OrgM

A jython macro for automated measurements organoid size (diameter and area) and shape (roundess and circularity) from brightfield images

Jython = Java + python - the language of imageJ

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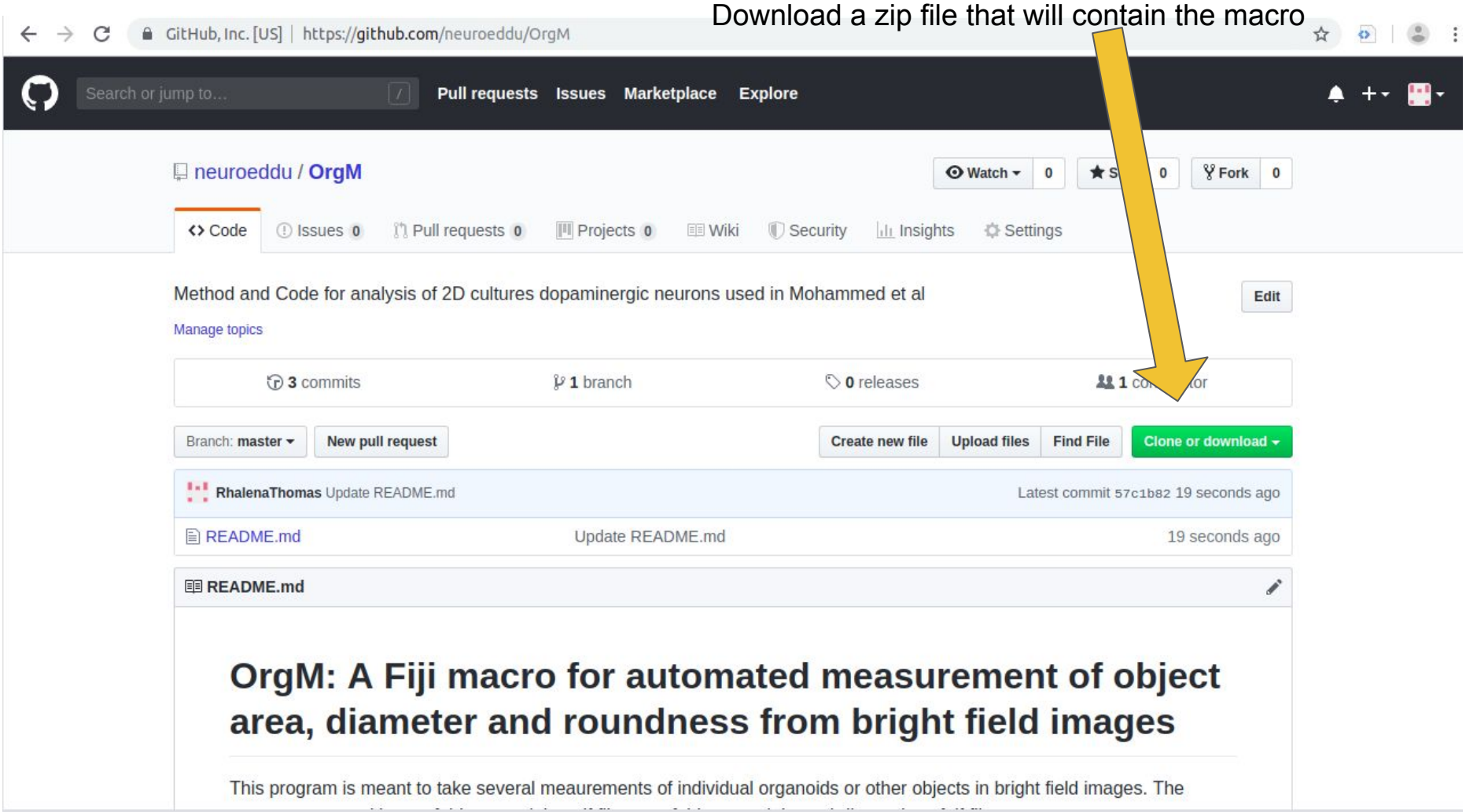
Overview: How to use orgM to measure organoids size

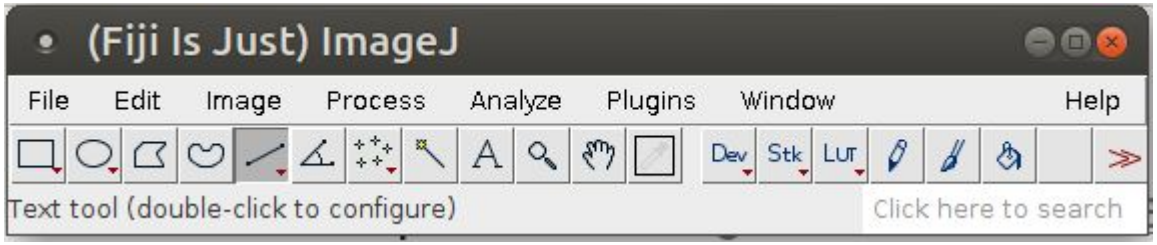
- ➔ Take brightfield images of your organoids with a single organoid per image.
  - ◆ If you have images with items in the background or multiple organoids see “pre-processing” options at the end of this document.
- ➔ Download the Github repository
  - ◆ https://github.com/neuroeddu/OrgM
- ➔ Open FIJI (Fiji is just ImageJ) or download and install FIJI 
  - ◆ https://imagej.net/Fiji/Downloads
- ➔ Drag the file “OrgM.py” into FIJI application or open Macro
  - ◆ Press run inside the editor.
- ➔ Follow the instructions in the windows that open.
  - ◆ Step by step instructions are are the next slides.
- ➔ When all the images have been measured you will find a csv file in the output directory you selected.
  - ◆ Open the file and check if the default threshold are appropriate for your data.

Download the Github repository https://github.com/neuroeddu/OrgM

For more informtion on github https://guides.github.com/activities/hello-world/



Open or install Fiji



Find information about ImageJ and Fiji here:

https://imagej.nih.gov/ij/docs/guide/146-2.html

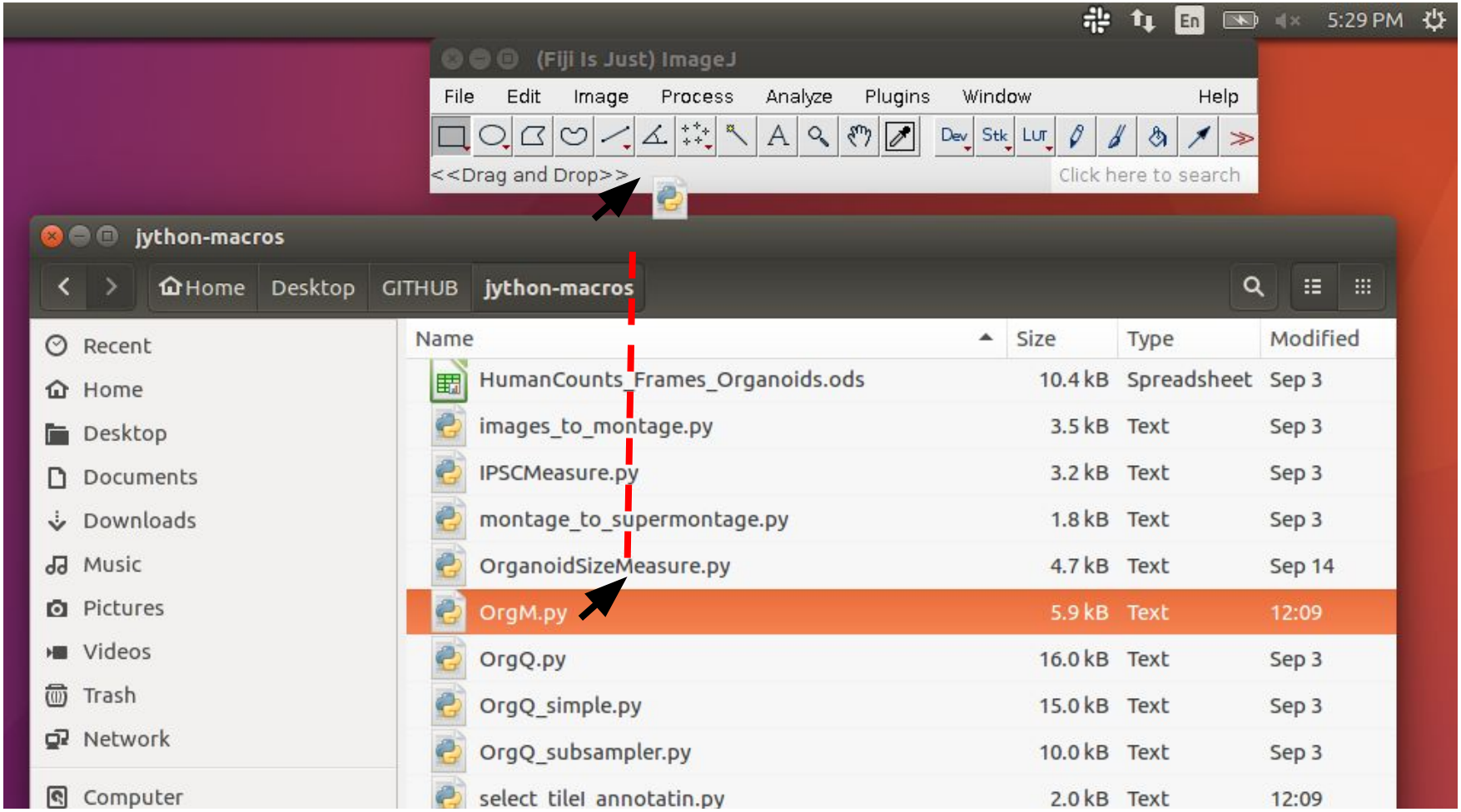
Download the version for your operating system

https://imagej.net/Fiji/Downloads

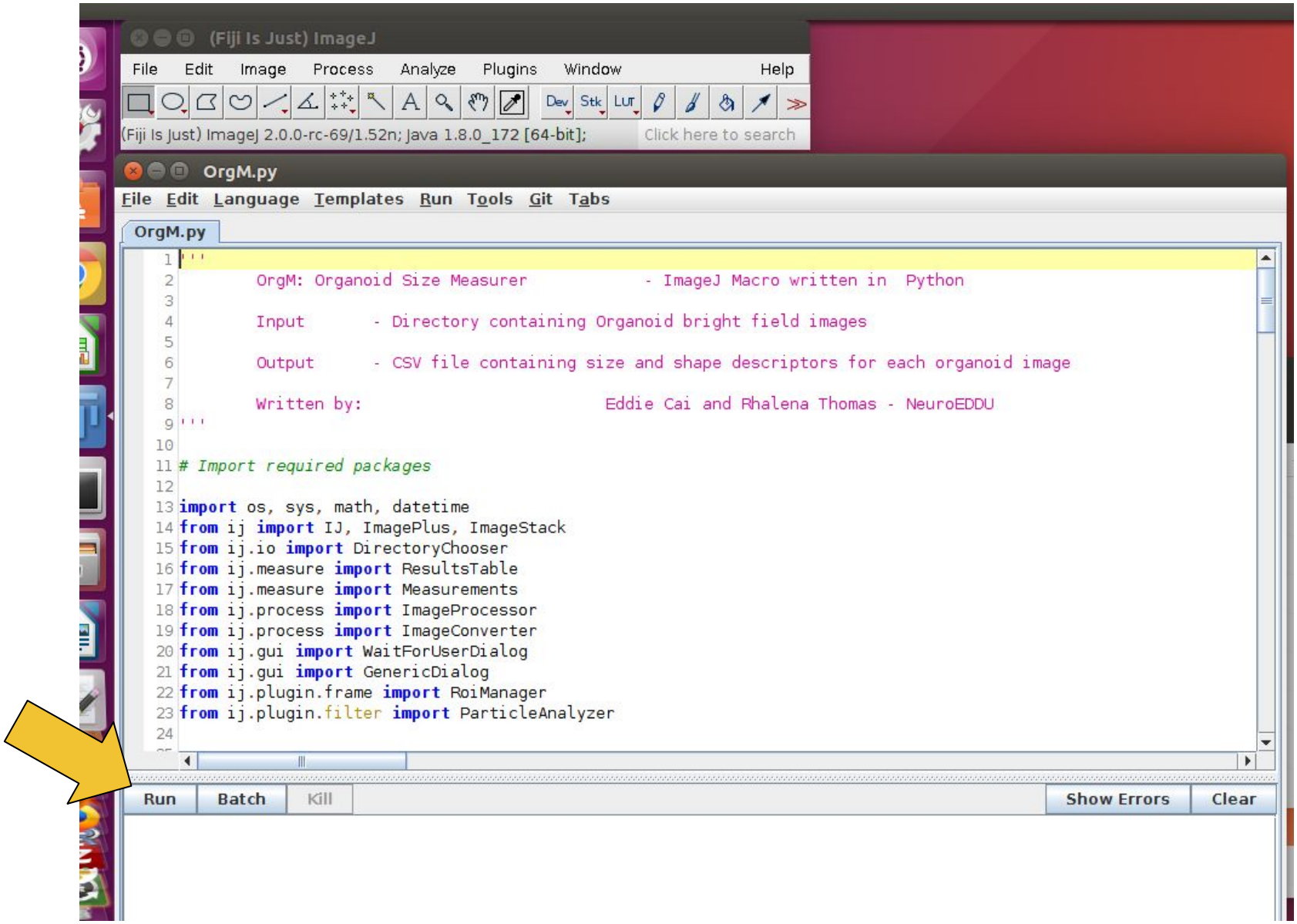
The macro OrgM requires Fiji

Extract your files from the Github OrgM folder

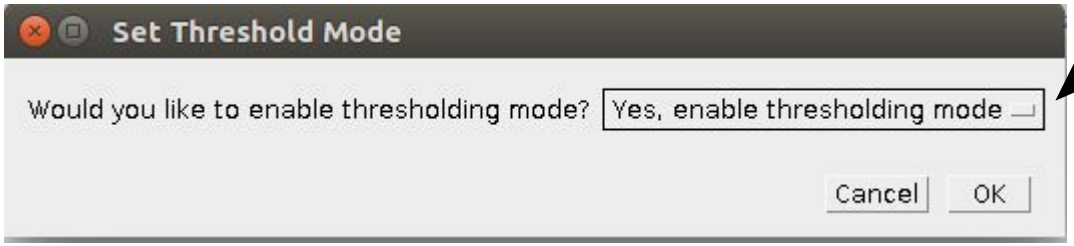
Drag OrgM.py into Fiji to open macro editor



