ΒΙΟΛ-491 Ειδικά Κεφάλαια Βιοτεχνολογίας και Απεικονιστικής Φυτών

https://www.imbb.forth.gr/en/research-en/plant-molecular-biology/item/4119-panagiotis-n-moschou

https://pmoschoulab.blog/home/

FORTH
INSTITUTE OF MOLECULAR BIOLOGY & BIOTECHNOLOGY

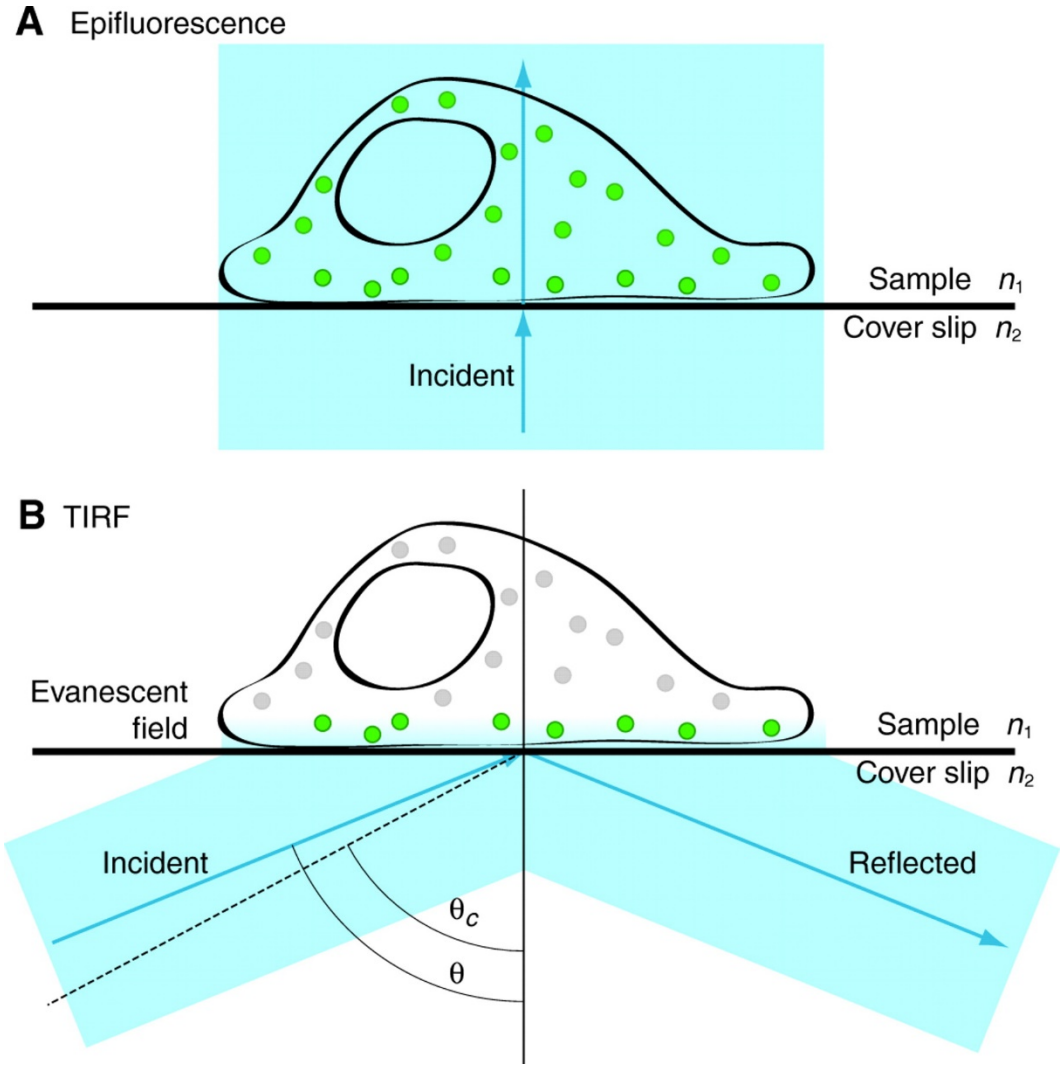
https://twitter.com/Panosmoschou

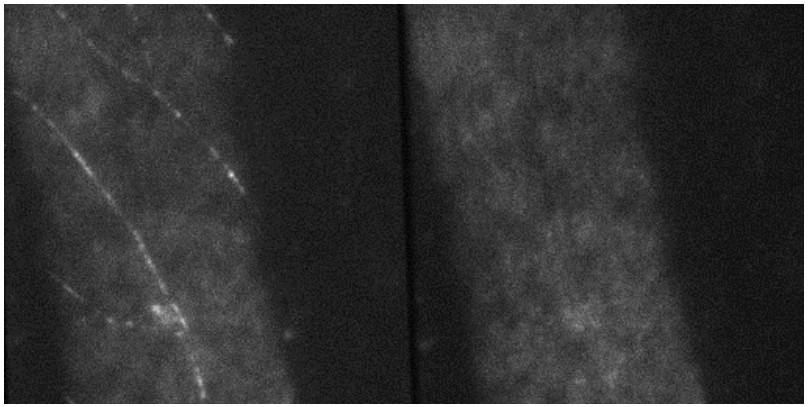
ΕΥΡΩΠΗ

ΒΙΟΛ-491 Ειδικά Κεφάλαια Βιοτεχνολογίας και Απεικονιστικής Φυτών-ΔΙΑΛΕΞΗ 4

ΕΡΓΑΣΙΑ 1

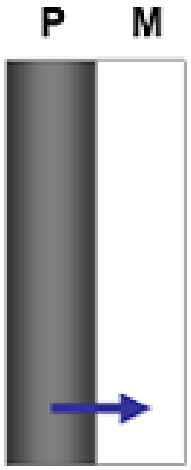
TOTAL INTERNAL REFLECTION MICROSCOPY





Ρυθμός διάχυσης (Diffusion rate)

Νόμος του FICK



P - polymeric material
M - contacting medium

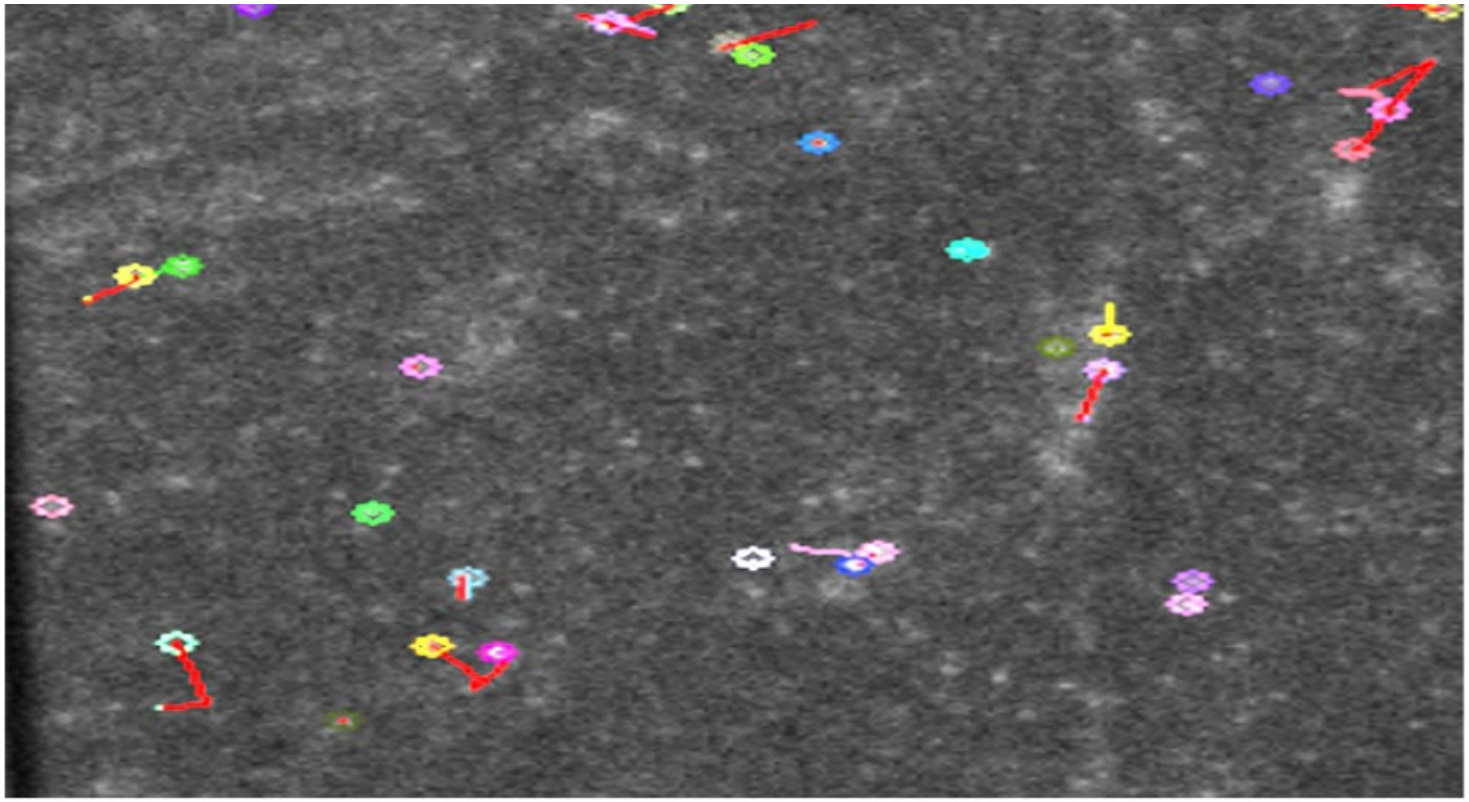
$$\frac{\partial c}{\partial t} = D \cdot \frac{\partial^2 c}{\partial x^2}$$

c - concentration
t - time
x - distance
D - diffusion coefficient

AKTS

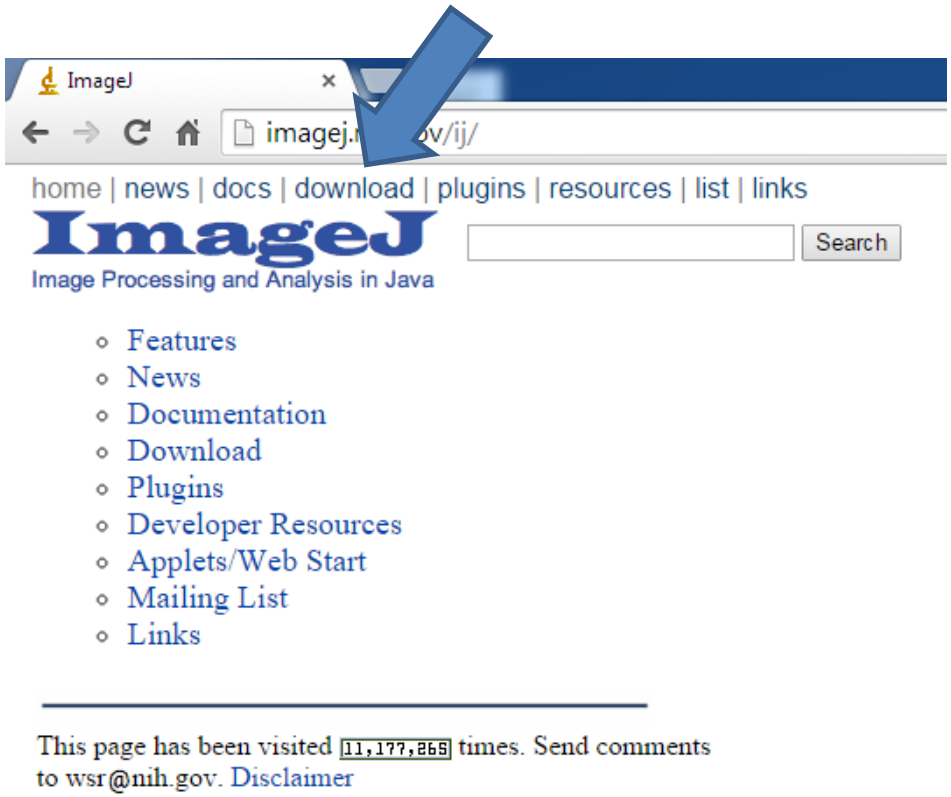
mdctec systems

Tracks (τροχιές)



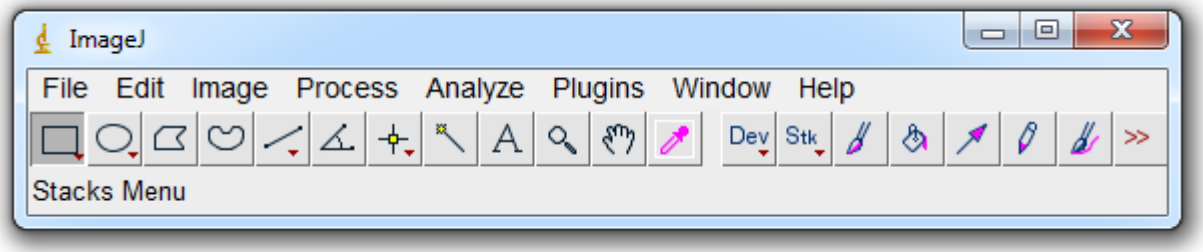
Πώς ξεκινάμε

Step 1

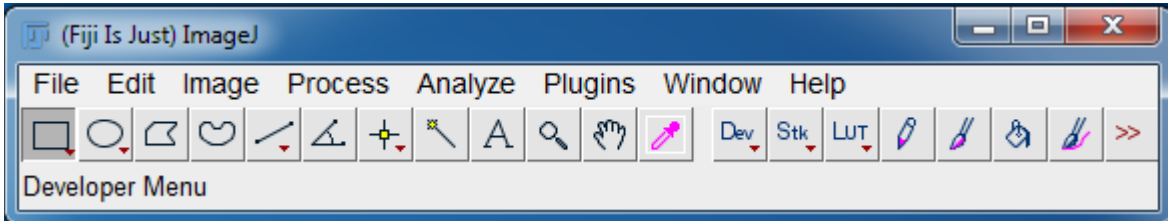


Step 2

Πώς μοιάζει το περιβάλλον του



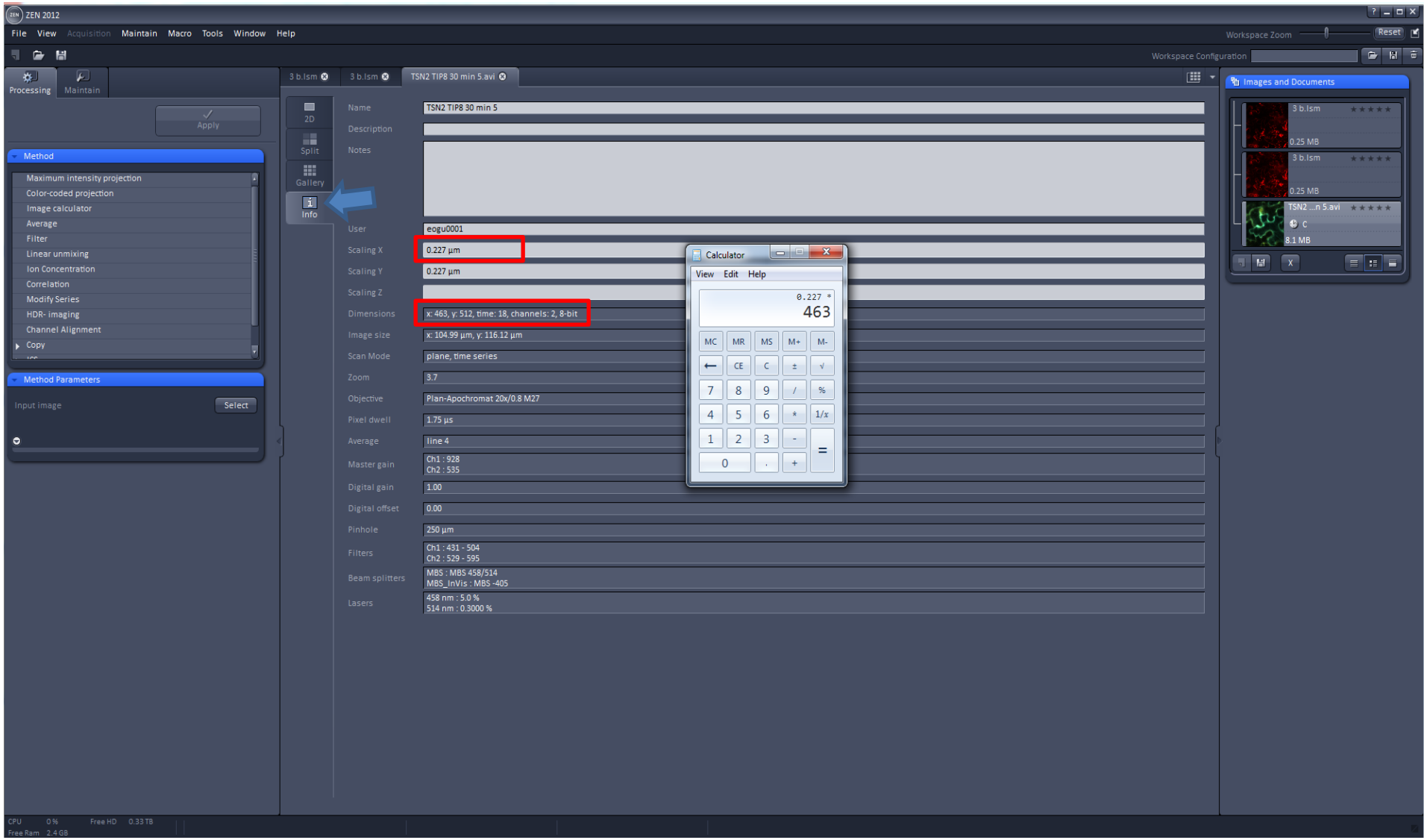
OR?



Έστω ότι έχουμε μια εικόνα χρονικής αλληλουχίας, τι κάνουμε μετά;

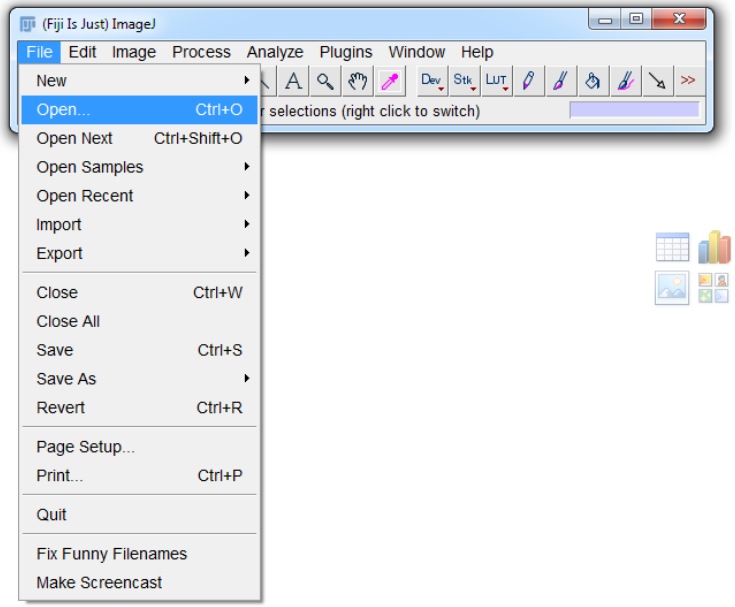
- Έλεγχος εικόνας (ανάλυση 512x512 dpi κατά τη λήψη; Δες και French et al., 2008)
- Χρειάζεται να κάνουμε ανάλυση;

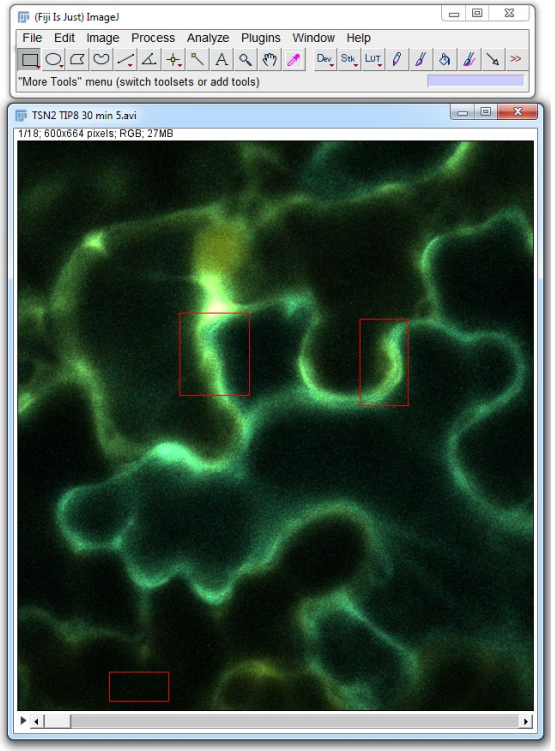
Πώς μπορώ να βρω το μέγεθος της εικόνας μου;



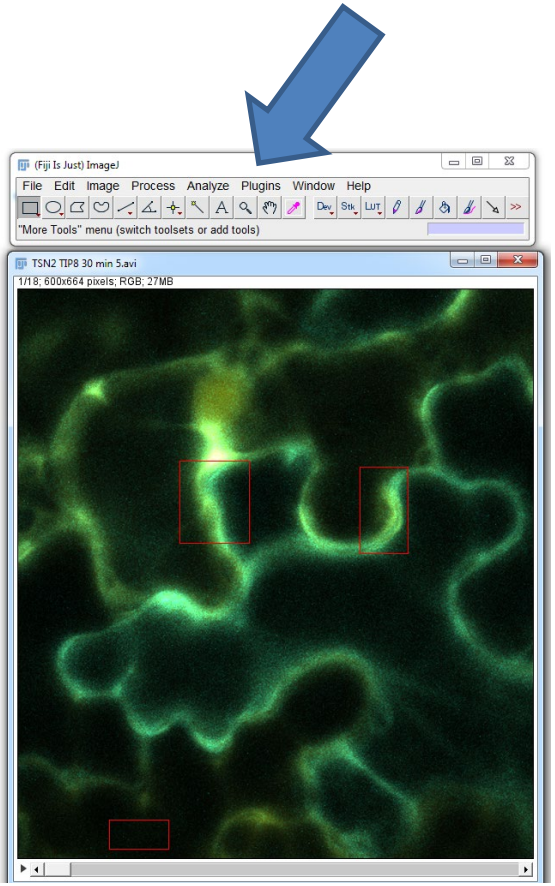
Άνοιξε το αρχείο με το FIJI

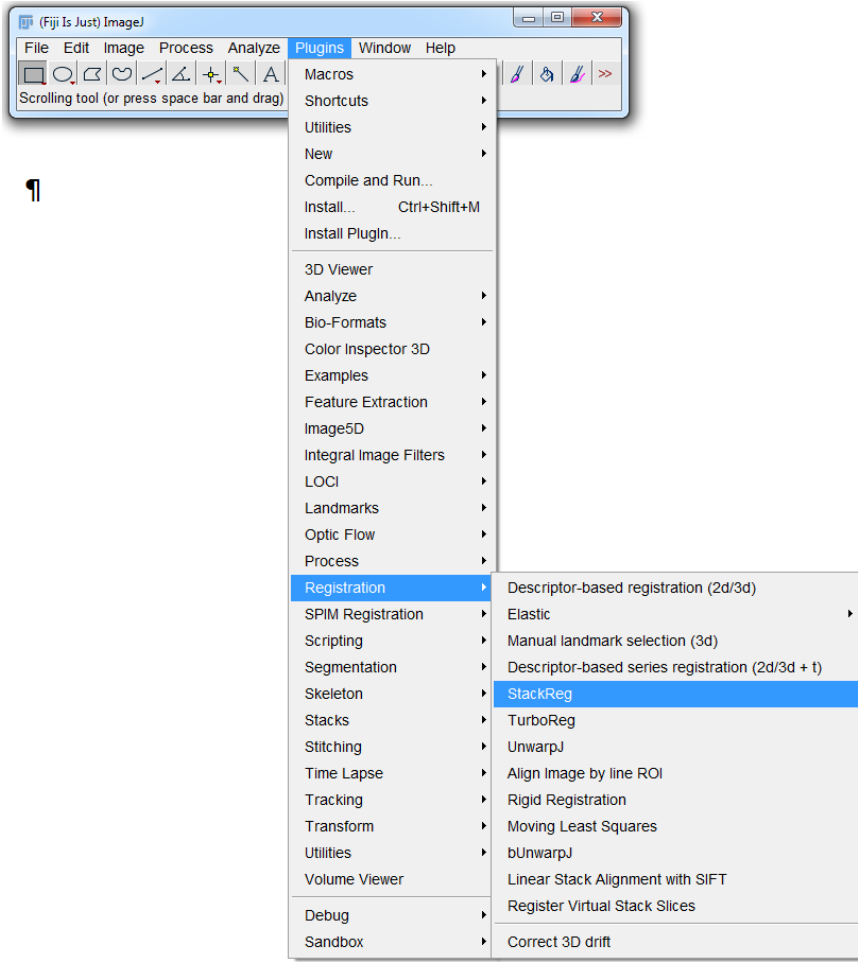
- Το FIJI ανοίγει πολλούς τύπους αρχείων!!!!





