

# **3D Cell Explorers**

**STEVE** Refractive Index maps and Data Processing



**Exporting data from STEVE for Data Processing** 

### **General Objective**

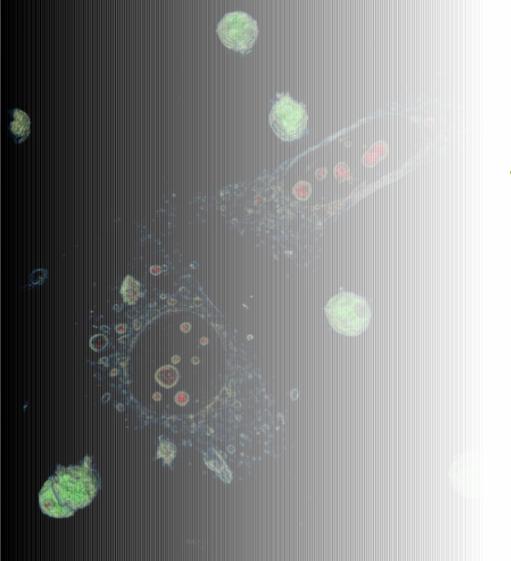
The goal of this document is to present simple ways to export Refractive Index data generated by the 3D Cell Explorer (STEVE) into Excel to perform data calculations. These methods can be extended to any other scientific application and will allow you to work in a quantitative way on the Refractive Index data distribution maps generated by STEVE.

In this document, we are using Fiji (ImageJ), an open source platform to process the RI information. We are presenting only few Fiji basic commands that help in this process, but many other alternatives can be defined with more advanced tools.

The data sets used in these procedures are for illustration only, meaning that the RI information provided are just for information.

Nanolive is actively developing more advanced technics to process RI datasets. Please do not hesitate to contact us for more advanced needs.





# **3D Cell Explorers**

## 1. Tool Installation

- 2. General Workflow
- 3. Create a colour map
- 4. Advanced Operations
- 5. Correlate Refractive Index and Fluorescence information



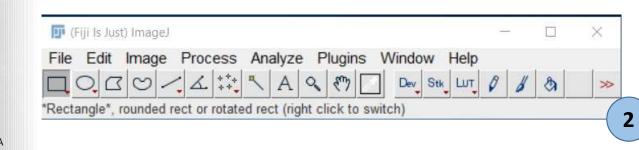
# How to export tomographic RI data for data processing ?

**Tools & Installation** 

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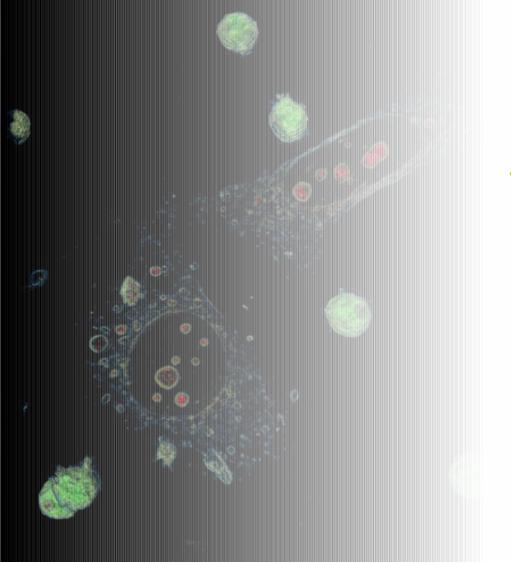
Install Fiji (ImageJ). This open source software package can be found here: <u>https://imagej.net/Fiji/Downloads</u> (1)

Once installed, when Fiji is launched a tool bar is visible on the PC desktop (2).



Slide /4





# **3D Cell Explorers**

- 1. Tool Installation
- 2. General Workflow
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# STEVE

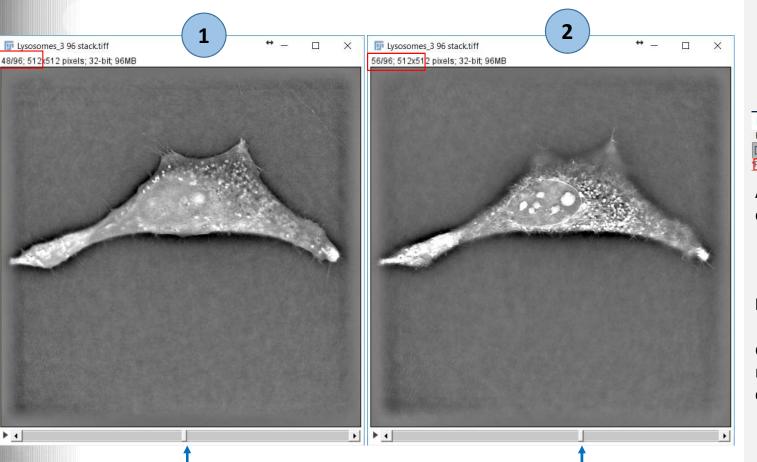
#### **Export Data in Tiff**

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Nanolive SA 2014-2017			

From STEVE, **Export** the RI data in a .tiff format. Either use the export button or the File> Export command. In the Export dialog box : Select RI Volume Select Tiff format Select a folder and define a filename Click OK

Note: the exported Tiff file is made of 96 stacked layers, one layer per STEVE tomographic slice. STEVE layers are numbered from -47 to +48. Tiff layers are numbered from 0 to 96.





Import a Tiff file for scientific analysis

In Fiji, **Open** the exported .tiff file with the File> Open command.

