



# 3D Cell Explorers

STEVE Refractive Index maps and 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.

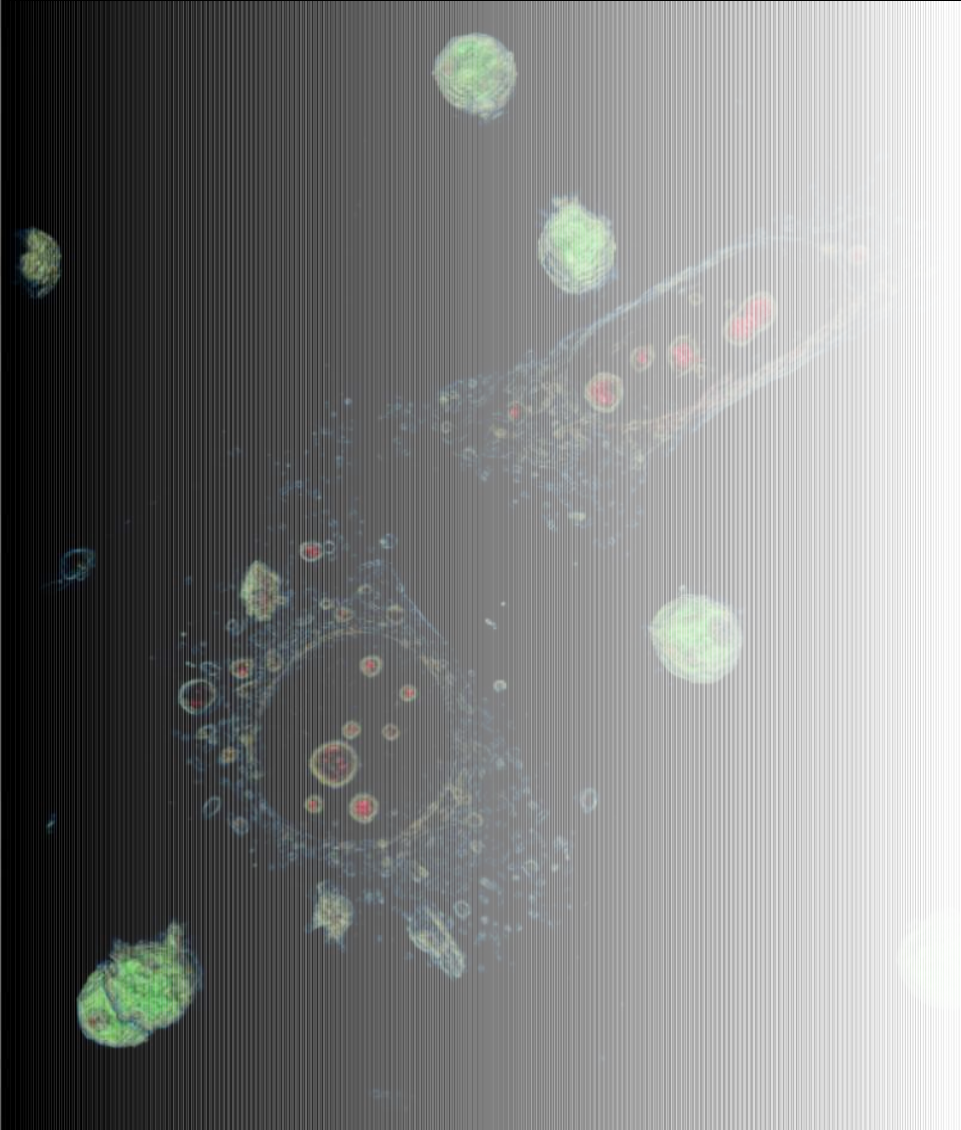
These methods can be extended to any other scientific application and will allow you to work in a quantitative way on the Refractive Index data distribution maps generated by STEVE.

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

The screenshot shows the Fiji (ImageJ) website's 'Downloads' page. The website header includes the 'IMAGEJ' logo, a search bar, and navigation links: ABOUT, DOWNLOADS, LEARN, DEVELOP, NEWS, EVENTS, STATUS, HELP. The main content area is titled 'Fiji/Downloads' and contains a description: 'Fiji is a distribution of ImageJ which includes many useful plugins contributed by the community.' Below this is a section for downloading Fiji for different operating systems, with icons for Windows (64-bit), macOS, and Linux (64-bit). There is also an 'Other downloads' section with icons for Windows (32-bit), No JRE, and Linux (32-bit). To the right of the download section is a 'Contents' table of contents with links to System requirements, Installation, Troubleshooting, Source code, and Other downloads (including Life-Line Fiji versions for Java 8 and Java 6, and a 'See also' link).

Below the website screenshot is a screenshot of the Fiji software interface. The window title is '(Fiji Is Just) ImageJ'. The menu bar includes File, Edit, Image, Process, Analyze, Plugins, Window, and Help. The toolbar contains various icons for image manipulation, including a rectangle tool. A tooltip for the rectangle tool is visible, stating: '\*Rectangle\*, rounded rect or rotated rect (right click to switch)'. A blue circle with the number '2' is overlaid on the bottom right corner of the software interface screenshot.

## Install Fiji (ImageJ).

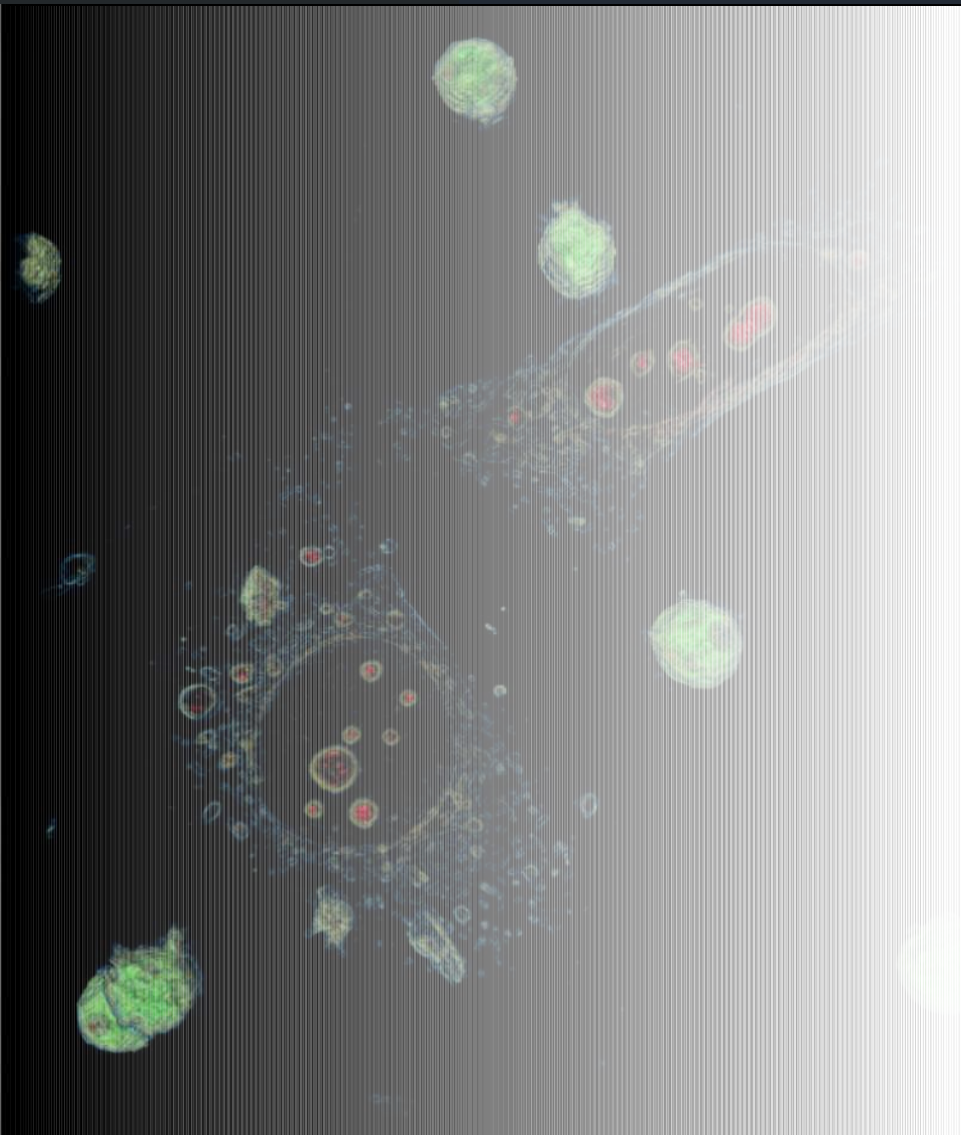
This open source software package can be found here:

<https://imagej.net/Fiji/Downloads> (1)

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

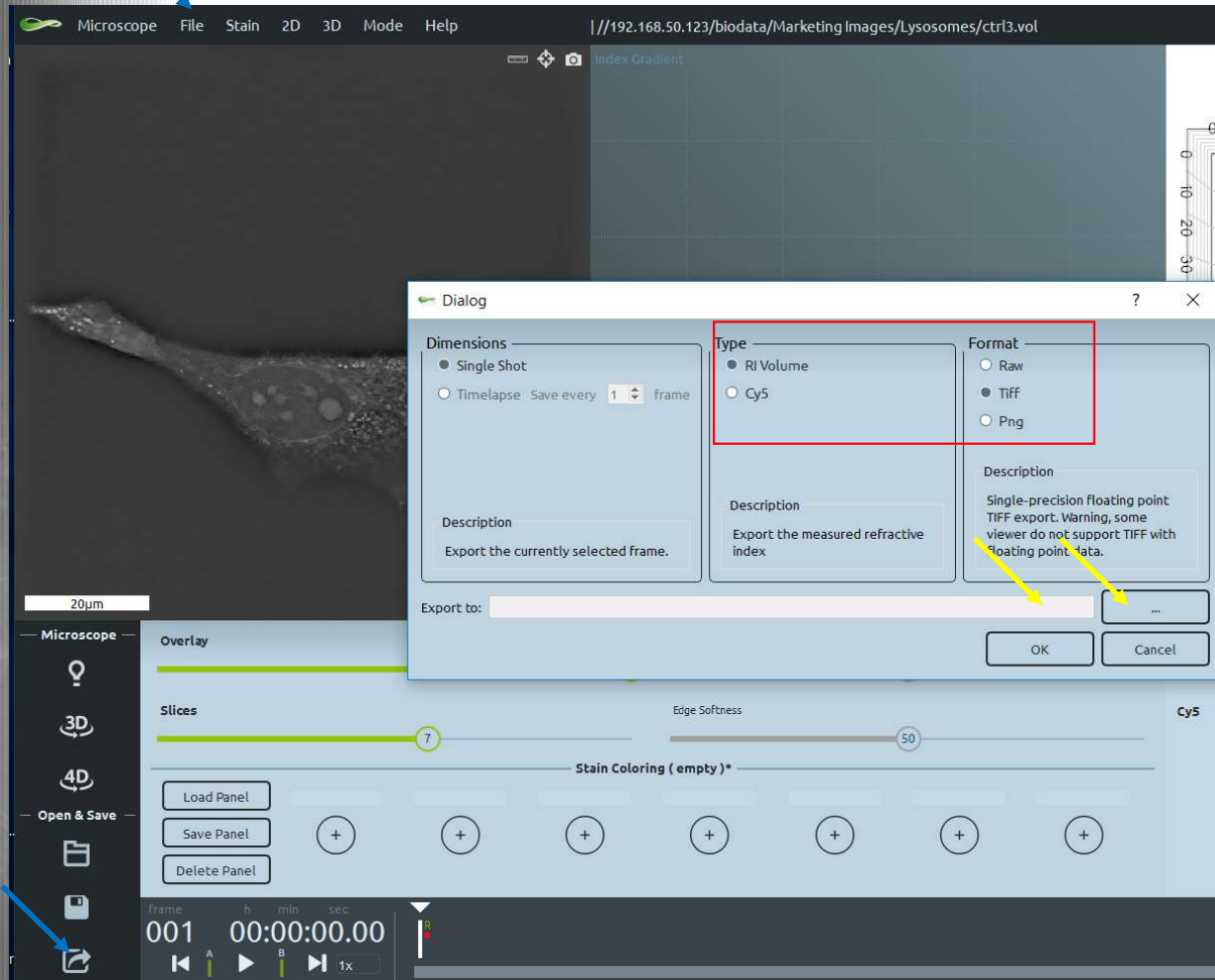
1

2



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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

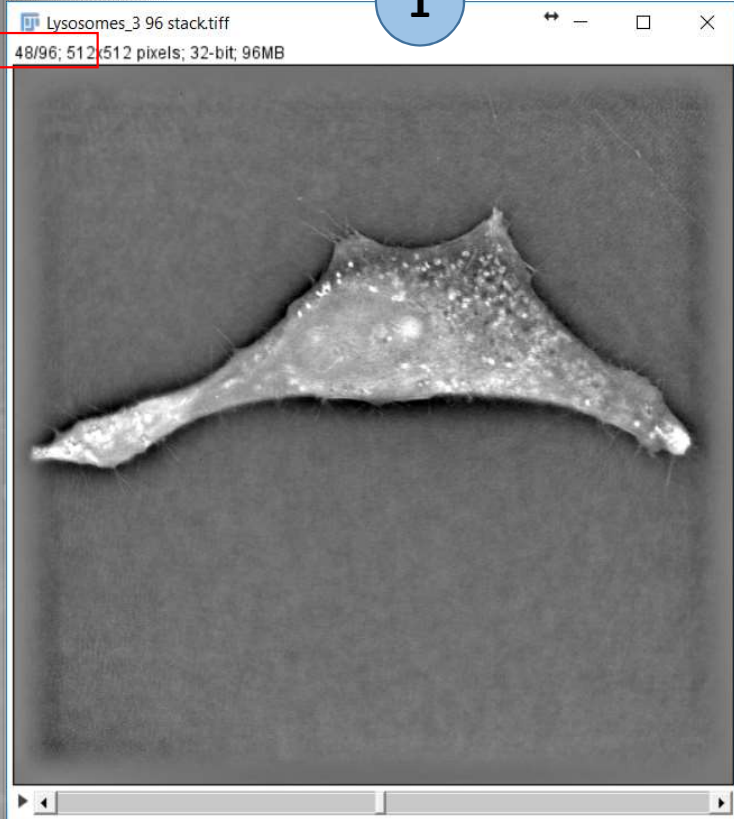
**Note:** the exported Tiff file is made of 96 stacked layers, one layer per STEVE tomographic slice.