Η εικόνα μου κουνιέται, τι μπορώ να κάνω;



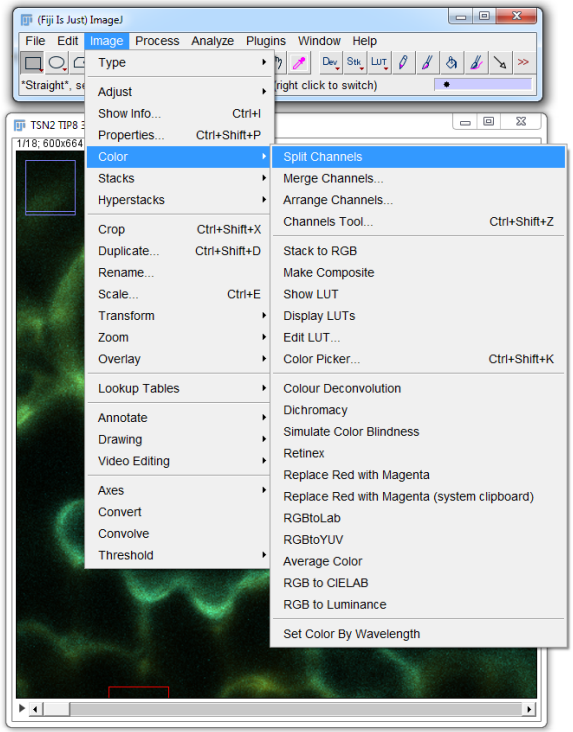


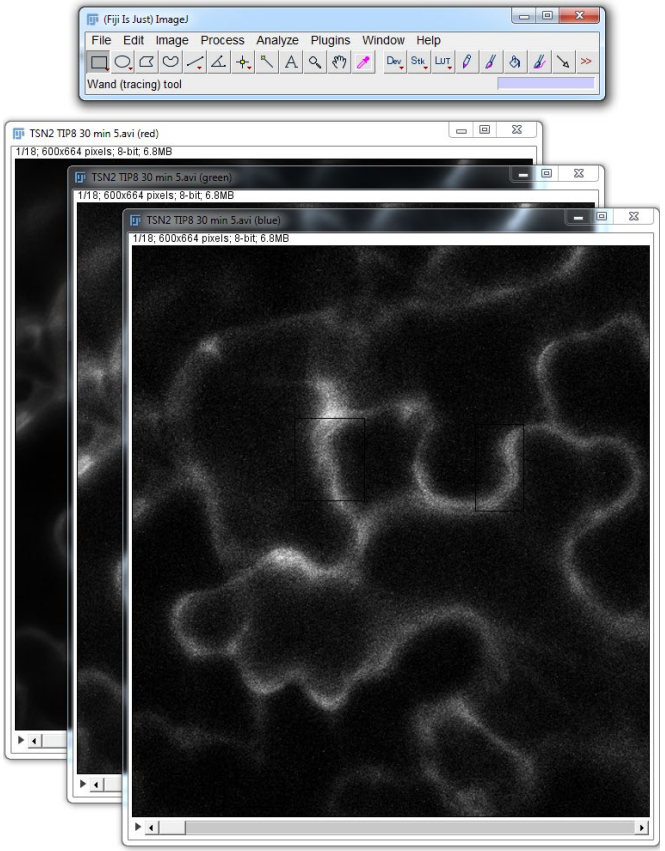
¶

How does it work?

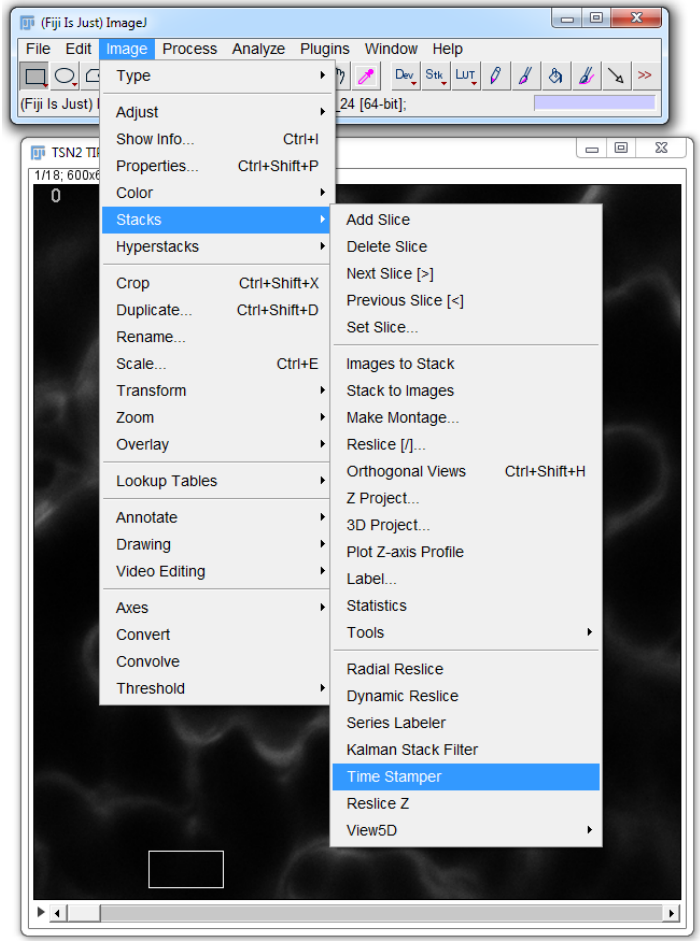
- We are using Rigid body transformations
- http://bigwww.epfl.ch/thevenaz/stackreg/

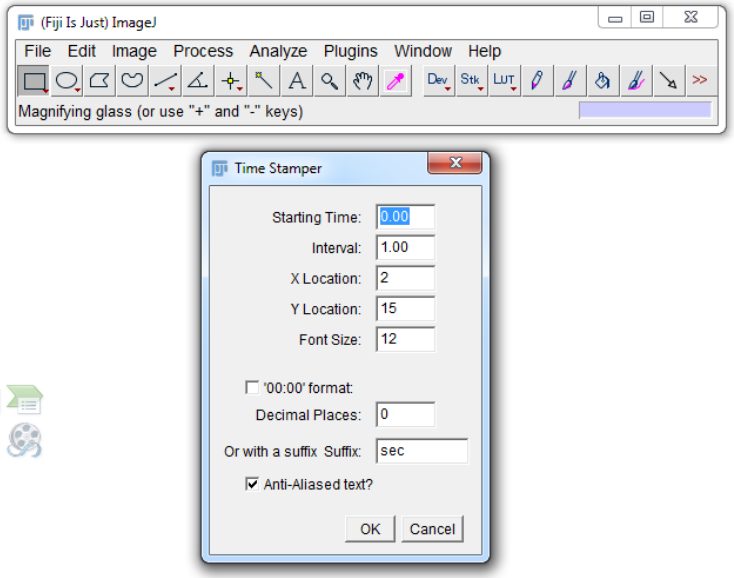
Αν έχεις διάφορα κανάλια μπορείς να τα διαχωρίσεις



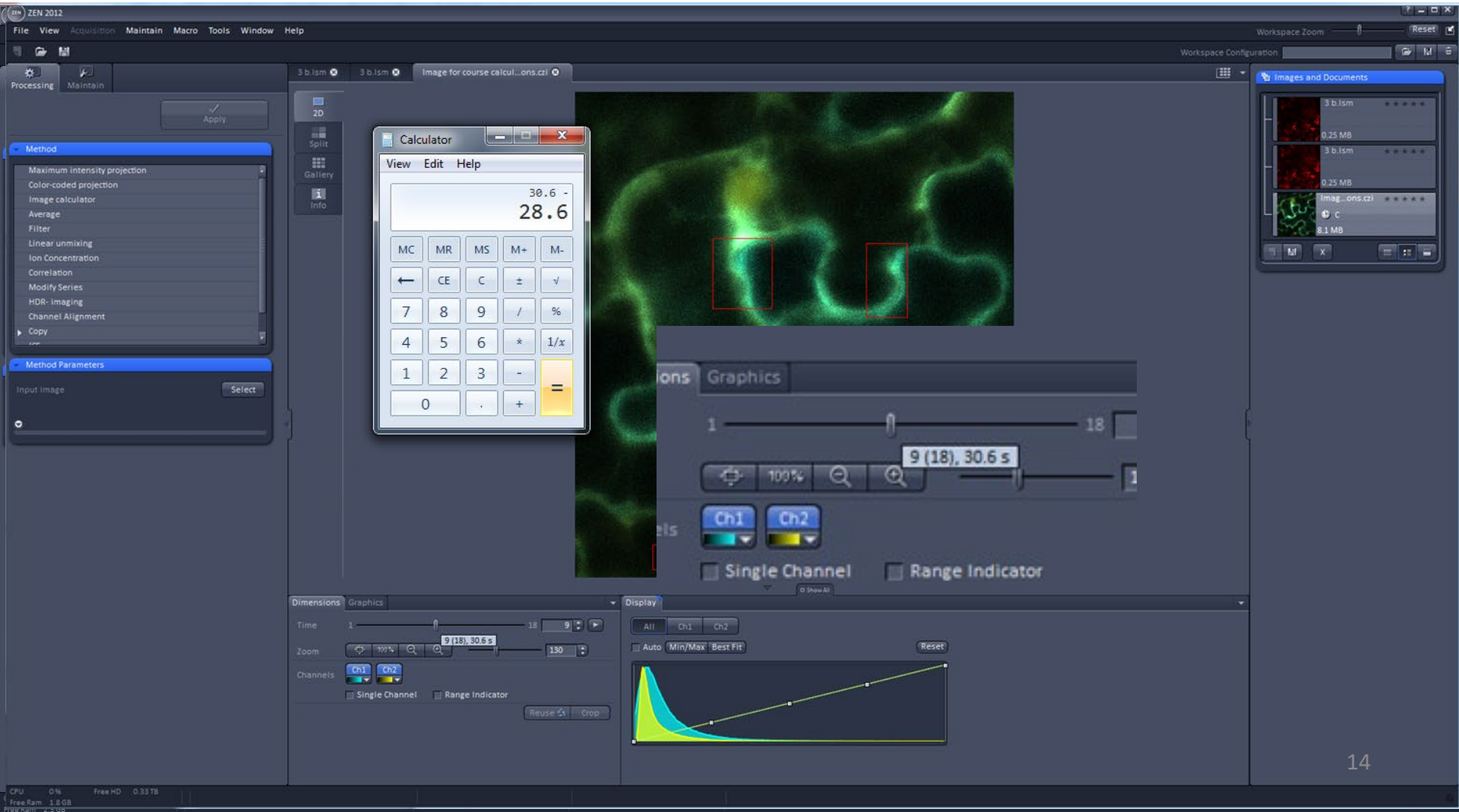


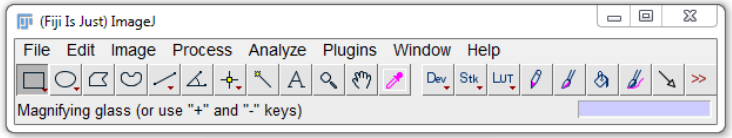
Μπορούμε να βάλουμε χρονικές ενδείξεις

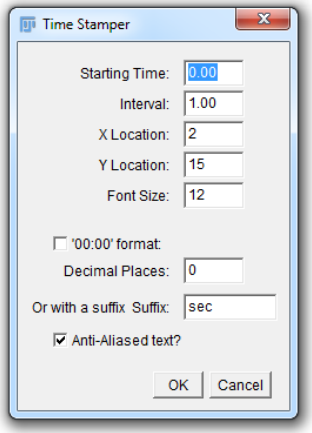


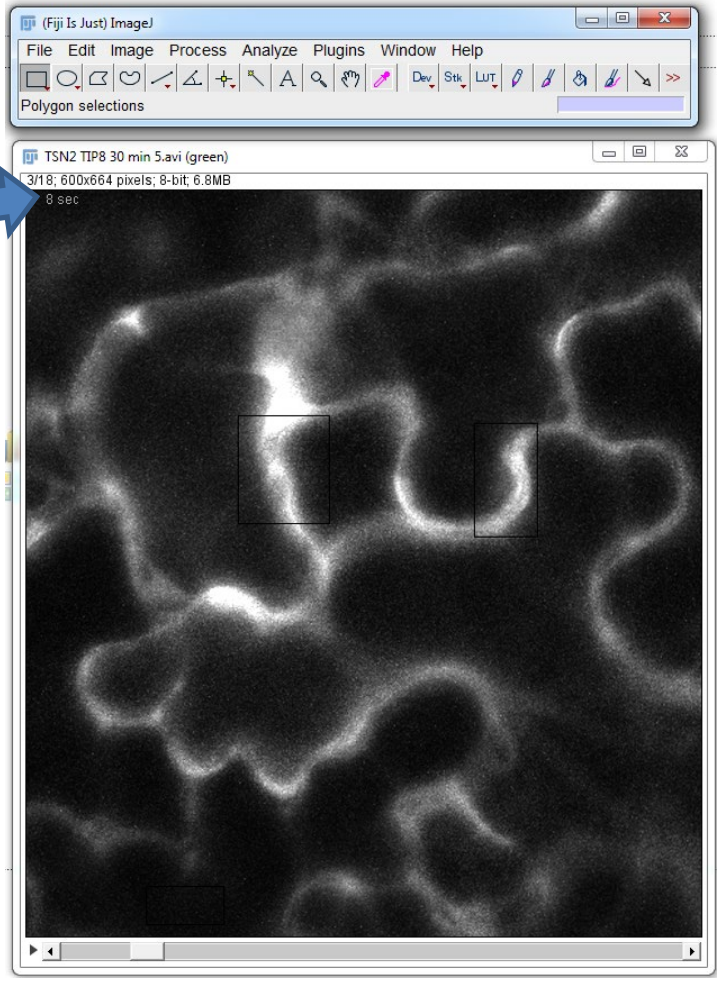


Υπολογισμός ενδιάμεσου χρόνου

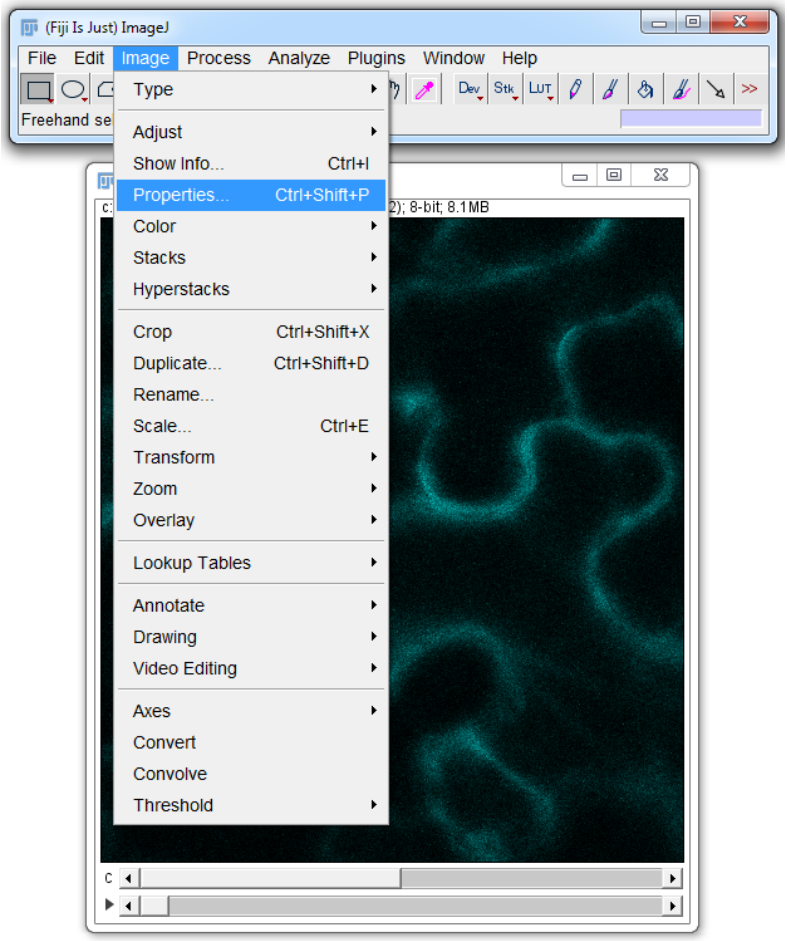


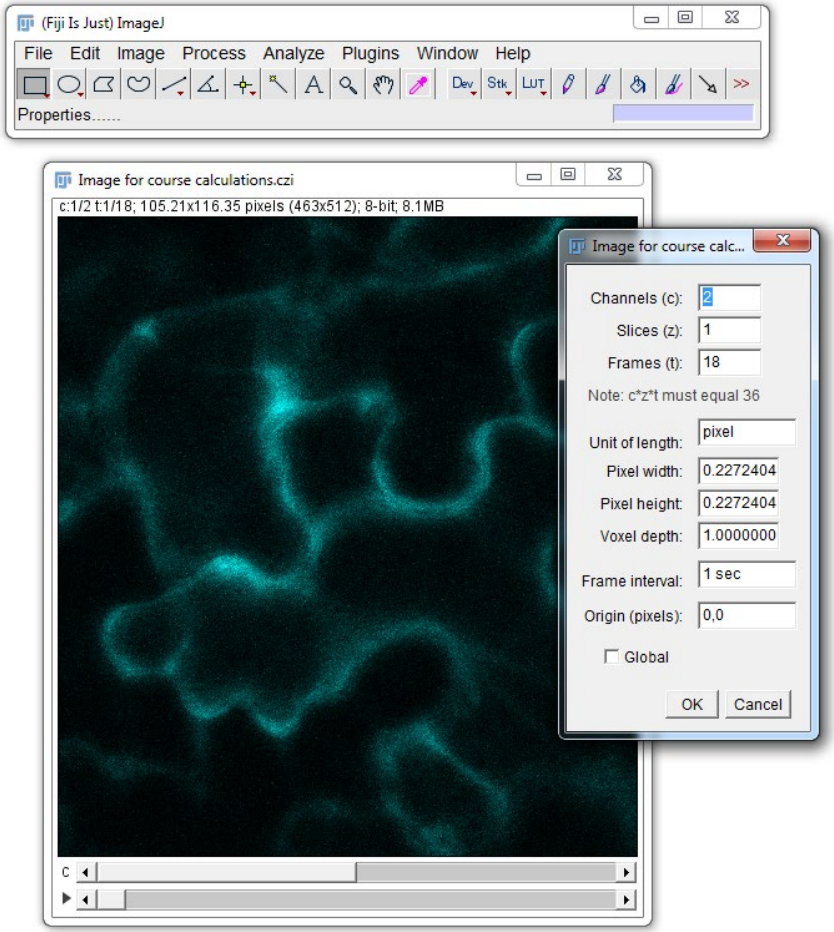

(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Magnifying glass (or use "+" and "-" keys)

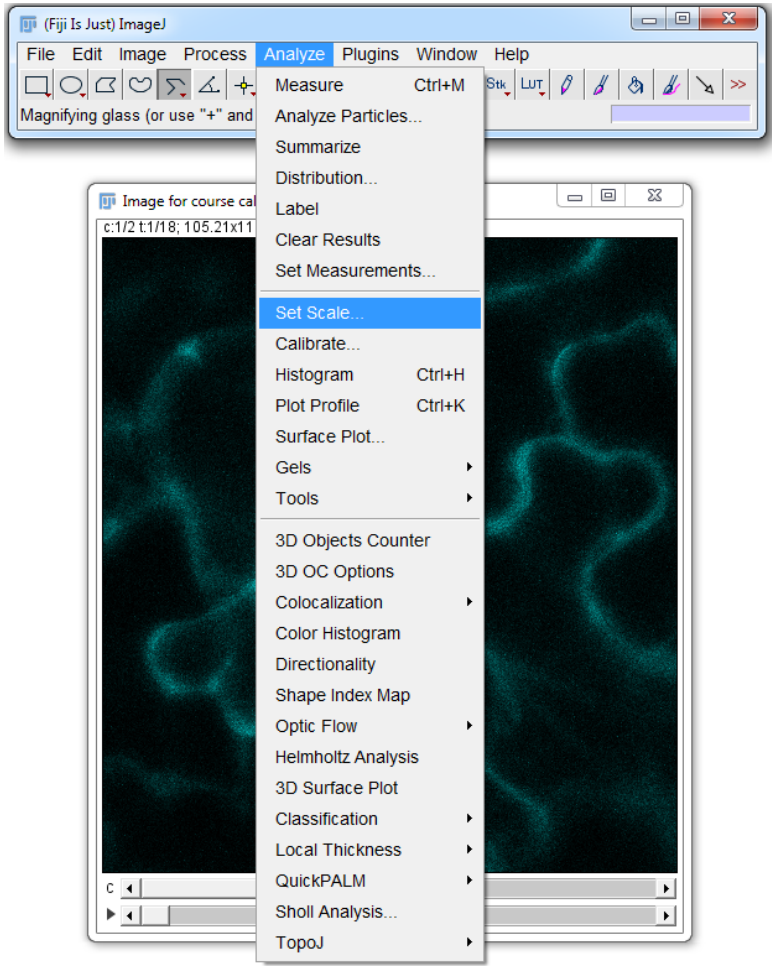

Time Stamper
Starting Time: 0.00
Interval: 1.00
X Location: 2
Y Location: 15
Font Size: 12
'00:00' format
Decimal Places: 0
Or with a suffix Suffix: sec
Anti-Aliased text?
OK
Cancel


(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

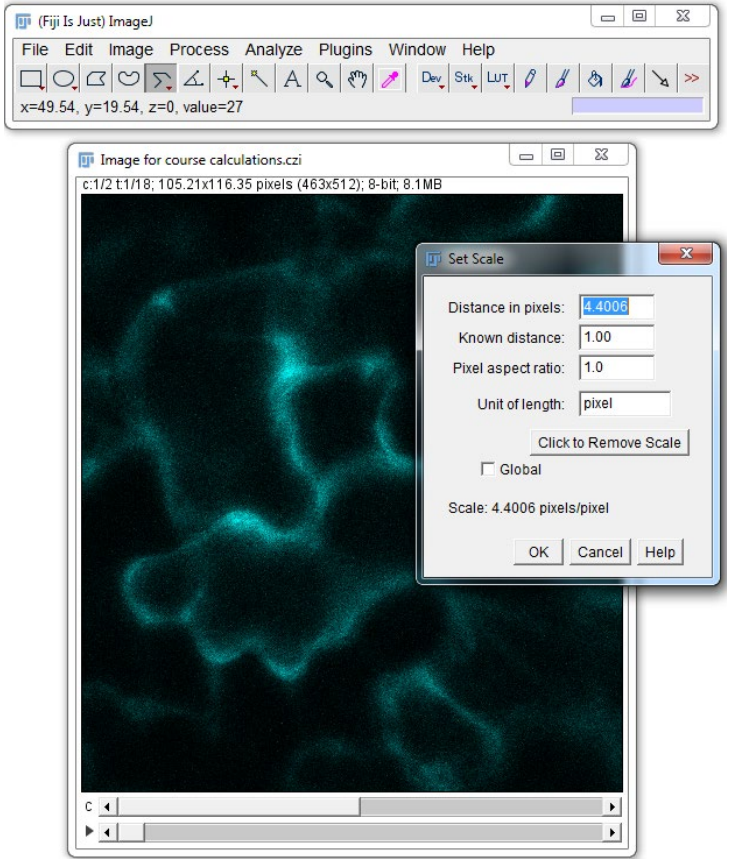
Πώς βάζω κλίμακα;



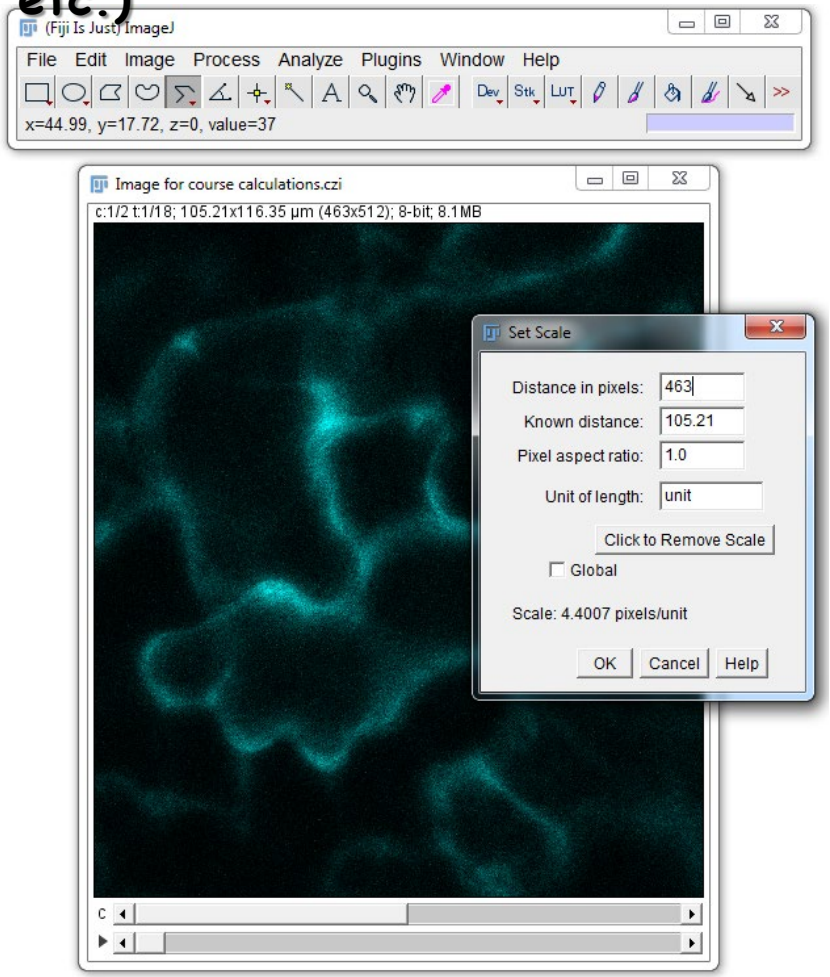




1. Εάν έχεις το αυθεντικό αρχείο



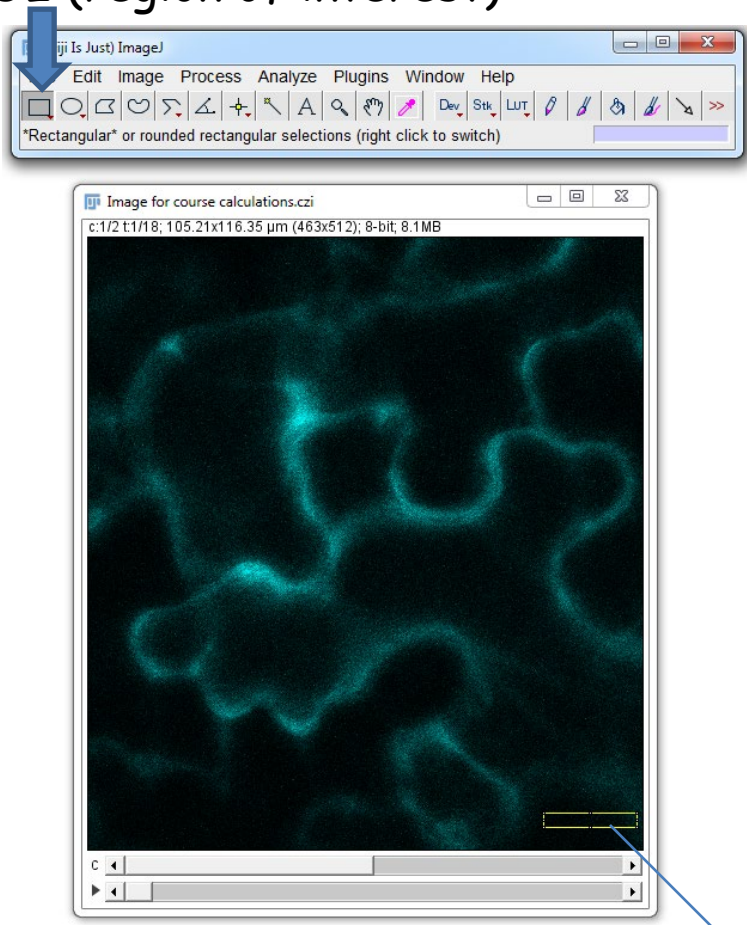
2. Εάν δεν έχεις το αρχείο στην αρχική του μορφή (π.χ. 'avi' files etc.)