In Fiji macro editor and press run

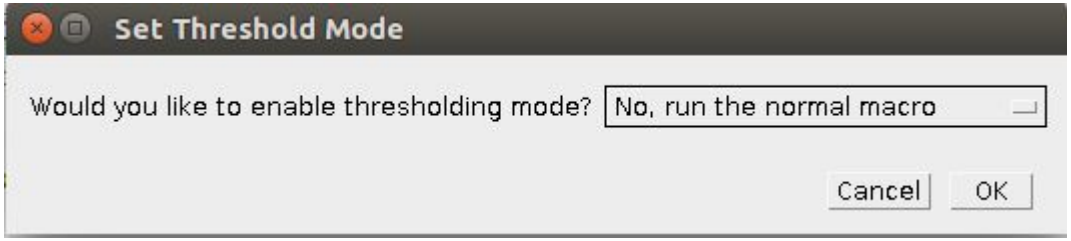


Set Threshold Mode: decide if you want to see the images



This is a drop down menu
If you don't select an option the threshold mode is the default

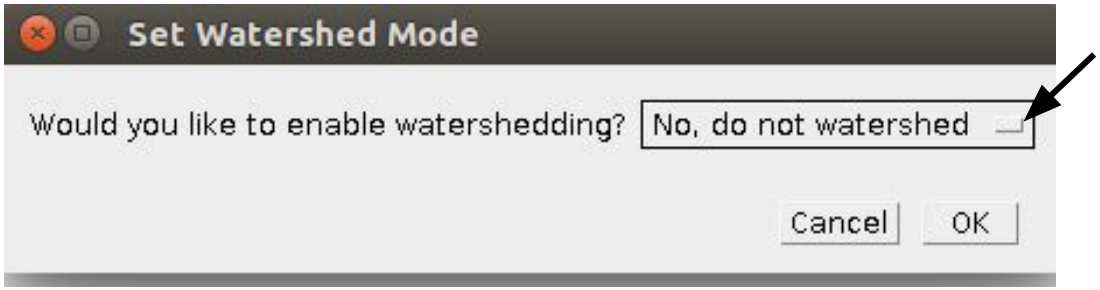
In normal macro mode: images will be auto-thresholded and they will not be opened. This mode should be used after you have checked some images.

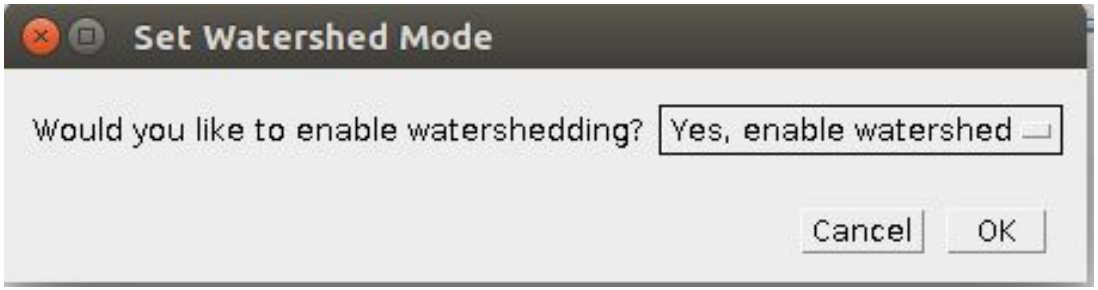


Threshold mode will show you the autothrehsold and allow you to change the threshold. You will be able to see the images and what region is being measured. The mode is not practical for large amounts of images.

We recommend using Threshold mode to check your images and if you have organoids/objects that are at the edge of the plate and irregular (not circular) in shape you may need to analyze images in this mode.

Set Watershed Mode: Do you want to apply a watershed



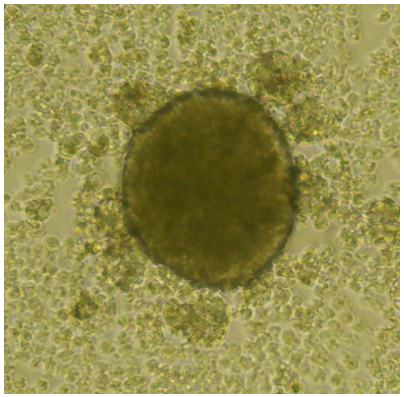


This is a drop down menu
If you don't select an option" no watershed" is the default

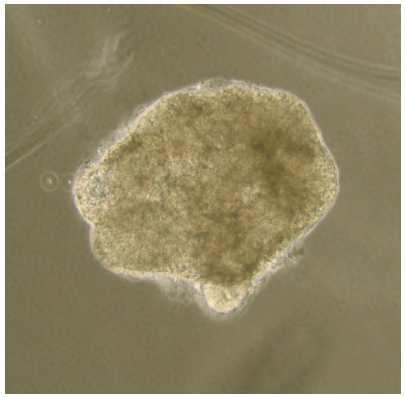
The benefit of watershed is that it will cut off background artifacts and large buds
However, in vary oblongue and bumpy organoids watershed might cut them in half (not wanted).

We recommend running "threshold mode" and watershed to see if your oganoids are being cut or not.

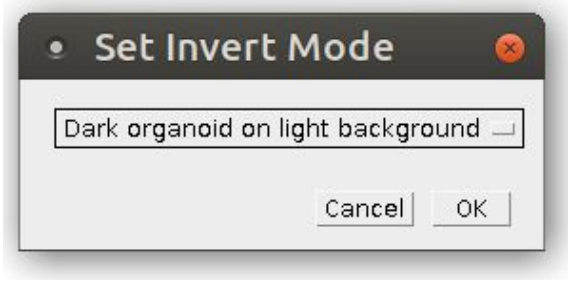
Set Invert Mode



Dark with light background



Light with dark background

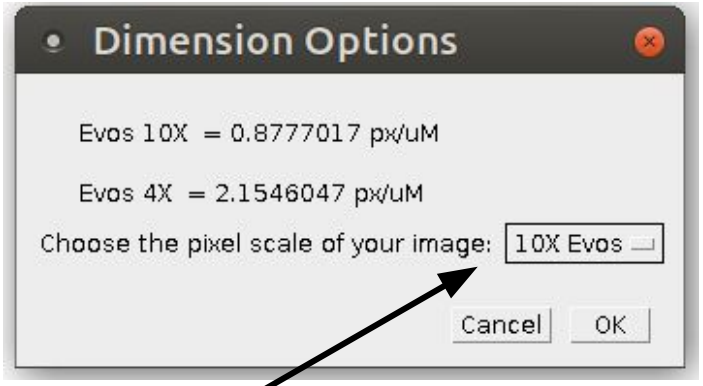


Select you if you have dark or light organoids

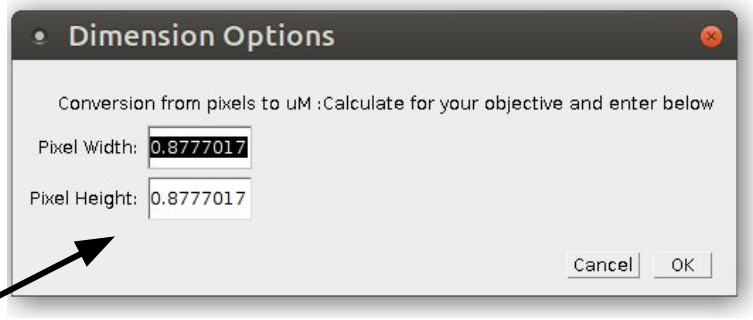
- ➔ For you organoids to be detected a threshold is used to apply a mask
- ➔ You need to know if your organoid is darker on a light background or light on a dark background
- ➔ If you have the wrong selection the organoid will not be detected

