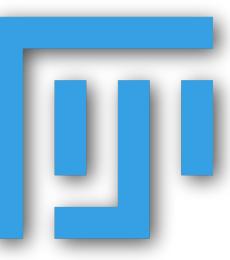
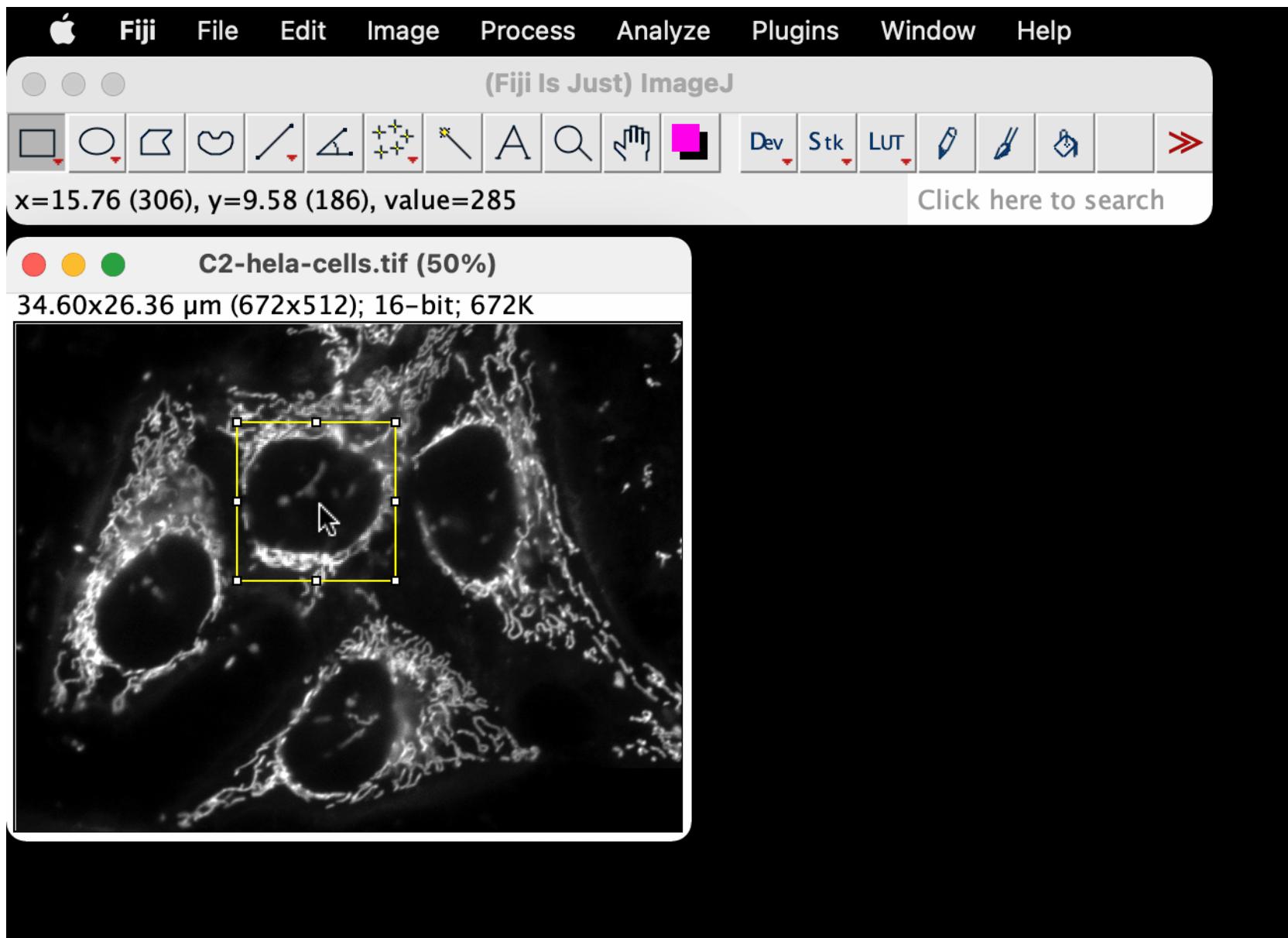
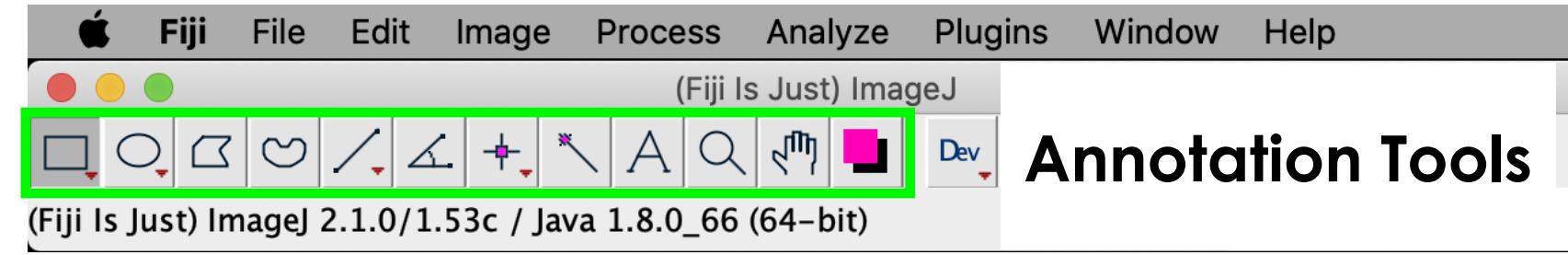


# ROI Manager

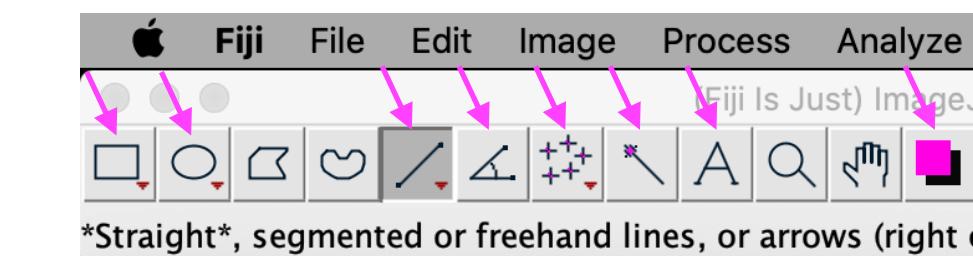
Annotation Tools  
*\*see also Edit > Selection*



# Region Of Interest (ROI)



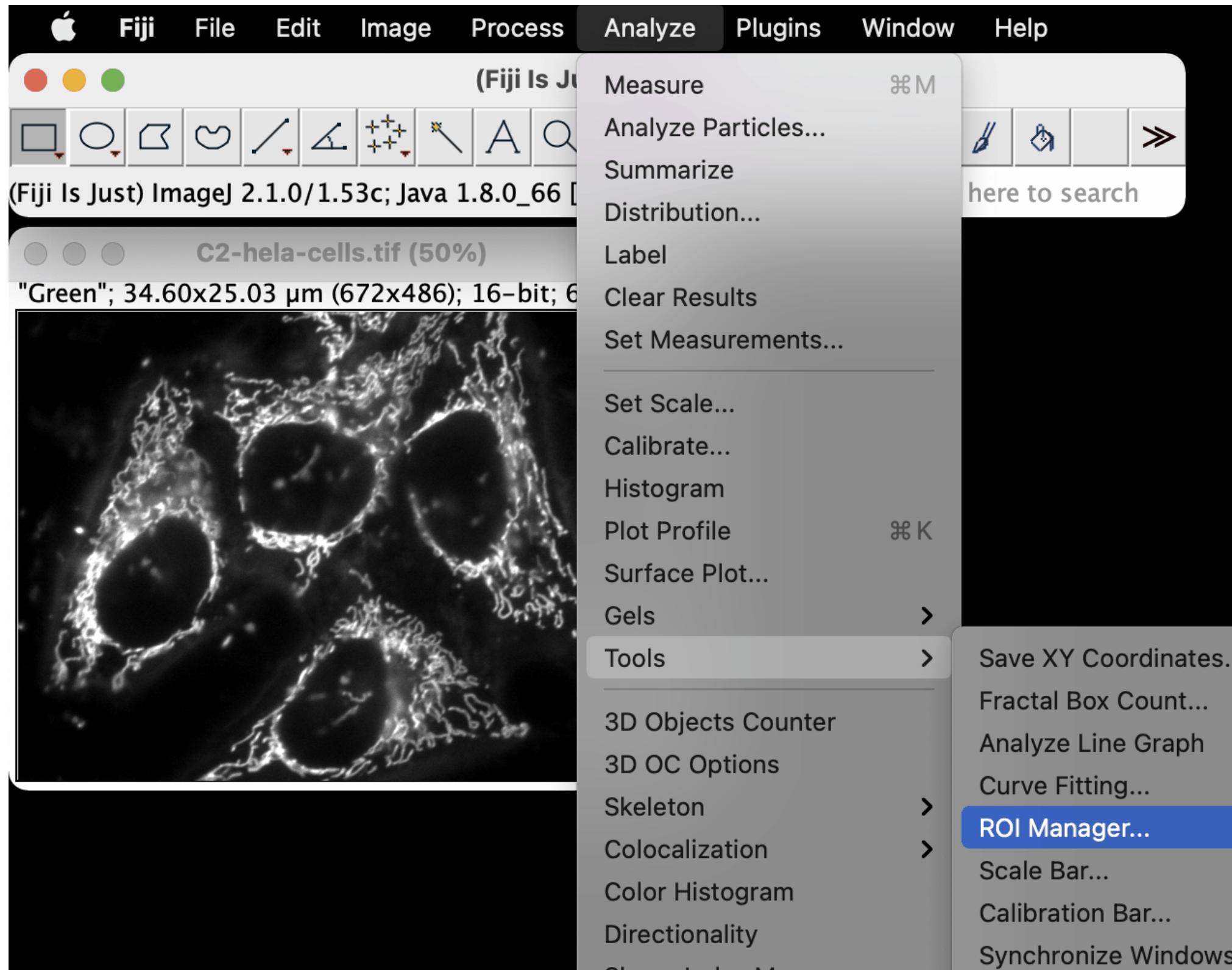
Secondary/Right Click  
for more options



Double Click to  
set/change properties

\*Straight\*, segmented or freehand lines, or arrows (right c

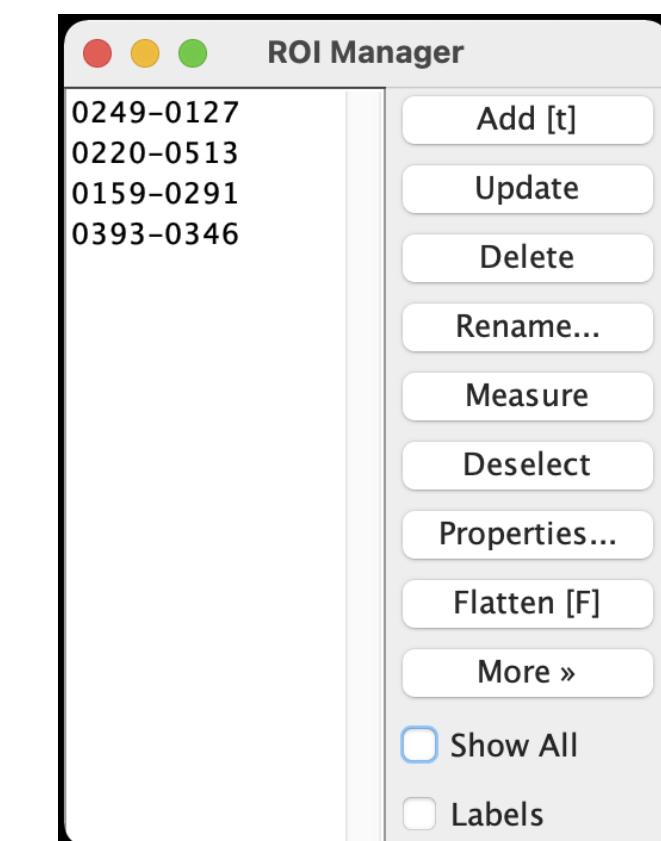
# ROI Manager



Analyze > Tools > ROI Manager...

t \*

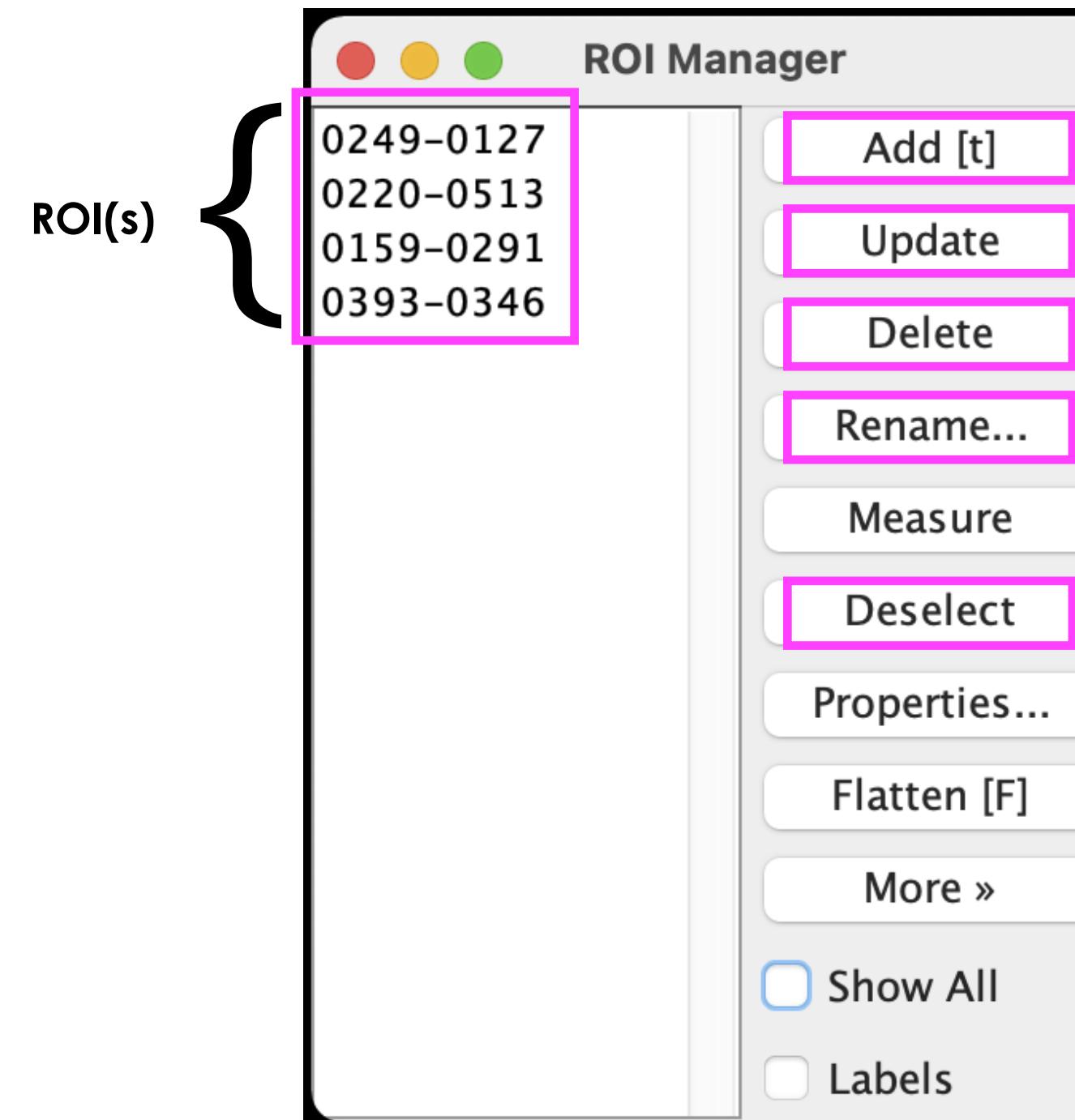
**The ROI Manager is a tool for working with *multiple* selections (ROIs).**



**\*(cmd) + t can be used to both to open the ROI Manager and/or add a new ROI to the Manager.**

**\*\*shift + e can be used to draw the last ROI.**

# ROI Manager



**Add new ROI (t).**

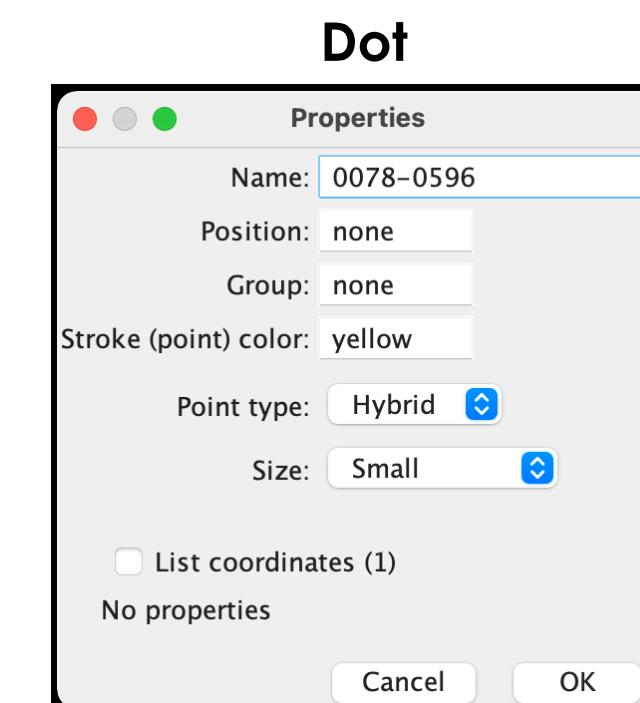
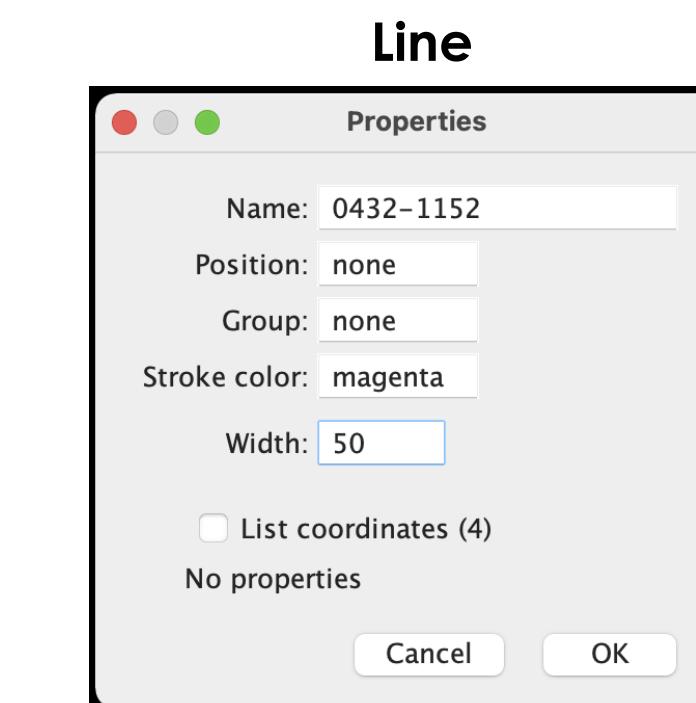
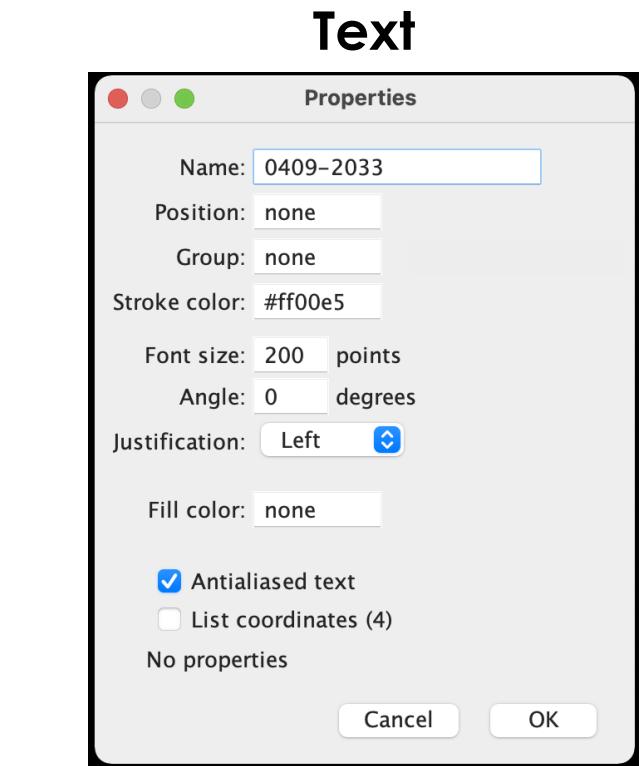
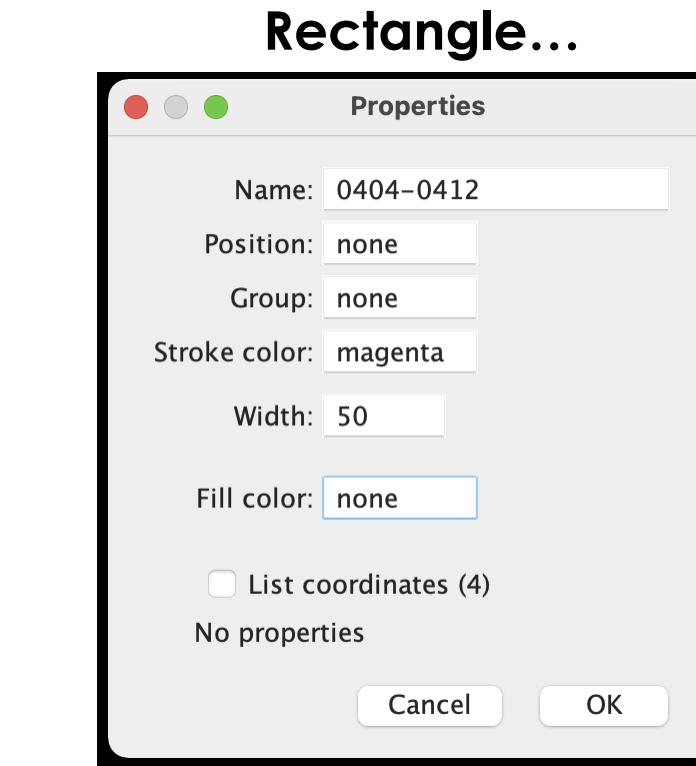
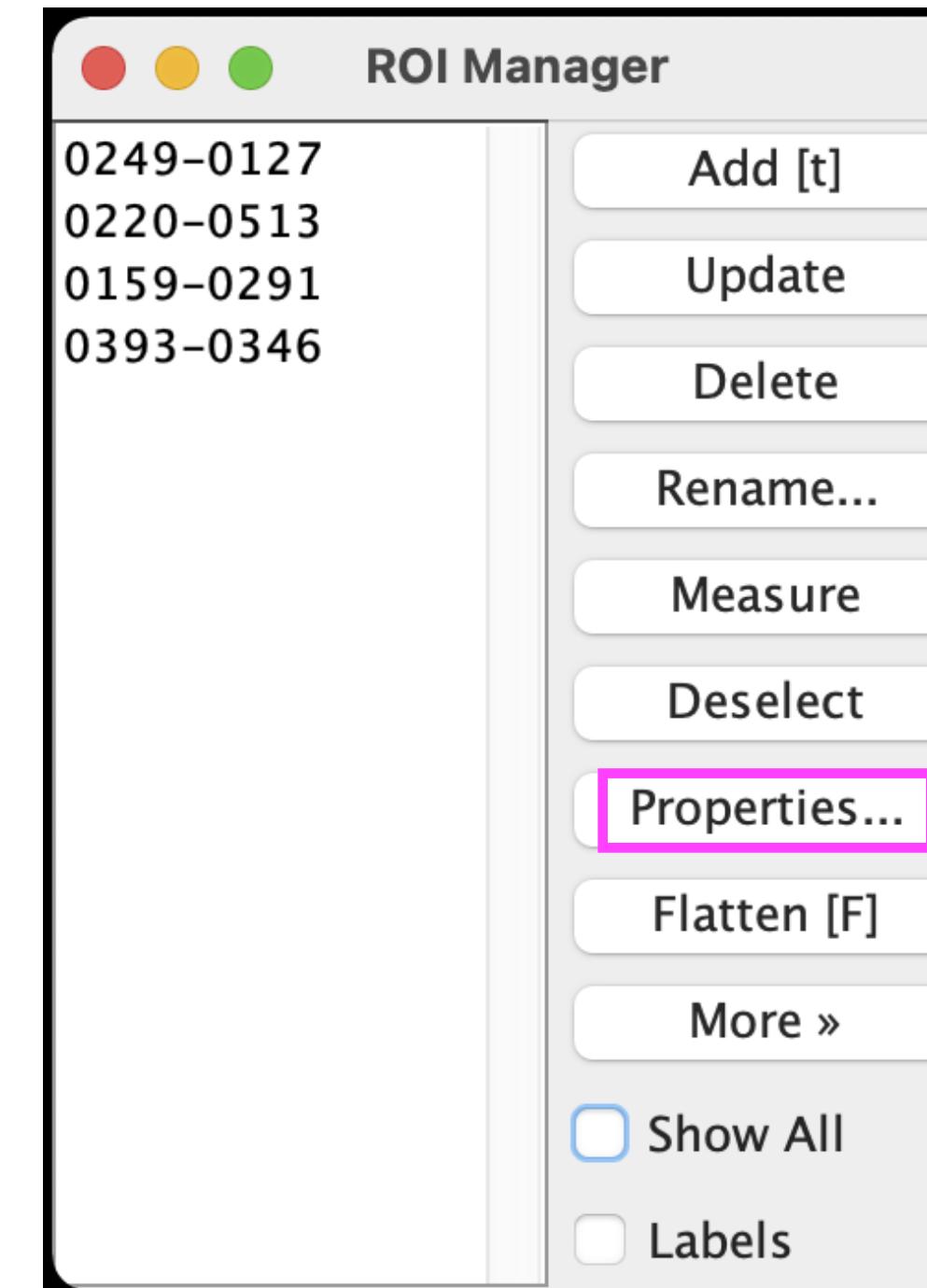
After modifying a ROI, you can use **Update** to save the changes.

**Delete selected ROI.** If none is selected, delete all.

**Rename selected ROI.**

**Deselect one or more selected ROI.**

# ROI Manager

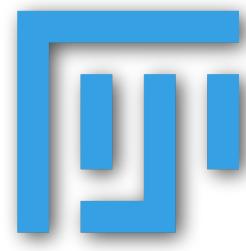


Change the properties of the selected ROI(s)

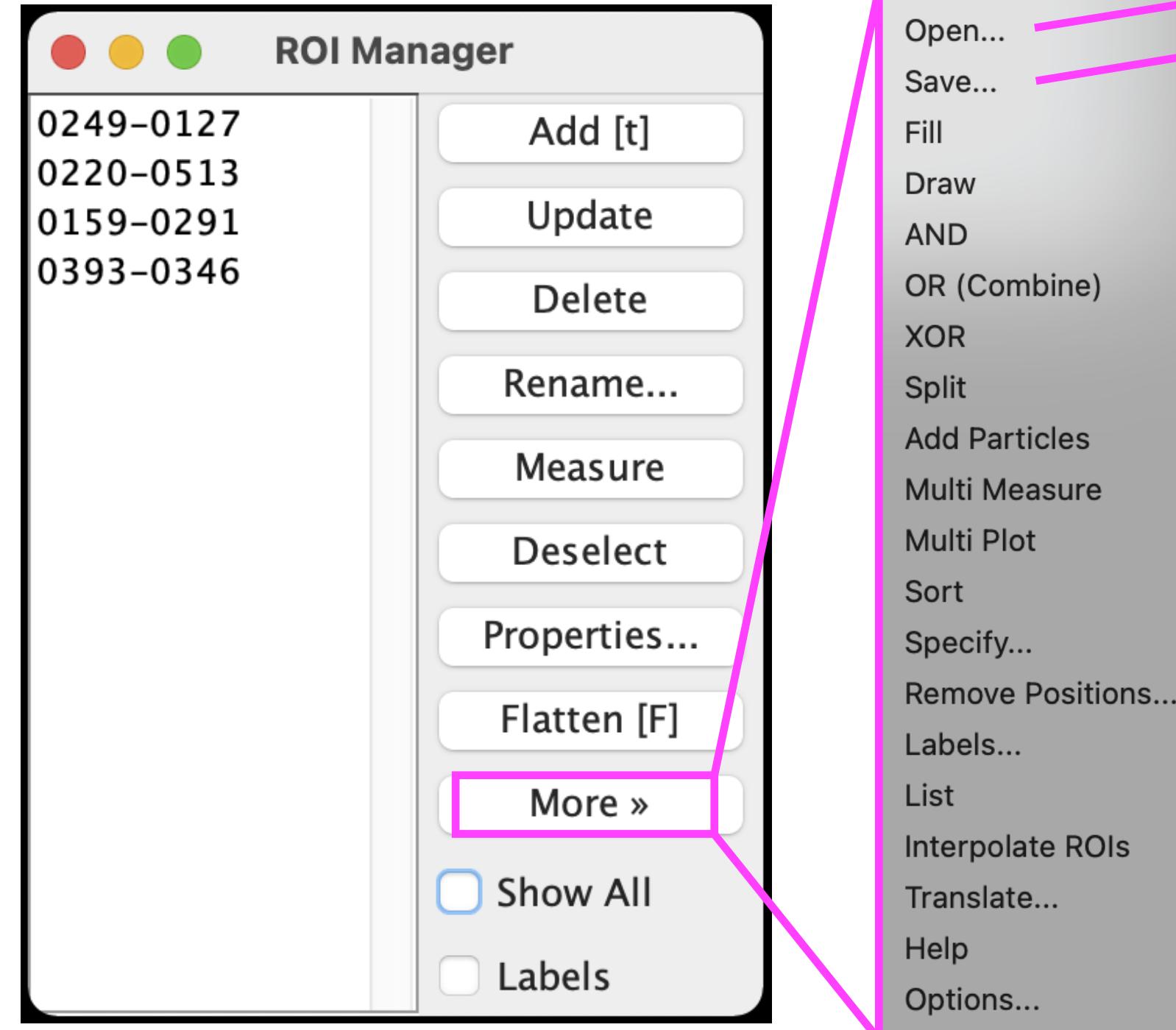


“Analyze” menu

“Edit” menu



# ROI Manager



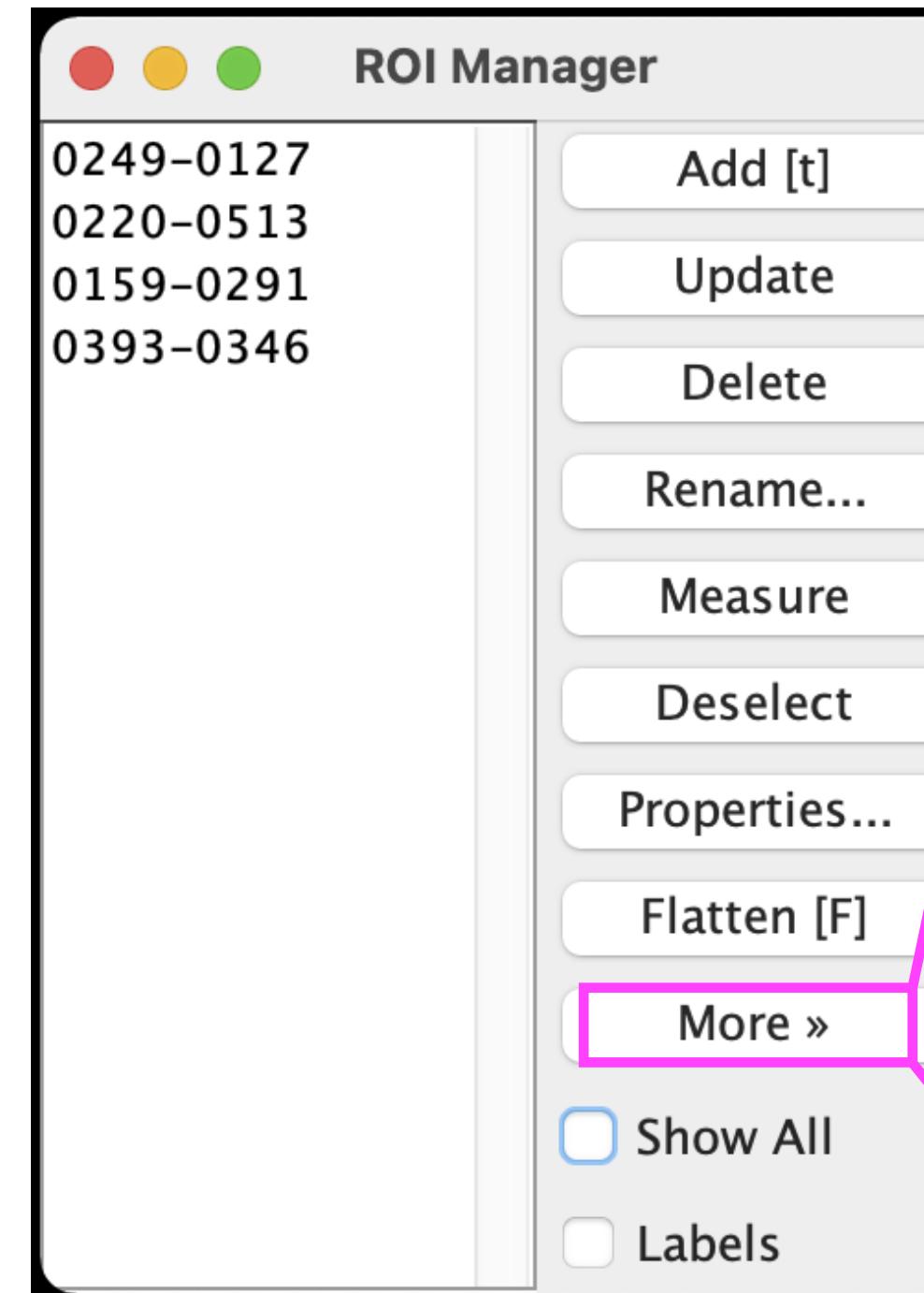
Open a saved ROI.zip file (drag & drop works as well)

