

Instructions to operate the software ImageJ® software

1. Open the ImageJ® software.
2. Select File > Open from the drop down menu.
3. Select the image file to be analyzed.
4. Trace a line over your scale bar. Select Analyze > Set scale. Fill in the "Known distance" box for the length of your scale bar, and configure the "Units." Tracing the line over the scale bar tells ImageJ® how many pixels correspond to a given distance (the length of your scale bar). This enables the software to determine the size, and hence the area, of different features that appear in your image.
5. Click Image > Adjust > Threshold. For the Threshold Color, select B&W. Adjust the Hue, Saturation, and Brightness levels until you obtain your bacterial colonies in black and the background agar in white.
6. From the toolbar, select the "Circle" image. Draw a circle around the edge of the Petri dish to define the precise area from which the software will measure the bacteria.
7. Next, from the Analyze menu, select "Analyze Particles." Set "upper limit" size to 4 cm² (generally bacterial colonies would not be larger than 4 cm²). "Circularity" should be 0-1, and select Nothing from the "Show" drop down menu. Select "Summarize" from the checklist. Click OK.
8. The Summary window opens and provides data on the area of the Petri dish that is covered in bacterial colonies. This data is found under the column "Total Area," and is given in square units of what you entered in step 5. The Summary window also displays the "Area fraction." Students may record the area fraction (given as a % of the total area measured) and use this as their data for the amount of the Petri dish covered by bacteria.
9. If you chose, you can save the adjusted photo by File > Save As > "new name."