Using ImageJ and FIJI

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Learning outcomes of the course

- What is ImageJ and Fiji
- Basic features of image analysis using this software
  - Add annotations
  - Intensity measurements
  - Drift corrections
  - Measure distances
  - Co-localization analyses
  - Basic features of analyzing time-lapse images

What is ImageJ and FIJI?

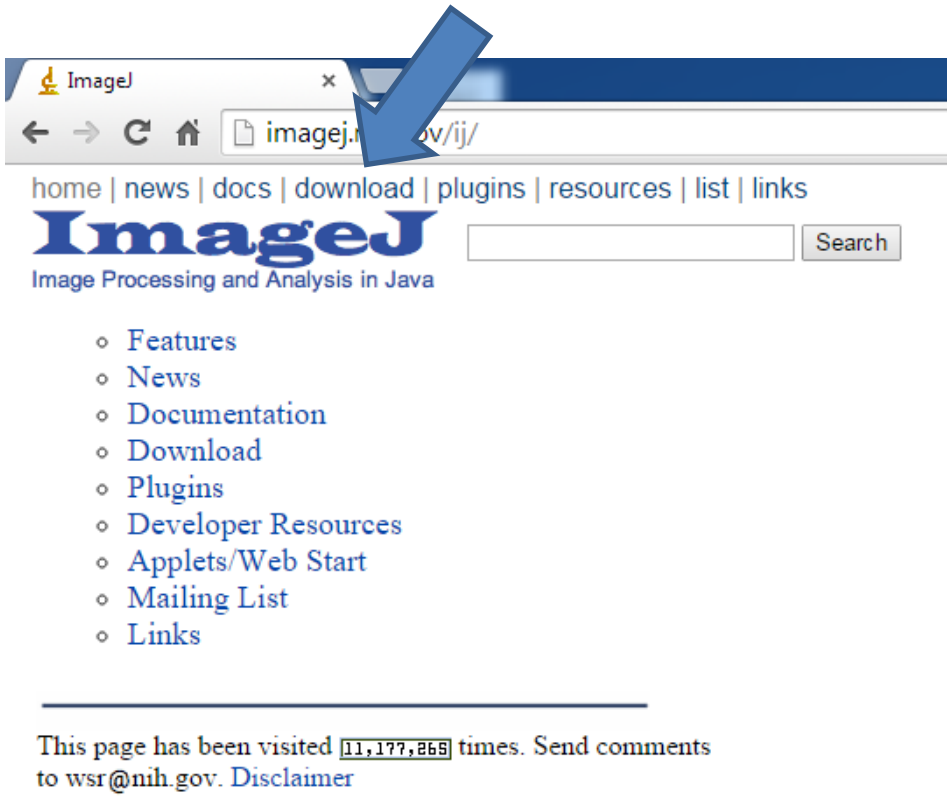
- ImageJ and FIJI are freeware used for image analysis in life sciences
- Freeware: nobody will ask you money for its use.

Collaboration

The Fiji project is driven by a strong desire to improve the tools available for life sciences to process and analyze data. To this end, Fiji collaborates closely with the following projects:

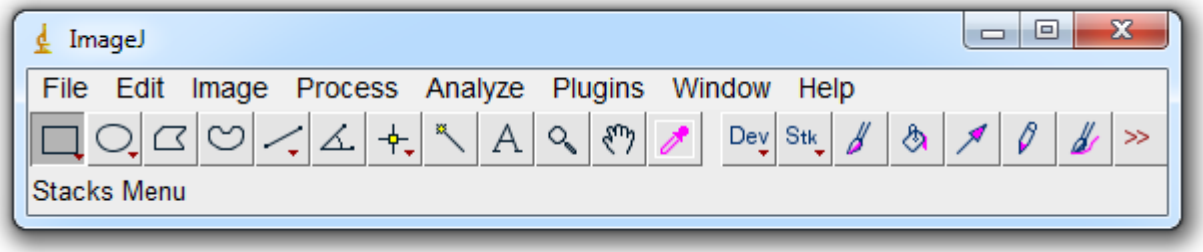
How to get started?

Step 1

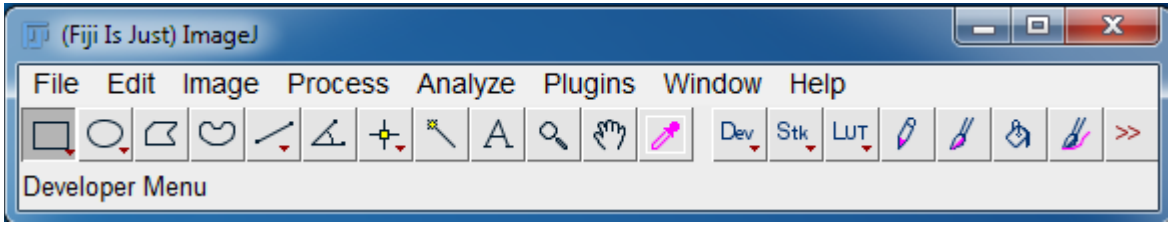


Step 2

Interface of the software



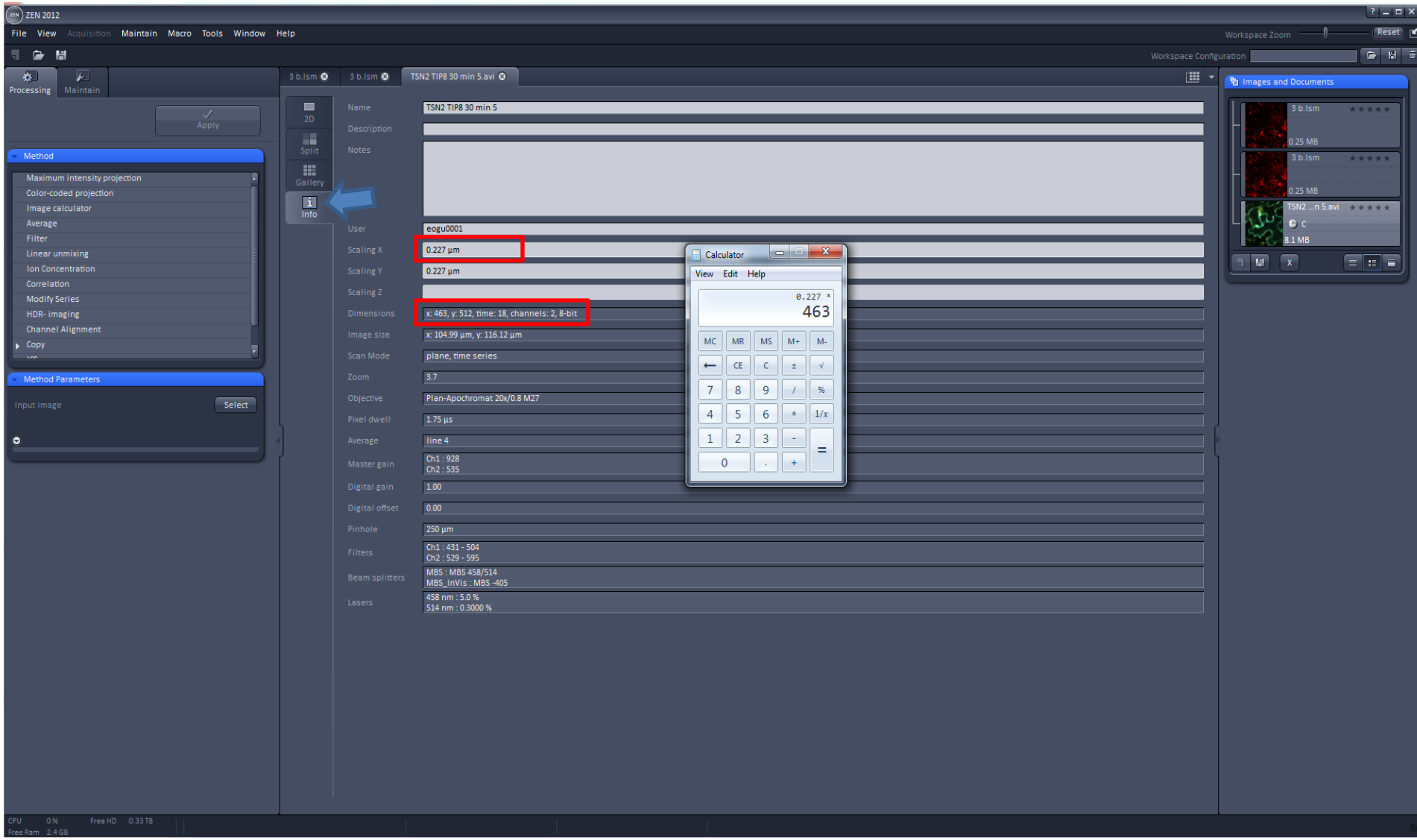
OR?



Assume that you have a time-lapse image. So what is next?

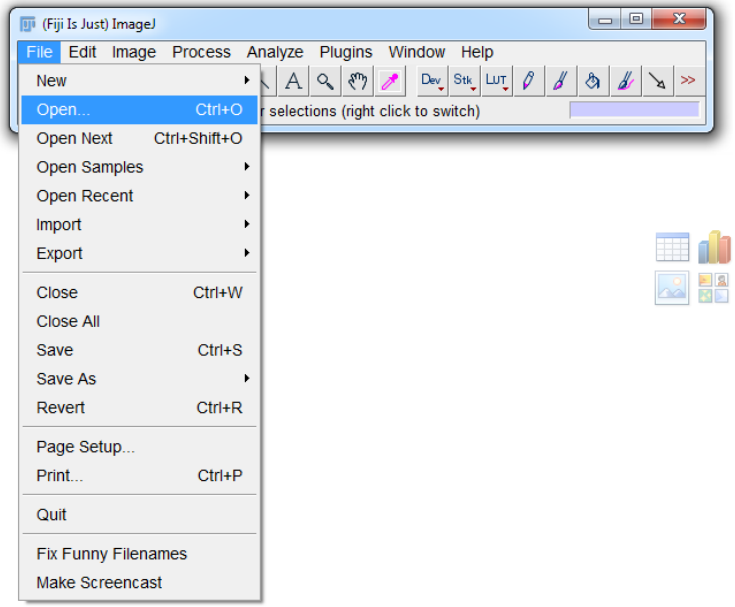
- Check the size of the image (dimensions should be at least 512x512 dpi during image acquisition; French et al., 2008-course literature)
- Do you need to analyze it?

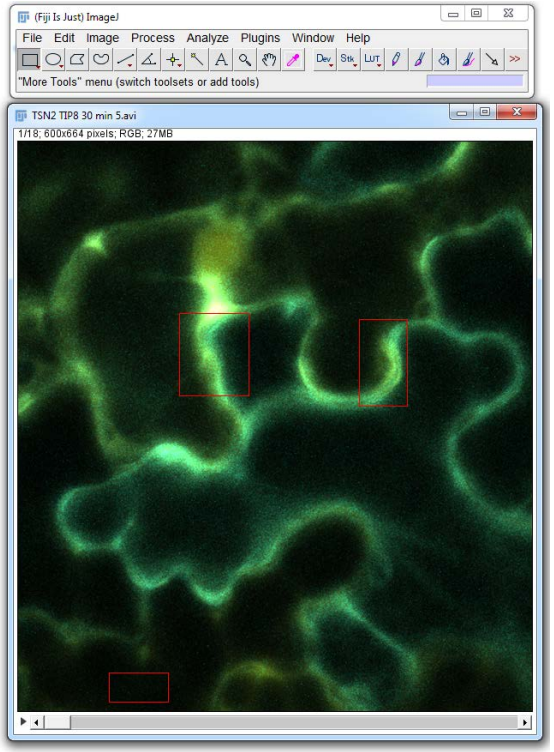
How do I know which is the size of my image



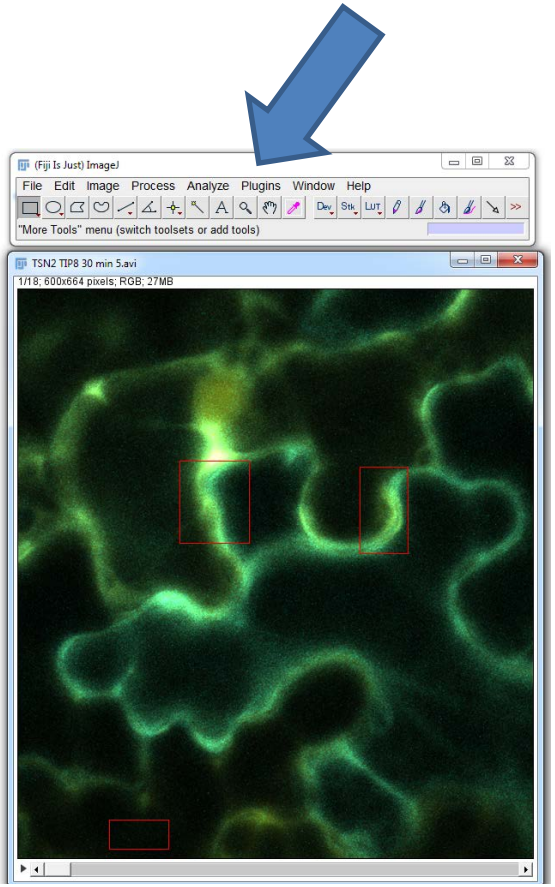
Open your file with FIJI

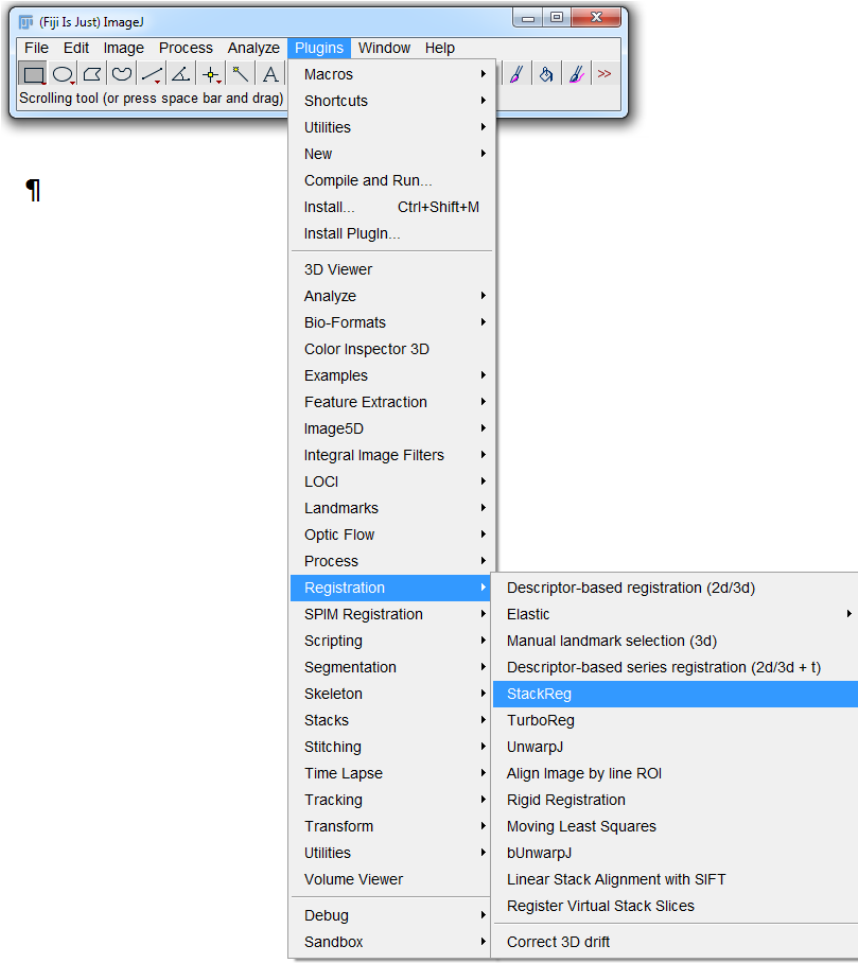
- FIJI is compatible with 'czi'!!!!





My image is 'drifting'. What shall I do?





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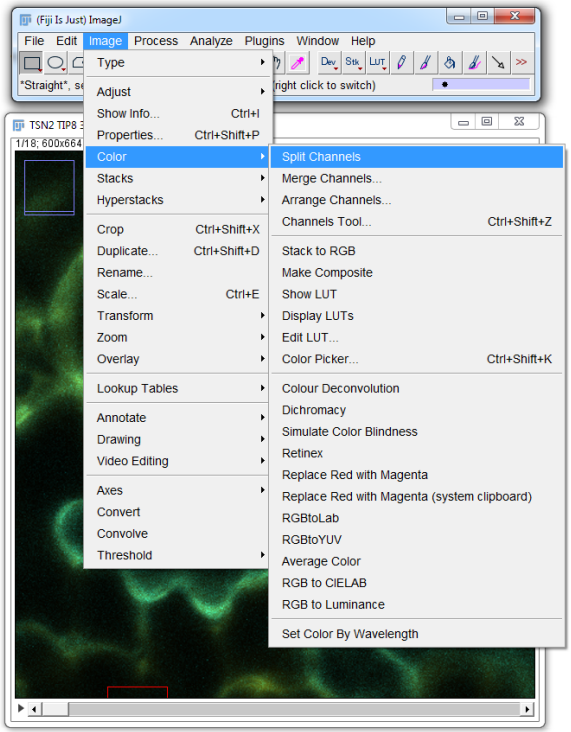
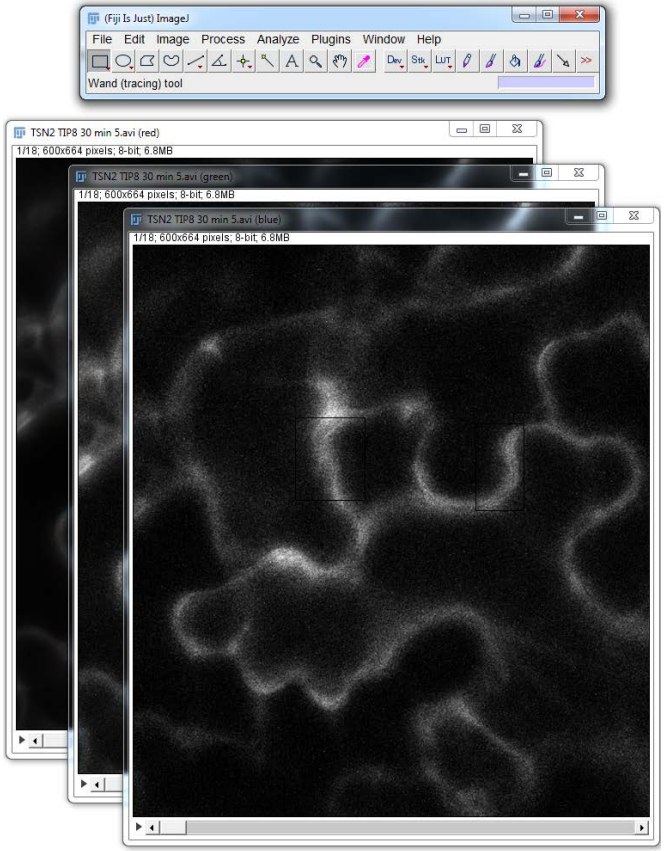
How does it work?

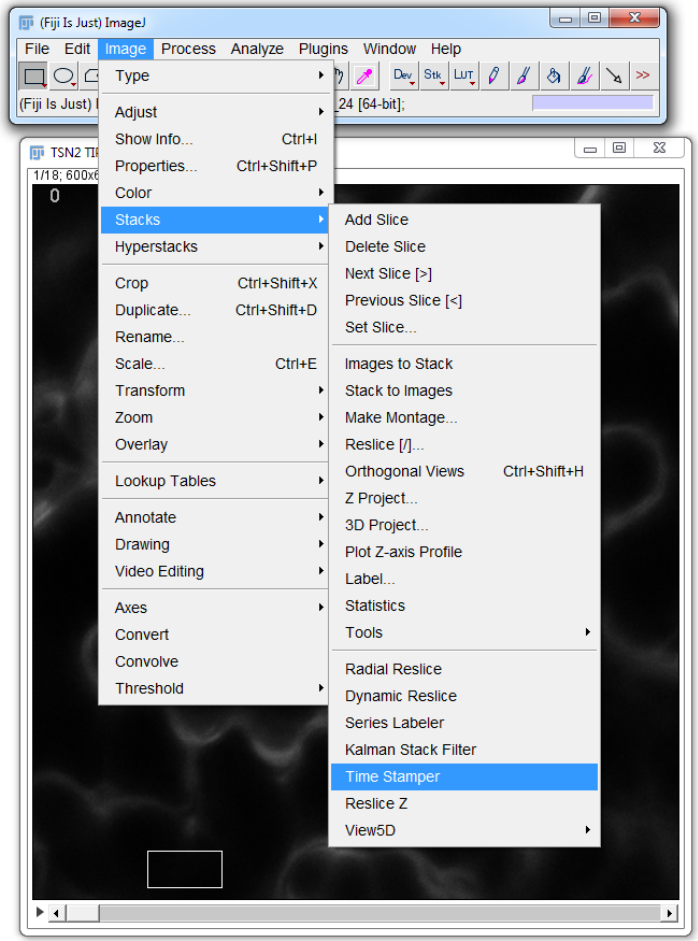
- We are using Rigid body transformations
- http://bigwww.epfl.ch/thevenaz/stackreg/

