

OrgM-Multi

A Fiji macro to count and measure organoids in brightfield images

This macro is adapted from OrgM, created by Eddie Cai and Rhalena Thomas

Overview

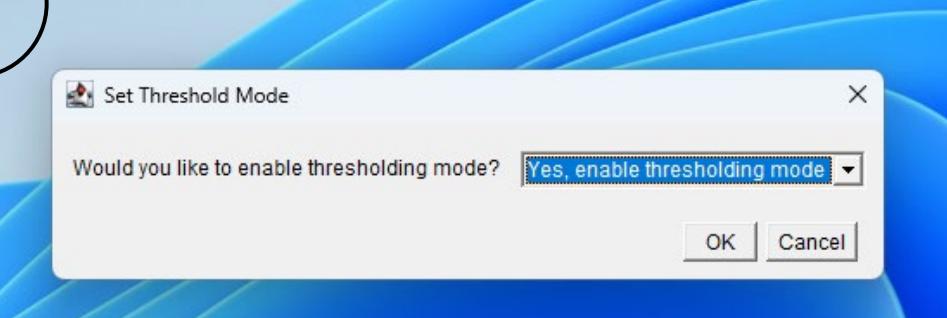
- This macro is designed to
 - Identify multiple organoids in a brightfield image
 - Assess those organoids on biological characteristics set by the user (area, roundness, circularity)
 - Create an image outlining the ROIs (i.e., organoids) that were identified and met the defined biological characteristics
 - Create a new folder and save ROI images
 - Create a CSV file with multiple measures of each organoid in the image, along with the total number of organoids that met all defined criteria in that image

Process

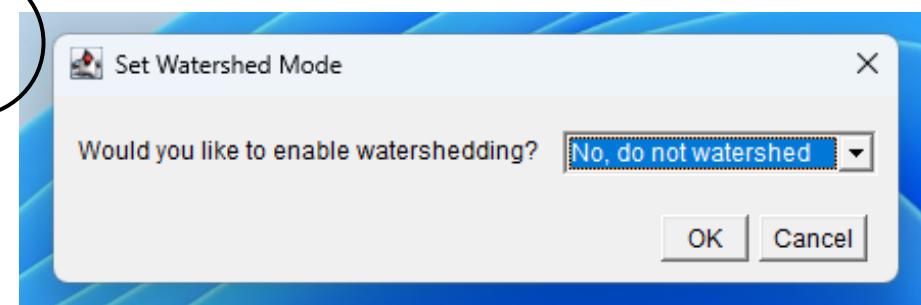
1. Take brightfield images of organoids
2. Download OrgM-Multi.py from Github
3. Open Fiji
4. Drag the OrgM-Multi.py into Fiji or select Plugins → Macros → Run and select OrgM-Multi.py from your computer
5. Push Run and follow the on-screen instructions
6. **Important note:** make sure to save your output location in a different location than your input image directory
7. The next pages will go over some of the options as well as ways to edit the script for various organoid applications