STEVE layers are numbered from -47 to +48. Tiff layers are numbered from 0 to 96.

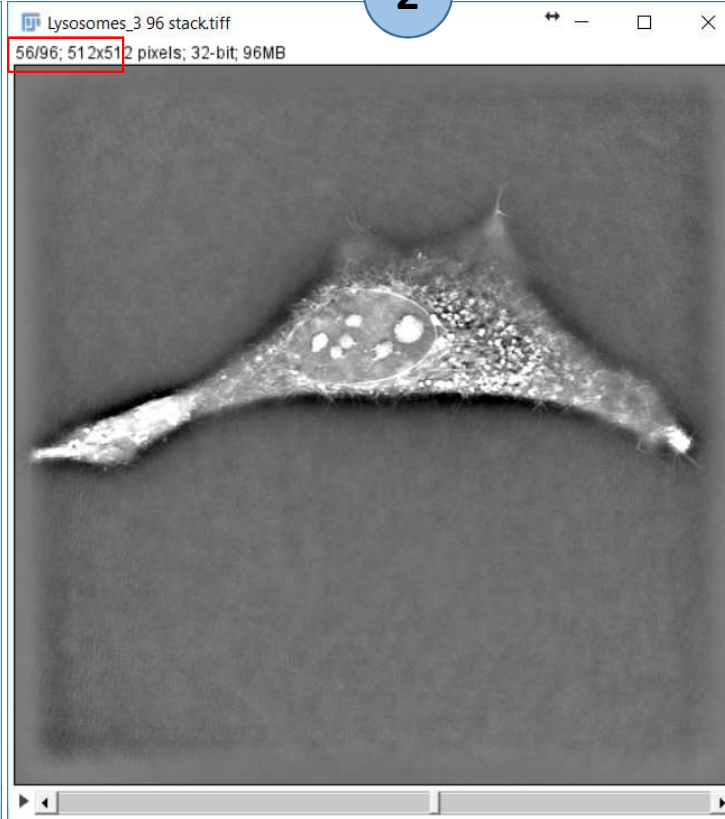


## Import a Tiff file for scientific analysis

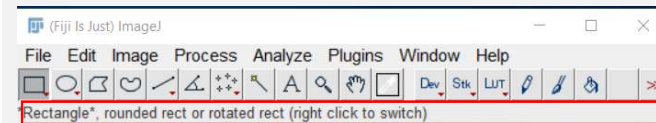
1



2



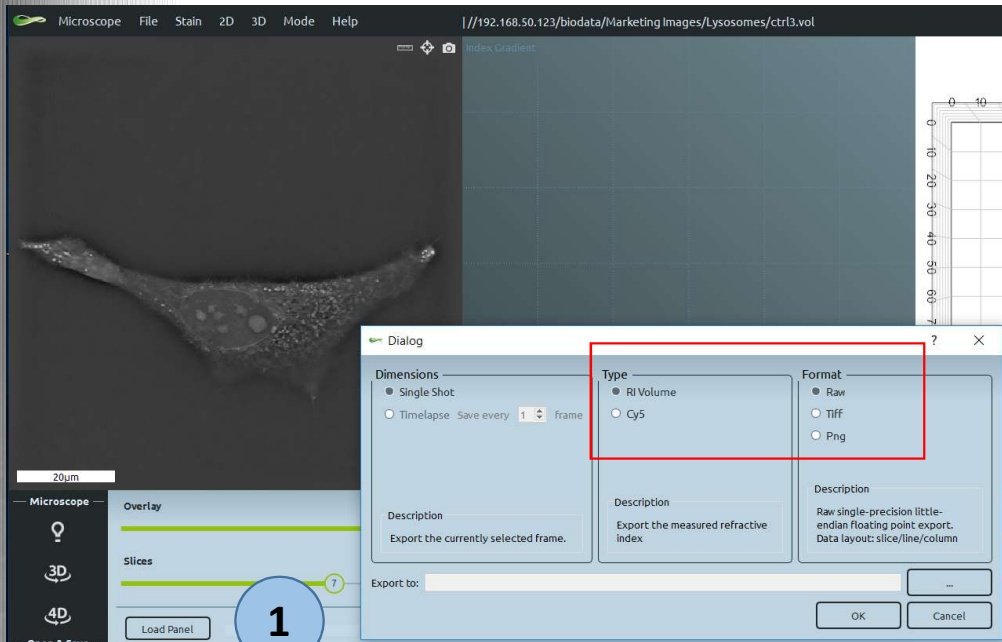
In Fiji, **Open** the exported .tiff file with the File> Open command.  
Or drag and drop the file directly in the box below the Fiji tool bar  
(red rectangle below)



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

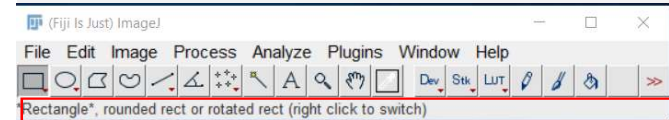
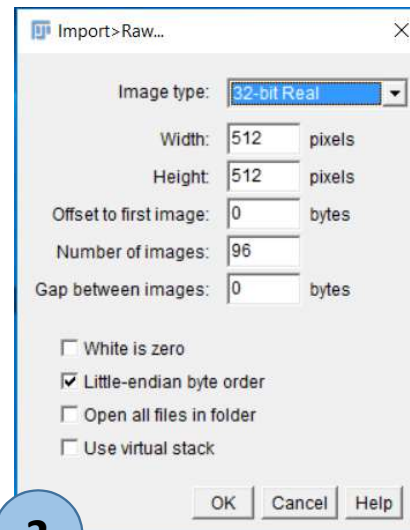
**Note:** moving the slider at the bottom of the window allows to visualize the different layers in the TIFF file, the layer reference is displayed in the upper left corner, here layers 48 and layer 56.

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



In STEVE, **Export** the RI volume in raw format (1).

In Fiji, **Open** the exported .raw file with the File> Open command.  
Or drag and drop the file directly in the box below the Fiji tool bar (red rectangle below)

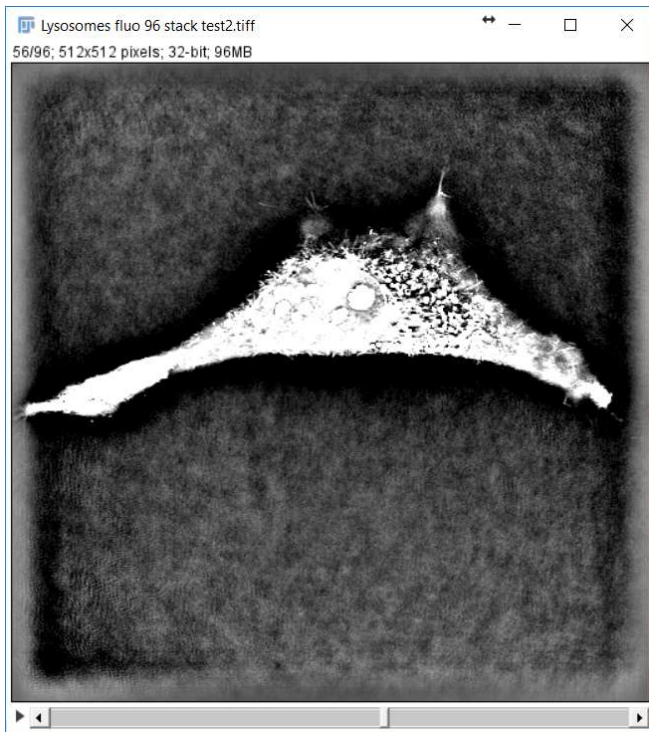


A new “Fiji Import>Tiff ” pops-up (2),  
Fill the following import parameters:  
IMAGE TYPE: 32-bit Rel  
WIDTH 52  
HEIGHT 512  
OFFSET TO FIRST IMAGE 0  
NUMBER OF IMAGES 96  
GAP BETWEEN IMAGES 0  
Select LITTLE-ENDIAN BYTE ORDER

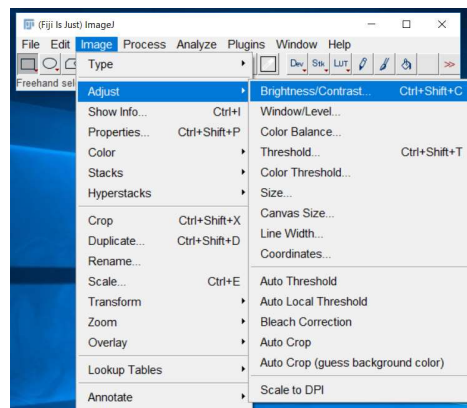


This operation allows to improve the visualization in term of Brightness and Contrast by adjusting the image histogram. Please note that this operation do not modify the RI values inside the image, it makes the image just looking better.

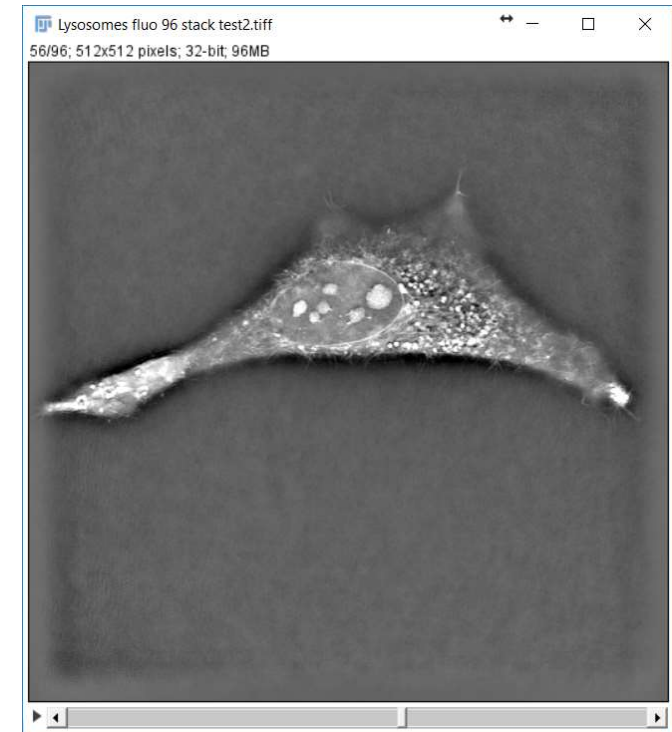
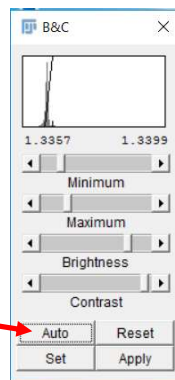
1. Run the command Image > Adjust > Brightness/Contrast



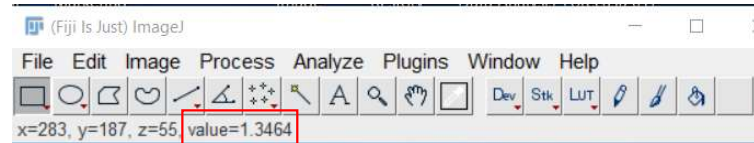
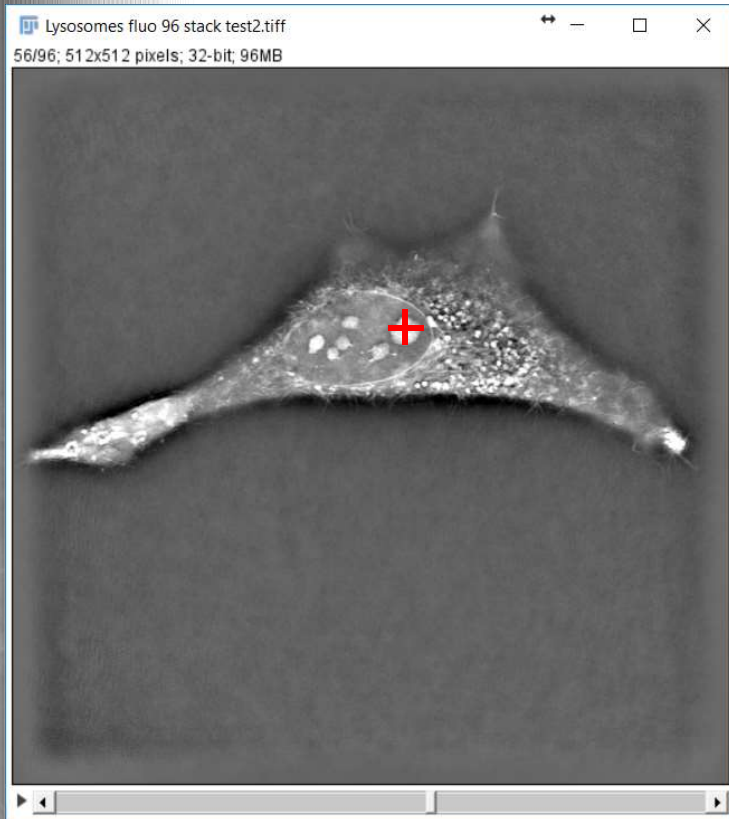
Before



