

Beliveau Lab Summer Project: Cell Profiler Pipeline for ADAMTS5 and DAPI Downstream Analysis
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Background:

This was a small project aimed at designing a pipeline to robustly identify mRNA puncta from ADAMTS5 stained images given a medium quality image input. This pipeline will be useful in the future for Yuzhen Liu's project in the Beliveau Lab. Another aspect of this projects is combining two coordinate spaces given two magnification images—40x and 60x images.

Methods

ADAMTS5 Pipeline

Important files for this pipeline can be found in my folder in Github. The ADAMTS5 pipeline is in the folder labelled Image Processing, and the file is called “adam_pipeline.cpproj”. One python script is used in this pipeline, labelled image_adam_dapi.py.

```
import os
cwd = os.getcwd()
print(cwd)

import javabridge
import bioformats
import imageio

javabridge.start_vm(class_path = bioformats.JARS)

path = "100x1.45_175_488_1.5x032.nd2"
stacks = 21

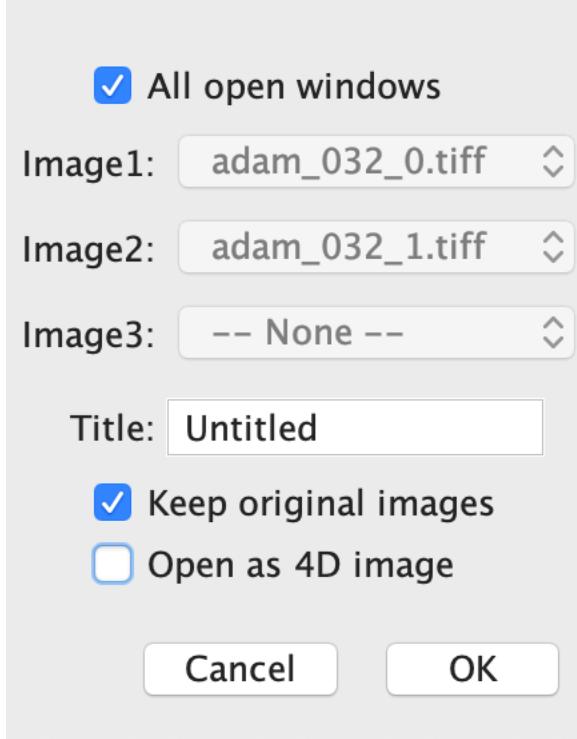
for i in range(0, stacks, 1):
    img = bioformats.load_image(path, c=0, z=i) #if c = 1, change name to adam in next
line
    imageio.imwrite("/net/beliveau/vol1/home/jumanaf/image_analysis/dapi_032_" +
str(i) + ".tiff", img, format = "tiff")
    print("one image done!")
```

The first step is open the image on FIJI, and check to see if there are DAPI and ADAMTS5 images in the file. Typically, this is in an nd2 file. Unless you know already, this is a good place to check how many stacks there are for reference in future steps.

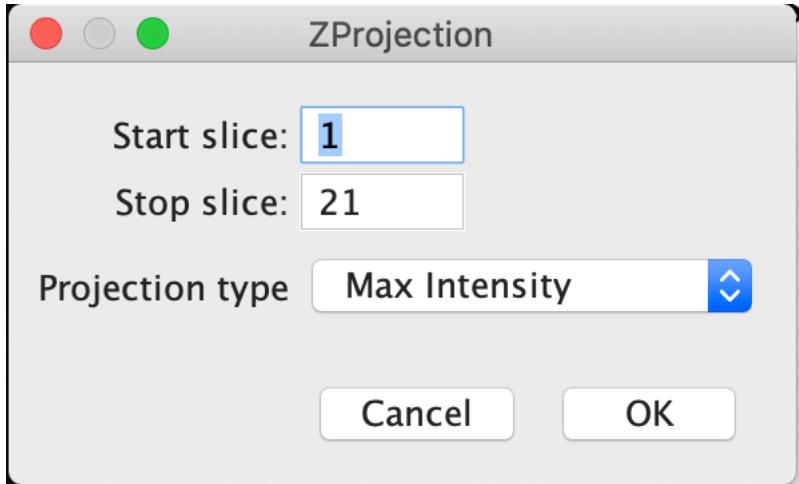
Next, change line 10 that contains the name of the image file, and change the number of stacks (line 12) depending on how many there are in that image. Depending on if your DAPI or ADAMTS5 images are first in your stack, you can set c=0 or 1, and change the respective file name in the line with imageio.imwrite. In this case, the first image in my stack was a DAPI stained image, so I set c=0 and named my output file respectively.

Run this code to produce separate DAPI images, then change c=1 and the file name and re-run the code to get separate ADAMTS5 images.