Dimension options

- This window is to select the magnification you used
- You need the conversion for the uM in the length/width of one pixel
- Some microscopes have the converse info saved in the file
- If this info is absent you must take an image of a hemocytometer and measure it (instruction at the end)
- We have put the conversion we measured for our images for the EVOS system 10X and 4X conversion
- Select other if you have used a different microscope

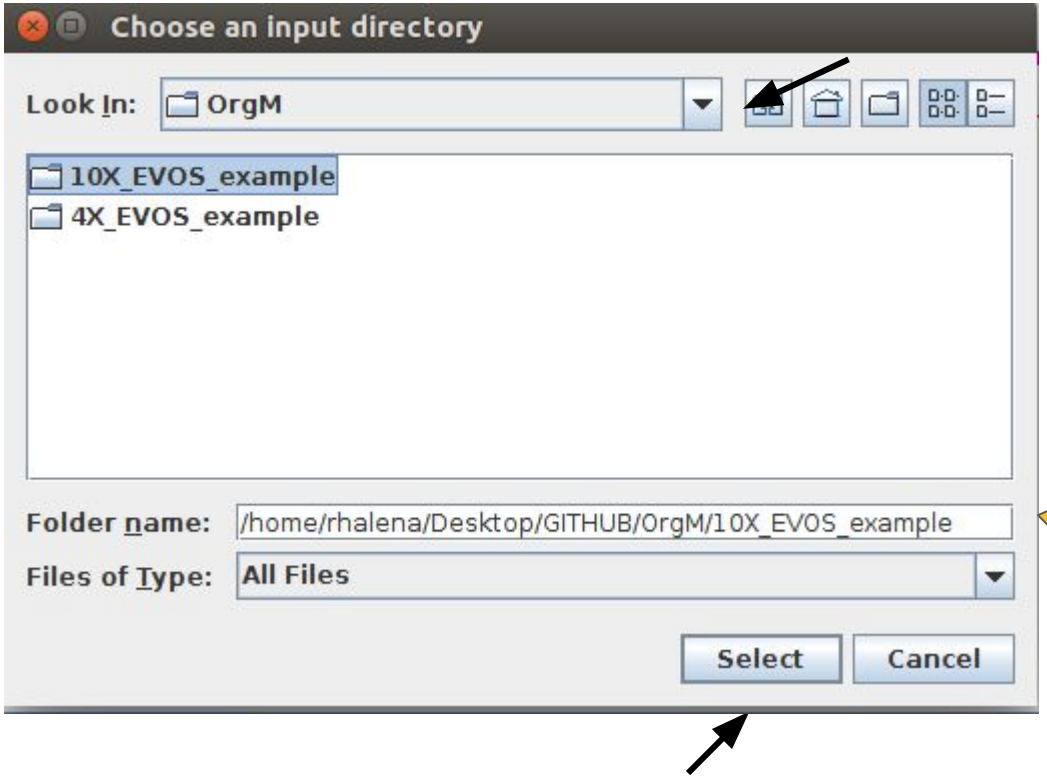


10X is the default
Click the menu to change your magnification



Fill uM length of one pixel in both windows. You will need to know the conversion

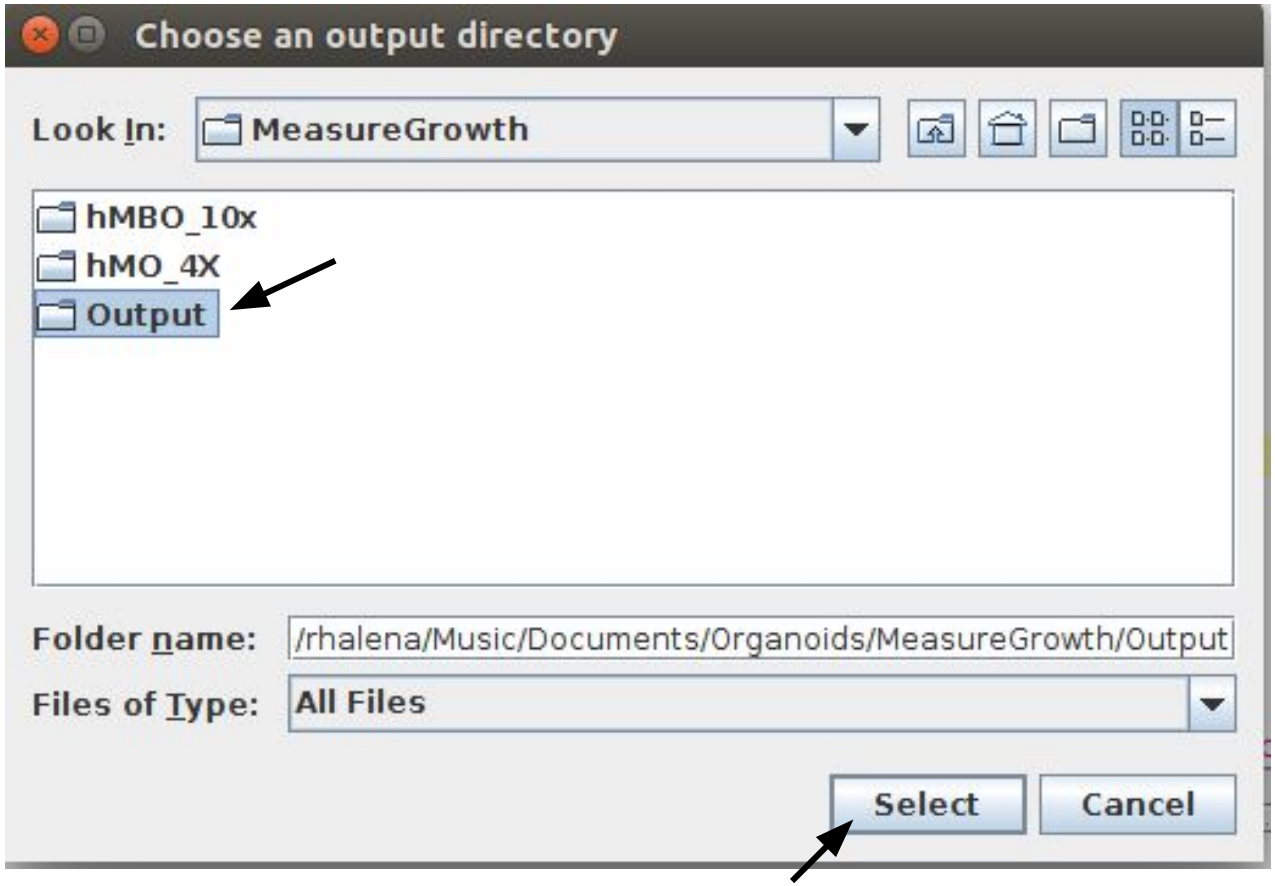
Choose where you have your images:



You can choose a folder of images OR A folder of folders with images But they need to be the same magnification

