

OrgM

A jython macro for automated measurements
organoid size (diameter and area) and shape
(roundness 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.

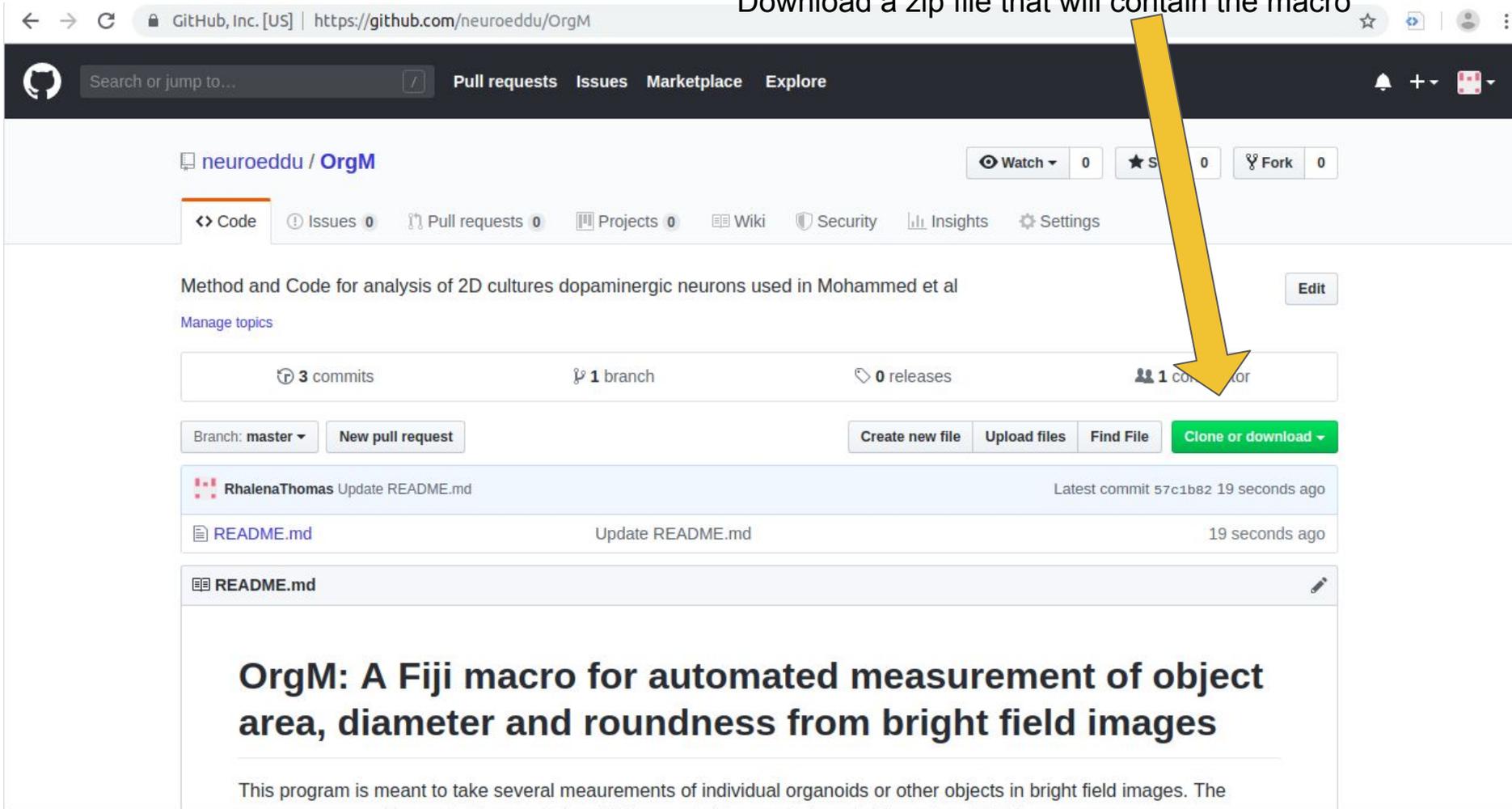
Download the Github repository

<https://github.com/neuroeddu/OrgM>

For more information on github

<https://guides.github.com/activities/hello-world/>

Download a zip file that will contain the macro



The screenshot shows the GitHub repository page for 'neuroeddu / OrgM'. The page includes a navigation bar with 'Pull requests', 'Issues', 'Marketplace', and 'Explore'. Below the repository name, there are statistics for 'Watch', 'Stars', and 'Fork'. The main content area shows the repository description: 'Method and Code for analysis of 2D cultures dopaminergic neurons used in Mohammed et al'. Below this, there are statistics for '3 commits', '1 branch', and '0 releases'. A yellow arrow points to the 'Clone or download' button, which is highlighted in green. The repository content shows a commit by 'RhaleaThomas' updating the README.md file. The README content is visible at the bottom of the page.

neuroeddu / OrgM

Watch 0 Stars 0 Fork 0

Code Issues 0 Pull requests 0 Projects 0 Wiki Security Insights Settings

Method and Code for analysis of 2D cultures dopaminergic neurons used in Mohammed et al

Manage topics

3 commits 1 branch 0 releases 1 contributor

Branch: master New pull request Create new file Upload files Find File Clone or download

RhaleaThomas Update README.md Latest commit 57c1b82 19 seconds ago

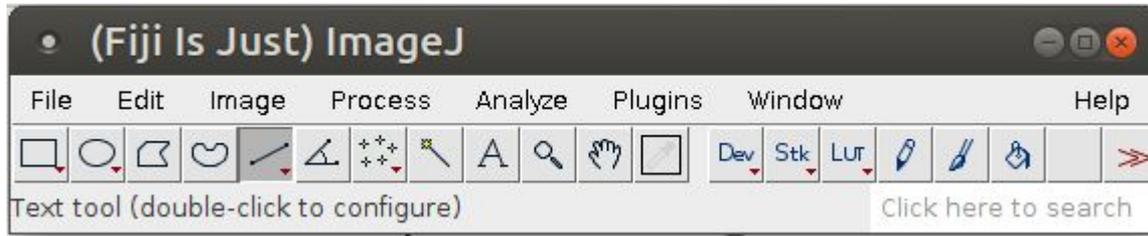
README.md Update README.md 19 seconds ago

README.md

OrgM: A Fiji macro for automated measurement of object area, diameter and roundness from bright field images

This program is meant to take several measurements of individual organoids or other objects in bright field images. The

Open or install Fiji



Find information about ImageJ and Fiji here:

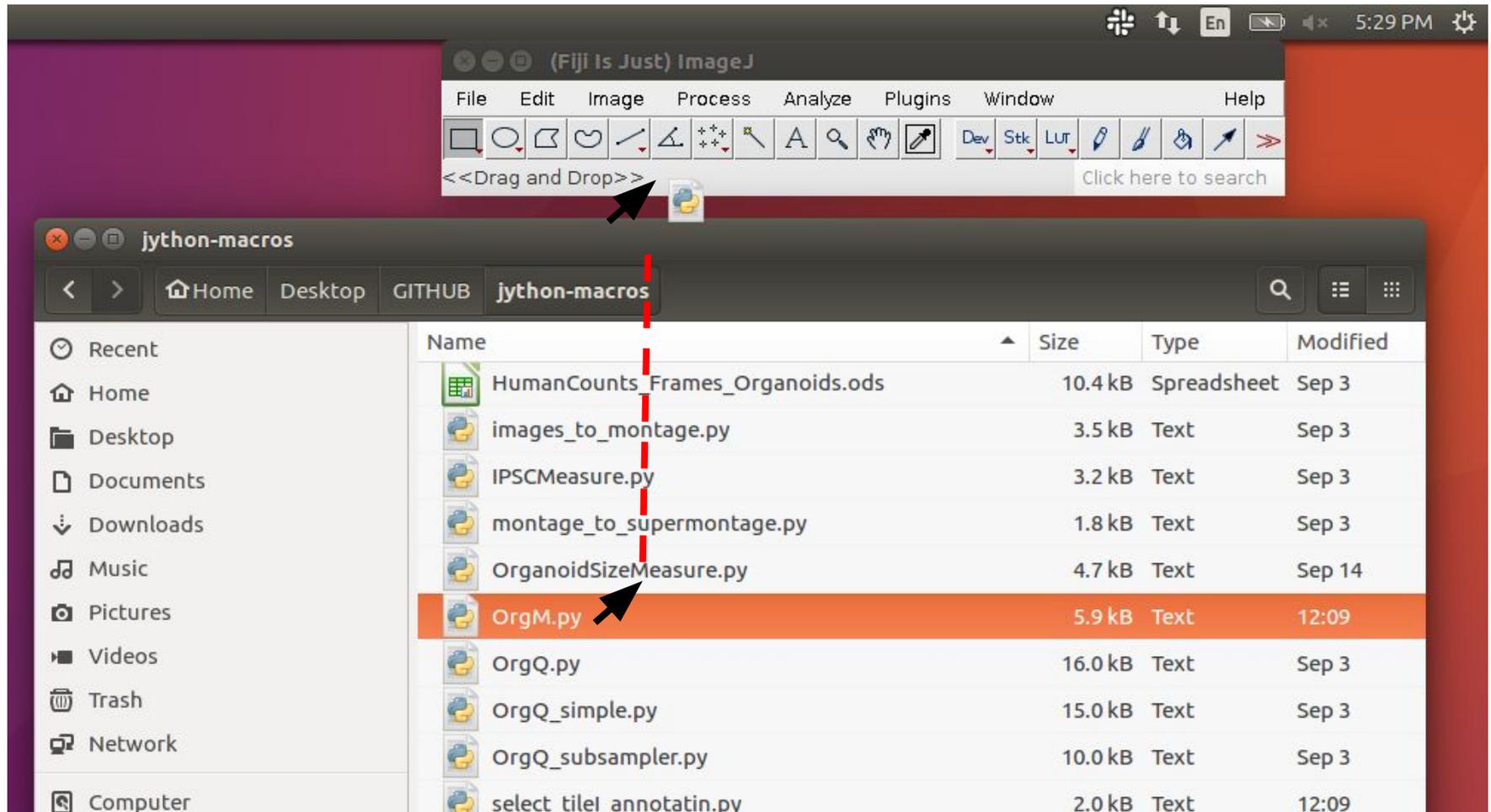
<https://imagej.nih.gov/ij/docs/guide/146-2.html>

