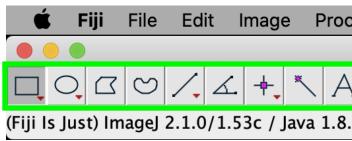
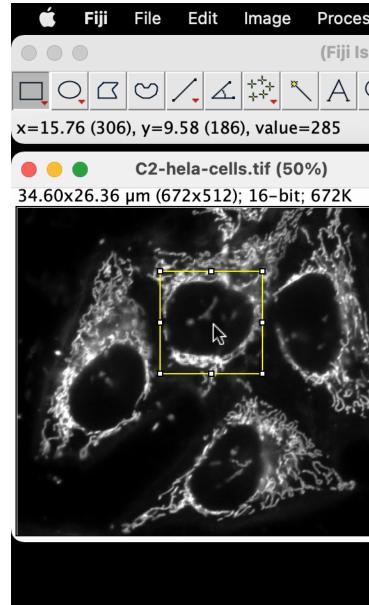


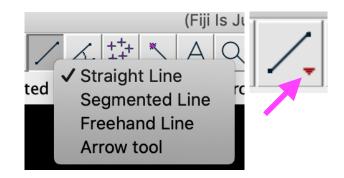
ROI Manager

Annotation Tools *see also Edit > Selection

Region Of Interest (ROI)







Secondary/Right Click for more options

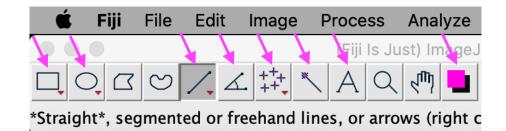


https://imagej.net

https://imagej.nih.gov/ij/

| ess | Analyze | Plug | ins | Window | Help | |
|---------|-------------|------|-----|--------|-------------|--|
| (Fiji I | s Just) Ima | geJ | | | | |
| | . Lui | Dev_ | Α | nnota | ition Tools | |
| 0_66 | (64-bit) | | | | | |

| S | Analyze | Plugins | Window | Help | | |
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| Jus | st) ImageJ | | | | | |
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Double Click to set/change properties





https://fiji.sc/

<u>https://imagej.net/Fiji</u>

ROI Manager

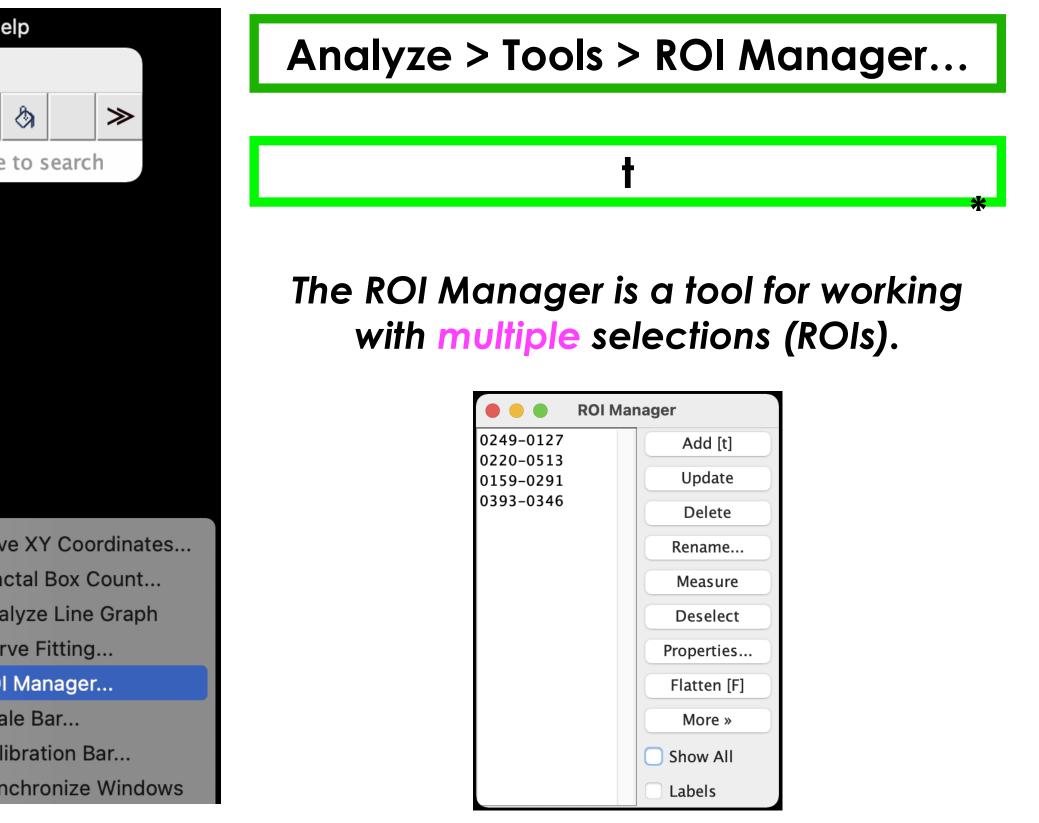
| 🗯 Fiji File Edit Image | Process | Analyze | Plugins | Window | He |
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| C2-hela-cells.tif (50 | %) | Label | | _ | |
| "Green"; 34.60x25.03 µm (672x486) |); 16-bit; 6 | Clear Res | ults | | |
| The second second | 105 | Set Meas | urements | | |
| An other Eller | \$ 31.67 (1.10) | Set Scale | | | |
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| - 5-2-49 mart | 1. 8 | Plot Profi | | жĸ | |
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| | 8 | Tools | | > | Save |
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| | | Colocaliza | ation | > | ROI |
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*(cmd) + t can be used to both to open the ROI Manager and/or add a new ROI to the Manager.

**<u>shift + e</u> can be used to draw the last ROI.



https://imagej.net https://imagej.nih.gov/ij/



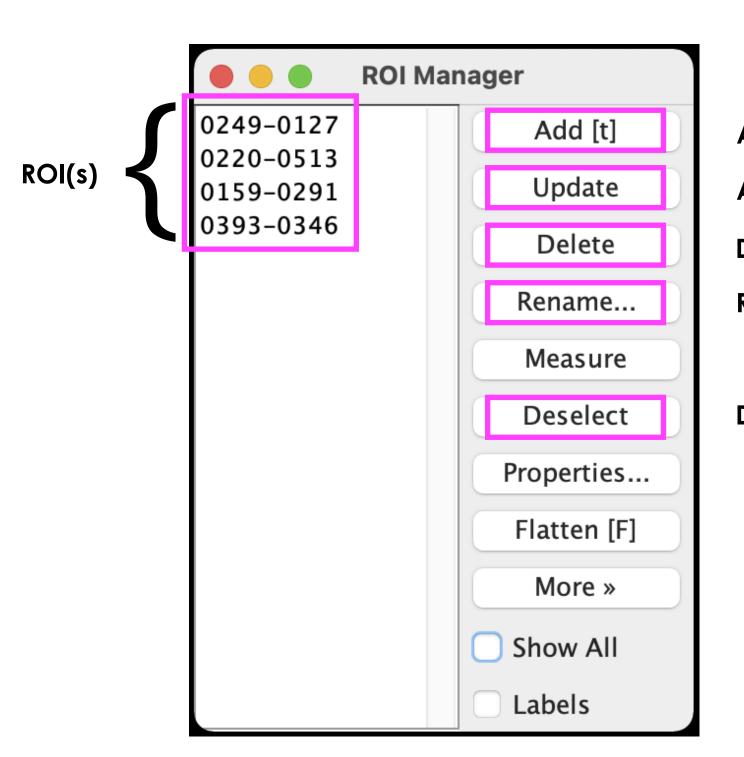
https://fiji.sc/

<u>https://imagej.net/Fiji</u>



"Analyze" menu

ROI Manager





https://imagej.net

https://imagej.nih.gov/ij/

Add new ROI (†).

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.



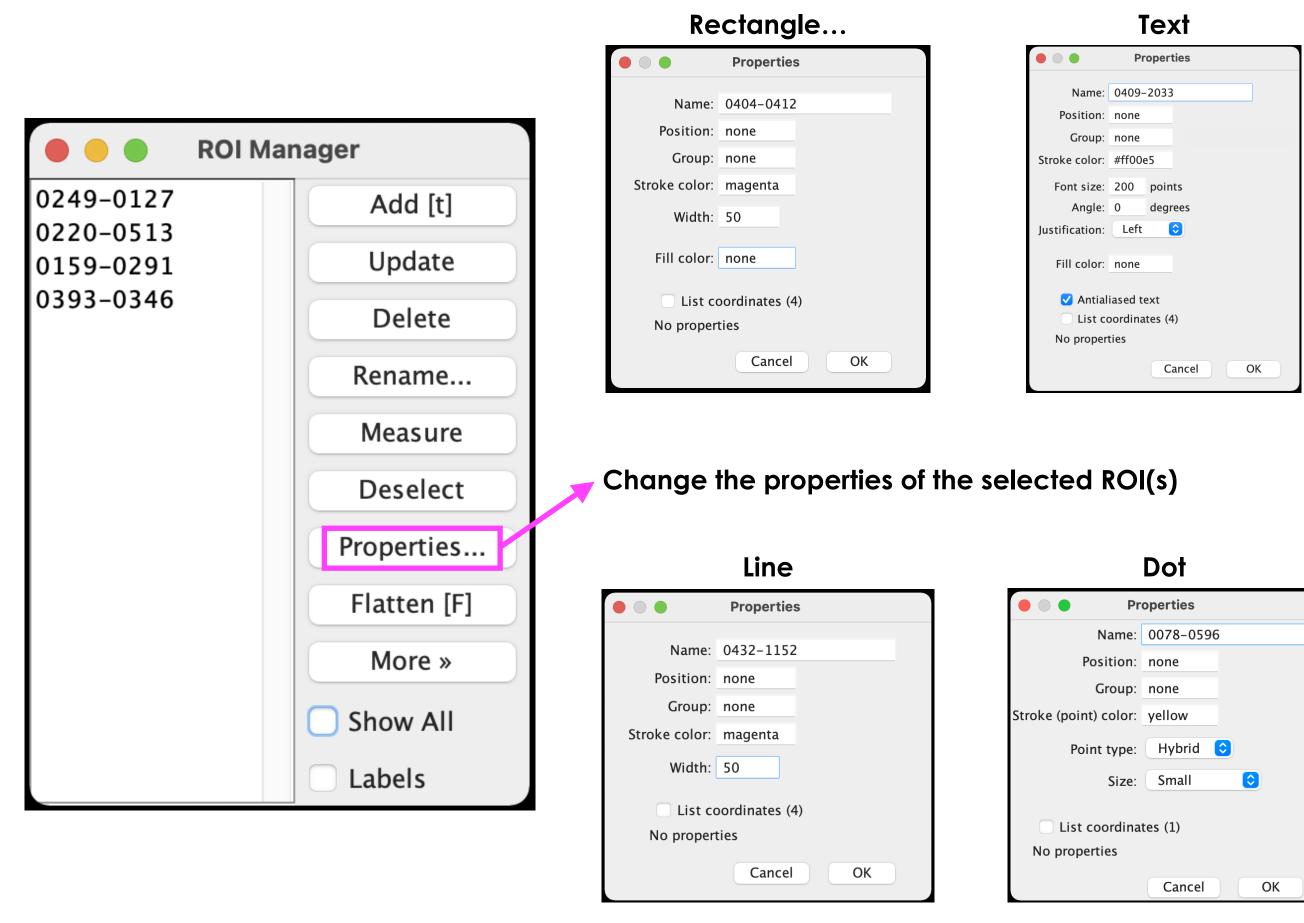


https://fiji.sc/

<u>https://imagej.net/Fiji</u>

"Analyze" menu

ROI Manager





https://imagej.net

https://imagej.nih.gov/ij/

| | Line | | Dot | | |
|-----------------------|-------------------------------------|--------------------------------|-----------|------------|--|
| | Properties | • • Pr | operties | | |
| N | 0422 1152 | Name: | 0078-0596 | , | |
| | 0432-1152 | Position: | none | | |
| Position: | none | Group: | none | | |
| Group: | none | Stroke (point) color: | yellow | | |
| roke color: Width: | | Point type: | Hybrid | • | |
| | | Size: | Small | \bigcirc | |
| List co | oordinates (4) ties Cancel OK | List coordina No properties | | | |
| | | | Cancel | OK | |

