



# Introduction to image processing and analysis with ImageJ / Fiji.

## Part 3

### Simple measurements in Fiji

Course by Dale Moulding



# Session 3

1 hour

30 minute lecture

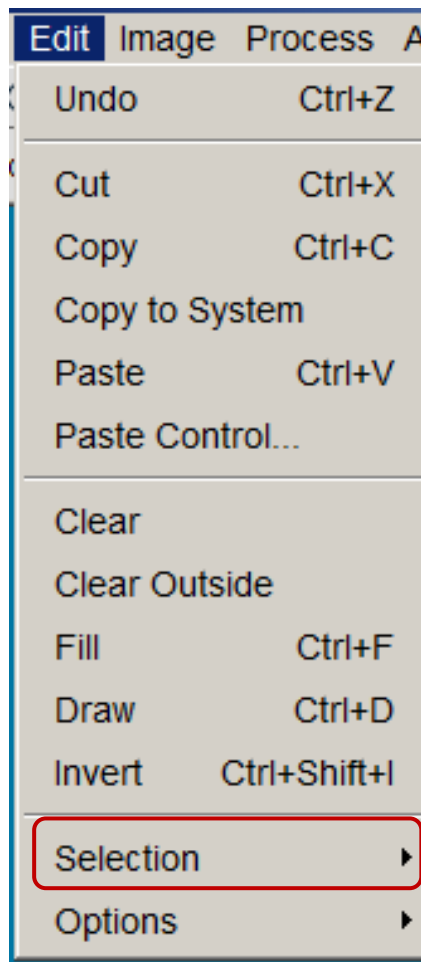
30 minutes exercises

## Learning objectives:

- Make, edit and use ROIs for measurements
- Use the ROI manager for measuring multiple objects
- Draw a teddy bear!
- Define ROIs manually and semi-automatically
- Set scale and use scalebars
- Measure parameters including distances, intensity, area...



## Some common commands and functions in Fiji



- Not always available

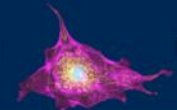
- Copy to the internal clipboard

- the content of the internal clipboard

(Can check by File > New > System Clipboard – Ctrl + Shift + V)

- clear the selection and replace by background colour
- clear outside the selection and replace by background colour
- fill the selection with foreground color
- outlines the selection (I prefer ROI manager for drawing)
- creates a “negative image”

Next slide...



## Some common commands and functions in Fiji

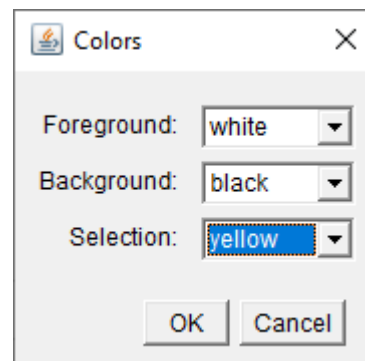
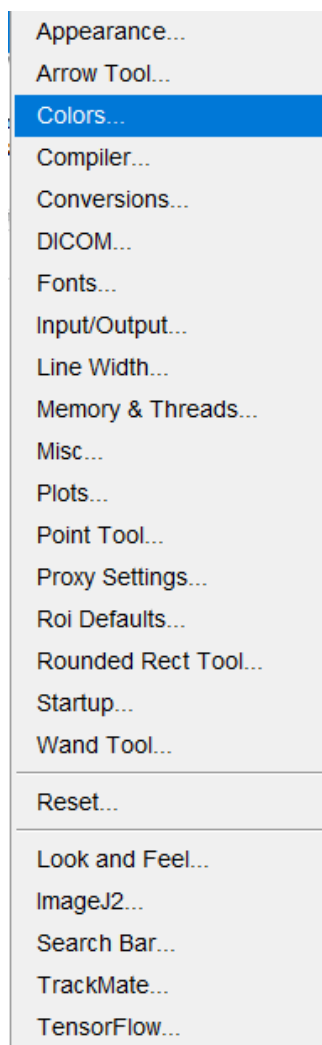
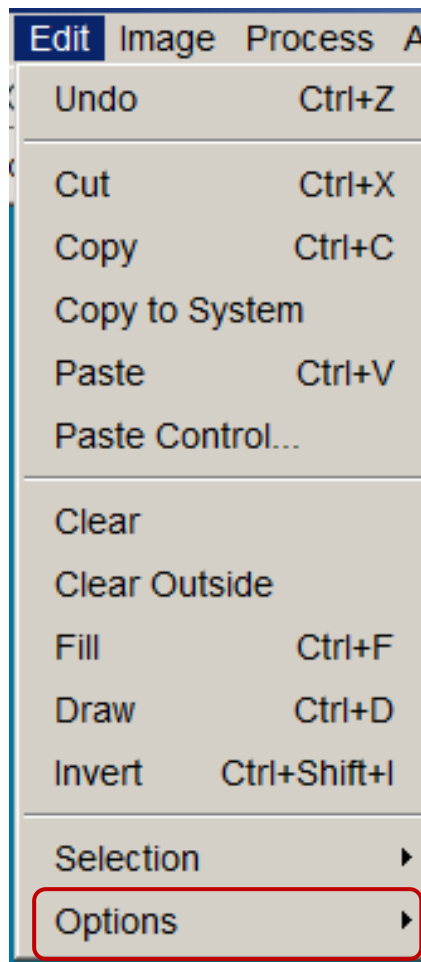
### *Edit / Selection*

Select All	Ctrl+A
Select None	Ctrl+Shift+A
Restore Selection	Ctrl+Shift+E
Fit Spline	Transfer ROI
Fit Circle	between images
Fit Ellipse	
Fit Rectangle	
Interpolate	
Convex Hull	
Make Inverse	Change a mask (binary image) to a selection (ROI) & ROI to mask
Create Selection	
Create Mask	