Or drag and drop the file directly in the box below the Fiji tool bar (red rectangle below)

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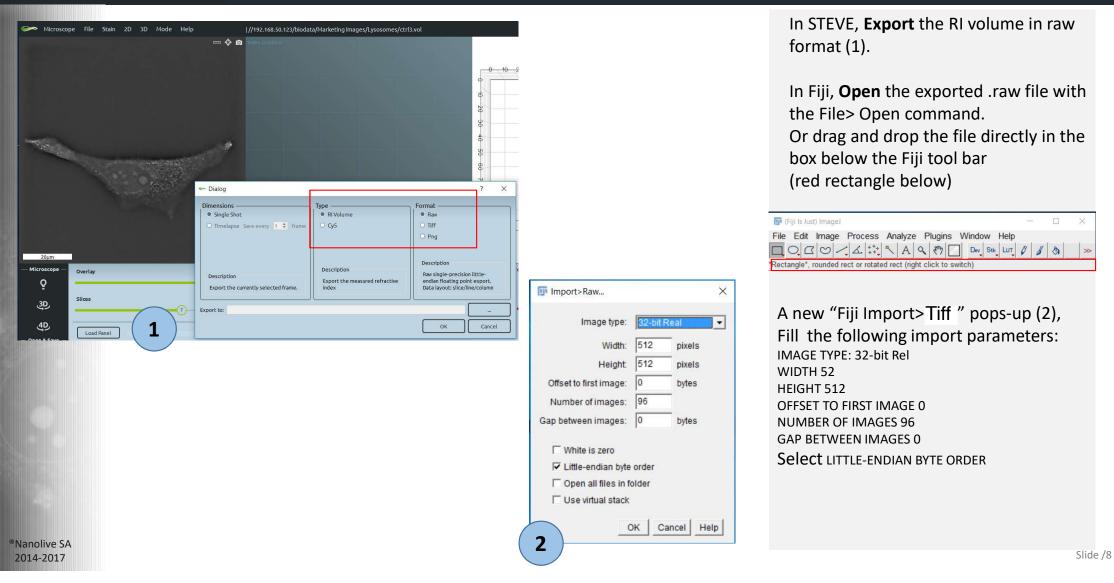
A new Fiji window displays the first layer of the TIFF file (1)

**Note:** moving the slider at the bottom of the window allows to visualize the different layers in the TIFF file, the layer reference is displayed in the upper left corner, here layers 48 and layer 56.



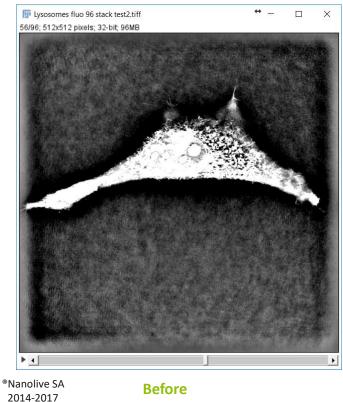
### Export/Import a raw file (volume) for scientific analysis

Fiji





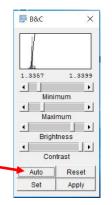
This operation allows to improve the visualization in term of Brightness and Contrast by adjusting the image histogram. Please note that this operation do not modify the RI values inside the image, it makes the image just looking better.

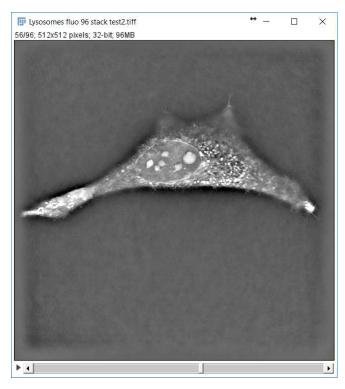


1. Run the command Image > Adjust > Brightness/Contrast

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	Overlay	,	Auto Crop		
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2. A window B&C appears, then click on "Auto". Do **NOT** click on "Apply" that will modify the data in the image.

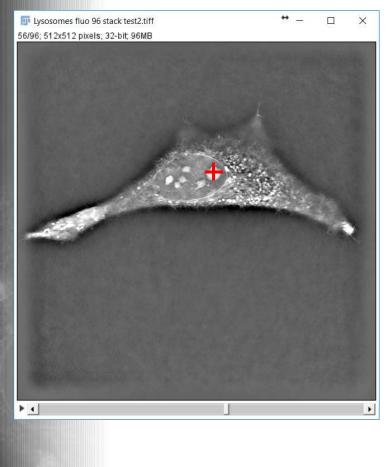




After

Slide /9



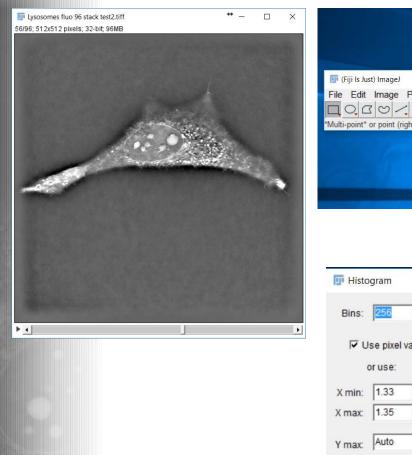


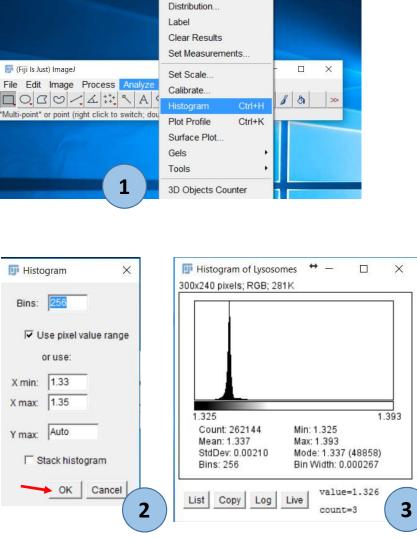
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The Refractive Index values can be visualized by **moving** the mouse cursor over the image. The RI value is displayed in the display

window of the Fiji tool bar (see red rectangle)







### Fiji Display RI Data <u>Histogram</u>

**Run** the command Analyze > Histogram (1)

A pop-up window histogram is showing up (2) to adjust histogram settings (bins, min, max...).

