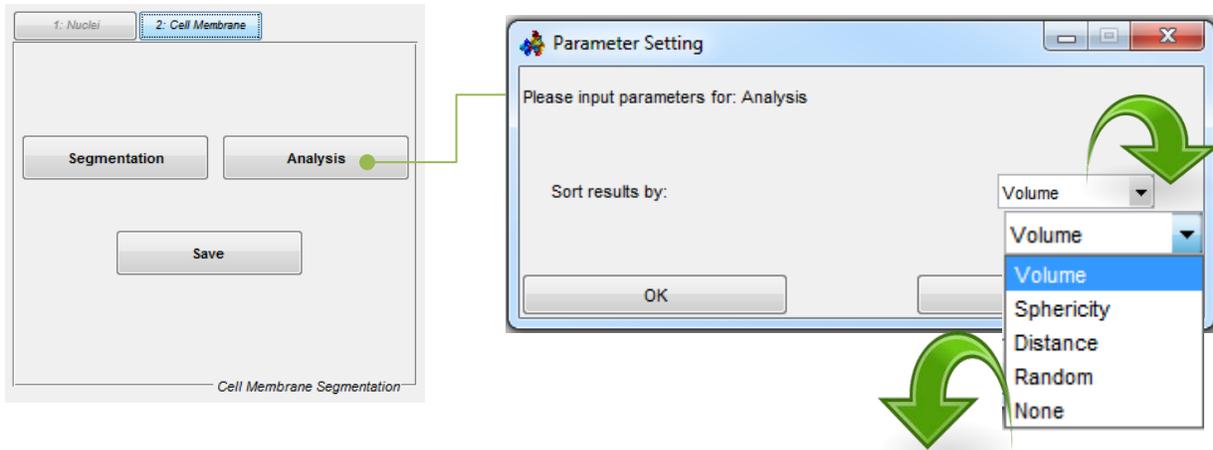
Select the correct membrane stack image file that associated with nuclei stack image.



Components	Description
Global threshold level	User set the threshold level in between 0 and 1. Threshold level with 0.1 is recommended to be set.



Analysis



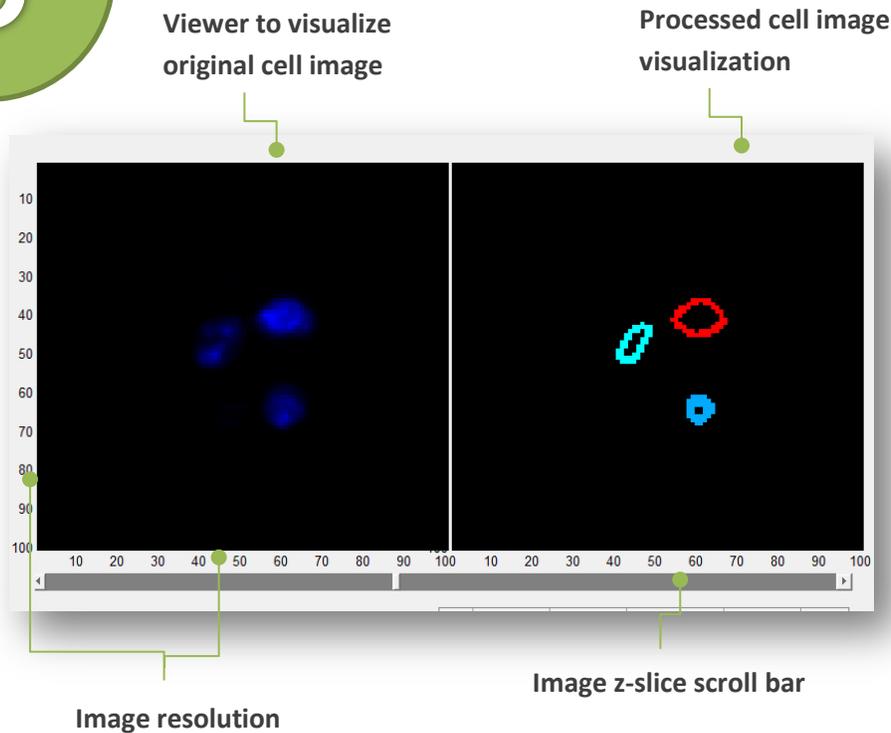
	Cell Volume (um ³)	Cell Sphericity	Nearest neighbor D (pix)	Total Intensity
20	0.0208	0.7698	15.6849	727.6549
21	0.0260	0.9242	19.6936	2.1574e+03
22	0.0288	0.7861	21.2882	716.1765
23	0.0304	0.6711	13.2008	1.1963e+03
24	0.0304	0.8588	18.3376	1.1249e+03
25	0.0318	0.8031	15.8673	1.0843e+03
26	0.0414	0.7313	13.2008	1.7994e+03

Analysis parameter setting	Description
Sort results by	Select the preferred quantitative measurements of cell for quick analysis.



3

Visualization panel



Components	Description
Cell viewer	Visualize original cell image.  Tips: Single left clicks and drags on the cell image viewer to measure the targeted cell. Left click and drags on the cell to adjust the brightness contrast.
Preprocessed viewer	Preview all processed cell image after parameters change.  Tips: User can insert and remove the seed to the processed image viewer if the seed detection is not accurate.
Image z-slice scroll bar	Scroll along the scroll bar to select and view the original cell image and processed image (both viewer is synchronized).
Image resolution	Numbering indicate to the 2D image resolution (width and height).



4

Information panel

Configuration and statistics

Loaded File	PreProcessing	Mask, seeds & segmentation	Statistics
Pathname	F:\Research ... Smoothing	Gaussian	<nearest nei... 9.7939
Filename	100714_NS5... Contrast adju... off	Mask Treshold c...	<Nuclei Vol.>... 169.7432
xy resolution	0.6500 Window size=	9 Seeds sensitivity	<Sphericity> 0.9735
z_res	1.5000 background r...	15 Number of Seeds	sorted by Volume
reducedsize	1 L_low	0.0046 Watershed bas...	N_postprocc... 0
Enhanced	L_high	1.0000 Number of Nuclei	

	Vol (um ³)	Sphericity	Nearest neighbor D (px)	Intensity
1	34.3281	1.0055	13.2588	0.1859
2	36.2505	1.0009	13.2588	0.2429
3	149.6706	0.9379	14.3308	0.2852
4	166.4228	1.0253	20.7151	0.3876
5	166.9720	0.9550	14.3308	0.2715
6	168.3451	0.9677	19.1960	0.2351
7	184.5480	0.9580	17.2853	0.3123



Tips: Average value of volume, sphericity, and nearest neighbor

Quantitative viewer

Components	Description
<p>Configuration and statistics viewer</p> 	<p>List of the parameters setting value for reference</p> <p><i>Tips: All setting value will be saved in the results directory and named as Table_parameter.txt</i></p>
<p>Quantitative viewer</p> 	<p>List of quantitative information for each segmented cells. The measurements included volume, sphericity, nearest neighbor and intensity.</p> <p><i>Tips: All quantitative results of each segmented cell will be save and named as Table_Results.txt</i></p>