Properties...	Ctrl+Y
Scale...	
Rotate...	
Enlarge...	
Make Band...	
Specify...	
Straighten...	
To Bounding Box	
Line to Area	
Area to Line	ROI manager –
Image to Selection...	My Favourite!
Add to Manager	Ctrl+T
Fit Circle to Image	
Select Bounding Box	
Select Bounding Box (guess background color)	
Points from Mask	
Make rectangular selection rounded	
Fill ROI holes	



## Some common commands and functions in Fiji



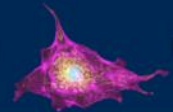


## Some common commands and functions in Fiji

### *Image*

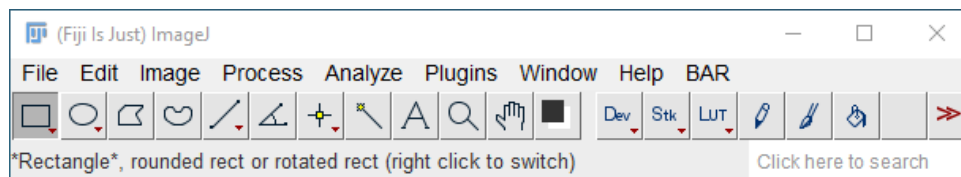
Image	Process	Analyze	Plugins
Type			▶
Adjust			▶
Show Info...		Ctrl+I	
Properties...		Ctrl+Shift+P	
Color			▶
Stacks			▶
Hyperstacks			▶
Crop		Ctrl+Shift+X	
Duplicate...		Ctrl+Shift+D	
Rename...			
Scale...		Ctrl+E	
Transform			▶
Zoom			▶
Overlay			▶
Lookup Tables			▶
Annotate			▶
Drawing			▶
Video Editing			▶
Axes			▶

- Switch between bit depths and colour type
- Many functions in here...
- Image meta-data
- Dealing with multi-channel images
- Dealing with multichannel, 3D & time series
- Remember this shortcut – work on a duplicate image!
- Rotate, flip etc
- Apply colour to an image

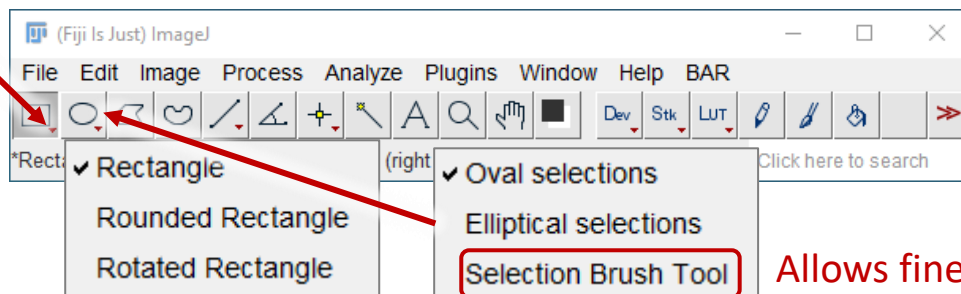


## Selection tools – Drawing an ROI

Left click

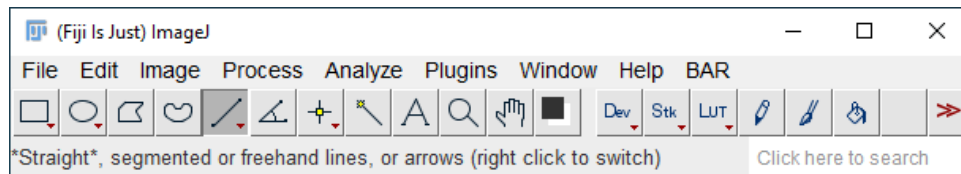


Right click  
for more tools



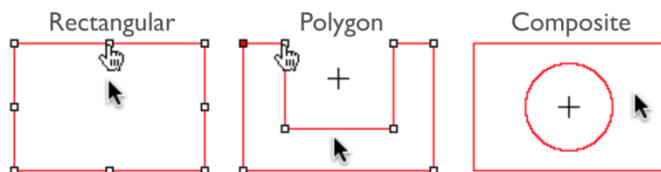
Allows fine  
tuning of ROIs

Double left click  
for tool options





## Editing a selection (ROI)

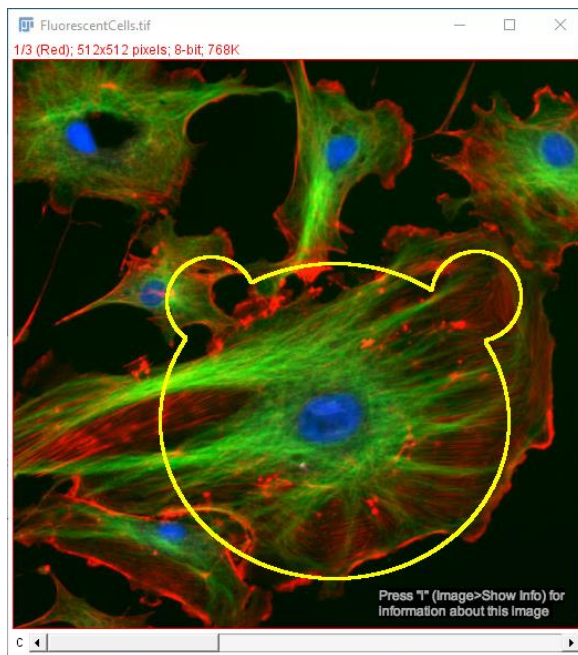


- + Cursor outside selection
- Selection can be moved
- Selection can be resized
- Edge can be moved, deleted or added

