Appendix C: User manual for performing image analysis in experiment of monitoring E-coli growth

ImageJ user manual

A. Recommended Browser for ImageJ

Browser	Version
Internet Explorer	5+
Google Chrome	3
Mozilla Firefox	3
Opera	4.x
Safari	4.x

B. Setting up the Browser

1. Java Runtime Environment (JRE) is required for ImageJ. Access "http://imagej.nih.gov/ij/download.html" and click "Agree and Continue"to ensure that you have the recommended version of Java installed on your computer. Otherwise, download Java Runtime Environment.
   Link : http://www.oracle.com/technetwork/java/javase/downloads/jre8-downloads-2133155.html

2. Open "Control Panel" and select "Program" → "Java" , "Java Control Panel" will pop up as shown in Fig.B1.
3. Select "Security" → "Edit Site List...".

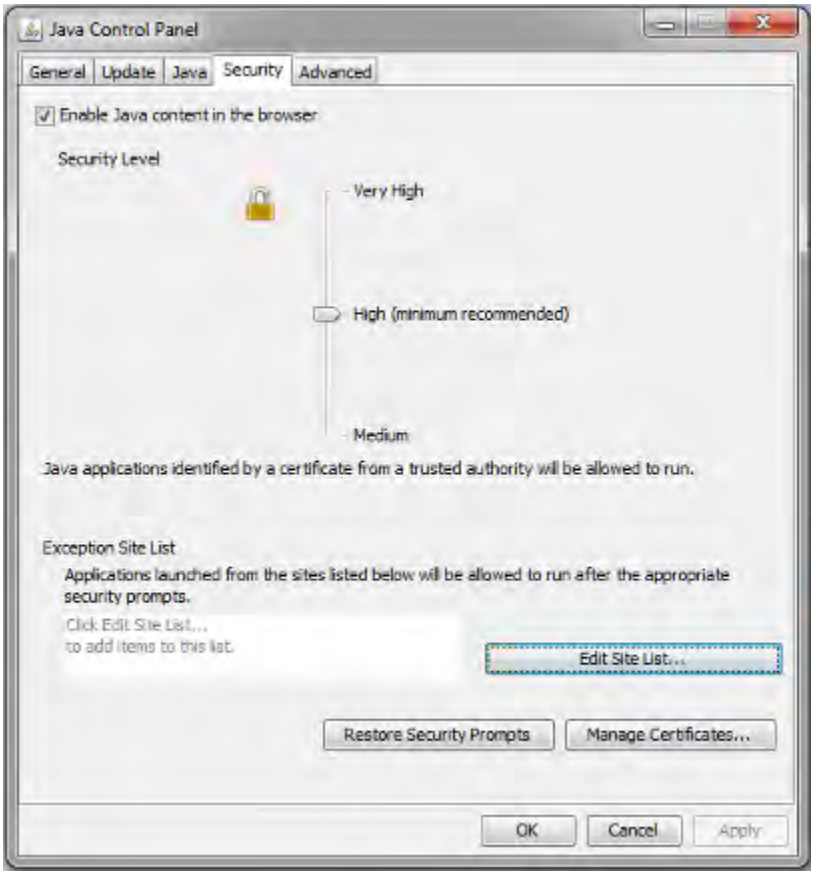

Fig.B1

4. Add "http://imageja.sourceforge.net/applet.html" as shown in Fig.B2. Warning pop-up as Fig.B3 is shown and click "Continue" allows this web-page in the exception list, then click "OK".

