

Worm Measuring Protocol

Step 1: Download ImageJ



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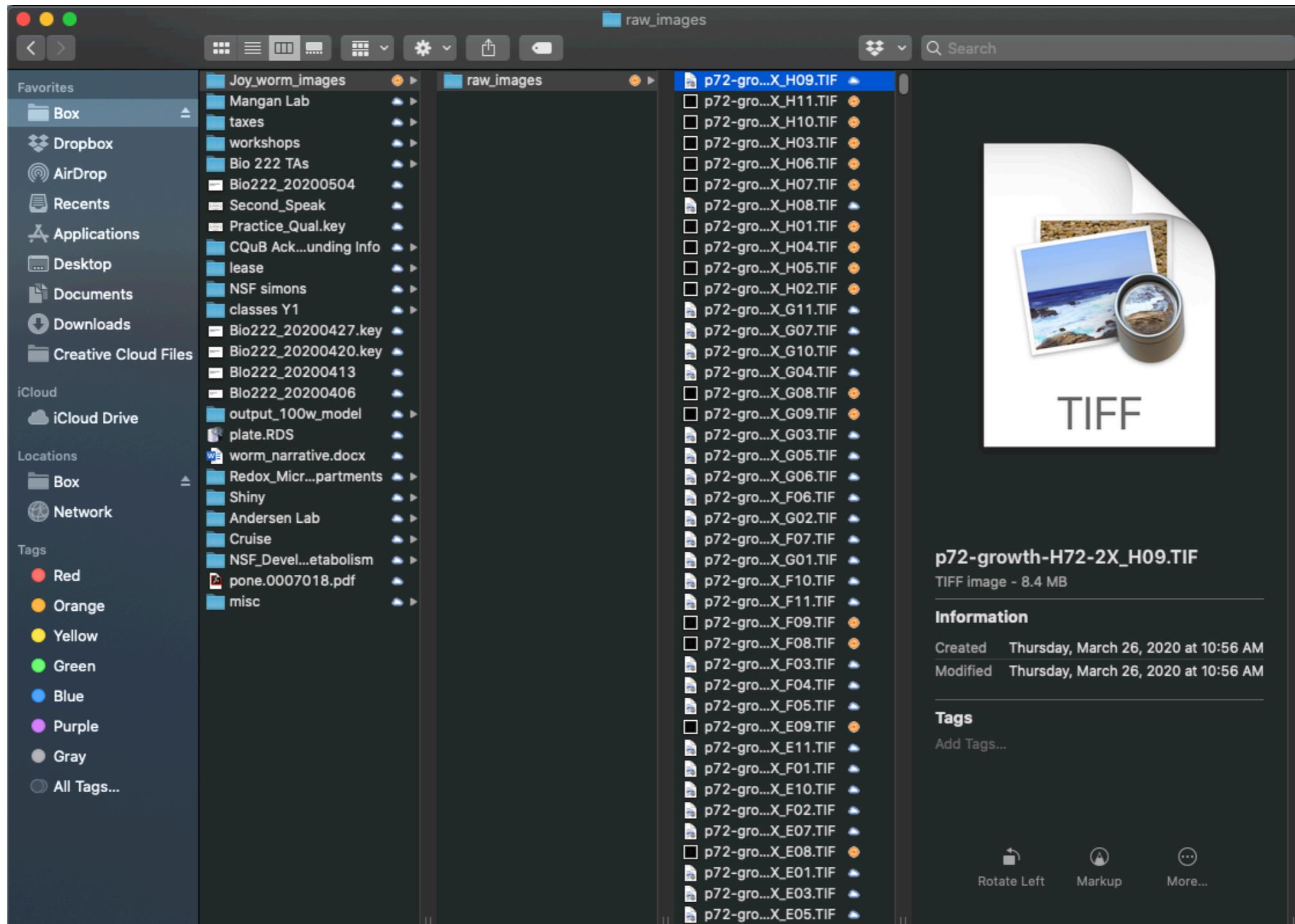
ImageJ

Image Processing and Analysis in Java

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Support is available on the [mailing list](#) and on the [image.sc](#) forum. [Disclaimer](#)

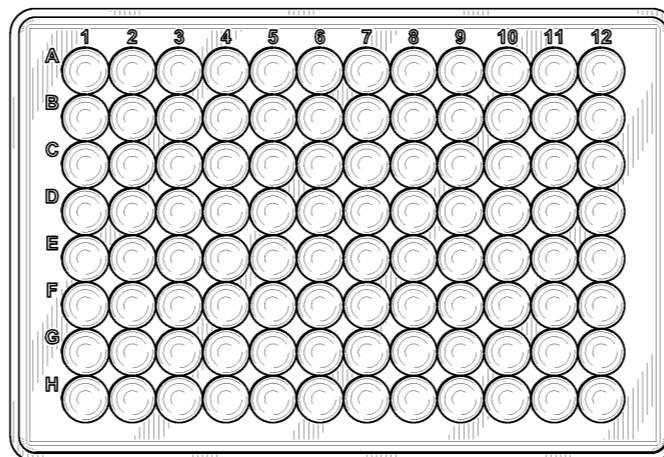
Step 2: Find image folder on Box



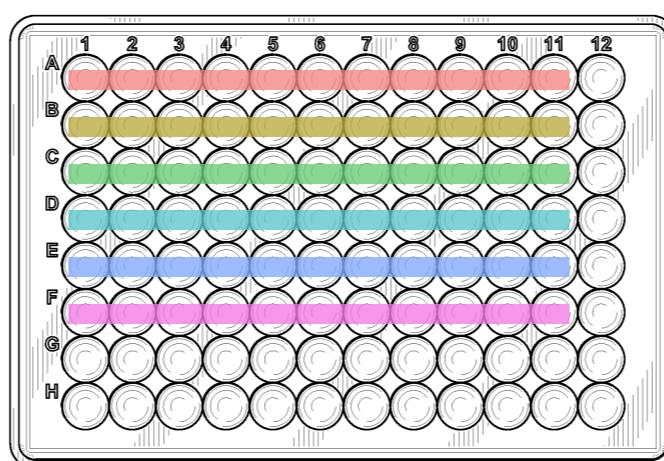
You should have access to my shared folder

You will not be able to preview images on your device before opening in ImageJ

When I performed this experiment I imaged a single plate every hour.

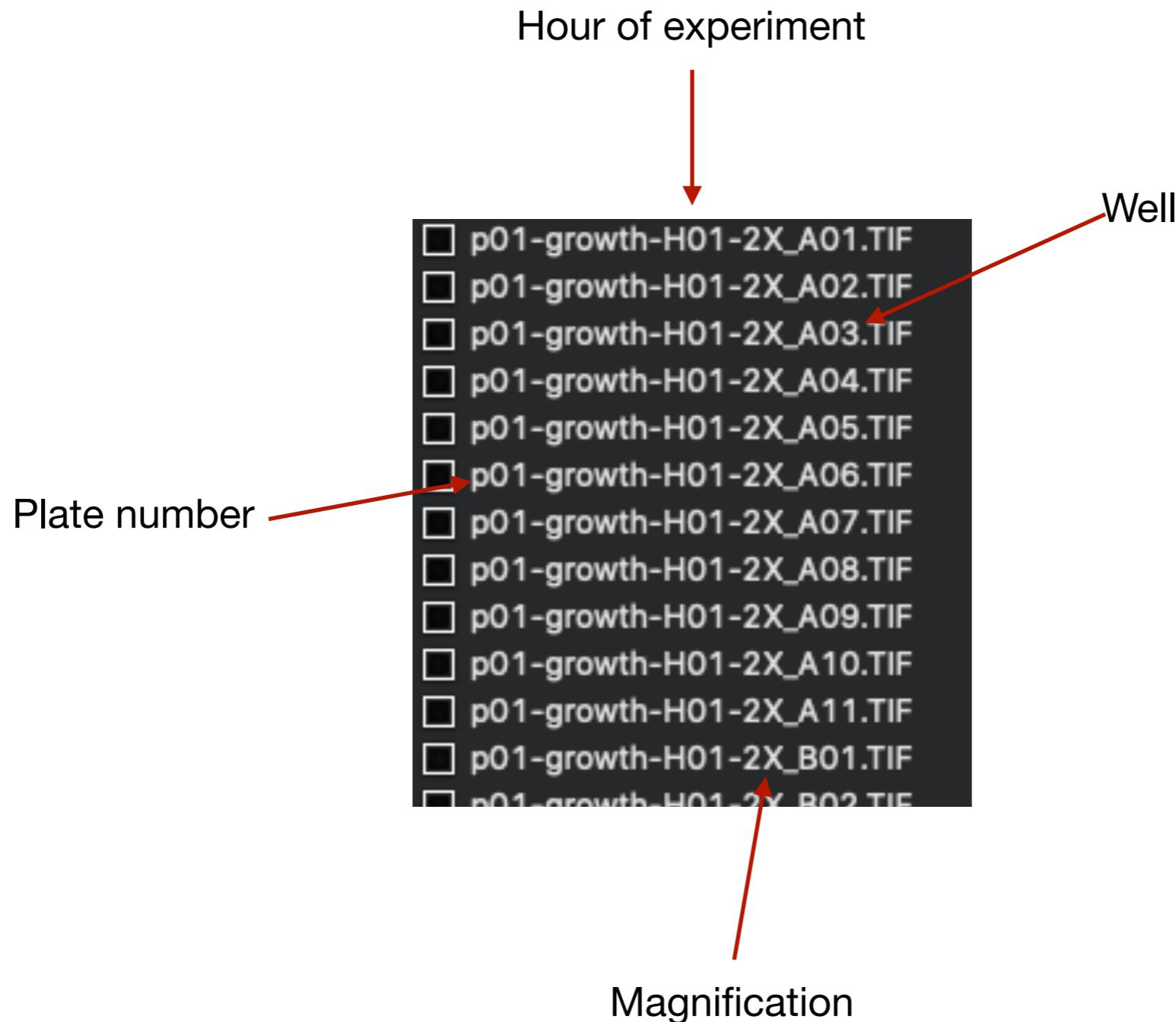


This plate contains data for 6 independent replicates (A - F)