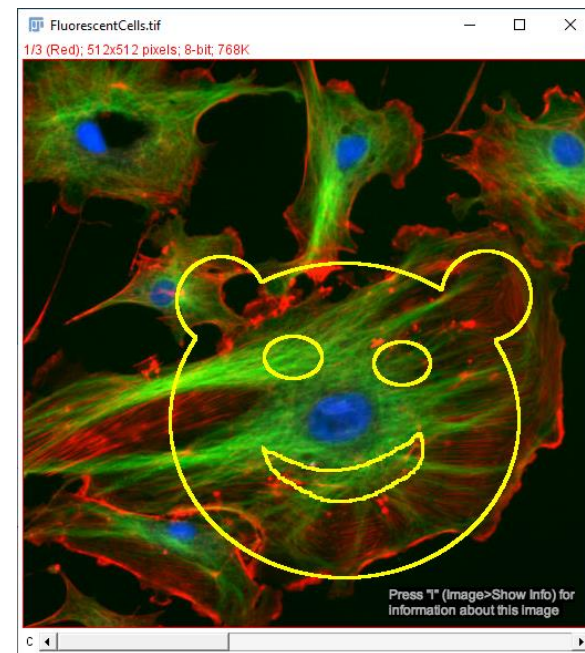


Hold 'Shift' key  
before drawing,  
adds to the  
current selection.

'Shift' key held  
after starting  
drawing constrains  
the drawing  
(square, circle, line  
angled at 0,45,90  
degrees)



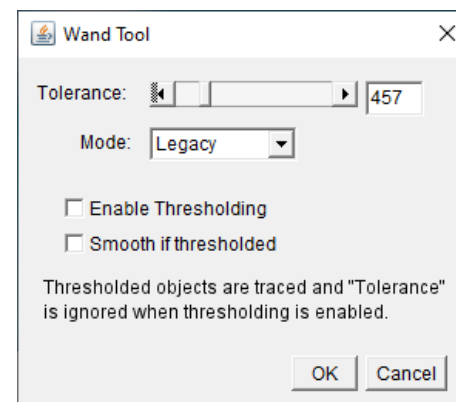
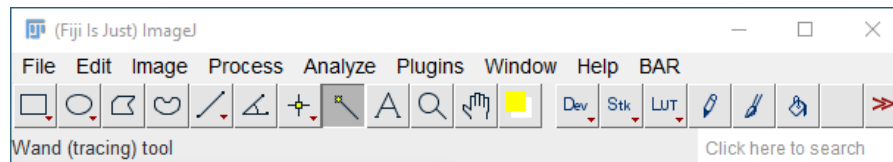
Hold 'Alt' key  
before drawing,  
subtracts from  
the current  
selection.







## Wand tool (semi – automatic ROIs)

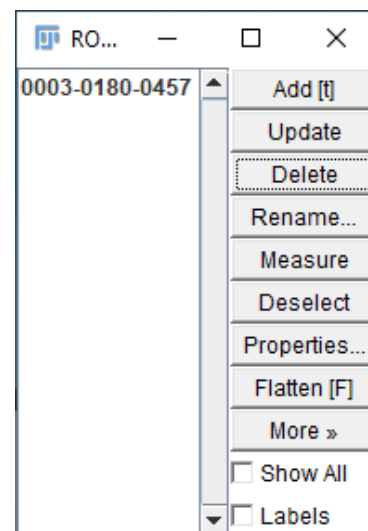


- Click the middle (intensity not position) of an object.
- Grow the ROI by increasing the Tolerance.



## ROI manager

Add to ROI manager by pressing 'T'



- ROIs can be renamed
- Measured...
- Any colour or line width
- Drawn (Flatten) on the image (irreversible)
- More>>

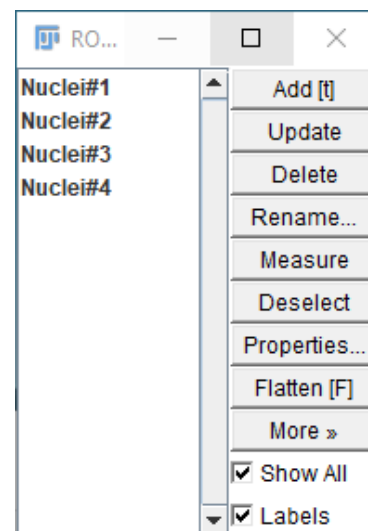
ROIs can be saved, combined, interpolated

- ROIs can be text, arrows, any selected shape.



## ROI manager

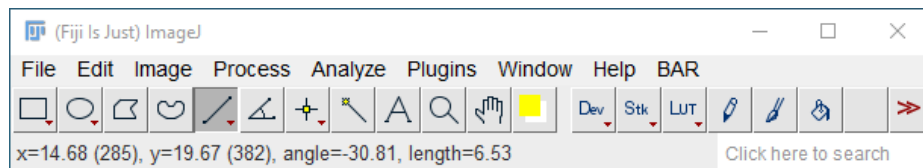
Add to ROI manager by pressing 'T'



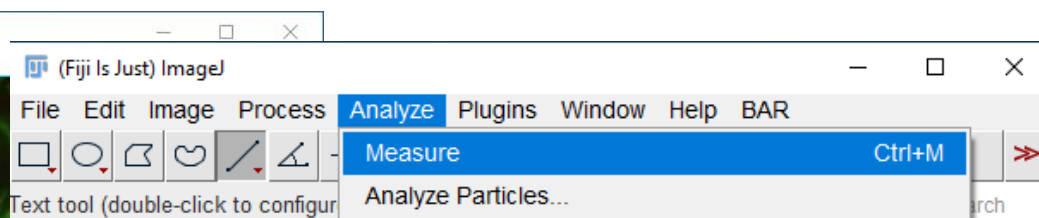
- Multiple ROIs added
- Renamed
- Properties changed
- Show All & Labels