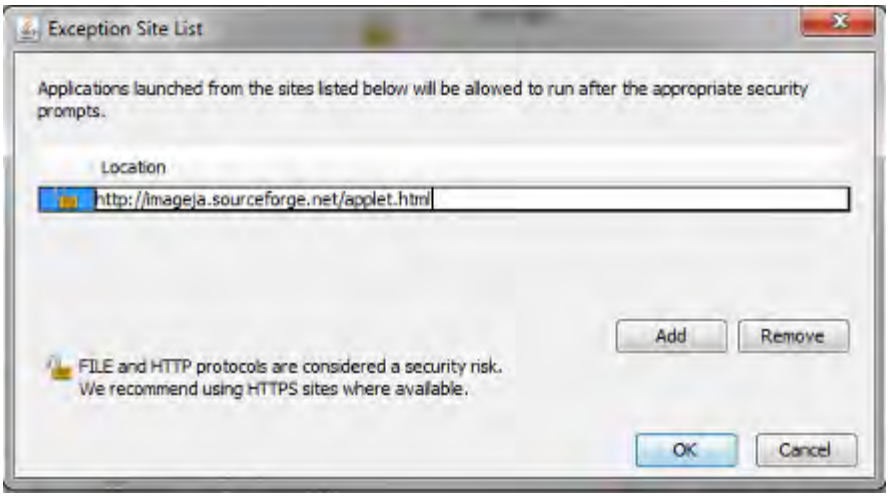

Fig.B2

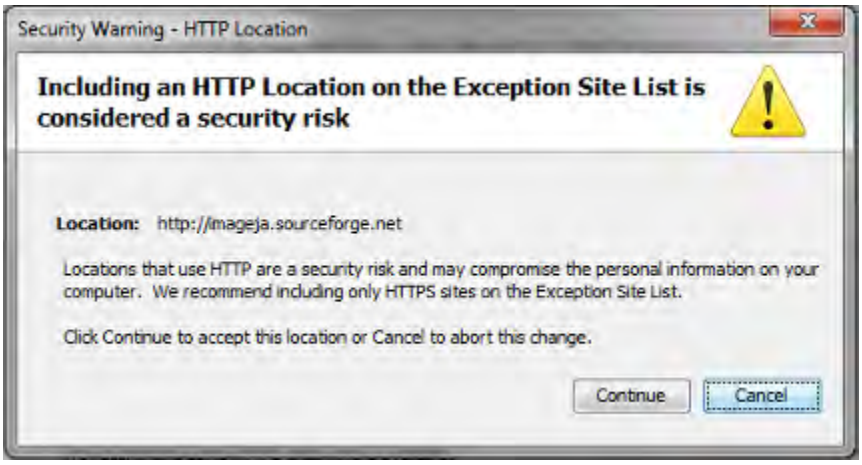

Fig.B3

5. Enter the experiment page, you may see a security prompt as shown in Fig.B4 from Java. Select "I accept the risk and want to run this application." and click "Run". The pop-up "Open URL" shows, click "x" or "OK" to ignore this error. "ImageJ Applet" interface is shown.

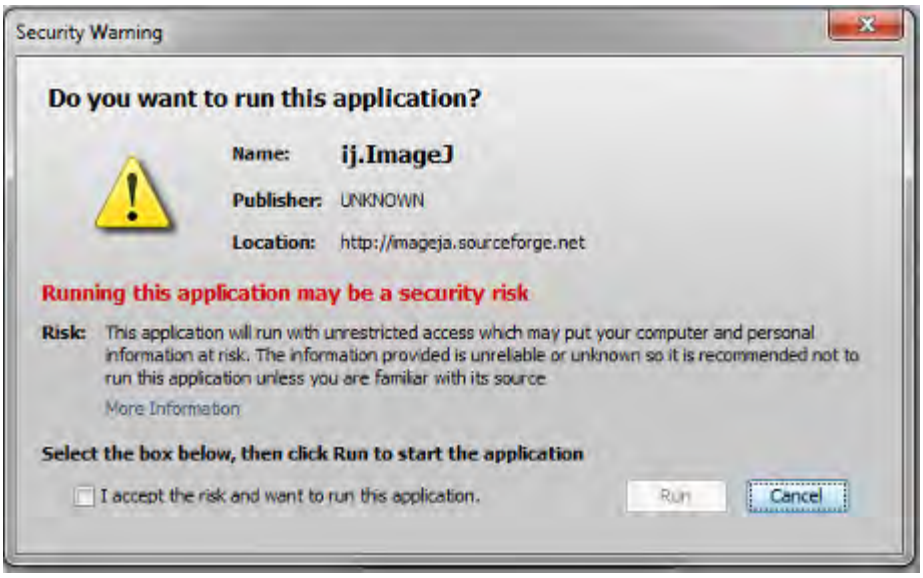

Fig.B4

**Remark : If you want to use the applet version of ImageJ should open in a separate "ImageJ" window, just repeat step 2 to 4 but add "http://rsb.info.nih.gov/ij/signed-applet/" to the exception list or Download ImageJ according to the operating system of your computer.*
Link : http://imagej.nih.gov/ij/download.html

C. Set scale bar in ImageJ

The scale must be set, otherwise we may not know the actual size of cell.

1. Download the image for calibration here.
   Link :
   http://158.132.254.133/petridish/images/experiment/interference/labmanual/reference.jpg.

   Start ImageJ and open the image.

2. Select the straight line selection tool and draw a straight line that defines a known distance on your calibration image (1 cm in this case).