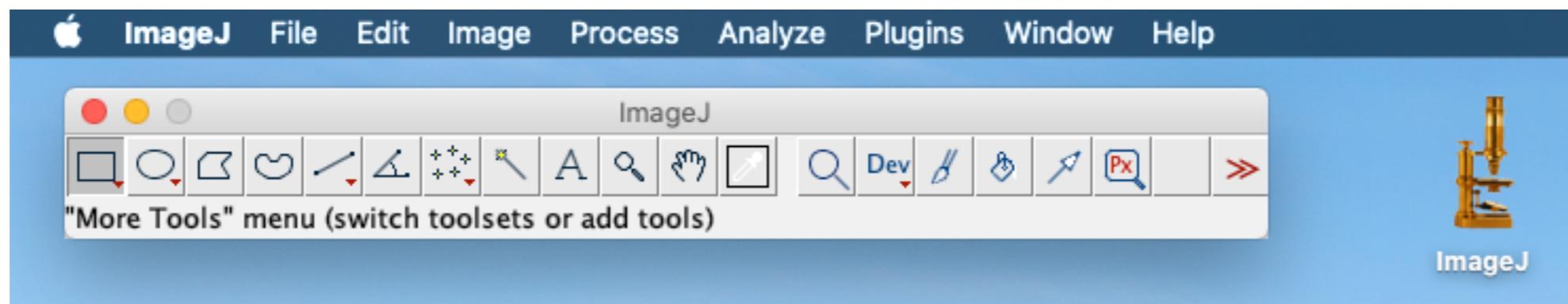


** Note: column 12 will have no images, it was used as a wash well
Disregard images for row G - H

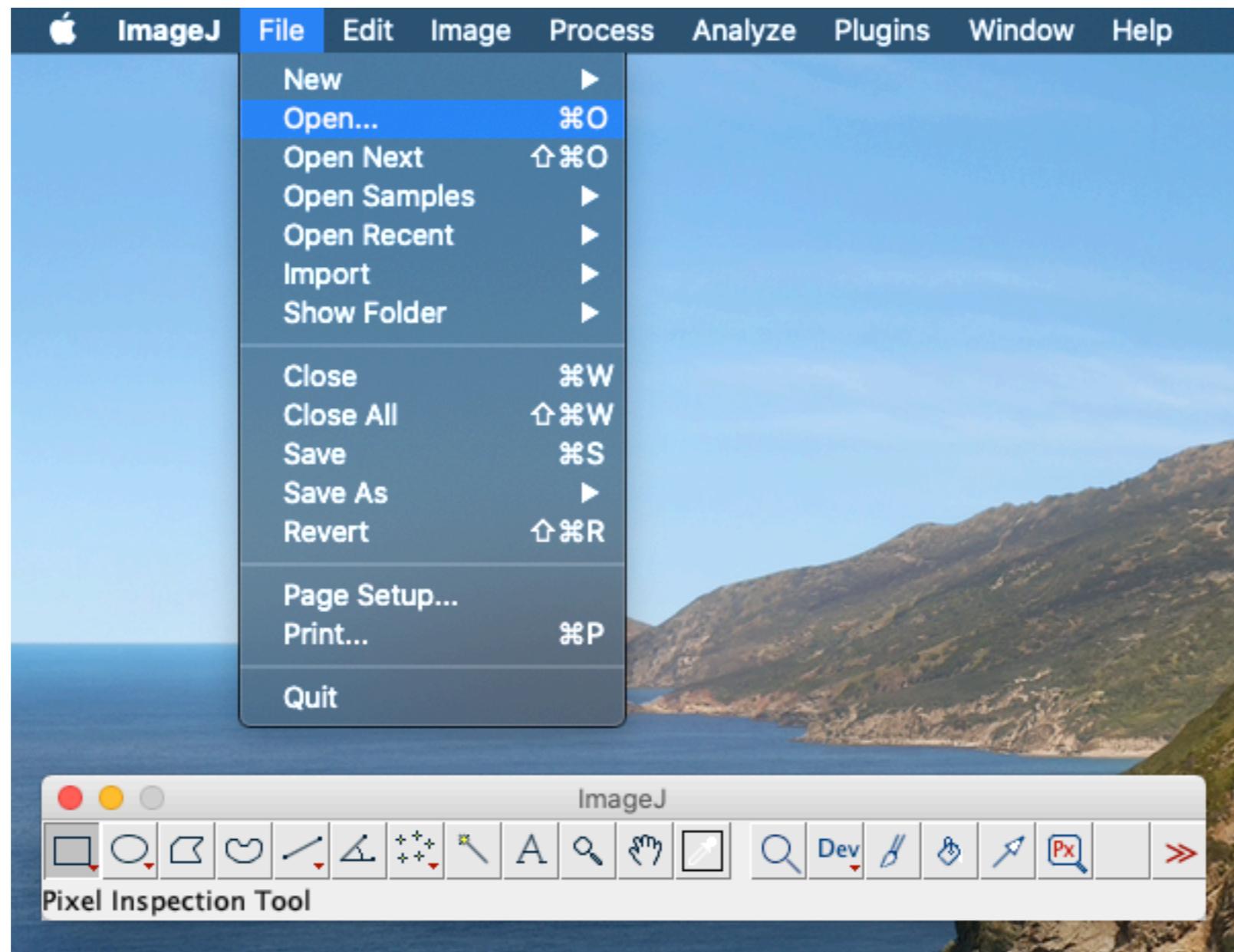
You will notice that each file is an image of a single well.



