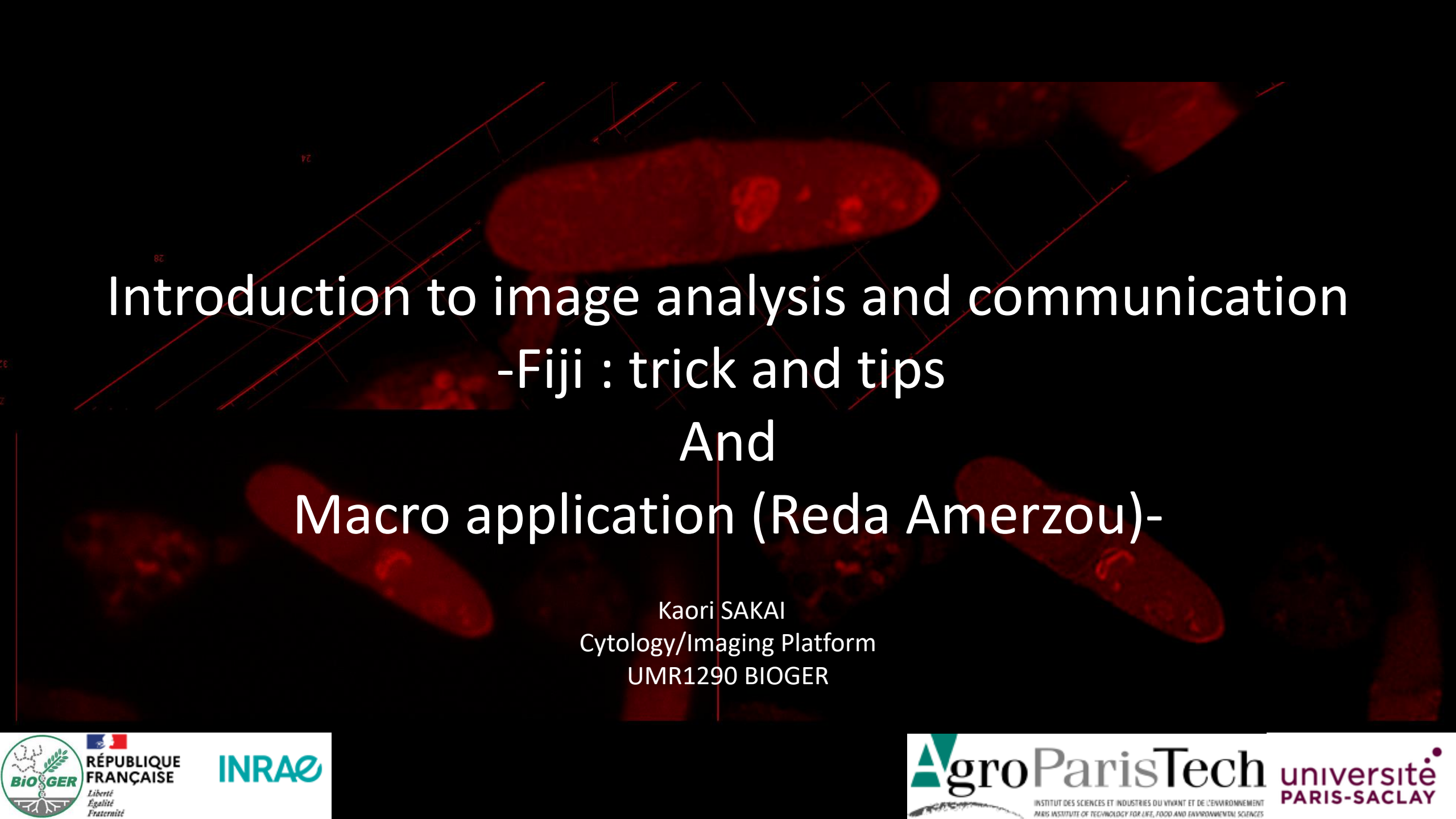




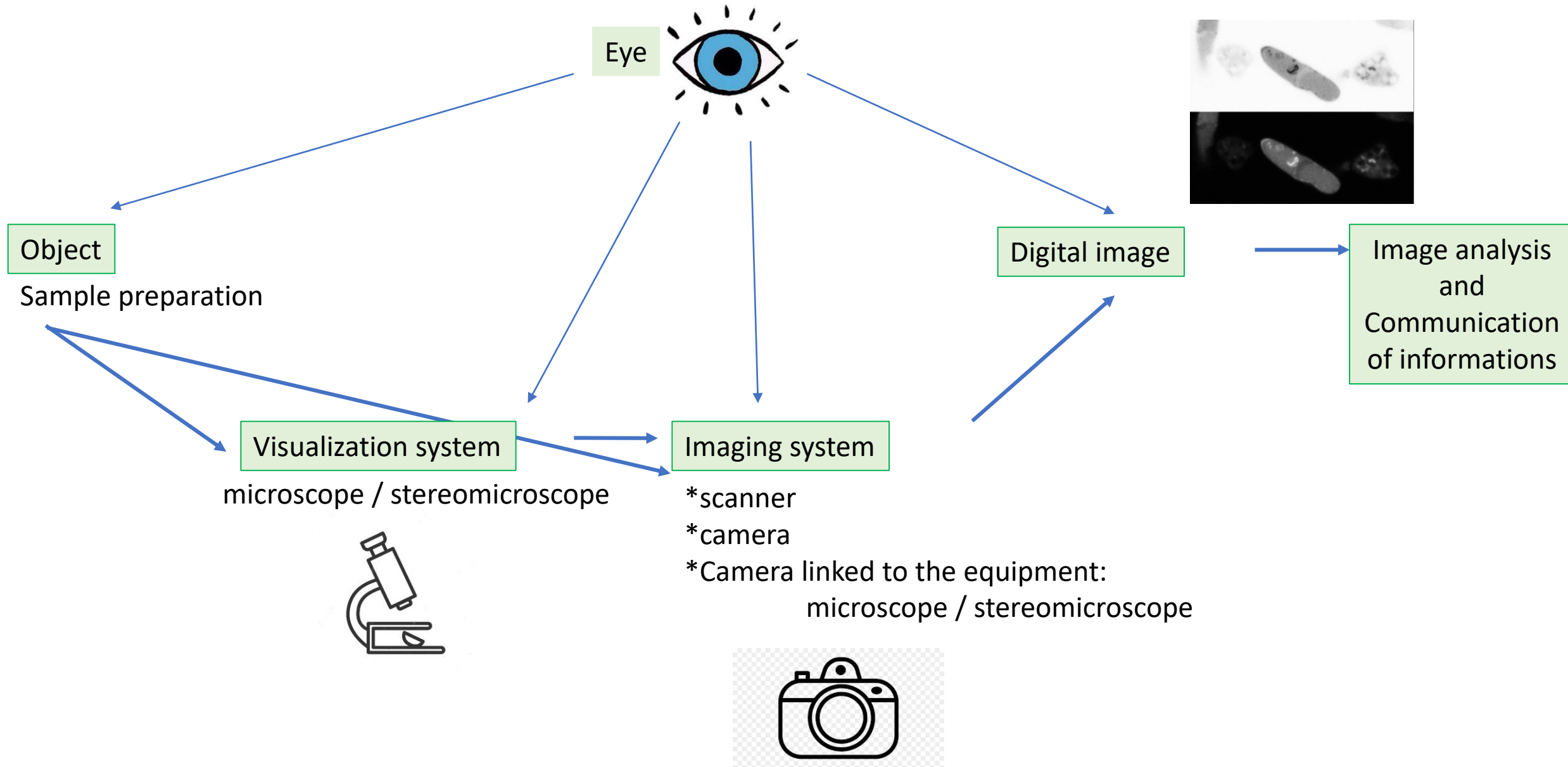
Introduction to image analysis and communication -Fiji : trick and tips And Macro application (Reda Amerzou)-

Kaori SAKAI
Cytology/Imaging Platform
UMR1290 BIOGER



Reminder : What is an image?

An image is a partial representation of reality



Reminder...

Samples preparation

*Mounting media



Immersion media:
refraction index

Visualization system

Light path should be clean
No dust !!

Imaging system (camera)

Exposure time
File format when saving data

Analyse et manipulation des images

Attention !! Improper handling of images can destroy information because it is based on "false" results.

(conserve as much as possible raw data!!)

Plastic surgery of image can change the conclusion of image analysis!!

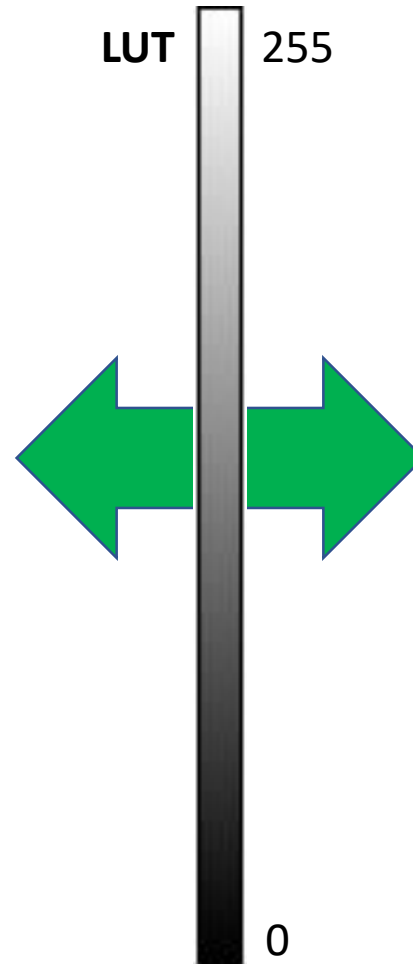
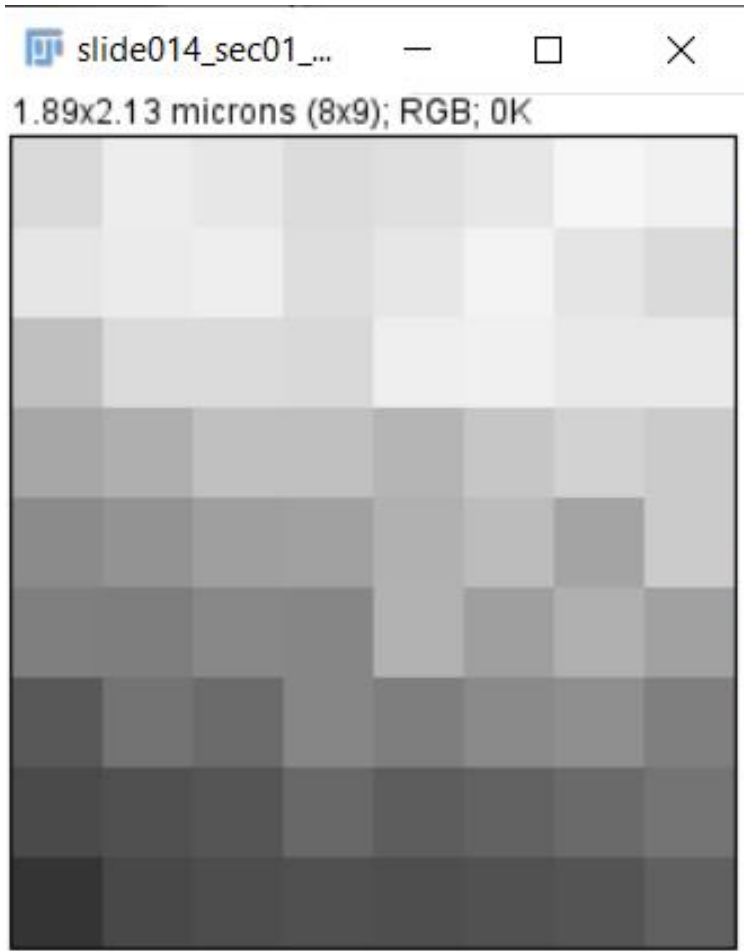
https://www.youtube.com/watch?v=c_Oi2HKom_Y

In Defense of Image Data & Analysis Integrity

Reminder : What's a digital image?

LUT (Lookup Table) : Correlation table

Allows to correlate numerical value and the visual aspect of the image.



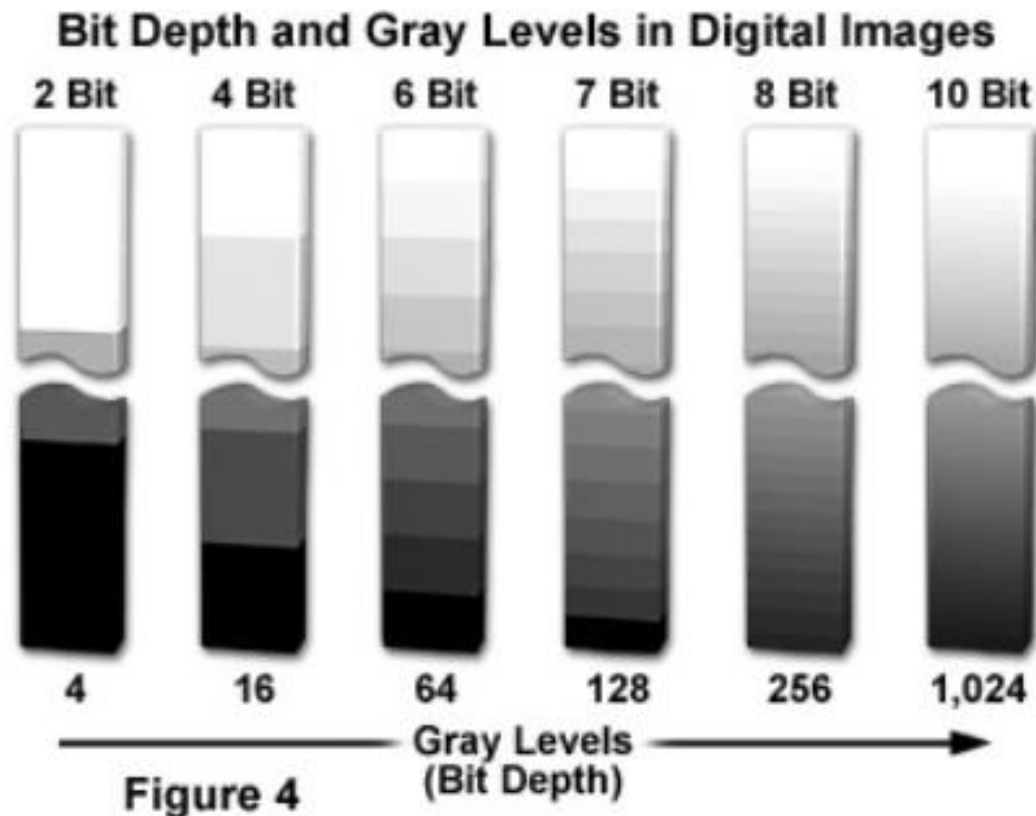
2 Pixels: (PICTure ELeMENT)

Grey level

234	218	237	231	220	224	231	246	241
216	230	235	238	222	231	243	229	218
189	192	219	219	217	239	240	233	233
148	167	175	192	192	181	198	210	203
137	139	147	159	161	177	188	164	203
119	127	126	136	134	178	159	176	161
92	88	115	107	134	126	138	143	127
55	74	80	85	104	93	98	106	116
64	52	72	77	80	78	81	84	96

Reminder : Image dynamic of a digital image

Notion of bit (Binary digit) : image dynamic



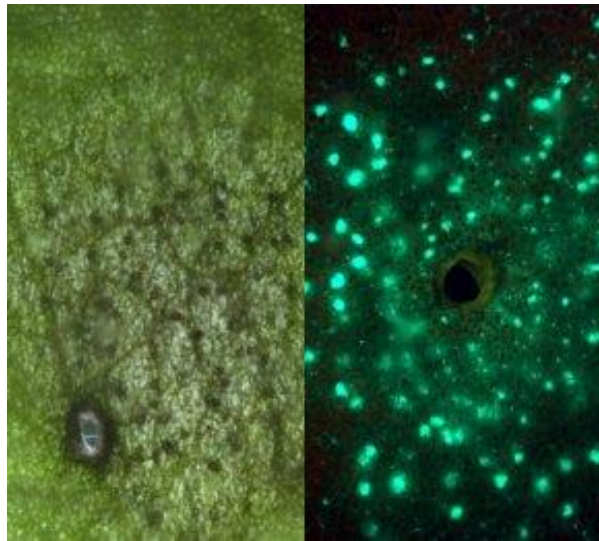
A n bit image can contain up to 2^n bits of different grey levels.

... 16bit

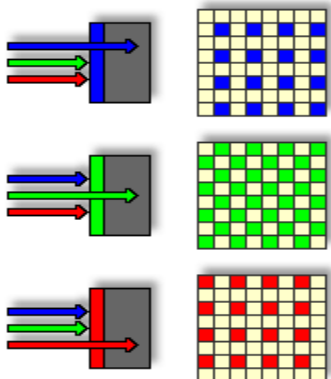
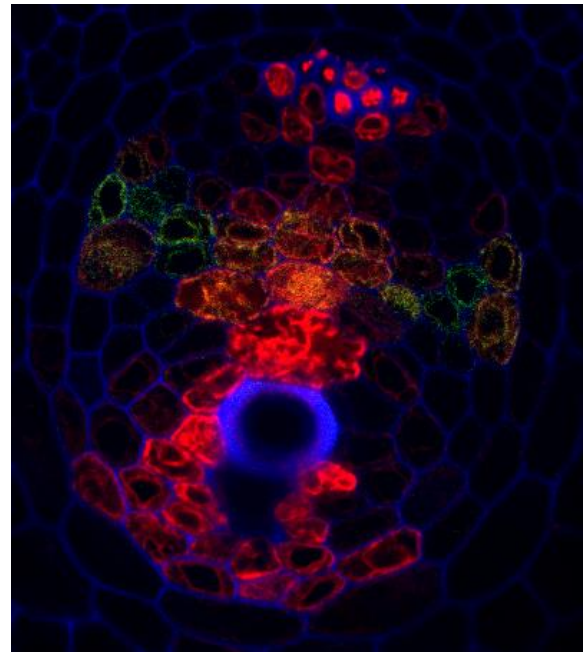
<http://zeiss-campus.magnet.fsu.edu/articles/basics/digitalimaging.html>

Reminder : « colour » and « false-color » image

« Colour » image

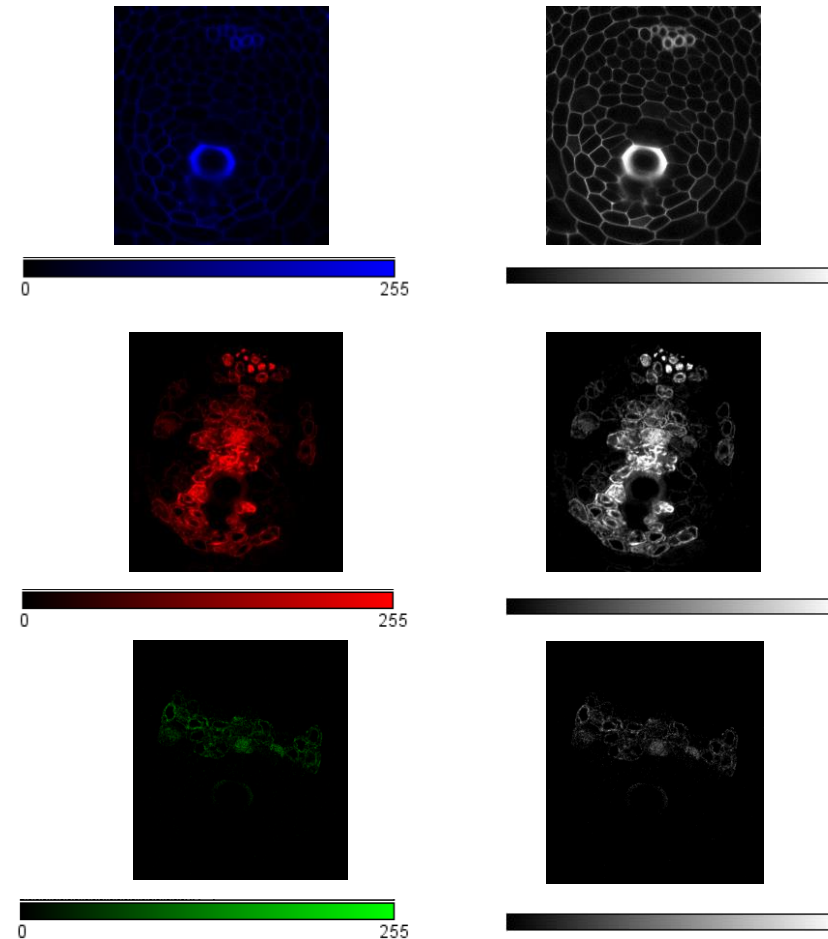


« False-colour » image



Light filter

An RGB image contains informations fom R + G + B channels.



Playing with LUTs, some information is better revealed!

Image format

(Major reason for mistakes during image analysis!!)

Prioritize manufacturer format or OME tiff format when analysing image data or want to publish images.

Otherwise

Attention: 2 type of tiff format

OME Tiff (Metadata kept) : Open Microscopy Environment

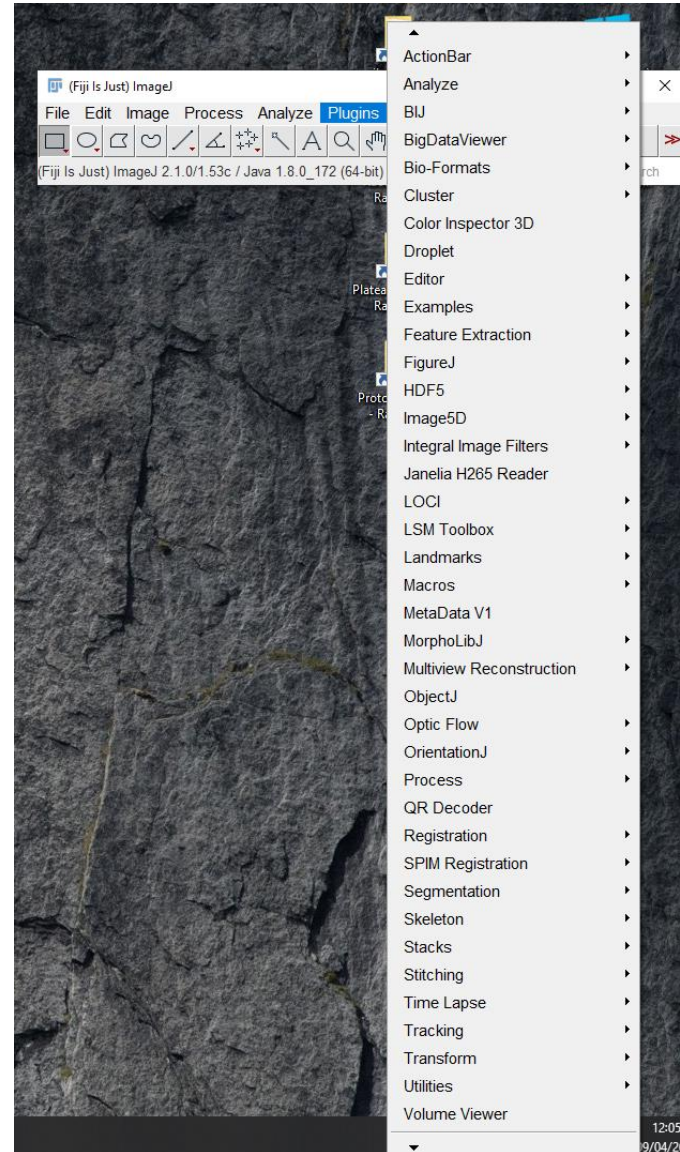
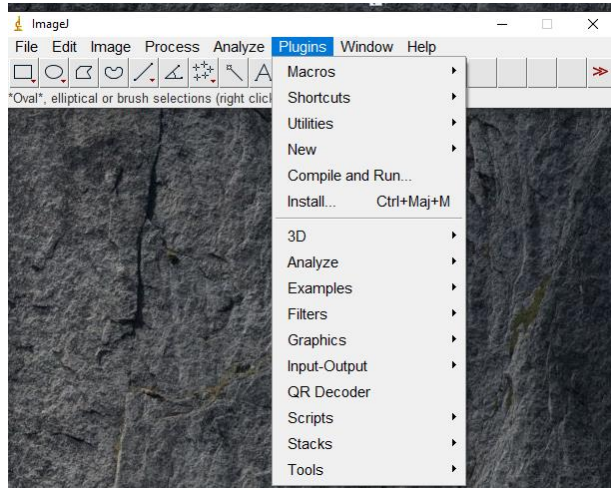
Tiff : Metadata lost

Illustration : png/jpeg

Compressed format OK, you don't need details and it allows also to avoid a heavy file like over 200Mo.

