AutoSeg v0.1

This auto segmentation pipeline operates using three main python files. The pipeline can be summarised as: Machine auto-segmentation -> Human edits -> Improved auto-segmentation. This guide will go through how to use this pipeline.

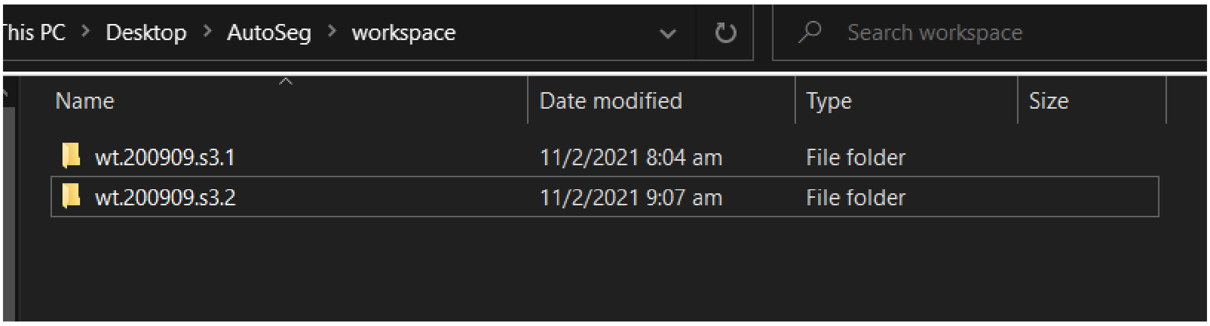
Step 0: Installation of required libraries (on windows)

1. Please ensure that Python (version 3.8 preferable) is installed and that the py and pip path variables are set up to your preferred environment.
2. Either use command prompt and type `pip install -r requirements.txt` (you need to change directory) or click on the install_requirements.bat file.
3. If there are no errors, you should be done with the setup. If some errors appear, you may need to manually install a few libraries. In that case, go to seg1.py, seg2.py and editor.py and ensure the utilised libraries are installed on your python environment.

Step 1: Machine auto-segmentation

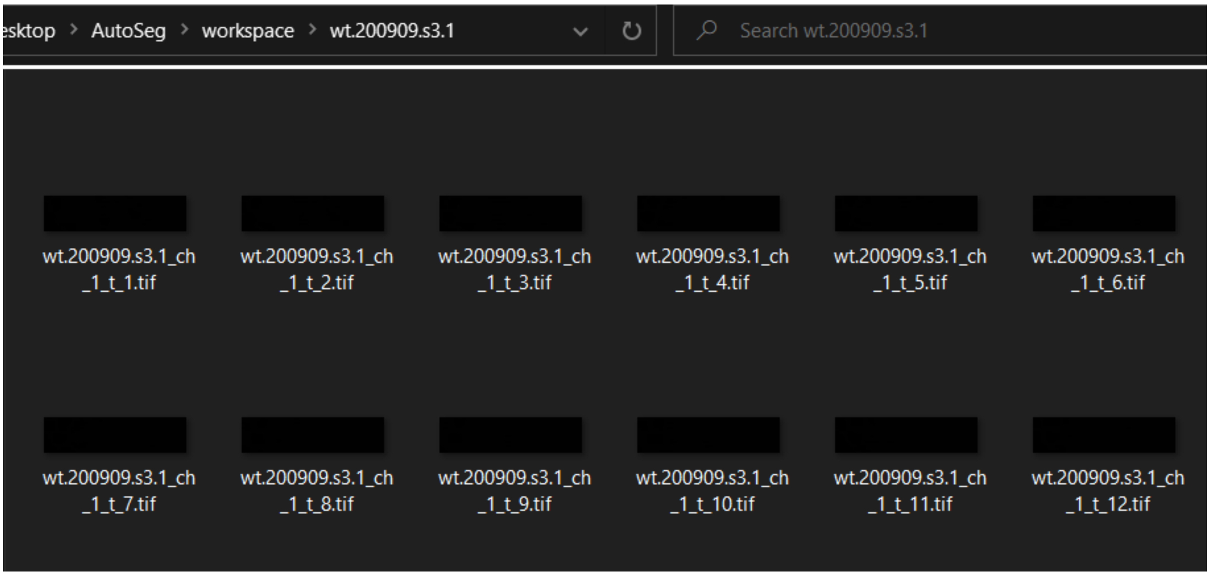
The first step in the pipeline would be to run an auto-segmentation on your files and to convert them into a suitable format.