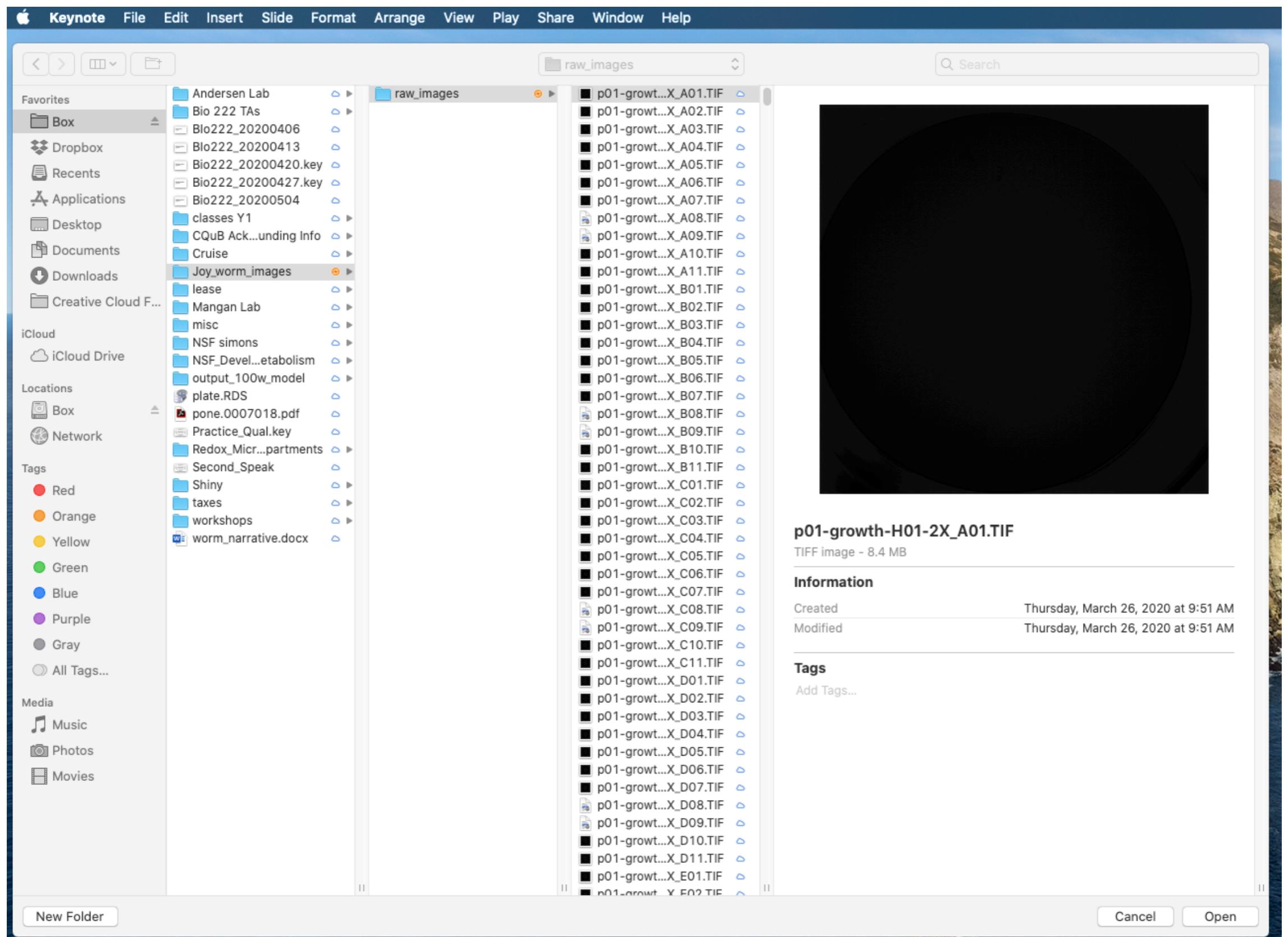
Step 3: Open ImageJ software



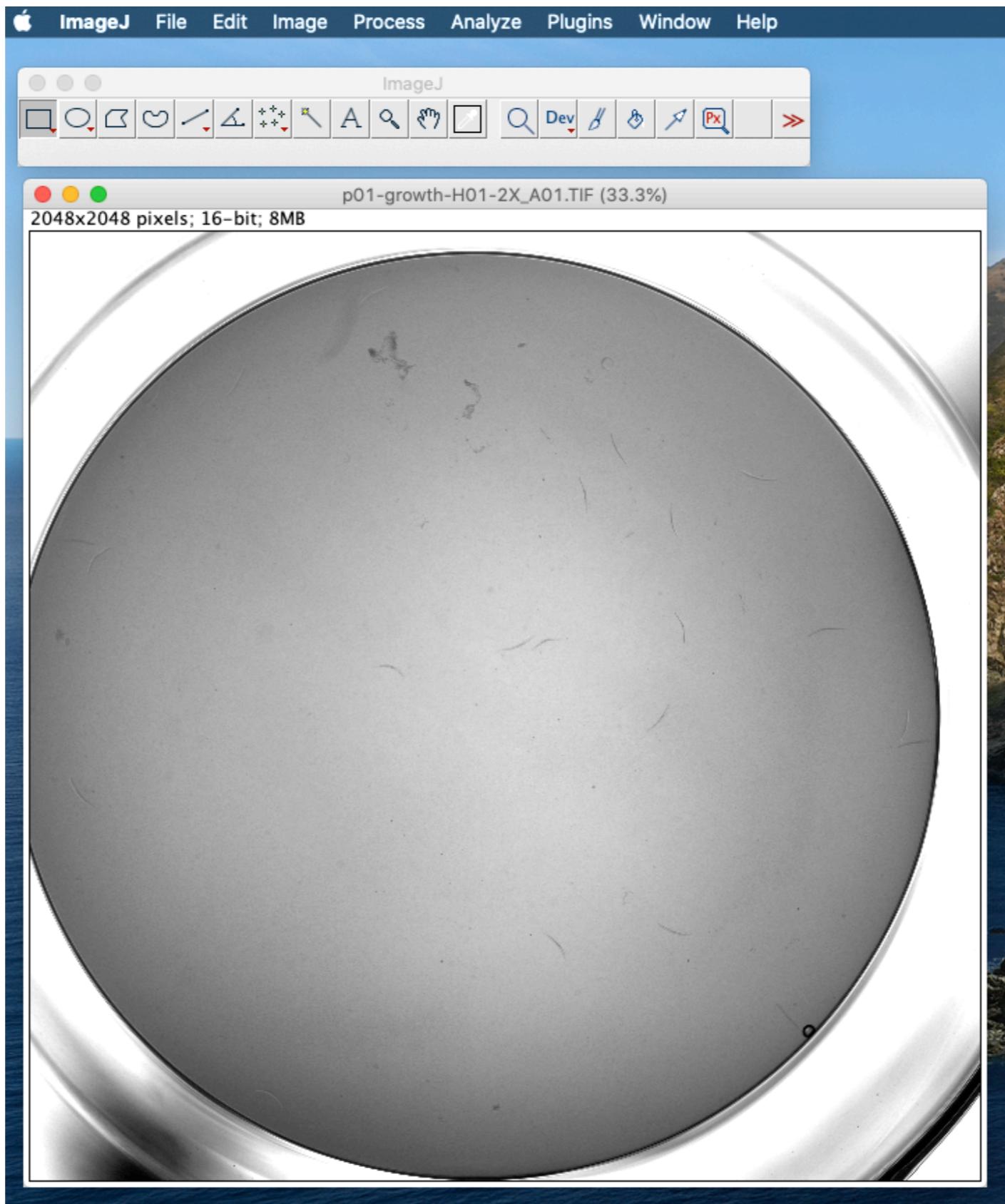
Step 4: Now open the first image



Step 4: Now open the first image

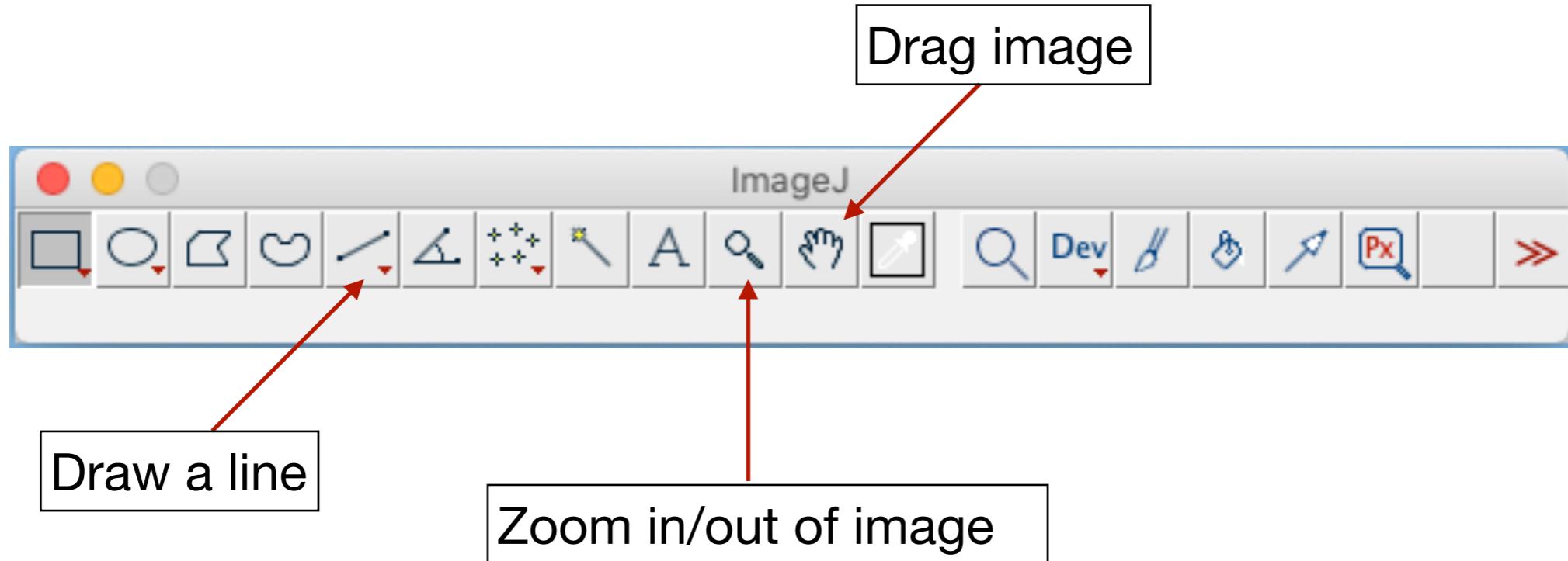


Step 4: Now open the first image



Step 5: Navigating ImageJ

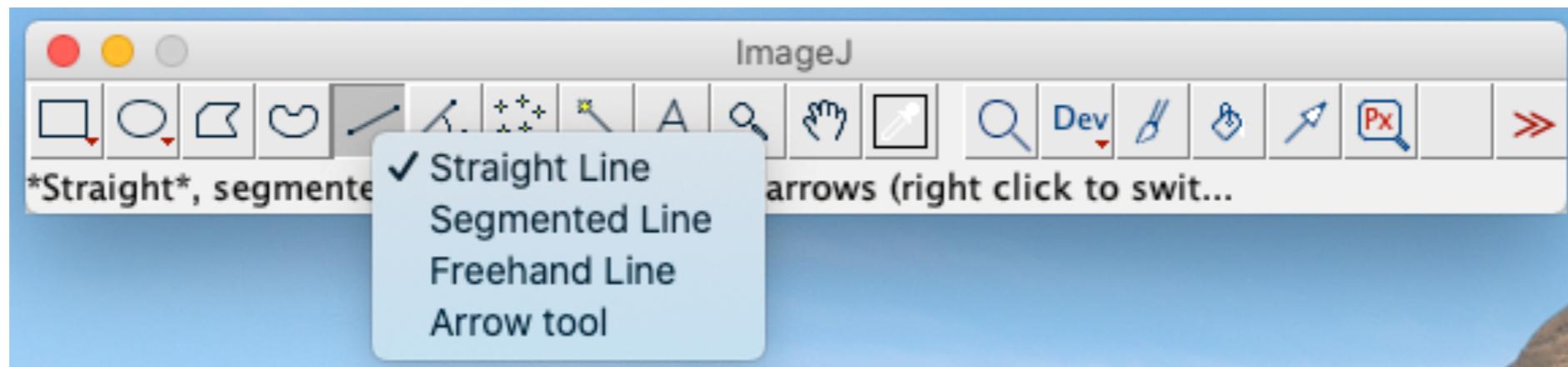
We will mainly use functions on this toolbar



I have also found that using “+” and “-” keys allows me to zoom

Step 5: Navigating ImageJ

Right click on line tool to open up more line options.