Download the version for your operating system

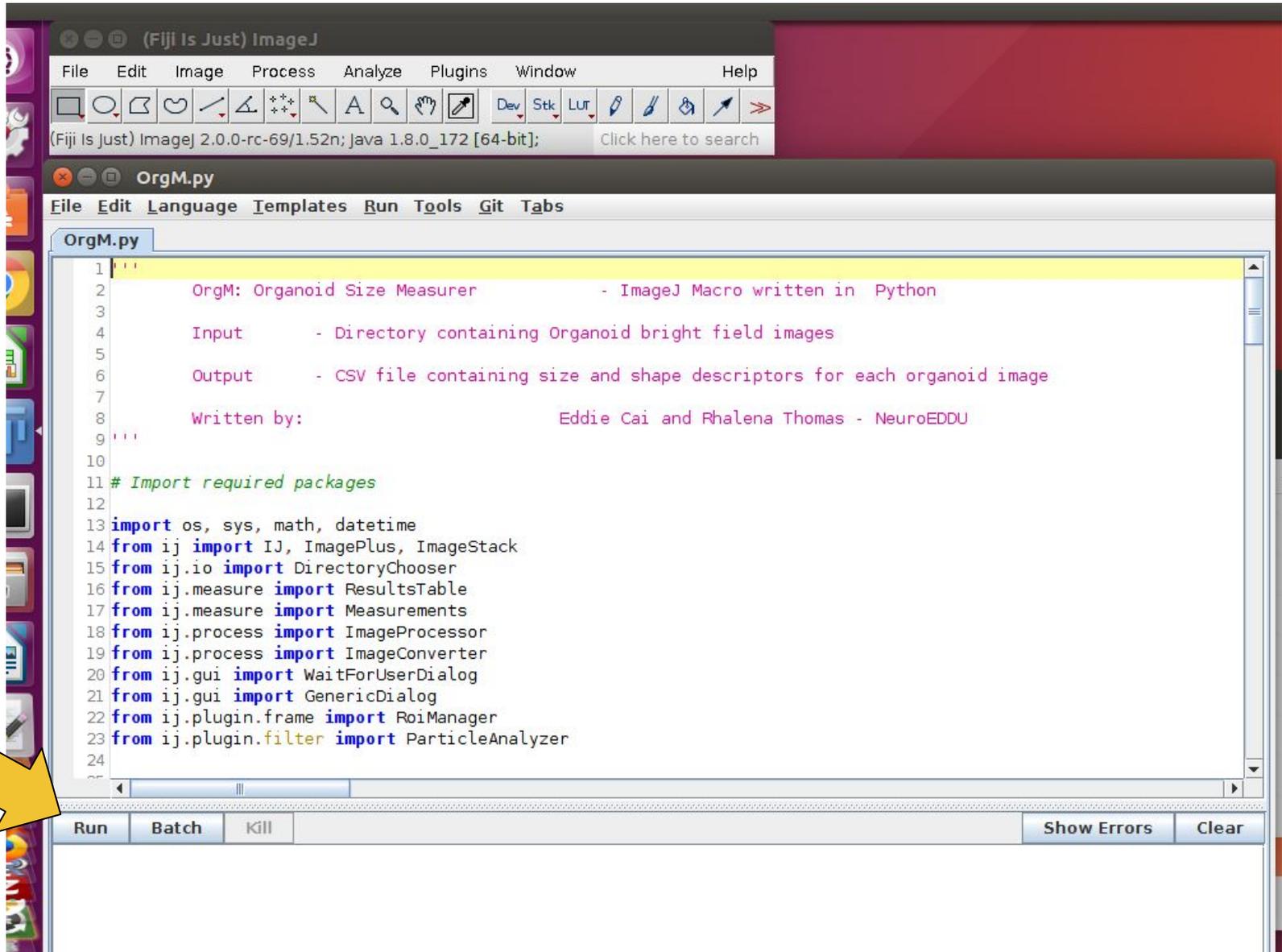
<https://imagej.net/Fiji/Downloads>

The macro OrgM requires Fiji

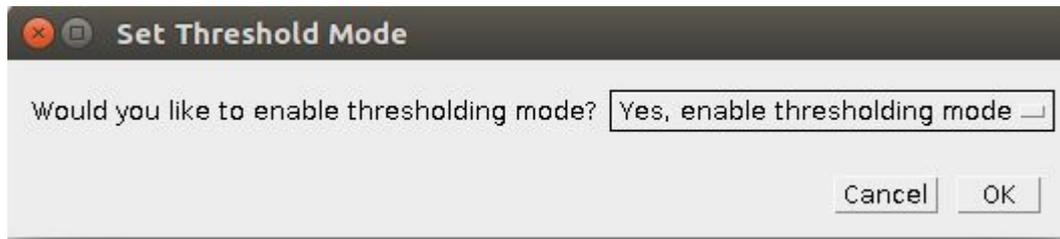
Extract your files from the Github OrgM folder
Drag **OrgM.py** into Fiji to open macro editor



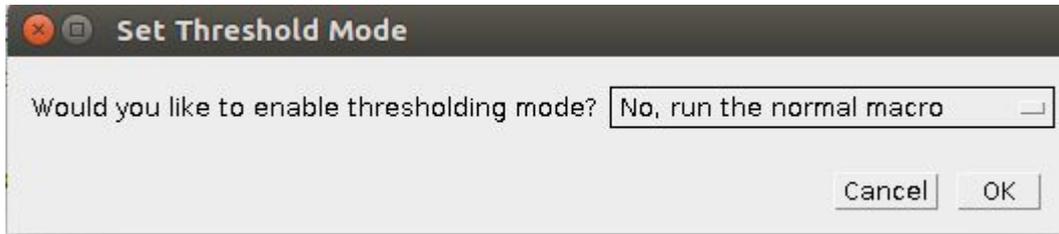
In Fiji macro editor and press run



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



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.

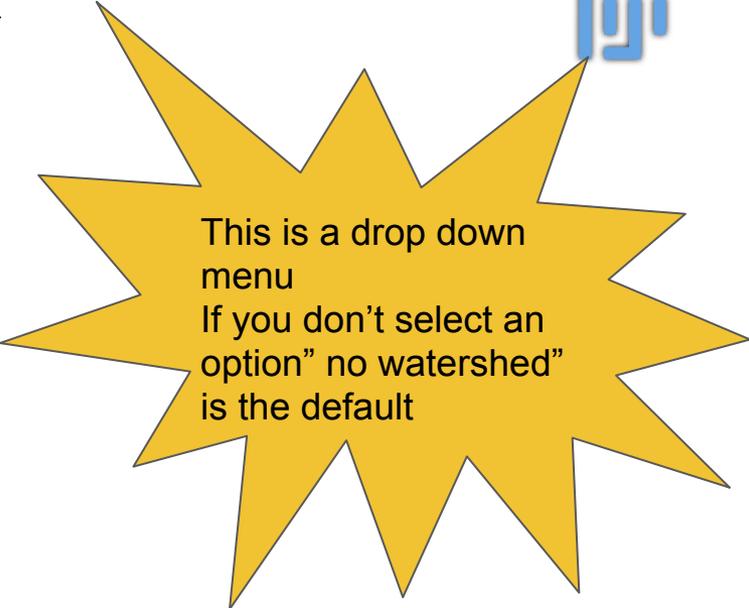
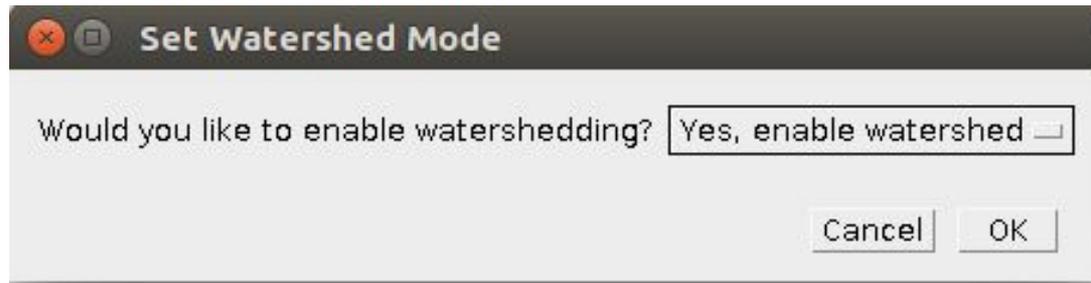
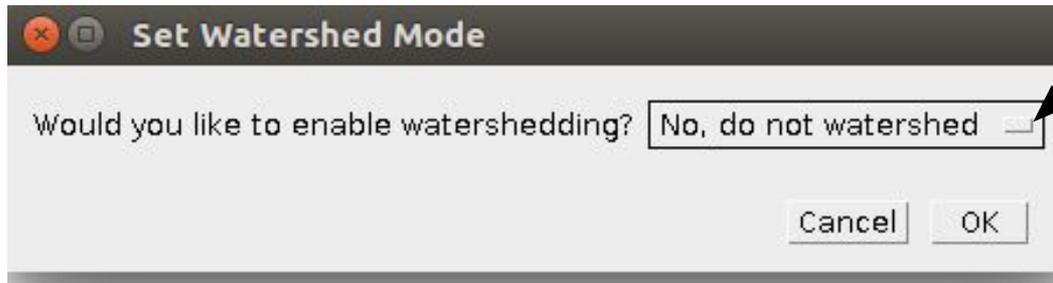


This is a drop down menu
If you don't select an option the threshold mode is the default

Threshold mode will show you the autothreshold 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