Options

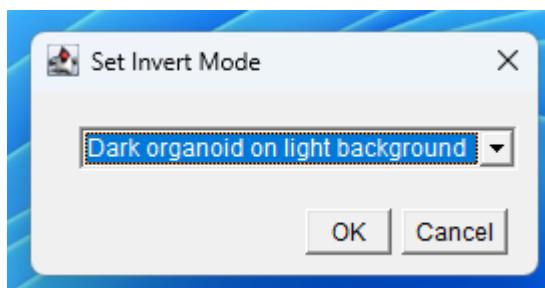
1



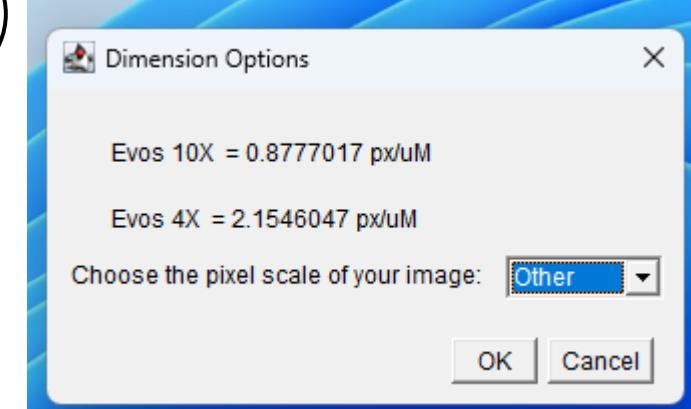
2



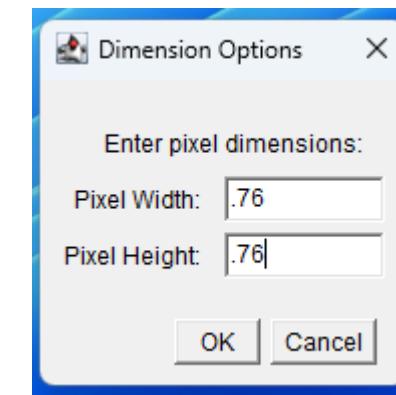
3



4

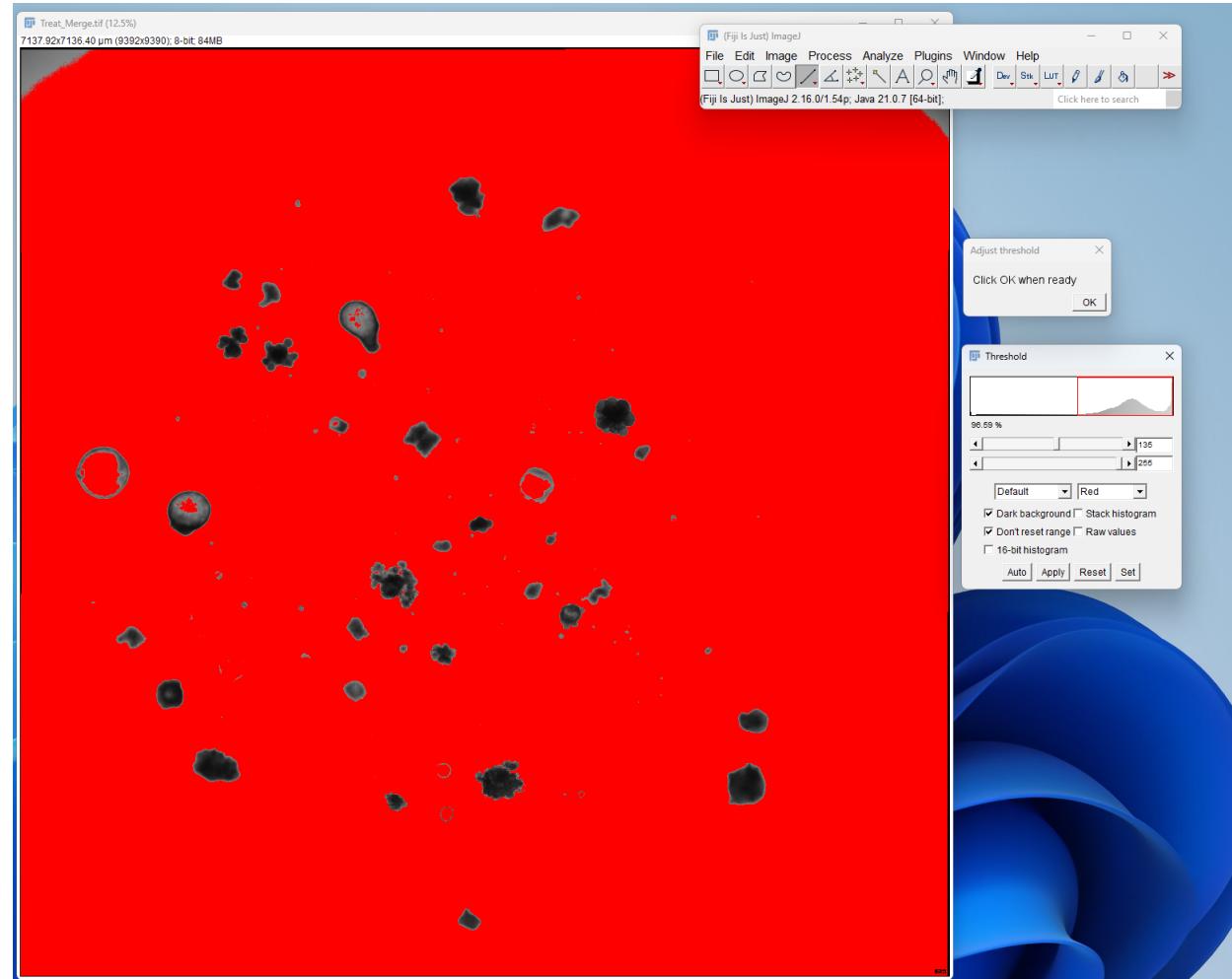


5



Options: Thresholding

Thresholding allows you to set the image threshold to discriminate between objects and background. If you select **No**, Fiji will do it automatically. If you prefer more control, choose **Yes** and you will be prompted to set the threshold for each image. Fiji will open up the image along with a threshold panel. Once happy with the threshold, click OK on the “Click Ok when ready” window.