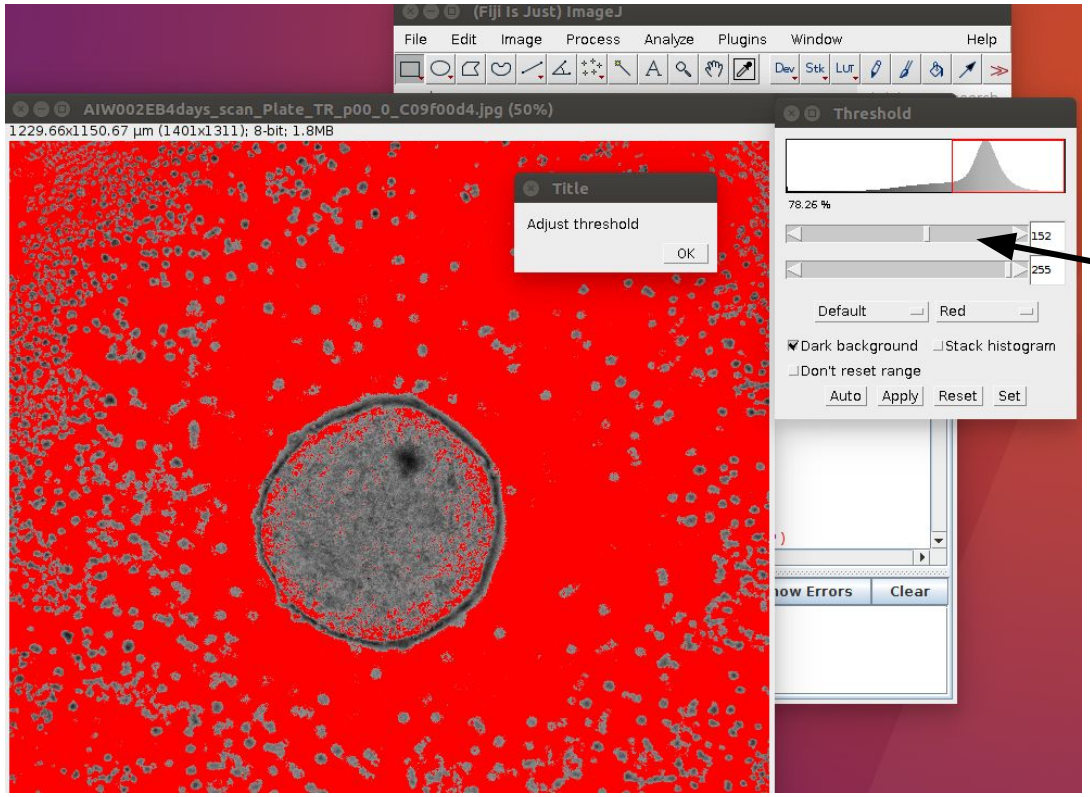
The macro will keep the file navigator open..
Navigate to where you have your images or folders of images
Press select.

Choose where you want to put your results:



The macro will keep the file navigator open..
Navigate to where you want your csv output file to go and click the folder.
You may make a folder in the navigator.
Press select.

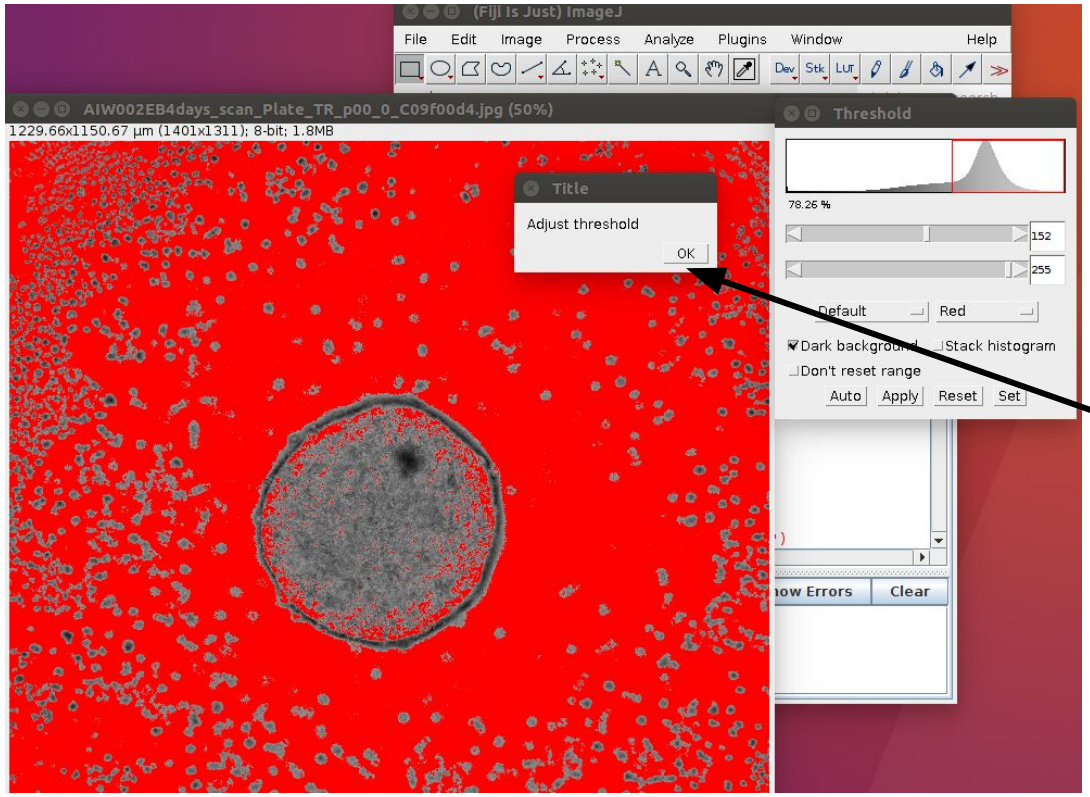
Running the macro: Threshold mode no watershed



Adjust threshold here

The auto-threshold image will appear.
You can press okay to see what will be is selected or adjust the threshold.

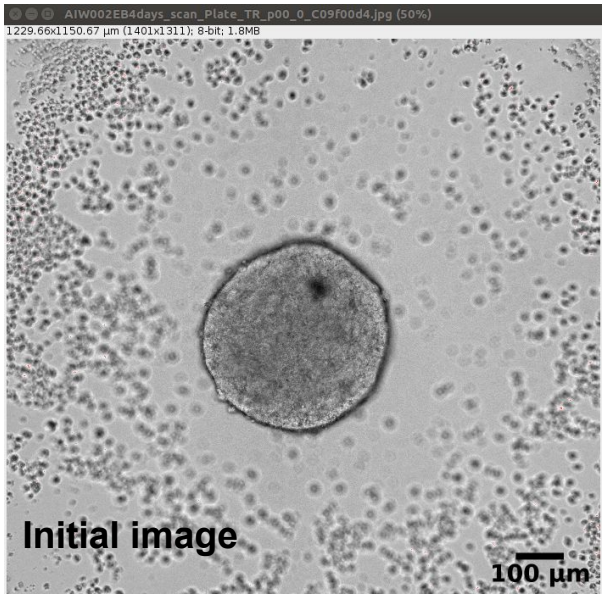
Running the macro: Threshold mode no watershed