Save ROI(s) as a .zip file

“Analyze” menu

“Image” menu

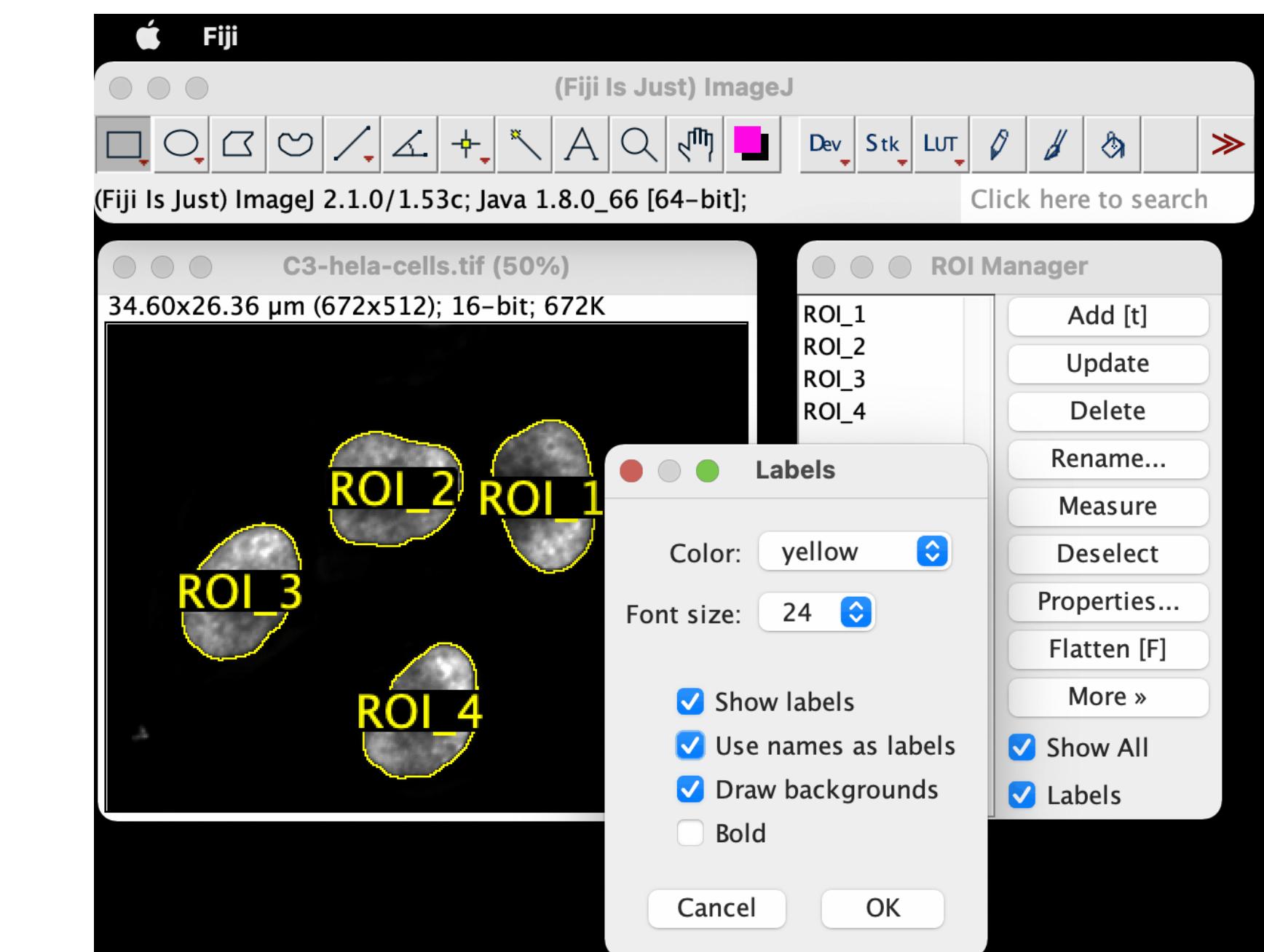
# ROI Manager



- Open...
- Save...
- Fill
- Draw
- AND
- OR (Combine)
- XOR
- Split
- Add Particles
- Multi Measure
- Multi Plot
- Sort
- Specify...
- Remove Positions...
- Labels...
- List
- Interpolate ROIs
- Translate...
- Help
- Options...

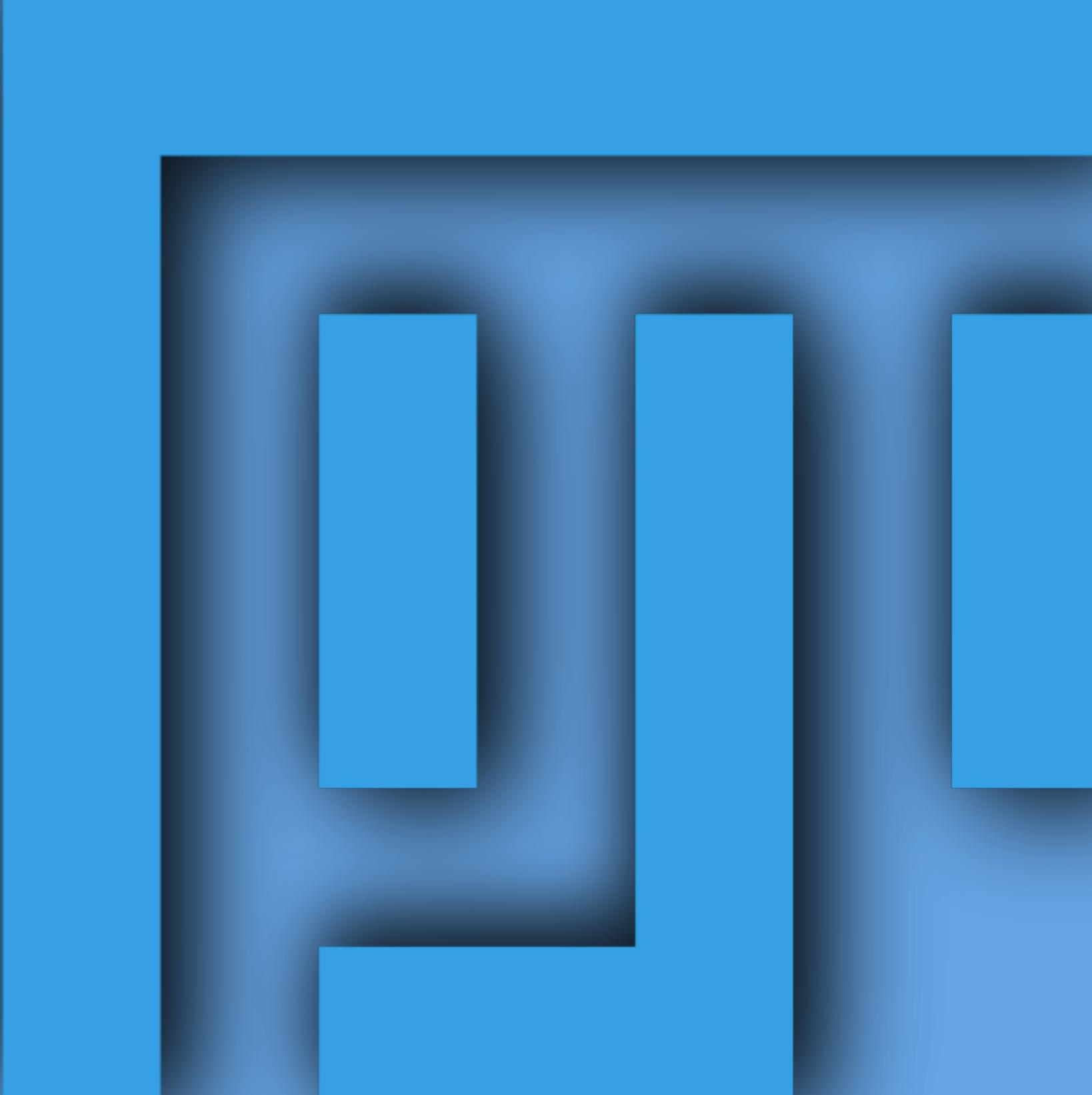
Open a saved ROI.zip file (drag & drop works as well)  
Save ROI(s) as a .zip file

Set ROI(s) label options



\*Labels option can also be found under “Image > Overlay”

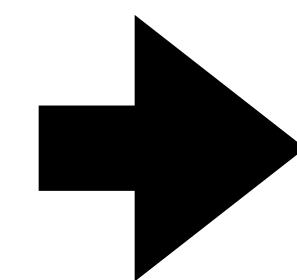
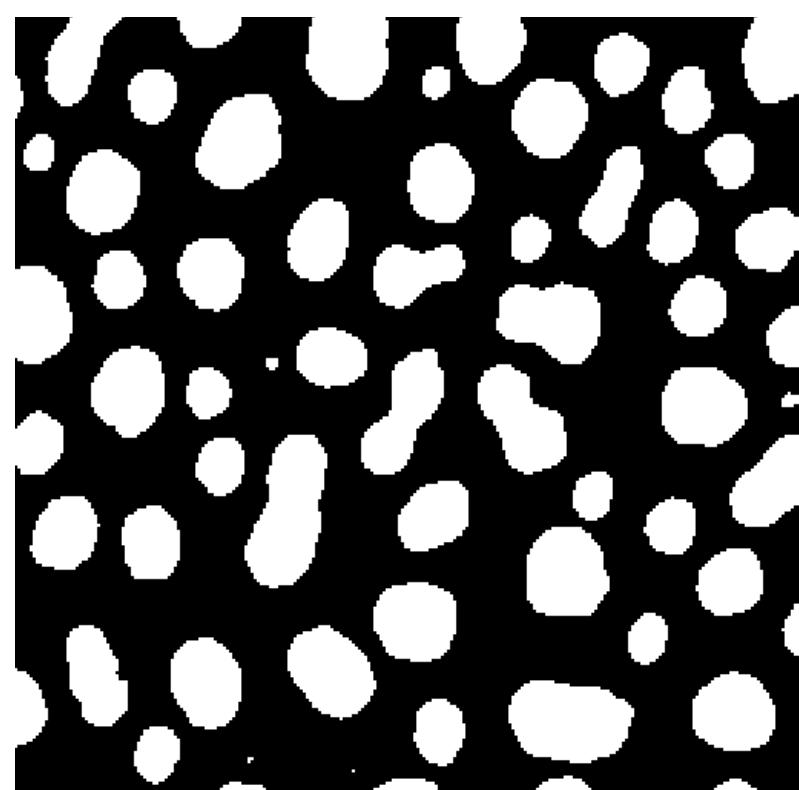
\*\*“Use names as labels” can be also found under “More>Options...”



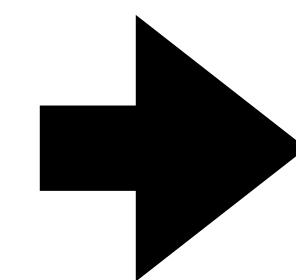
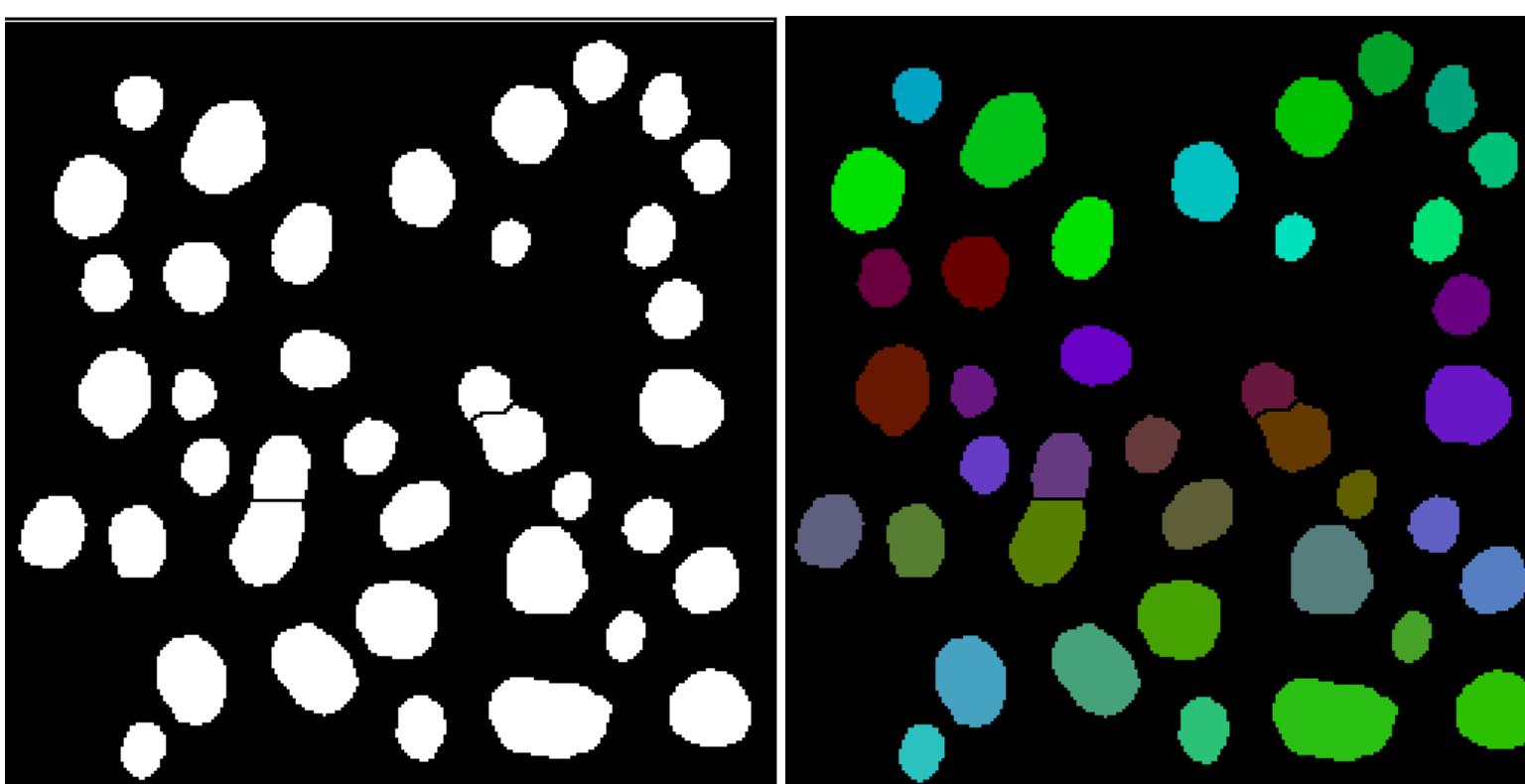
**Live Demo of ROI manager**

# Segmentation And Measurements

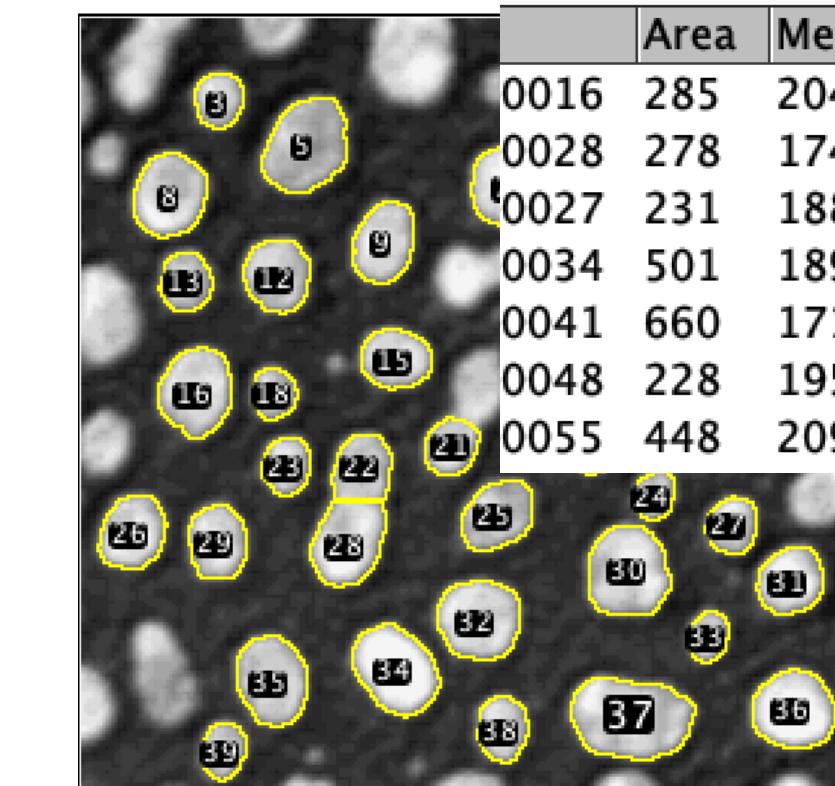
Binary mask



Process and segment

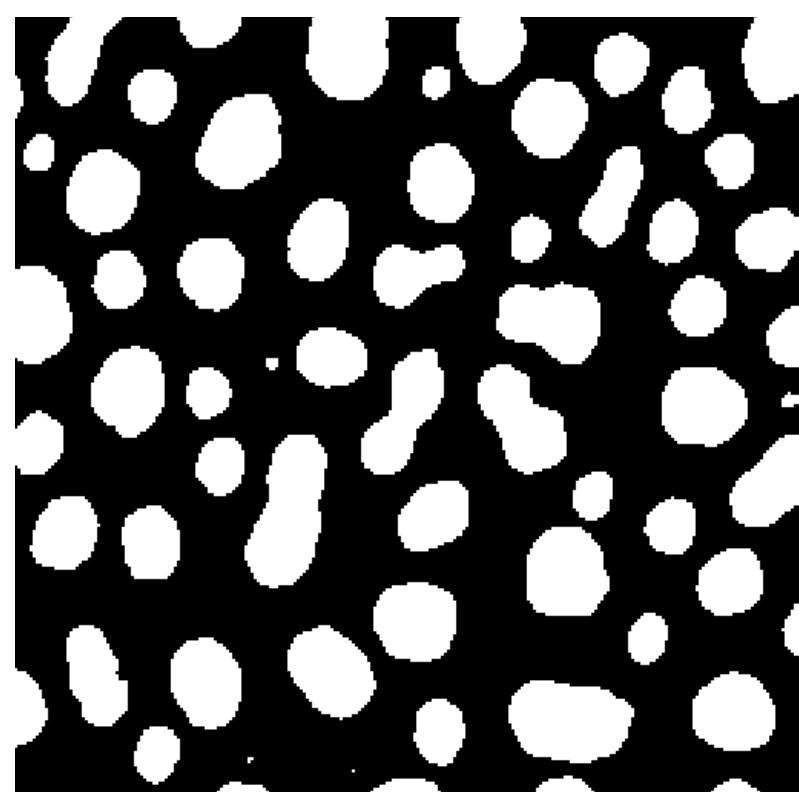


Export and Measure

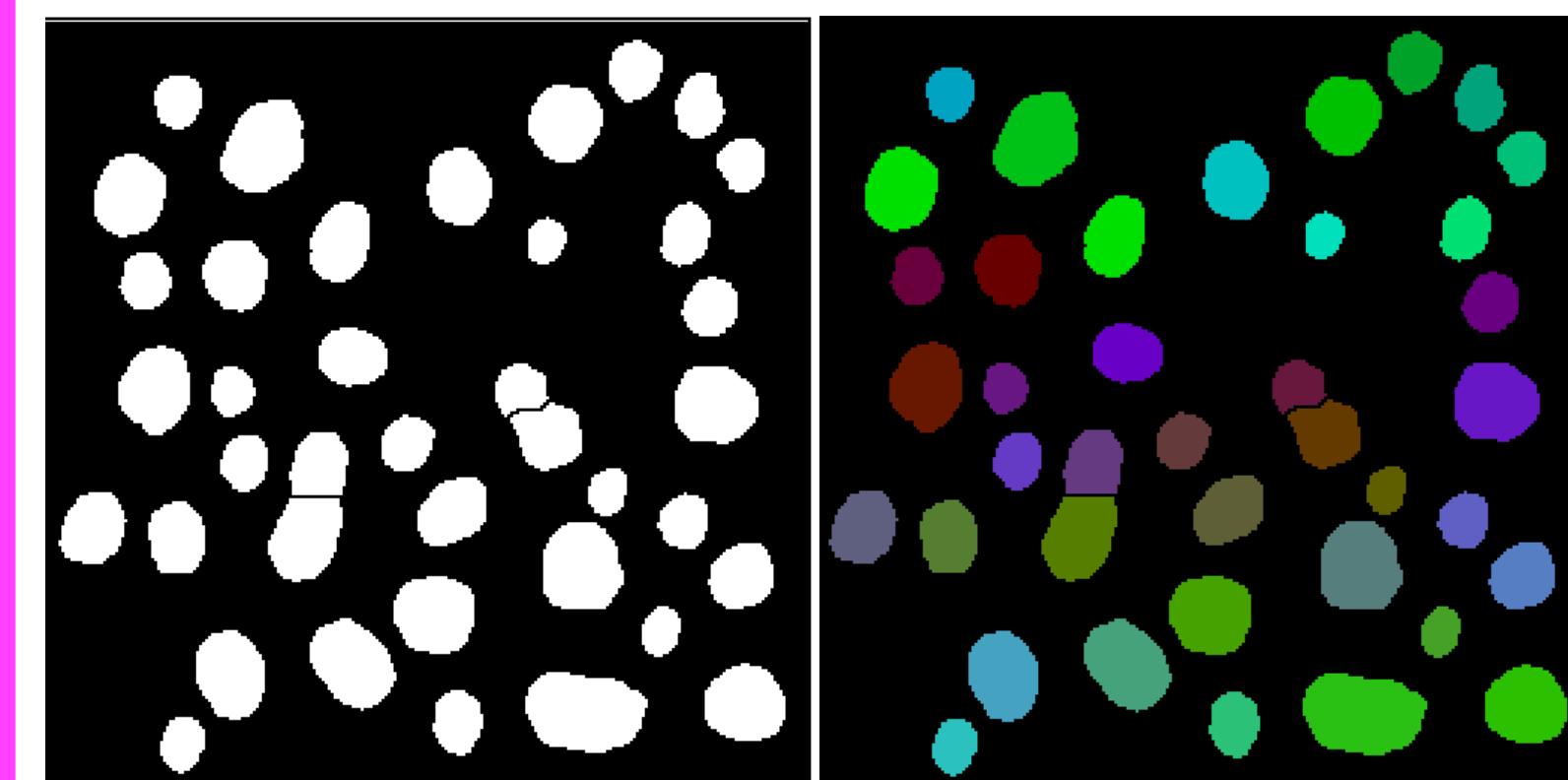


Results			
	Area	Mean	X
0016	285	204.29474	197.
0028	278	174.84892	219.
0027	231	188.46753	45.0
0034	501	189.14172	174.
0041	660	171.69697	73.8
0048	228	195.89474	233.
0055	448	209.03571	138.

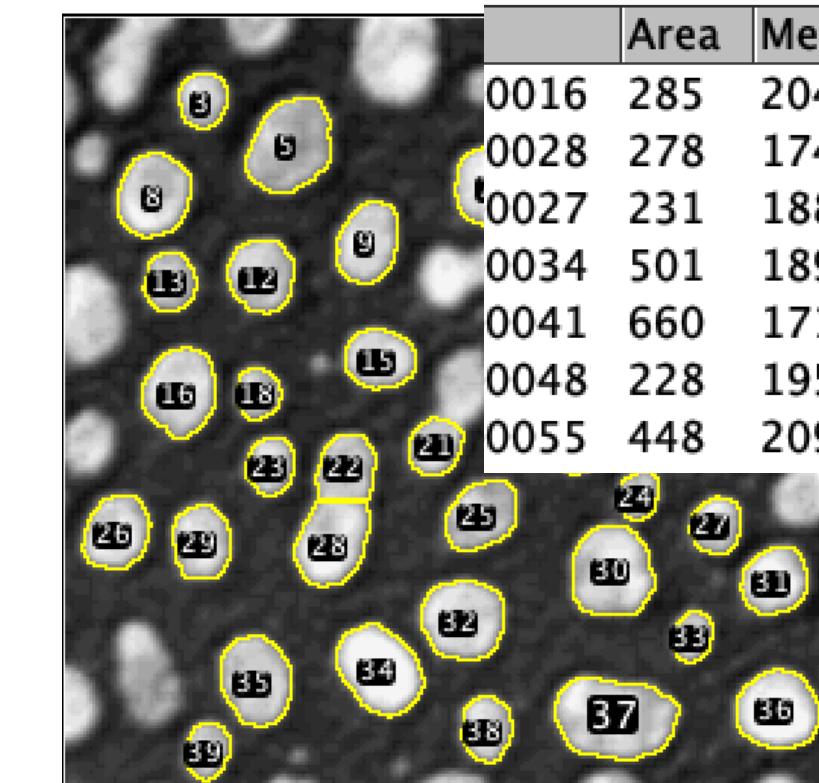
Binary mask



Process and segment



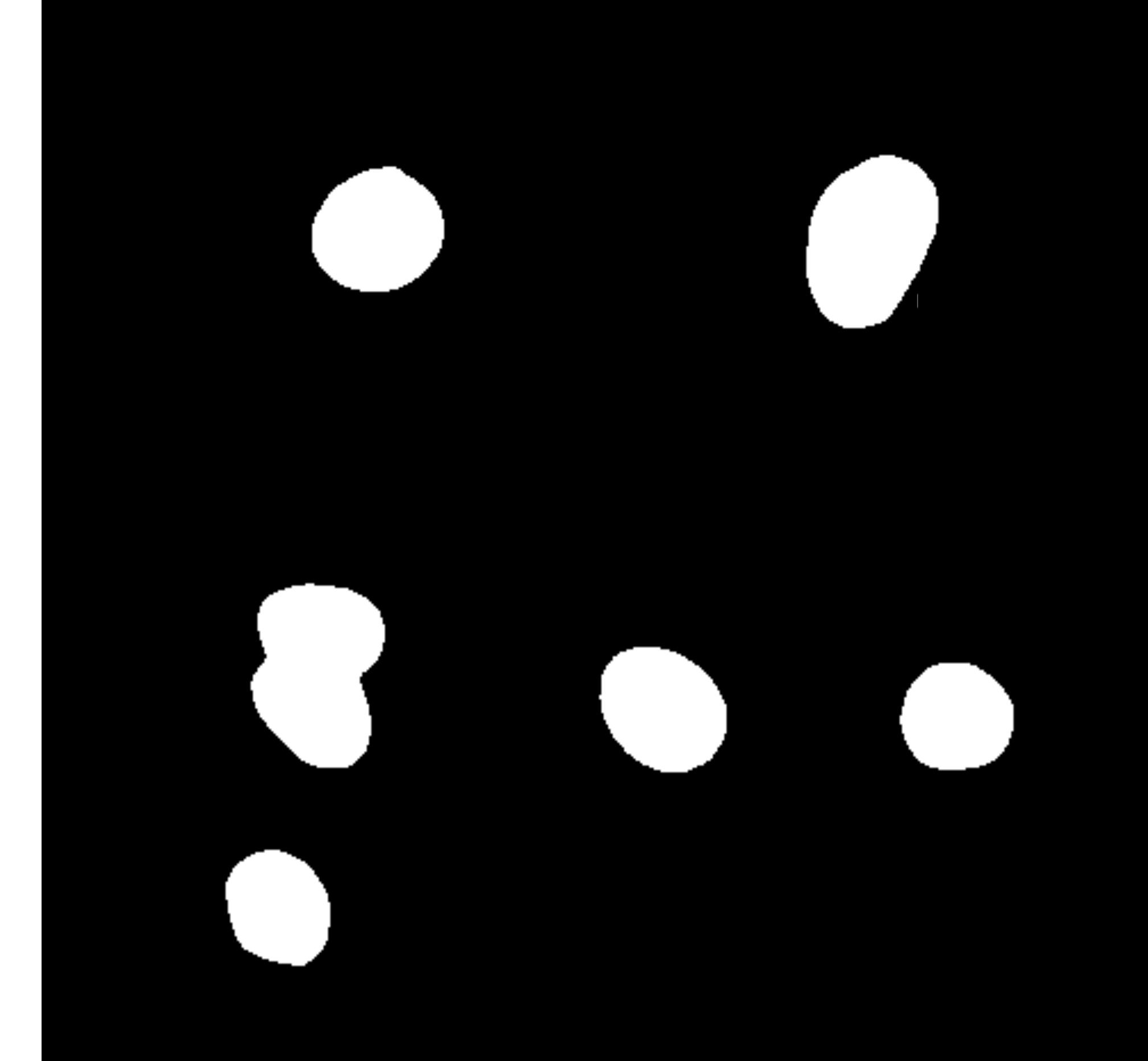
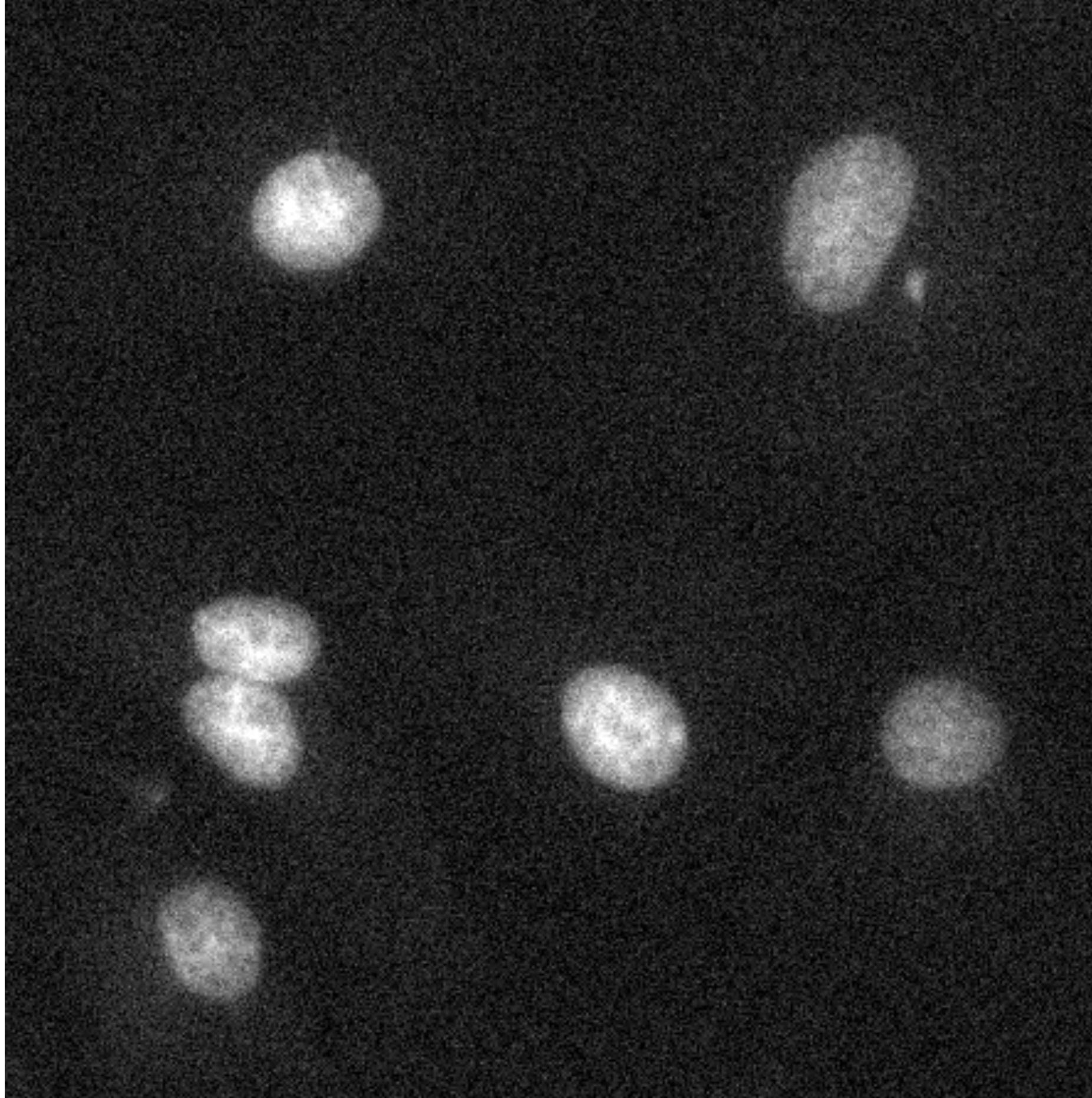
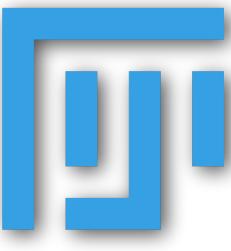
Export and Measure



Results			
	Area	Mean	X
0016	285	204.29474	197.
0028	278	174.84892	219.
0027	231	188.46753	45.0
0034	501	189.14172	174.
0041	660	171.69697	73.8
0048	228	195.89474	233.
0055	448	209.03571	138.