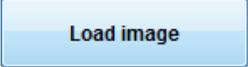


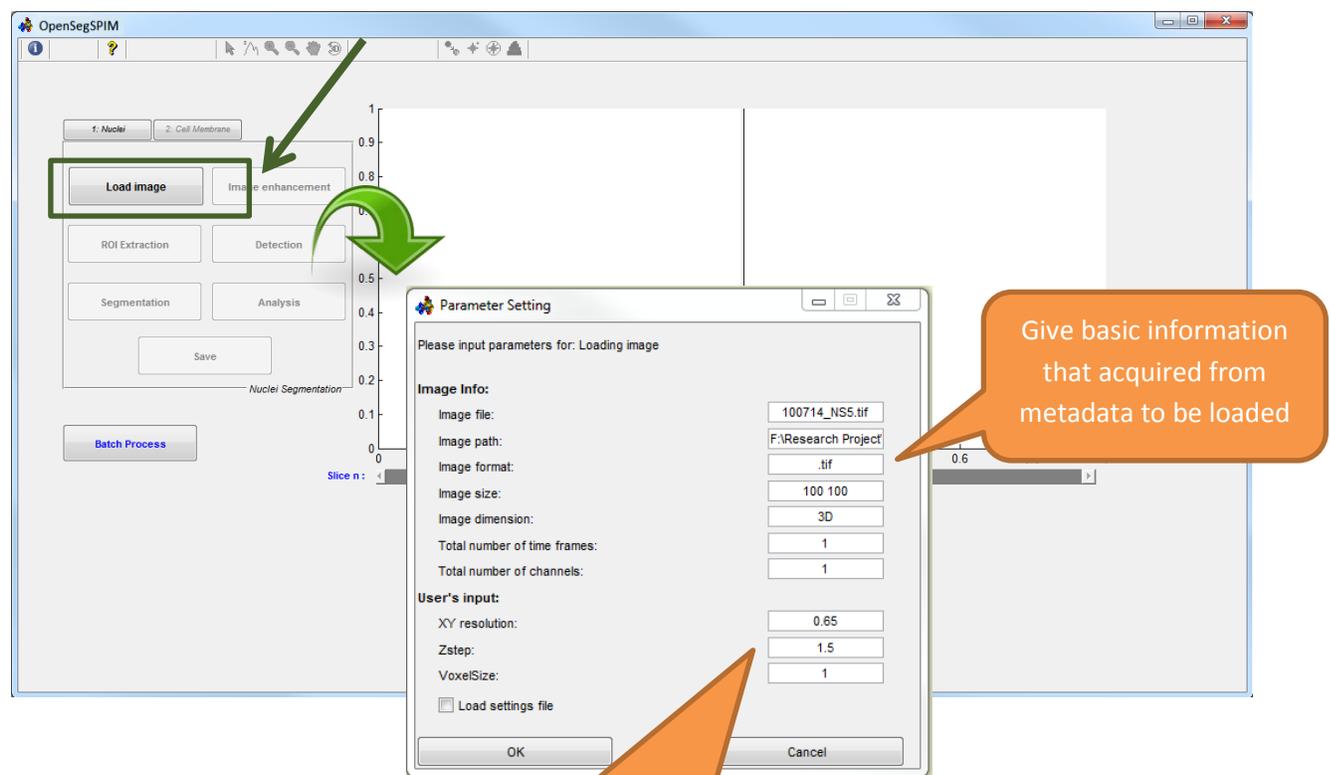
OpenSegSPIM

Chapter 2: Nucleus segmentation in action

Step 1: Select entrances of cell and nucleus image

The image processing pipeline in OpenSegSPIM is a linear design. Therefore, **each following steps is not accessible** (gray shaded) before the previous step is complete.

“ First step is to load the Nuclei 3D stack image by clicking Load Image . Each push button contains a tooltip attached when the cursor stays over it giving the user quick tips to progress through the software.



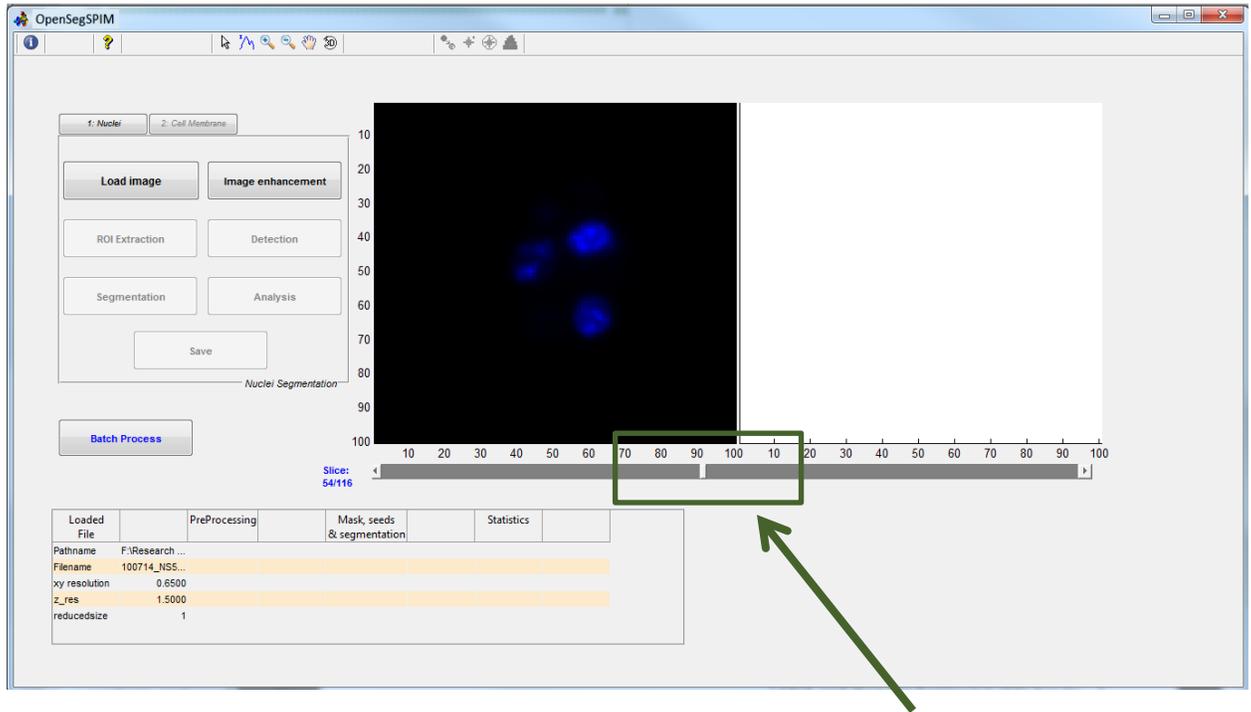
- Enter the x-y resolution (For example: 0.65 μm / pixel)
- Enter the Z-step of the 3D stack (1.5 μm step here)
- Change Voxel size to any value higher than 1 voxel will interpolate data with the specified step and reduce the size of the 3D image (uniformly along the 3 dimensions).



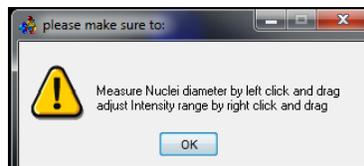
OpenSegSPIM

Step 2: Cell measuring and image enhancement

Interactive GUI design enable user fill up parameters in the quick way. Besides, user can select and view the image by scrolling the scroll bar as shown in following figure:



Please make sure the size of nucleus is determined before image enhance button is push.



“ User needs to adjust brightness by right click and drag. Once the nucleus appear clearly then user required to measure the nucleus size by right click and drag (preferred at [0,1]) in the original image viewer as shown in following. Both information will be automatically fill up the parameter dialog when the image enhancement button is clicked ”



Right click and drag to adjust contrast. Potential cell will be appearing from blue to green. Therefore, cells can visualize correctly by user

Average value of nucleus size <10>

Loaded File	Pre-processing	Mask, seeds & segmentation	Statistics
Pathname F:\Research ...			
Filename 100714_NS5...			
xy resolution 0.6500			
z_res 1.5000			
reducedsize 1			



Tips: Parameters used during each processing steps will be store in this table and save as a text file.

Left click and drag to draw a red line as rules. Size of all cells (median value) will be automatic filled in the dialog in the next process.

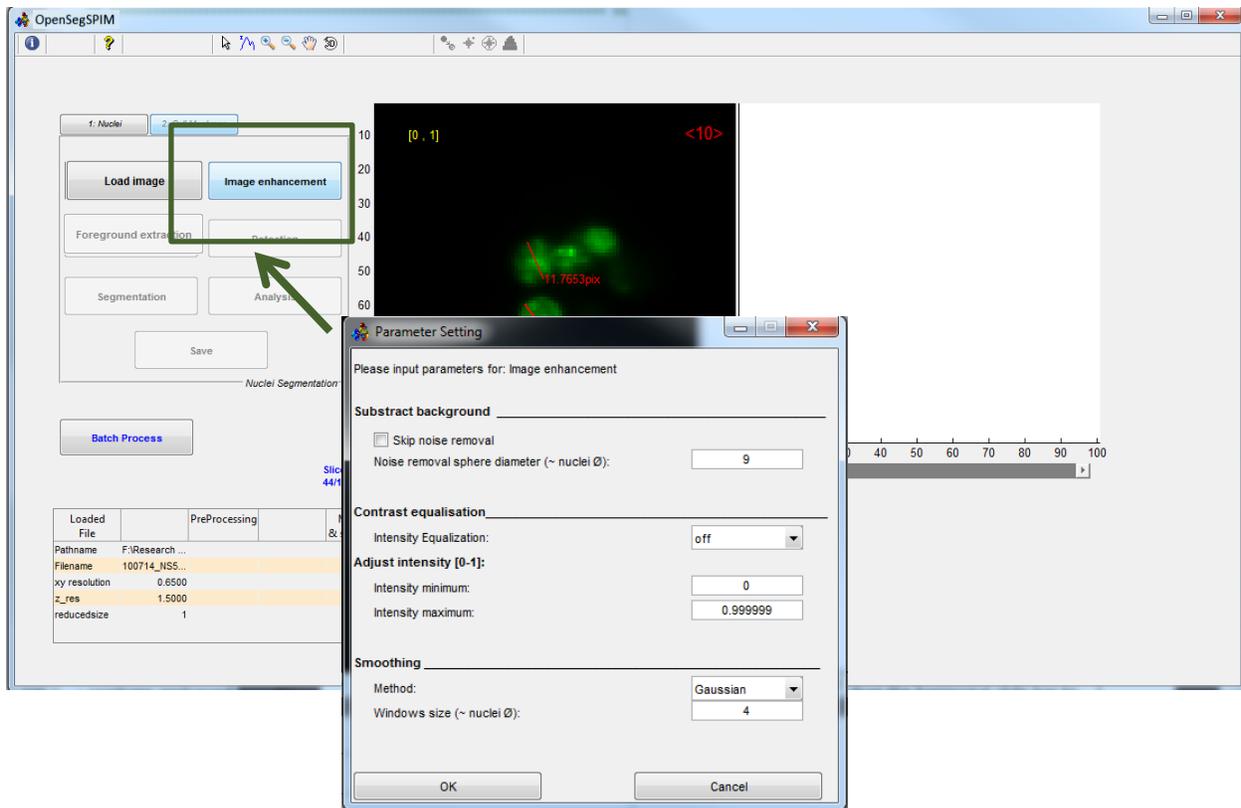


Image enhancement

Once the several sample cells have been measured. Click on **Image enhancement** button to start following process.