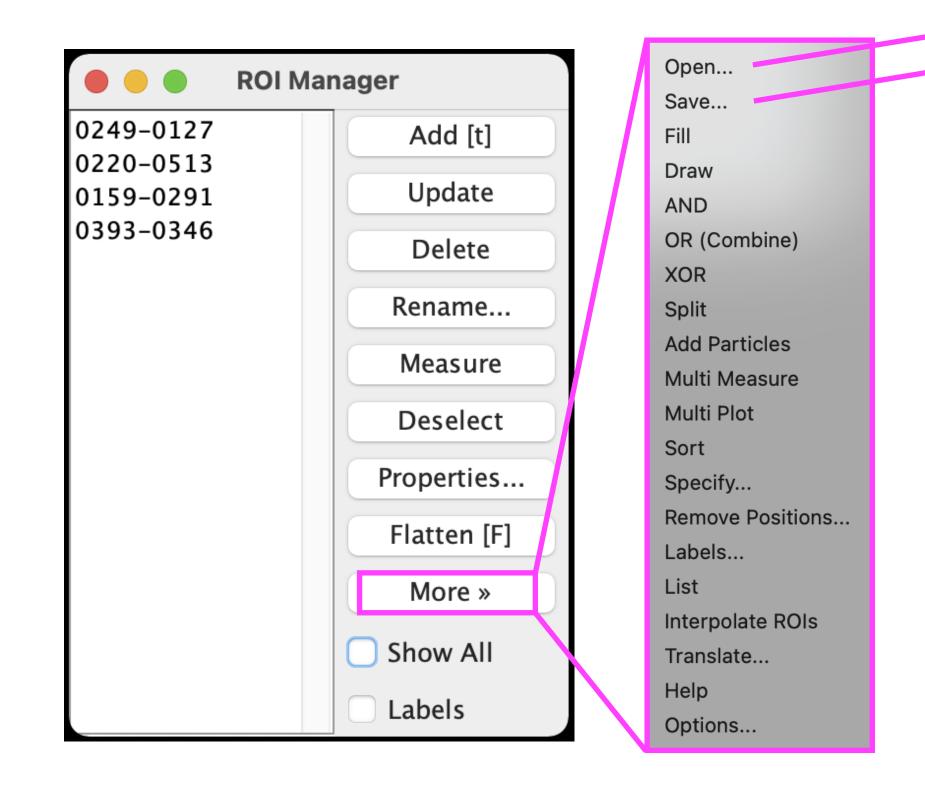




<u>https://fiji.sc/</u>

<u>https://imagej.net/Fiji</u>

"Analyze" menu "Edit" menu





https://imagej.net

https://imagej.nih.gov/ij/



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

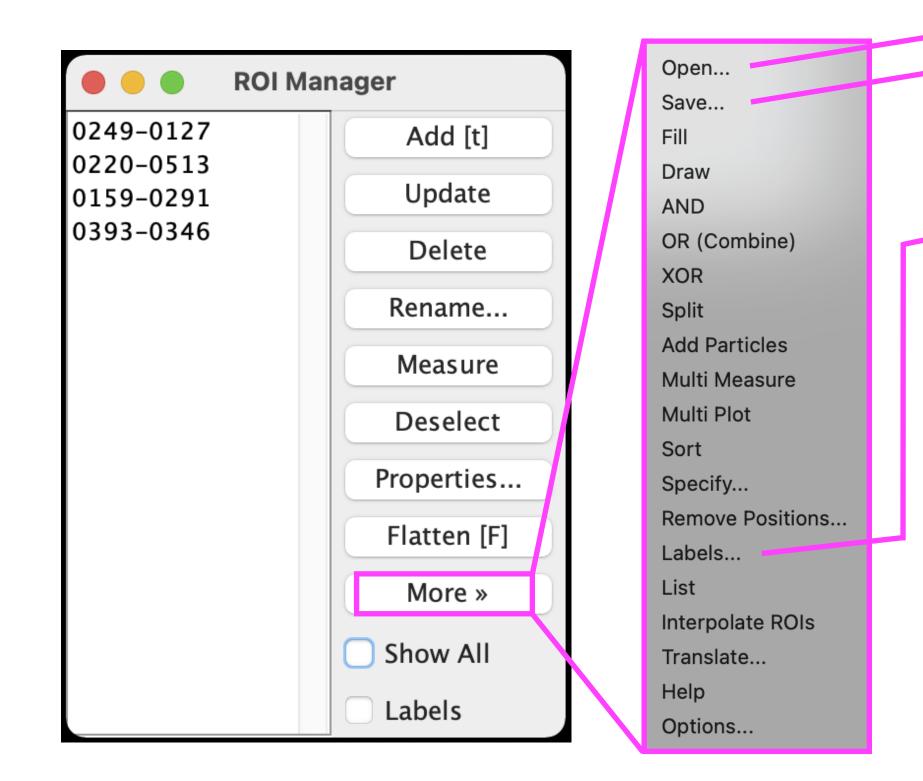




<u>https://fiji.sc/</u>

<u>https://imagej.net/Fiji</u>

"Analyze" menu "Image" menu



*Labels option can also be found under "Image > Overlay"

*"Use names as labels" can be also found under "More>Options..."



https://imagej.net

https://imagej.nih.gov/ij/



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

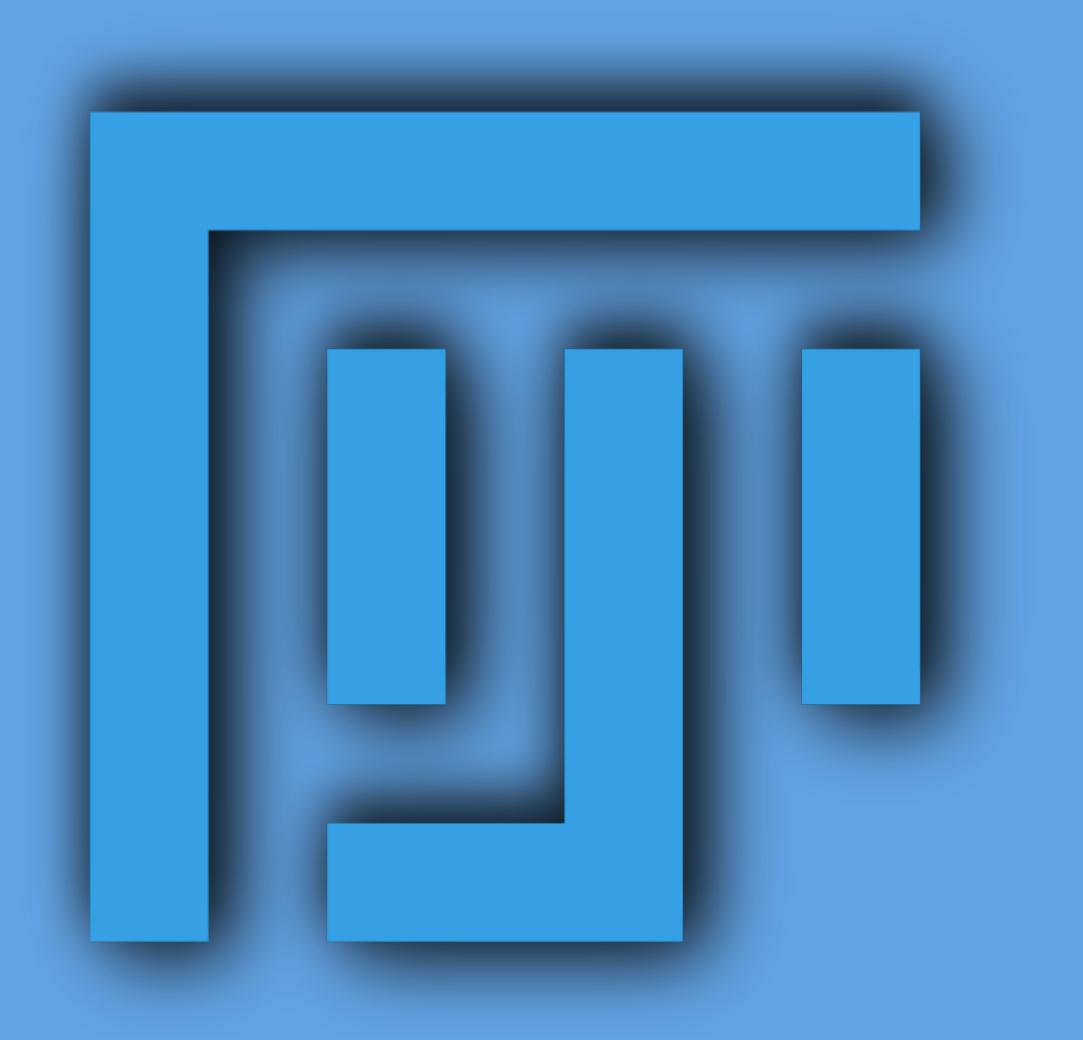
Set ROI(s) label options

| 🗯 Fiji | | |
|---|-----------------------|---------------------|
| (Fiji | ls Just) ImageJ | |
| $\Box, \bigcirc \Box \heartsuit / \measuredangle + \land A$ | Q IN LUT Dev Stk LUT | ∅ ∅ ७ ≫ |
| (Fiji Is Just) ImageJ 2.1.0/1.53c; Java 1.8.0_ | 66 [64-bit]; C | lick here to search |
| C3-hela-cells.tif (50%) | | Vanager |
| 34.60x26.36 µm (672x512); 16-bit; 672K | ROI_1 | Add [t] |
| | ROI_2 ROI_3 | Update |
| | ROI_4 | Delete |
| | Labels | Rename |
| ROI_2 ROI_1 | | Measure |
| | Color: yellow ᅌ | Deselect |
| ROI_3 | Font size: 24 ᅌ | Properties |
| | | Flatten [F] |
| ROI 4 | 🗹 Show labels | More » |
| | 🗹 Use names as labels | Show All |
| | 🗹 Draw backgrounds | 🔽 Labels |
| | Bold | |
| | Cancel OK | |



https://fiji.sc/

<u>https://imagej.net/Fiji</u>

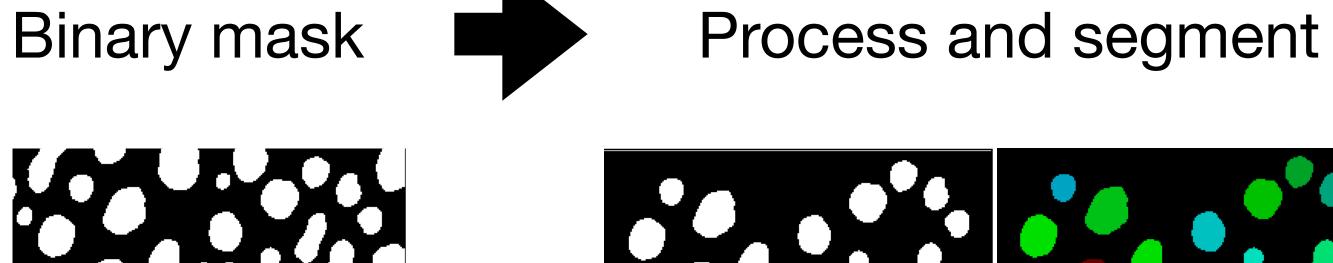


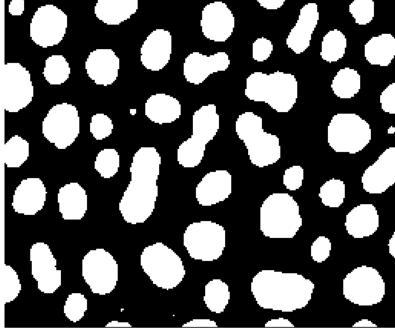
Live Demo of ROI manager

And

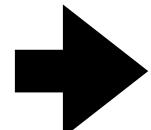
Segmentation Measurements





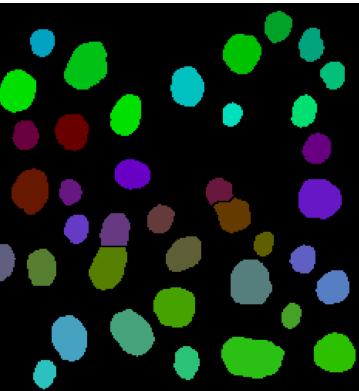


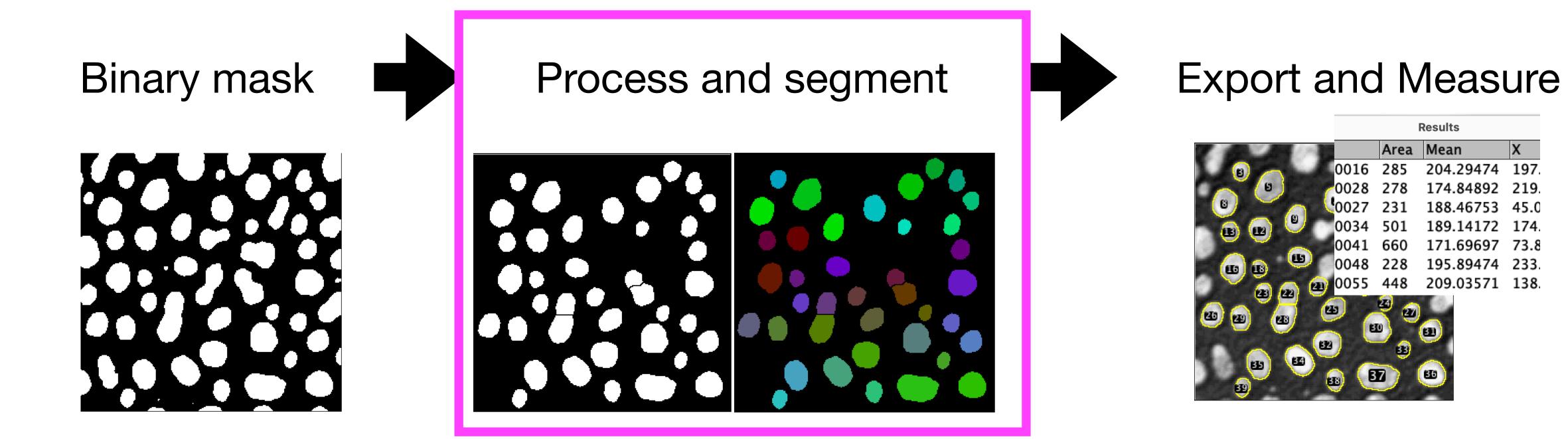




Export and Measure

| | | I | Results | |
|--------------|-----------|--------------|-------------|------|
| 1 4 10 | | Area | Mean | Х |
| B | 0016 | 285 | 204.29474 | 197 |
| 6 | 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 |
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| EB EB | | _ | | |
| 19 | - 🔁 🔁 | D) (| 6 10 | |
| | | 1 | 1990 C | |