We will primarily use Straight Line and Segmented Line

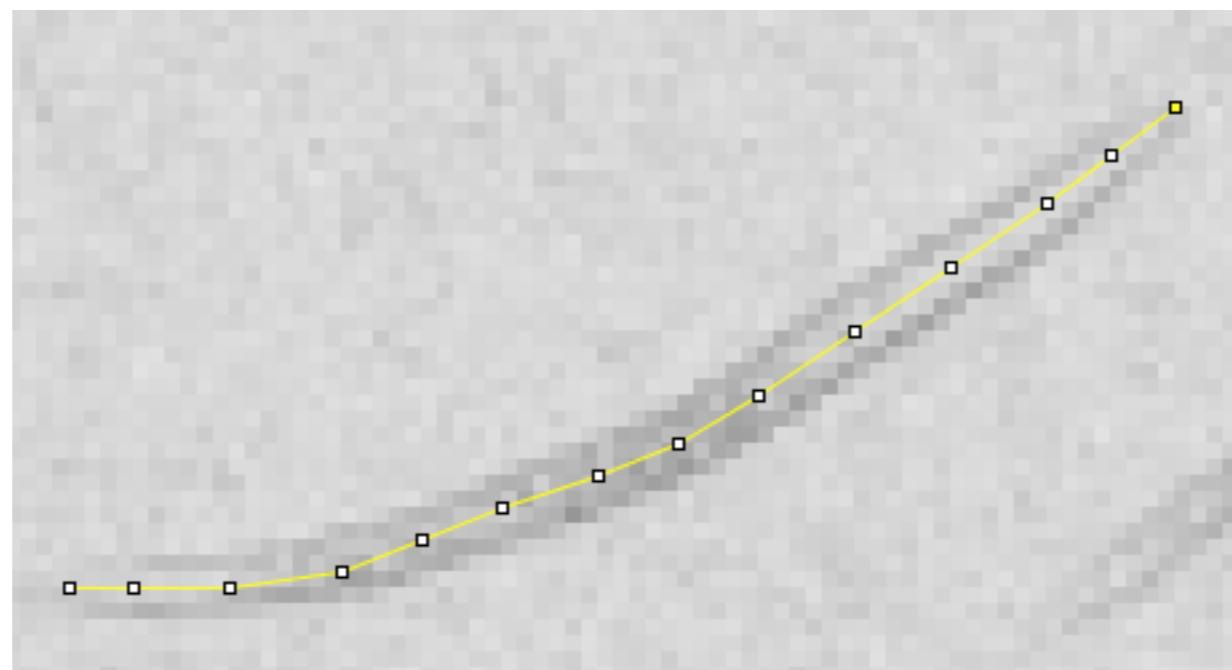
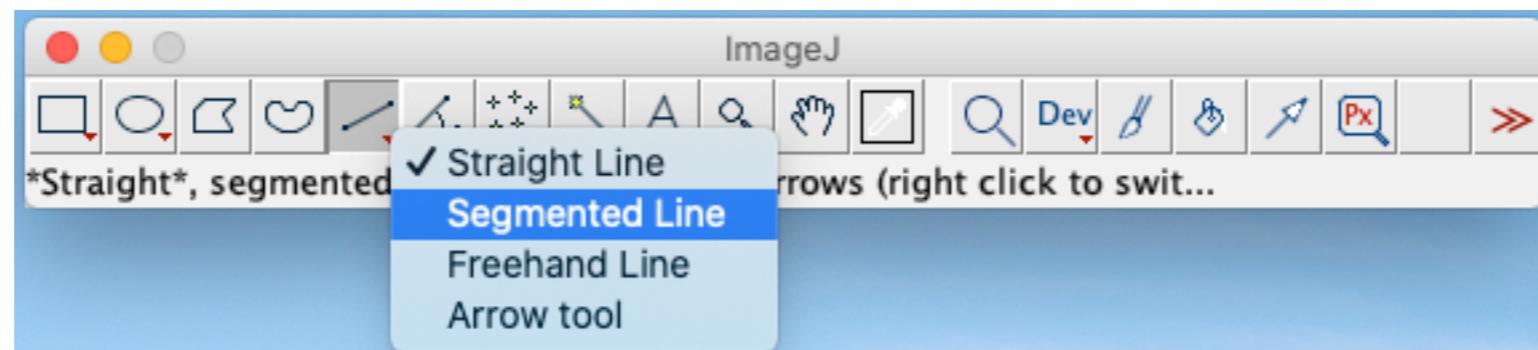
Step 6: Begin measurements

This will be most difficult with the smaller/younger worms

1. Zoom in to an individual animal (you can use “+” key)



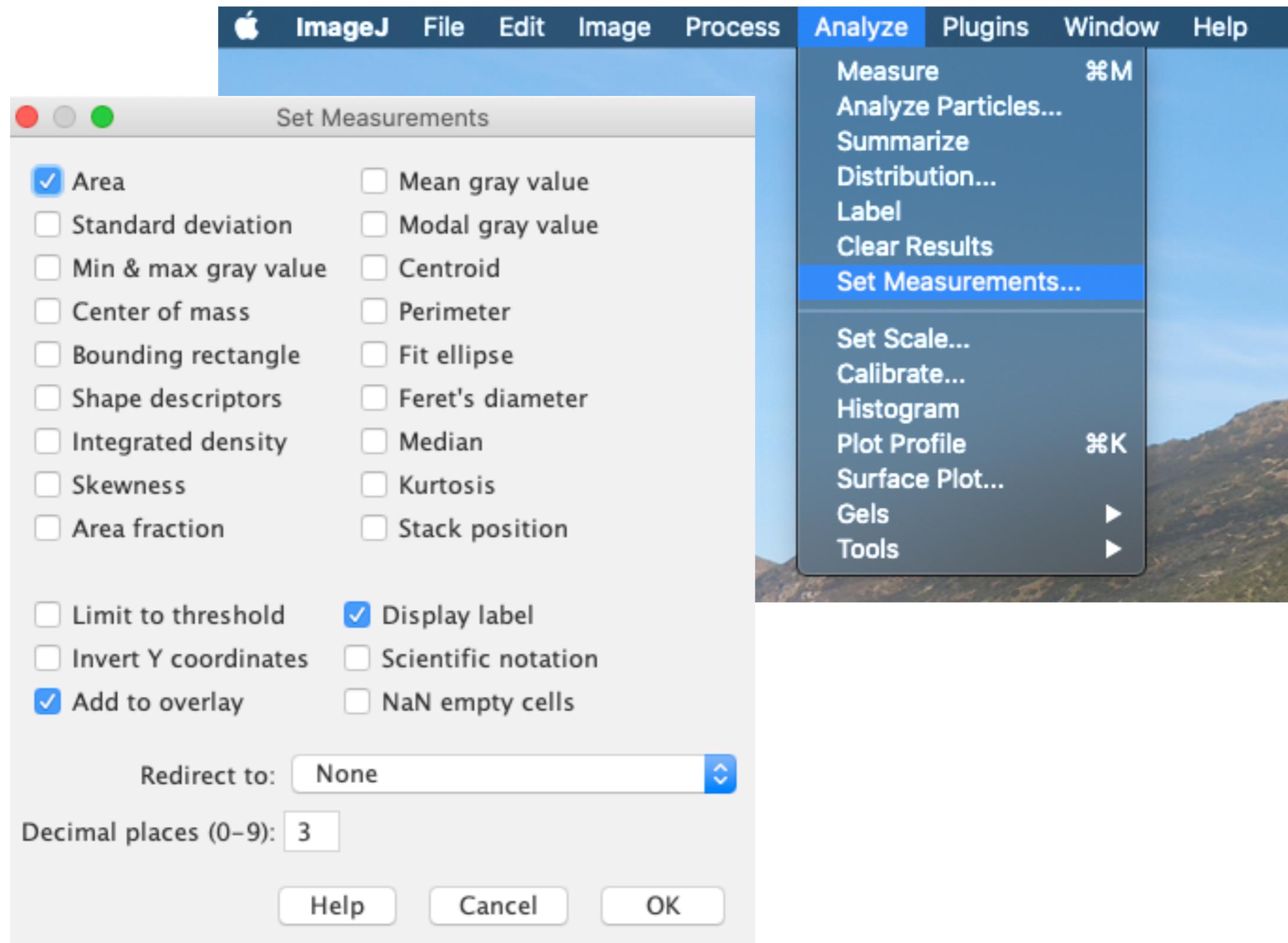
2. Use line tool to measure length of animal from head to tail along the midline.
 - a. If animal is not straight, use Segmented Line