Πώς βάζω τη μπάρα

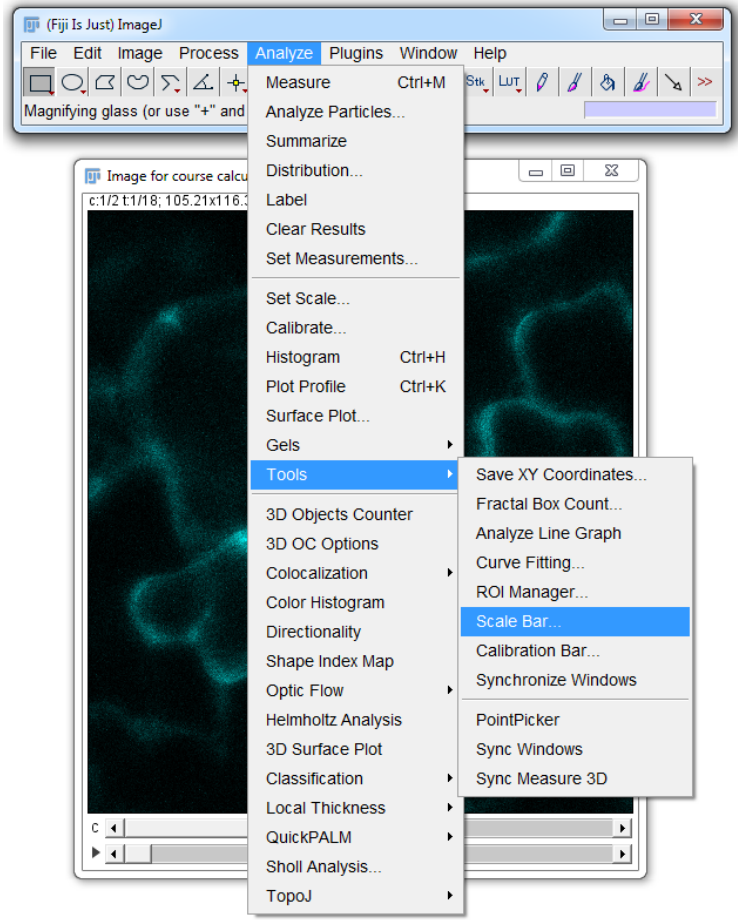
Step 1: πού θα μπεί

ROI (region of interest)

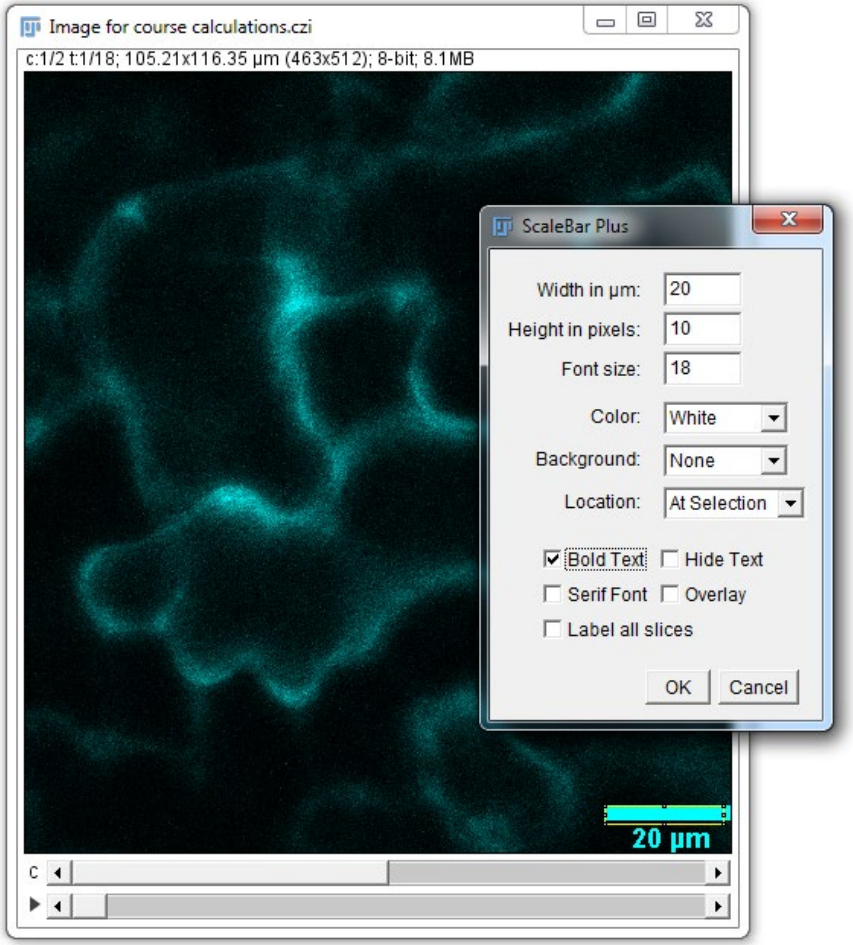


Εδώ

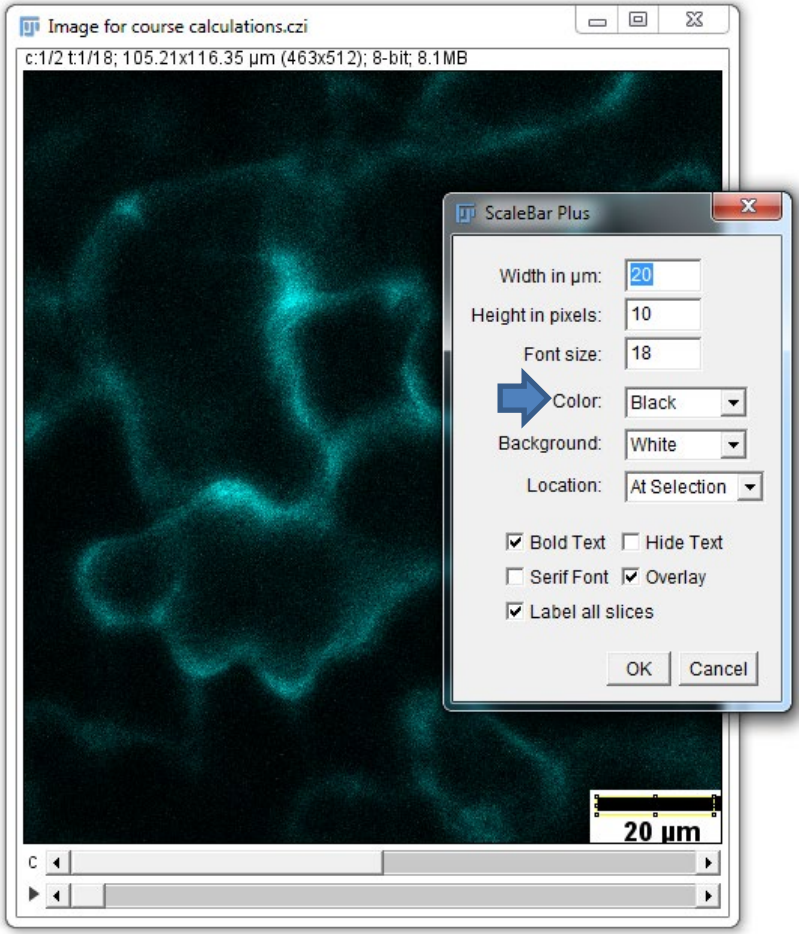
Step 2: κάνε τα εξής



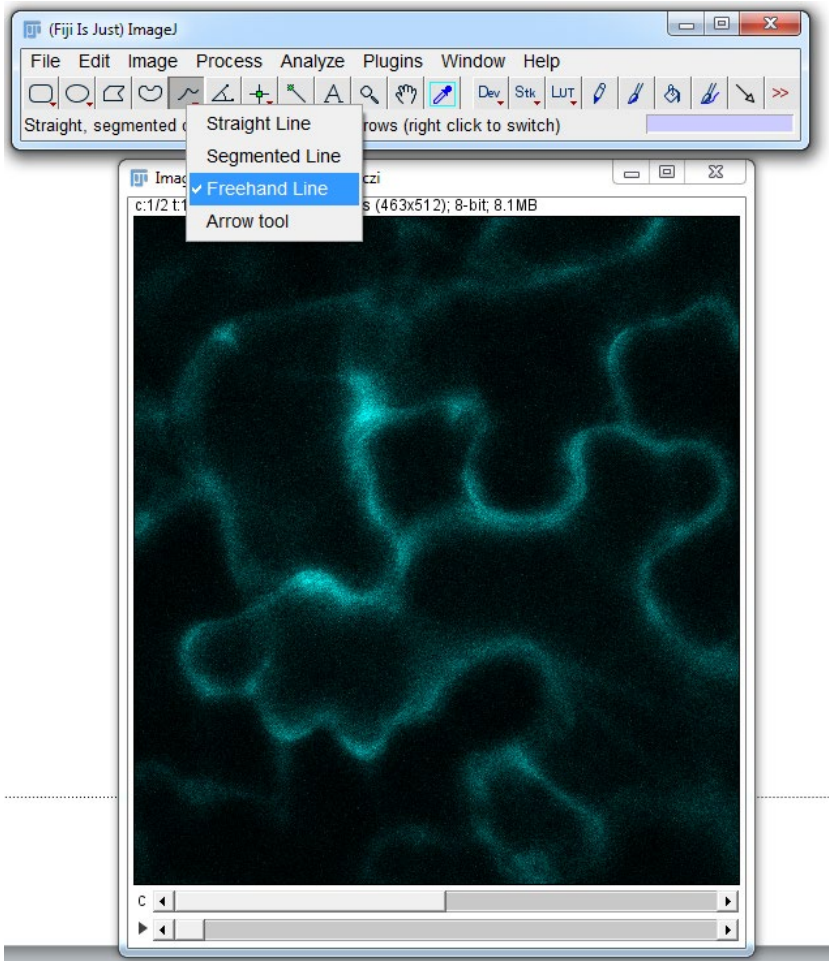
Step 3:

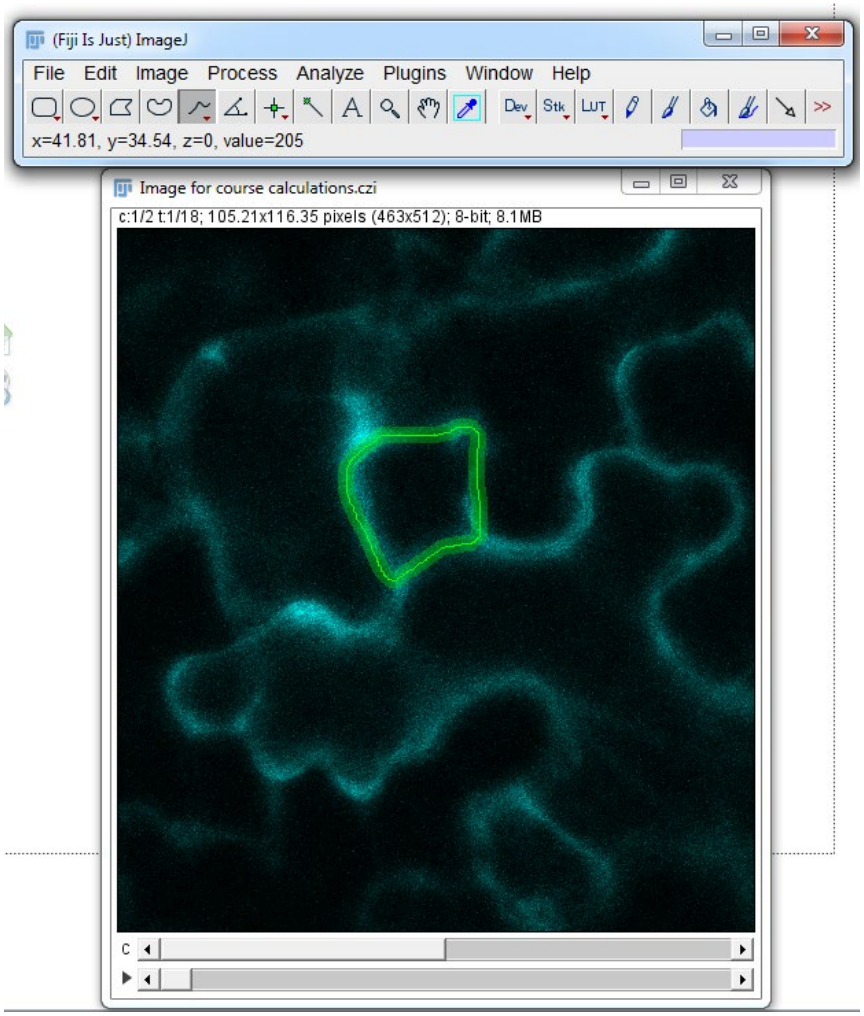


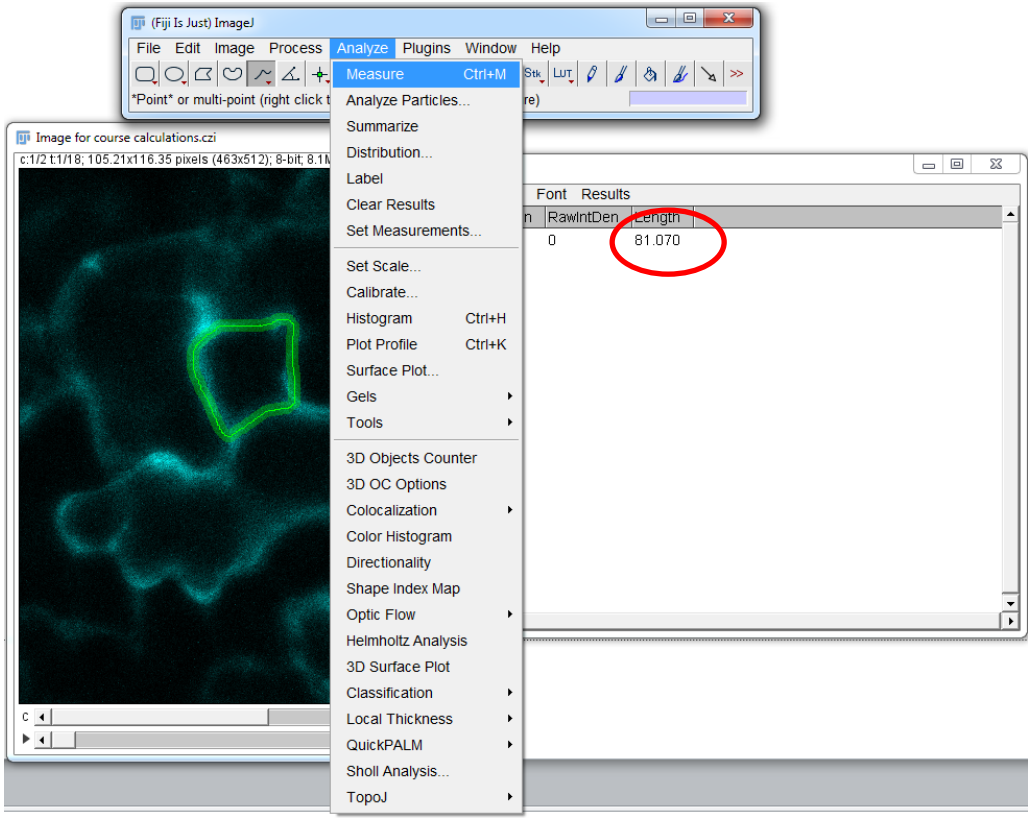
Step 4: Χρώμα κτλ.



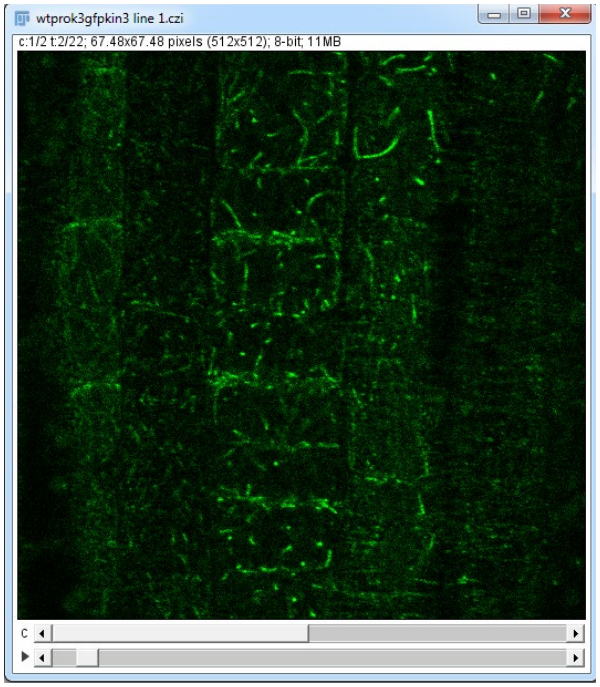
Τώρα μπορώ να μετρήσω αποστάσεις

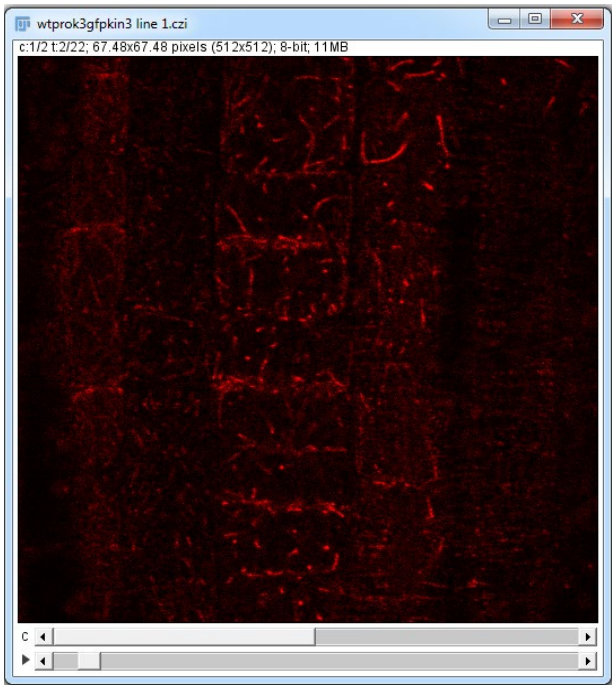


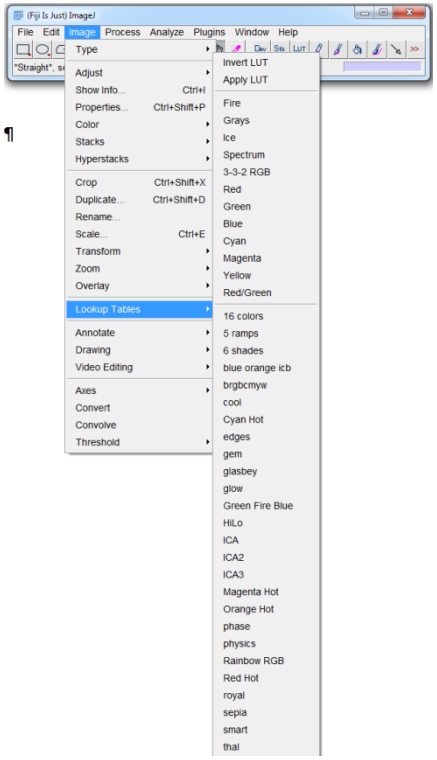



(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Measure Ctrl+M
Analyze Particles...
Summarize
Distribution...
Label
Clear Results
Set Measurements...
Set Scale...
Calibrate...
Histogram Ctrl+H
Plot Profile Ctrl+K
Surface Plot...
Gels
Tools
3D Objects Counter
3D OC Options
Colocalization
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

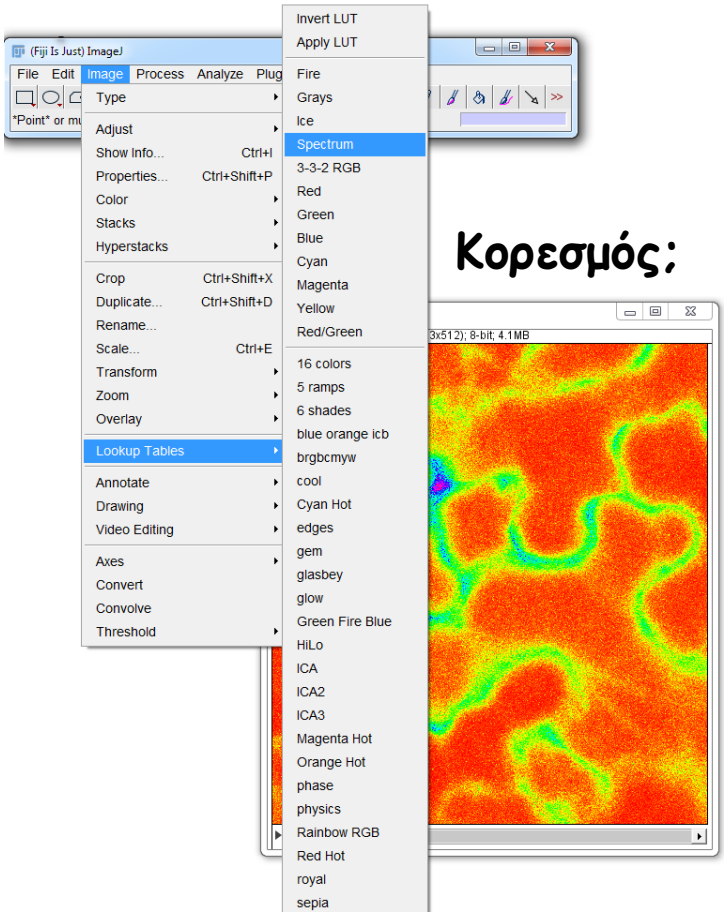
Πώς μπορώ να προσαρμόσω τα χρώματα;