**Download Fiji and
Open an image**

Why Fiji? And not ImageJ



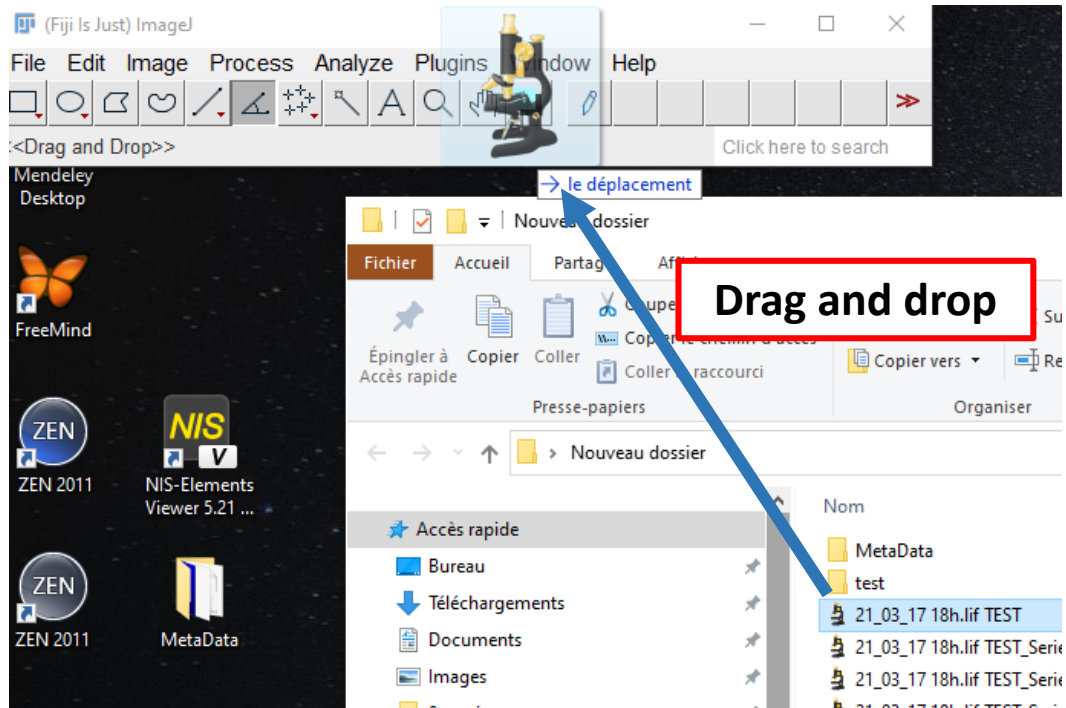
<https://imagej.net/Fiji>

Fiji is just ImageJ with options already installed.

For users - Fiji is easy to install and has an automatic update function, bundles a lot of plugins and offers comprehensive documentation.

For developers - Fiji is an open source project hosted in a Git version control repository, with access to the source code of all internals, libraries and plugins, and eases the development and scripting of plugins.

Open files : drag and drop



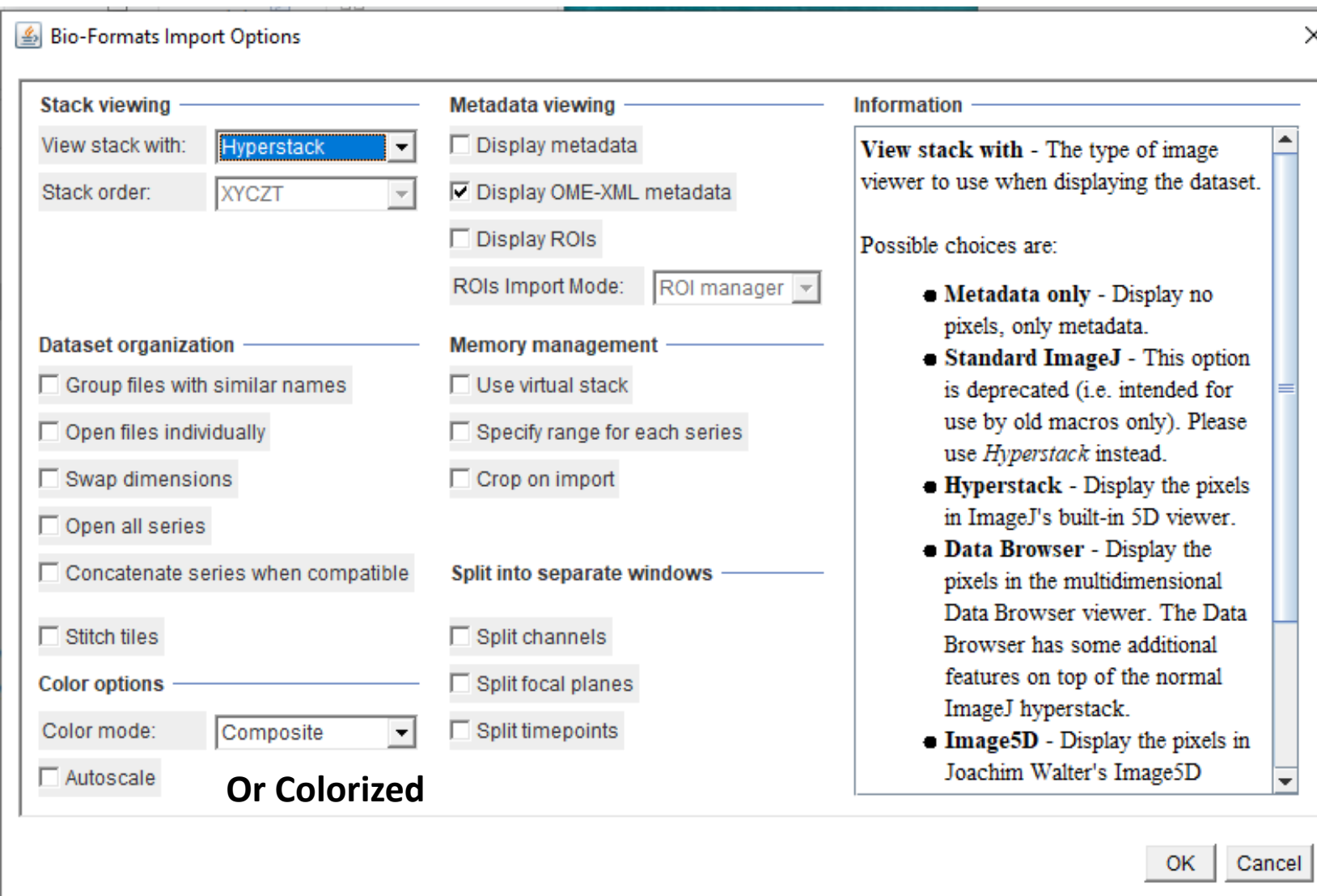
**If single image: directly open
image with 1 dimension**

This is the case for classical format :
png, jpeg, in some case tiff

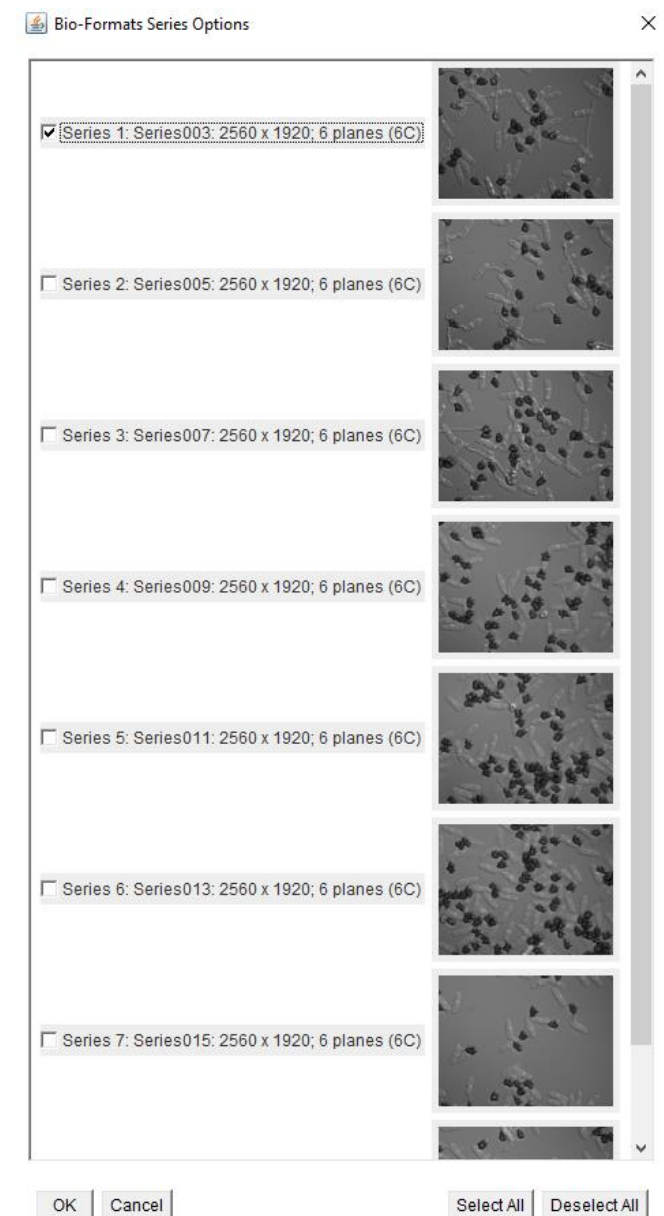
**If the file is in manufacturer format and
contains several images : 2 steps**

Manufacturer format is : lsm, lif, lef, nis etc...

Open file which contains several images : 2 steps

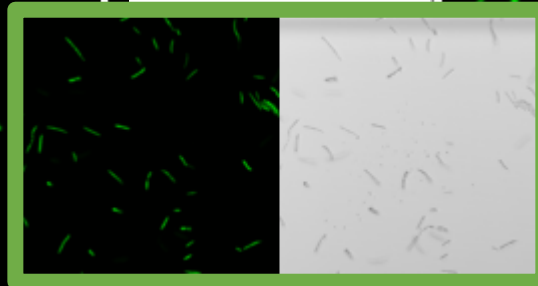
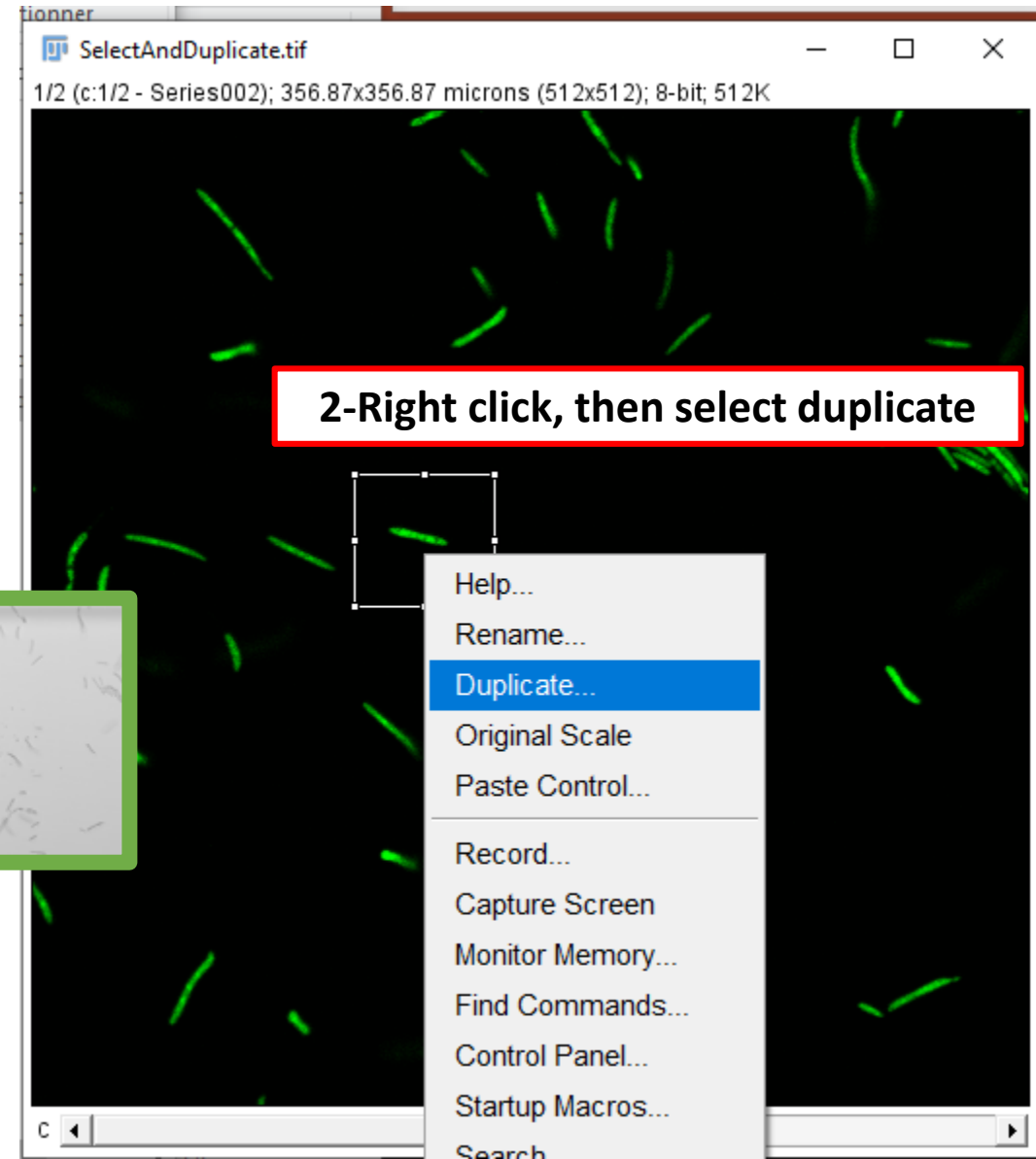
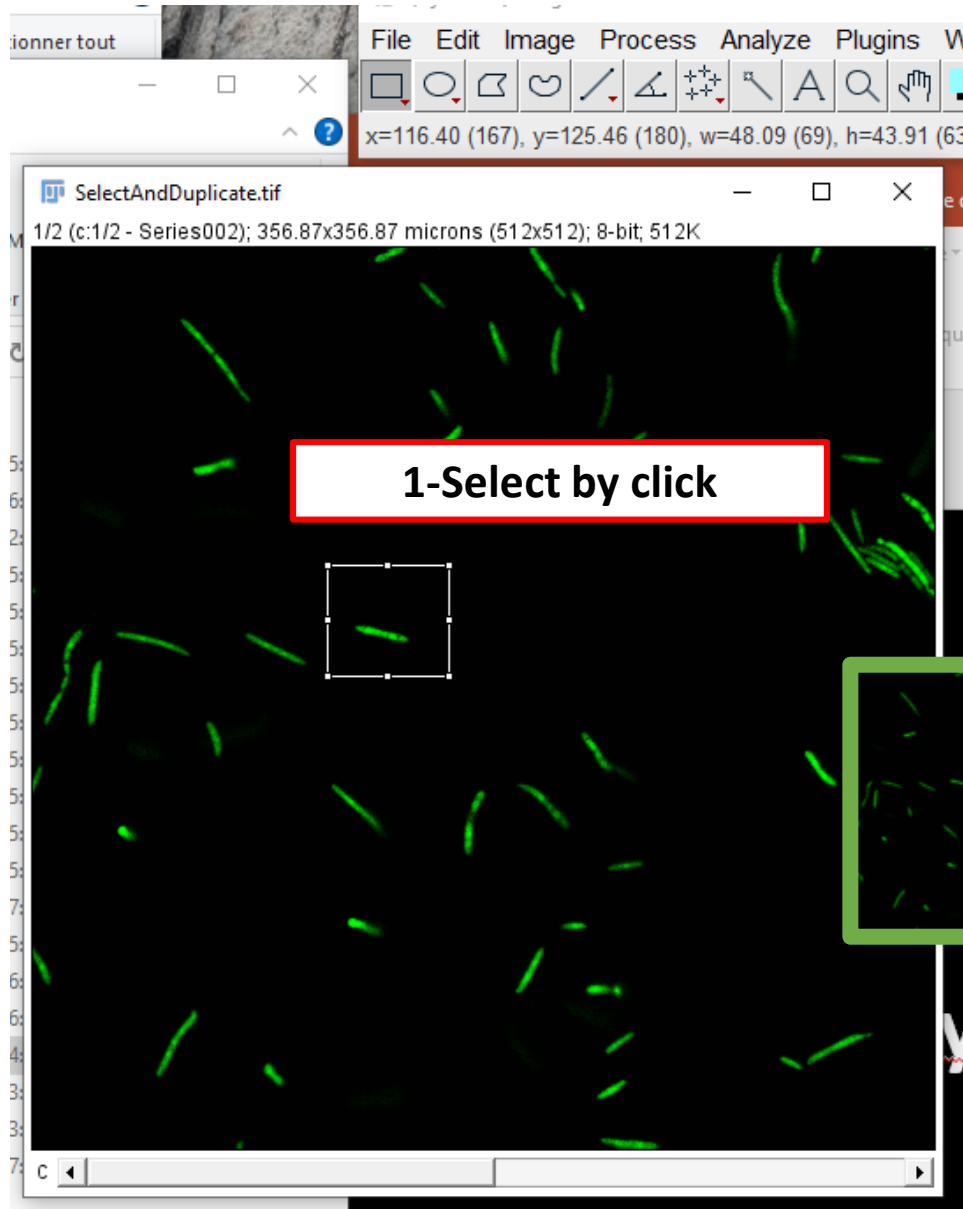


Or Colorized

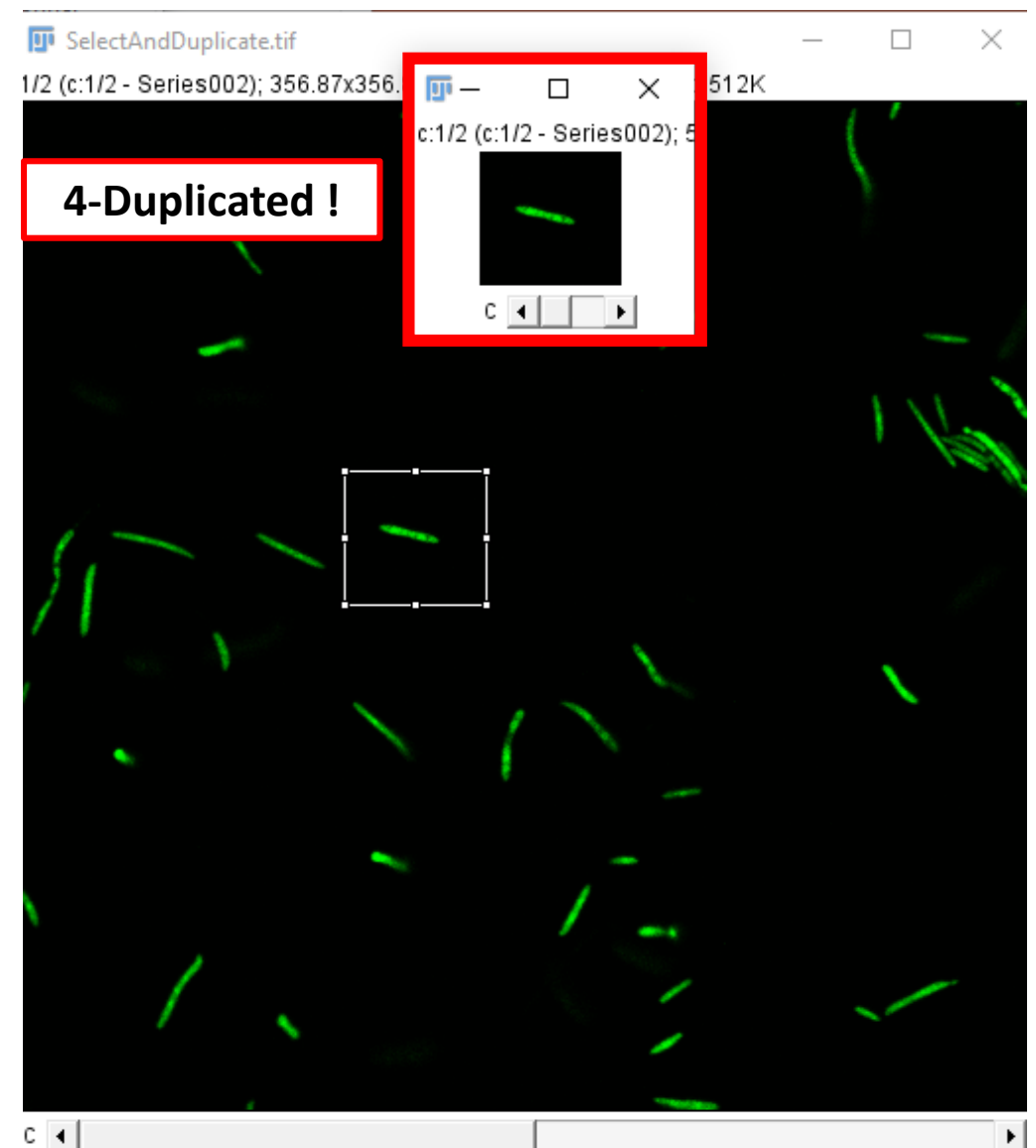
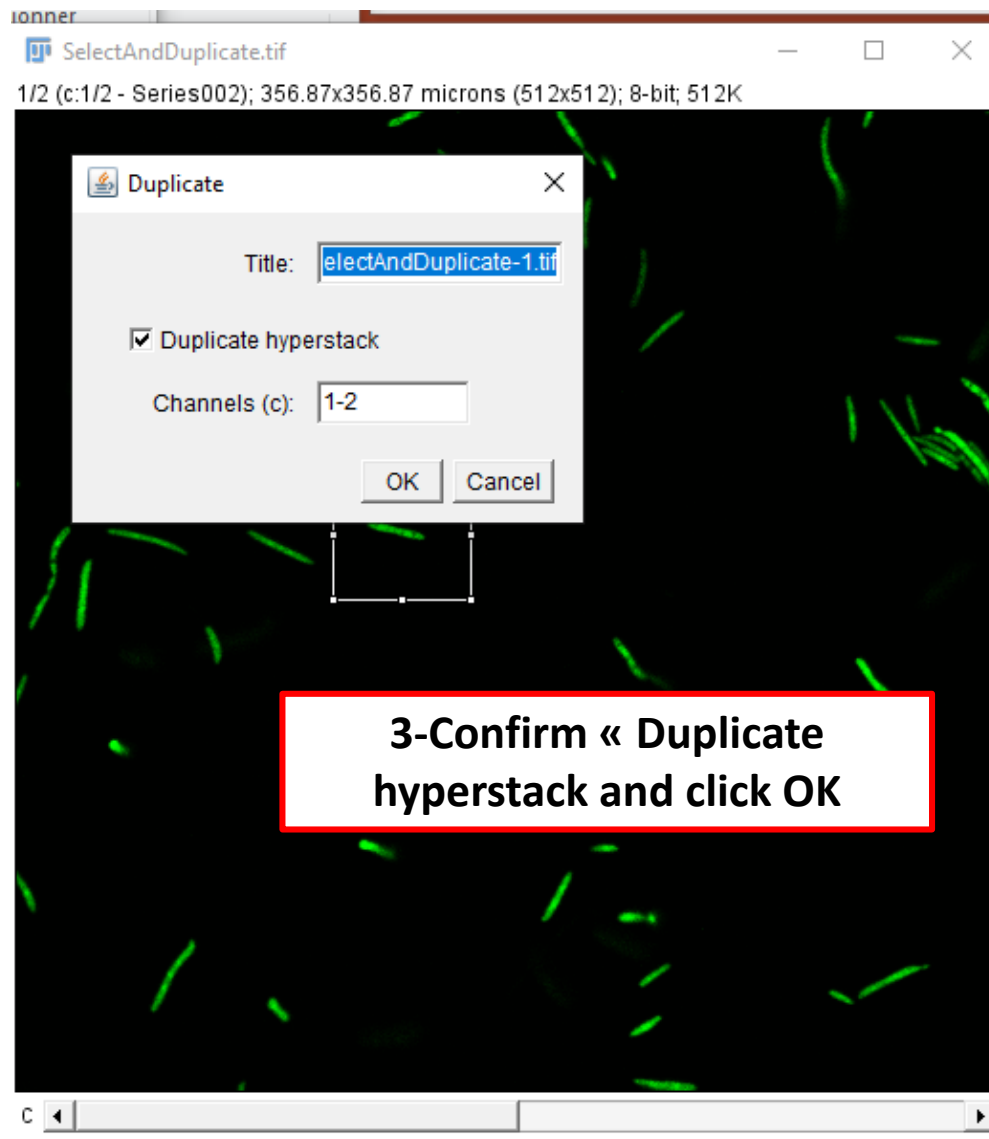