# Do you see an issue here?

Process and segment

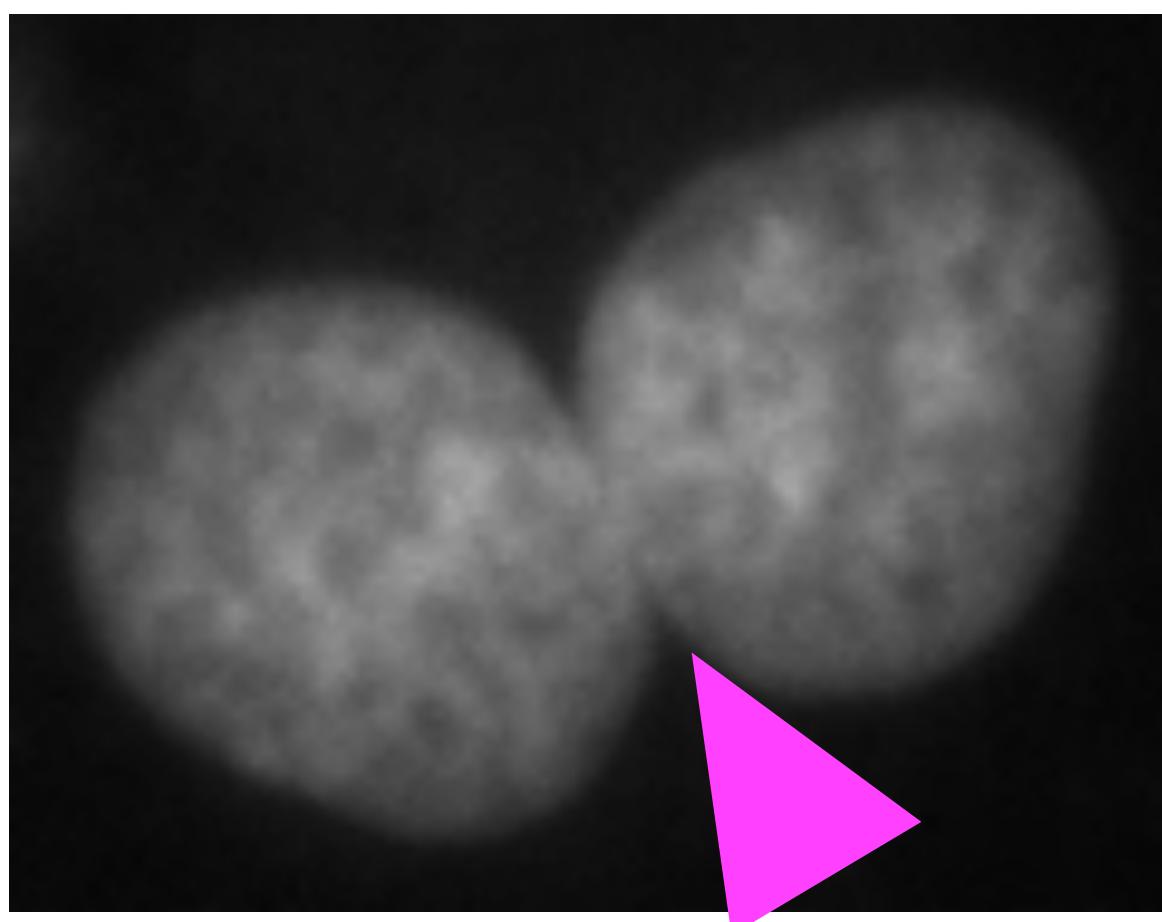


# Solution: Watershed

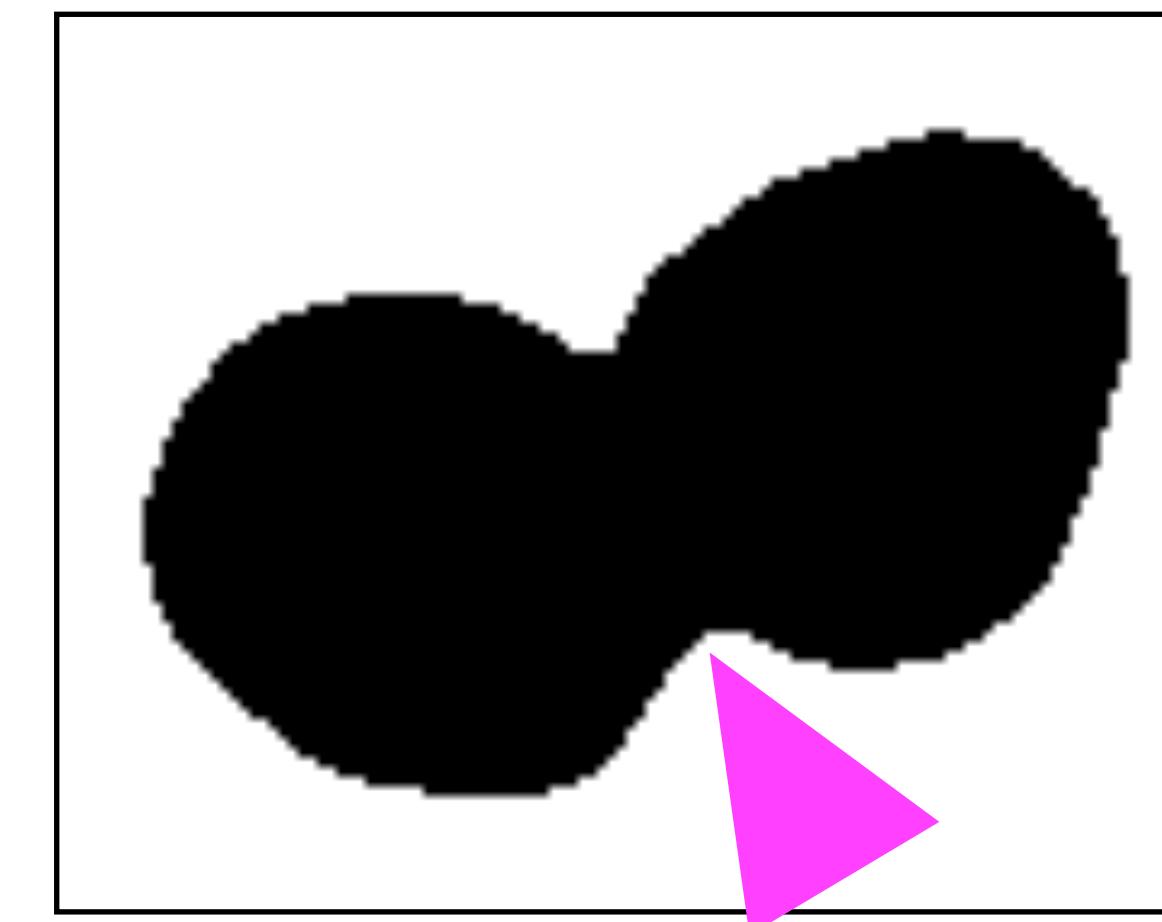
in **Fiji**: Process > Binary > Watershed

**Watershed** is a useful algorithm to try to **separate touching objects**.

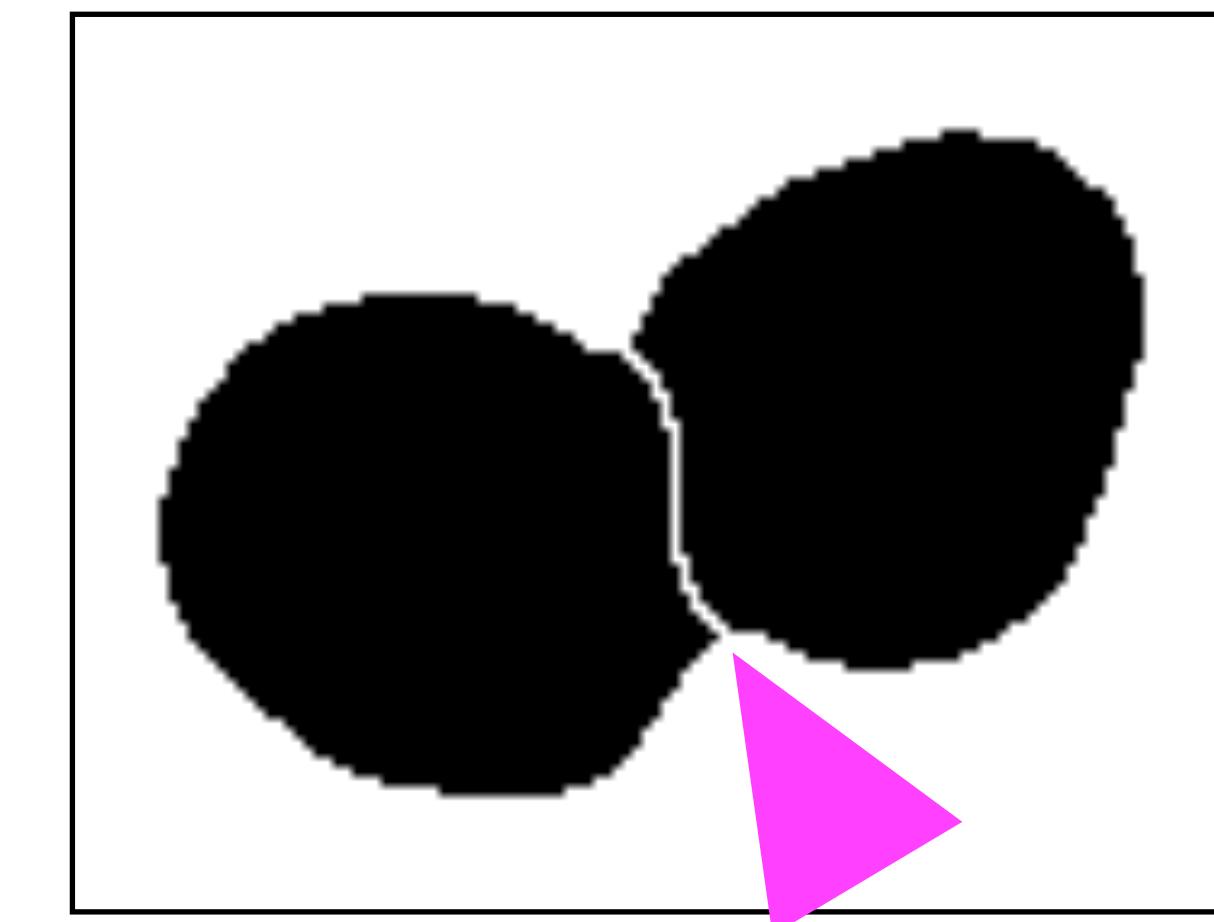
Image



Binary Mask

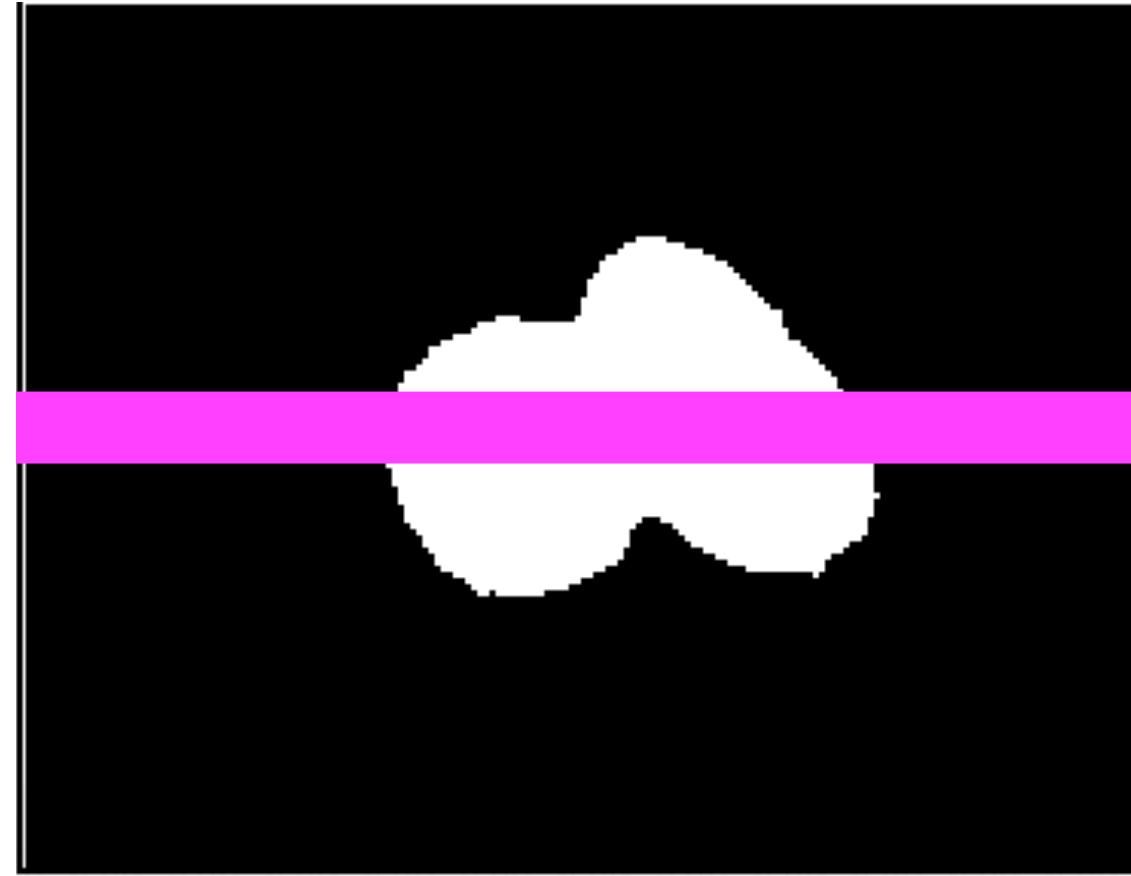


Watershed

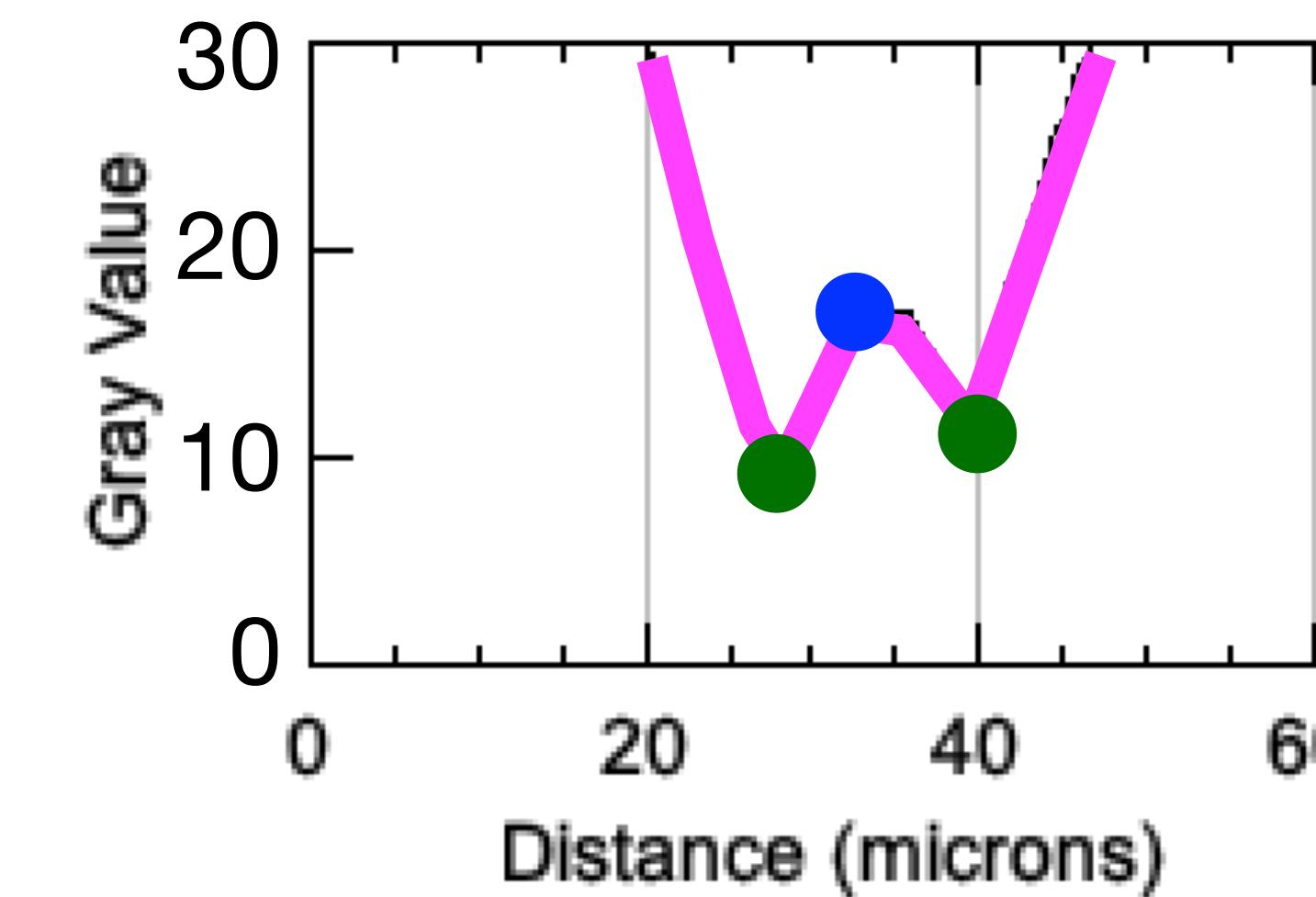
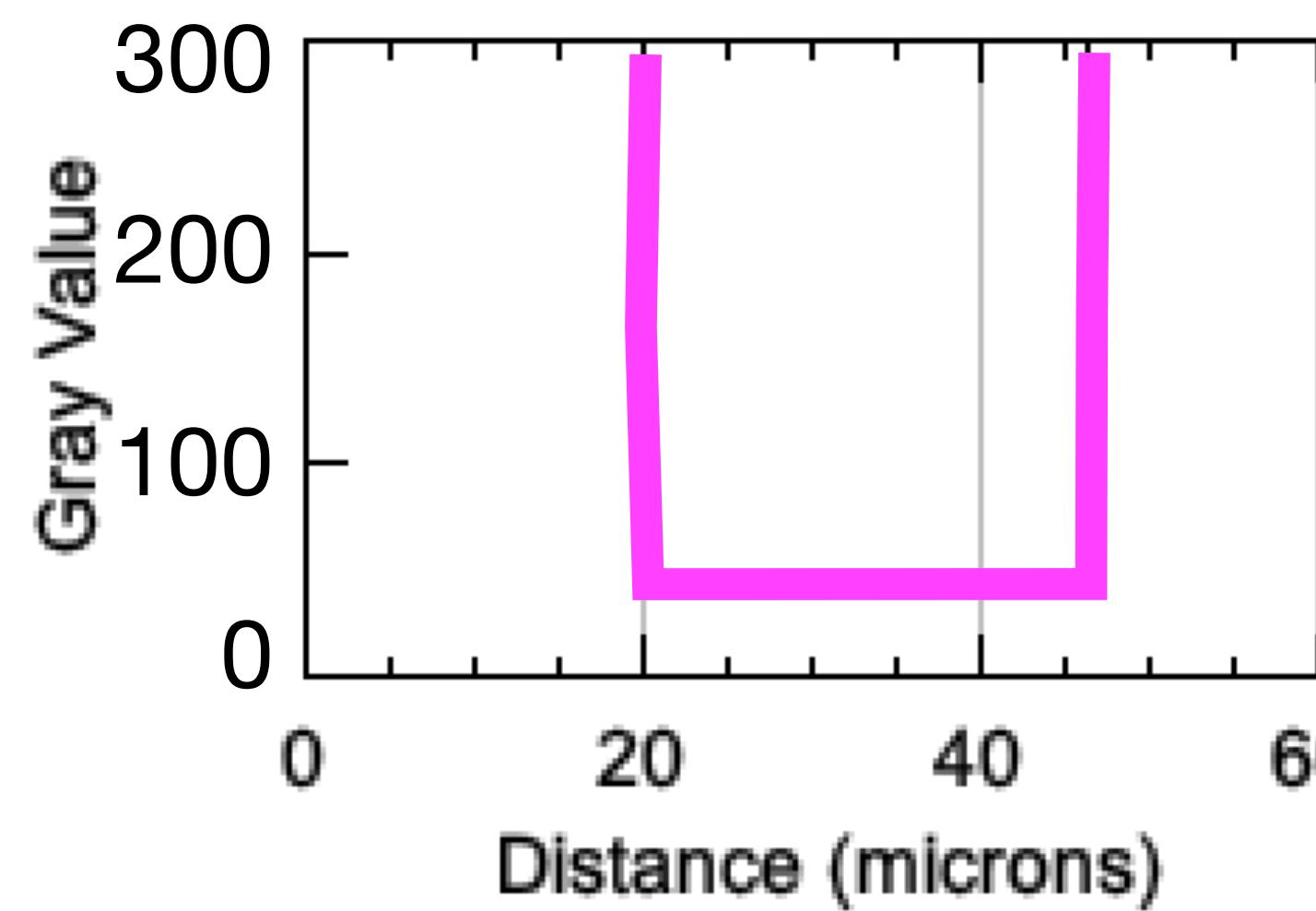
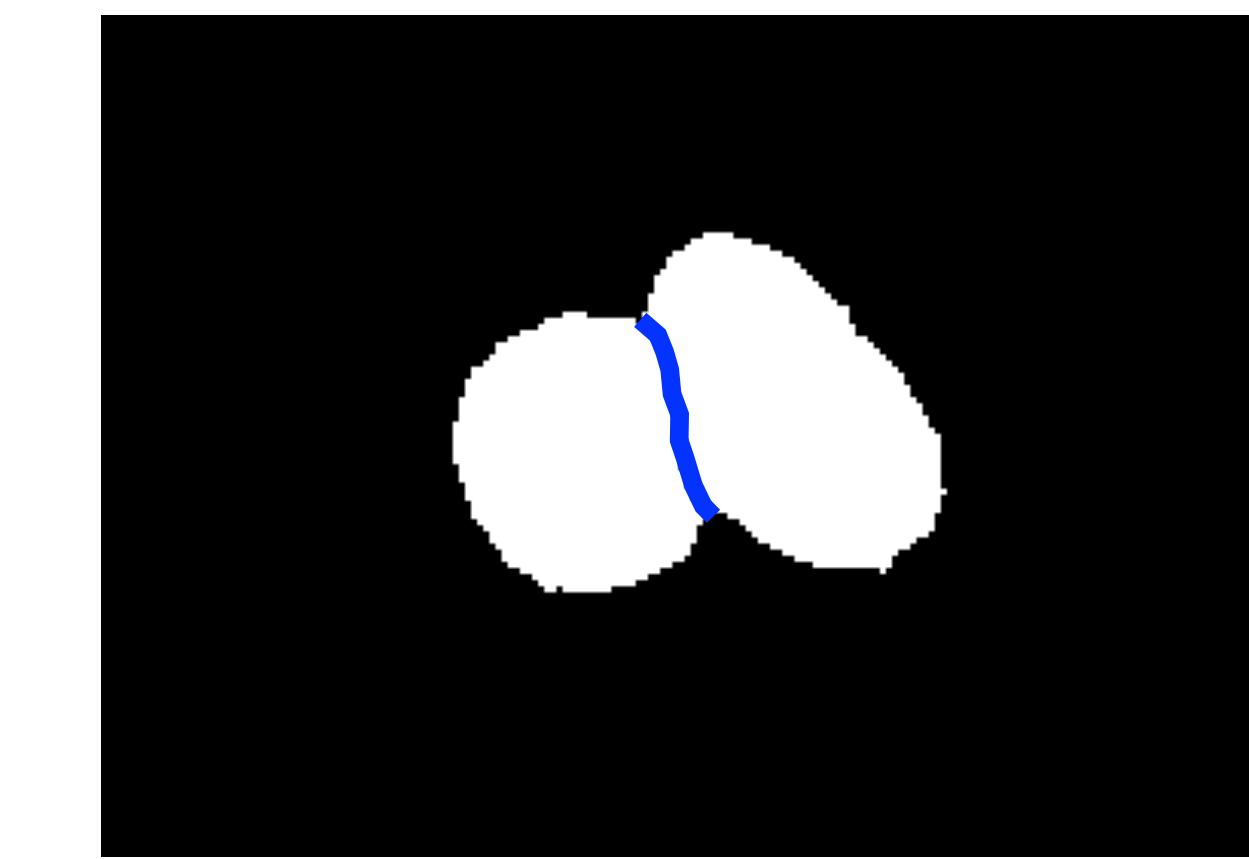
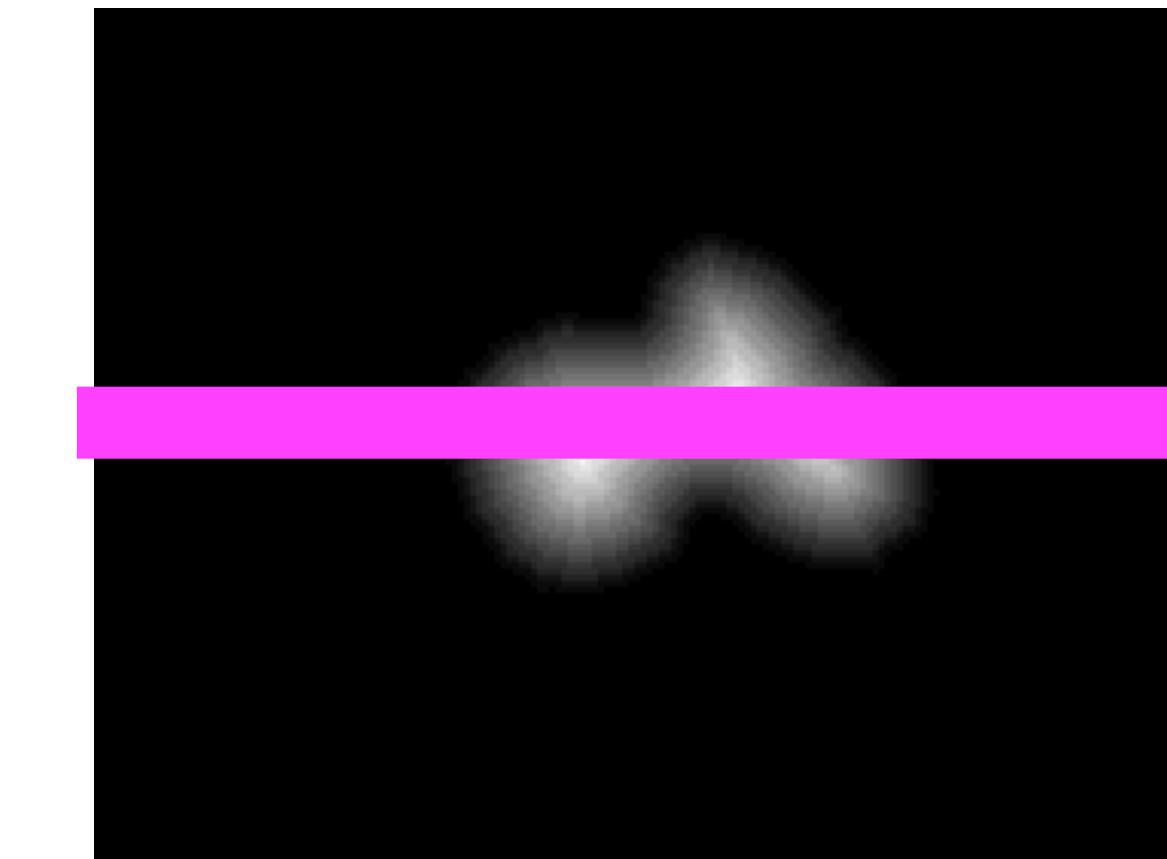