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

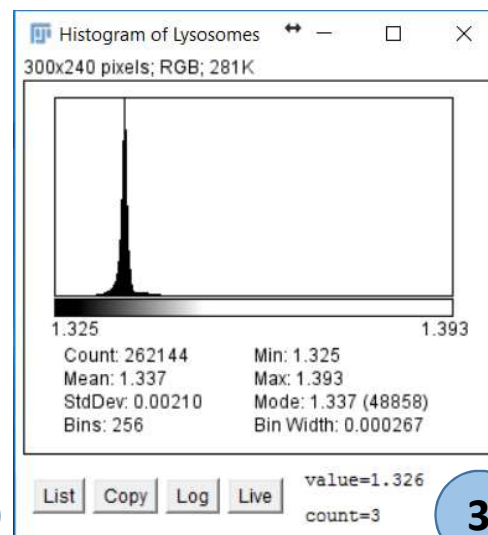
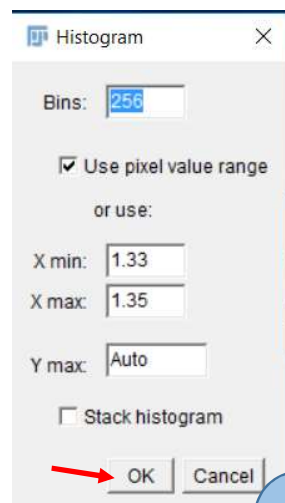
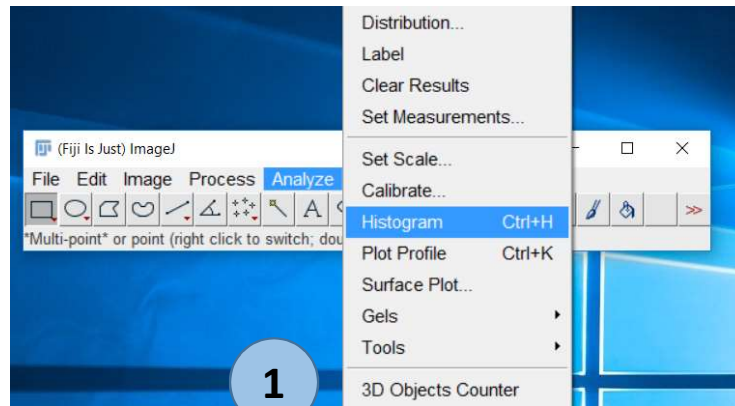
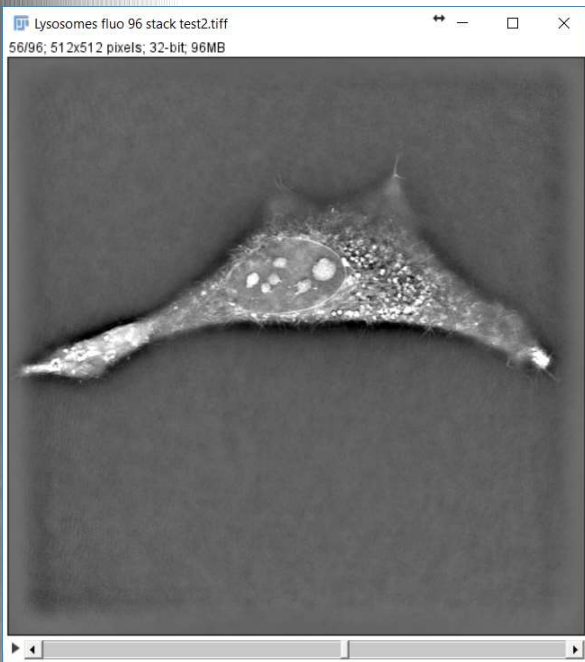


After



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)



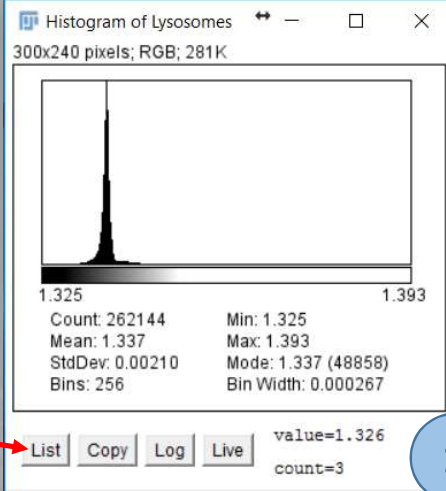
**Run** the command Analyze > Histogram (1)

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

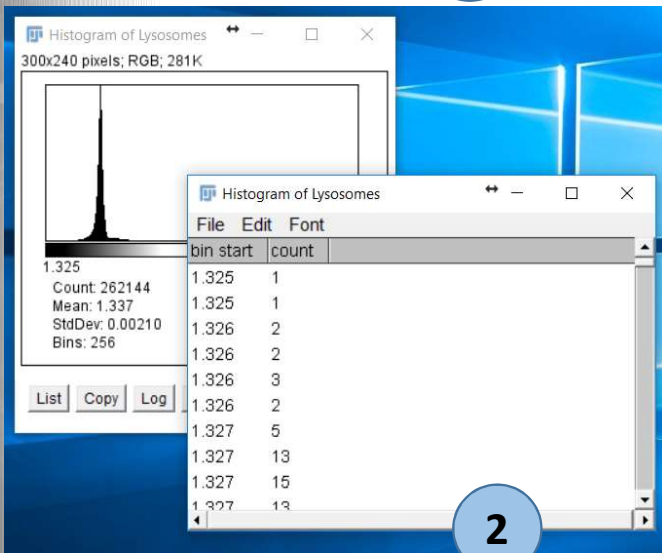
Keep default values and click on “OK”, the histogram is displayed (3).

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

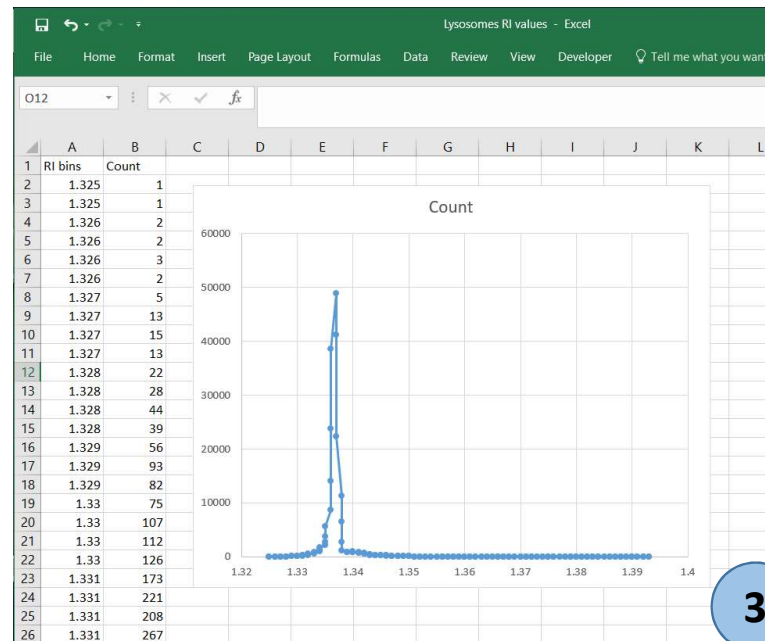
**Note2:** the focus plane is often defined in STEVE as the slice number 0 corresponding to the Tiff layer 48. Please consider that based on your experiment and samples, Tiff layers below 48 might be not relevant in term of data analysis.



1



2



3

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)

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

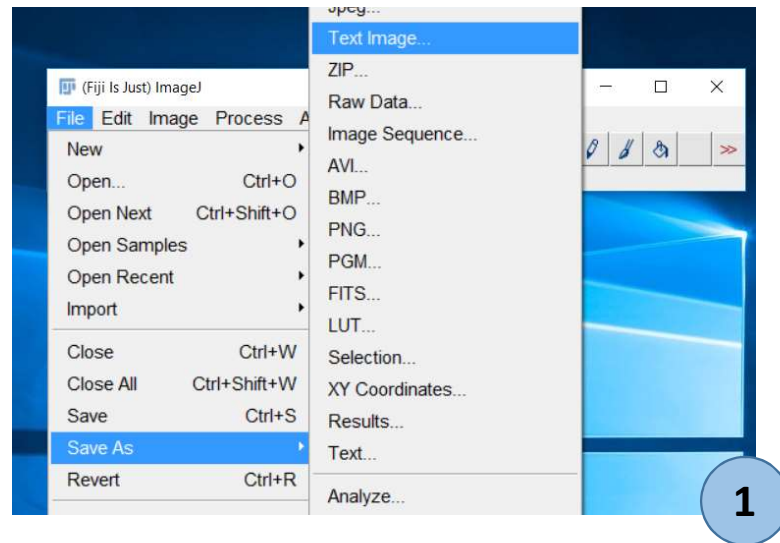
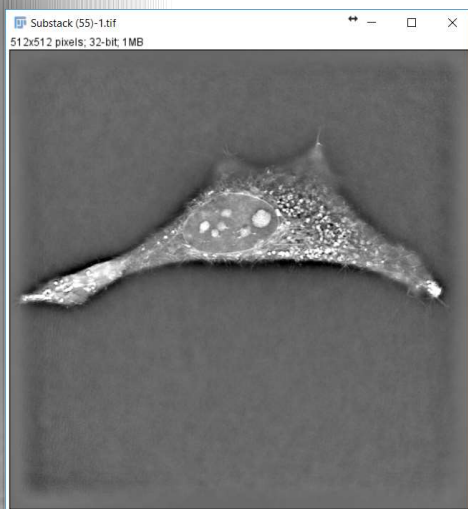
If it is needed to import all the RI values of a Tiff image in Excel, we provide two alternative methods:

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.



Export RI Data to Excel (rounded method)

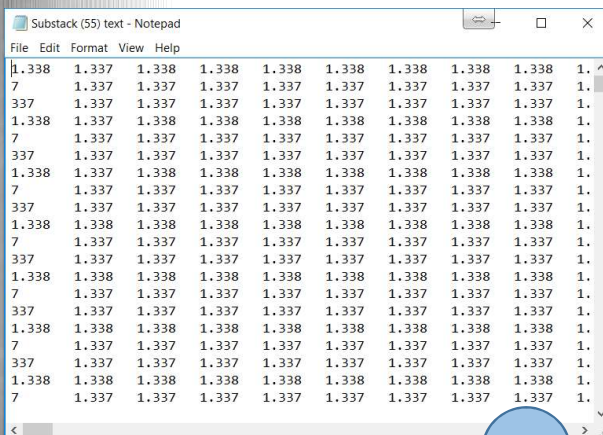


1

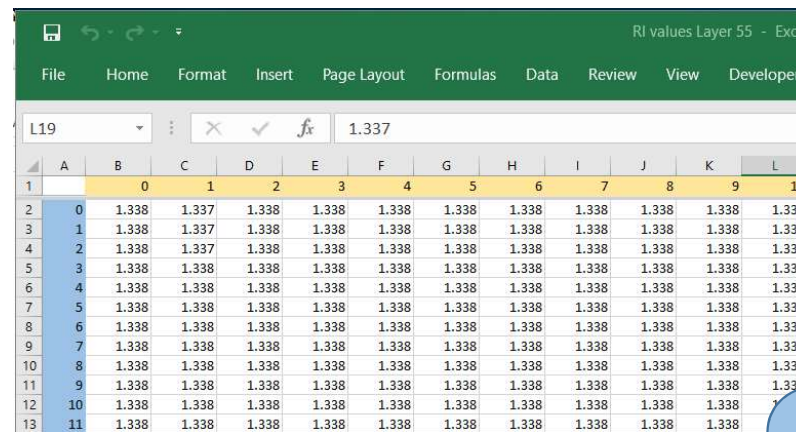
Load a Tiff file, **Run** the command File > Save as > Text Image (1) and save the file.

**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.



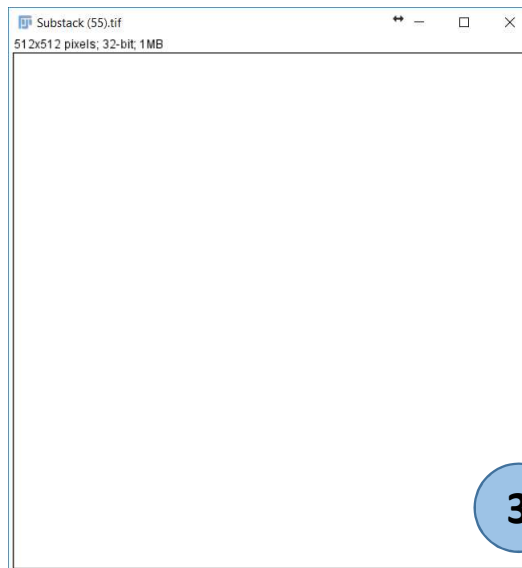
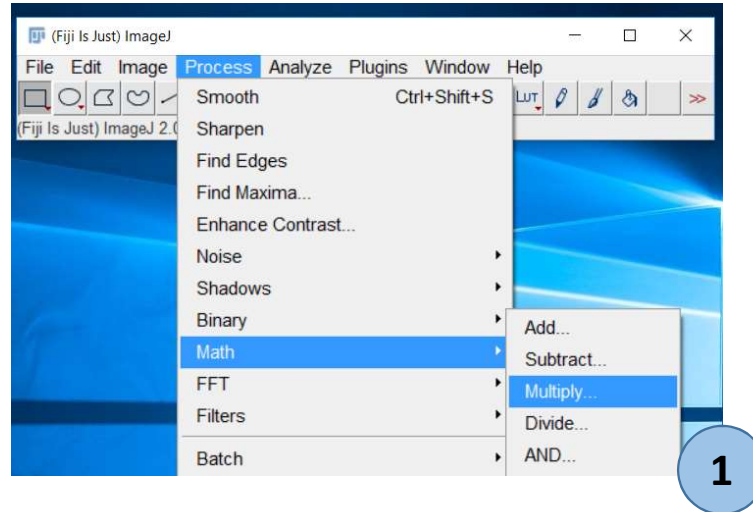
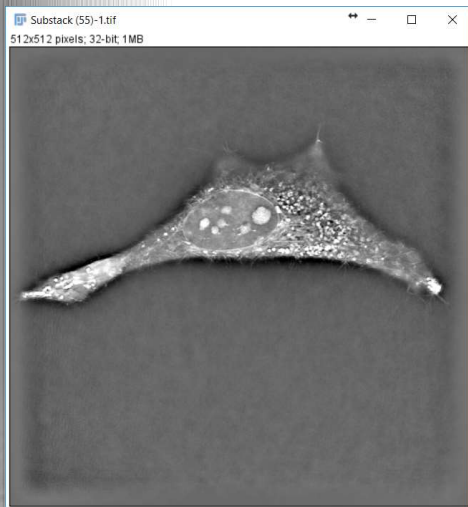
2



3

**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.





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).