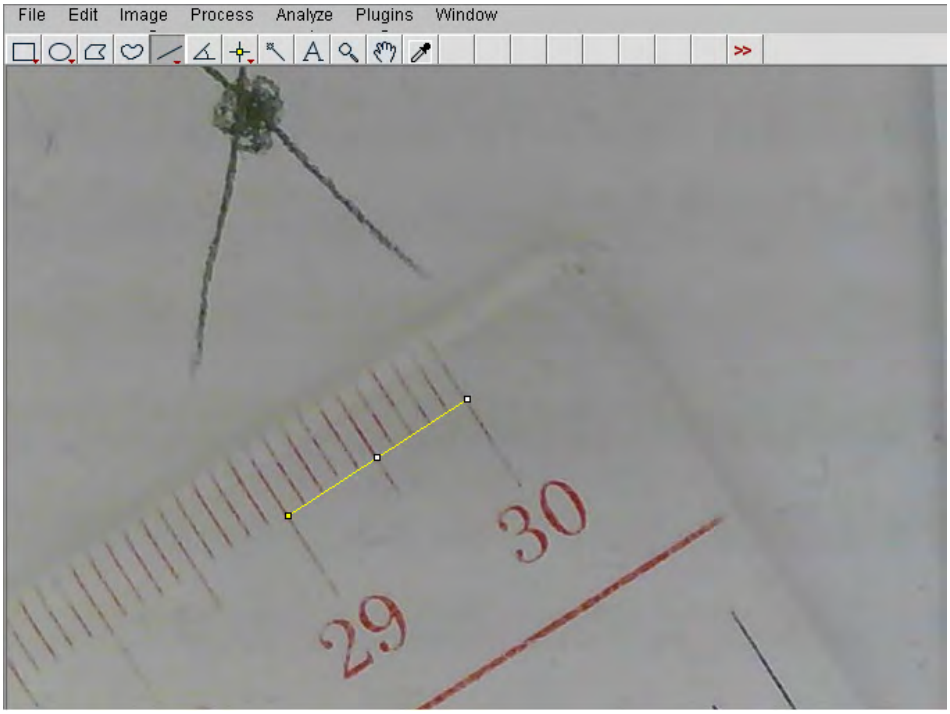

Fig.C1

3. In the "Analyze" menu, select "Set Scale". The following dialog box will pop up:

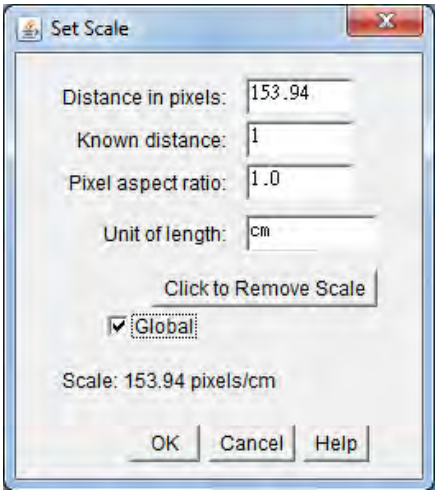

Fig.C2

4. Input the "known distance" (1cm in this case) without units and define the units of length in the "Unit of Length" field. Click on "Global" so that this calibration applies to all images that you open in this ImageJ session,the result can be shown in Fig.C7. Click "OK". After that the actual size of the object can be measured.
5. The distance in pixels will be recognized by ImageJ according the length of a straight line which you have drawn, the example is shown as Fig.C3.