**Select and duplicate
a region of your image**

Useful when you have a multichannel image or z-stack (1)

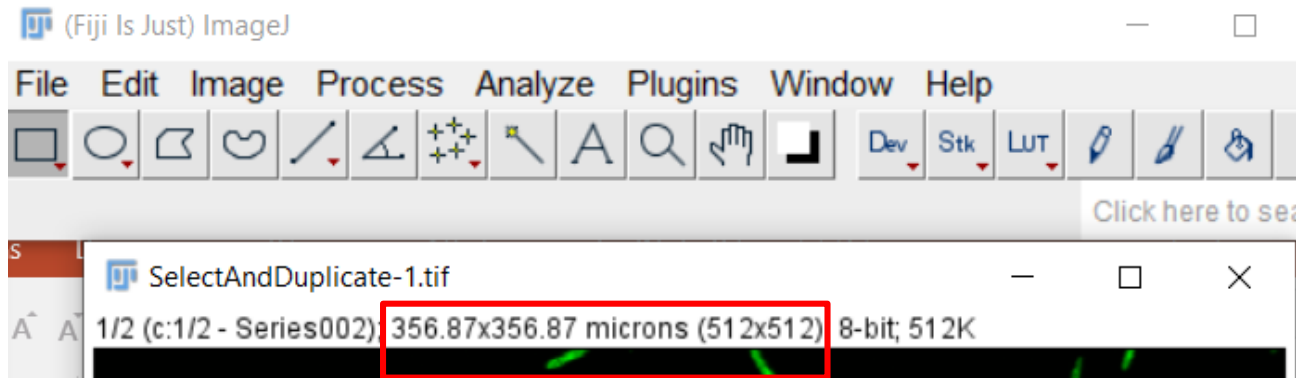


Useful when you have a multichannel image or z-stack (2)

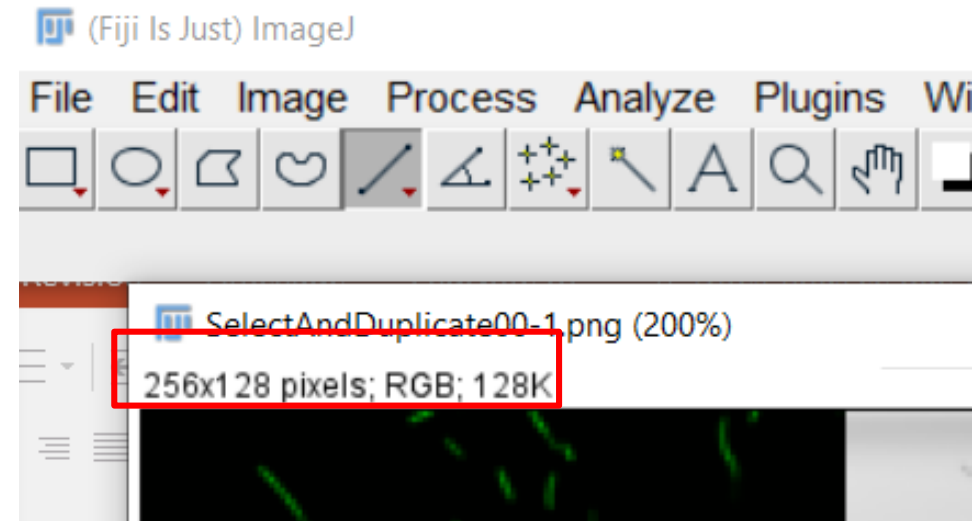


Set scale and show scale bar

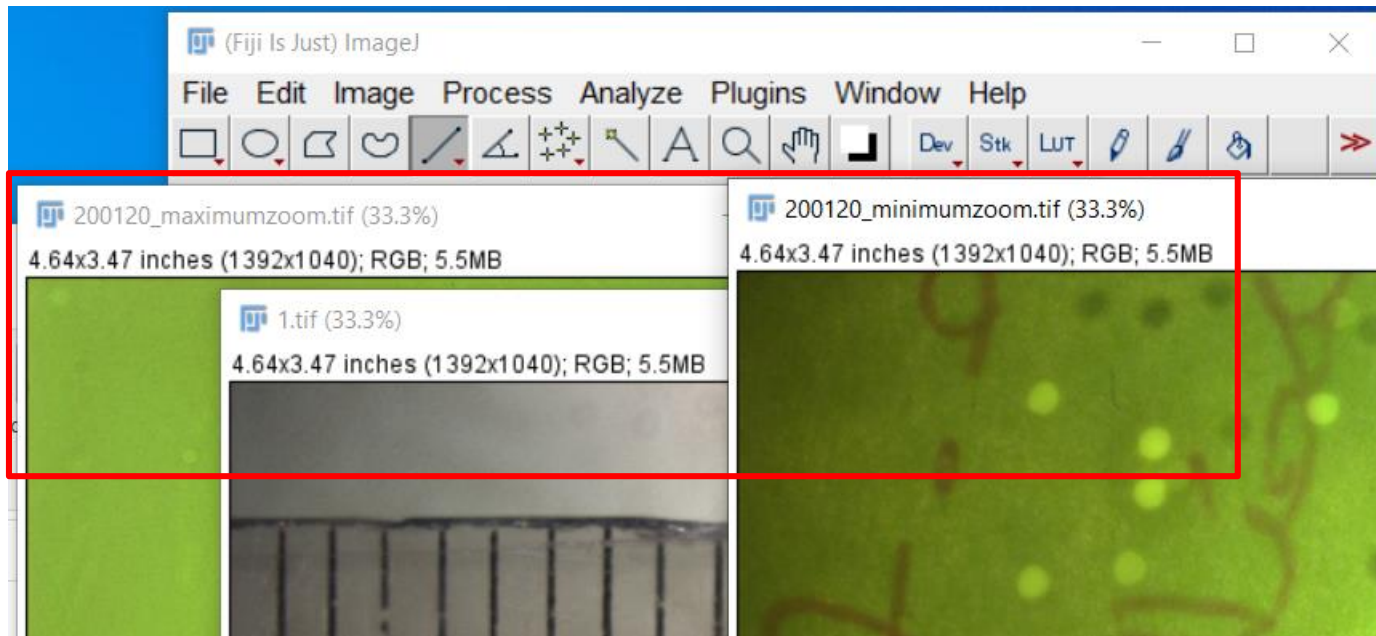
How to check if pixel size is already known or not



= 512 pixel = 356,87 microns



Pixel size not known

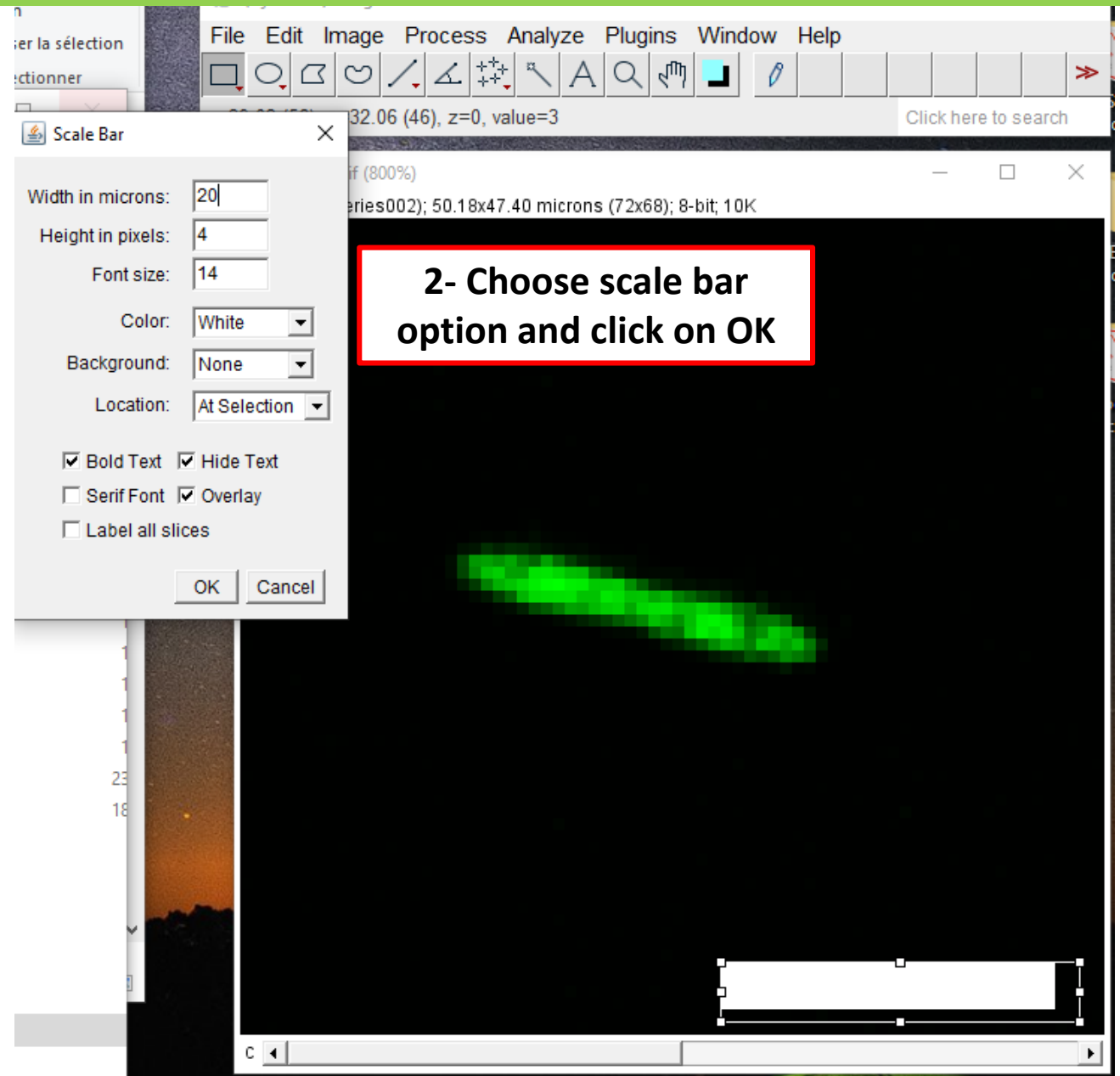
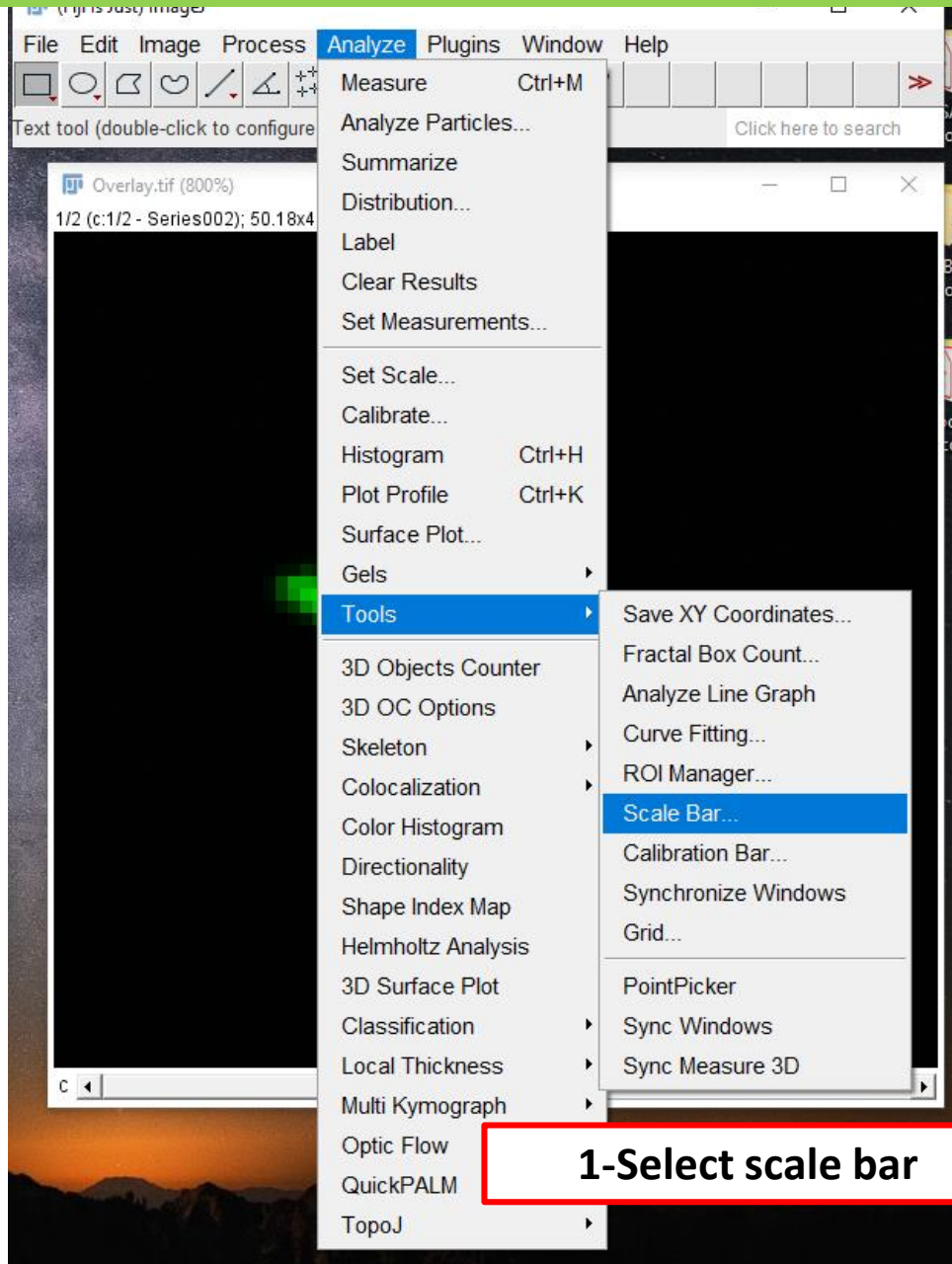


Attention!

When unit is in inch be careful!!

Ex. Stereomicroscope, with different zoom, same pixel size. This is NOT possible.

Show scale bar when pixel size is already known



Set Scale, in case pixel size is NOT known (1)

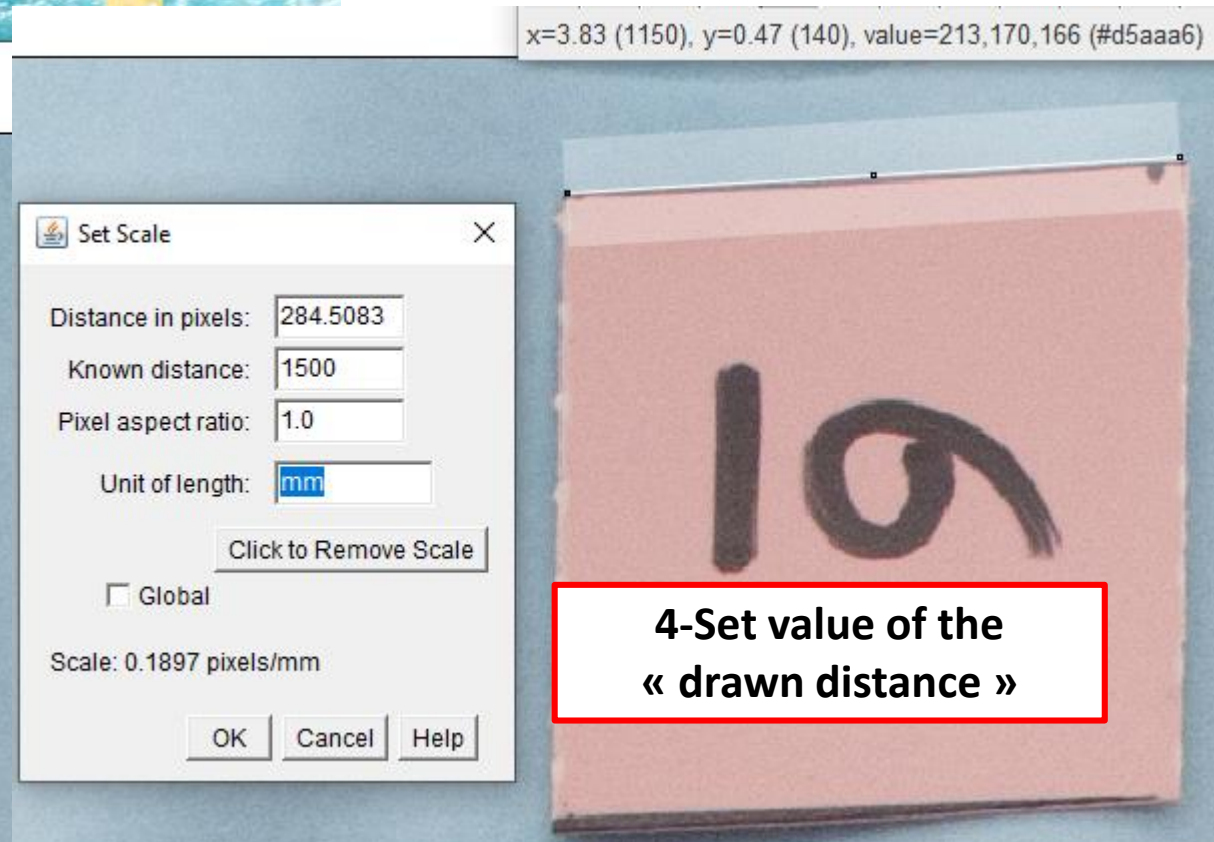
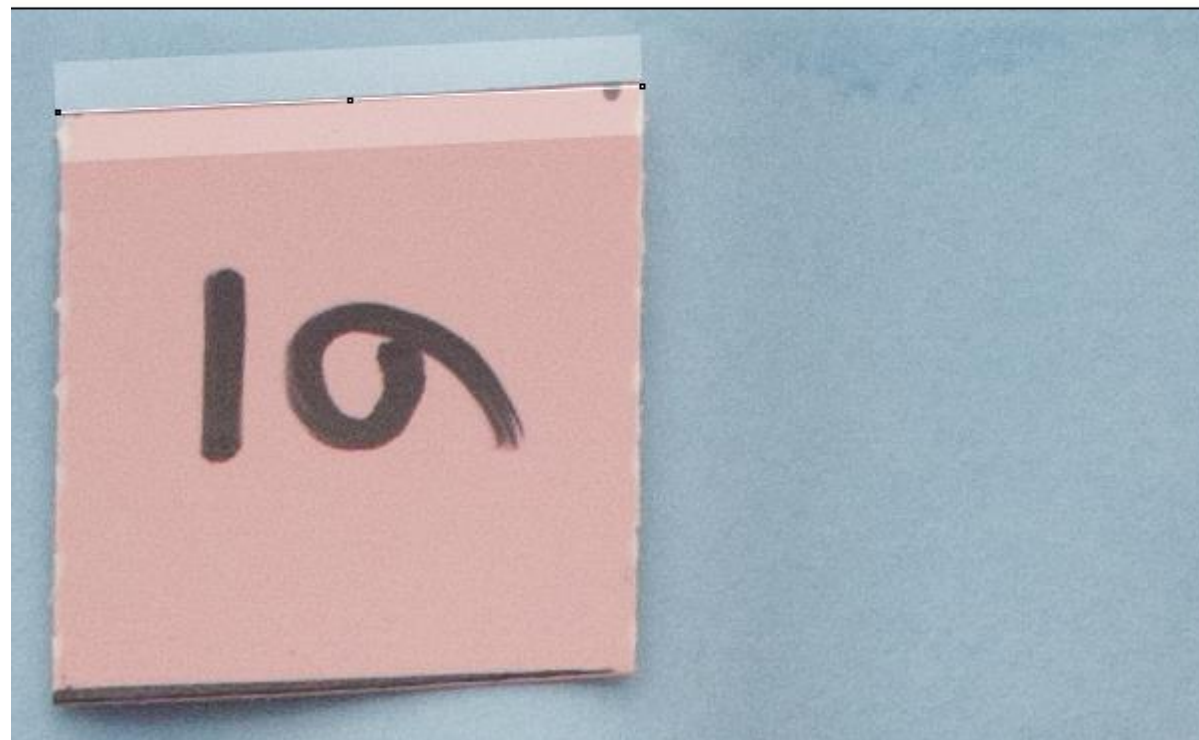
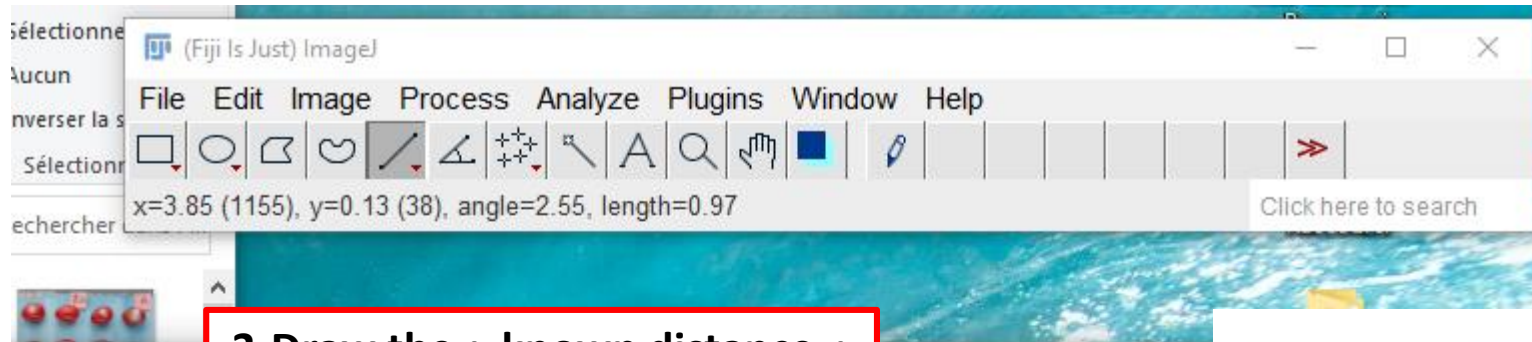
The image is a composite of three screenshots from the Fiji ImageJ software interface, illustrating the steps to set a scale for an image.

