

User's Guide for ImageJ Plugin Measure_Line

1. Download “Measure_Line.class” and “Measure_Line.java”, and copy them into the directory “ImageJ\plugins\Utilities”.
2. Before you start using Measure_Line plugin, make sure to set appropriate maximum amount of memory allowed to be used by ImageJ. The place to set this parameter is in menu “Edit/Options/Memory”. (Fig. 1) After resetting the memory parameter, **RESTART** ImageJ.

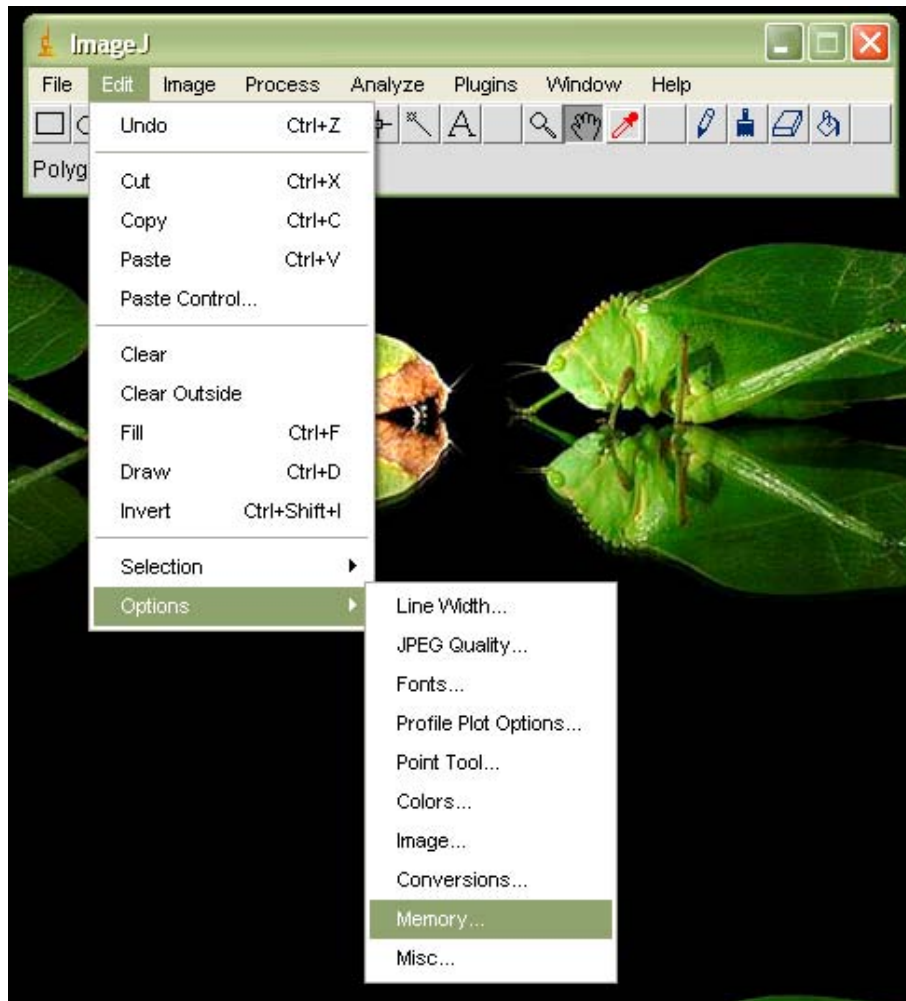


Figure 1. Set maximum allowed memory in ImageJ

3. Open the image that contains the pieces of the cochlea that you want to measure. If you would like to make the tracings on different pieces to be in different colors, make sure to change image mode to “RGB color” (in “Image/Type”).
4. Start the “Measure_Line” plugin from menu “Plugins/Utilities/Measure Line”. (Fig. 2)