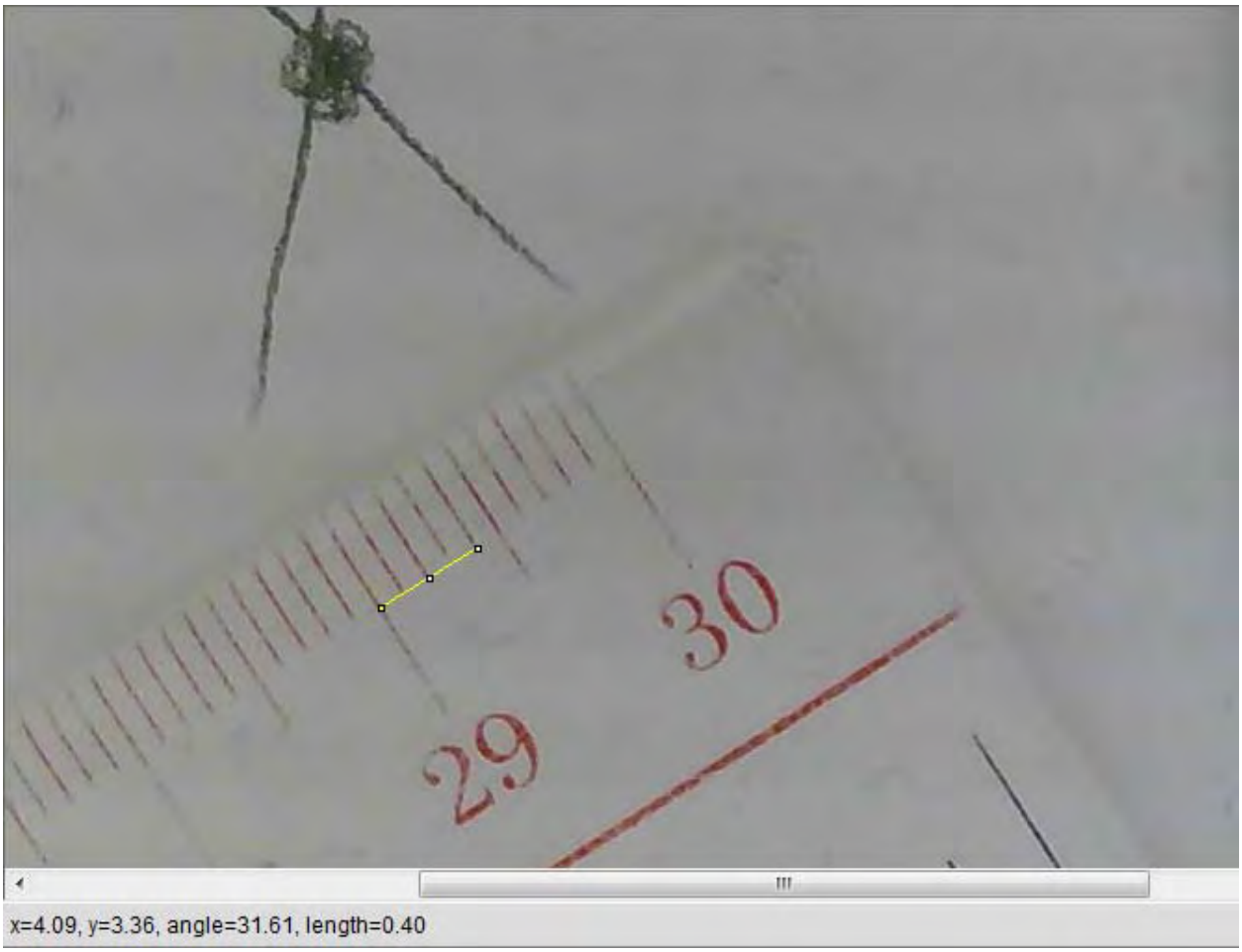

Fig.C3

6. You can set the scale bar by select "Analyze" → "Tool" → "Scale Bar". "ScaleBar Plus" dialog will pop up and a scale bar will appear on your image.