Keep default values and click on "OK", the histogram is displayed (3).

**Note1**: the histogram is calculated based on the RI values of the current layer, the layer displayed and active (here 56).

**Note2**: the focus plane is often defined in STEVE as the slice number 0 corresponding to the Tiff layer 48. Please consider that based on your experiment and samples, Tiff layers below 48 might be not relevant in term of data analysis.

<sup>®</sup>Nanolive SA 2014-2017



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X

Histogram of Lysosomes

### Fiji Import RI Data histogram in Excel

#### 300x240 pixels; RGB; 281K 1.393 1.325 Count: 262144 Min: 1.325 Mean: 1.337 Max: 1.393 StdDev: 0.00210 Mode: 1.337 (48858) Bin Width: 0.000267 Bins: 256 value=1.326 List Copy Log Live 1 count=3 Histogram of Lysosomes X 300x240 pixels; RGB; 281K ++ \_\_\_\_ F Histogram of Lysosomes X File Edit Font bin start count 1.325 1.325 Count 262144 1.325 1 Mean: 1.337 StdDev: 0.00210 1.326 2 Bins: 256 1.326 2 1.326 3 List Copy Log 1.326 2 5 .327 13 1.327 1.327 15 327 13 2

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25	1.331	208											- 5
26	1.331	267											

From the histogram window, click on List (1).

A pop-up window is showing up (2) with all the RI data values.

Save the RI values distribution in a text file, by running the File > "Save as" command. And import the text file in Excel.

Or directly click in the Histogram window, use the keyboard shortcut CTRL-A to select all RI values, then copy (shortcut CTRL-C) and paste directly in an Excel spreadsheet (shortcut CTRL-V).

Then create the suitable graph for your application (3)

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The process to import a RI histogram in Excel has been described in Slide 12.

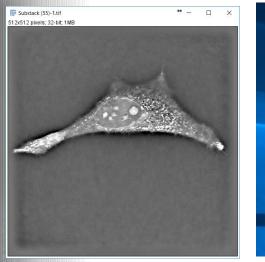
If it is needed to import all the RI values of a Tiff image in Excel, we provide two alternative methods:

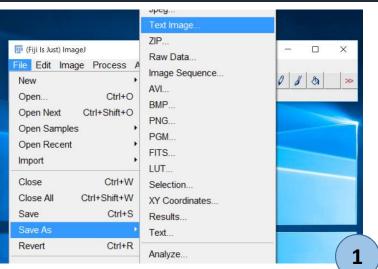
The first one, slide 14, is the simplest. It allows to export the file in text format and to copy it to Excel. With this method RI data are rounded during the text export.

The second method, slides 15,16,17, is an evolution of the first one and avoid this rounding effect, so you can get the exact values as they are in the RI file.



### Fiji Export RI Data to Excel (rounded method)





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7	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.
337	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.
1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.
7	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.
337	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.
1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.
7	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.
337	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.
1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.
7	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.
337	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.337	1.
1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.
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4	2	1.338	1.337	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.33
5	3	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.33
6	4	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.33
7	5	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.33
8	6	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.33
9	7	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.33
10	8	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.33
11	9	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.33
12	10	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	Y
13	11	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	1.338	(

Load a Tiff file, **Run** the command File > Save as > Text Image (1) and save the file.

**Open** the text file, select all data (shortcut CTRL-A), copy (CTRL-C) and paste (CTRL-V) the text in Excel (3).

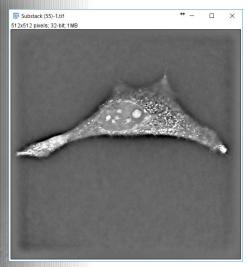
**Note1**: please note that the X,Y coordinates in the image in the upper left Corner and start at 0,0.

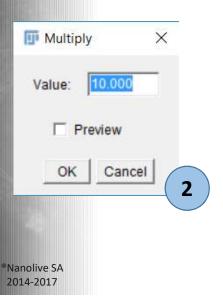
**Note2**: the RI values are rounded when they are saved in text format, meaning that the value 1.3375 will be changed in 1.338. To avoid this, if needed, use the alternative Not rounded method.



#### Export RI Data to Excel (NOT rounded method) Part 1 of 3

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When loading a Tiff file and trying to export it as a text file, RI values are rounded and truncated to 3 digits after the coma.

The way to avoid this is to multiply the RI values of the image by 10 before the Export and then to divide them by 10 in Excel.

Open the Tiff, image, **Run** the command Process> Math > Multiply (1).

In the pop-up window, insert the value 10 , then click OK (2)

The image becomes white, not visible, as all RI values are now multiplied by 10



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## Export RI Data to Excel (NOT rounded method) Part 2 of 3

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69	13.369	13.370	13.369	13.370	13.370
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3	13.376	13.375	13.375	13.376	13.375	13.376	13.376	13.376
4	13.377	13.376	13.376	13.377	13.376	13.377	13.377	13.377
5	13.377	13.376	13.376	13.377	13.376	13.377	13.377	13.377
5	13.376	13.376	13.376	13.377	13.376	13.377	13.377	13.377
7	13.377	13.376	13.377	13.377	13.377	13.377	13.377	13.378
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From theTiff file with RI values multiplied by 10, **Run** the command File > Save as > Text Image (1) and save the file.