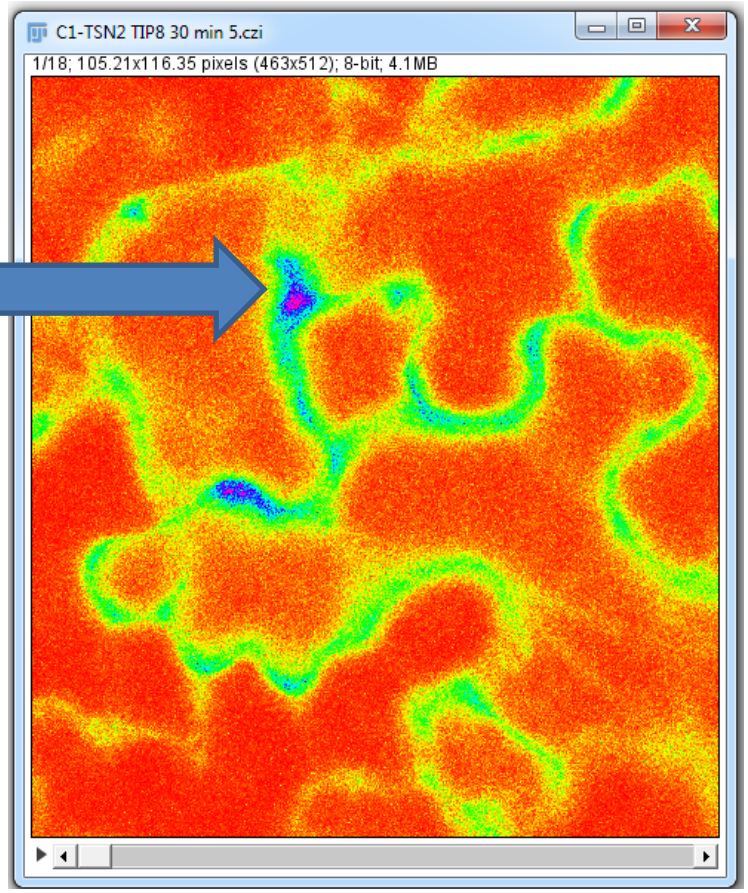




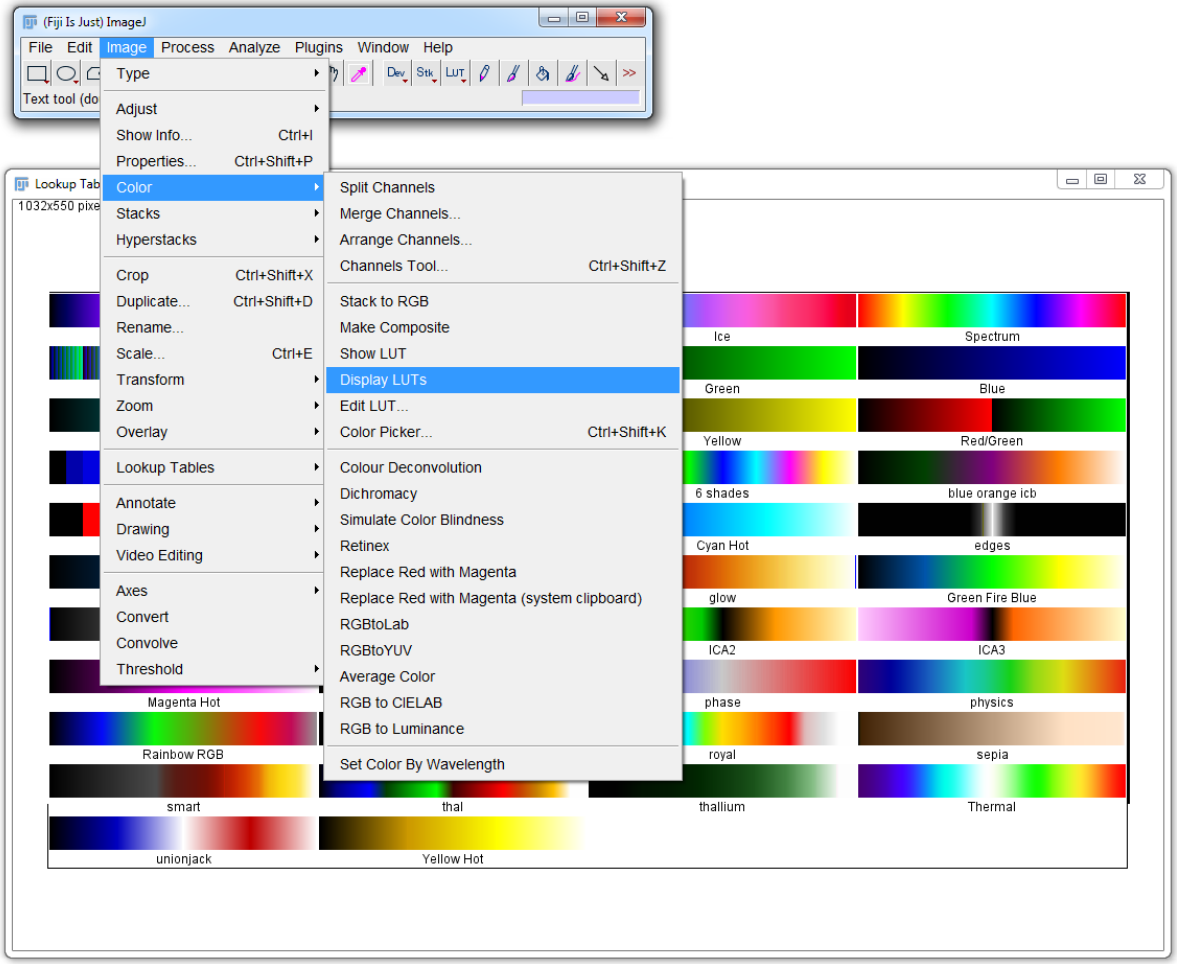
Λύση: αποχρώσεις του γκρι


(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

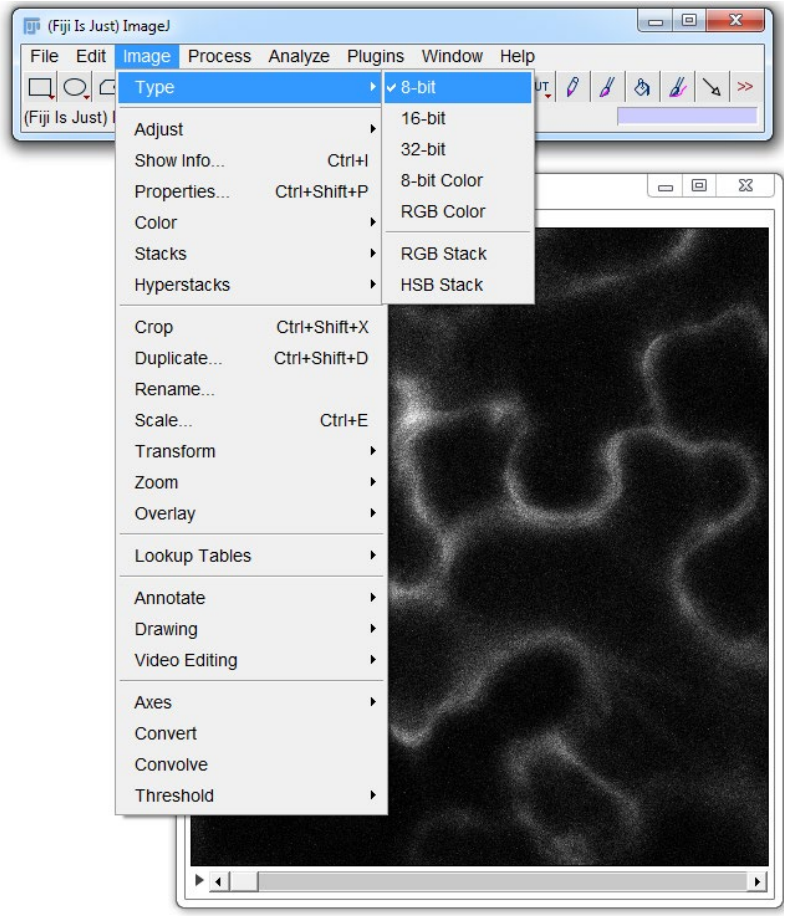
Κορεσμός:

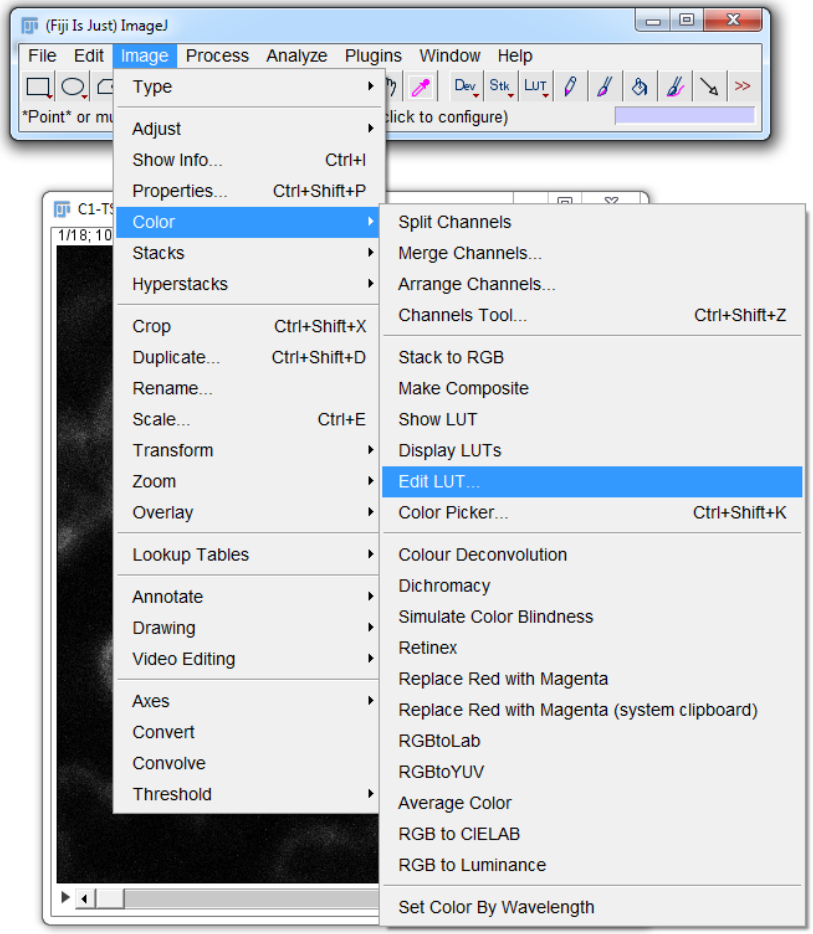

C1-TSN2 TIP8 30 min 5.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB

Τί είναι το Look Up Table (LUT)?

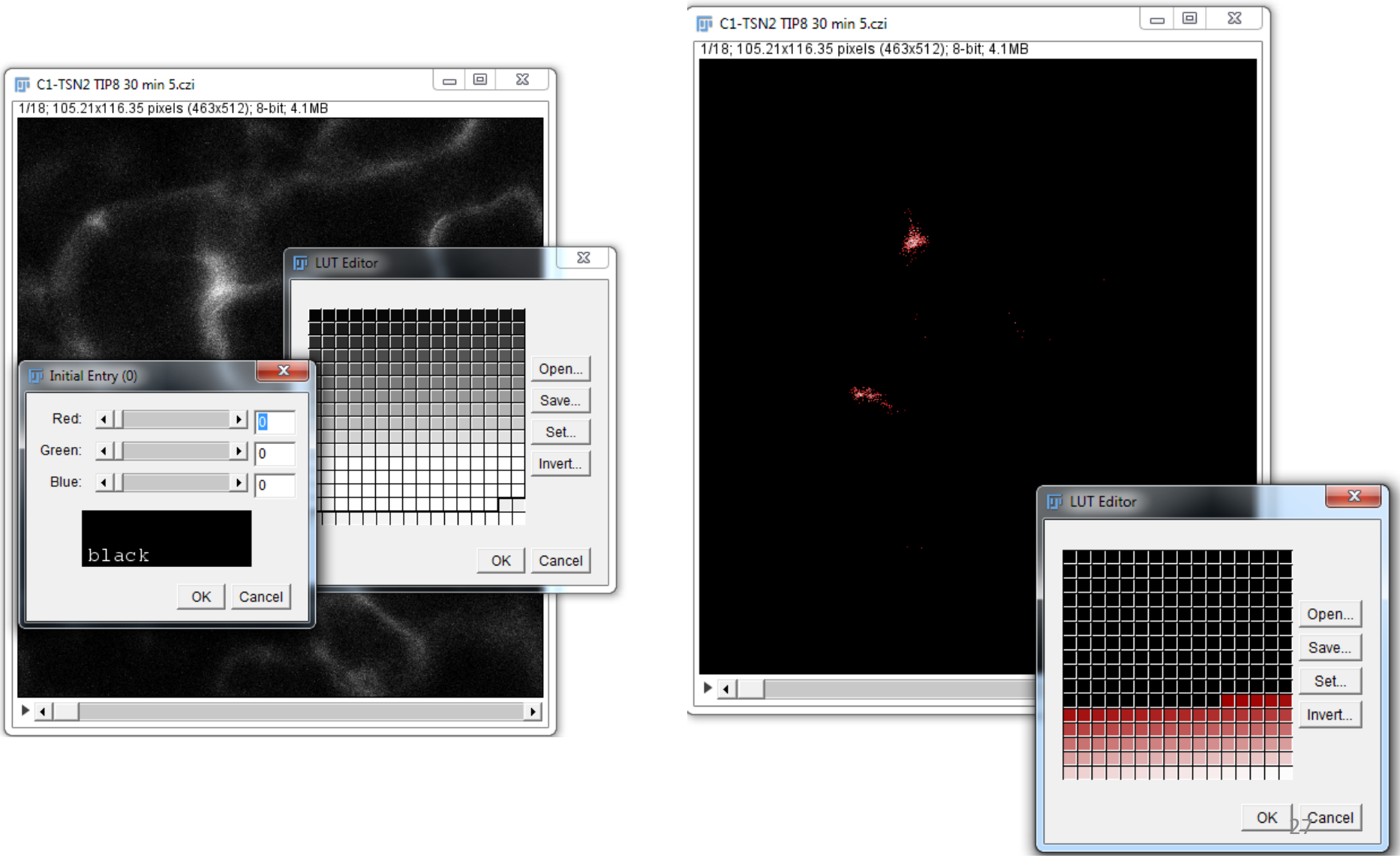


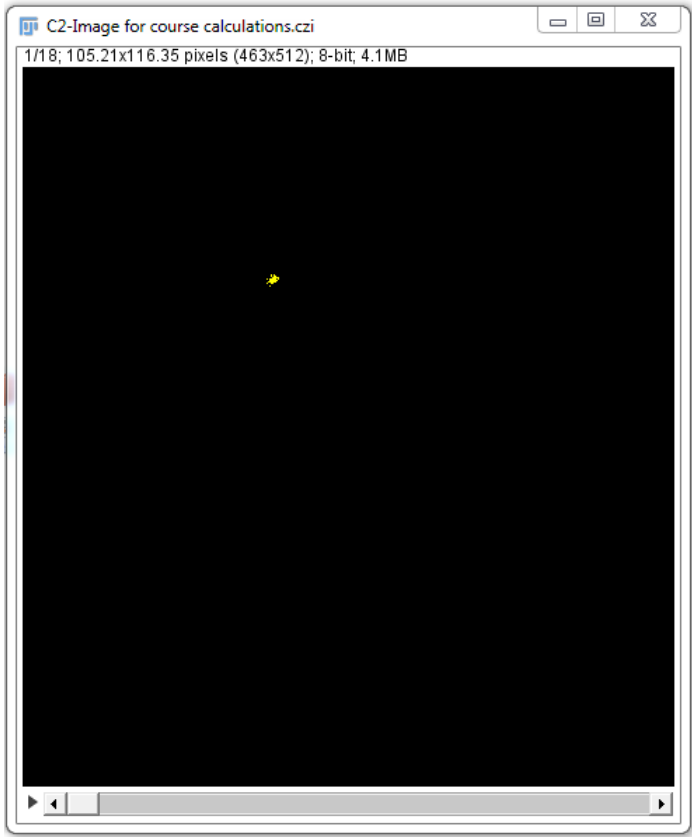
Μπορώ αν επισημάνω συγκεκριμένες εντάσεις

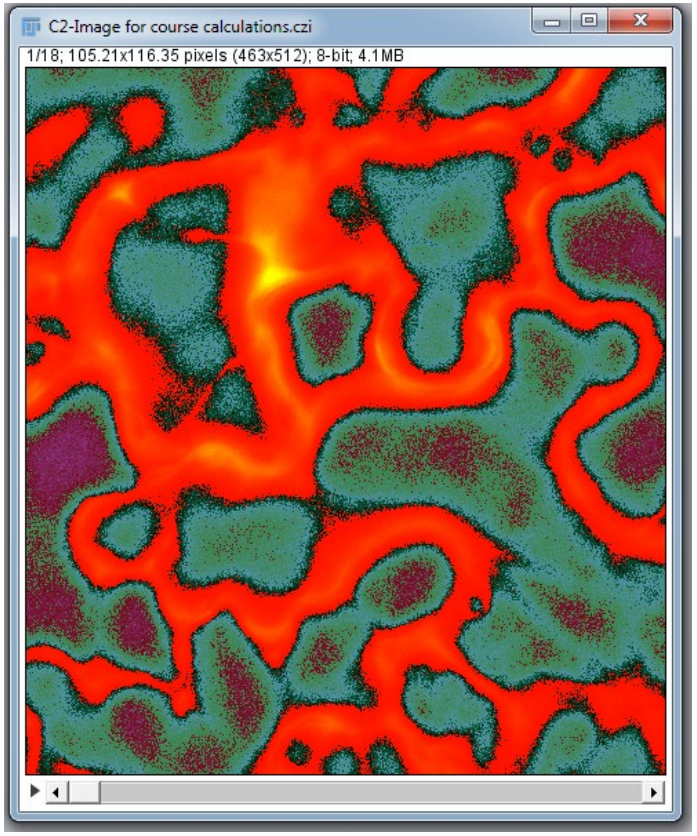




Είναι τα χρώματά μου δυναμικά;

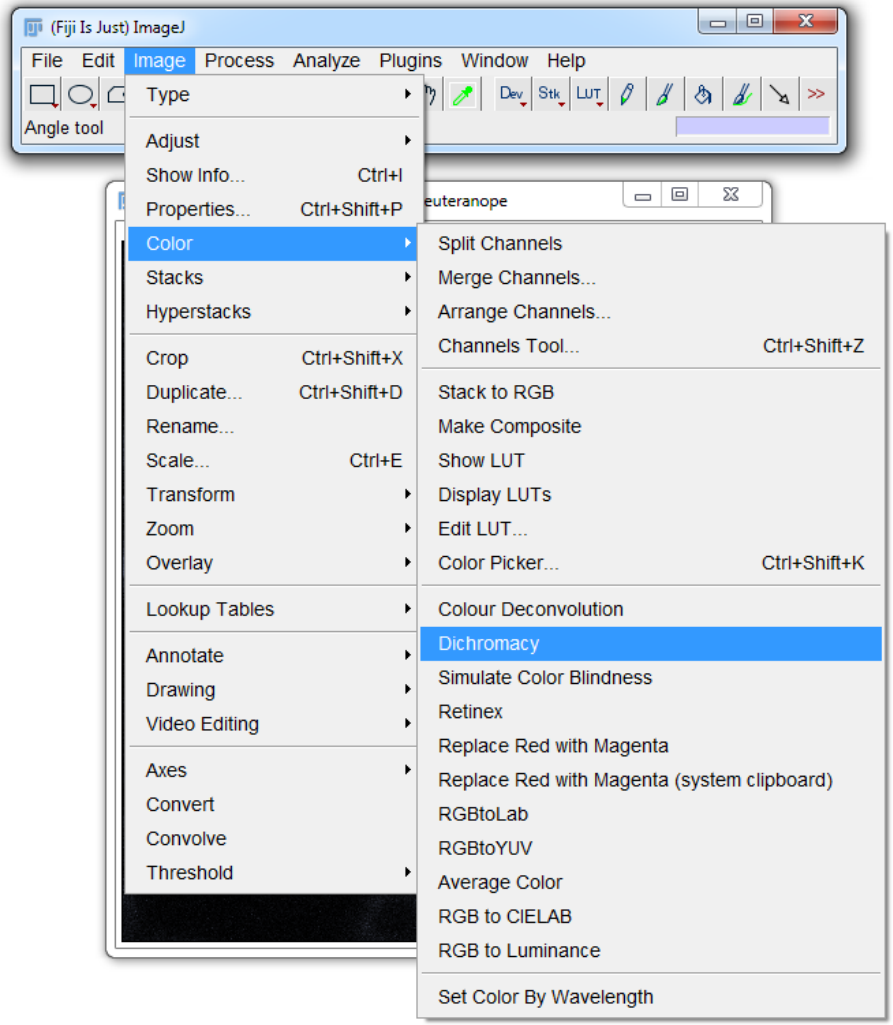


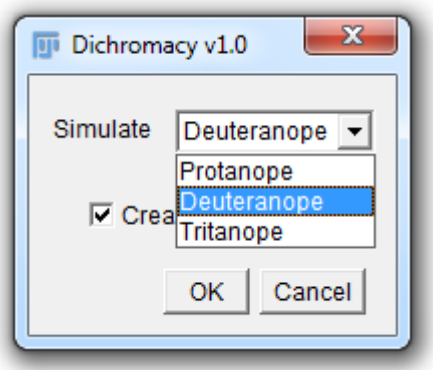

C2-Image for course calculations.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB

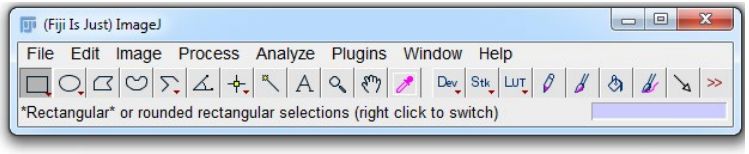

C2-Image for course calculations.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB

Τύποι αχρωματοψίας

Προσομοίωση αχρωματοψίας






(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Rectangular or rounded rectangular selections (right click to switch)

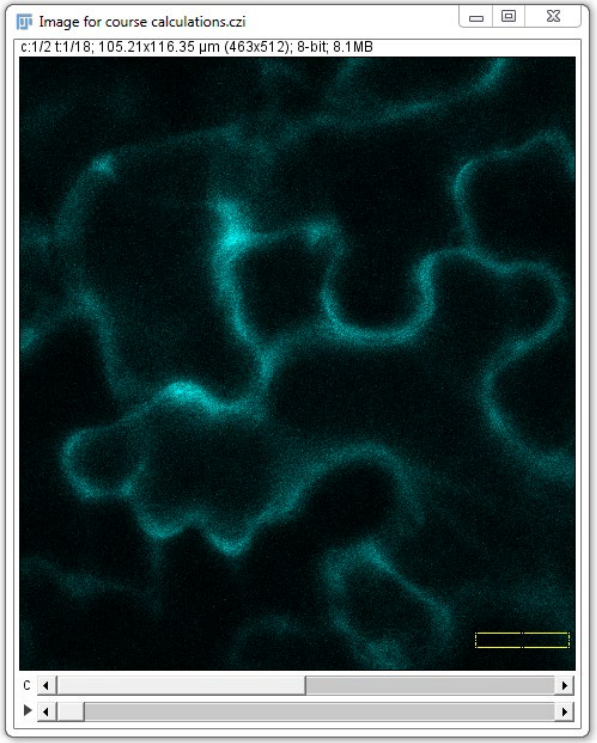
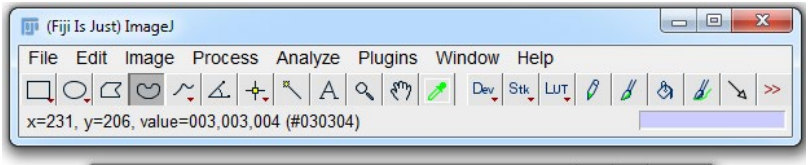
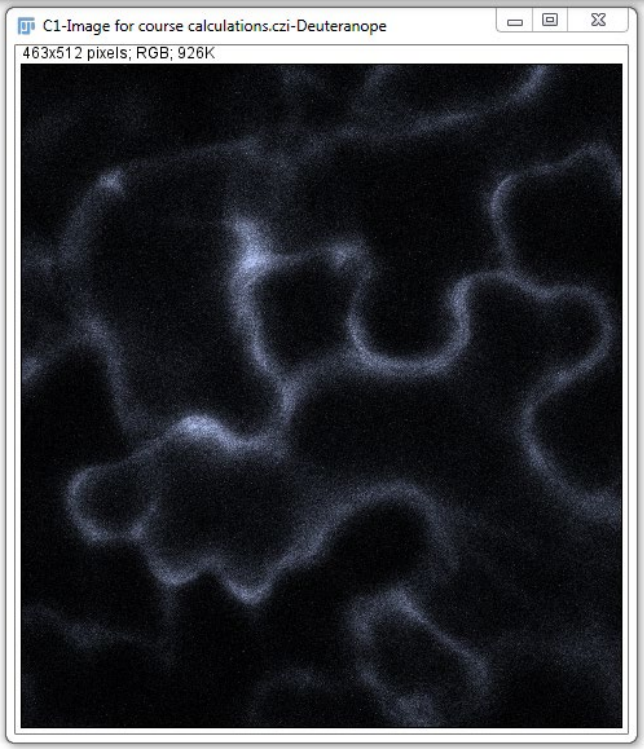
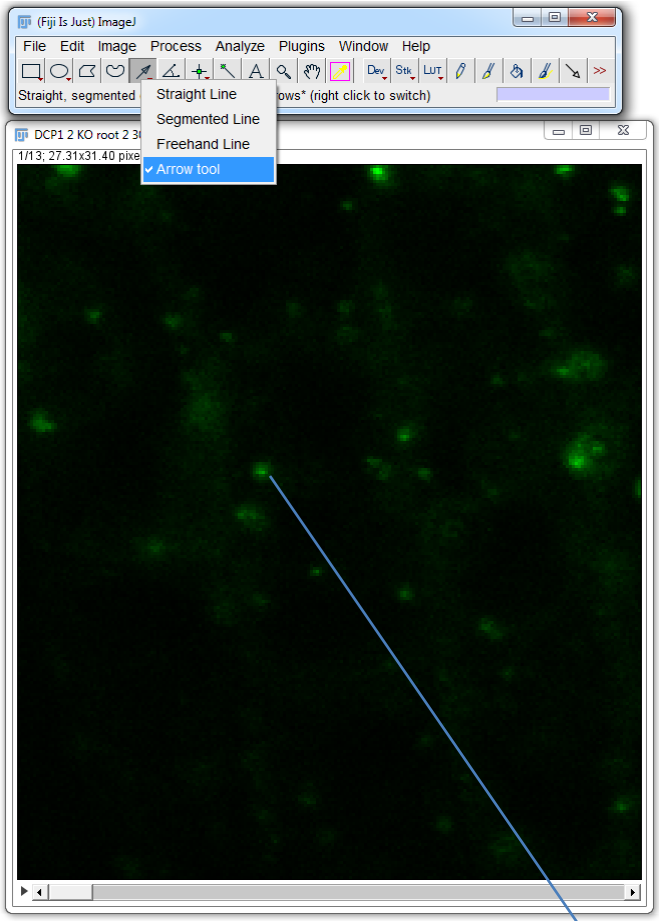


