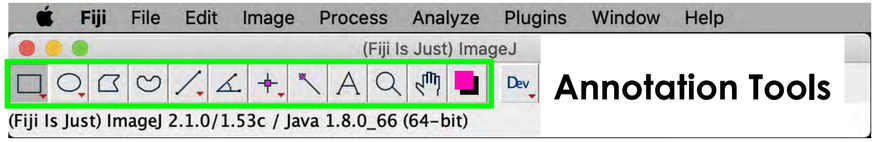
Image Analysis Collaboratory

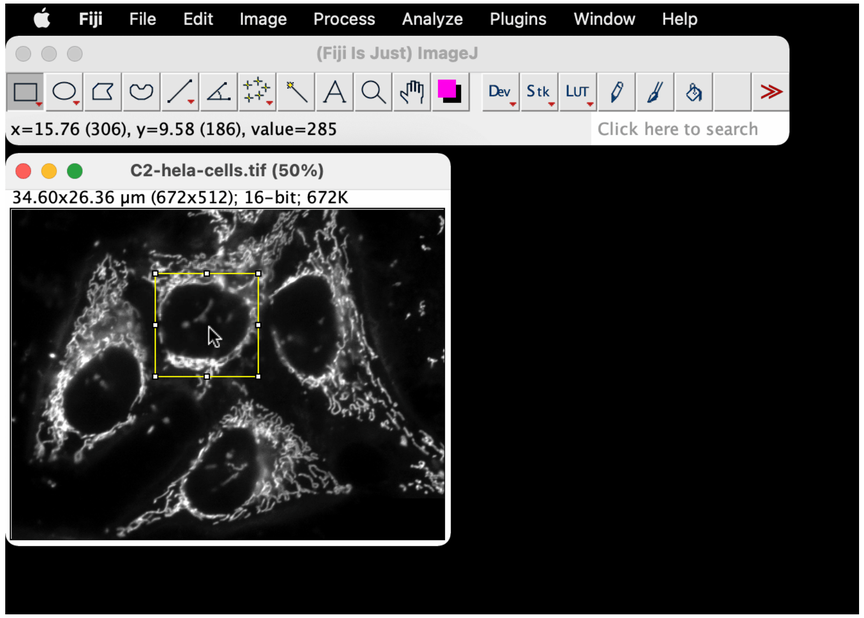
ROI Manager

Image Analysis Collaboratory

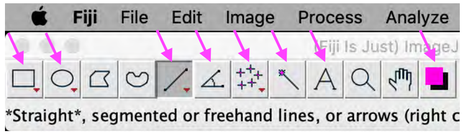
Annotation Tools
***see also Edit > Selection**

Region Of Interest (ROI)



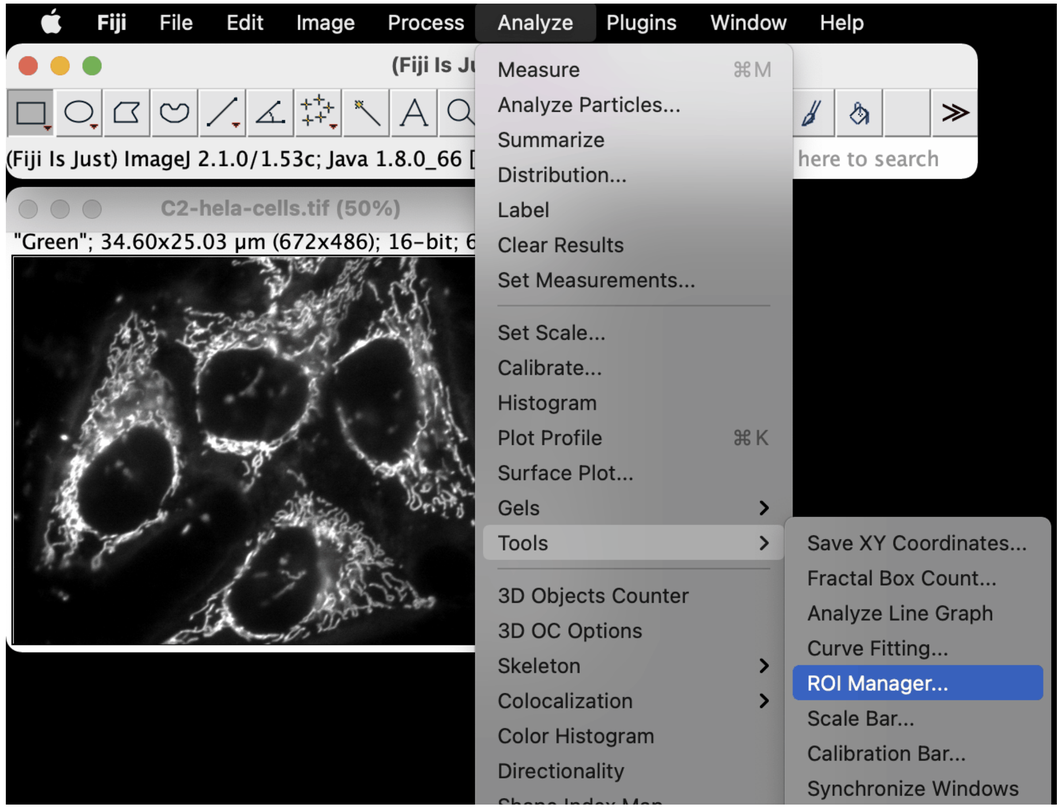


Secondary/Right Click for more options



Double Click to set/change properties

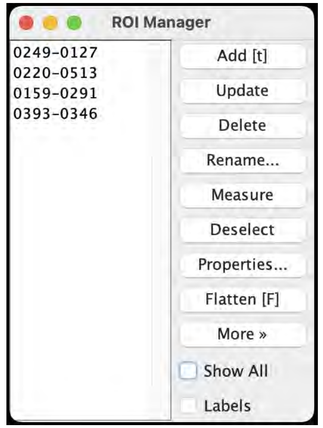
ROI Manager



Analyze > Tools > ROI Manager…

t*

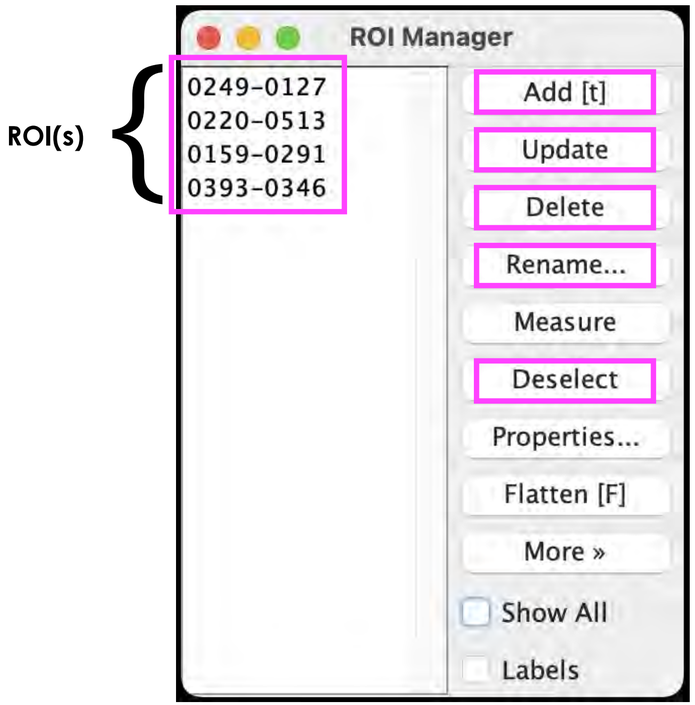
The ROI Manager is a tool for working with multiple selections (ROIs).



*(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



ROI(s)

Add new ROI (t).

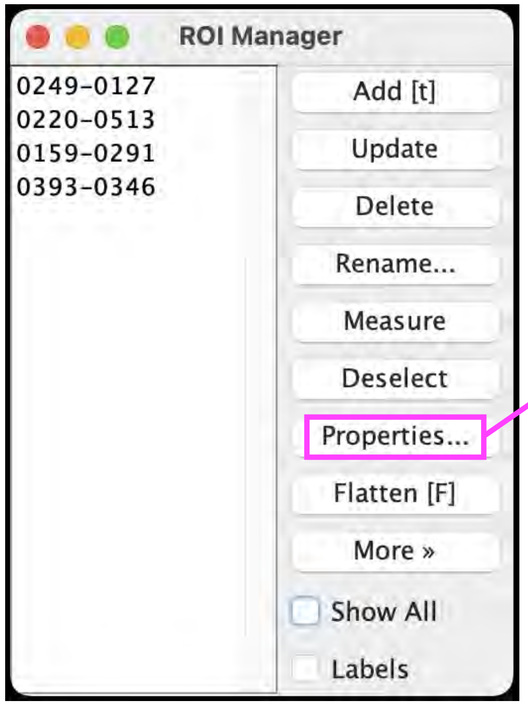
After modifying a ROI, you can use Update to save the changes.

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

Rename selected ROI.

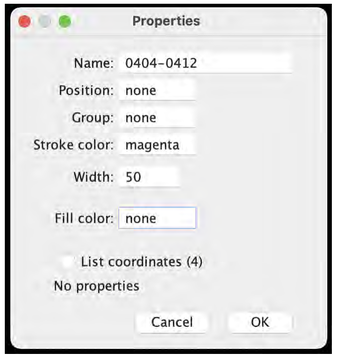
Deselect one or more selected ROI.

ROI Manager

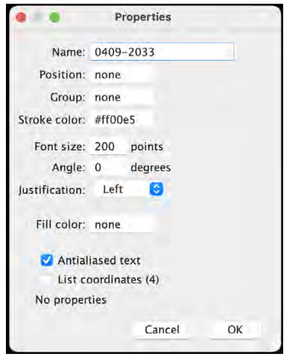


Change the properties of the selected ROI(s)

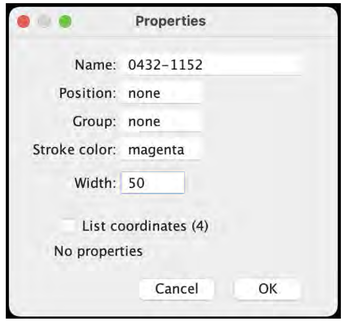
Rectangle…



Text



Line



Dot

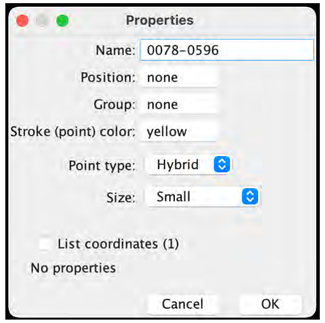
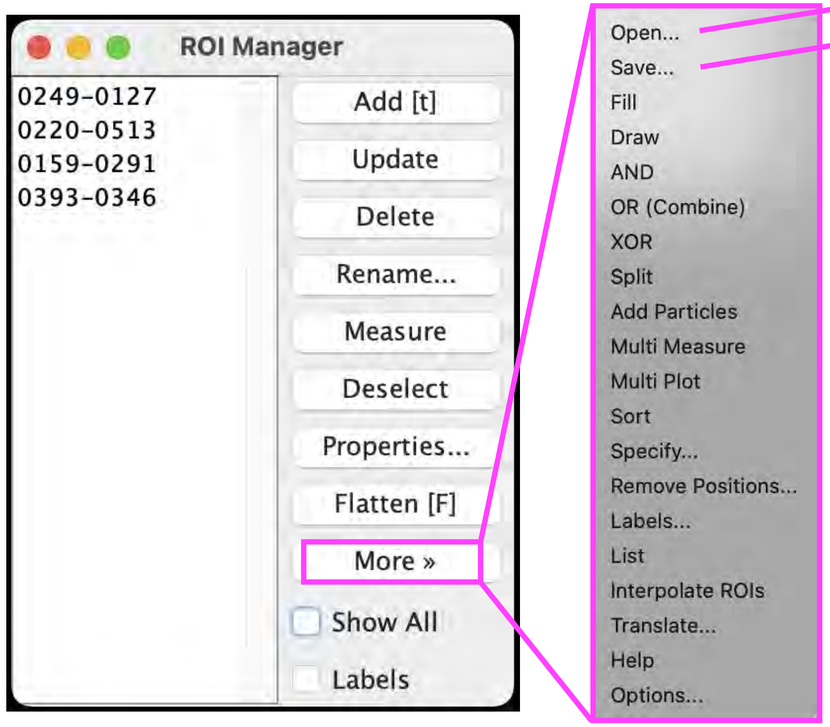


Image Analysis Collaboratory

"Analyze" menu

"Edit" menu

ROI Manager



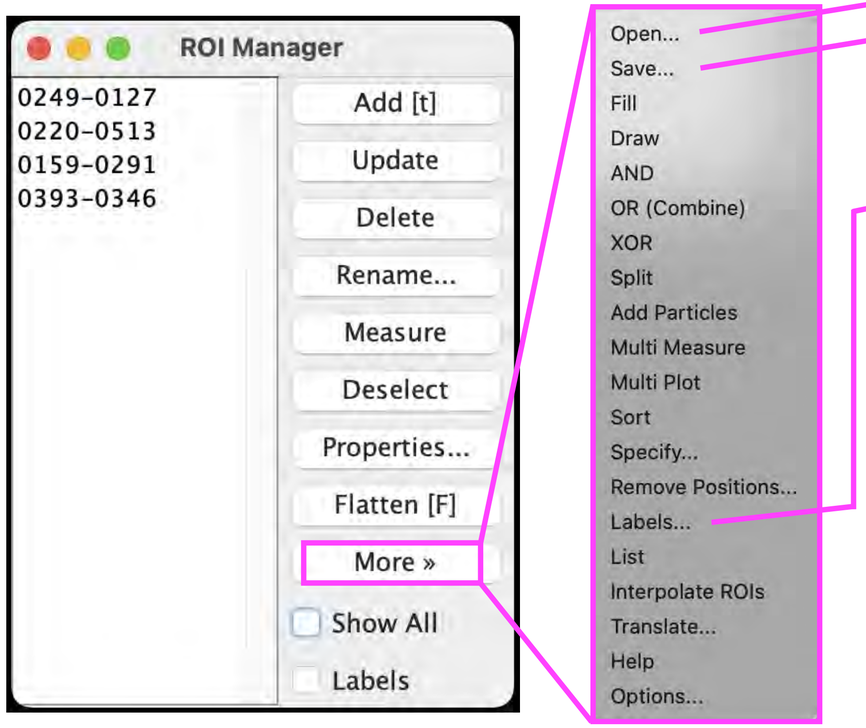
Open a saved ROI.zip file (drag & drop works as well)
Save ROI(s) as a .zip file