**Open** the text file, select all data (shortcut CTRL-A), copy (CTRL-C) and paste (CTRL-V) the text in Excel (2).

**Note1**: please note that the X,Y coordinates in the image in the upper left Corner and start at 0,0.

**Note2**: RI values are now in the range of 13.xxx as they have been multiplied by 10.



#### Export RI Data to Excel (NOT rounded method) Part 3 of 3

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9	13.379	13.378	13.378	13.378	13.377	13	.378	13.377	13.376	13.376	13.375
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1	13.38	13.378	13.378	13.379	13.378	13	.378	13.377	13.377	13.37	
2	13.38	13.378	13.379	13.38	13.378	13	.378	13.378	13.377	13.37	2
3	13.38	13.378	13.379	13.379	13.378	13	.378	13.378	13.376	13.376	
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3	1	1.3375	1.3374	1.3375	1.3375	1.3376	1.3376	1.3376	1.3376
4	2	1.3376	1.3375	1.3375	1.3376	1.3375	1.3376	1.3376	1.3376
5	3	1.3377	1.3376	1.3376	1.3377	1.3376	1.3377	1.3377	1.3377
6	4	1.3377	1.3376	1.3376	1.3377	1.3376	1.3377	1.3377	1.3377
7	5	1.3376	1.3376	1.3376	1.3377	1.3376	1.3377	1.3377	1.3377
8	6	1.3377	1.3376	1.3377	1.3377	1.3377	1.3377	1.3377	1.3378
9	7	1.3377	1.3376	1.3378	1.3377	1.3376	1.3377	1.3376	1.3376
10	8	1.3378	1.3377	1.3377	1.3378	1.3377	1.3377	1.3377	1.3376
11	9	1.3378	1.3377	1.3377	1.3378	1.3377	1.3377	1.3376	1.3377
12	10	1.3378	1.3377	1.3378	1.3378	1.3377	1.3378	1.3377	1.3377
13	11	1.3378	1.3377	1.3377	1.3378	1.3377	1.3377	1.3377	1.3377
14	12	1.3378	1.3378	1.3377	1.3377	1.3377	1.3377	1.3377	1,7
15	13	1.3379	1.3378	1.3378	1.3378	1.3377	1.3377	1.3377	1 1
16	14	1.3379	1.3377	1.3378	1.3378	1.3377	1.3377	1.3377	1
17	15	1.3378	1.3377	1.3378	1.3378	1.3377	1.3378	1.3377	1.3

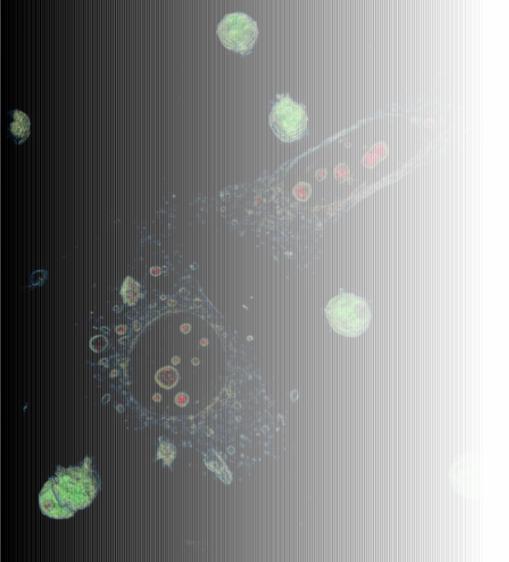
In Excel, in a blank cell, enter the value 10 (1) and copy it in the clipboard (CTRL-C).

Select all your RI values and open the menu with a right click on the mouse and "Select Paste special". The paste special options are displayed. Select "Divide" (2)

Click on OK, then all the selected values are divided by the value stored in the Clipboard, here the value 10 (3).

**Note**: This method can be used also when exporting an histogram.





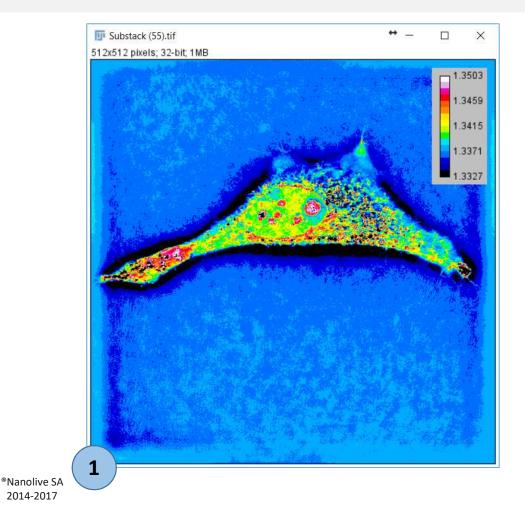
# **3D Cell Explorers**

- 1. Tool Installation
- 2. General Workflow
- 3. Create a colour map
- 4. Advanced Operations
- 5. Correlate Refractive Index and Fluorescence information



## Fiji Intensity / Colour map distribution

This operation allows to create a coloured RI distribution map with a colour scale. This helps to quickly visualize the RI distribution.



🗊 Calibration Bar	×
Location:	Upper Right 💌
Fill color:	Light Gray 💌
Label color:	Black 🔻
Number of labels:	5
Decimal places:	4
Font size:	12
Zoom factor:	1.0
Bold text	Overlay
2	OK Cancel

In Fiji, Load a tiff image in grey scale.

Adjust the thresholds to remove unwanted values if necessary (here values below 1.33), Run Image > Adjust > threshold.