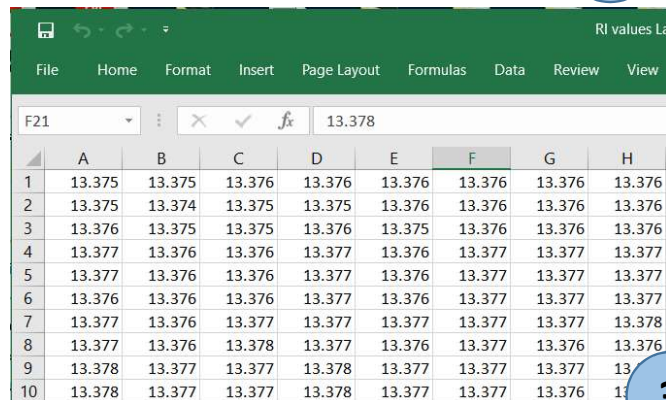
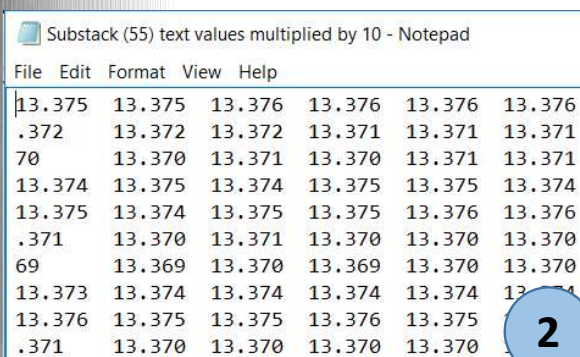
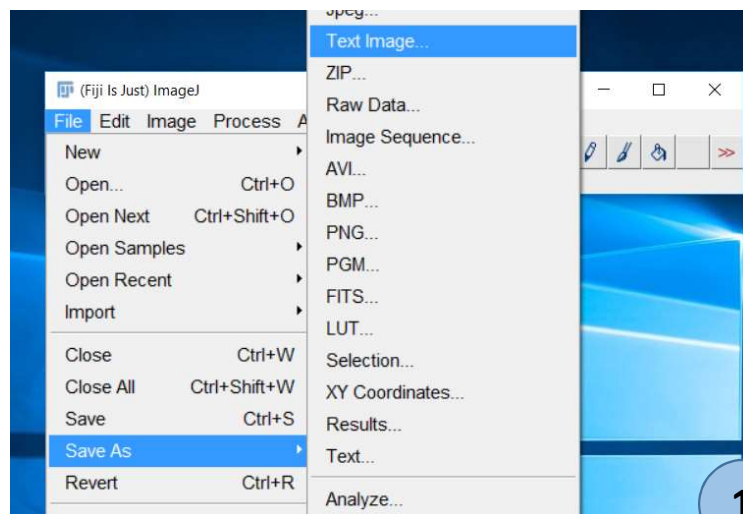
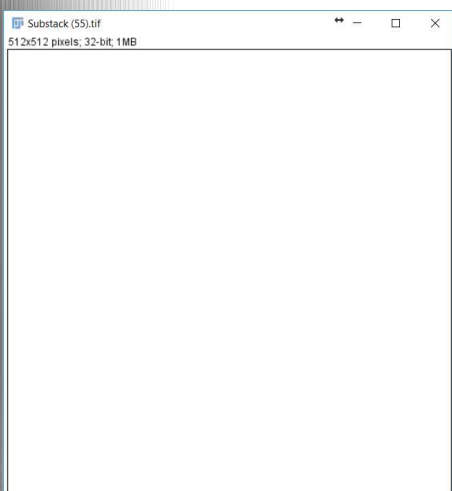
In the pop-up window, insert the value 10 , then click OK (2)

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

1

2

3

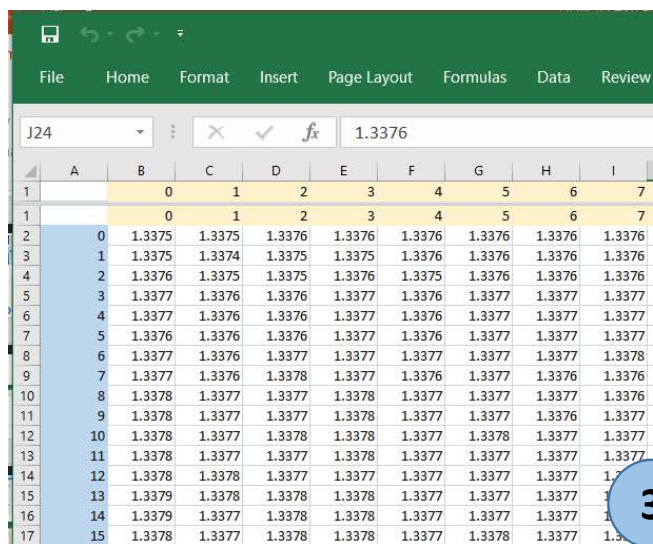
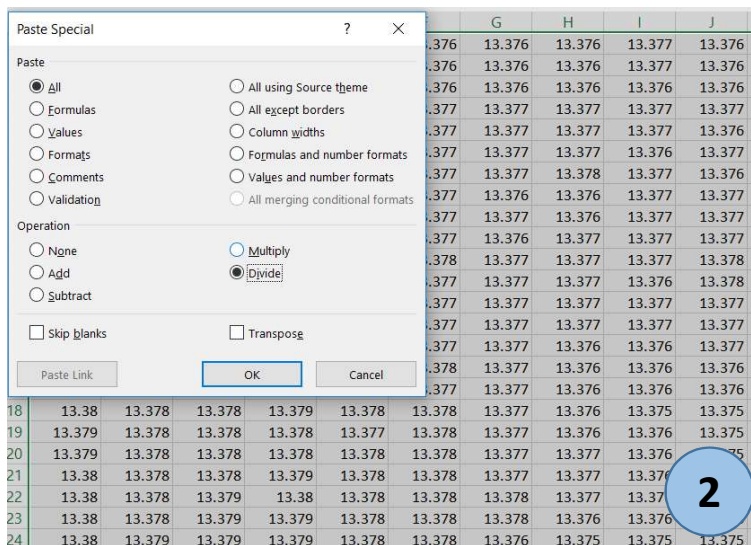
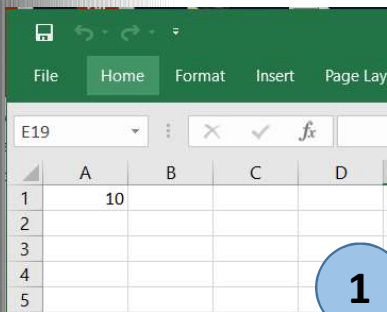


From the Tiff file with RI values multiplied by 10, **Run** the command File > Save as > Text Image (1) and save the file.

**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.

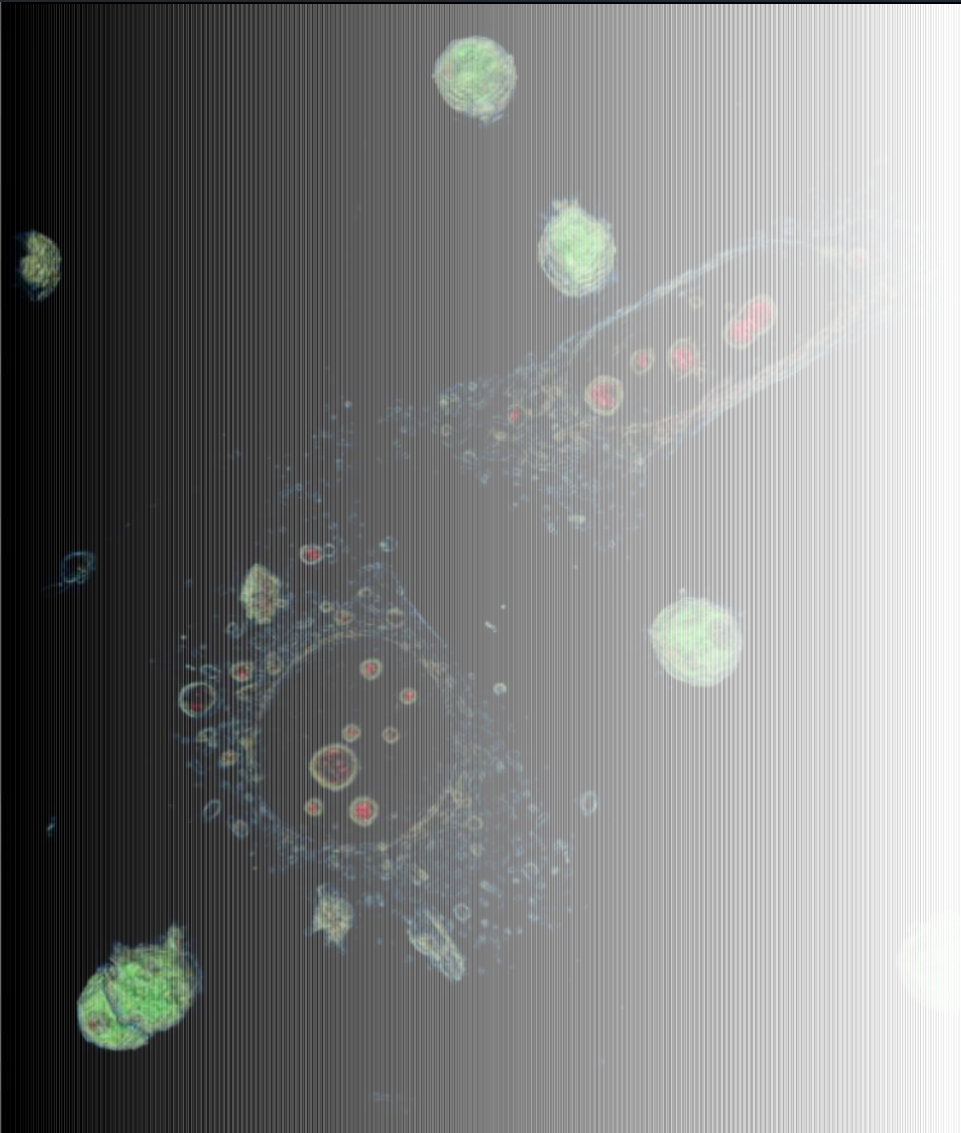


In Excel, in a blank cell, enter the value 10 (1) and copy it in the clipboard (CTRL-C).

**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.

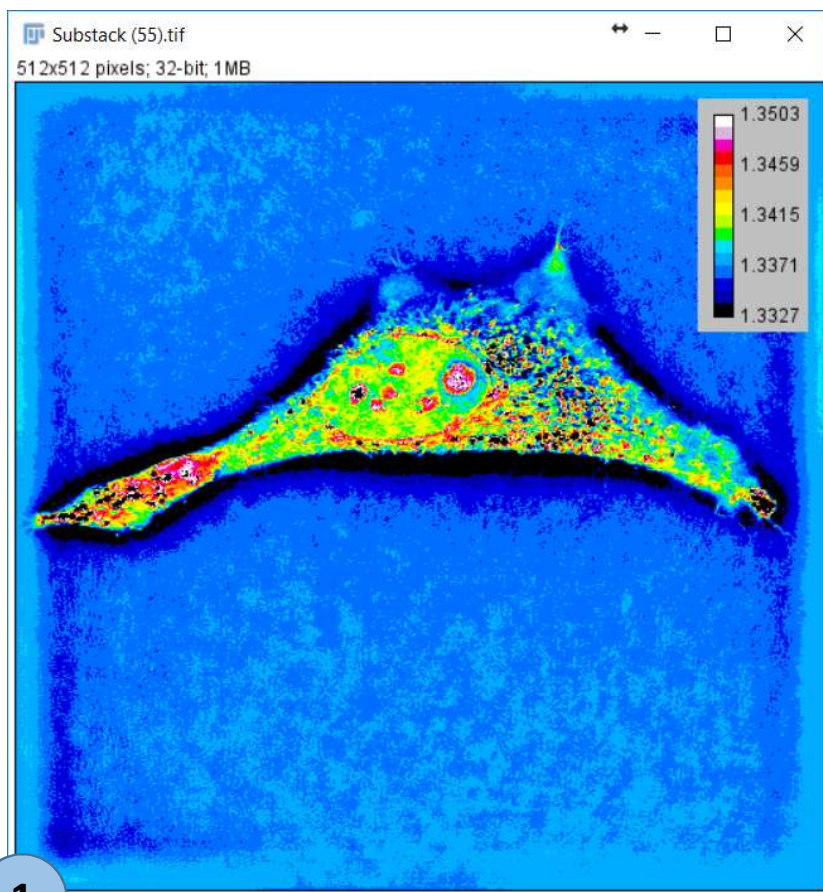


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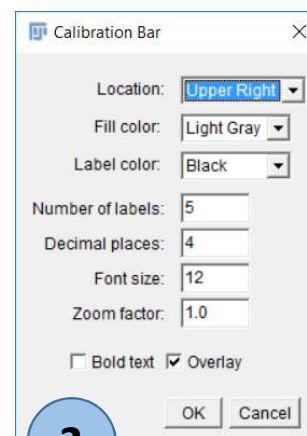
1. Tool Installation
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This operation allows to create a coloured RI distribution map with a colour scale. This helps to quickly visualize the RI distribution.