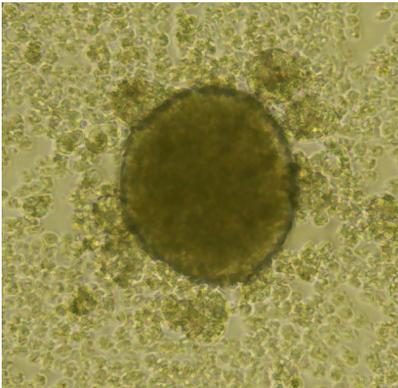


This is a drop down menu
If you don't select an option" no watershed" is the default

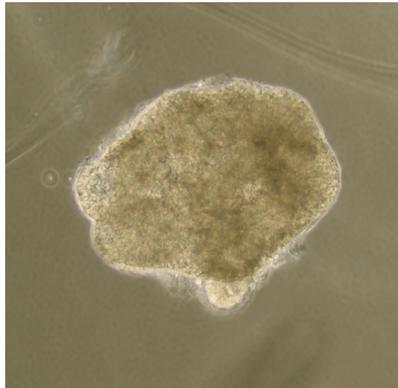
The benefit of watershed is that it will cut off background artifacts and large buds
However, in vary oblongue and bumpy organoids watershed might cut them in half (not wanted).

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

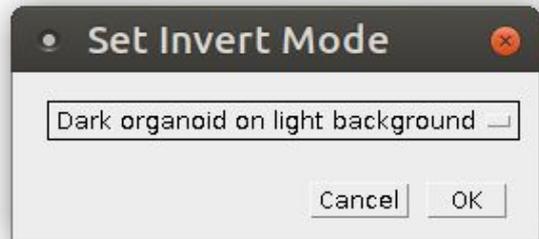
Set Invert Mode



Dark with light background



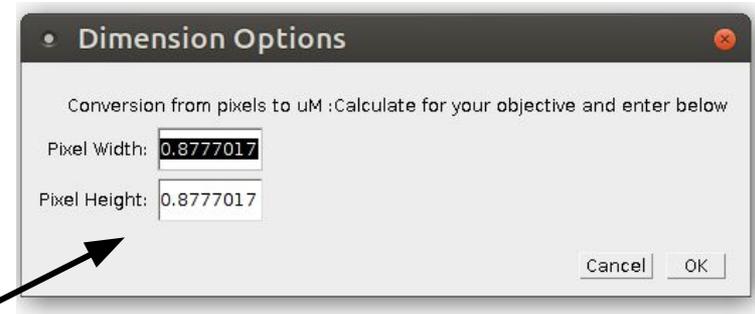
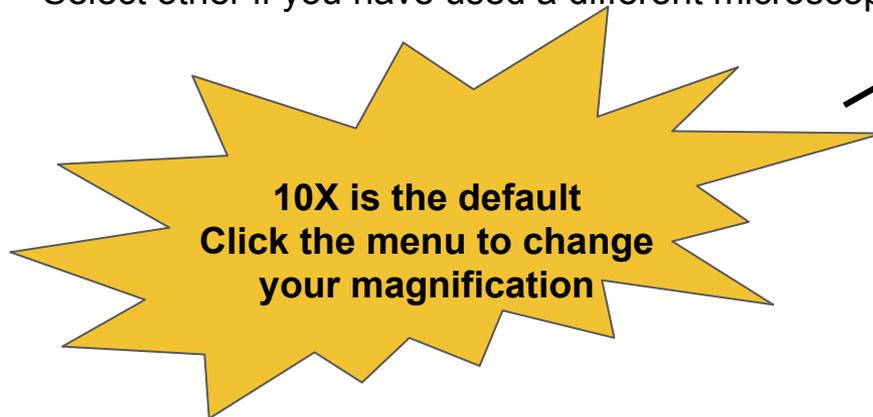
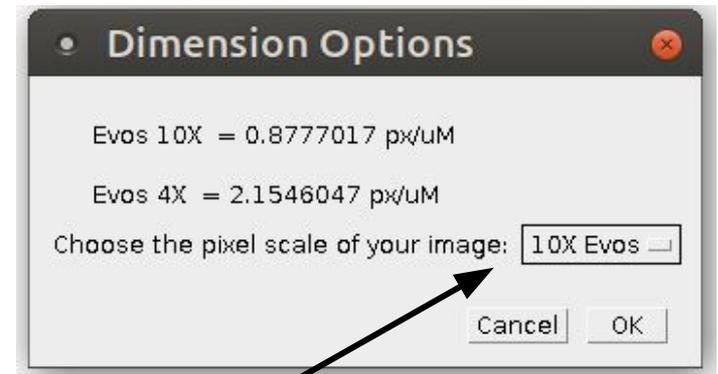
Light with dark background



- 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

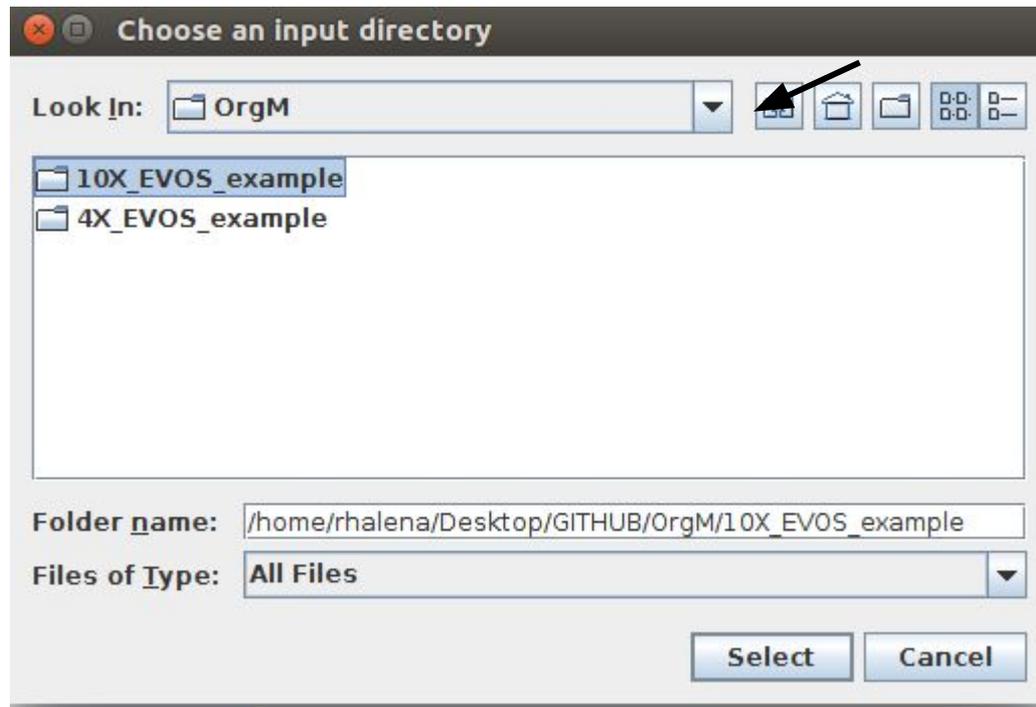
Dimension options

- This window is to select the magnification you used
- You need the conversion for the μM 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