Top Left Screenshot: Shows the 'Analyze' menu open. The 'Set Scale...' option is highlighted. A red box with the text '1-Select « Set scale »' points to this option.

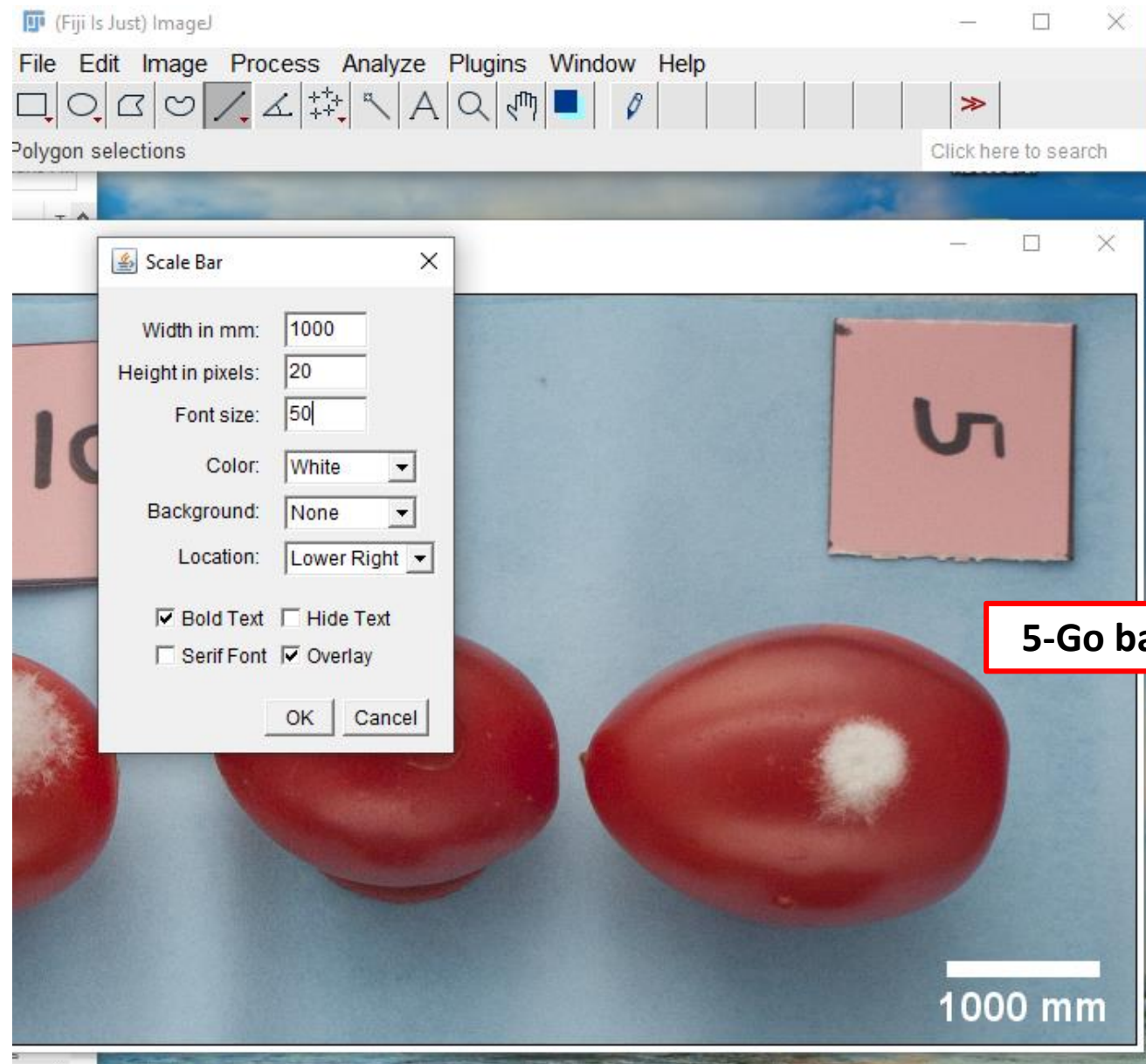
Top Right Screenshot: Shows the 'Set Scale' dialog box. The 'Distance in pixels' field is set to 300, 'Known distance' is 1.00, 'Pixel aspect ratio' is 1.0, and 'Unit of length' is 'inch'. A red box with the text '2- Here you have « Default Value »' points to the 'Distance in pixels' field.

Bottom Screenshot: Shows the 'Set Scale' dialog box with the same settings as the top right screenshot. The 'Scale' is displayed as '300 pixels/inch'.

Set Scale, in case pixel size is NOT known (2)



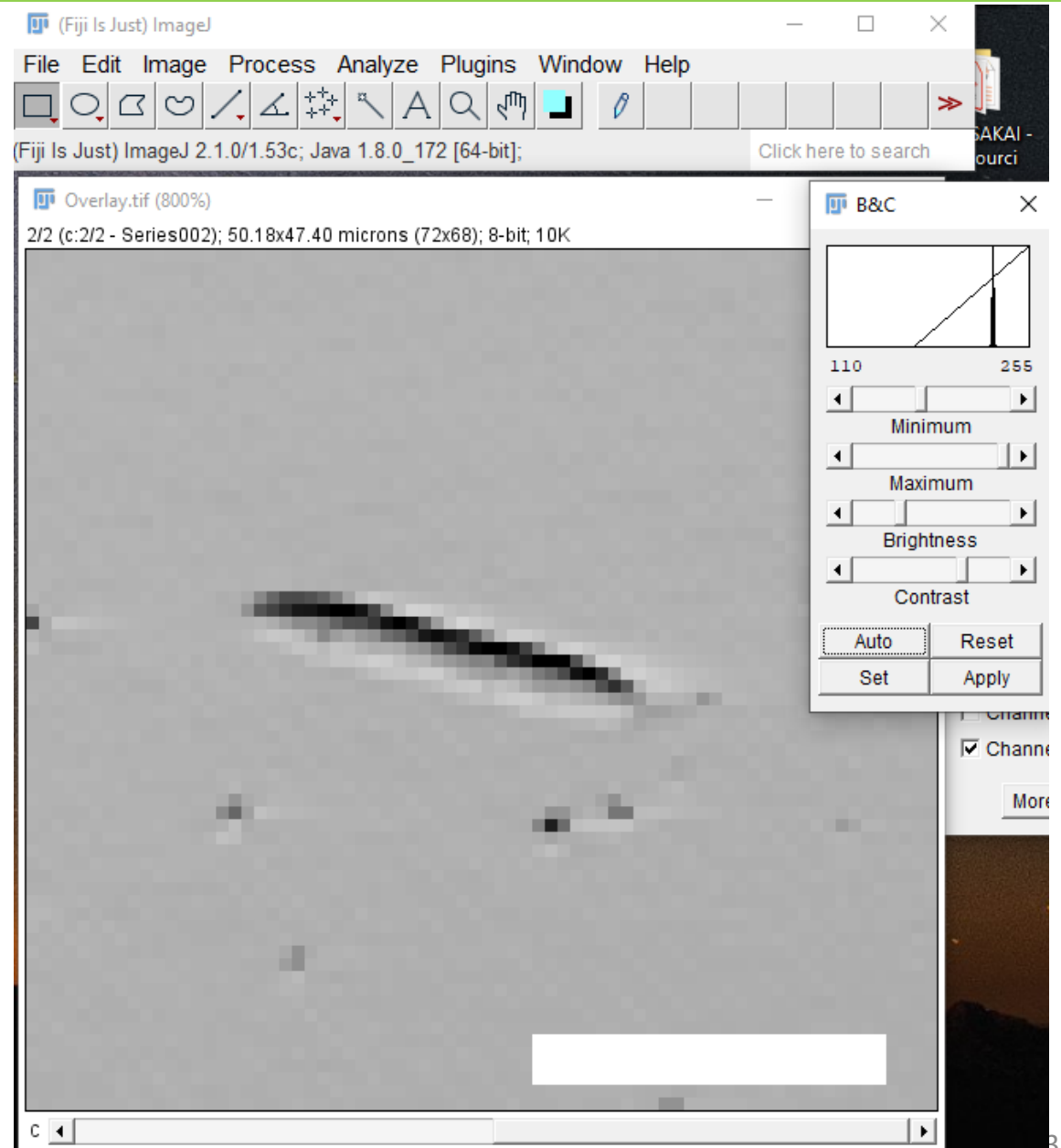
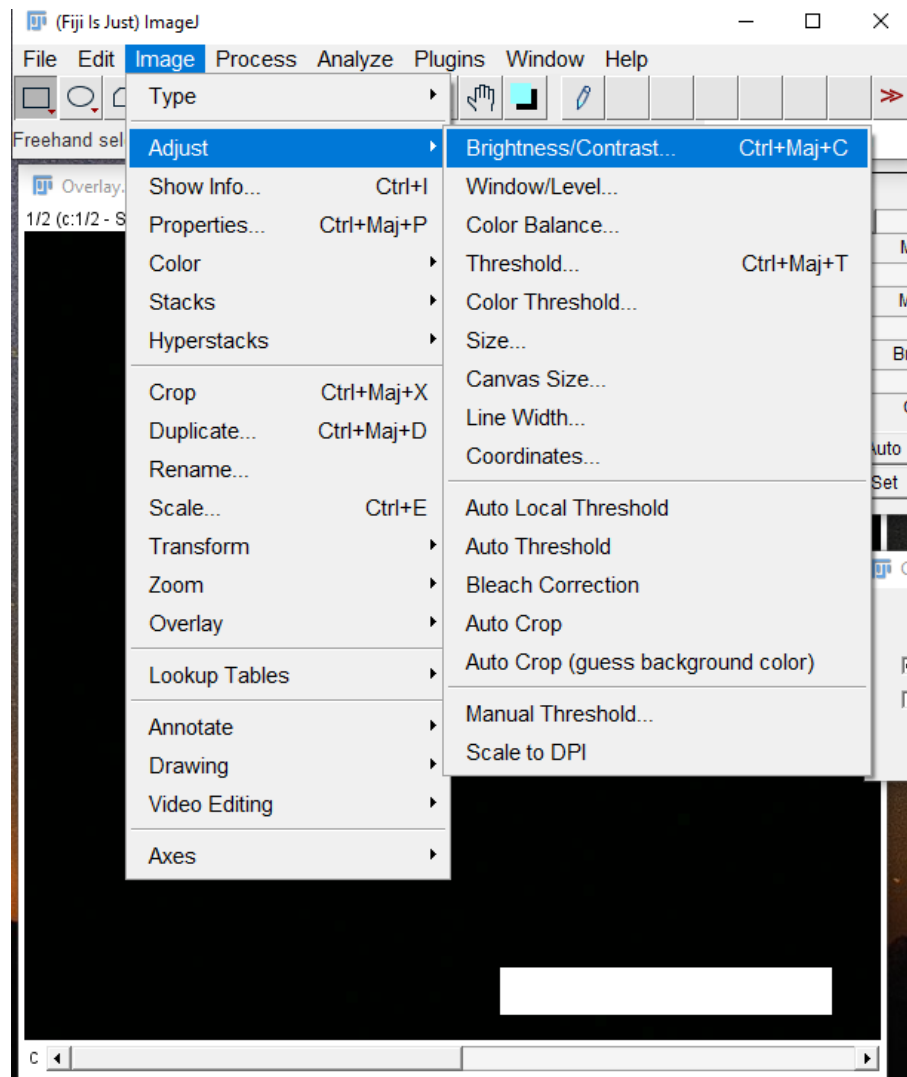
Set Scale, in case pixel size is NOT known (3)



5-Go back to scale bar option

Brightness and contrast

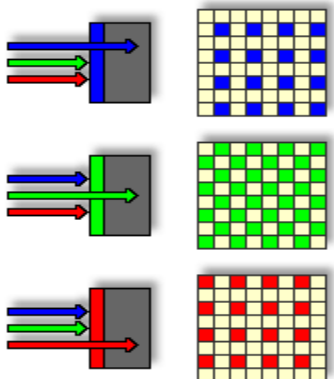
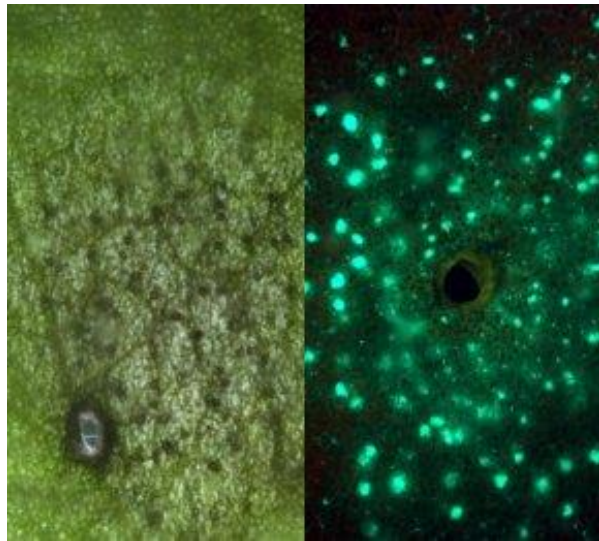
Brightness and contrast



« Overlay channels »
False color images (B/W or detector)
VS
Color images (RGB camera)

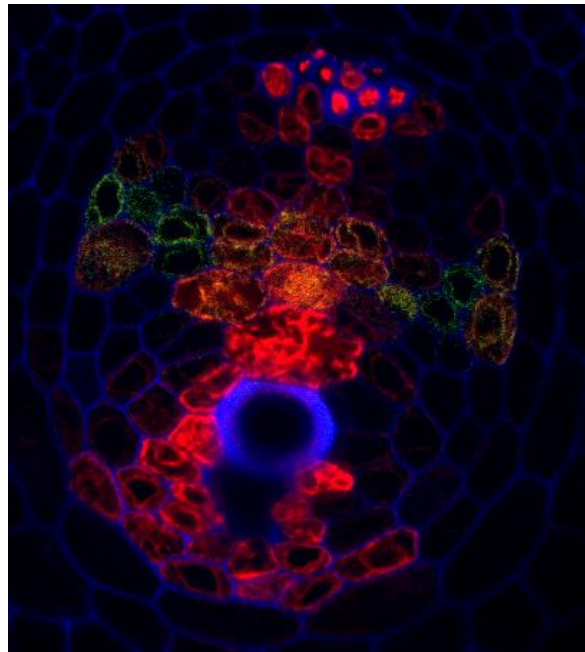
Reminder : « colour » and « false-color » image

« Colour » image

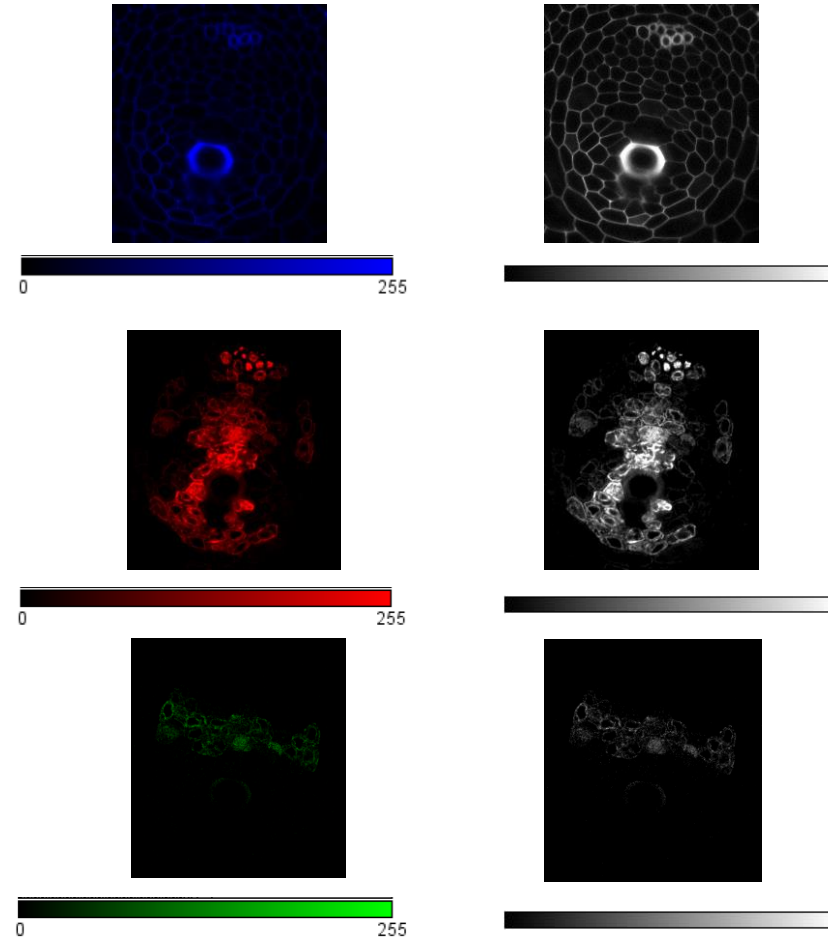


Light filter

« False-colour » image
(8-16bit)



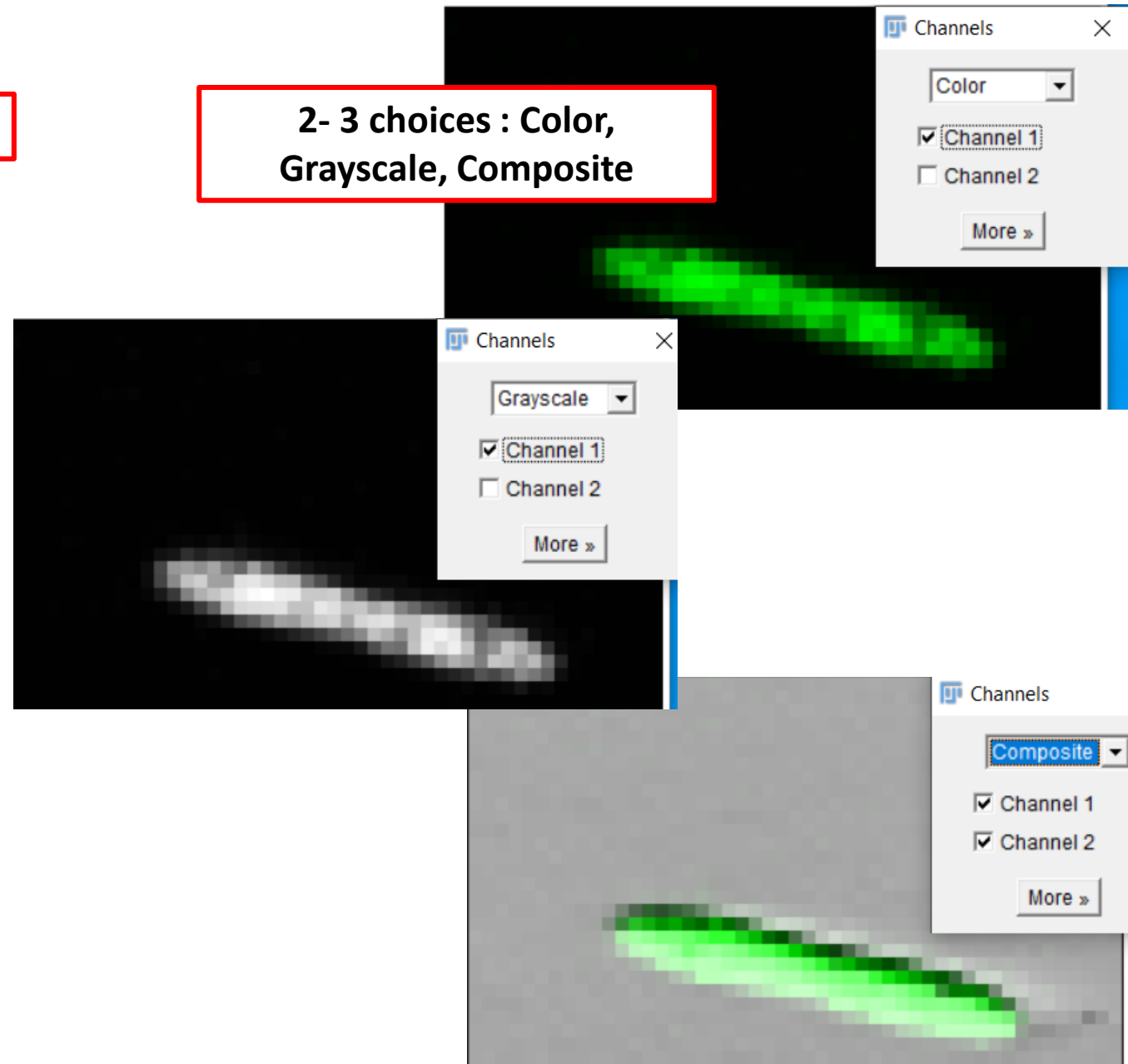
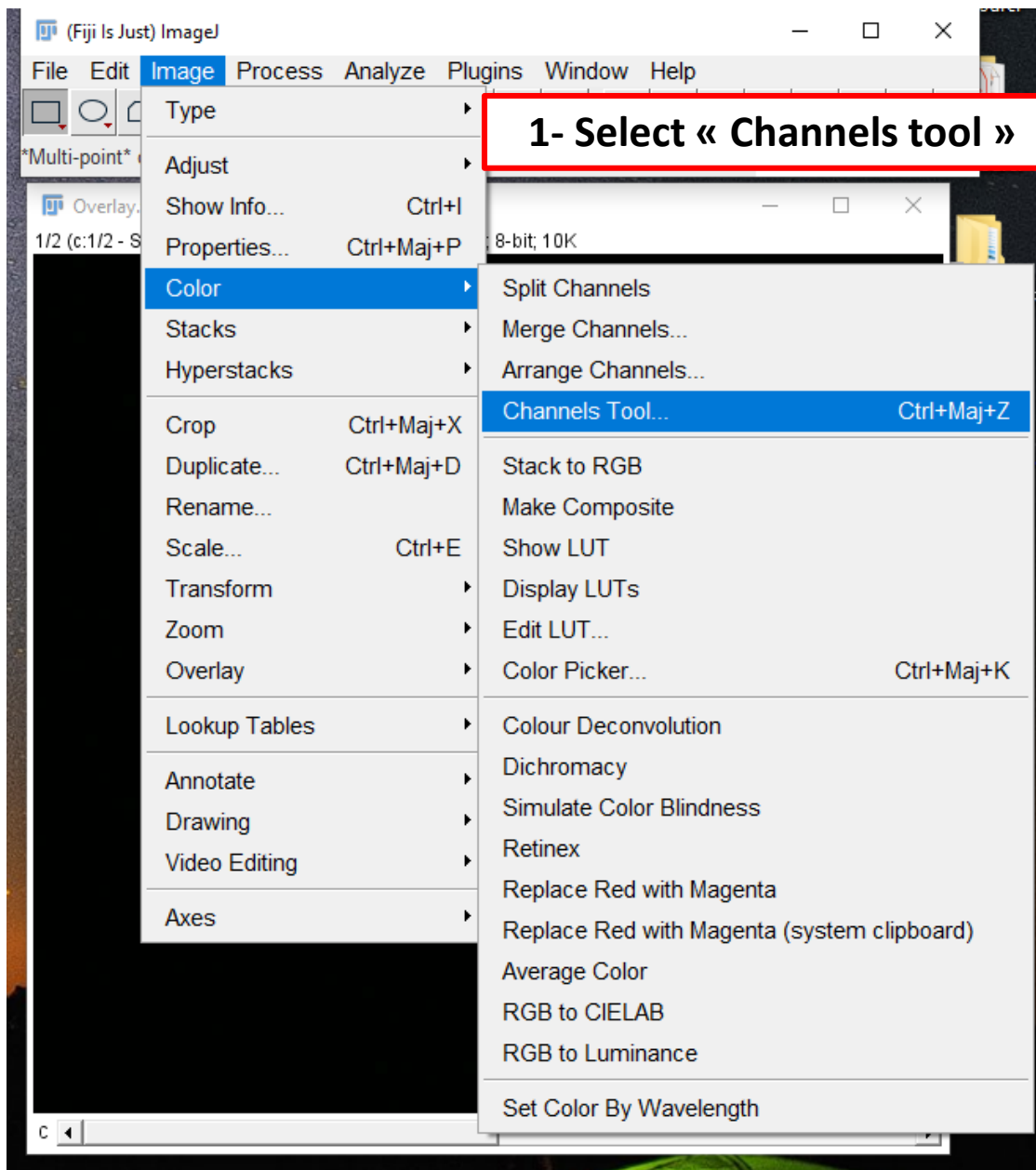
An RGB image contains informations
from R + G + B channels = 3 different
channels form 1 image.