Next, open all the ADAMTS5 images in FIJI, and select Image > Stacks > Tools > Concatenate. The popup window should look something like this:



Make sure to select “All Open Windows” and name the file with an appropriate title. Once the stacked image comes up, go to Image > Stacks > Z-Project. The popup window should look like this:



Click “OK”, and when the new image pops up, make sure to save the image using File > Save As.

Finally, input this ADAMTS5 maximum intensity image into the Cell Profiler pipeline. Drag and drop the image into an open pipeline file in the “Images” tab, and make sure to change the file locations in the tabs labelled “Save Images” and “Export To Spreadsheet”. Then, press “Analyze Images”, and the output images can be found in the file location specified in the “Save Images” tab.

Overlaying Different Magnifications

Initially, this was conducted through imageJ with the plug-in called Landmark Correspondences. Two images, 40x and 60x, were opened on ImageJ, and the 60x image was cropped so that only relevant information was in the 60x image. Landmarks were then made using the multipoint tool in imageJ, and were then overlayed using the plugin. More information can be found at https://imagej.net/Landmark_Correspondences.

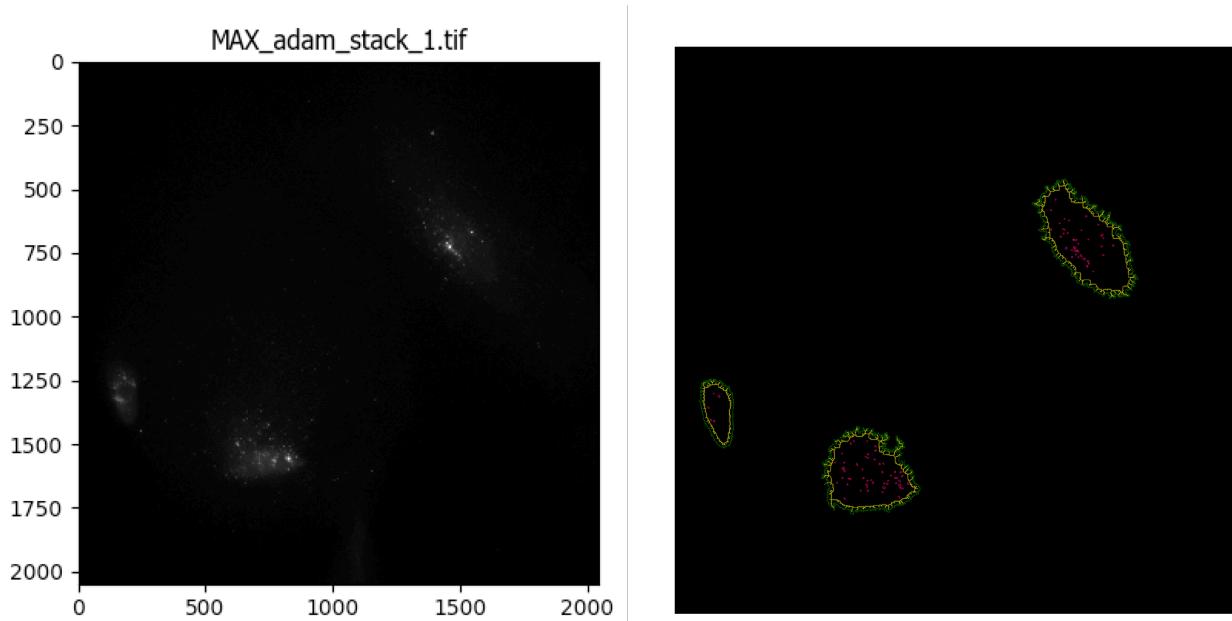
However, to automate this, either macros or python code must be written. I approached this issue by writing python script using OpenCV. The script is not complete as of yet (09/28/2020), due to some minor bugs in the script. This script is included in the lab repository labelled resizing.py.