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

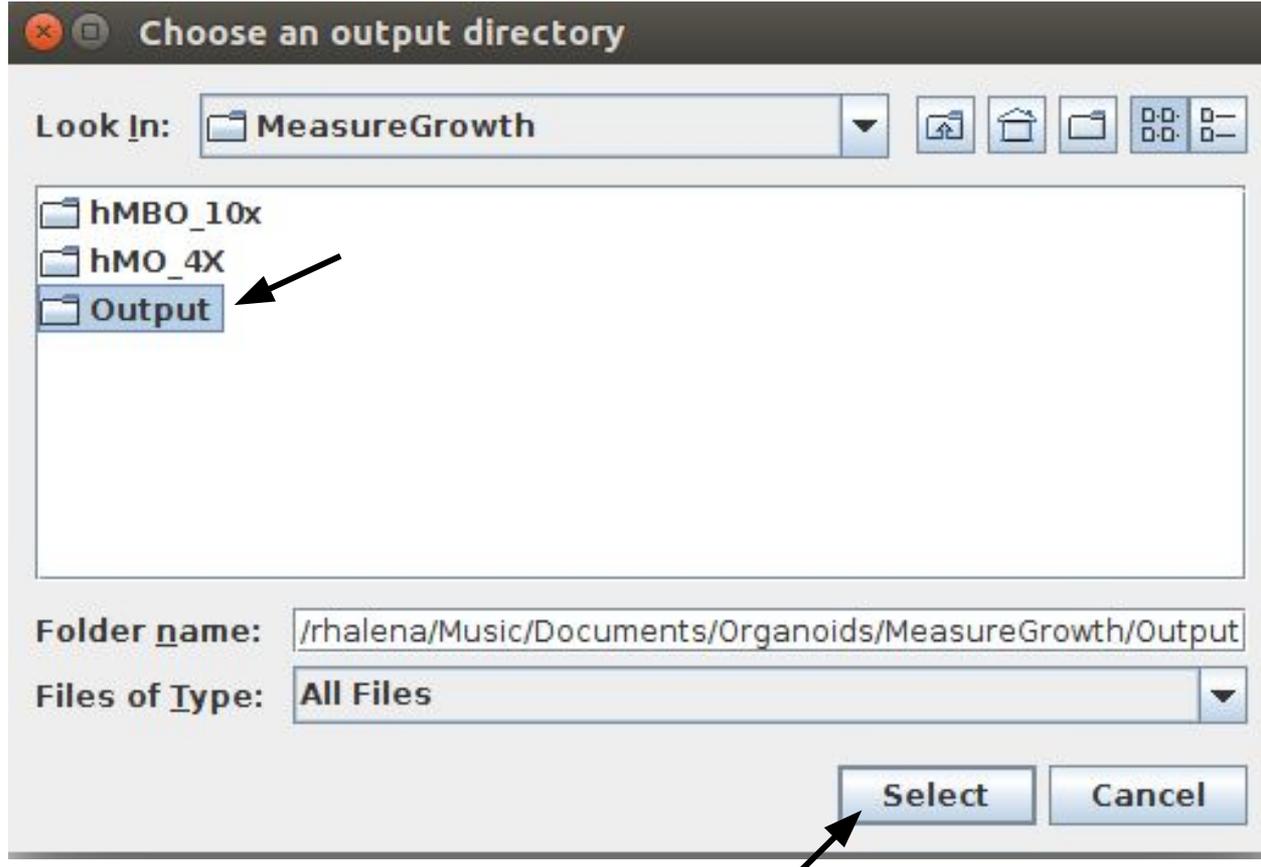
Choose where you have your images:



**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:



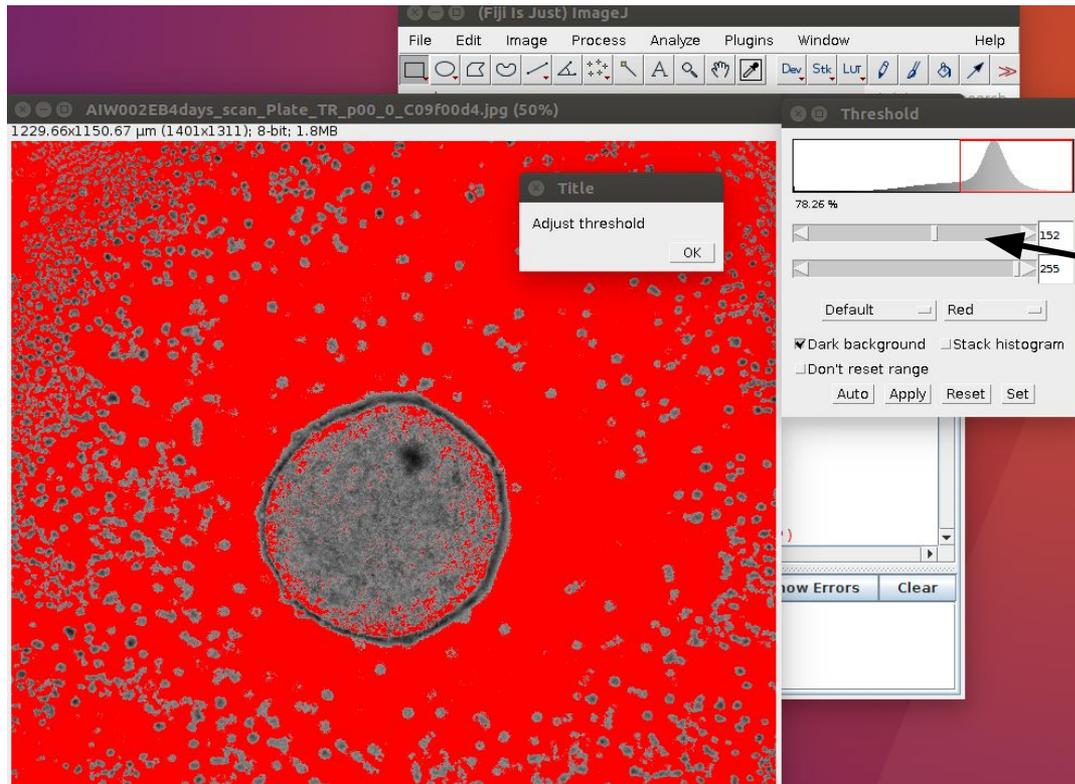
The macro will keep the file navigator open..