Tips: Parameter setting dialog of image enhancement are filled automatically, For example: intensity minimum and maximum acquired from brightness contrast (right click and drag) interactive approach. Noise removal parameter is automatically filled based on the diameter all cells (median value).



*Typically the **non-uniform background removal diameter** should be slightly larger than the nuclei size whereas the **smoothing window size** should be slightly smaller than the nucleus diameter (odd integers only). A wide range of different type of 3D smoothing can be applied. Most useful are **Gaussian** and **ellipsoid** ones.*



Image processing note: Click on intensity profile button  allow the user to visualize the 2D intensity profile along a line to have an idea of the intensity range (between 0 and 1). Line is drawn by a left click anywhere on the image , right click to end the line and display the intensity profile.



Tips: Intensity profile tool is provided to help user understand intensity value crossing along the dotted line and plot intensity profile. It is useful and a guideline to set the low and min intensity for preprocessing adjustment.



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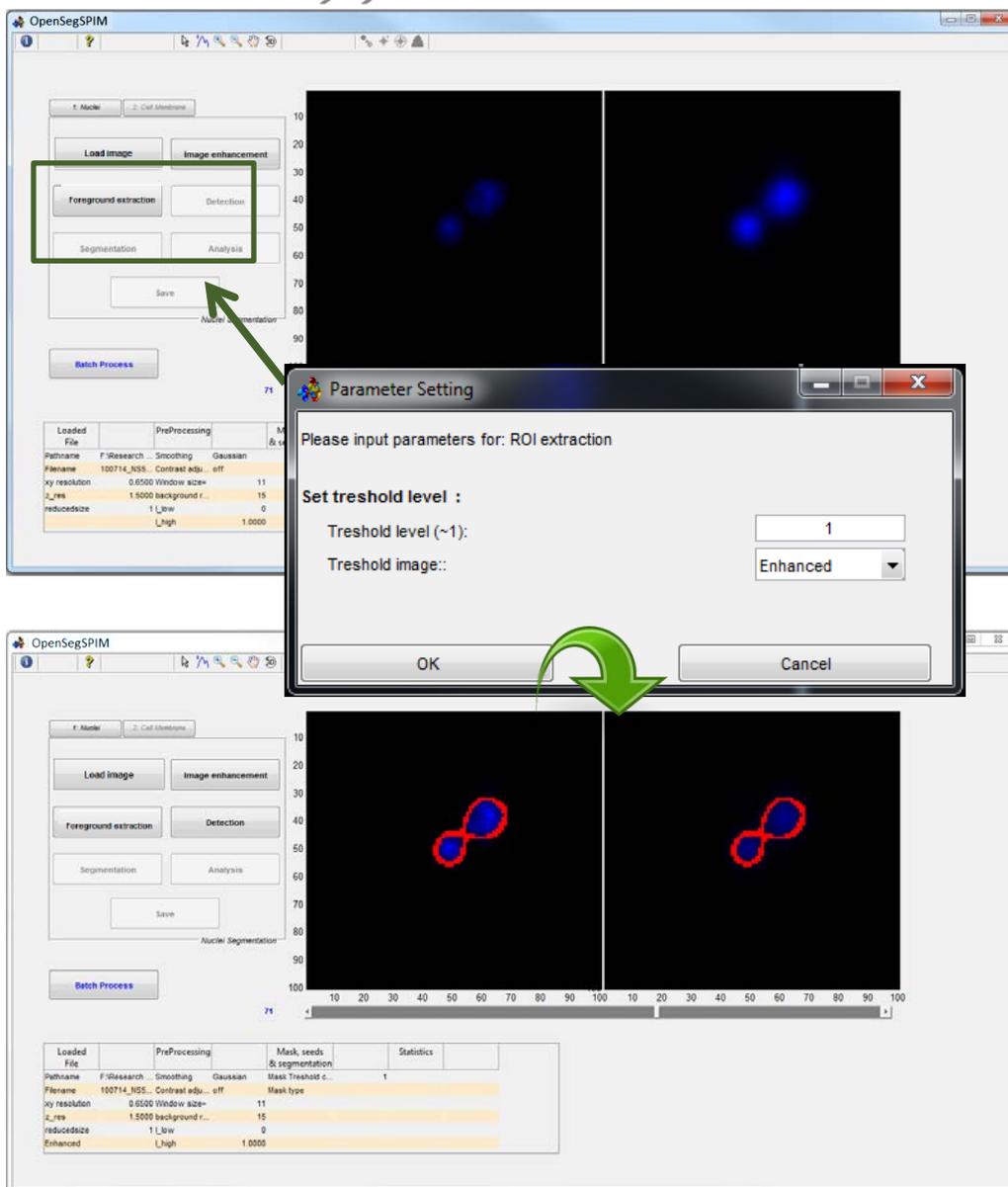
Step 3: Foreground extraction

Enhanced image in previous step will be displayed in the right viewer. We further process the

Foreground extraction

foreground extraction using the enhanced image by click on **Foreground extraction** button. Click ok once the parameters value has been decided.

“ Foreground Extraction use a Otsu Method Thresholding . User can adjust The Threshold level value. It is a coefficient multiplied to the Threshold value found automatically (can be any positive value). 1 is typically a good value. ”





OpenSegSPIM

Step 4: Detection

Detection

A boundary annotation is overlaid in red on the processed image. Click on **Detection** to open seeds detection parameter setting dialog. Click ok to process the seeds detection once the parameters value defined. This seeds detection process will produce reliable seeds shown as green dots as following figure.

Parameter Setting

Please input parameters for: Nuclei detection

Local intensity maxima detection:

Method: Intensity

Intensity based detection sensitivity [0-100] %: 95

Shape based detection sensitivity (~nuclei ray) : 1

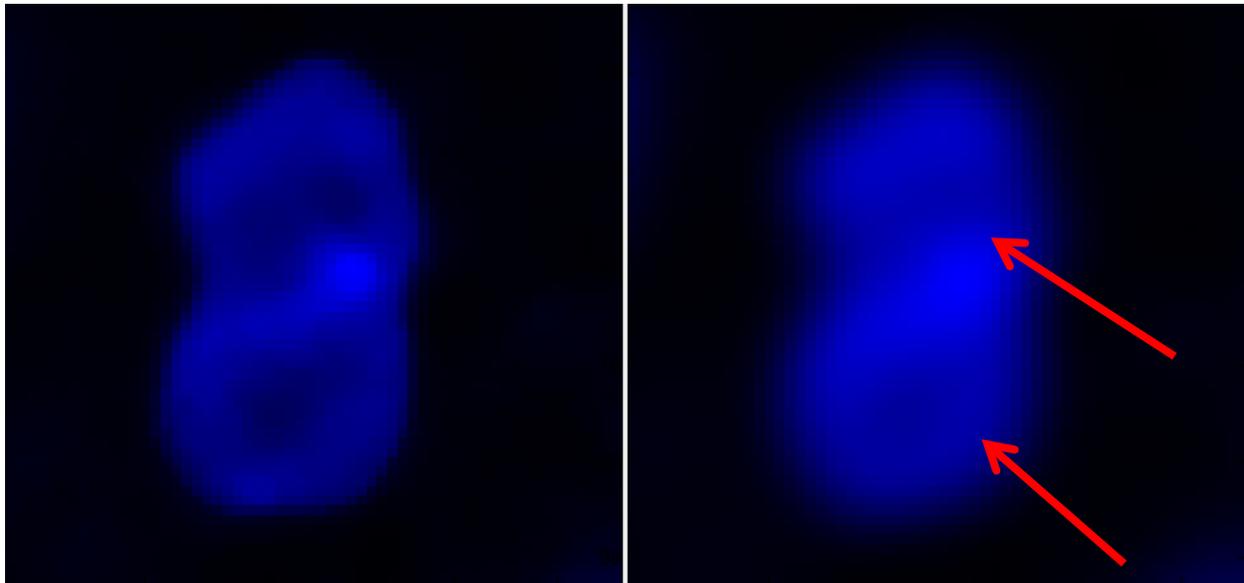
Loaded File	PreProcessing	Mask, seeds & segmentation	Statistics
Pathname F:\Research ... Smoothing	Gaussian	Mask Threshold c.	1
Filename 100714_N55... Contrast adju...	off	Seeds detection... Intensity	
xy resolution 0.6500 Window size=		11 Seeds sensitivity	10
z_res 1.5000 background r...		15 Number of Seeds	10
reducedsize 1 Low		0	
Enhanced L_high		1.0000	