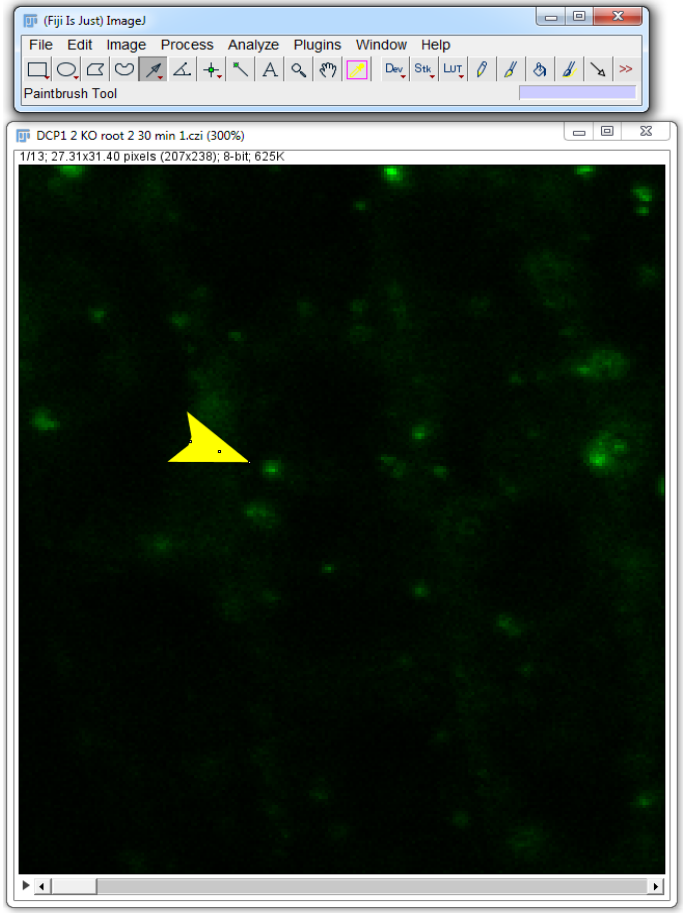
Image for course calculations.czi
c:1/2 t:1/18; 105.21x116.35 µm (463x512); 8-bit; 8.1MB


(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

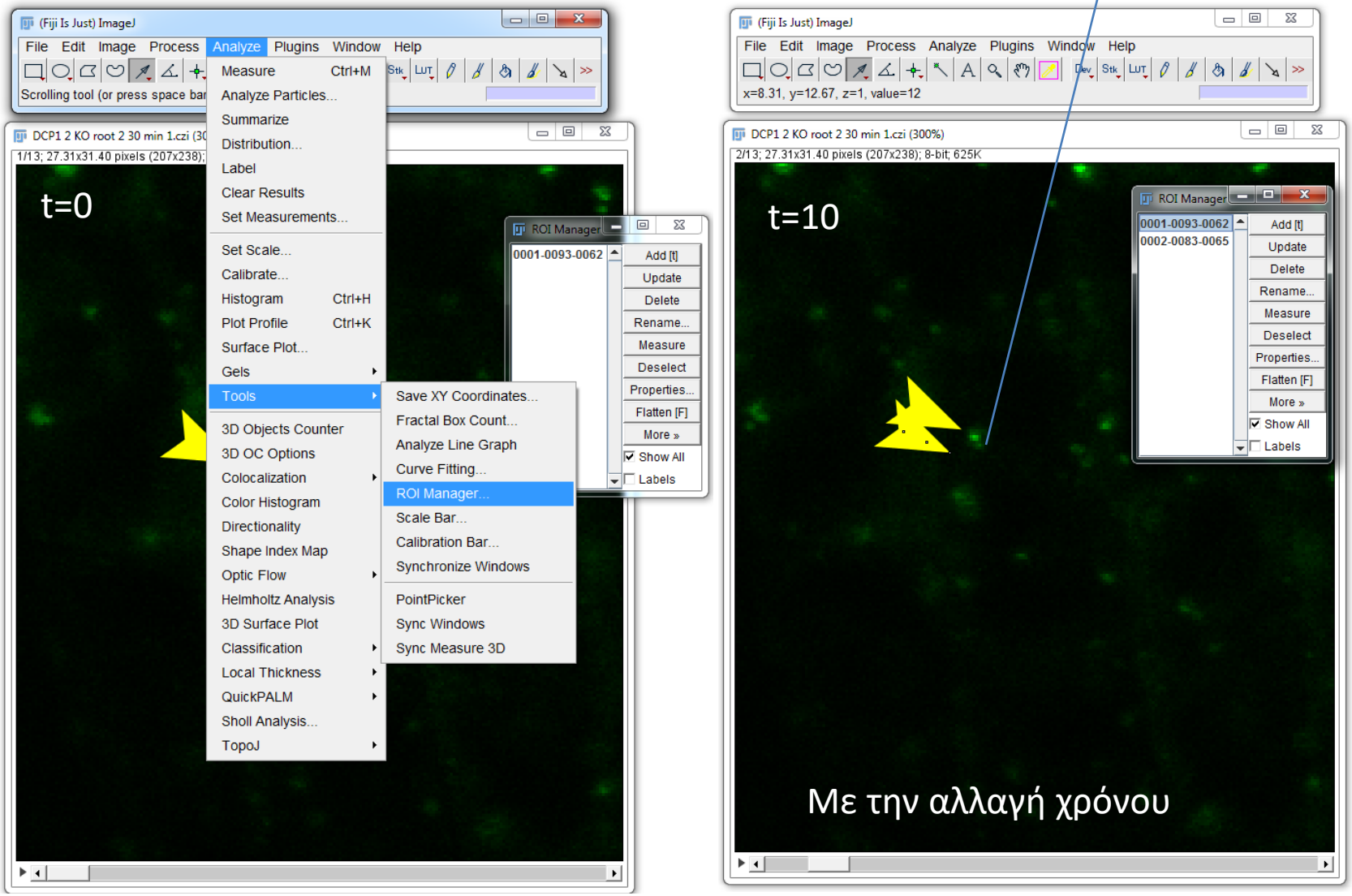
Πώς μπορώ να σχεδιάσω δομές στις εικόνες μου;

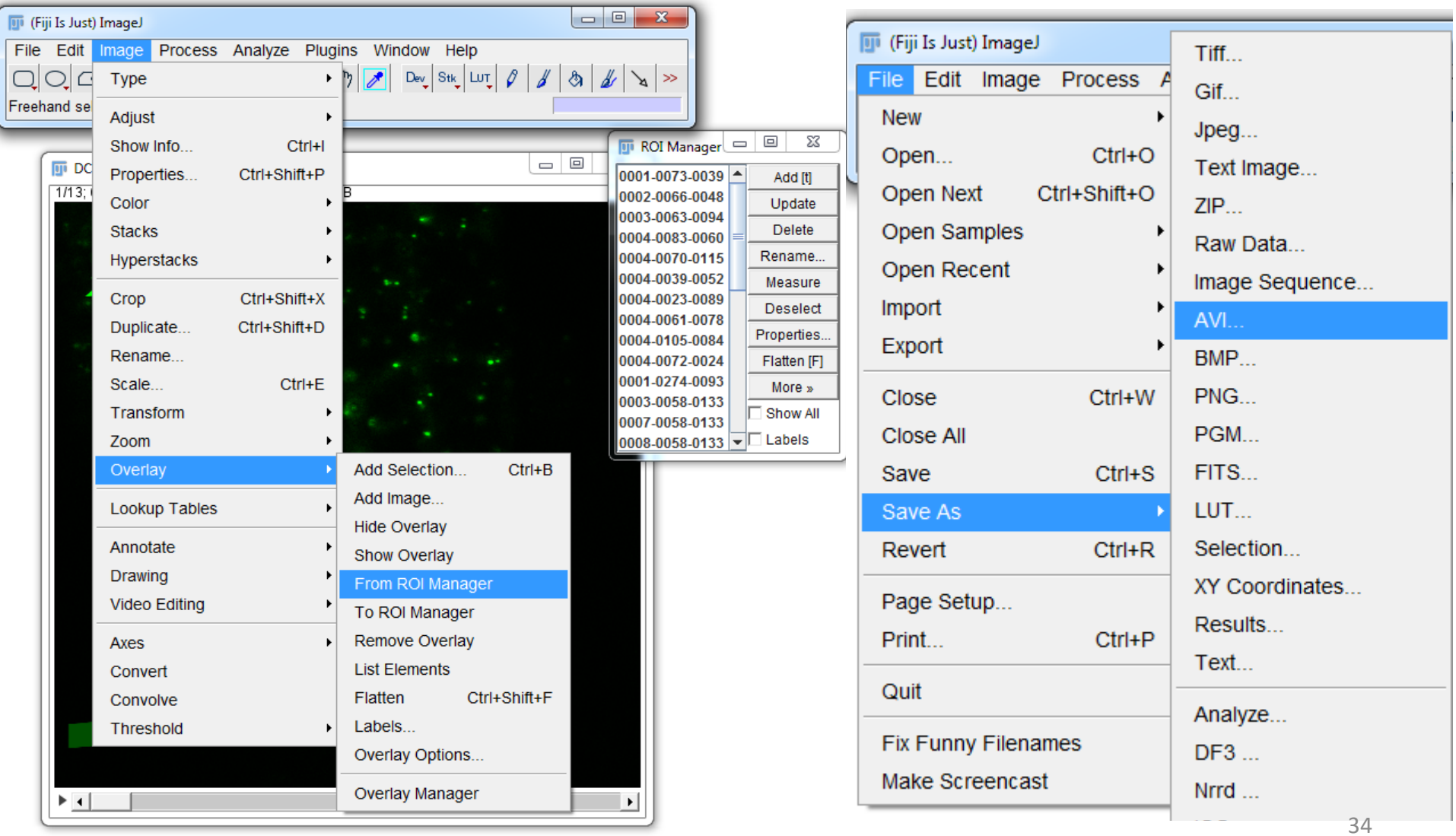


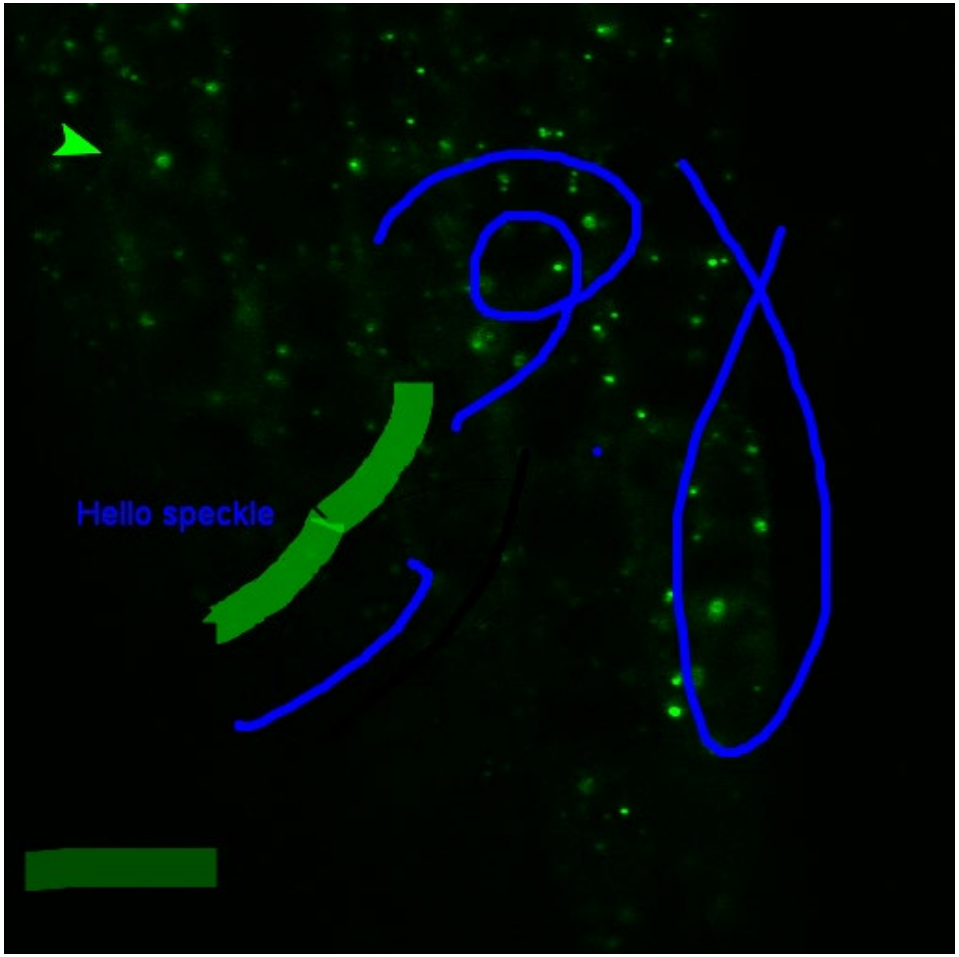


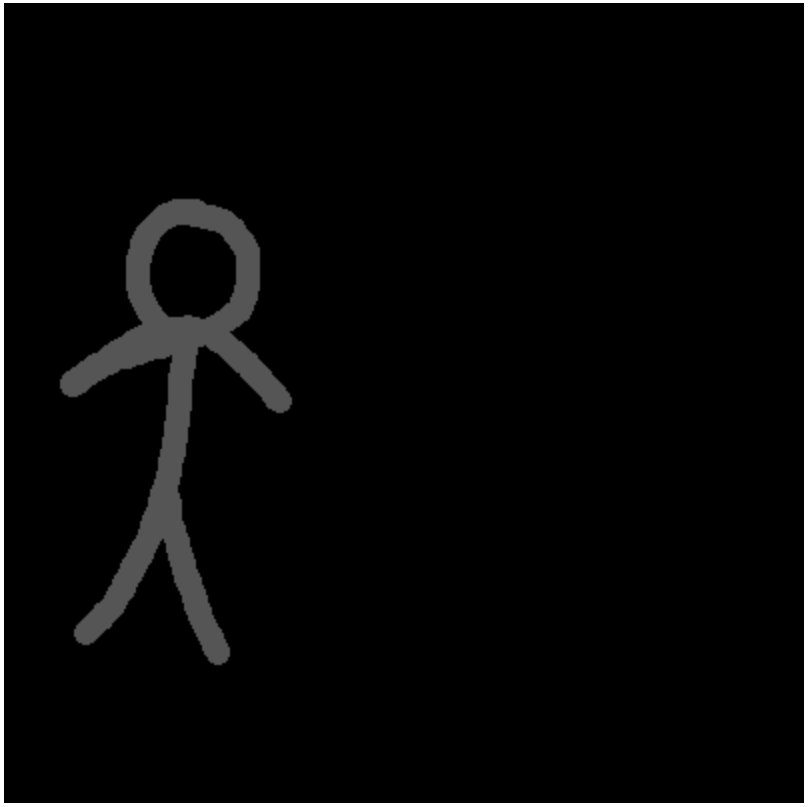
Με ενδιαφέρει αυτό το σημείο

Το σημείο κινείται…




(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]
Update
Delete
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



Συνεντοπίζονται οι δομές μου;

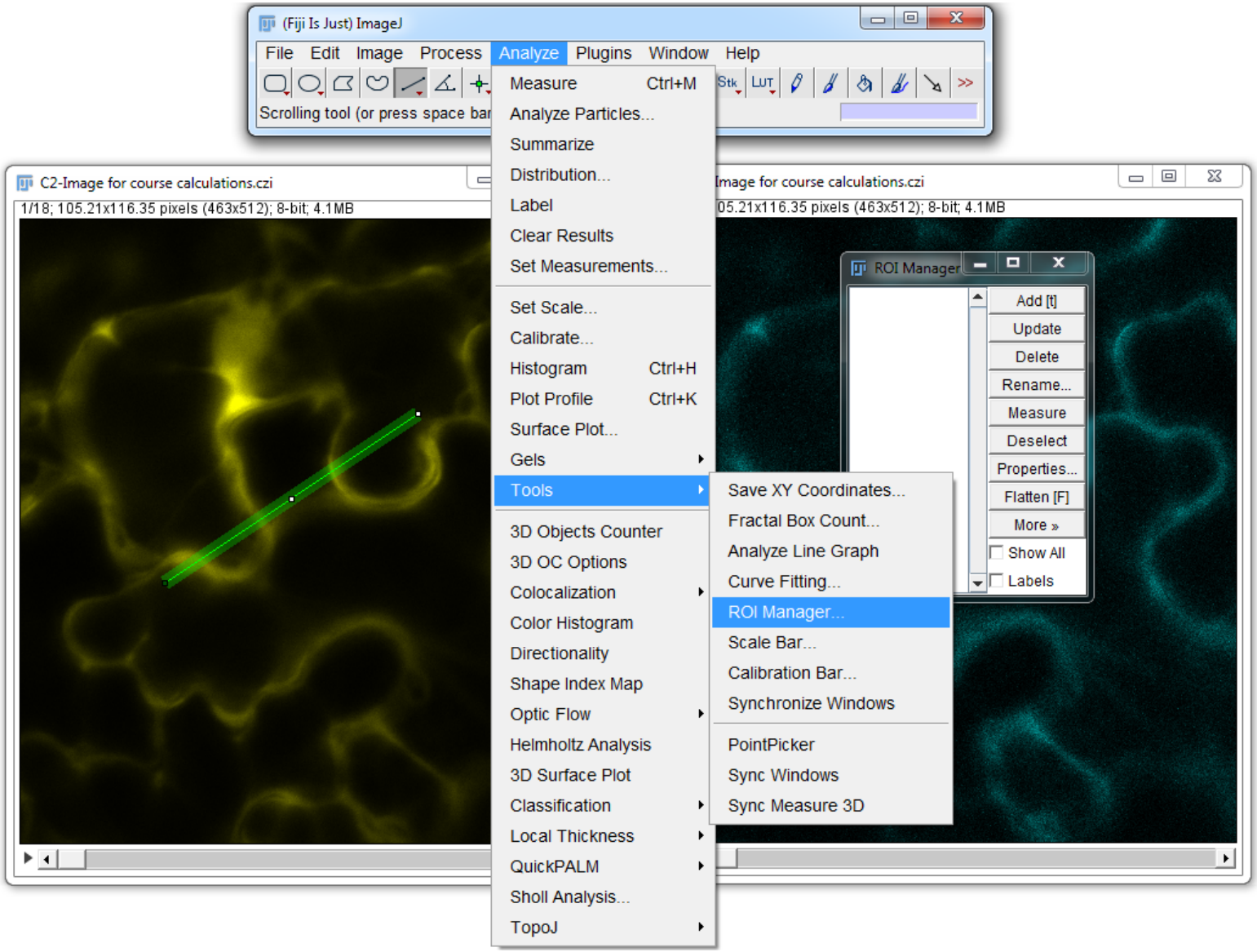
Συσχέτιση μεταξύ χρωμάτων σε δύο κανάλια:

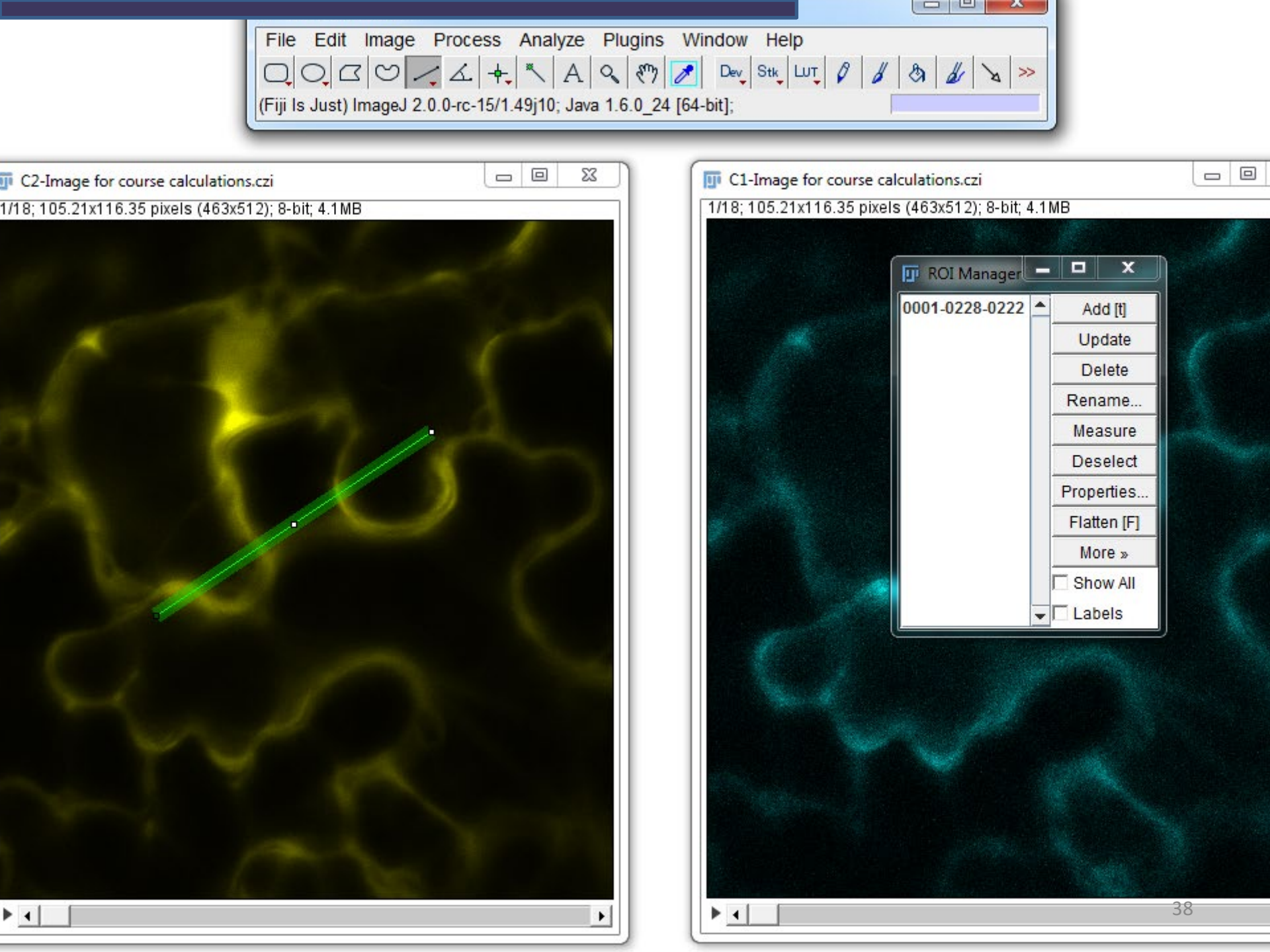
Pearson's correlation coefficient. Απαραίτητη η γραμμική σχέση μεταξύ των χρωμάτων.