1



2

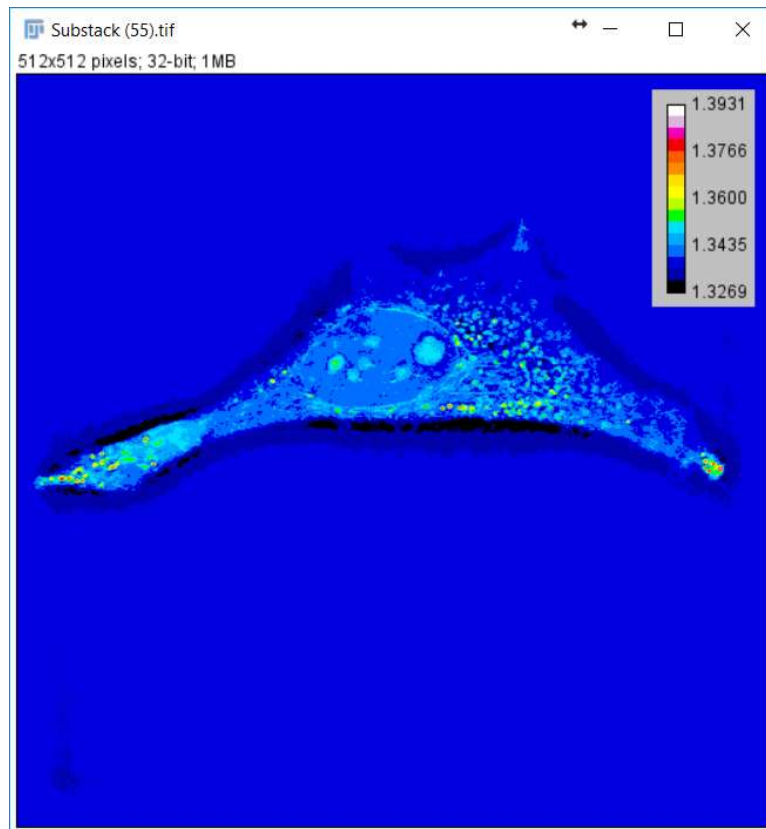
In Fiji, **Load** a tiff image in grey scale.

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

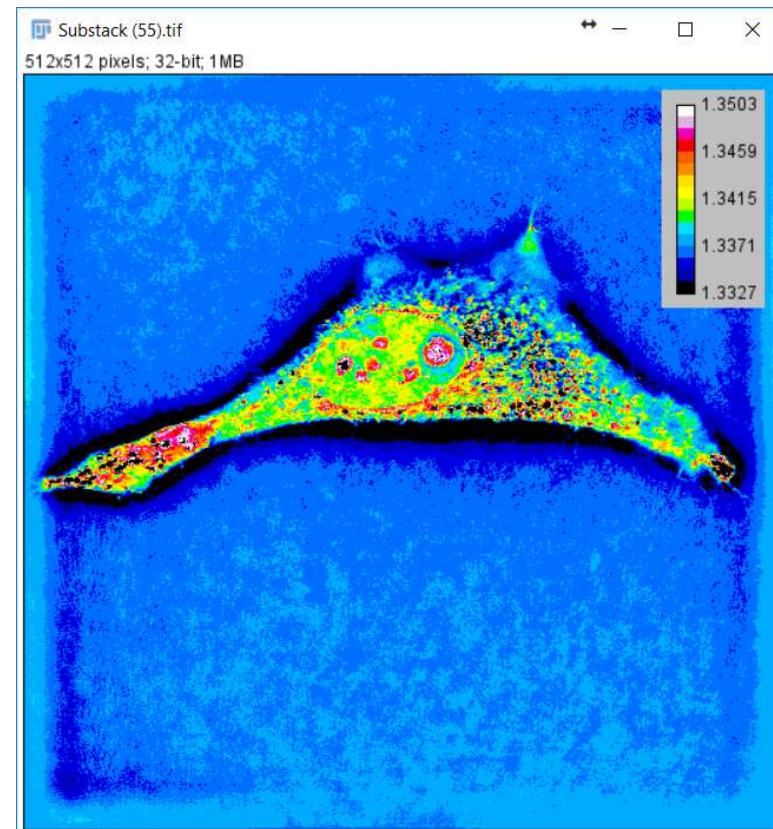
**Transform** the gray scale image in colour map by applying a LUT, **Run** Image > Lookup tables > 16 colours (1)

**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)

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.

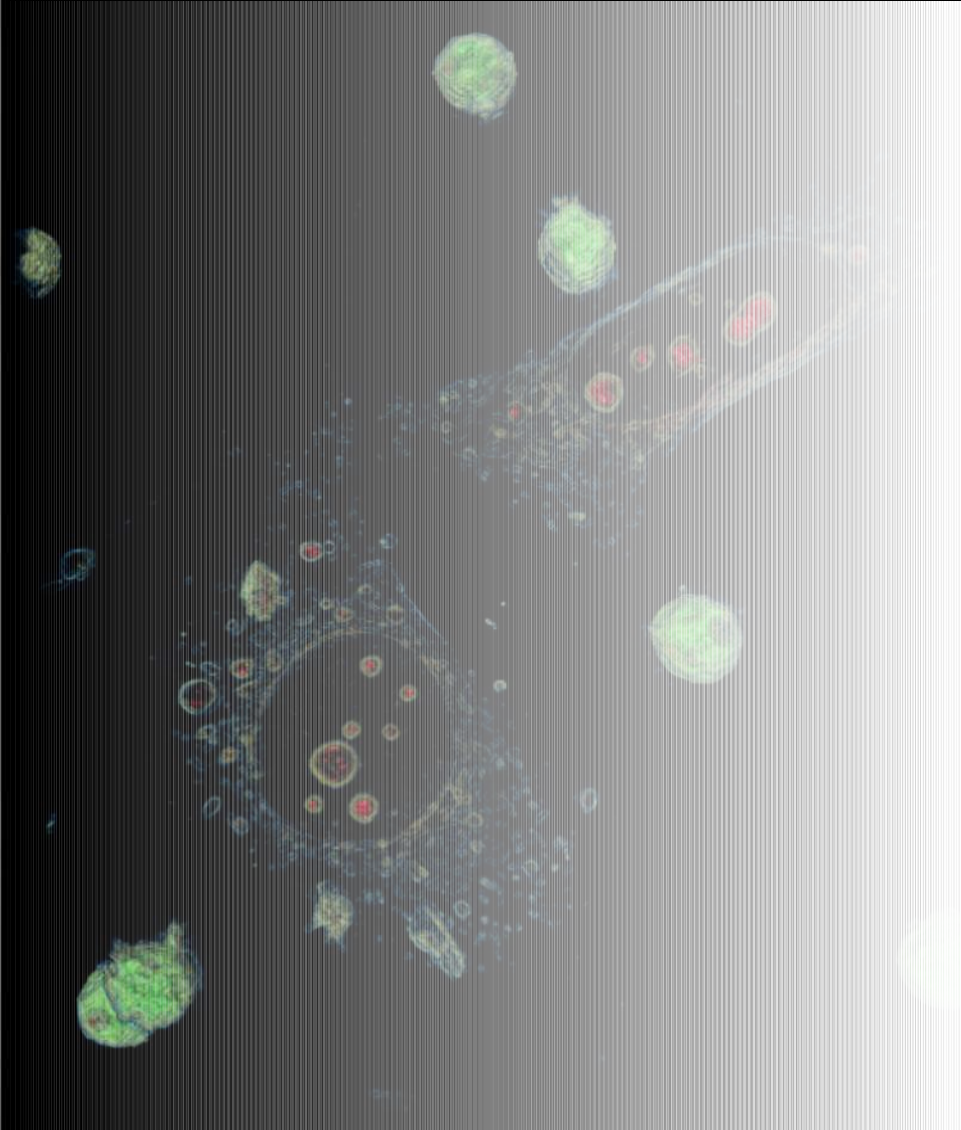


Thresholding done with max value: 1.39 and a full range.



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



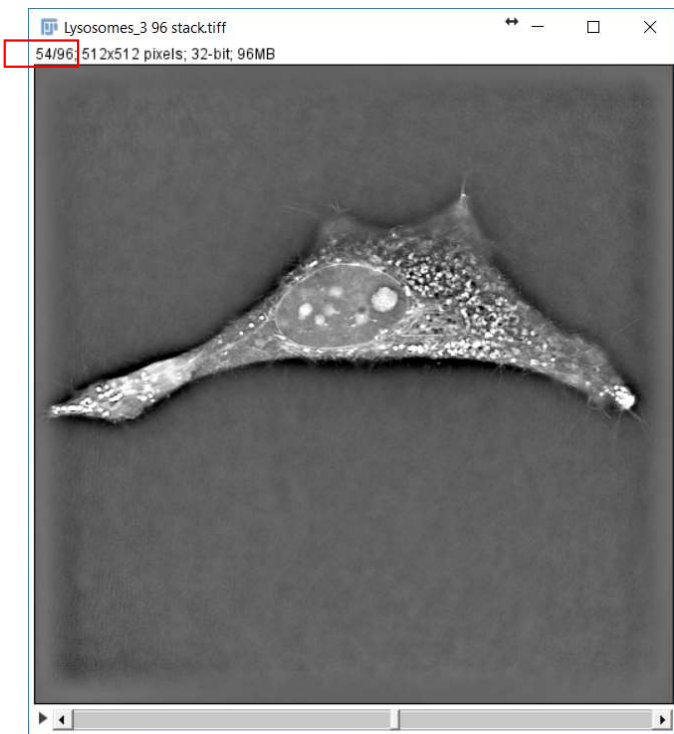
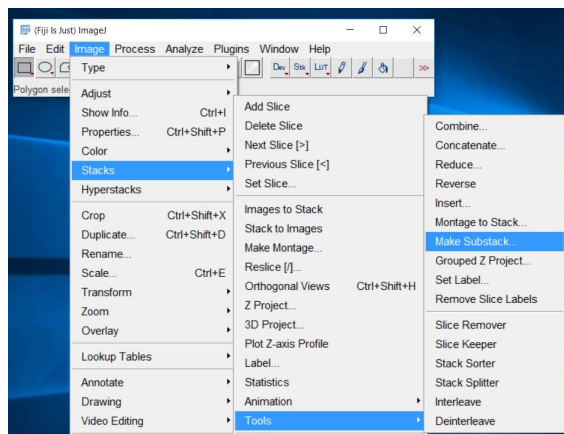


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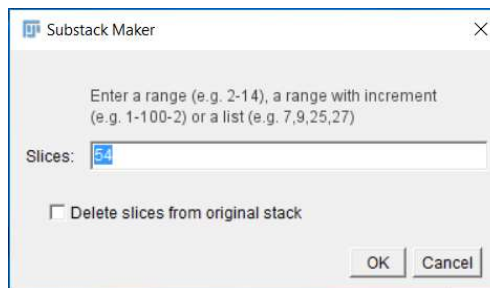
This operation allows to extract one layer from the exported Tiff file that contains 96 layers and to save it as an independent file.

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

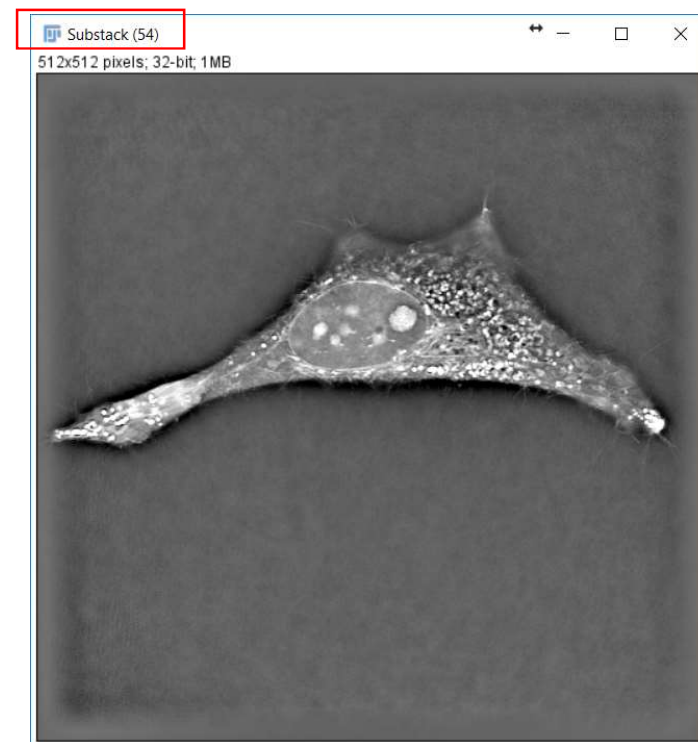


Before

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



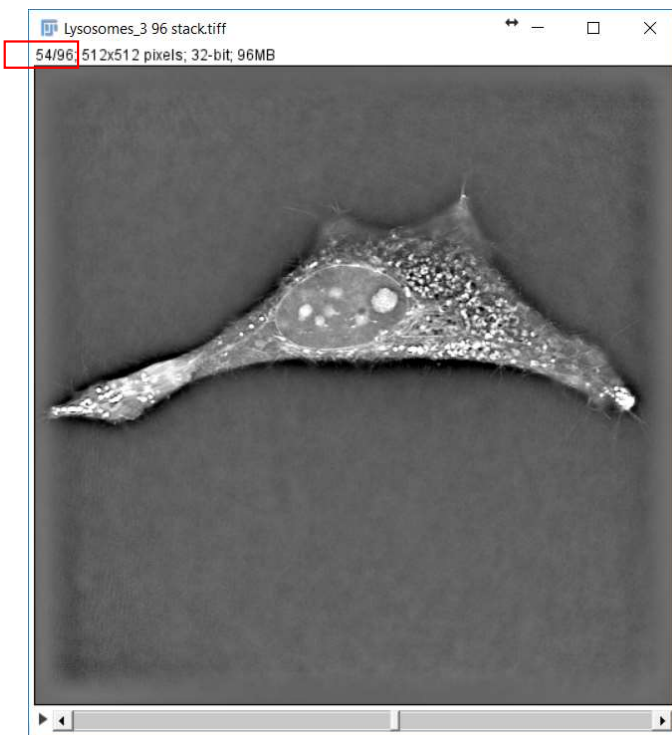
3. A new window pops-up with the name Substack(xx), here xx = 54. Save the file by File > Save as > Tiff. The new file contains now only 1 layer.