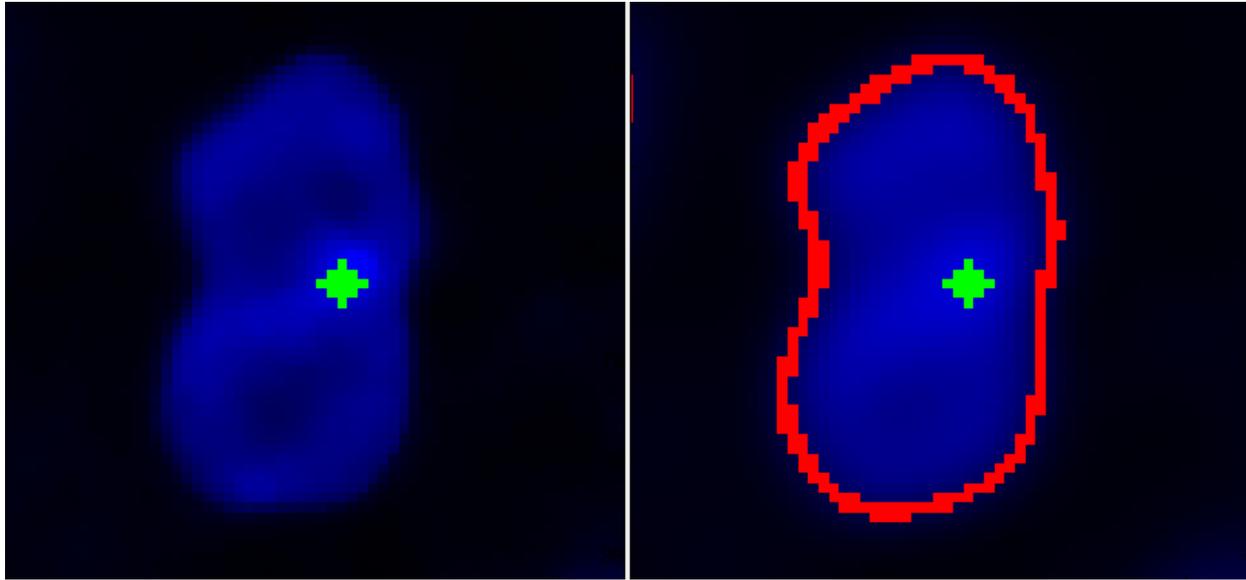
Number of seeds detected is specified in the bottom parameter table.



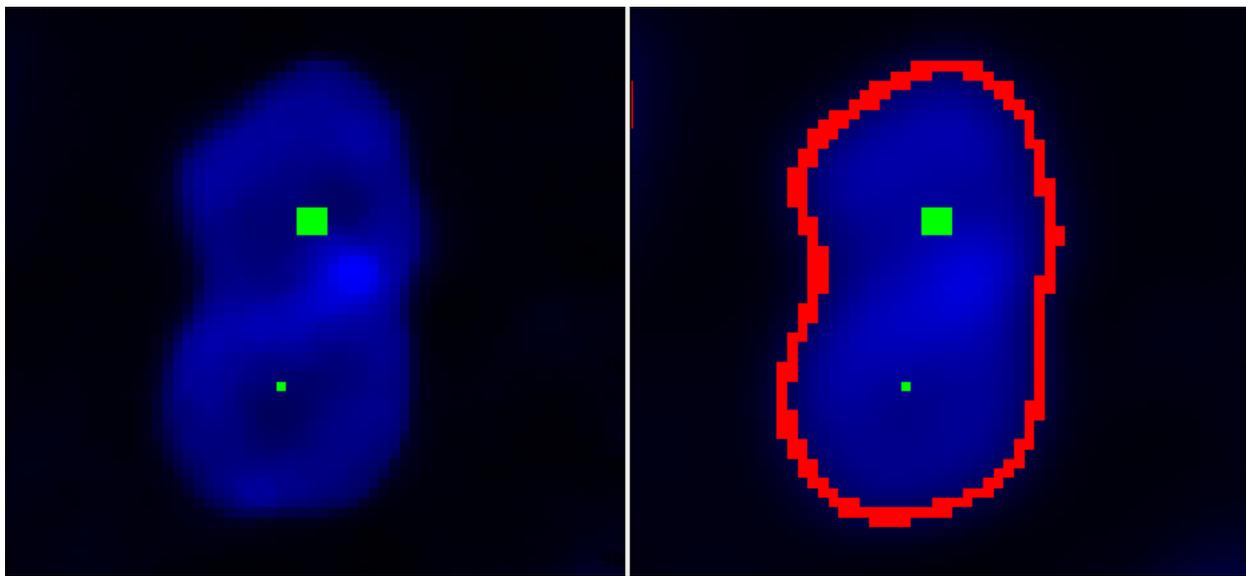
Tips: using intensity or shape is very depend to the images, if you find the nuclei with visible “hole” in the image as shown in following. This is because of nuclei stain is not distribute evenly. You should consider using shape instead of intensity. Visible “hole” in the object is because of low value of pixel intensity compare to high value of pixel intensity surrounding. Therefore, distance of shape is computed to finding the good seed during detection process.

*For example: Given image in following is containing two nucleuses, Two “holes” is visible after enhancement process as pointed by **red arrow**. In the detection process, choosing intensity might not appropriate seem it only can detect one seed but in fact, it is two nucleus in this case. User is advised to perform detection method based on shape if image in above as input. Following are the example of detection results based on shape detection*





Detection result based on *intensity* method that is consider wrong detection since there are two nucleuses.



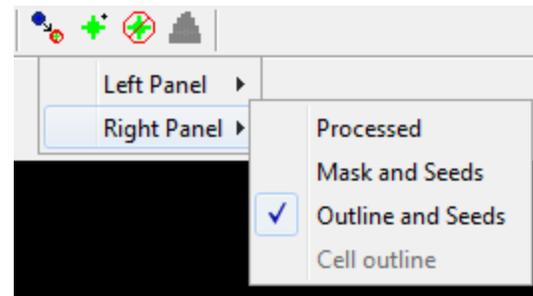
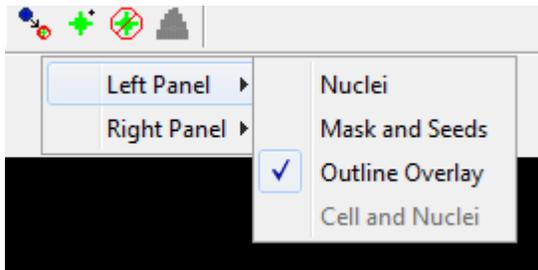
Detection result based on *shape* method.



OpenSegSPIM



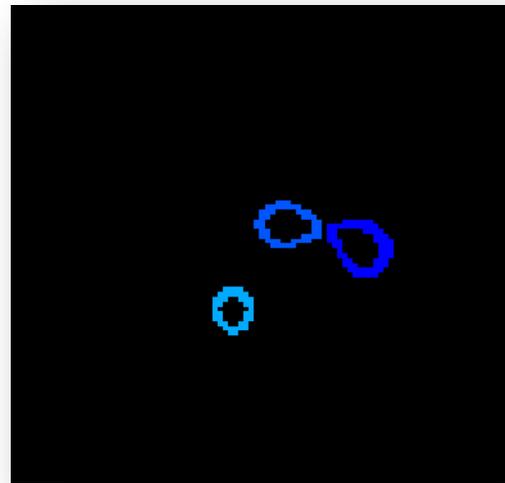
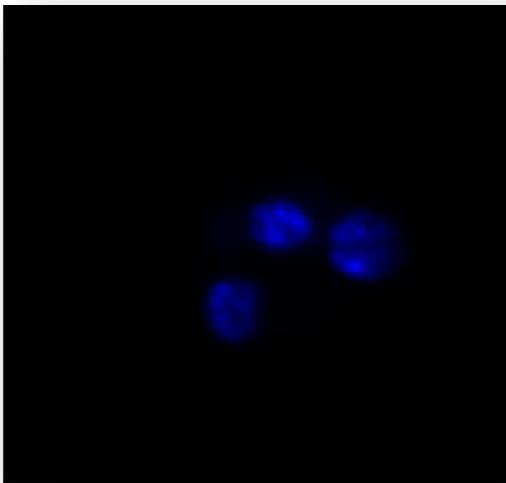
Tips: user can define the view panel based on their preferred display modes. Five different display modes are provided to users. They are included Nuclei, Processed foreground and seeds, Outline Overlay, Cell and Nuclei. Example of these modes provided in following:



*User can choose the display mode from the **change display**  from menu bar.*

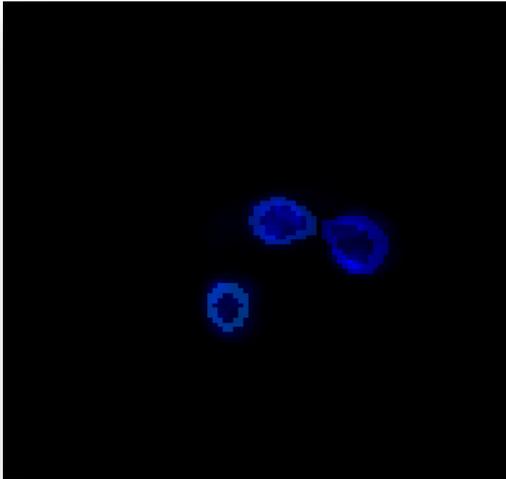
Left panel -> Nuclei

Right panel -> Processed

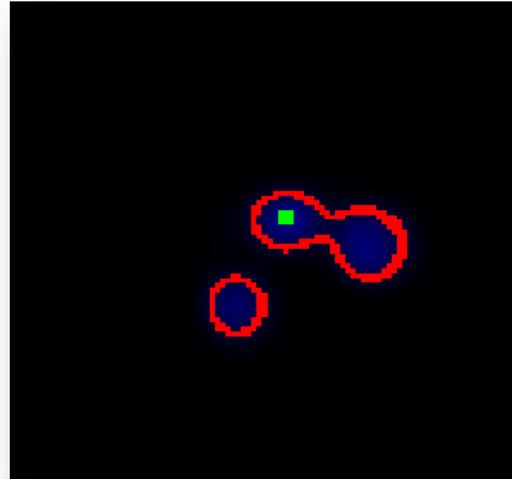




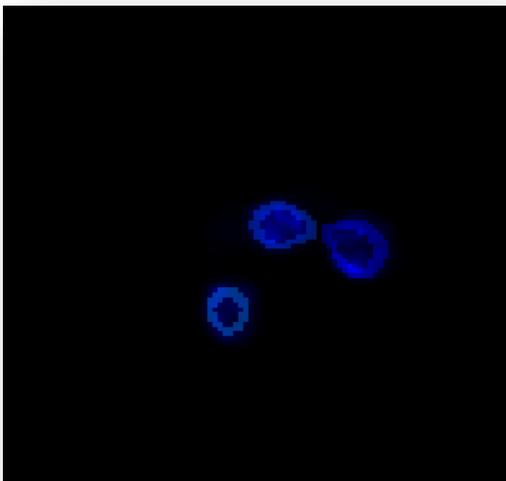
Left panel ->Mask and seeds



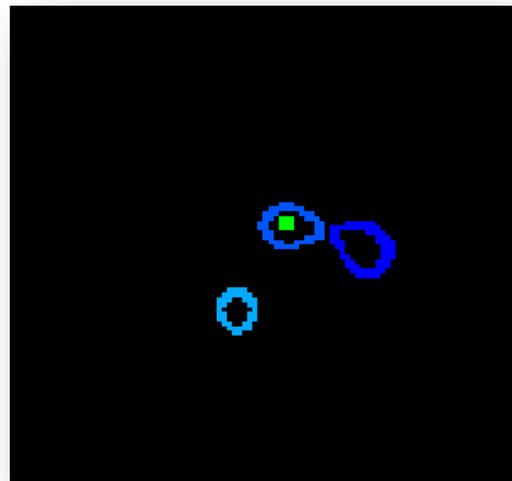
Right panel ->Mask and seeds



Left panel ->Outline overlay



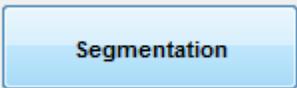
Right panel ->outline and seeds





OpenSegSPIM

Step 5: Segmentation



Click on the Segmentation button to prompt a dialog to select proffered approach (Watershed with intensity feature or shape distance feature) to segment the nucleus.

The screenshot shows the OpenSegSPIM interface. The 'Segmentation' button is highlighted with a green box. A 'Parameter Setting' dialog box is open, showing the 'Method' dropdown set to 'Shape'. A green arrow points from the dialog box back to the main interface.

Loaded File	PreProcessing	Mask, seeds	Statistics
Pathname	F:\Research\Smoothing	Gaussian	Mask segmentation
Filename	100714_N55... Contrast adju...	Mask Threshold c...	1
xy resolution	0.6500 Window size=	Seeds detection... Intensity	
z_res	1.5000 background r...	11 Seeds sensitivity	10
reducedsize	1_low	15 Number of Seeds	10
Enhanced	L_high	0 Watershed bas... Intensity	
		1.0000 Number of Nuclei	7

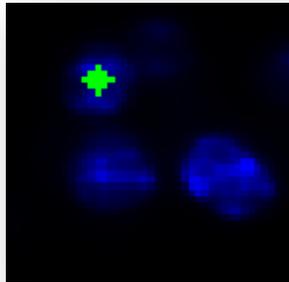


OpenSegSPIM



Tips: User can annotating the seed by using seed editing tool  (It is only available after **analysis process is complete**), in order to improve the quality of segmentation. It is strongly recommender user should base on statistical information (Step 6: Analysis) to add/remove the missing/unreliable seeds respectively. This is because missing/incorrect seeds easy to identify from the result list instead of detecting every slice images.

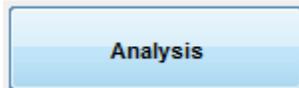
To add a new seed



Click and select  then click and locate at the nucleus that is suspected missing the seed (Please ensure this by sliding a few slice, z-position to ensure no seed is allocated during detection process). Click

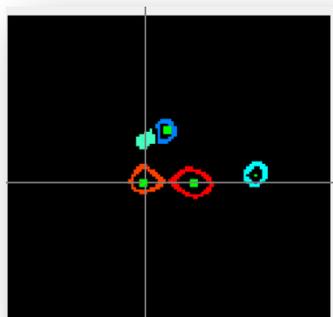


to re-run the results to segment the missing nucleus. Click on



to check the update results.

To remove a seed





OpenSegSPIM

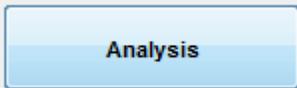
Click and select  then click and locate at the nucleus that is false positive. The green dot will be disappearing. User required performing segmentation process to remove selected entire nucleus by click

on . Click on  to check the update results.



OpenSegSPIM

Step 6: Analysis

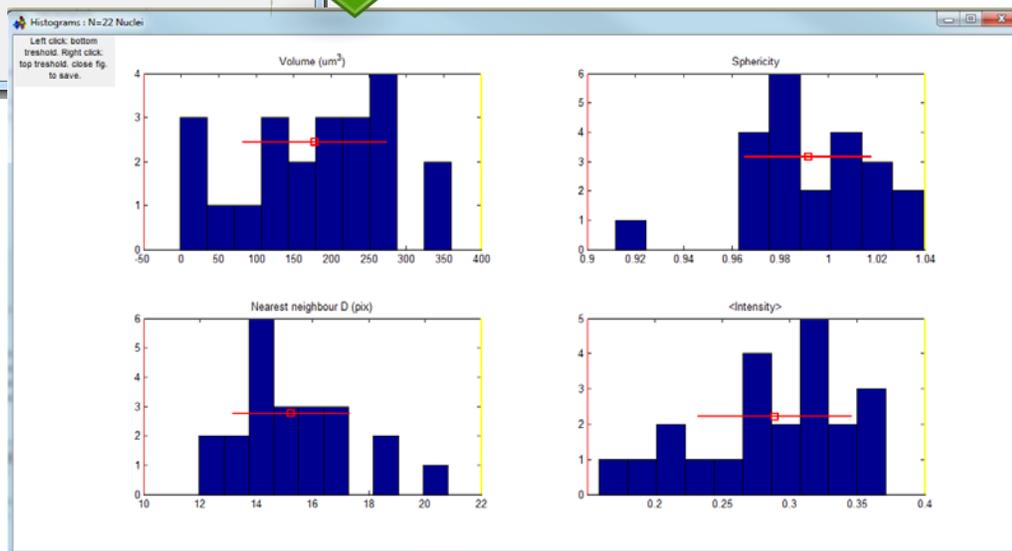


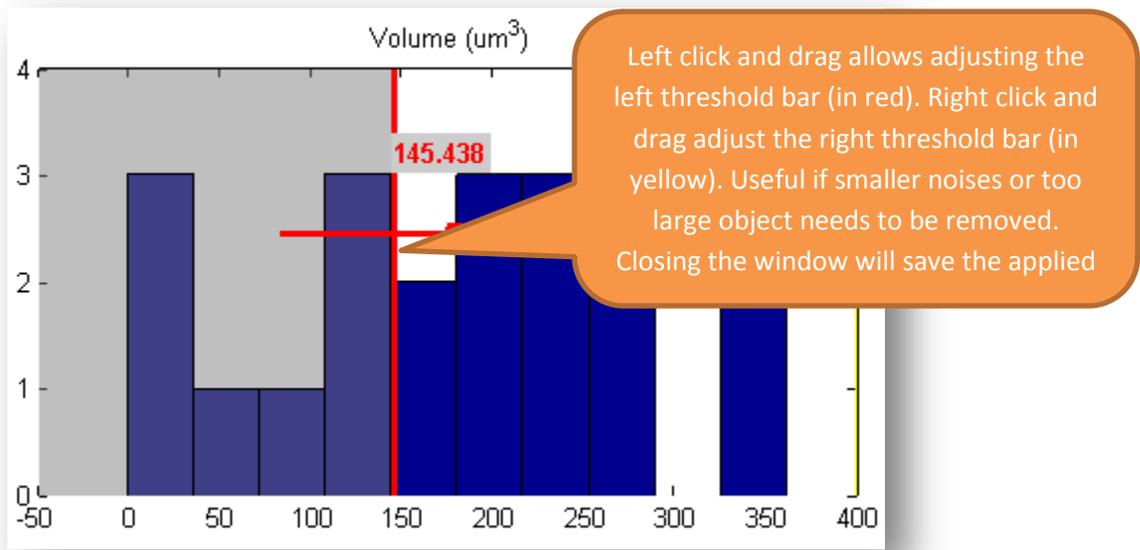
Click on the **Analysis** button to prompt a dialog to set the number of bins for histogram. Results can be sorted according to different preferred quantitative information. Histogram analysis is then enabling to let user to access for further analysis.

The main interface shows a segmentation workflow on the left, a central image view with two panels (original and segmented), and a statistics table at the bottom. A red box highlights the 'Analysis' button in the top toolbar. A green box highlights the statistics table.