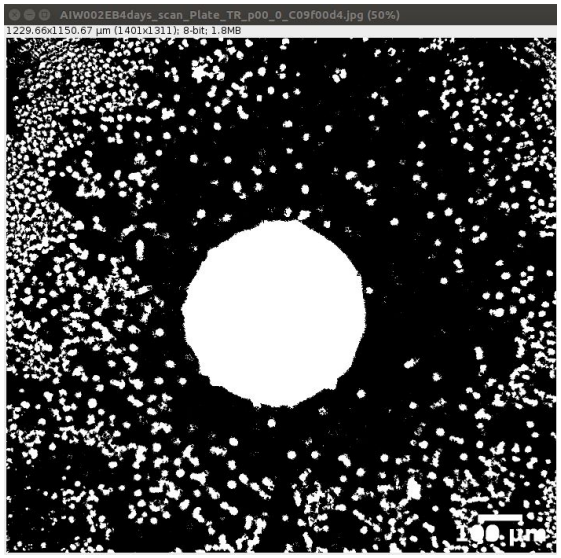


Press okay when you are finished

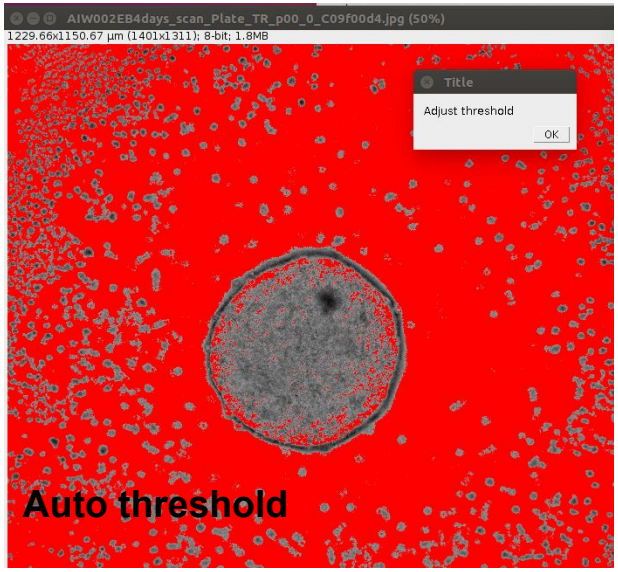
The auto-threshold image will appear.
You can press okay to see what will be is selected or adjust the threshold.

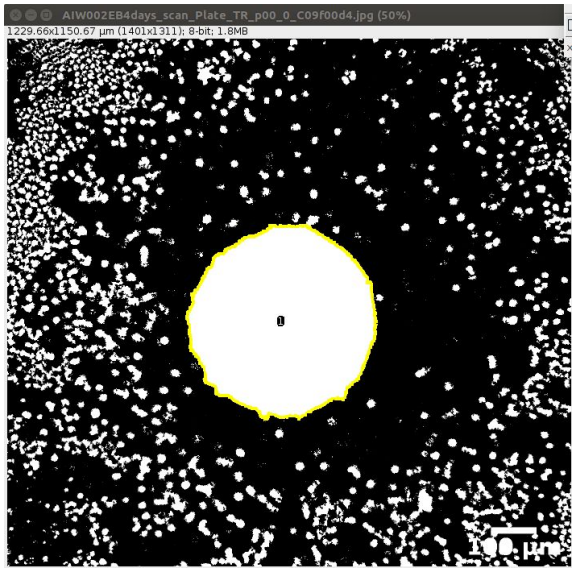
Running the macro: Threshold mode no watershed





Convert to mask

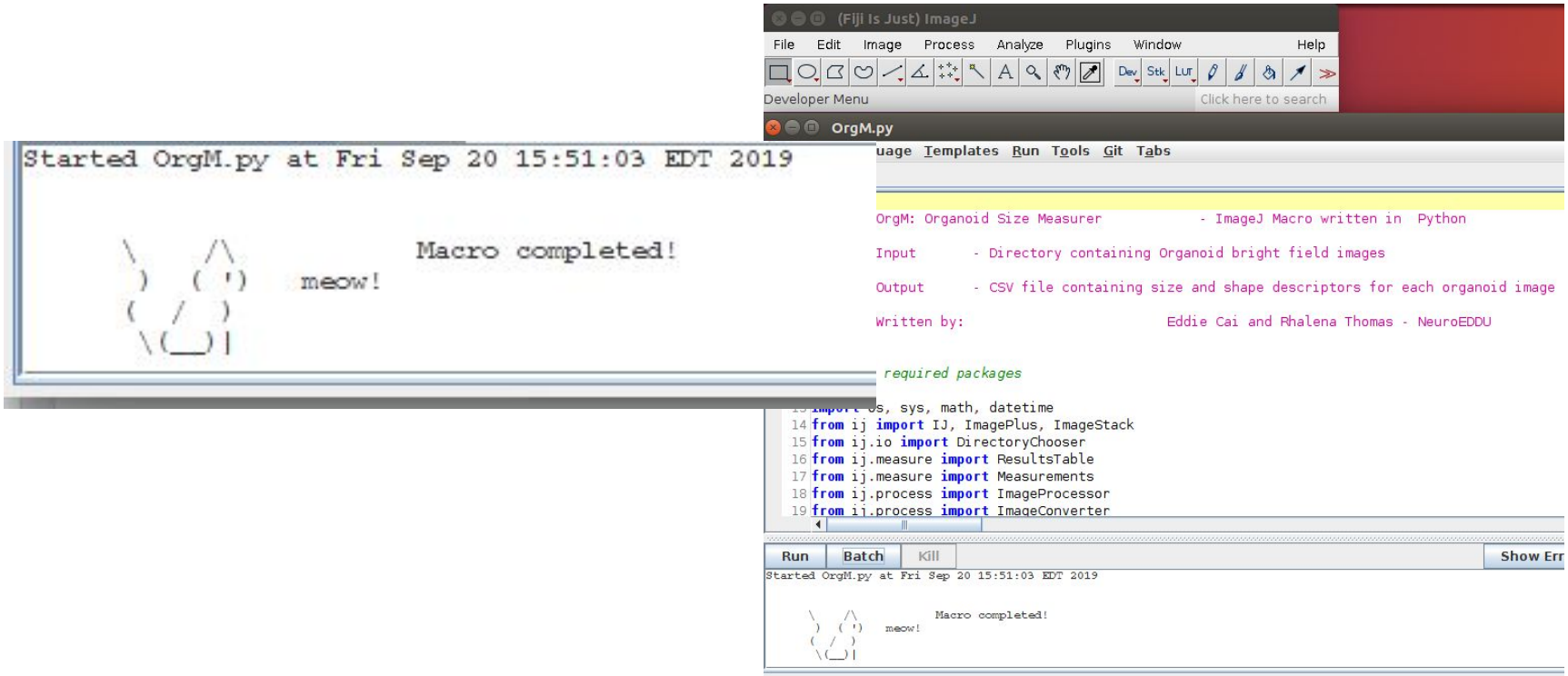




The object selected to measure is outlined in yellow

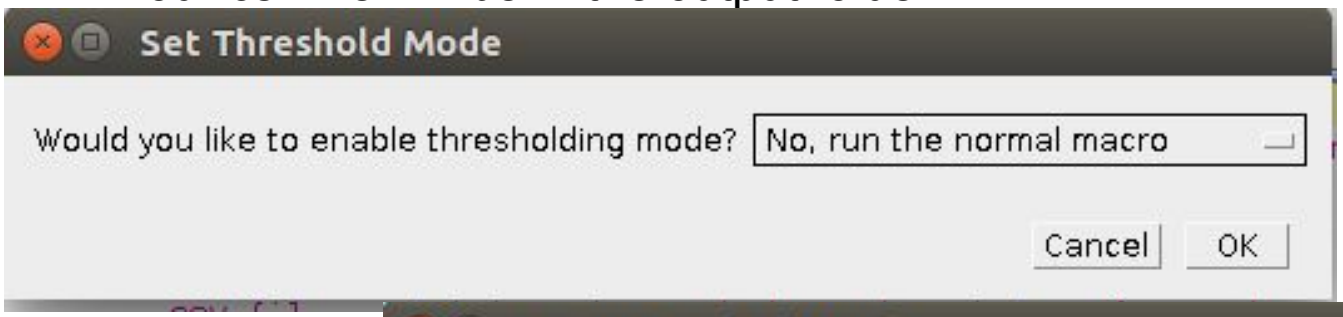
Running the macro: Threshold mode no watershed