1. First, add the cells you would like to work on into the workspace folder (Figure 1)



Name	Date modified	Type	Size
wt.200909.s3.1	11/2/2021 8:04 am	File folder	
wt.200909.s3.2	11/2/2021 9:07 am	File folder	

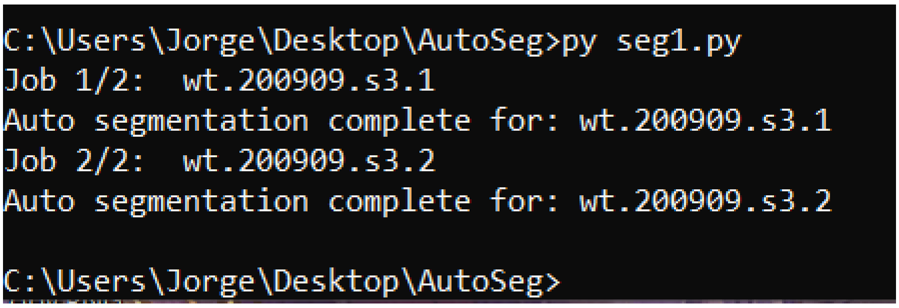
Figure 1: Place the cell folders into the workspace. You may add multiple cell folders to be worked on at a time if you wish. Remember to remove cell folders only after you finish all steps. I suggest working on a few cells at a time.



wt.200909.s3.1_ch_1_t_1.tif
wt.200909.s3.1_ch_1_t_2.tif
wt.200909.s3.1_ch_1_t_3.tif
wt.200909.s3.1_ch_1_t_4.tif
wt.200909.s3.1_ch_1_t_5.tif
wt.200909.s3.1_ch_1_t_6.tif
wt.200909.s3.1_ch_1_t_7.tif
wt.200909.s3.1_ch_1_t_8.tif
wt.200909.s3.1_ch_1_t_9.tif
wt.200909.s3.1_ch_1_t_10.tif
wt.200909.s3.1_ch_1_t_11.tif
wt.200909.s3.1_ch_1_t_12.tif

Figure 2: Each of the cell folders should contain multiple tif files, each representing one time point for the cell. It is assumed that the tif files each contain 2 channels (nucleus and membrane) and multiple layers. It is further assumed that the focal cell is always in the centre of the x,y and z axes. [TLDR: Mario’s format]

2. Click the seg1.bat file and the auto-segmentation script will run on its own.
    a. If the .bat file does not work (e.g. using mac/linux or incorrect path for py), you may use the command prompt/terminal to run the seg1.py script.
    b. If you are running the seg1.py script using command prompt/terminal, ensure that the current working directory is changed to the main directory of this program (e.g. Desktop/AutoSeg).
3. Once the command prompt either terminates on its own or the script stops running without error, this step is complete.
    a. This step would have generated two files. A raw segmentation .gif file that would be in the /raw directory and an original image .gif file that would be in the /original directory.



```
C:\Users\Jorge\Desktop\AutoSeg>py seg1.py
Job 1/2:  wt.200909.s3.1
Auto segmentation complete for: wt.200909.s3.1
Job 2/2:  wt.200909.s3.2
Auto segmentation complete for: wt.200909.s3.2

C:\Users\Jorge\Desktop\AutoSeg>
```

Figure 3: Example of a successful run.

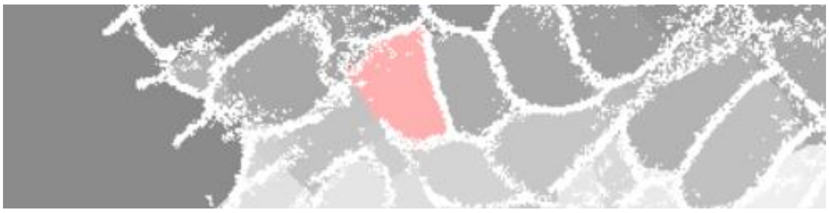


Figure 4. Example of a frame of the automatic segmentation.