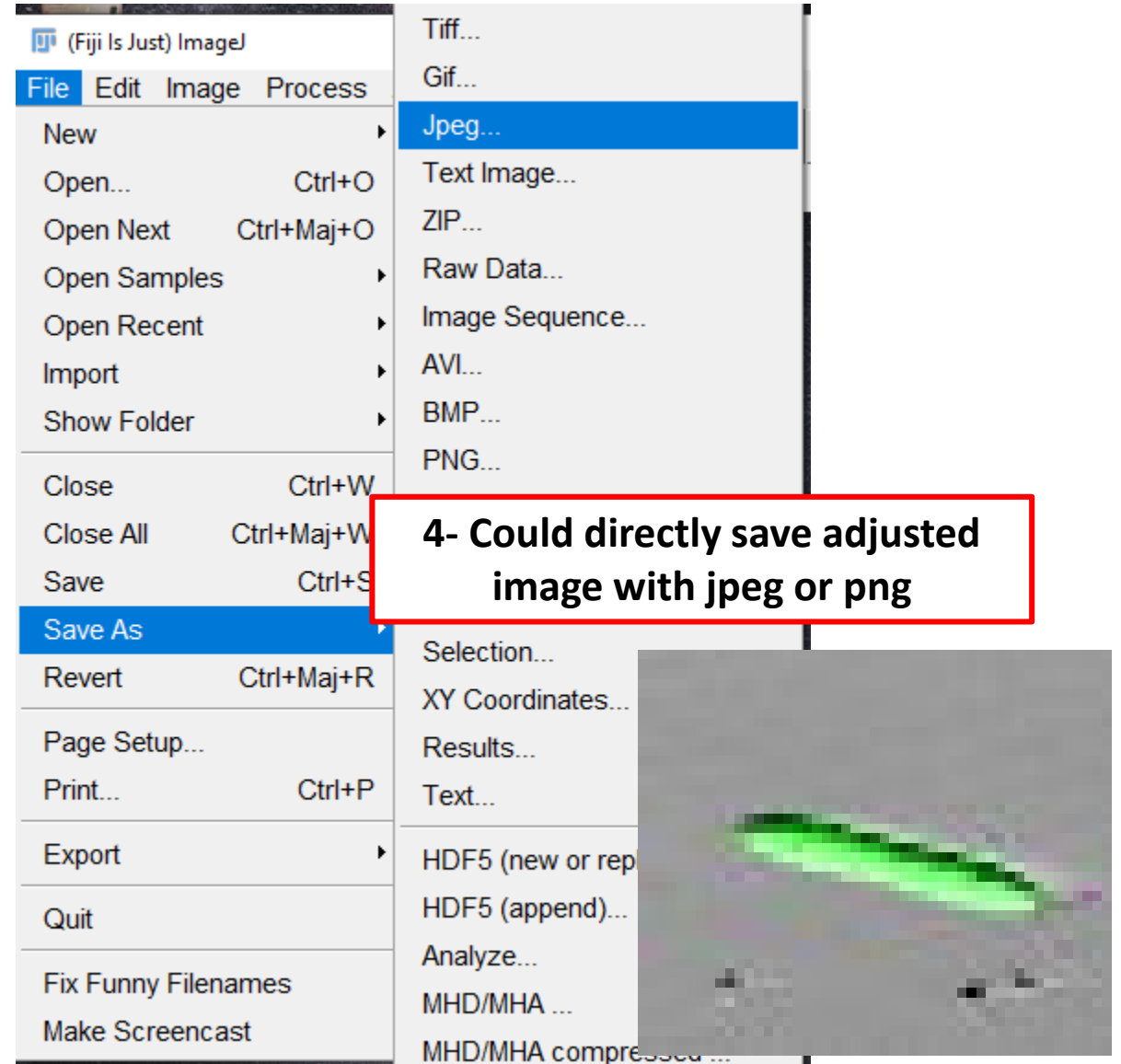
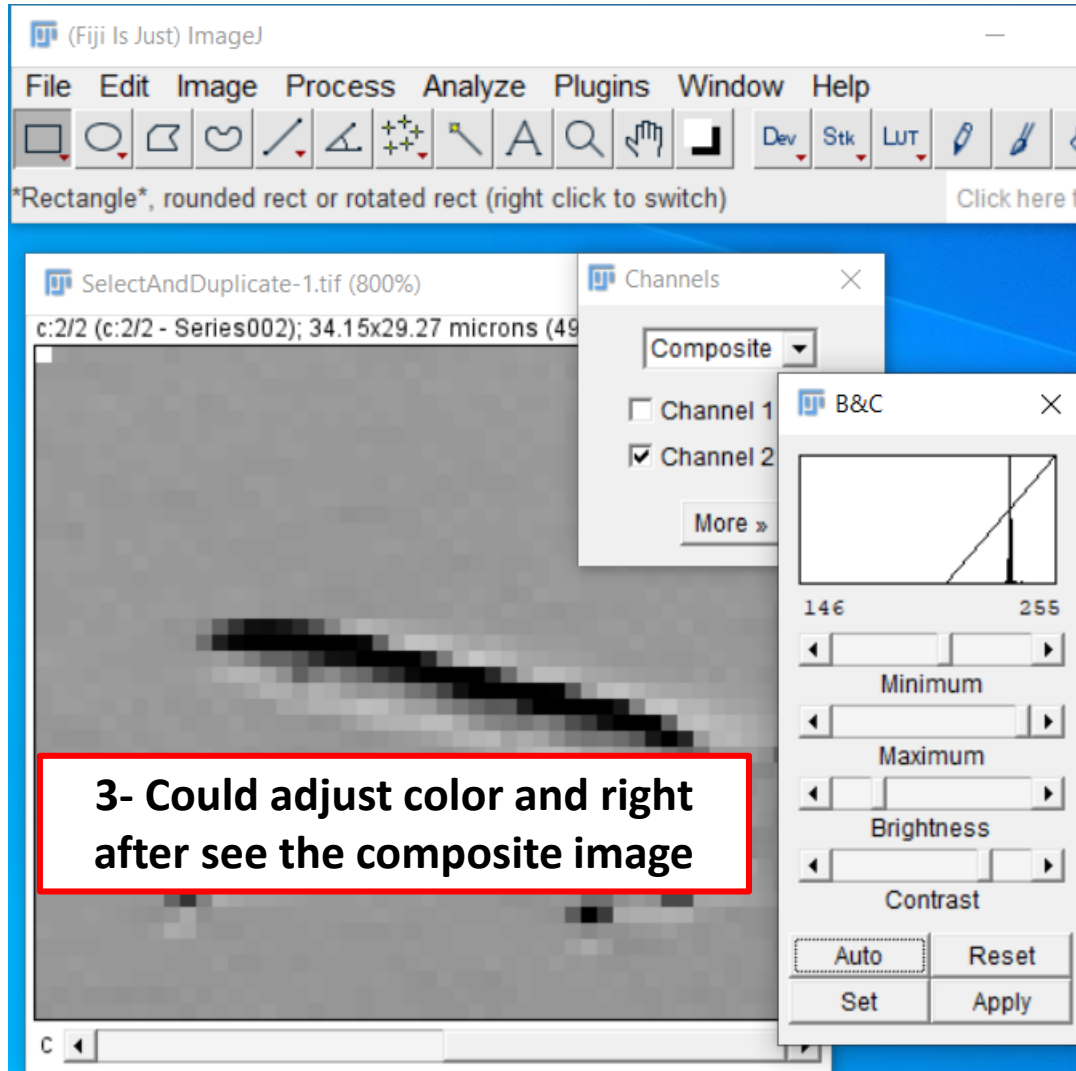
Playing with LUTs, some information is better revealed!

RGB = 3 channels
8-16 bit= 1 channel

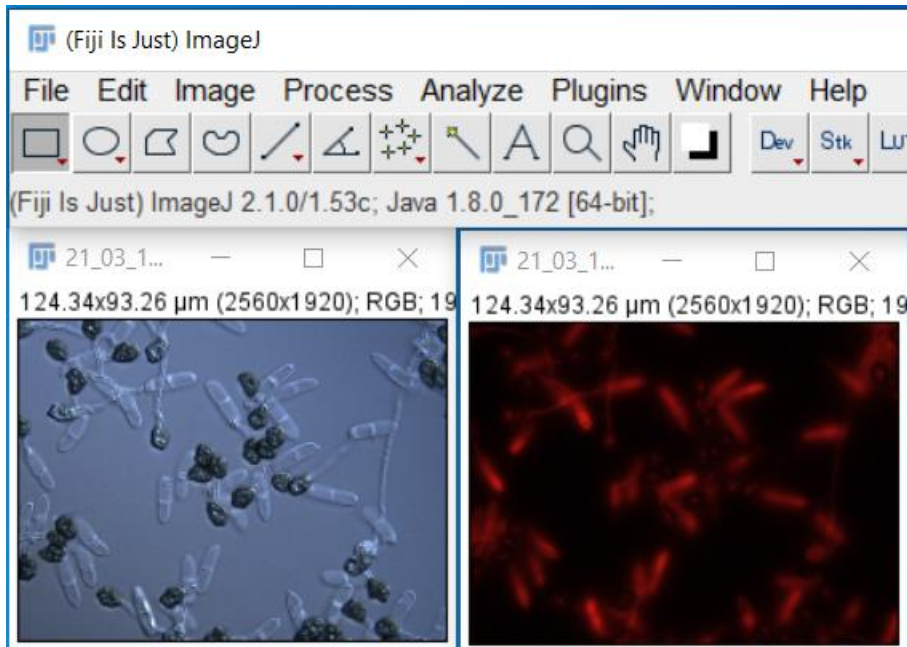
Channel tool for B/W images and LUT : X bit images (1)



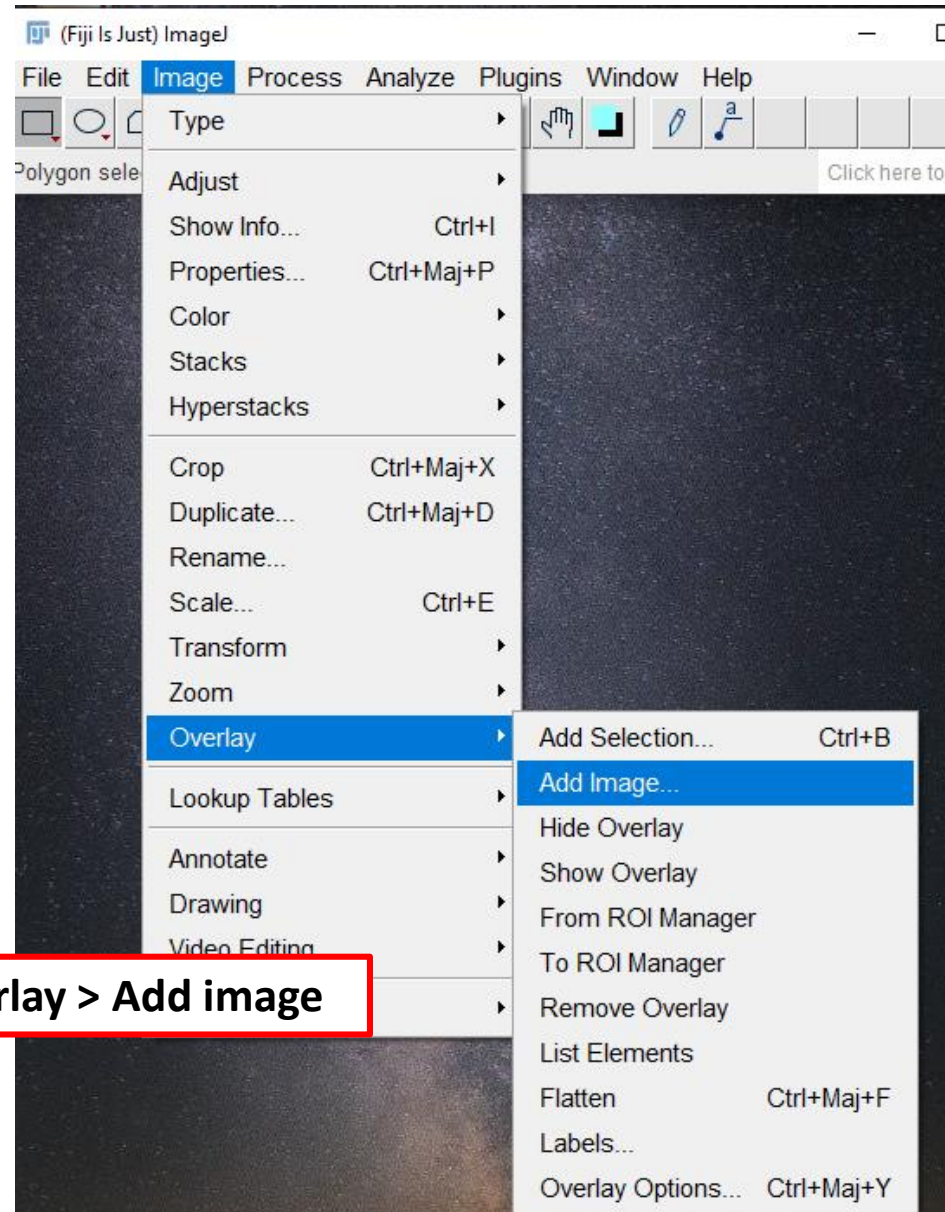
Channel tool for B/W images and LUT : X bit images (2)



Overlay RGB images is possible but...1by1 (1)

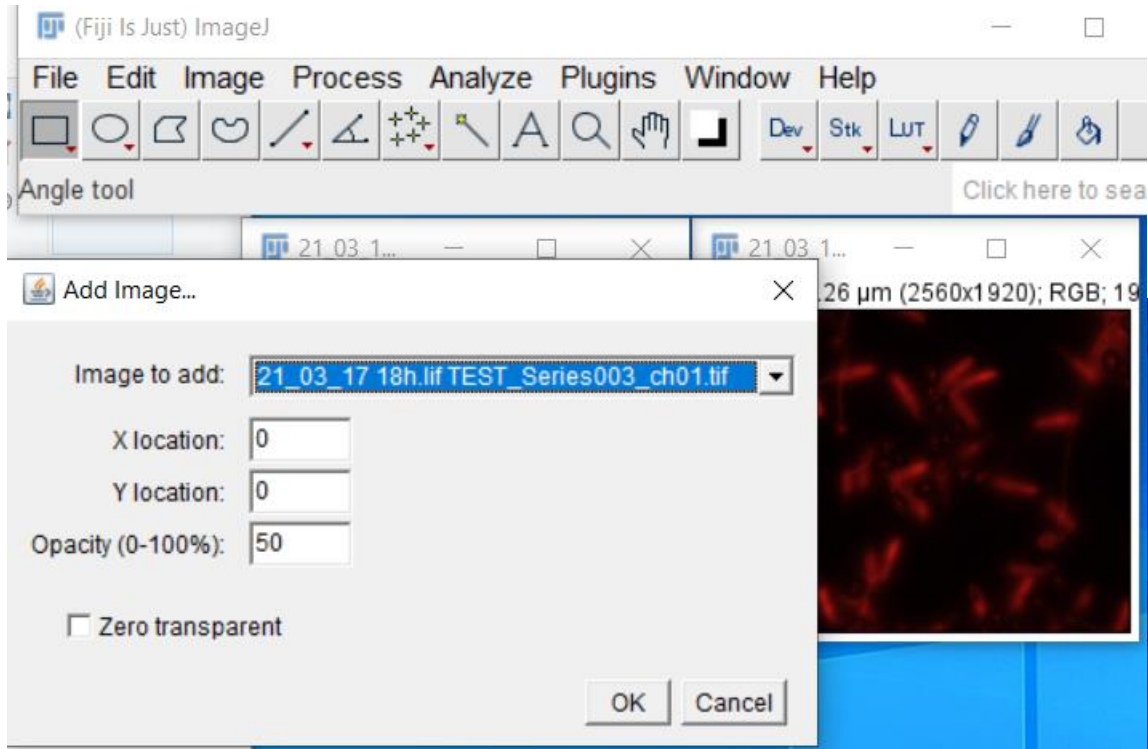


1-Open all RGB images to overlay

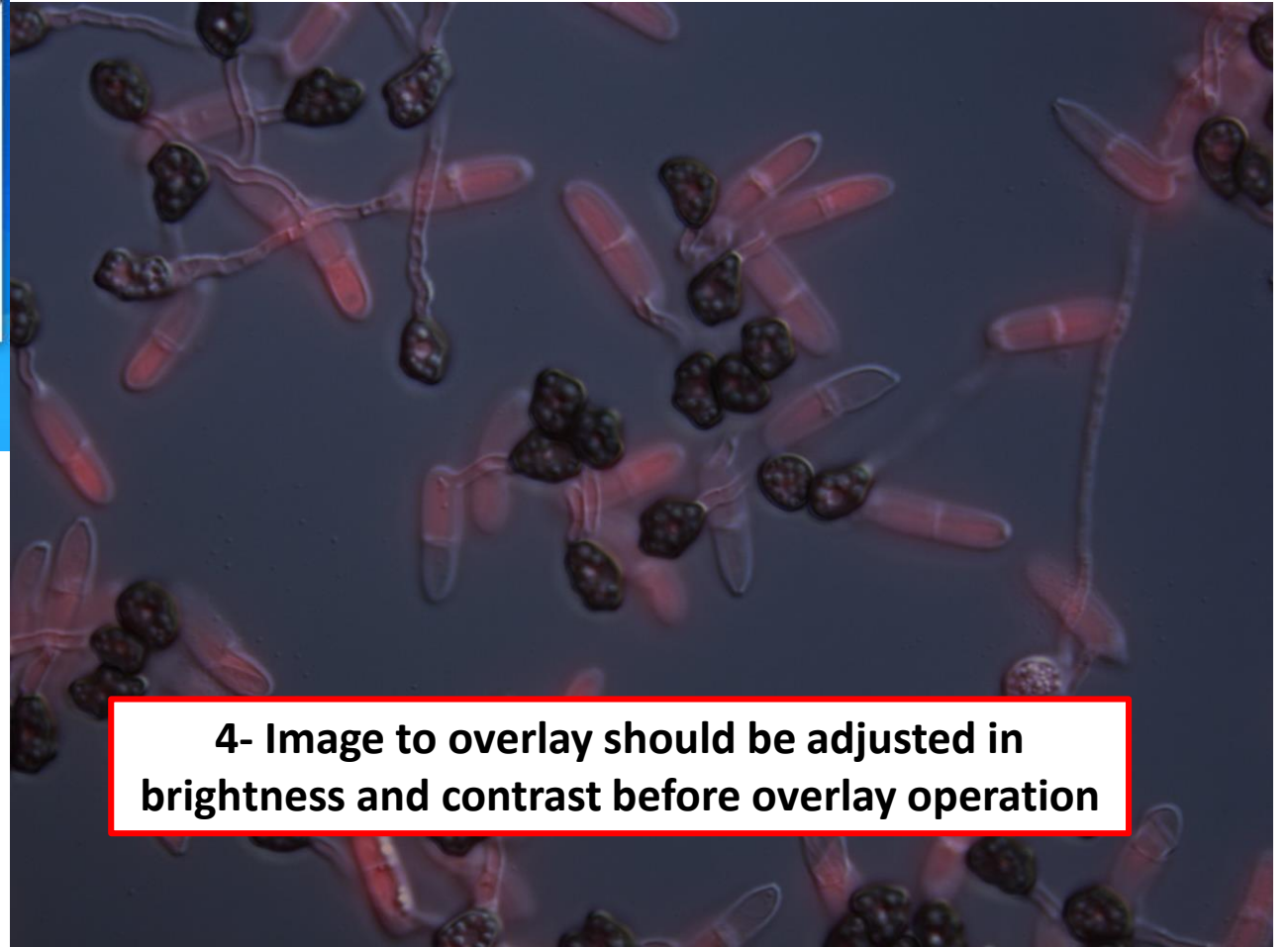


2- Choose Overlay > Add image

Overlay RGB images is possible but...1by1 (2)

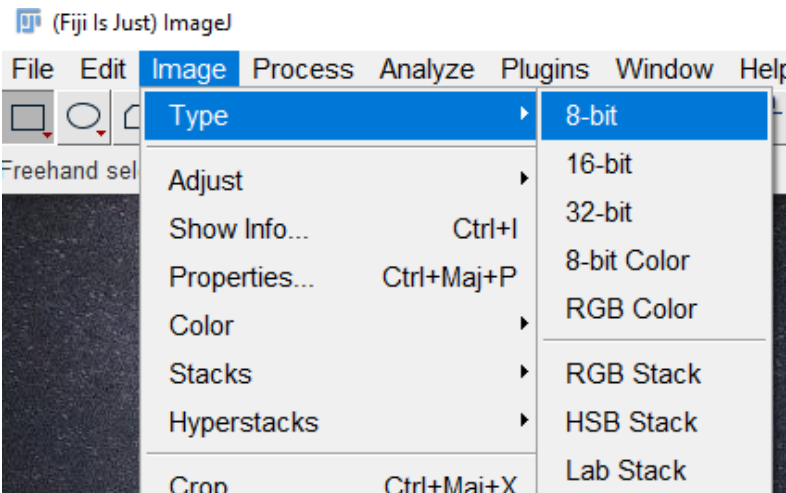


3- Choose the image to add

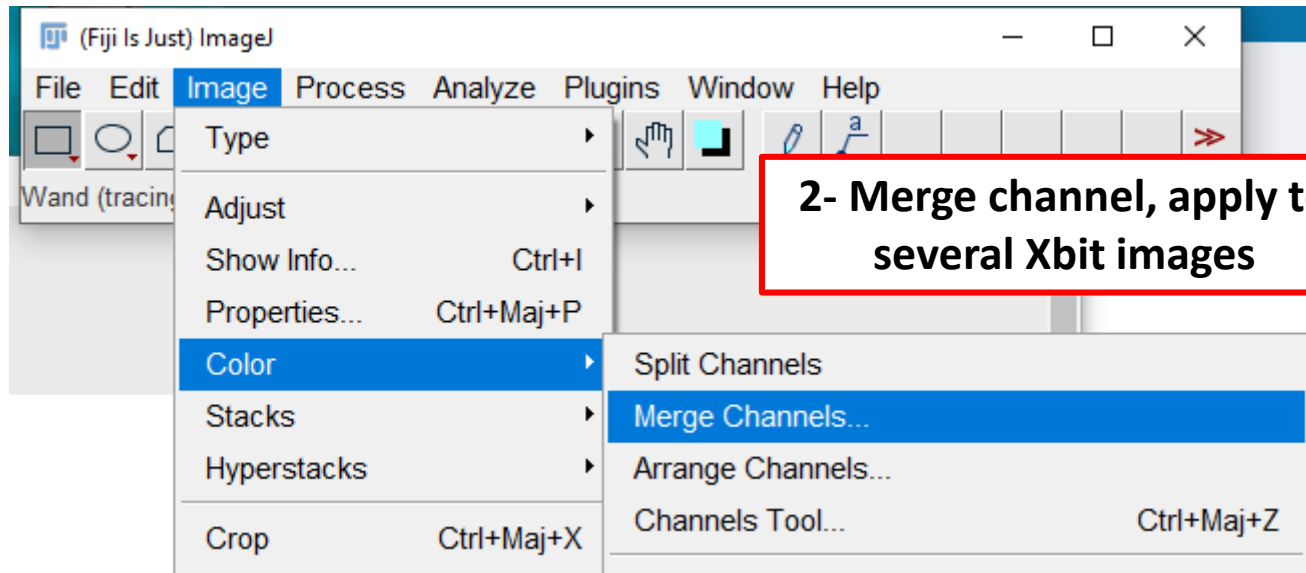


4- Image to overlay should be adjusted in brightness and contrast before overlay operation