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.

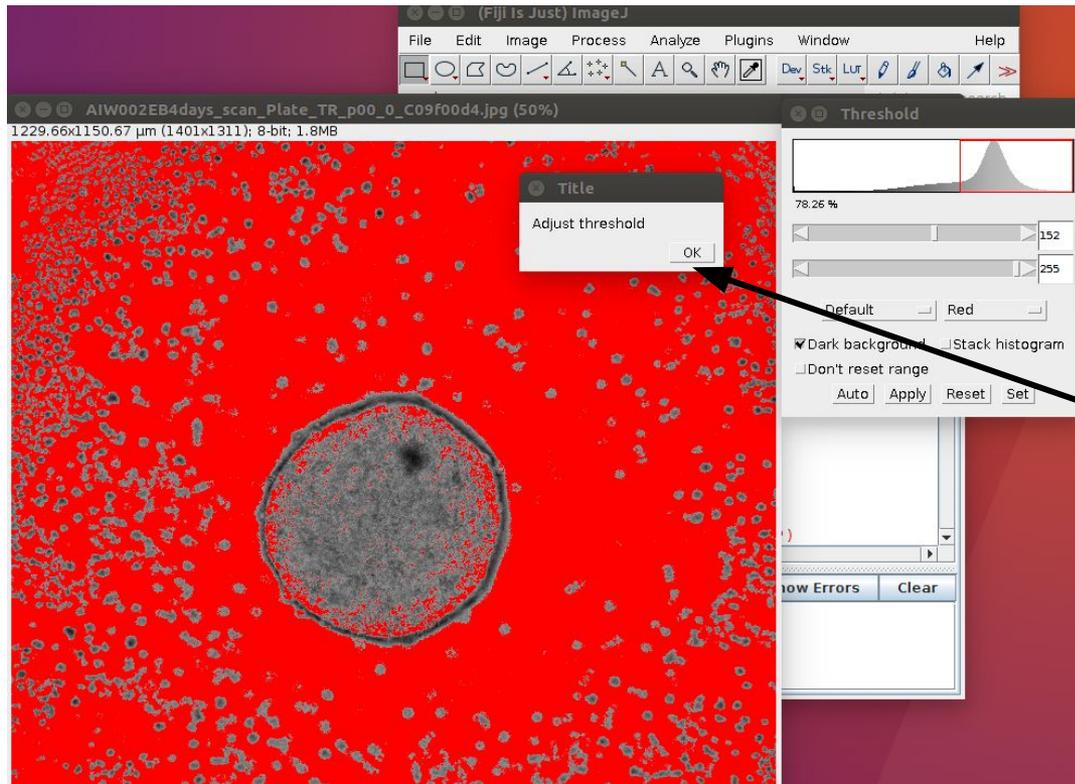
Running the macro: Threshold mode no watershed



The auto-threshold image will appear.

You can press okay to see what will be selected or adjust the threshold.

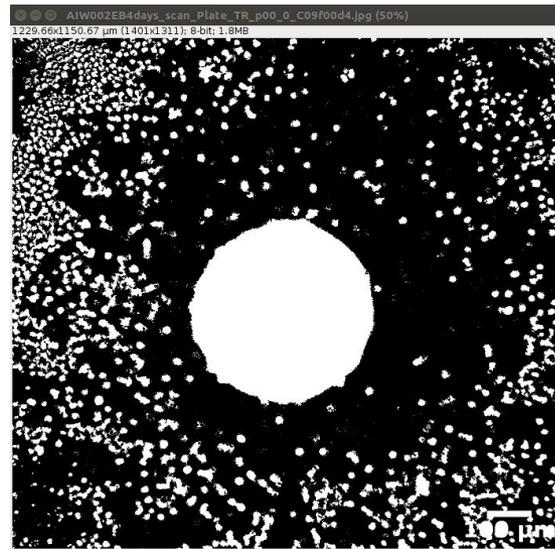
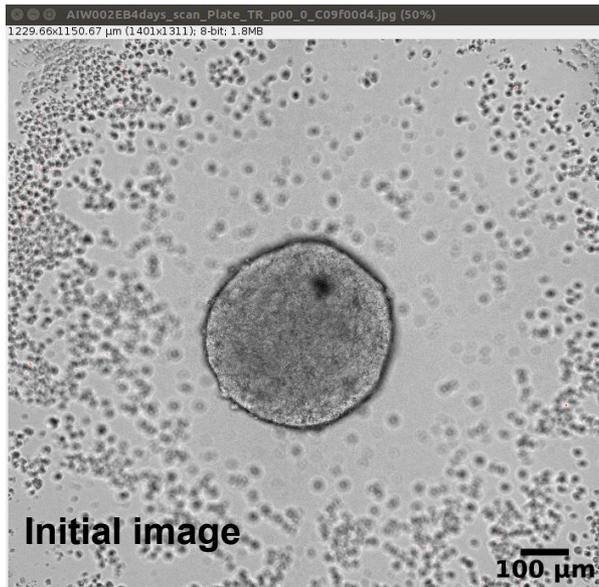
Running the macro: Threshold mode no watershed



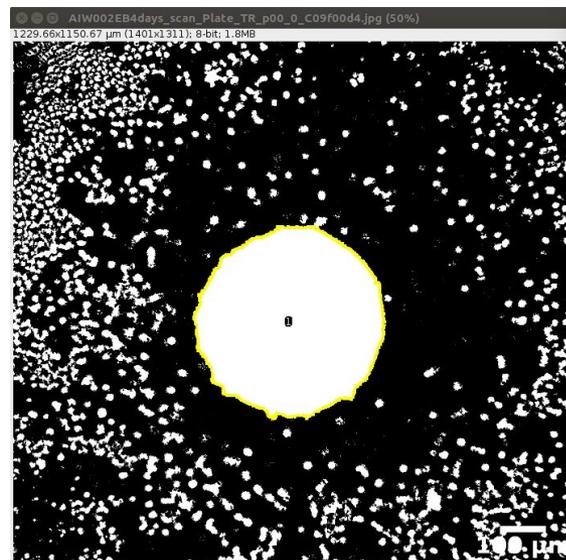
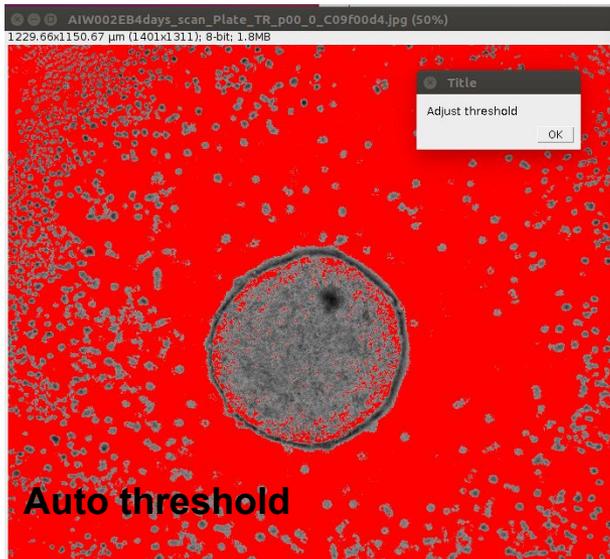
Press okay when you are finished