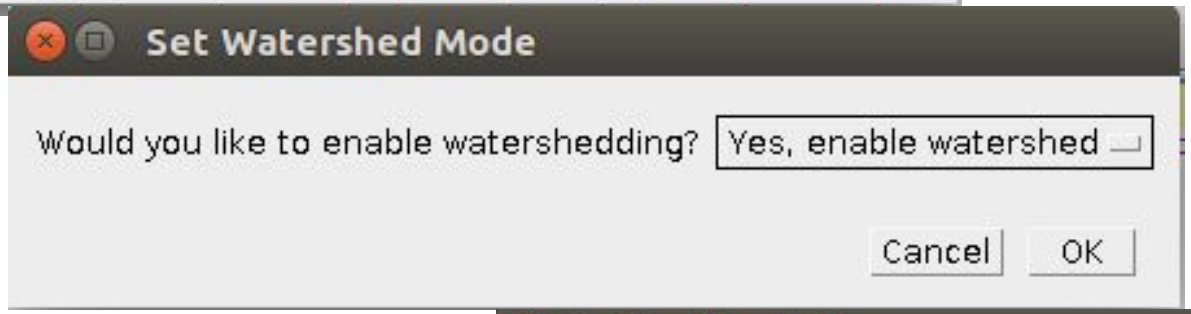
- The images will close and the next image will automatically open
- The “Adjust Threshold” window will open for each image
- When the macro is complete the last image will close
- In the macro editor window “Macro completed!” will appear
- Your csv file will be in the output folder

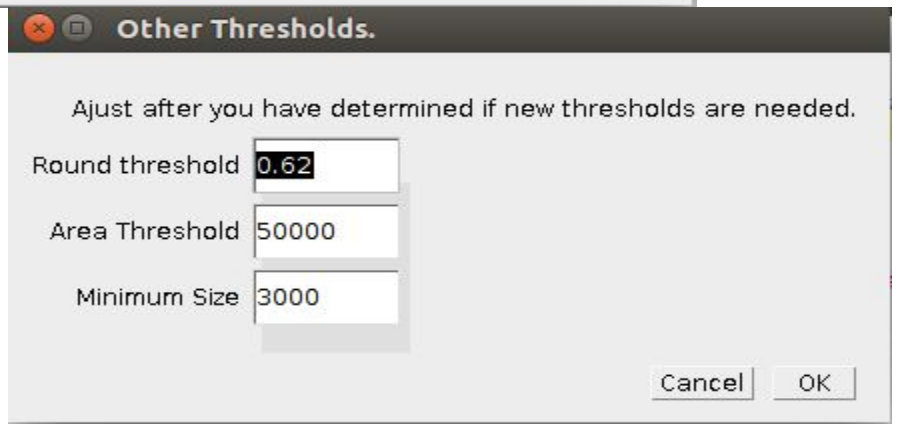


Running the macro: Normal mode

- The images will not open
- In the macro editor window “Macro completed!” will appear when the macro is finished
- Your csv file will be in the output folder







Example of the csv output file from 10X organoids

A	B	C	D	E	F	G	H	I	J	K	L	M
Subfolder	File Name	Max Diameter uM	Min Diameter uM	Diameter uM	Area	Equivalent Circle Diameter	Ellipse Major	Ellipse Minor	Circularity	Bumpy	Solidity	Meets Criteria
	cOrg3.jpg	830.37	731.71	781.04	412664.28	512.55	752.52	698.22	0.27	0.93	0.92	True
	cOrg1.jpg	766.99	698.90	732.94	417310.32	515.43	760.81	698.39	0.37	0.92	0.97	True
	cOrg2.jpg	1059.18	870.75	964.96	546351.06	589.76	911.33	763.32	0.21	0.84	0.81	True
	cOrg4.jpg	902.23	698.18	800.21	407568.35	509.38	779.60	665.64	0.24	0.85	0.92	True

If you have multiple folders the subfolder name will be included

Original file name

Diameter at the largest distance Fiji function “Ferret”

Shortest diameter (Fiji MinFerret)

Diameter Mean of Longest and shortest diameters (Feret and MinFeret)

Total Area uM^2

A circle is drawn around the object and this is the diameter of that circle

An Ellipse is drawn to fit the object. This is the largest line that fits in the ellipse.

An Ellipse is drawn to fit the object. This is the smallest line that fits in the ellipse.

This is how close the area is to that of a shape.

This is how not “bumpy” it is. The fiju function used is Roundness

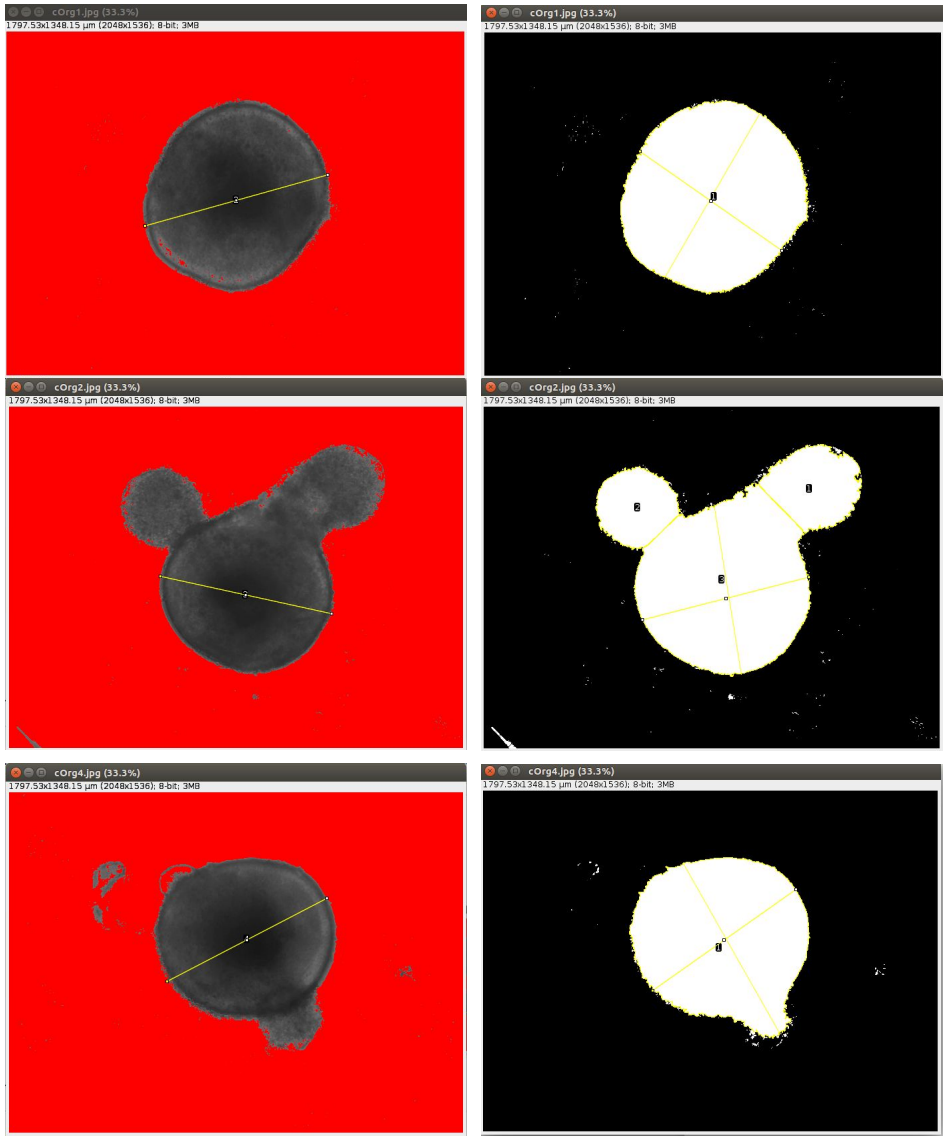
This is the percent area of the same shape if we filled in the concave areas.