# Solution: Watershed

Binary mask

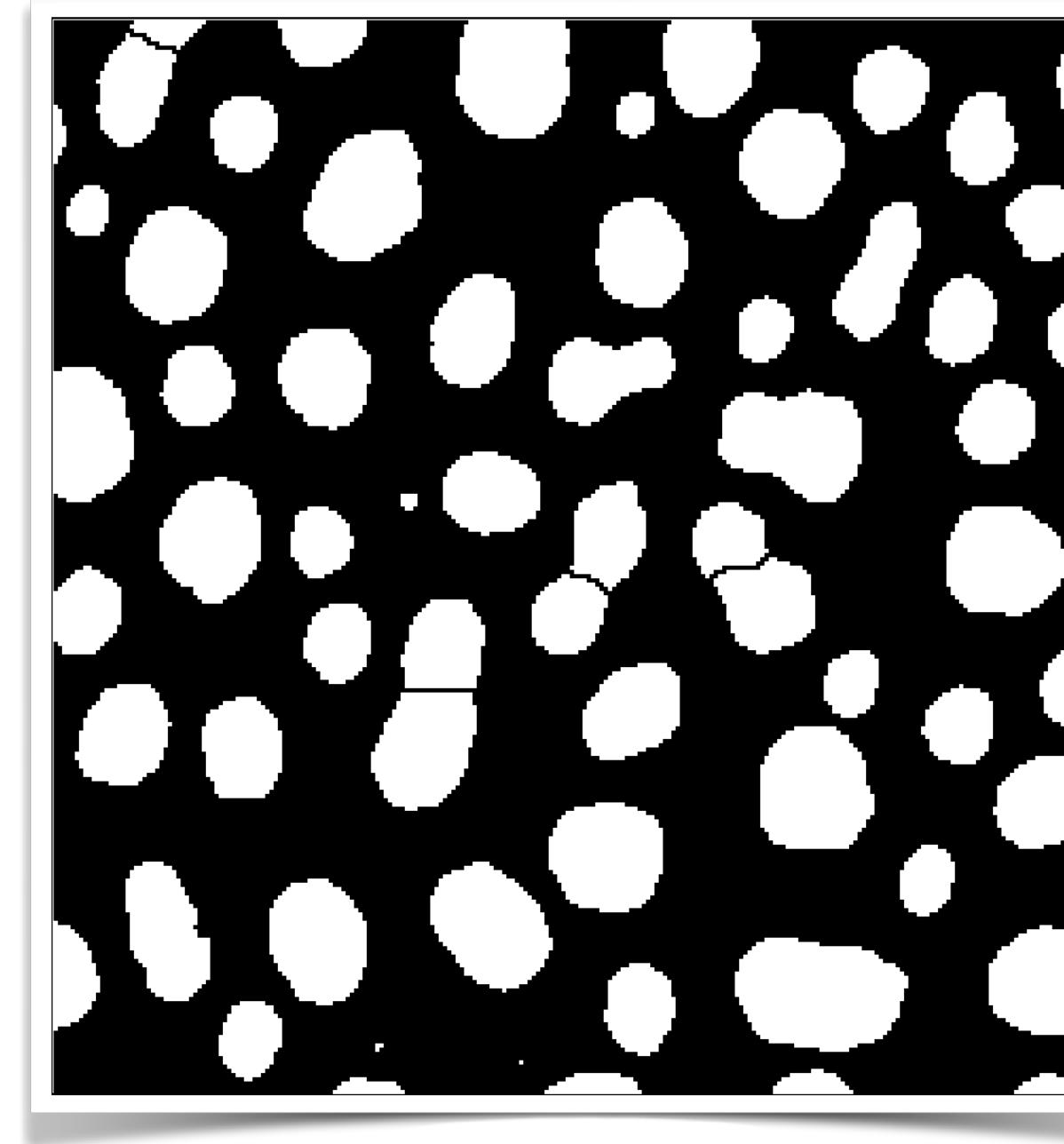


Distance transform

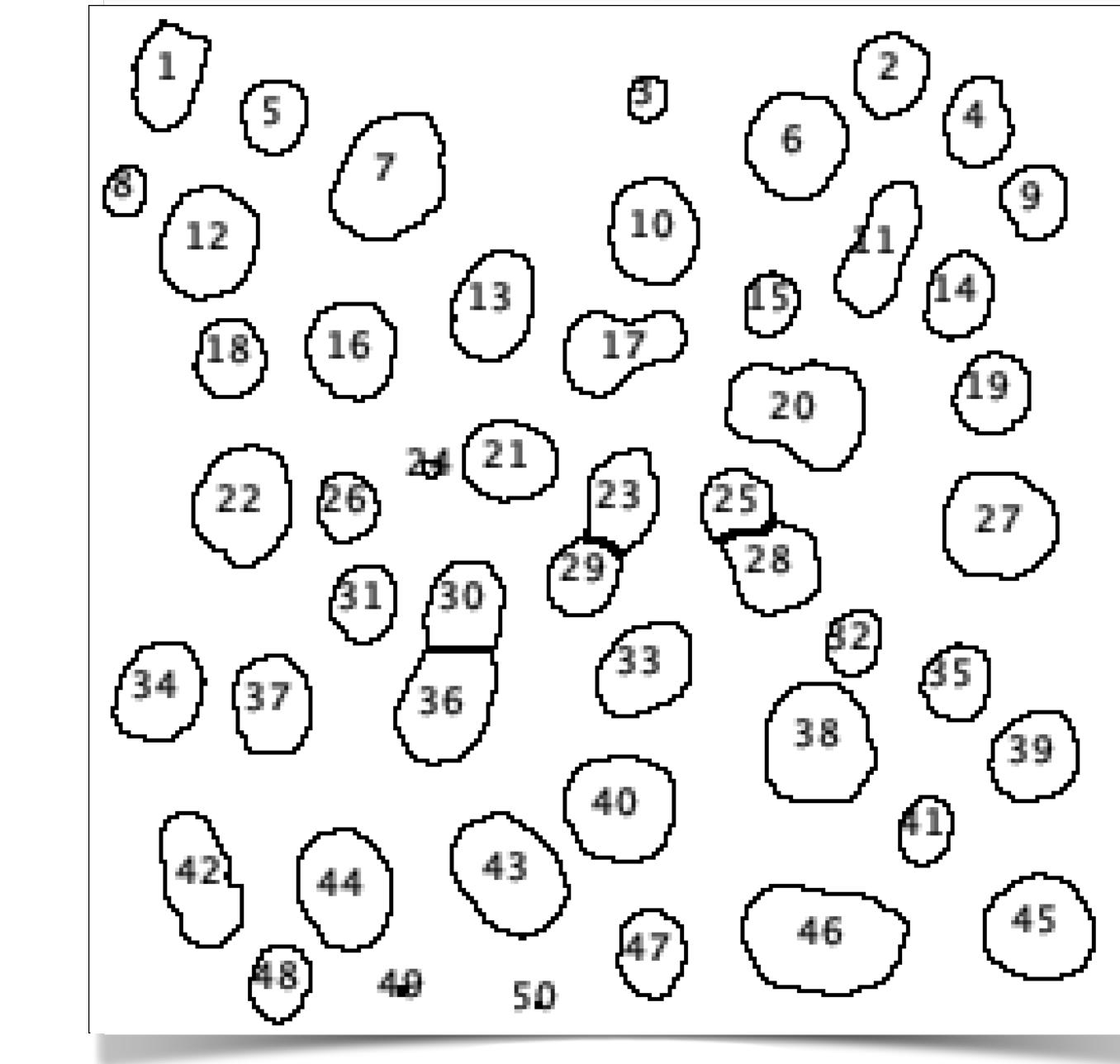


# From binary image to instance segmentation

Process and segment



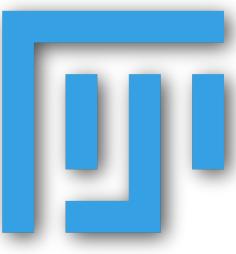
Binary mask



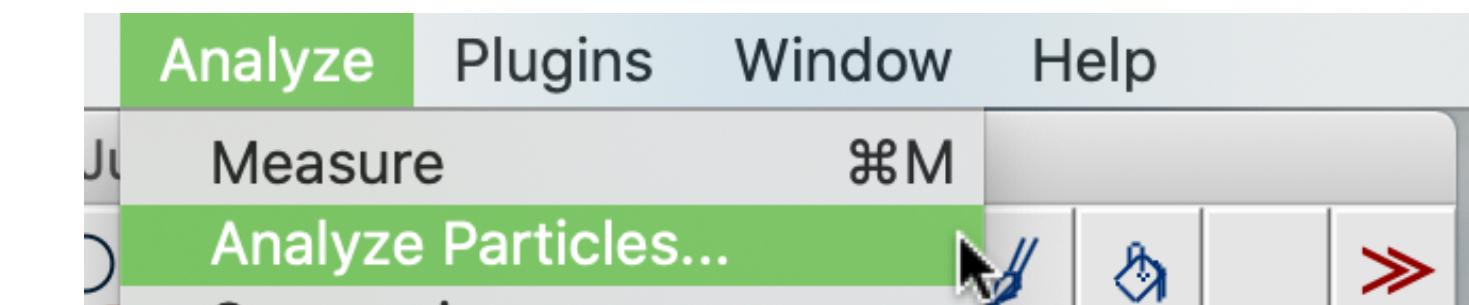
Instance segmentation

# Analyze particles

Process and segment

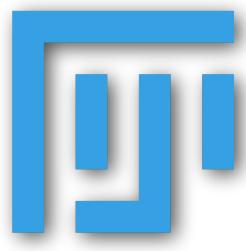


in **Fiji**: **Analyze > Analyze Particles...**

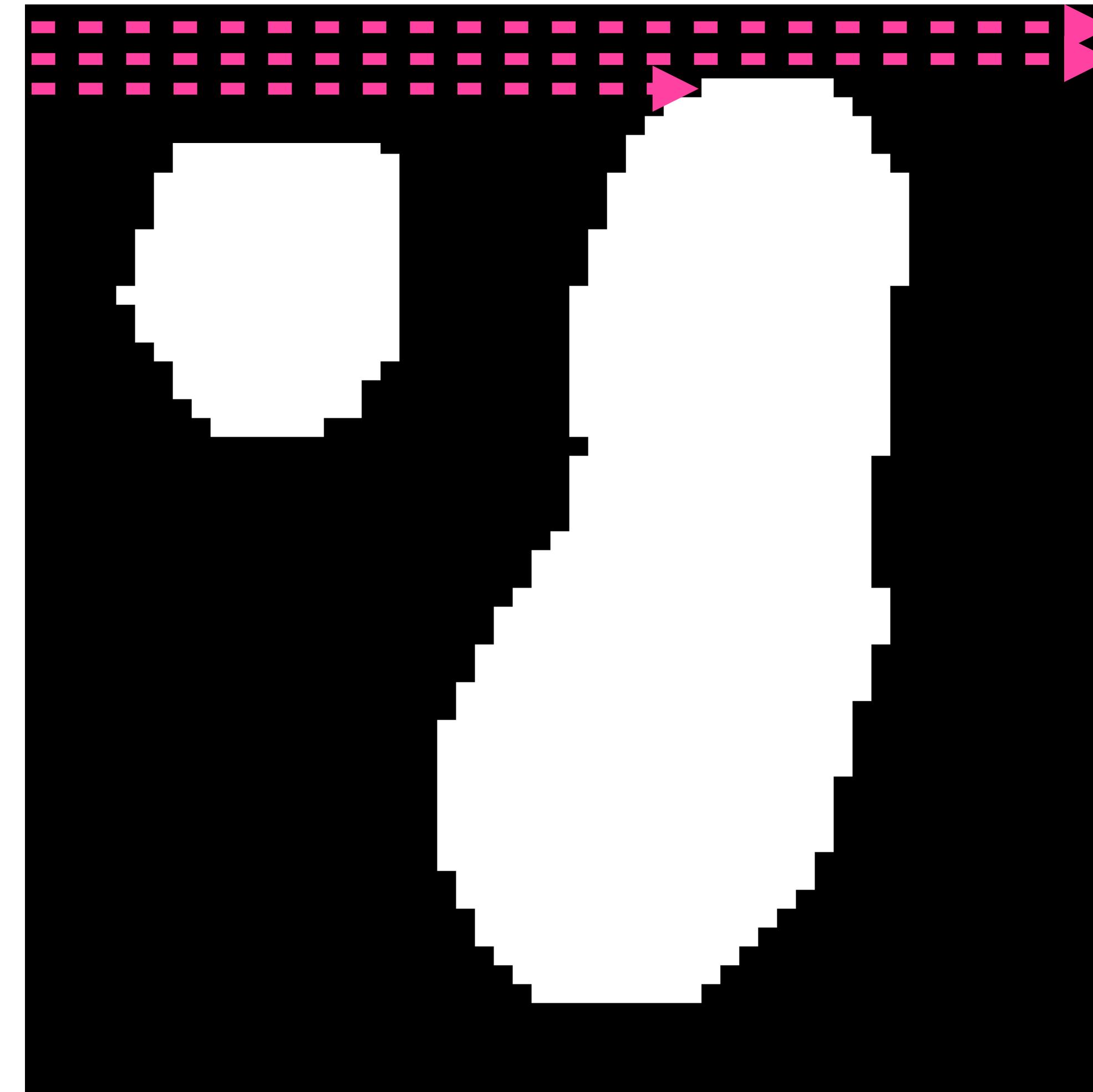


# Analyze particles — underlying principles

Process and segment



in Fiji:  
**Analyze**  
  > **Analyze Particles...**

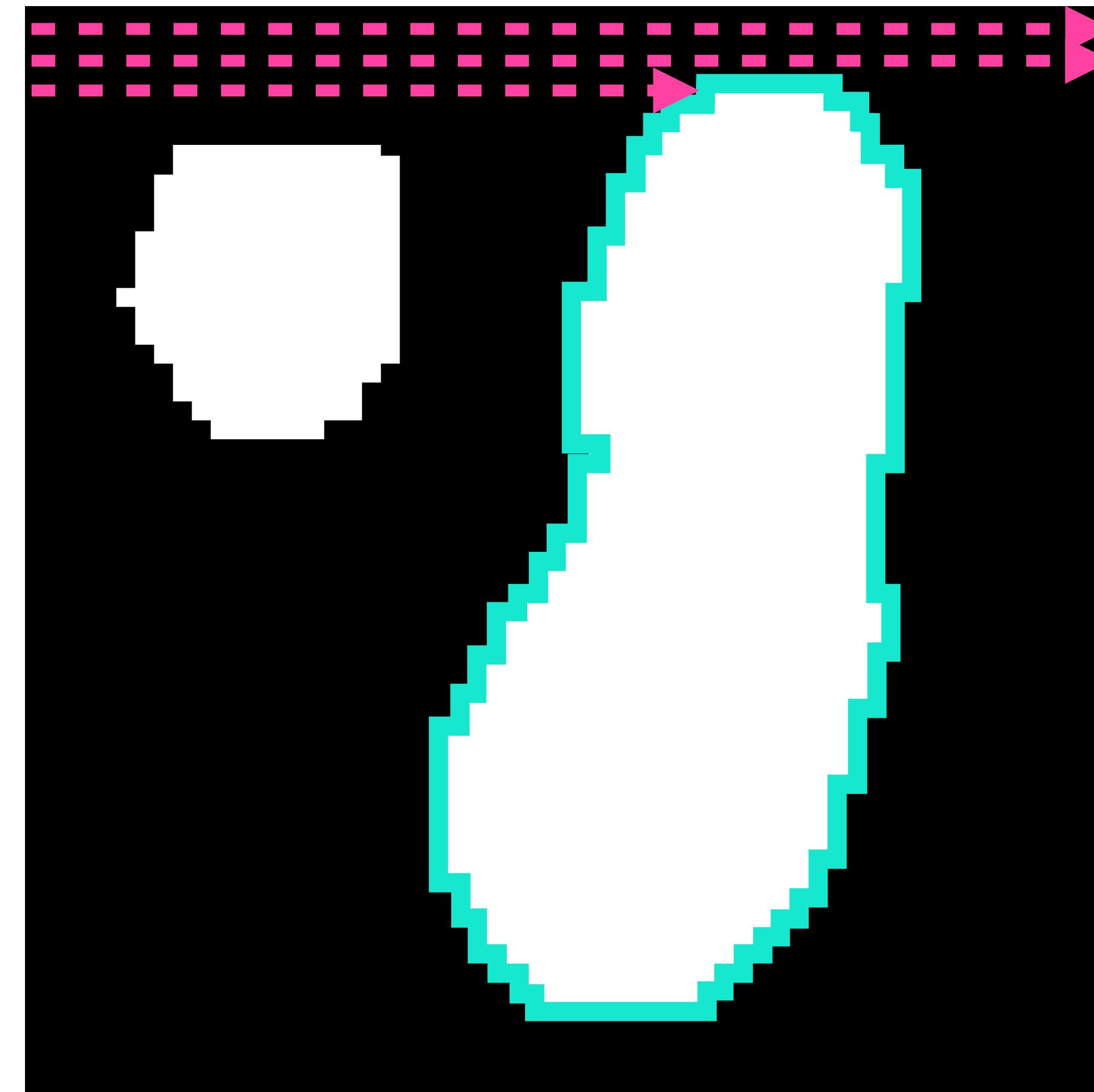


# Analyze particles — underlying principles

Process and segment

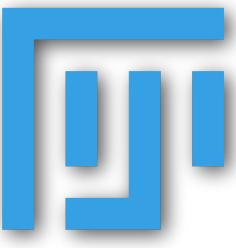


in Fiji:  
**Analyze**  
-> **Analyze Particles...**

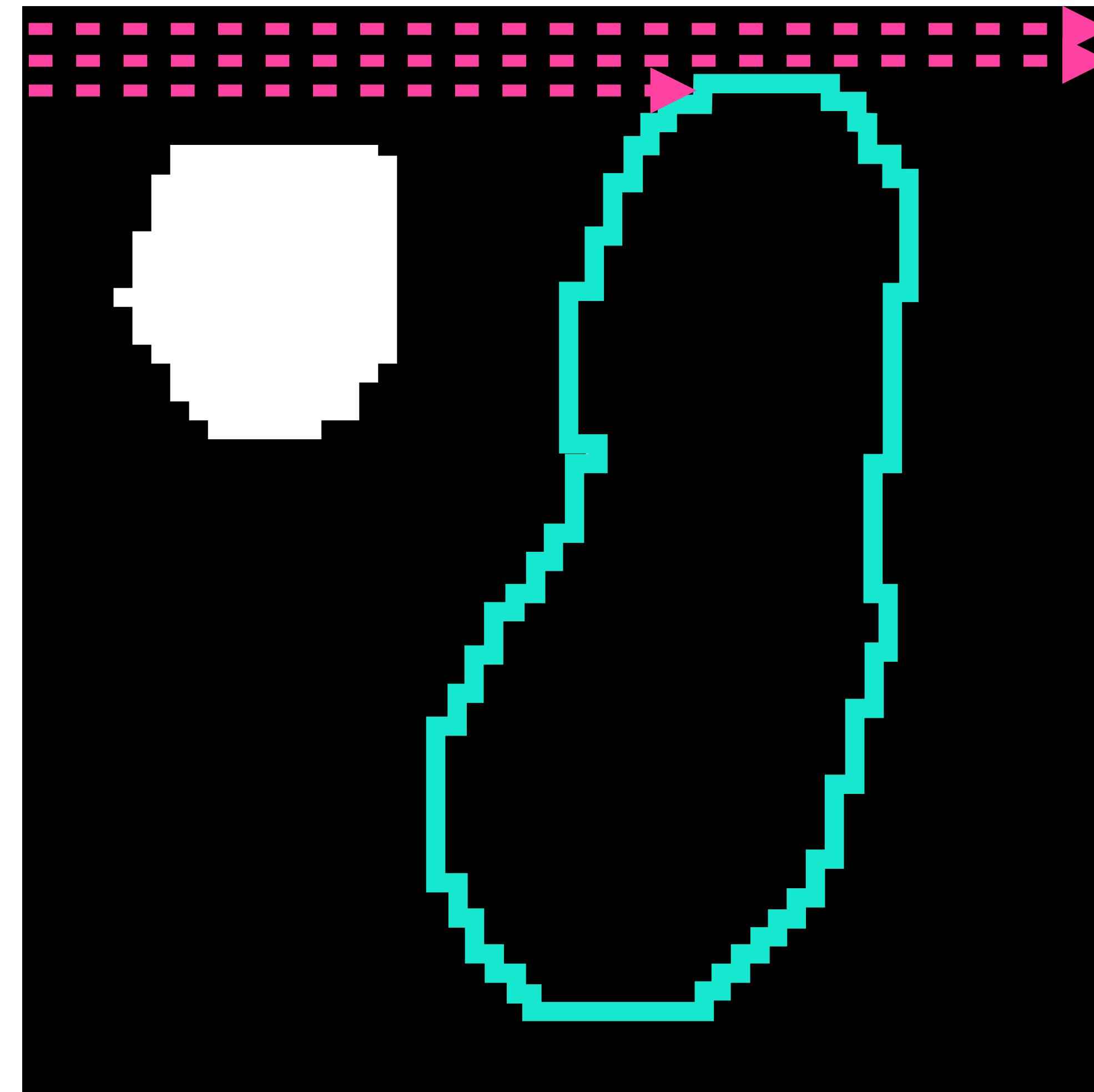


# Analyze particles — underlying principles

Process and segment

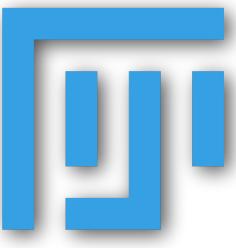


in Fiji:  
**Analyze**  
-> **Analyze Particles...**

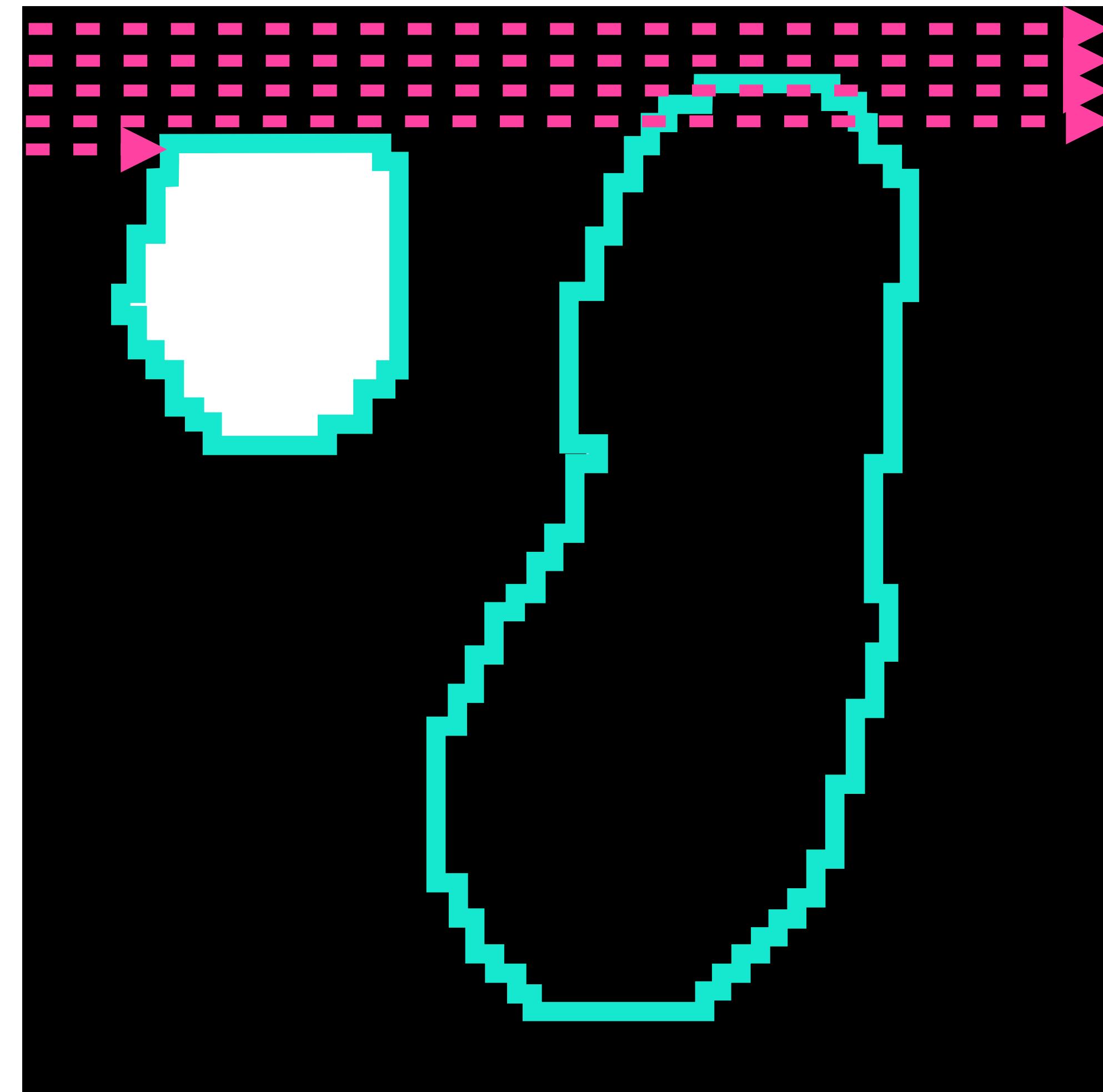


# Analyze particles — underlying principles

Process and segment

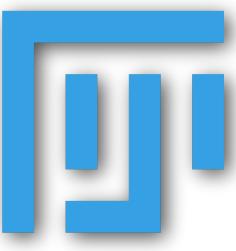


in Fiji:  
**Analyze**  
-> **Analyze Particles...**

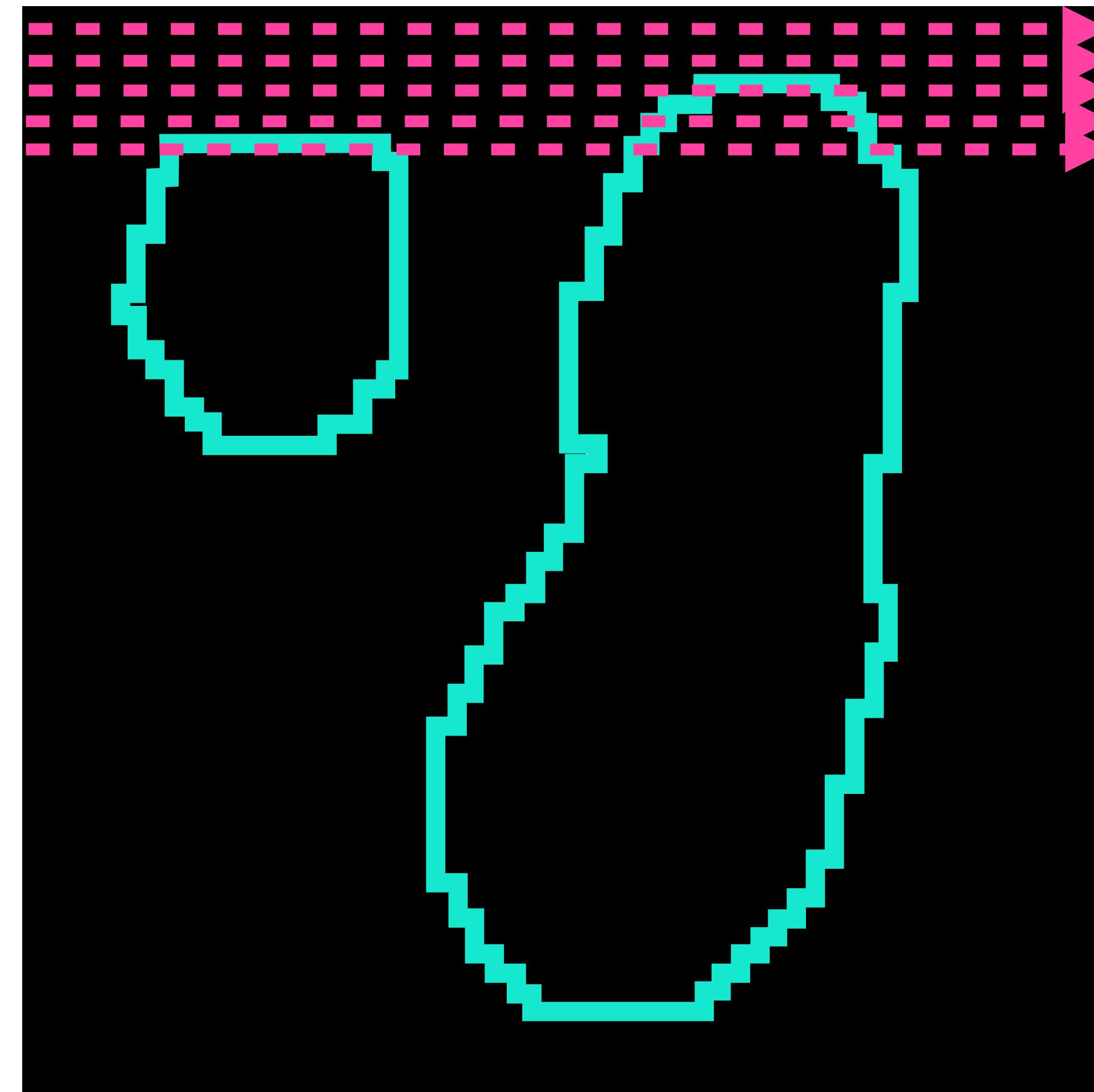


# Analyze particles — underlying principles

Process and segment

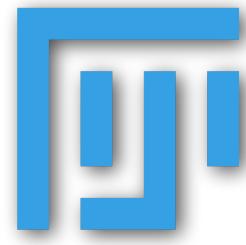


in Fiji:  
**Analyze**  
-> **Analyze Particles...**

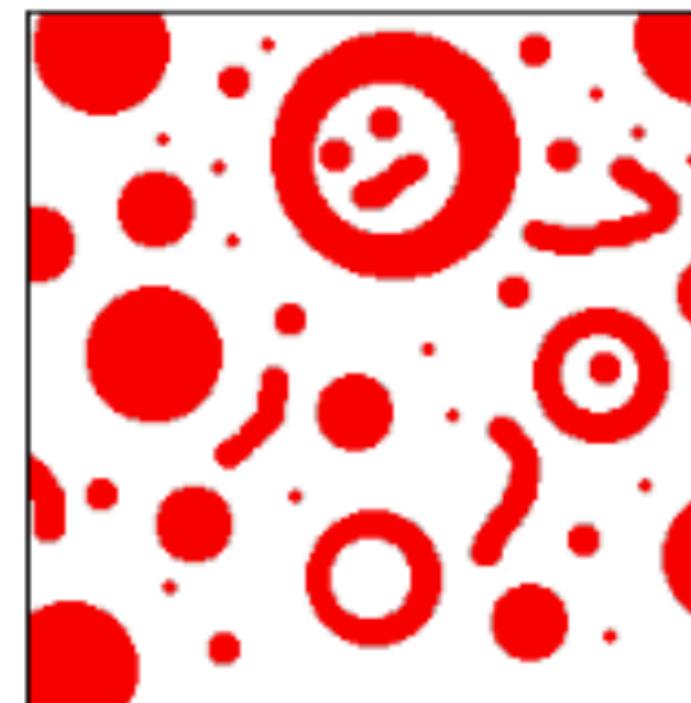


# Analyze particles: Select for morphology

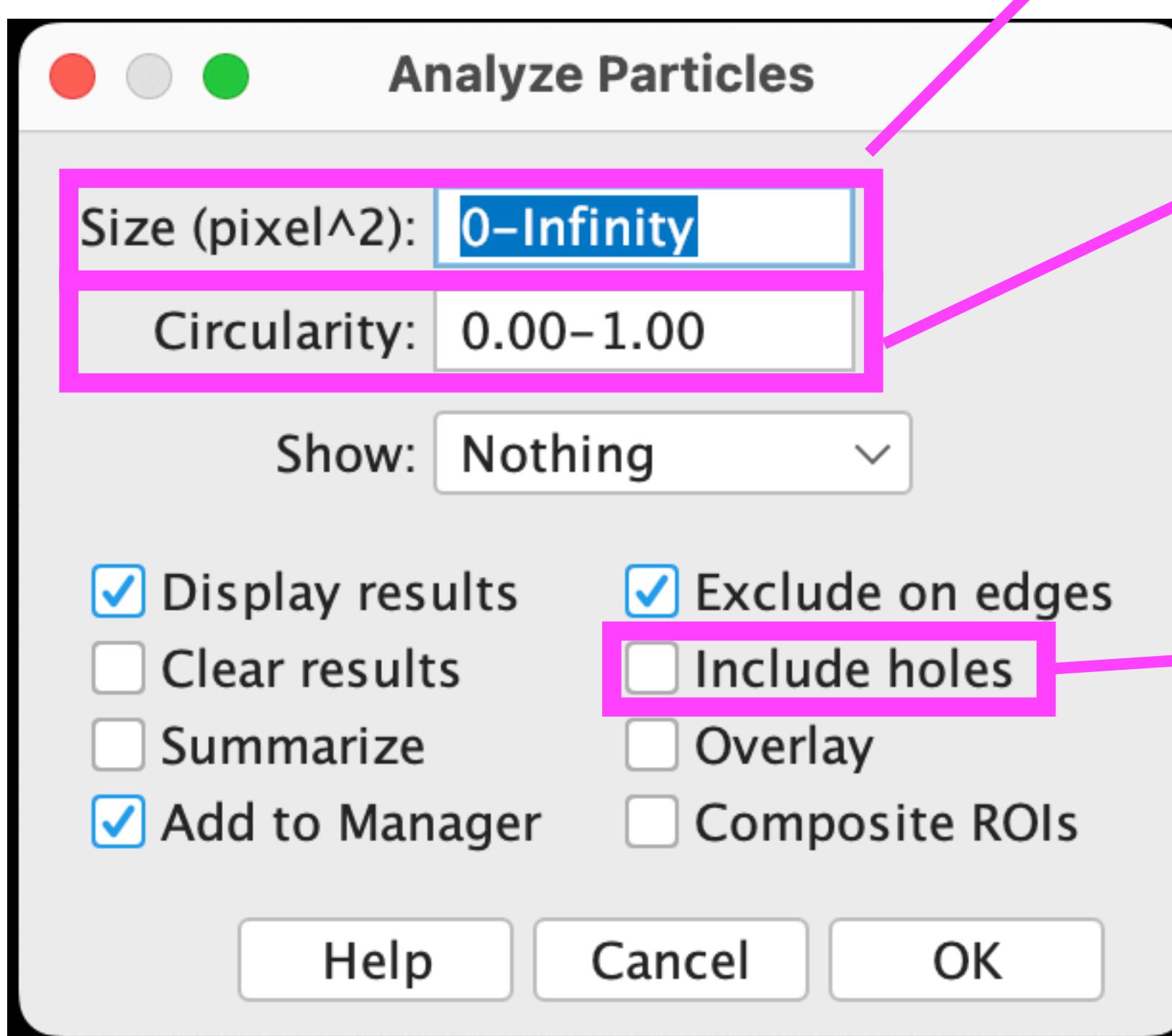
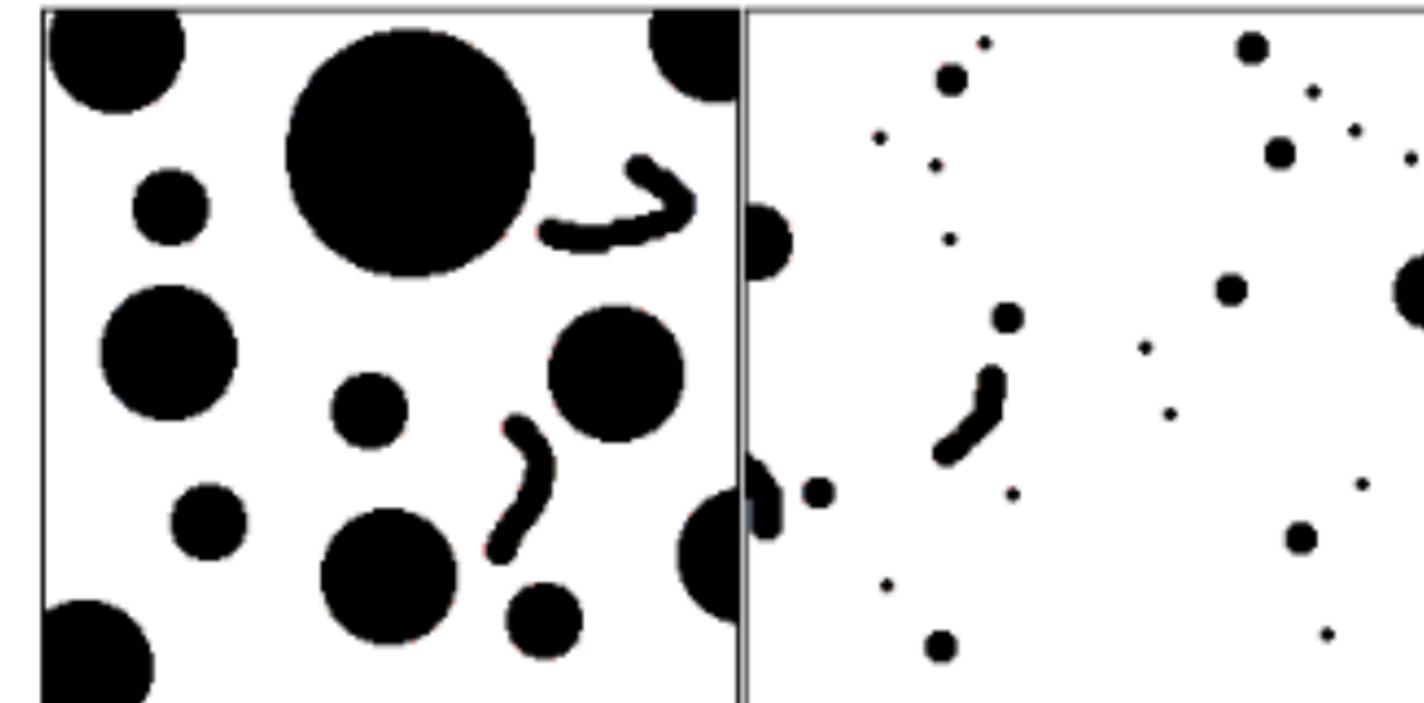
Process and segment



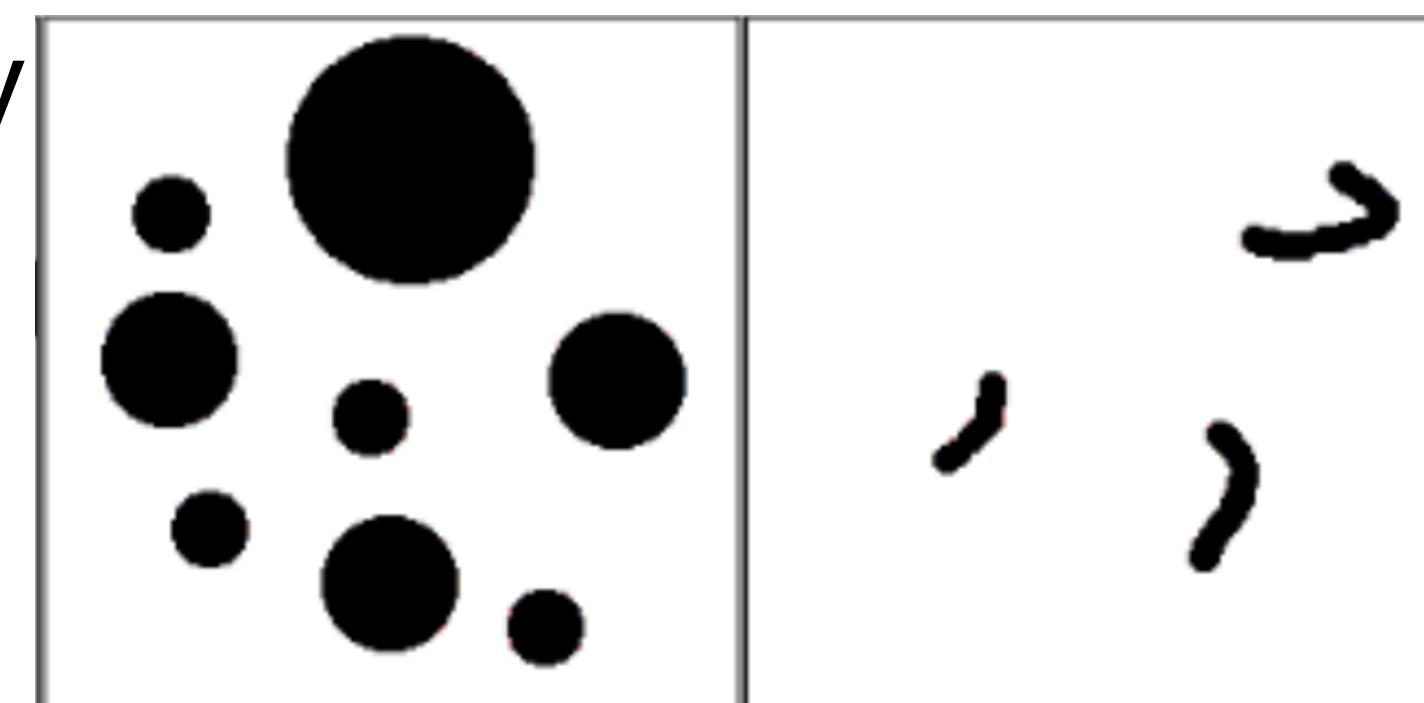
in Fiji:  
Analyze  
> Analyze Particles...