Mander's coefficient. Αναλογική με την ένταση σήματος.

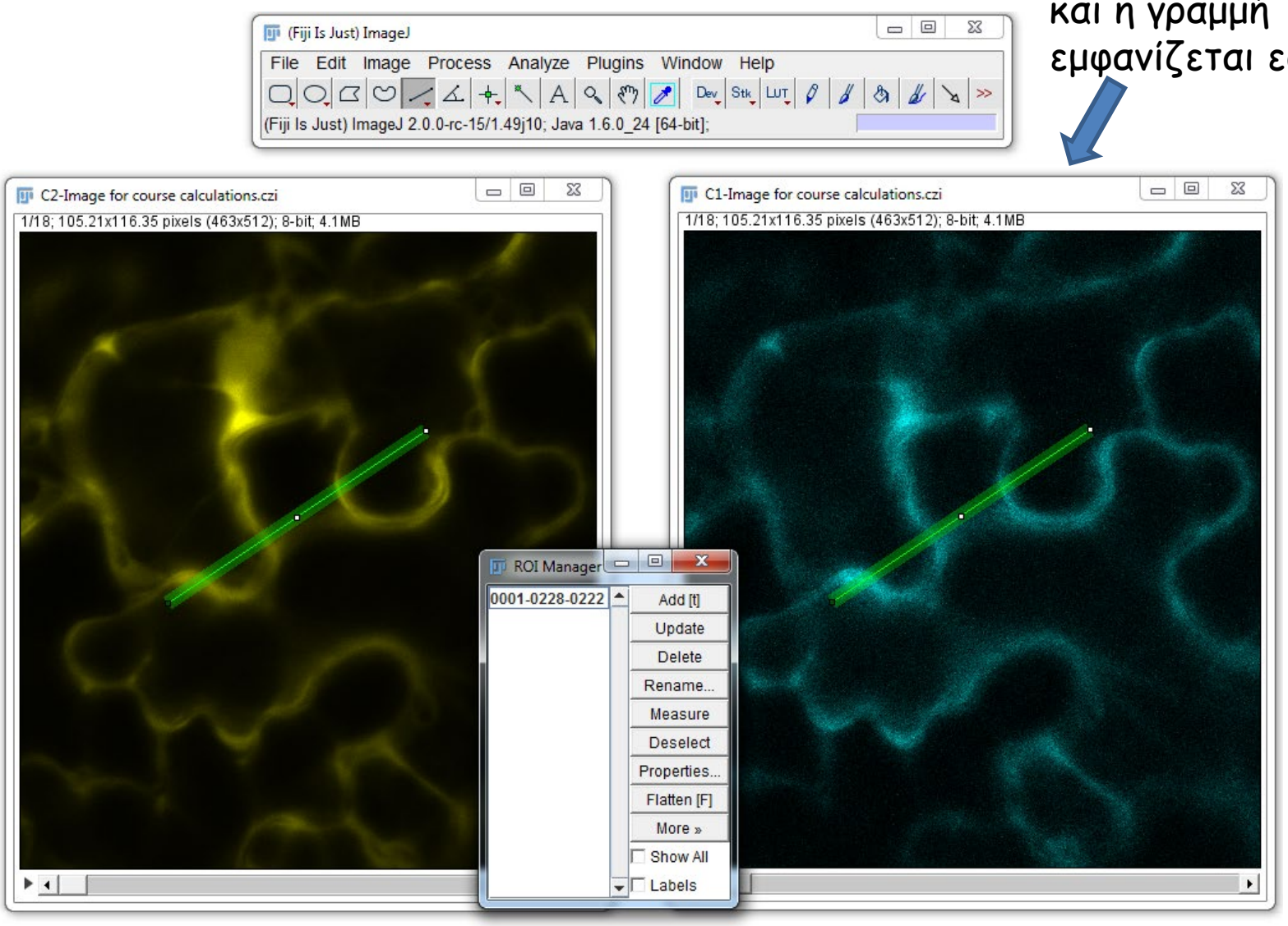
Spearman's rank coefficient. Κατατάσσει σε μία σειρά

Διάγραμμα χωρικών εντάσεων

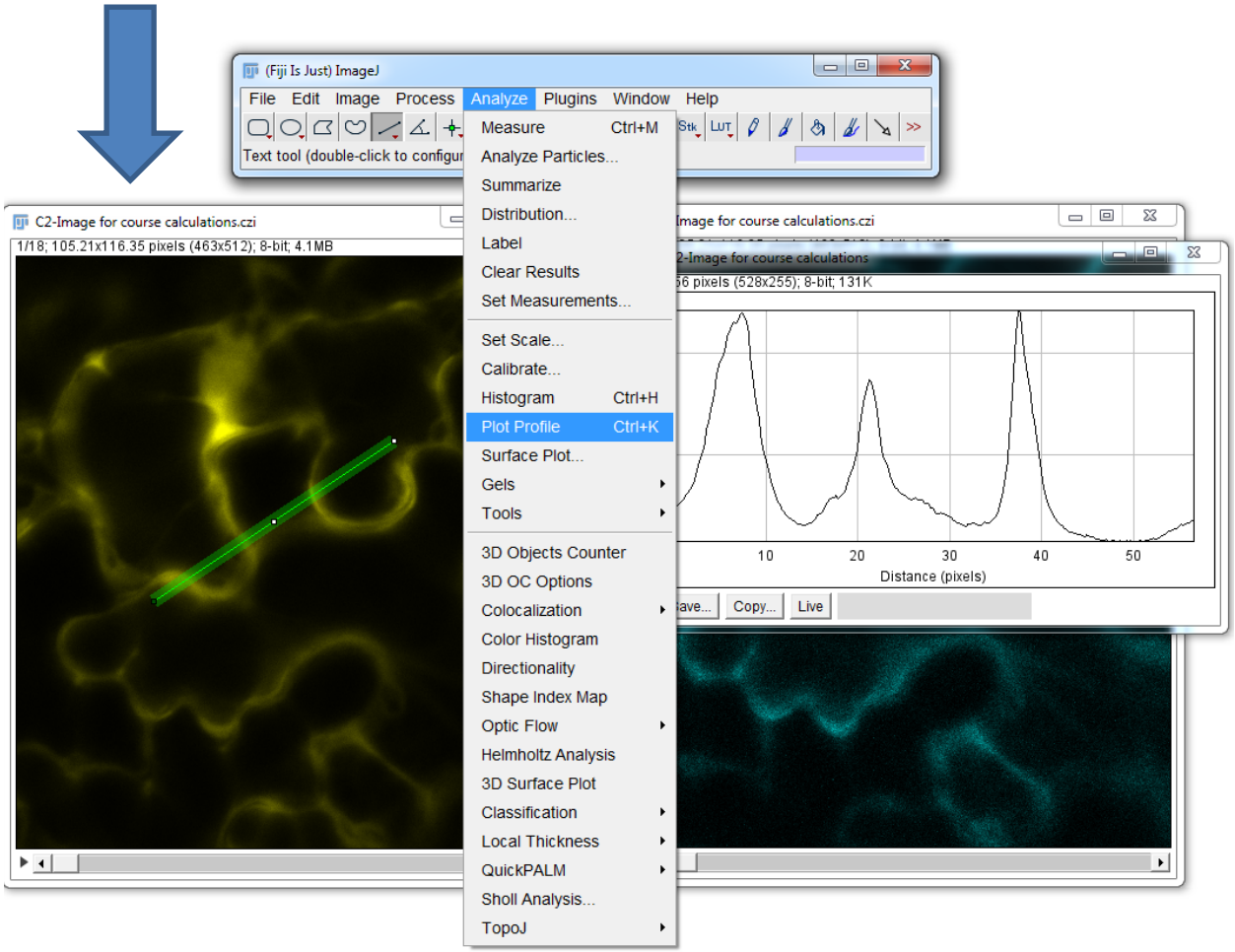


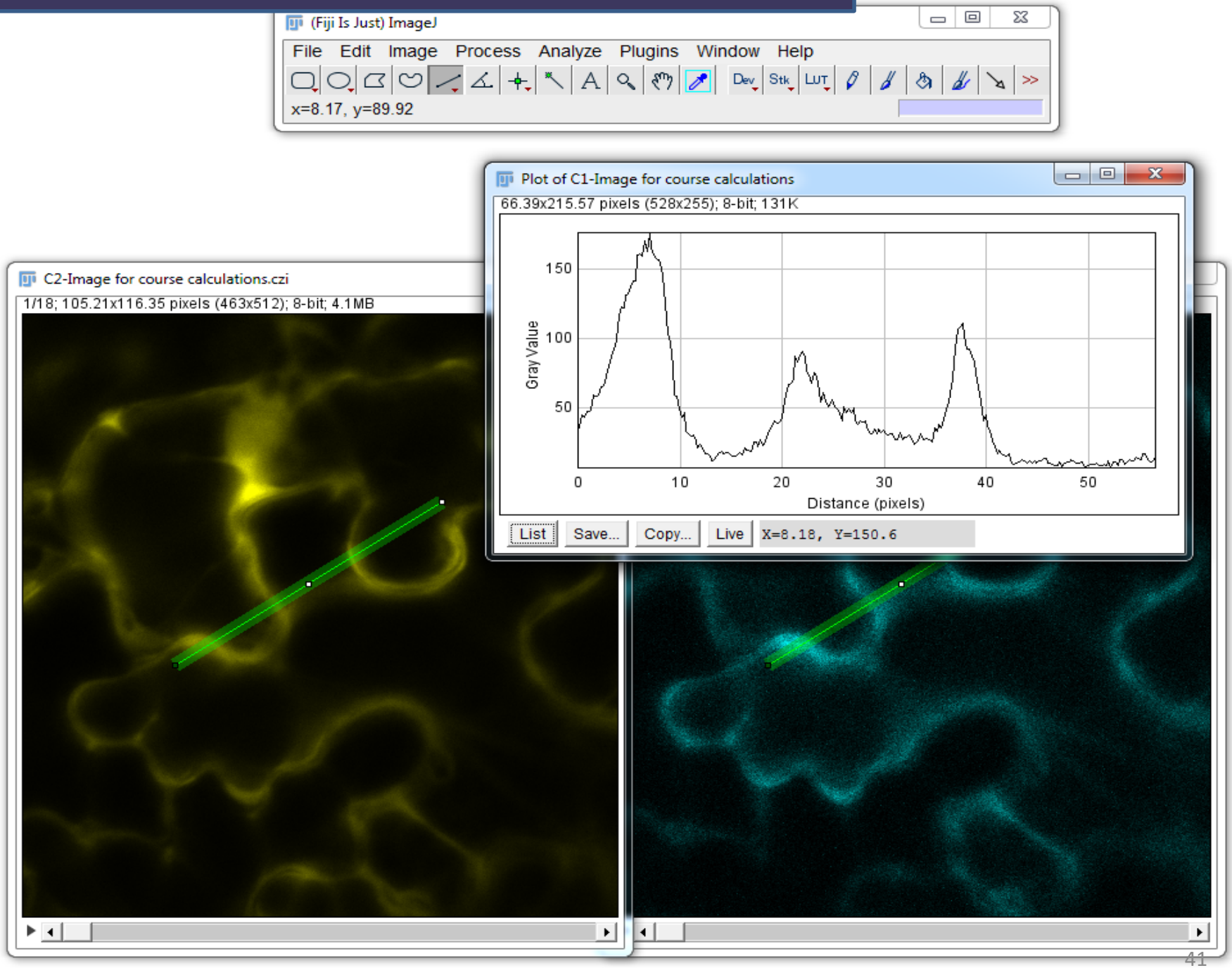

File Edit Image Process Analyze Plugins Window Help
(Fiji Is Just) ImageJ 2.0.0-rc-15/1.49j10; Java 1.6.0_24 [64-bit];
C2-Image for course calculations.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB
C1-Image for course calculations.czi
1/18; 105.21x116.35 pixels (463x512); 8-bit; 4.1MB
ROI Manager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

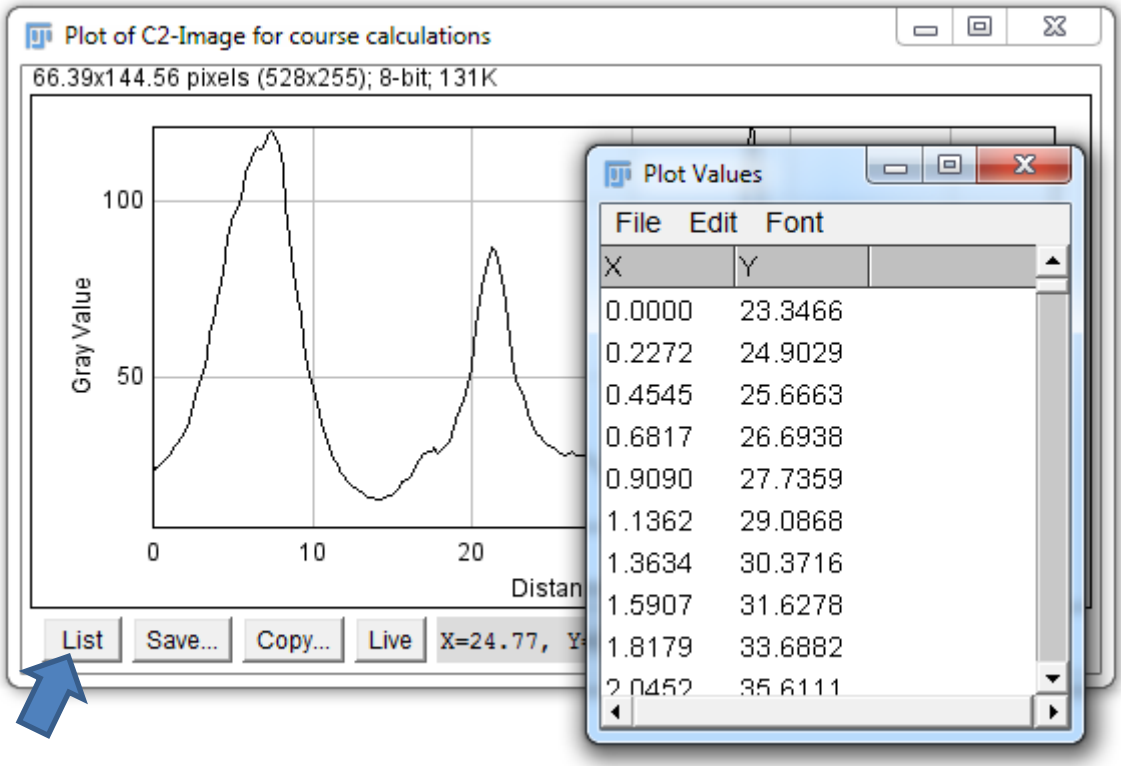
Επιλέγω αυτό το παράθυρο και το ROI από το ROI manager και η γραμμή εμφανίζεται εδώ



Επέλεξε κάθε παράθυρο και 'analyze'->'plot profile'

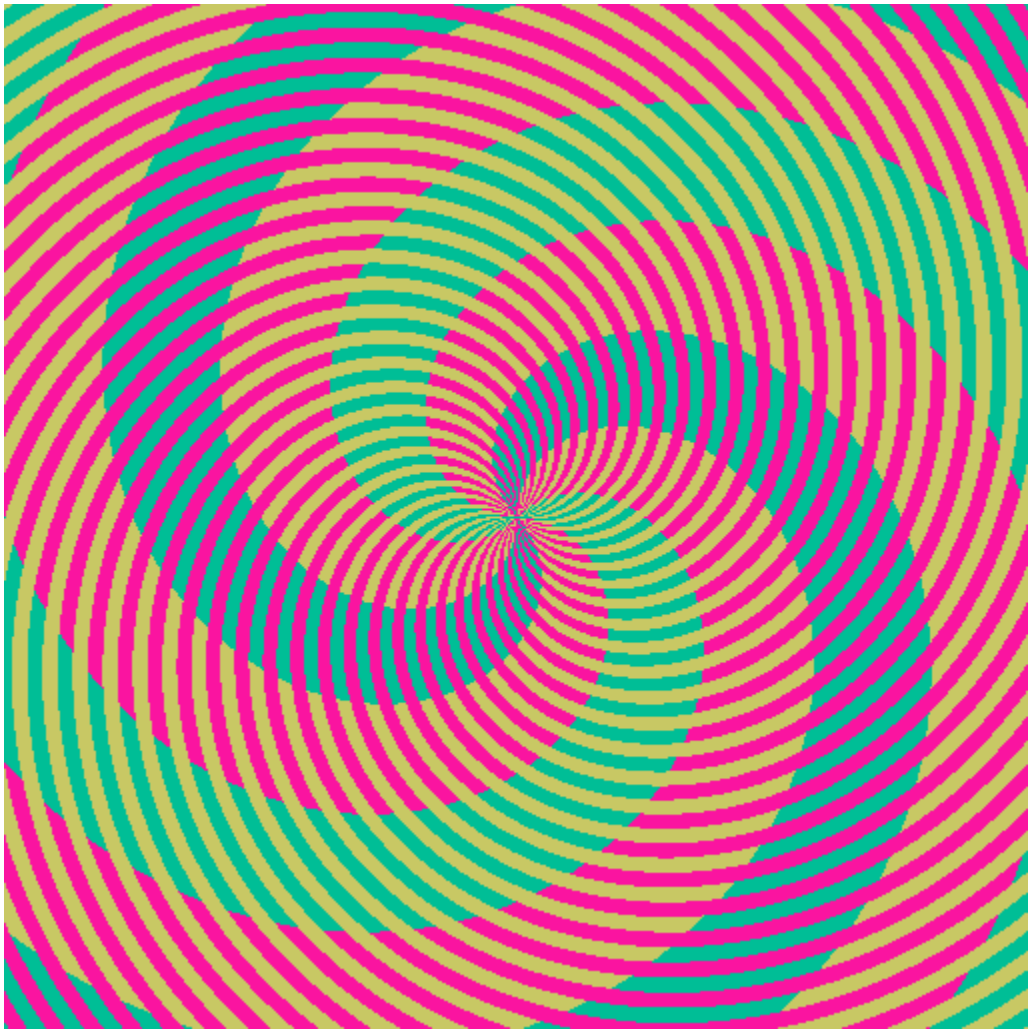


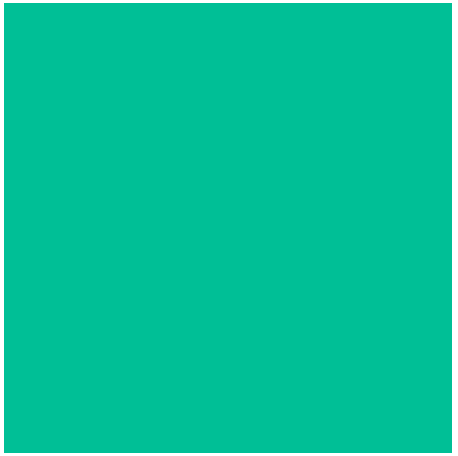

(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
x=8.17, y=89.92
Plot of C1-Image for course calculations
66.39x215.57 pixels (528x255); 8-bit; 131K
Gray Value
150
100
50
0
10
20
30
40
50
Distance (pixels)
List
Save...
Copy...
Live
X=8.18, Y=150.6
C2-Image for course calculations.czi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

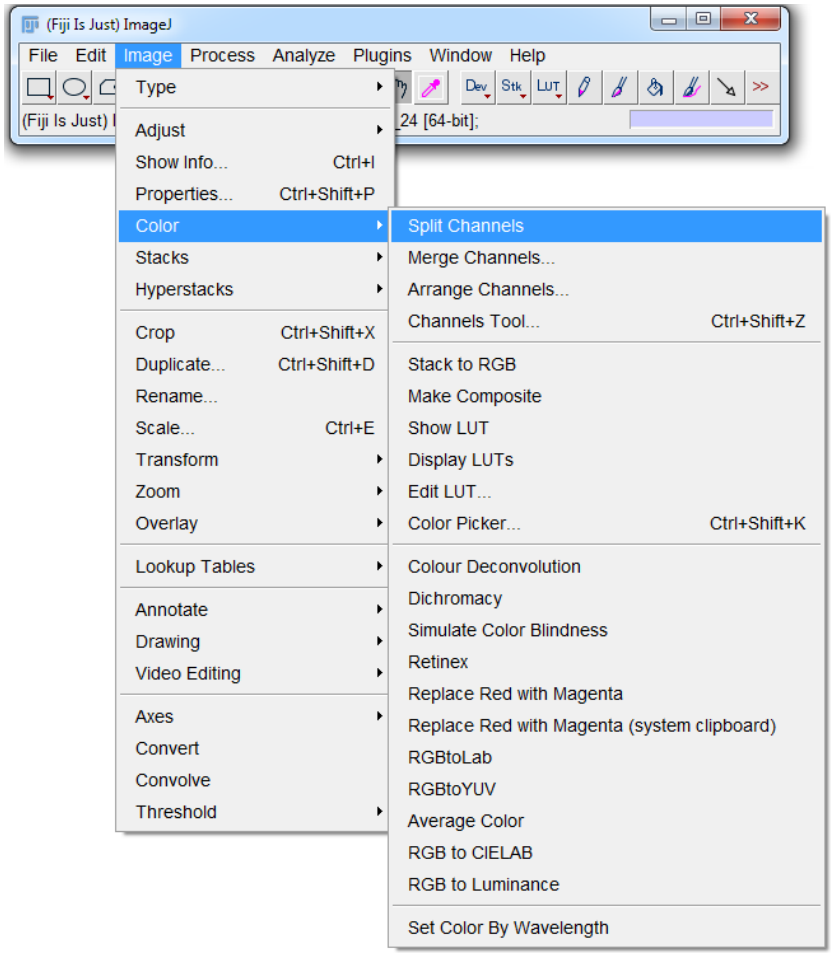


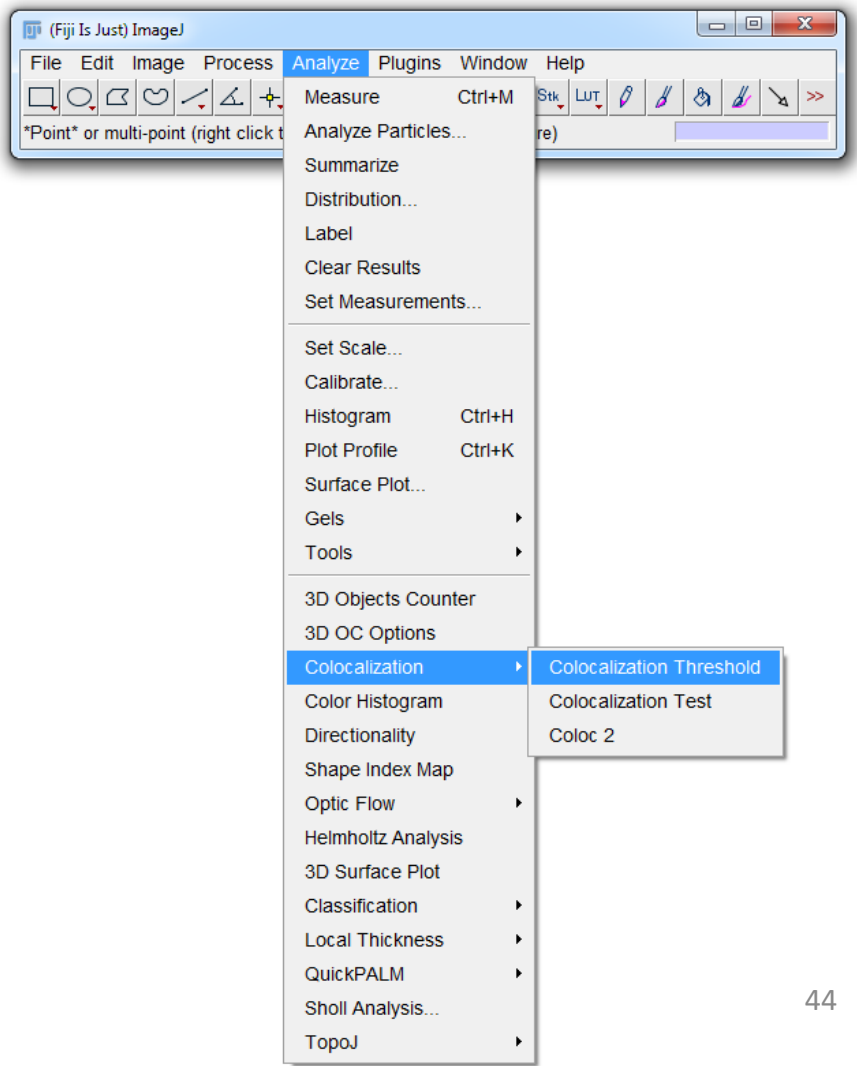
Επέλεξε το list button, πάρε τις τιμές και μετέφερέ τις στο Excel

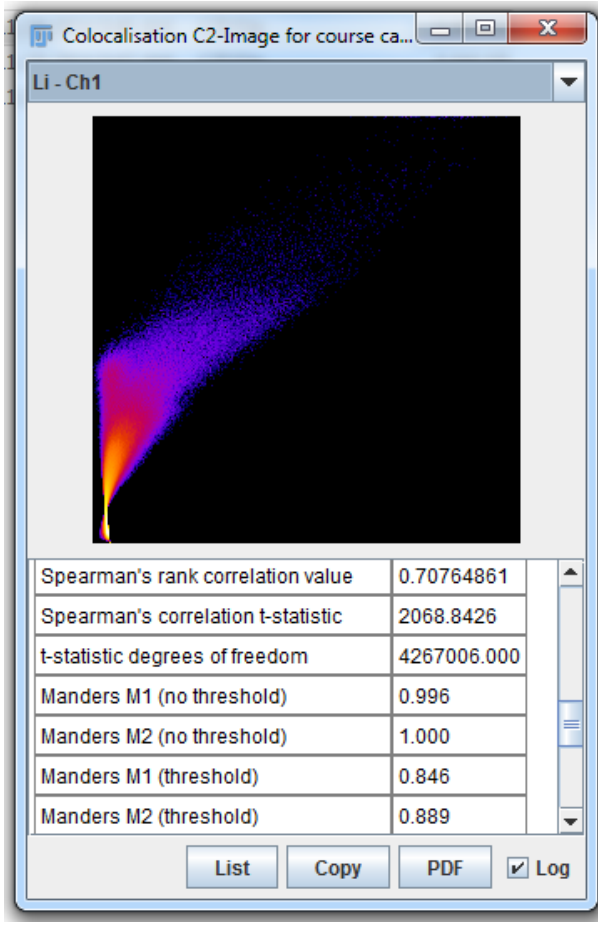
Ok! Πάμε για μετρήσεις!