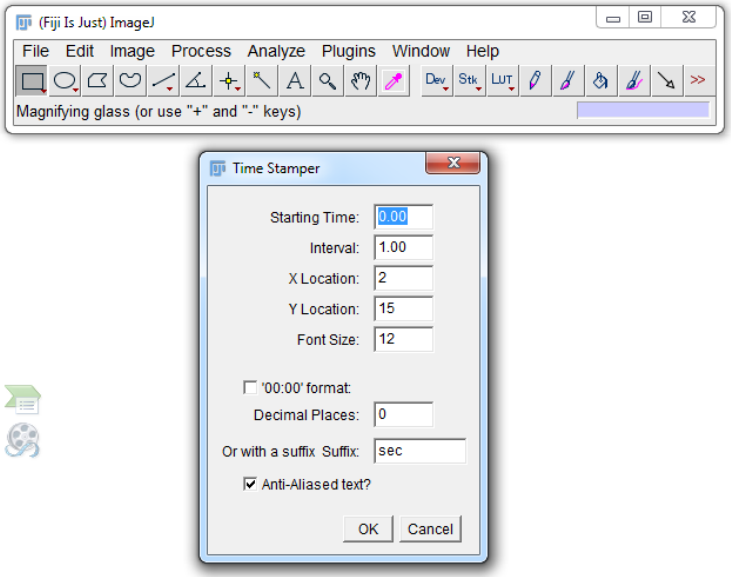
If you have channels you can split them



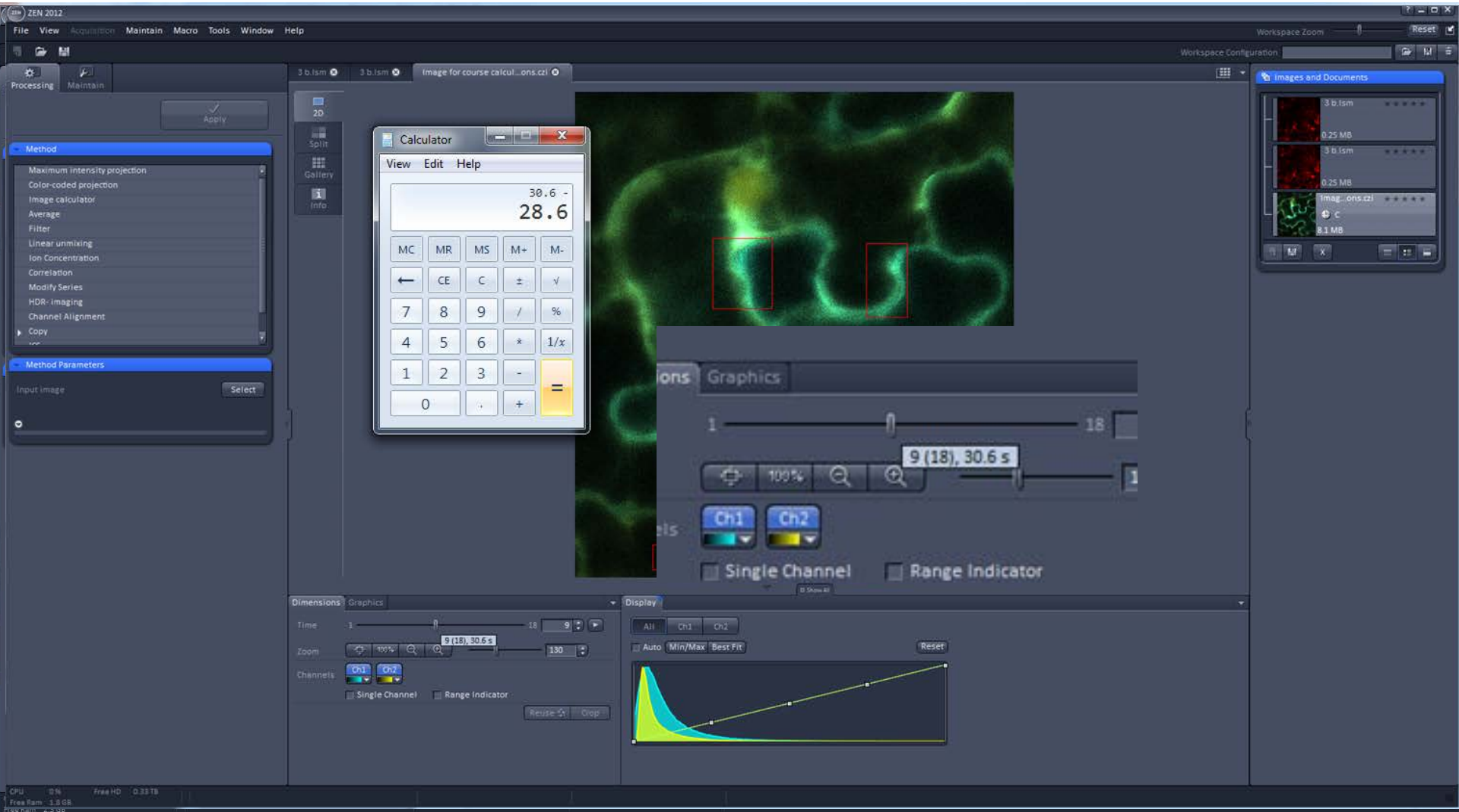


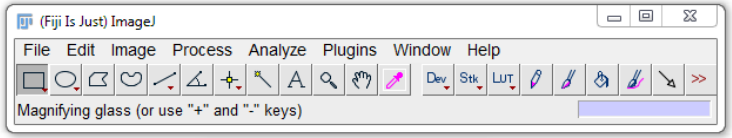
Lets add time stamps

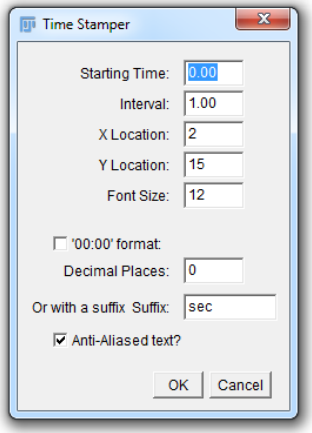


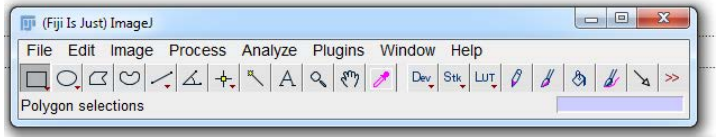


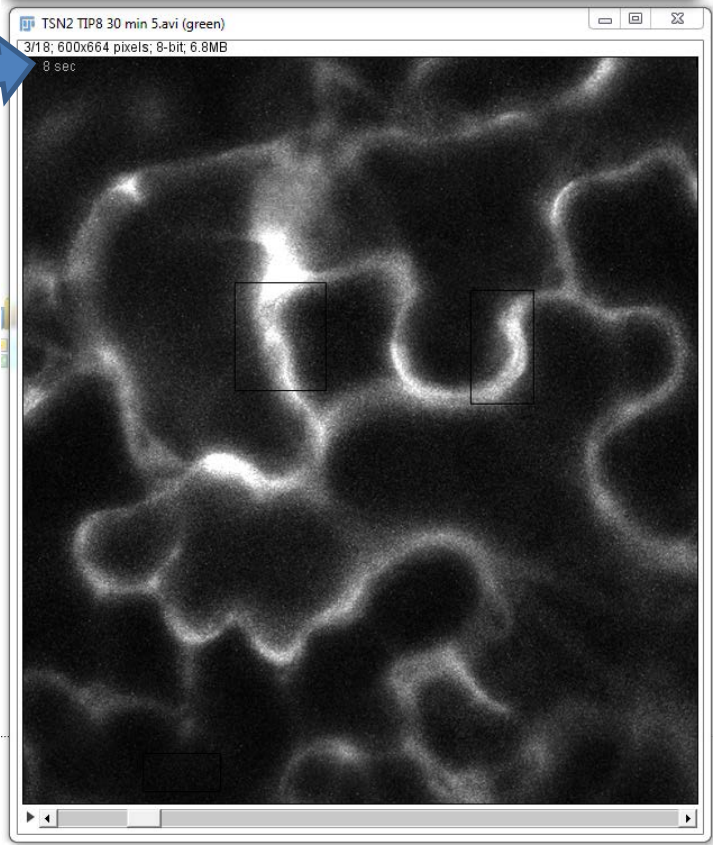
Calculation of interval time



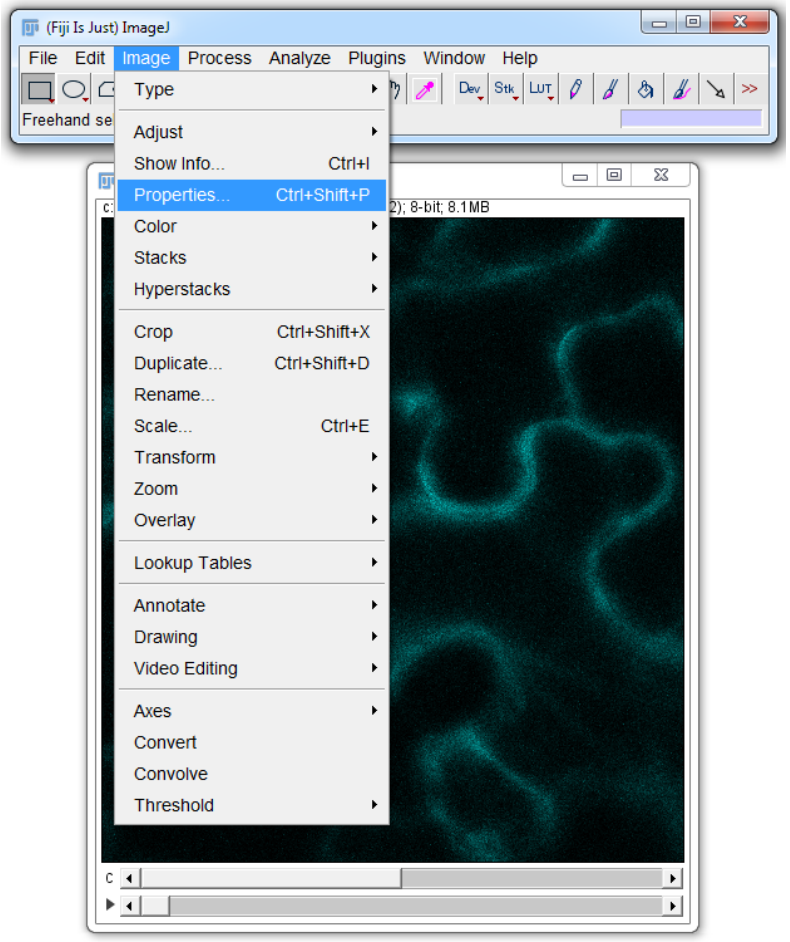

(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Magnifying glass (or use "+" and "-" keys)

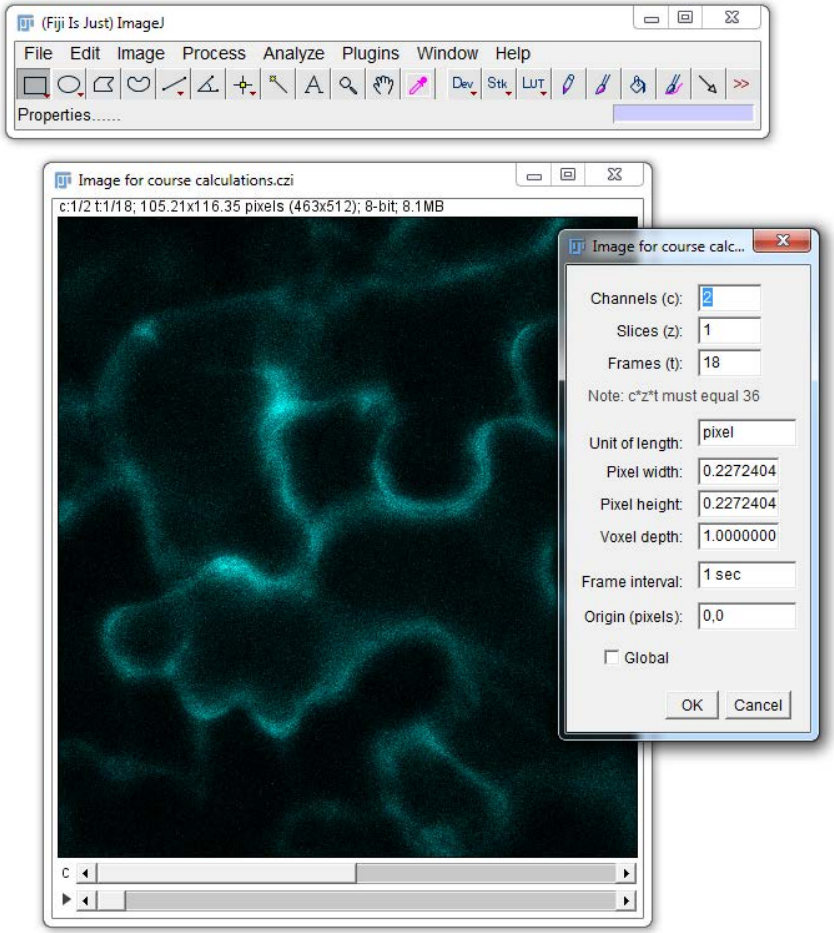

Time Stamper
Starting Time: 0.00
Interval: 1.00
X Location: 2
Y Location: 15
Font Size: 12
'00:00' format:
Decimal Places: 0
Or with a suffix Suffix: sec
Anti-Aliased text?
OK
Cancel


(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Polygon selections

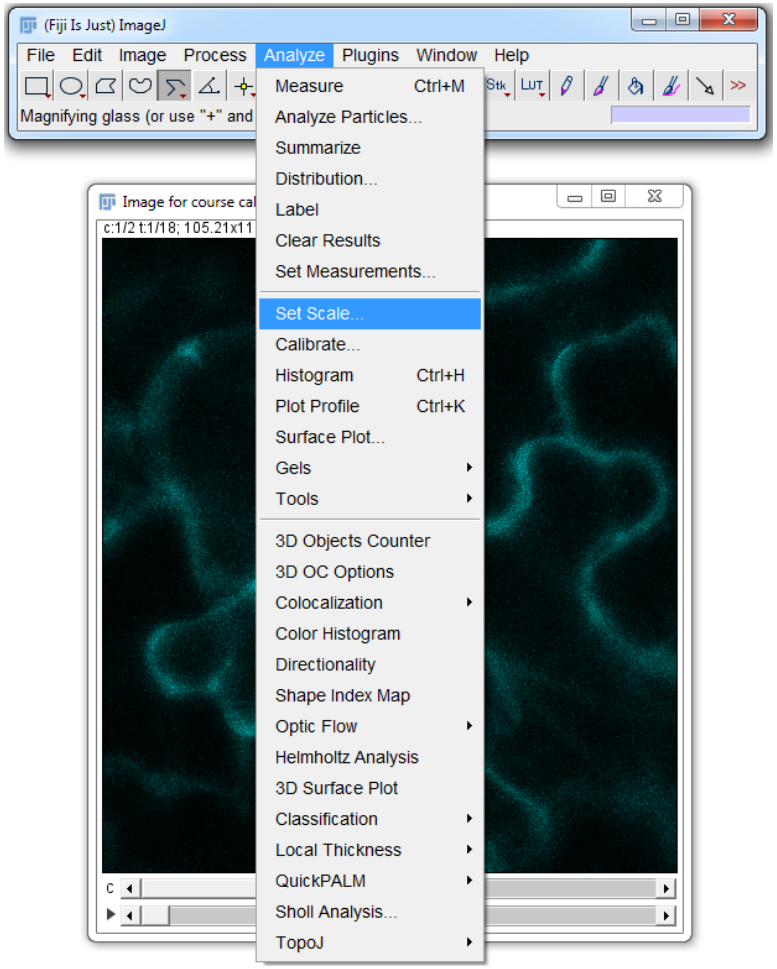

TSN2 TIP8 30 min 5.avi (green)
3/18; 600x664 pixels; 8-bit; 6.8MB
8 sec

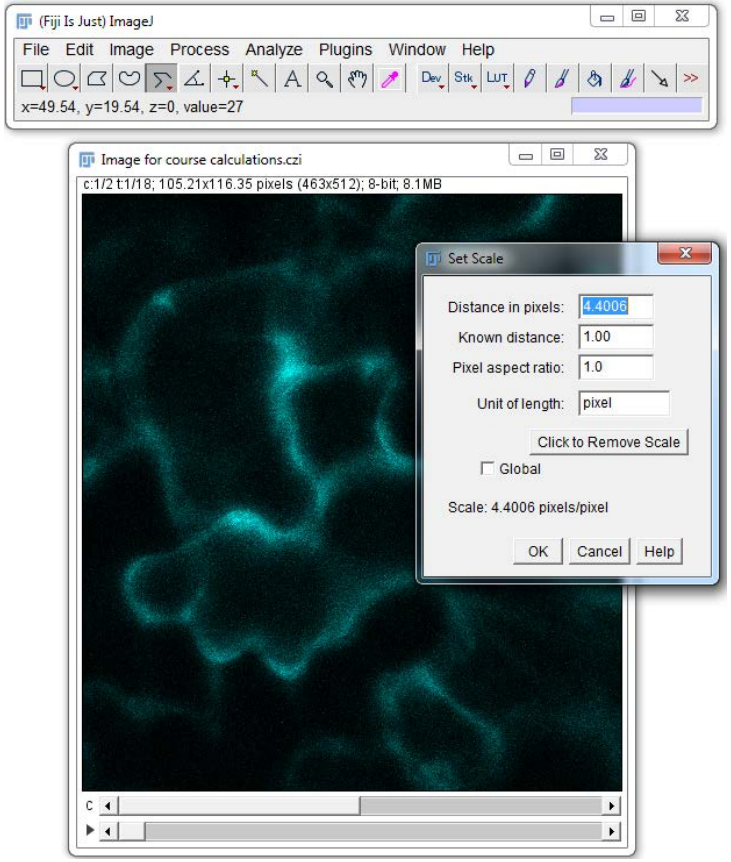
Set up scale bars



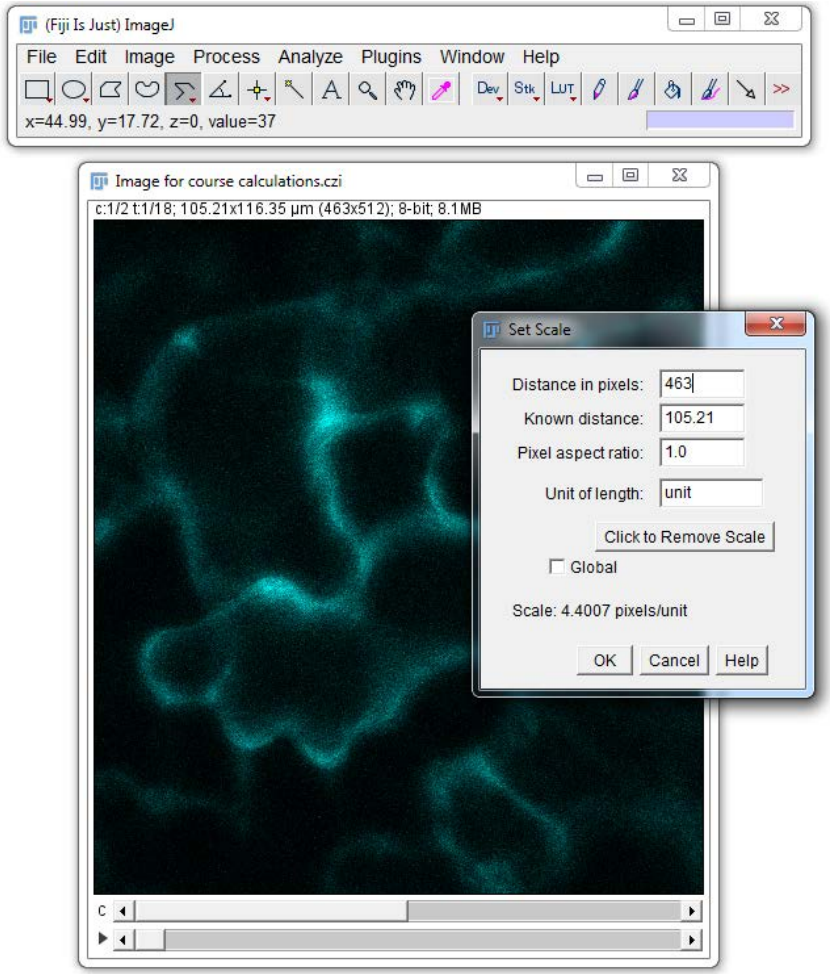


1. If you have 'CZI' files





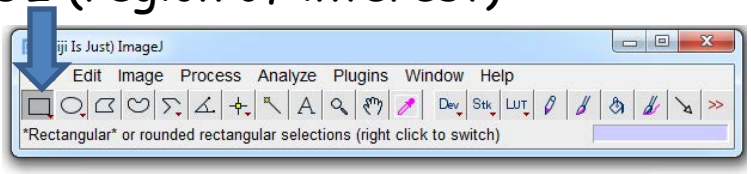
2. If you do not have 'CZI' files (e.g. 'avi' files etc.)

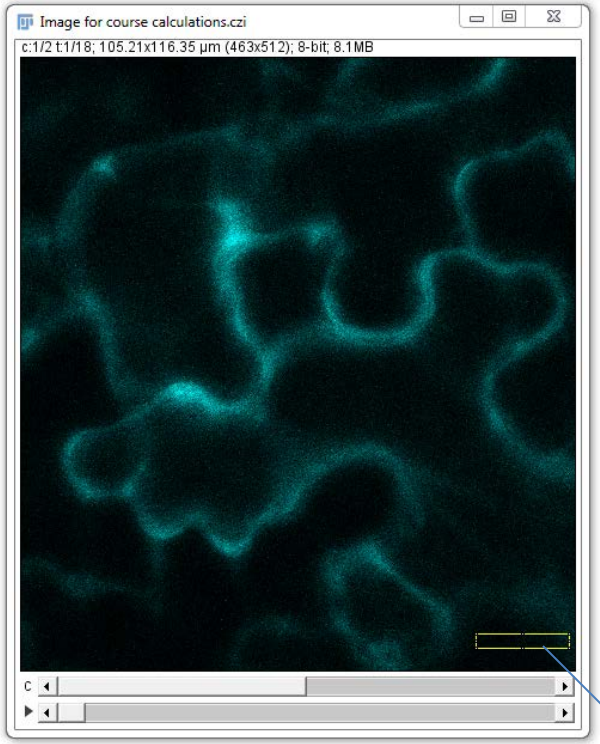


Adding the scale bar on the image

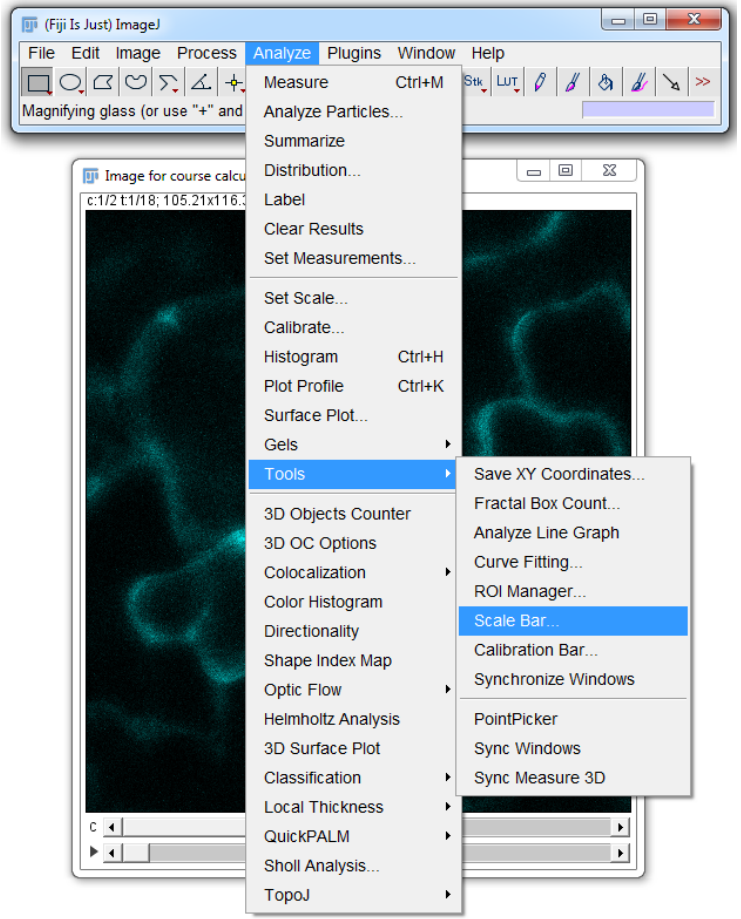
Step 1: select where you want the scale bar

ROI (region of interest)



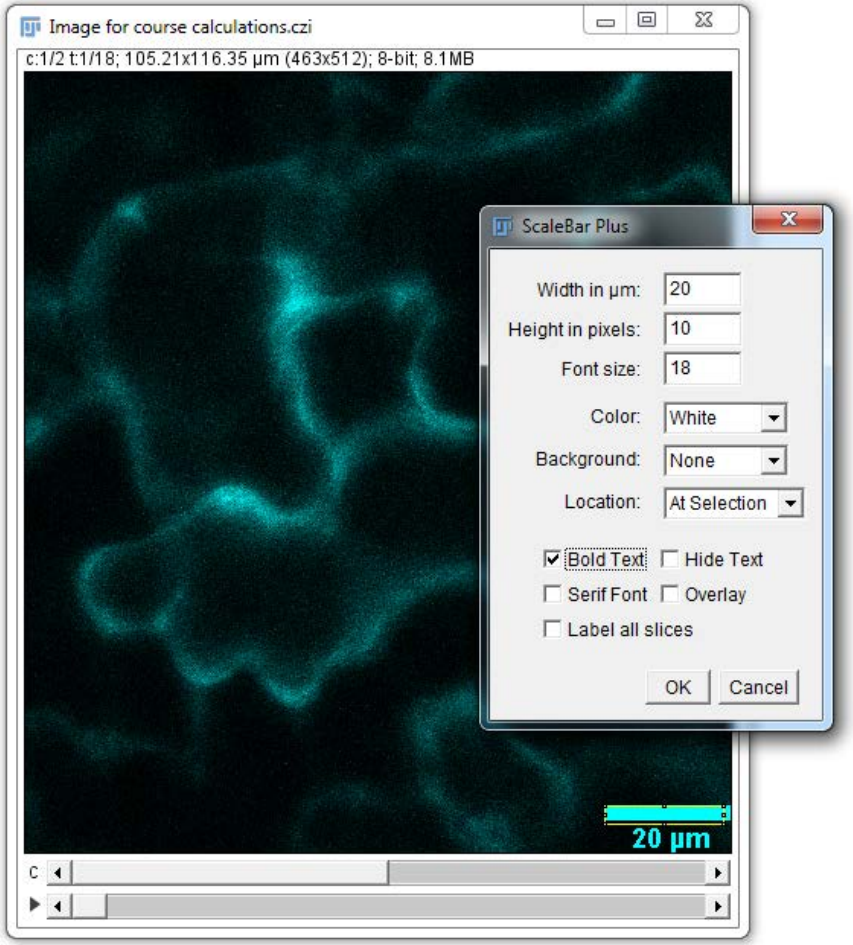


Step 2: do the following

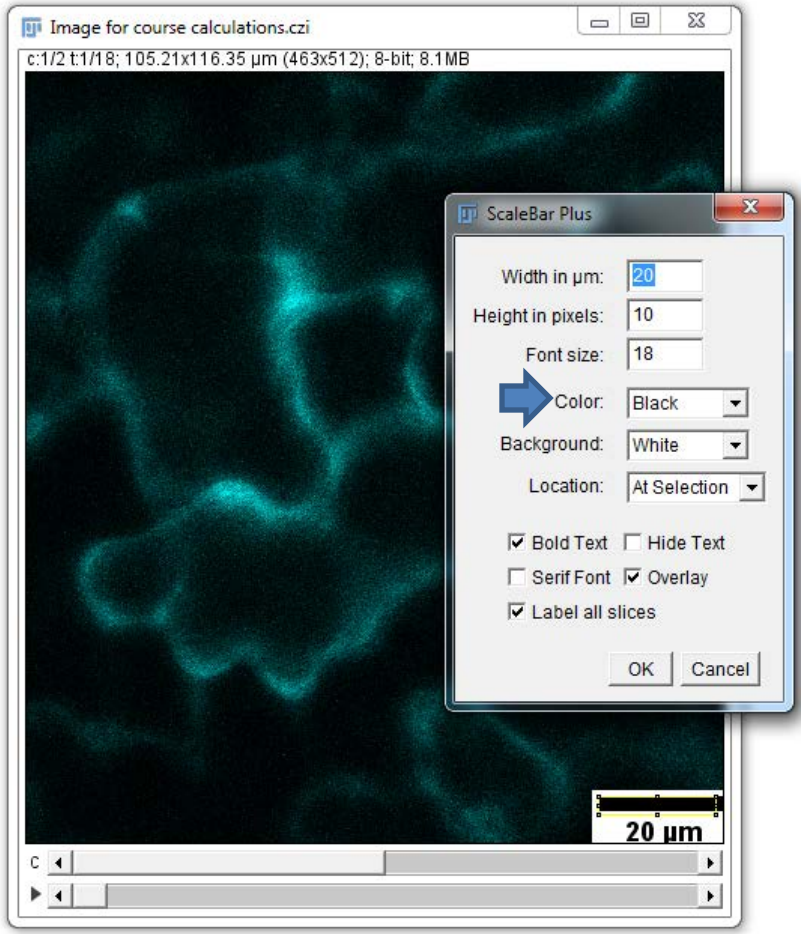


I want my scale bar here

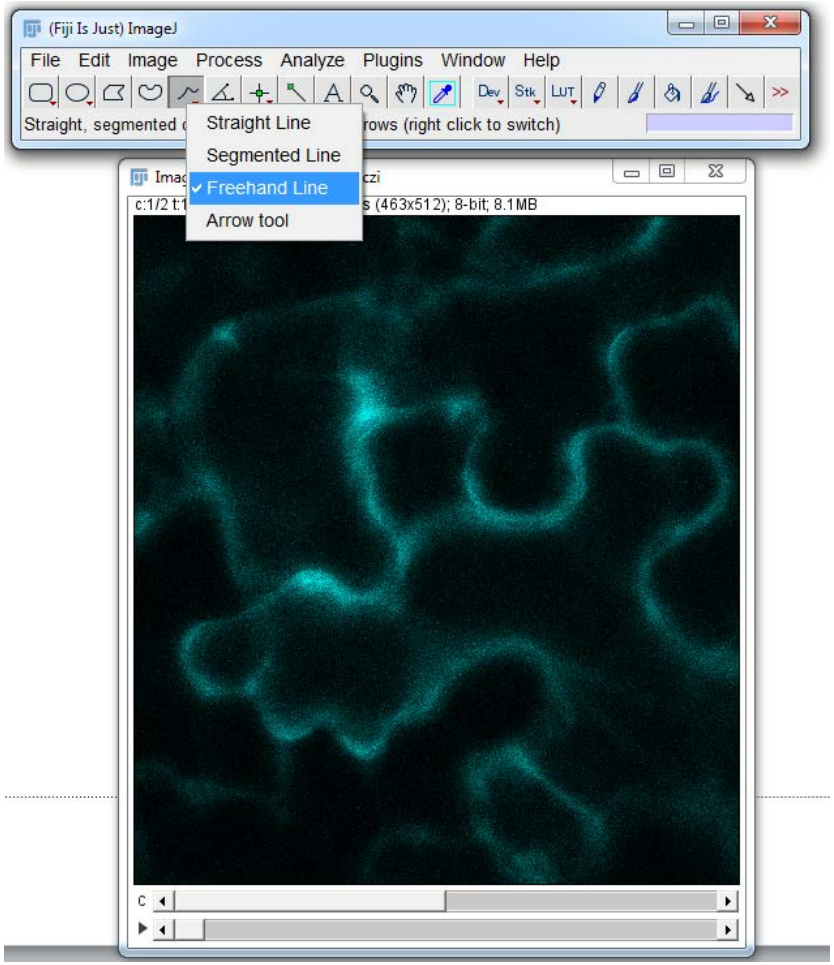
Step 3: this box appears.