**Transform** the gray scale image in colour map by applying a LUT, Run Image > Lookup tables > 16 colours (1)

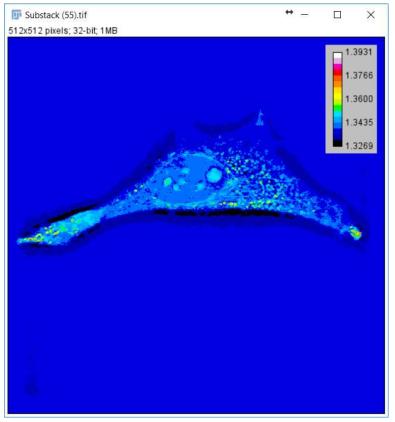
Add the values scale in the upper right corner, Run Analyse > tools > calibration bar. In the pop-up window, enter the value 4 for the number of decimal places (2)

Slide /19

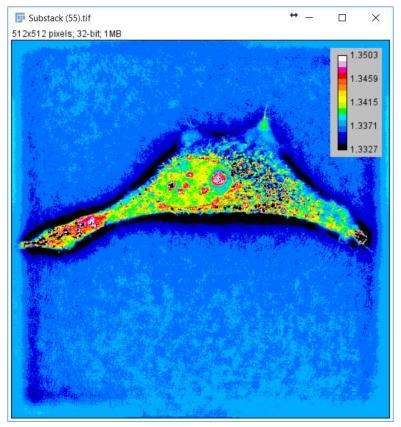


## Fiji Examples of colour maps distribution

By adjusting the threshold values, the range of RI displayed is modified and images are looking differently. Below, two examples of the same image with two different ranges.



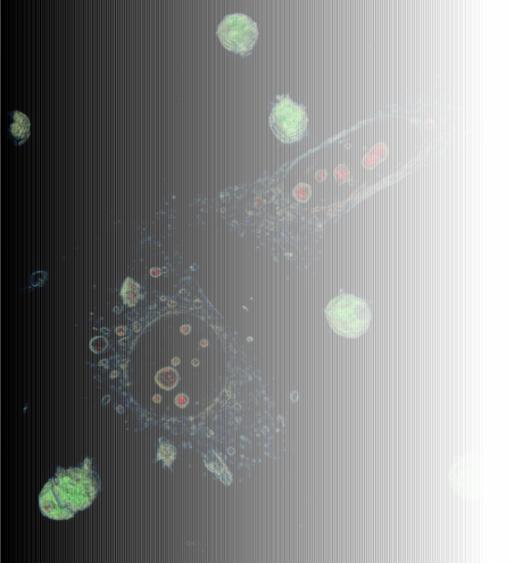
Thresholding done with max value: 1.39 and a full range.



Thresholding done with max value: 1.35 and a reduced RI range

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# **3D Cell Explorers**

- 1. Tool Installation
- 2. General Workflow
- 3. Create a colour map

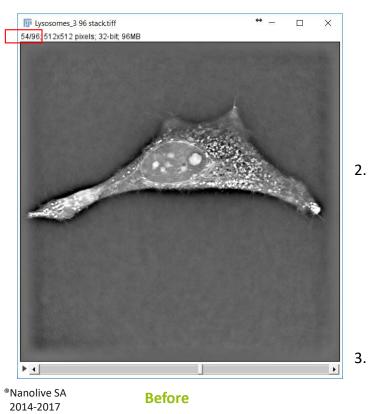
## 4. Advanced Operations

5. Correlate Refractive Index and Fluorescence information

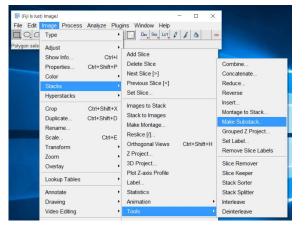


### Fiji Extract one layer from the Exported Tiff file

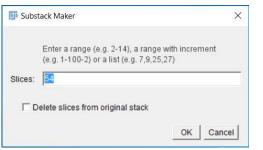
This operation allows to extract one layer from the exported Tiff file that contains 96 layers and to save it as an independent file.



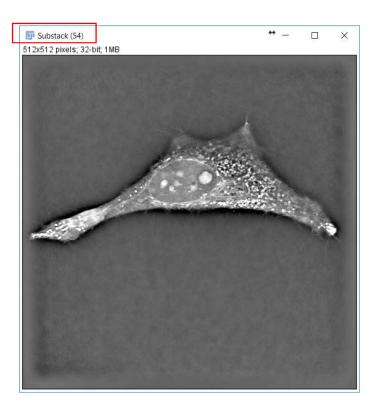
1. Run the command Image > Stacks > Tools > Make Substack



2. A window "Substack Maker" appears, then indicate your layer reference (here 54) and click OK



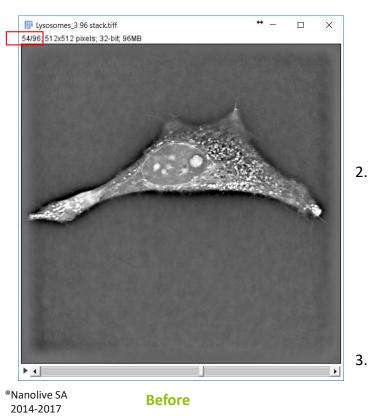
A new window pops-up with the name Substack(xx), here xx = 54. Save the file by File > Save as > Tiff. The new file contains now only 1 layer.



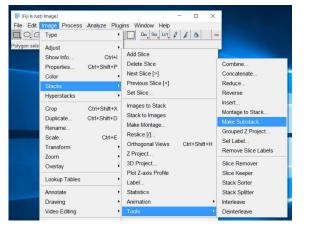
Slide /22



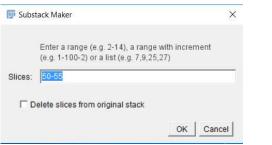
This operation allows to extract several layers of interest from the exported Tiff file that contains 96 layers and to save this new stack of layers as an independent file.



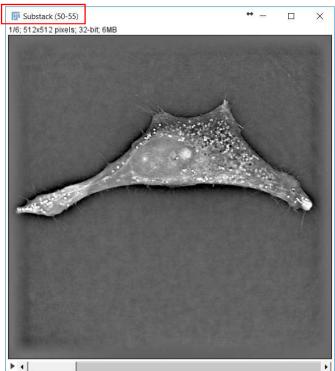
1. Run the command Image > Stacks > Tools > Make Substack



2. A window "Substack Maker" appears, then indicate your layer reference (here 50-55) and click OK



A new window pops-up with the name Substack(xx), here xx = 50-55. Save the file by File > Save as > Tiff. The new file contains now only 6 layers.