Vol (um ³)	Sphericity	Nearest neighbor D (pix)	Intensity
1 191.1390	0.9610	14.2957	0.2437
2 199.9270	1.0190	21.4195	0.3350
3 211.1896	0.9378	14.2528	0.2385
4 235.6283	0.9688	17.0931	0.2571
5 268.3563	0.9109	17.0931	0.2433
6 340.8095	0.9501	14.2067	0.2791
7 109.3453	0.8562	15.3927	0.2508

Parameter Setting dialog box with the text: "Please input parameters for: Bin size". The "Set bin size:" field contains the value "20". An "OK" button is at the bottom.





Close the histogram dialog, you will notice that quantitative information (red rectangle) by volume is filter according to the threshold value **145.4** as shown in figure above.

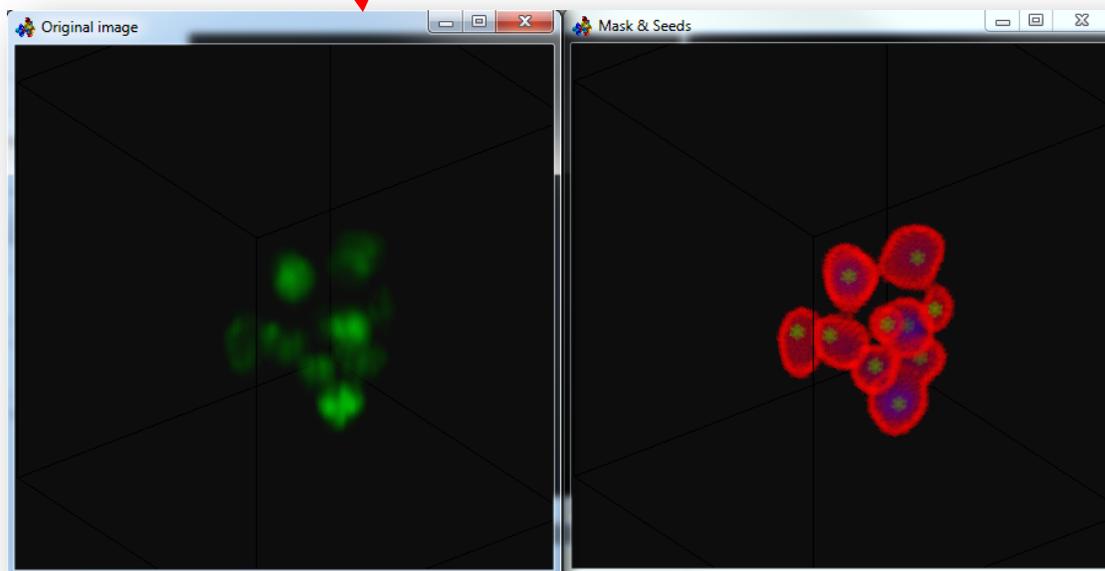
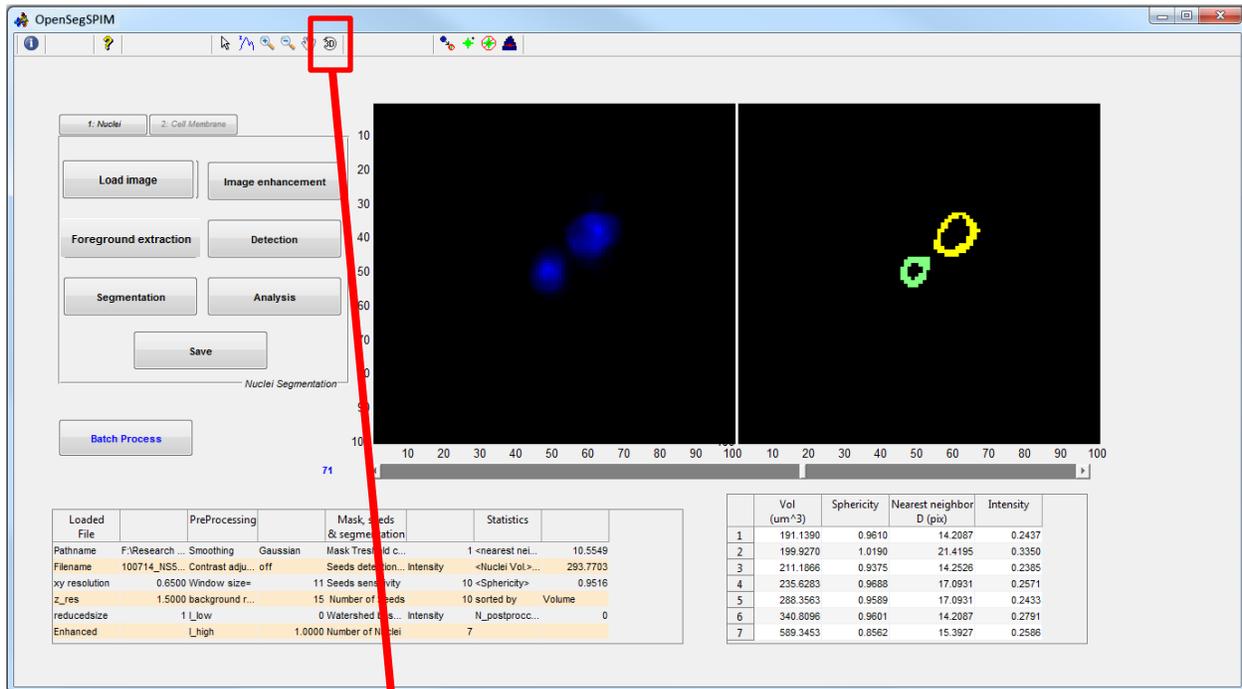
	Vol (μm^3)	Sphericity	Nearest neighbor D (pix)	Intensity
1	199.9270	1.0190	21.4195	0.3350
2	211.1866	0.9375	14.2526	0.2385
3	235.6283	0.9688	17.0931	0.2571
4	288.3563	0.9589	17.0931	0.2433
5	340.8096	0.9601	14.2087	0.2791



OpenSegSPIM

Step 7: 3D visualization

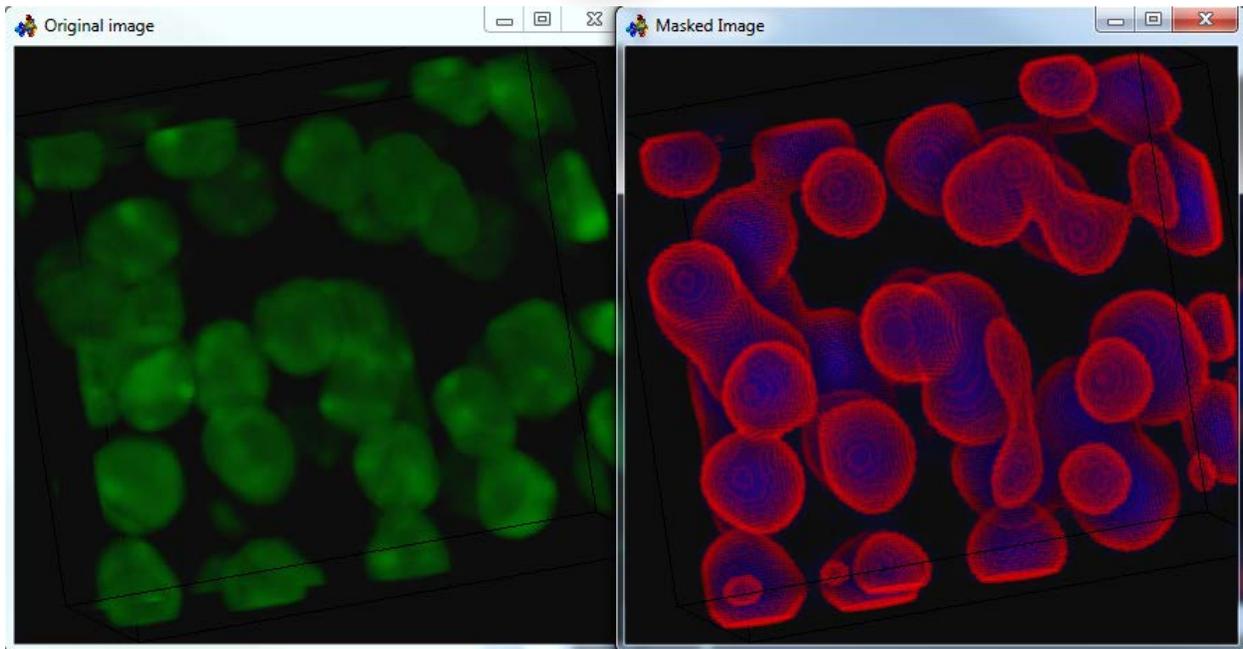
Click on  to perform 3D reconstruction and visualize the segmented cells in 3D space as shown in following:



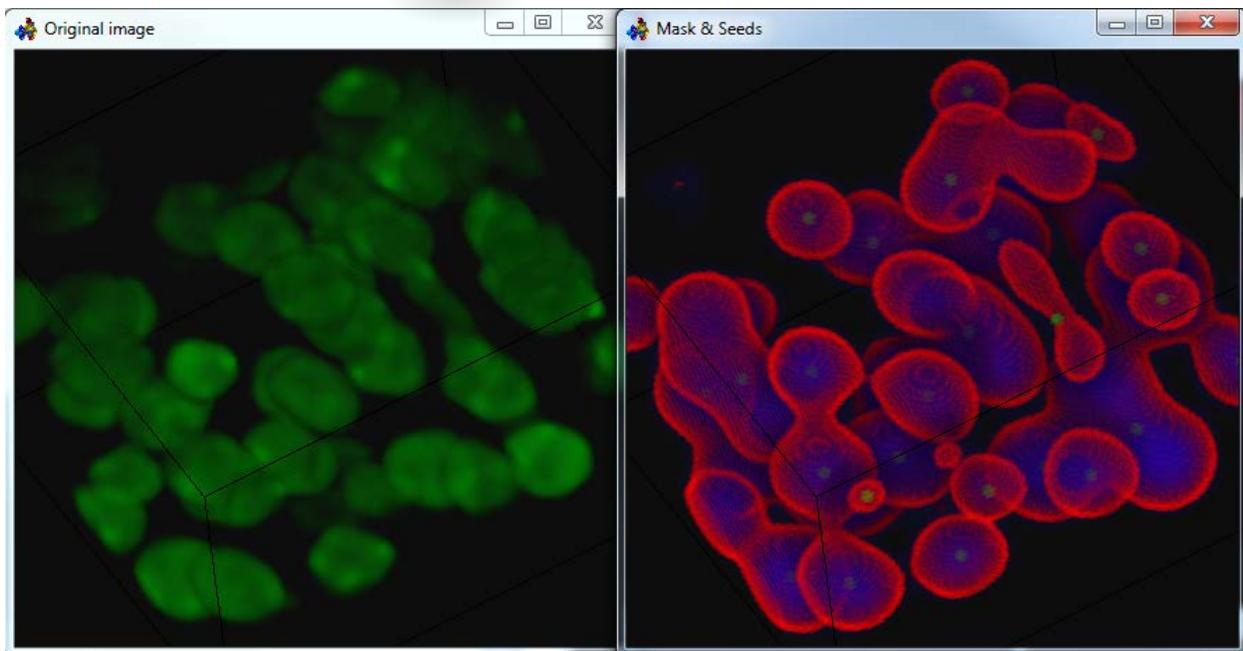


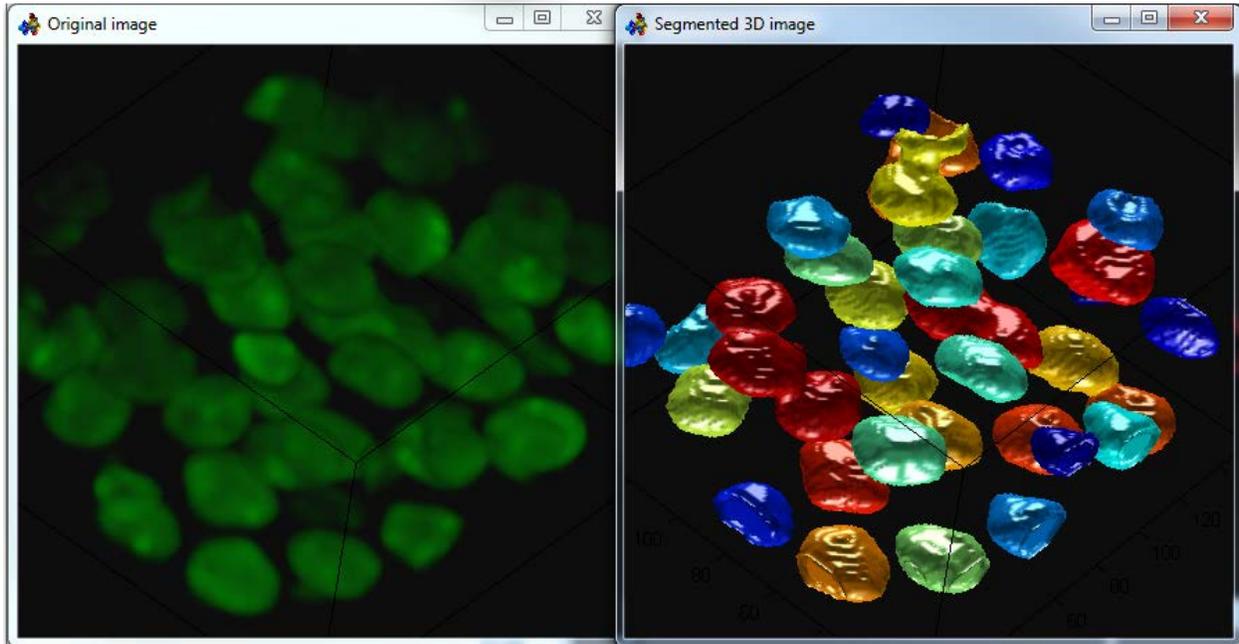
Tips: Visualize the 3D object in different processing state

After foreground extraction processing state



After detection processing state







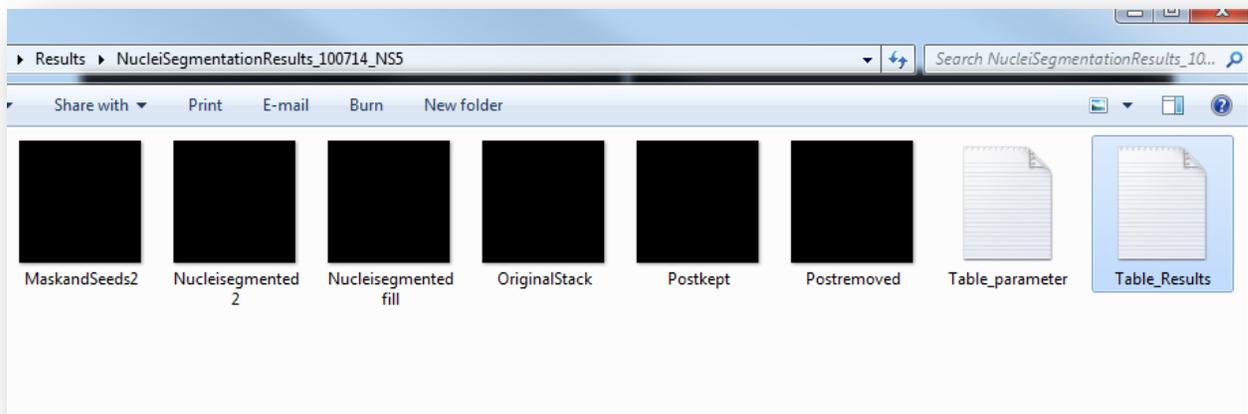
OpenSegSPIM

Step 8: Save (Export results)



Click on the , image of tiff format are exported and saved at the directory selected by users. The several saved files as listed in following:

- 2 text files containing the applied parameters and the measurements of each nucleus are saved.
- 1 original interpolated image.
- 1 smoothed image with overlaid mask and seeds
- 2 segmented labelled image (fill and outlines)
- 2 binary images that contained with filtered nucleus and non-filtered nucleus.



“ The computational quantitative result and configuration file will be **saved as a txt file under the same folder**. The name of the result file will be “Table_Results.txt” and their configuration file containing parameters setting named as” Table_parameter.txt” ”

	A	B	C	D	E	F	G	H
1	Volume (um ³)	Sphericity	Nearest neighbour D (pix)	<Intensity>	X	Y	Z	
2	1.3005	1.0164	34.515	0.31182	37.3333	136.2917	68.0417	
3	14.577	0.83555	22.3912	0.33801	150.9544	49.3485	8.3485	
4	16.4736	0.71744	30.1701	0.2495	61.7986	49.9618	8.3576	
5	17.0697	0.76213	13.5599	0.24316	150.0068	90.5171	68.5788	



Tips: x, y, z coordinate value is indicate the centroid of each nucleus that only saved in text files.



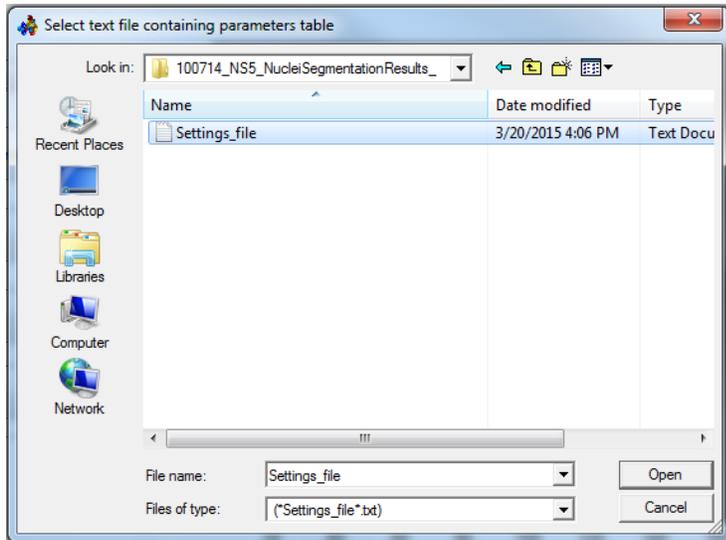
OpenSegSPIM

Step 9: Batch processing

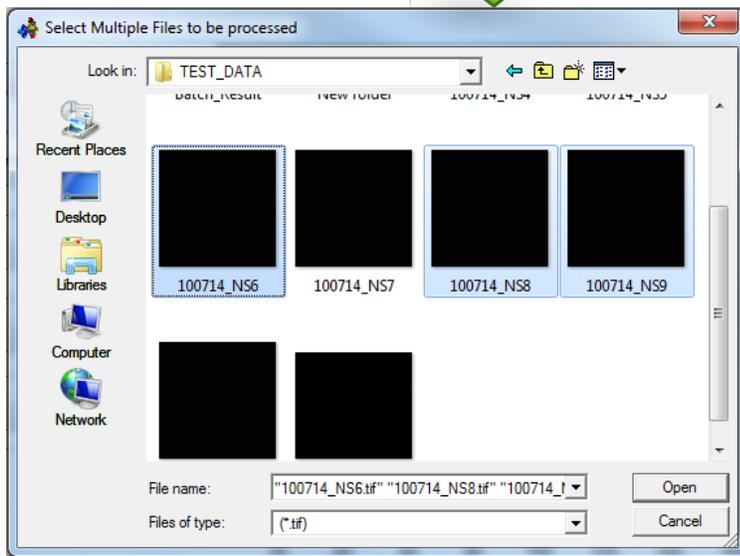


Click on **Batch Process** button to select the configuration file and stack images need to perform the quantitative analysis.

