

ROI Manager





Annotation Tools *see also Edit > Selection

Region Of Interest (ROI)







Secondary/Right Click for more options

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(Fiji Is	s Just) Imag	leJ		
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0_66	(64-bit)			

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Double Click to set/change properties





ROI Manager

Fiji File Edit Image Process Image Image Image Image Image Image	Analyze Plugins Measure	Window H	lelp	Analyze >	> Tools >	ROI Ma	nager
(Fiji Is Just) ImageJ 2.1.0/1.53c; Java 1.8.0_66 [C2-hela-cells.tif (50%) "Green"; 34.60x25.03 μm (672x486); 16-bit; 6	Analyze Particles Summarize Distribution Label Clear Results Set Measurements Set Scale	here	te to search	The ROI Mo with m	t anager is	s a tool for lections (F	* working
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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.







ROI Manager

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.







ROI Manager





"Edit" menu





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







"Image" menu



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

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



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 Is Just) ImageJ	
	0 1 3 >
(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%)	lanager
34.60x26.36 μm (672x512); 16-bit; 672K ROI_1	Add [t]
ROI_2 ROI_3	Update
ROI_4	Delete
	Rename
ROI_2 ROI_1	Measure
Color: yellow 📀	Deselect
ROI 3 Font size: 24 😒	Properties
	Flatten [F]
ROL 4 Show labels	More »
Use names as labels	Show All
✓ Draw backgrounds	✓ Labels
Bold	
Cancel OK	





Live Demo of ROI manager



And

Segmentation Measurements









Export and Measure





X







Export and Measure

		Results	
	Area	Mean	X
0016	285	204.29474	197.
B 0028	278	174.84892	219.
B C C C C C C C C C C	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.
	24 22 33 27	ED	













Image Analysis Solution: Watershed

in Fiji: Process > Binary > Watershed

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

Image





Process and segment

Binary Mask



Watershed





Solution: Watershed

Binary mask





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Process and segment

Distance transform











From binary image to instance segmentation



Binary mask



Instance segmentation





in Fiji: Analyze > Analyze Particles...







in Fiji: Analyze

> Analyze Particles...







in Fiji: Analyze

> Analyze Particles...









in **Fiji**: **Analyze**

> Analyze Particles...







in **Fiji**: **Analyze**

> Analyze Particles...







in **Fiji**: **Analyze**

> Analyze Particles...







Analyze particles: Select for morphology

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



ize (pixel^2):	0-In	finity		
Circularity:	0.00	-1.00		
Show:	Noth	ing	\sim	
Display res	ults	Excl	ude on ed	ges
Clear result	5	Inclu	ude holes	
Summarize		Over	rlay	

Process and segment



Circularity







Image Analysis Analyze particles: Size

in Fiji: Analyze > Analyze Particles...

ize (pixel^2):	0–Infinity
Circularity:	0.00-1.00
Show:	Nothing ~
Display res Clear result	ults Seclude on edges

Size range of the particles that you want to detect.









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







Analyze particles: Circularity

in Fiji: Analyze > Analyze Particles...

Size (pixel^2):	0–Infinity
Circularity:	0.00-1.00
Show:	Nothing ~
Display rest	ults 🗹 Exclude on edges
Clear result	s Include holes
Summarize	Overlay
Add to Man	ager Composite POIs



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





Analysis Analyze particles: Circularity











Image Analysis Collaboratory Analysis















Analysis Analyze particles: Circularity

















Analyze particles: Exclude on Edges

in Fiji: Analyze > Analyze Particles...

ize (pixel^2):	0-In	finity		
Circularity:	0.00	-1.00		
Show:	Noth	ning	Ý	
Display res	ults	Exc	lude o	n edge
Clear result	5	Inc	lude ho	oles
Summarize		Ov	erlay	
		Dr.		- DOI-

Process and segment

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













Export and Measure

		Results	
	Area	Mean	X
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.
	24 20 33 37	63) 650	







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

Export and Measure

	Results		
	Area	Mean	X
0016	285	204.29474	197.
B 0028	278	174.84892	219.
⁸ 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.
	24 20 33 37	E D	







Analyze particles: Save the segmented image

in Fiji: Analyze > Analyze Particles...