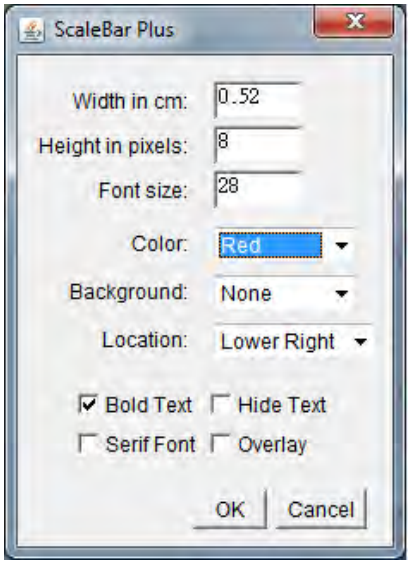

Fig.C4

7. You can modified the size, color and location of the scale bar. Click "OK" and save your image.

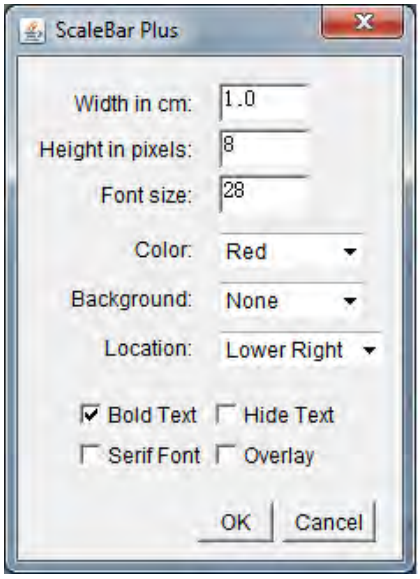

Fig.C5

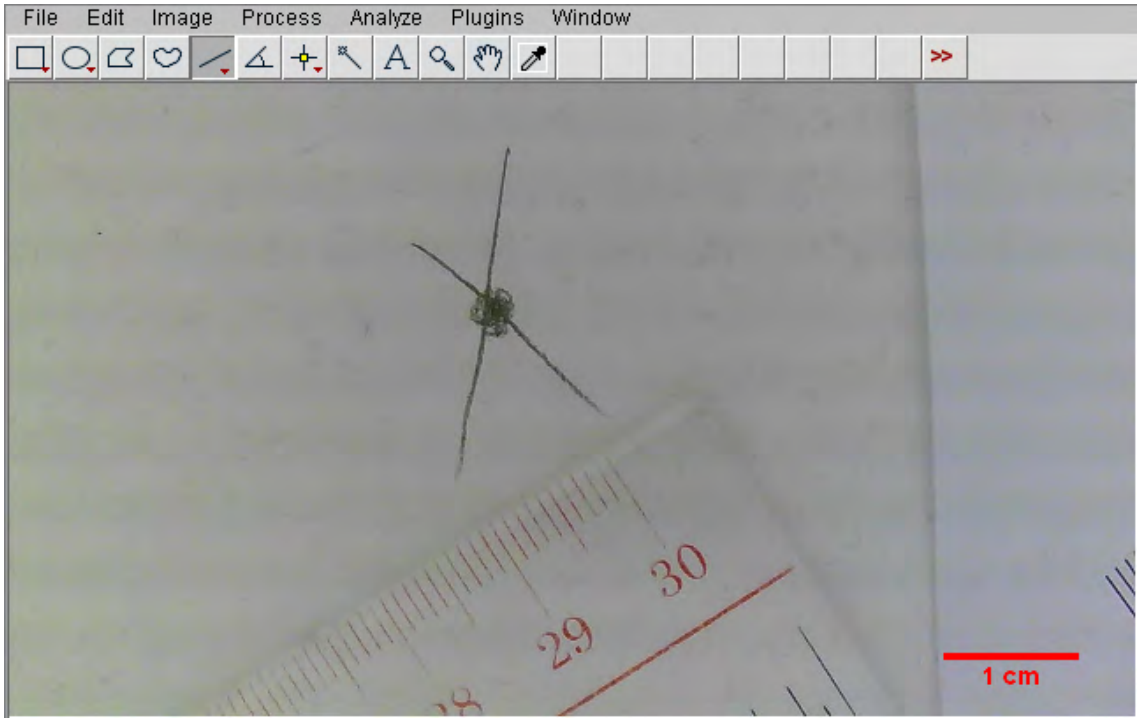

Fig.C6

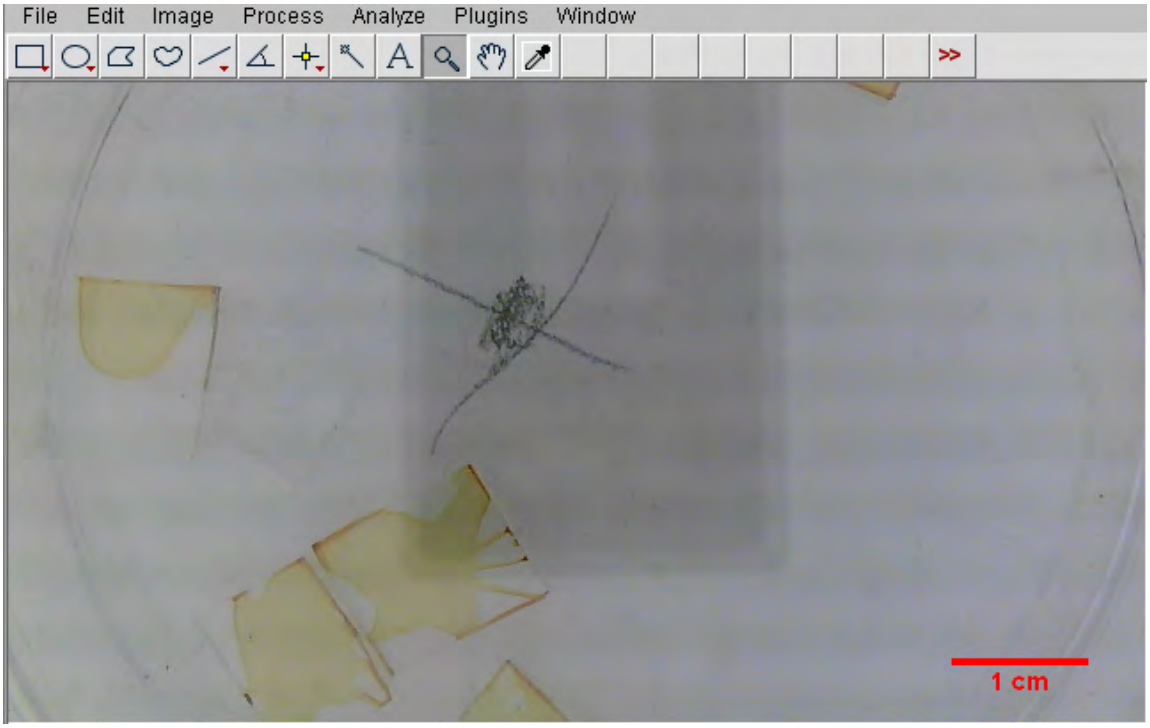

Fig.C7

D. Automated Counting of Single Color Images

1. Open the specified image to be processed, If it is a color image (RGB), as in Fig. D1, it has to be converted into a greyscale image. Check that you have set Edit Options Conversions to “scale when converting.” Then use Image Type 16-bit to convert to greyscale.