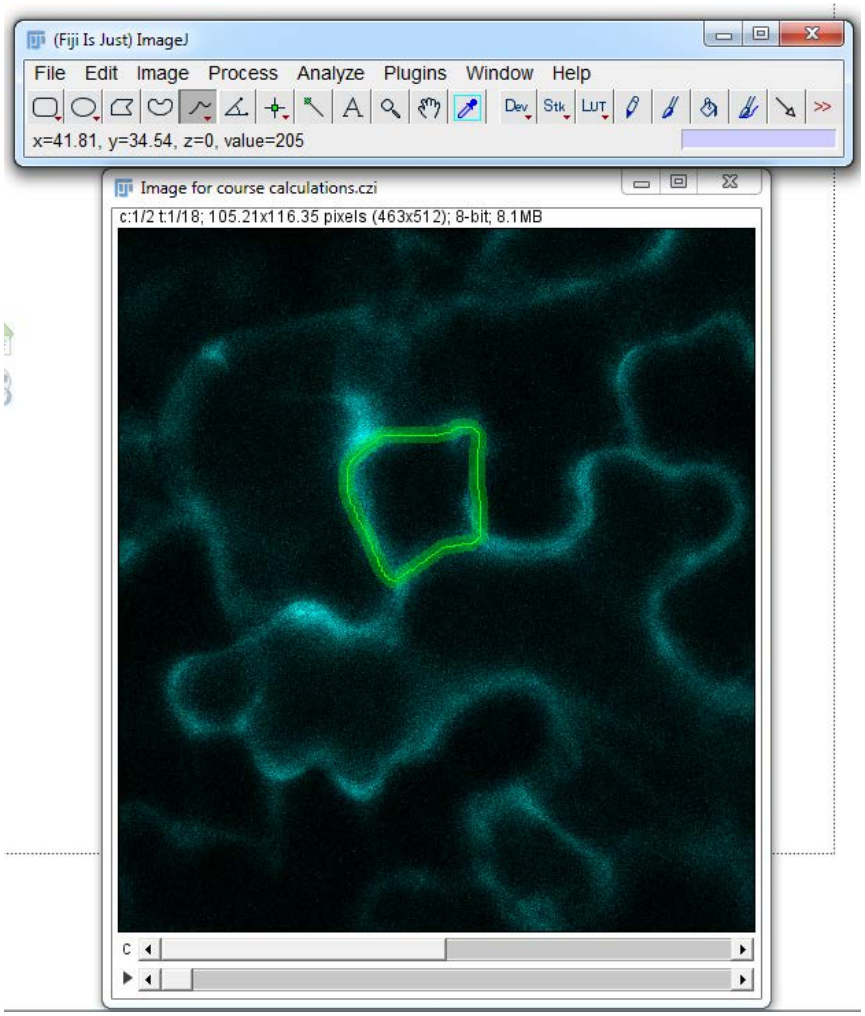


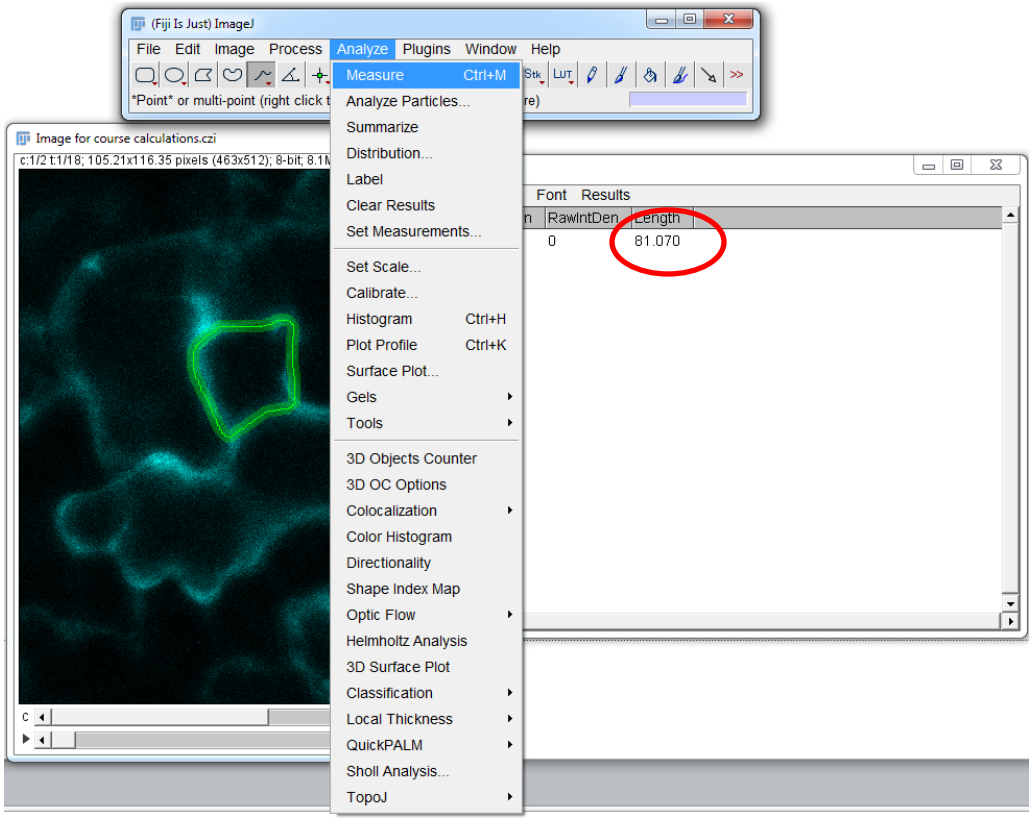
Step 4: adjust 'color' and…done.



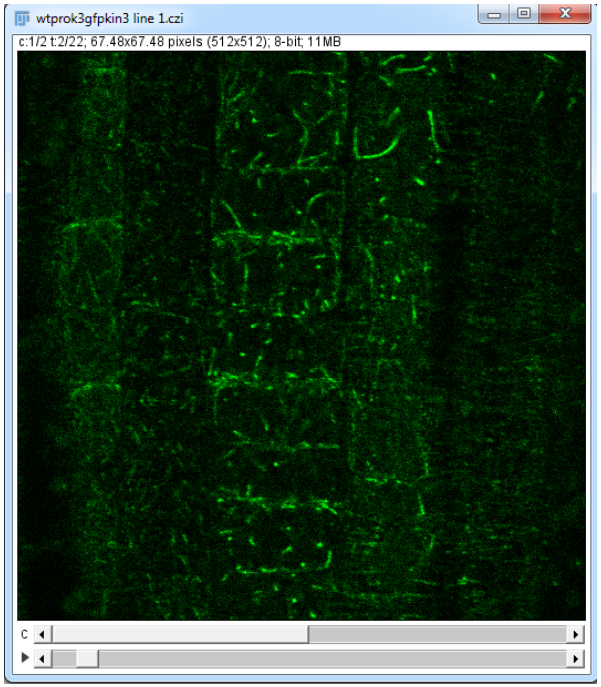
Now I can make dimension measurements

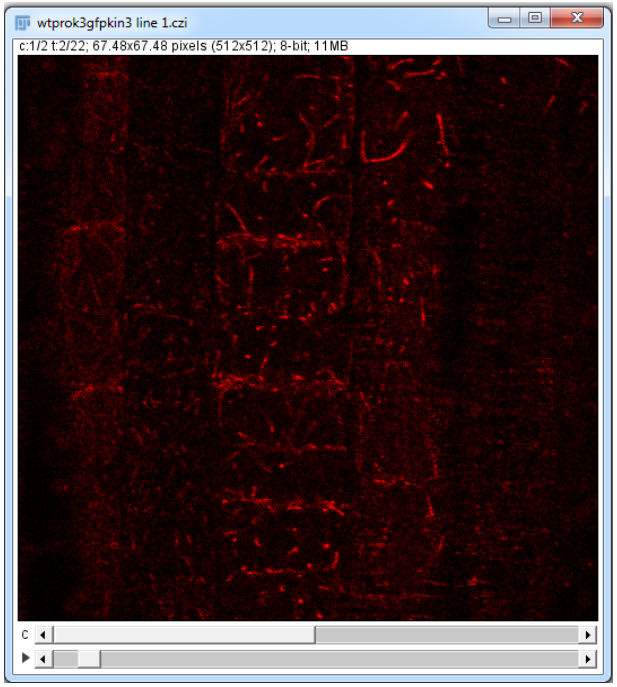


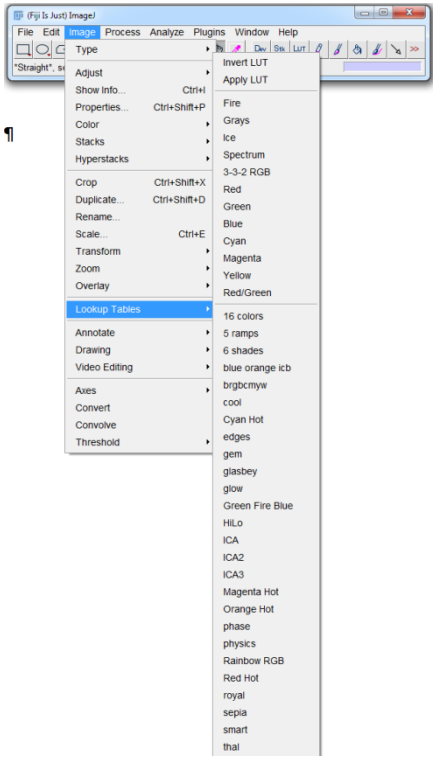



(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Measure Ctrl+M
Analyze Particles...
Summarize
Distribution...
Label
Clear Results
Set Measurements...
Set Scale...
Calibrate...
Histogram Ctrl+H
Plot Profile Ctrl+K
Surface Plot...
Gels
Tools
3D Objects Counter
3D OC Options
Colocalization
Color Histogram
Directionality
Shape Index Map
Optic Flow
Helmholtz Analysis
3D Surface Plot
Classification
Local Thickness
QuickPALM
Sholl Analysis...
TopoJ
Image for course calculations.czi
Font Results
RawIntDen
Length
0
81.070

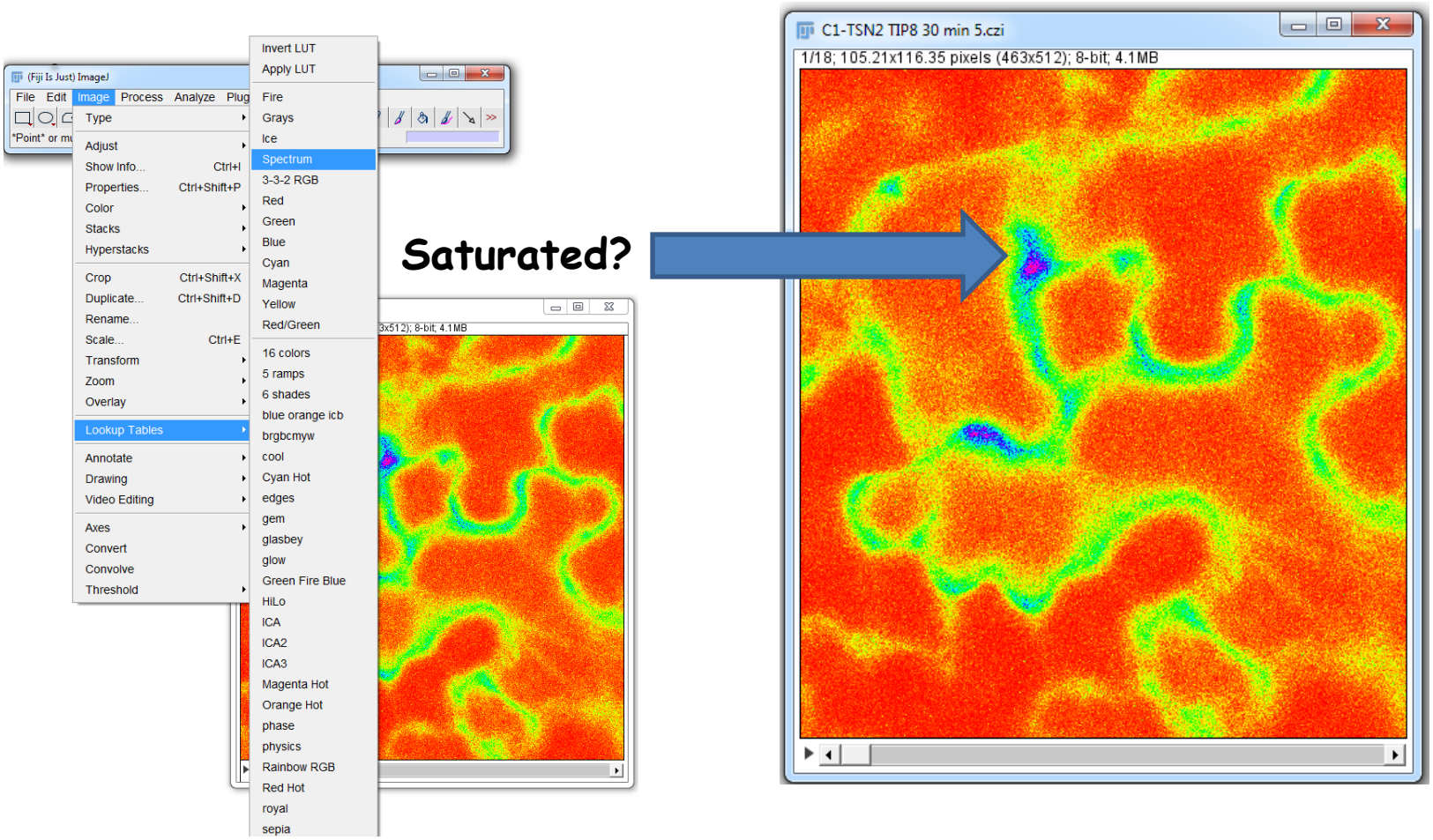
Adjusting colors



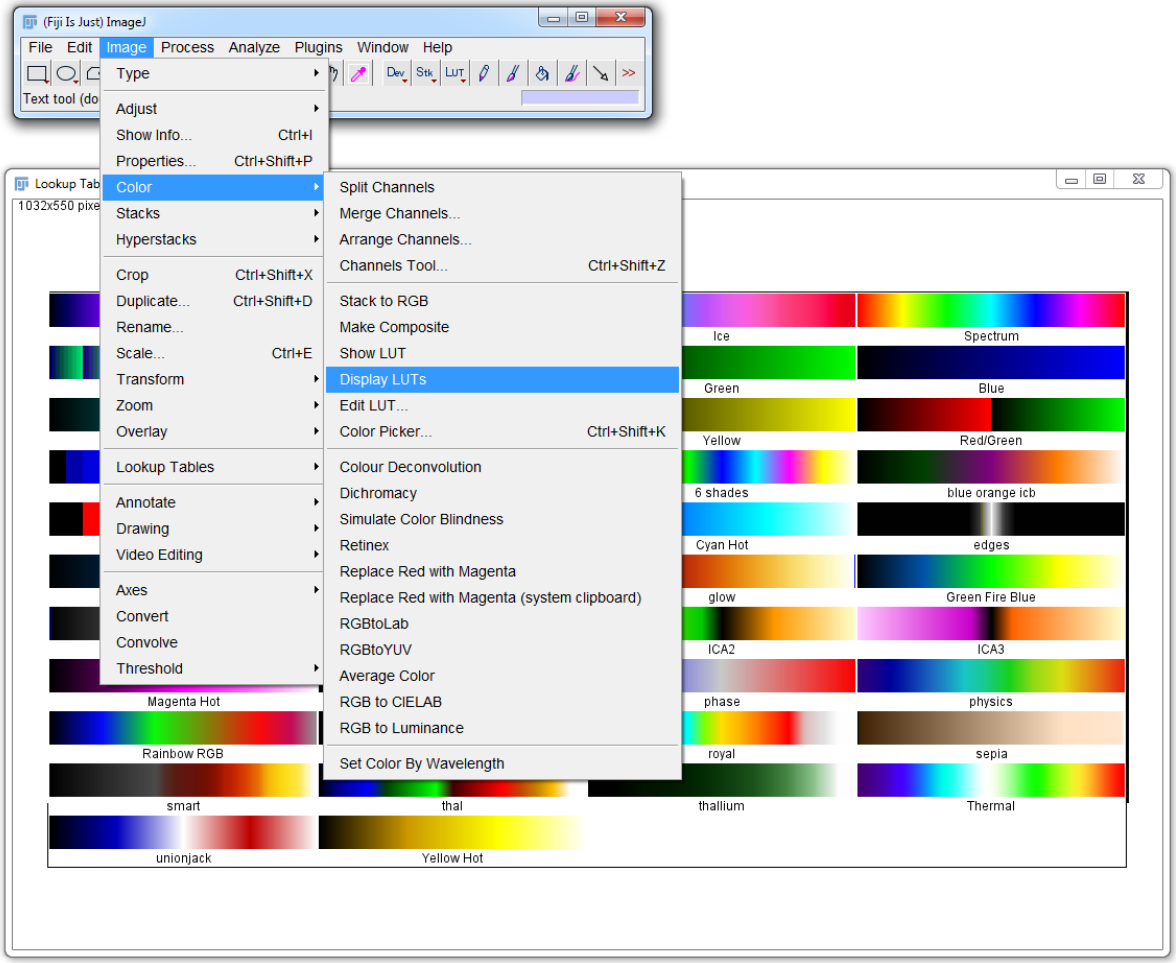




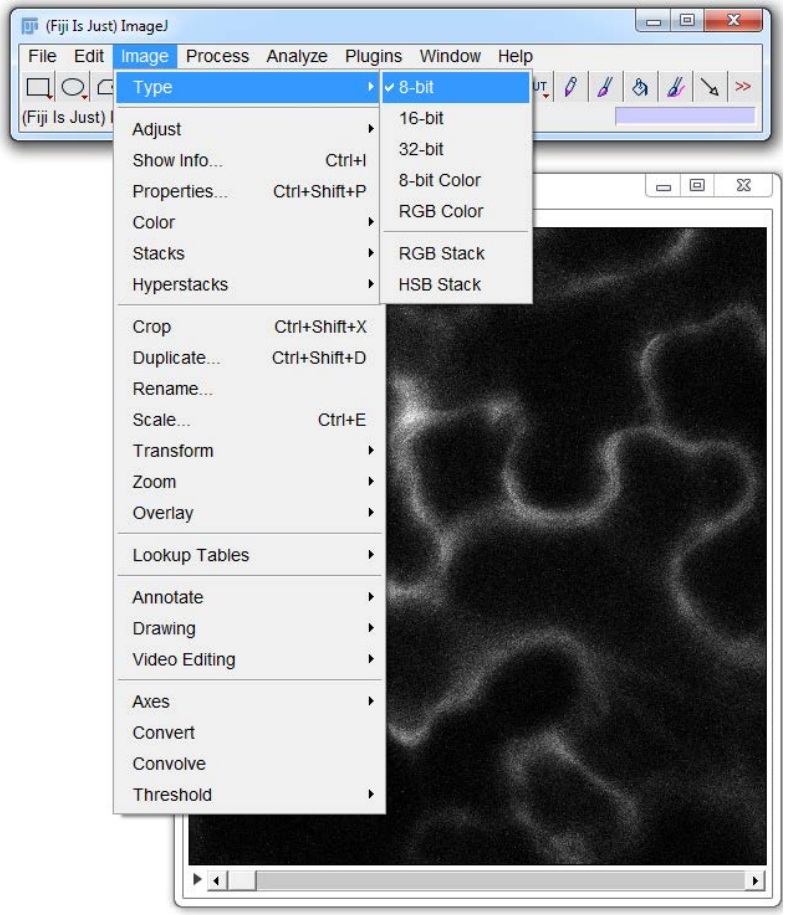
Solution: Use grey scale images

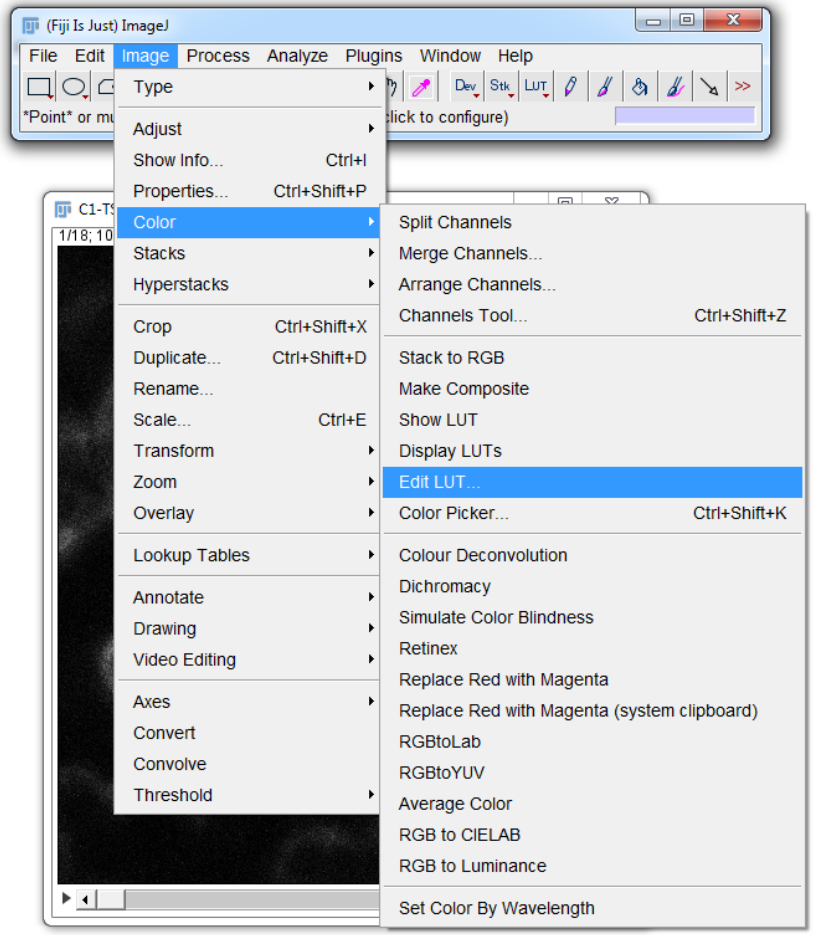

(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plug
Type
Adjust
Show Info... Ctrl+I
Properties... Ctrl+Shift+P
Color
Stacks
Hyperstacks
Crop Ctrl+Shift+X
Duplicate... Ctrl+Shift+D
Rename...
Scale... Ctrl+E
Transform
Zoom
Overlay
Lookup Tables
Annotate
Drawing
Video Editing
Axes
Convert
Convolve
Threshold
Invert LUT
Apply LUT
Fire
Grays
Ice
Spectrum
3-3-2 RGB
Red
Green
Blue
Cyan
Magenta
Yellow
Red/Green
16 colors
5 ramps
6 shades
blue orange icb
brgbcmyw
cool
Cyan Hot
edges
gem
glasbey
glow
Green Fire Blue
HiLo
ICA
ICA2
ICA3
Magenta Hot
Orange Hot
phase
physics
Rainbow RGB
Red Hot
royal
sepia
Saturated?
C1-TSN2 TIP8 30 min 5.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB

What is a Look Up Table (LUT)?