*Double click at the end of animal to stop selection

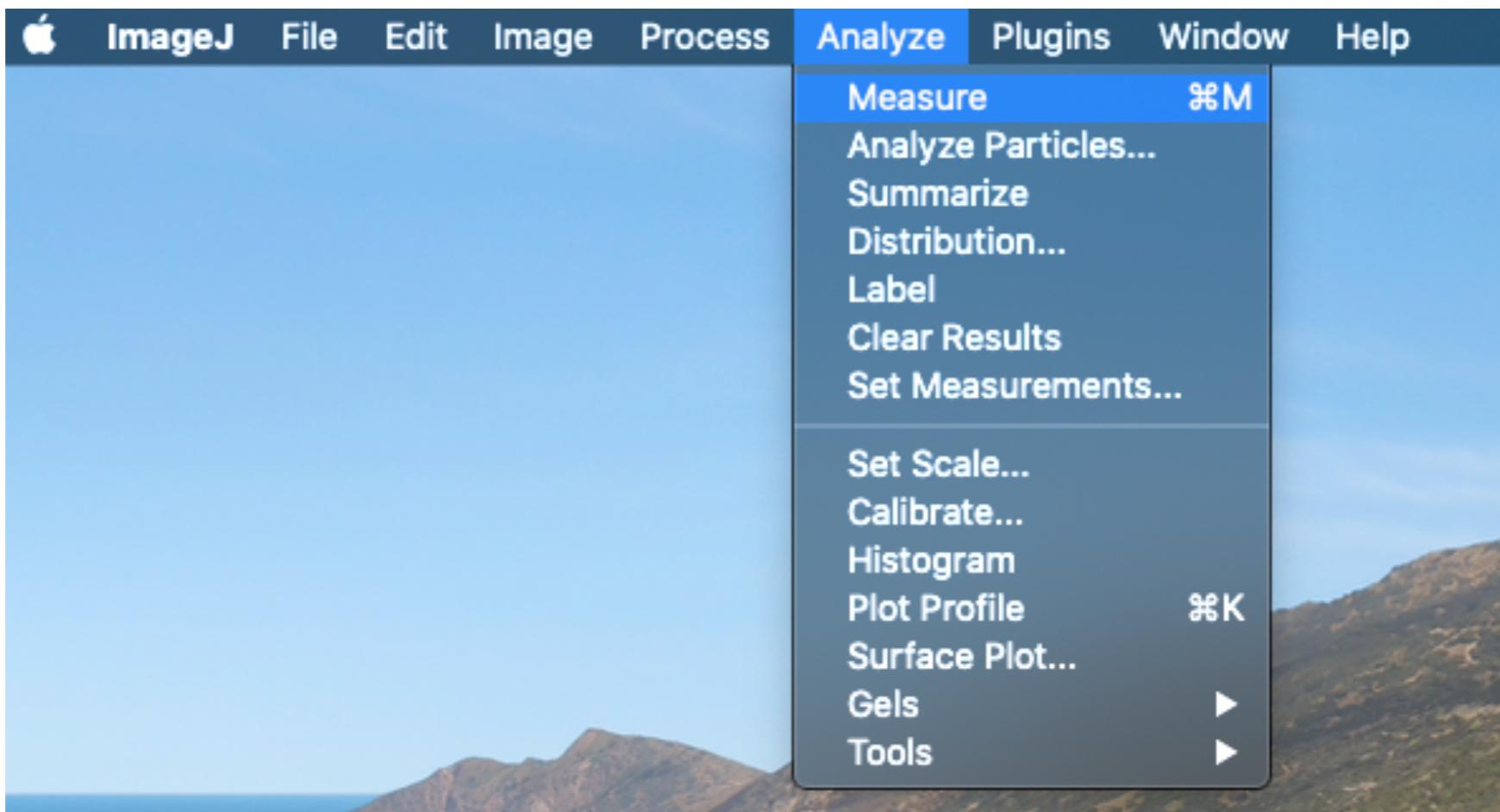
3. Collect measurement of worm

a. First Set Measurements



3. Collect measurements

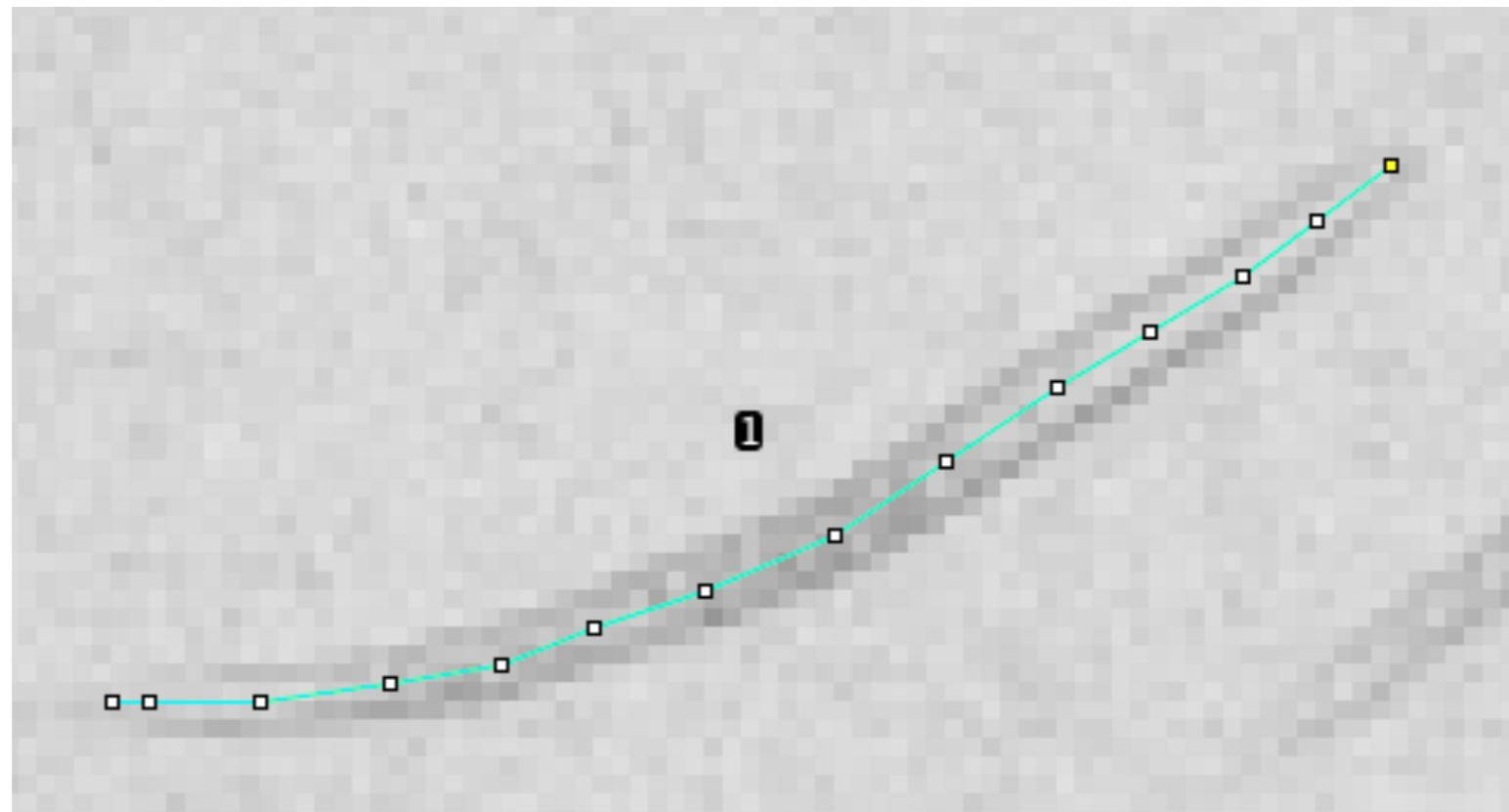
b. Collect data



Results				
	Label	Area	Length	
1	p01-growth-H01-2X_A01.TIF	79	78.999	

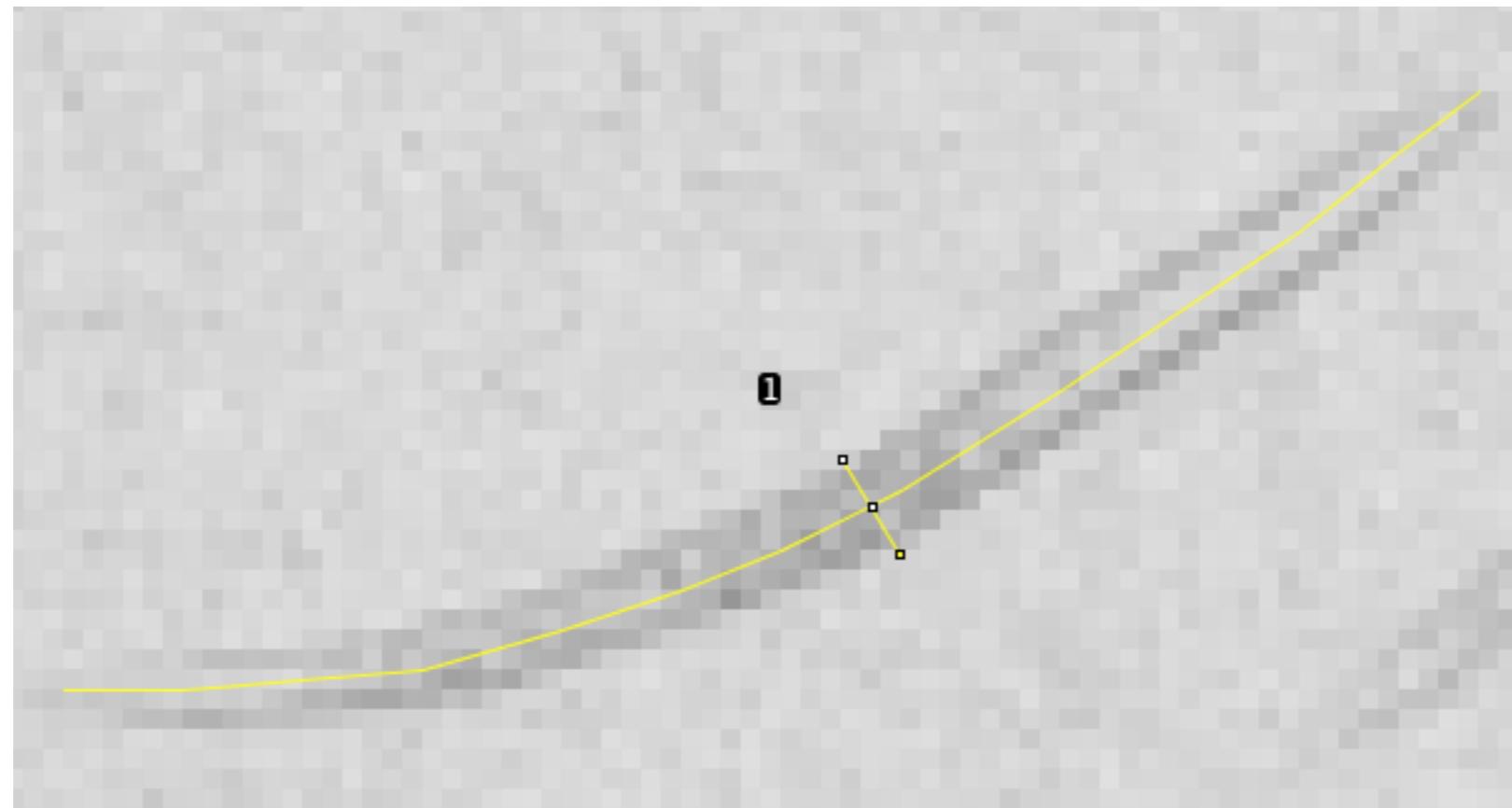
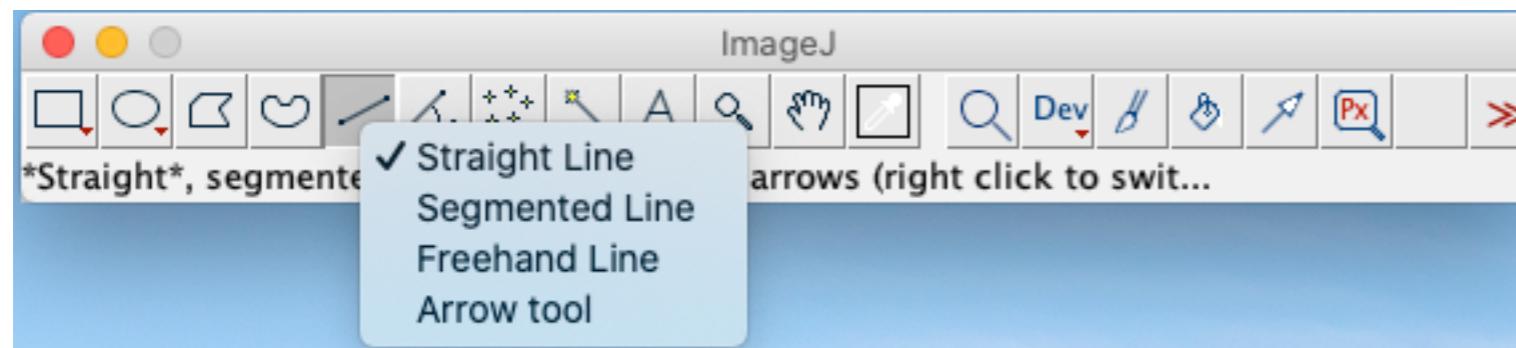
3. Collect measurements