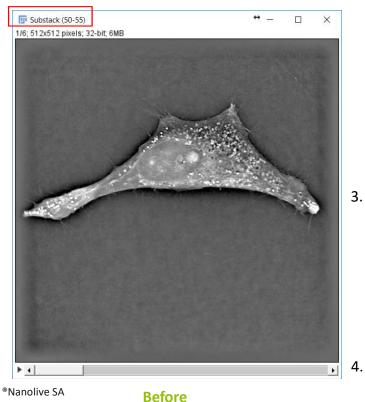




This operation allows to create a synthetic image by combining RI information contained in a set of tomographic layers .

📴 (Fiji Is Just) ImageJ

- 1. Create a substack with the selected tomographic layers.
- 2. Run the command Image > Stacks > Z Project



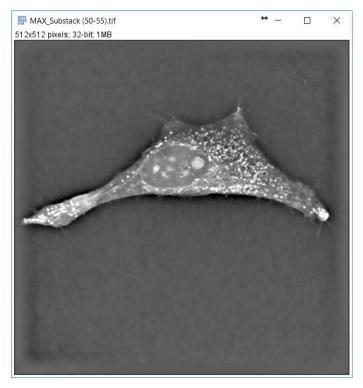
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File Edit Image Process Analyze Plugins Window Help О.С. Туре Dev Stk LUT 0 8 3 Fiji Is Just) li [64-bit] Adjust Show Info Ctrl+ Add Slice Properties. Ctrl+Shift+P Delete Slice Color Next Slice [>] Previous Slice [<] Hyperstacks Set Slice. Ctrl+Shift+X Crop Images to Stack Duplicate. Ctrl+Shift+D Stack to Images Rename Make Montage Scale.. Ctrl+E Reslice [/] .. Transform Orthogonal Views Ctrl+Shift+H Zoom Overlay 3D Project.

3. A window "ZProjection" appears, then select the projection method (here : Max Intensity)

Trojection	×
Start slice: Stop slice:	6
Projection type	Max Intensity
	OK Cancel

A new image MAX\_Substack (50-55) is created. Adjust the Brightness/Contrast for a better visibility. Save the file by File > Save as > Tiff.



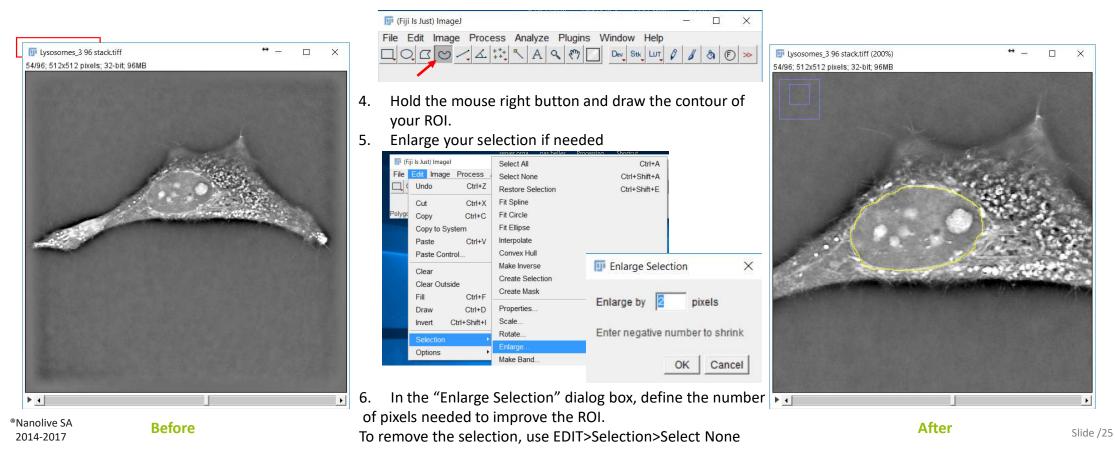
After

Slide /24



This operation allows to select a region of Interest.

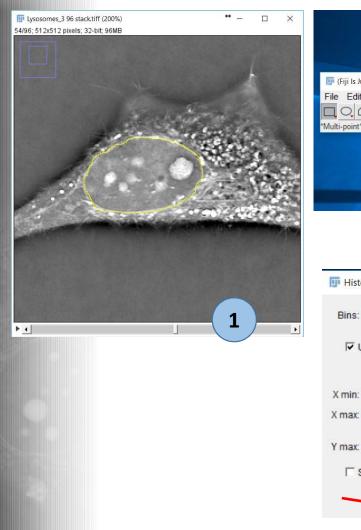
- 1. Zoom in your image to improve visualization of the ROI. (SHIFT + or SHIFT- ).
- 2. Move the ROI by holding SHIFT and moving the mouse cursor.
- 3. Select the free hand tool by example

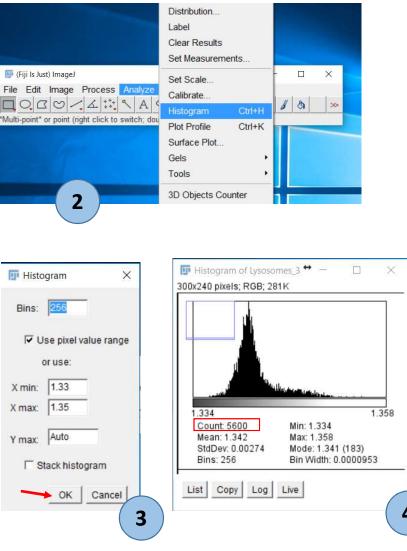




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Fiji Display RI Data histogram of a ROI

> Define a ROI, Region Of Interest in the image (1).
> Run the command Analyze > Histogram (2)
> A pop-up window histogram is showing up (2) to adjust histogram settings (bins, min, max...) (3)
> Click on "OK" and the histogram is
> displayed (4).