The auto-threshold image will appear.
You can press okay to see what will be 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

The screenshot displays the Fiji ImageJ interface with the OrgM.py macro editor and console windows open. The macro editor window shows the following text:

```
Started OrgM.py at Fri Sep 20 15:51:03 EDT 2019  
  
  \  ^  
   ) ( ' meow!  
  ( / )  
  \_ |
```

The console window shows the following output:

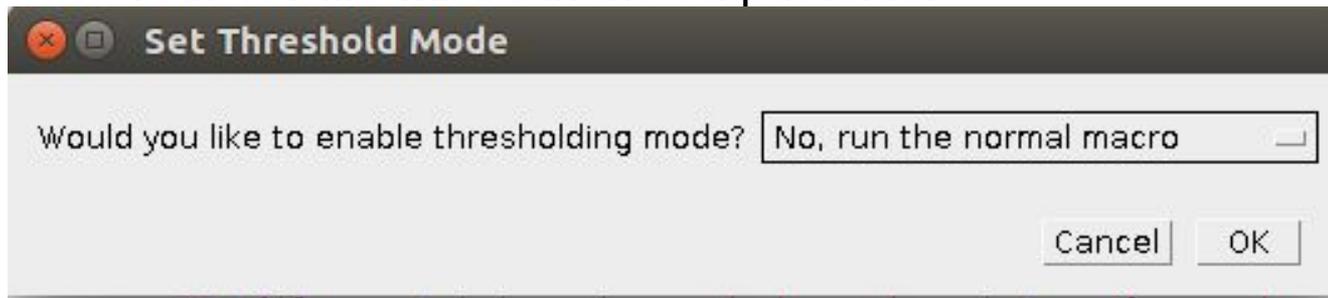
```
OrgM: Organoid Size Measurer - ImageJ Macro written in Python  
  
Input - Directory containing Organoid bright field images  
Output - CSV file containing size and shape descriptors for each organoid image  
Written by: Eddie Cai and Rhalena Thomas - NeuroEDDU  
  
required packages  
13 import os, sys, math, datetime  
14 from ij import IJ, ImagePlus, ImageStack  
15 from ij.io import DirectoryChooser  
16 from ij.measure import ResultsTable  
17 from ij.measure import Measurements  
18 from ij.process import ImageProcessor  
19 from ij.process import ImageConverter
```

The console window also shows the following text:

```
Started OrgM.py at Fri Sep 20 15:51:03 EDT 2019  
  
  \  ^  
   ) ( ' meow!  
  ( / )  
  \_ |
```

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 μM	Min Diameter μM	Diameter μM	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

Original file name

Diameter at the largest distance
Fiji function "Ferret"

Shortest diameter (Fiji MinFerret)

Diameter Mean of Longest and shortest diameters (Ferret and MinFerret)

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.

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

This is how not "bumpy" it is. The fiji function used is Roundness

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

Test if the object meets the criteria set in the "Other Thresholds" Window: area and "Bumpyness"

If you have multiple folders the subfolder name will be included

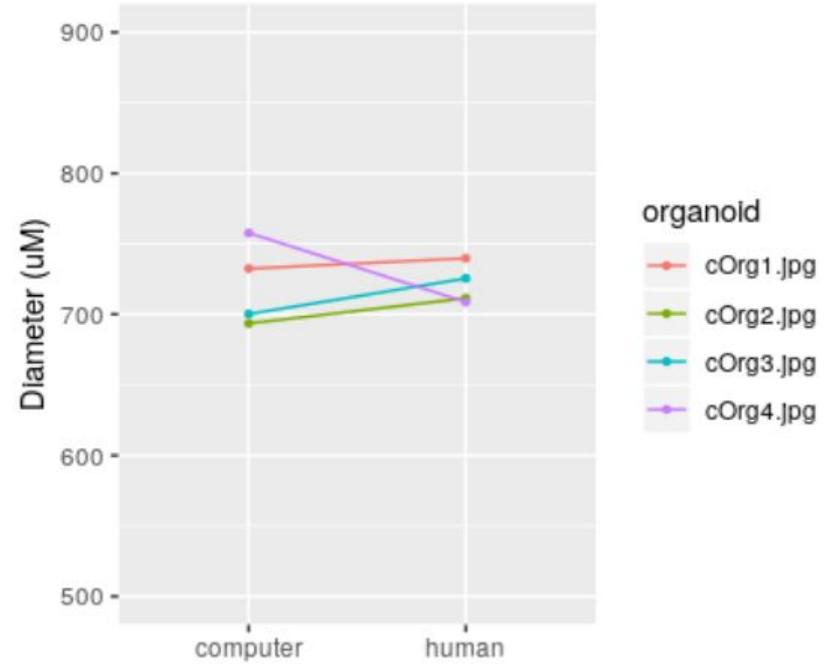
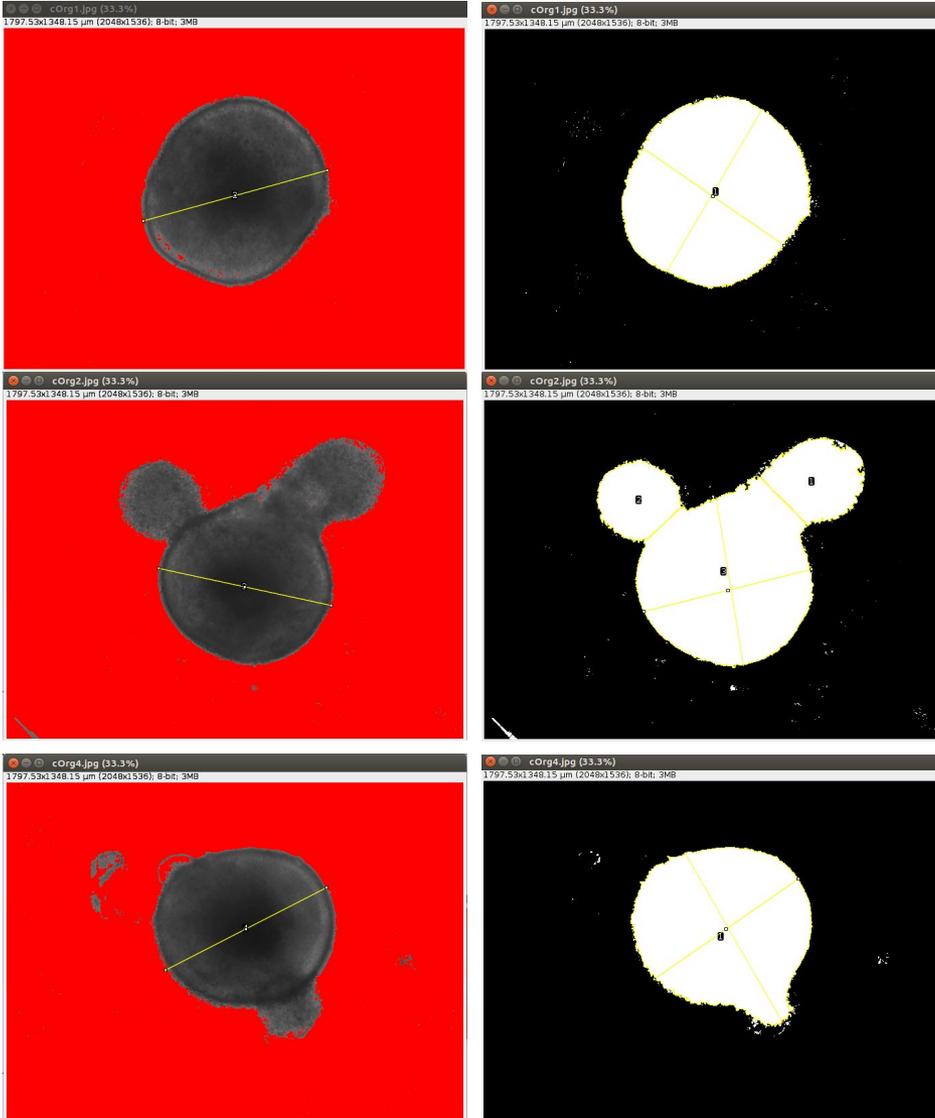
Total Area μM^2