Image Analysis Collaboratory

"Analyze" menu

"Image" menu

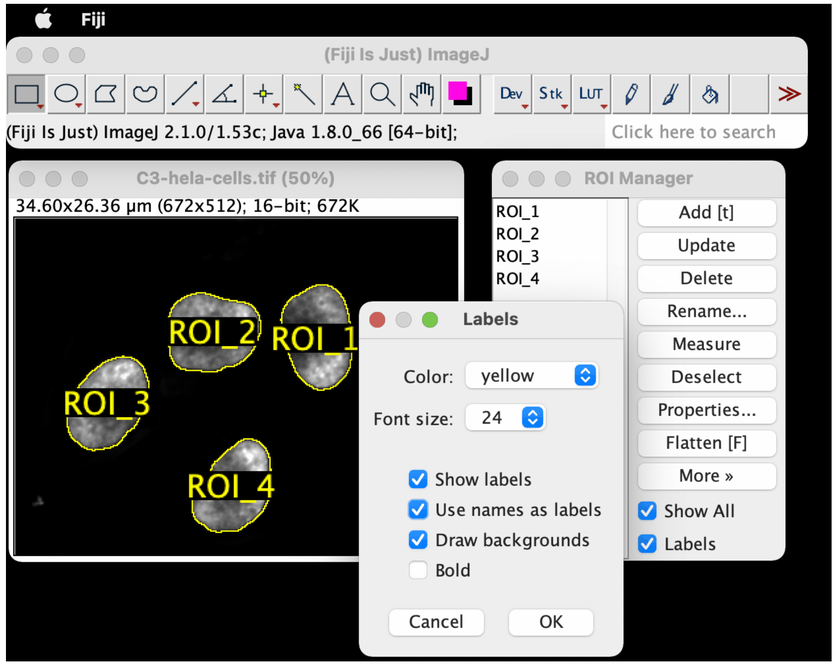
ROI Manager



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…"

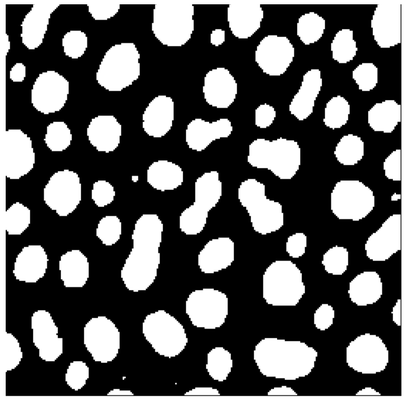
Live Demo of ROI manager

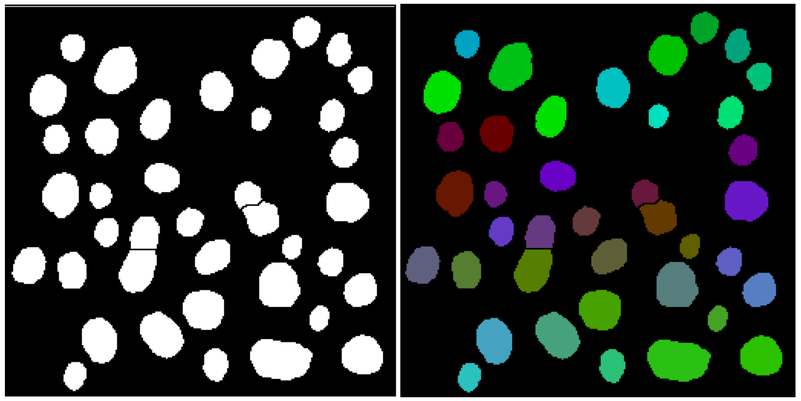
Image Analysis Collaboratory

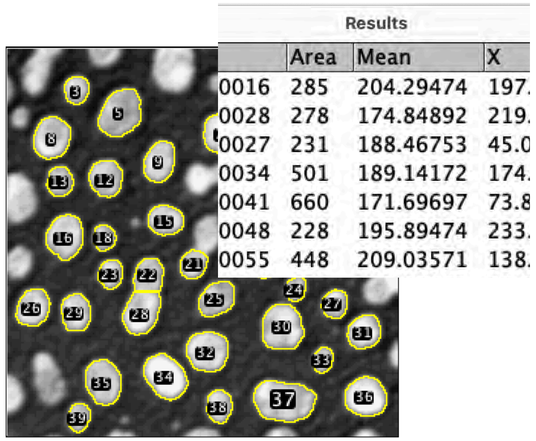
Segmentation And Measurements

Image Analysis Collaboratory

Binary mask → Process and segment → Export and Measure





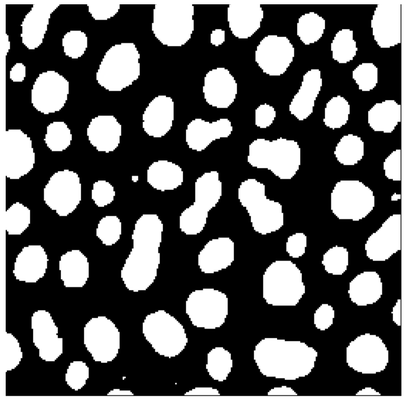


Results

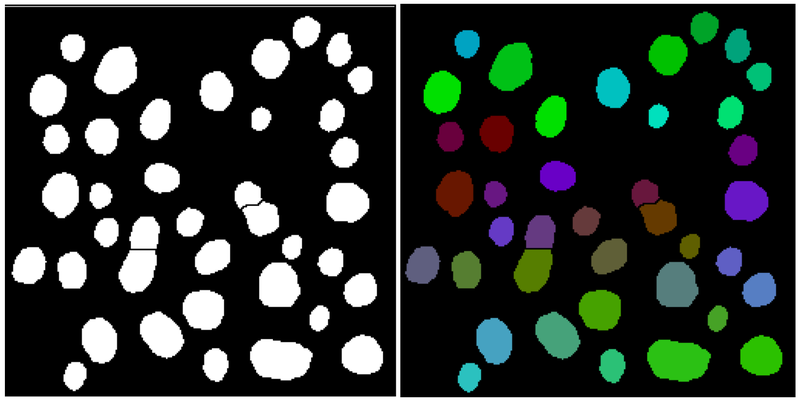
	Area	Mean	X
0016	285	204.29474	197.
0028	278	174.84892	219.
0027	231	188.46753	45.0
0034	501	189.14172	174.
0041	660	171.69697	73.8
0048	228	195.89474	233.
0055	448	209.03571	138.

Image Analysis Collaboratory

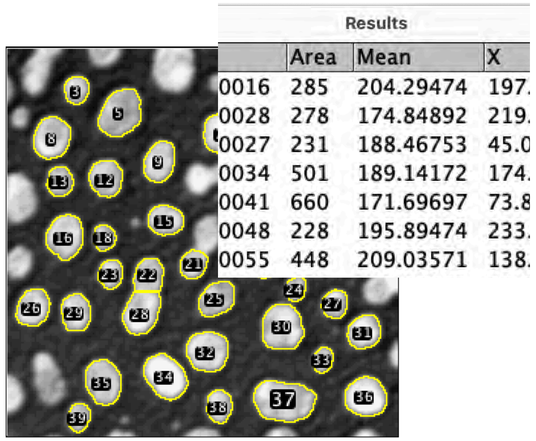
Binary mask



Process and segment



Export and Measure



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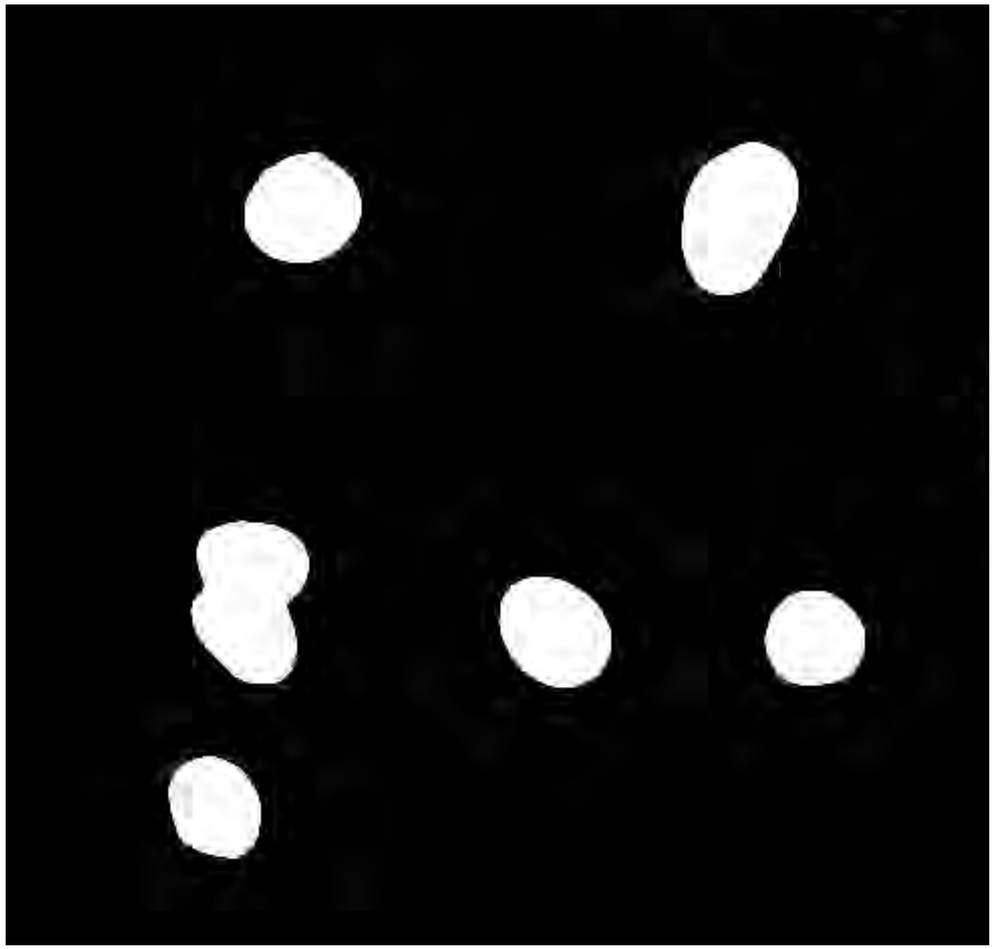
0055	448	209.03571	138.

Do you see an issue here?

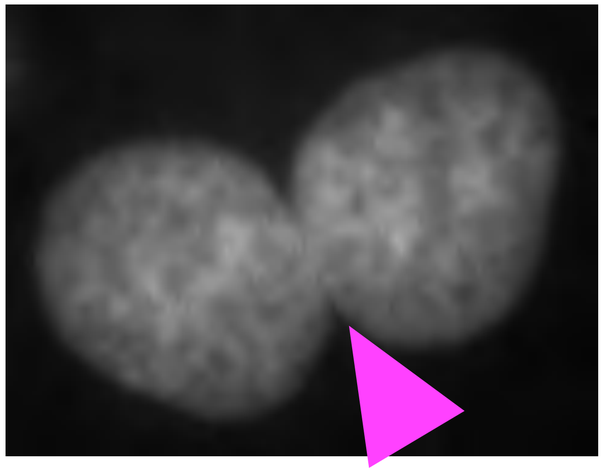


Solution: Watershed

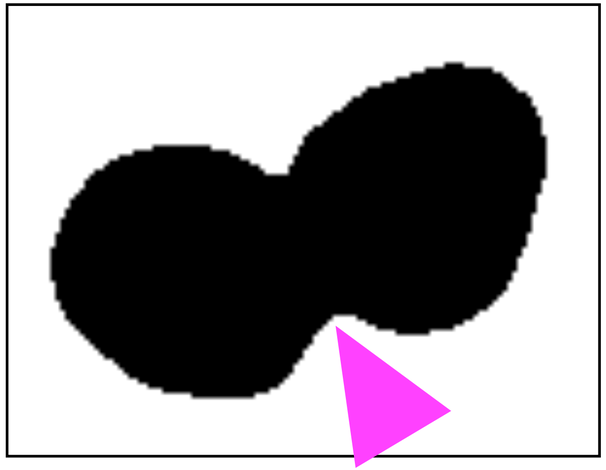
in **Fiji**: **Process** > **Binary** > **Watershed**