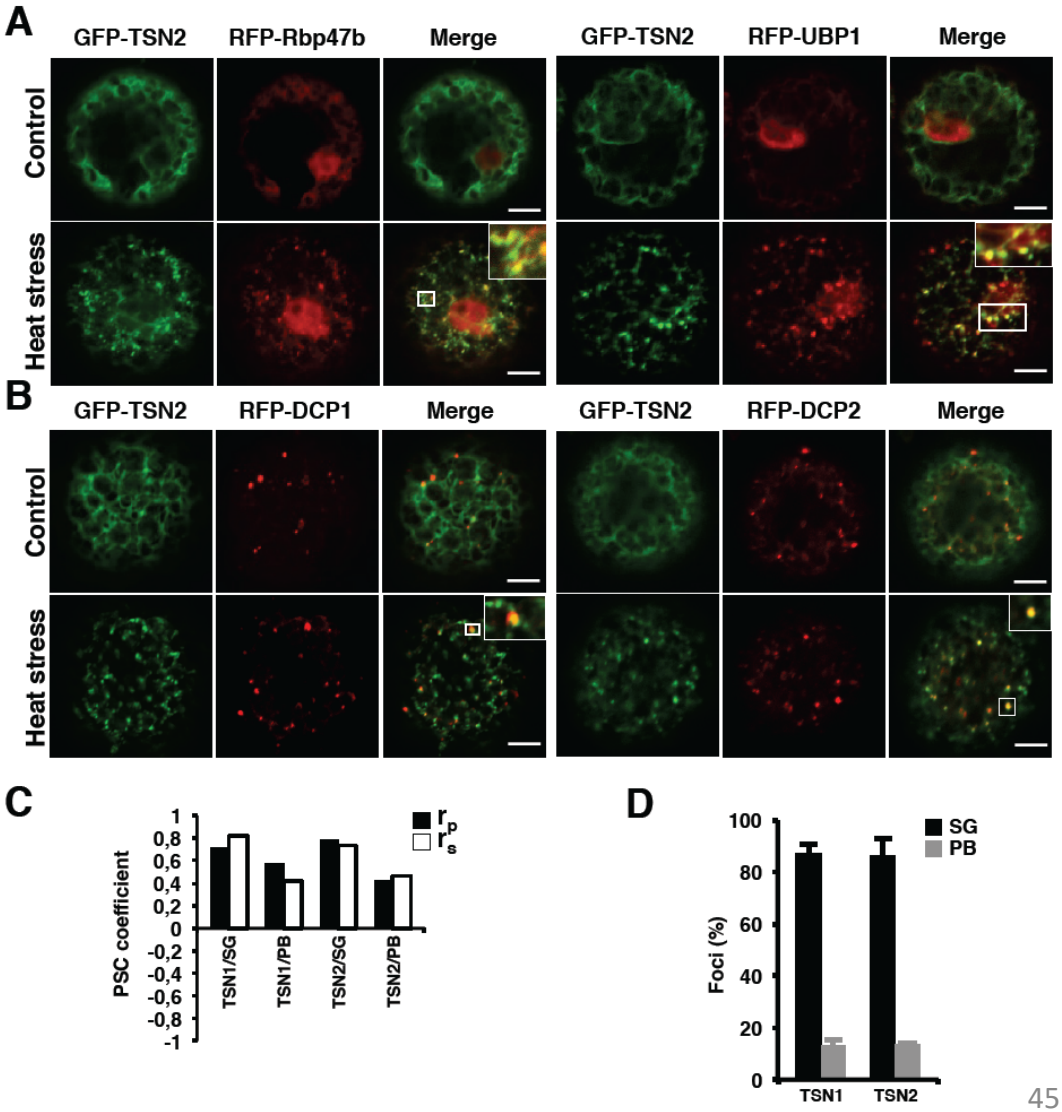




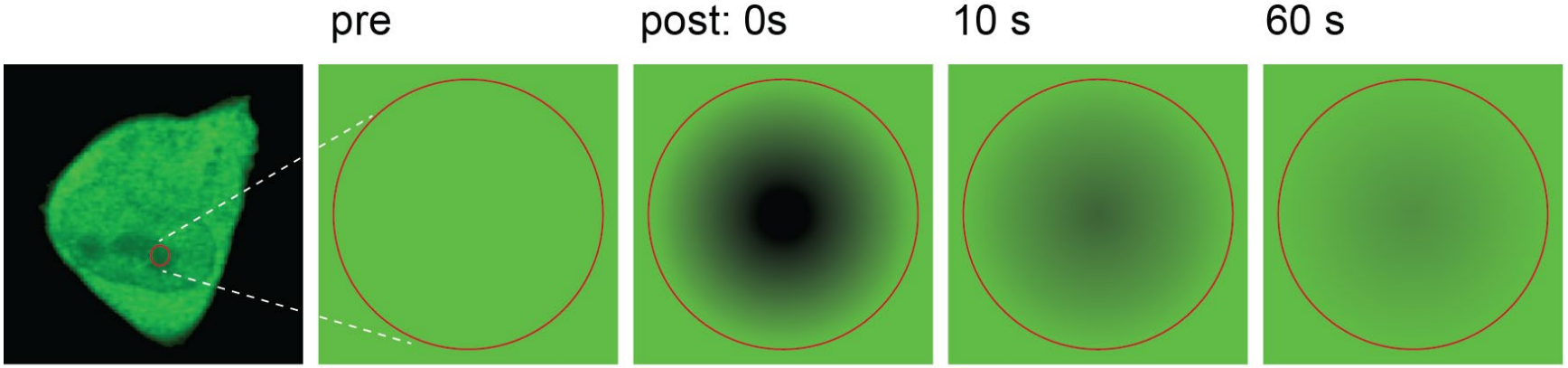

(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Type
Adjust
Show Info... Ctrl+I
Properties... Ctrl+Shift+P
Color
Stacks
Hyperstacks
Crop Ctrl+Shift+X
Duplicate... Ctrl+Shift+D
Rename...
Scale... Ctrl+E
Transform
Zoom
Overlay
Lookup Tables
Annotate
Drawing
Video Editing
Axes
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

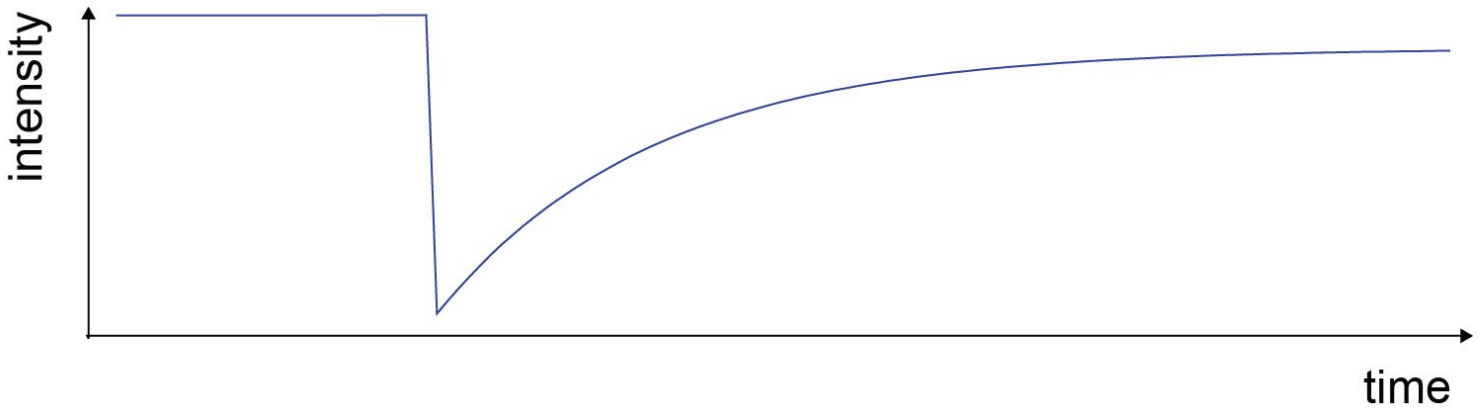

(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Measure Ctrl+M
Analyze Particles...
Summarize
Distribution...
Label
Clear Results
Set Measurements...
Set Scale...
Calibrate...
Histogram Ctrl+H
Plot Profile Ctrl+K
Surface Plot...
Gels
Tools
3D Objects Counter
3D OC Options
Colocalization
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

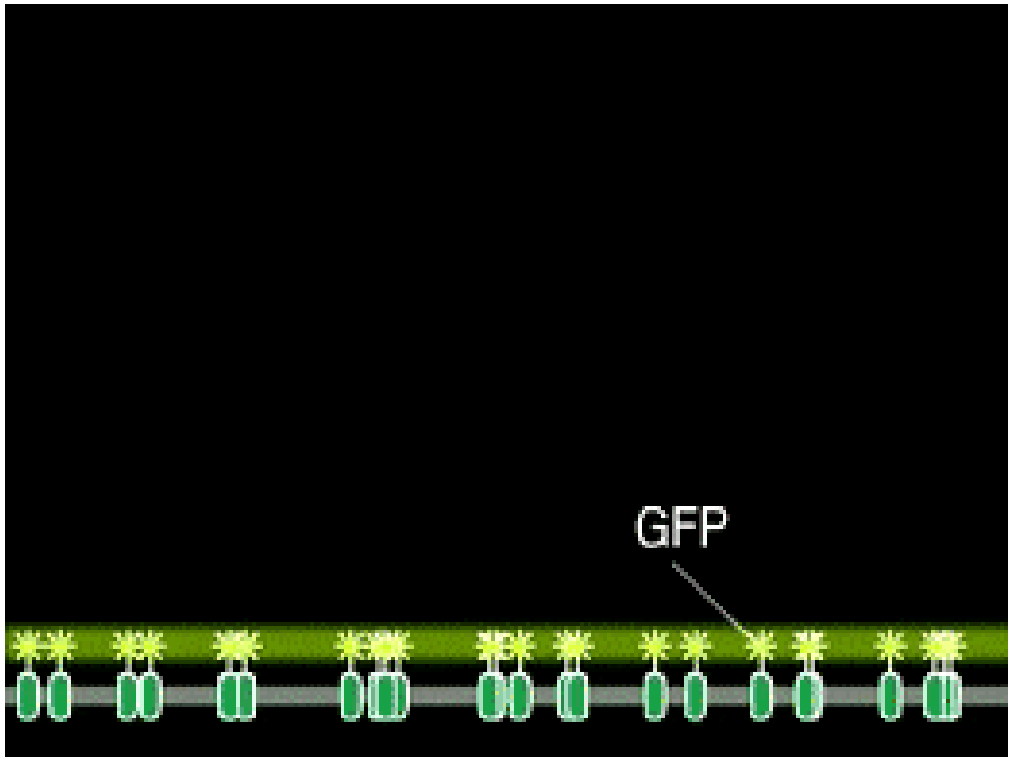

Colocalisation C2-Image for course ca...
Li - Ch1
Spearman's rank correlation value 0.70764861
Spearman's correlation t-statistic 2068.8426
t-statistic degrees of freedom 4267006.000
Manders M1 (no threshold) 0.996
Manders M2 (no threshold) 1.000
Manders M1 (threshold) 0.846
Manders M2 (threshold) 0.889
List
Copy
PDF
Log

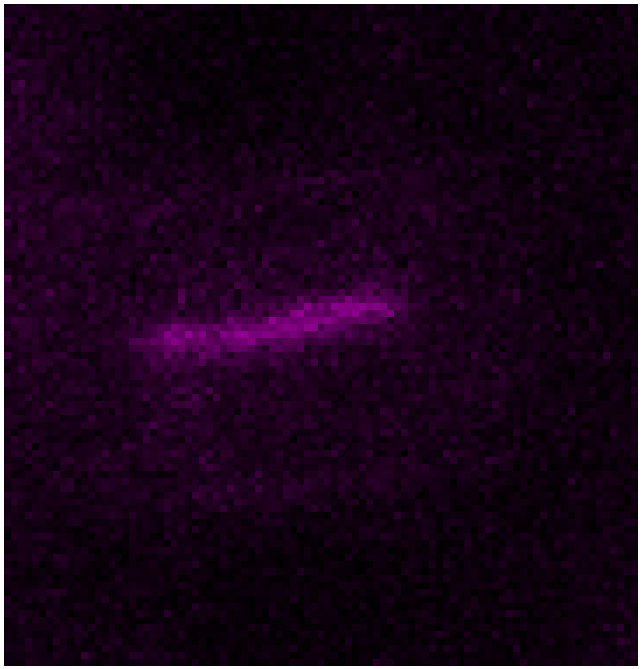

A
GFP-TSN2
RFP-Rbp47b
Merge
GFP-TSN2
RFP-UBP1
Merge
Control
Heat stress
B
GFP-TSN2
RFP-DCP1
Merge
GFP-TSN2
RFP-DCP2
Merge
Control
Heat stress
C
PSC coefficient
1
0,8
0,6
0,4
0,2
0
-0,2
-0,4
-0,6
-0,8
-1
TSN1/SG
TSN1/PB
TSN2/SG
TSN2/PB
r_p
r_s
D
Foci (%)
100
80
60
40
20
0
TSN1
TSN2
SG
PB

Fluorescence recovery after photo-bleaching (FRAP)



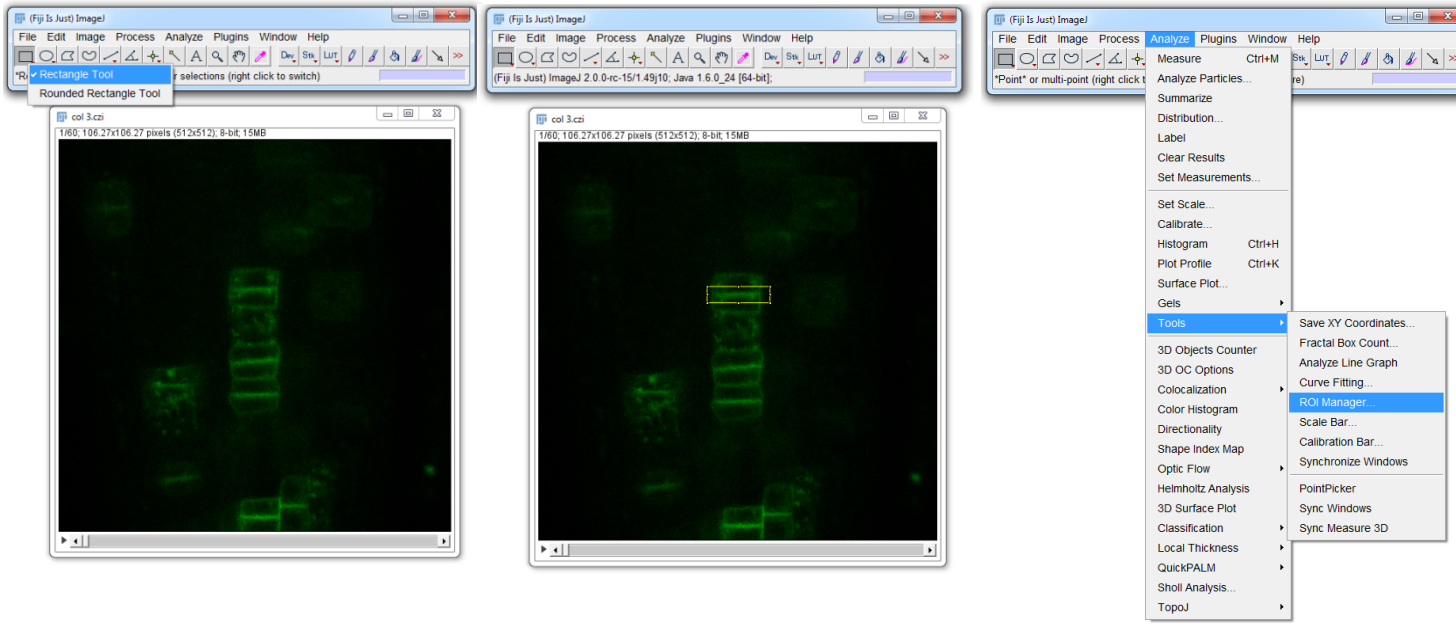



GFP

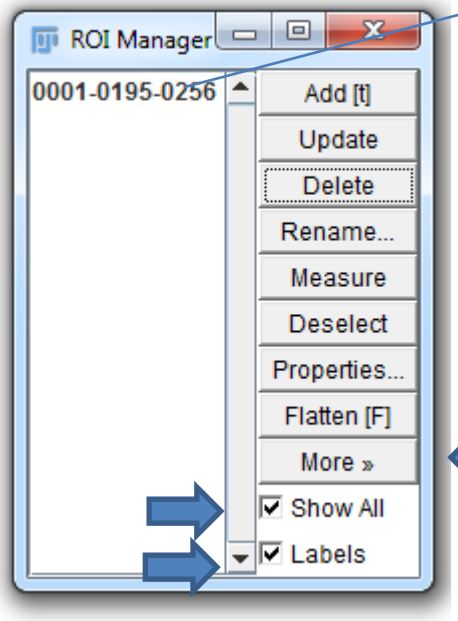


Ανάλυση δεδομένων FRAP

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

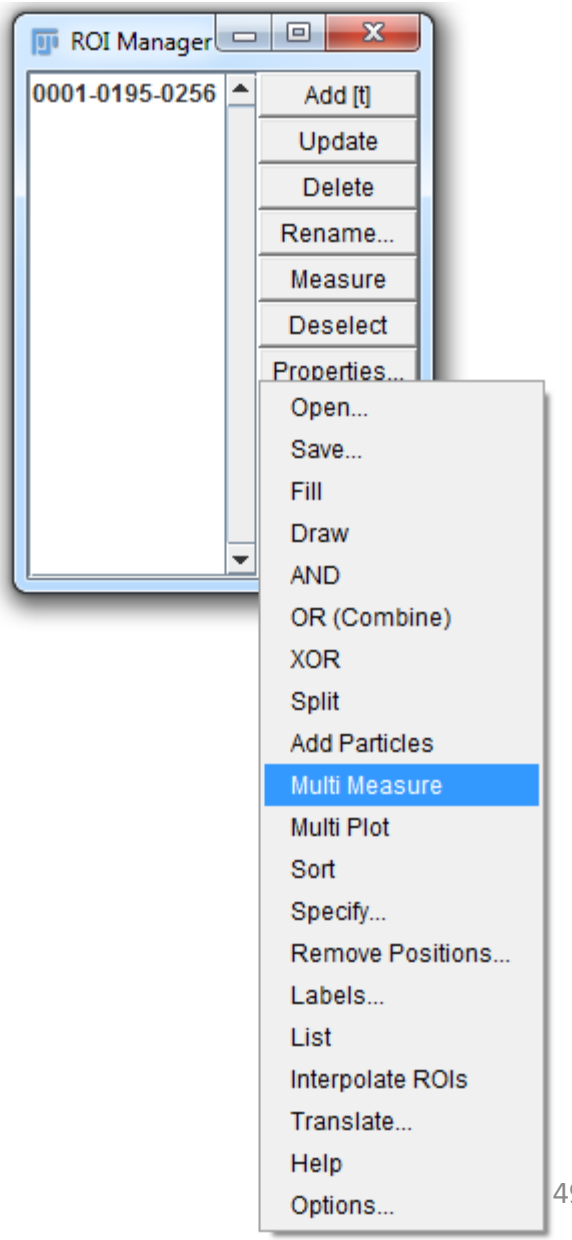


2. 'show all' και 'labels'



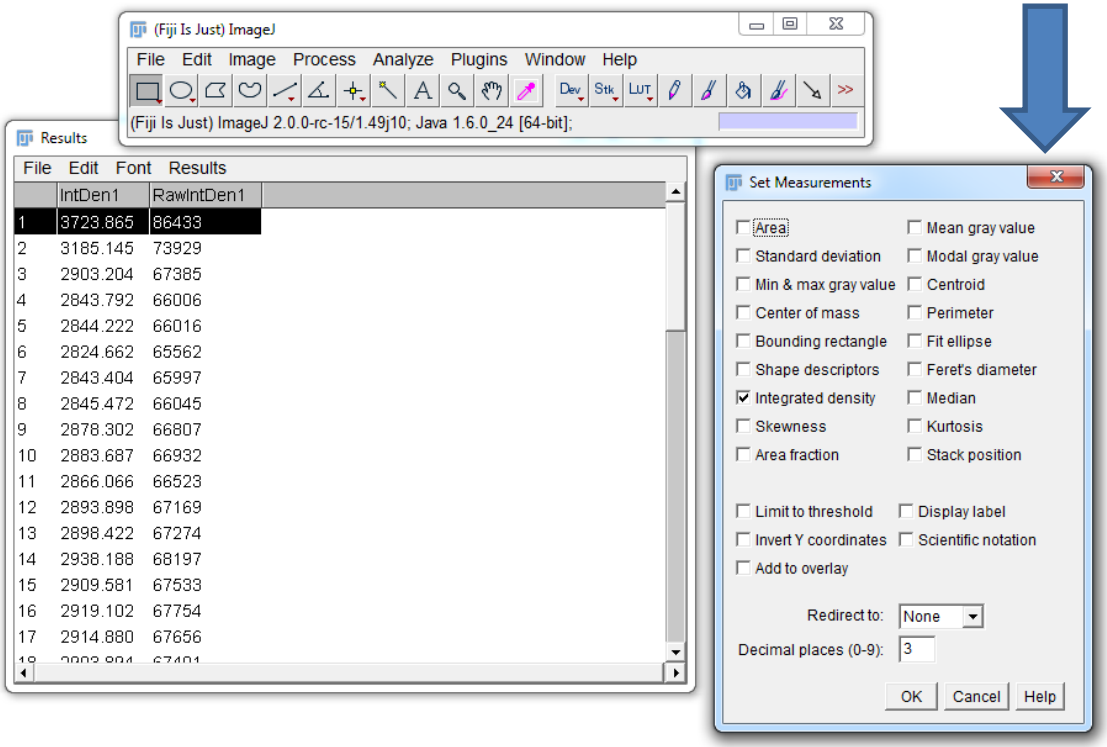
Αυτό είναι το ROI

3. επέλεξε 'more' και 'Multi measure'



- # 4. καταλήγεις με μετρήσεις εντάσεων

'integrated density' πρέπει να επιλεχθεί (Results-> Set measurements…)