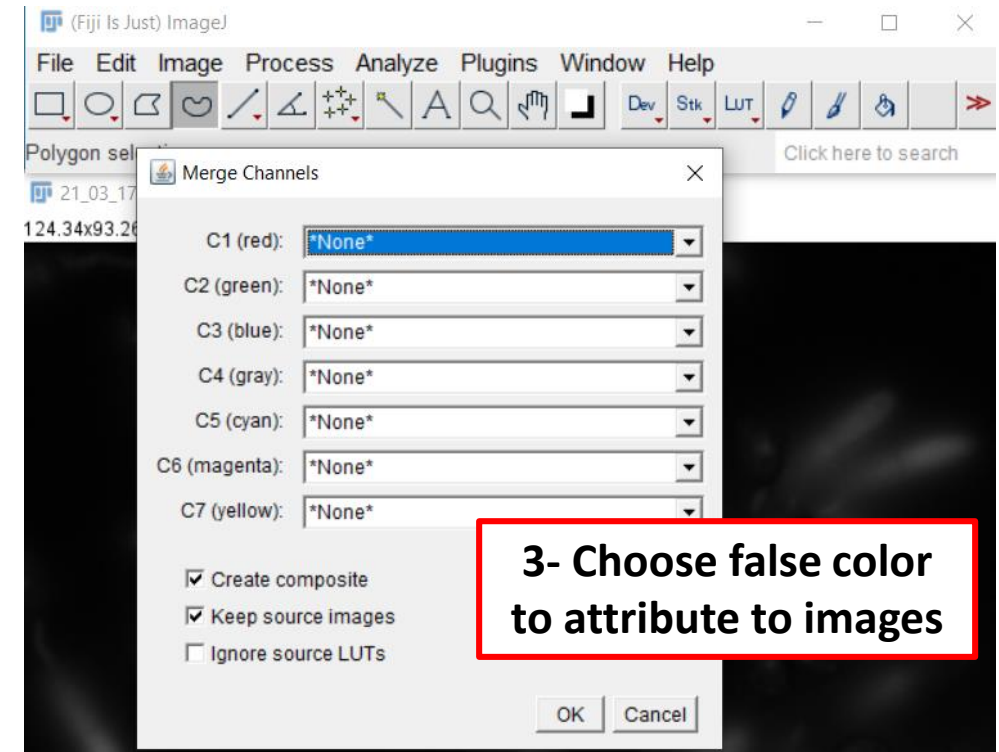
If color information is not needed : transform image type to X bit



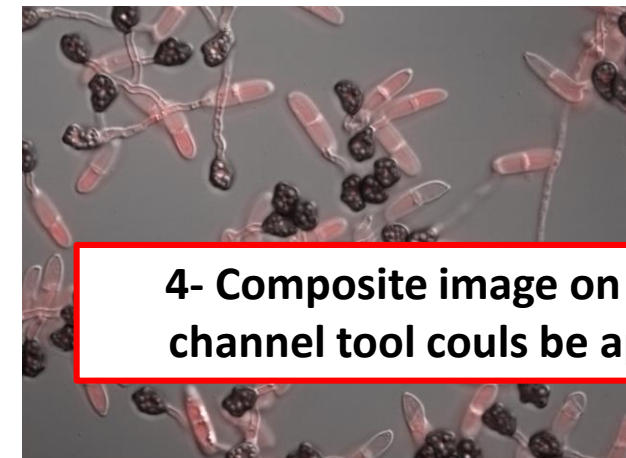
1- Transform image type to Xbit image



2- Merge channel, apply to several Xbit images



3- Choose false color to attribute to images

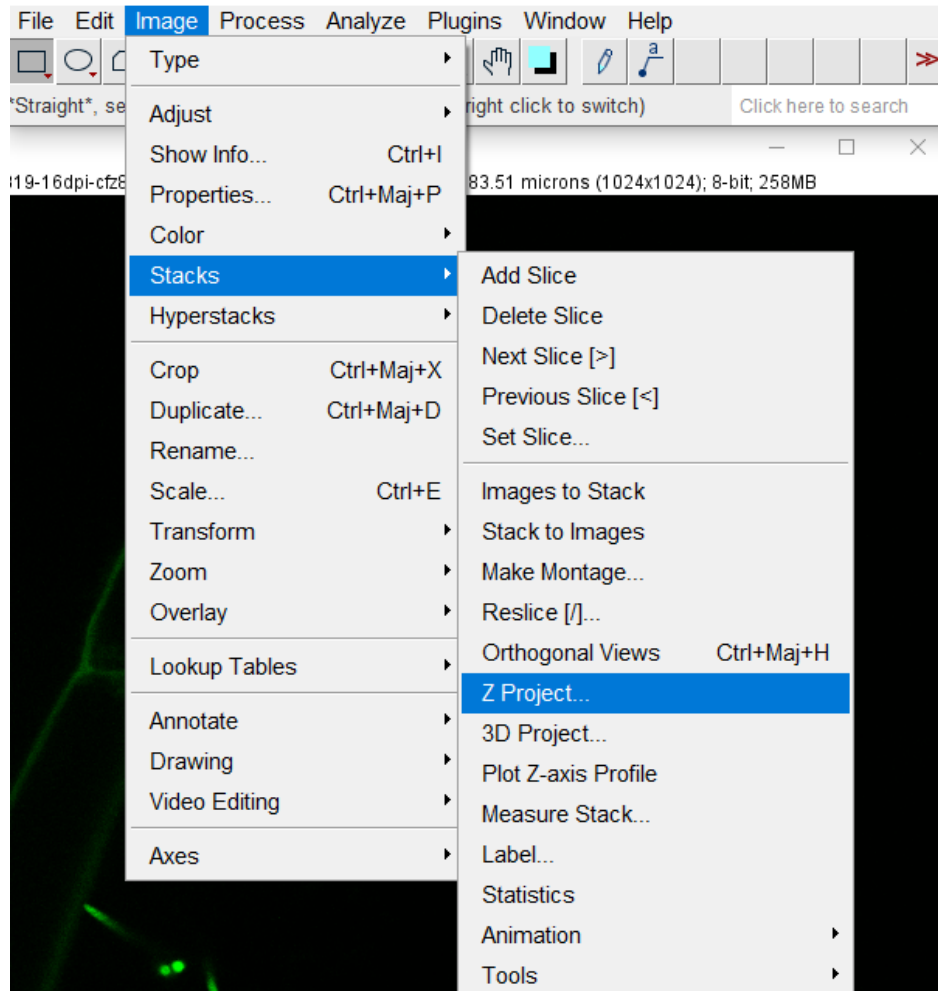


4- Composite image on which channel tool could be applied

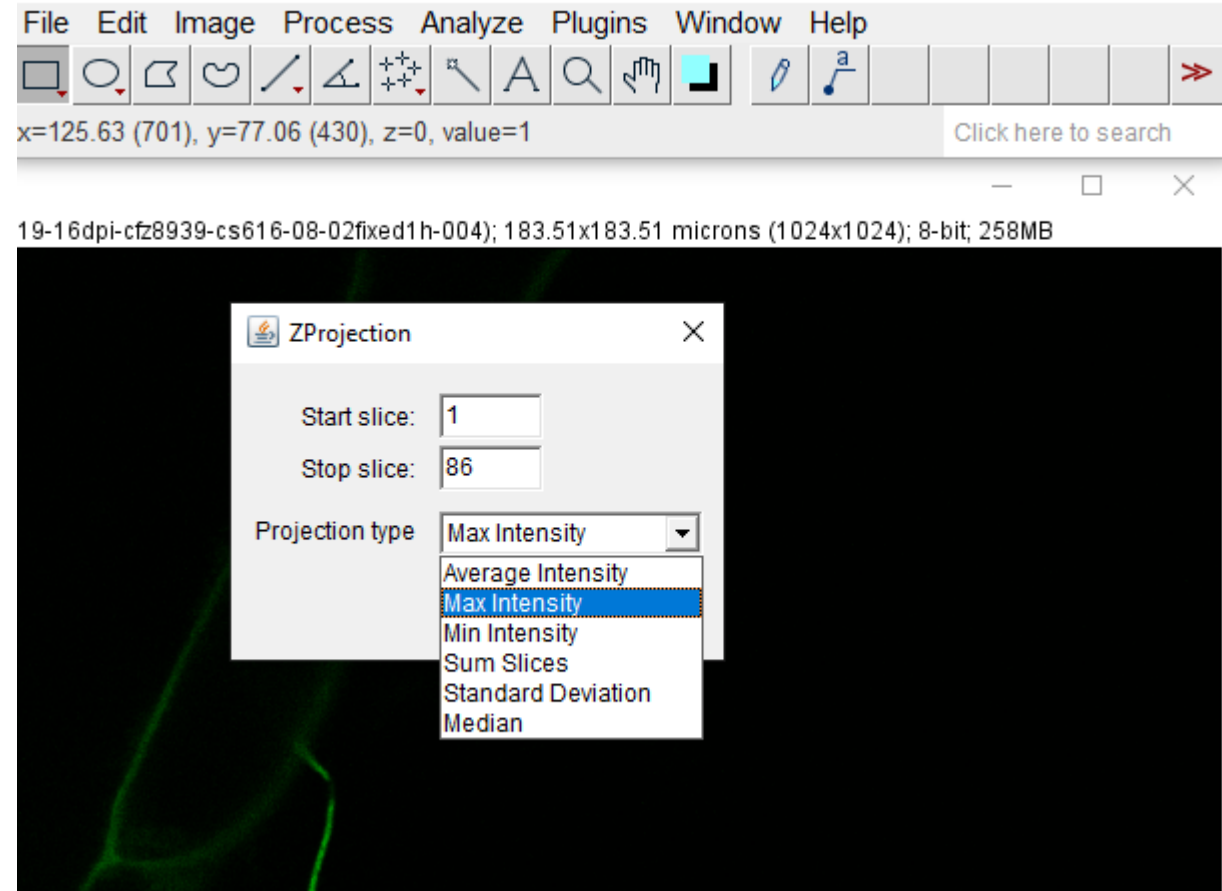
Stack : Z-project, Montage

Z-project : to get an overview of your stack (1)

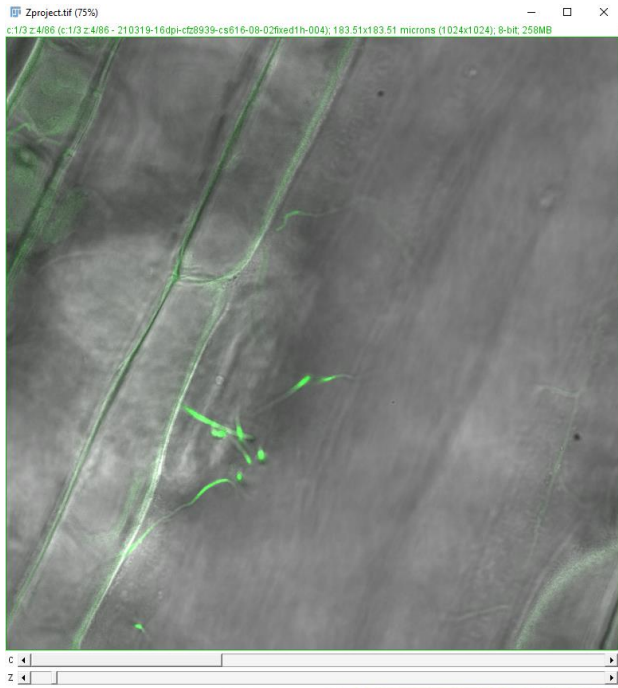
1- Select Stacks > Z project



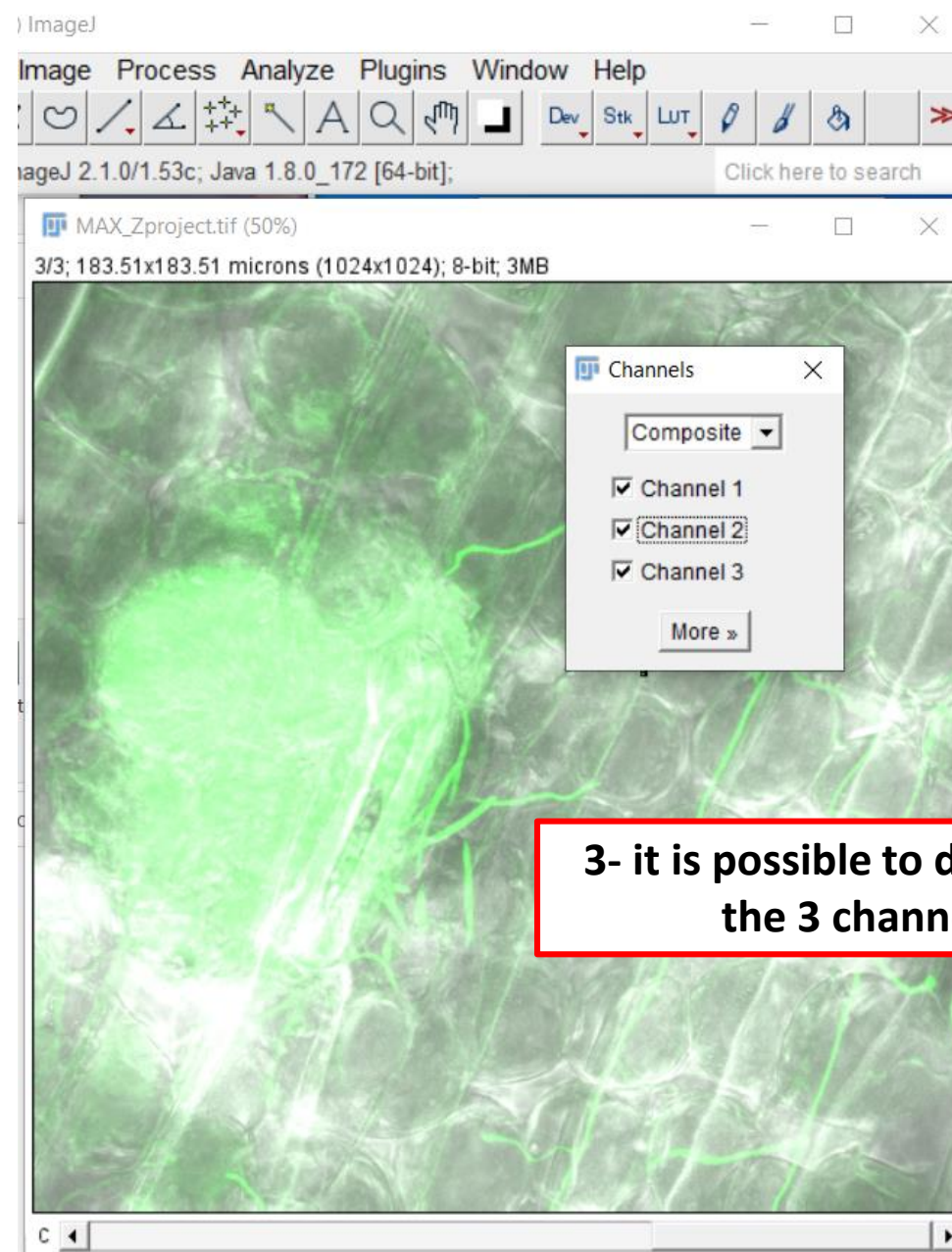
2- Select max intensity



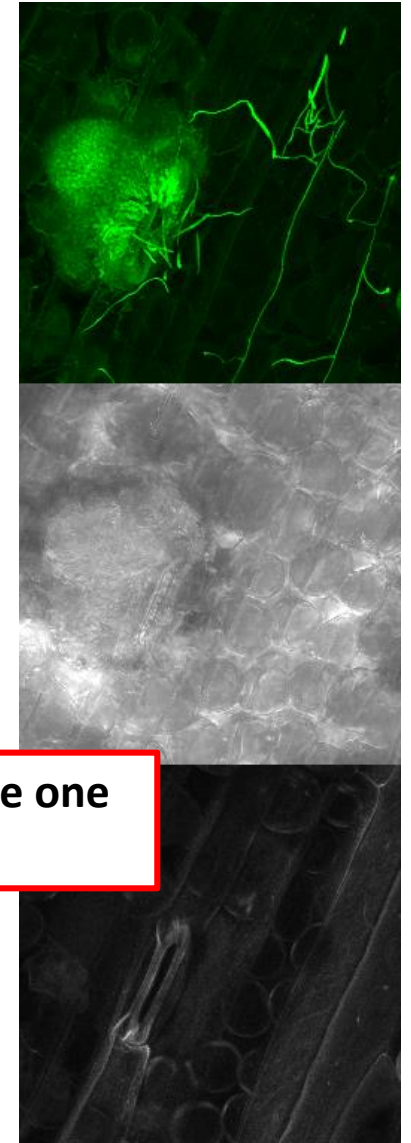
Z-project : to get an overview of stack (2)



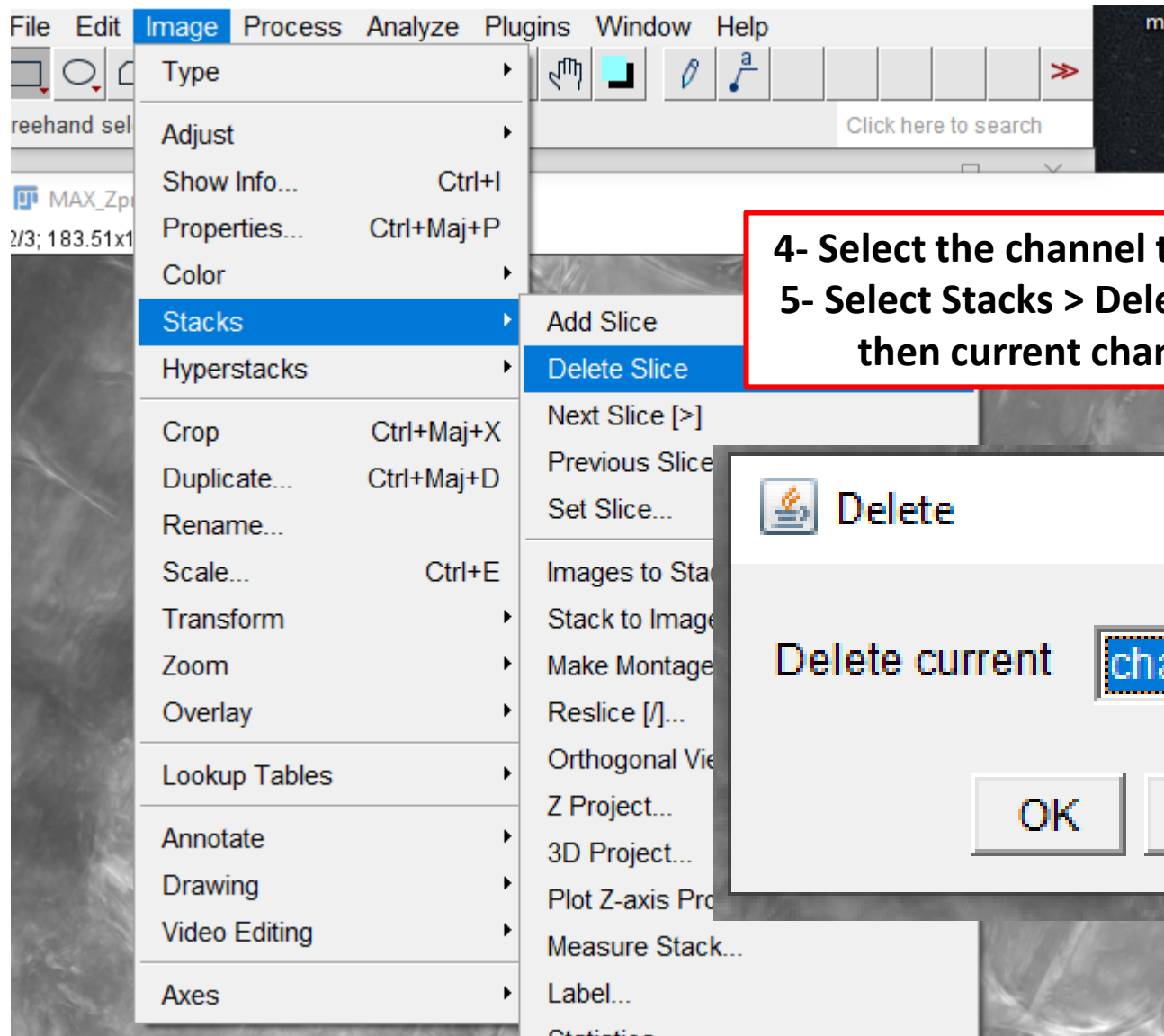
Z project



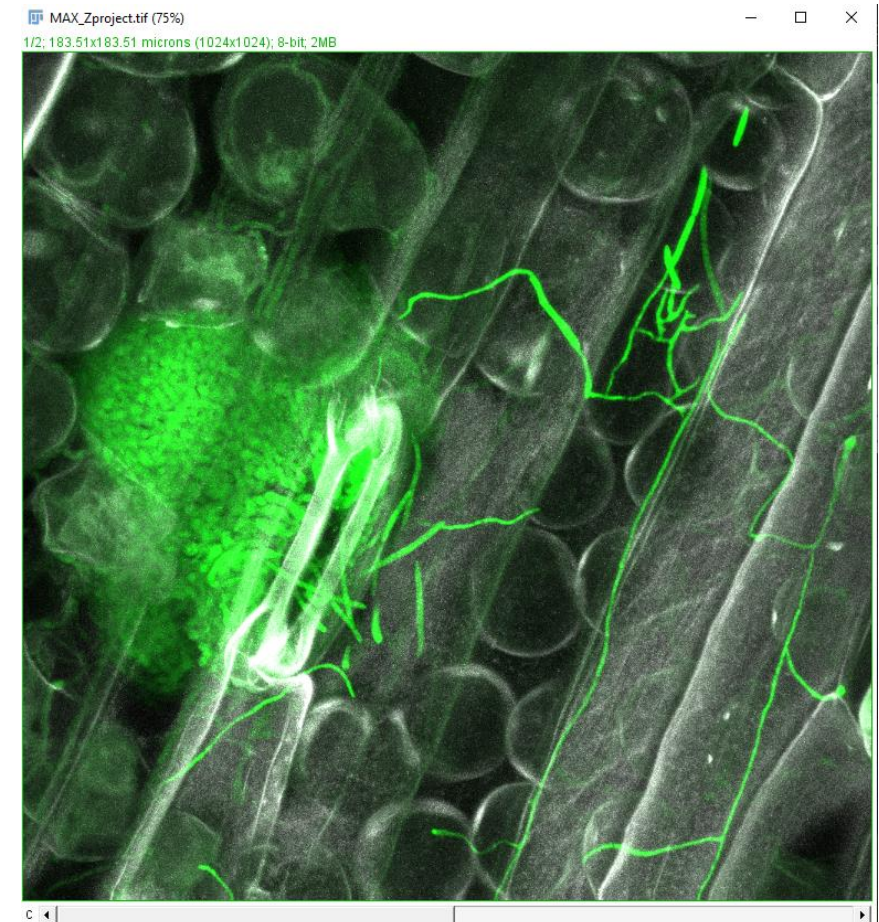
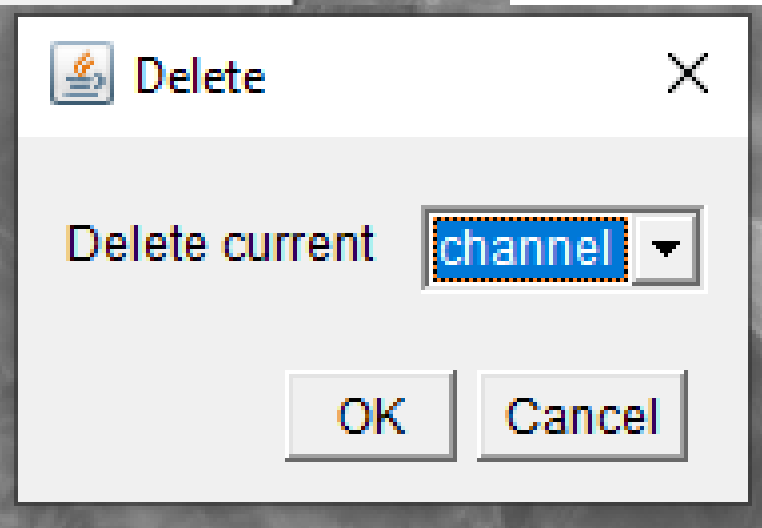
**3- it is possible to delete one
the 3 channels**