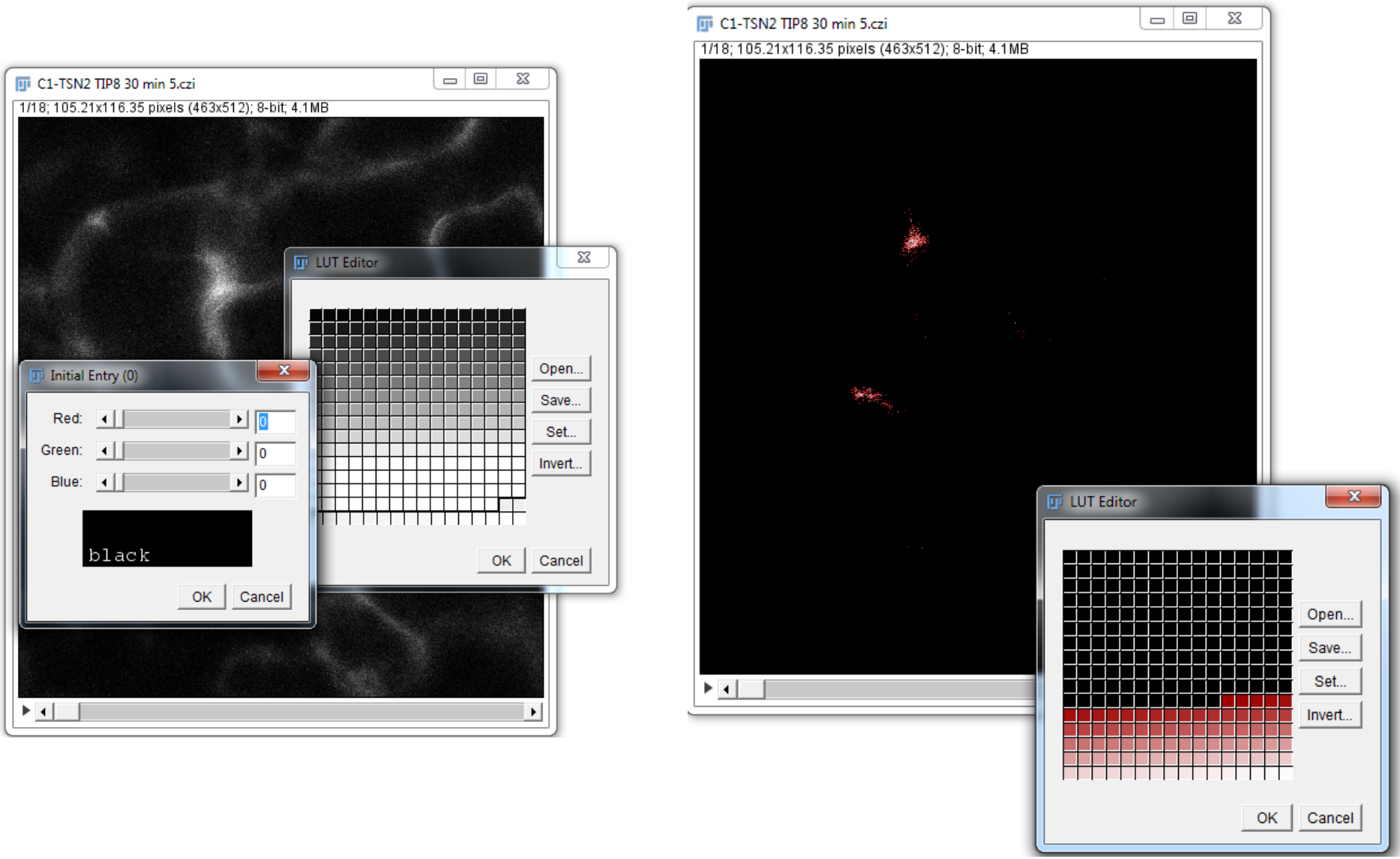


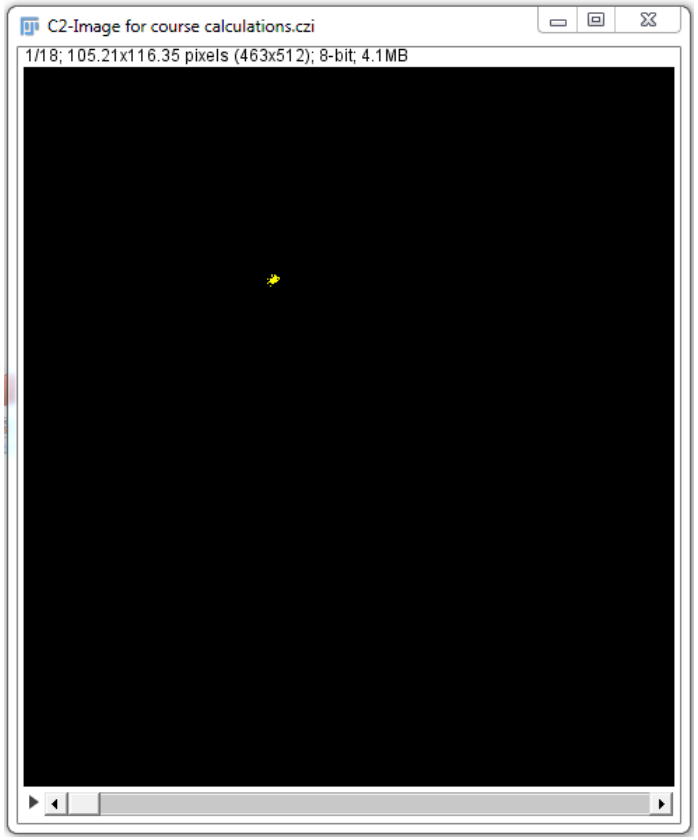
Highlighting certain signal intensities

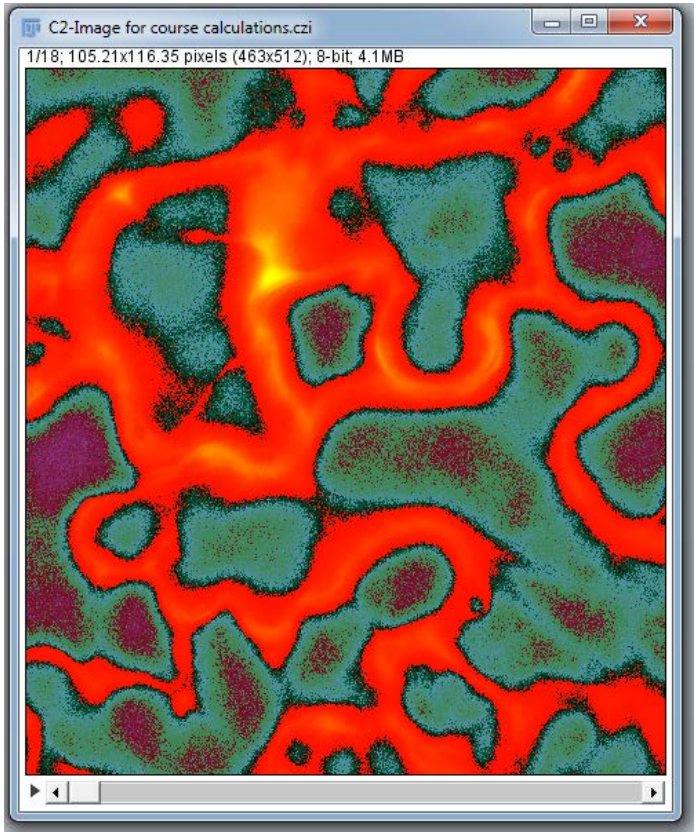




Are my colors in linear range?

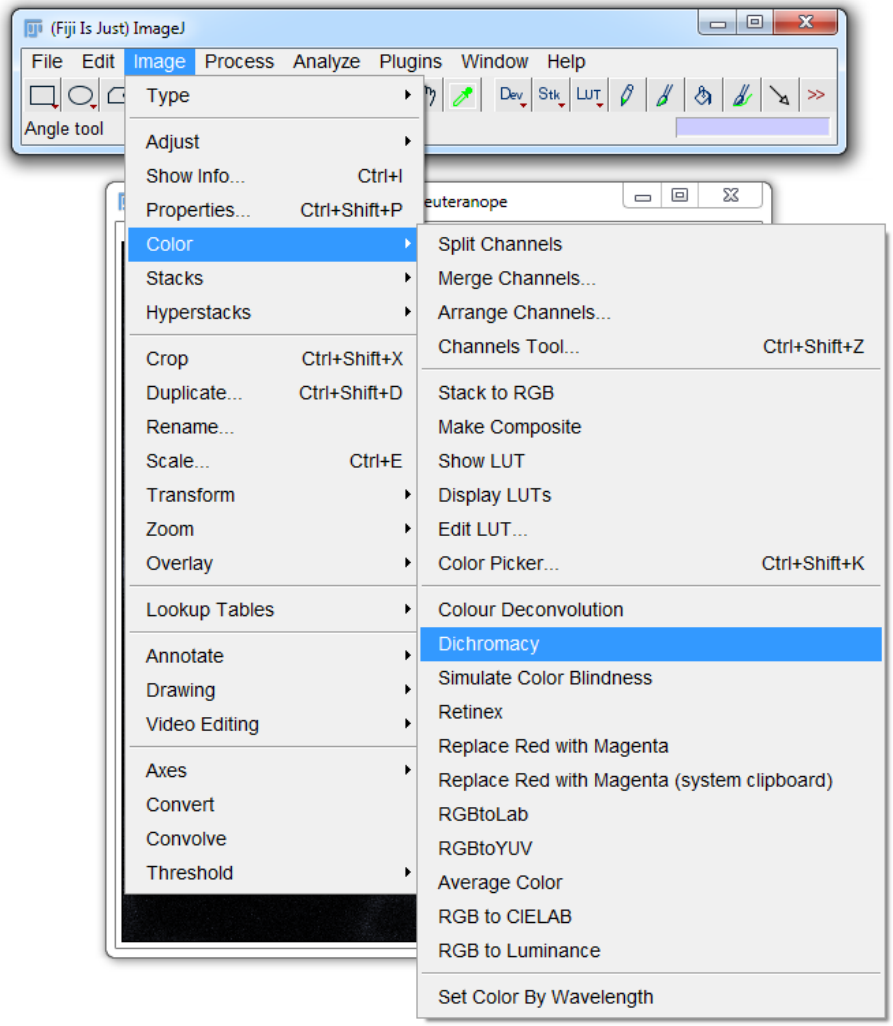


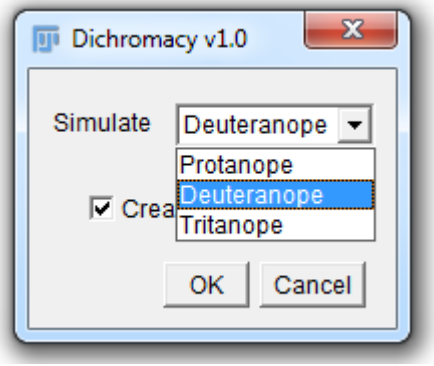

C2-Image for course calculations.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB

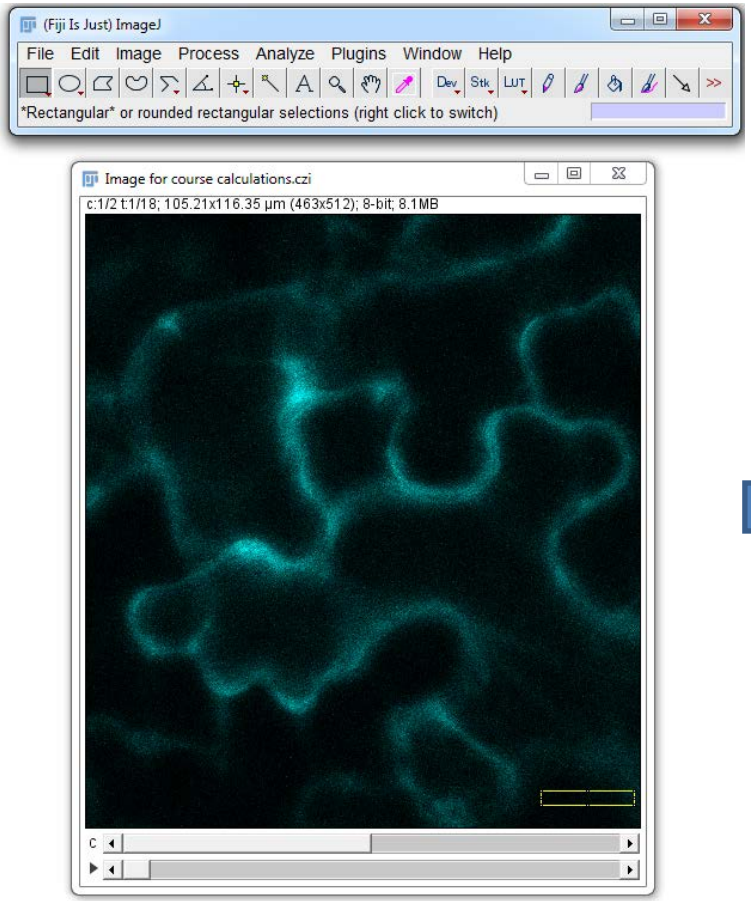

C2-Image for course calculations.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB

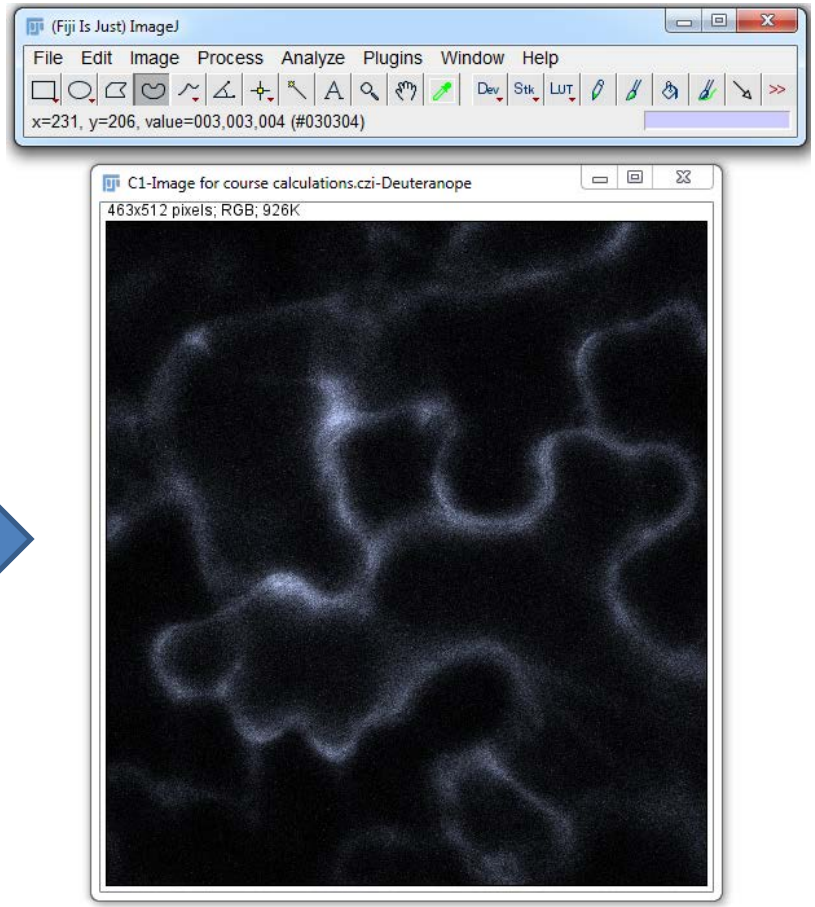
Types of color blindness

Simulate color blightness

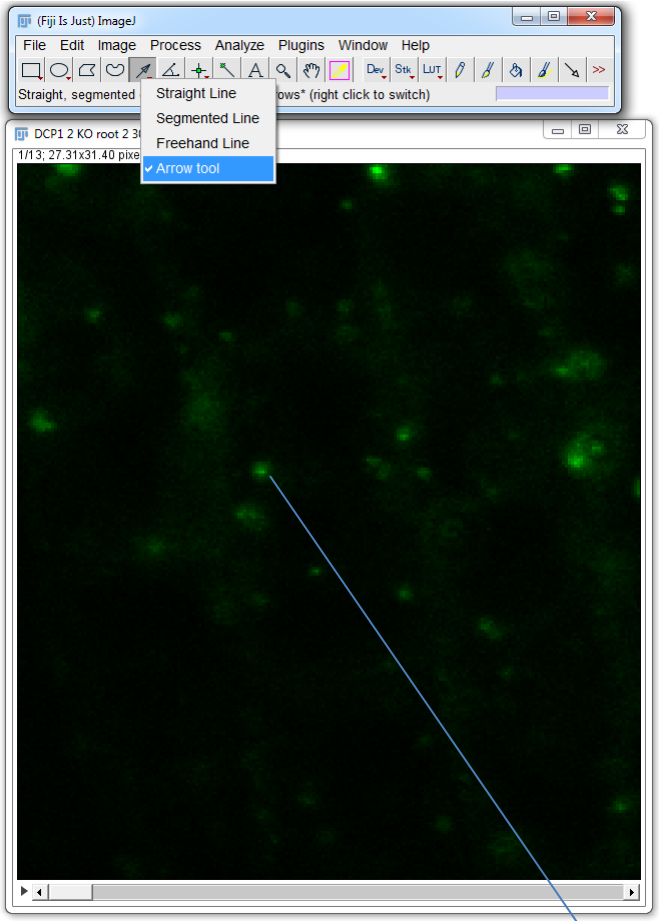


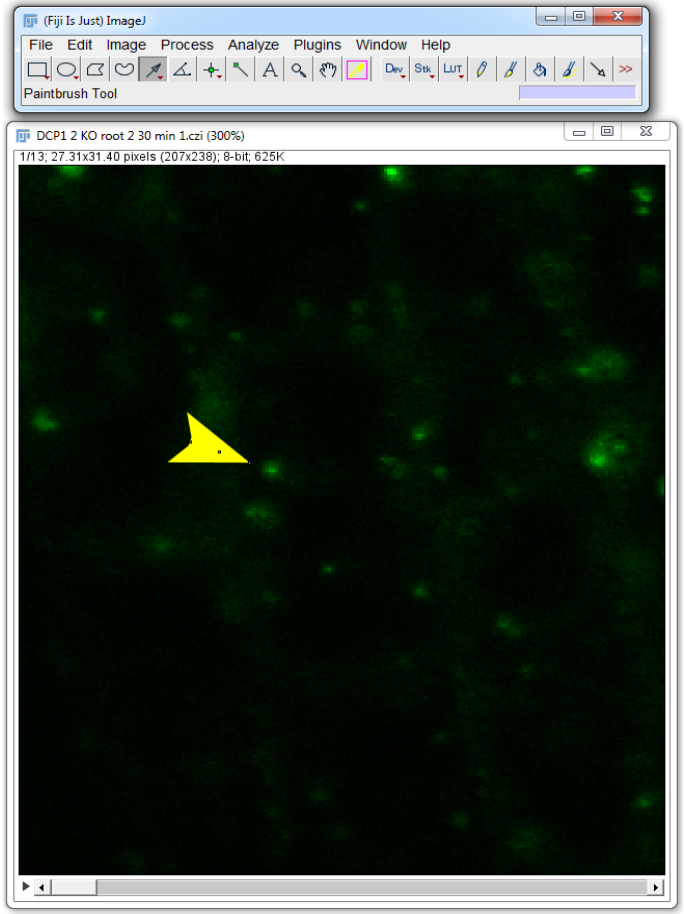



(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Rectangular or rounded rectangular selections (right click to switch)
Image for course calculations.czi
c:1/2 t:1/18; 105.21x116.35 µm (463x512); 8-bit; 8.1MB

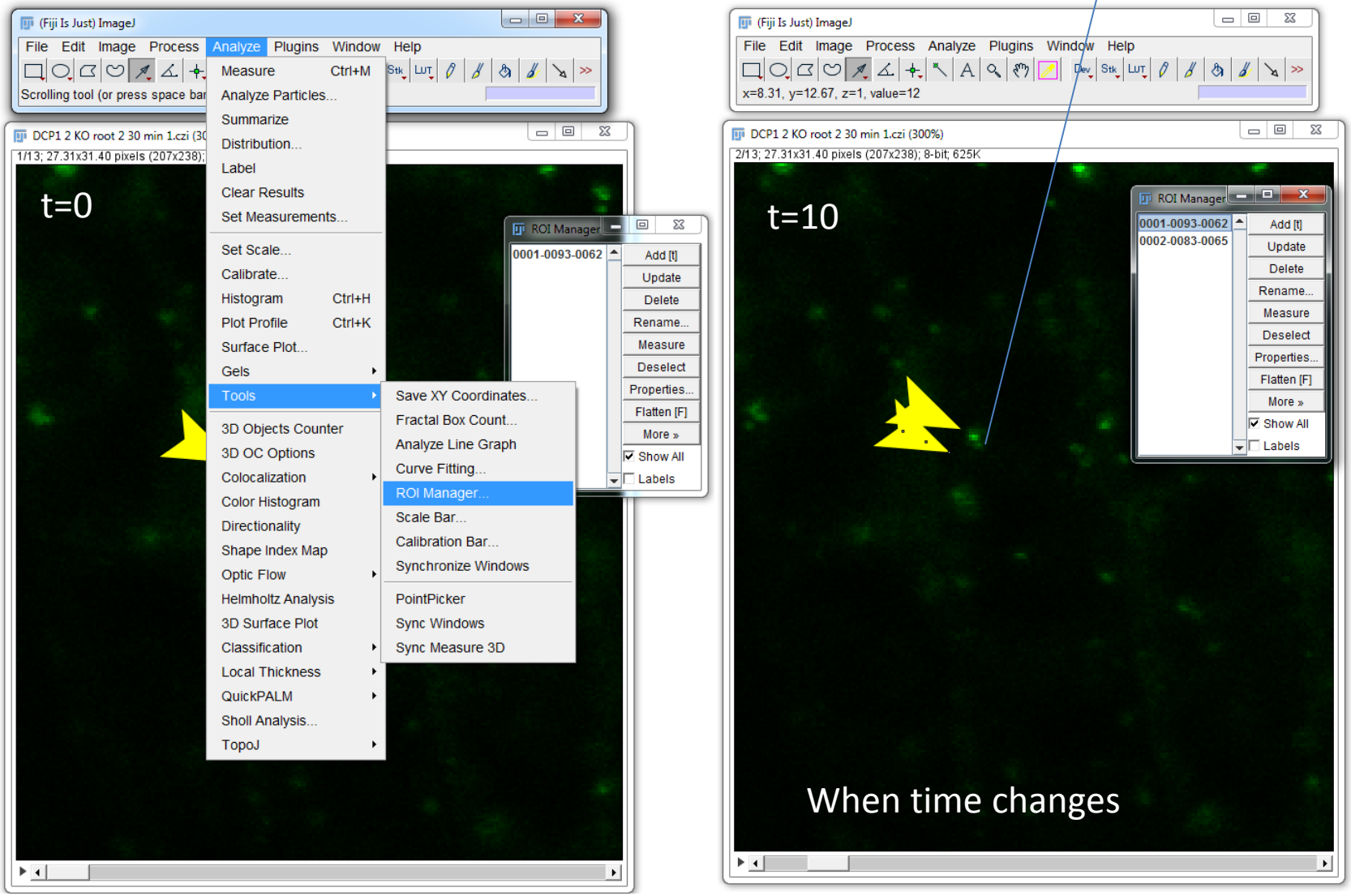

(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
x=231, y=206, value=003,003,004 (#030304)
C1-Image for course calculations.czi-Deuteranope
463x512 pixels; RGB; 926K

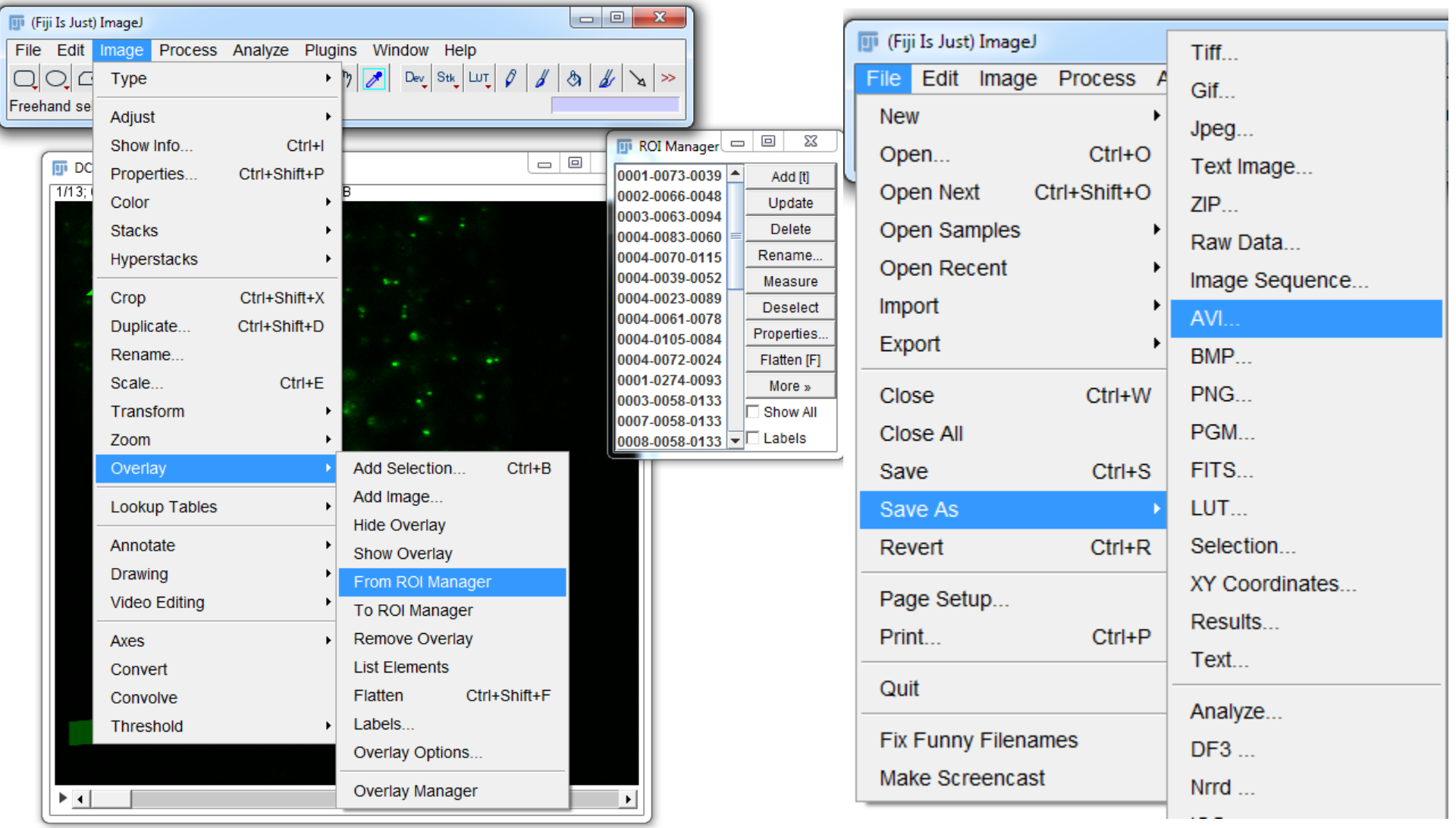
Designate structures on your images





I am interested on this spot


My spot is moving…
(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Scrolling tool (or press space bar
DCP1 2 KO root 2 30 min 1.czi (30
1/13; 27.31x31.40 pixels (207x238);
t=0
Measure Ctrl+M
Analyze Particles...
Summarize
Distribution...
Label
Clear Results
Set Measurements...
Set Scale...
Calibrate...
Histogram Ctrl+H
Plot Profile Ctrl+K
Surface Plot...
Gels
Tools
3D Objects Counter
3D OC Options
Colocalization
Color Histogram
Directionality
Shape Index Map
Optic Flow
Helmholtz Analysis
3D Surface Plot
Classification
Local Thickness
QuickPALM
Sholl Analysis...
TopoJ
Save XY Coordinates...
Fractal Box Count...
Analyze Line Graph
Curve Fitting...
ROI Manager...
Scale Bar...
Calibration Bar...
Synchronize Windows
PointPicker
Sync Windows
Sync Measure 3D
ROI Manager
0001-0093-0062
Add [t]
Update
Delete
Rename...
Measure
Deselect
Properties...
Flatten [F]
More »
Show All
Labels
(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
x=8.31, y=12.67, z=1, value=12
DCP1 2 KO root 2 30 min 1.czi (300%)
2/13; 27.31x31.40 pixels (207x238); 8-bit; 625K
t=10
ROI Manager
0001-0093-0062
0002-0083-0065
Add [t]
Update
Delete
Rename...
Measure
Deselect
Properties...
Flatten [F]
More »
Show All
Labels
When time changes