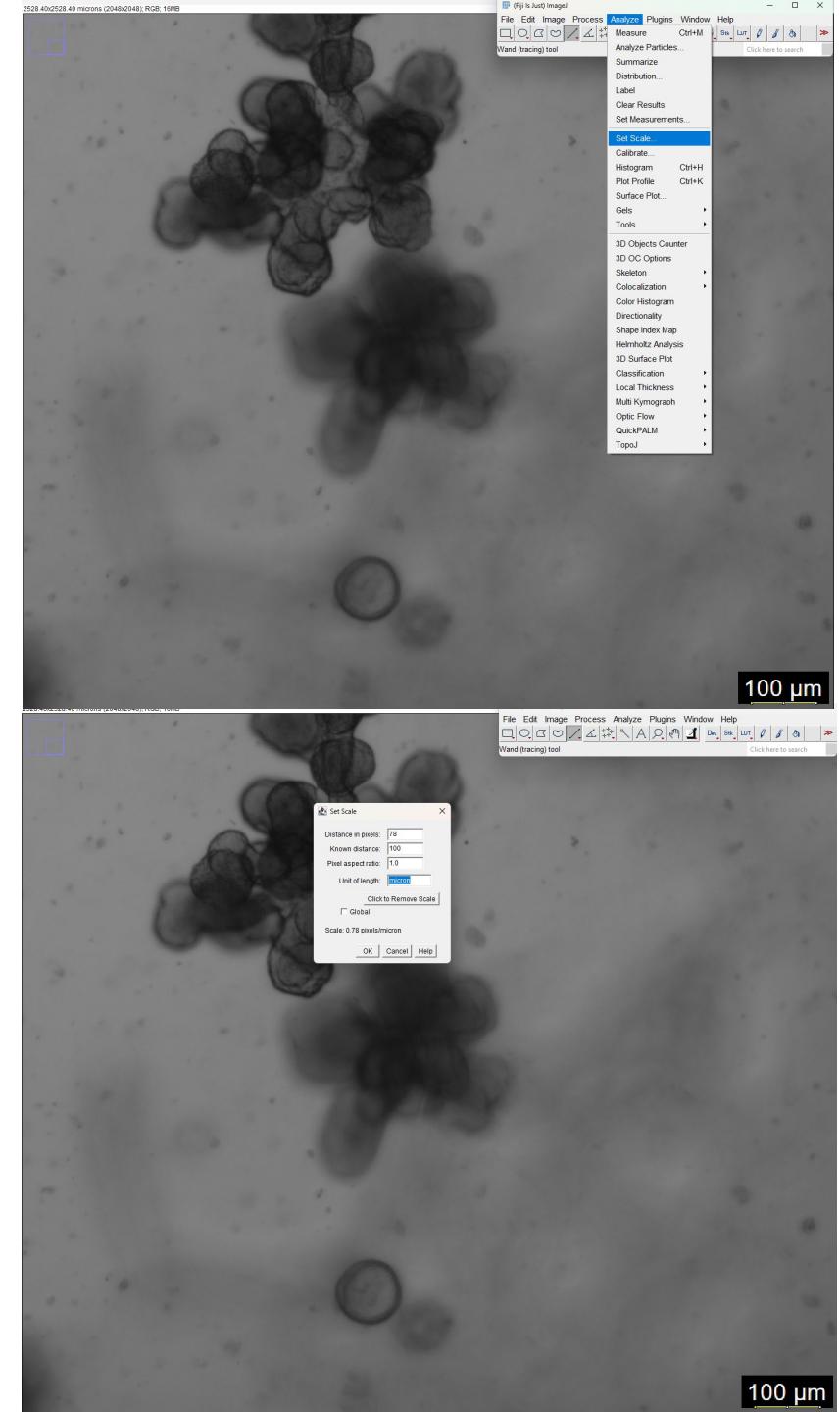


Options: Watersheding

Watersheding will segment the objects. This is useful when two organoids are especially close together or there are many overlaying one another. If you have background objects or organoids that are more spread out, this could be problematic and count non-organoids as ROIs or count one organoid as multiple smaller organoids. We typically select **No** but suggest new users try it both ways with their application.