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

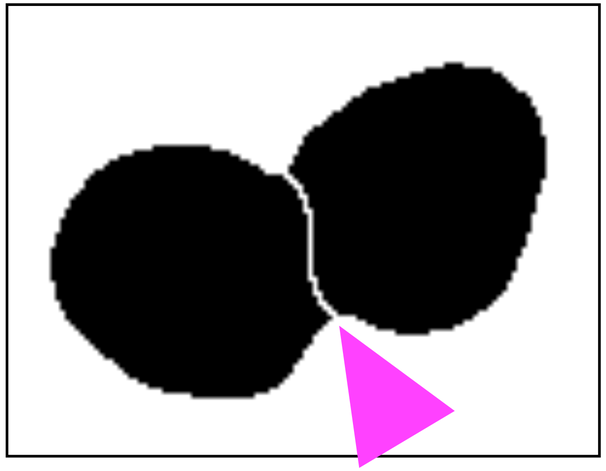
Image



Binary Mask

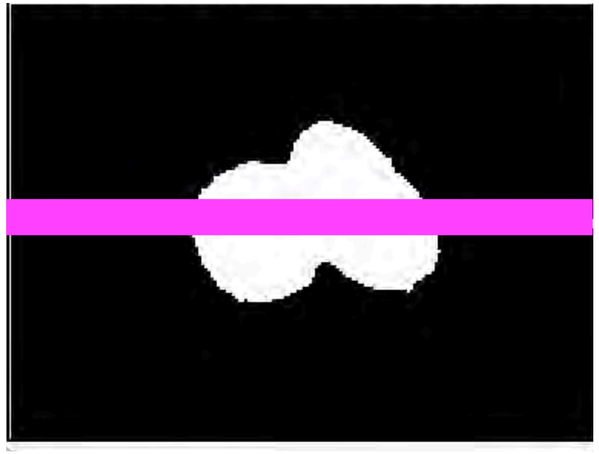


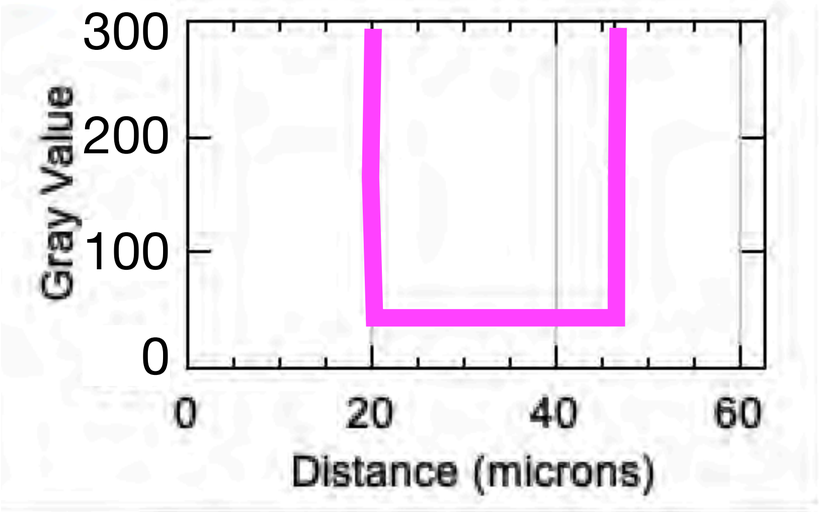
Watershed



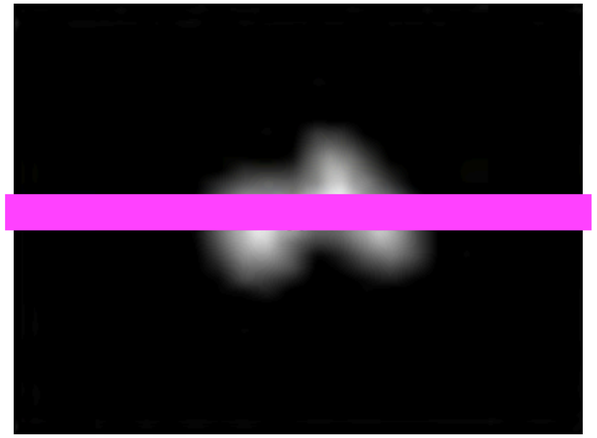
Solution: Watershed

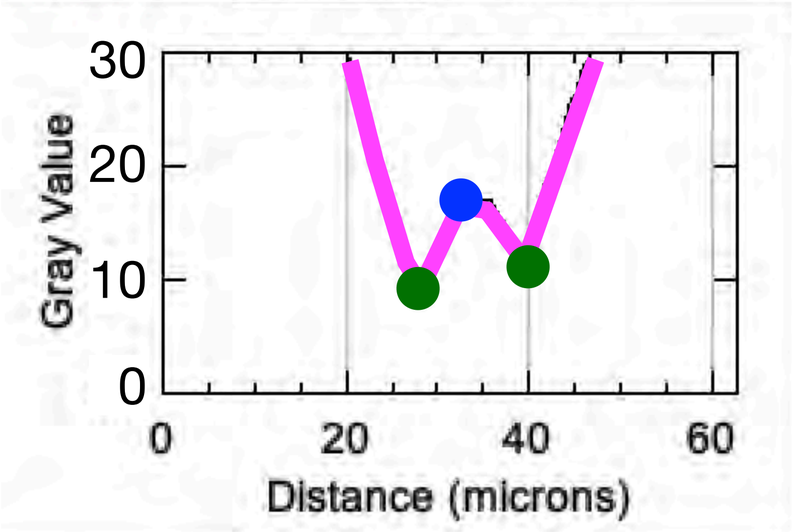
Binary mask

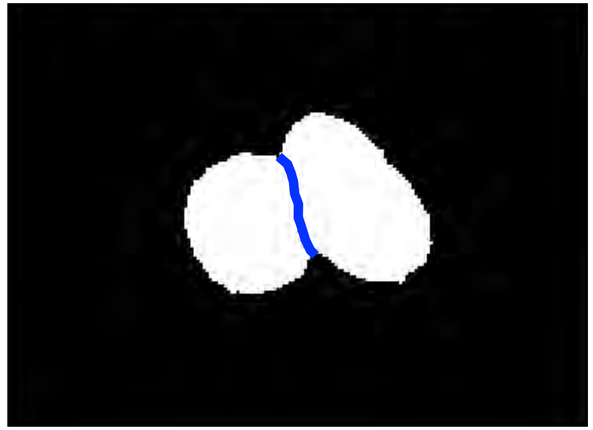




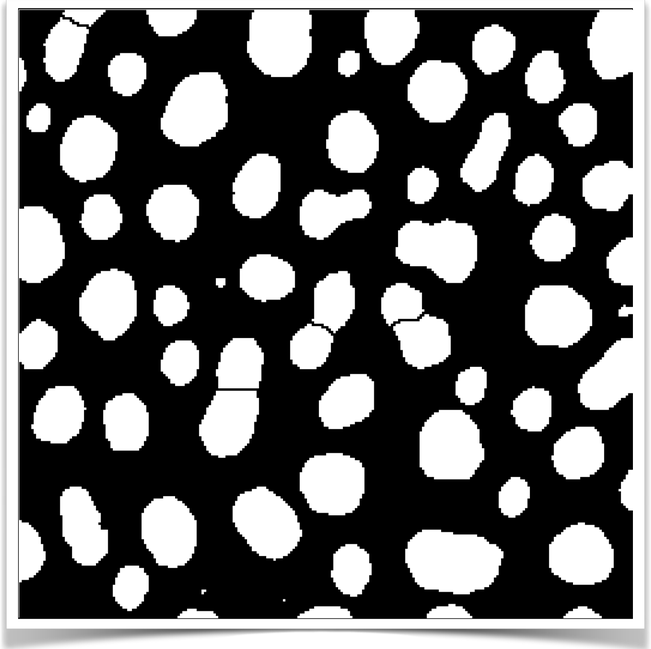
Distance transform



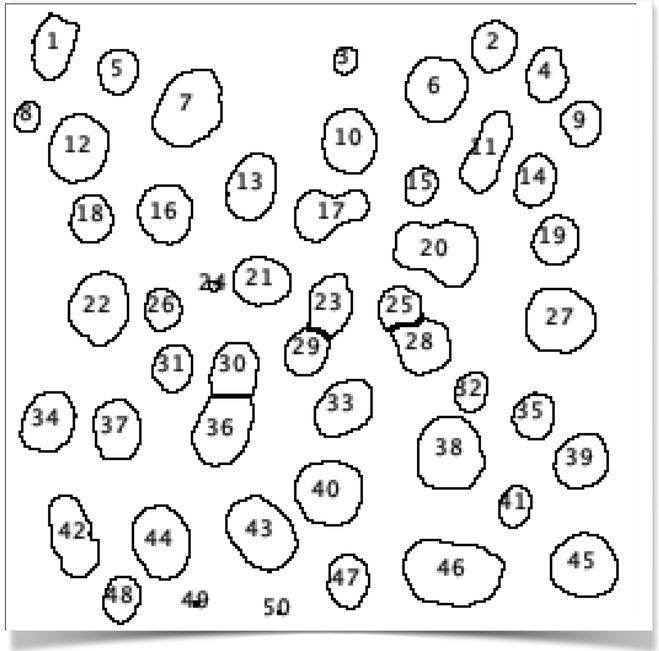




From binary image to instance segmentation



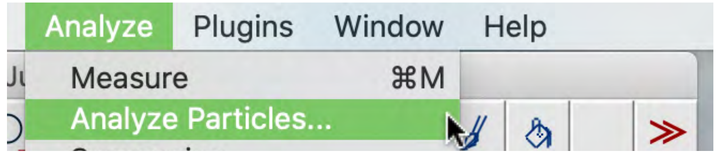
Binary mask



Instance segmentation

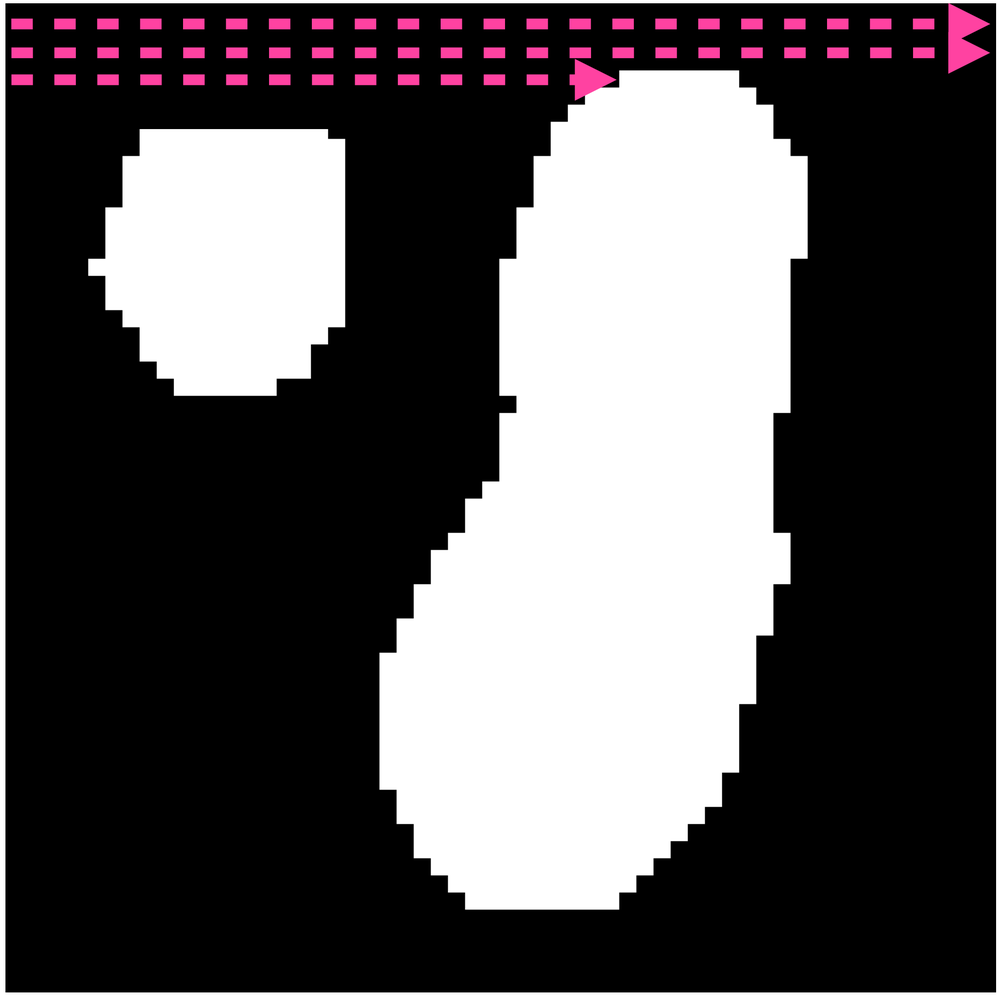
Analyze particles

in Fiji: **Analyze** > **Analyze Particles…**



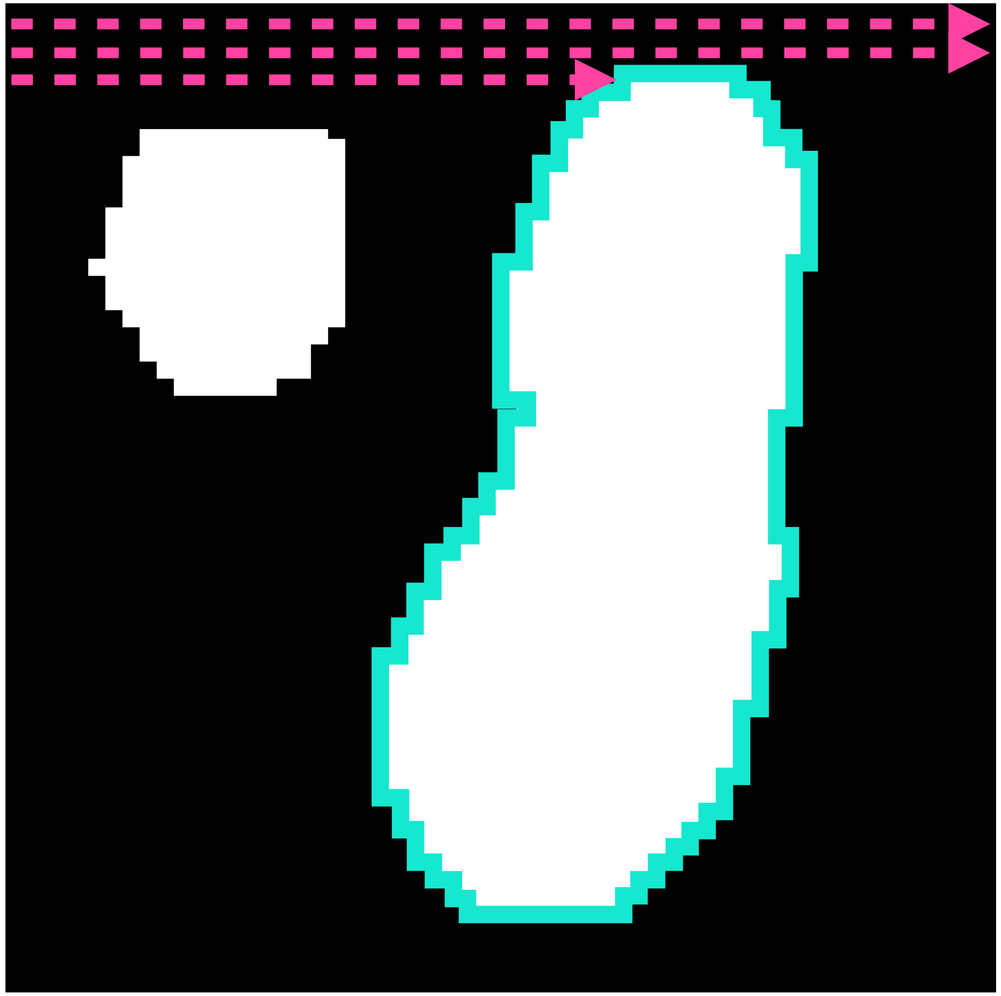
Analyze particles — underlying principles