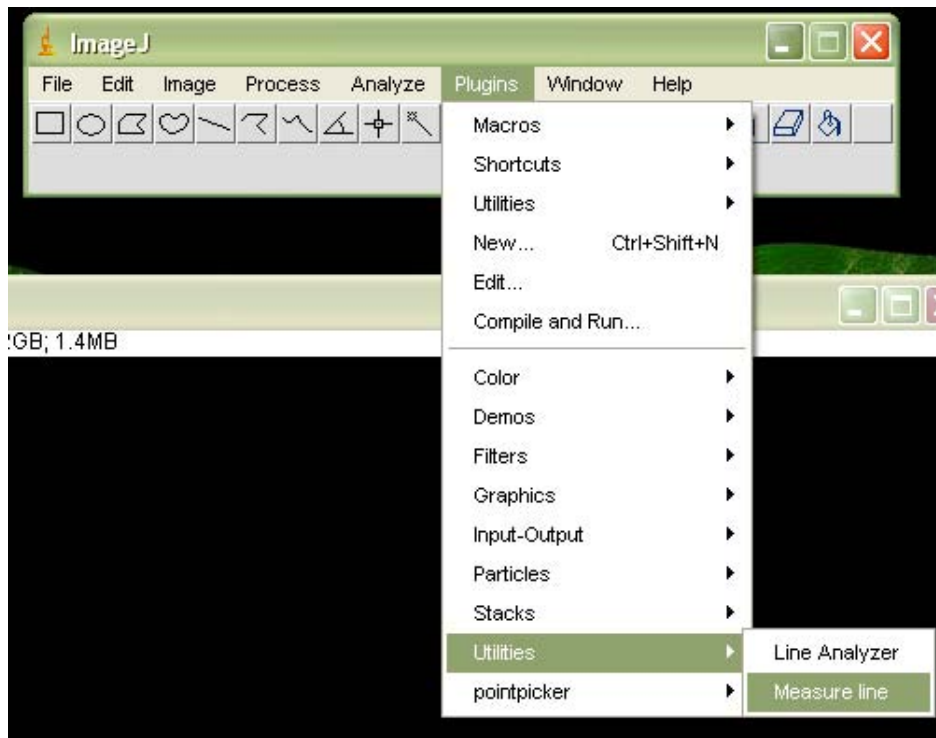


Figure 2. Start “Measure_Line” plugin.

5. Use the “Segmented Line” tool (Fig. 3) to trace the first cochlear piece. When you are using the “Segmented Line” tool, left click along the line that you would like to trace, and right click or double click to finish the line. After you finished tracing, Press “d” (for drawing) on the keyboard, then a window will pop up asking which piece it is (1= base). Input the piece number and click “OK”. (Fig. 4). The “Measure_Line” plugin will automatically do a spline fit to smooth the tracing and assign a color to it. (1 = red, 2 = green, 3 = blue, 4 = cyan, 5 = magenta, 6 or larger = black). Repeat this procedure for all the pieces.

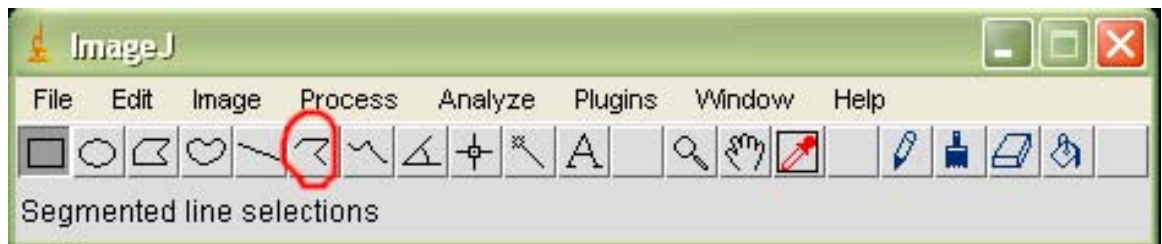


Figure 3. Segmented Line tool.

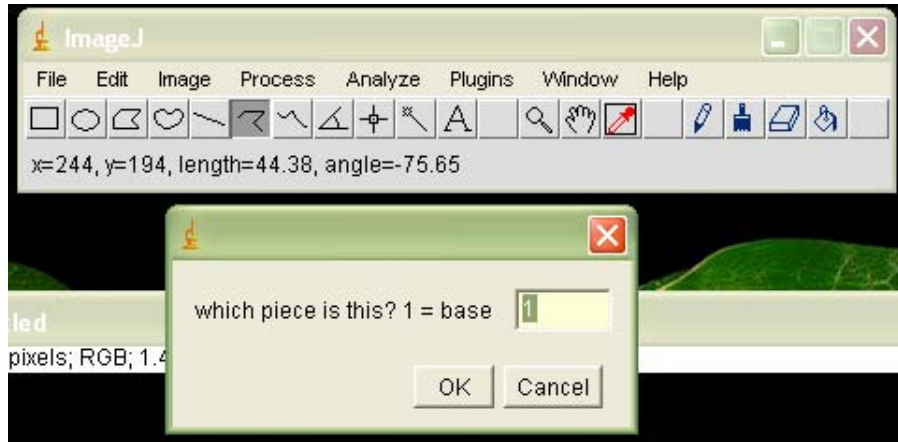


Figure 4. Input piece number.

6. Go to menu “Edit/Options/Colors” to select the foreground color you desire. (Fig. 5).



Figure 5. Set foreground color.

7. After you finished tracing all the cochlear pieces, press “a” (for annotation) on the keyboard. A window will pop up asking you which species it is. (Fig. 6). Input the species number and click “OK”. The nine points with octave and half-octave frequencies will be labeled on the cochlear tracings in the foreground color you just chose.

