Hand counting bacterial plates is a tedious task and the color blind may not be capable of performing this task at all. However, processing the image with computer software such as ImageJ could speed up the process and eliminate issues associated with color blindness.

**ImageJ Procedure**

After sample incubation, the plates were photographed using the macro setting on a Canon Powershot A470 (7.1 mp) camera. The macro setting improves clarity and resolution of the base image. The digital images of the Coliform plate were then cropped using Adobe Photoshop so that only the area to be counted remained in the image. This reduces the amount of processing power that the computer and software must utilize to render the image. It also simplifies the image processing procedure by reducing complexity of the image.

The image was then imported into the ImageJ software. The image is then converted from full color to 8-bit black and white also known as grayscale. This is done by converting the image into 8bit under the Image Type menu (Figure 2). By converting to grayscale the image will be comprised of 256 different shades of gray, which allows for easier manipulation and isolation of data.

The colonies of interest are then isolated from the rest of the image using the Threshold Tool of ImageJ’s. This tool is accessed from the Image Adjust menu. The Threshold Tool allows the user to select the range or ranges of gray that are visible in the image (Figures 3 and 4). When counting colonies the image is adjusted to display the shades of gray that correspond to the bacterial colonies of interest.

Once the proper threshold values have been set the Particle Analysis Tool can be used. This tool will automatically identify and count the colonies within the image (Figures 5 and 6). The identification can be adjusted to account for size of the colonies (pixel)’s. In this analysis, size of the colony was not considered. Therefore, even a one-pixel’ colony would be identified and counted.

In addition to the ImageJ counts, the coliform plates were hand-counted independently by both a novice and an expert. The results of ImageJ count were then compared to the hand counts and a percent difference calculated.

The most unexpected result of this project was that the ImageJ counts were typically smaller than both the novice and expert counts. The cause of this is unclear but it may be due to the grid lines on the filter paper interfering with colony identification. This result could be further explored by conducting a series of experiments using unlined filter paper.

Consistent and high quality images are imperative to the effectiveness and accuracy of the ImageJ counts. The thresholding process is the most critical part of this procedure and is heavily impacted by the base image. When the hued and lighting of each image varies, even slightly, different threshold values are required to isolate the colonies on each plate. High quality images are produced by most modern digital cameras that have a macro setting for close range photography and a self-timer. Achieving consistent image quality requires a tripod, standardized background, and non-glare lighting. The lighting is the most important but also the most difficult to achieve. In the next phase of this study, a light box will be used when taking photos. If the image consistency issues can be solved, a standard set of threshold values could be developed for any procedure that requires the identification and counting of colored bacterial colonies.

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