How to use Image J and Trainable Weka Segmentation

1. Open Fiji Program (Image J Processor)
2. Download Cell Movie File from Dropbox Folder
3. Drag and Drop Movie file onto Fiji Program
4. Select Hyperstack, and keep the default information on the Import Screen
5. Once the Image pops up, select Command Shift D to duplicate the image, and change the range to 1-16 (This is the max allowable storage before Java Heap Space Error)
6. Hit Plugin, Segmentation, Trainable Weka Segmentation (NOT 3-D)
7. There are 2 default classes set, add a third class by clicking Create New Class on the Left Side
8. Separate the classes between the cell membrane, inside the cell membrane, and the surrounding area outside the cell membrane
9. Add each of these items to a separate class, and use the toolbar drawing tool as appropriate for each section
10. Hit Train Classifier
11. After it loads click create result, and a new image will pop up
12. Click Image, Type, 8-bit to change to greyscale
13. Click Image, Adjust, Threshold and change the threshold to B&W and move the bars around to eye it to where you can see the difference between the cell membranes, then hit the exit
14. Click Analyze, Analyze Particles, and it will ask what size, choose 0-(A set Value) – I choose 100, but its trial and error, also make sure the Display Results box is checked
15. Then you will receive a CSV, and save that file into a folder to test on the python Program