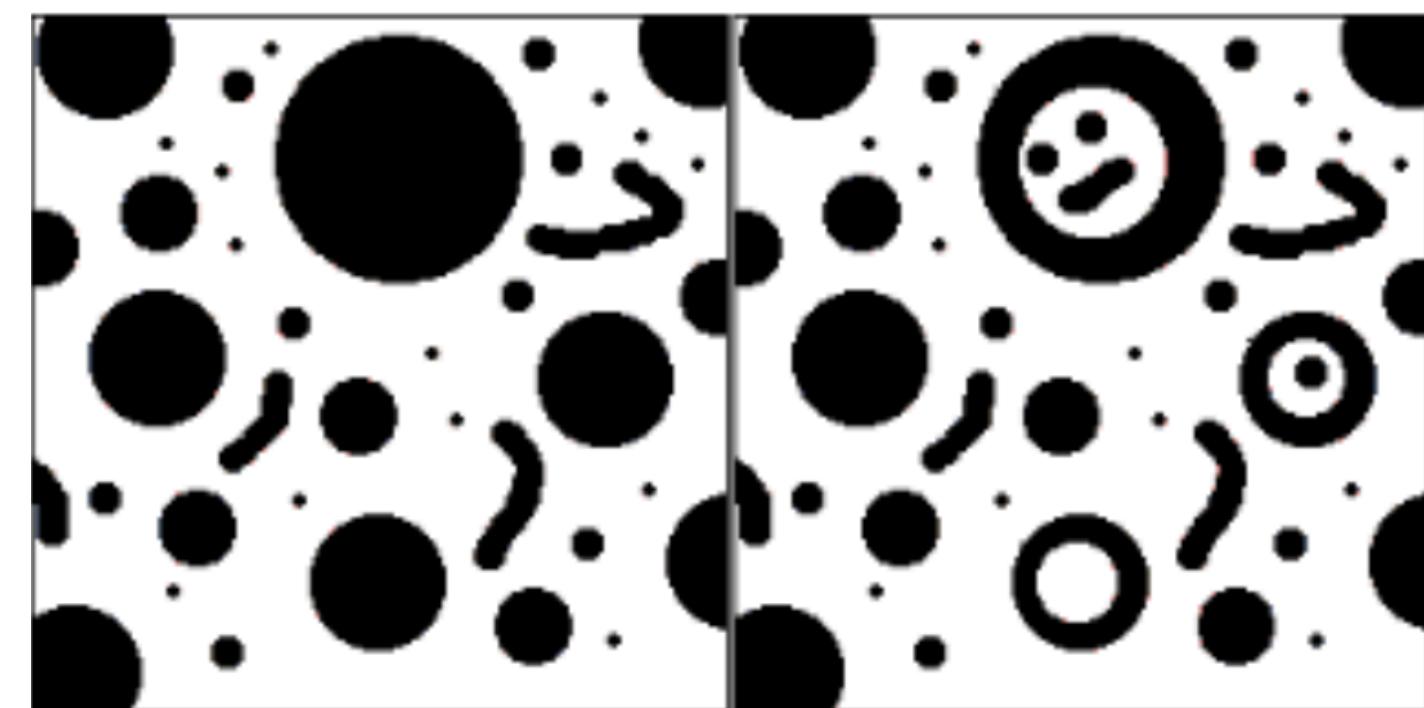
Size



Circularity

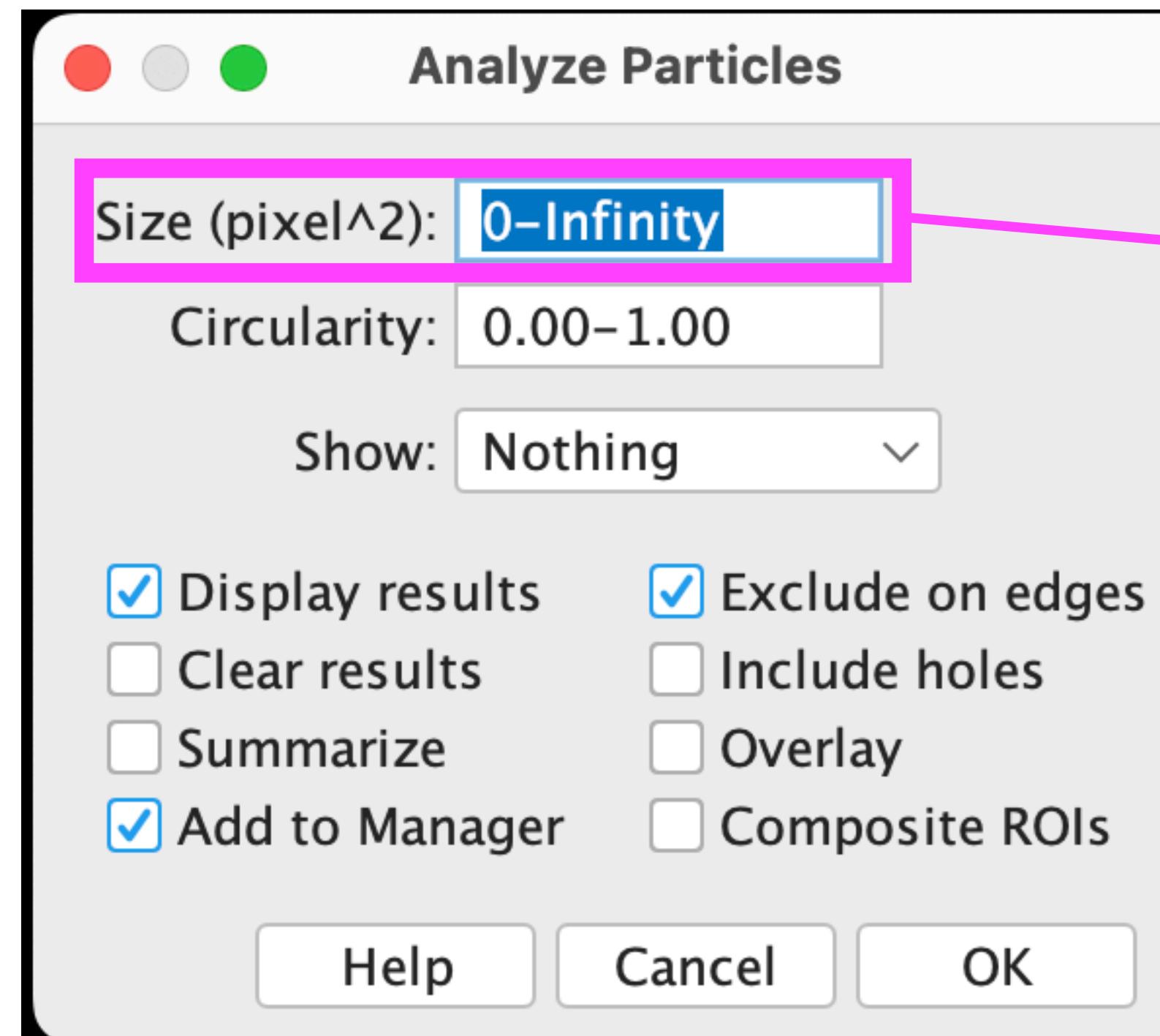


Fill

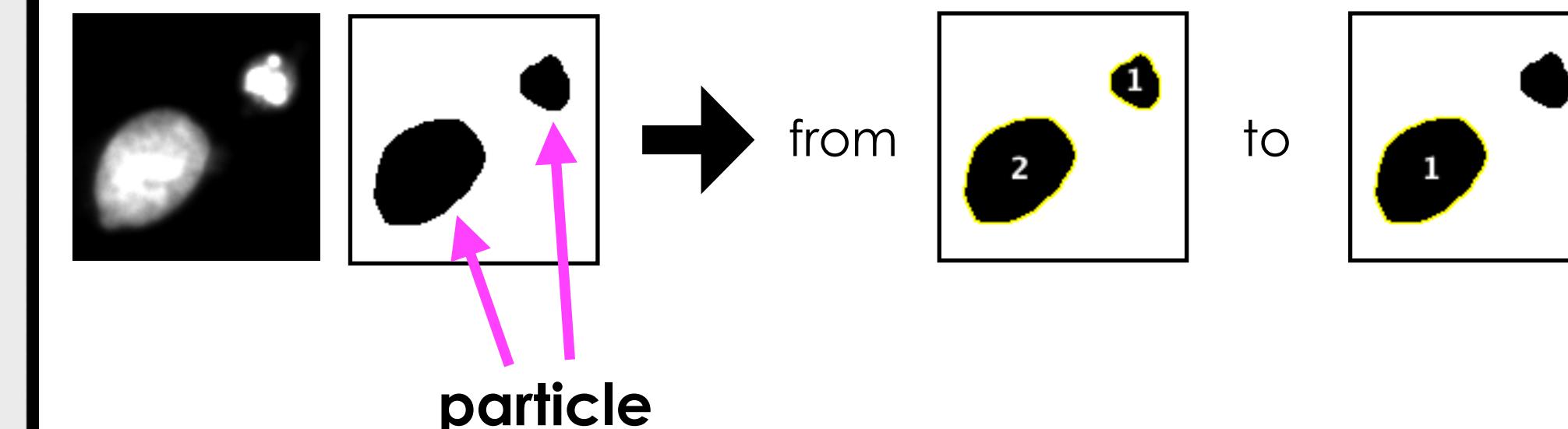


# Analyze particles: Size

in Fiji: Analyze > Analyze Particles...

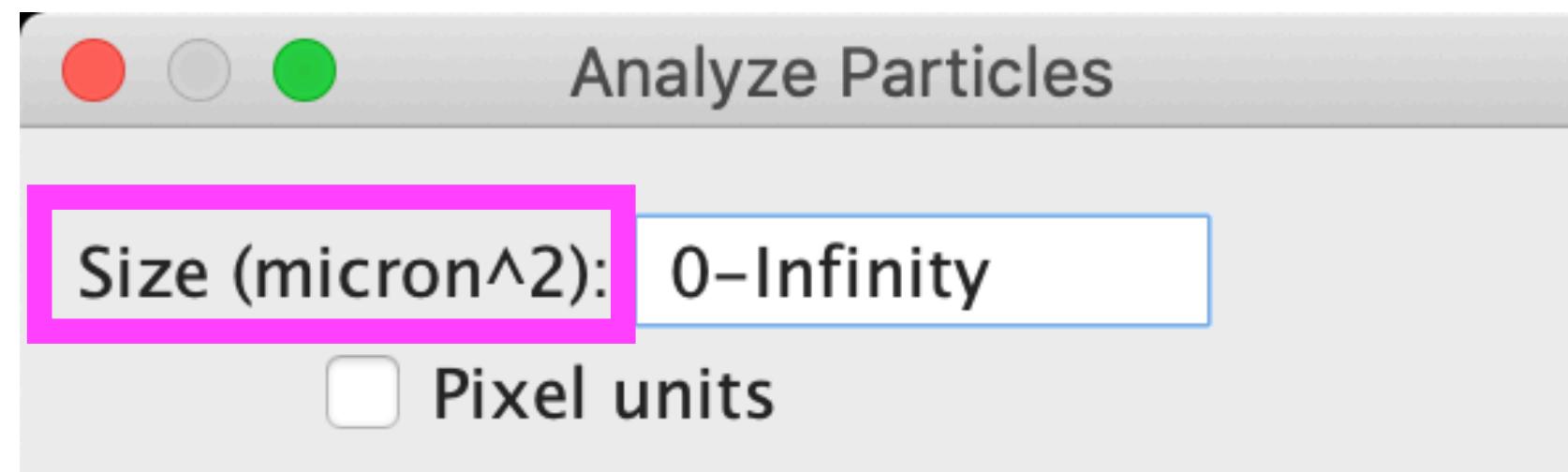


Size range of the particles that you want to detect.

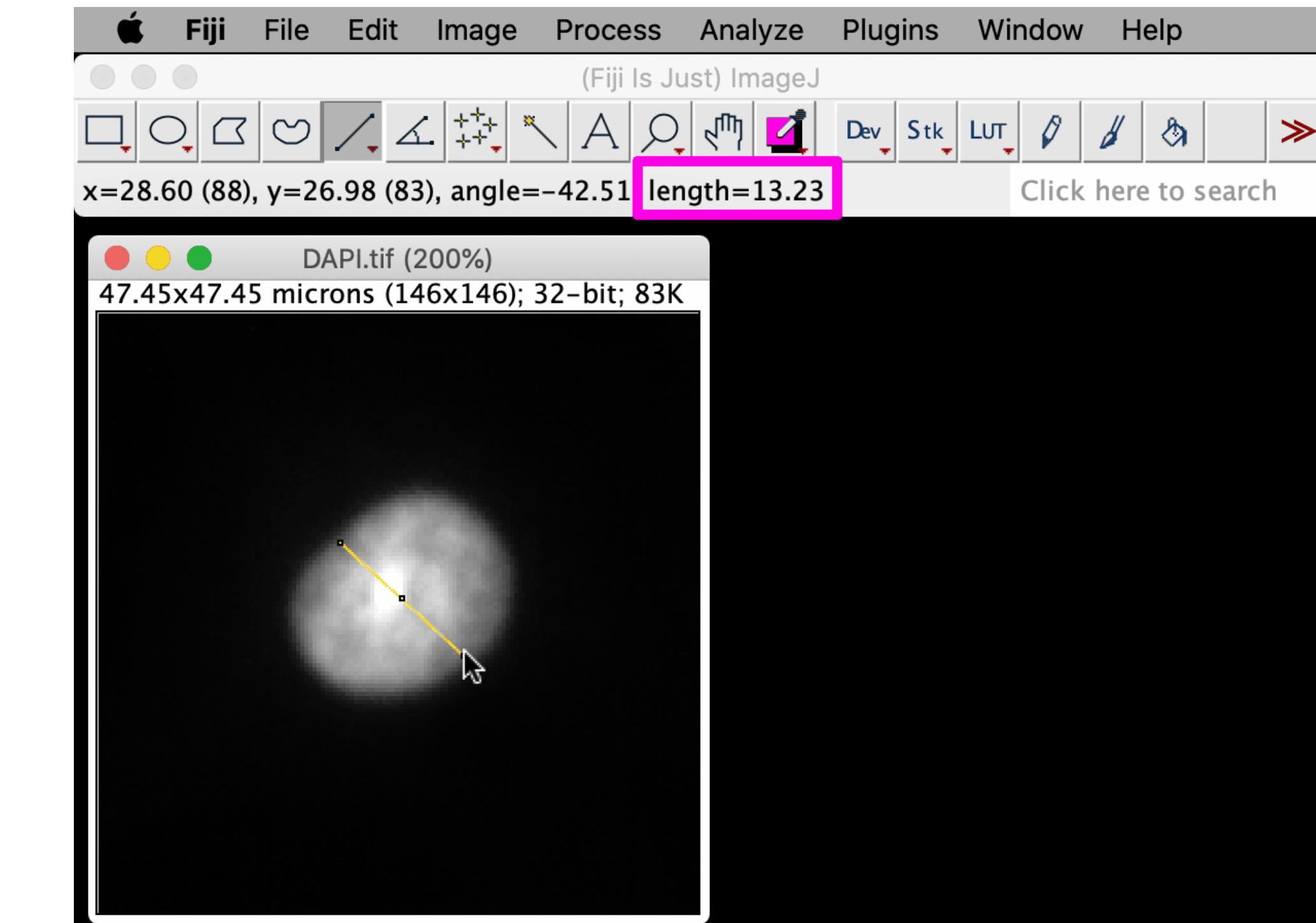


# Analyze particles: Size

Process and segment

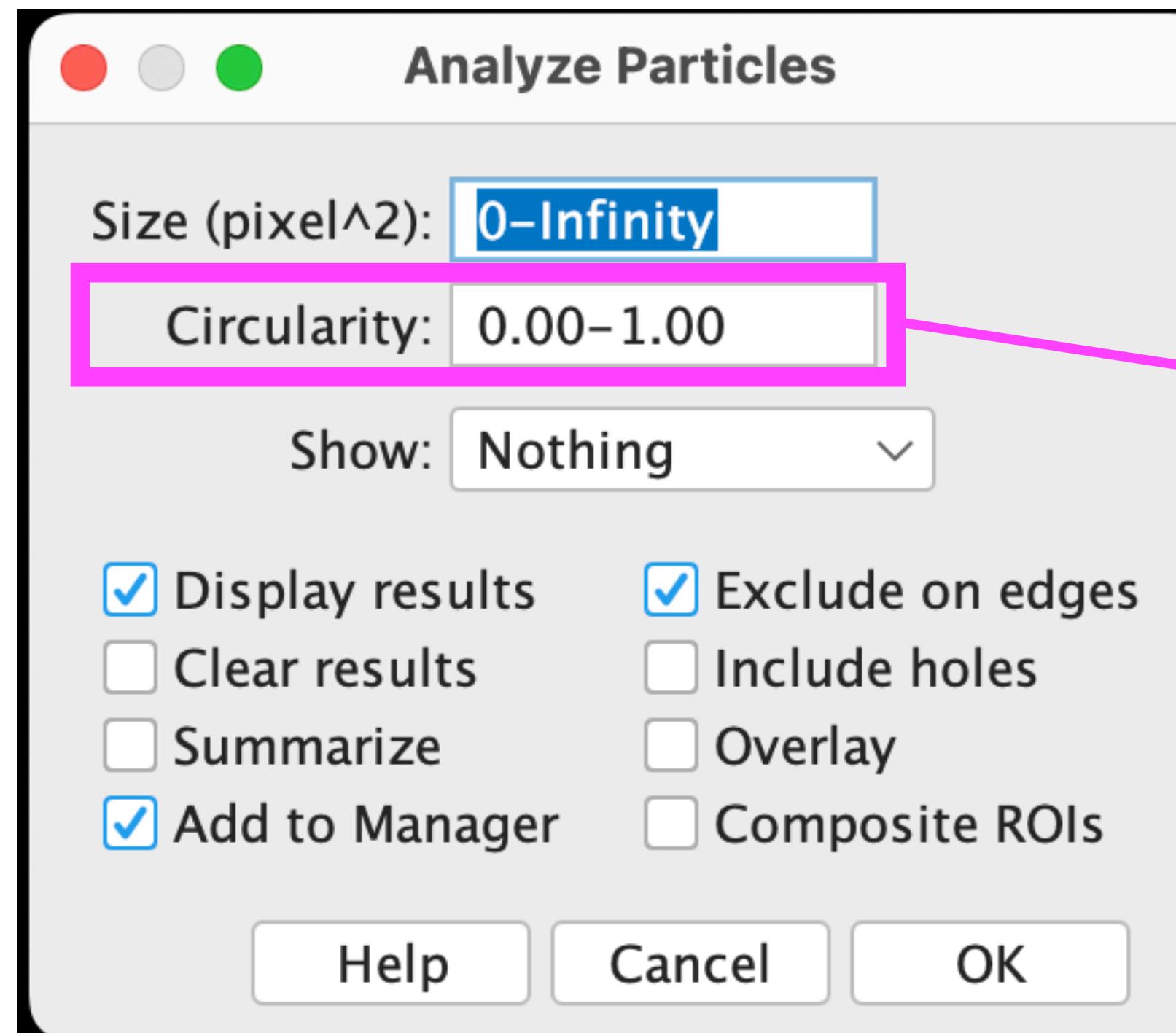


the **size range** of the **particles** that you want to detect.



# Analyze particles: Circularity

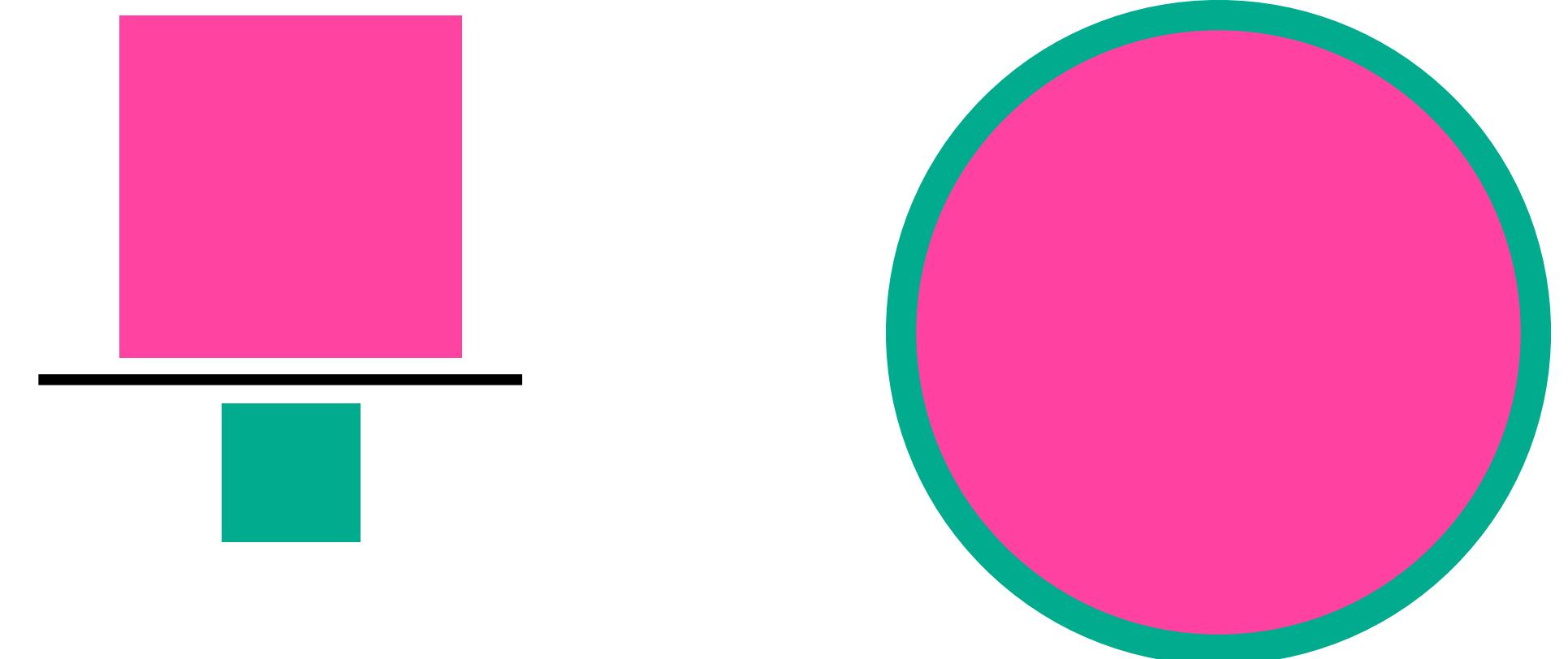
in Fiji: Analyze > Analyze Particles...



Circularity of the **particles** that you want to detect.

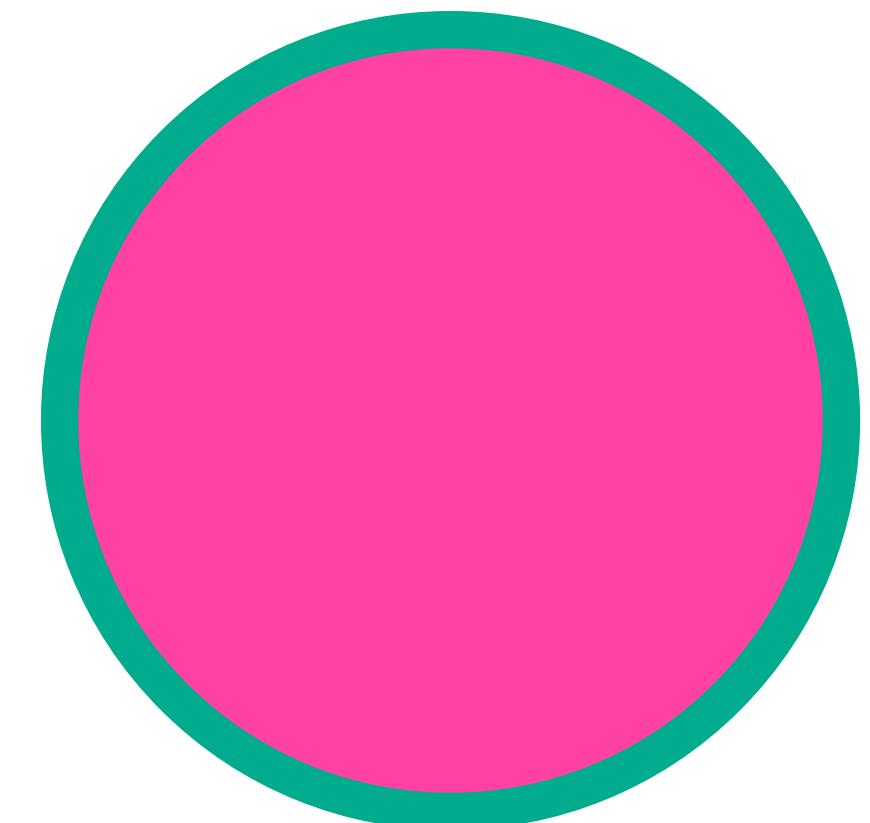
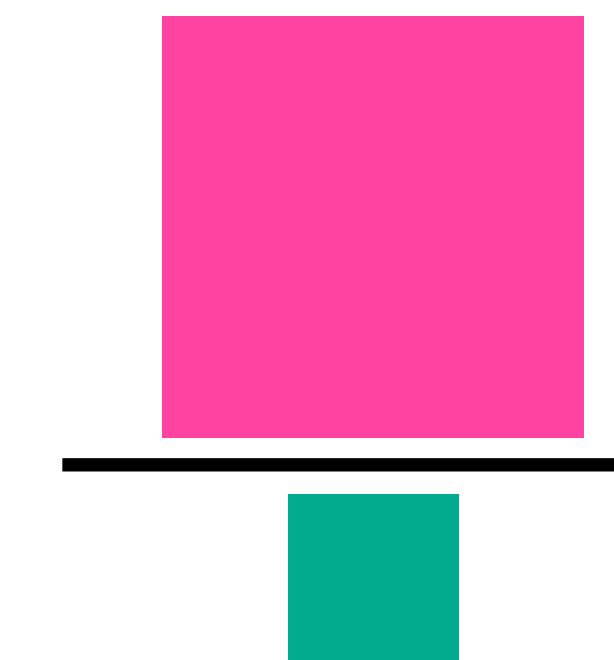
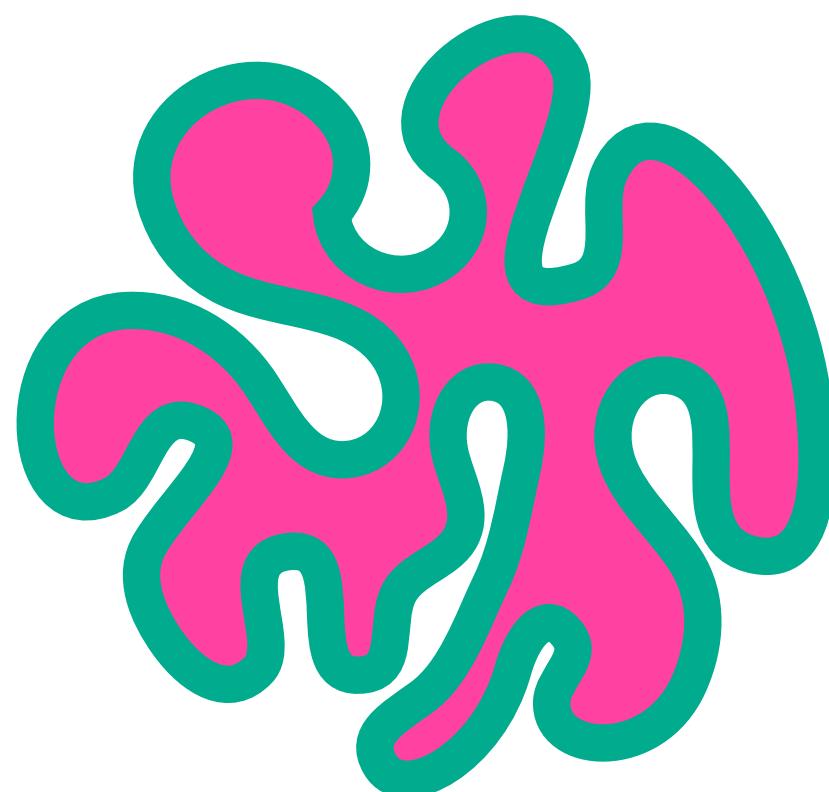
# Analyze particles: Circularity