c. Selection should now be saved and labelled



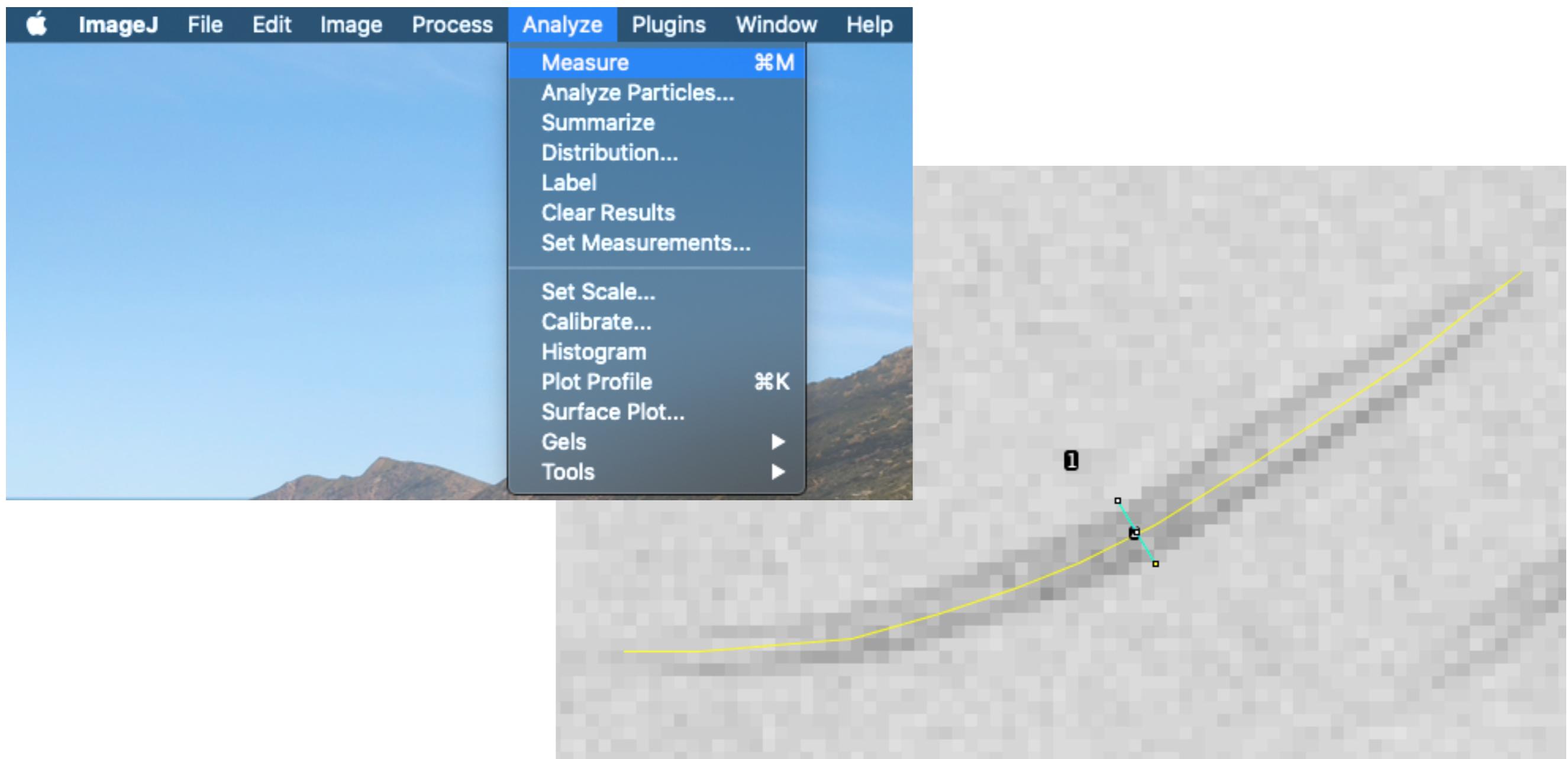
5. Use Straight Line to measure width of animal

- Draw a single line cross the center of the animal



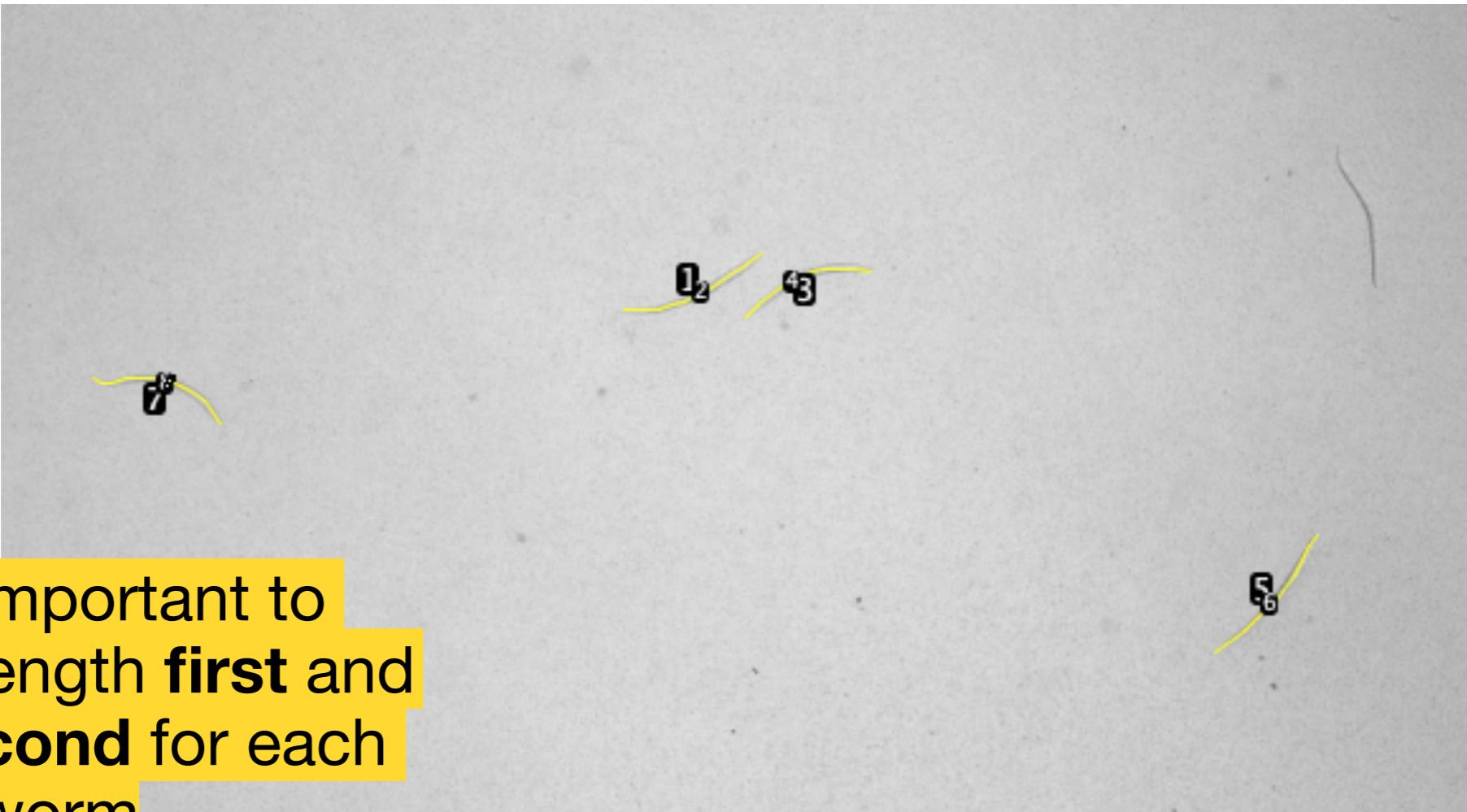
5. Use Straight Line to measure width of animal

b. Take measurement as before



	Label	Area	Angle	Length
1	p01-growth-H01-2X_A01.TIF	79	0.000	78.999
2	p01-growth-H01-2X_A01.TIF	7	120.964	5.831

6. Repeat for 5 animals in image



** it is important to measure length **first** and width **second** for each worm

	Label	Area	Angle	Length
1	p01-growth-H01-2X_A01.TIF	79	0.000	78.999
2	p01-growth-H01-2X_A01.TIF	7	120.964	5.831
3	p01-growth-H01-2X_A01.TIF	75	0.000	74.168
4	p01-growth-H01-2X_A01.TIF	6	126.870	4.680
5	p01-growth-H01-2X_A01.TIF	83	0.000	82.056
6	p01-growth-H01-2X_A01.TIF	6	-33.690	4.670
7	p01-growth-H01-2X_A01.TIF	78	0.000	77.605
8	p01-growth-H01-2X_A01.TIF	6	-116.565	4.924

For larger/older animals this will be easier...