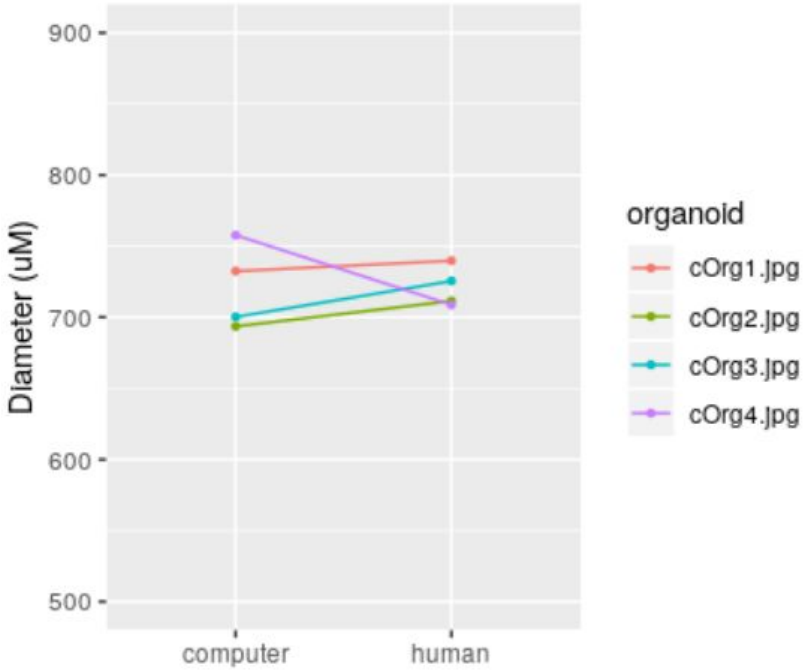
Test if the oject meets the criteria set in the “Other Thresholds” Window: area and “Bumpyness”

Comparison of OrgM with human measurements

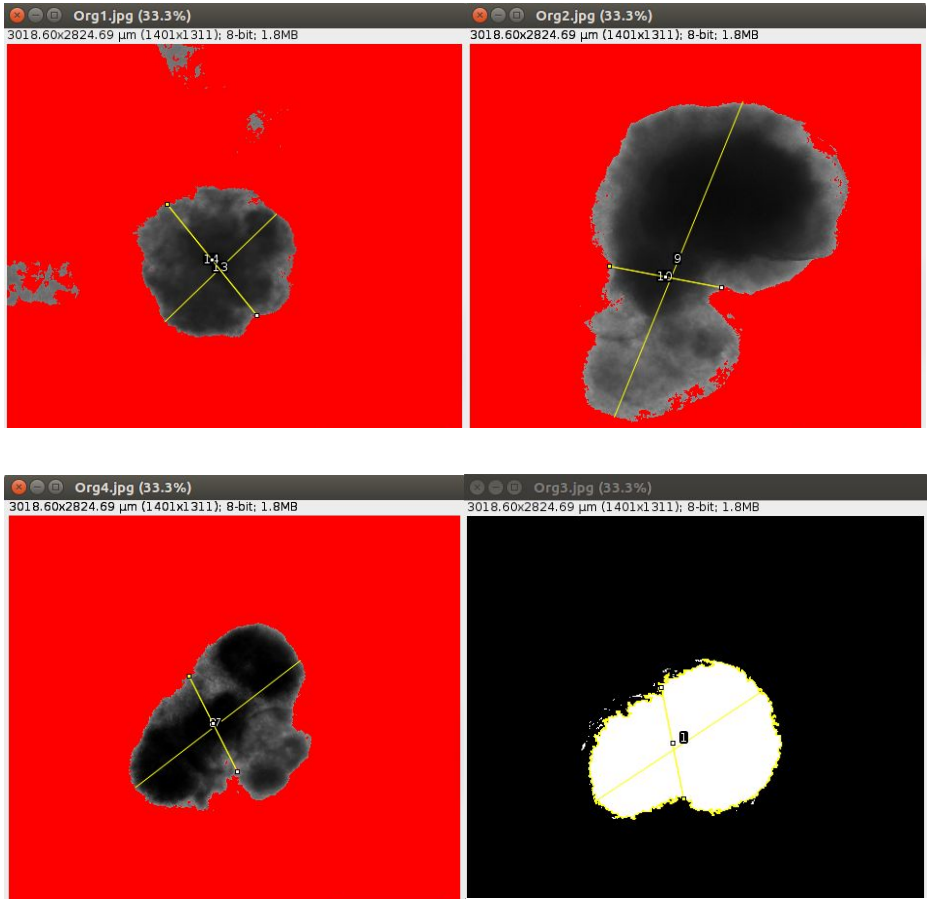
Human measurement

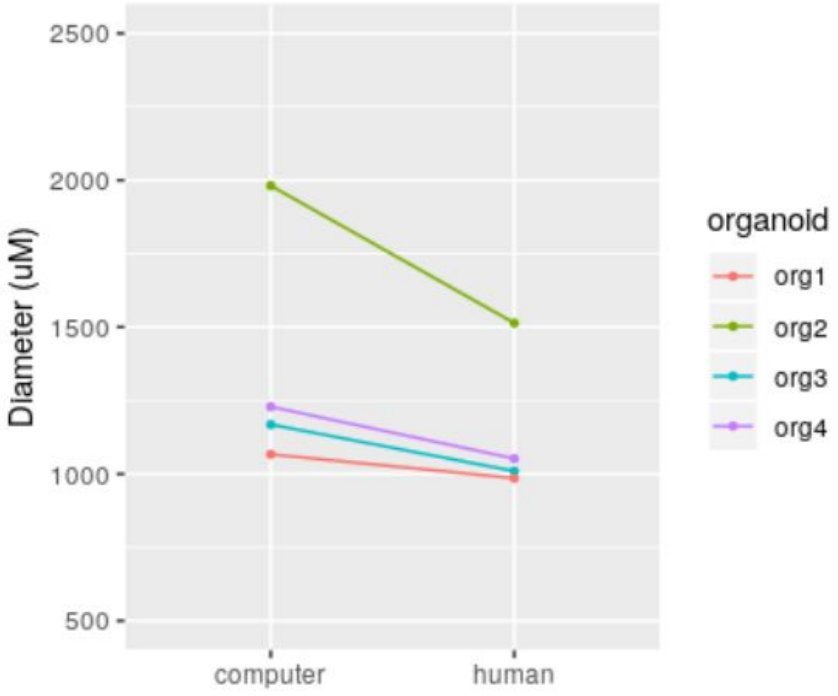
Computer measurement





Comparison of OrgM with human measurements for 4x images





In the case of these irregular shapes the area is a much better measurement than diameter.

Preprocessing of data to remove background items

- ➔ We have provided another macro to cut out the part of an image you want to analyze
  - Step1_keep_area.ijm
  - Open it in FIJI just like OrgM
- ➔ The macro functions by:
  - Opens an image
  - The user selects the area they want to keep (cut out the background stuff)
  - User selects okay
  - New image is save
- ➔ Take these images and run them in OrgM