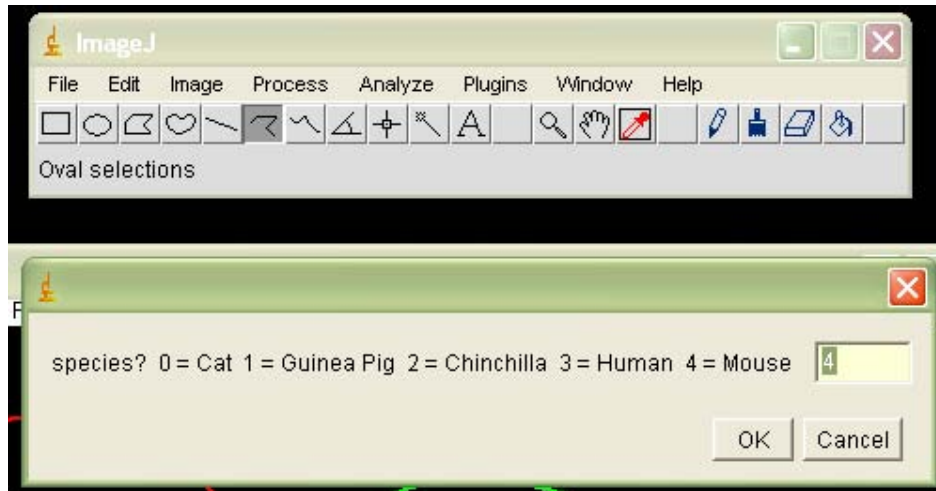


Figure 6. Input species number.

8. Press **“m” (for measurement)** on the keyboard, a “Measurements” window will pop up which gives you the length of each cochlear piece and the total length of all the pieces. (Fig. 7). If you have already done the calibration, the lengths will be in whatever units you have calibrated, otherwise they will be in pixels. For information about how to do calibration, please refer to Attachment A.
9. Press **“p” (for pointing)** on the keyboard, the program will turn into “Pointing” mode. Place the cursor on any point on the cochlear tracings, the frequency of that point will be shown on the menu bar. Click the left mouse button, the frequency of that point will be labeled right next to where the point is. (Change the foreground color as described before can change the color of the labels.) (Fig. 7).