## Measurements



Line tool gives distance  
and angle



Other measurements are made via:  
**Analyze / Measure (Ctrl + M)**

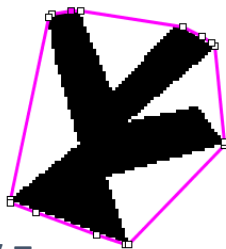


## Measurements

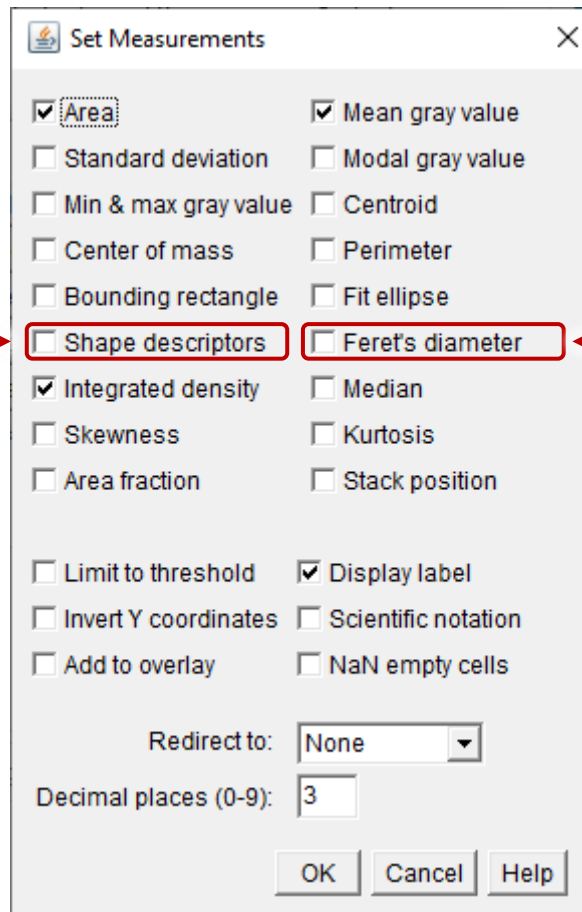
Select your measurements:

**Analyze / Set Measurements...**

Circularity, Aspect Ratio,  
Roundness, Solidity



Solidity =  
Area / Convex Hull Area  
(Elastic band around object)



**Set Measurements**

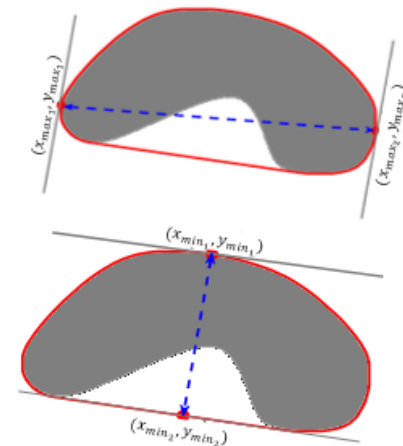
<input checked="" type="checkbox"/> Area	<input checked="" type="checkbox"/> Mean gray value
<input type="checkbox"/> Standard deviation	<input type="checkbox"/> Modal gray value
<input type="checkbox"/> Min & max gray value	<input type="checkbox"/> Centroid
<input type="checkbox"/> Center of mass	<input type="checkbox"/> Perimeter
<input type="checkbox"/> Bounding rectangle	<input type="checkbox"/> Fit ellipse
<input type="checkbox"/> Shape descriptors	<input type="checkbox"/> Feret's diameter
<input checked="" type="checkbox"/> Integrated density	<input type="checkbox"/> Median
<input type="checkbox"/> Skewness	<input type="checkbox"/> Kurtosis
<input type="checkbox"/> Area fraction	<input type="checkbox"/> Stack position
<input type="checkbox"/> Limit to threshold	<input checked="" type="checkbox"/> Display label
<input type="checkbox"/> Invert Y coordinates	<input type="checkbox"/> Scientific notation
<input type="checkbox"/> Add to overlay	<input type="checkbox"/> NaN empty cells

Redirect to:

Decimal places (0-9):

OK Cancel Help

Feret's diameter



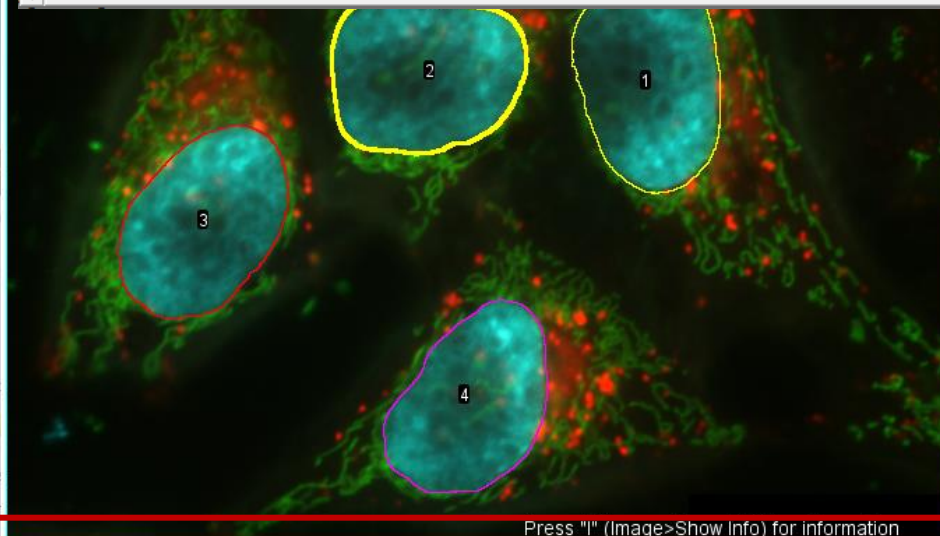
Think: Callipers  
Longest & shortest  
distance between  
parallel lines on the  
convex hull