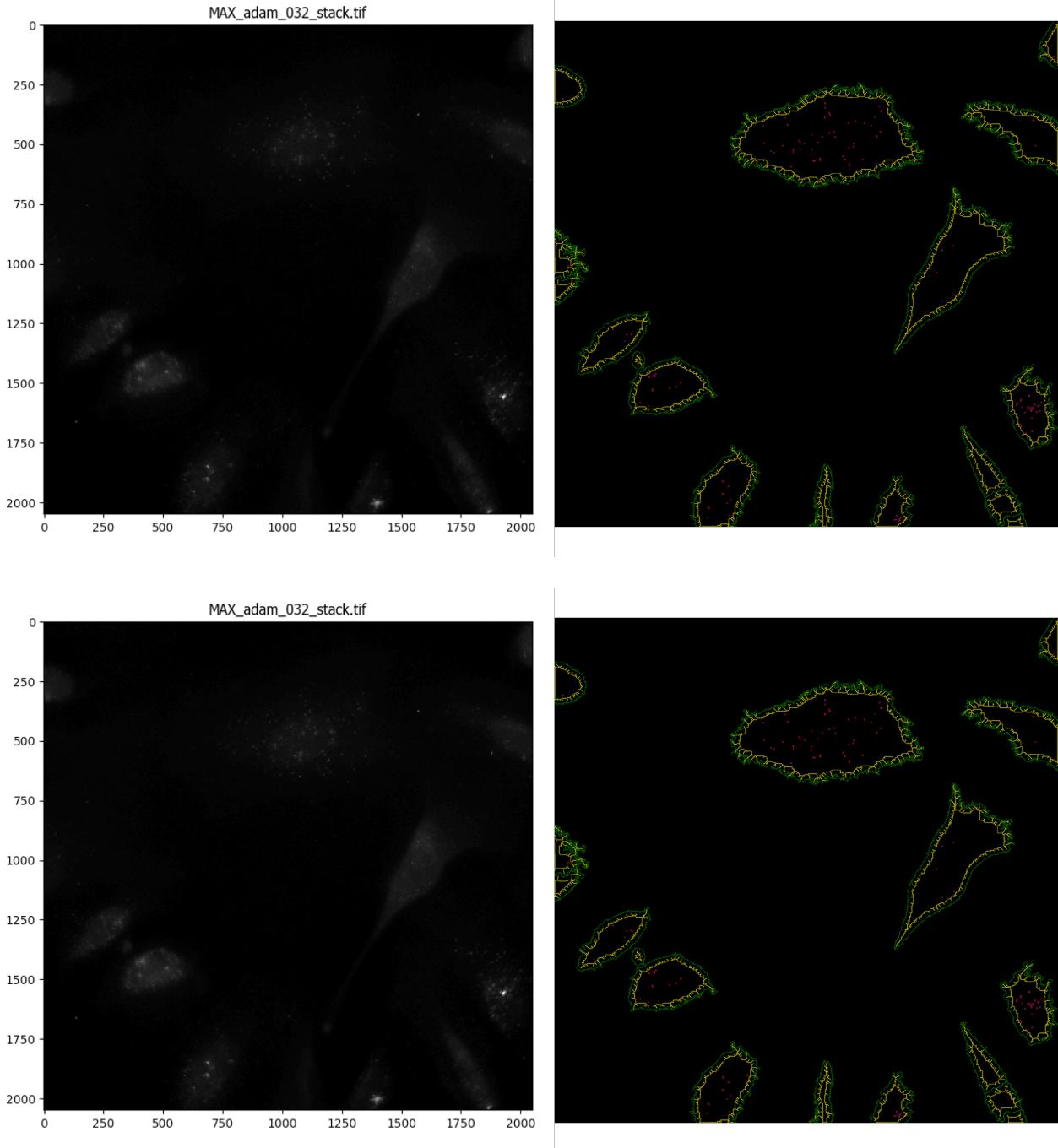
The pipeline for this step is as follows: firstly, the maximum intensity of the ADAMTS5 and DAPI stacks are obtained, as explained above. Next, the 60x image is resized on imageJ such that it is the same size as the 40x image. Finally, the two images are processed using the Python script, resizing.py.

Results

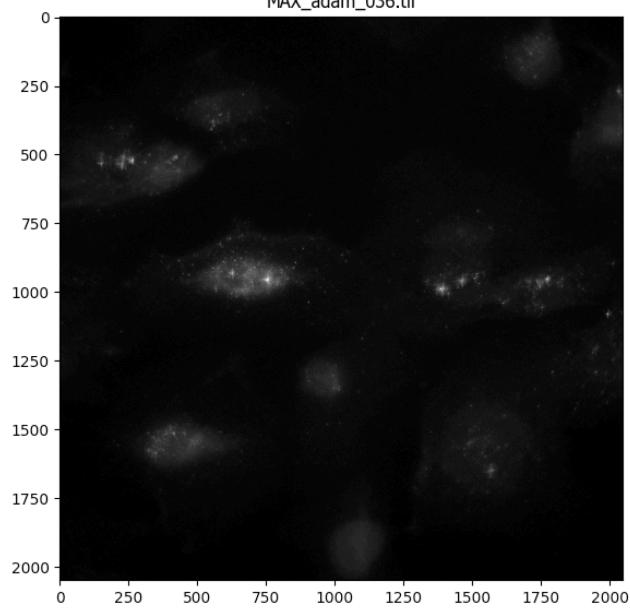
ADAMTS5 Pipeline

This pipeline produces images that are segmented with mRNA puncta, as well as overall cell segmentation. The images produced can be seen below.

