Computer vision-based analysis of *Pseudomonas putida* growth rates

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Introduction

Pseudomonas putida is a species of bacteria found in brook trout intestines, and it produces a polysaccharide substance called biofilm that acts as protection against antibiotics. *P. putida* is related to the bacteria that causes infections in cystic fibrosis patients. *P. putida* is related to *P. aeruginosa*, one of the bacteria that cause infections in cystic fibrosis patients. Learning how different concentrations of antibiotics affect the growth of *P. putida* could help suggest the specific dosage of antibiotics needed to successfully combat infections in people suffering from cystic fibrosis.

Goal

Use computer vision to accurately measure and analyze the growth of the bacteria *P. putida* and the production of biofilm in response to varying dosages of antibiotics. Current biochemical analysis is time consuming and cannot provide continuous measurements of biofilm development.

Key Scientific Questions

- What is the rate of biofilm production in response to specific levels of antibiotics?
- How can we measure biofilm production accurately and automatically?
- How can we measure the thickness of semi-transparent materials?

Bio-Imager

The Bio-Imager is an imaging system used for taking time-lapse photographs of bacteria under stable lighting conditions. The optical design of this imaging system is similar to a simple microscope, with cold-cathode lights placed underneath the bacteria specimen and a camera placed directly above the specimen.

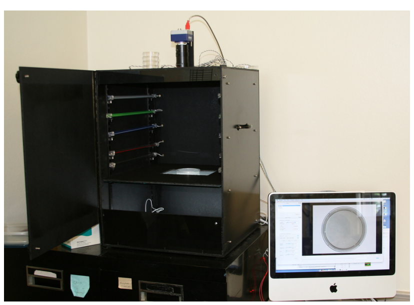

Figure 1. The Bio-Imager imaging system for capturing images of bacteria.

Noise Reduction

Thermal noise and fixed-pattern noise are both accounted for in our imaging system in an effort to improve the accuracy of image analysis. Thermal noise in the camera is reduced by calibration of a dark current image. Fixed-pattern noise and illumination irregularities in the lighting setup are reduced by calibration of a flat field image.

Measuring Bacteria Area

Computer vision techniques are used to automatically determined the area of each bacteria colony. We calculated the rate of growth of the bacteria colonies using a Gaussian filter to smooth noise and estimate the derivative of area with respect to time.

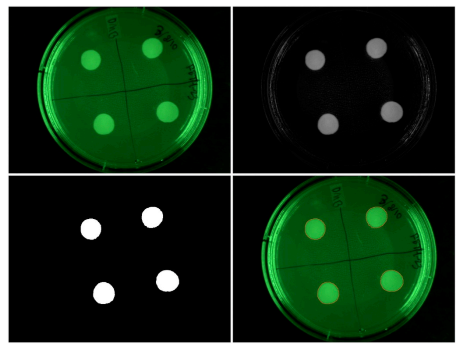

Figure 2. The process of finding regions in an image that represent bacteria colonies. 1. Image from camera. 2. Background-subtracted image. 3. Binarized image. 4. Region image.

Measuring Bacteria Thickness

Measuring bacteria colony thickness can be used to estimate bacteria growth in terms of volume rather than just area. In order to measure thickness, the camera first must be calibrated. The camera calibration results confirm the linear relationship between transmission and camera intensity measures, meaning that it is possible to measure light transmission very accurately. The next step in research is to measure the transmission of light through biofilm in order to determine the relationship between light transmission and thickness.

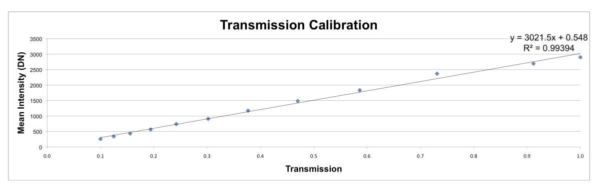

Figure 3. Graph of average transmission of light through a stepped neutral density filter.

Software System

This project involved creating a software system to allow for a variety of image analysis tasks. This software system was created in a maintainable and easily extensible way so that new image analysis tools could be added on with minimal effort.

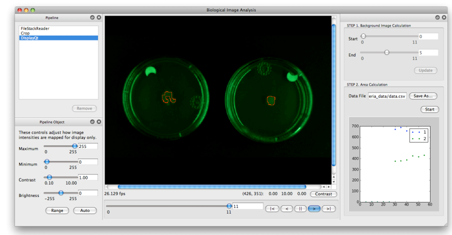

Figure 4. A screenshot of the image analysis software.

Conclusion

The imaging setup and software captured and analyzed the two-dimensional rates of growth of the bacteria colonies. Current research is working on calculating the three-dimensional volume of bacteria colonies by measuring the transmission of light through the colonies to estimate thickness. By combining area and thickness measurements of bacteria colonies, we hope to gain a more accurate understanding of the growth patterns and biofilm production of *P. putida*.

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