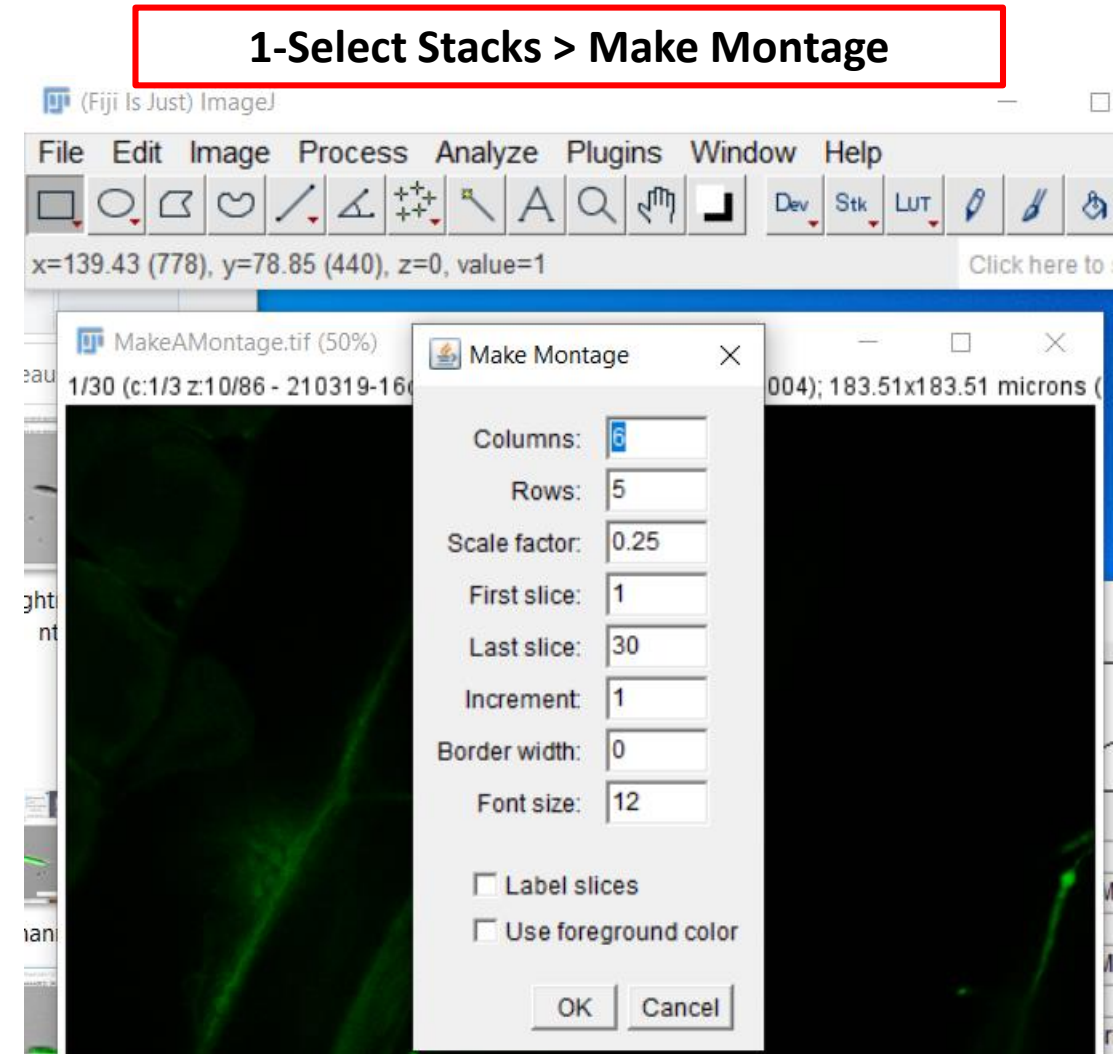
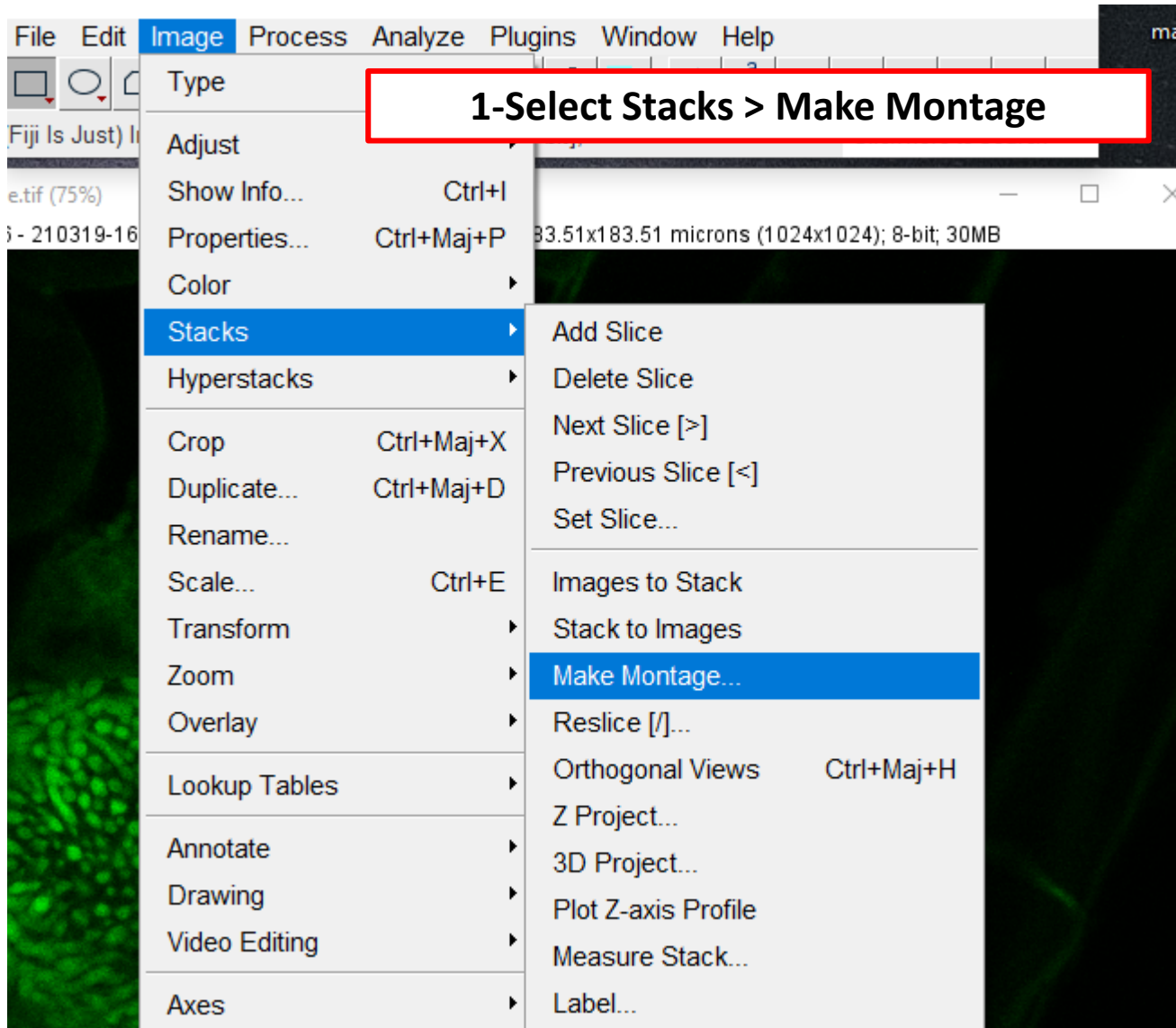
Z-project : to get an overview of stack (3)



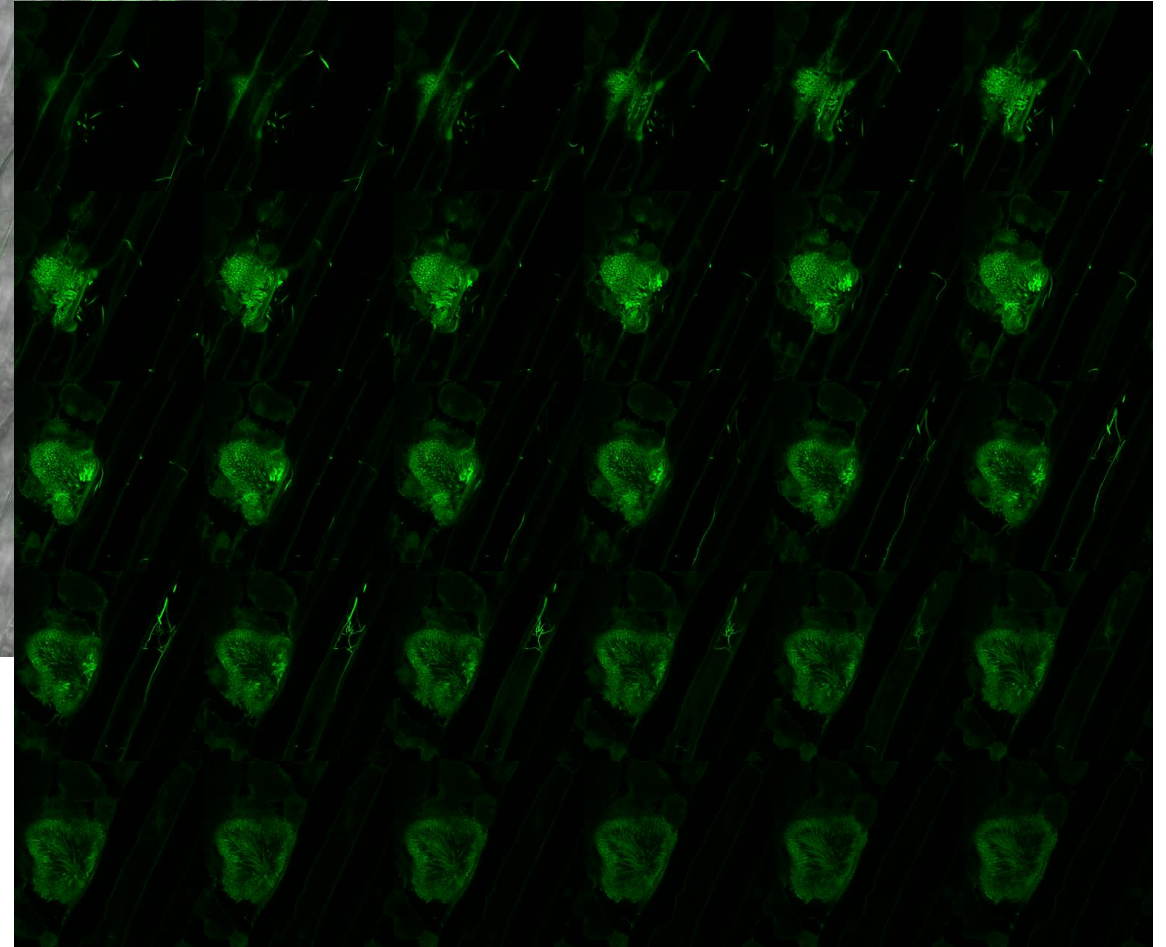
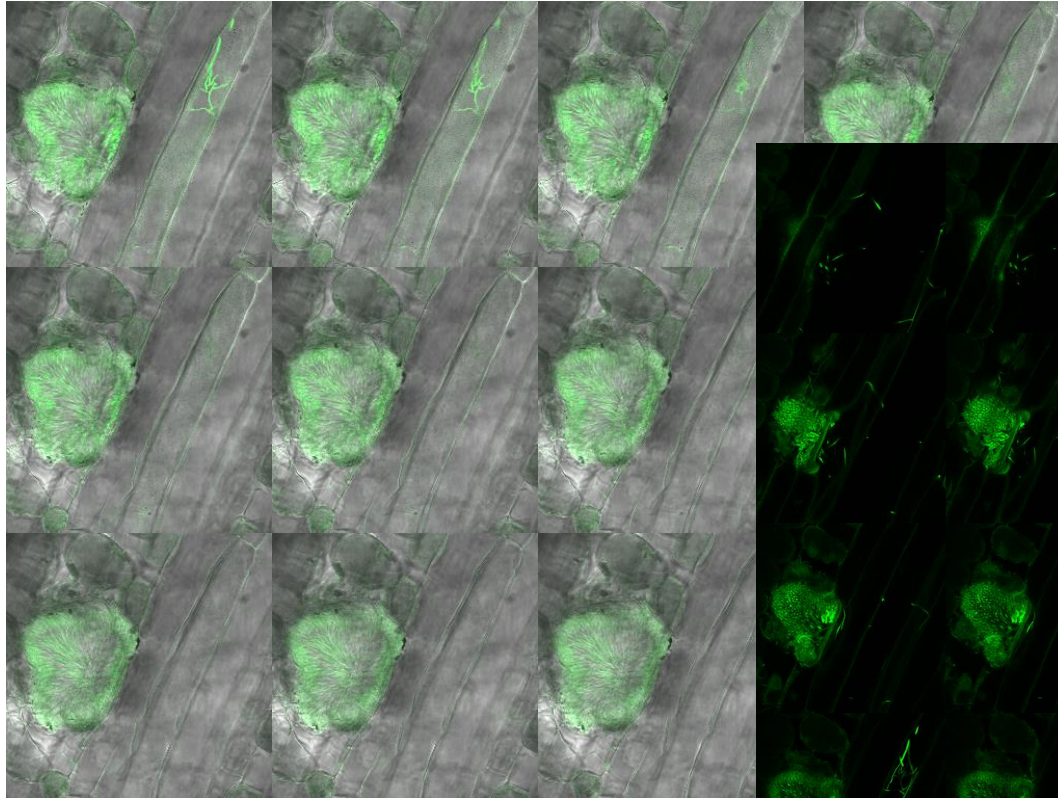
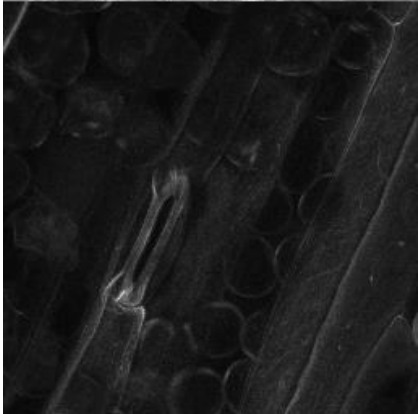
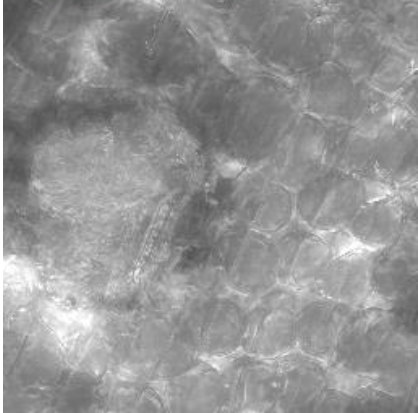
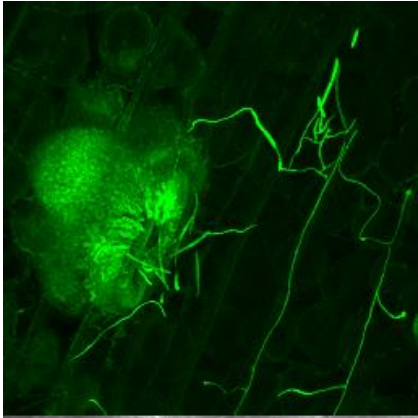
4- Select the channel to delete
5- Select Stacks > Delete slice,
then current channel



Make a montage : to show an overview of stack / channels (1)

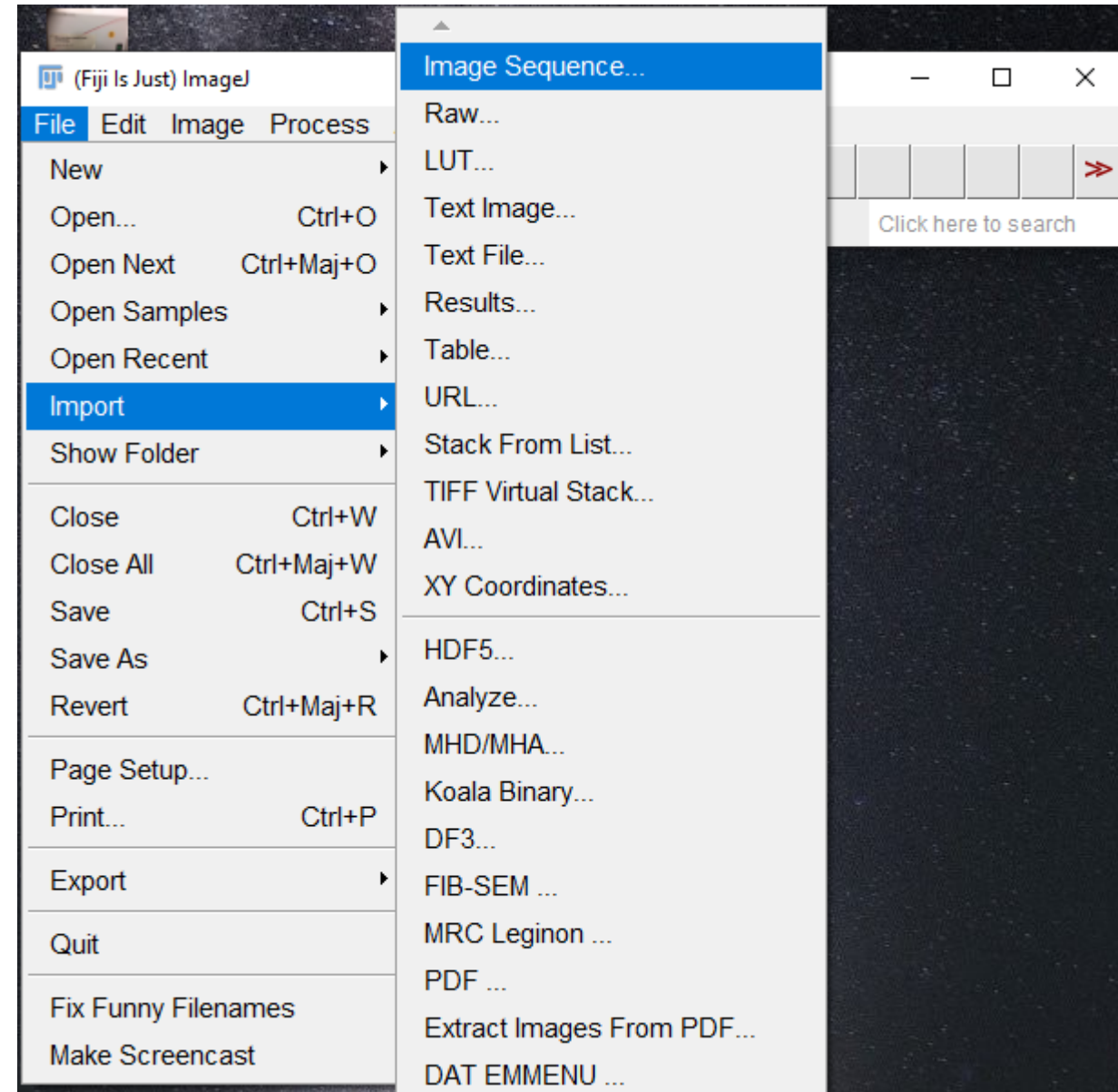
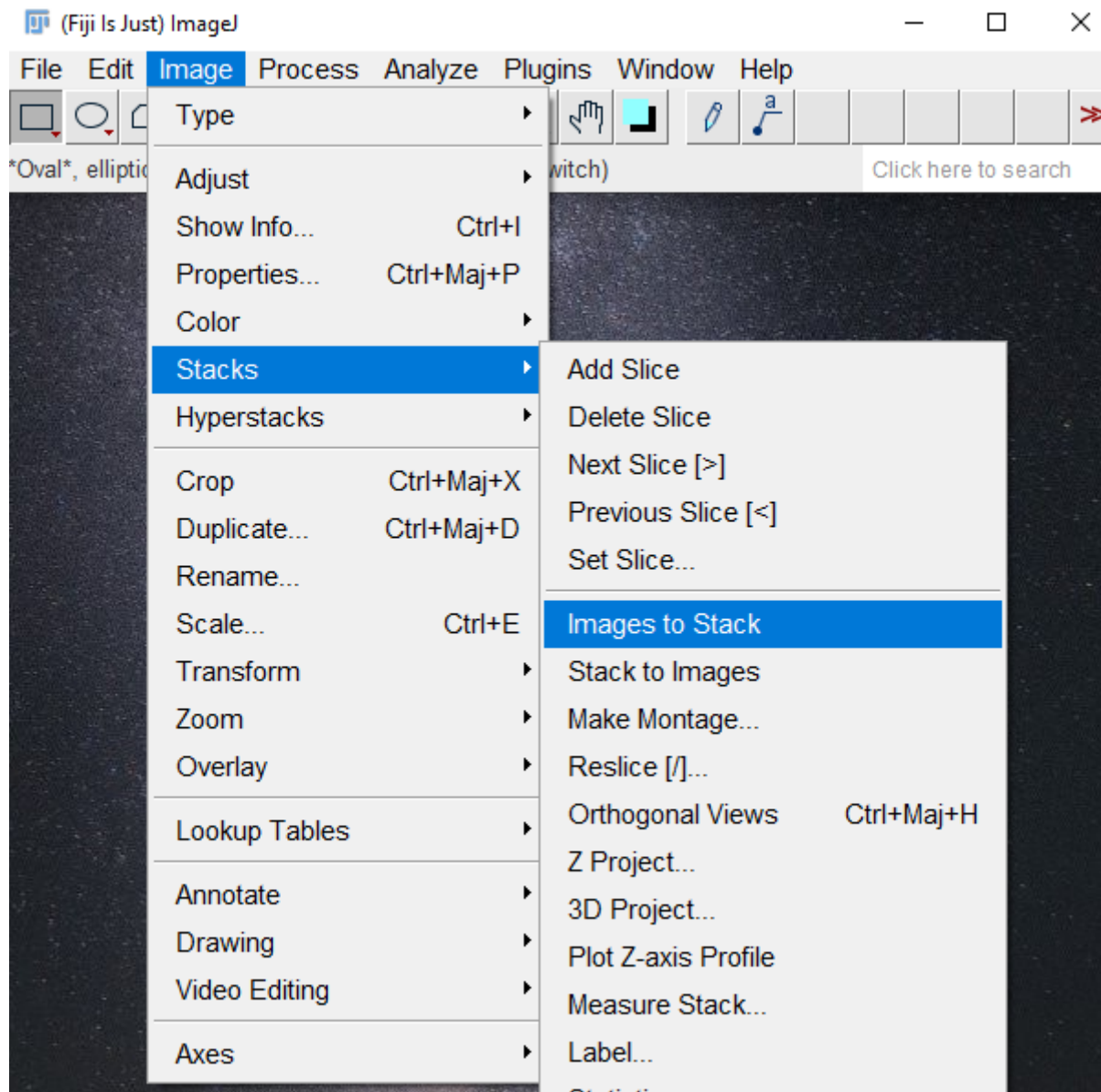