## Measurements

Measure a single channel (press M)  
or in ROI manager press Measure

Results															
File	Edit	Font	Results												
	Label	Area	Mean	Circ.	Feret	IntDen	RawIntDen	FeretX	FeretY	FeretAngle	MinFeret	AR	Round	Solidity	
1	hela-cells.tif:Nuclei#1:Blue	34.701	1118.525	0.810	8.247	38813.643	14638134.000	461	260	87.495	5.375	1.537	0.651	0.981	
2	hela-cells.tif:Nuclei#2:Blue	34.998	1110.516	0.846	7.569	38865.526	14657701.000	240	217	30.228	6.040	1.226	0.815	0.979	
3	hela-cells.tif:Nuclei#3:Blue	32.012	1233.912	0.838	7.860	39500.077	14897015.000	104	347	58.392	5.182	1.505	0.665	0.981	
4	hela-cells.tif:Nuclei#4:Blue	30.482	1233.273	0.823	7.792	37592.809	14177710.000	294	471	60.725	5.154	1.533	0.652	0.976	



$\text{IntDen} = \text{Area} * \text{Mean}$

$\text{RawIntDen} = \text{sum of pixel values}$

$\text{FeretAngle} = \text{direction of longest axis}$





## Measurements


Measure **all channels**

In ROI manager press More>> Multi Measure

Results

File Edit Font Results

	Label	Area	Mean	Circ.	Feret	IntDen	RawIntDen	Ch	FeretX	FeretY	FeretAngle	MinFeret	AR	Round	Solidity
1	hela-cells.tif:Nuclei#1:Red	34.701	448.026	0.810	8.247	15546.819	5863310.000	1	461	260	87.495	5.375	1.537	0.651	0.981
2	hela-cells.tif:Nuclei#2:Red	34.998	459.965	0.846	7.569	16097.733	6071081.000	1	240	217	30.228	6.040	1.226	0.815	0.979
3	hela-cells.tif:Nuclei#3:Red	32.012	444.736	0.838	7.860	14236.925	5369298.000	1	104	347	58.392	5.182	1.505	0.665	0.981
4	hela-cells.tif:Nuclei#4:Red	30.482	460.846	0.823	7.792	14047.570	5297885.000	1	294	471	60.725	5.154	1.533	0.652	0.976
5	hela-cells.tif:Nuclei#1:Green	34.701	361.306	0.810	8.247	12537.596	4728415.000	2	461	260	87.495	5.375	1.537	0.651	0.981
6	hela-cells.tif:Nuclei#2:Green	34.998	360.021	0.846	7.569	12599.926	4751922.000	2	240	217	30.228	6.040	1.226	0.815	0.979
7	hela-cells.tif:Nuclei#3:Green	32.012	352.280	0.838	7.860	11277.230	4253082.000	2	104	347	58.392	5.182	1.505	0.665	0.981
8	hela-cells.tif:Nuclei#4:Green	30.482	362.599	0.823	7.792	11052.796	4168439.000	2	294	471	60.725	5.154	1.533	0.652	0.976
9	hela-cells.tif:Nuclei#1:Blue	34.701	1118.525	0.810	8.247	38813.643	14638134.000	3	461	260	87.495	5.375	1.537	0.651	0.981
10	hela-cells.tif:Nuclei#2:Blue	34.998	1110.516	0.846	7.569	38865.526	14657701.000	3	240	217	30.228	6.040	1.226	0.815	0.979
11	hela-cells.tif:Nuclei#3:Blue	32.012	1233.912	0.838	7.860	39500.077	14897015.000	3	104	347	58.392	5.182	1.505	0.665	0.981
12	hela-cells.tif:Nuclei#4:Blue	30.482	1233.273	0.823	7.792	37592.809	14177710.000	3	294	471	60.725	5.154	1.533	0.652	0.976



Press "I" (Image>Show Info) for information

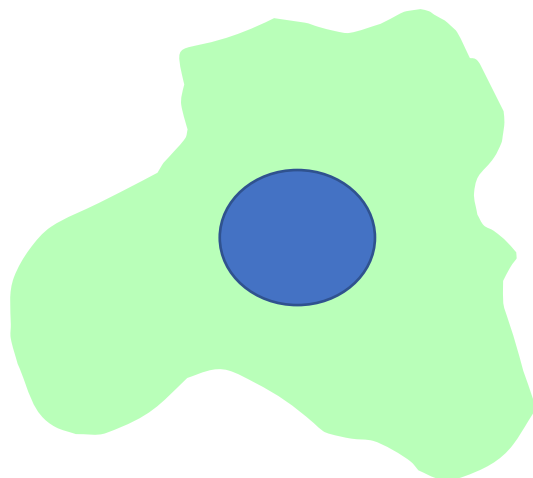
RO...

Nuclei#1  
Nuclei#2  
Nuclei#3  
Nuclei#4

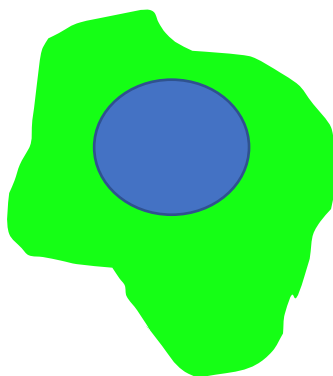
Add [t]  
Update  
Delete  
Rename...  
Measure  
Deselect  
Properties...  
Flatten [F]  
More »  
Show All  
Labels



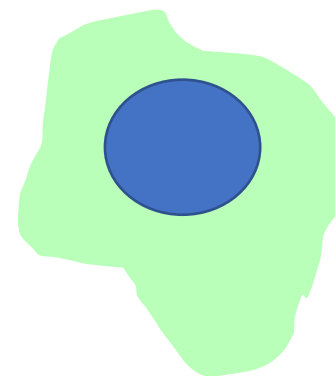
## Measuring intensity – Mean or Integrated density?