Setting the scale

- OrgM has built in scales for 4x and 10x EVOS. If you use any other microscope or imager, you'll need to set your own scale. This is easily achieved by including a scale bar in an image and then following these instructions:
 - Open a brightfield organoid image with scale bar
 - Select the line tool and draw a line over the scale bar (zoom in to be as accurate as possible)
 - Click Analyze → Set Scale
 - Don't change the pixel value, but do add the known distance and the unit
 - Click OK
 - Make sure all images you will analyze with the script are at the same scale!



Output: CSV File

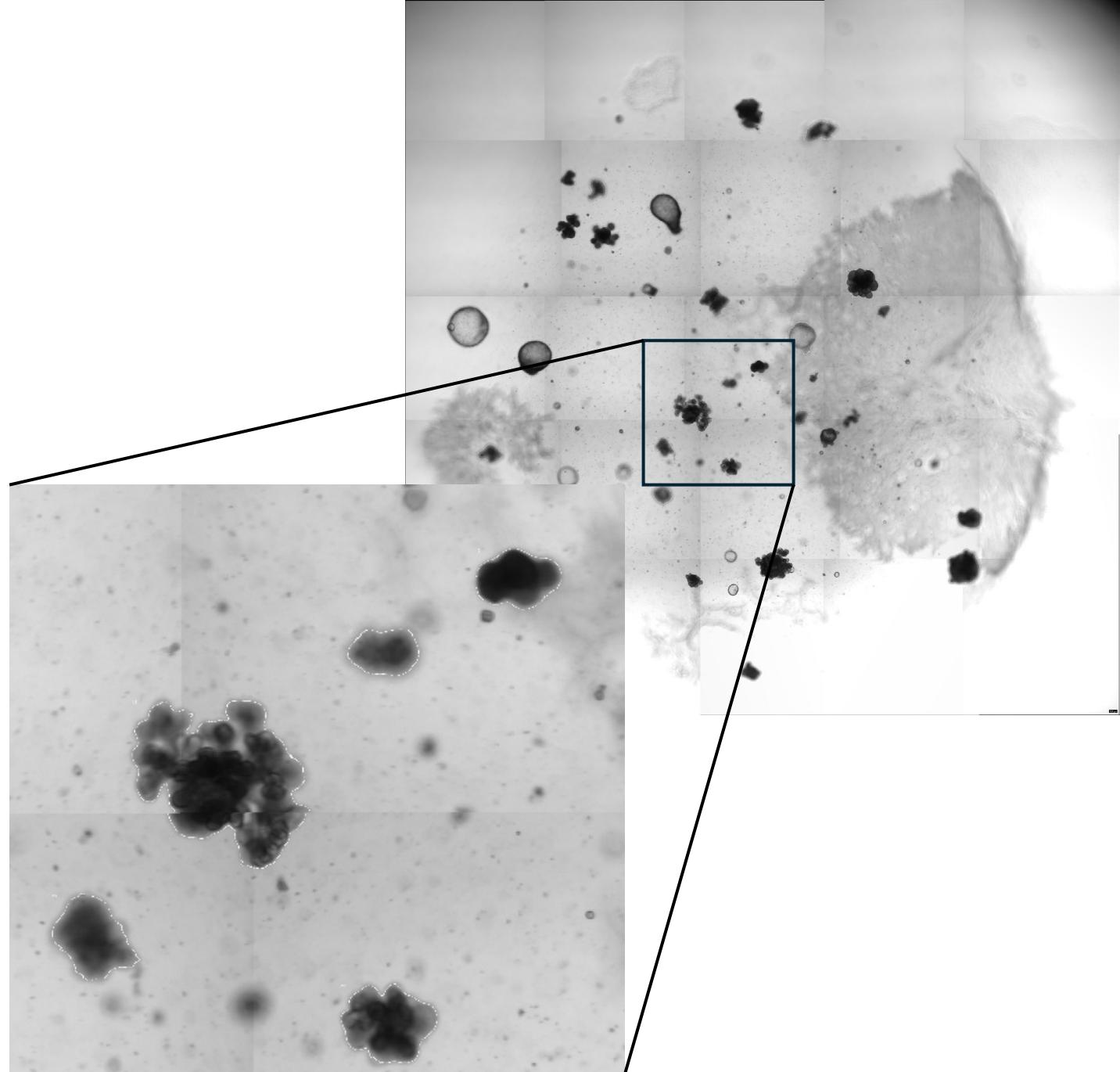
Output file will include the following:

1. File Name
2. ROI Index = an identifier for each identified organoid
3. NumOrganoid = the number of organoids in that file that met the defined characteristics
4. Feret = Longest diameter
5. MinFeret = Shortest diameter
6. Average Feret = Mean of Feret and MinFeret
7. Area = total organoid area
8. Equivalent Circle Diameter = If you draw a circle around the object, this is the diameter of the circle
9. Major = If you draw an ellipse that fits the object inside, this is the longest line that fits inside the ellipse.
10. Minor = If you draw an ellipse that fits the object inside, this is the smallest line that fits inside the ellipse.
11. Circularity = Smoothness (a smooth oval has higher circularity than a wrinkled or bumpy oval)
12. Roundness = How close to a circle it is (more elongated = lower roundness, regardless of bumpiness)
13. Solidity = The percent of actual area compared to the area if you were to fill in all the concave shapes. A perfect circle with no indent would have a value of 1
14. MeetsCriteria = Does it meet the criteria you set (size, roundness, circularity)? This is redundant in our script as we excluded all ROIs that did not meet criteria. So, this should always be True, but it can be modified to set the ROI analysis separate from the "IsOrganoid" analysis

Subfolder	File Name	ROI Index	NumOrga	Feret	MinFeret	Average Feret	Area	Equivalent	Major	Minor	Circularity	Roundnes	Solidity	MeetsCriteria
Treat_Mer		1	32	345.3052	257.1612	301.2332	64322.69	286.1786	325.2158	251.8272	0.594186	0.774339	0.939395	TRUE
Treat_Mer		2	32	351.0155	197.4867	274.2511	45412.64	240.4603	328.0951	176.233	0.324986	0.53714	0.915051	TRUE
Treat_Mer		3	32	186.5487	153.0205	169.7846	20099.32	159.9727	176.1789	145.2572	0.613006	0.824487	0.943713	TRUE
Treat_Mer		4	32	229.7288	173.394	201.5614	27100.41	185.7561	209.2135	164.9287	0.491834	0.788327	0.894777	TRUE
Treat_Mer		5	32	457.5964	278.3738	367.9851	91094.45	340.5658	444.621	260.8627	0.636808	0.586708	0.943928	TRUE
Treat_Mer		6	32	284.8226	217.0986	250.9606	40838.05	228.0277	267.5221	194.3638	0.506148	0.726534	0.877633	TRUE
Treat_Mer		7	32	312.3119	246.3183	279.3151	50386.36	253.2862	276.9463	231.6475	0.45636	0.836435	0.823961	TRUE
Treat_Mer		8	32	372.4659	301.8334	337.1496	77229.74	313.5793	333.8468	294.5422	0.336241	0.882268	0.907997	TRUE
Treat_Mer		9	32	181.3966	124.2466	152.8216	17499.55	149.2686	177.8109	125.308	0.574837	0.704726	0.965488	TRUE
Treat_Mer		10	32	308.6283	235.645	272.1367	52835.96	259.3701	282.6427	238.0137	0.504502	0.842101	0.896322	TRUE
Treat_Mer		11	32	162.656	114.6318	138.6439	13644.07	131.8035	153.2677	113.3453	0.525837	0.739525	0.954424	TRUE
Treat_Mer		12	32	425.1118	400.2015	412.6567	130874.9	408.2097	409.9987	406.4284	0.814052	0.991292	0.992884	TRUE
Treat_Mer		13	32	343.356	273.7279	308.5419	62868.87	282.926	300.0359	266.7918	0.365274	0.8892	0.936357	TRUE
Treat_Mer		14	32	363.0136	323.4662	343.2399	92455.28	343.1002	359.8348	327.1438	0.818374	0.90915	0.985519	TRUE
Treat_Mer		15	32	194.4724	131.7839	163.1282	18661.1	154.143	190.9939	124.4022	0.702968	0.651341	0.953643	TRUE
Treat_Mer		16	32	125.9767	88.16	107.0684	7197.474	95.72935	116.1838	78.87594	0.559334	0.678889	0.919801	TRUE
Treat_Mer		17	32	159.7158	104.0882	131.902	12938.24	128.3491	158.6712	103.8215	0.612159	0.654318	0.957715	TRUE
