

Using Image J in Your Laboratory Course

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Introduction

An image is more than just a beautiful picture. For scientists, images contain a wealth of information. Image processing can complement many of the current biology laboratories. Image J is a free, easy to use image processing program, developed by the National Institutes of Health, widely used in research applications. Image J also supports standard imaging processing functions allowing students to crop, manipulate contrast or subtract background. Java plugins and macros have been developed by Image J users to expand the functionality of the program. Multiple tutorials as well as YouTube videos are available to help users with almost every aspect of Image J. At the University of California Irvine, Image J is used in the Developmental and Cell biology labs to give students an appreciation for microscopic quantitative techniques. Students analyze their own images and images obtained from research. Measurements, conversion from pixels to micrometers, area determinations, density profiles, histograms and particle counts are typical Image J applications. The mini-workshop presented during the 2014 ABLE conference is similar to material presented to undergraduate students in a scientific writing course that is a prerequisite to all biology laboratories.

Objective

Students in biology laboratory courses frequently use microscopes to visualize experimental results but rarely analyze the results. Introducing students to Image J, a free simple image processing program, can promote quantitative skills into the visualization process. These skill can be later used by the students in research laboratories.

Materials

Image J program downloaded from <http://rsbweb.nih.gov/ij/>. The Image J program can be downloaded into a flash drive to facilitate distribution.

Set of practice images. Practice images should include an image of an object with a centimeter ruler (Figure 1), a microscope image of a stage micrometer with a scale bar included, (Figure 2) images of objects seen under the microscope at the same magnification as the stage micrometer slide (Figures 3-5), image with numerous items to count (Figure 6) and an image of a gel (Figure 7).



Figure 1. Photograph of a trilobite fossil with a centimeter ruler.

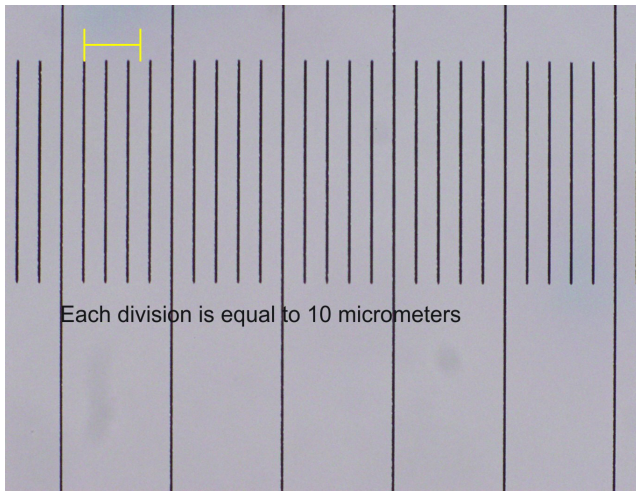


Figure 2. Microscope image of a stage micrometer taken with a 40x objective lens. Each division of the stage micrometer is equal to 10 micrometers. Scale bar is equal to 250 micrometers.



Figure 3. Microscope image of *Echschozia californium* (California poppy) pollen germinating. The image was taken with the same objective lens and microscope as the image in Figure 2.

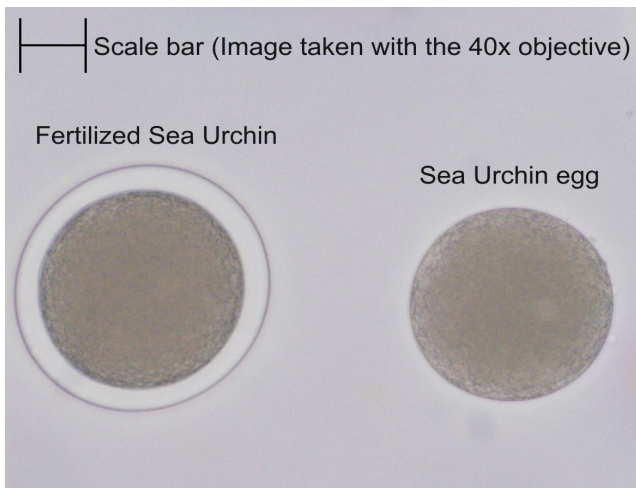


Figure 4. Microscope image of a fertilized (left) and an unfertilized sea urchin egg. The image was taken with the same objective lens and microscope as the image in Figure 2.