Area ( $\mu\text{m}^2$ ) = 200  
Mean = 100  
IntDen = 20,000



Area ( $\mu\text{m}^2$ ) = 100  
Mean = 200  
IntDen = 20,000



Area ( $\mu\text{m}^2$ ) = 100  
Mean = 100  
IntDen = 10,000

**Mean : Concentration**

**IntDen: Total amount**

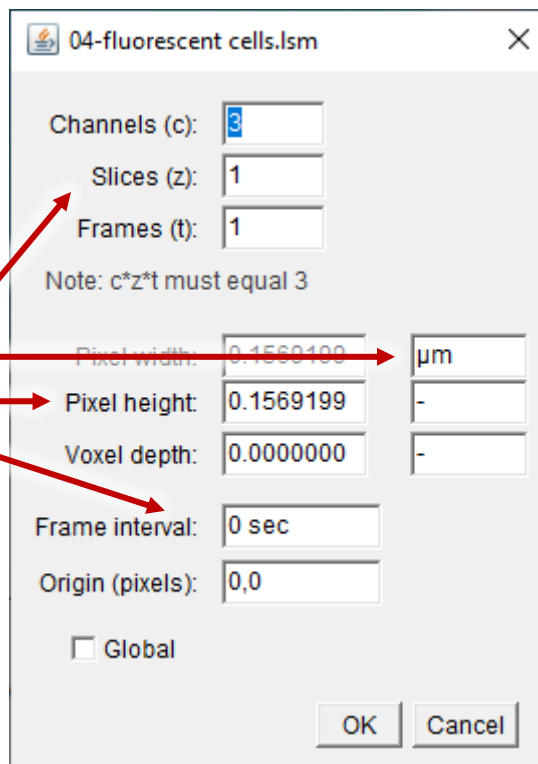




## What units are you measuring? Setting image properties & scale

**Image / Properties**  
(Ctrl + Shift + P)

Metadata



04-fluorescent cells.lsm

Channels (c): 3

Slices (z): 1

Frames (t): 1

Note: c\*z\*t must equal 3

Pixel width: 0.1569199 μm

Pixel height: 0.1569199 -

Voxel depth: 0.0000000 -

Frame interval: 0 sec

Origin (pixels): 0,0

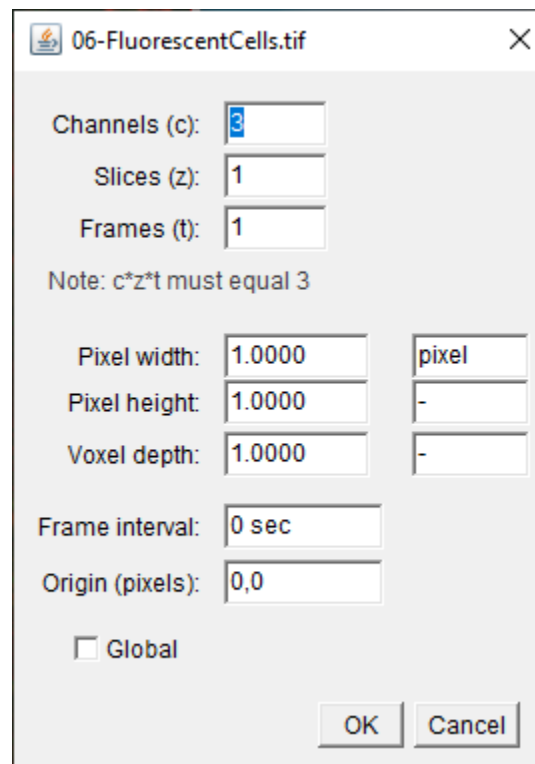
☐ Global