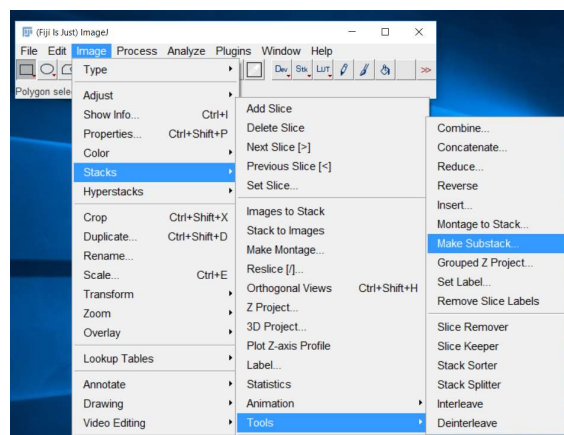
After

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.

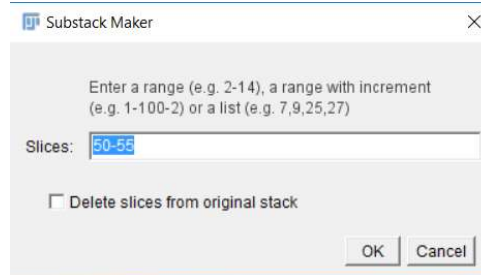
1. Run the command Image > Stacks > Tools > Make Substack



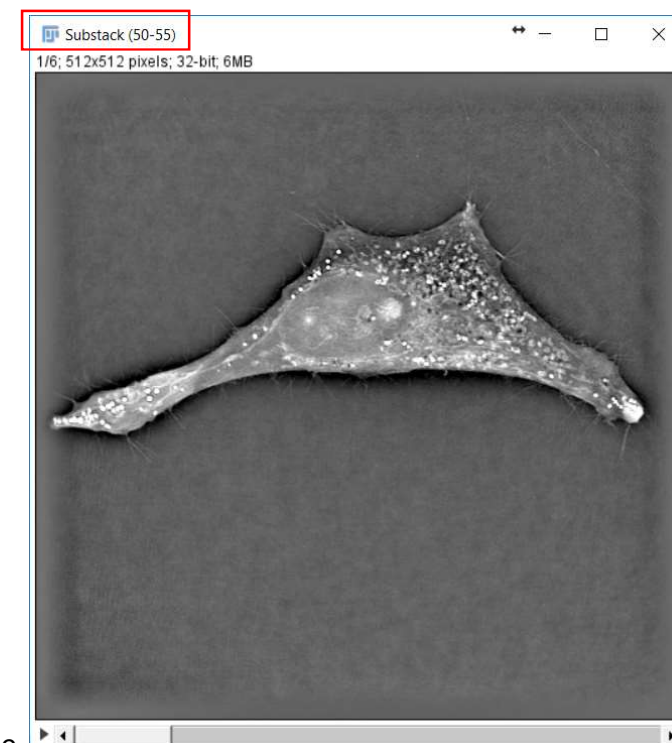
Before



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



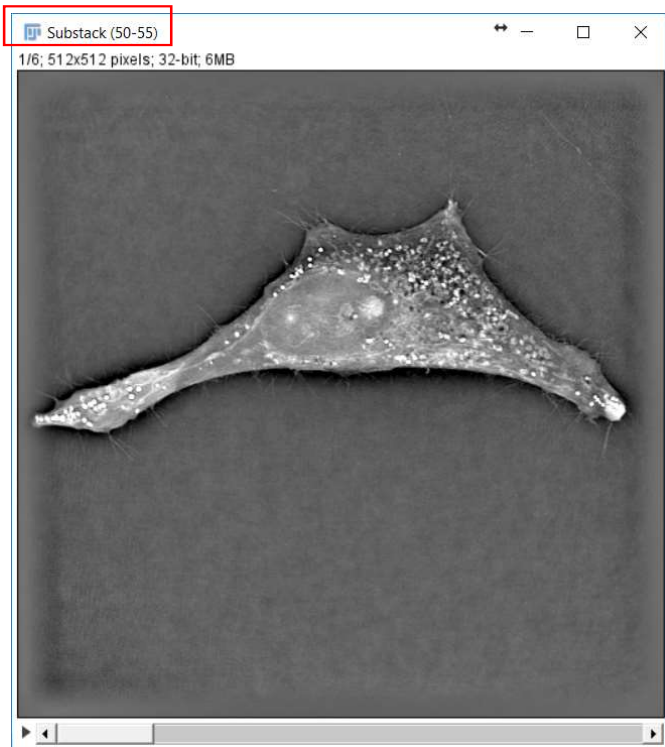
3. A new window pops-up with the name Substack(xx), here xx = 50-55. Save the file by File > Save as > Tiff. The new file contains now only 6 layers.



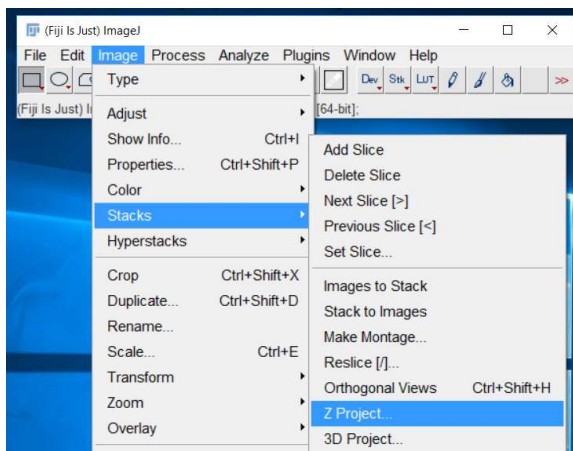
After

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

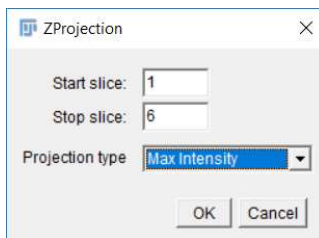
1. Create a substack with the selected tomographic layers.
2. Run the command Image > Stacks > Z Project



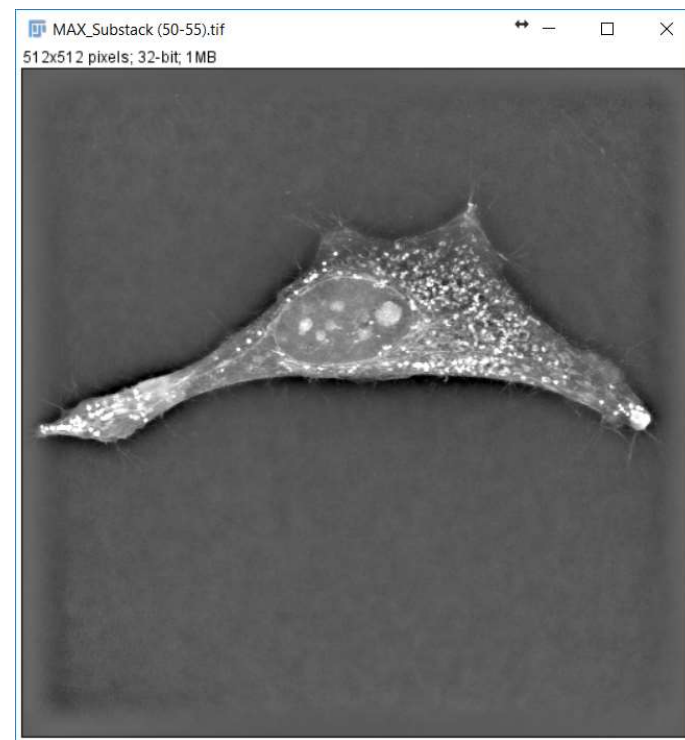
Before



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



4. 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.

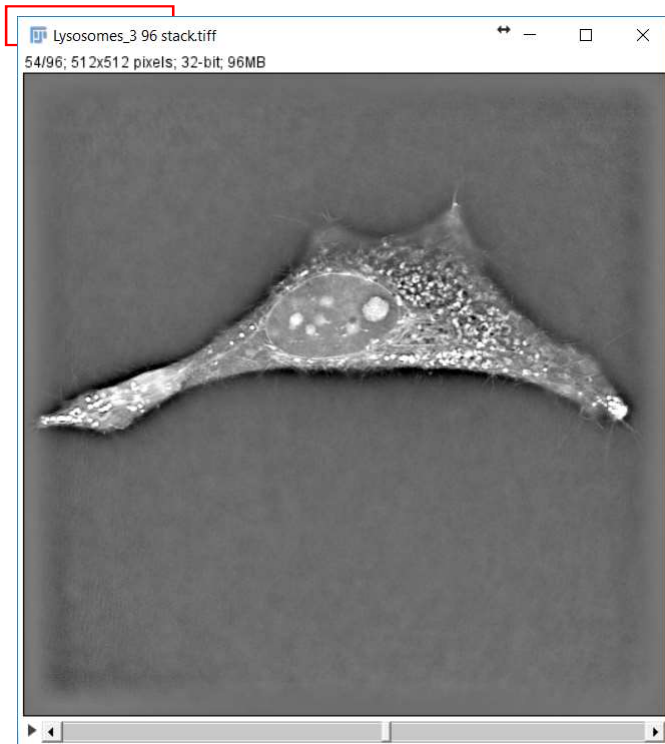


After

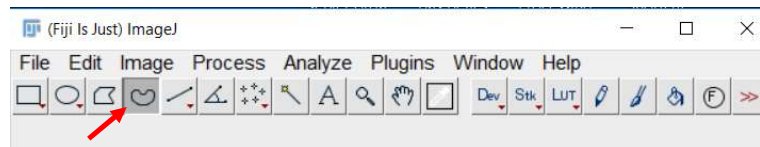


This operation allows to select a region of Interest.

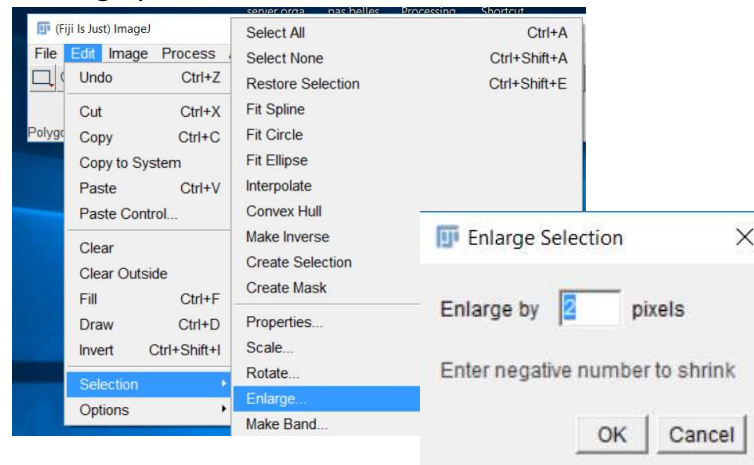
1. Zoom in your image to improve visualization of the ROI. (SHIFT + or SHIFT-).
2. Move the ROI by holding SHIFT and moving the mouse cursor.
3. Select the free hand tool by example