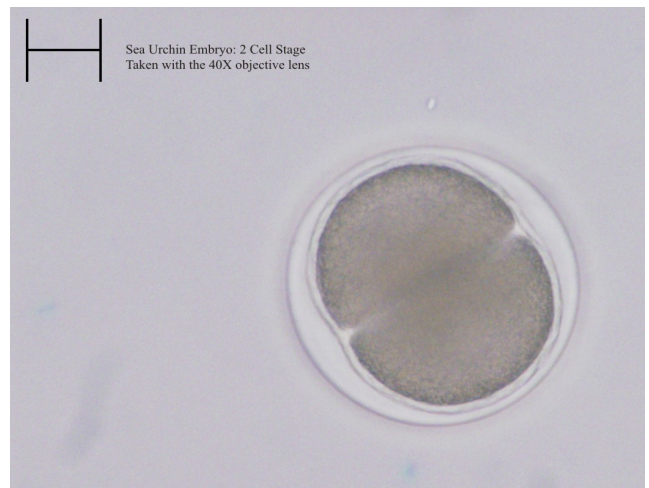


Figure 5. Microscope image of a sea urchin embryo at the 2 cell stage. The image was taken with the same objective lens and microscope as the image in Figure 2.

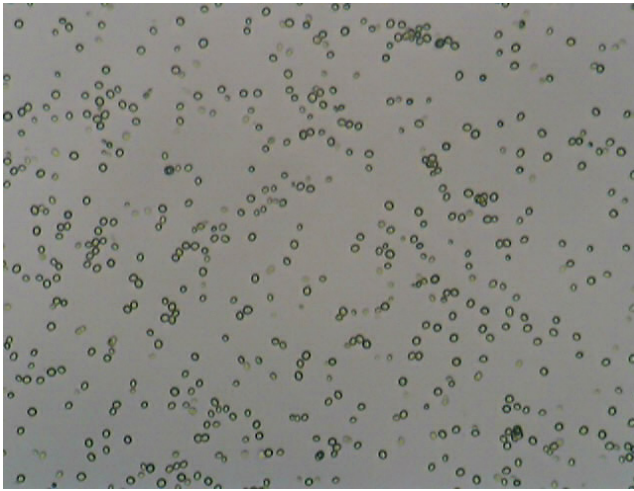


Figure 6. Microscope image of *Saccharomyces cerevisiae* in a solution of water.

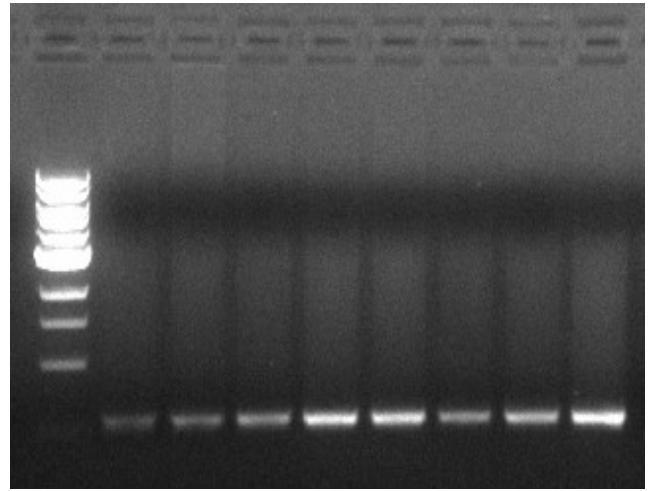


Figure 7. Electrophoresis Gel of PCR products.

Methods

The Image J commands for each of the process are outlined below. An image must be opened in Image J for the program to function correctly. The Image J program window consists of two bars, a menu command bar with pull downs and a tool bar. The menu commands bar consists of the major commands: File, Edit, Image, Process, Analyze, Plugins, Window and Help. Mac based computers will have the Menu command bar at the top of the screen. PC based computers will have the command bar and the tool bar attached somewhere within the screen.

Change Line Color and Width

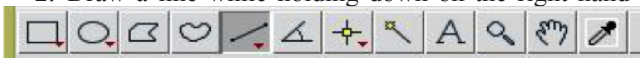
The default line width is one and the color is yellow. For some images, either the line width or the color may need to be change to allow for better visualization.

1. *Edit > Options > Line Width*
2. *Edit > Options > Colors > Selection*

Length Measurements

Length measurements are the most common measurements made in most biology laboratory activities. If the measurement is for a straight object, the Straight Line Tool should be used. The Segmented Line Tool is used for measurements of nonlinear objects. The Freehand Line Tool is available from the pull down menu of the Straight Line Tool with a icon of a wavy line instead of a straight line. Figures 1, 2, and 3 should be used to demonstrate length measurements. Figure 3 will require the use of the Freehand Line Tool.