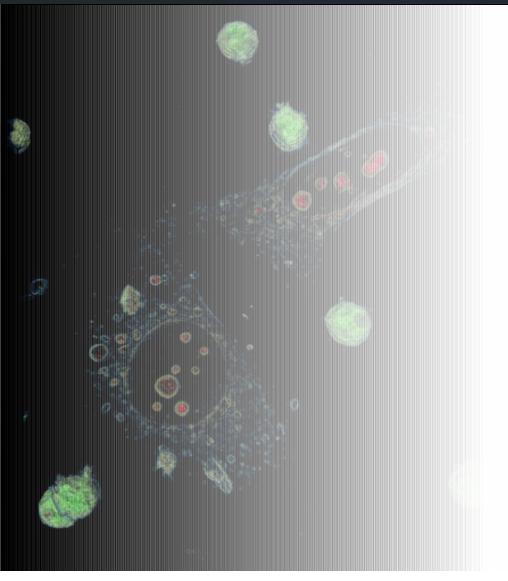
**Note1**: the histogram is calculated based on the ROI values. The count indicates the number of pixel information considered, a full image includes 262144 pixels (512\*512).

**Import** the histogram data in Excel (slide11)

**Note2**: Please note that this ROI is a 2D ROI only.

Slide /26





# **3D Cell Explorers**

- 1. Tool Installation
- 2. General Workflow
- 3. Create a colour map
- 4. Advanced Operations
- 5. Correlate Refractive Index and Fluorescence information



Select RI data based on a fluo marker.

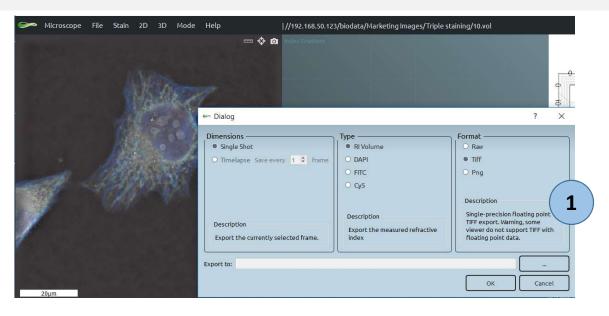
The 3D Cell Explorer-fluo is a master piece for Advanced Research activities as both RI and fluorescence information can be correlated.

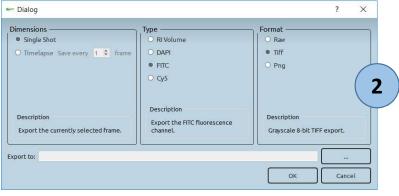
The method hereafter describes how to use the fluo signal from marked organelles as a selection mask to collect RI information in a 2D RI distribution map generated by STEVE.



Create a selection mask from a fluo signal Part 1 of 7

This operation describes how to export information from STEVE.





From STEVE, **Export** the RI data in a .tiff format. Either use the export button or the File> Export command. In the Export dialog box : Select RI Volume Select Tiff format Select a folder and define a filename Click OK (1)

**Note**: the exported Tiff file is made of 96 stacked layers, one layer per STEVE tomographic slice.

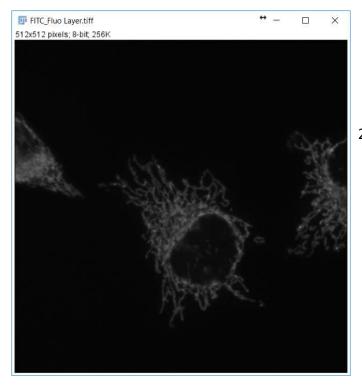
Then, from STEVE, **Export** the Fluo data in a .tiff format. Select the appropriate Fluo channel to export. Select a folder and define a filename Click OK (2)

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Create a selection mask from a fluo signal Part 2 of 7

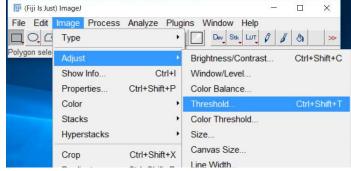
This operation allows to define precisely the ROI that will be selected by adjusting a threshold level to select or not pixels.



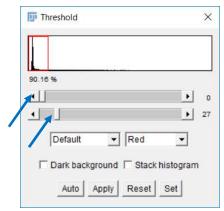
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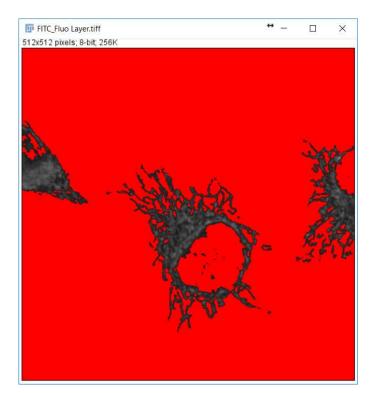
Before

 From the Tiff image of the fluo layer, run the command Image> Adjust > Threshold.



2. The image is becoming red where are the unselected pixels. In the pop-up window "Threshold" adjust the cursors to enlarge or narrow the number of pixels that will be part of the ROI.





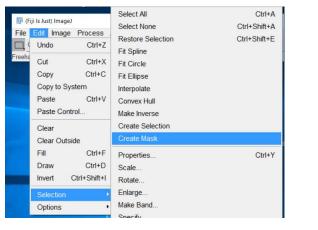
After



Create a selection mask from a fluo signal Part 3 of 7

This operation allows to create the mask from the selected pixels.

1. From the Tiff image where a threshold has been applied, Run the command Edit > Selection > Create mask.



- 2. A new image appears, named "mask". The black region are the unselected pixels, value is 255. The white region are the selected pixels, value is 0.
- 3. Close the "red" source image.