$$4\pi \times \frac{\text{area}}{\text{perimeter}^2} = 1$$



# Analyze particles: Circularity

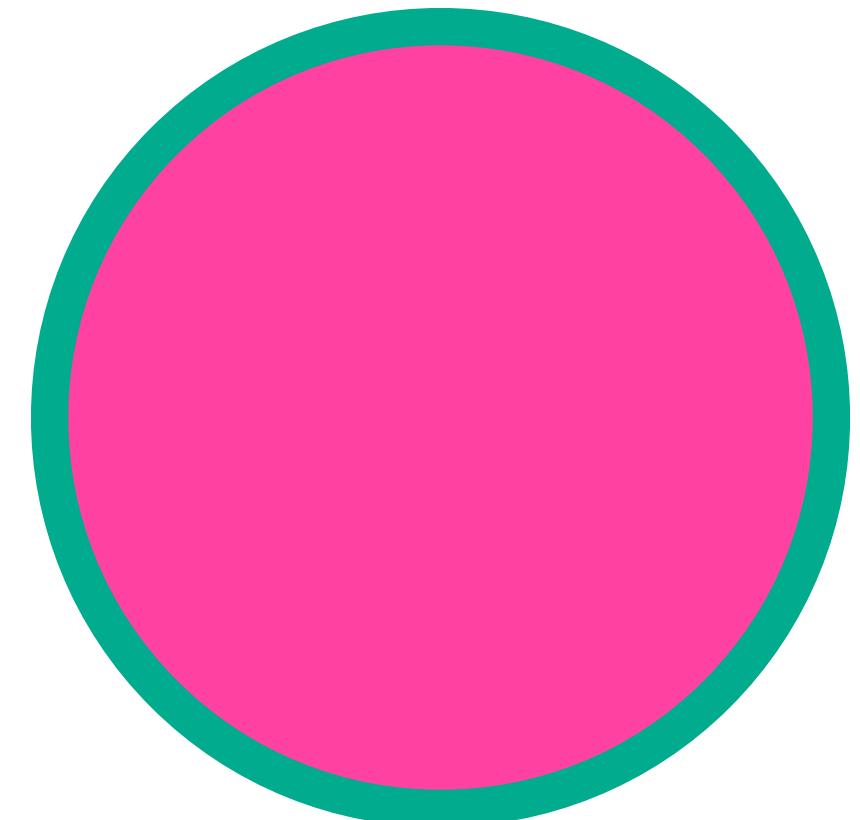
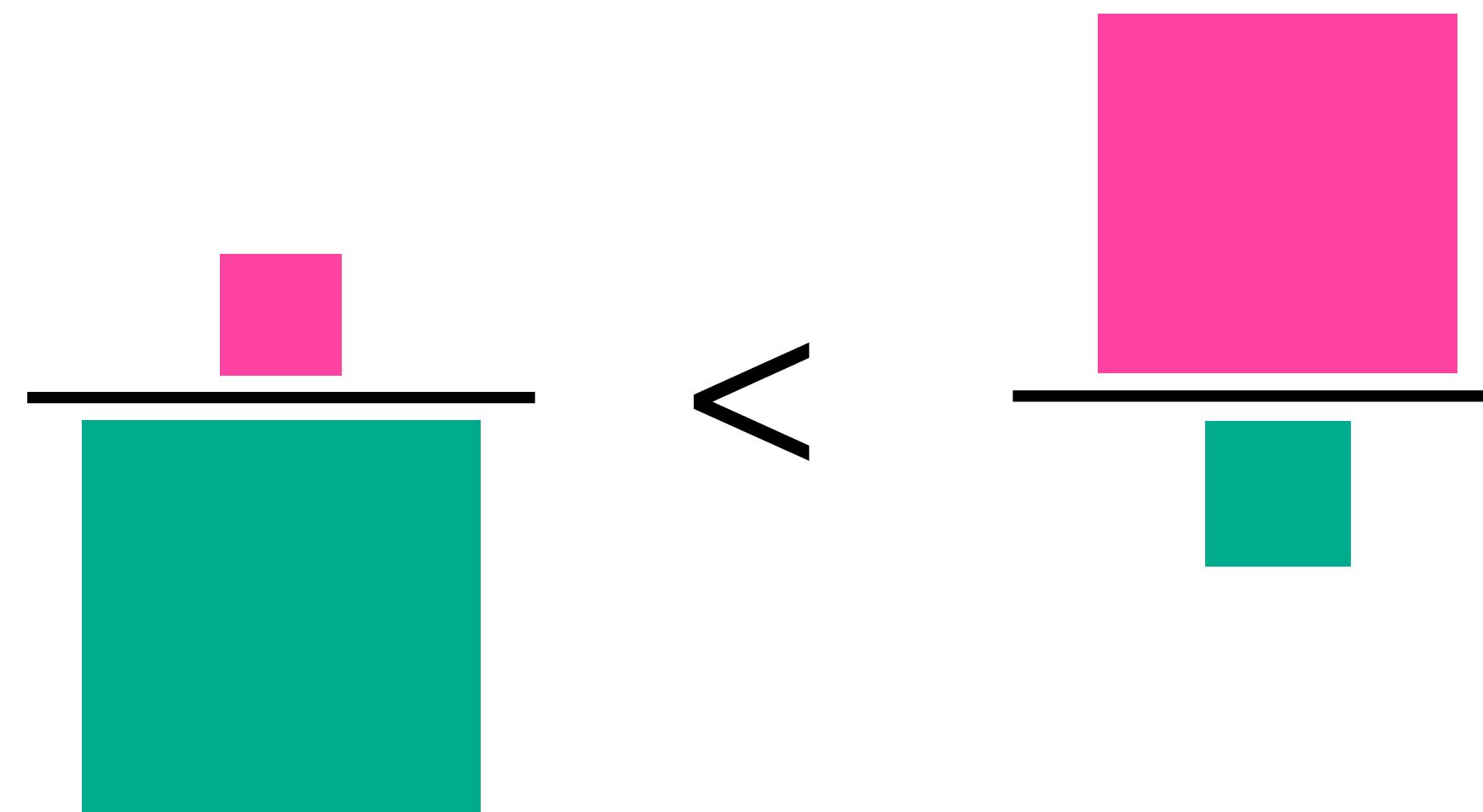
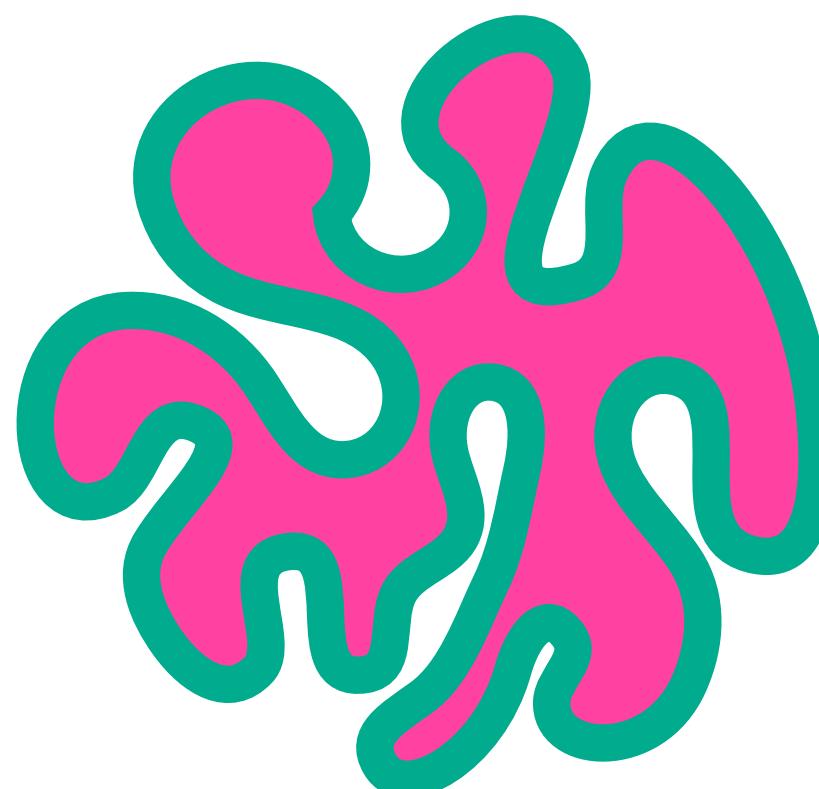
$$4\pi \times \frac{\text{area}}{\text{perimeter}^2} = 1$$



# Analyze particles: Circularity

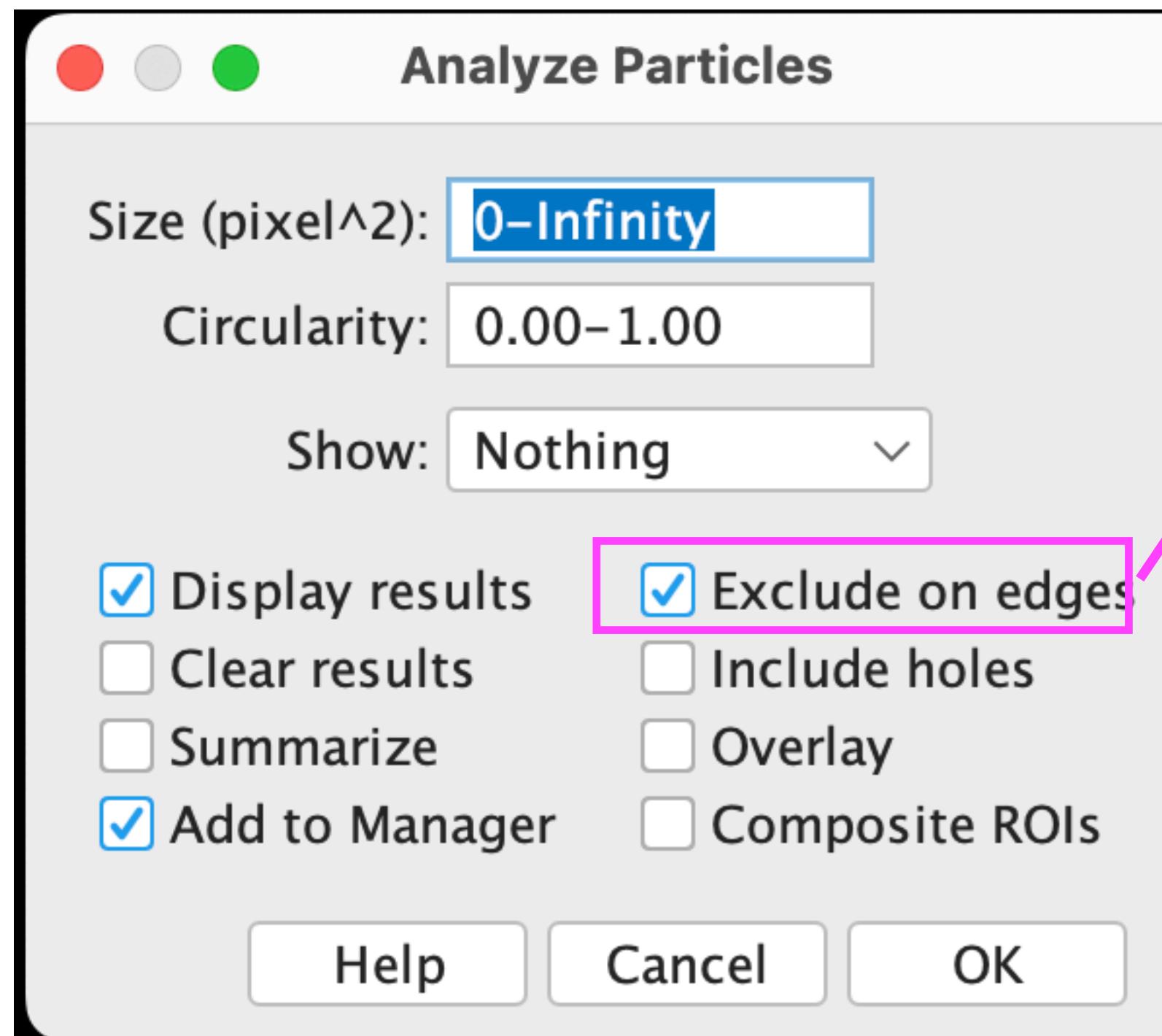
$$4\pi \times \frac{\text{area}}{\text{perimeter}^2} < 1$$

$$4\pi \times \frac{\text{area}}{\text{perimeter}^2} = 1$$

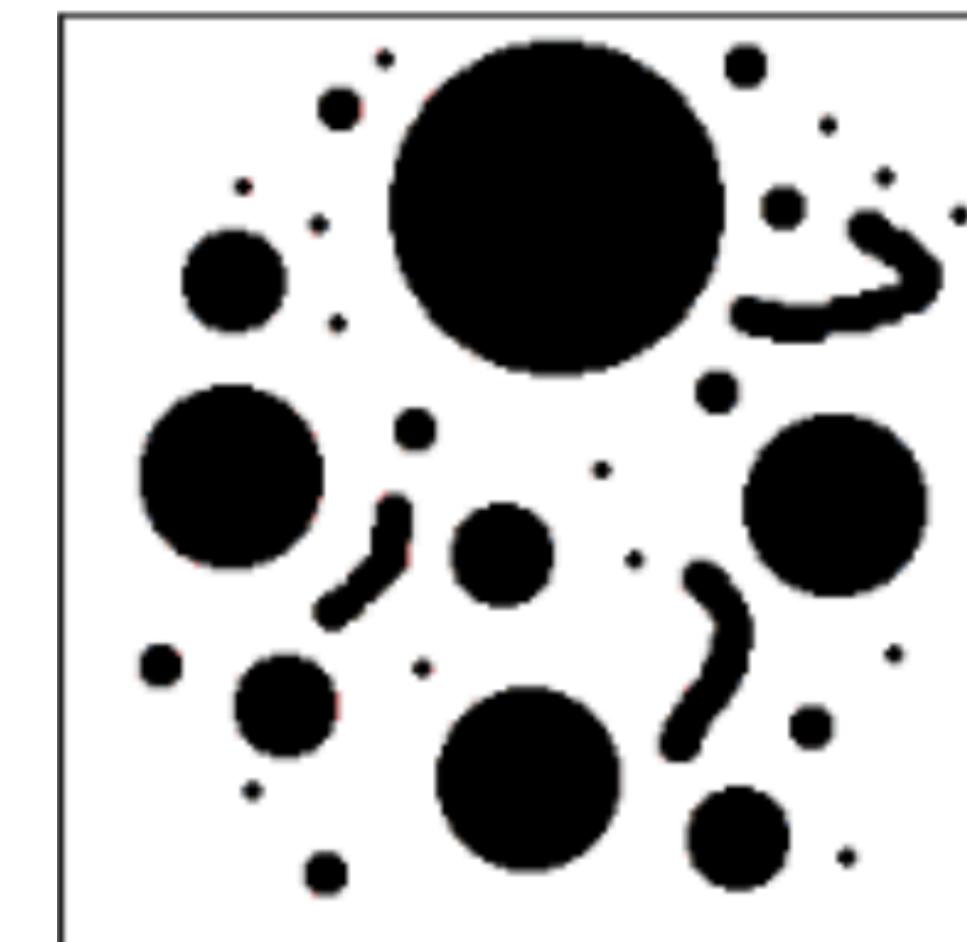
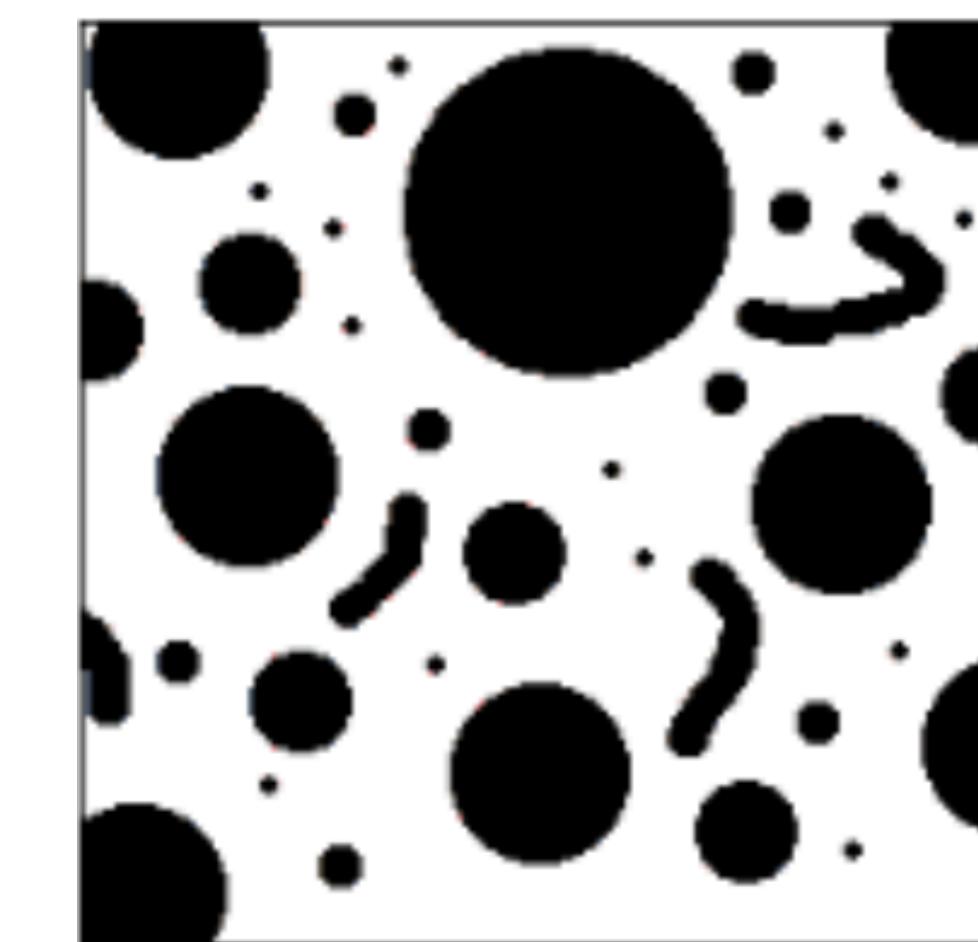


# Analyze particles: Exclude on Edges

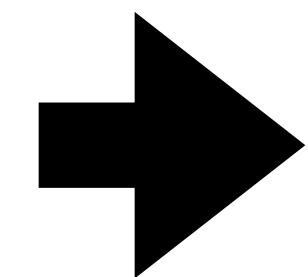
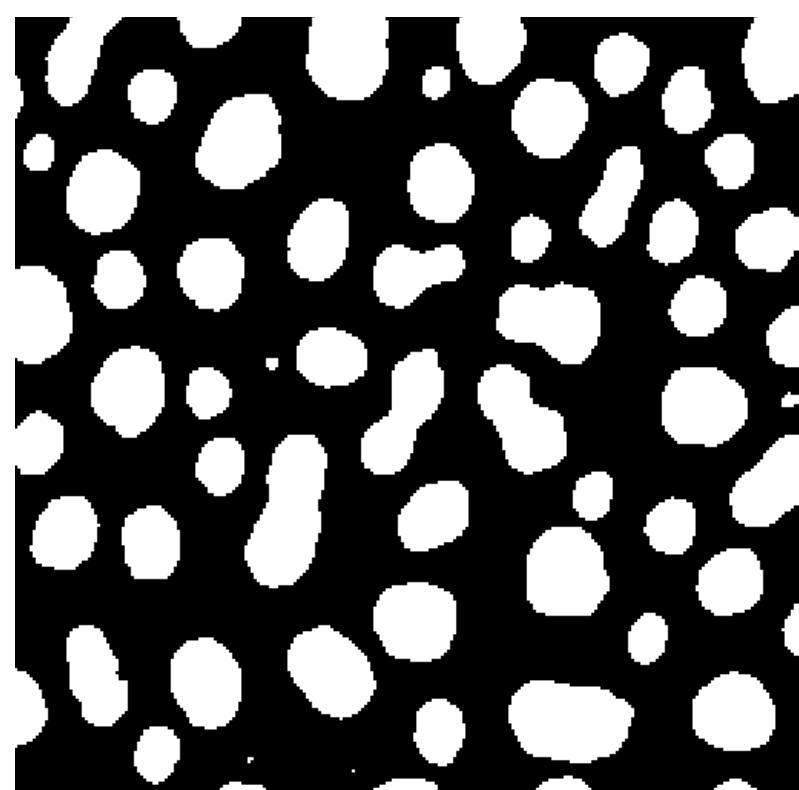
in Fiji: Analyze > Analyze Particles...



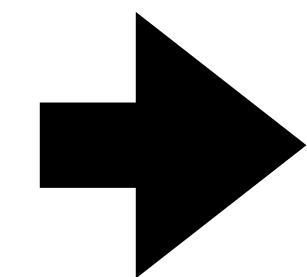
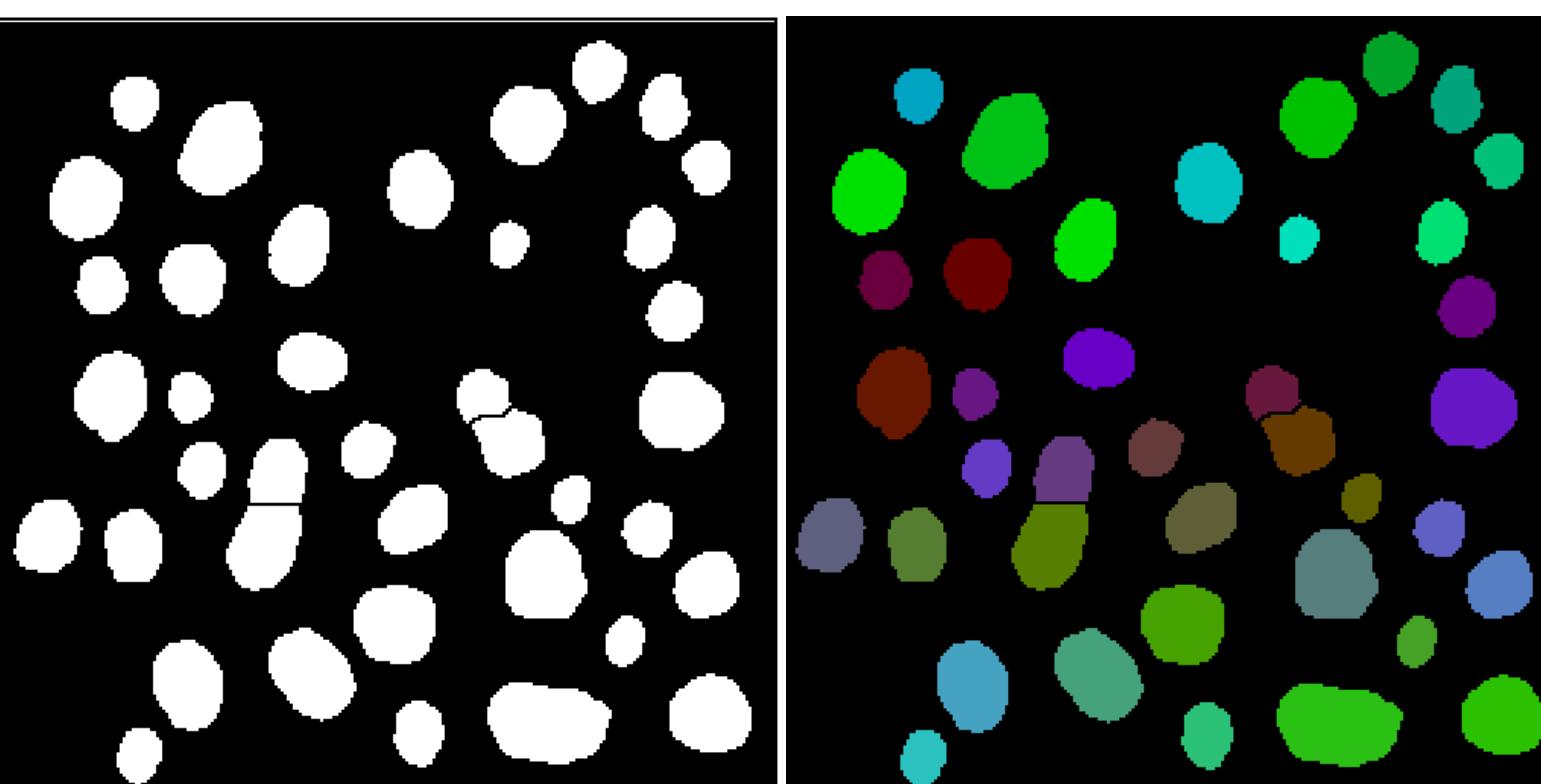
**“Exclude on edges”** excludes objects that are touching the borders of the image.



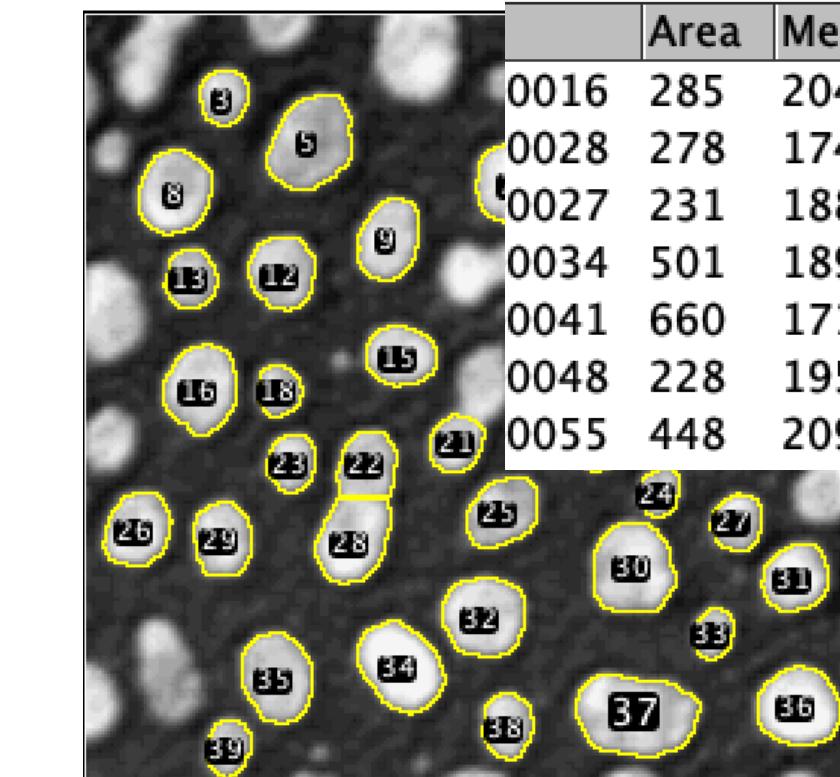
Binary mask



Process and segment



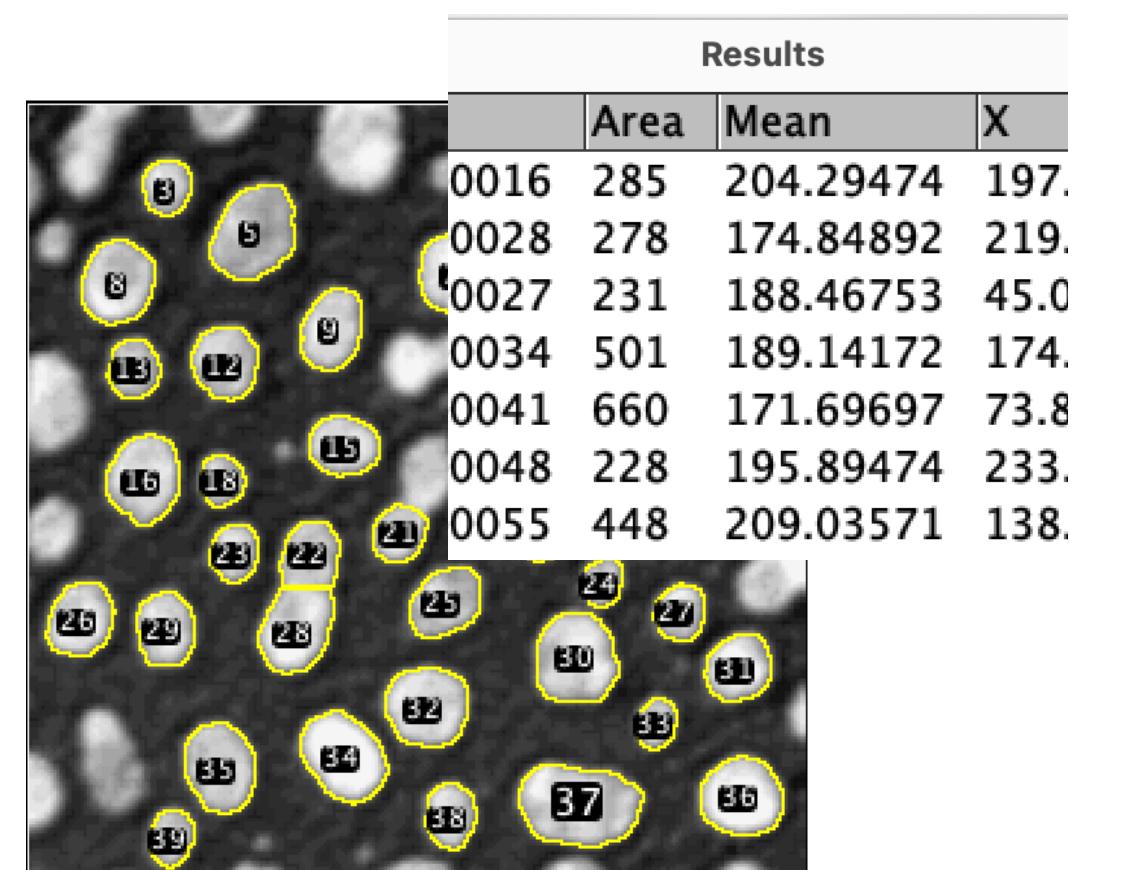
Export and Measure



Results			
	Area	Mean	X
0016	285	204.29474	197.
0028	278	174.84892	219.
0027	231	188.46753	45.0
0034	501	189.14172	174.
0041	660	171.69697	73.8
0048	228	195.89474	233.
0055	448	209.03571	138.

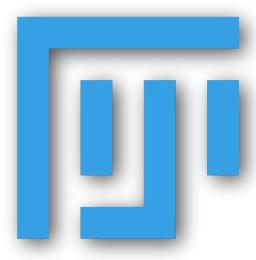
- Save the segmented image
- Add to ROI manager
- Generate and export measurements