** it is important to measure length **first** and width **second** for each worm

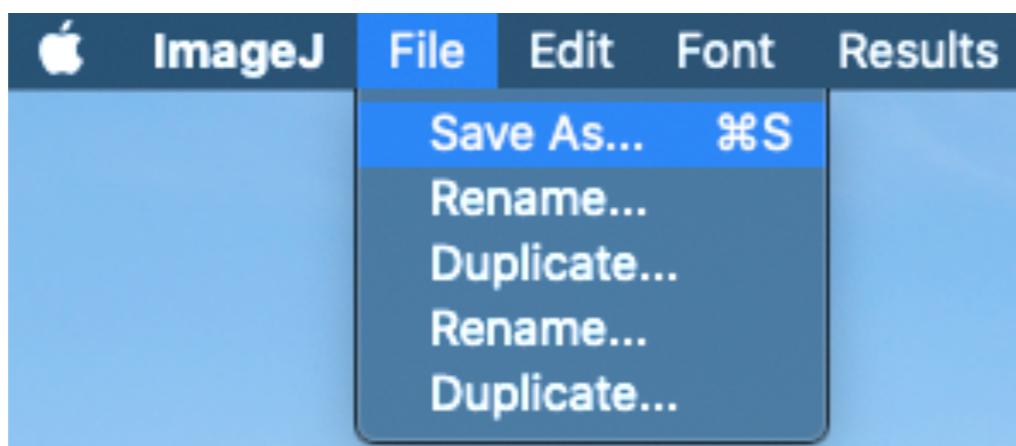
	Label	Area	Angle	Length
1	p01-growth-H01-2X_A01.TIF	79	0.000	78.999
2	p01-growth-H01-2X_A01.TIF	7	120.964	5.831
3	p01-growth-H01-2X_A01.TIF	75	0.000	74.168
4	p01-growth-H01-2X_A01.TIF	6	126.870	4.680
5	p01-growth-H01-2X_A01.TIF	83	0.000	82.056
6	p01-growth-H01-2X_A01.TIF	6	-33.690	4.670
7	p01-growth-H01-2X_A01.TIF	78	0.000	77.605
8	p01-growth-H01-2X_A01.TIF	6	-116.565	4.924
9	p55-growth-H55-2X_A01.TIF	354	0.000	354.070
10	p55-growth-H55-2X_A01.TIF	17	135.000	16.028
11	p55-growth-H55-2X_A01.TIF	323	0.000	322.690
12	p55-growth-H55-2X_A01.TIF	18	132.510	16.651
13	p55-growth-H55-2X_A01.TIF	362	0.000	362.102
14	p55-growth-H55-2X_A01.TIF	18	128.157	17.414
15	p55-growth-H55-2X_A01.TIF	371	0.000	371.168
16	p55-growth-H55-2X_A01.TIF	19	33.690	17.903

Step 7: Save measurements

Either right click in Results

Results				
	Label	Area	Angle	Length
1	p01-growth-H01-2X_A01.TIF	79	0.000	78.999
2	p01-growth-H01-2X_A01.TIF	7	120.964	5.831
3	p01-growth-H01-2X_A01.TIF	75	0.000	74.168
4	p01-growth-H01-2X_A01.TIF	6	126.870	4.680
5	p01-growth-H01-2X_A01.TIF	83	0.000	82.056
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15	p55-growth-H55-2X_A01.TIF	371	0.000	371.168
16	p55-growth-H55-2X_A01.TIF	19	33.690	17.903

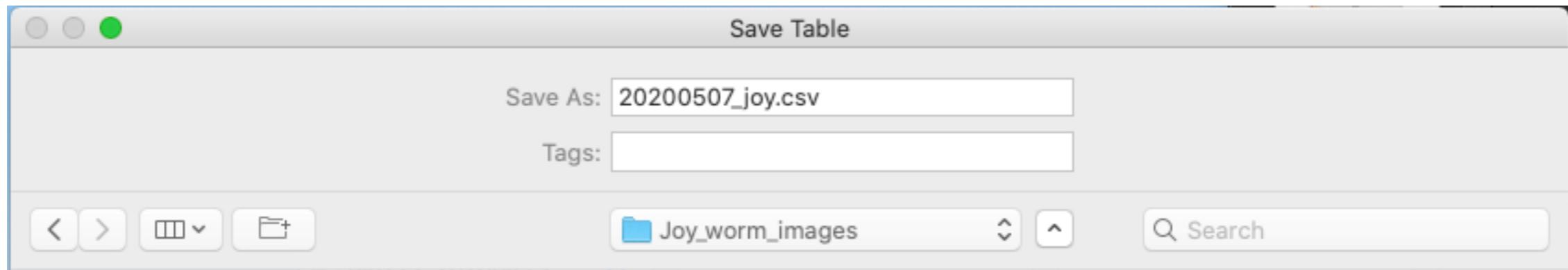
Save As...
Cut
Copy
Clear
Select All
Clear Results
Summarize
Distribution...



Or use drop down menu

7. Save measurements

a. Save as date_yourname.csv



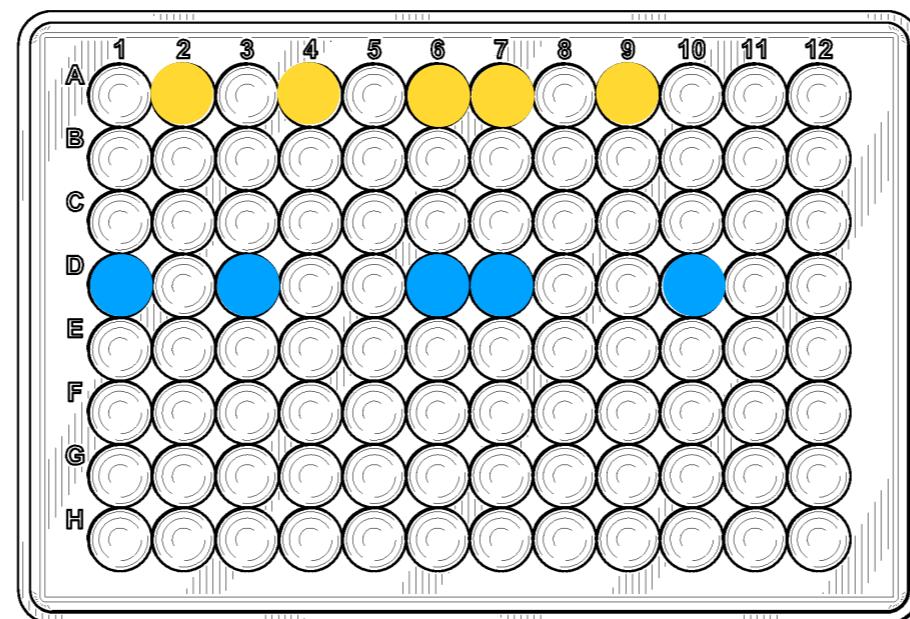
You can create a folder inside Joy_worm_images to store your files

Your responsibilities...