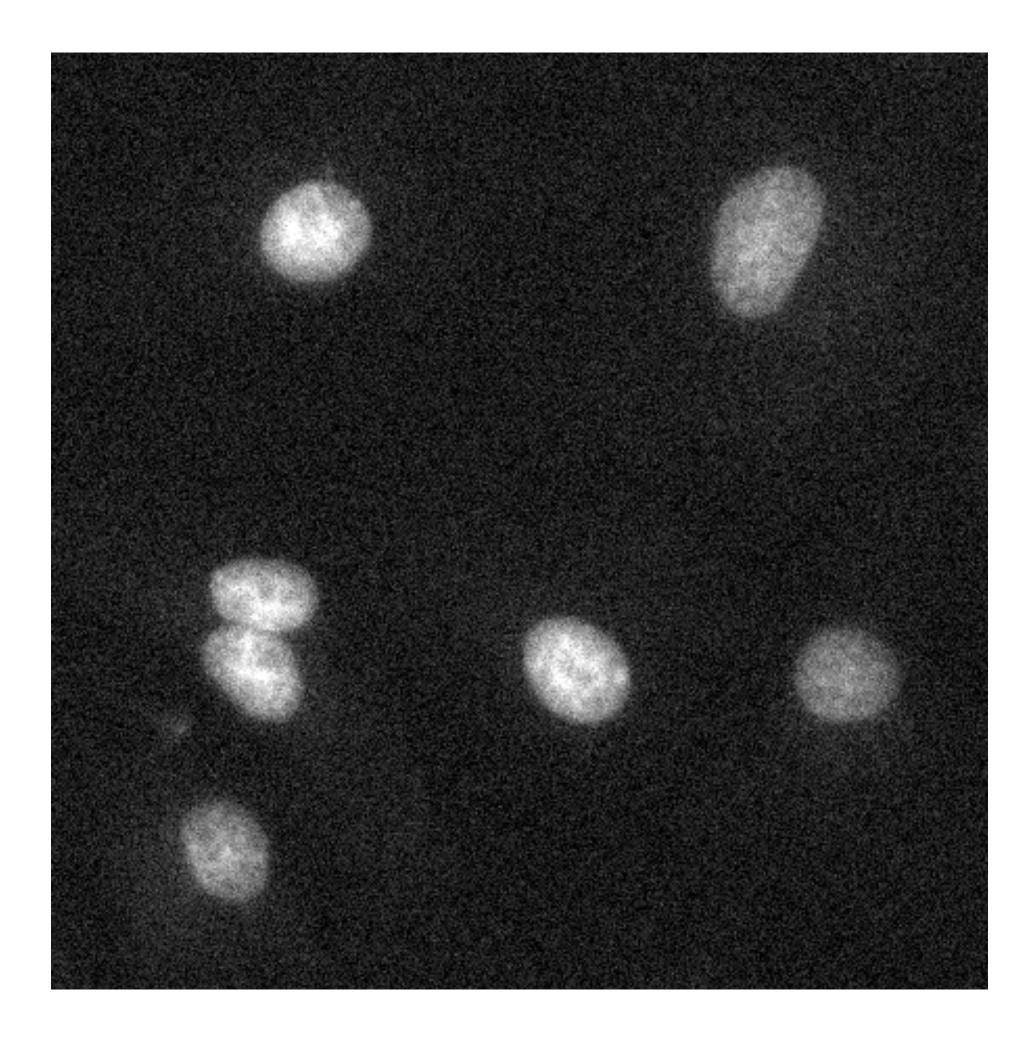






Х

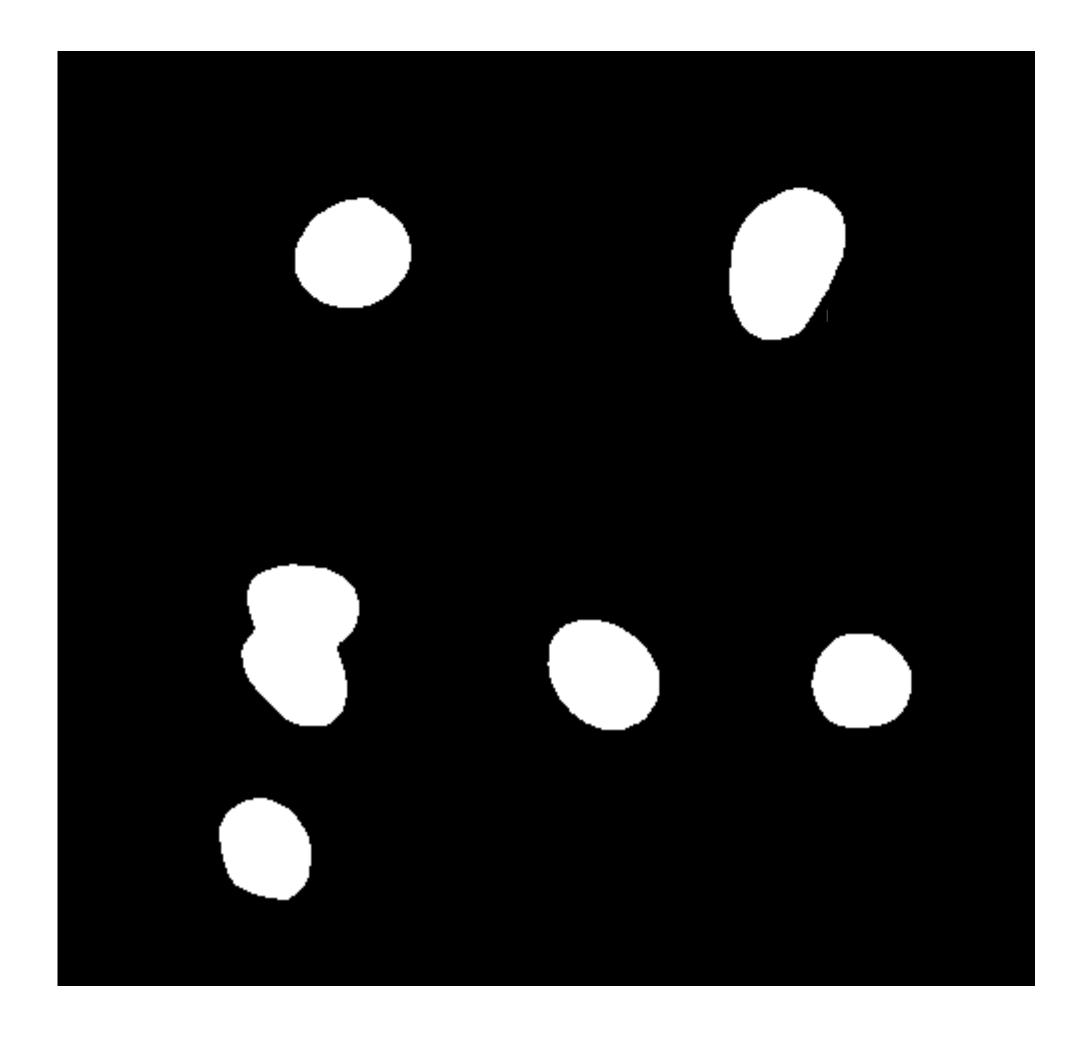
Do you see an issue here?





<u>https://imagej.net</u>

<u>https://imagej.nih.gov/ij/</u>



<u>https://fiji.sc/</u>

<u>https://imagej.net/Fiji</u>

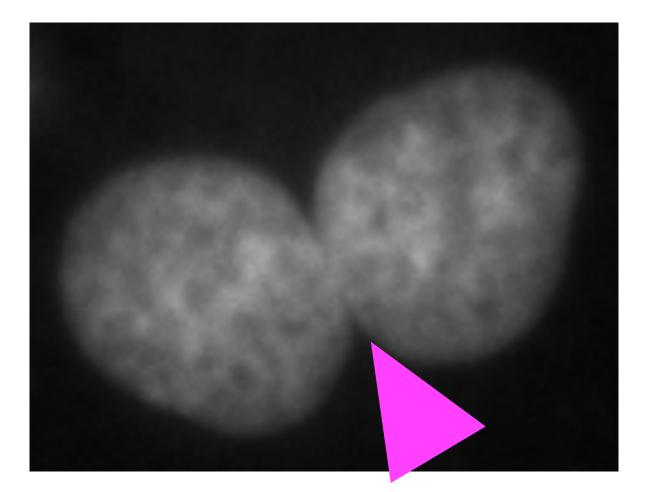


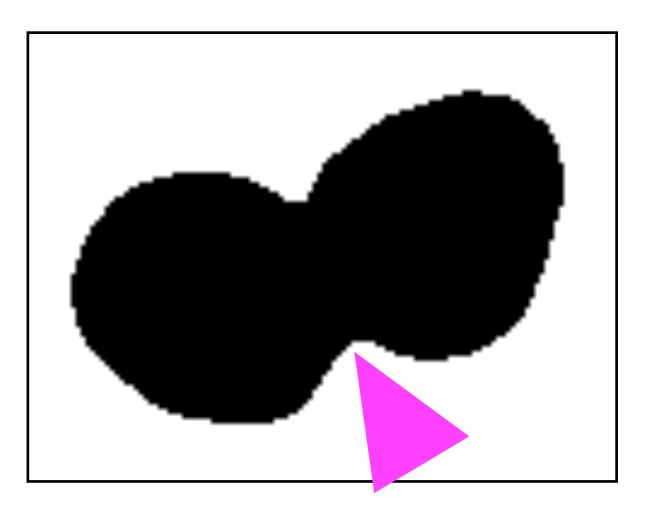
Process and segment Solution: Watershed

in Fiji: Process > Binary > Watershed

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

Image







1

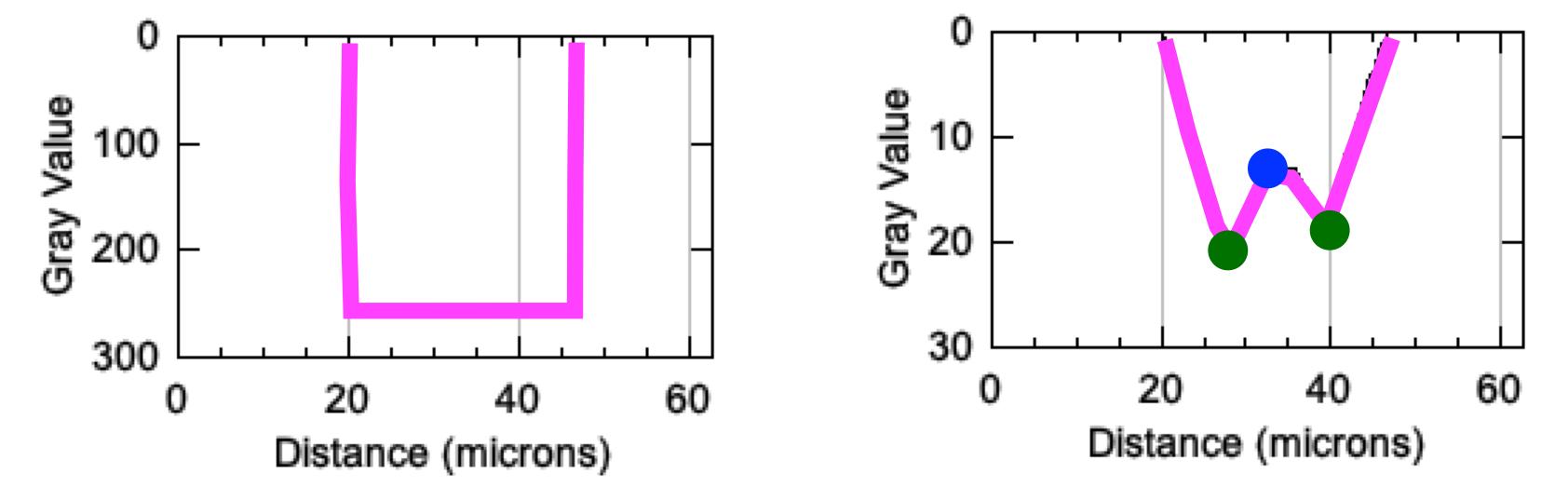
Binary Mask

Watershed

Process and segment Solution: Watershed

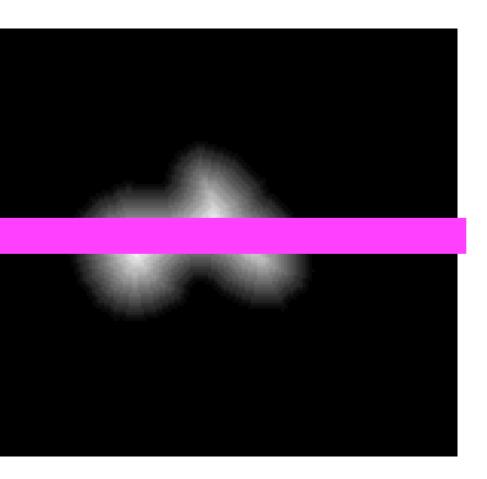
Binary mask

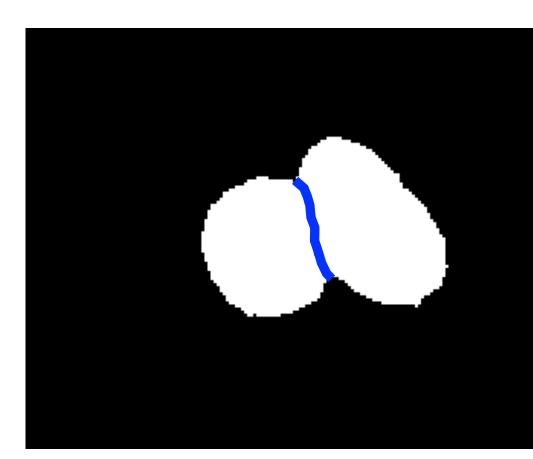






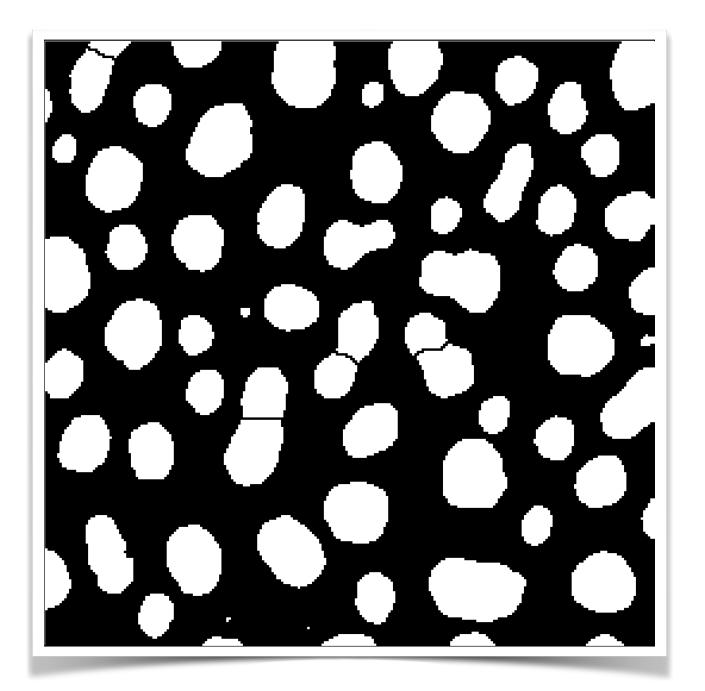
Distance transform





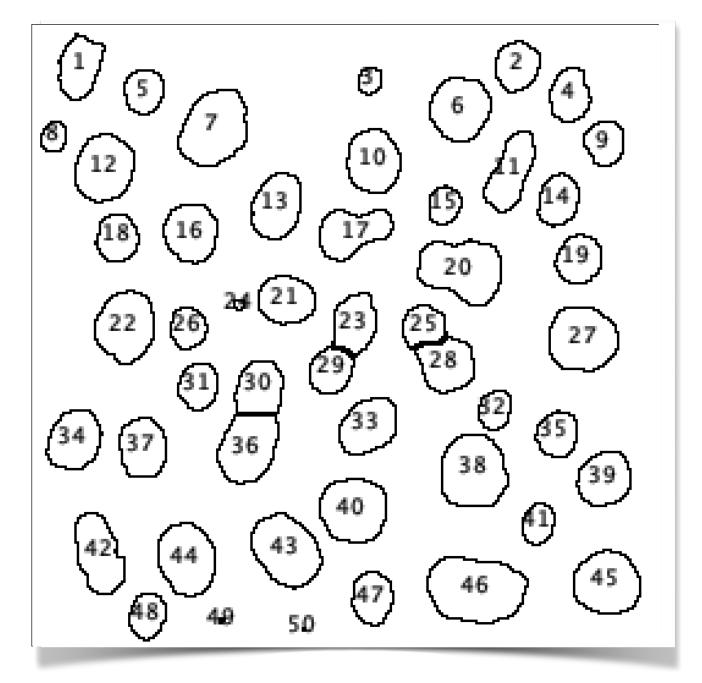


Process and segment From binary image to instance segmentation



Binary mask



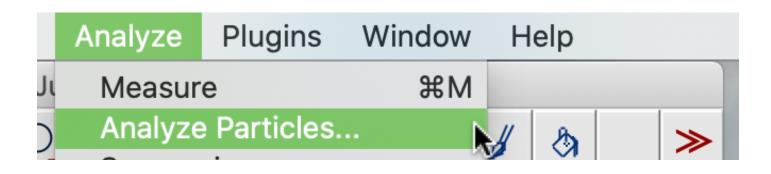


Instance segmentation

Solution: Analyze particles

in Fiji: Analyze > Analyze Particles...

