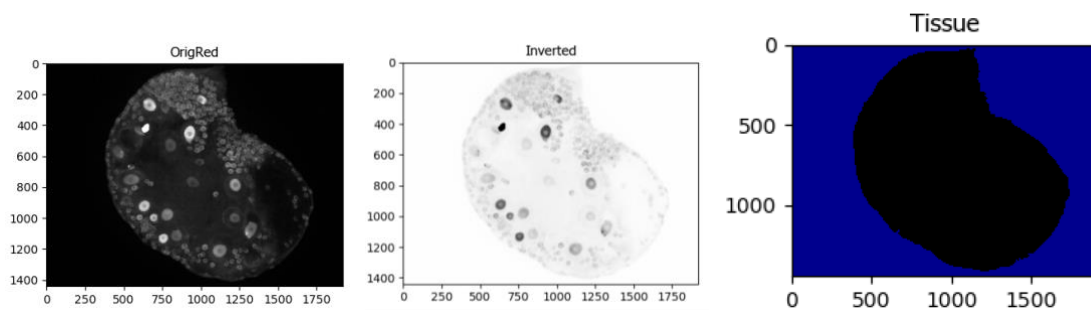# Segmentation And Classification Of Individual Oocytes

Goal: The goal is to segment individual oocytes and classify them based on size.

Images: Single color image.

Pipeline: This pipeline illustrates the segmentation of individual oocytes and then classifies them according to size. This pipeline shows how to create a mask of the region of interest (in this case the tissue), to isolate a colony of oocytes, identify and segment individual oocytes, and then filter them based on size. The workflow is as follows:
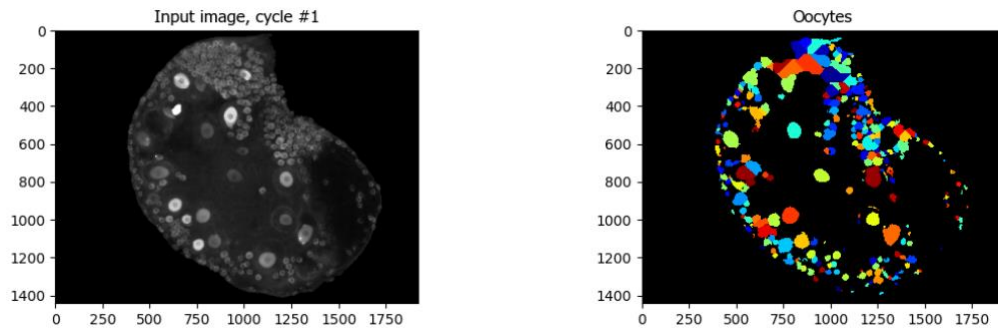
1. Open **CellProfiler**.

2. Click on **Images**. Highlight the image listed. Right click and Clear File list. Go to the downloaded Input images folder, drag and drop the image in the appropriate CellProfiler window. The original image maintains the folder structure of the original computer used to create the pipeline. If the image is not reloaded from your computer an error will occur.

3. **Metadata** information is not used in this example.

4. **NamesAndTypes** is used to name the single color image used in this example as Orig.

5. **ColorToGray** is used to convert the color RGB image into grey scale. In this example the conversion method "split" is used so only the red channel is converted to grey since the oocytes are tagged using a red fluorophore.

6. **ImageMath** is used to invert the image. This is done so in a later step a mask of the tissue containing the oocytes can easily be created.

7. **IdentifyPrimaryObjects** is used to identify and segment the image.

   a. **Typical Diameter of Objects, in pixels** was set to 1000-10,000 to capture the background as a single object, creating a mask of the tissue. To get an idea of object size, go to the "Images" module, right click on an image and select "Show Selected Image". With the new image window selected, select "Tools", "Measure length" from the toolbar pull down menu. Drag your mouse over an object and view it's size in the lower right hand of the image window.

   b. **Thresholding method** "Manual" was used to create a simple cut off where only the inverted background signal is captured.

c. **Manual Threshold** of 0.94 was ideal to capture the inverted background, while eliminating any signal from within the tissue.

d. **Method to distinguish clumped objects** was set to "none" since only one object is detected.

8. **ConvertObjectsToImage** is used to convert the image from the previous step into an object so the mask of the tissue can be saved to your hard drive in a later step. This will allow you to easily use the mask again in the future, if desired.
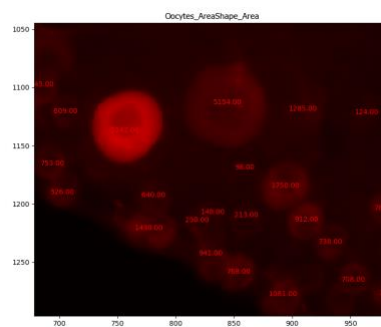

9. **MaskImage** uses the mask created above on the raw image that was converted to greyscale. This ensures only objects within the mask are identified and segmented. In this step "Objects" is selected to mask the object (background) identified in Step 7. In the future if you use the Image created in step 8, you would select "Image" to achieve the same results without first having to run step 7 which is time consuming.