in **Fiji**:
Analyze
> **Analyze Particles…**



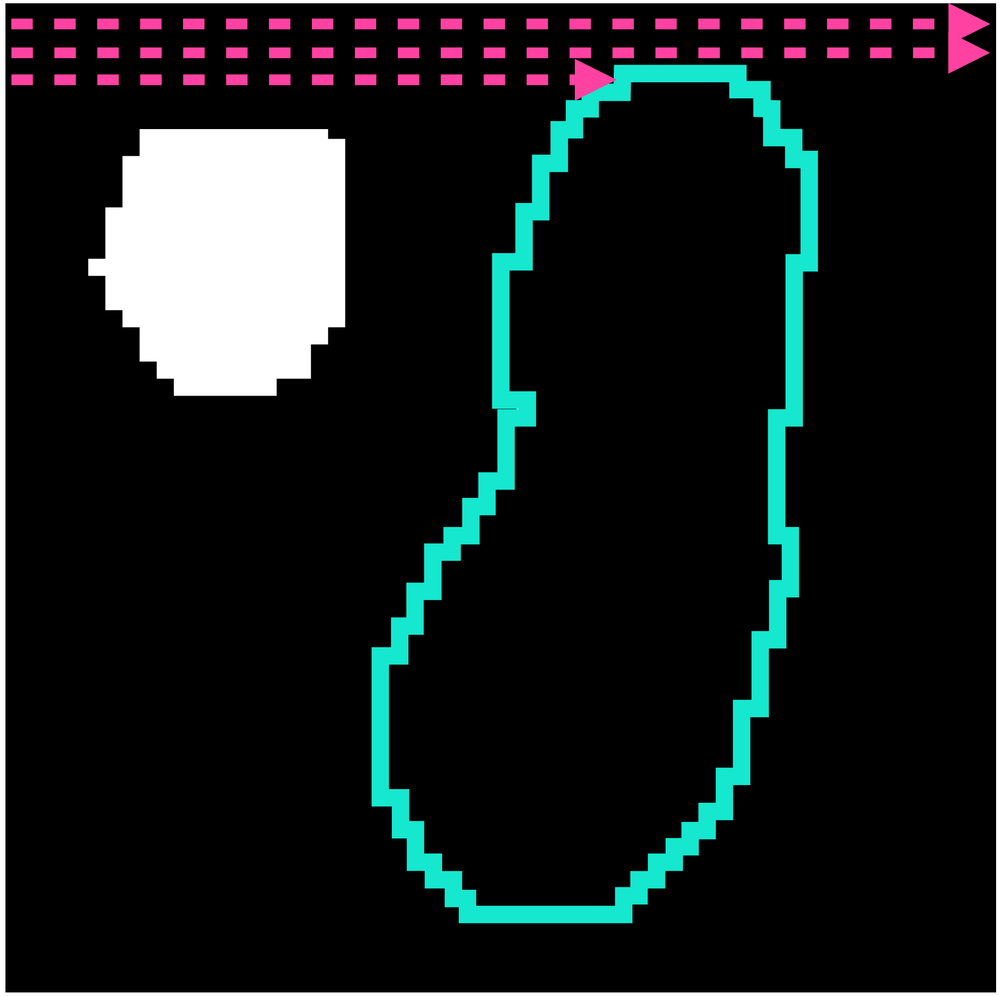
Analyze particles — underlying principles

in **Fiji**:
Analyze
> **Analyze Particles…**



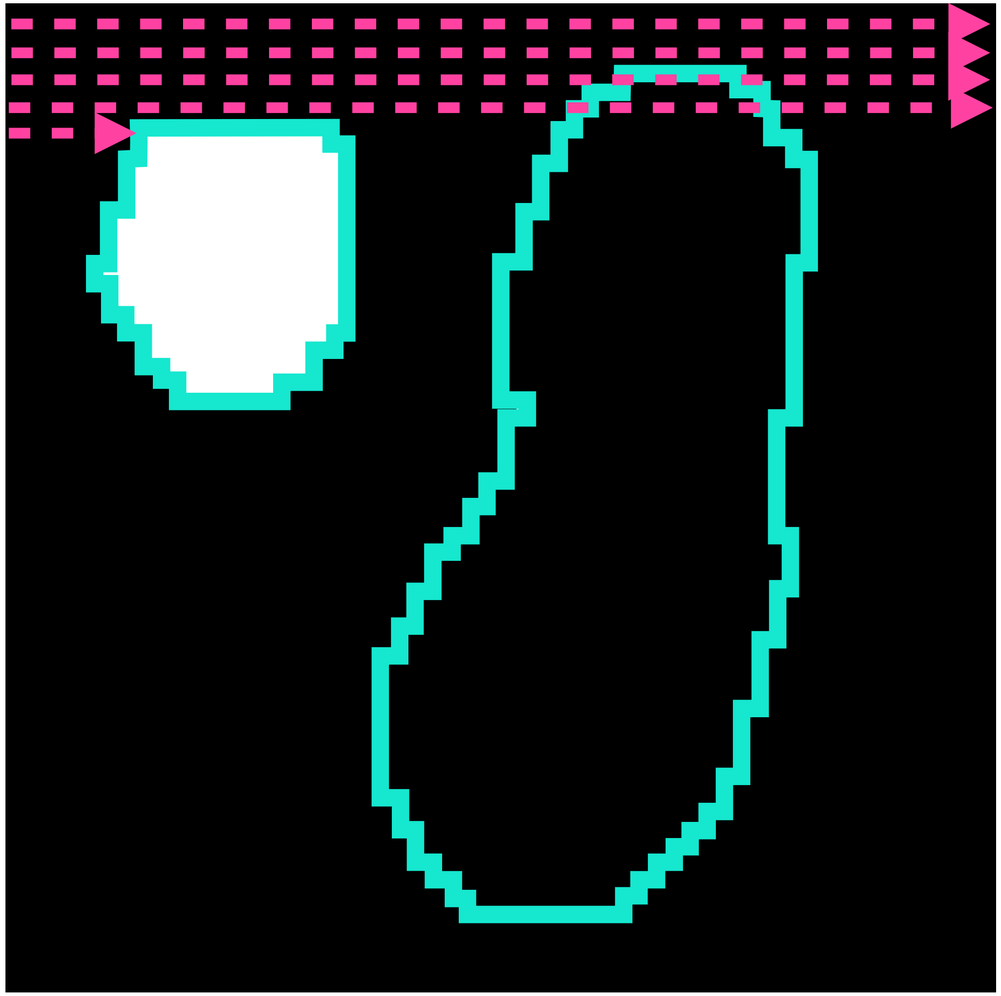
Analyze particles — underlying principles

in **Fiji**:
Analyze
> **Analyze Particles…**



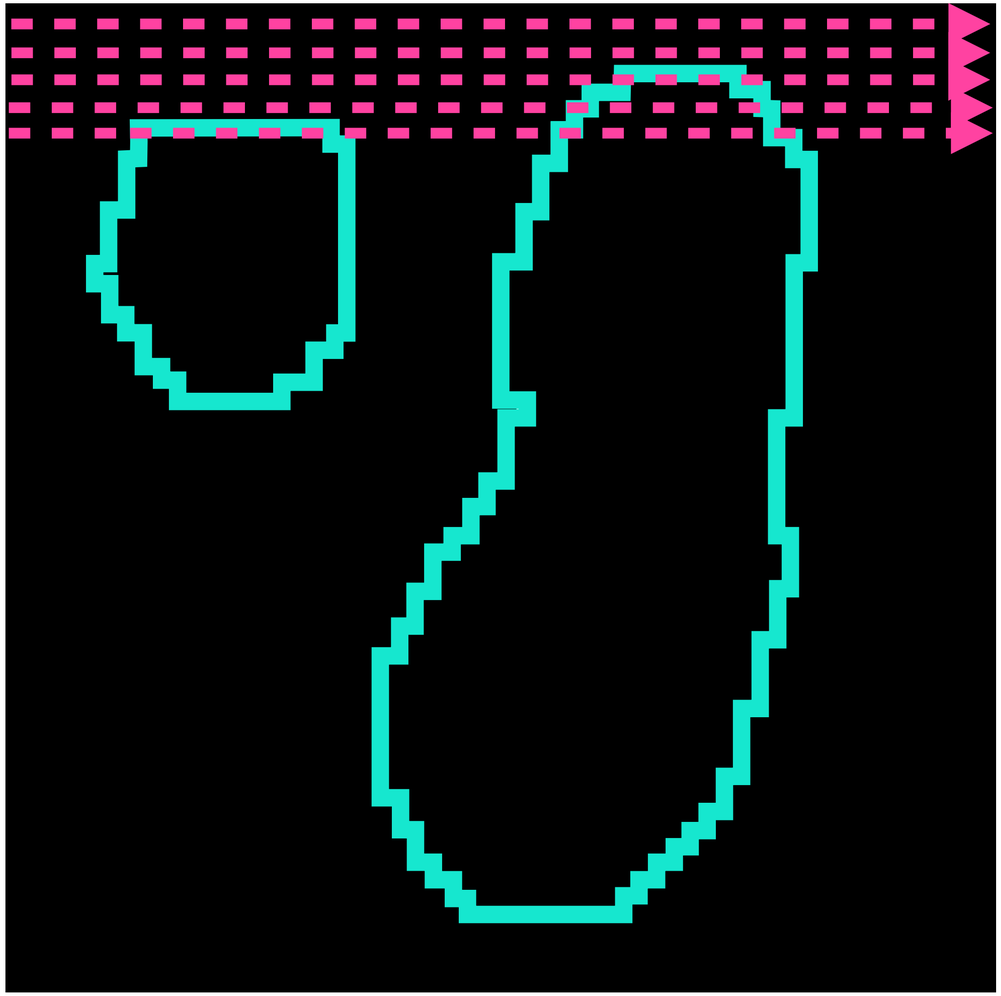
Analyze particles — underlying principles

in **Fiji**:
Analyze
> **Analyze Particles…**



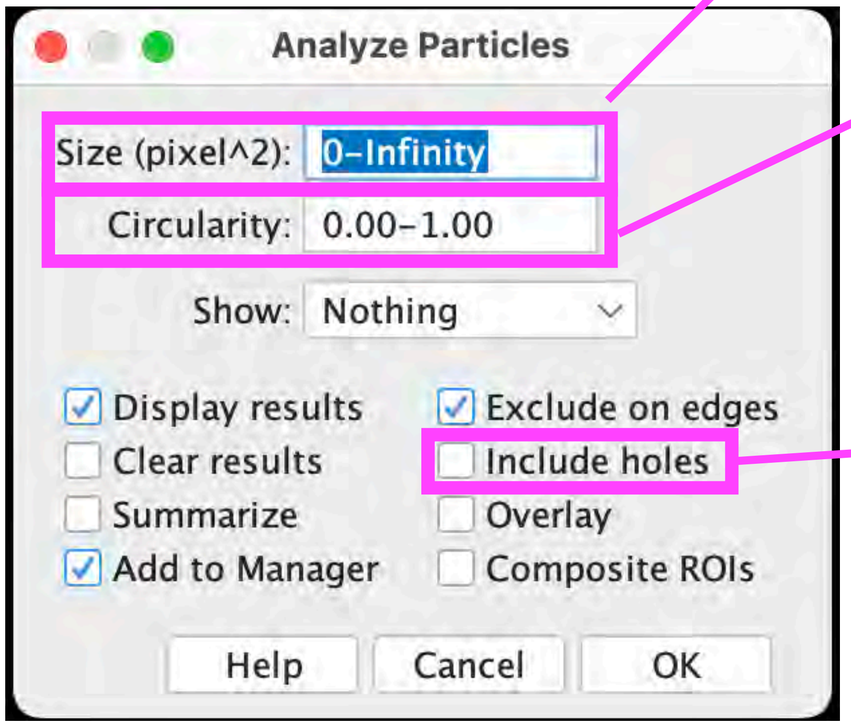
Analyze particles — underlying principles

in **Fiji**:
Analyze
> **Analyze Particles…**

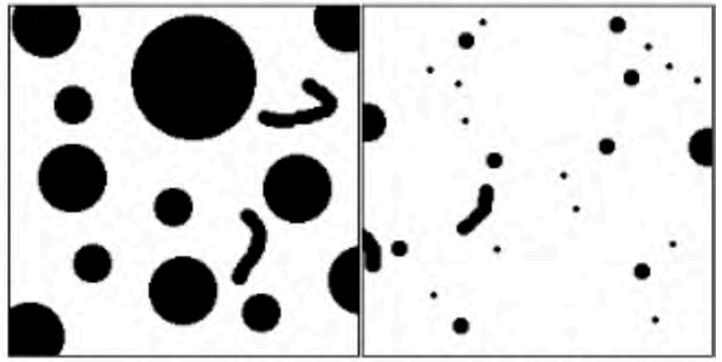


Analyze particles: Select for morphology

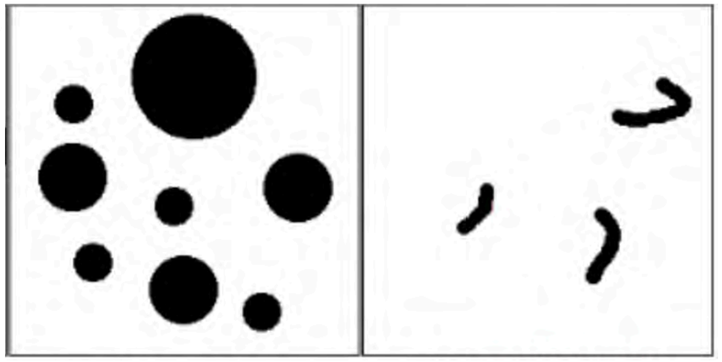
in **Fiji**:
Analyze
> **Analyze Particles…**