- You are responsible for a single replicate (A - F)
- For each Hour (H01 - H72) you should measure **5 worms** per well for **5 total wells**

<input type="checkbox"/> p01-growth-H01-2X_A01.TIF
<input type="checkbox"/> p01-growth-H01-2X_A02.TIF
<input type="checkbox"/> p01-growth-H01-2X_A03.TIF
<input type="checkbox"/> p01-growth-H01-2X_A04.TIF
<input type="checkbox"/> p01-growth-H01-2X_A05.TIF
<input type="checkbox"/> p01-growth-H01-2X_A06.TIF
<input type="checkbox"/> p01-growth-H01-2X_A07.TIF
<input type="checkbox"/> p01-growth-H01-2X_A08.TIF
<input type="checkbox"/> p01-growth-H01-2X_A09.TIF
<input type="checkbox"/> p01-growth-H01-2X_A10.TIF
<input type="checkbox"/> p01-growth-H01-2X_A11.TIF
<input type="checkbox"/> p01-growth-H01-2X_B01.TIF
<input type="checkbox"/> p01-growth-H01-2X_B02.TIF

For example



● Student 1

● Student 2

You can choose whichever wells are best
but it must be for your replicate (A - F)

In the end you should have measured 25 worms per hour

** it is important to measure
length **first** and width **second**
for each worm

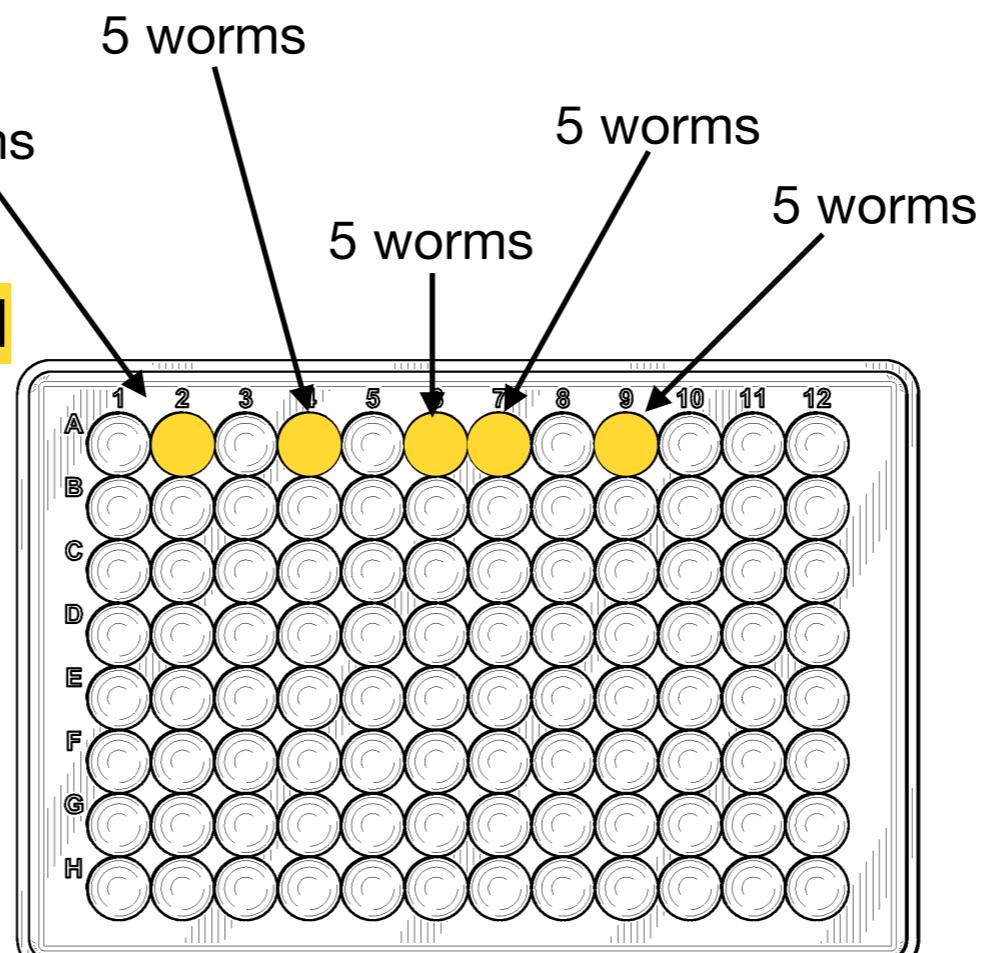
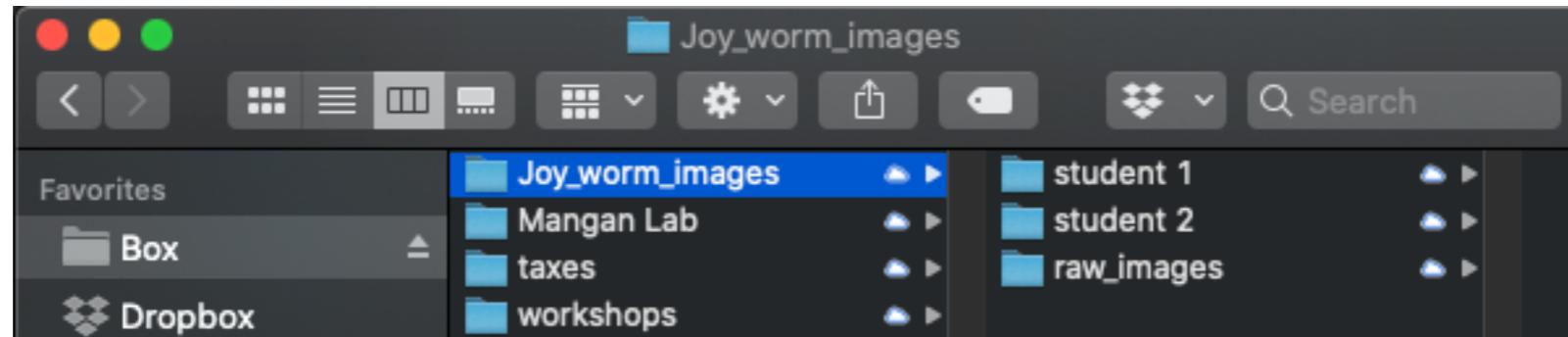


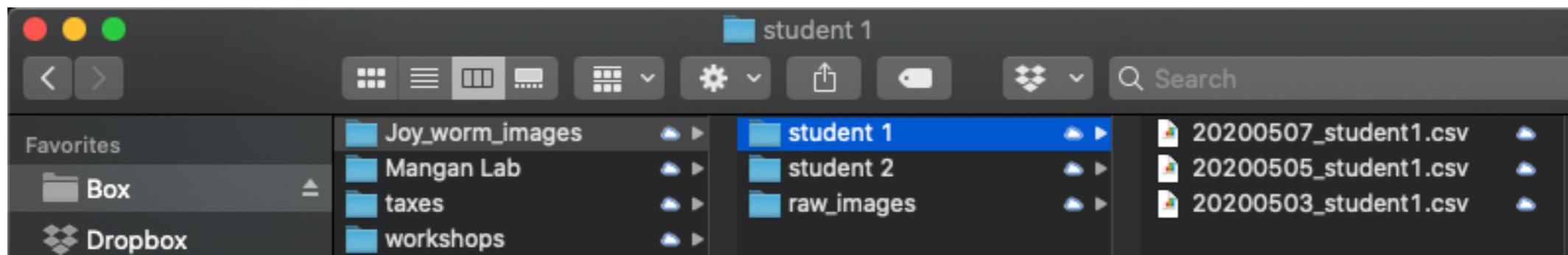
Plate 1, Hour 1

- p01-growth-H01-2X_A01.TIF
- p01-growth-H01-2X_A02.TIF
- p01-growth-H01-2X_A03.TIF
- p01-growth-H01-2X_A04.TIF
- p01-growth-H01-2X_A05.TIF
- p01-growth-H01-2X_A06.TIF
- p01-growth-H01-2X_A07.TIF
- p01-growth-H01-2X_A08.TIF
- p01-growth-H01-2X_A09.TIF
- p01-growth-H01-2X_A10.TIF
- p01-growth-H01-2X_A11.TIF
- p01-growth-H01-2X_B01.TIF
- p01-growth-H01-2X_B02.TIF

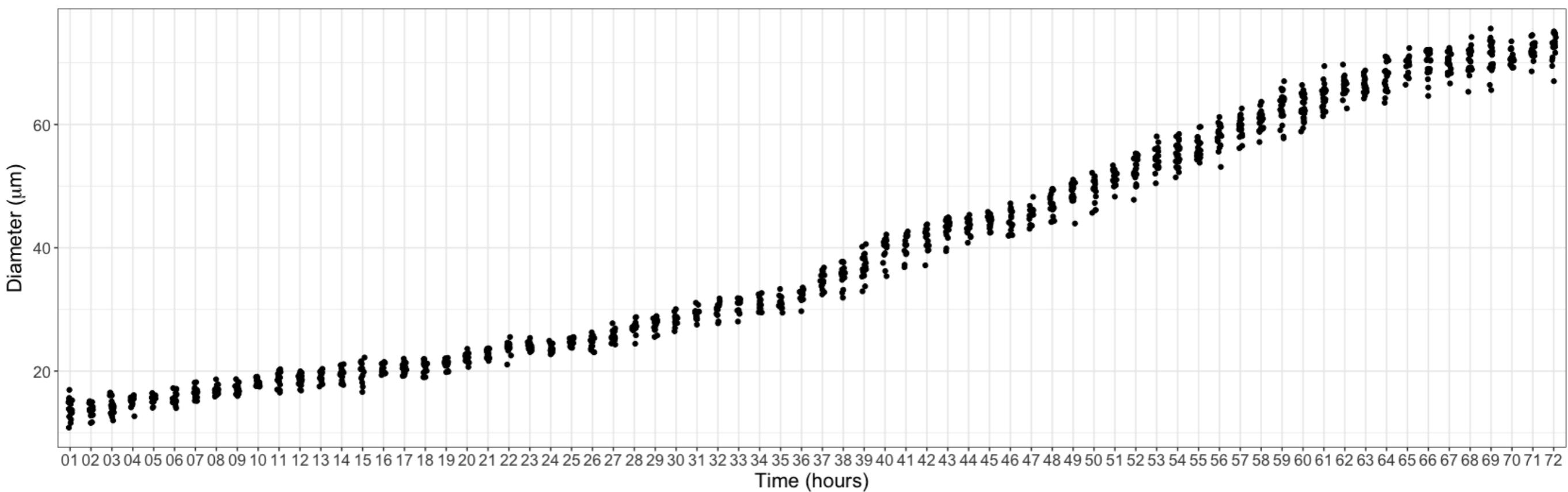
Feel free to create a folder in Joy_worm_images to save the .csv files of your measurements.



I would recommend saving a new .csv file with your data from each day you take measurements



I have also collected measurements for a single replicate, but only for width. Put together this is what my data looked like.



With your help I will be able to significantly extend this dataset and even calculate worm volume.