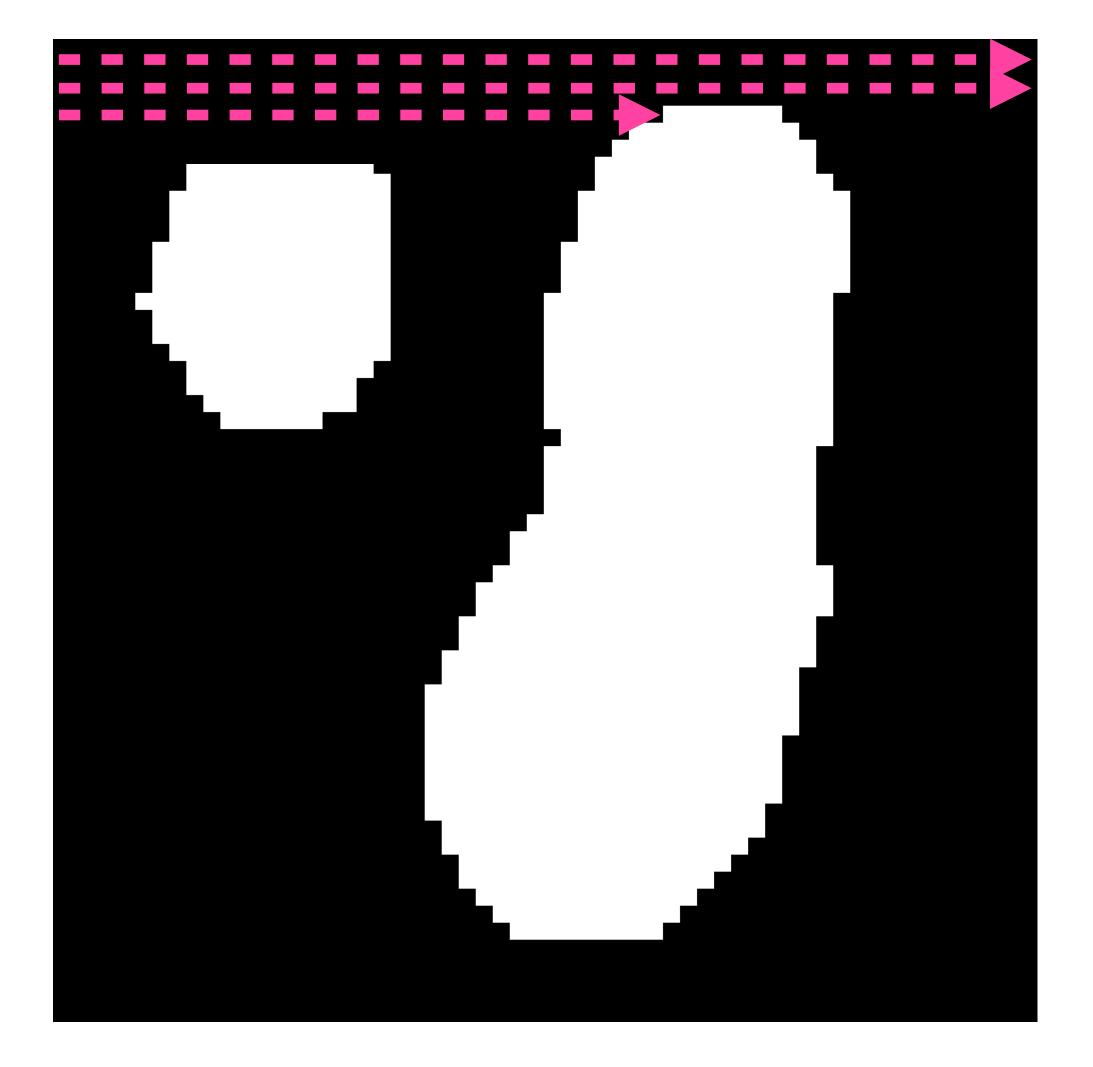




Analyze particles — underlying principles

in Fiji:

Analyze

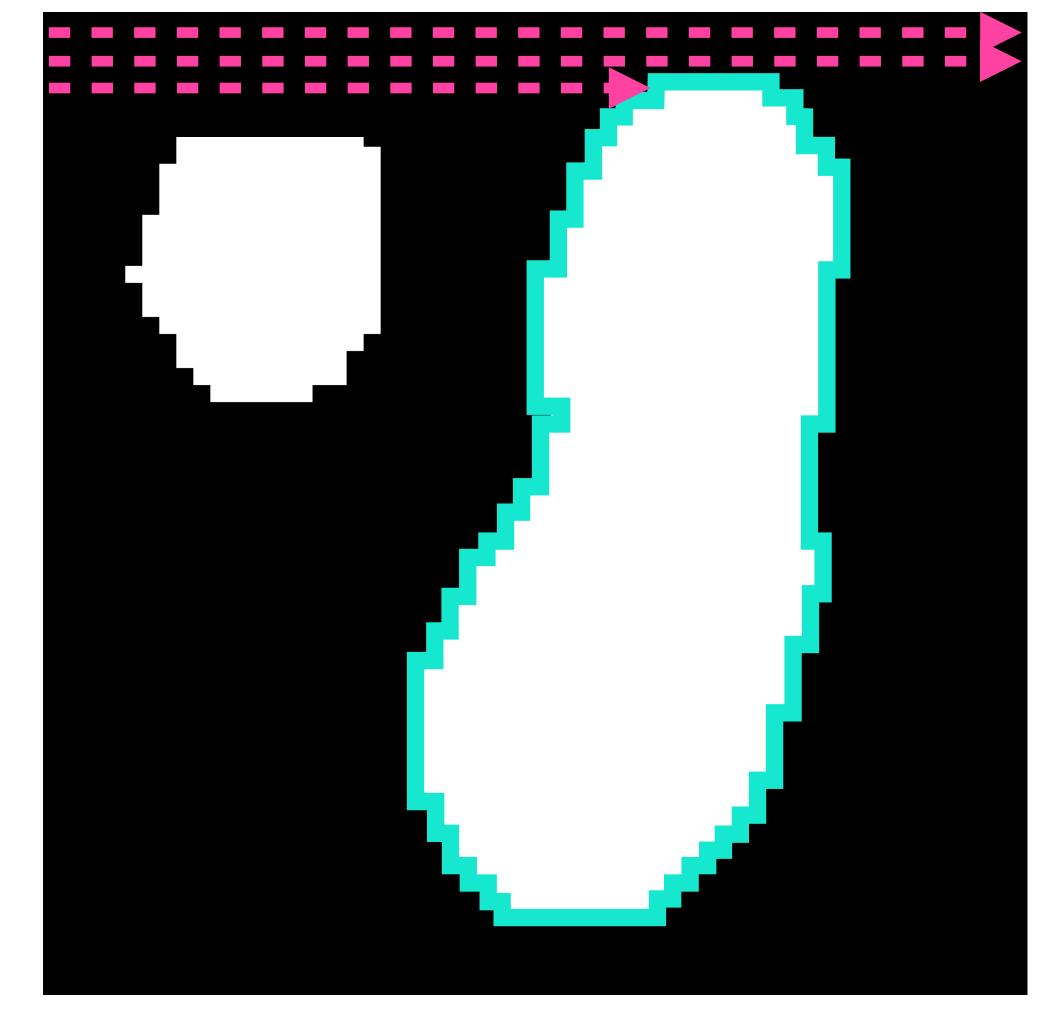




Analyze particles — underlying principles

in **Fiji**:

Analyze



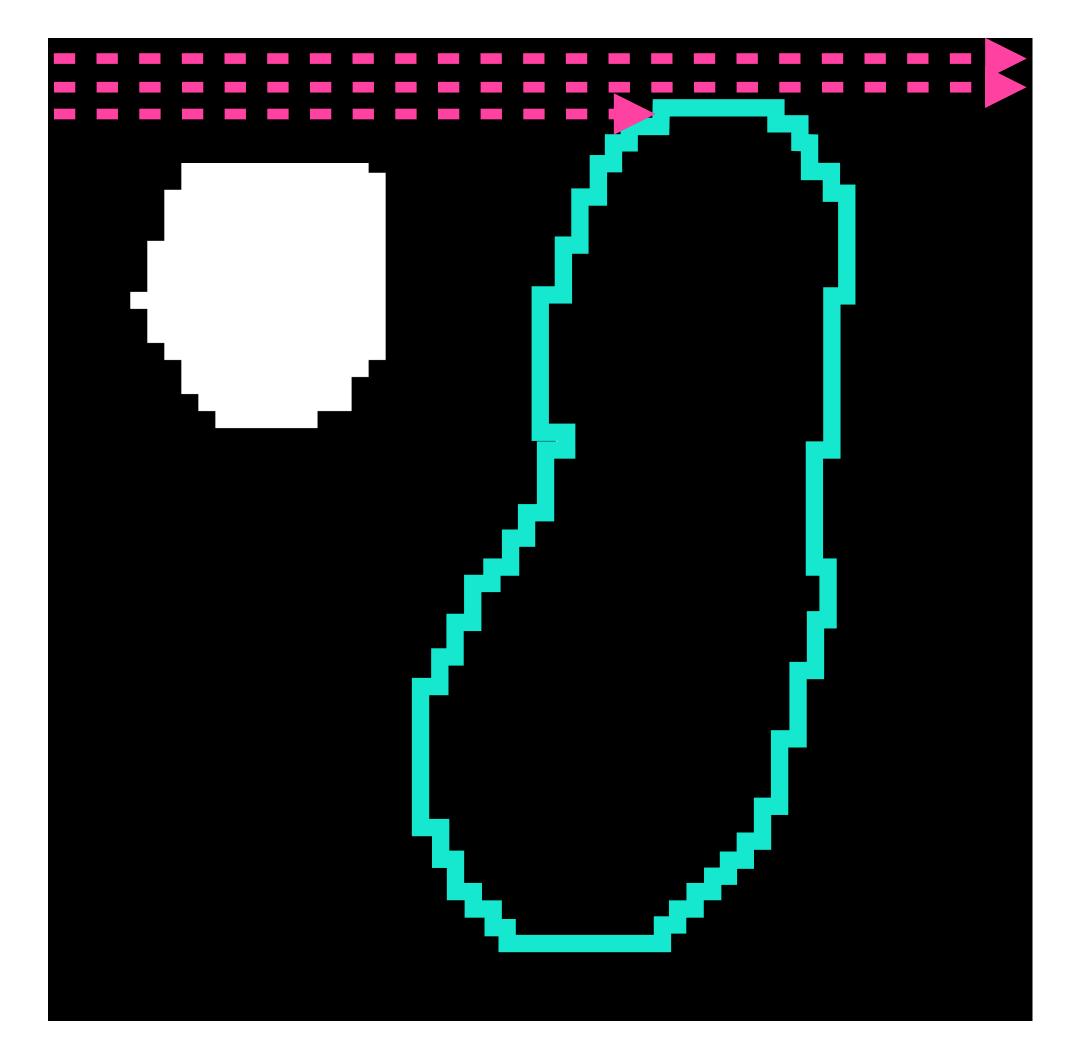




Analyze particles — underlying principles

in Fiji:

Analyze

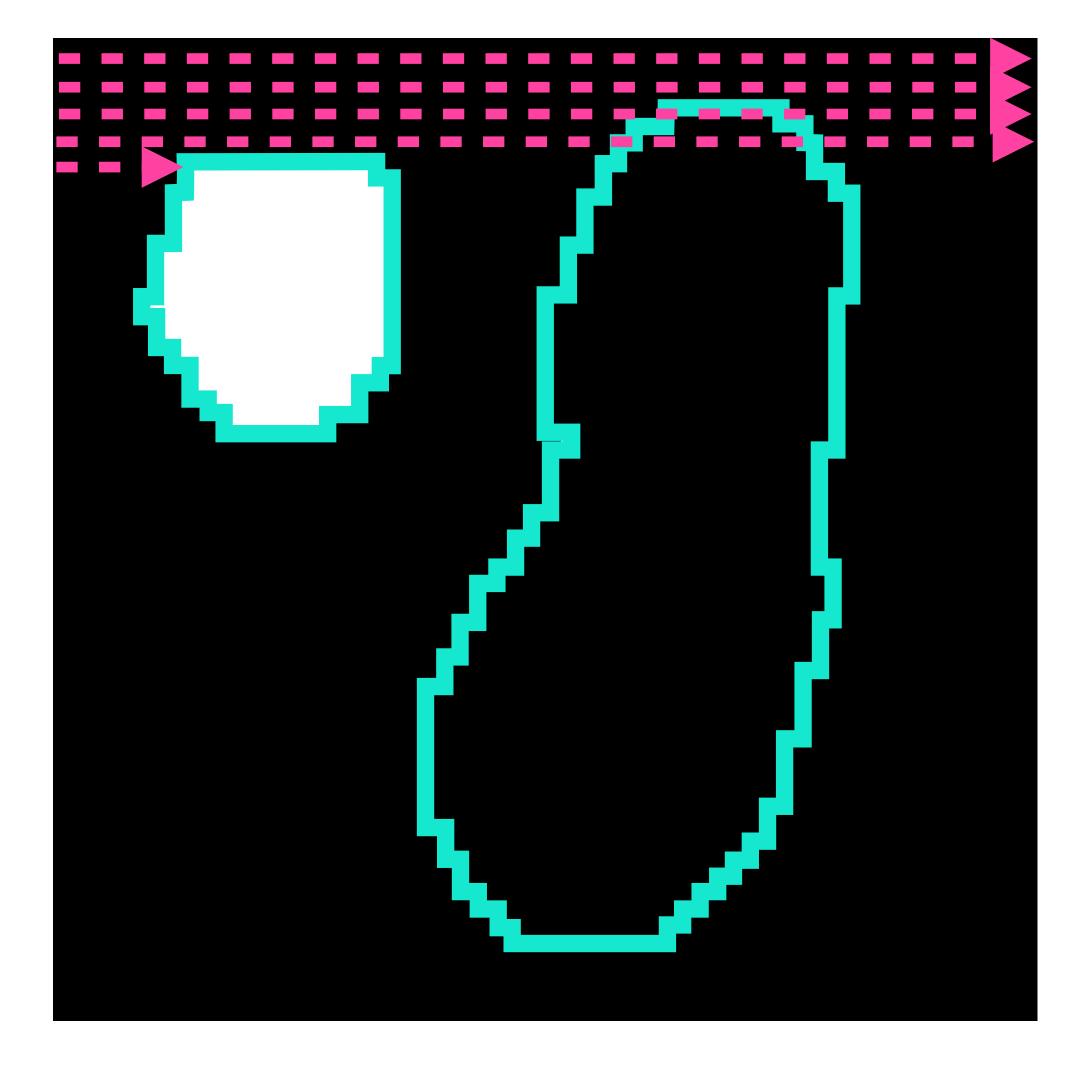




Analyze particles — underlying principles

in Fiji:

Analyze





Analyze particles — underlying principles

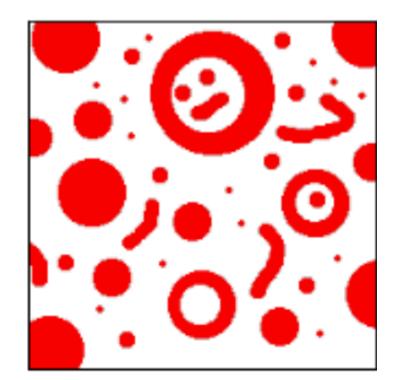
in Fiji:

Analyze

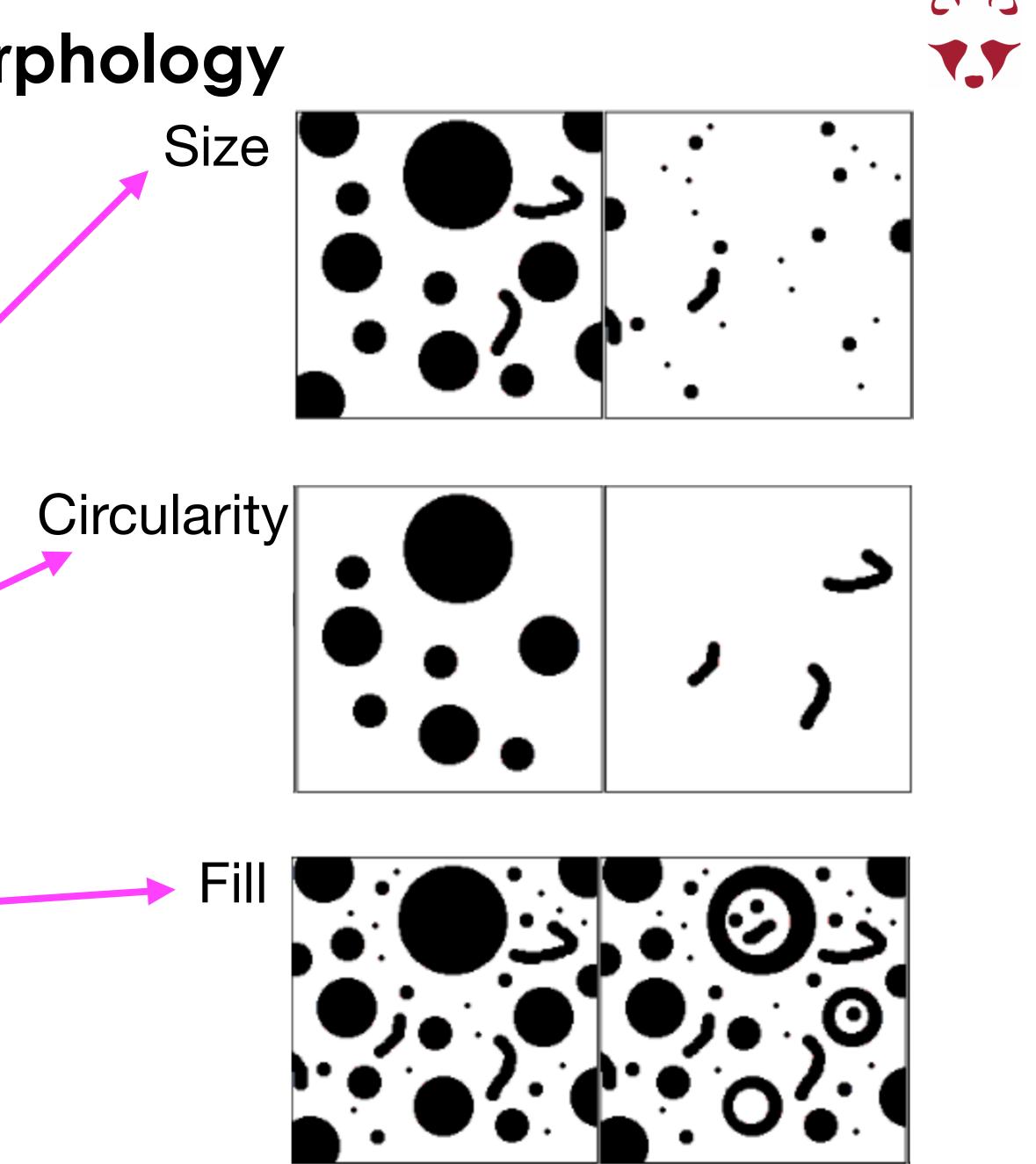




Analyze particles: Filter for morphology



| Analyze Particles |
|---|
| Size (pixel^2): <mark>0–Infinity</mark> |
| Circularity: 0.00-1.00 |
| Show: Nothing ~ |
| Display results Exclude on edges |
| Clear results Include holes Summarize Overlay |
| Add to Manager Composite ROIs |
| Help Cancel OK |





Analyze particles: Size

in Fiji: Analyze > Analyze Particles...

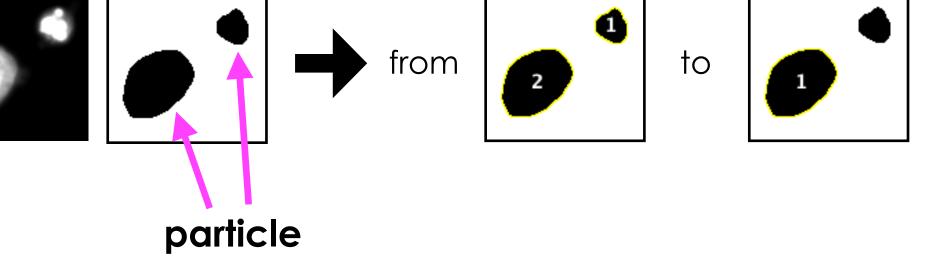
| Analyze Particles | |
|---|----|
| Size (pixel^2): 0–Infinity | L |
| Circularity: 0.00-1.00 | |
| Show: Nothing ~ | ١. |
| Display results Clear results Summarize Add to Manager Exclude on edges Include holes Overlay Composite ROIs | |
| Help Cancel OK | |



https://imagej.net

https://imagej.nih.gov/ij/

Size range of the particles that you want to detect.

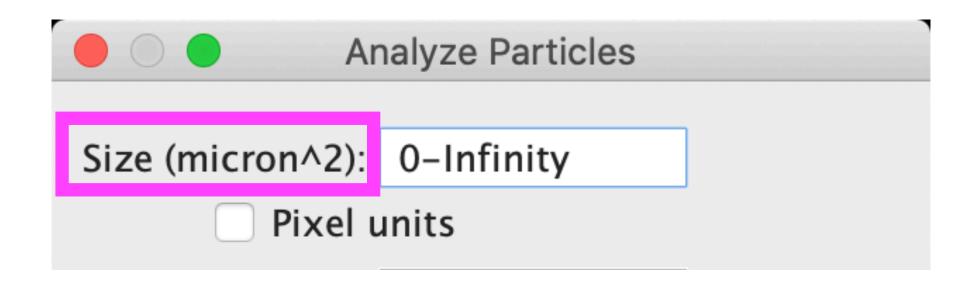




<u>https://fiji.sc/</u>

<u>https://imagej.net/Fiji</u>

Analyze particles: Size

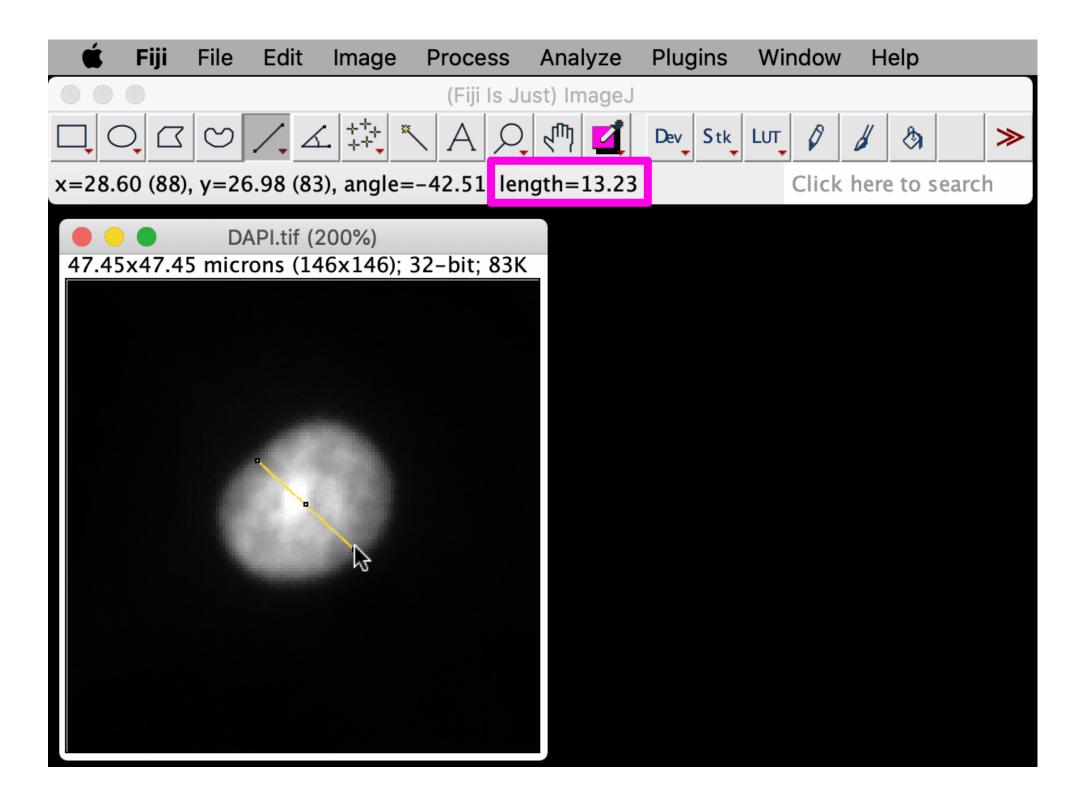




<u>https://imagej.net</u>

<u>https://imagej.nih.gov/ij/</u>

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





<u>https://fiji.sc/</u>

https://imagej.net/Fiji

Analyze particles: Circularity

in Fiji: Analyze > Analyze Particles...

| Analyze Particles | | | | | | |
|---|--|--|--|--|--|--|
| Size (pixel^2): 0–Infinity | | | | | | |
| Circularity: 0.00-1.00 | | | | | | |
| Show: Nothing ~ | | | | | | |
| Display results Clear results Summarize Add to Manager Exclude on edges Include holes Overlay Composite ROIs | | | | | | |
| Help Cancel OK | | | | | | |