(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Type
Adjust
Show Info... Ctrl+I
Properties... Ctrl+Shift+P
Color
Stacks
Hyperstacks
Crop Ctrl+Shift+X
Duplicate... Ctrl+Shift+D
Rename...
Scale... Ctrl+E
Transform
Zoom
Overlay
Lookup Tables
Annotate
Drawing
Video Editing
Axes
Convert
Convolve
Threshold
Add Selection... Ctrl+B
Add Image...
Hide Overlay
Show Overlay
From ROI Manager
To ROI Manager
Remove Overlay
List Elements
Flatten Ctrl+Shift+F
Labels...
Overlay Options...
Overlay Manager
ROI Manager
0001-0073-0039
0002-0066-0048
0003-0063-0094
0004-0083-0060
0004-0070-0115
0004-0039-0052
0004-0023-0089
0004-0061-0078
0004-0105-0084
0004-0072-0024
0001-0274-0093
0003-0058-0133
0007-0058-0133
0008-0058-0133
Add [t]
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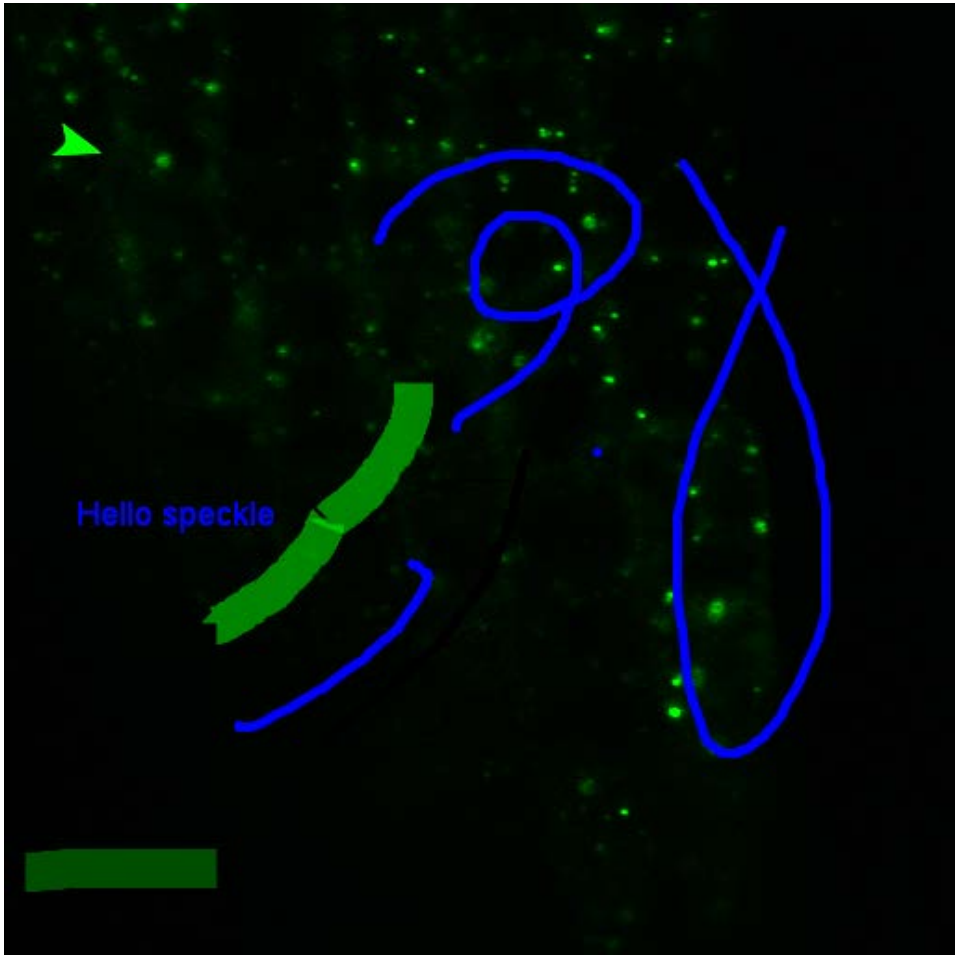
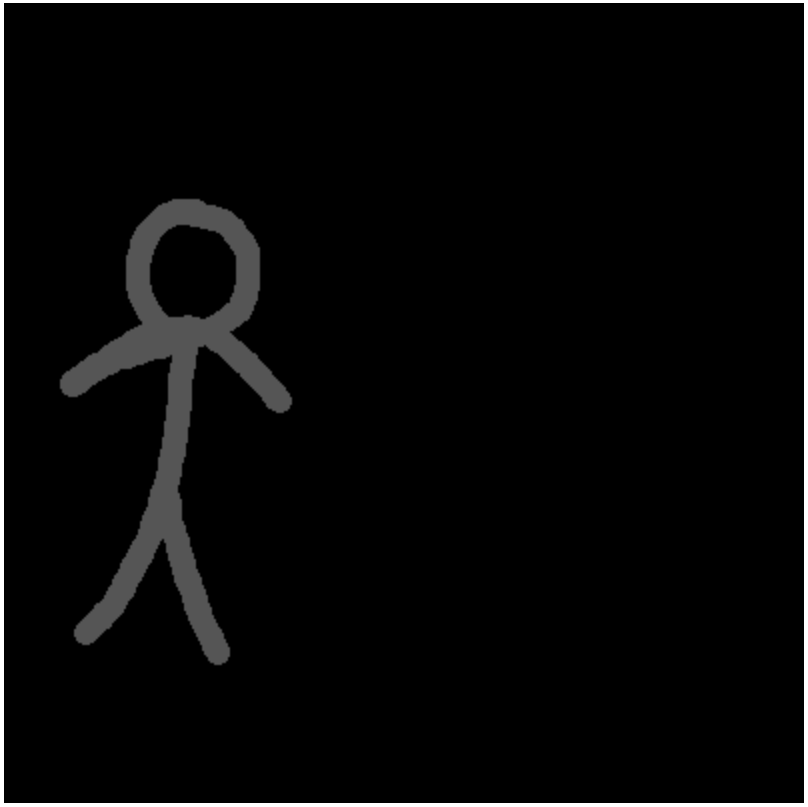
Rename...
Measure
Deselect
Properties...
Flatten [F]
More »
Show All
Labels
(Fiji Is Just) ImageJ
File Edit Image Process
New
Open... Ctrl+O
Open Next Ctrl+Shift+O
Open Samples
Open Recent
Import
Export
Close Ctrl+W
Close All
Save Ctrl+S
Save As
Revert Ctrl+R
Page Setup...
Print... Ctrl+P
Quit
Fix Funny Filenames
Make Screencast
Tiff...
Gif...
Jpeg...
Text Image...
ZIP...
Raw Data...
Image Sequence...
AVI...
BMP...
PNG...
PGM...
FITS...
LUT...
Selection...
XY Coordinates...
Results...
Text...
Analyze...
DF3 ...
Nrrd ...


Hello speckle



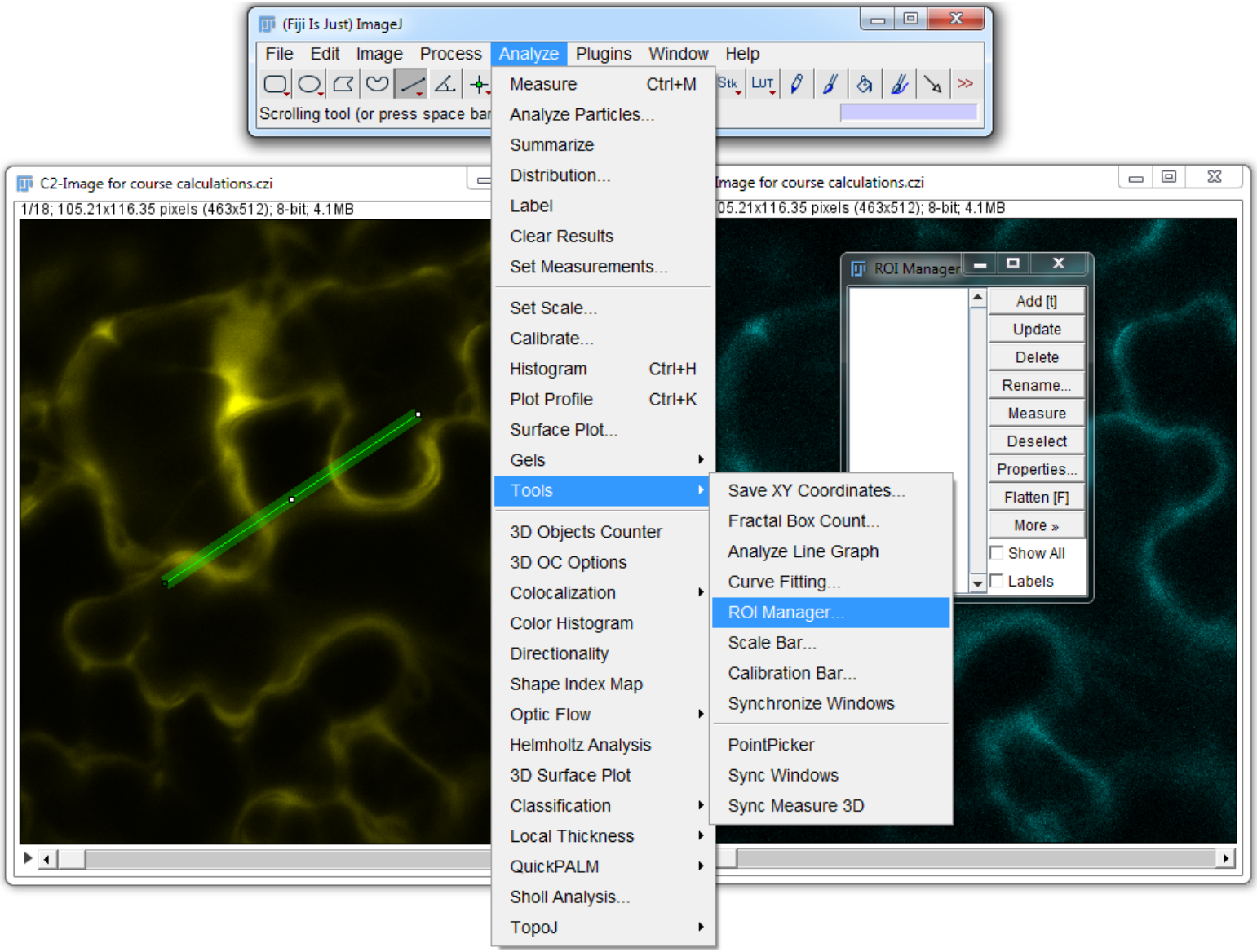
Do my structures co-localize?

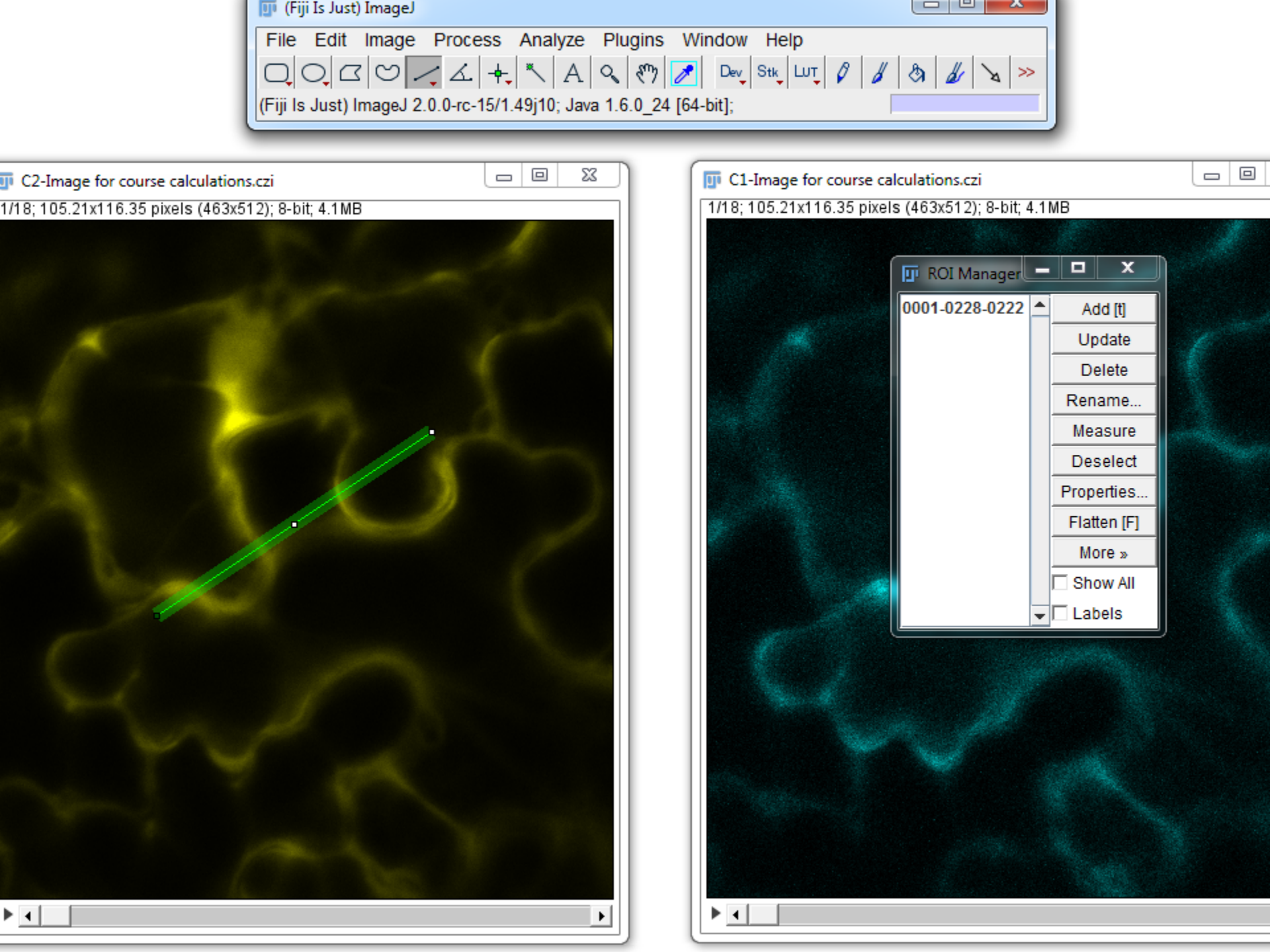
Are the intensities of green and red correlating:

Pearson's correlation coefficient. Requires linear relationship between the two channel intensities.
Mander's coefficient. Proportional to the amount of fluorescence of the co-localizing objects in each component, which is dependent on the intensities of the signals.

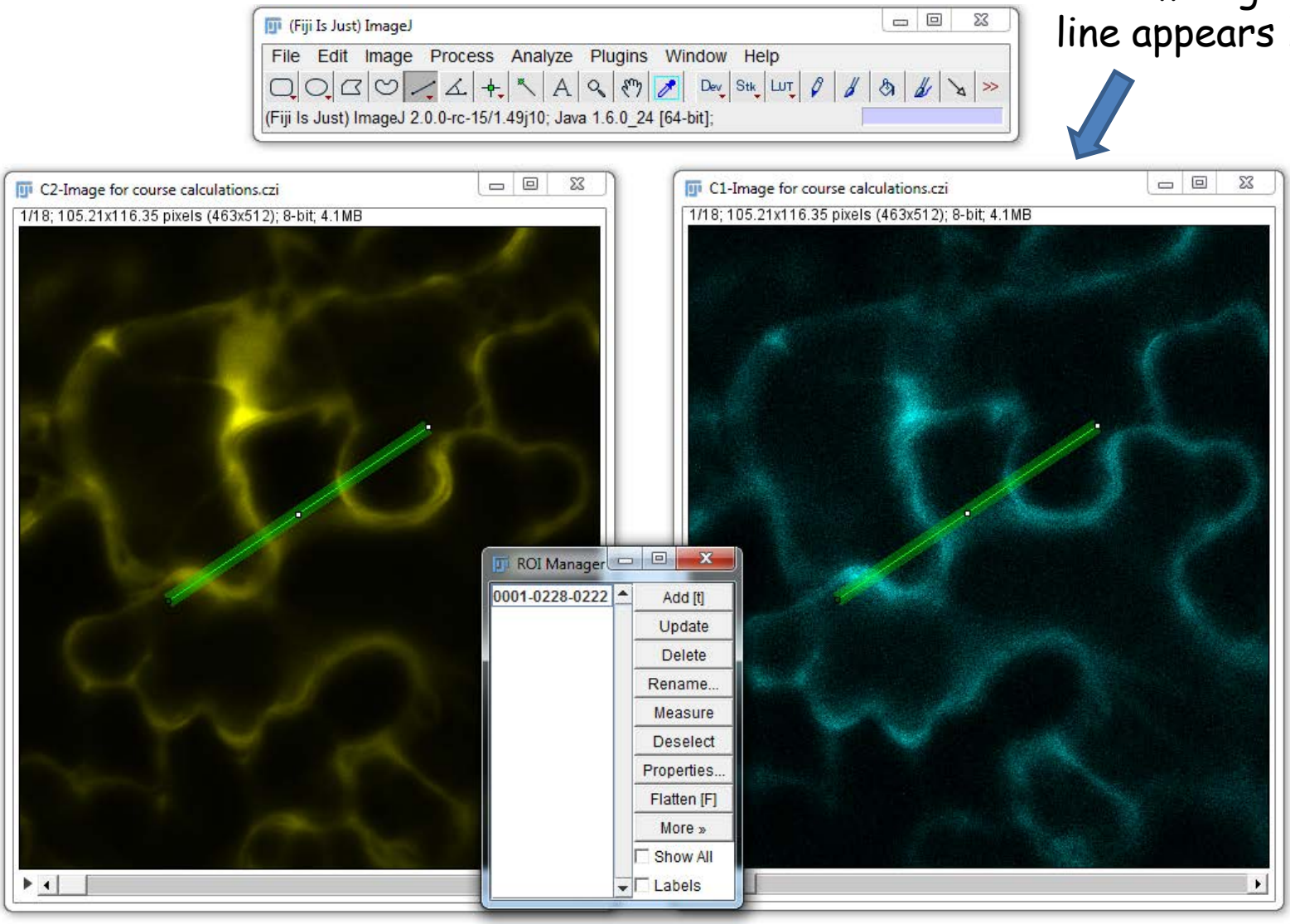
Spearman's rank coefficient. Pearson correlation coefficient coefficient between ranked variables

Plot profiles of the intensities

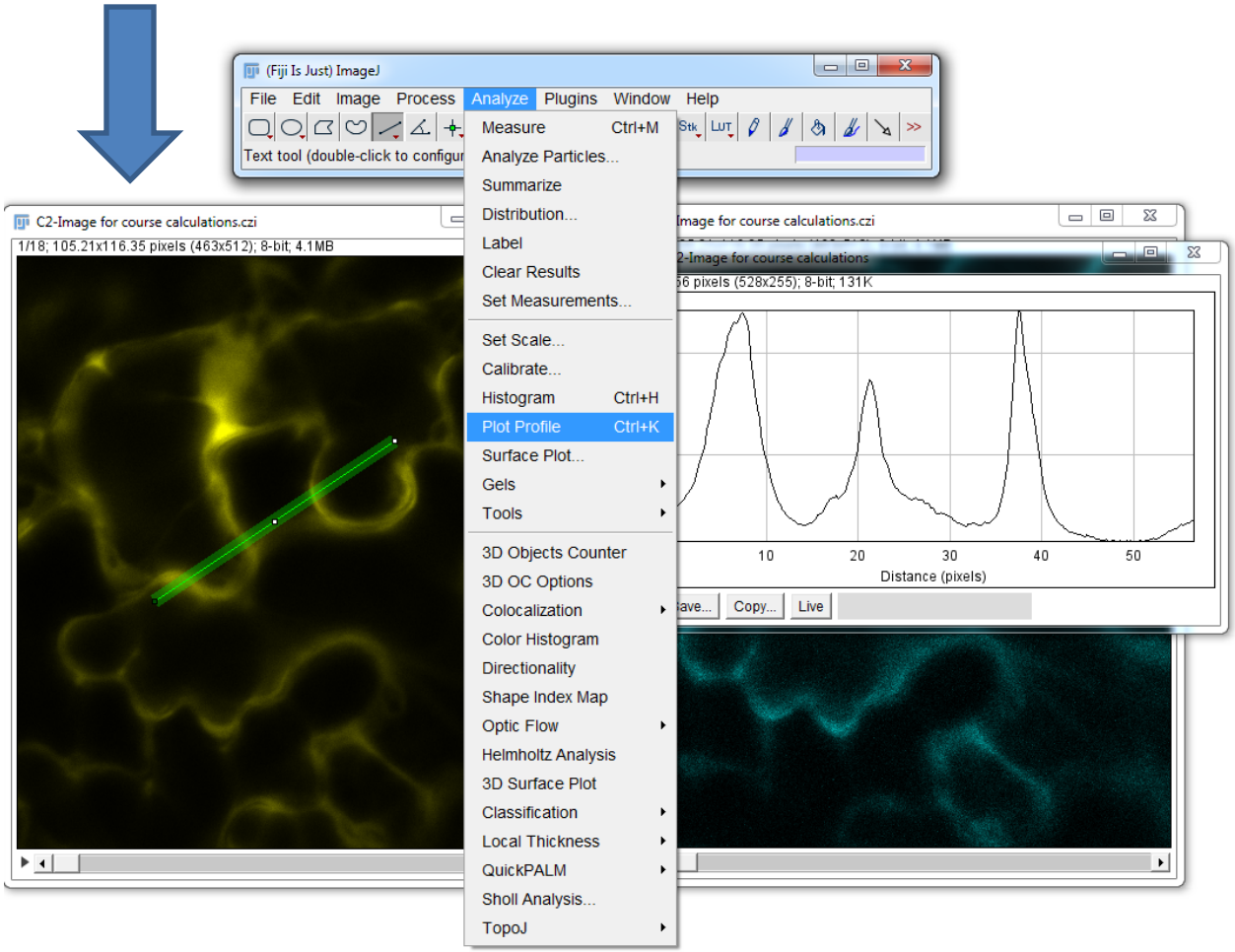


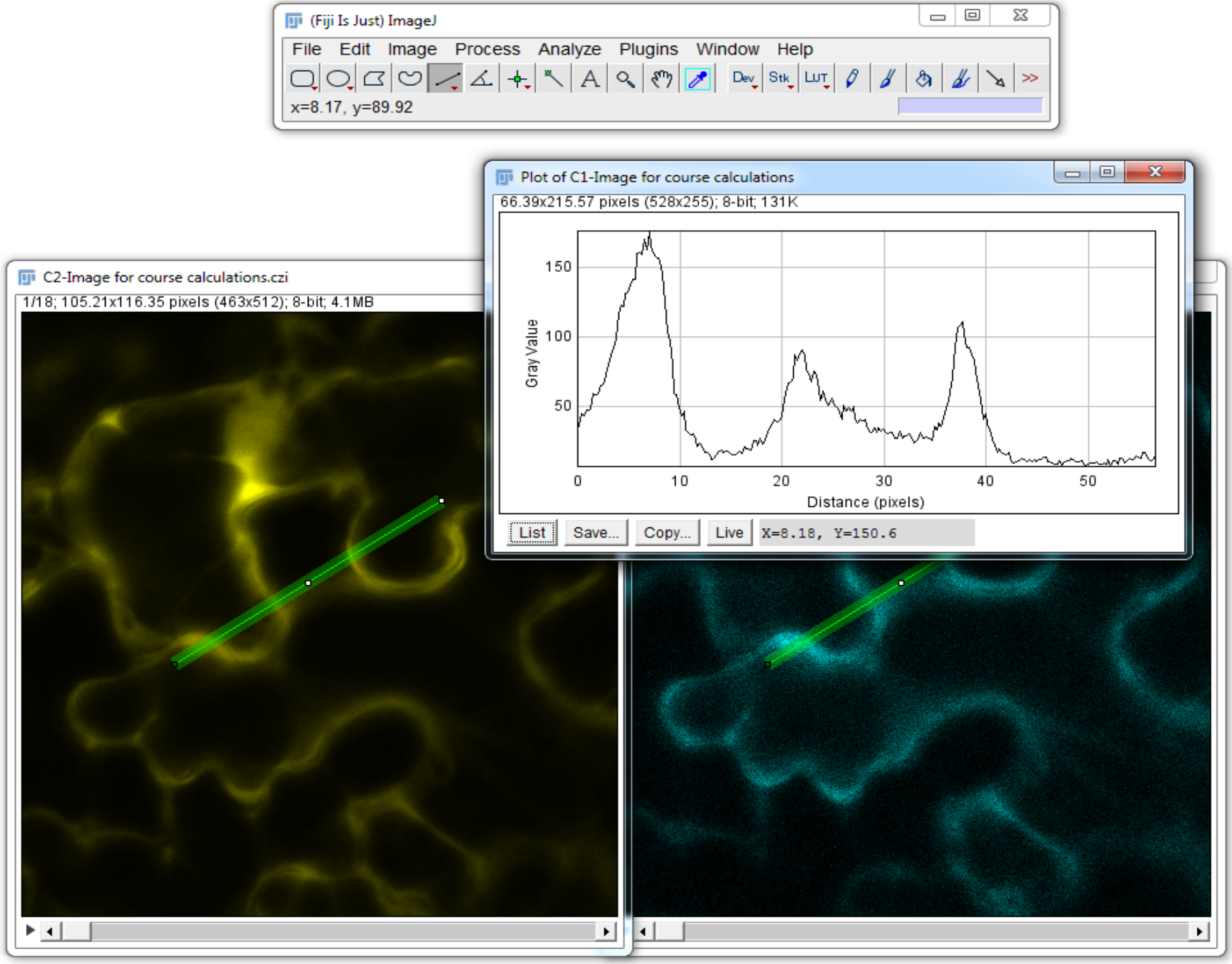

(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Dev Stk LUT >>
(Fiji Is Just) ImageJ 2.0.0-rc-15/1.49j10; Java 1.6.0_24 [64-bit];
C2-Image for course calculations.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB
C1-Image for course calculations.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB
ROI Manager
0001-0228-0222
Add [t]
Update
Delete
Rename...
Measure
Deselect
Properties...
Flatten [F]
More »
Show All
Labels