Before

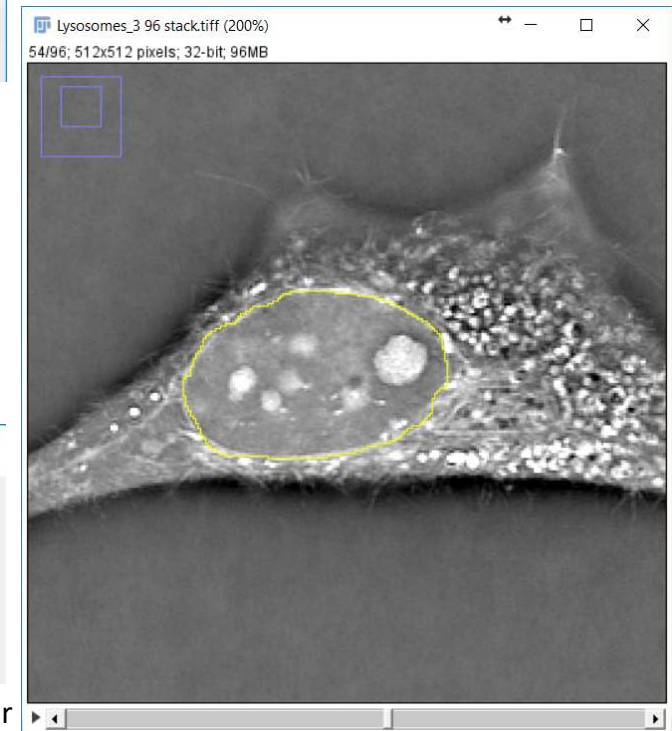


4. Hold the mouse right button and draw the contour of your ROI.
5. Enlarge your selection if needed



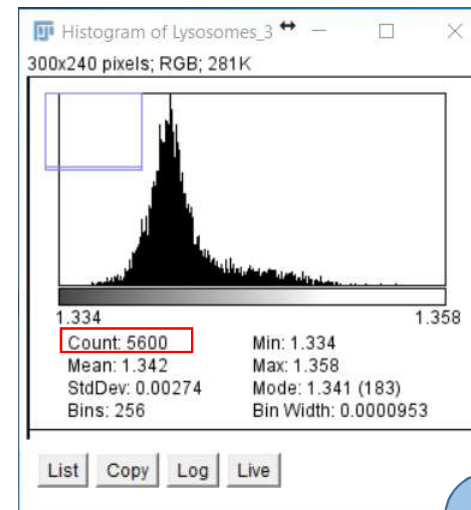
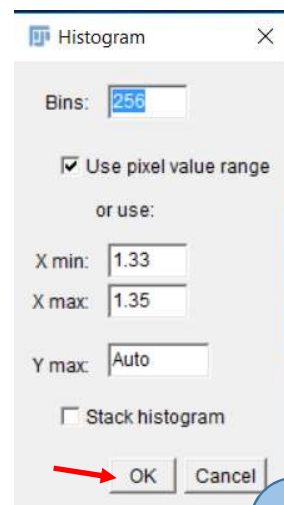
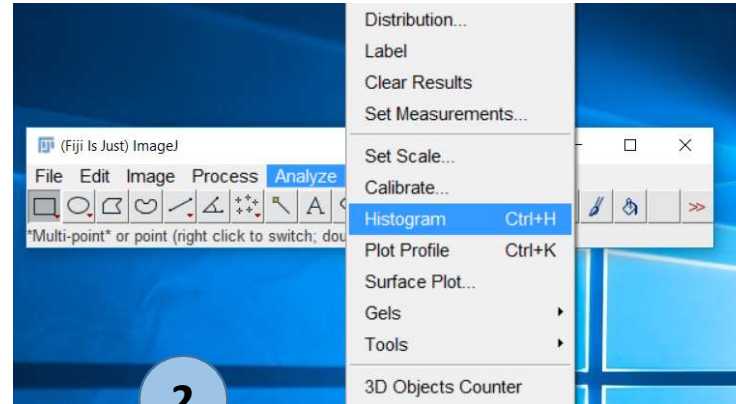
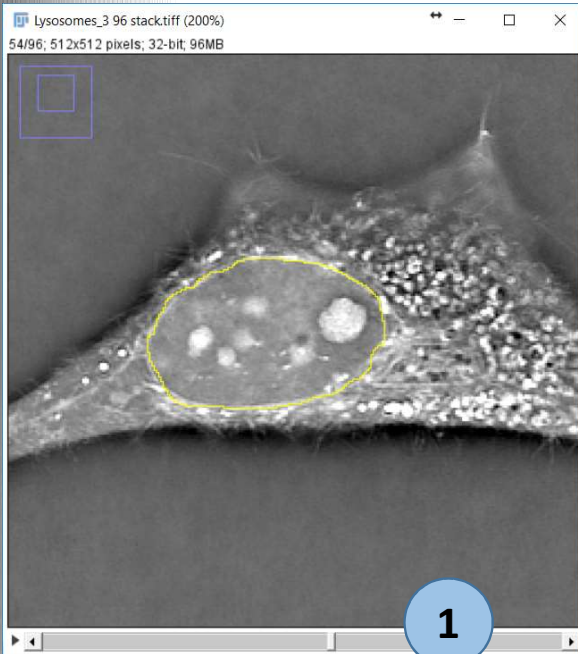
6. In the "Enlarge Selection" dialog box, define the number of pixels needed to improve the ROI.

To remove the selection, use EDIT>Selection>Select None



After

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)

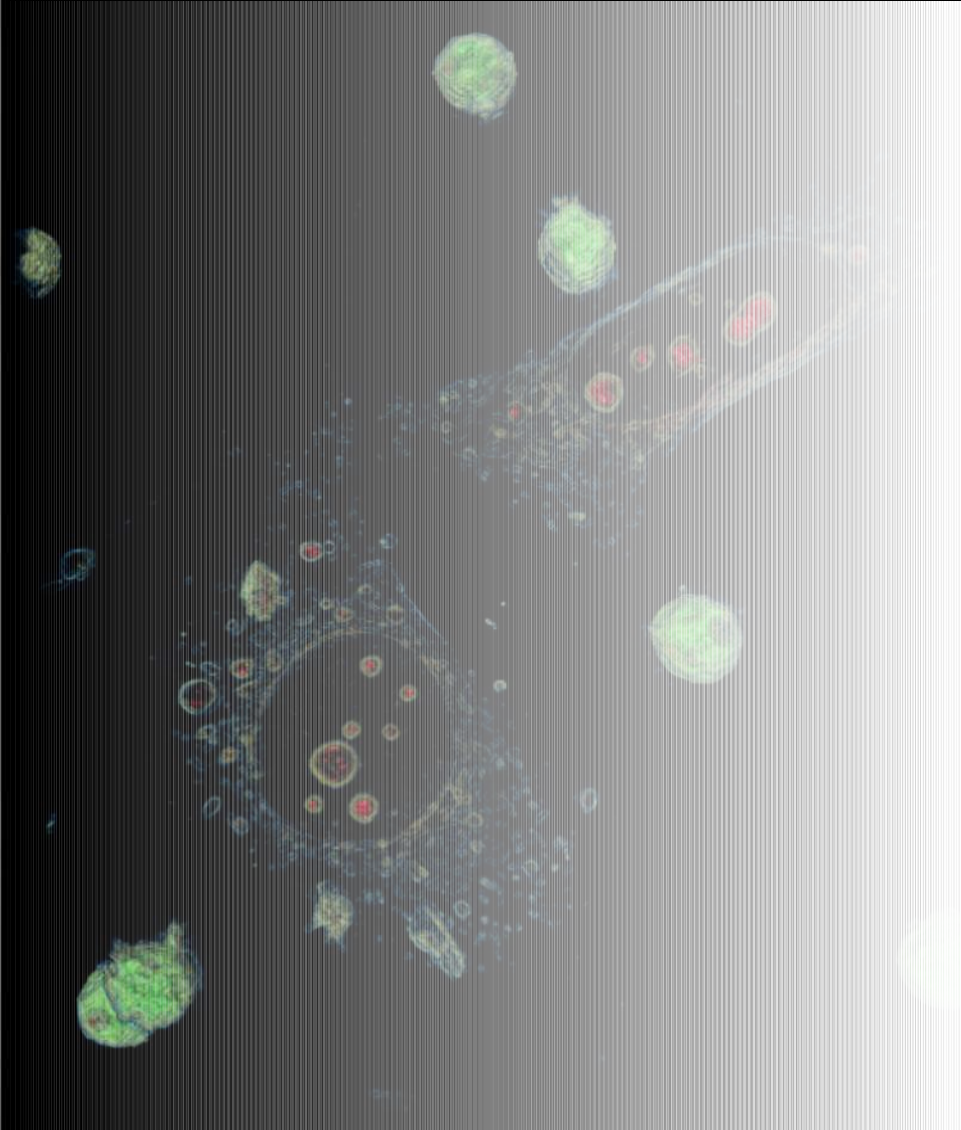
Click on "OK" and the histogram is displayed (4).

**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.





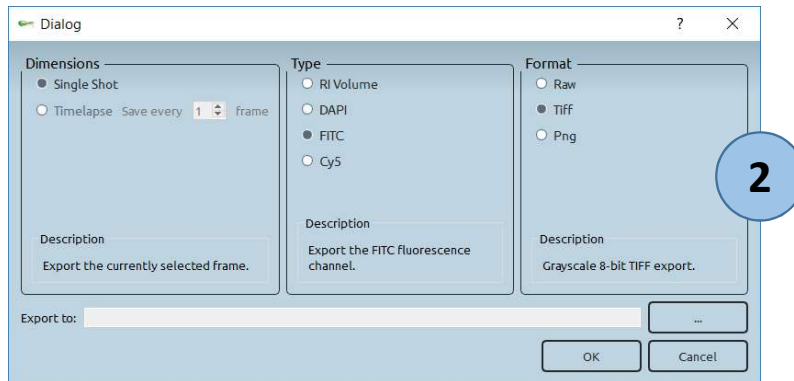
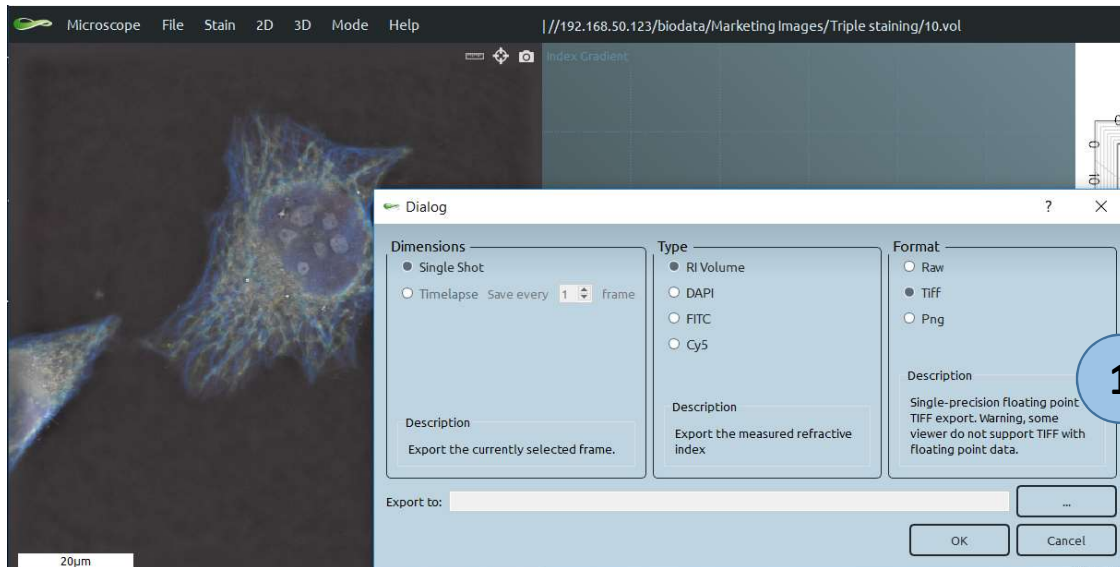
## 3D Cell Explorers

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

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.

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.

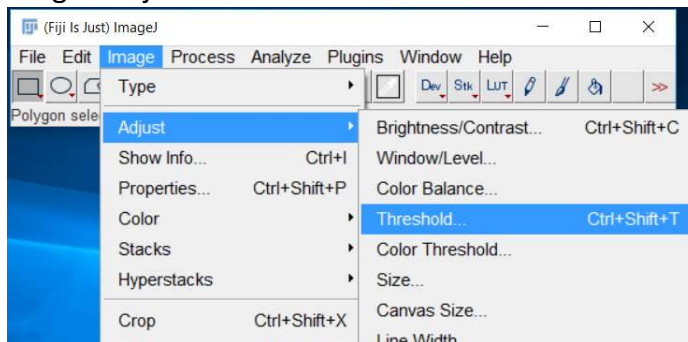
Then, from STEVE, **Export** the Fluo data in a .tiff format. Select the appropriate Fluo channel to export.

Select a folder and define a filename

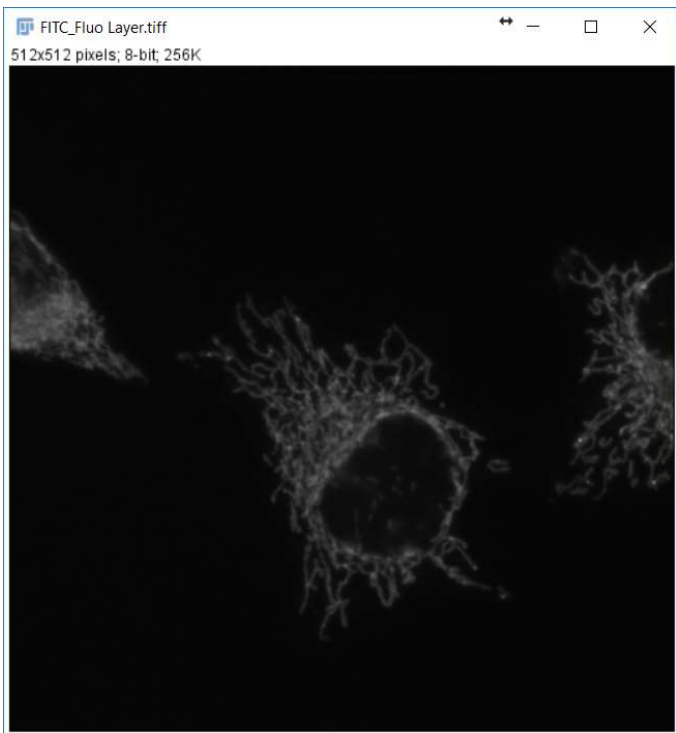
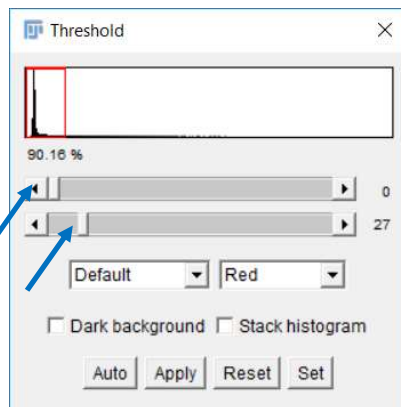
Click OK (2)

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

1. 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.