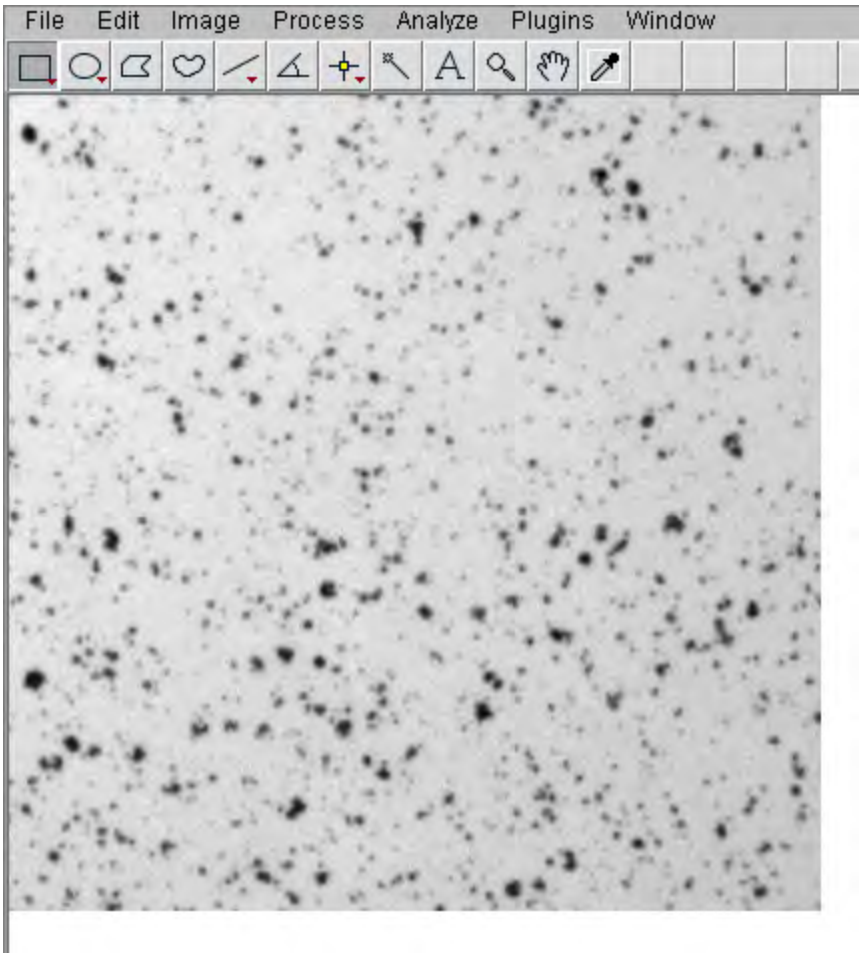

Fig.D1

2. Once the image is in greyscale use "Image" → "Adjust" → "Threshold" (Ctrl + Shift + T) to highlight all of the structures you want to count. To highlight, either use the sliders or use the “set” button to type in a known range of pixel intensities (if you want to preset the threshold for a whole set of images the same way, for instance).

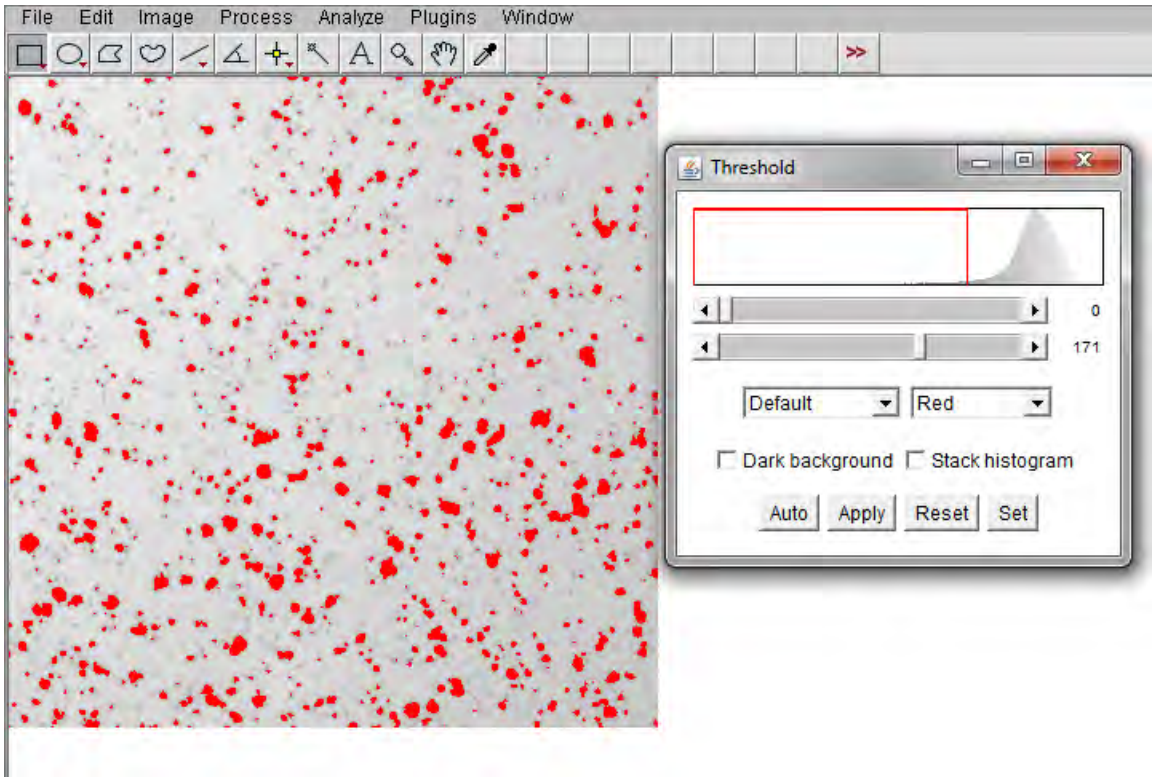
File Edit Image Process Analyze Plugins Window
Threshold
0
171
Default
Red
Dark background
Stack histogram
Auto
Apply
Reset
Set