10. **MedianFilter** is applied to the masked image to smooth the signal and improve segmentation in the following step.

11. **IdentifyPrimaryObjects** is used to identify and segment the oocytes.

a. **Typical Diameter of Objects, in pixels** is set to 10-100 since the oocytes are in a large range. Tightening this range will result in fewer oocytes identified, while expanding the range will include more objects. To get an idea of object size, go to the "Images" module, right click on an image and select "Show Selected Image". With the new image window selected, select "Tools", "Measure length" from the toolbar pull down menu. Drag your mouse over an object and view it's size in the lower right hand of the image window.

b. **Thresholding method** "Minimum cross entropy" was the most appropriate thresholding method to use since the signal difference between background and the oocytes is not large, and the intensity distribution is variable.

c. **Threshold correction factor** was adjusted down slightly to 0.8 to include slightly more pixels.

d. **Lower and upper bounds on threshold** was adjusted slightly so that the lower bound was raised to 0.07 to reduce the amount of background pixels identified as relevant objects.

e. **Method to identify clumping of objects** was set to "shape" since the intensity distribution is variable.

f. Automatically **calculate size of smoothing filter for declumping** was turned off and set to 20 to give greater control over declumping. The value is set just above the lower value of the object diameter range for ideal declumping.
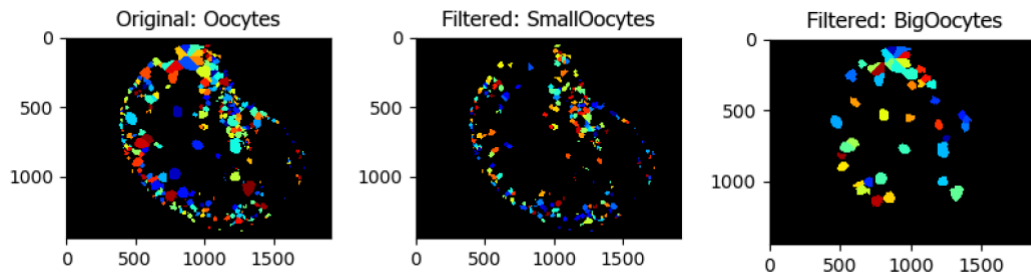

12. **MeasureAreaSizeShape** is used to calculate the area of each segmented oocytes. These values will be used to filter the oocytes based on size in a later step.

13. **DisplayDataOnImage** is used to display the computed area of each oocyte directly on the tissue image. This can be used to help determine the best cut off value for large vs small oocytes.
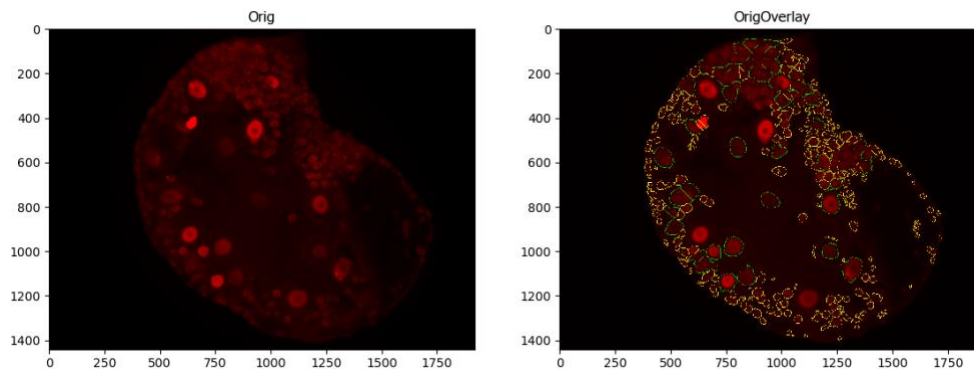
14. **FilterObjects** is used to create a population of smaller oocytes whose area doesn't exceed 2000 and name them SmallOocytes.

15. **FilterObjects** is used to create a population of larger oocytes whose area is larger than 2000 and name them BigOocytes.

16. **MeasureObjectSizeShape** is used to separately report the size and shape of the SmallOocyte and BigOoctye populations.

17. **OverlayOutlines** is used to overlay an outline of the segmented objects onto the original image.

18. **SaveImages** saves the OverlayOultines image to your hard drive.

19. **SaveImages** saves the mask image created in Step 8 to your hard drive.

20. **ExportToSpreadsheet** exports all calculated values for identified objects into a .csv file.