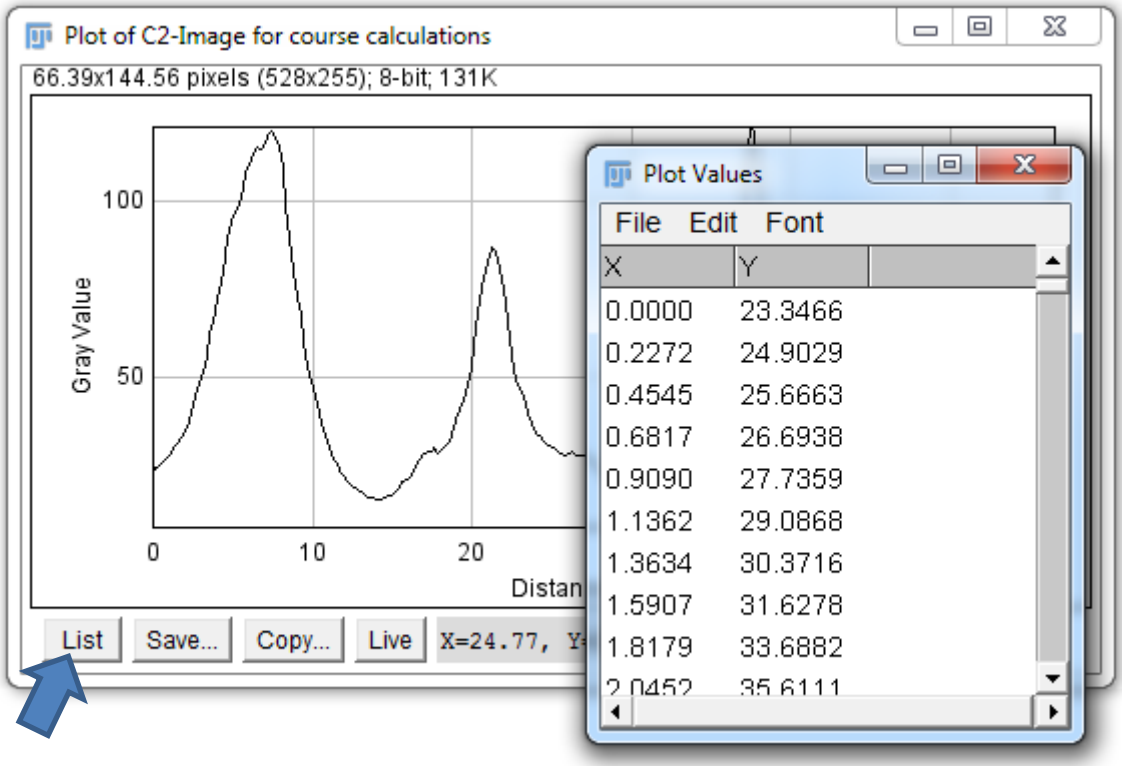
I select this window and the ROI from the ROI manager and the line appears here



Select each window and then 'analyze'->'plot profile'

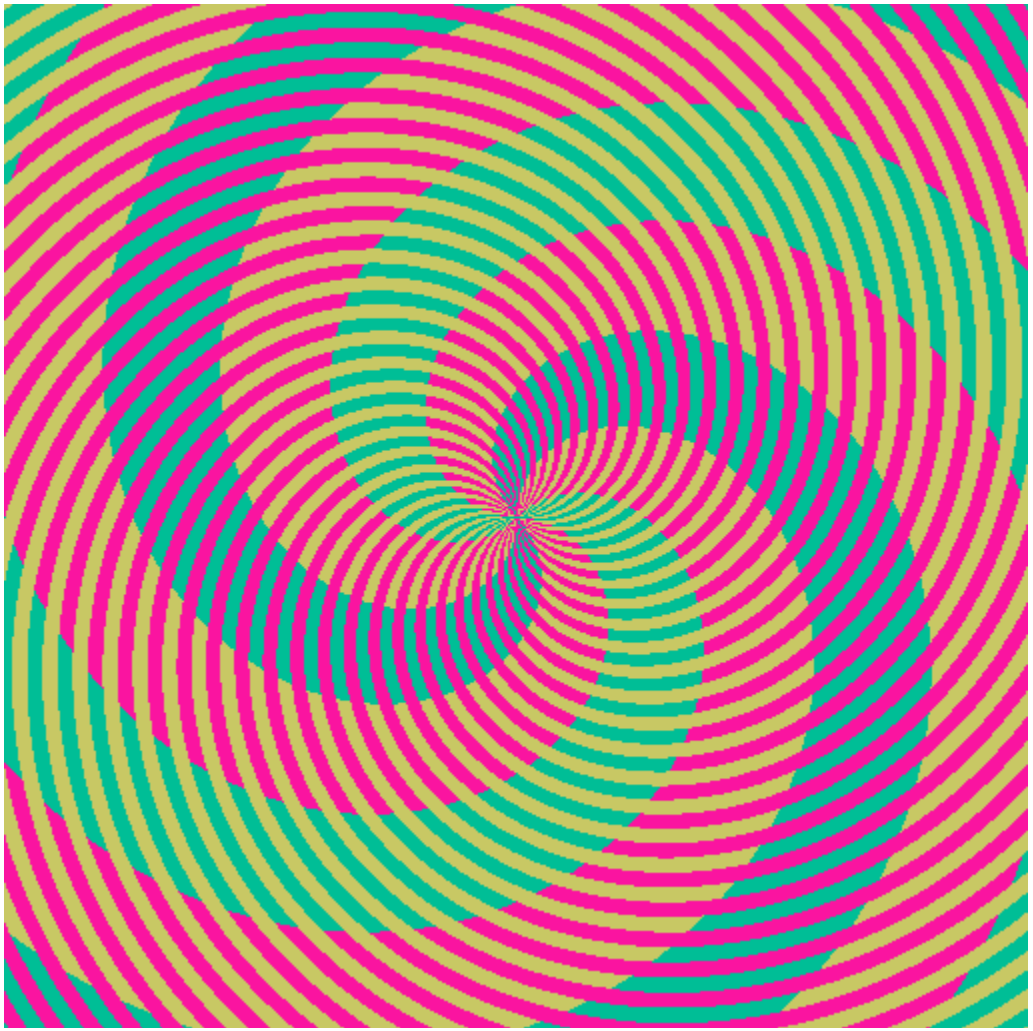


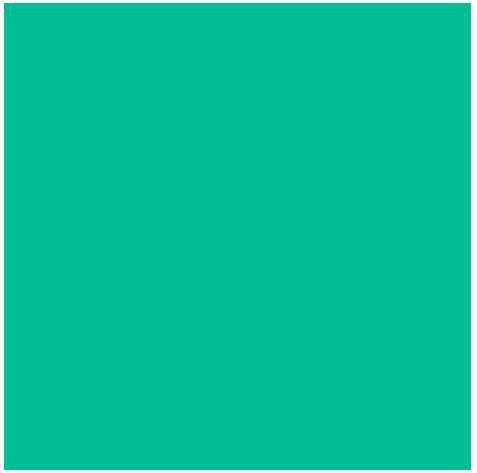

(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
x=8.17, y=89.92
Plot of C1-Image for course calculations
66.39x215.57 pixels (528x255); 8-bit; 131K
Gray Value
150
100
50
0
10
20
30
40
50
Distance (pixels)
List
Save...
Copy...
Live
X=8.18, Y=150.6
C2-Image for course calculations.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB

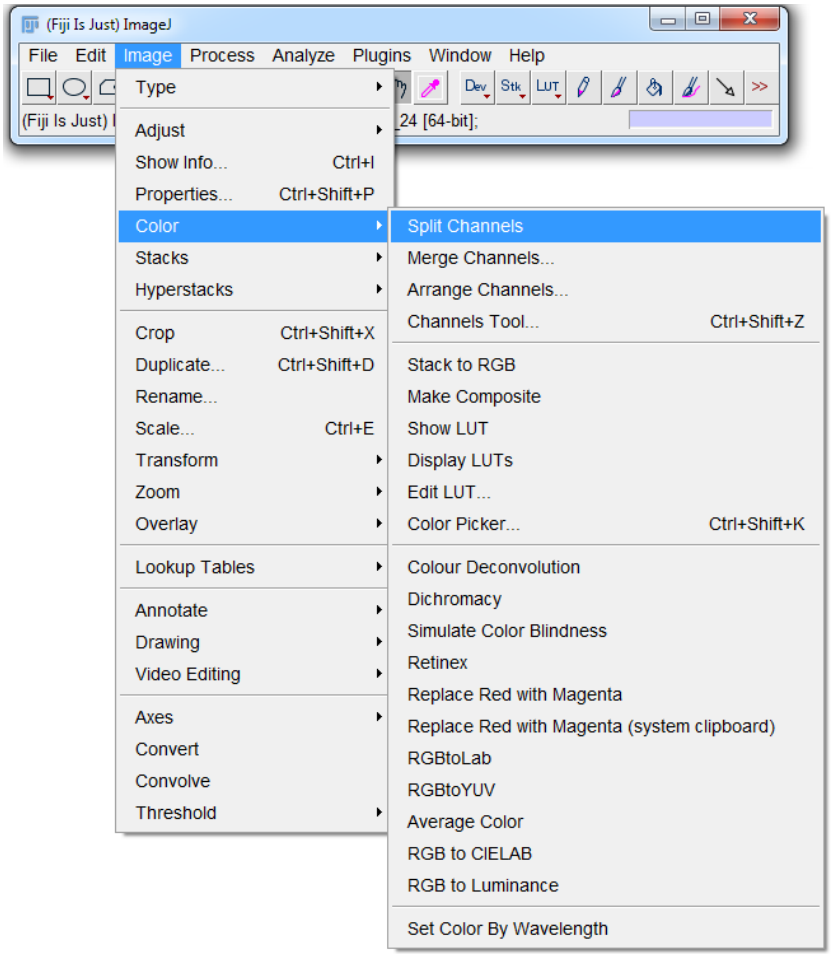


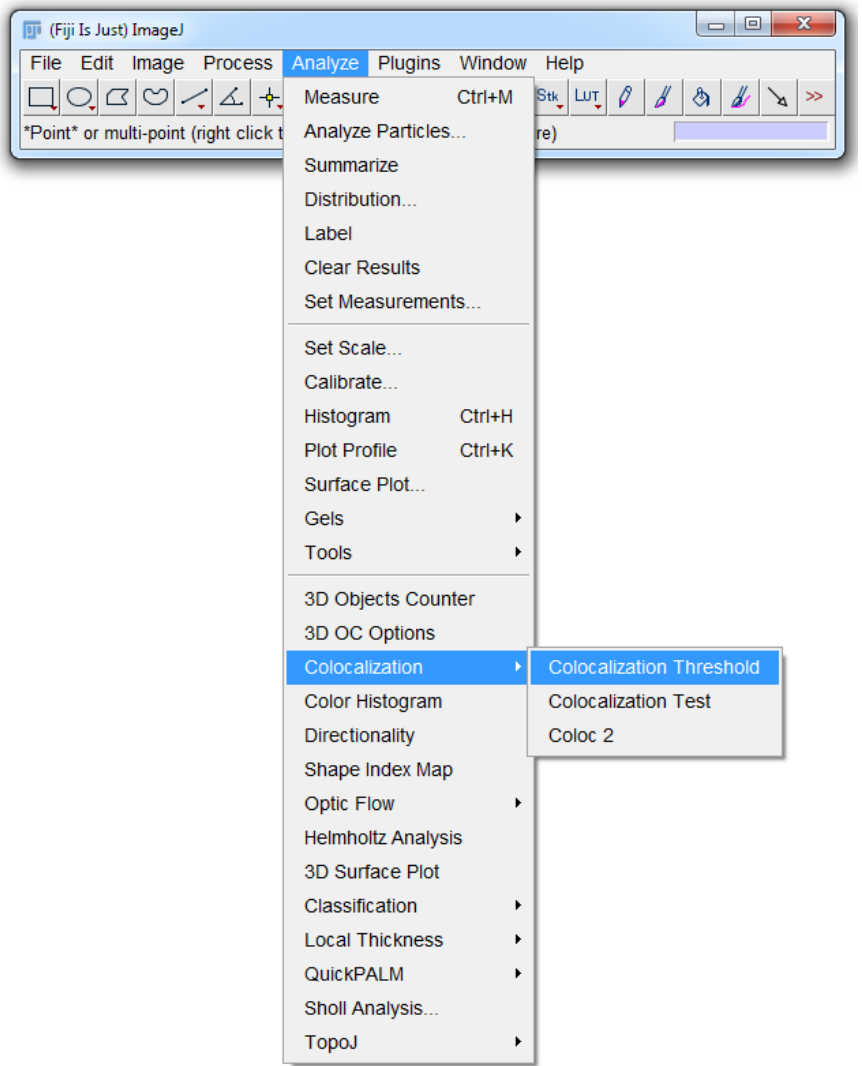
Click on the 'list' button and take the values that appear in the 'plot value' box to Excel sheet and plot them

Ok! I need some math now!

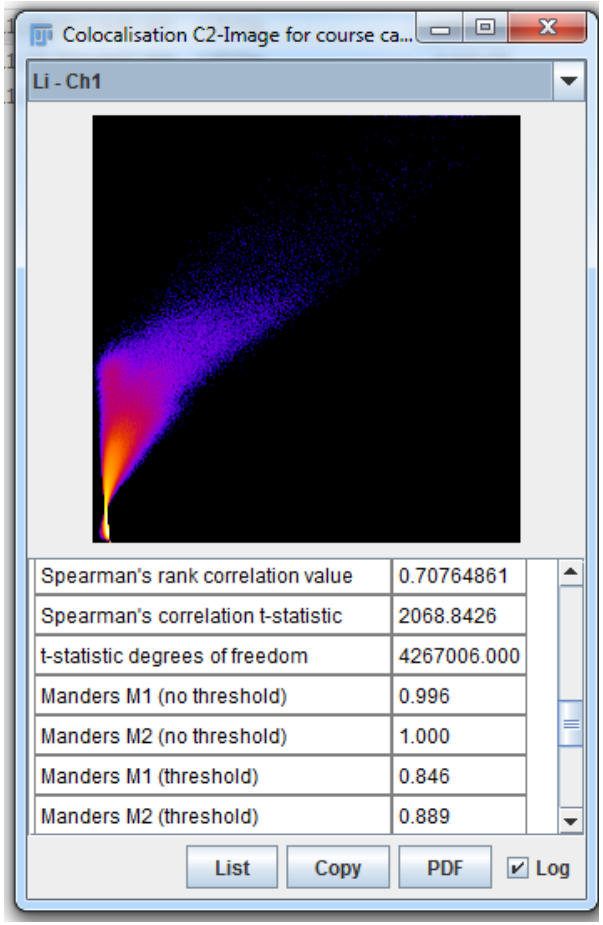


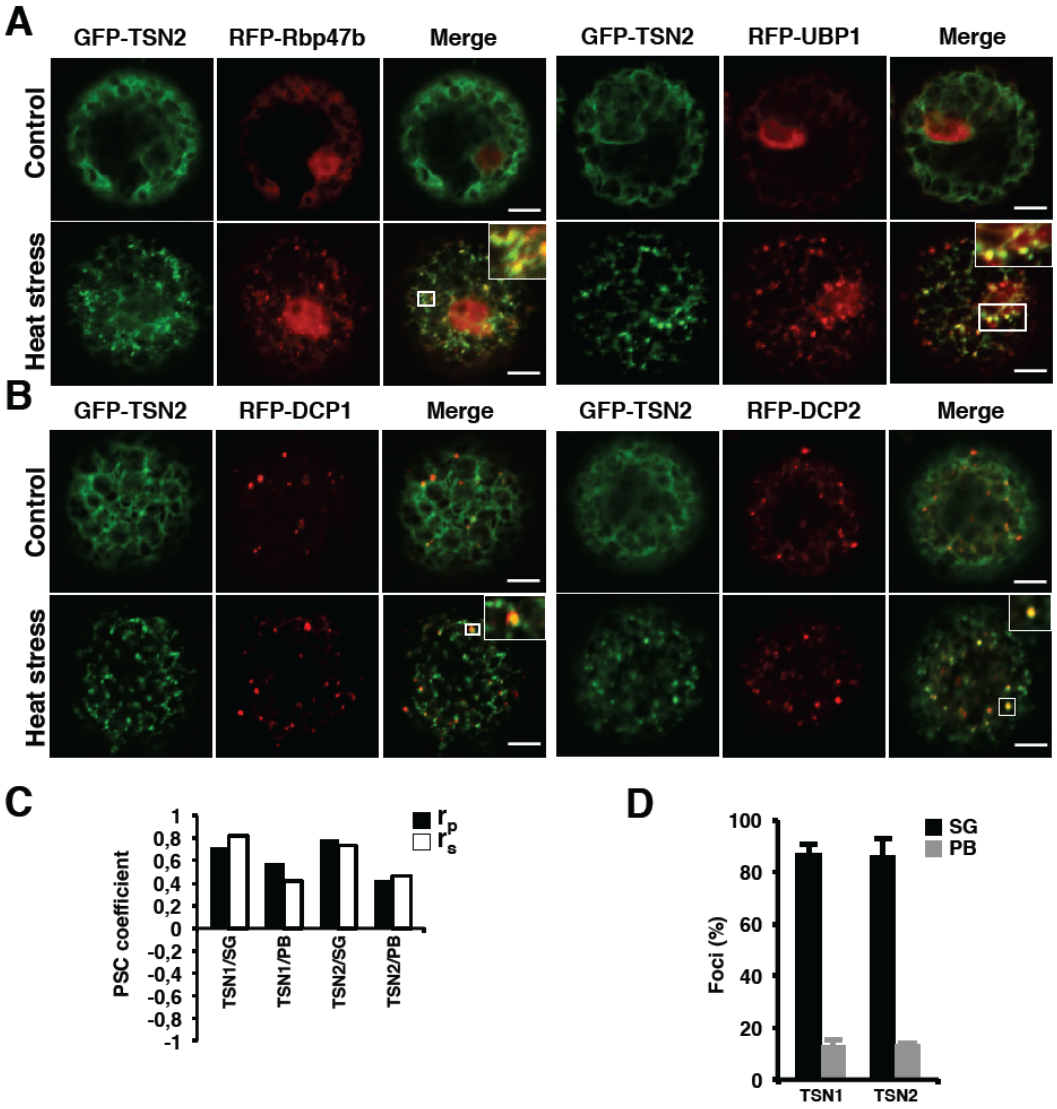



(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Type
Adjust
Show Info... Ctrl+I
Properties... Ctrl+Shift+P
Color
Stacks
Hyperstacks
Crop Ctrl+Shift+X
Duplicate... Ctrl+Shift+D
Rename...
Scale... Ctrl+E
Transform
Zoom
Overlay
Lookup Tables
Annotate
Drawing
Video Editing
Axes
Convert
Convolve
Threshold
Split Channels
Merge Channels...
Arrange Channels...
Channels Tool... Ctrl+Shift+Z
Stack to RGB
Make Composite
Show LUT
Display LUTs
Edit LUT...
Color Picker... Ctrl+Shift+K
Colour Deconvolution
Dichromacy
Simulate Color Blindness
Retinex
Replace Red with Magenta
Replace Red with Magenta (system clipboard)
RGBtoLab
RGBtoYUV
Average Color
RGB to CIELAB
RGB to Luminance
Set Color By Wavelength

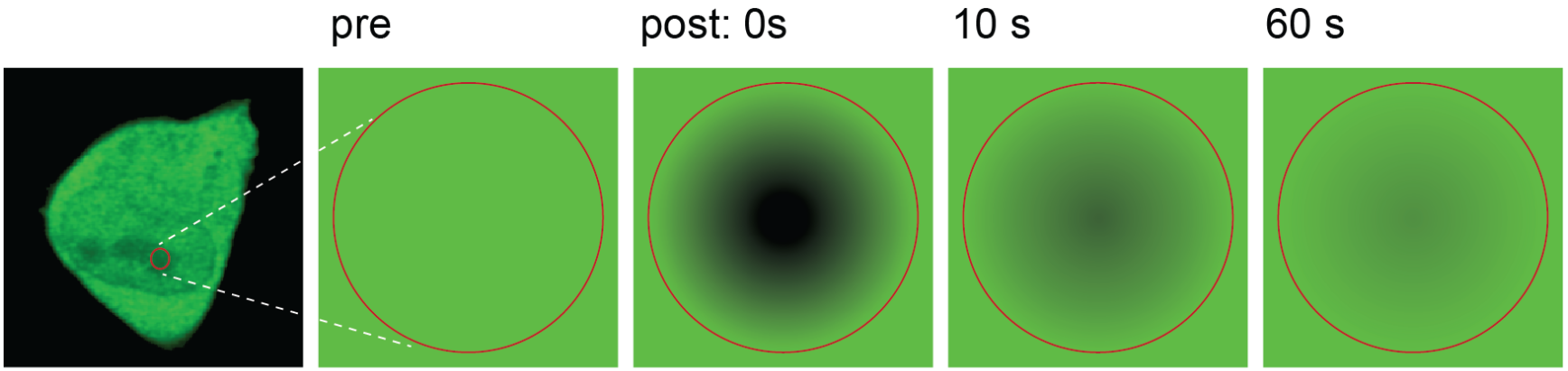

(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Measure Ctrl+M
Analyze Particles...
Summarize
Distribution...
Label
Clear Results
Set Measurements...
Set Scale...
Calibrate...
Histogram Ctrl+H
Plot Profile Ctrl+K
Surface Plot...
Gels
Tools
3D Objects Counter
3D OC Options
Colocalization
Color Histogram
Directionality
Shape Index Map
Optic Flow
Helmholtz Analysis
3D Surface Plot
Classification
Local Thickness
QuickPALM
Sholl Analysis...
TopoJ
Colocalization Threshold
Colocalization Test
Coloc 2

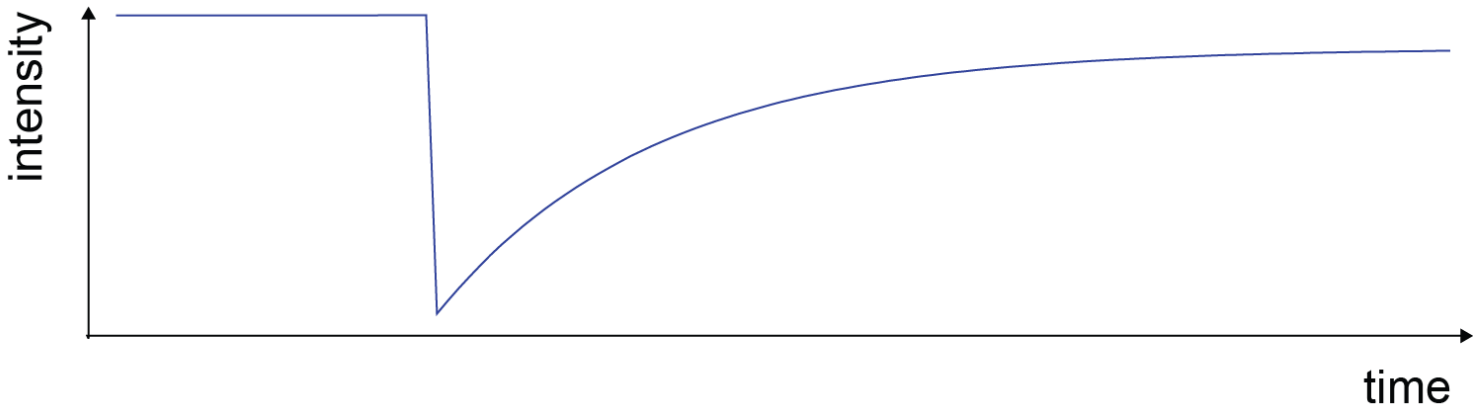
Data presentation of colocalization and some regular