https://imagej.net

https://imagej.nih.gov/ij/

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





<u>https://fiji.sc/</u>

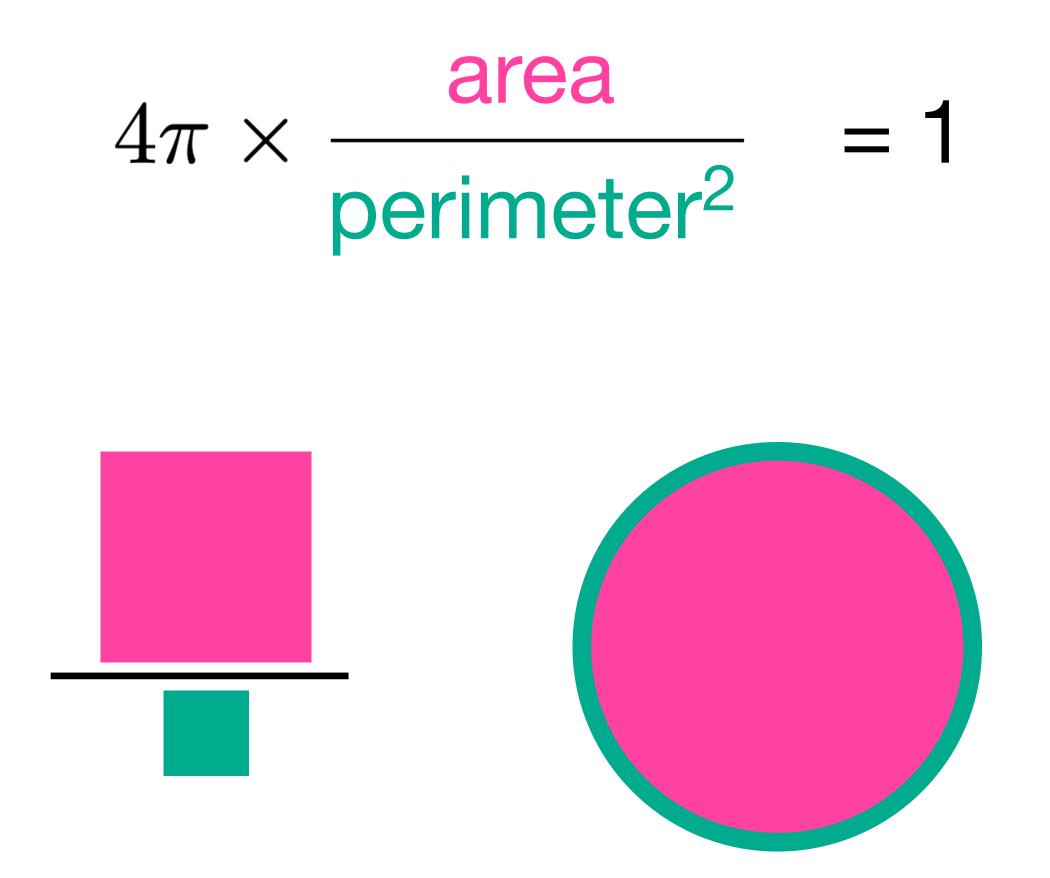
<u>https://imagej.net/Fiji</u>

Analyze particles: Circularity



<u>https://imagej.net</u>

<u>https://imagej.nih.gov/ij/</u>

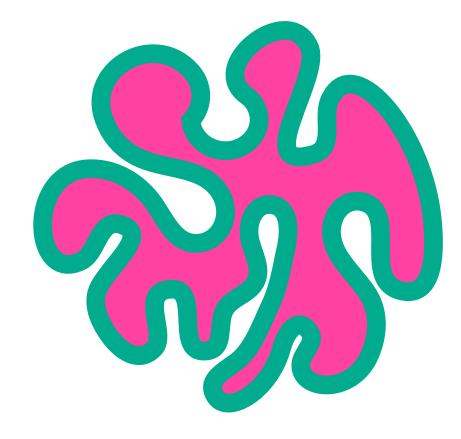


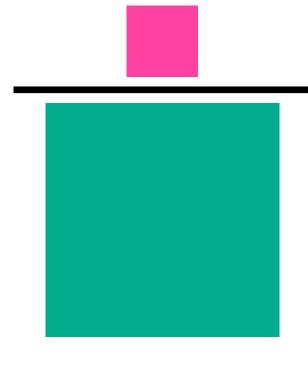


<u>https://fiji.sc/</u>

https://imagej.net/Fiji

Analyze particles: Circularity

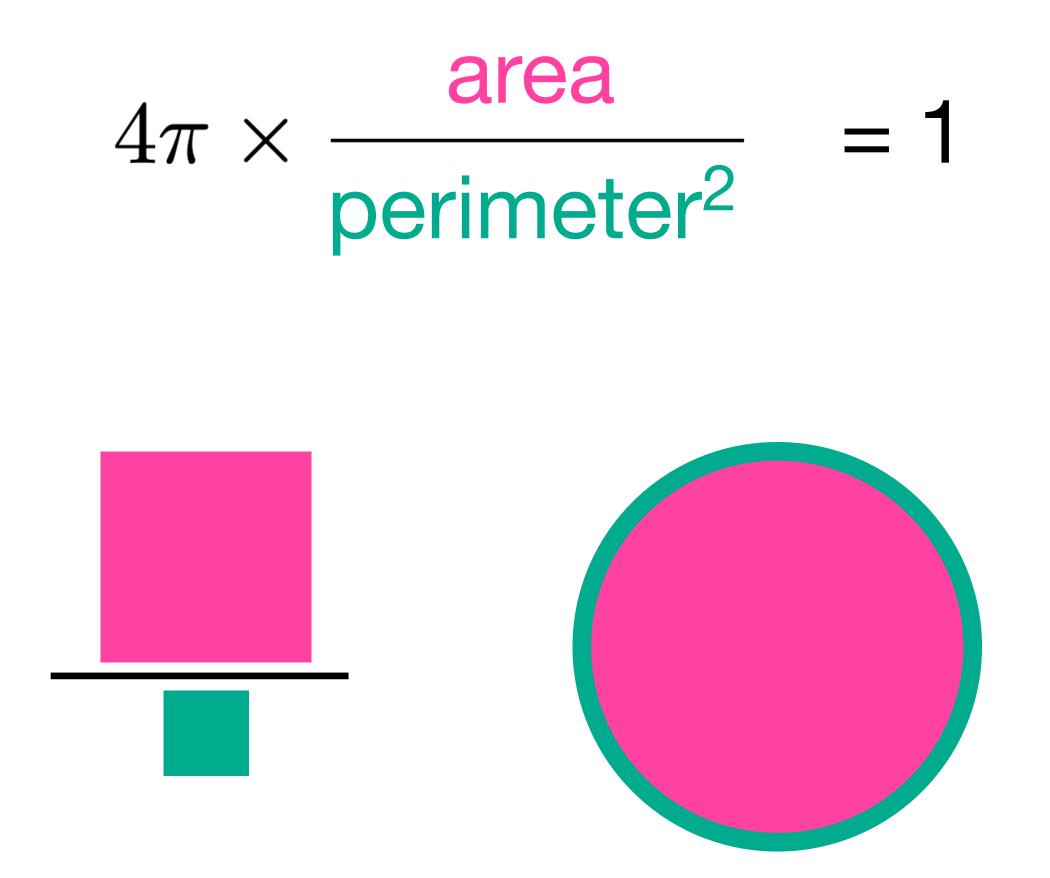






https://imagej.net

https://imagej.nih.gov/ij/



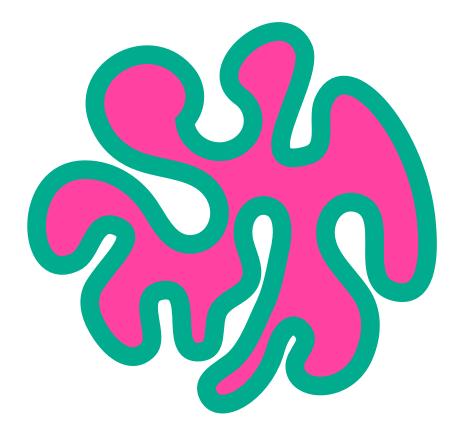


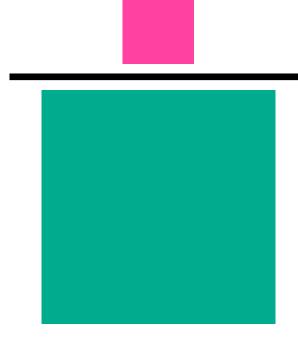
<u>https://fiji.sc/</u>

<u>https://imagej.net/Fiji</u>

Analyze particles: Circularity



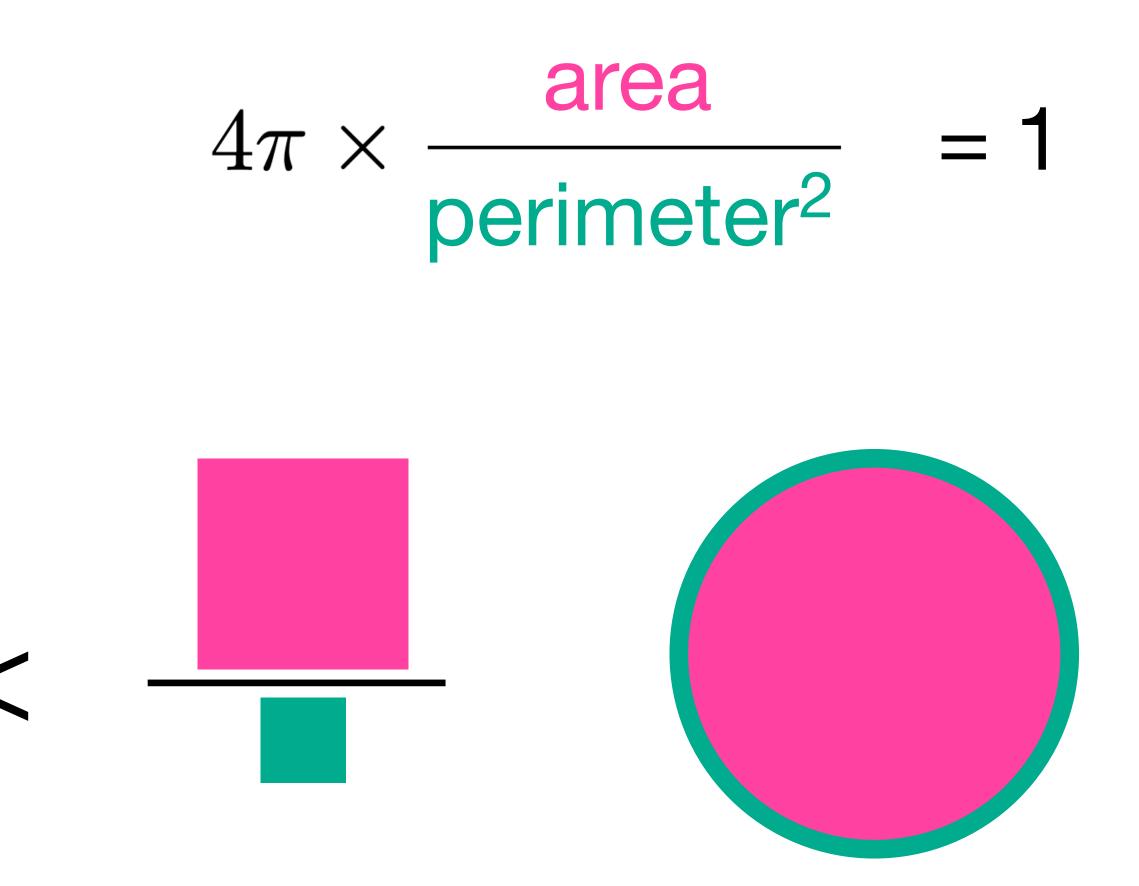






https://imagej.net

https://imagej.nih.gov/ij/





https://fiji.sc/

https://imagej.net/Fiji

Analyze particles: Exclude on Edges

in Fiji: Analyze > Analyze Particles...

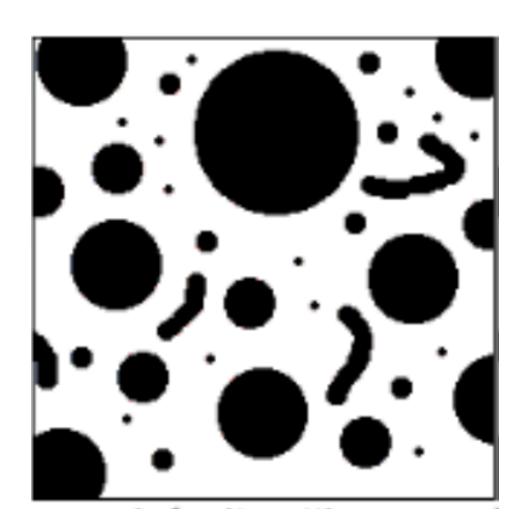
| | nalyze Particles |
|--|--------------------------------------|
| Size (pixel^2): Circularity: | <mark>0–Infinity</mark> 0.00–1.00 |
| Show: | Nothing |
| Display res Clear result Summarize | |
| Add to Man | |
| Help | Cancel OK |

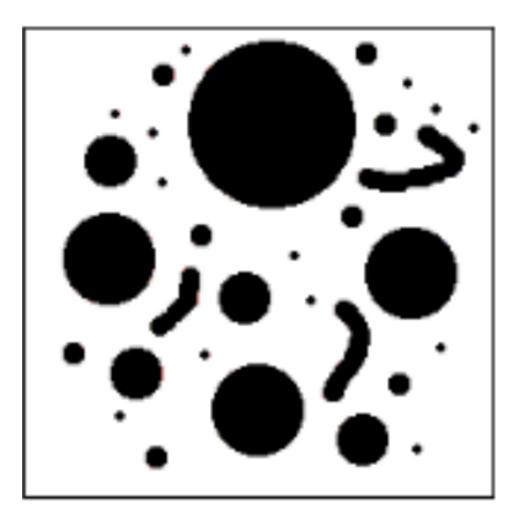


https://imagej.net

https://imagej.nih.gov/ij/

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

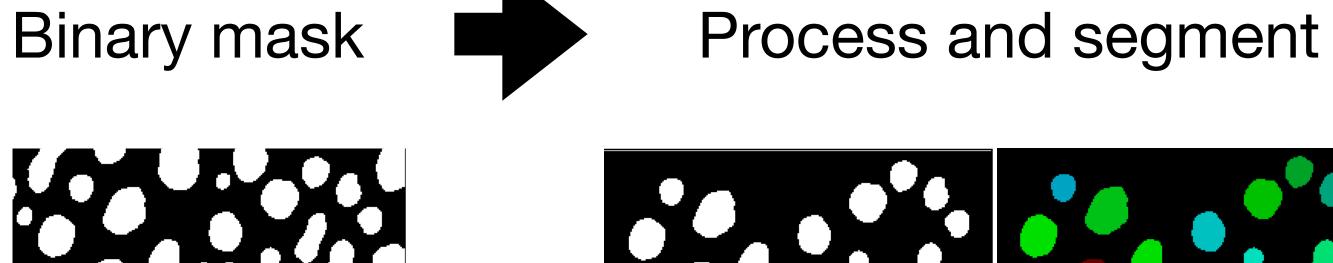


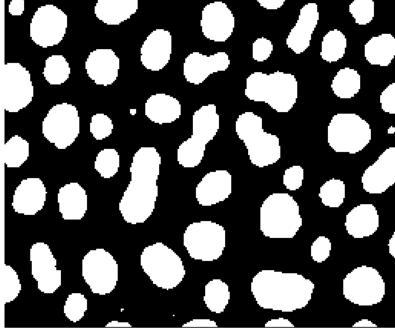


<u>https://fiji.sc/</u>

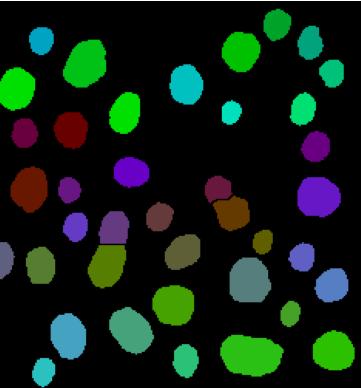
<u>https://imagej.net/Fiji</u>

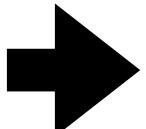












Export and Measure

| | 1 | Results | |
|-------------|------------|-----------|------|
| | Area | Mean | Х |
| 0016 | 285 | 204.29474 | 197. |
| B 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. |
| | _ <u>8</u> | E1) | |



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



Export and Measure

| | | R | esults | |
|--------------|-------------|------|-----------|------|
| | | Area | Mean | Х |
| B 00 |)16 | 285 | 204.29474 | 197. |
| | | 278 | 174.84892 | 219. |
| |)27 | 231 | 188.46753 | 45.0 |
| - 👝 📵 😐 oc |)34 | 501 | 189.14172 | 174. |
| 00 |)41 | 660 | 171.69697 | 73.8 |
| 🗖 💼 💼 🛄 🛛 00 |)48 | 228 | 195.89474 | 233. |
| |)55 | 448 | 209.03571 | 138. |
| | 8 8 8 | 8 | 31) | |