Anal	lyze Particles	
Size (micron^2):	0–Infinity nits	
Circularity:	0.00-1.00	
Show:	Count Masks	
 Display result Clear results Summarize Add to Manage 	Nothing Overlay Overlay Masks Outlines Bare Outlines Ellipses	edges es ROIs
Help	Masks	ik
	Count Masks	

Export



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





Analyze particles: Add to ROI Manager

in Fiji: Analyze > Analyze Particles...

Size (micron^2): 0-In	finity
Pixel units	
Circularity: 0.00	-1.00
Show: No	othing
Display results	Z Exclude on edges
Clear results	include holes
Summarize	Overlay
Add to Manager	Composite ROIs

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

😑 💿 ROI M	anager
	Add [t]
	Update
	Delete
	Rename
	Measure
	Deselect
	Properties
	Flatten [F]
	More »
	Show All
	Labels





Analyze particles: Add to ROI Manager

in Fiji: Analyze > Analyze Particles...

Size (micron^2): 0-1	nfinity
Pixel units	
Circularity: 0.0	0-1.00
Show: N	othing 😂
Display results	Exclude on edges
Clear results	Include holes
Summarize	Overlay
	 Annotation and the second se Second second seco







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

Export and Measure

		Results	
	Area	Mean	X
0016	285	204.29474	197.
B 0028	278	174.84892	219.
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0034	501	189.14172	174.
0041	660	171.69697	73.8
0048	228	195.89474	233.
0055	448	209.03571	138.
	24 20 33 37	61) 61)	





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

Export and Measure

		Results	
	Area	Mean	X
0016	285	204.29474	197.
B 0028	278	174.84892	219.
B C C C C C C C C C C	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.
	24 20 33 37	63) 65)	





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Collaboratory

Analyze > Set Measurements...

	aromonto
🗹 Area	🗹 Mean gray value
Standard deviation	Modal gray value
Min & max gray value	Centroid
Center of mass	Perimeter
Bounding rectangle	Fit ellipse
Shape descriptors	Feret's diameter
Integrated density	Median
Skewness	Kurtosis
Area fraction	Stack position
 Limit to threshold Invert Y coordinates Add to overlay 	 Display label Scientific notation NaN empty cells
Redirect to: D/	API_14.tif 🗸
Decimal places (0-9): 9	
Help	Cancel OK

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

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'- D	API.nd2 (series	14)"; 260.00x260.00) mi "– DAPI.nd2 (se	eries 14)";	: 260.00x20	50.00 mi
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100	lister	h Ause	results	Min	Mari	
1		Area	Mean	MIN 2270	Max 7102	
2	DAPI 14 Hif	142 805007140	6100 802004092	3275	0411	
2	DAPI 14 tif	173 964383608	8027 766848816	2965	12624	
4	DAPI 14 tif	143 438757172	5482 965390280	2305	7660	





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, ...)

• • •	Set Me	easur	ements	
	Redirect to:	DAP	l_14.tif ∨	
Decimal	places (0-9):	9		
	Help	, 11	Cancel	ОК

Export





Manage Analysis Side note: other kinds of measurements

Analyze > Plot Profile

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Collaboratory

(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

Set Meas	urements
📝 Area	🗹 Mean gray value
Standard deviation	Modal gray value
Min & max gray value	Centroid
Center of mass	Perimeter
Bounding rectangle	Fit ellipse
Shape descriptors	Feret's diameter
Integrated density	Median
Skewness	Kurtosis
Area fraction	Stack position
Limit to threshold	Display label
Invert Y coordinates	Scientific notation
Add to overlay	NaN empty cells
Redirect to: DA	API_14.tif 🗸
Decimal places (0-9): 9	
Help	Cancel OK

e e Ana	lyze Particles
Size (micron^2):	0–Infinity hits
Circularity:	0.00-1.00
Show:	Overlay Masks 🖂
 Display result Clear results Summarize Add to Manage 	er Composite ROIs
Help	Cancel OK

Export





Image Analysis Collaboratory

Measure — Option 3: Using the ROI manager



- 1. Select the "ORIGINAL" image.
- 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 <u>"Analyze Particle" step</u> form <u>ONE</u> of the workflow exercises below.

4.1 DAPI segmentation with thresholding

<u>OR</u>

4.3 - DAPI segmentation with Labkit