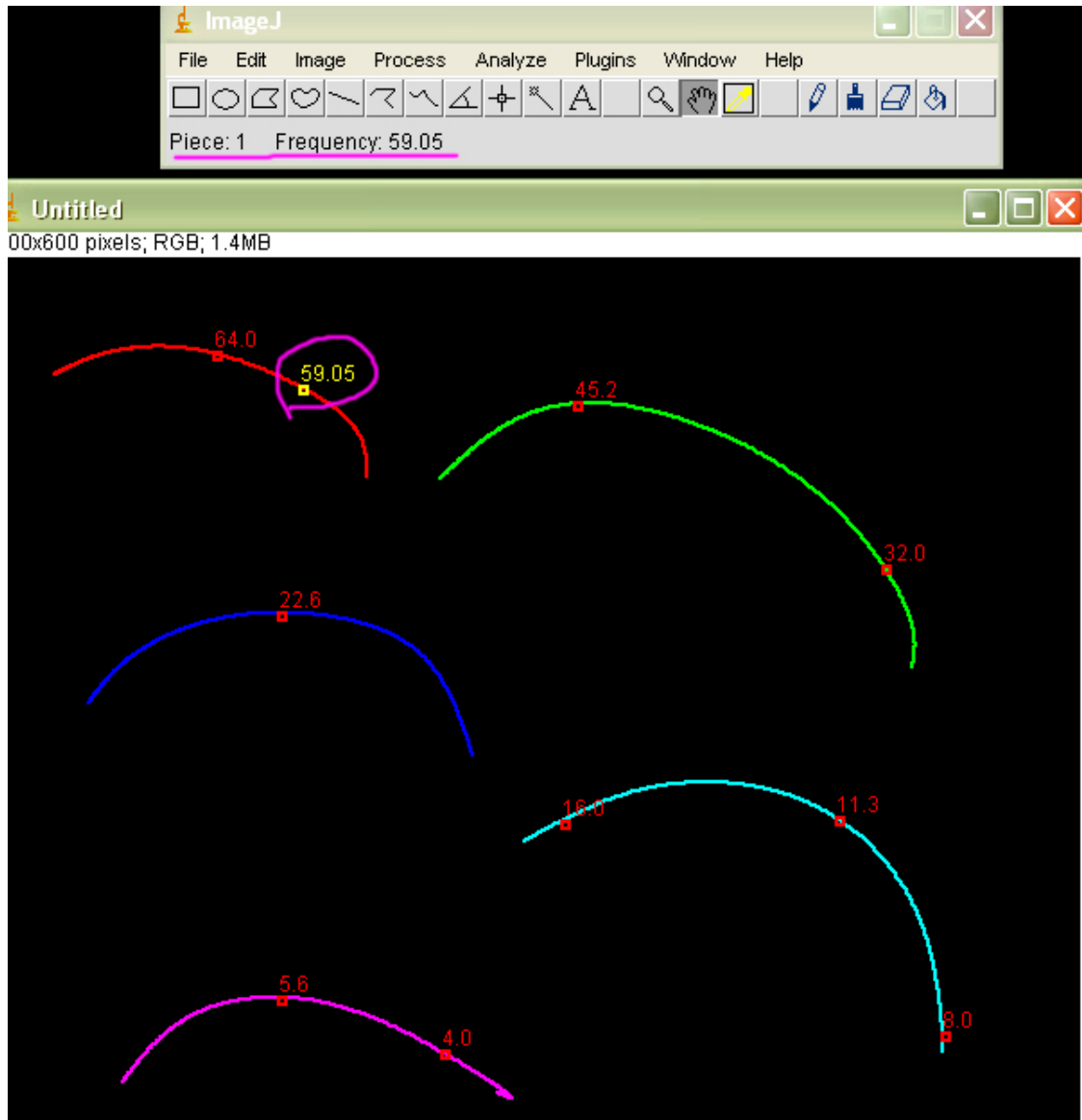


Figure 7. Pointing mode.

Attachment A: How to perform calibration

1. Before doing calibration, make sure you have a scale bar on the image that you would like to calibrate.
2. Open the image in ImageJ. Using the "Straight Line" tool to draw a line along the scale bar.
3. Go to Menu "Analyze/Set Scale", a calibration window will pop up. Input the Known Distance, which is "1" in the example, and the Unit of Length, which is "mm" in the example. (Fig. 8). Click "OK", and the calibration will be set to the image. If you check the "Global" check box, the calibration will be set to all the images opened during this execution of ImageJ.

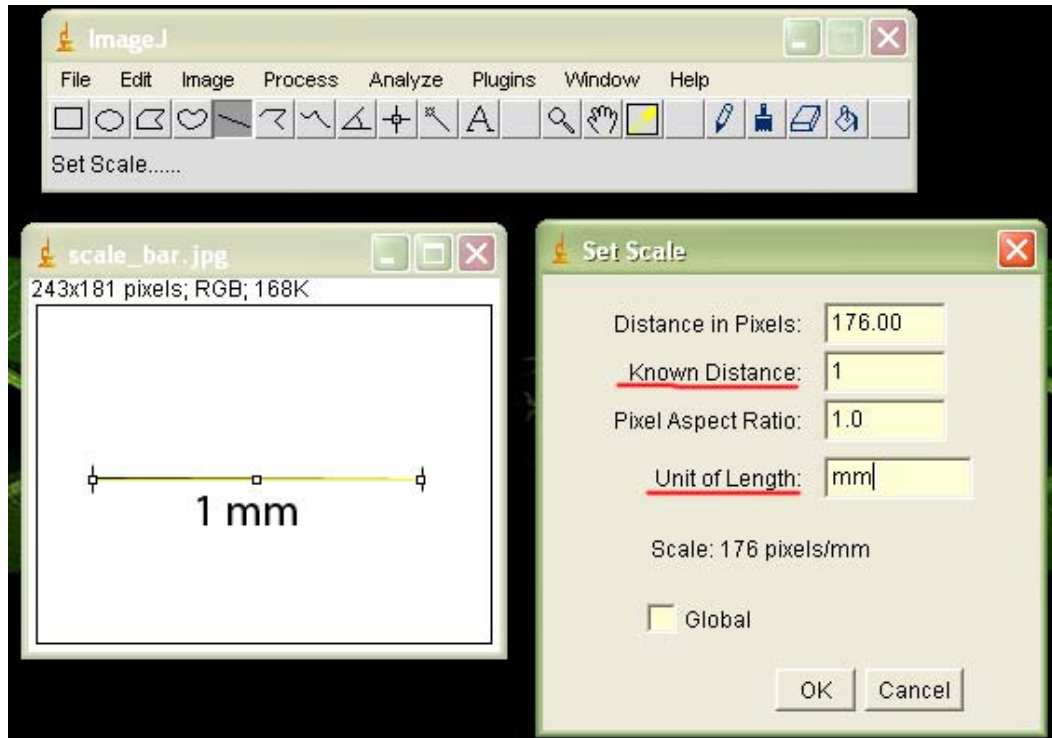


Figure 8. Perform calibration.