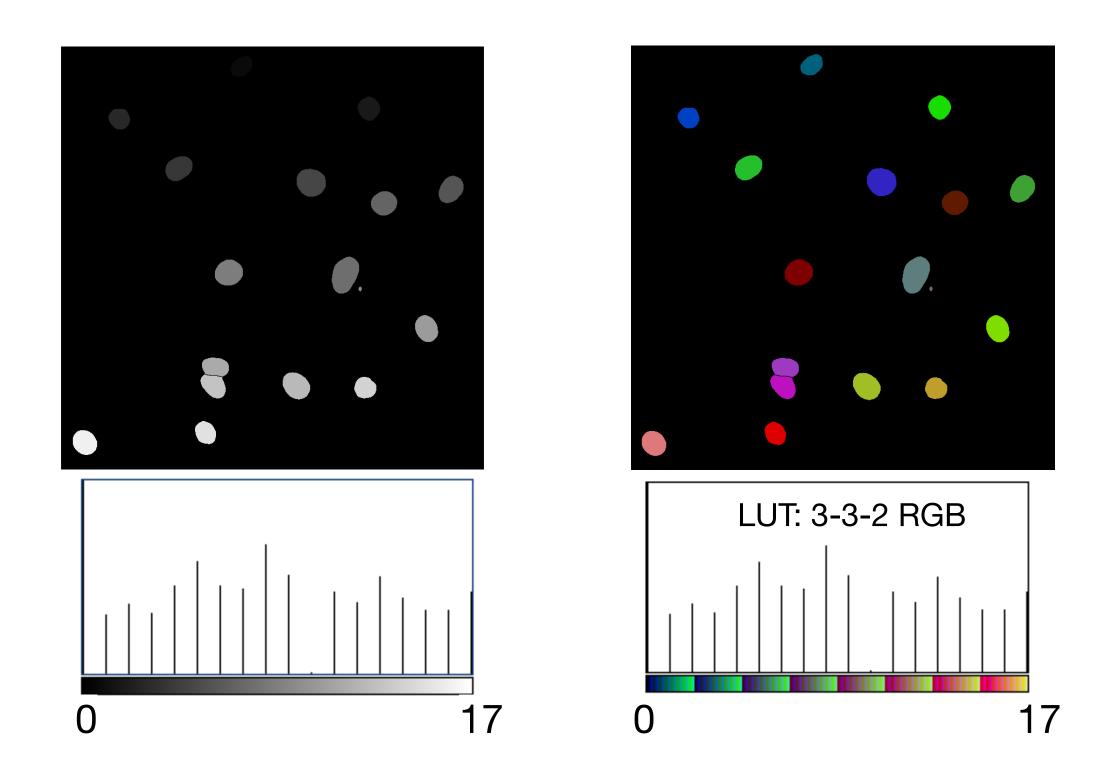
Export Analyze particles: Save the segmented image

in Fiji: Analyze > Analyze Particles...



https://imagej.net

https://imagej.nih.gov/ij/



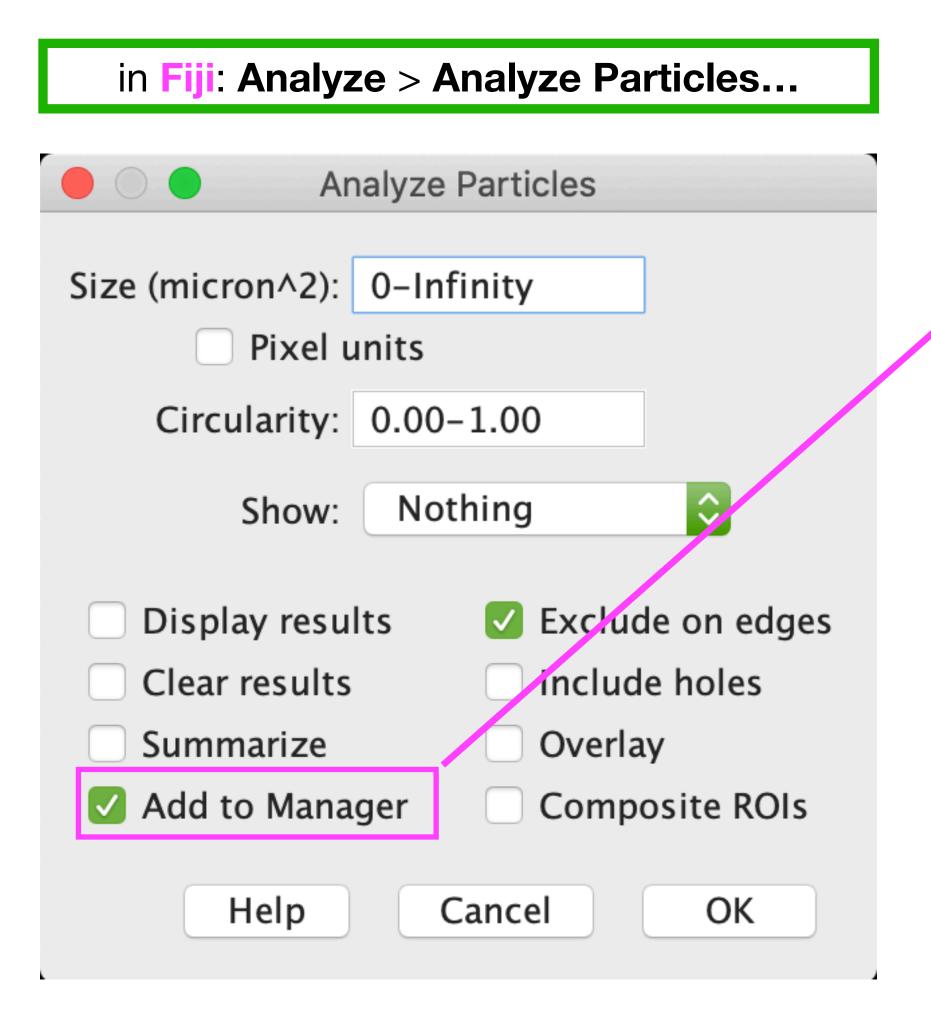
"Count Masks" encodes object identity as gray value.

<u>https://fiji.sc/</u>

<u>https://imagej.net/Fiji</u>



Export Analyze particles: Add to ROI Manager





https://imagej.net

https://imagej.nih.gov/ij/

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

| 🔴 🕘 🗧 ROI Manager | | |
|-------------------|-------------|--|
| | Add [t] | |
| | Update | |
| | Delete | |
| | Rename | |
| | Measure | |
| | Deselect | |
| | Properties | |
| | Flatten [F] | |
| | More » | |
| | Show All | |
| | Labels | |

https://fiji.sc/

https://imagej.net/Fiji

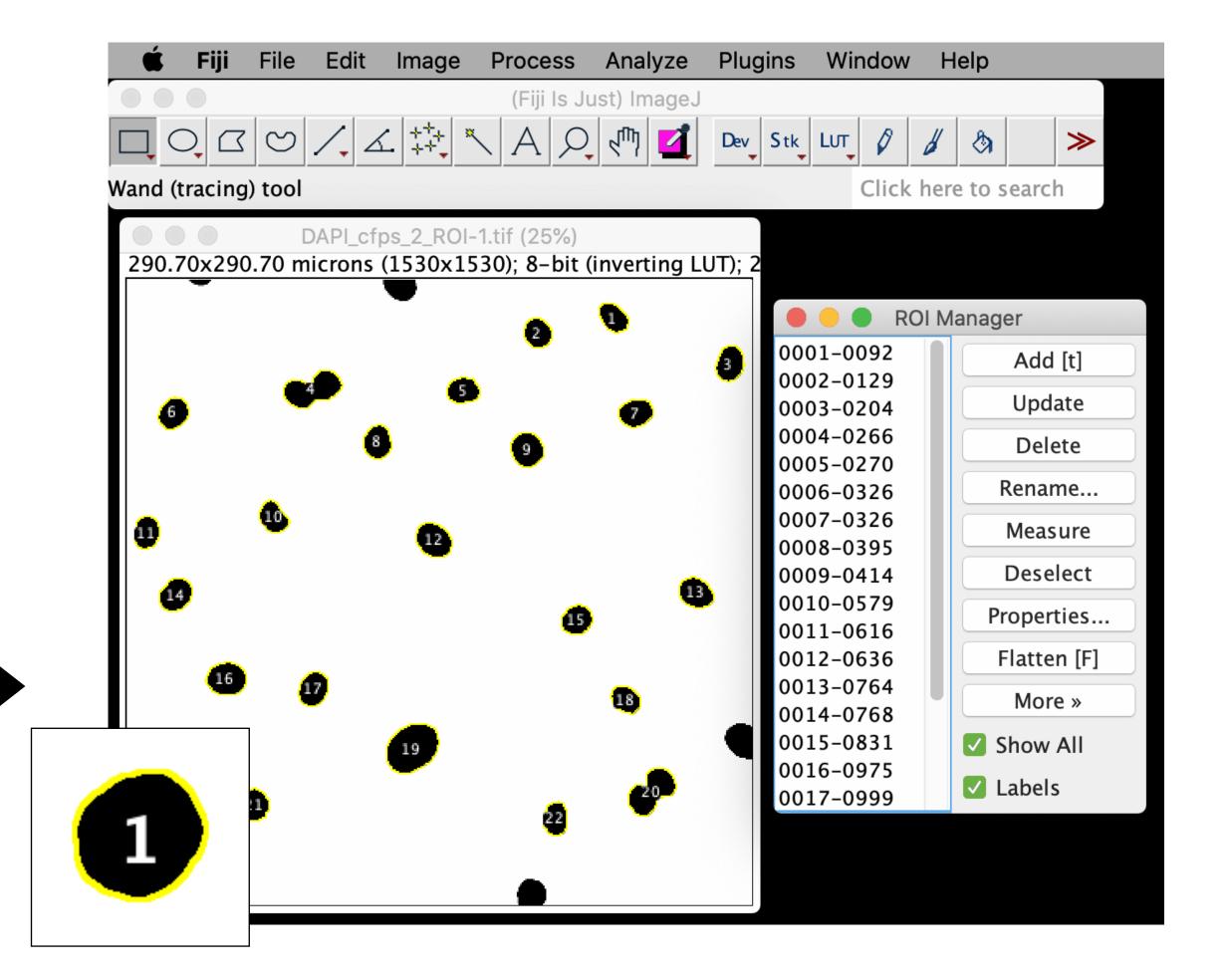
Export Analyze particles: Add to ROI Manager

| in Fiji: Analyze > Analyze Particles | | |
|---|--|--|
| | | |
| Analyze Particles | | |
| Size (micron^2): 0-Infinity Pixel units Circularity: 0.00-1.00 Show: Nothing | | |
| Display results Clear results Summarize Add to Manager Composite ROIs Help Cancel OK | | |



https://imagej.net

https://imagej.nih.gov/ij/



https://imagej.net/Fiji



https://fiji.sc/

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



Export and Measure

| | F | Results | |
|---------------|-------|-----------|------|
| | Area | Mean | Х |
| 0016 | 285 | 204.29474 | 197. |
| B 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. |
| 🖉 🖉 😰 🙆 🙆 🙆 🙆 | 448 | 209.03571 | 138. |
| | 24 27 | 100. | |
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| <u>(12)</u> | ້ສ່ | <u> </u> | |
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| an 🚽 🚽 🔒 🗳 | | <u>50</u> | |
| | | 100 C | |

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



Export and Measure

| | | R | lesults | |
|-------------------|------|-------|-----------|------|
| | | Area | Mean | Х |
| B (| 0016 | 285 | 204.29474 | 197. |
| | 028 | 278 | 174.84892 | 219. |
| | 0027 | 231 | 188.46753 | 45.0 |
| | 0034 | 501 | 189.14172 | 174. |
| | 0041 | 660 | 171.69697 | 73.8 |
| B B B | 048 | 228 | 195.89474 | 233. |
| | 055 | 448 | 209.03571 | 138. |
| 20 21 23 23 E2 | 8 | | 31 | |
| | | - × - | 55 | |

Export Select what to measure: Set Measurements

Analyze > Set Measurements...

| Set Measurements | | |
|--|--|--|
| Area Standard deviation Modal gray value Min & max gray value Center of mass Center of mass Perimeter Bounding rectangle Shape descriptors Feret's diameter Integrated density Median Skewness Kurtosis | | |
| Area fraction Limit to threshold Invert Y coordinates Add to overlay NaN empty cells | | |
| Redirect to: DAPI_14.tif ∨ Decimal places (0-9): 9 Help Cancel OK | | |



https://imagej.net

https://imagej.nih.gov/ij/

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

| ć | Fiji | | | | | |
|-------|-----------------------|---------------------|--------------|-----------------|----------------------|----------|
| | (Fiji Is Just) ImageJ | | | | | |
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| -100 | | .60 (48), value=0 | | <u> </u> | Click here to search | <u>⊸</u> |
| =109 | .20 (330), y=13. | .00 (48), value=0 | | | Click here to search | |
| 00 |) 🔘 DAPI_14.t | if (33.3%) | 000 | mask (33. | 3%) | |
| '- D/ | API.nd2 (series 1 | .4)"; 260.00x260.00 |) mi DAPI.nd | 2 (series 14)"; | 260.00x260.00 m | ni |
| | 10 B | | | • 2 | 0 | |
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| 8 | | | 10 | | • | |
| | | | | | | |
| | 0 0 | | Results | | | |
| | Label | Area | Mean | Min | Max | |
| 1 | DAPI 14.tif | 166.148133307 | 4870.167832 | | 7193 | |
| 2 | DAPI_14.tif | 142.805007140 | 6199.803994 | | 9411 | |
| 3 | DAPI_14.tif | 173.964383698 | 8027.766848 | | 12624 | |
| 4 | DAPI 14 tif | 143 438757172 | 5482 965390 | | 7660 | |