## Export and Measure

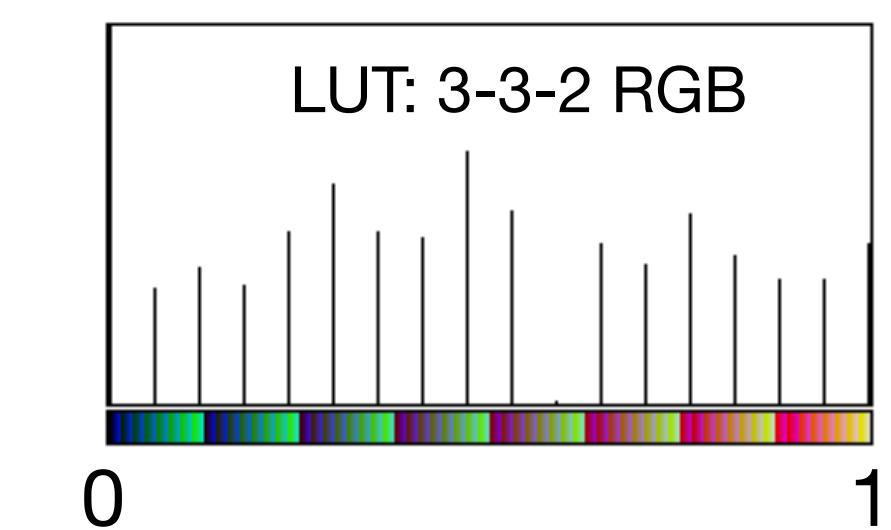
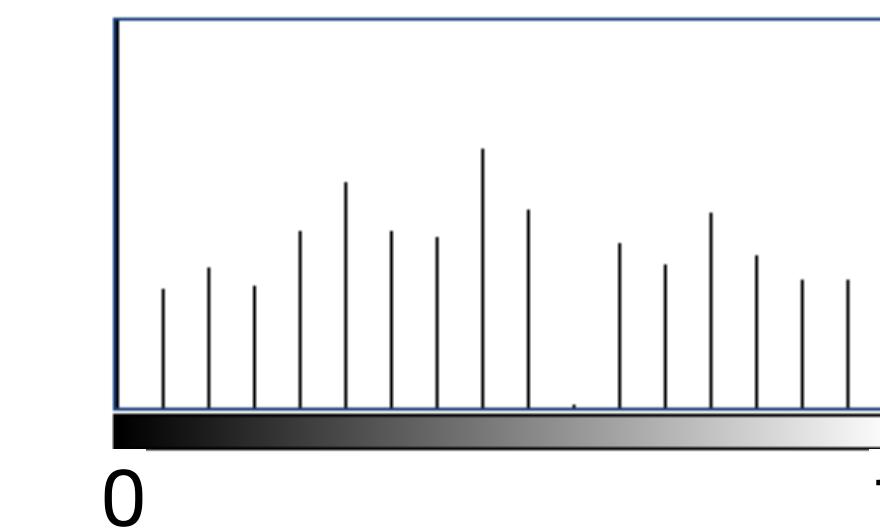
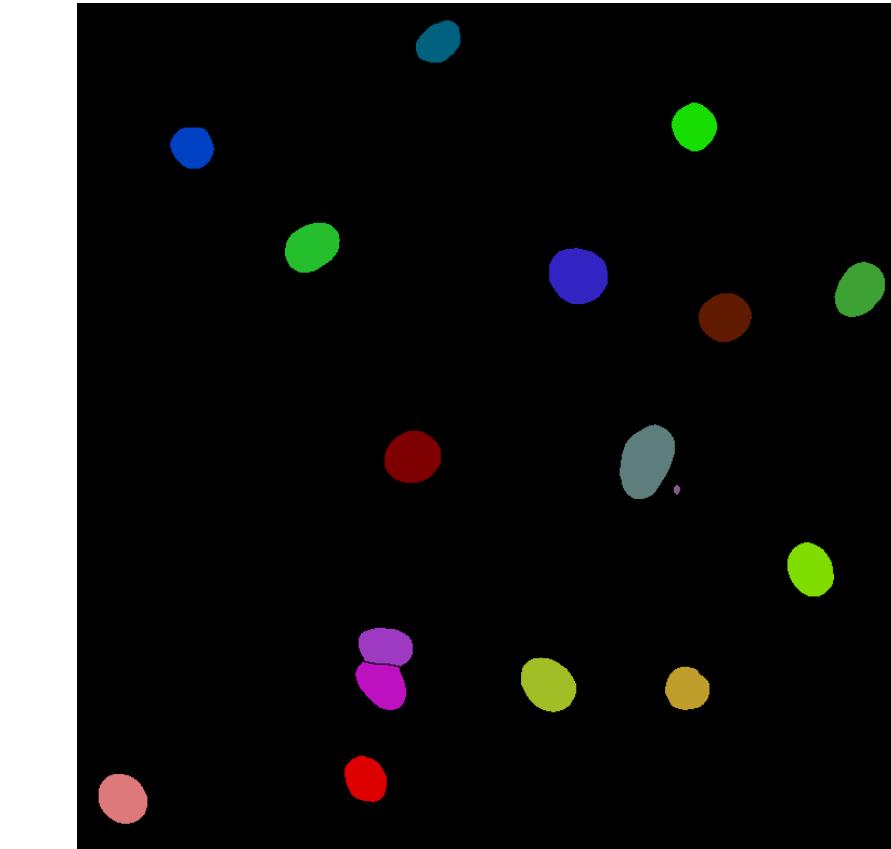
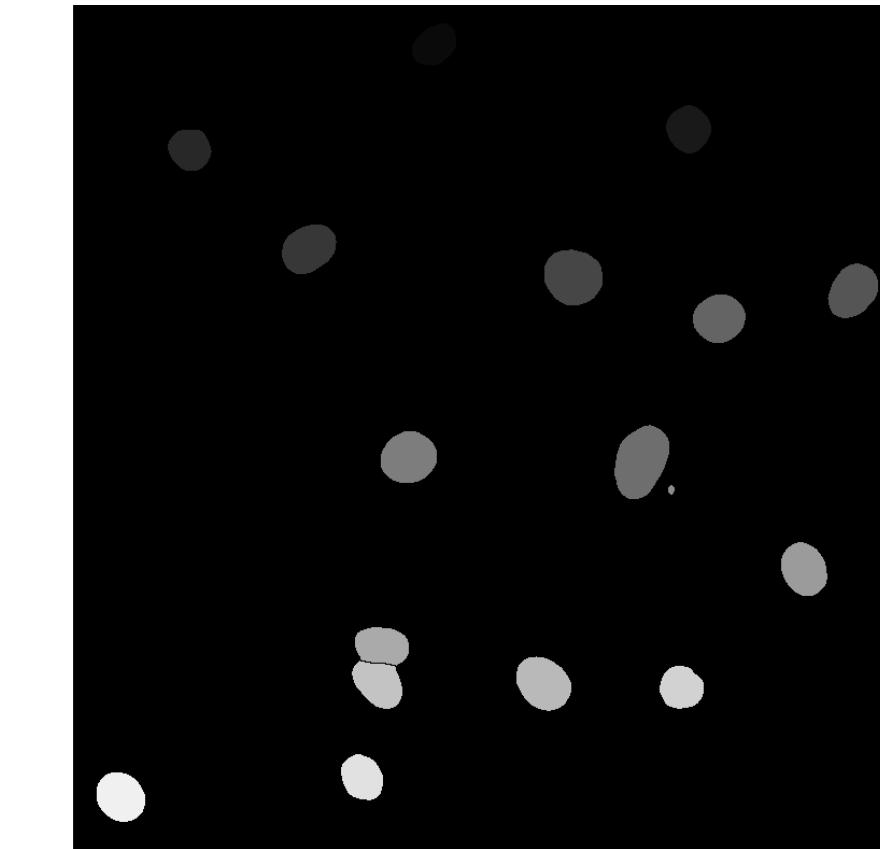
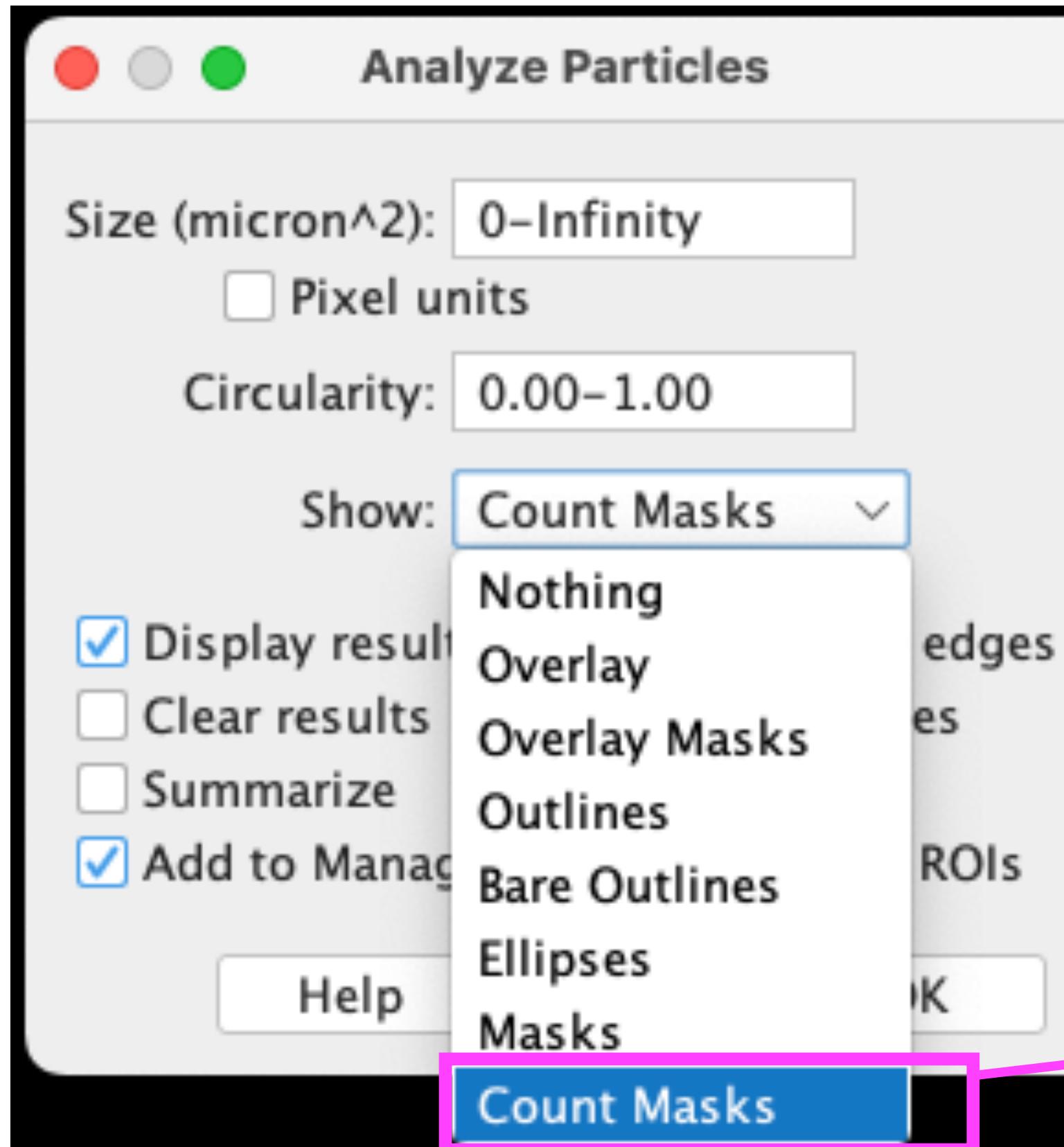


# Analyze particles: Save the segmented image

Export



in Fiji: Analyze > Analyze Particles...

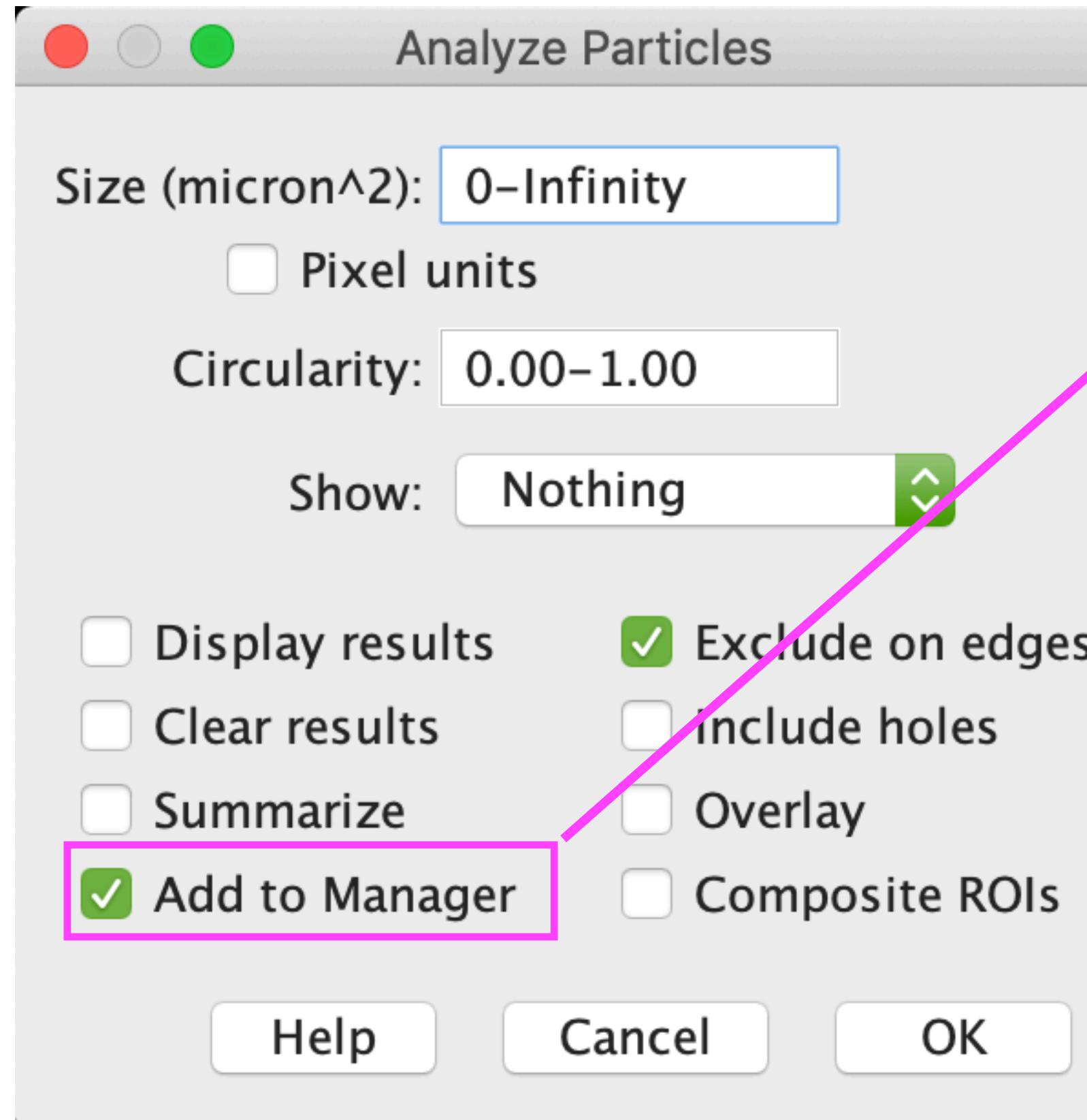


**"Count Masks"** encodes object identity as gray value.

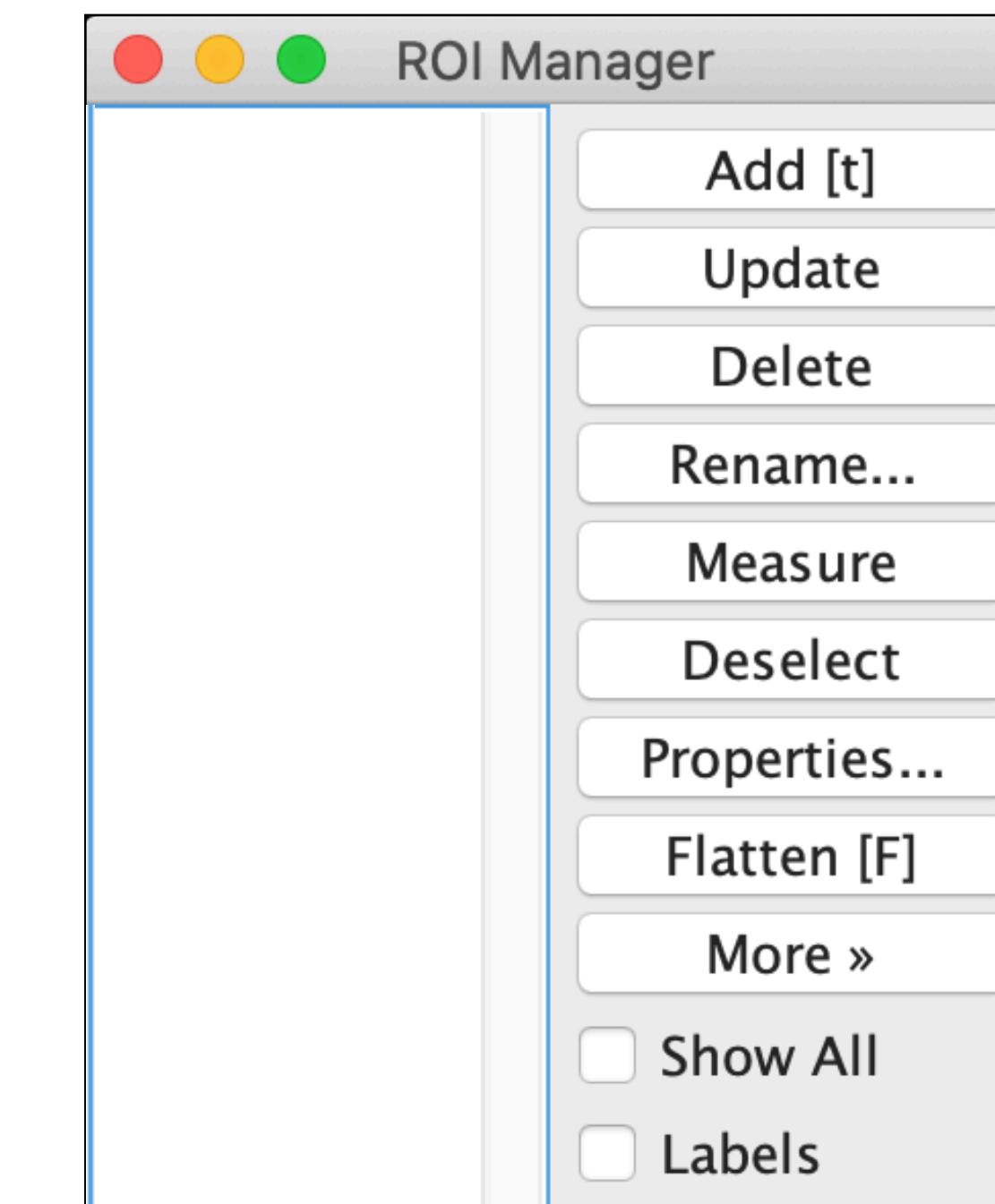
# Analyze particles: Add to ROI Manager

[Export](#)

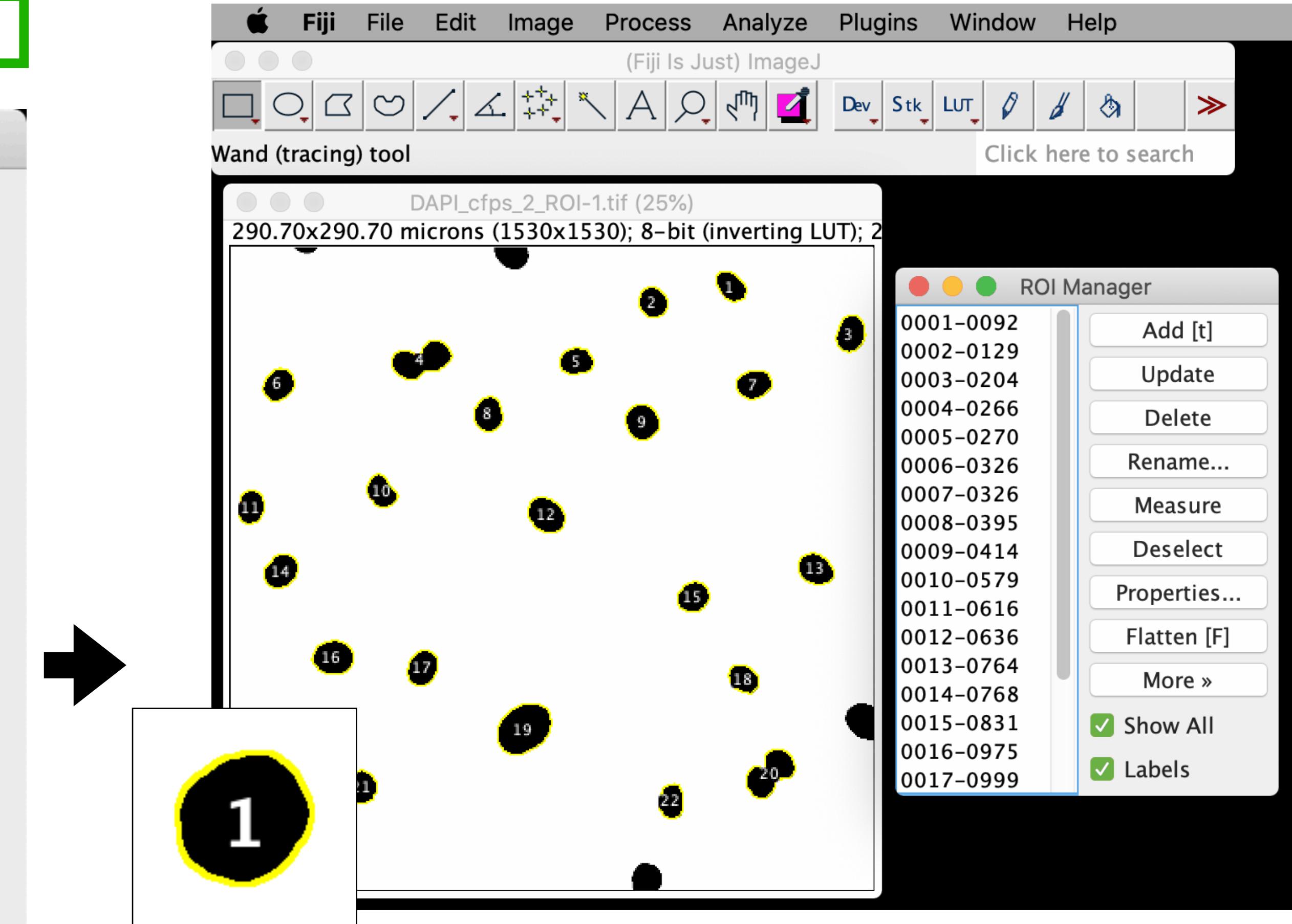
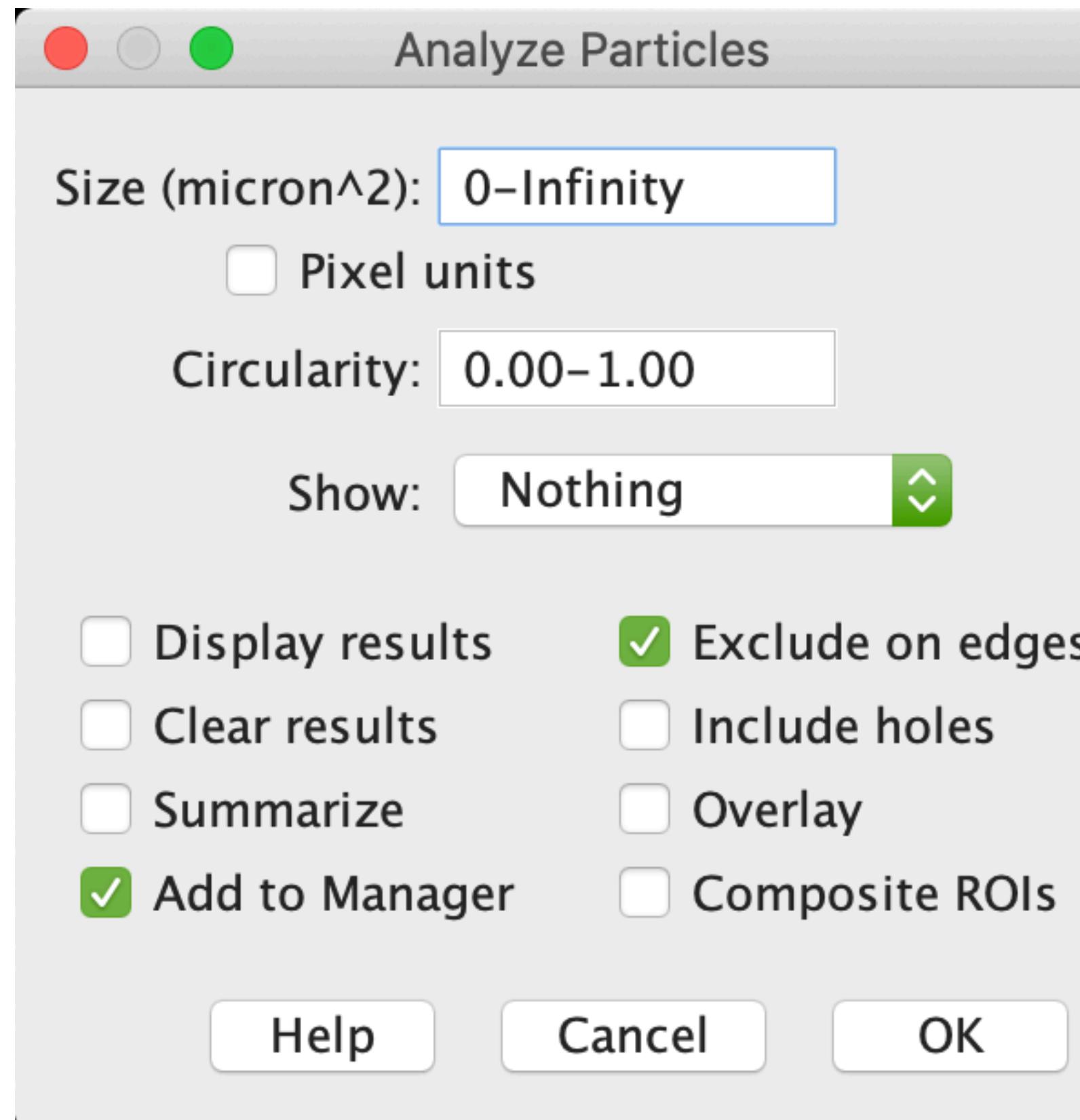
in **Fiji**: **Analyze > Analyze Particles...**



**"Add to Manager"** stores all found objects in the **ROI Manager**.

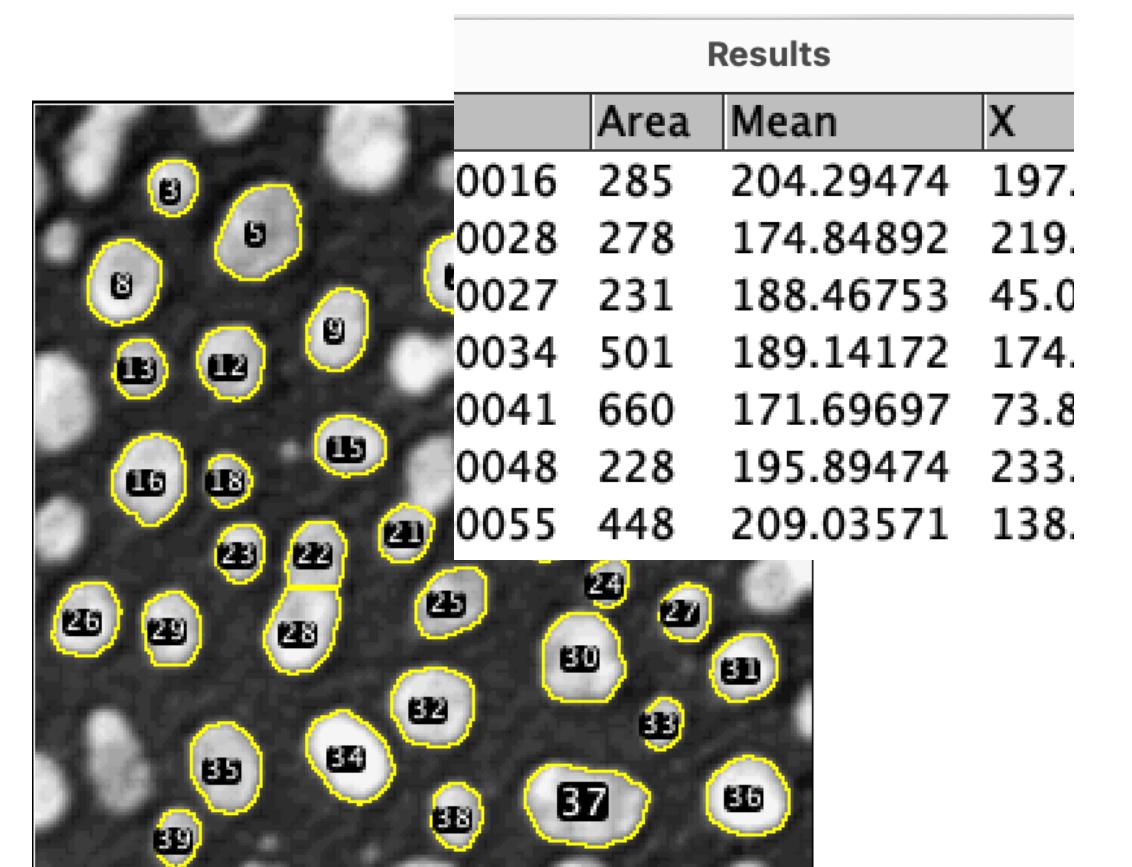


# Analyze particles: Add to ROI Manager

[Export](#)in **Fiji: Analyze > Analyze Particles...**

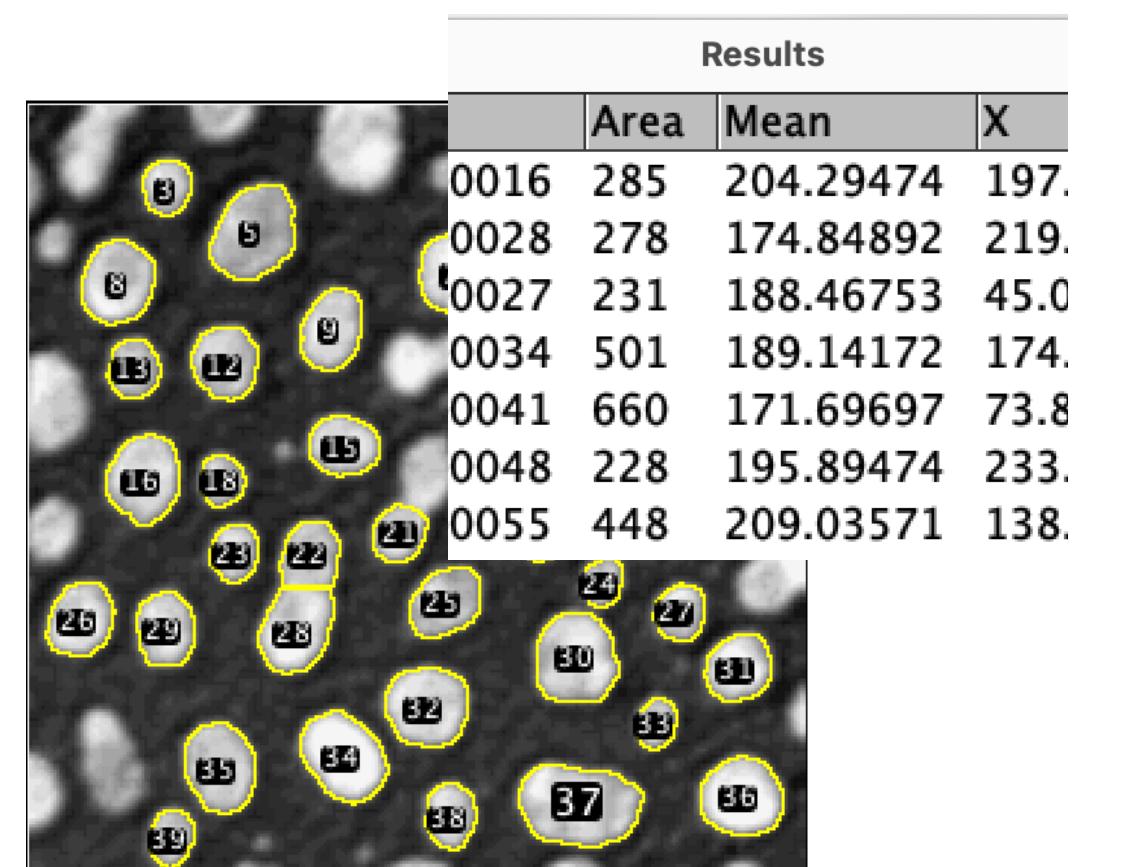
- Save the segmented image
- Add to ROI manager
- Generate and export measurements

## Export and Measure

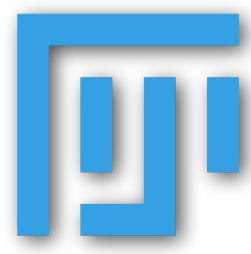


- Save the segmented image
- Add to ROI manager
- Generate and export measurements
  - Select what to measure
  - Measure

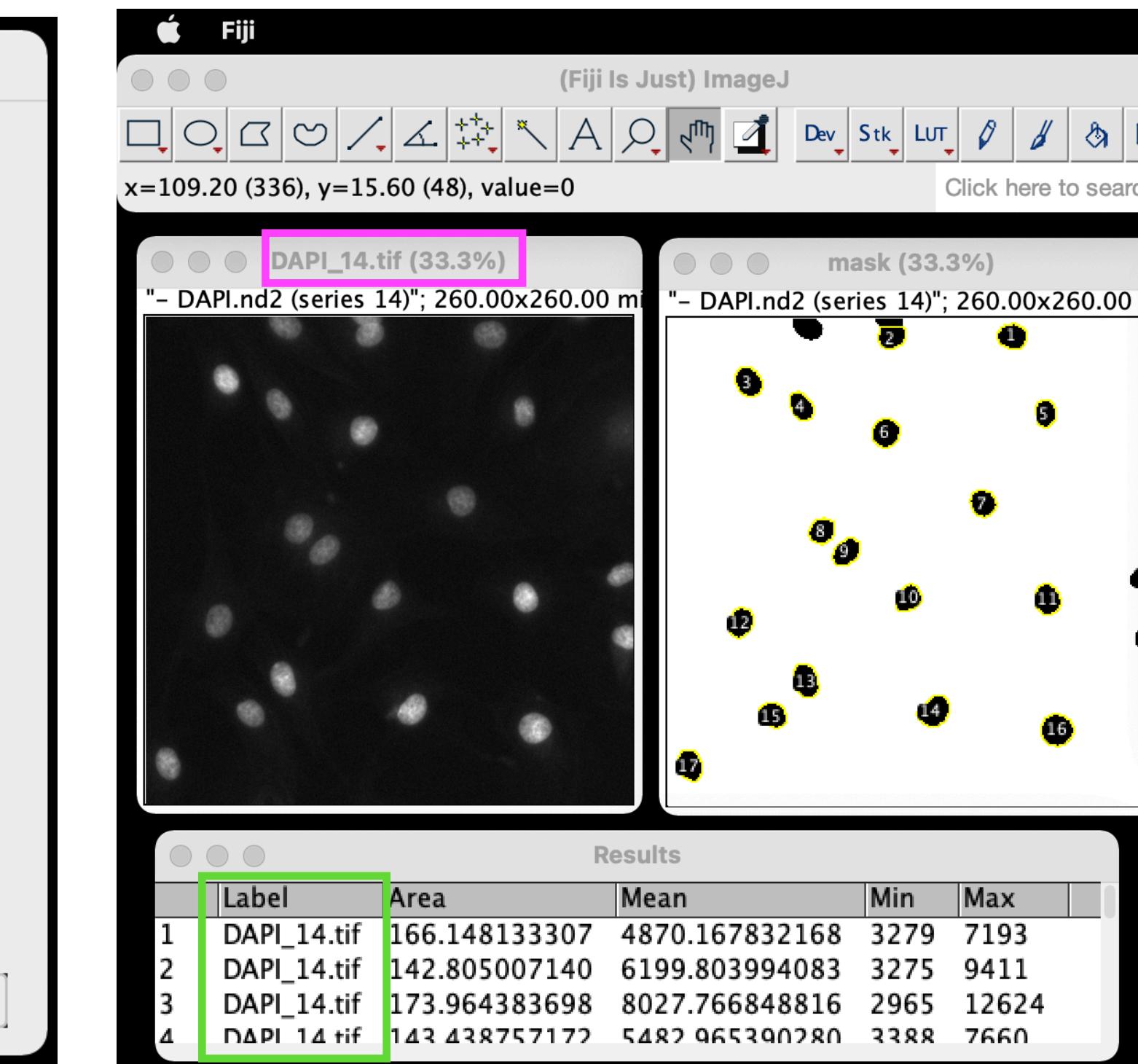
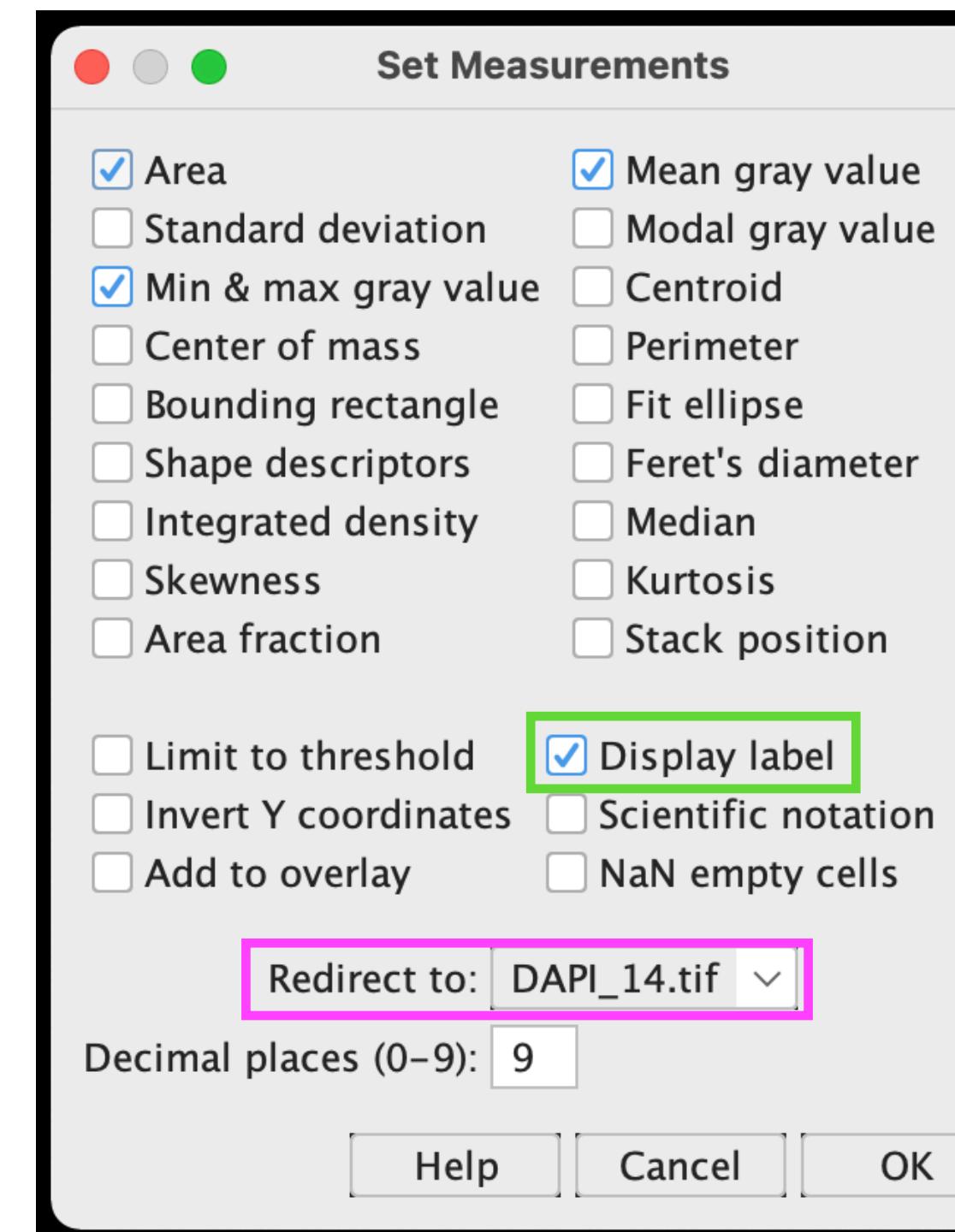
## Export and Measure