Treat_Mer		18	32	440.6748	335.6831	388.179	93974.36	345.9073	411.5125	290.7612	0.356633	0.706567	0.847935	TRUE
Treat_Mer		19	32	182.9265	142.9169	162.9217	19027.3	155.648	176.5266	137.2389	0.476741	0.77744	0.953583	TRUE
Treat_Mer		20	32	207.48	144.3886	175.9343	22687.55	169.9608	197.0651	146.5845	0.520842	0.743838	0.92241	TRUE
Treat_Mer		21	32	253.218	179.9845	216.6013	29755.06	194.6415	227.9286	166.2158	0.505237	0.729245	0.929739	TRUE
Treat_Mer		22	32	215.2733	171.7792	193.5262	26589.82	183.9978	204.7481	165.3506	0.63704	0.807581	0.928921	TRUE
Treat_Mer		23	32	256.3094	219.8292	238.0693	42476.13	232.556	241.8039	223.6617	0.779123	0.924971	0.981809	TRUE
Treat_Mer		24	32	199.997	168.55	184.2735	24699.91	177.3384	189.8802	165.6249	0.548266	0.87226	0.964412	TRUE
Treat_Mer		25	32	247.0047	198.0321	222.5184	36855.5	216.6238	247.1456	189.8714	0.707881	0.768257	0.967147	TRUE
Treat_Mer		26	32	389.8985	267.9714	328.935	76183.71	311.4484	384.2855	252.4168	0.611082	0.656847	0.947464	TRUE
Treat_Mer		27	32	411.7412	295.7645	353.7528	79105.21	317.364	348.7741	288.7826	0.386048	0.827993	0.863527	TRUE
Treat_Mer		28	32	373.5066	311.5191	342.5129	81653.58	322.4354	338.2209	307.3866	0.681615	0.908834	0.960012	TRUE
Treat_Mer		29	32	129.702	121.4527	125.5773	12096.68	124.1047	126.9771	121.2972	0.807283	0.955268	0.981167	TRUE
Treat_Mer		30	32	192.7376	133.1597	162.9486	17187.64	147.9324	174.575	125.3558	0.665244	0.718062	0.92459	TRUE
Treat_Mer		31	32	118.8033	102.6445	110.7239	9511.917	110.0498	117.7587	102.8455	0.893923	0.873358	0.986817	TRUE
Treat_Mer		32	32	190.4327	148.0739	169.2533	21048.32	163.7057	185.4604	144.5029	0.725876	0.779158	0.962838	TRUE

Output: ROI Images

OrgM-Multi will create an output folder entitled “ROI_Images” that will include a PNG of each analyzed image with the ROI boundaries overlaying the original image with a white line



Modifications: Defining ROIs

OrgM-Multi.py settings for defining valid ROIs:

```
round_threshold = 0.33  
area_threshold = 6000  
minimum_size = 6000
```

round_threshold is the minimum roundness a roi must have to be considered an organoid and counted as a valid ROI. Ours are rather elongated, so we have a permissive cutoff of 0.33. For round organoids, a range of 0.5-0.8 may be suitable.

area_threshold is the minimum area an ROI must have to be considered an organoid and counted as a valid ROI

minimum_size is the minimum area to be considered an ROI in ParticleAnalyzer

area_threshold and minimum_size are in particles. This will need to be determined for your specific use case. For us, 6000 pixels represents an object with 100-micron diameter

Modifications: Biological Characteristics

In this part of the script, we filter out ROIs that do not meet our biological criteria. These are the same as the previous screen, except you can add a circularity cutoff as well

```
# =====
# FILTER ROIs BY BIOLOGICAL CRITERIA
# You can filter ROIs by biological criteria, including area, roundness and circularity
# =====

valid_indices = []
for i in range(table.size()):
    area = table.getValue("Area", i)
    roundness = table.getValue("Round", i)
    circularity = table.getValue("Circ.", i)
    if (area > area_threshold) and (roundness > round_threshold) and (circularity <=1):
        valid_indices.append(i)

numOrganoids = len(valid_indices)
```