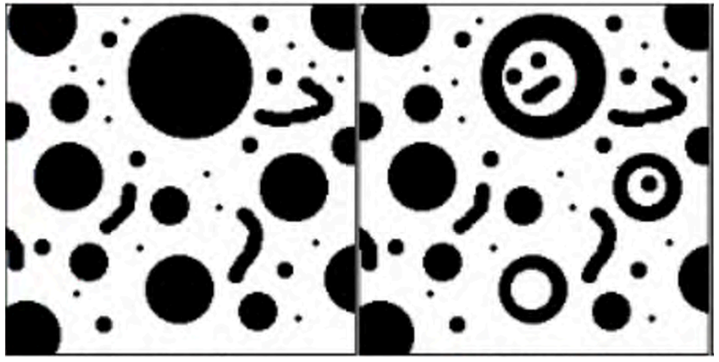
Size



Circularity

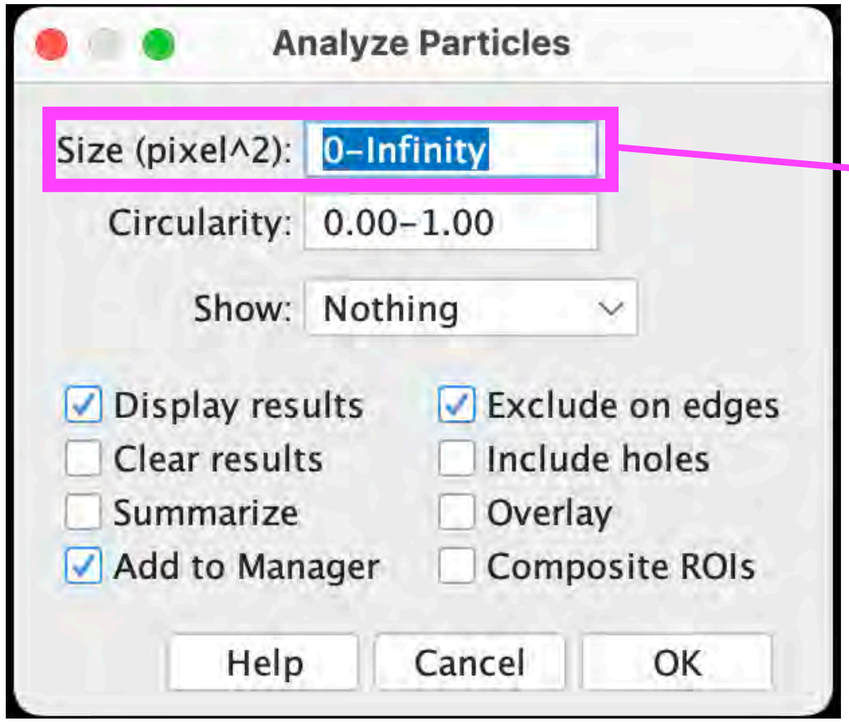


Fill

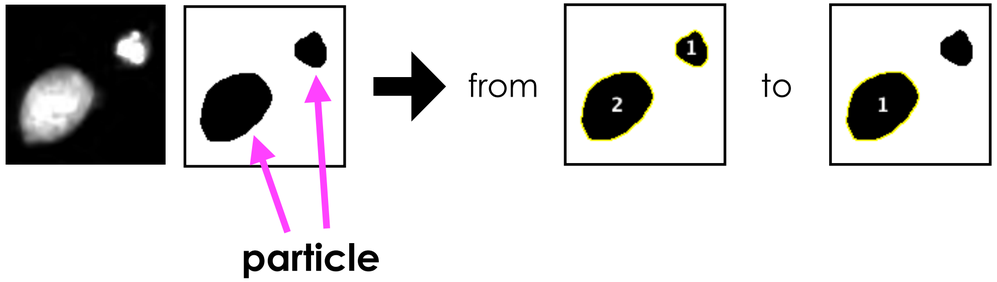


Analyze particles: Size

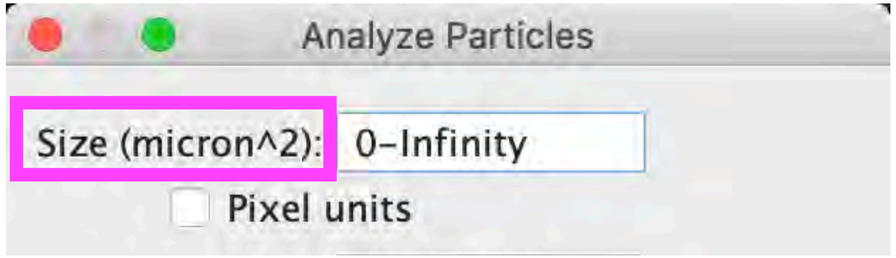
in **Fiji**: **Analyze** > **Analyze Particles…**



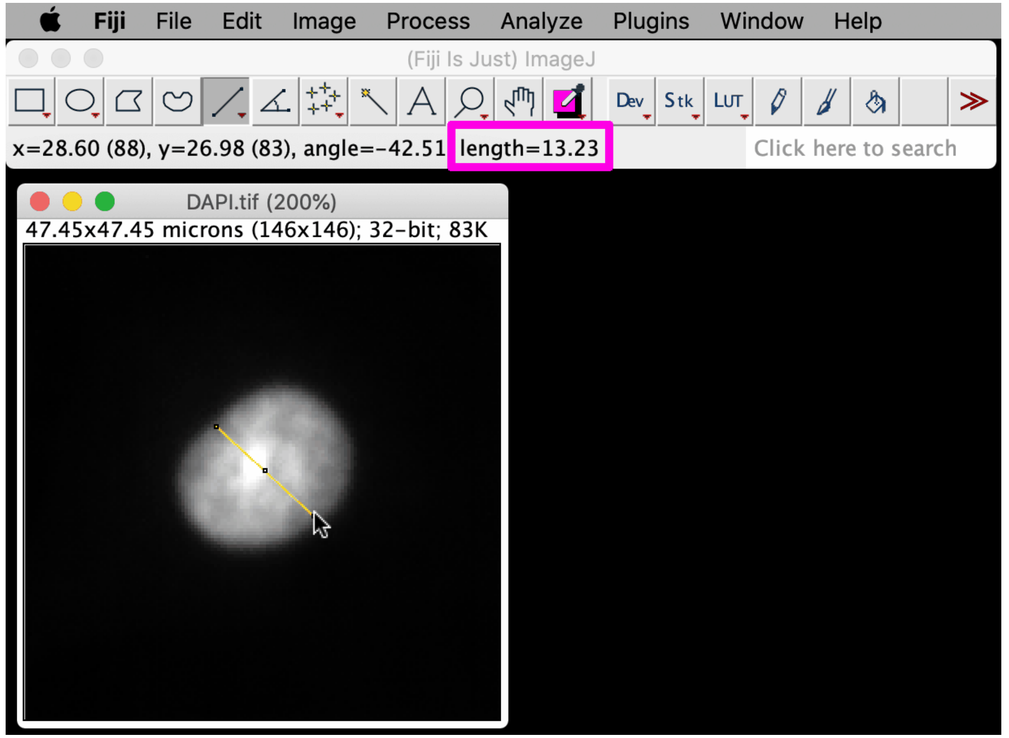
Size range of the **particles** that you want to detect.



Analyze particles: Size

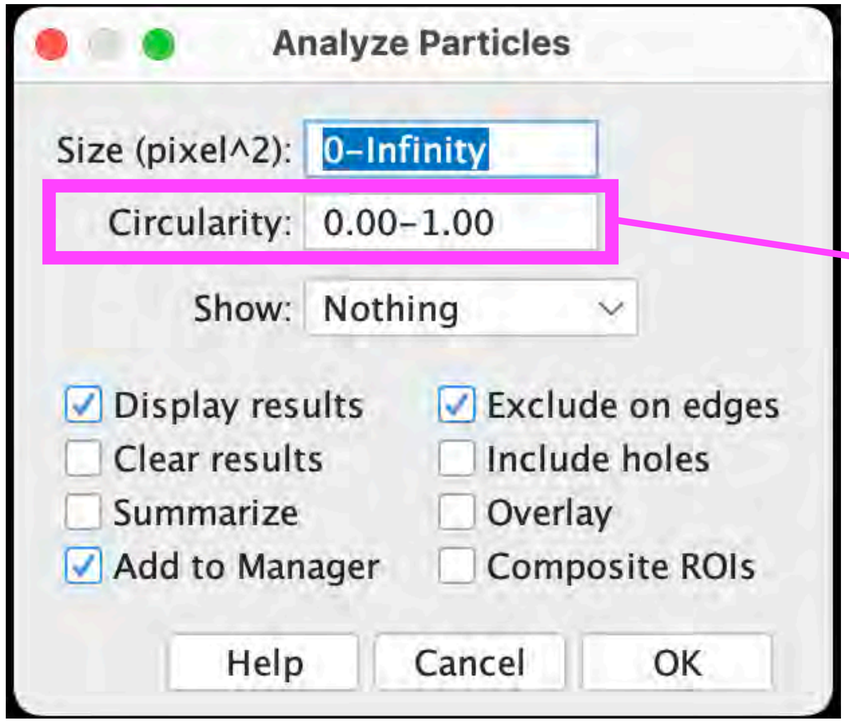


the **size range** of the **particles** that you want to detect.



Analyze particles: Circularity

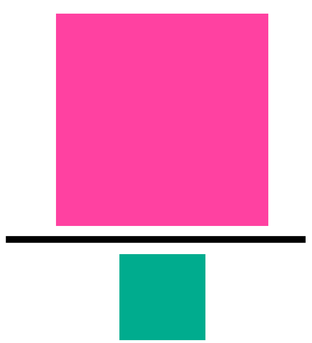
in **Fiji**: **Analyze** > **Analyze Particles…**

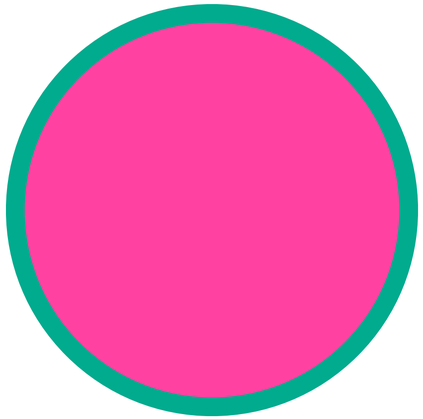


Circularity of the **particles** that you want to detect.

Analyze particles: Circularity

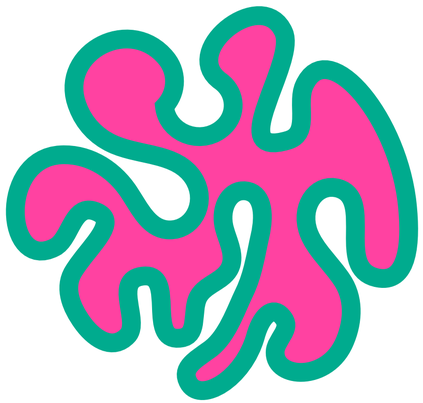
$$4\pi \times \frac{\text{area}}{\text{perimeter}^2} = 1$$

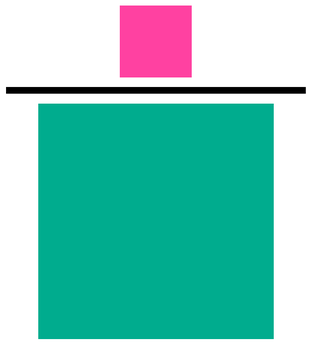


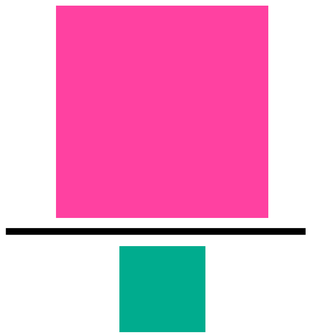


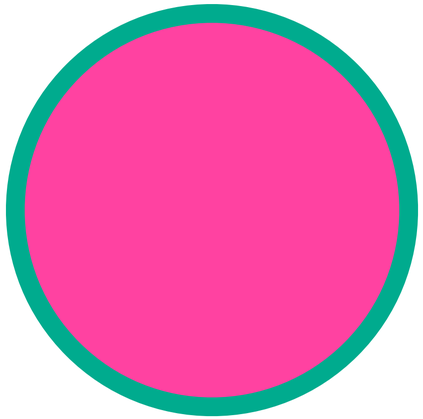
Analyze particles: Circularity

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





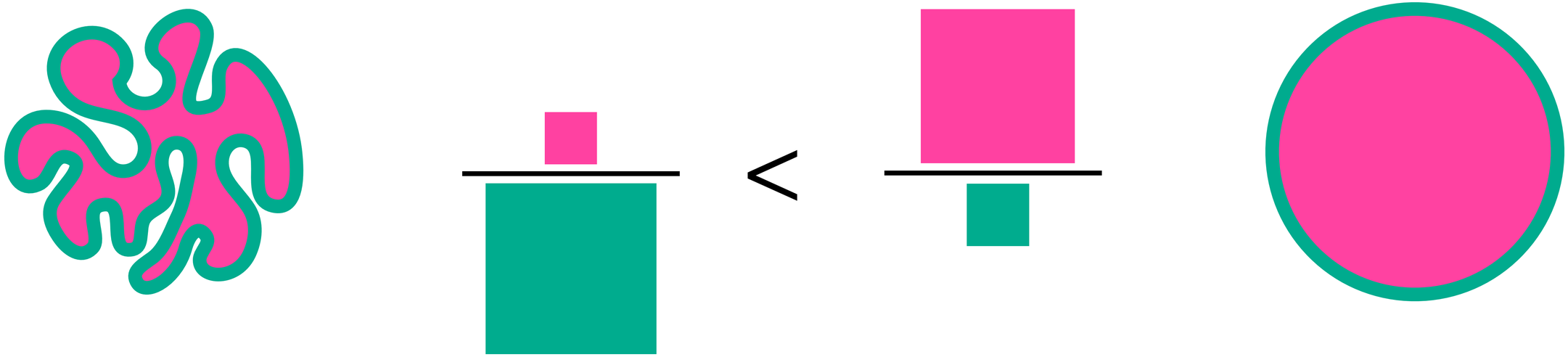




Analyze particles: Circularity

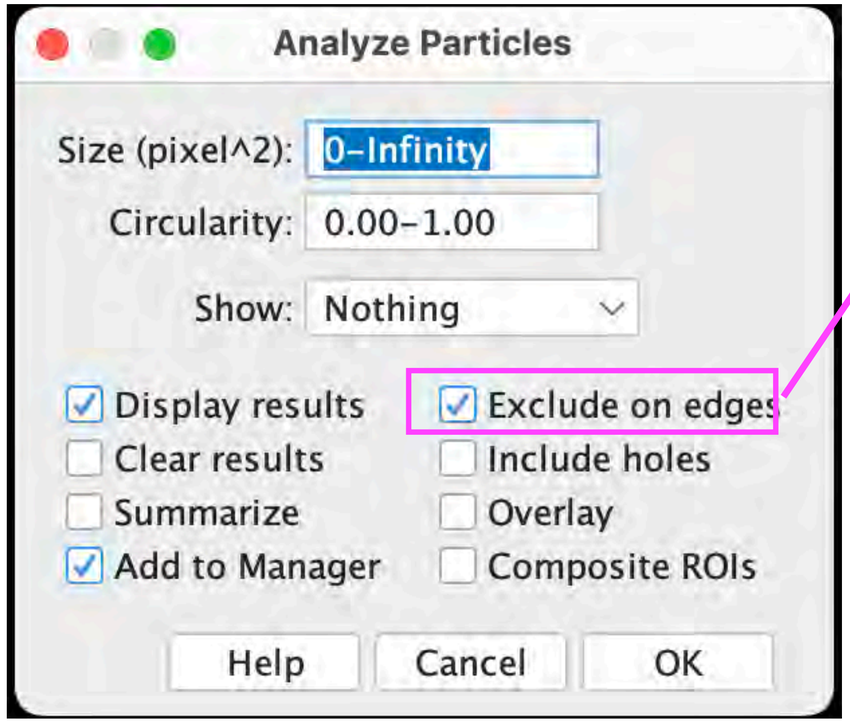
$$4\pi \times \frac{\text{area}}{\text{perimeter}^2} < 1$$