OK Cancel

You can edit the  
values here

Measurements in μm  
Areas as μm<sup>2</sup>  
Volumes as μm<sup>3</sup>

No Metadata



06-FluorescentCells.tif

Channels (c): 3

Slices (z): 1

Frames (t): 1

Note: c\*z\*t must equal 3

Pixel width: 1.0000 pixel

Pixel height: 1.0000 -

Voxel depth: 1.0000 -

Frame interval: 0 sec

Origin (pixels): 0,0

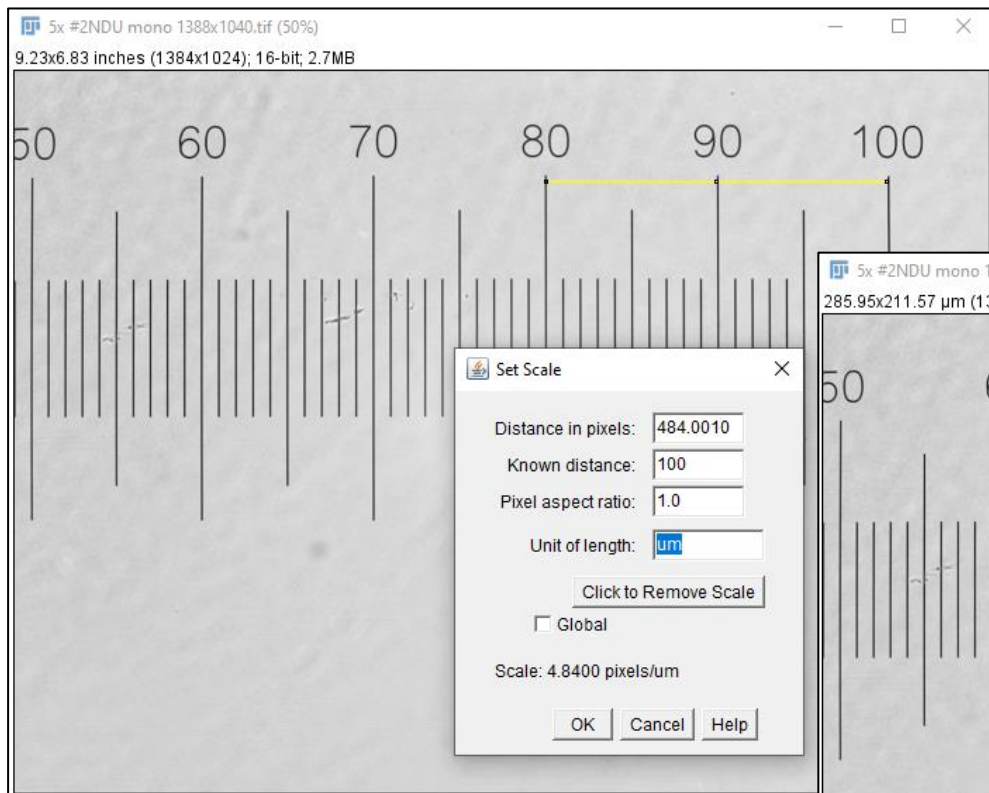
☐ Global

OK Cancel

Measurements in pixels

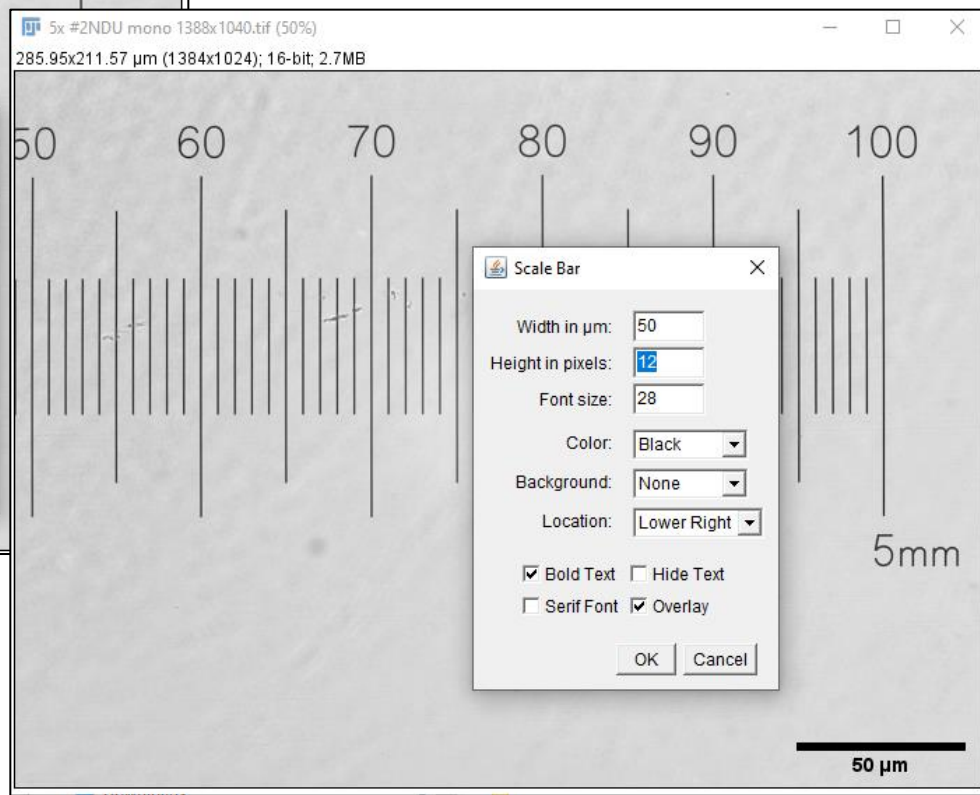


## What units are you measuring? Setting image properties & scale



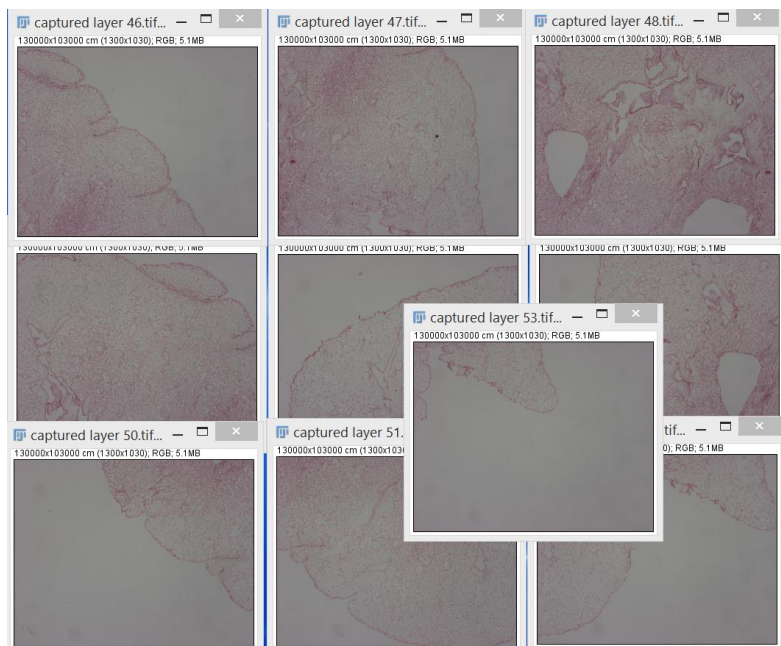
*Analyze / Set Scale...*

*Analyze / Tools > Scale bar...*





## Image Stitching Pt1



Homework:

***Plugins / Stitching / MosaicJ***

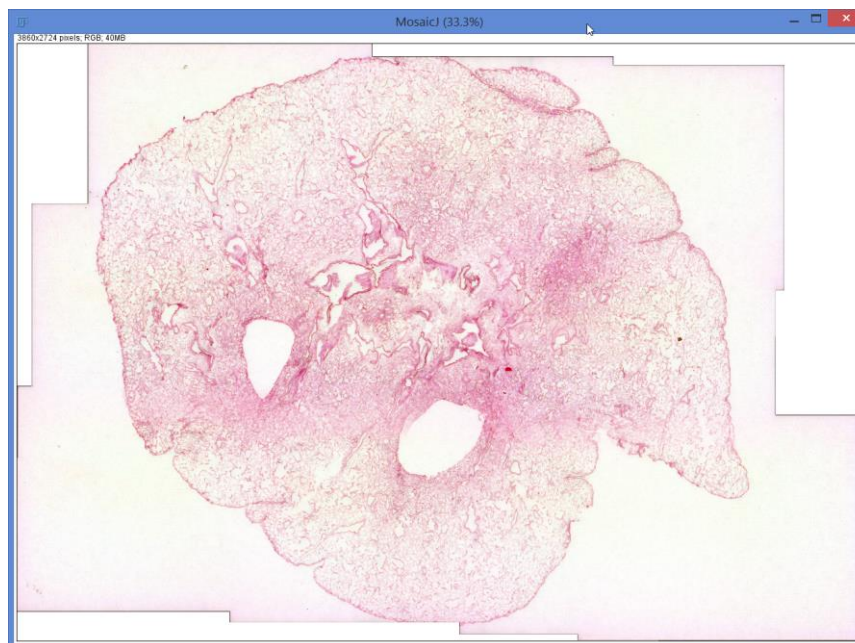
Open Image Sequence

First image in 2D Stitching folder...

Click each image,

move it to roughly the right place

File / Create mosaic...

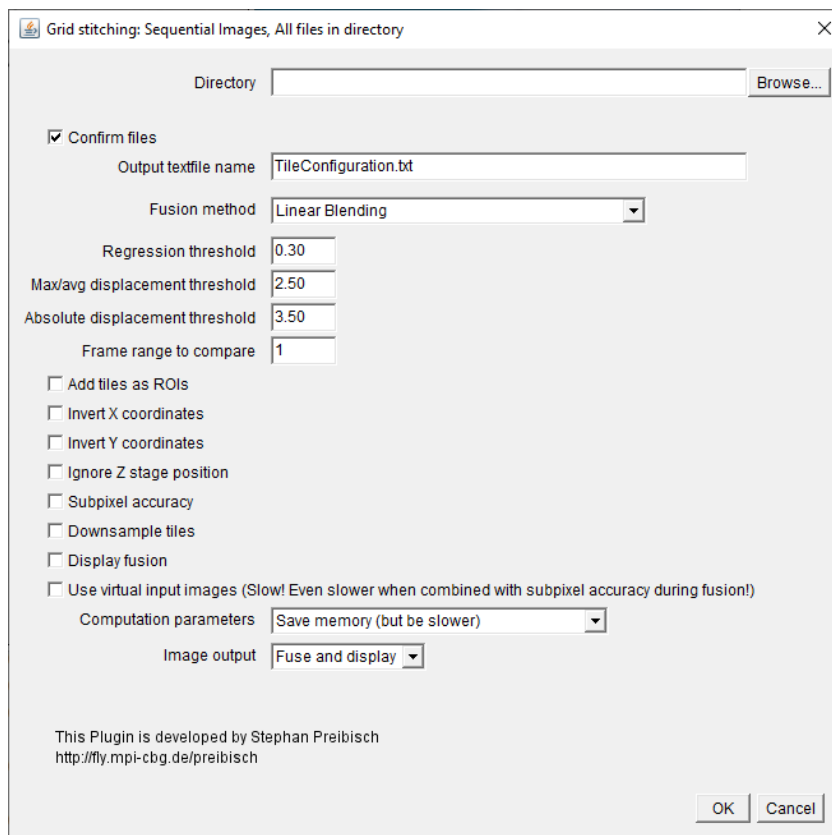
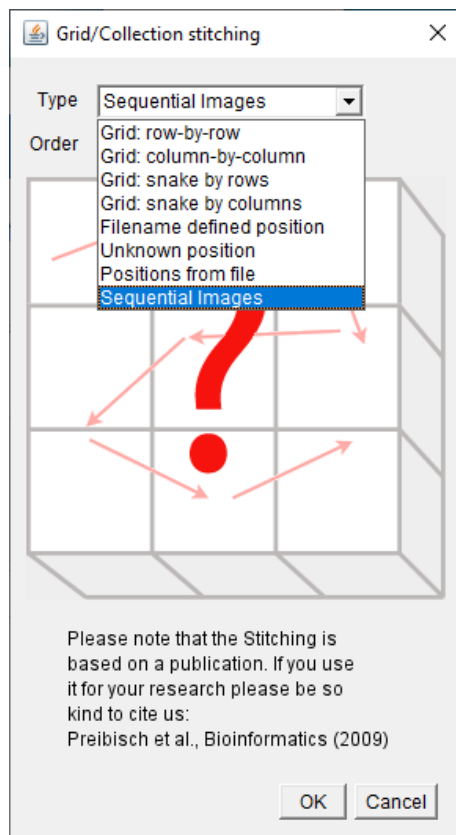




## Image Stitching Pt2

Lazy way:

***Plugins / Stitching / Grid Collection Stitching...***



The plugin will stitch any set of images (2D or 3D) as long as there is an overlap between adjacent images.

Simply have all your files in a single folder, and direct the plugin to the directory. Leave all variables at default (unless it fails).



## Exercises using Fiji. Session 3 – Simple measurements

Make ROIs and measure them...

- 6) Drawing ROIs manually.
- 7) Wand tool to select regions. Make measurements.
- 8) Why z-projections as Maximum Intensity should not be used for measurements.