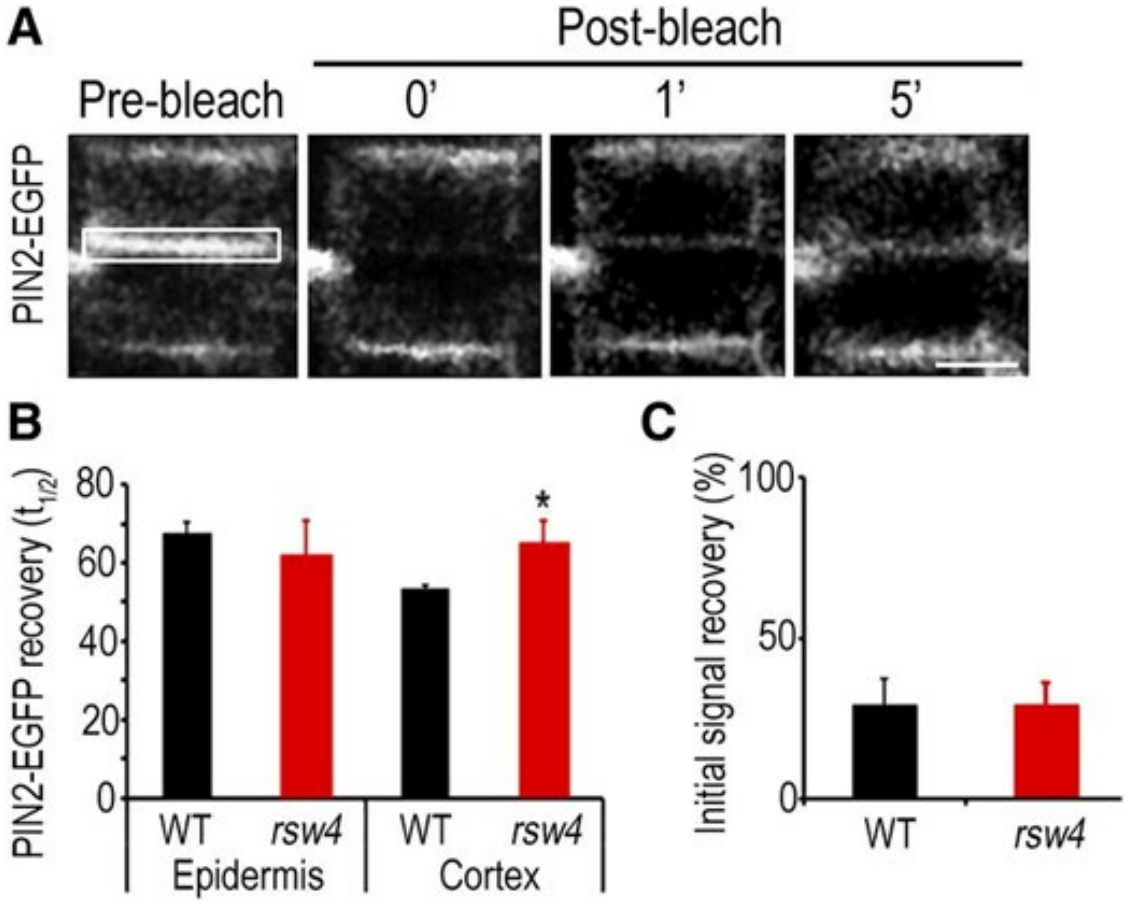
- 5. στο Excel

Χρόνος

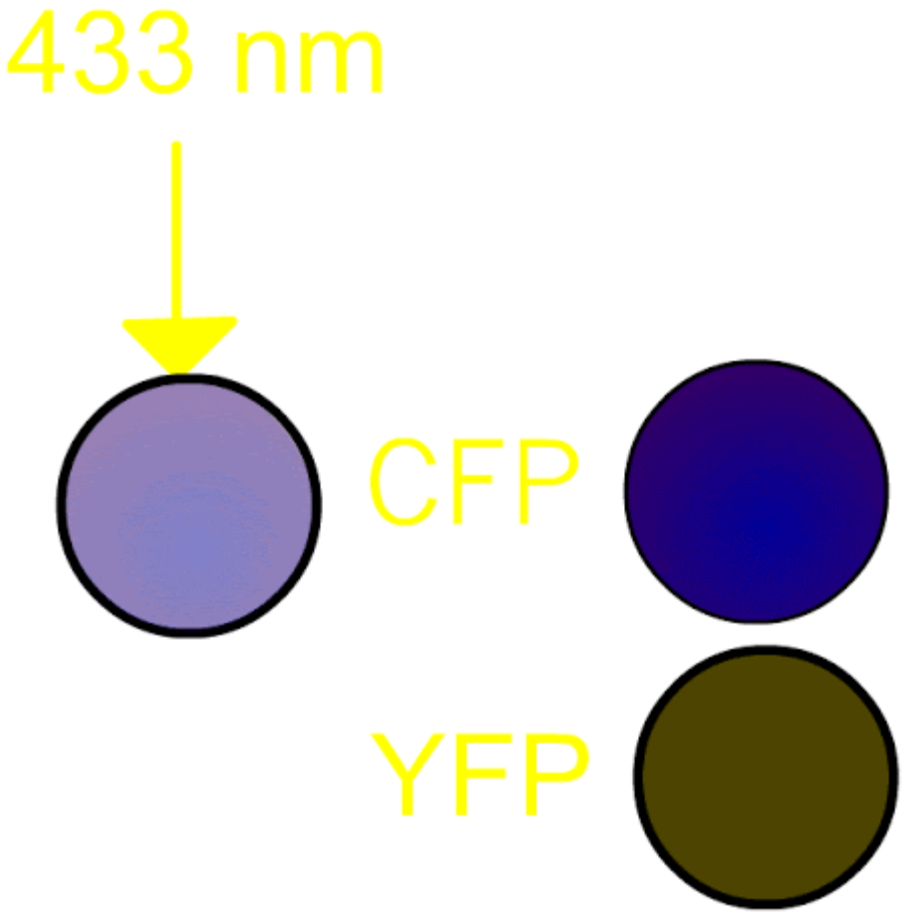
B	C	D	E	F	G	H
WT						
GFP-Ubp						
ROOT1	Value	IntDen	background		Time	
	1	194,068,542,827	46,903,794,809	147,164,748,018		
	2	64,253,054,902	35,820,844,291	28,432,210,611	0	28
	3	75,289,241,916	40,871,302,754	34,417,939,162	3	34
	4	81,892,248,723	42,638,963,217	39,253,285,506	6	39
	5	82,836,871,509	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

Κανονικοποίηση με το θόρυβο (my intensity-background)

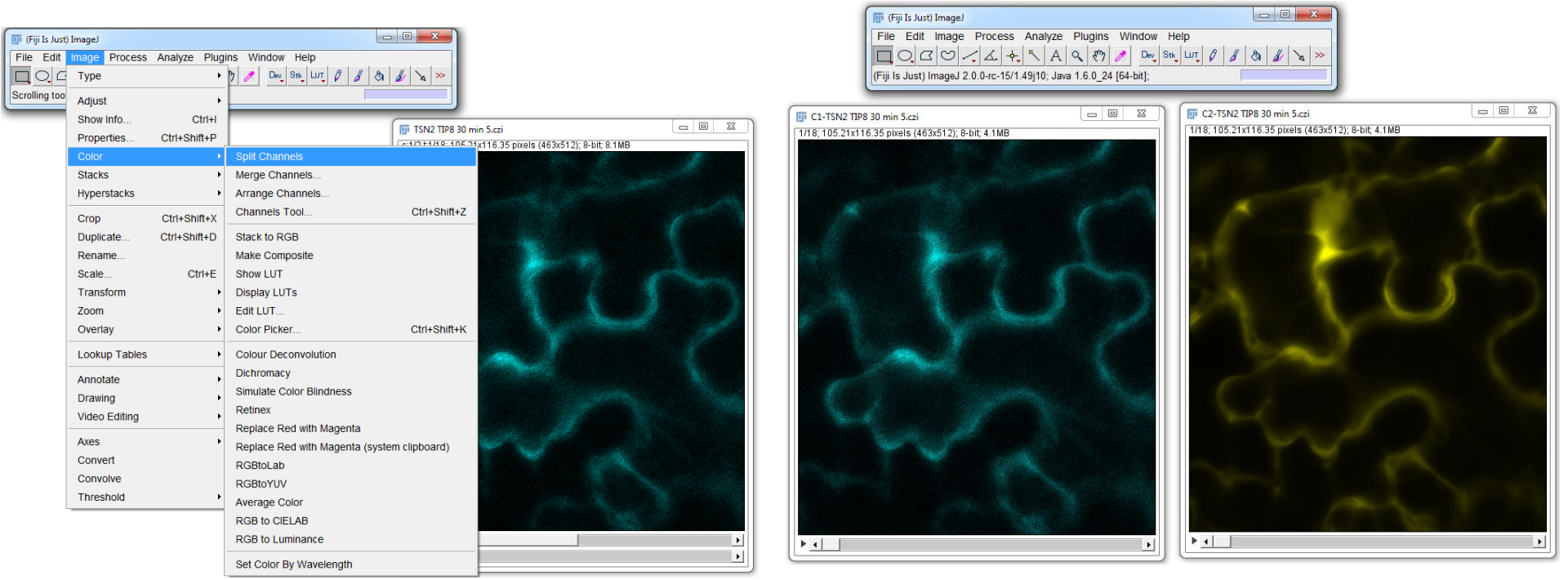
Παρουσίαση FRAP

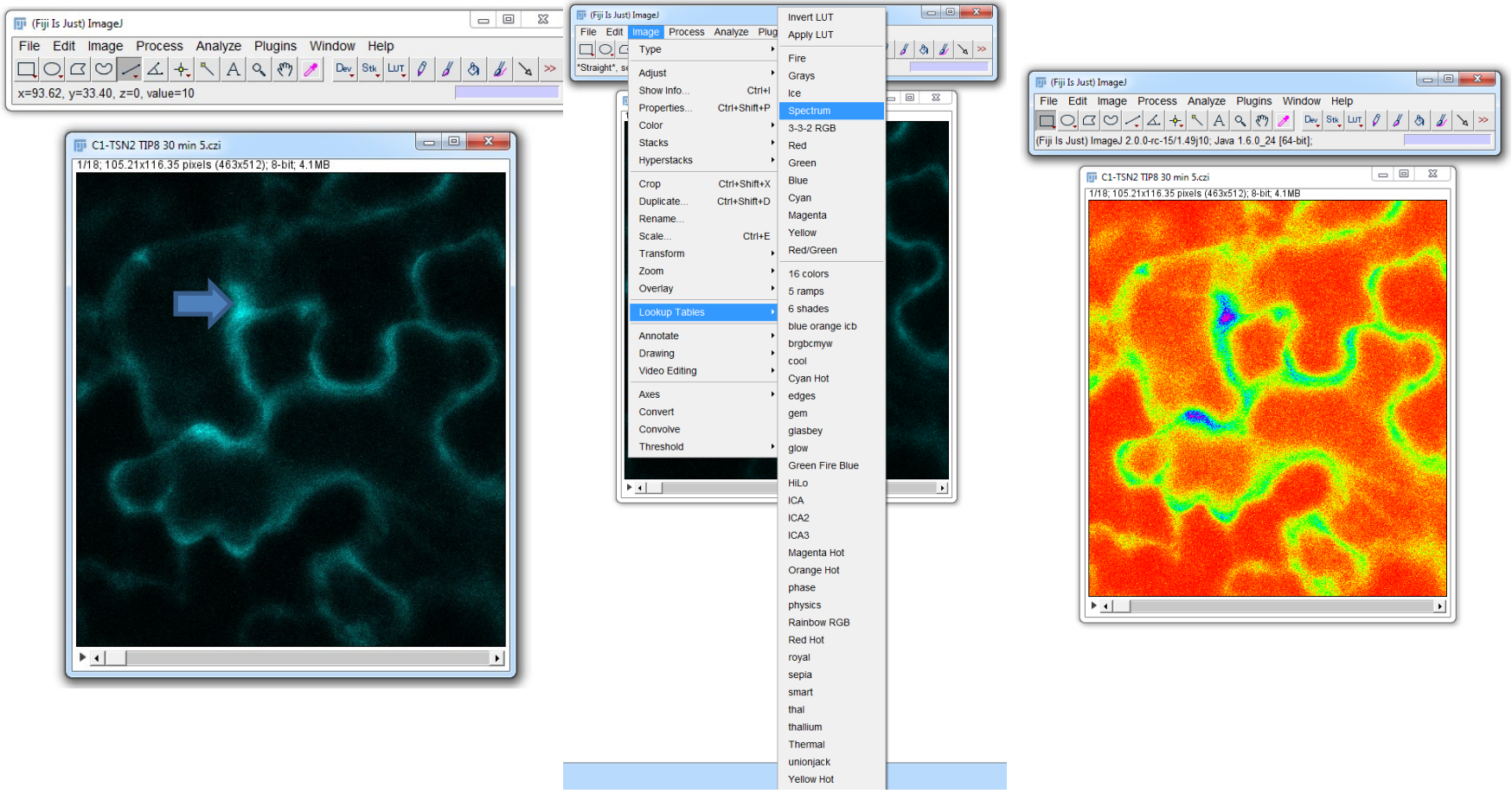


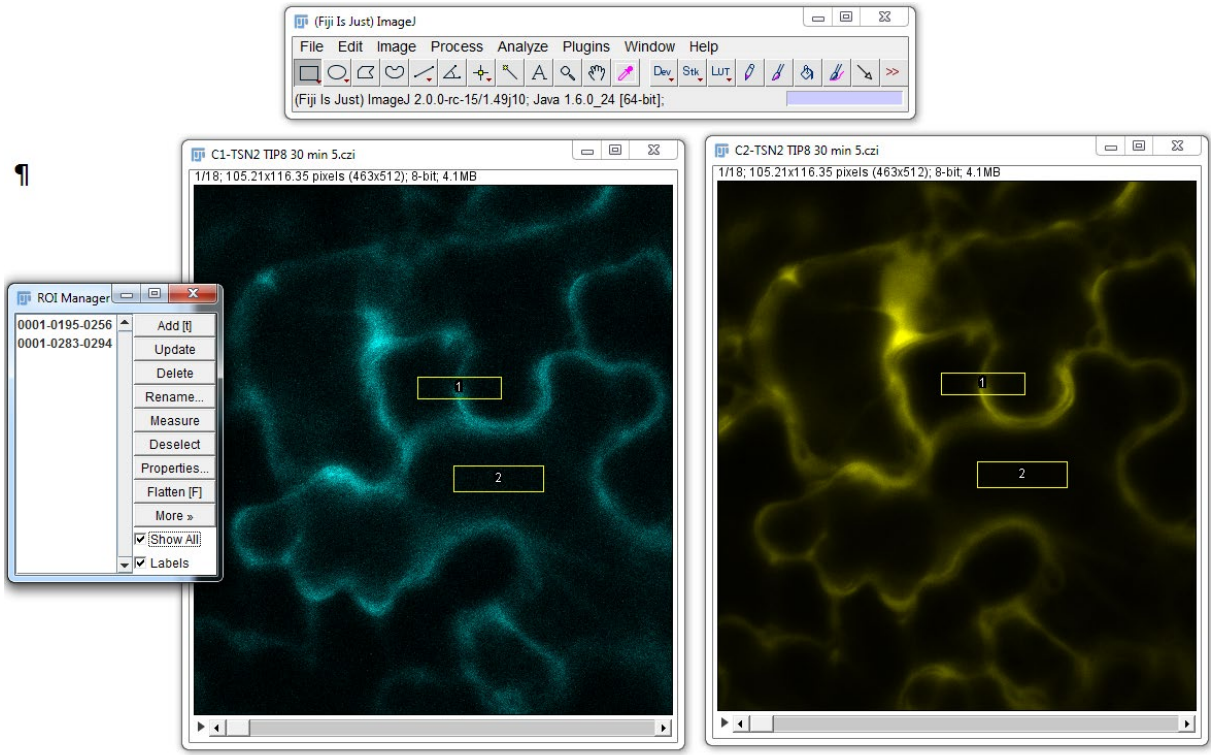
Försters resonance energy transfer (FRET)



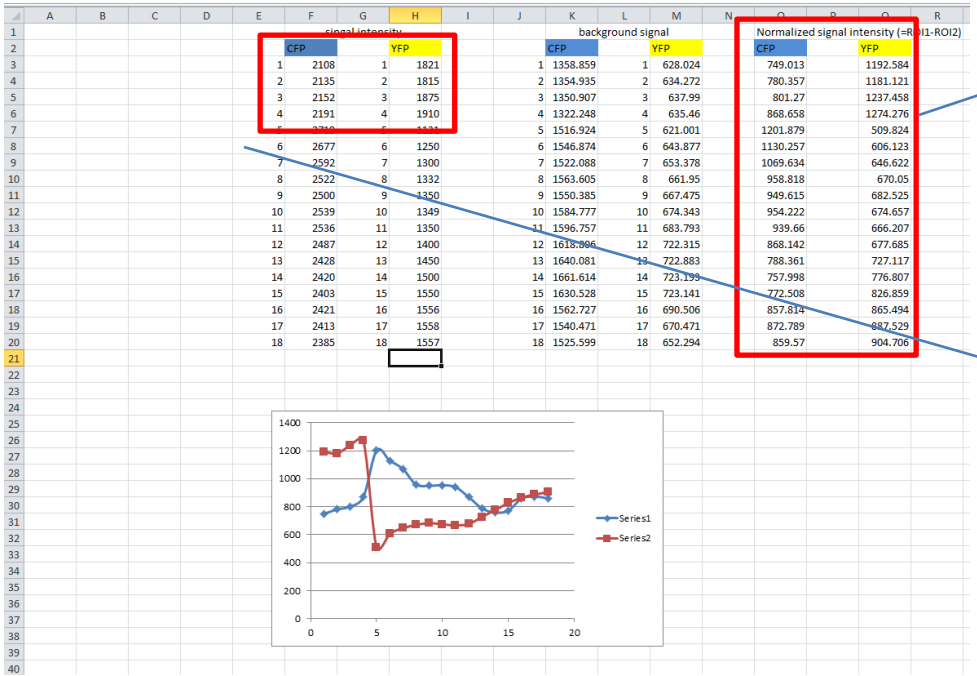
Acceptor photo-bleaching




(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];


(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]
Update
Delete
Rename...
Measure
Deselect
Properties...
Flatten [F]
More »
Show All
Labels

Όχι σημαντικές διαφορές στην ένταση



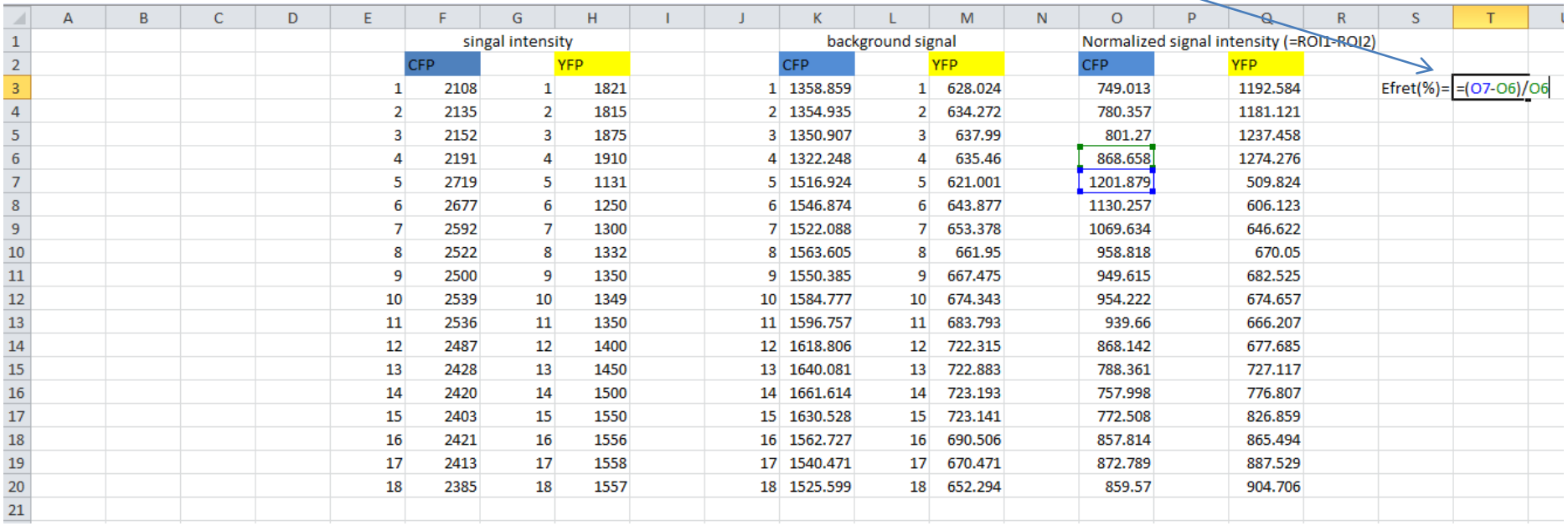
	signal intensity				background signal				Normalized signal intensity (=ROI1-ROI2)	
	CFP		YFP		CFP		YFP		CFP	YFP
1	2108	1	1821	1	1358.859	1	628.024		749.013	1192.584
2	2135	2	1815	2	1354.935	2	634.272		780.357	1181.121
3	2152	3	1875	3	1350.907	3	637.99		801.27	1237.458
4	2191	4	1910	4	1322.248	4	635.46		868.658	1274.276
5	[illegible]	5	[illegible]	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	1618.806	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	723.199		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

Γράφημα

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

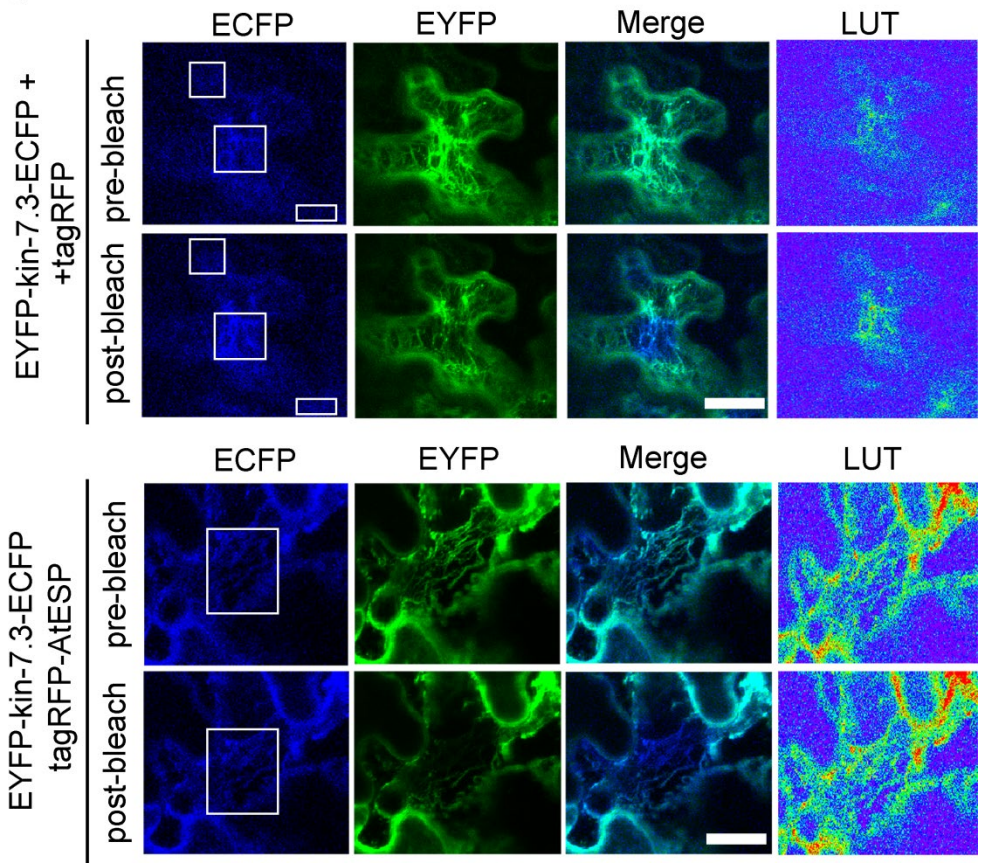
Υπολογισμός απόδοσης κατά FRET

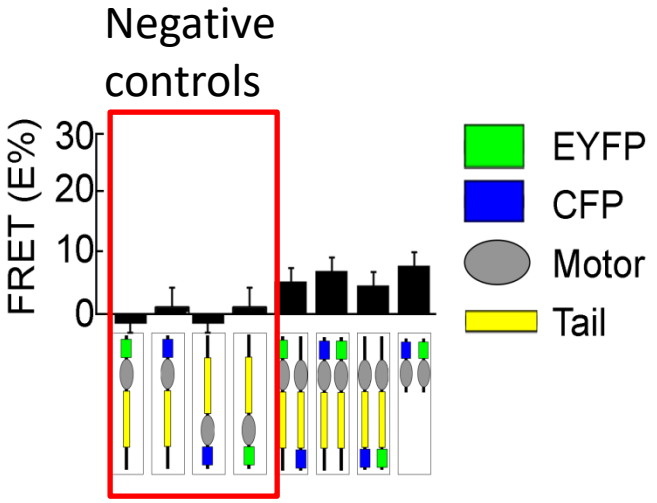
Efret(%)=(Τελική ένταση-Αρχική ένταση)/(Τελική ένταση)

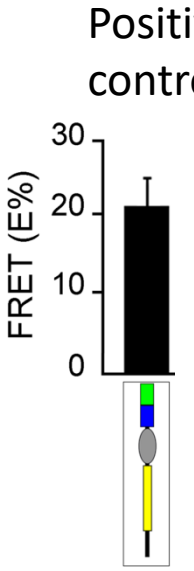


	singal intensity				background signal				Normalized signal intensity (=ROI1-ROI2)			
	CFP		YFP		CFP		YFP		CFP		YFP	
1	2108	1	1821	1	1358.859	1	628.024		749.013		1192.584	Efret(%)= =(O7-O6)/O6
2	2135	2	1815	2	1354.935	2	634.272		780.357		1181.121	
3	2152	3	1875	3	1350.907	3	637.99		801.27		1237.458	
4	2191	4	1910	4	1322.248	4	635.46		868.658		1274.276	
5	2719	5	1131	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	1618.806	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	

Πώς παρουσιάζω δεδομένα FRET







Επιπλέον διάβασμα

- 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/
- Θεωρία FRAP (Balinski paper) http://jcs.biologists.org/content/114/21/3885.full.pdf+html