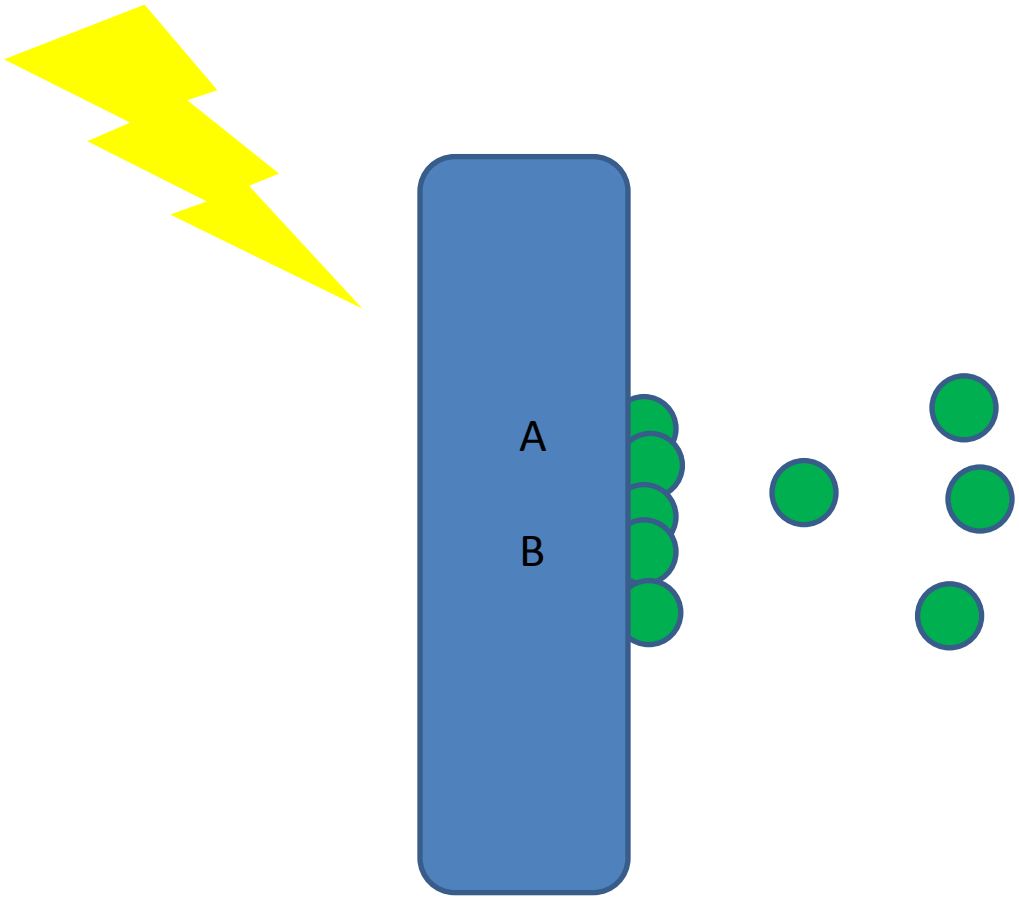


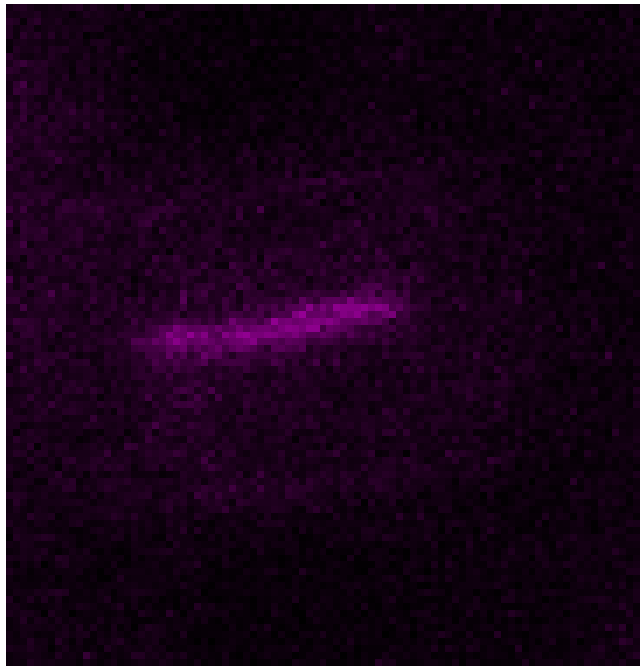


Fluorescence recovery after photo-bleaching



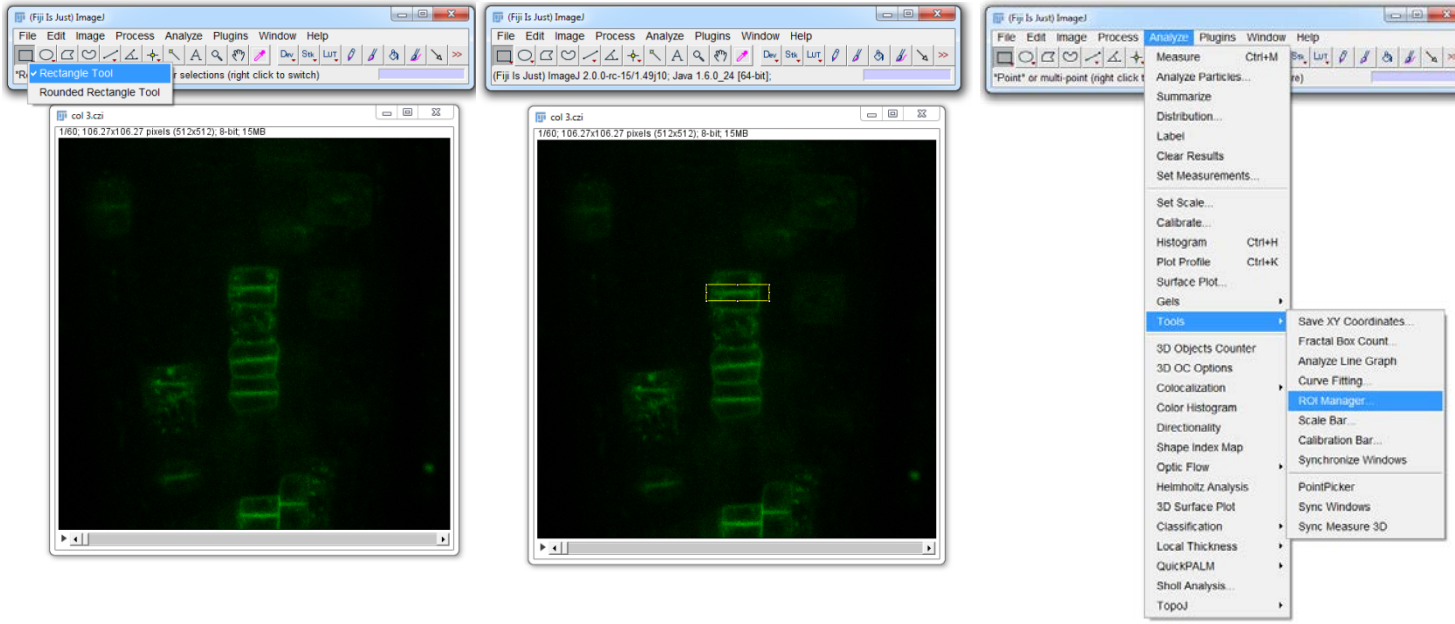



A
B



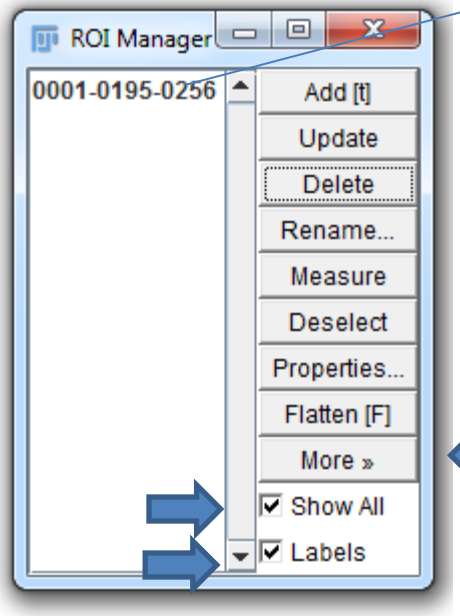
Analyzing FRAP data

Select the ROI → Use the rectangle tool to select the ROI → Select the ROI manager

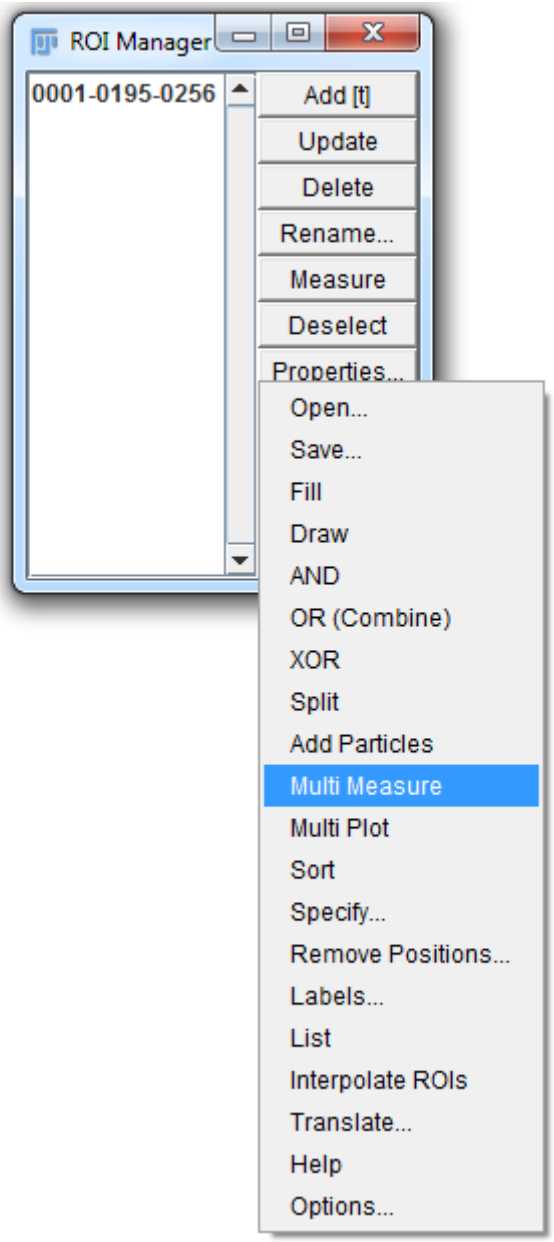


2. Click on 'show all' and 'labels'

This is my ROI

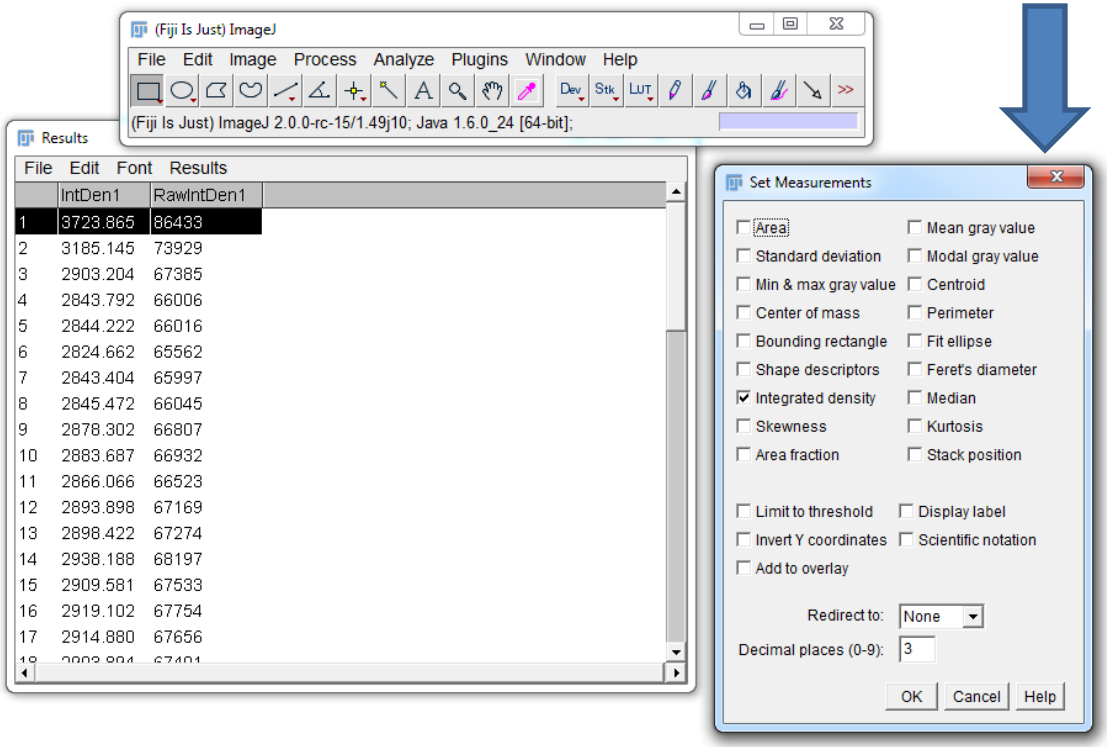


3. Select 'more' and on drop-down menu select 'Multi measure'



- 4. You end up with an intensity measurement table

Make sure that 'integrated density' is selected (Results-> Set measurements…)



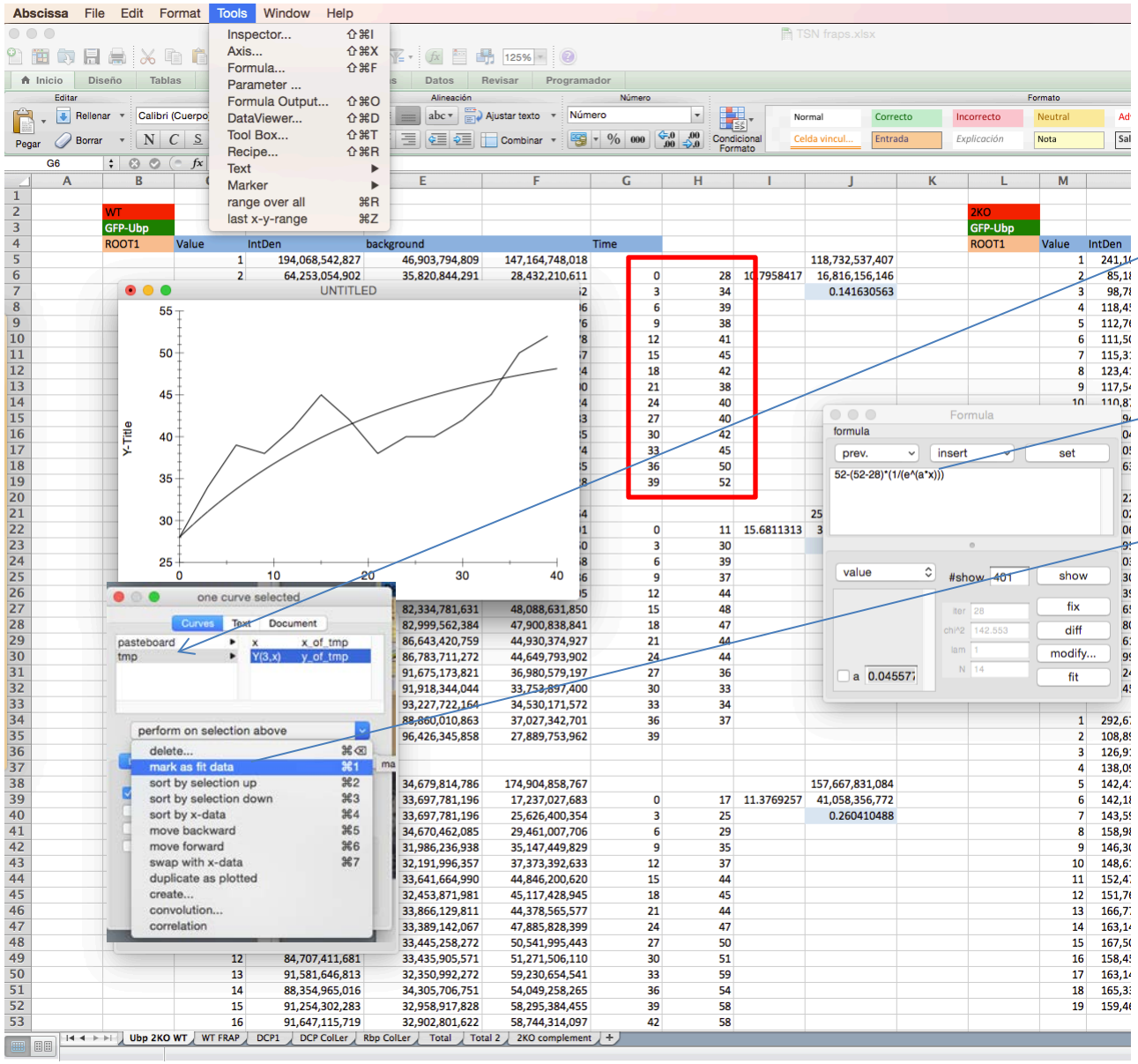
- 5. Copy and paste the data from the table to an Excel file

Time in sec (or min)

B	C	D	E	F	G	H
WT						
GFP-Ubp						
ROOT1	Value	IntDen	background		Time	
	1	194,068,542,827	46,903,794,809	147,164,748,018		
	2	64,253,054,902	35,820,844,291	28,432,210,611	0	28
	3	75,289,241,916	40,871,302,754	34,417,939,162	3	34
	4	81,892,248,723	42,638,963,217	39,253,285,506	6	39
	5	82,836,871,509	43,901,577,833	38,935,293,676	9	38
	6	85,820,383,084	44,696,557,406	41,123,825,678	12	41
	7	93,050,020,848	47,801,654,091	45,248,366,757	15	45
	8	91,413,298,197	48,690,160,673	42,723,137,524	18	42
	9	88,990,948,675	50,888,045,375	38,102,903,300	21	38
	10	91,114,011,770	50,579,406,246	40,534,605,524	24	40
	11	91,937,049,445	49,662,841,562	42,274,207,883	27	40
	12	89,813,986,350	50,785,165,665	39,028,820,685	30	42
	13	85,988,731,699	53,609,681,325	32,379,050,374	33	45
	14	85,876,499,289	54,470,129,804	31,406,369,485	36	50
	15	87,662,865,153	52,524,768,025	35,138,097,128	39	52

Normalized intensity (my intensity-background)

Use curve fitting



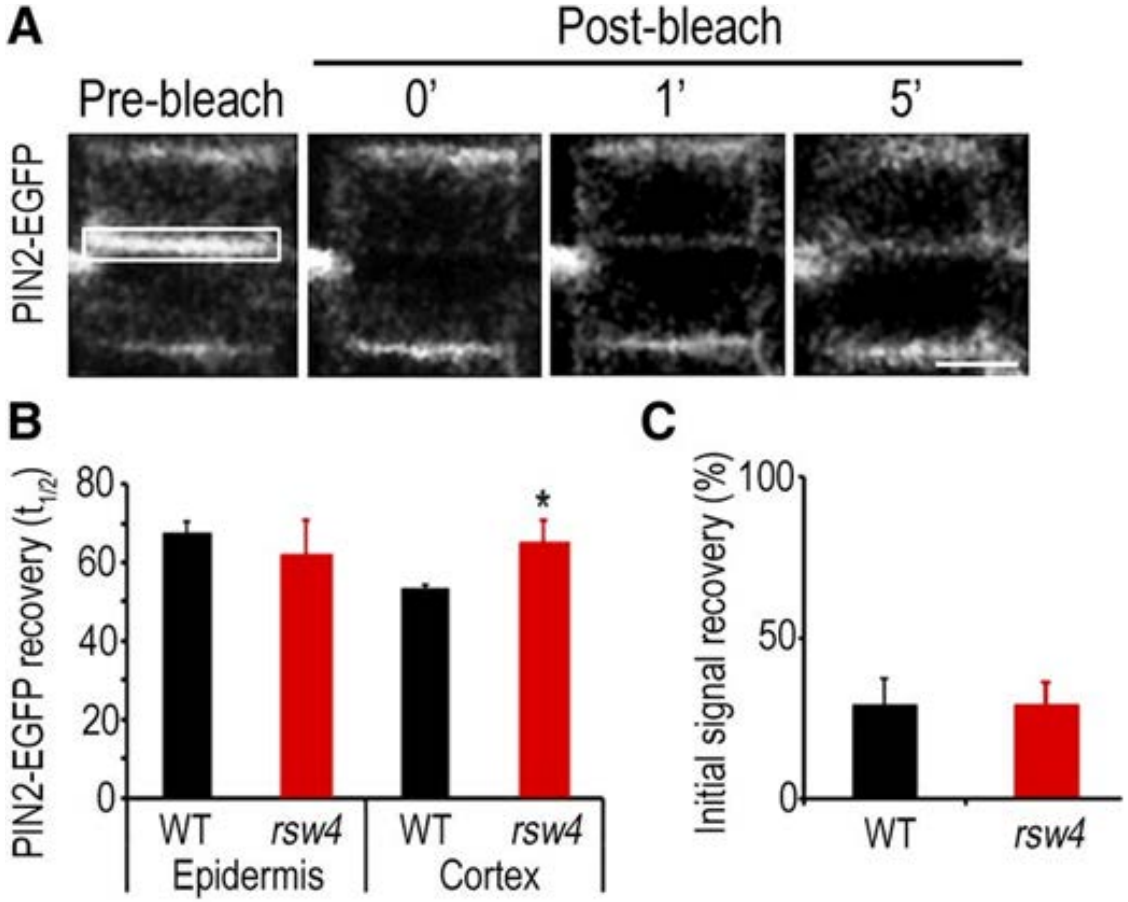
7.Paste your data here

8. Set up your equation

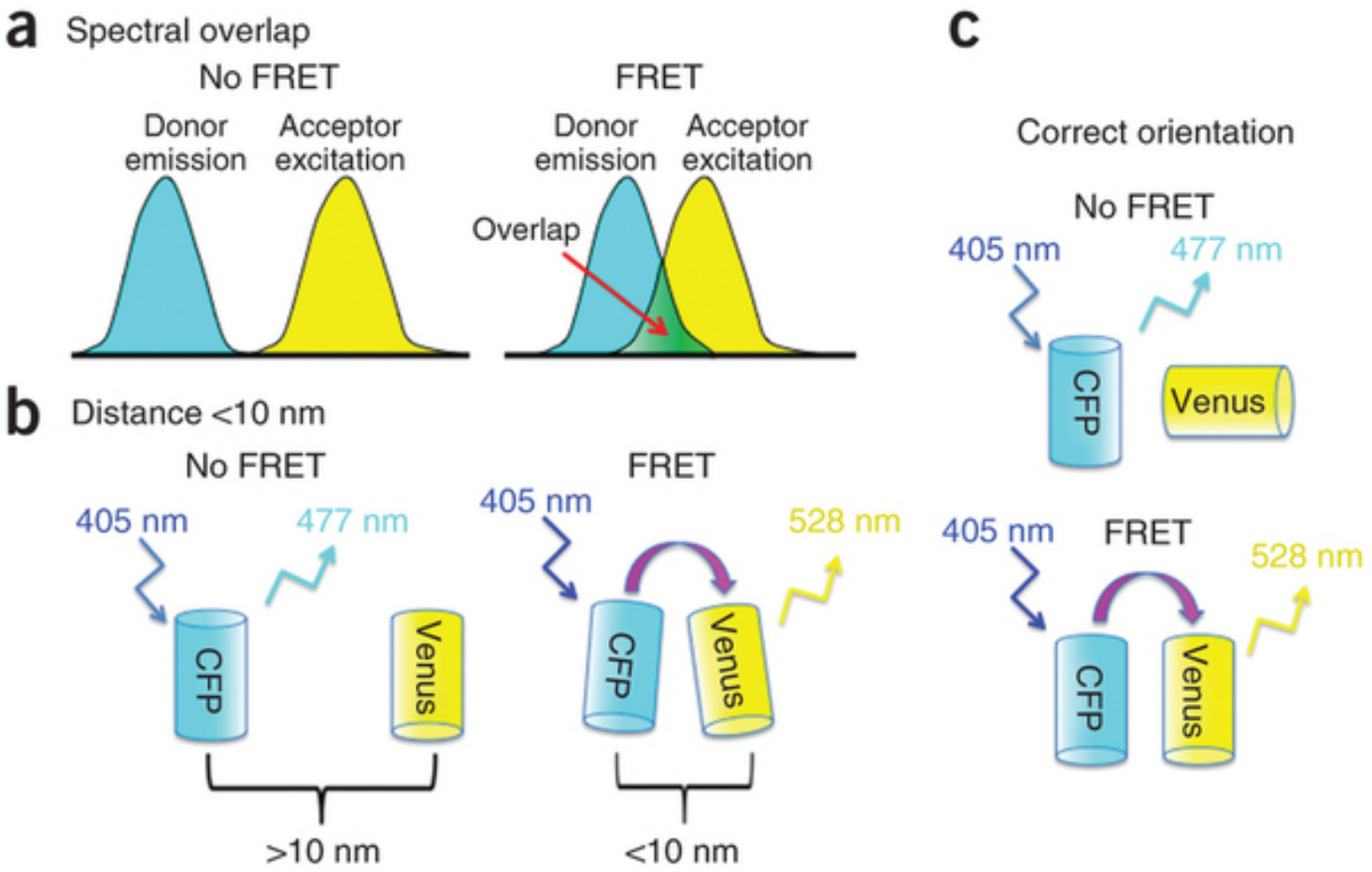
$F(t) = F_{inf} - (F_{inf} - F_0)*\exp(-t*\boldsymbol{\alpha})$

8. Select 'mark as fit data'

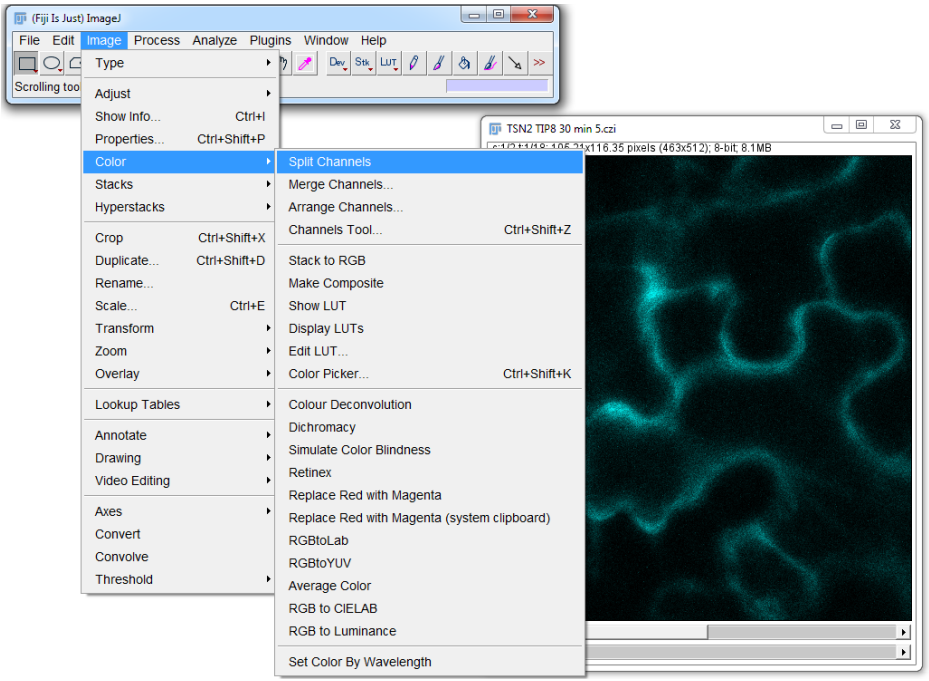
Presentation of FRAP data and control experiments required