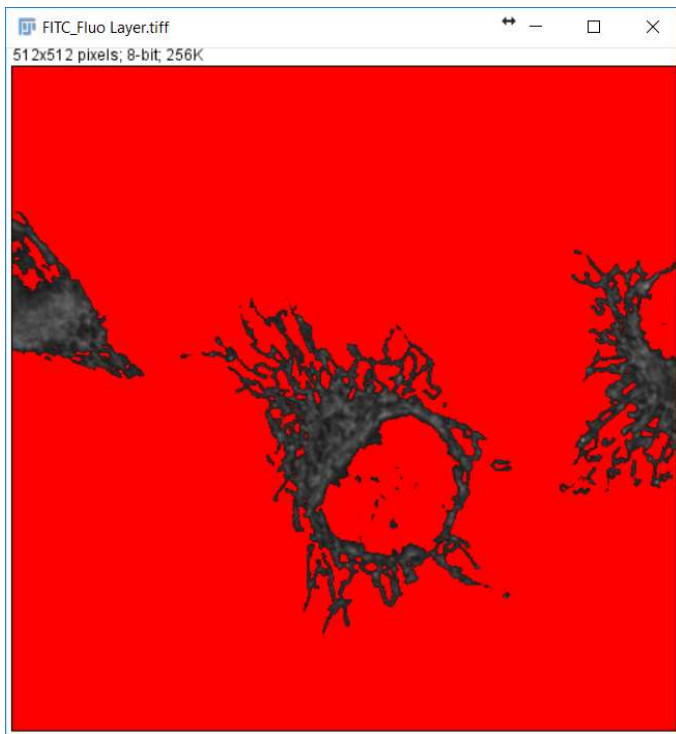
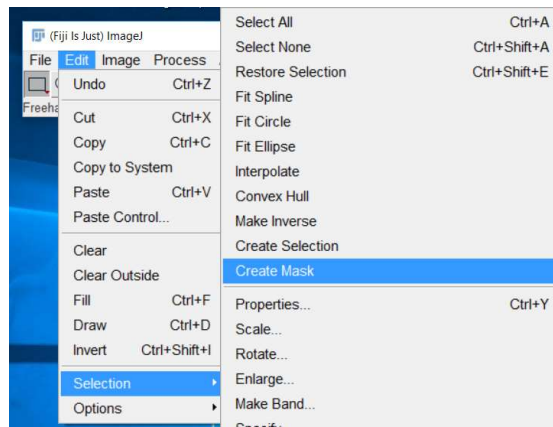
Before



After

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.



Before

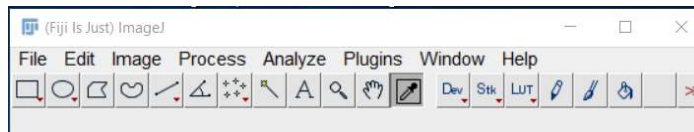


After

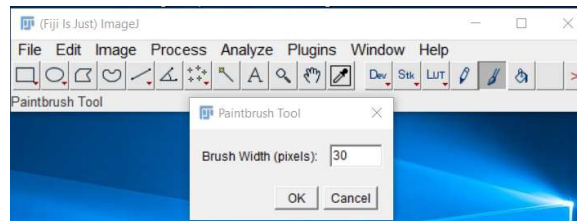
2. A new image appears, named "mask". The black region are the unselected pixels, value is 255. The white region are the selected pixels, value is 0.
3. Close the "red" source image.

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.



2. Select a black zone in the mask image.
3. In the Fiji tool bar, select the “brush” tool. By a right click, adjust the brush size.



4. Re-paint the unwanted white areas in black.



Before

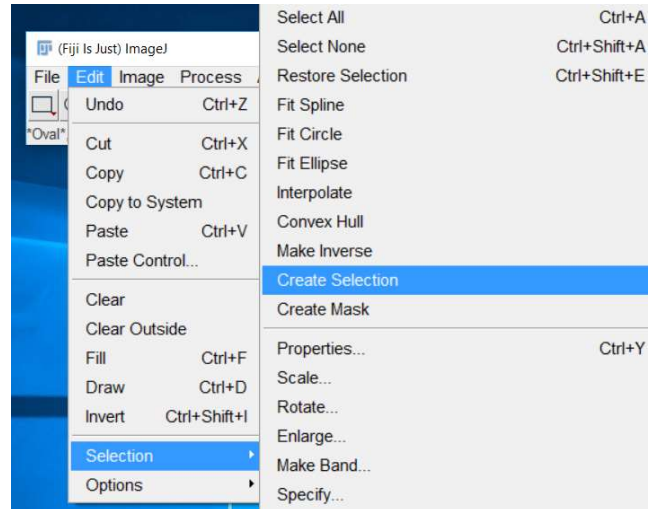


After

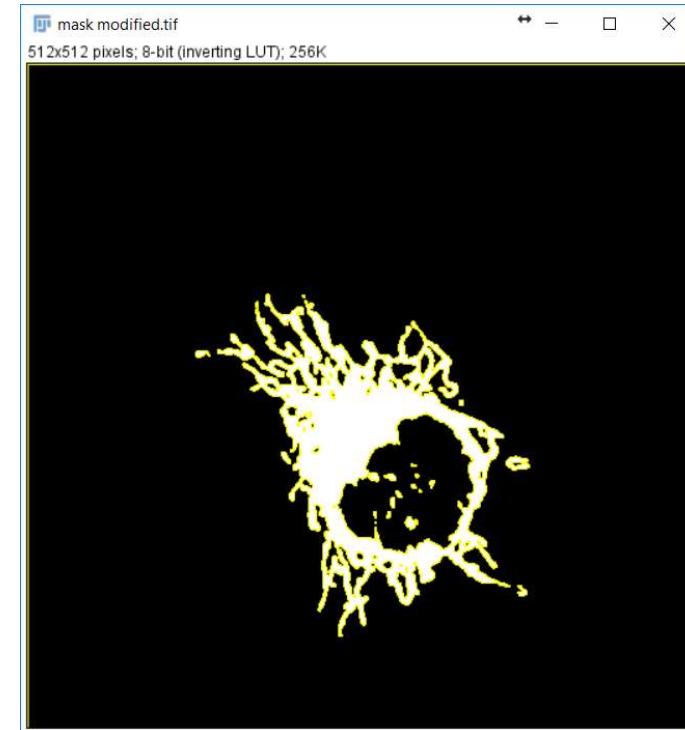


This operation allows to define the ROI contour inside the mask image.

1. Run the command Edit > Selection > Create Selection

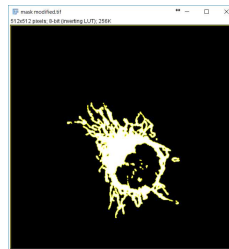
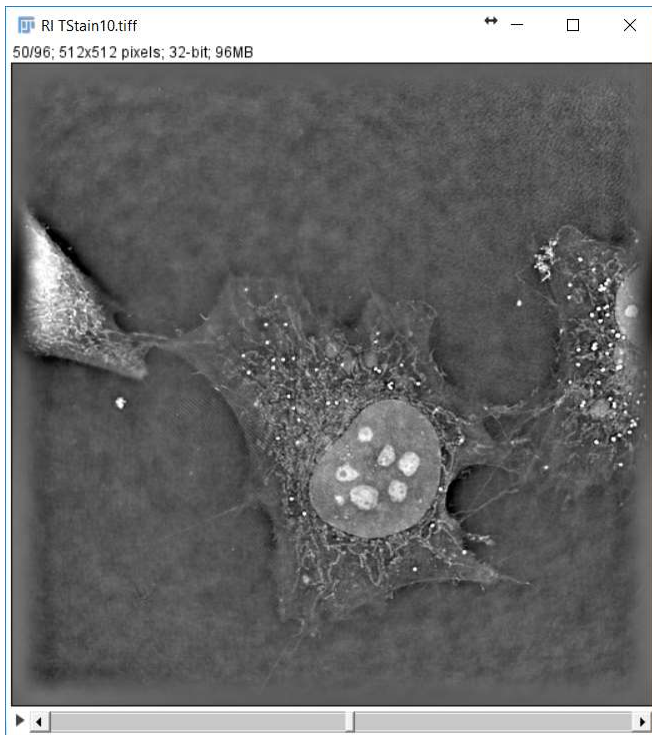


The borders of the ROI is highlighted in yellow now

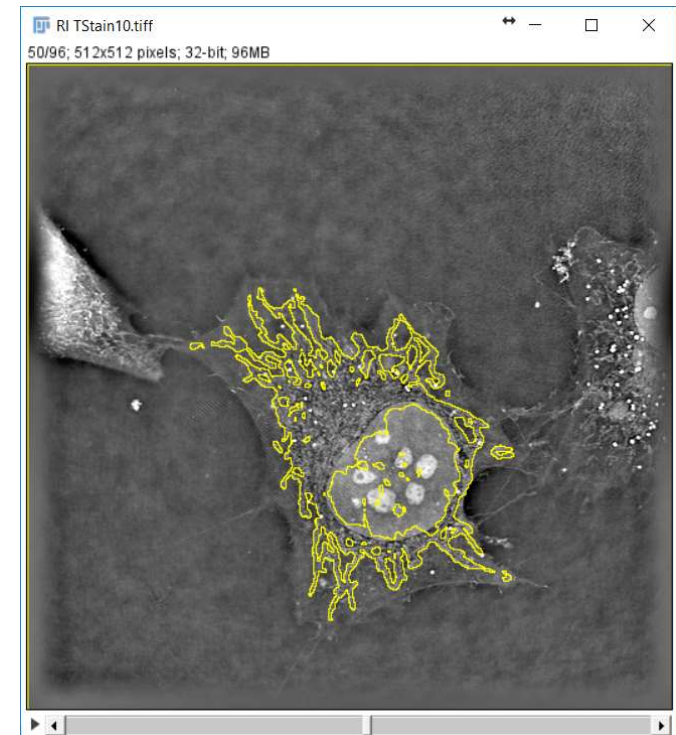


This operation allows to apply the ROI contour on the Refractive Index Image.

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 described in this presentation).
3. 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.



# Correlate Refractive Index and Fluorescence information

## Display RI Data Histogram

**1**

**2**

**3**

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

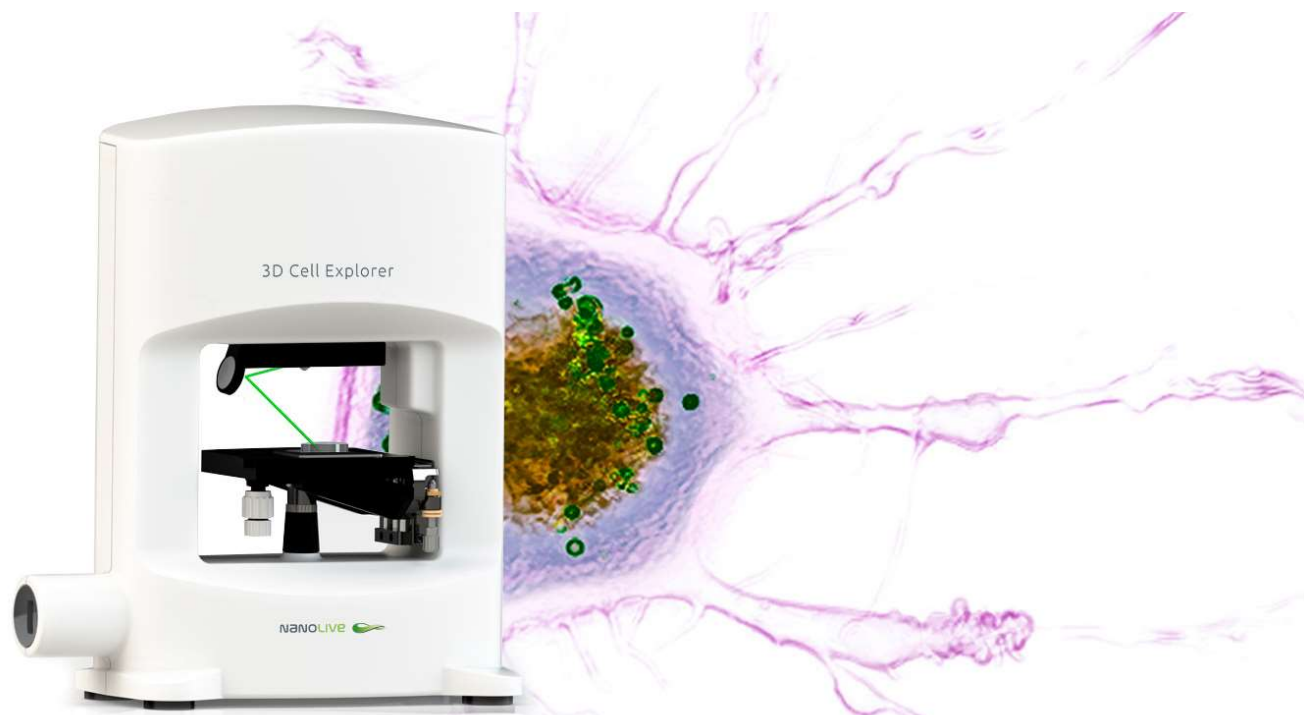
A pop-up window histogram is showing up (2) to adjust histogram settings (bins, min, max...).

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.



Awards



Partners