Fig.D2

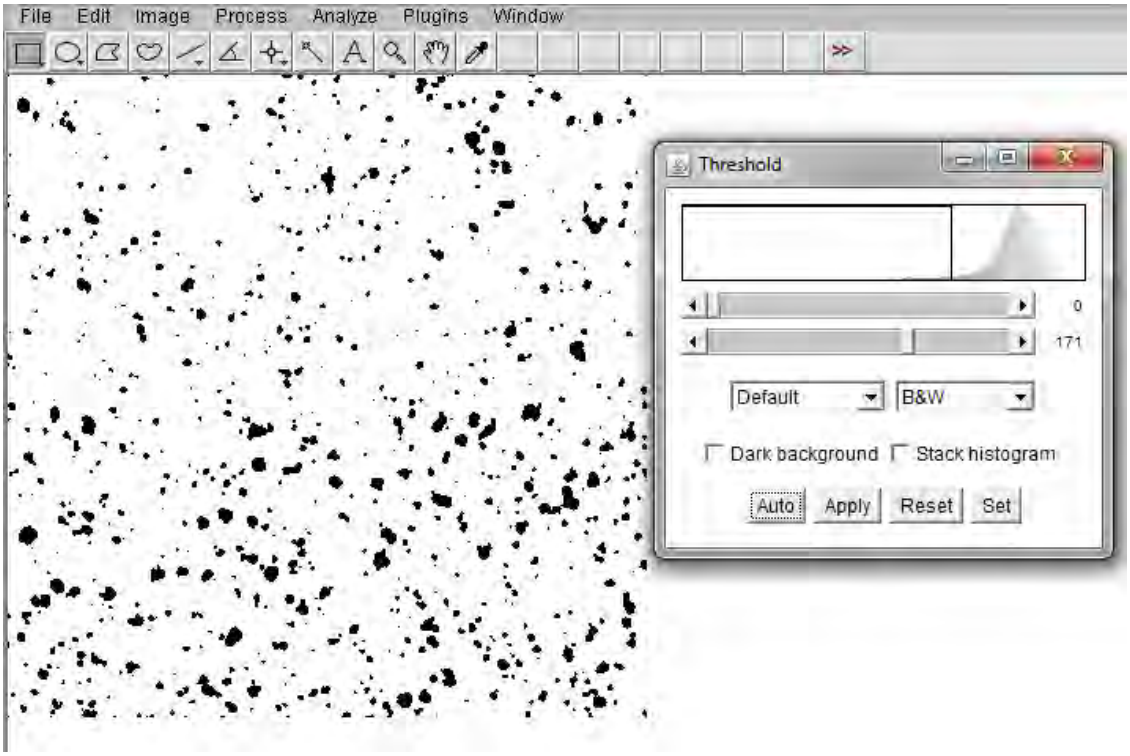
File Edit Image Process Analyze Plugins Window
Threshold
0
171
Default
B&W
Dark background
Stack histogram
Auto
Apply
Reset
Set

Fig.D3

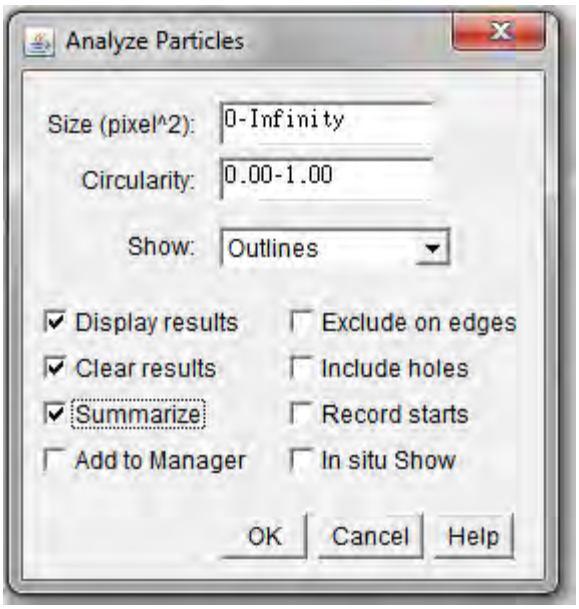

Fig.D4

3. If you have particles that have merged together, Process Binary Watershed can often (but not always) accurately cut them apart by adding a 1 pixel thick line where it feels the division should be. The example as Fig.D5a has been thresholded, turned into a binary image with “apply” and then run through the watershed program,the result is shown as Fig.D5c. For more information on other binary image tools, such as fill holes, see the Menu Commands section of the ImageJ Documentation page.
   Link : http://rsbweb.nih.gov/ij/docs/index.html

Fig.D5a Original image

Fig.D5b Thresholded image

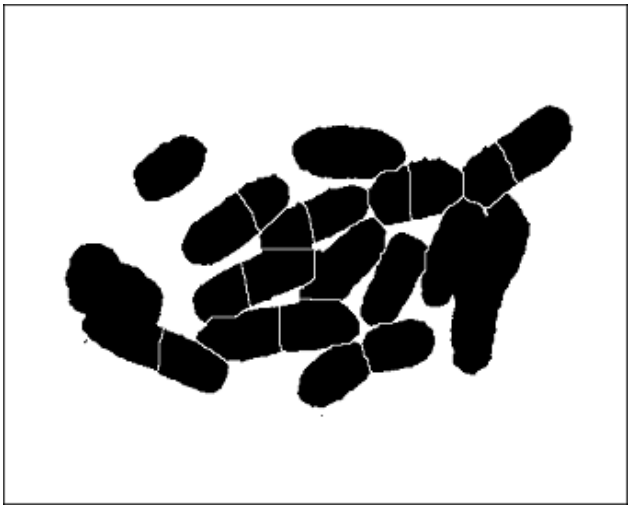

Fig.D5c Watersheded image

4. Once you have a binary image of the particles you wish to count, go to "Analyze" → "Analyze Particles". There are some choices here that can affect the counts from your images. Size will affect what size particles to count. It will either be in pixels, or, if your image is calibrated, in a unit of measurement^2 (check under Image → Properties (Ctrl + Shift + P).