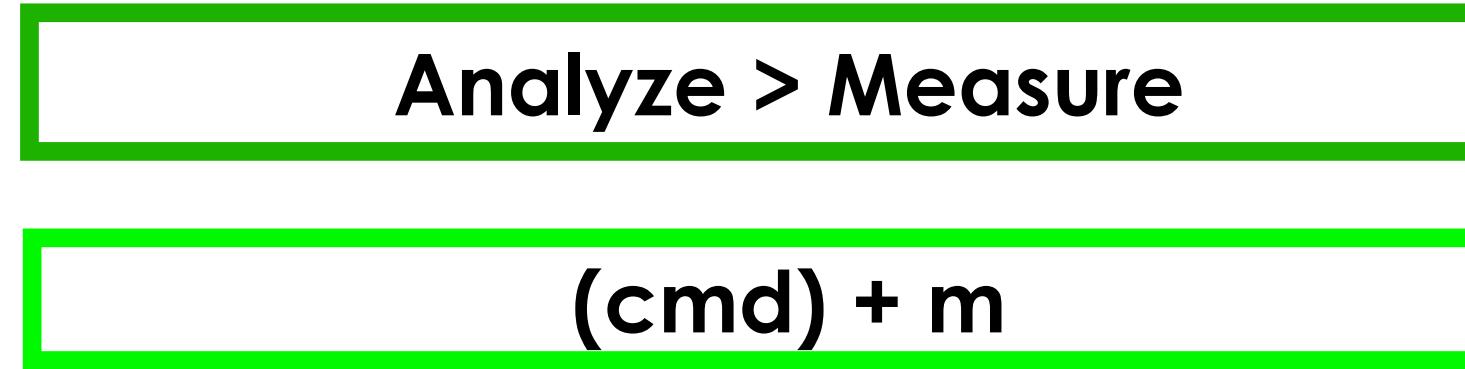
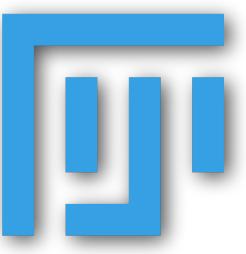
# Select what to measure: Set Measurements

[Export](#)**Analyze > Set Measurements...**

**Specifies which measurements have to be performed  
(e.g. area, mean grey value, max and min grey values, ...)**

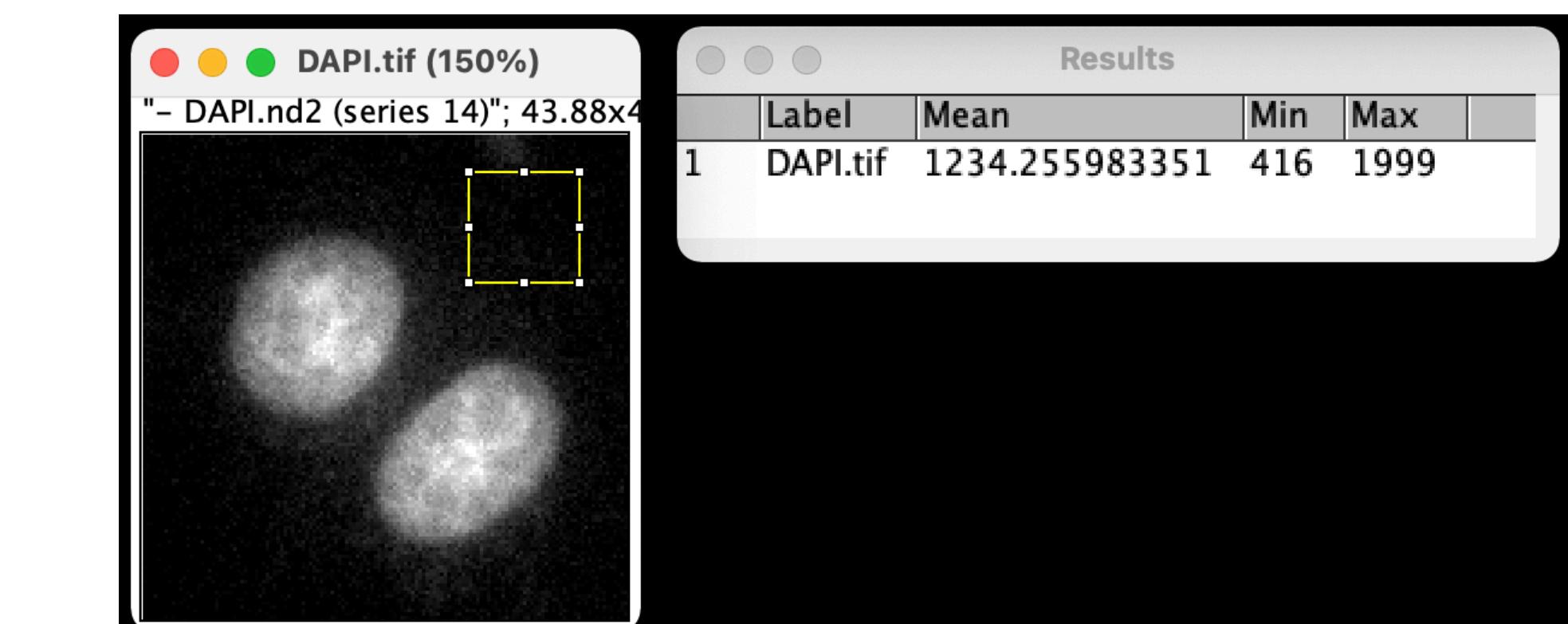
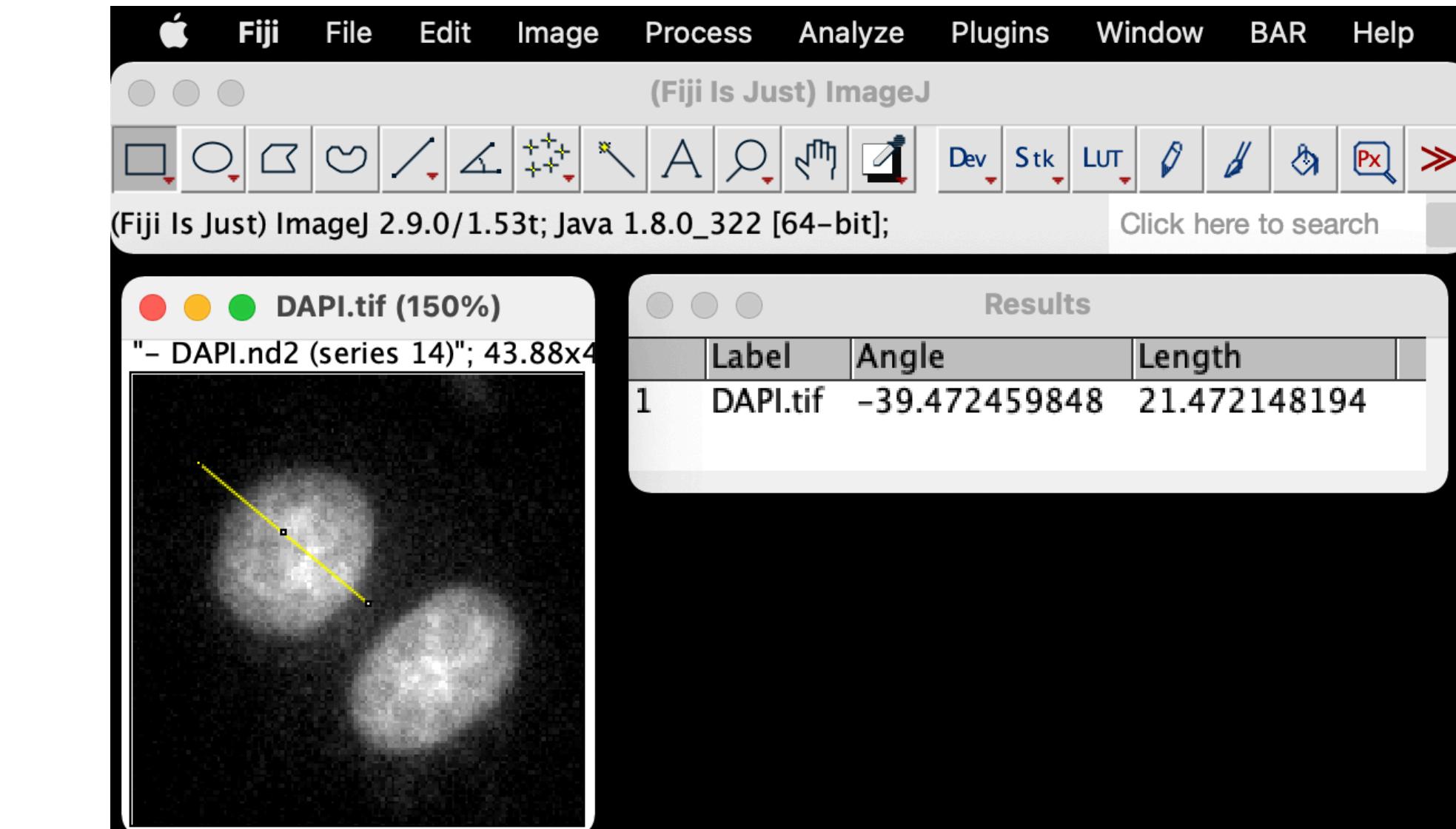
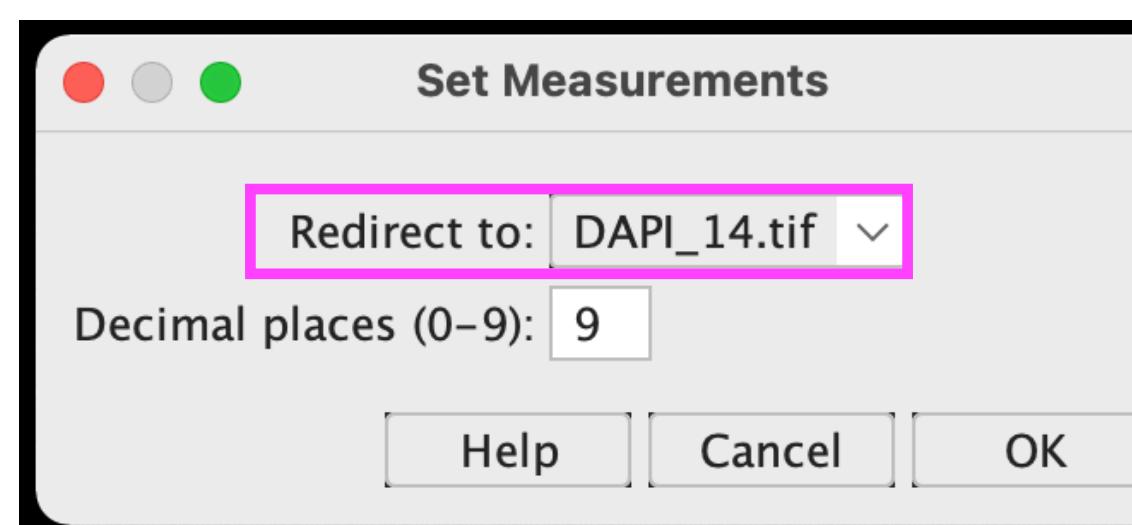


# Measure — Option 1: Manually

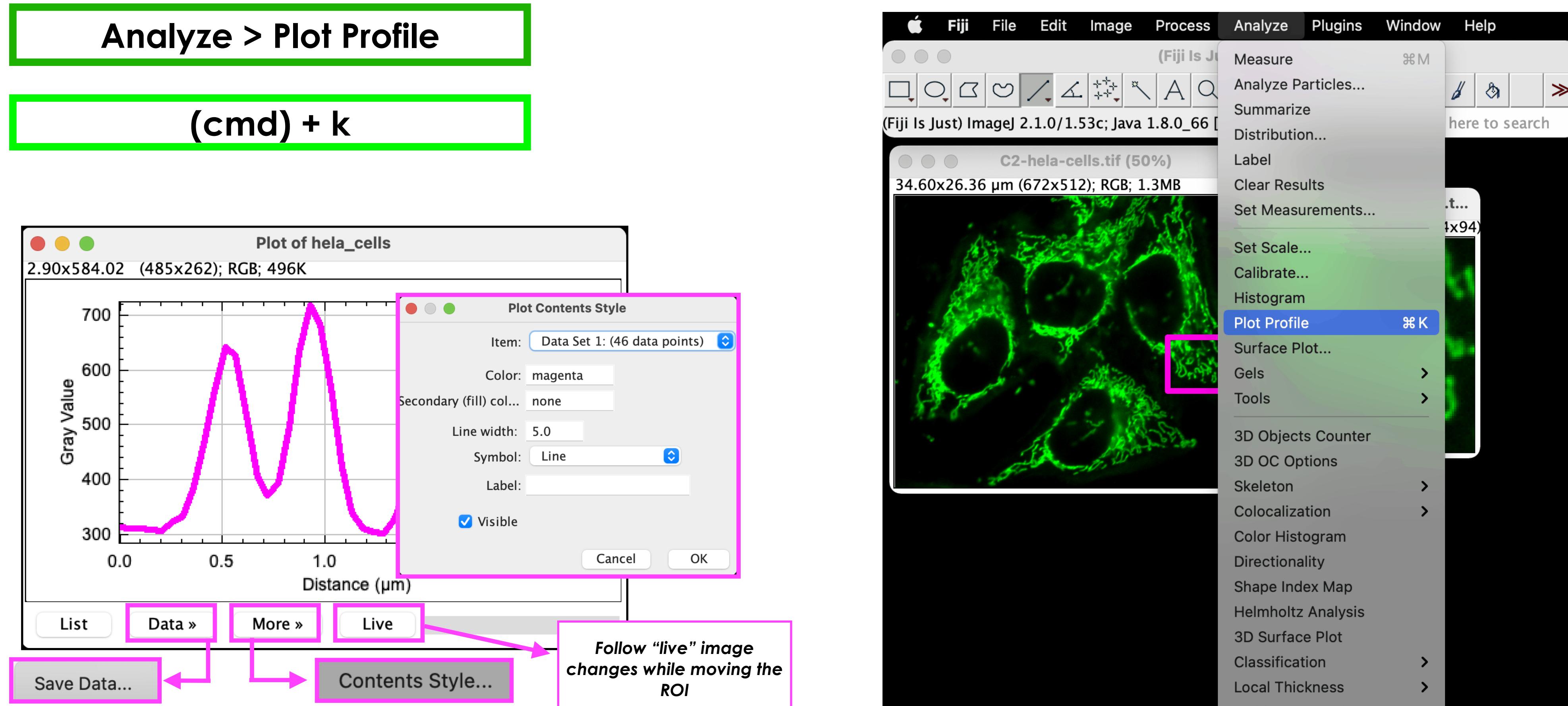
[Export](#)

*Measures the parameters chosen under  
“Analyze > Set Measurements...”  
in relation to the selected ROI.*

*Results are displayed in a Result Table  
(which can be saved as .csv, .xlsx, ...)*



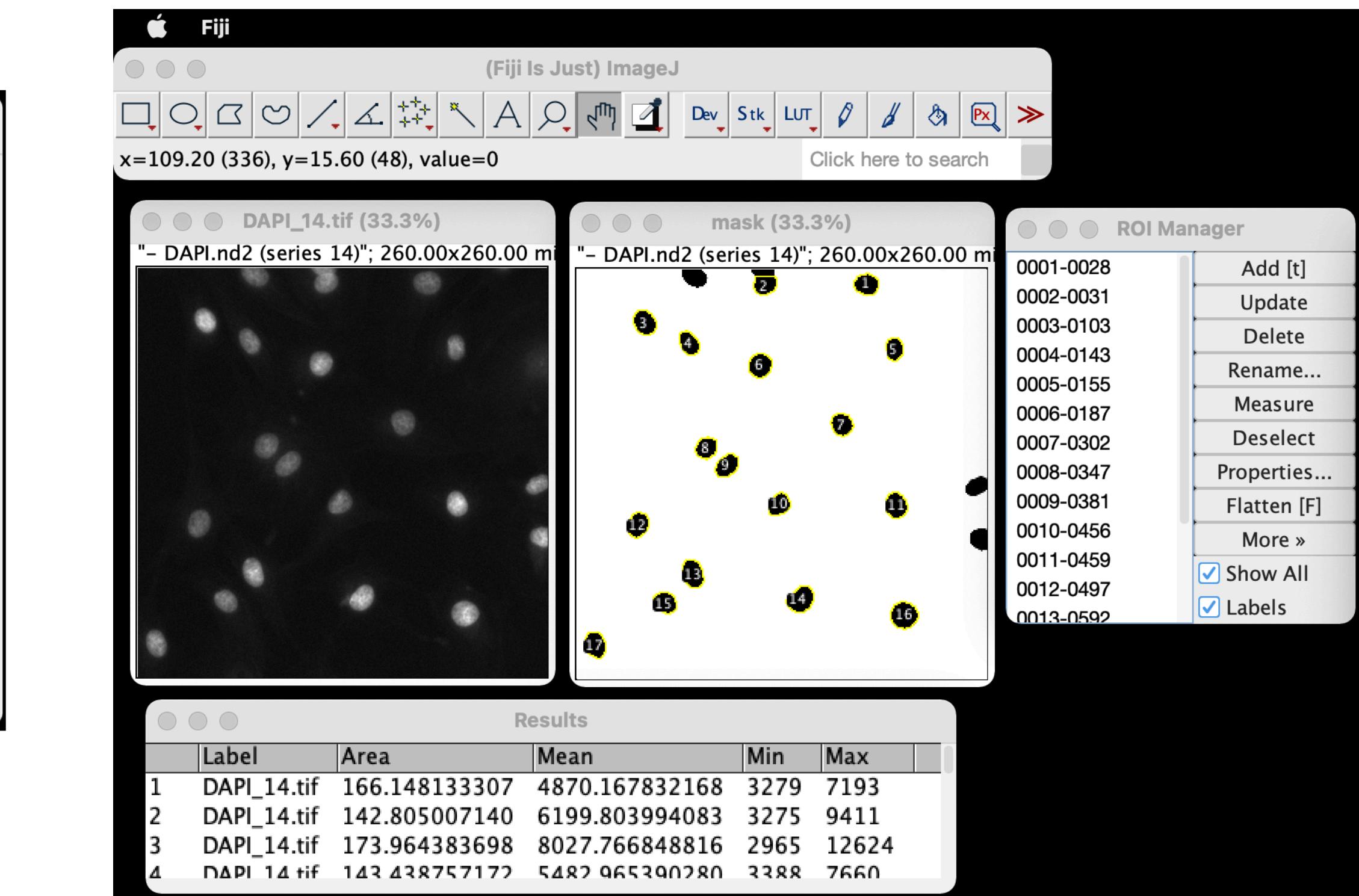
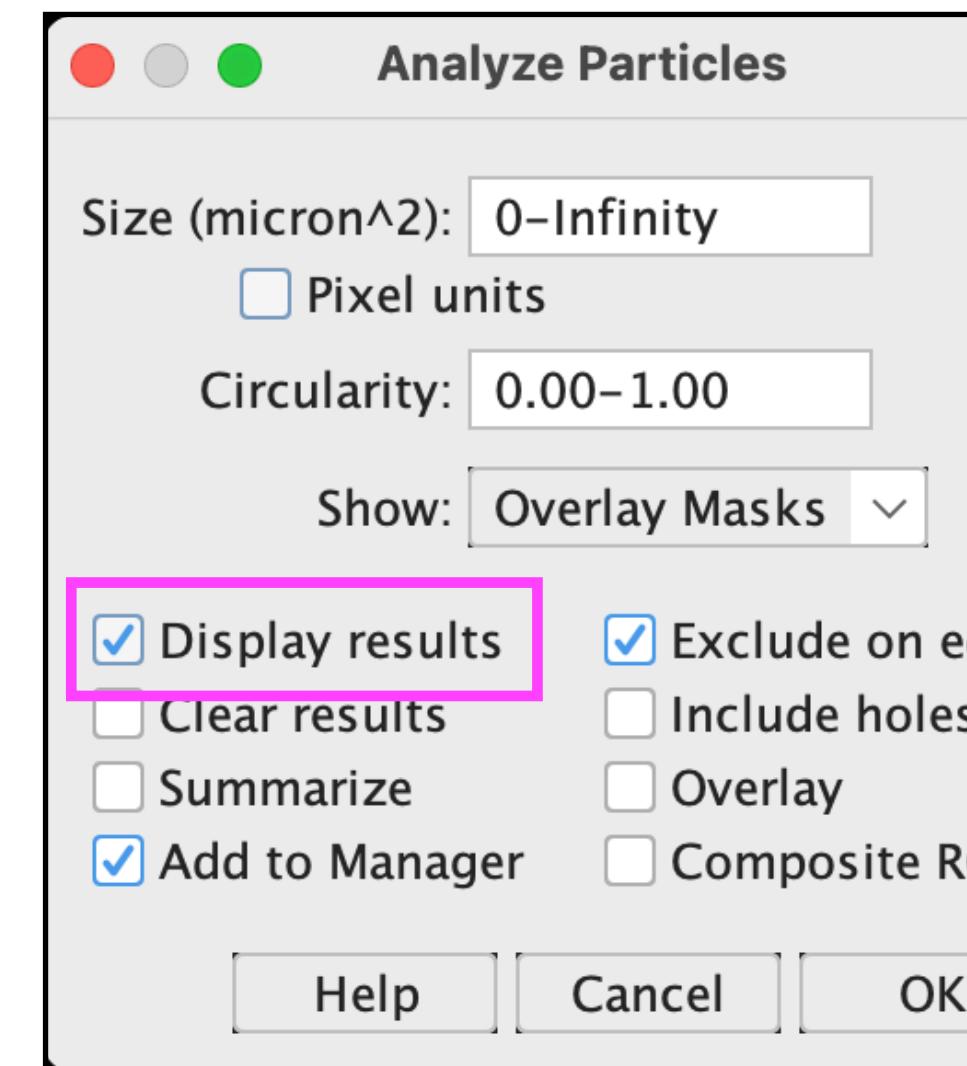
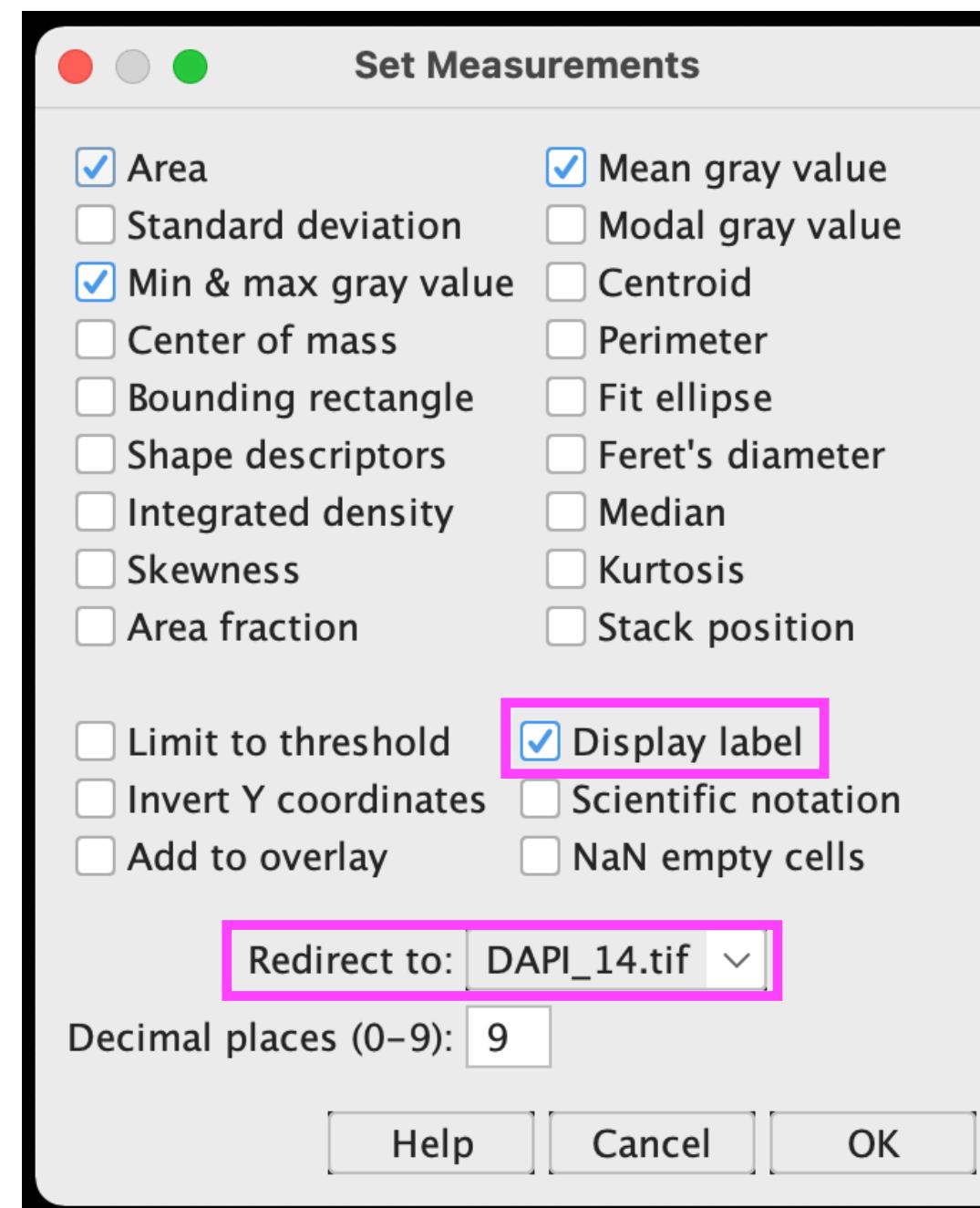
# Side note: other kinds of measurements



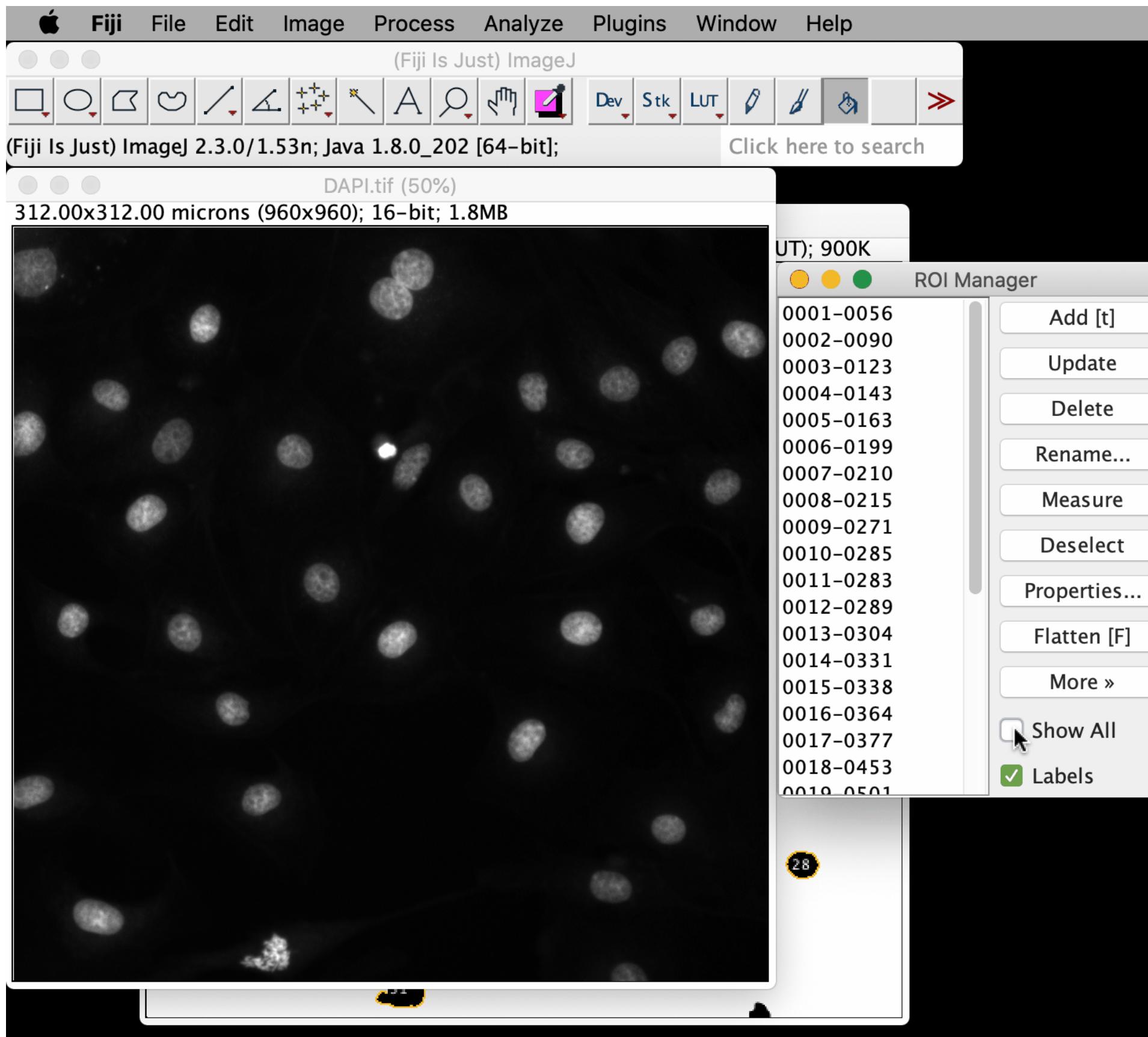
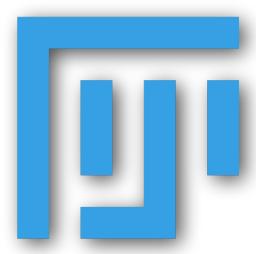
\*Plots can be saved as .csv file ("Save Data...") and also as images (e.g. "File > Save AS > PNG")

# Measure — Option 2: from Analyze particles

Export



# Measure — Option 3: Using the ROI manager

[Export](#)

1. Select the “ORIGINAL” image.
2. To measure all the ROIs stored in the “ROI Manager”, **no ROI has to be selected** in the “ROI Manager”. Use the “Deselect” button.
3. Click on the “**Measure**” button.
4. A “**Results**” table will appear.

# Segmentation with pixel based classifier—exercises



Continue with the “Analyze Particle” step  
form ONE of the workflow exercises below.

4.1 DAPI segmentation with thresholding

OR

4.3 - DAPI segmentation with Labkit