

Computational Approaches to detect and segment Biofilm Regions from Microscopy Images probed by Fluorescence In Situ Hybridization (FISH)

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Abstract

Biofilms are communities of microorganisms that colonize and grow on surfaces such as living tissues, medical implant devices, water systems, and natural aquatic systems. Biofilms are key to many biological processes. Microscopic imaging of biofilms is one of the important tools used for characterizing biofilms. In this work we propose to develop computational approaches using both traditional methods such as thresholding and edge detection to distinguish and segment biofilm regions that were studied by fluorescence in situ hybridization (FISH).

We plan to study FISH images of biofilms formed by *Pseudomonas aeruginosa*, an opportunistic pathogen; these images were formed over a 10 day starvation period of each biofilm, in order to characterize the differences as biofilm starvation occurs. Our methodology will facilitate automatic detection of biofilm boundaries to extract fluorescent intensity values which will provide valuable data to quantitatively analyze biofilm heterogeneity.

Introduction

Biofilms are communities of microorganisms that colonize and grow on surfaces such as living tissues, medical implant devices, water systems, and natural aquatic systems. Biofilms are key to many biological processes. Since biofilm bacteria are physiologically heterogeneous, treating an

infection with generic antibiotics can prove to be difficult. The efficacy of antibiotics depends on the physiologically state of the bacteria, causing tolerance to one type of bacteria and resistance to another.

Microscopic imaging of biofilms is one of the important tools used for characterizing biofilms. Using Carl Zeiss Image microscopes which produce CZI files for computer vision analysis, we were able to visualize and segment biofilm regions. The overarching goal of microscopic imaging of biofilm is to understand the physiological heterogeneity and how that influences treatment of infections.

A part of the strategy to determine physiological heterogeneity is the cellular concentration of ribosomes. Ribosomes are the most essential molecules for all life forms, and biofilm bacteria are not different. The cellular concentration of ribosomes varies depending on the physiological status of the cells, with actively growing cells having high concentrations of ribosomes and dormant cells having a low amount but maintainable enough for the cell. The concentration in dormant cells also relies on small ribosome-binding protein, hibernation promoting factor (HPF), which protects a number of ribosomes from degradation. Therefore, because the cellular concentration of ribosomes changes depending on the physiological state of the biofilm bacteria, the concentration can be used as an indicator of the physiological status.

Ribosomes are complex molecules that contain three inherent ribosome RNA species (rRNA). The three species found in bacteria are 5S, 16S, and 23S rRNAs. Since ribosomes

contain rRNA, a method to determine the relative cellular concentration is to probe the bacteria for their rRNA. With this research study, FISH is used to probe biological heterogeneity of single species biofilms, based on the fluorescent intensity of 16S rRNA within cells.

In this work, we will be using FISH image data of biofilms formed by *Pseudomonas aeruginosa*, PAO1 which is the wild type (WT) along with three PAO1 mutants, HPF, HPFc, relAspoT. The HPF mutants were created by knocking out the HPF (hibernation promoting factor), HPFc was created by inserting the HPF gene back into the HPF mutant. Finally, relAspoT, a double mutant, was created by knocking off both relA and spoT genes from the WT. For each of these strains, the biofilm images were produced at day intervals (2, 6, 10) where they undergo starvation. In PAO1 biofilm starvation, dispersal movement starts within 5

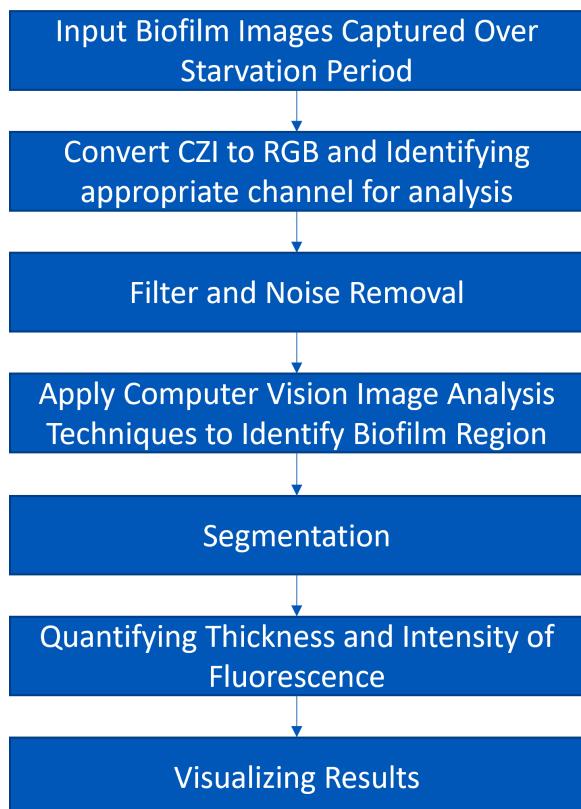
minutes and about 60% of the original biomass will be depleted in 24 hours.

Motivation

Software packages currently available to analyze microscopic images such as FIJI, Napari, and ImageJ are not ideal to analyze the large volume of datasets. They work on individual images and the analysis on each image needs to be parametrized to generate optimal results. This is time-consuming, laborious, tedious, and not ideal. Automated approaches that can handle large volumes of data that we have is required.