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



Analyze particles: Exclude on Edges

in **Fiji**: **Analyze** > **Analyze Particles…**



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

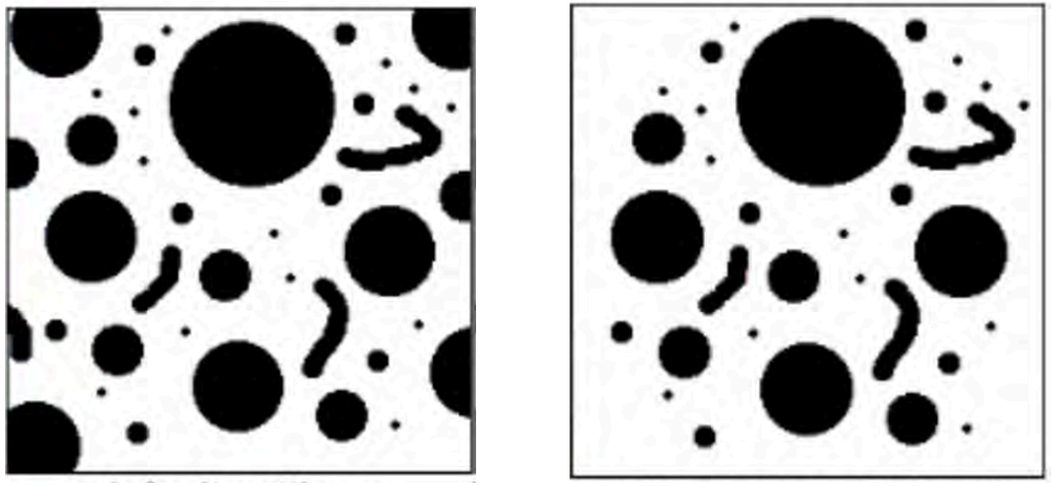
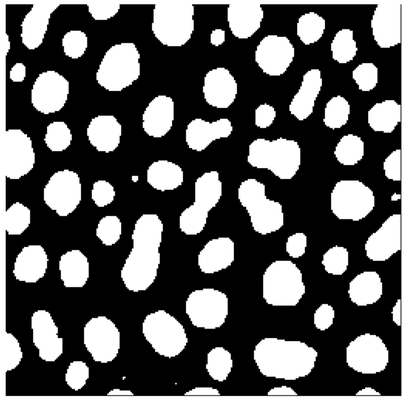
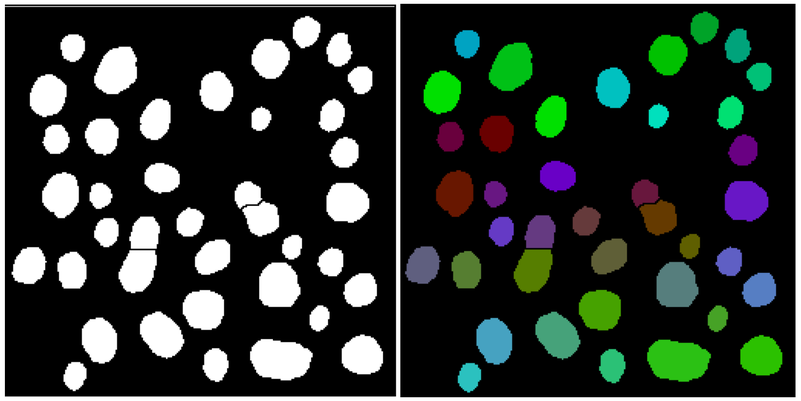


Image Analysis Collaboratory

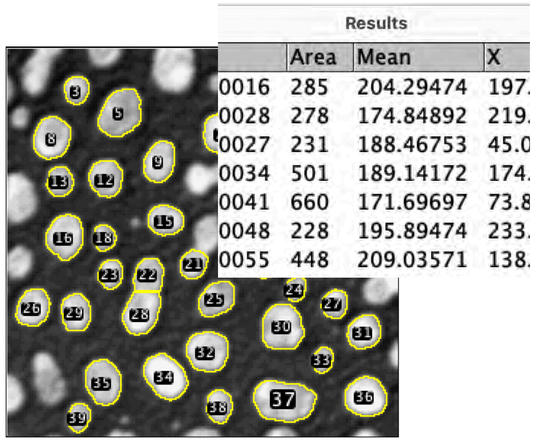
Binary mask



Process and segment



Export and Measure



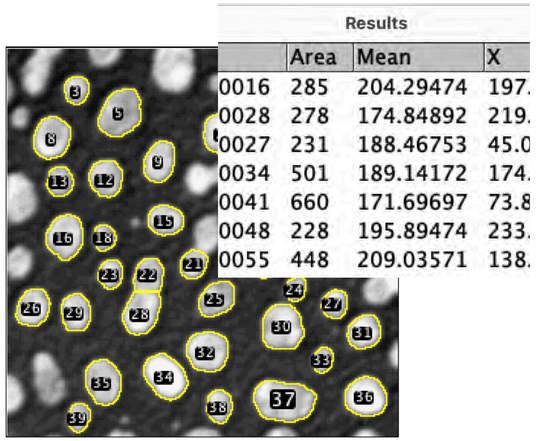
Results

	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.

Image Analysis Collaboratory

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

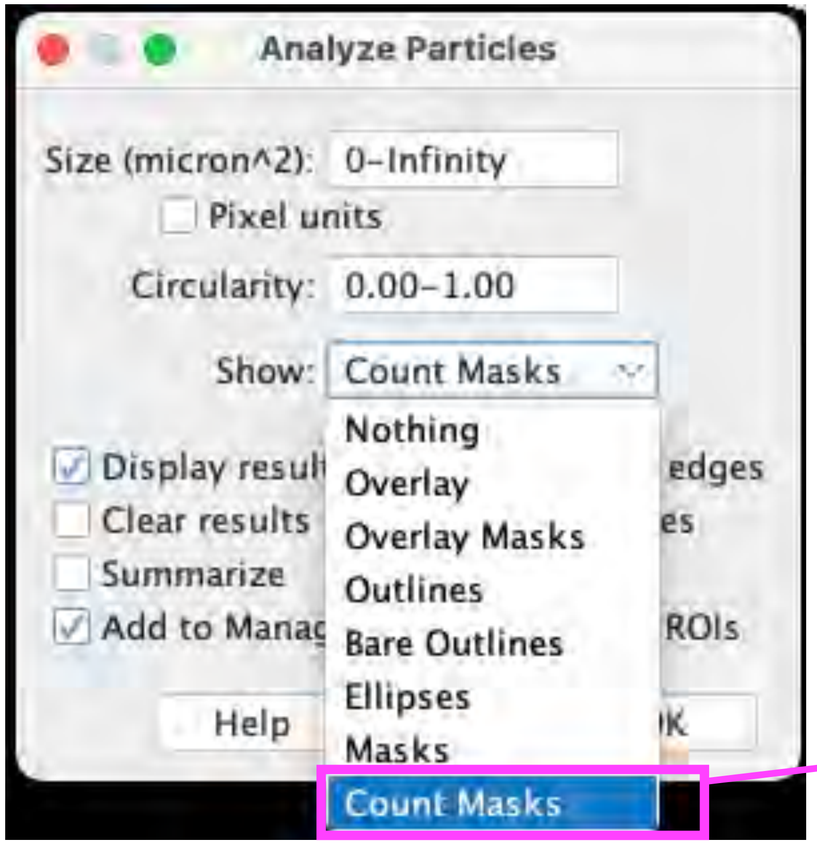
Export and Measure

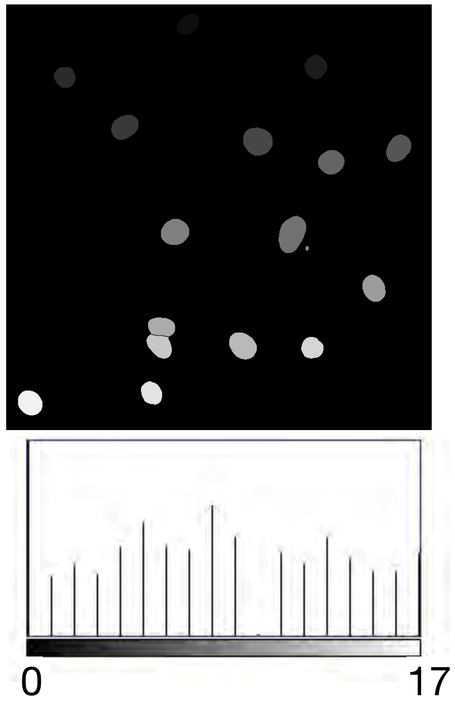


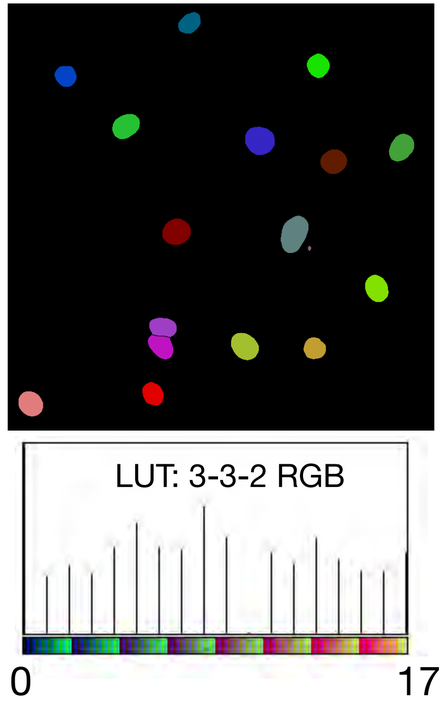
Analyze particles: Save the segmented image

Export

in **Fiji**: **Analyze** > **Analyze Particles…**



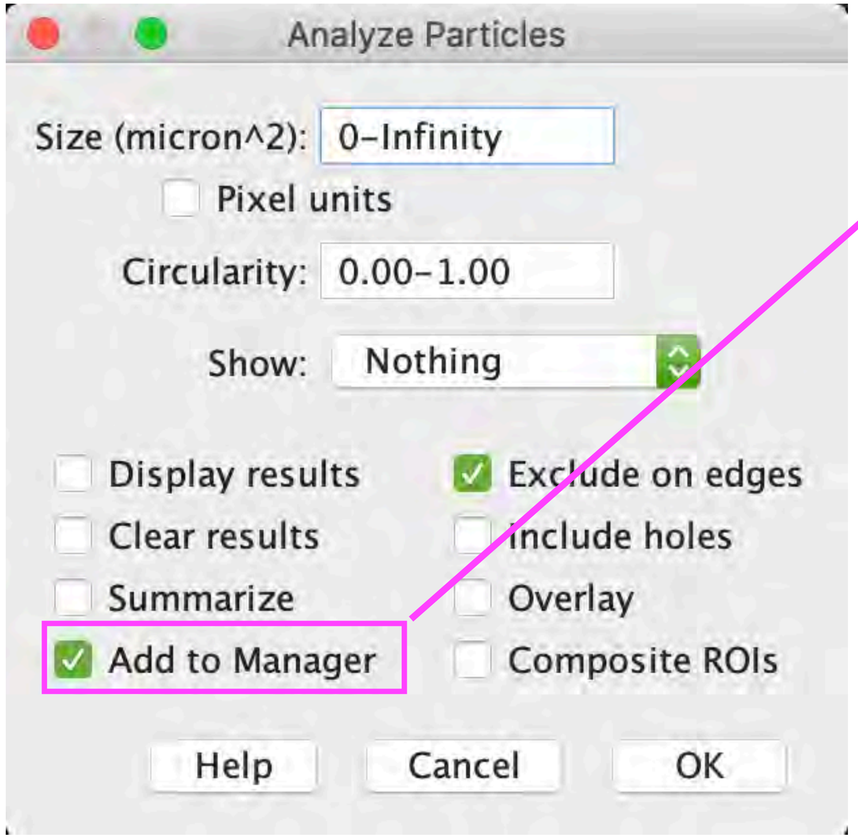




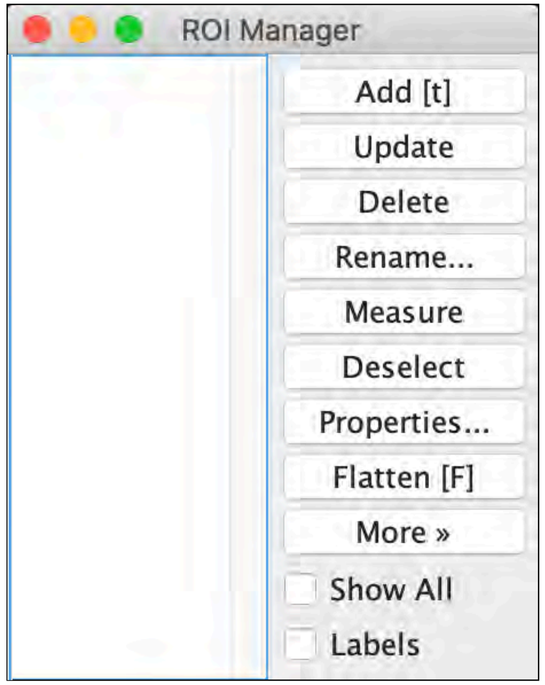
"**Count Masks**" encodes object identity as gray value.