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Before

After



Create a selection mask from a fluo signal Part 4 of 7

This operation allows to rework the mask to remove unwanted areas (e.g. adjacent cells).

1. In the Fiji tool bar, select the "color picker" tool.

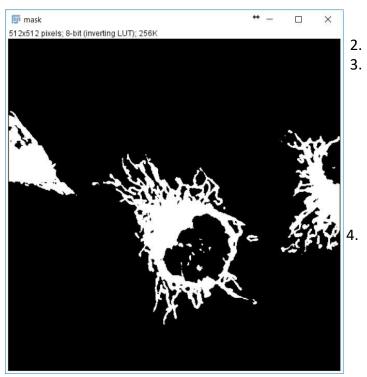
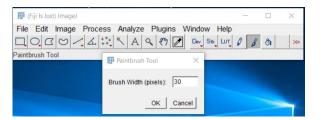


 Image
 ×

 File
 Edit
 Image
 Process
 Analyze
 Plugins
 Window
 Help

 Image
 Image

- Select a black zone in the mask image.
- . In the Fiji tool bar, select the "brush" tool. By a right click, adjust the brush size.



Re-paint the unwanted white areas in black.



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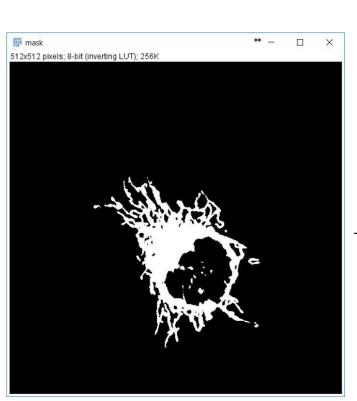
Before

After

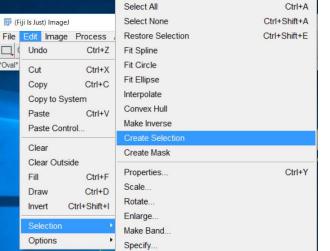


Create a selection mask from a fluo signal Part 5 of 7

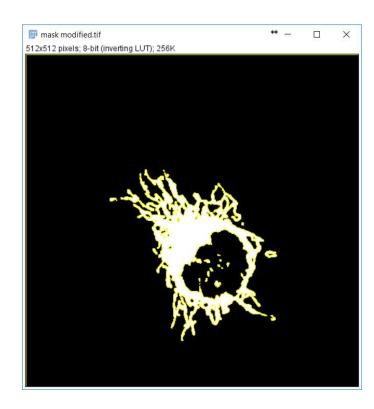
This operation allows to define the ROI contour inside the mask image.



1. Run the command Edit > Selection > Create Selection



The borders of the ROI is highlighted in yellow now



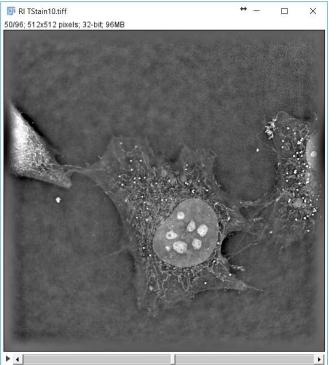
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Create a selection mask from a fluo signal Part 6 of 7

This operation allows to apply the ROI contour on the Refractive Index Image.

- 1. Open the Tiff file containing RI values in grey levels and select the appropriate layer by moving the slicer at the bottom of the image.
- 2. Adjust the Contrast and Brightness (see procedure
- 3. described in this presentation).

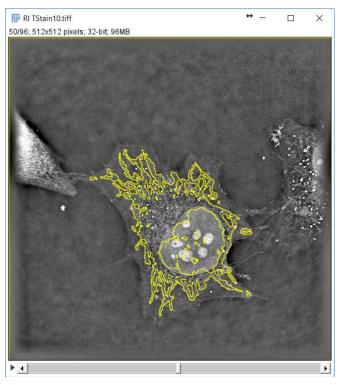


4. Click first on the mask image below to select the window



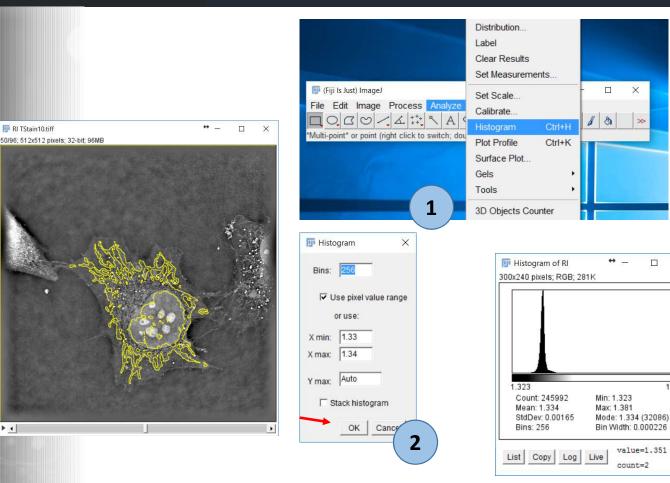
5. Then, Click on the RI grey levels image

6. Type SHIFT and E. The yellow contour is now applied to The RI grey level image.



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**Display RI Data Histogram** 

X

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3

**Run** the command Analyze > Histogram (1)

A pop-up window histogram is showing up (2) to adjust histogram settings (bins, min, max...).

Keep default values and click on "OK", the histogram is displayed (3).

Note1: the histogram is calculated based on the ROI selection. The count indicates the number of pixel information considered, a full image includes 262144 pixels (512\*512).

**Import** the histogram data in Excel (slide11)

**Note2**: Please note that this ROI is a 2D ROI only.

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# 3D Cell Explorer



Thank you for your attention !

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Slide /36