Neurolucida cell counting

1. Take images of AON and piece them together in photoshop. Make sure the image is oriented such that lateralis is on the right (as opposed to left) side. You may need to go to Image -> Image rotation -> Flip canvas horizontal. Save as a TIFF file.
2. Open Neurolucida
3. Click somewhere to mark reference point
4. Go to -> File -> Image Open -> Select your image
5. Zoom out/in to see the whole image
6. Draw outside boundary of AON starting at boundary between medialis and vp and going clockwise (Go to Trace -> check Contour mapping) Click AON outside boundary from the drop down menu. Click on Freehand option to right. When done right-click -> end open contour
7. Draw inside boundary of AON starting at same boundary between medialis and vp and going clockwise. Click AON inside boundary from the drop down menu. When done right-click -> end open contour.
8. Using the drop down contour menu, draw a line with two points for each of the following boundaries that are present [lateralis-dorsalis, dorsalis-medialis, lateralis-vp]
9. Make sure the Image -> “Collect Luminance Information” button is checked
10. Change the drop down contour menu to “Cell” and the drop down to right of it from “Freehand” to “Circle”
11. Zoom in to clearly see your cells. Click on the center of a cell, then move the circle to be large enough that it touches the edge of the cell, but not so large that the circle includes tissue that is outside the cell. Repeat for all cells.
12. Zoom out and change to the “background” contour. Make 20 background measurements that are roughly the size of cells (check this by looking in the Contour Measurements window). Make sure your background measurements are in cell-free areas.
13. Save your data file