Analyze particles: Add to ROI Manager

in **Fiji**: **Analyze** > **Analyze Particles…**



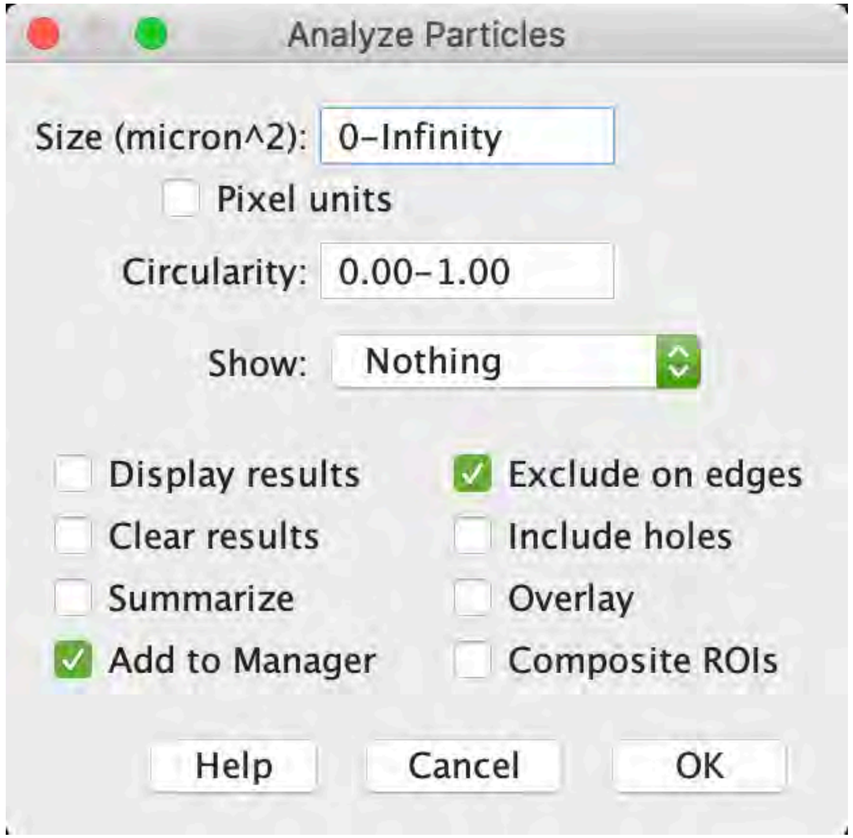
"**Add to Manager**" stores all found objects in the **ROI Manager**.



Analyze particles: Add to ROI Manager

Export

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



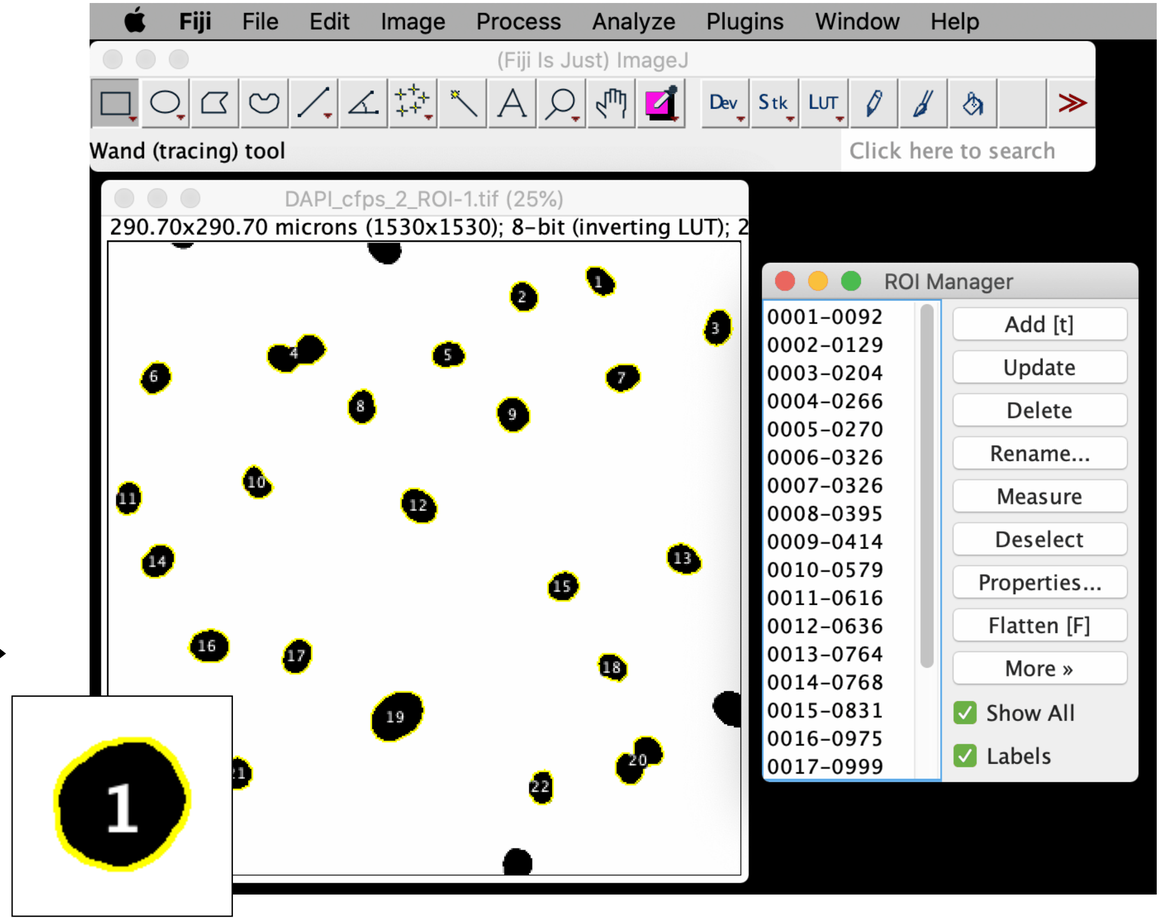
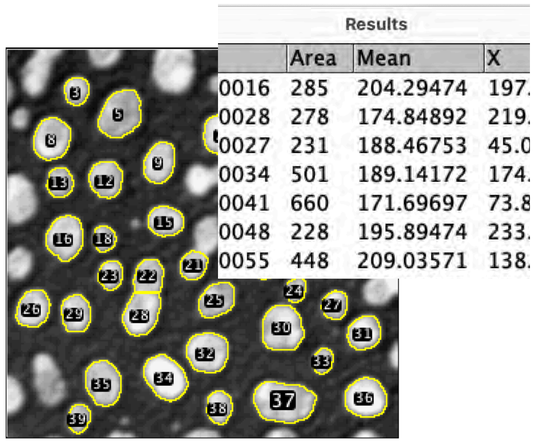


Image Analysis Collaboratory

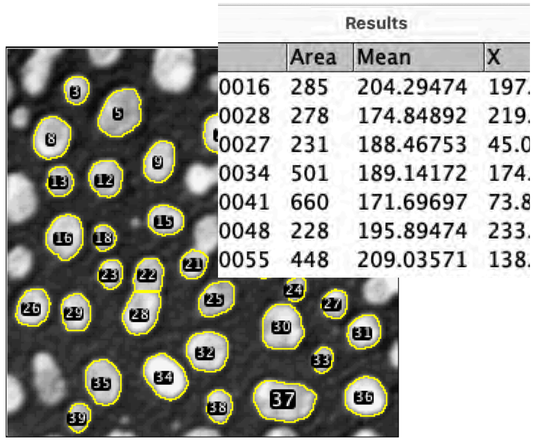
- Save the segmented image
- Add to ROI manager
- Generate and export measurements

Export and Measure



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

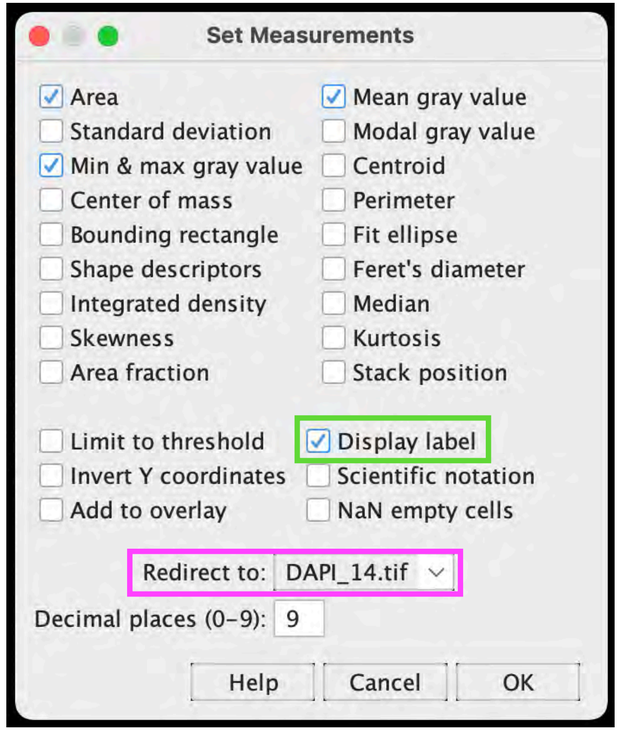
Export and Measure

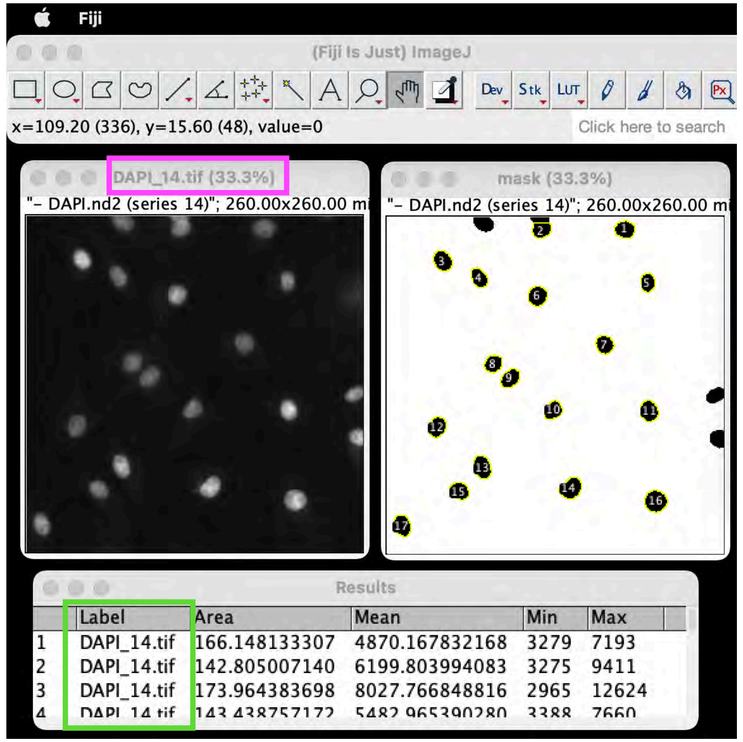


Select what to measure: Set Measurements

Analyze > Set Measurements…

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





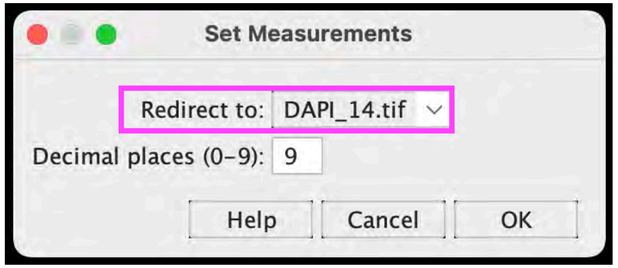
Measure — Option 1: Manually

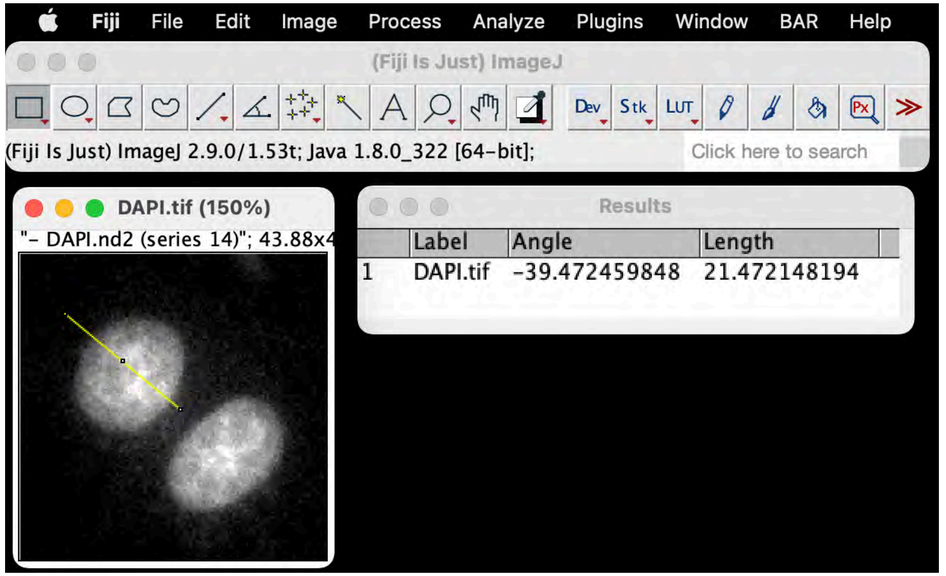
Analyze > Measure

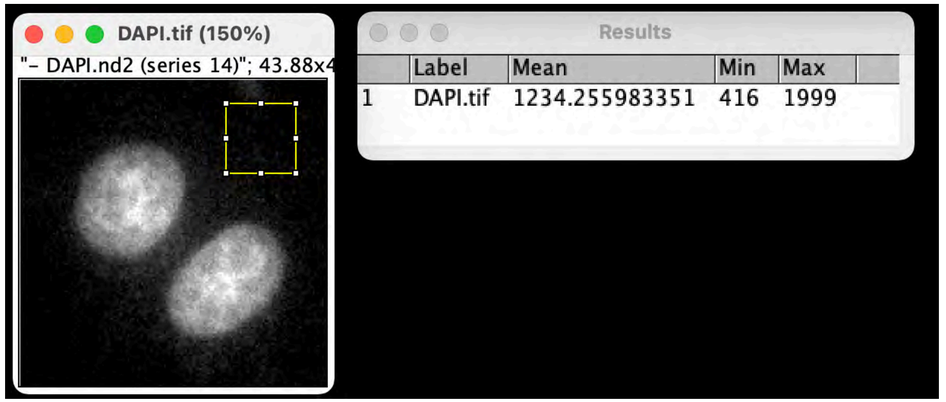
(cmd) + m

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, …)