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

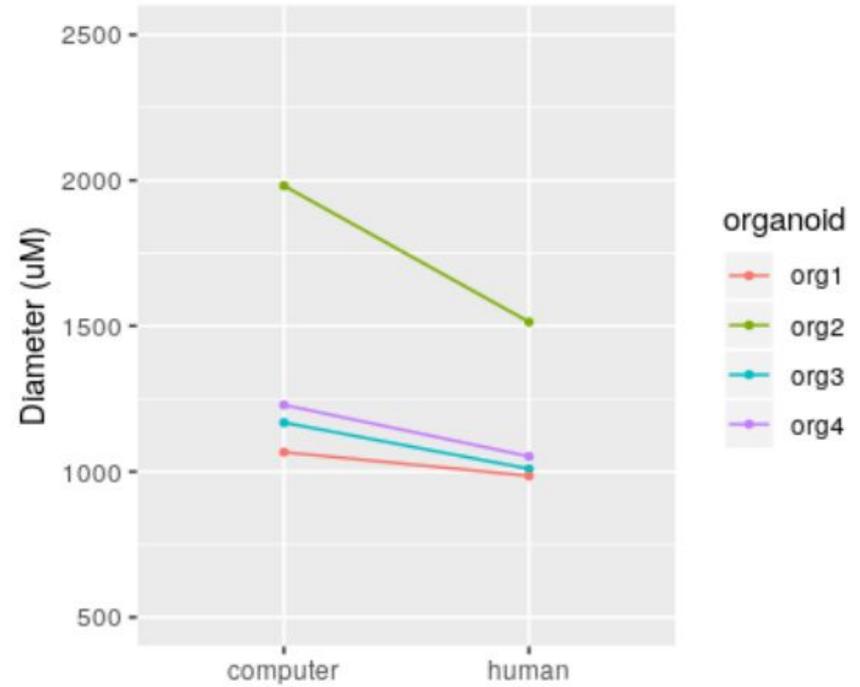
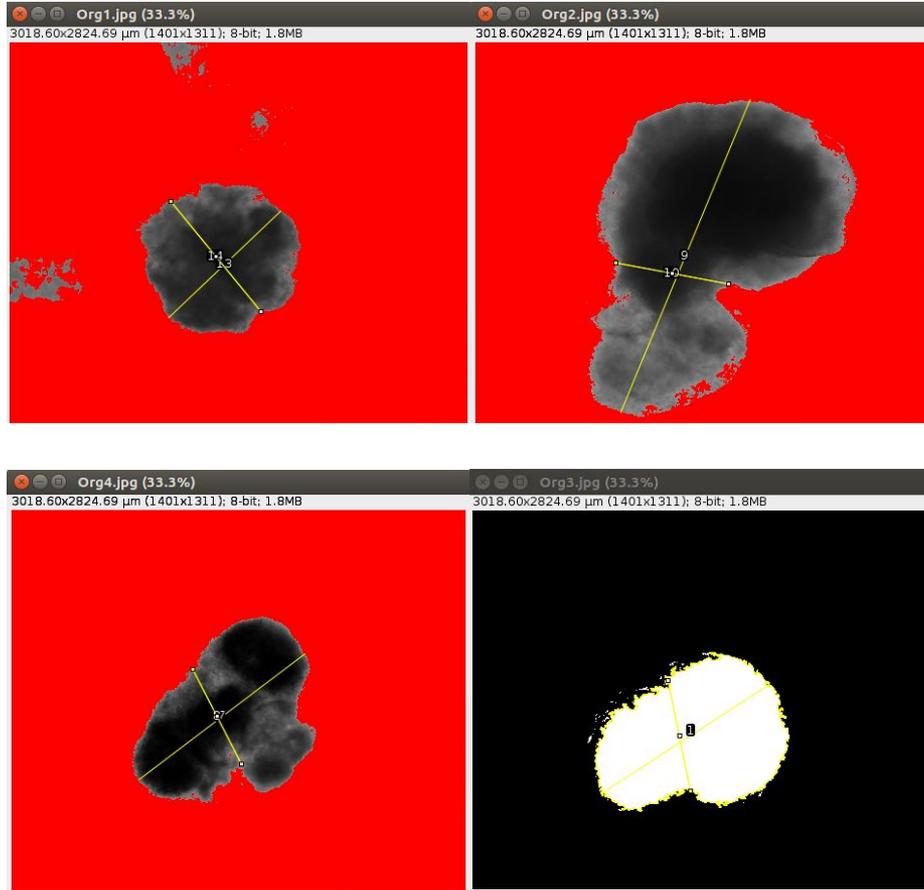
Comparison of OrgM with human measurements

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