1. Select Straight Line Tool.
2. Draw a line while holding down on the right hand



mouse button. (Ctrl click for Mac)

3. Click on *Analyze > Measure* (Shortcut key: Ctrl or Command M)
4. Length in pixels will be displayed in a Results window (window may be hidden behind image)
5. Other Line tools are Segmented and Freehand line tools (Right click to see line choices)

Set Scale

The default measurement value is in pixels. If the image has a ruler, stage micrometer or scale bar, the pixel values can be converted into metric measurements. Figure 1 of the Trilobite includes a centimeter ruler. Figure 2 is of a stage micrometer with the distance between each interval equal to 10 micrometers. Figure 3 of the California poppy pollen germination was taken with the same objective lens as figure 2. The value for the scale bar can be determined for Figures 3, 4 and 5 from figure 2. It is important to have students confirm their measurements. A simple confirmation that the set scale is working correctly is to measure the distance between 4 and 5 on the centimeter ruler in figure 1.

1. Select Straight Line Tool
2. Draw a line while holding down on the right hand mouse button across a ruler or scale bar.
3. Click on *Analyze > Set Scale*
4. Click on Global (Need to confirm scale next time you open a new image with a different magnification)
5. Confirm scale is correctly set by measuring ruler.

Area Measurements

Area measurements are similar to linear measurements. The most common tool used is the Freehand tool (icon is a heart shaped object). Figure 4 and 5 should be used for area measurements.

1. Use *Selection* tools (Rectangle, Oval, Polygon, Free-hand)



2. Same procedure as line measurements

Analyze Particle

Analyze particles can be used to count cells under a microscope or bacterial colonies on a plate. The image does not need to have a scale bar or ruler. The results from the analyze particles will include the total number of particles and the size and intensity of each particle. Figure 6 should be used for analyze particles.

1. Convert image to 8-Bit (*Image > Type > 8-bit*)
2. Remove background (*Process > Subtract Background*)
3. Determine particles of interest (*Image > Adjust > Threshold > Move bar until the particles are outlined > Apply*)
4. Click on *Analyze > Analyze Particles > Display Results > OK*
5. Results window will appear with a list of all the particles (Area, Minimum and Maximum intensity)

Region of Interest Manager (ROI Manager)

The ROI manager is valuable if you wish to analyze only a part of the image rather than the complete image. You can select more than one ROI for your analysis.

1. *Analyze > Tools > ROI Manager*
2. Use an *area tool* to mark region
3. *Type T* (Short cut for *Edit > Selection > Add to Manager*)
4. Repeat for each ROI

Gel Band Intensities

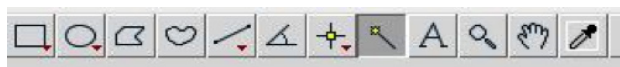
Gel band intensities can be used to determine the relative intensities between two or more electrophoresis bands or between the staining intensity between different regions of a cell or tissue. Figure 8 should be used for gel band intensities measurements.

1. *File > Open*
2. Enlarge using *Magnifying Glass* (or *Ctrl + or _ Keys*)
3. *Image > LookUp Table > Invert*
4. To highlight bands use the Rectangular tool
5. **Select by Command 1 (Mac) PC Ctrl 1**
6. Click on *Analysis > gel > gel analyzer option*
7. Uncheck invert peaks

8. Click on *Analyze > gel > plot lanes*

9. *Straight line tool*- isolate each peak

10. Use the peak *Wand tool* and click on each peak. Results will appear in a Results window.



11. Click on *Analysis > gel > label peaks* and the percentages will appear in the Results window.

Notes for the Instructor

Image J is relatively easy to use by students with little oversight. Students are given the accompanying PowerPoint to help them work through the set of images using the screen shots as part of an introduction to data analysis. Students are given a practice quiz (using figure 1-7) to help them determine if they are having any problems using image J before they are given a class quiz. The most common student errors are reporting an area measurement when doing linear measurements. The results table will include both an area and a linear measurement because the line used to determine the line has an area. Using the results from the practice or class quiz as the data to use when discussing the variability in class data has additional value. Student routinely use Image J for every lab activity in the UC Irvine Cell and Developmental laboratory and have reported they have used Image J in microbiology, molecular biology labs and their own independent research. The set of practice images were chosen to reflect the needs of the above mentioned laboratories. Area measurements of the eggs and embryos further demonstrate that the early embryos does not change its overall size during the early cleavage events. Different sets of images could be designed that are more closely related to other laboratory experiments.

Literature Cited

Documentation. Documentation. N.p., n.d. Web. 1 Oct. 2014. <http://rsb.info.nih.gov/ij/docs/>.

About the Author

Debra Mauzy-Melitz is an assistant teaching professor in the Department of Developmental and Cell Biology, UC Irvine. She teaches Scientific Writing and Developmental and Cell Biology laboratory. She received her degree in Developmental Molecular Genetics from Marquette University, Milwaukee, WI.

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