

Image Analysis

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A longitudinal study is one that allows the researcher to follow a certain group or specimen and track changes over time. Of importance when performing these experiments is to minimize artifacts in the experiment while taking measurements. When examining cells, phase contrast microscopy is often used, which allows the observer to watch a cell or group of cells over time. In order to study bacterial colonies in culture with time, a method must be used that has a similarly minimal effect on cell growth. It would be disadvantageous to add a dye or stain to the cells and introduce an artifact such as poisoning the cells. Instead, a dissecting scope can be used to with relatively low light intensity to capture images at certain time points along the life of the cell culture. Those images can be compared to determine what if any differences occurred over time or across groups.

While qualitative analysis can elucidate many characteristics of cell culture, it is often important to quantify those changes. Image analysis is a tool used in research to perform that function for a characteristic of an image. If cells were cultured on an agar plate, over time the number of cells would increase. However, in order to determine the amount of that increase, one could look at the area that the cell colonies occupied, or the size of a specific colony. While it is possible to graphically approximate the area of occupation, there are software packages available that automate this process. One such program, Image J, is available for general use and was developed, and is being continually updated by the National Institute of Health (NIH). The program is powerful, yet very simple in layout. It can be downloaded from the NIH website: rsb.info.nih.gov/ij/. Click on the program for your operating systems and follow the instructions to download.

Also on the website are a series of PDF help files. These give step-by-step instructions for various functions within the program. A simple area analysis may be useful for cells cultured on plates in various experiments, but many more analyses are available with Image J, such as directionality, and circularity, among others. These help files are very useful in trouble shooting problems as well as determining novel methodologies for studying cell cultures.

Image Capture

Dissecting Scope

The dissecting scope is a microscope used to observe objects near or just above the magnification of the unaided eye. To use the scope and the camera, open the Motic Image Plus software located on the desktop. Go to the file tab and open the image capture window. Click on the camera icon. This should bring up the viewing window. Be sure to place a white piece of paper under the Petri dish with a 1 cm line drawn on it in black that that it shows through the agar. The line should be away from the bacterial colony of interest. This is for scaling purposes during analysis.

The image may appear black. There is a toggle switch midway up the back of the scope. Switch it to the left position while looking at the front of the scope. If the image still appears black, turn on the light source and increase the light until the image is lit. Note that the box labeled “auto exposure” must be checked. Otherwise, the camera may not pick up the image. Use the knob on the front of the scope to set the magnification to the lowest setting. Then use the large knob to the rear of the scope to focus the image. Readjusting the light may be necessary at this point to reduce shadows. It is also important to use enough light to adequately illuminate the plate, but not so much as to bleach the image white. An example of a good image is shown in Figure 1 below.

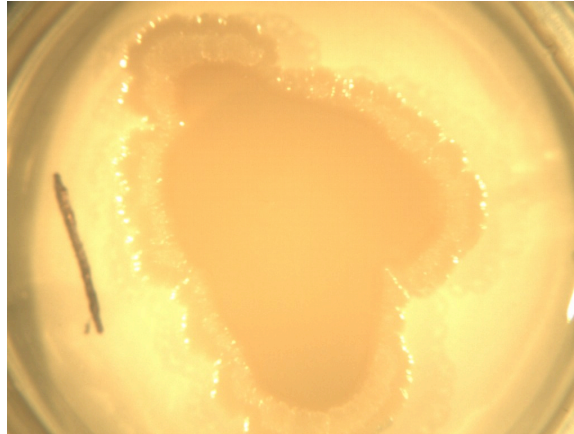


Figure 1: A cell culture was obtained with the dissecting scope. Notice the scaling line on the left hand side.

Once a good image is obtained click the capture image button in the program. Close the image capture window. The captured image will appear on the left of the screen. Click on the image of choice to open it. Save the image under a meaningful file name to be analyzed later. JPEG format works well for Image J. Repeat this process for as many images as are desired and transfer the files to your computer.

Scanner

The scanner may also be used to capture images. In addition to capturing an entire Petri dish, it is useful in producing good quality images of dried PAGE gels. To capture an image, click on the scanner icon on the desktop of the computer and click next. Position the dried gel on the scanner bed. It is helpful to put a book on the gel to flatten out any wrinkles in the gel. At this time preview the image. The gel can then be repositioned as necessary. In the preview window highlight only the gel. This will decrease the scanning time, and post processing required. Scan the image as a color document. Figure 2 shows an example of a scanned gel.

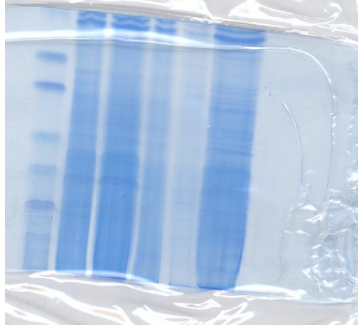


Figure 2: A scanned gel is shown. The image can be further prepared with any imaging program.

Analysis

Once the desired images are obtained, they can be analyzed. Open the image of choice in ImageJ. The first step is to scale the image. To accomplish this, there are several shape buttons on the left hand side of the menu. Choose the line symbol and draw a line on the 1 cm line that was placed in the image for scaling (see above). Then go to Analyze>set scale and set the known value to one centimeter and check the “global” box. The image then needs to be converted to grayscale. This can be done by going under the image tab > type> 8 bit. This puts all pixels of the image on the same color scale. Depending of the type of bacteria and the particular method of seeding, the bacteria may be lighter or darker than the background. It is important to have the bacteria darker. If they are lighter the image can be inverted so that dark areas become light and vice versa. Inversion of the image can be accomplished by going to Edit>invert. This step is not necessary if the bacteria are already darker than the agar.

Next crop the image. Use the circle selection tool to choose the area of interest. Then go to Edit>Clear outside. Be sure to eliminate the dish edges. A grayscale, cropped image is shown in Figure 3.



Figure 3: The image from Figure 1 has been converted to grayscale and cropped, leaving only the bacteria and nearby agar.

The program works by looking for a threshold. When determining area, points above the threshold it counts and points below it disregards. In order to make the threshold clear

the image is transferred into a binary (black and white) format. This is where you must distinguish between the points that are relevant and the ones that need not be counted. This can be done by adjusting the threshold of the image. Go to Image>Adjust>Threshold. Use the sliders to highlight only the bacteria. After thresholding the image, there may be some artifacts from reflections or shadows, which can be removed by using the paintbrush tool. White can be chosen by using the eye dropper tool and selecting a white portion of the picture. Care must be taken here not to introduce any error by eliminating bacteria colonies. Carefully studying the original image will aid in this step. The final prepared image can be seen in Figure 4.



Figure 4: The image has been reduced to a binary representation with the bacteria in black and the background in white.

In order to obtain the area measurement, use any of the selection tools to surround all of the black pixels (bacteria) of interest. Then use the Analysis>Analyze Particles option. Check the summary choice in the window. An area will appear in a new window. This can be exported to and analyzed in any statistical software for differences between time periods or treatment groups.

Reference:

rsb.info.nih.gov/ij/