5. To count all particles, leave it at the default of 0 – Infinity. If you are getting too many small “noise” pixels counted as objects, or you want to exclude particles based on size, adjust these numbers. Circularity excludes particles based on how close to perfectly round they are. To include everything, keep at the default 0.00 – 1.00. To exclude things, adjust these numbers, keeping in mind that 1.00 is a perfect circle and 0.00 is a straight line.
6. To get other information from the image besides just area, go to "Analyze" → "Set Measurements".
7. Check the boxes next to the information you want. For an explanation of any of these parameters, see the ImageJ documentation page.
   Link : http://rsbweb.nih.gov/ij/docs/menus/analyze.html#set
8. The information about the counted image as shown in Fig.D6 (such as total number of elements, total area, average size) can be obtained in “Summary” dialog box. "Result" dialog shows the size of each element.

9. All of the result can be saved "File" → "Save as". The type of data can be saved as text file or CSV (Comma Separated Values) format.

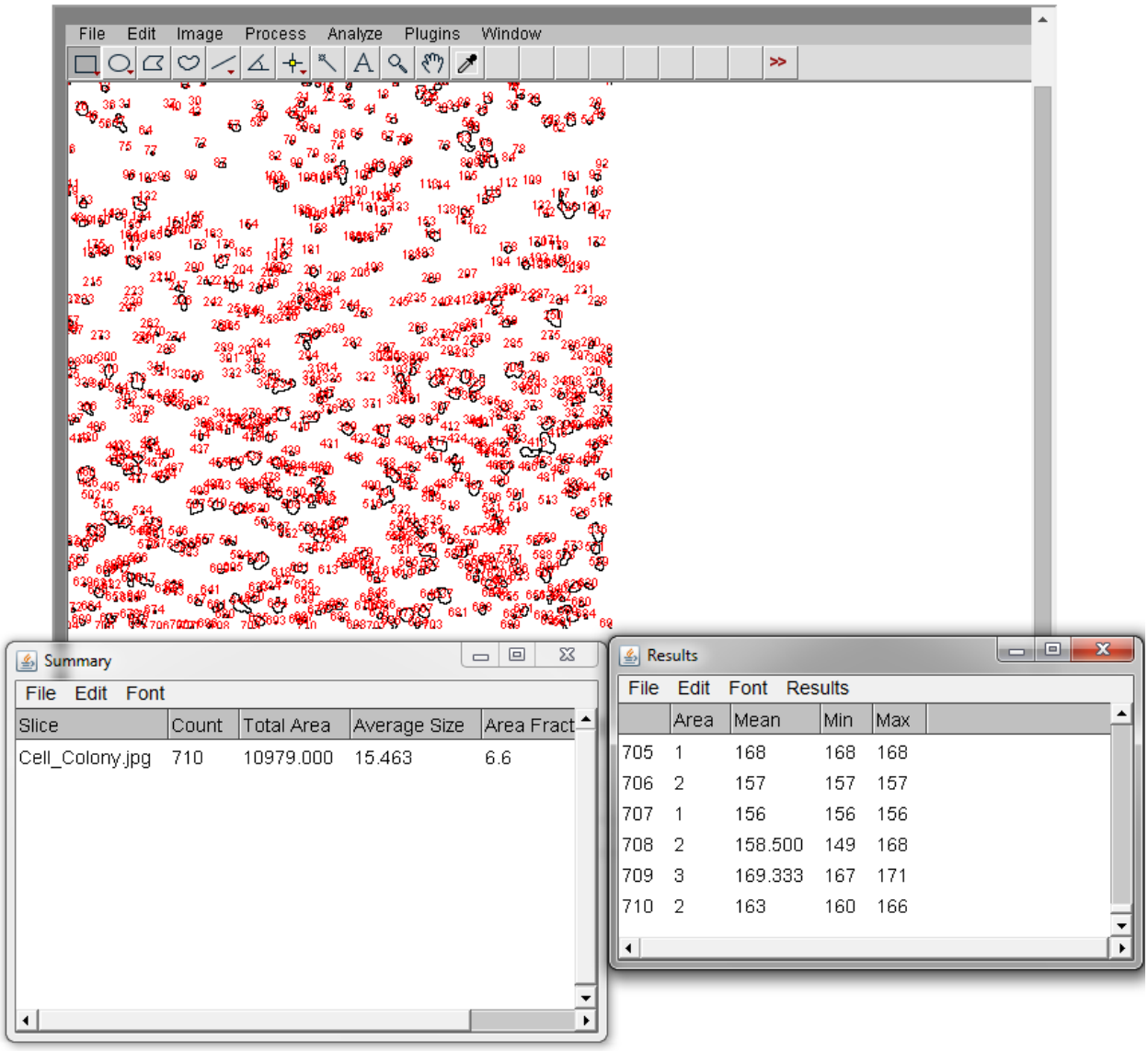

Fig.D6

E. References

Useful Websites

How do I enable Java in my web browser?

Two Ways to Count Cells with ImageJ

ImageJ

How to count cells using ImageJ - YouTube

Adding scale bars to images using ImageJ