Step 2: Membrane Editor

As it is virtually impossible to obtain perfect segmentations all the time (especially if the data is noisy), some manual edits will have to be made. A membrane editing tool is included in this package to facilitate this step.

1. Open the editor tool by clicking the editor.bat file or using python to open it. You should see a blank canvas and some tools.

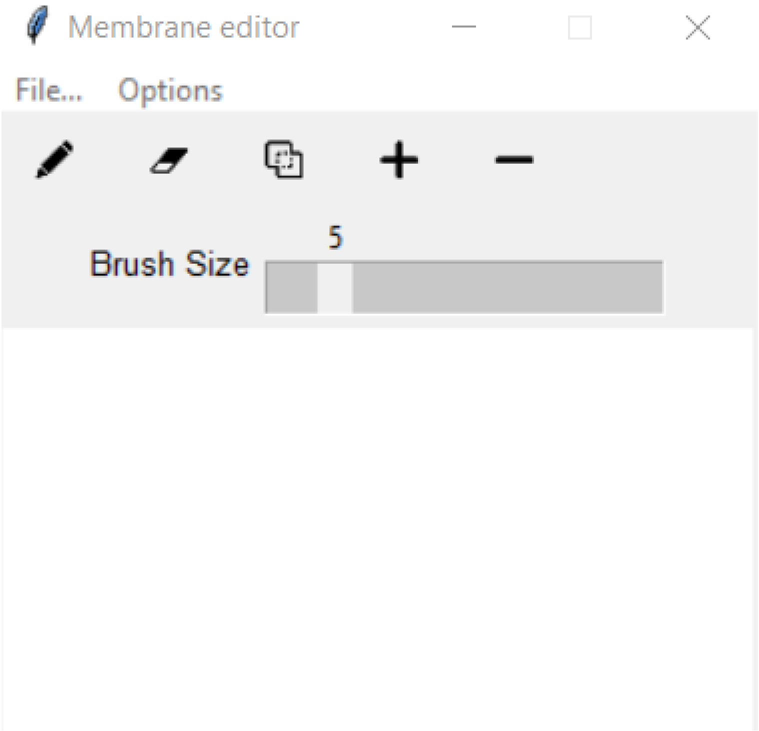


Figure 5. Screenshot of membrane editor tool. The editor allows users to make colour annotations that will assist the machine in making an improved segmentation. Clicking on the tool icons changes to the colour corresponding to the tool. From left to right, the tools are pen, eraser, merge, add, subtract.

Tools:

Pen: The pen tool can be used to draw membranes. This is useful for filling in gaps between membranes.

Eraser: The eraser tool can be used to erase membranes. This is useful when there is a lot of noise/artifacts.

Merge: The merge tool merges multiple watershed segments together. This is useful if you notice that the auto-segmentation chopped a cell into multiple parts (particularly the case for elongated cells)

Add: The add tool adds area to the cell segmentation. Regions painted with this tool will be treated as part of the final cell segmentation. Useful for making minor modifications.

Subtract: The subtract tool removes areas of the cell segmentation. Regions painted with this tool will not be treated as part of the final cell segmentation. Useful for making minor modifications.

Options:

Under options, you can clear annotations for a time frame. Doing so will reset the annotations for only that frame. If you make a mistake in the annotation process, use this.

File:

Save: The program autosaves your work under the temp folder whenever you change the frame. This function exists in case you want to force save your annotations. You should also use it if you are on the last frame of the project.

Import Raw Segmentation: Import a raw auto-segmentation using this tool. You can find the auto-segmentations in the /raw directory.

Import Original Images: Imports the original image for reference. You can find the converted original images in the /original directory.

2. After opening the editor, import your raw segmentation (file > Import Raw Segmentation) and the corresponding original image (file > Import Original Images).