Make a montage : to show an overview of stack / channels (2)

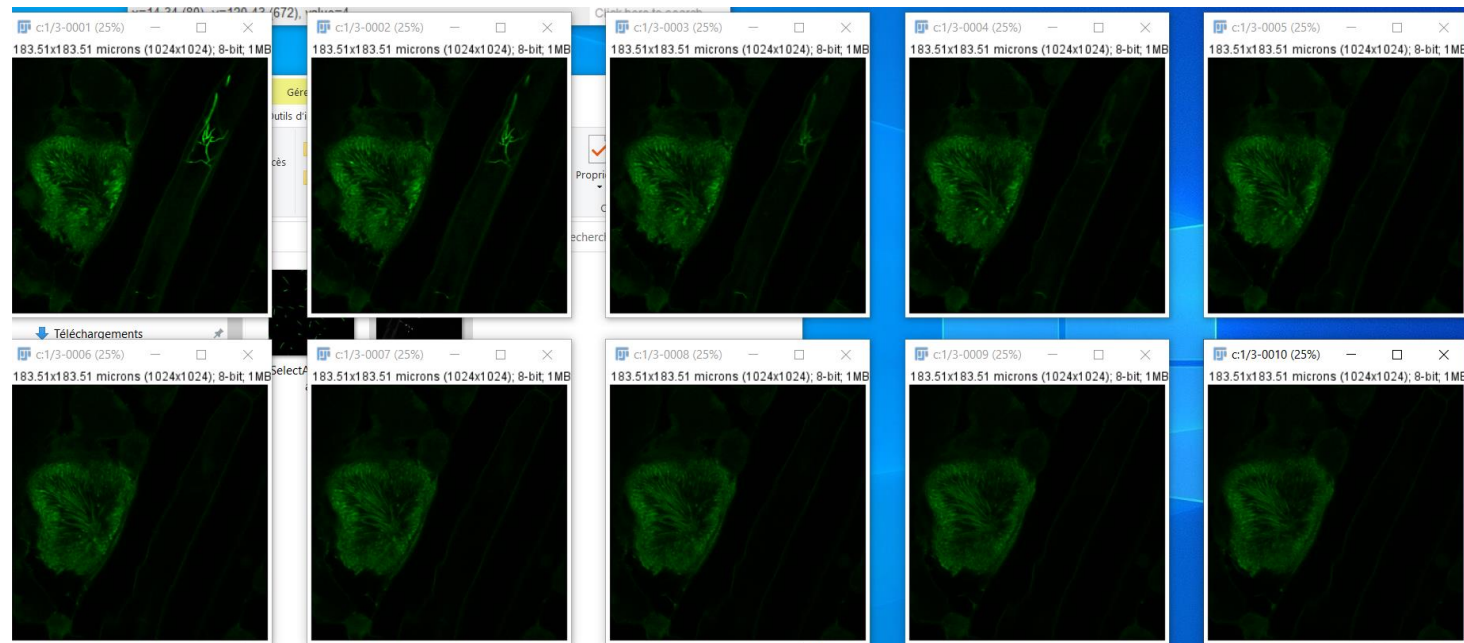
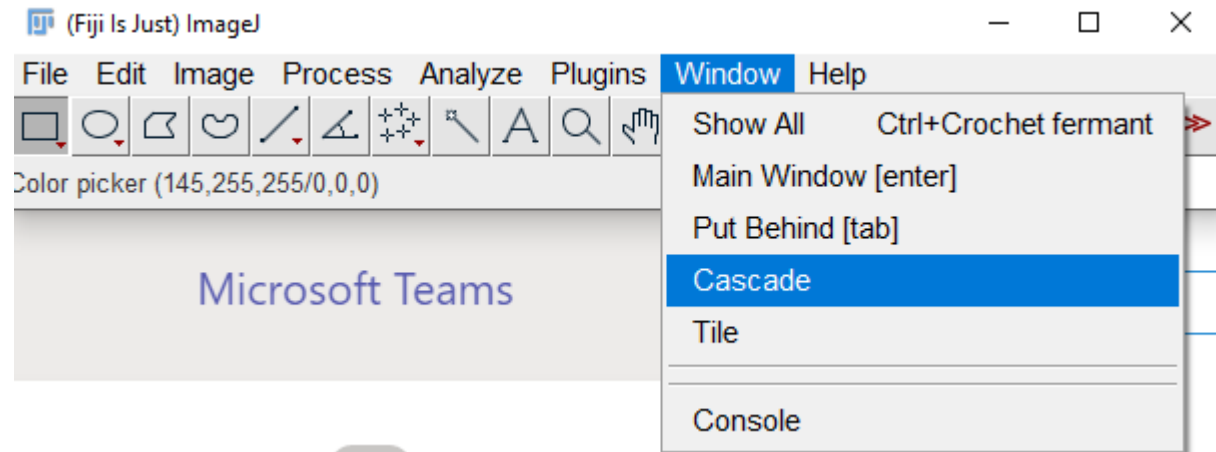


Organize images :
Image to stack / image sequence
tile and cascade

Image to stack / image sequence : group by stack

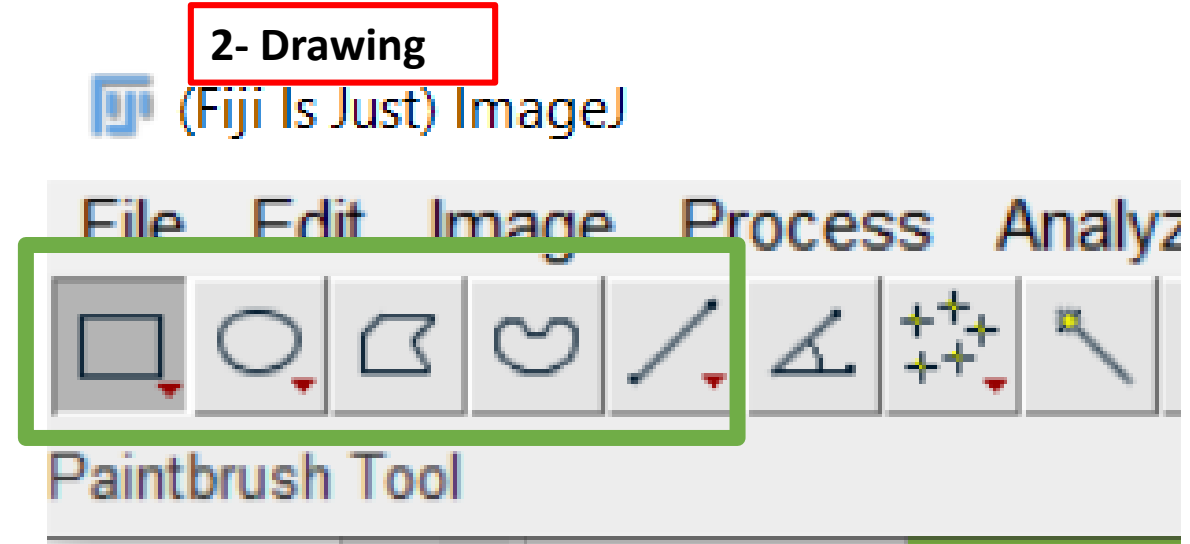
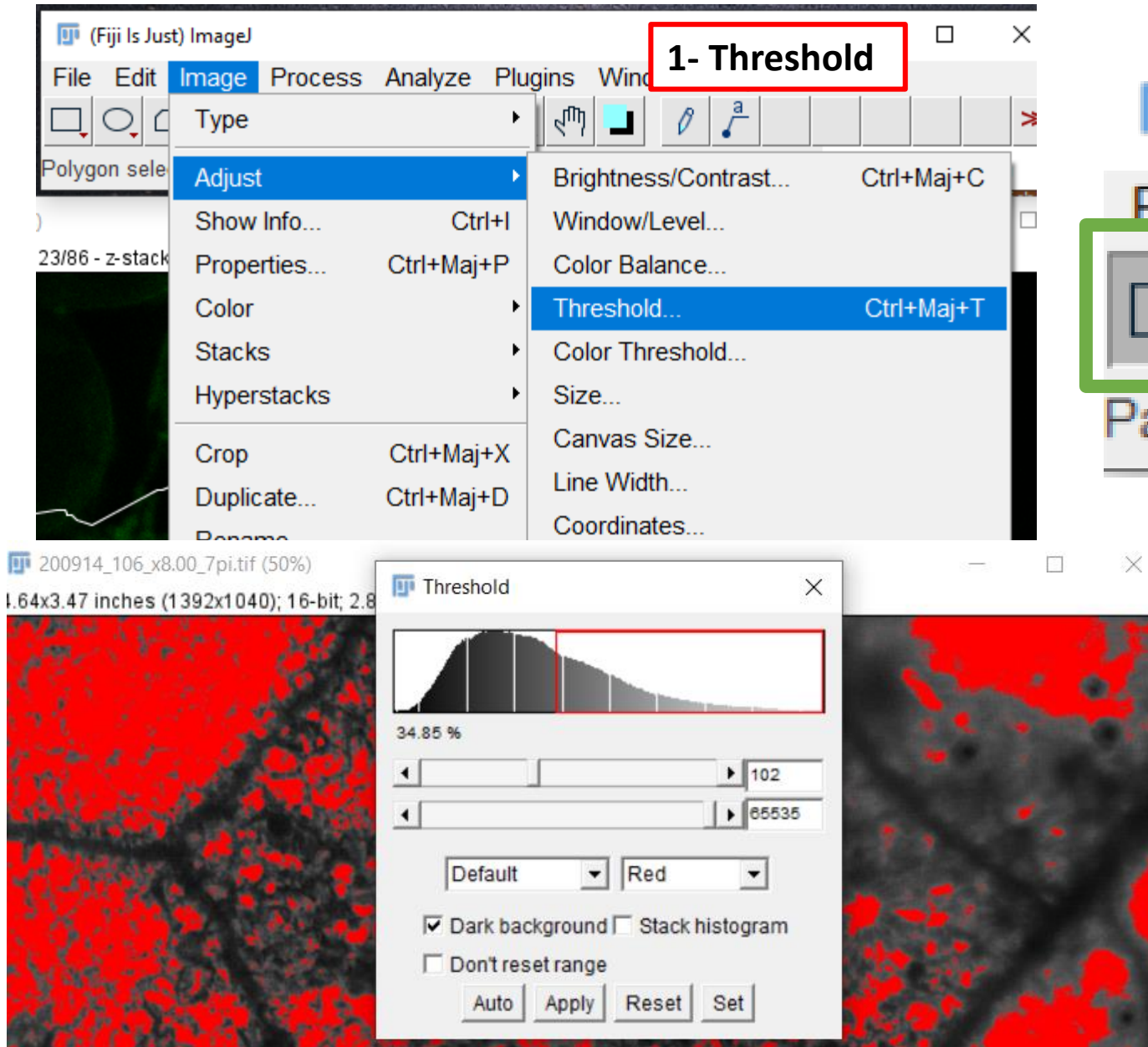


Tile and cascade

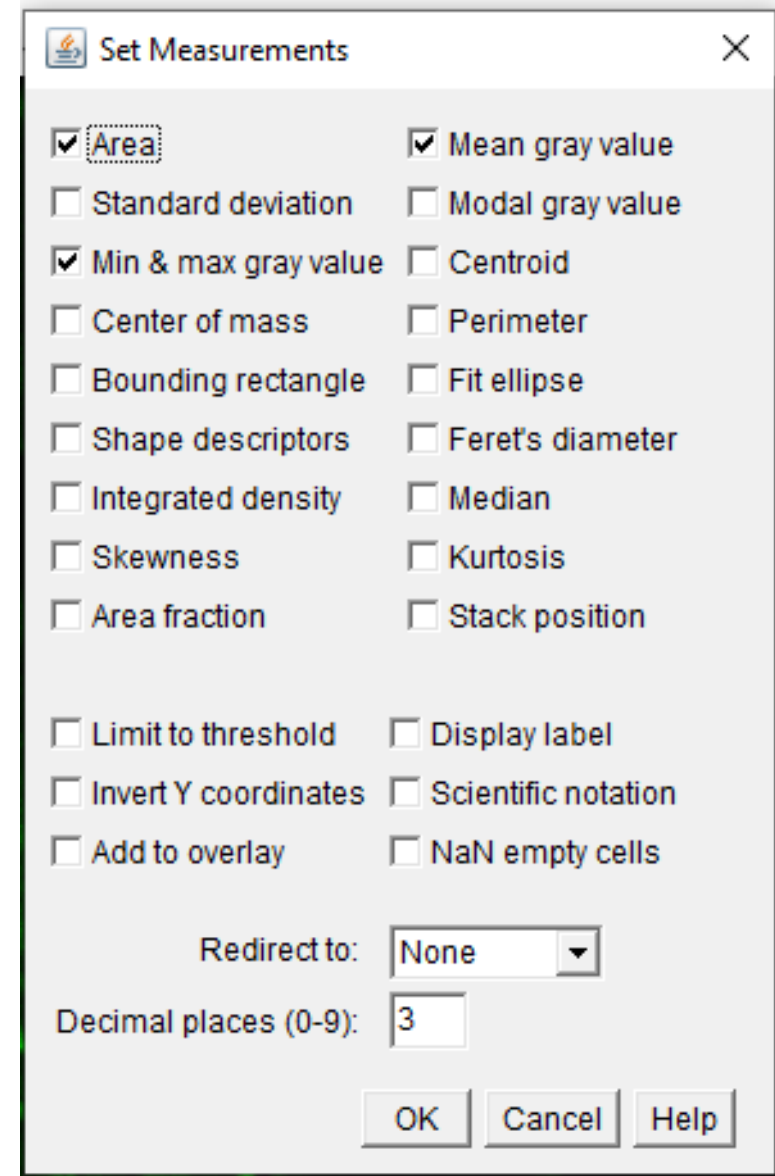
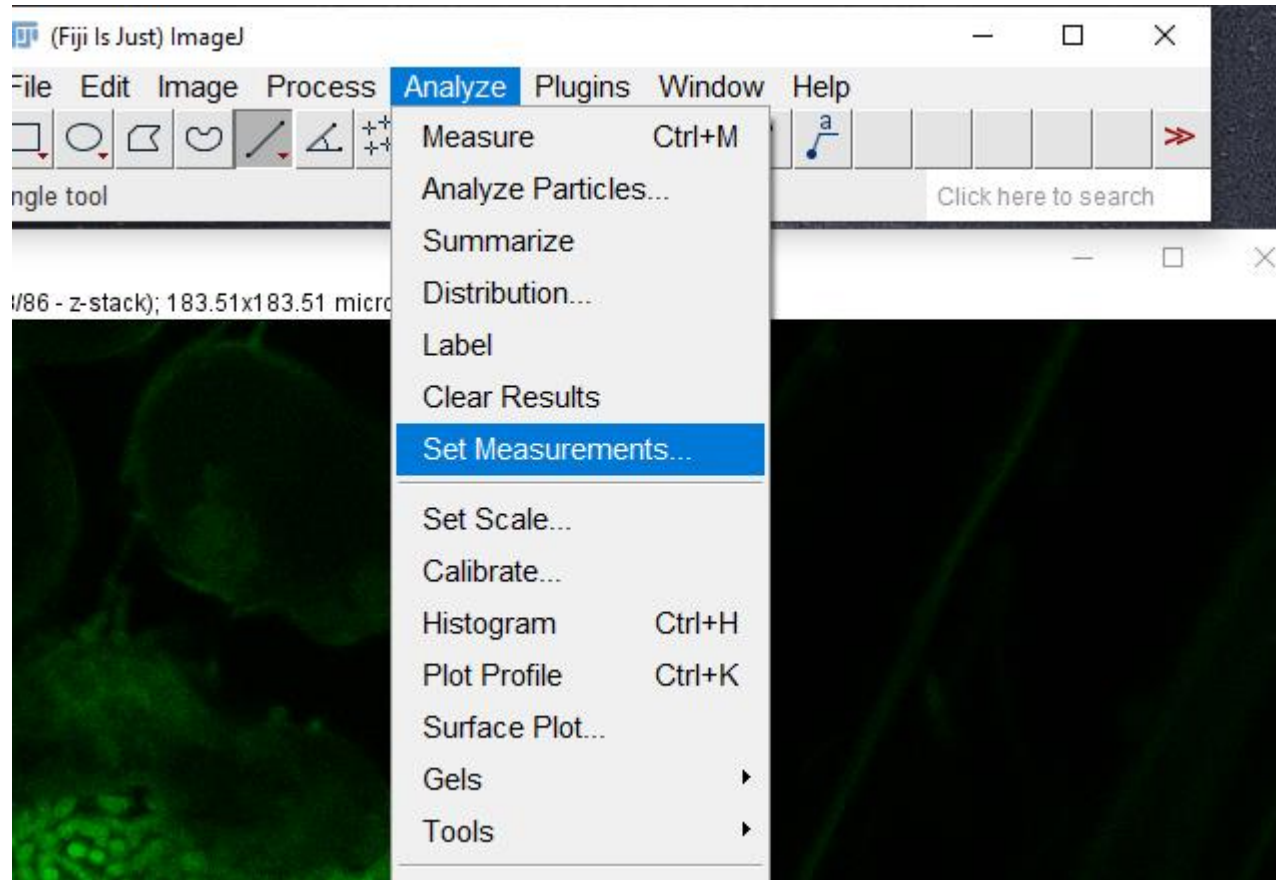


Analysis

Define region to analyse by using threshold or drawing (1)



Define region to analyse by using threshold or drawing (2)



1- Set measurements

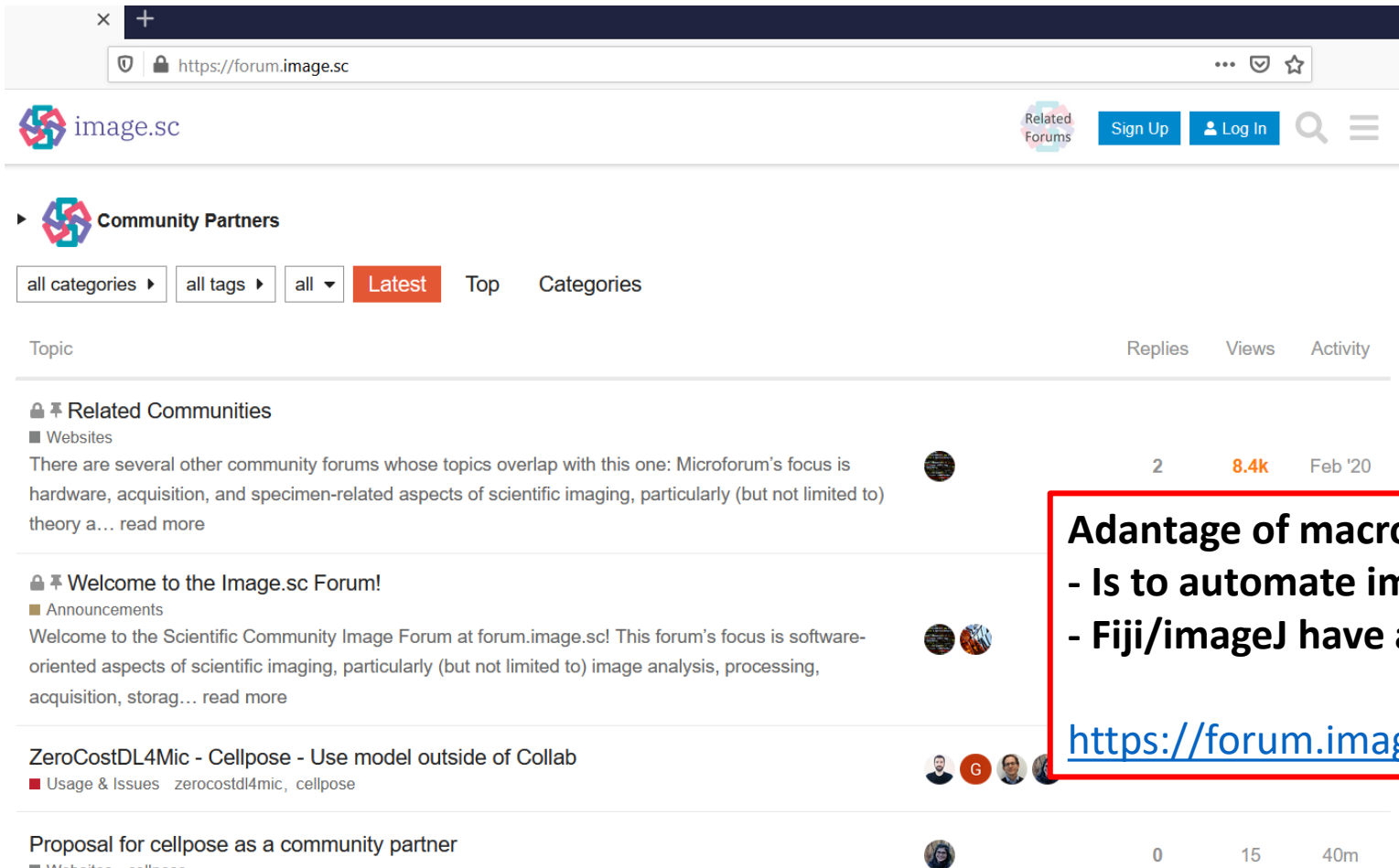
2-Measure to get result

Macro in Fiji or ImageJ

Example of macro in Fiji

FigureJ : to make figures

Cell counter : to count several classes



Adantage of macro with Fiji:

- Is to automate image analysis**
- Fiji/imageJ have a hage and active community of users**

<https://forum.image.sc/>

A microscopic image of cells, possibly bacteria or fungi, with a red overlay. A grid of thin red lines is superimposed on the image. The text "Now, Macro application By Reda Amerzou" is centered in white. There are some small numbers like "24" and "28" in the top left corner.

Now,
Macro application
By Reda Amerzou