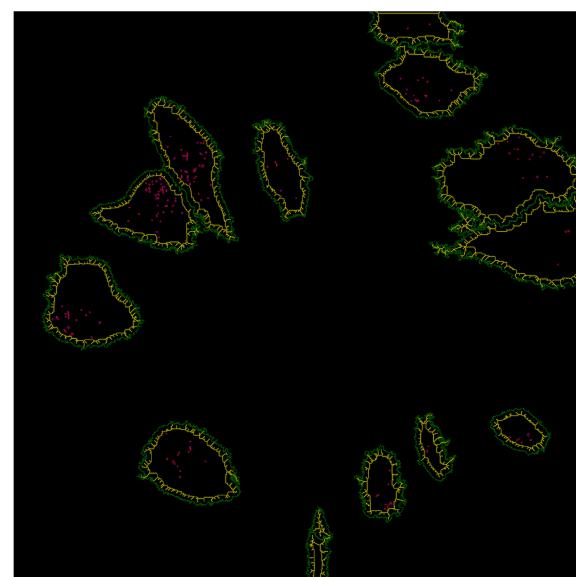
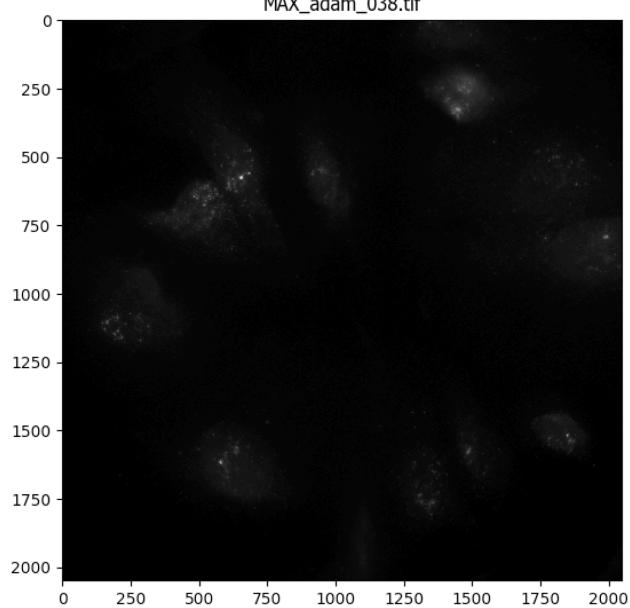


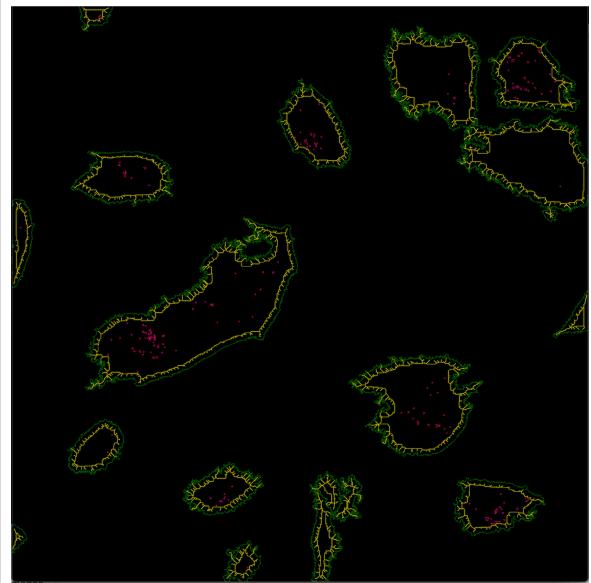
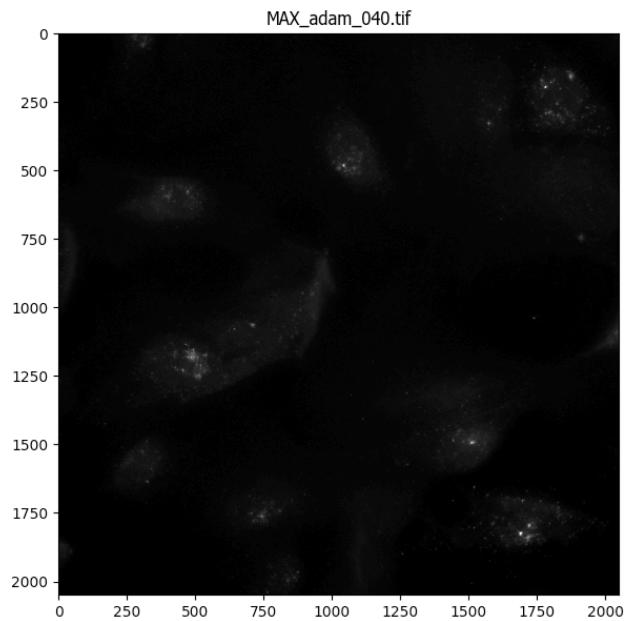


MAX_adam_036.tif



MAX_adam_038.tif





Next Steps

More images need to be tested for the