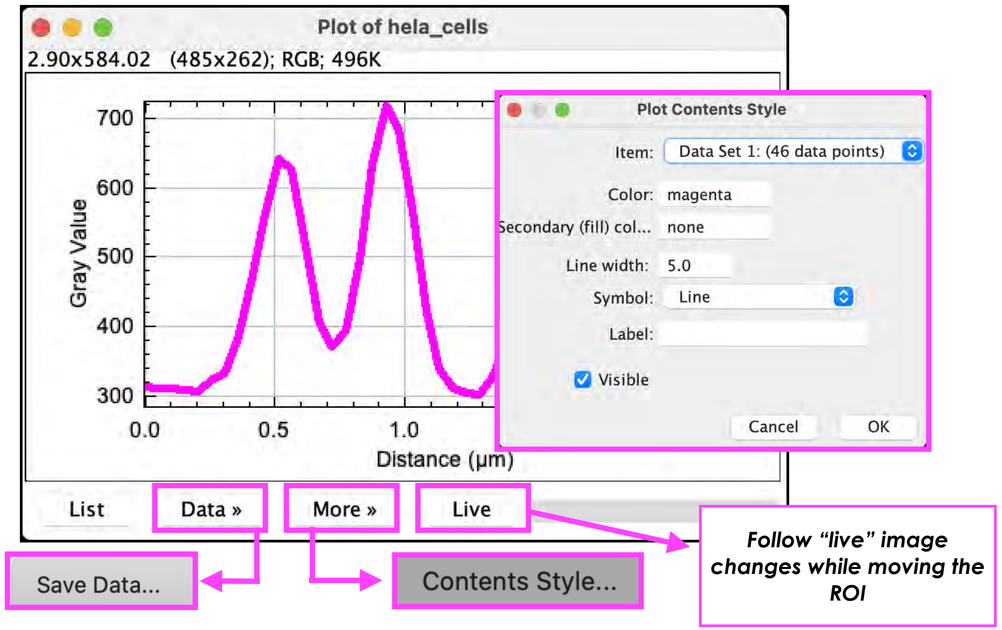


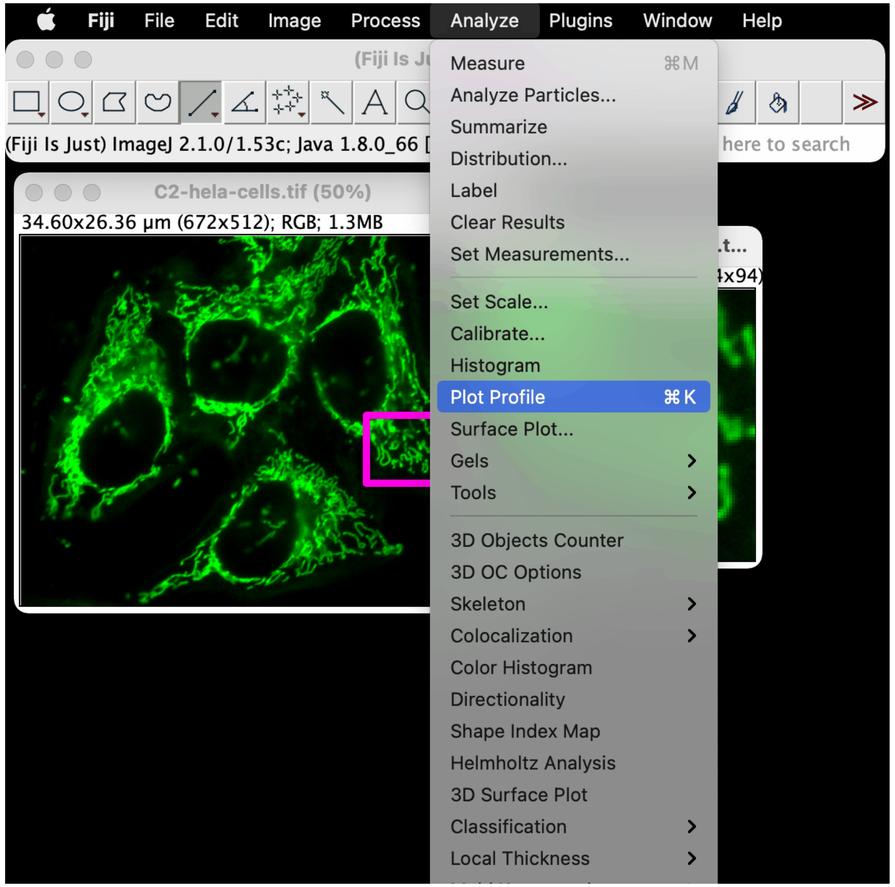


Side note: other kinds of measurements

Analyze > Plot Profile

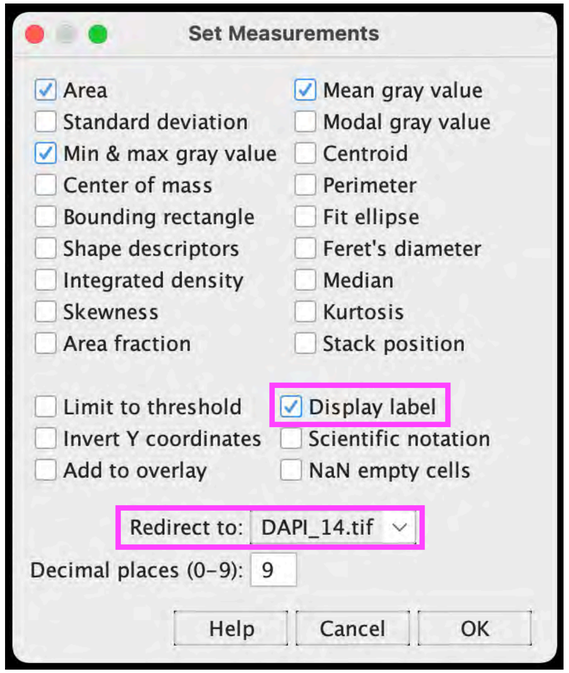
(cmd) + k

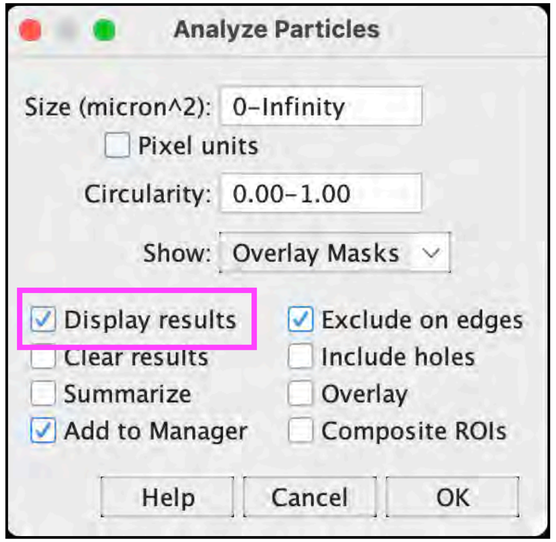


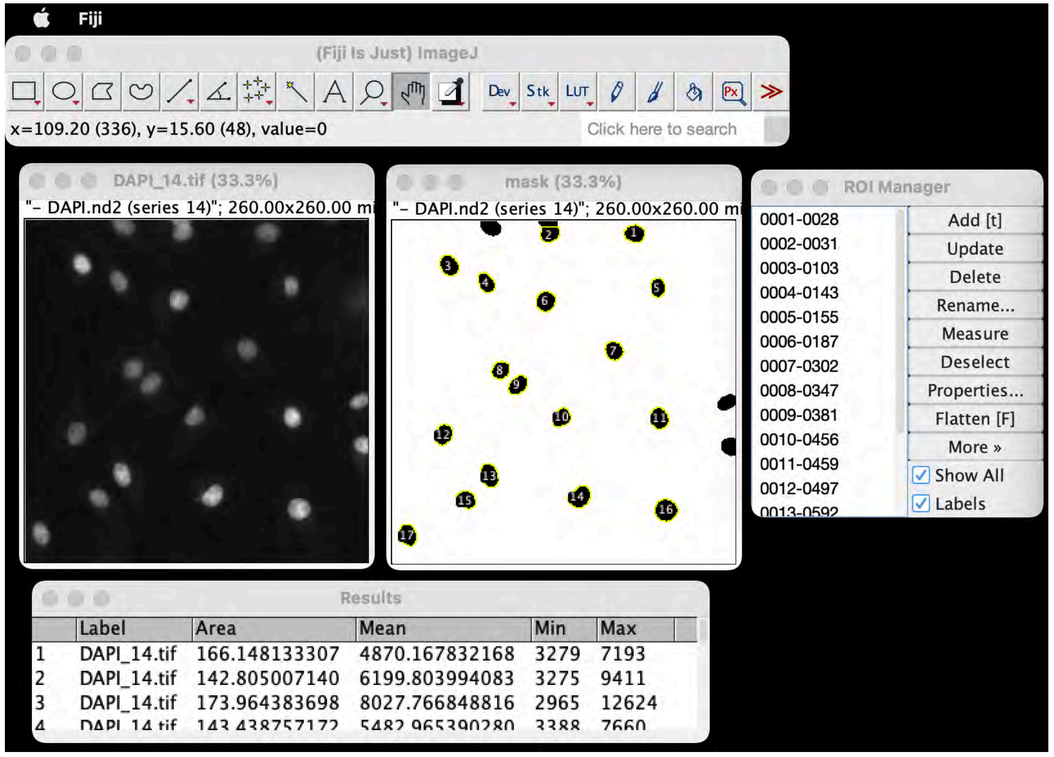


*Plots can be saved as .csv file ("Save Data…") and also as images (e.g. "File > Save AS > PNG")

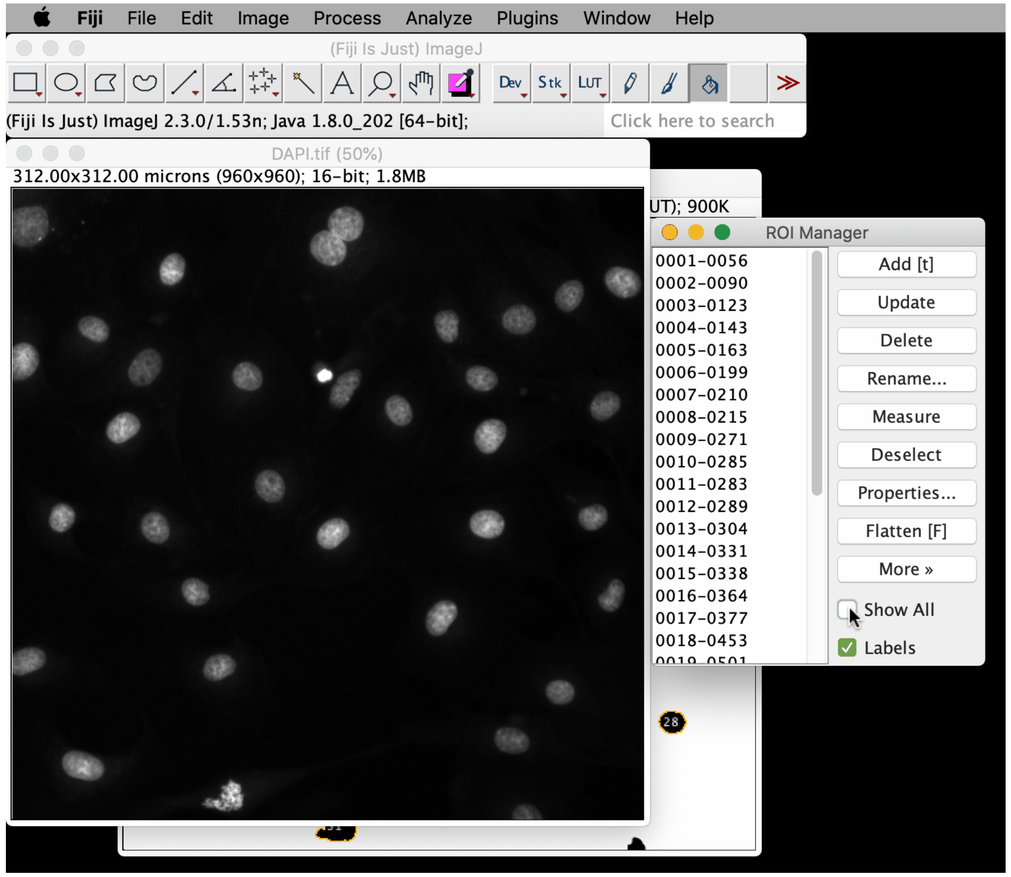
Measure — Option 2: from Analyze particles







Measure — Option 3: Using the ROI manager



1. Select the "ORIGINAL" image.

2. To measure <u>all</u> 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