Configuration file



Stack images





OpenSegSPIM

Process is execute

The execute batch process button show the number of processed image and total image to be processed

processing stack:3/5

Loaded File	Enhancement
Pathname	F:\Research Pr... Smoothing Gaussian
Filename	100714_NS7.tif Contrast adjust... Off
xy resolution	0.6500 Window size= 13
z_res	1.5000 background re... 19
reducedsize	1_low 0
Enhanced	L_high 1.0000

Process is complete

Once all stack images were done. The execute batch process button will turn into

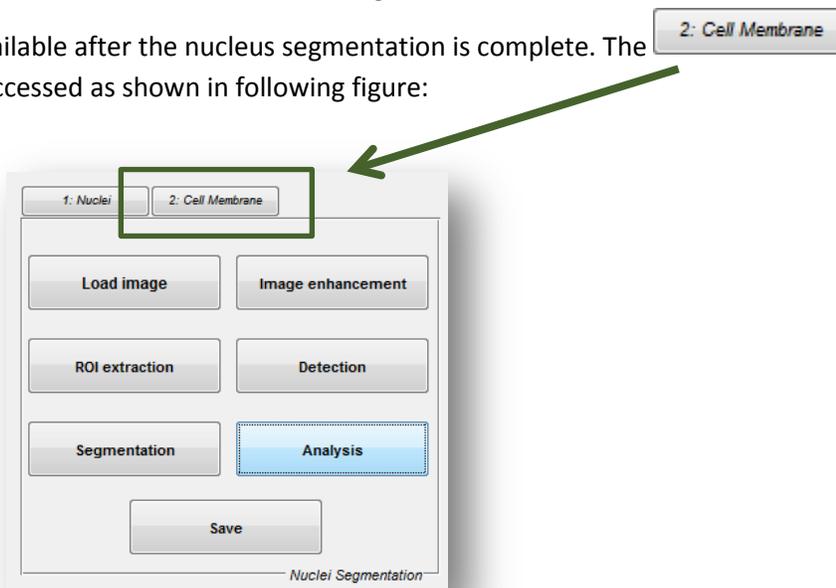
Batch run completed!

Loaded File	Enhancement	Detection & Segmentation
Pathname	F:\Research Pr... Smoothing Gaussian	Mask Threshold coef
Filename	100714_NS9.tif Contrast adjust... Off	Seeds detection M... Inten
xy resolution	0.6500 Window size=	13 Seeds sensitivity
z_res	1.5000 background re...	19 Number of Seeds
reducedsize	1_low	0 Watershed based ... Inten
Enhanced	L_high	1.0000 Number of nuclei



Cell membranes segmentation

OpenSegSPIM provide additional features to do membranes segmentation if the membrane cell data is available. This function only available after the nucleus segmentation is complete. The button will be available to be accessed as shown in following figure:



Click on **2: Cell Membrane** tab, following interface will be changed accordingly:



Click on the **Segmentation** button to load and select the membrane cell stack images as shown in following figure:



Parameter Setting

Please input parameters for: Cell stack error!

Error! Cell membrane channel not found.

Try to load another file containing the membrane stack ?

yes

OK Cancel

Pick image data

Look in: TEST_DATA

100714_N54 100714_N55 100714_N56 100714_N57 100714_N58 100714_N59

100714_N60 C4-dapi C4-dapi-1TEST Original3D_9_cell... Original3D_9_nuc... (green)

Select the membranes stack images

Parameter Setting

Please input parameters for: Cell Segmentation

Set threshold level:

Global threshold level (~0.1): 0.1

OK Cancel

Execute cell segmentation with thresholding method

Once the cell segmentation is done, results of segmented cell will be displayed on the right visualization viewer.

OpenSegSPIM

1. Nuclei 2. Cell Membrane

Segmentation Analysis

Save

Cell Membrane Segmentation

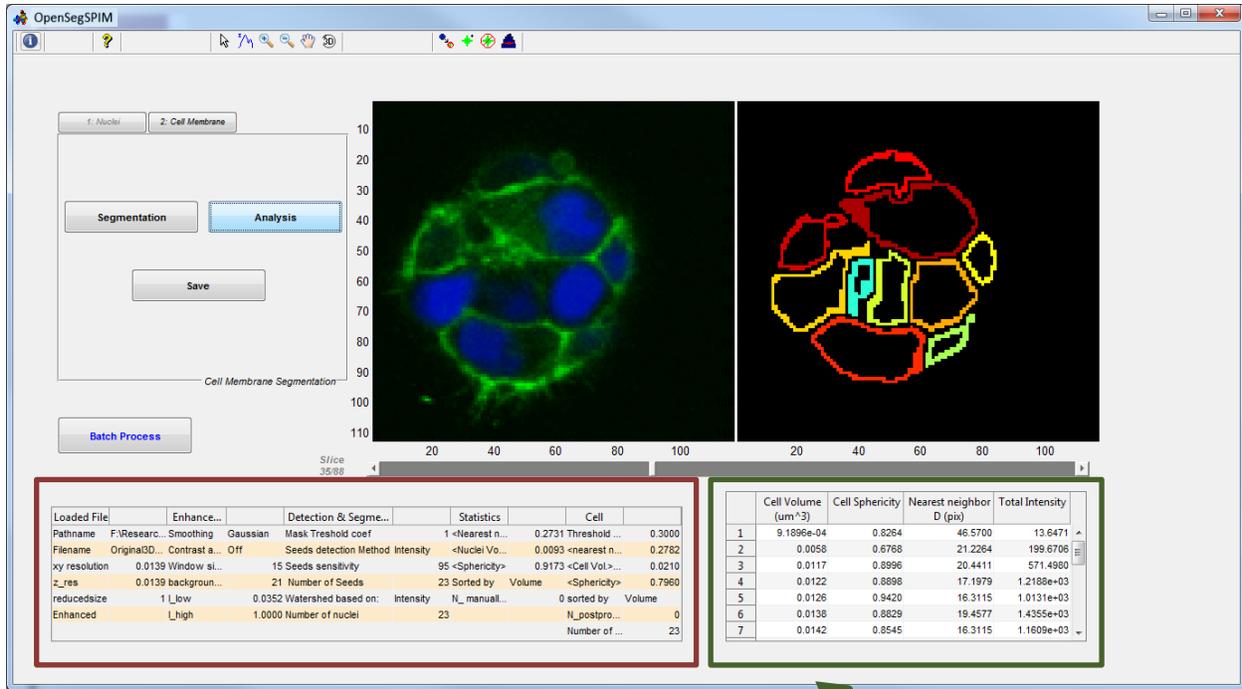
Batch Process

Loaded File	Enhance...	Detection & Segme...	Statistics	Cell	
Pathname	F:\Researc... Smoothing	Gaussian	Mask Threshold coef	1 <clearst n...	0.2731 Threshold ...
Filename	Original3D_... Contrast s...	Off	Seeds detection Method Intensity	<Nuclei Va...	0.0093
xy resolution	0.0139 Window si...	15	Seeds sensitivity	95 <Sphericity>	0.9173
z_res	0.0139 backgroun...	21	Number of Seeds	23 Sorted by	Volume
reducedsize	1 L_low	0.0352	Watershed based on:	Intensity	N_manuall...
Enhanced	L_high	1.0000	Number of nuclei	23	



Analysis

Click on **Analysis** to extract quantitative information from cell as shown in green rectangle in following figure:



Save

The **Save** button is similar to nucleus segmentation, it allow user to export and save the binary image and results in text file. The files including:

- Cell_measurement_results.txt – Quantitative results from cell.
- Cellsegmentedfill.tiff – labeled grayscale images that containing cell objects.
- CellSegments.tiff – labeled grayscale images that containing boundary of cell object.
- MaskandSeeds2.tiff – labeled color images that containing cell and seed object.
- OriginalStack.tiff – Original stack images.
- Settings_file.txt – a text file that store the information as shown in red rectangle.



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