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<u>https://fiji.sc/</u>

<u>https://imagej.net/Fiji</u>

Export Measure — Option 1: Manually

Analyze > Measure

(cmd) + m

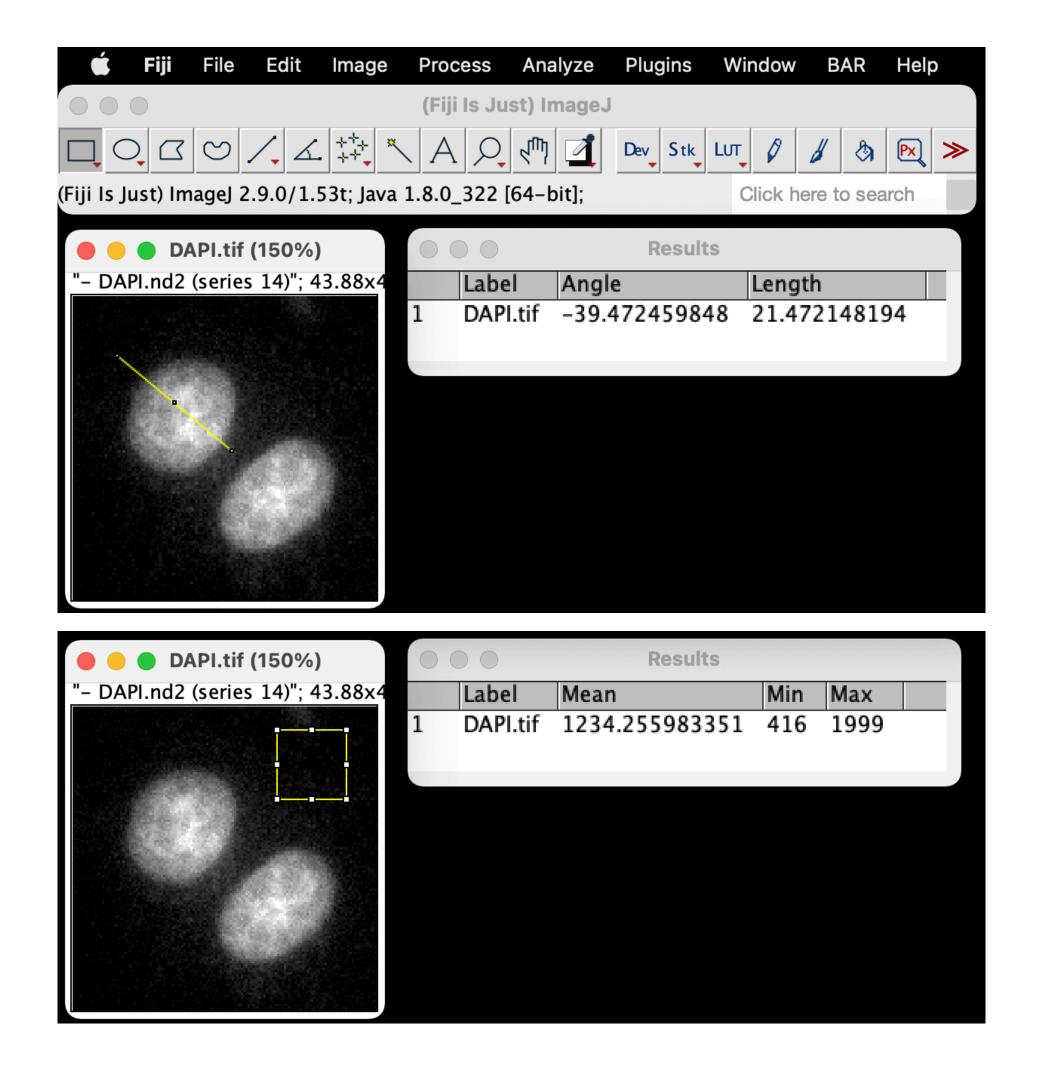
Measures the parameters chosen under "Analyze > Set Measurements..." in relation to the selected ROI.

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



https://imagej.net

https://imagej.nih.gov/ij/



https://fiji.sc/

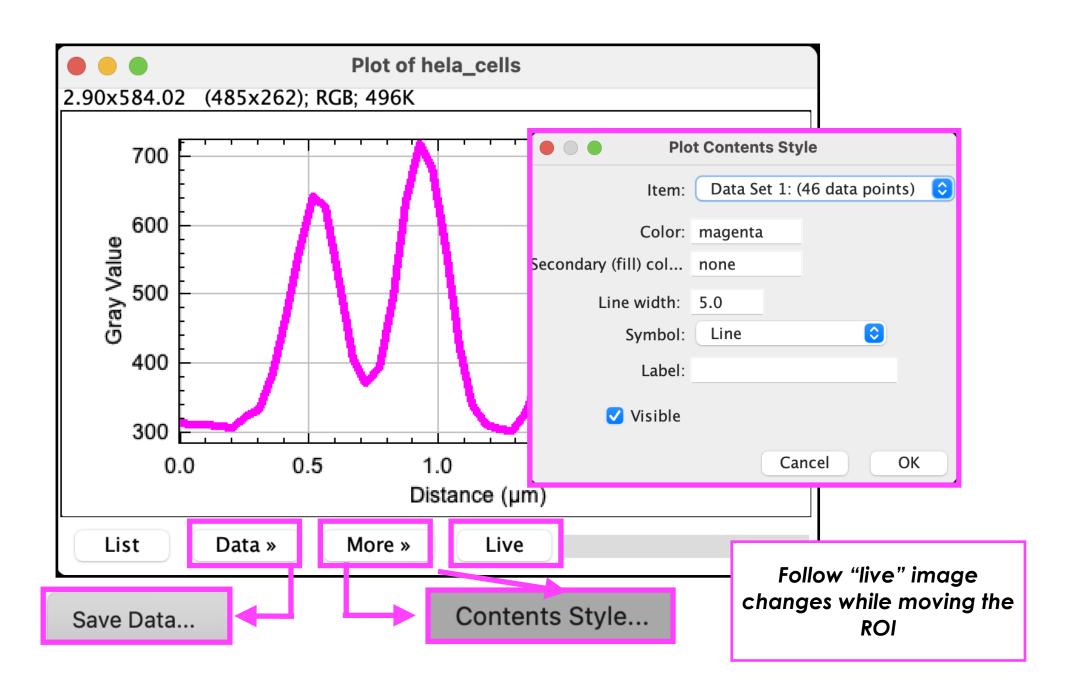
https://imagej.net/Fiji



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")



<u>https://imagej.net</u>

https://imagej.nih.gov/ij/

| 🗯 Fiji File Edit Image Process | Analyze Plugins | Window | Help |
|--|--------------------|--------|----------------|
| O O (Fiji Is Ju | Measure | ЖМ | |
| $\Box, O, \Box \heartsuit / \checkmark + + \land A Q$ | Analyze Particles | | ∦ & ≫ |
| (Fiji Is Just) ImageJ 2.1.0/1.53c; Java 1.8.0_66 [| Summarize | | here to search |
| (Fiji 13 jušt) imagėj 2.1.0/ 1.550, java 1.0.0_00 [| Distribution | | nere to search |
| C2-hela-cells.tif (50%) | Label | | |
| 34.60x26.36 µm (672x512); RGB; 1.3MB | Clear Results | | + |
| The second of th | Set Measurements | | .t 1x94) |
| AND CONTRACT AND AND | Set Scale | | |
| | Calibrate | | |
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| - Stand Real | Plot Profile | ЖК | |
| | Surface Plot | | ¥. |
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| and the second second | 3D OC Options | 4 | |
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| | Colocalization | > | |
| | Color Histogram | | |
| | Directionality | | |
| | Shape Index Map | | |
| | Helmholtz Analysis | | |
| | 3D Surface Plot | | |
| | Classification | > | |
| | Local Thickness | > | |

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<u>https://fiji.sc/</u>

https://imagej.net/Fiji

Export Measure — Option 2: from Analyze particles

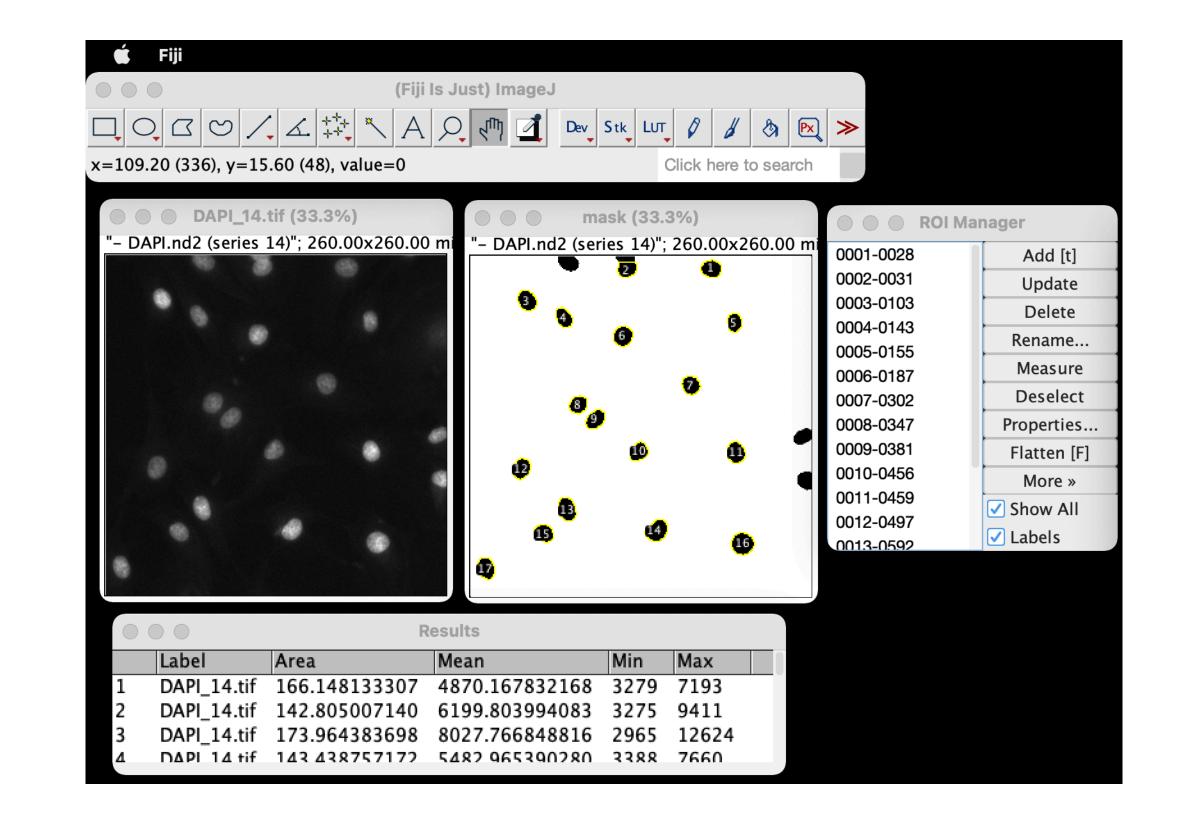
| Set Measurements | | | |
|--|---|--|--|
| Area Standard deviation Min & max gray value Center of mass Bounding rectangle Shape descriptors Integrated density Skewness Area fraction | Mean gray value Modal gray value Centroid Perimeter Fit ellipse Feret's diameter Median Kurtosis Stack position | | |
| Limit to threshold Invert Y coordinates Add to overlay | Stack position Display label Scientific notation NaN empty cells I_14.tif | | |

| 🔴 🔵 🌒 🛛 Anal | yze Particles |
|------------------|----------------------|
| Size (micron^2): | - |
| Circularity: | 0.00-1.00 |
| Show: | Overlay Masks 🗸 |
| 🗹 Display result | s 🗹 Exclude on edges |
| Clear results | Include holes |
| 🗌 Summarize | Overlay |
| 🗹 Add to Manag | er 🗌 Composite ROIs |
| Help | Cancel OK |



https://imagej.net

https://imagej.nih.gov/ij/



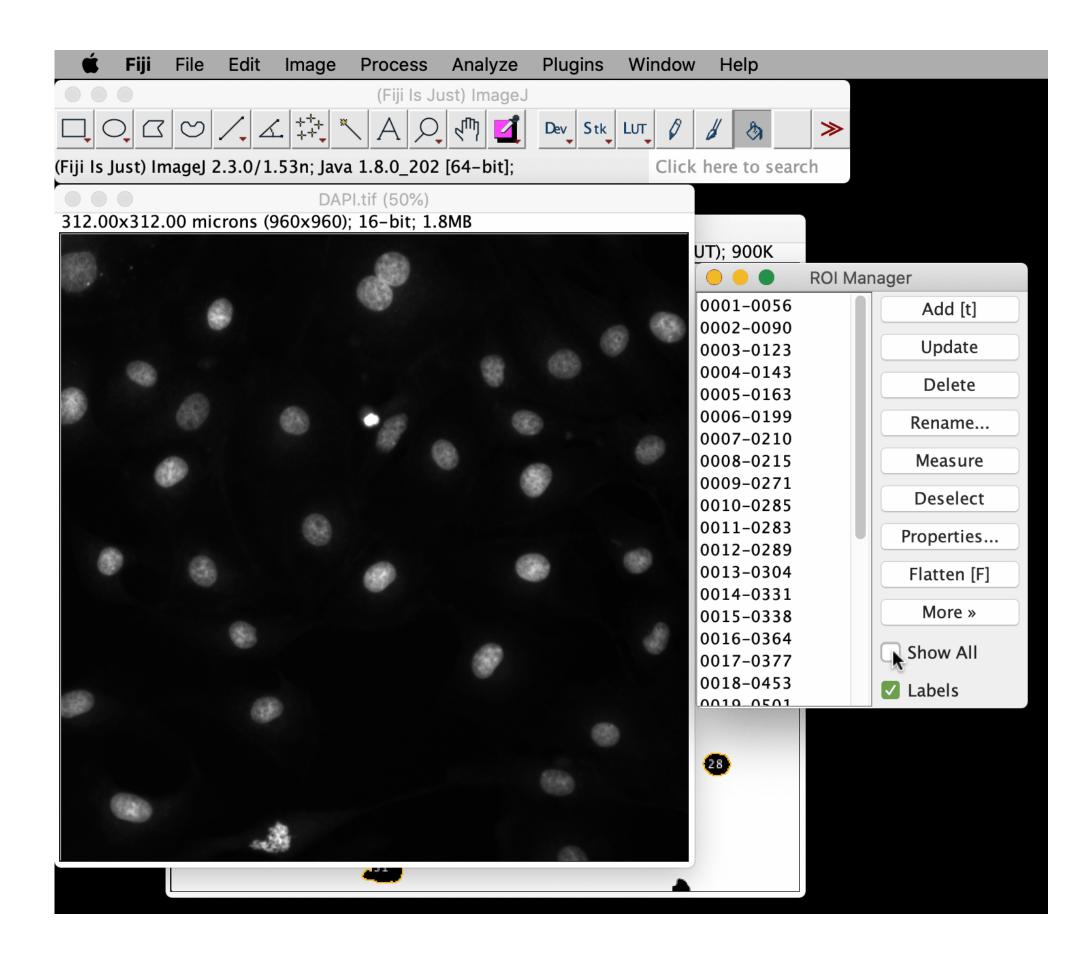
https://fiji.sc/

https://imagej.net/Fiji



Export

Measure — Option 3: Using the ROI manager





https://imagej.net

https://imagej.nih.gov/ij/

- 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.





https://fiji.sc/

https://imagej.net/Fiji

Segmentation with pixel based classifier—exercises



- Continue with the <u>"Analyze Particle" step</u> form <u>ONE</u> of the workflow exercises below.
- 7.1 segmentation with thresholding
 - OR
- 7.2 segmentation with Weka