Telencephalon Volume measurement using NH Image 1.63

Quick notes:
1. You must use OS 9.2.2.to be able to use this program.
2. If you are in OS X:
    -Select the Apple from the Taskbar
    -Click System Preferences
    -Click Startup Disk
    -Select 9.2.2 from the options
3. You can use this protocol to measure the area/volume of hippocampus, HVC or any other closed space directly from the slides.

Protocol:

1. To calibrate the computer program for your measurement, place a ruler under the microscope.
2. Turn on the camera light, and add an extension tube to the camera if necessary.
3. Select NIH Image 1.63 from the Apple pull-down screen.
4. Select "Special" from the taskbar, and then select "start capturing".
5. Rotate the camera lens until you clearly see the ruler. This also may involve lowering the mounted camera.
6. From the two columns of icons on the left, select the diagonal line (2nd column, 4th row).
7. Drag the two points together on the screen. In this case, the two points will be whichever two markings you decide to measure from- for example, the "1 mm" and "10 mm" markings.
8. From the pulldown menu select "analyze", then "set scale". Choose whatever units you used in the initial measurement from the ruler.
9. In the "known distance" category, choose "1", then hit "okay".
10. Hold down the "apple" and "G" buttons on the keyboard (this will unselect what you have on the screen).
11. Place your image on the camera bed.
12. From the "map" menu on the screen, click the icon on the bottom right (the 2nd of two icons lining the bottom). The screen should turn grainy.
13. From the "LUT" taskbar on the screen, drag the black and white bar until the optimum setting is found to ensure no gaps in the image.
14. Select "Analyze" from the pulldown menu, then "options". Ensure that the boxes next to "include interior holes" and "wand auto-measure" are checked. Next to decimals, type "4".
15. Click the paint bucket from the list of icons, then click inside your image so the entire image turns black.
16. Select the wand (1st column, 9th row). Click your blackened image.
17. Under "info" (on the bottom left), your data will be displayed.
18. To clear the entire screen and start over, select the "Apple" and "G" keys.