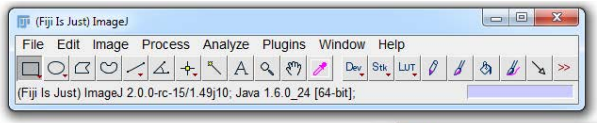


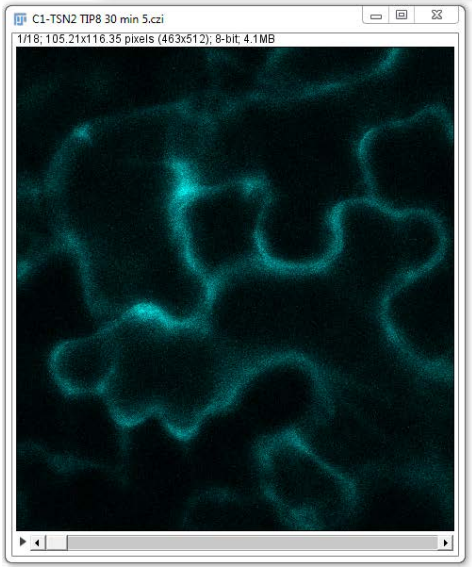
Försters resonance energy transfer (FRET)

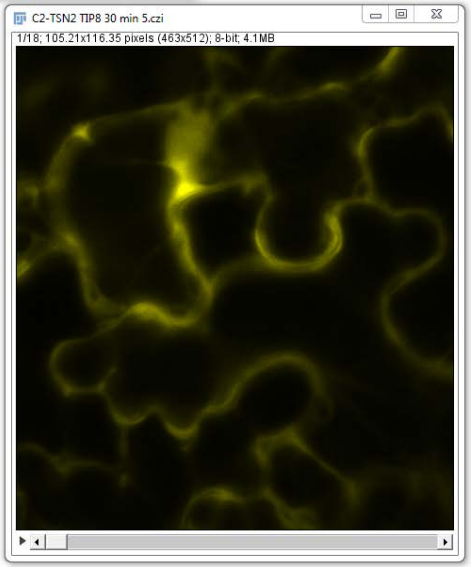


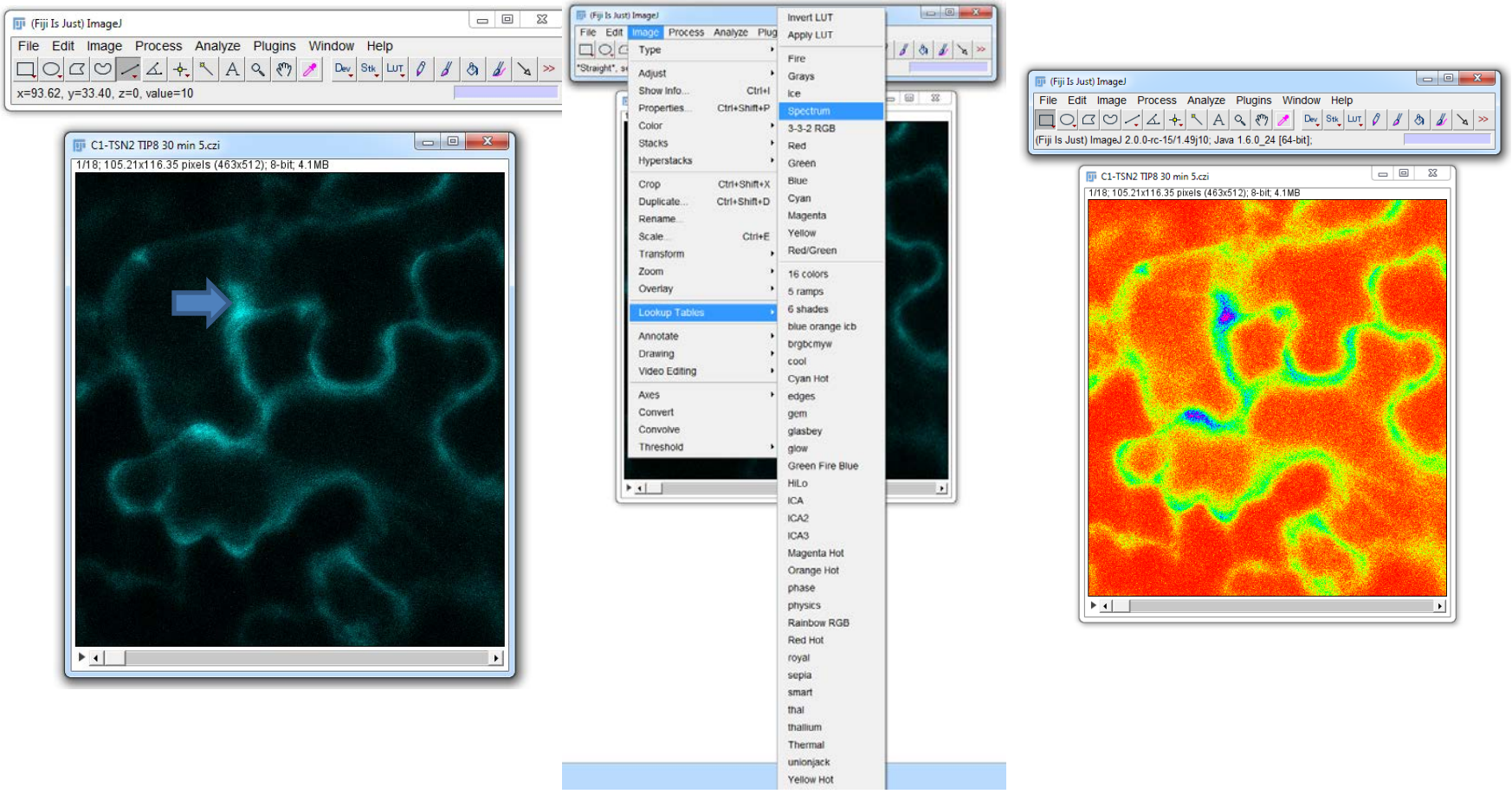
Acceptor photo-bleaching










(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
x=93.62, y=33.40, z=0, value=10
C1-TSN2 TIP8 30 min 5.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB
Type
Adjust
Show Info... Ctrl+I
Properties... Ctrl+Shift+P
Color
Stacks
Hyperstacks
Crop Ctrl+Shift+X
Duplicate... Ctrl+Shift+D
Rename...
Scale... Ctrl+E
Transform
Zoom
Overlay
Lookup Tables
Annotate
Drawing
Video Editing
Axes
Convert
Convolve
Threshold
Invert LUT
Apply LUT
Fire
Grays
Ice
Spectrum
3-3-2 RGB
Red
Green
Blue
Cyan
Magenta
Yellow
Red/Green
16 colors
5 ramps
6 shades
blue orange icb
brgbcmyw
cool
Cyan Hot
edges
gem
glasbey
glow
Green Fire Blue
HiLo
ICA
ICA2
ICA3
Magenta Hot
Orange Hot
phase
physics
Rainbow RGB
Red Hot
royal
sepia
smart
thal
thallium
Thermal
unionjack
Yellow Hot
(Fiji Is Just) ImageJ 2.0.0-rc-15/1.49j10; Java 1.6.0_24 [64-bit];
C1-TSN2 TIP8 30 min 5.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB

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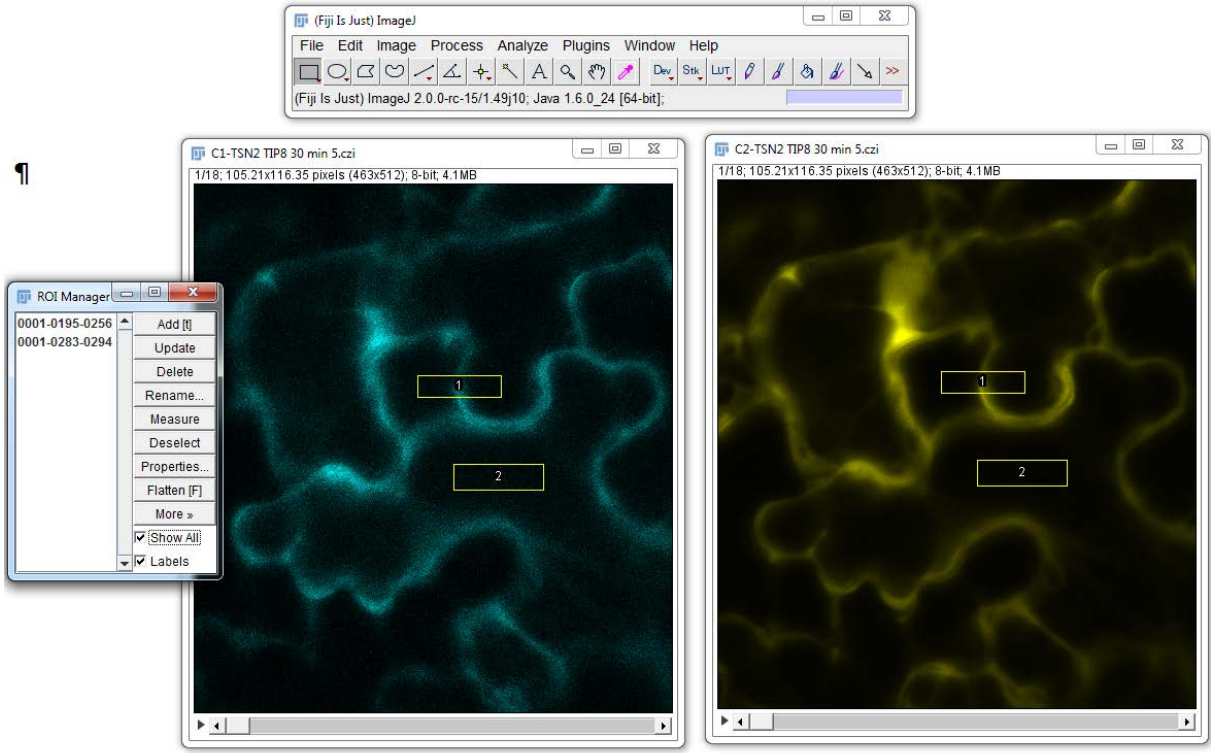
(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
(Fiji Is Just) ImageJ 2.0.0-rc-15/1.49j10; Java 1.6.0_24 [64-bit];
C1-TSN2 TIP8 30 min 5.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB
1
2
C2-TSN2 TIP8 30 min 5.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB
1
2
ROI Manager
0001-0195-0256
0001-0283-0294
Add [t]
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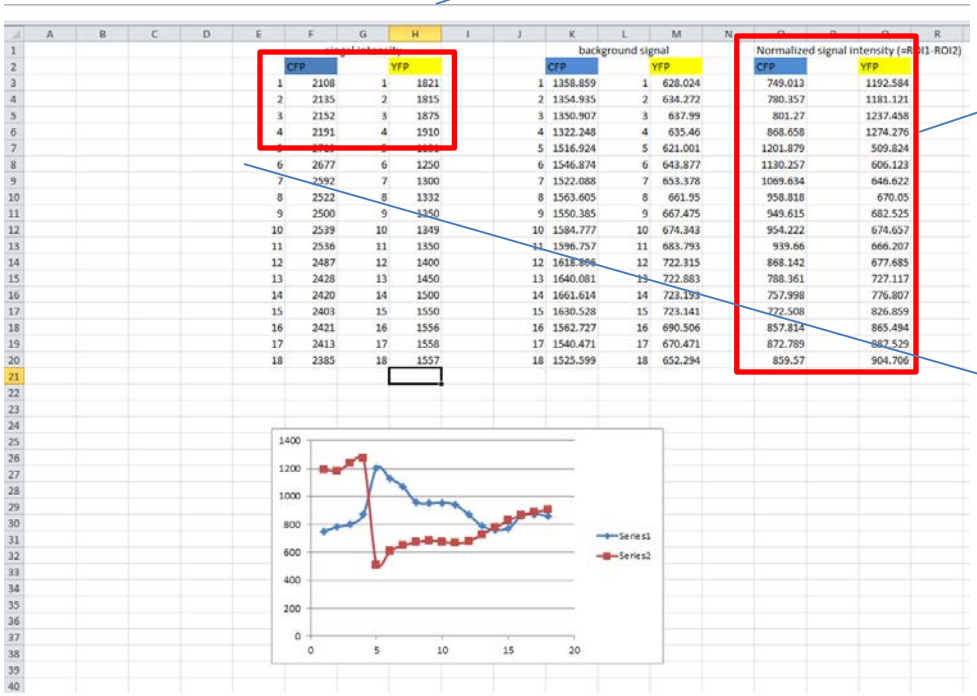
Delete
Rename...
Measure
Deselect
Properties...
Flatten [F]
More »
Show All
Labels

No significant changes in intensity



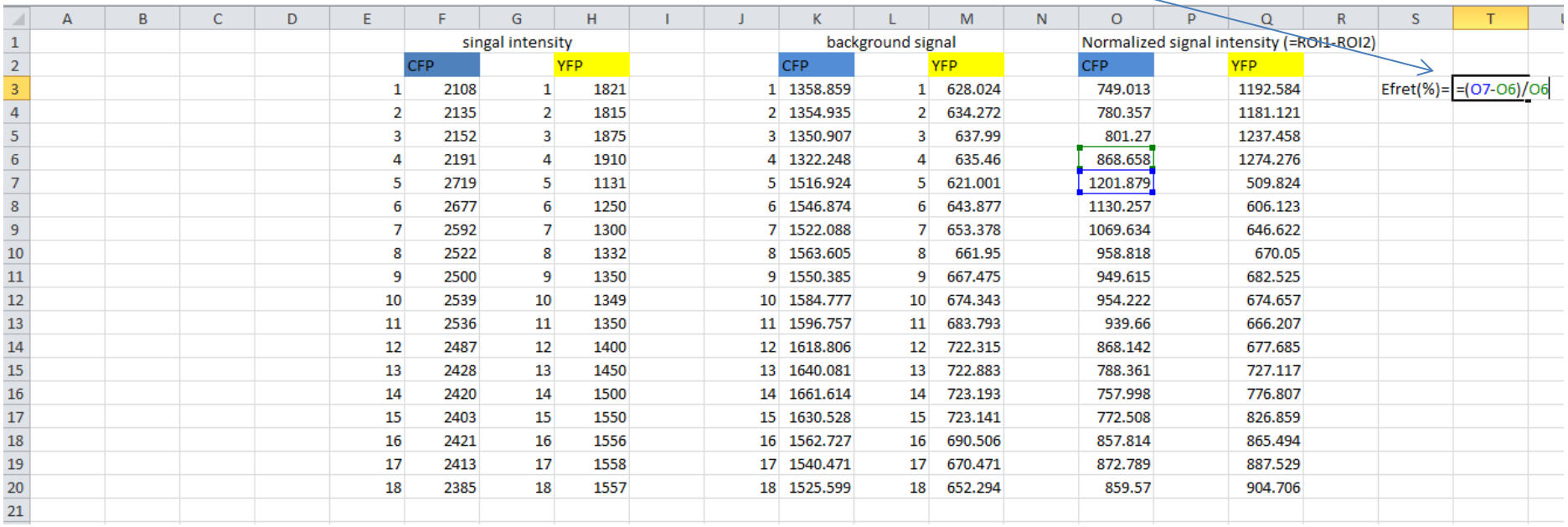
	CFP		YFP		CFP		YFP		CFP	YFP
	signal intensity				background signal				Normalized signal intensity (=ROI1-ROI2)	
1	2108	1	1821	1	1358.859	1	628.024		749.013	1192.584
2	2135	2	1815	2	1354.935	2	634.272		780.357	1181.121
3	2152	3	1875	3	1350.907	3	637.99		801.27	1237.458
4	2191	4	1910	4	1322.248	4	635.46		868.658	1274.276
5	2719	5	2091	5	1516.924	5	621.001		1201.879	509.824
6	2677	6	1250	6	1546.874	6	643.877		1130.257	606.123
7	2592	7	1300	7	1522.088	7	653.378		1069.634	646.622
8	2522	8	1332	8	1563.605	8	661.95		958.818	670.05
9	2500	9	1350	9	1550.385	9	667.475		949.615	682.525
10	2539	10	1349	10	1584.777	10	674.343		954.222	674.657
11	2536	11	1350	11	1596.757	11	683.793		939.66	666.207
12	2487	12	1400	12	1618.866	12	722.315		868.142	677.685
13	2428	13	1450	13	1640.081	13	722.883		788.361	727.117
14	2420	14	1500	14	1661.614	14	723.193		757.998	776.807
15	2403	15	1550	15	1630.528	15	723.141		772.508	826.859
16	2421	16	1556	16	1562.727	16	690.506		857.814	865.494
17	2413	17	1558	17	1540.471	17	670.471		872.789	887.529
18	2385	18	1557	18	1525.599	18	652.294		859.57	904.706

I plot these data

Keep in mind that FRAP takes some time (depending on the iterations). Therefore, I should always check that the increased intensity is not due to ‘relaxation’ of the CFP

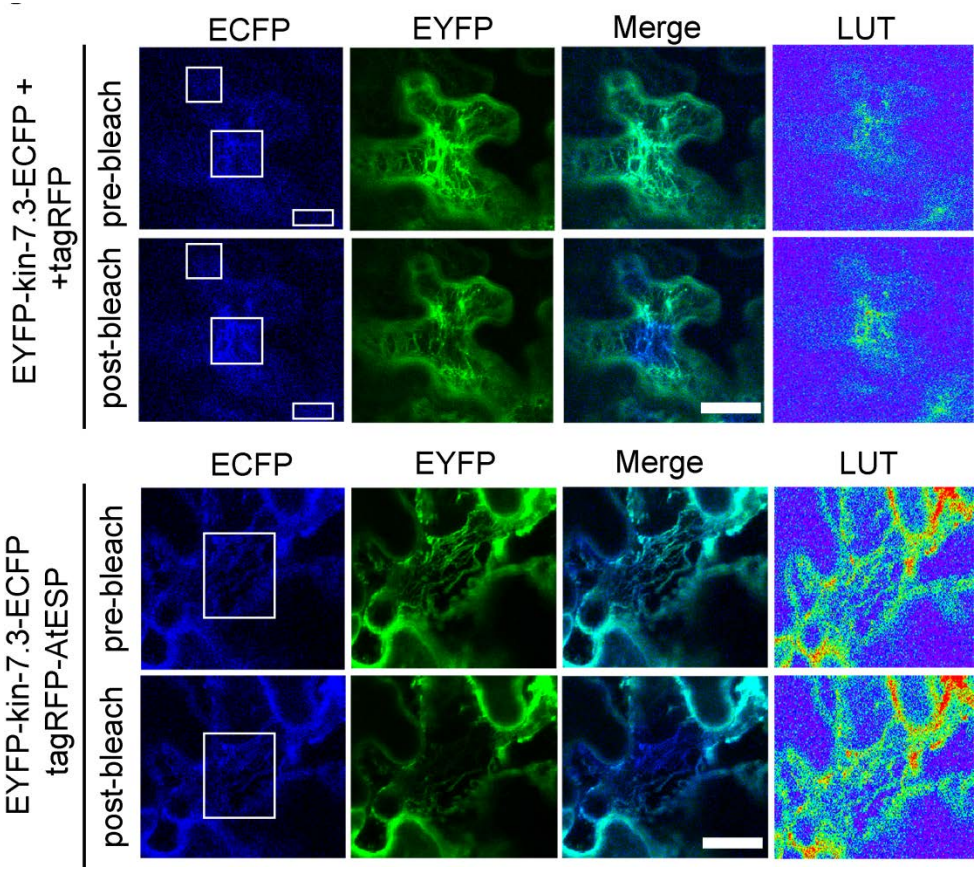
Calculate FRET efficiency

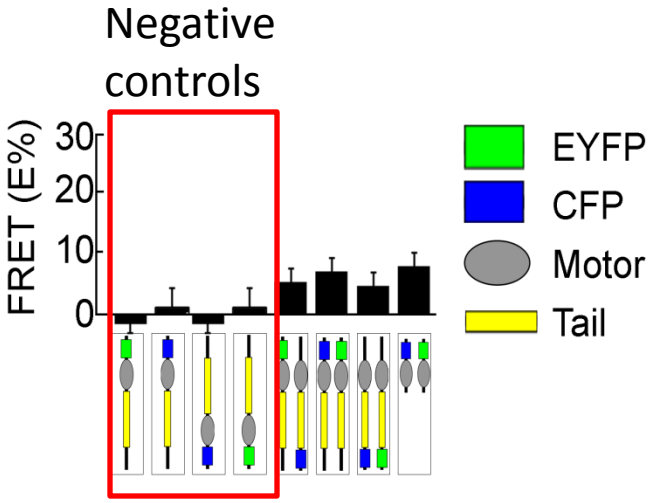
Efret(%)=(Int. final-Iint. initial)/(Int. final)

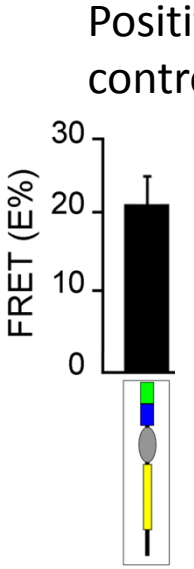


		singal intensity			background signal			Normalized signal intensity (=ROI1-ROI2)		
		CFP		YFP	CFP		YFP	CFP	YFP	
1	1	2108	1	1821	1	1358.859	1	628.024	749.013	1192.584	Efret(%)= =(O7-O6)/O6
2	2	2135	2	1815	2	1354.935	2	634.272	780.357	1181.121	
3	3	2152	3	1875	3	1350.907	3	637.99	801.27	1237.458	
4	4	2191	4	1910	4	1322.248	4	635.46	868.658	1274.276	
5	5	2719	5	1131	5	1516.924	5	621.001	1201.879	509.824	
6	6	2677	6	1250	6	1546.874	6	643.877	1130.257	606.123	
7	7	2592	7	1300	7	1522.088	7	653.378	1069.634	646.622	
8	8	2522	8	1332	8	1563.605	8	661.95	958.818	670.05	
9	9	2500	9	1350	9	1550.385	9	667.475	949.615	682.525	
10	10	2539	10	1349	10	1584.777	10	674.343	954.222	674.657	
11	11	2536	11	1350	11	1596.757	11	683.793	939.66	666.207	
12	12	2487	12	1400	12	1618.806	12	722.315	868.142	677.685	
13	13	2428	13	1450	13	1640.081	13	722.883	788.361	727.117	
14	14	2420	14	1500	14	1661.614	14	723.193	757.998	776.807	
15	15	2403	15	1550	15	1630.528	15	723.141	772.508	826.859	
16	16	2421	16	1556	16	1562.727	16	690.506	857.814	865.494	
17	17	2413	17	1558	17	1540.471	17	670.471	872.789	887.529	
18	18	2385	18	1557	18	1525.599	18	652.294	859.57	904.706	

Data presentation for FRET







Further reading

- http://fiji.sc/Cookbook
- http://fiji.sc/Time_Stamper
- http://fiji.sc/Annotating_Images
- http://occm.otago.ac.nz/resources/Making-a-Look-Up-Table---LUT.pdf
- http://fiji.sc/Image_Intensity_Processing
- Abscissa software http://rbruehl.macbay.de/
- FRAP theory (Balinski paper in the course literature) http://jcs.biologists.org/content/114/21/3885.full.pdf+html

Where does ‘F(t) = Finf – (Finf – F0)*exp(–t*a)’ comes?

Assuming that molecule A can bind reversibly to a spatially-fixed site B to form a complex AB, then the reaction equilibrium is given by

$$A + B \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} AB \qquad \text{(A1)}$$

The forward reaction rate (units: M s^{-1}) is

$$r_{on} = k_{on}[A][B] \qquad \text{(A2)}$$

where k_{on} is the bimolecular association rate constant (units: M^{-1} s^{-1}) and the reverse reaction rate (units: M s^{-1}) is

$$r_{off} = k_{off}[AB] \qquad \text{(A3)}$$

where k_{off} is the unimolecular dissociation rate constant (units: s^{-1}) and all the bracketed quantities are the molar concentrations of the indicated species. At equilibrium these two rates are equal (r_{on}=r_{off}) and the corresponding association equilibrium constant, K, is defined by

$$K = \frac{k_{on}}{k_{off}} = \frac{[AB]_e}{[A]_e[B]_e} = \frac{1}{K_D} \qquad \text{(A4)}$$

where the subscript ‘e’ indicates the concentration at equilibrium. Given the reaction in Eqn A1, the rate of formation of AB at any arbitrary time is given by

$$\frac{d[AB]}{dt} = k_{on}[A][B] - k_{off}[AB] \,. \qquad \text{(A5)}$$

In the FRAP experiments, we use a brief laser pulse to photobleach the fluorescent species A when it is bound to B, so that at time t=0, [AB] will be 0. We assume that the photobleaching has little effect on the fluorescence of [A] because the bleached A subunits readily leave the bleached region by diffusion and are replaced by unbleached subunits at an equivalent concentration. Also, it is assumed that the amount of A that is bleached is small compared with the total amount in the cell. Assuming that Eqn A1 has reached equilibrium prior to the bleaching event at t=0, and that [A] and [B] are unaffected by the bleaching process, then

$$[A] = [A]_e \text{ and } [B] = [B]_e \qquad \text{(A6)}$$

so that by combining Eqns A4 and A6

$$K_{on} = \frac{k_{off}[AB]_e}{[A][B]} \,. \qquad \text{(A7)}$$

Substituting Eqn A7 into Eqn A5,

$$\frac{d[AB]}{dt} = k_{off}[A][B]_e - k_{off}[AB] \,. \qquad \text{(A8)}$$

Assuming that [AB]=0 at t=0, then the solution to equation A8 is given by

$$\frac{[AB]}{[AB]_e} = 1 - e^{-k_{off}t} \qquad \text{(A9)}$$

which applies regardless of the degree of binding site saturation at equilibrium. Therefore, given the stated assumptions, the rate constant for FRAP recovery is identical to the dissociation rate constant, k_{off}.

Balinski et al., 2001 (see course literature)