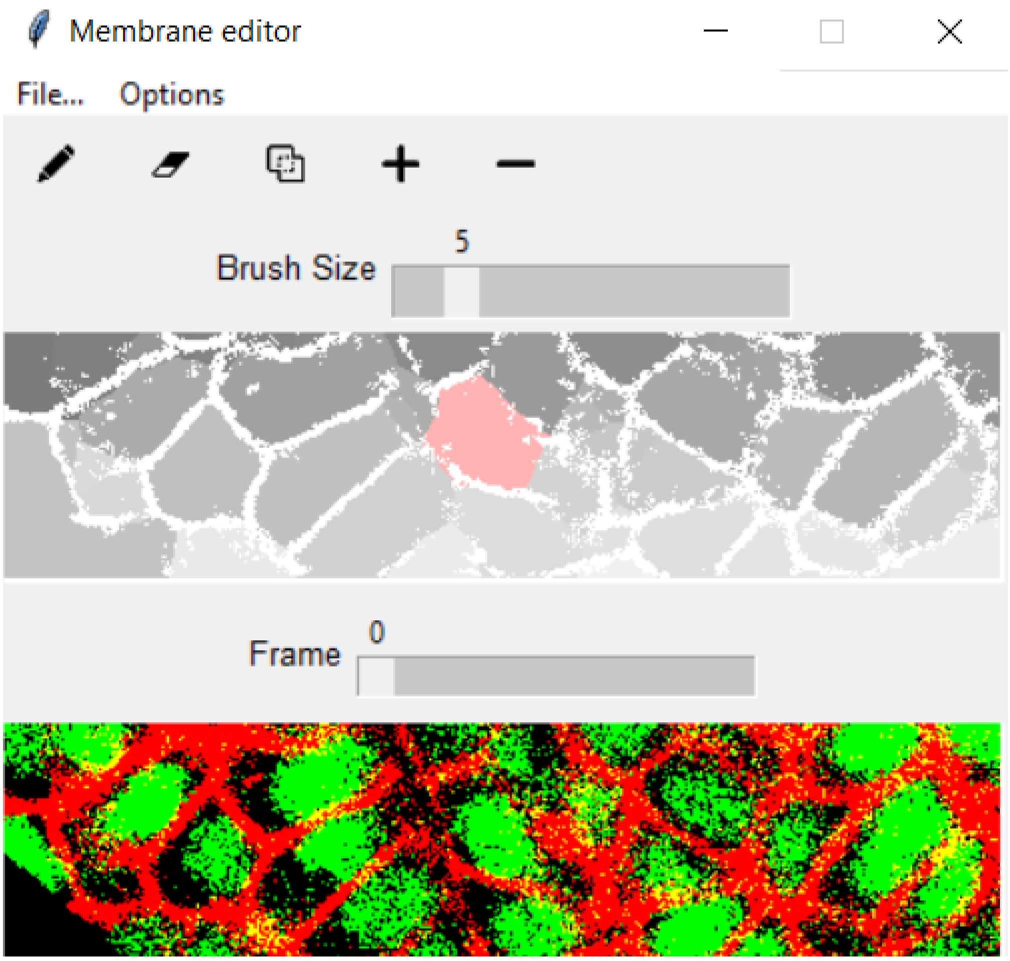


Figure 6. Screenshot of editor tool with both the raw auto-segmentation and original images opened.

3. You can scroll through the frames and use the tools to make whatever annotations you wish. Changing the frame automatically saves your annotations and you go back to make changes if you desire. If you think an auto-segmentation is good enough, you do not need to make any annotation for the frame.

4. After you are finished making annotations, go to file > save. All your annotations can be found in the /temp directory. The next step in this pipeline will use these annotations to make an improved segmentation.

Step 3: Improved Segmentation

With the annotations that have been made, you can use the seg2 script to run a second machine-driven segmentation that utilises your annotations to make an improved segmentation.

1. Click on the seg2.bat file or run the seg2.py script using python.

2. This script makes an improved auto-segmentation using the annotations that you have provided.

3. Two files are created by this script. One would be the final output file (a .gif file comprising of a mask of the cell. The other would be another raw auto-segmentation file (like the one generated by the seg1 script). The raw auto-segmentation file can be put back into the membrane editor script for more modifications if you desire.

4. The final output file can be found in the /processed directory.