Related Work

Luis E. Chávez de Paz's paper, *Image Analysis Software Based on Color Segmentation for Characterization of Viability and Physiological Activity of Biofilms*, the main purpose is to present bioImage_L, which enables *in situ* color segmentation without prior transformation of micrographs into monochrome channels. With the biofilm infection of dental plaque being the primary dataset of this research, the applicability of the software was tested to determine the baseline physiology of dental plaque grown in a mini-flow cell system and the changes to the physiological parameters when dental plaque was subjected to different stress conditions. Although a much more mathematically involved approach as stress conditions such as starvation was incorporated, the visualized images are graphed of population density and over a starvation period of only 24 hours. In our study, we maintain FISH images of PAO1 from a period of 2 days, to 10 days. A prolonged duration allows for a more consistent result of how starvation affects biofilm boundary thickness and cellular concentration,



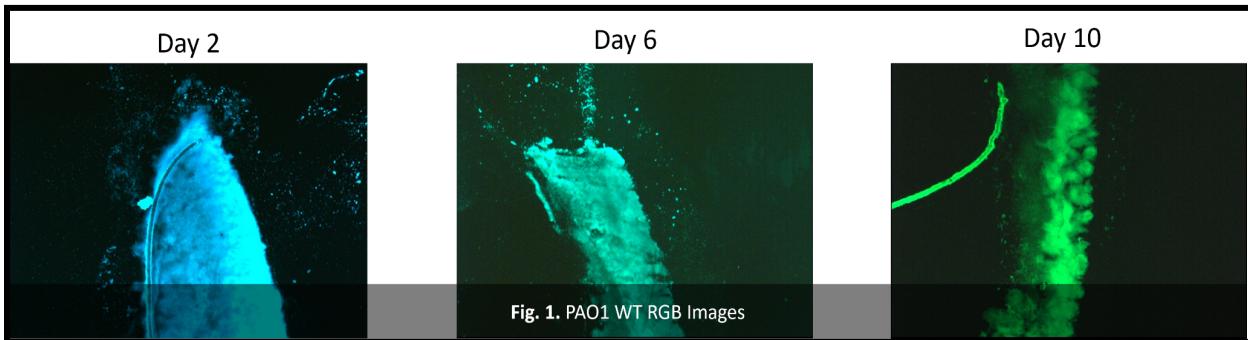
Methodology

1. Image preprocessing

A. Converting CZI to RGB

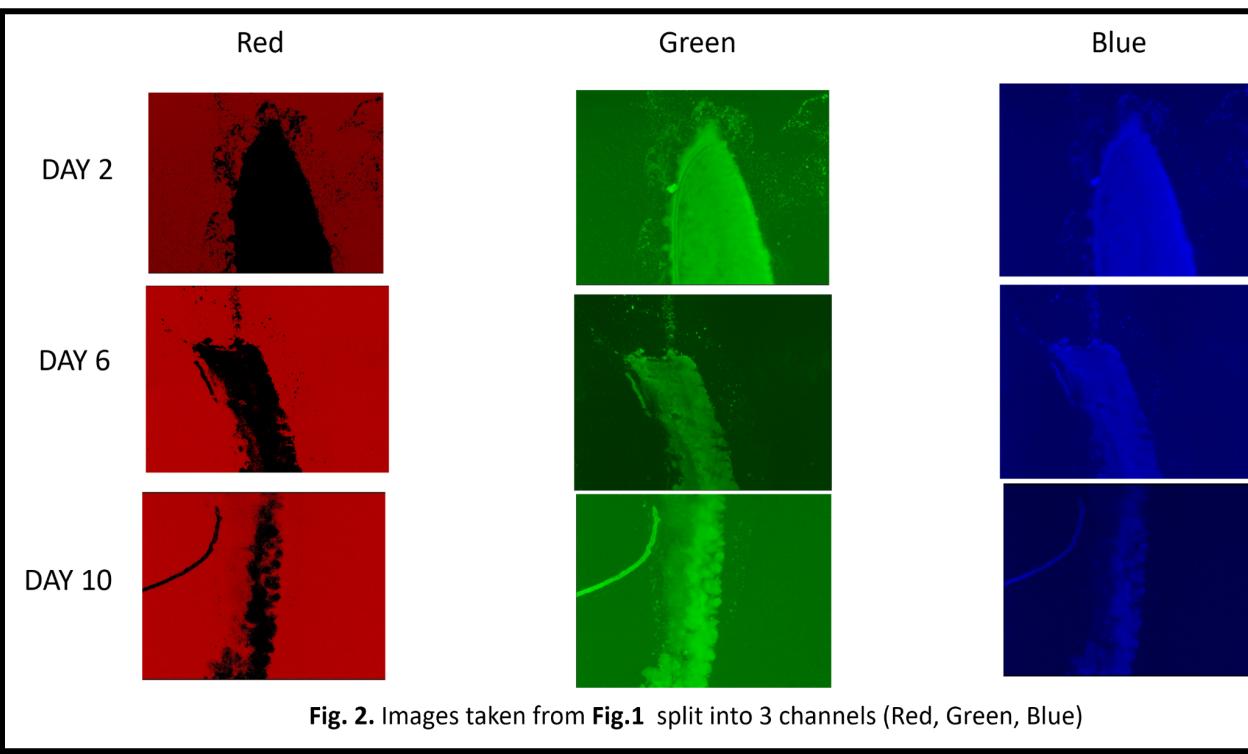
The first step is to convert the Carl Zeiss Image format from the microscope into a format that can be visualized and used on computer vision algorithms. Using the computer vision

Having to split up the RGB image into the three channels up red, green, and blue each have their own purpose in the image segmentation process. In the red channel, the high contrast between the black and red colors allows for a binary output of the boundaries of the biofilm region. The green and blue channels are



software Napari, we were able to visualize the microscope formatted image.

important in their ability to visualize the cellular concentration in terms of fluorescence intensity in the biofilm region.



2. Image Analysis

A. Apply computer vision techniques to identify biofilm region from preprocessed FITC images

- i. OTSU method to select background and foreground region

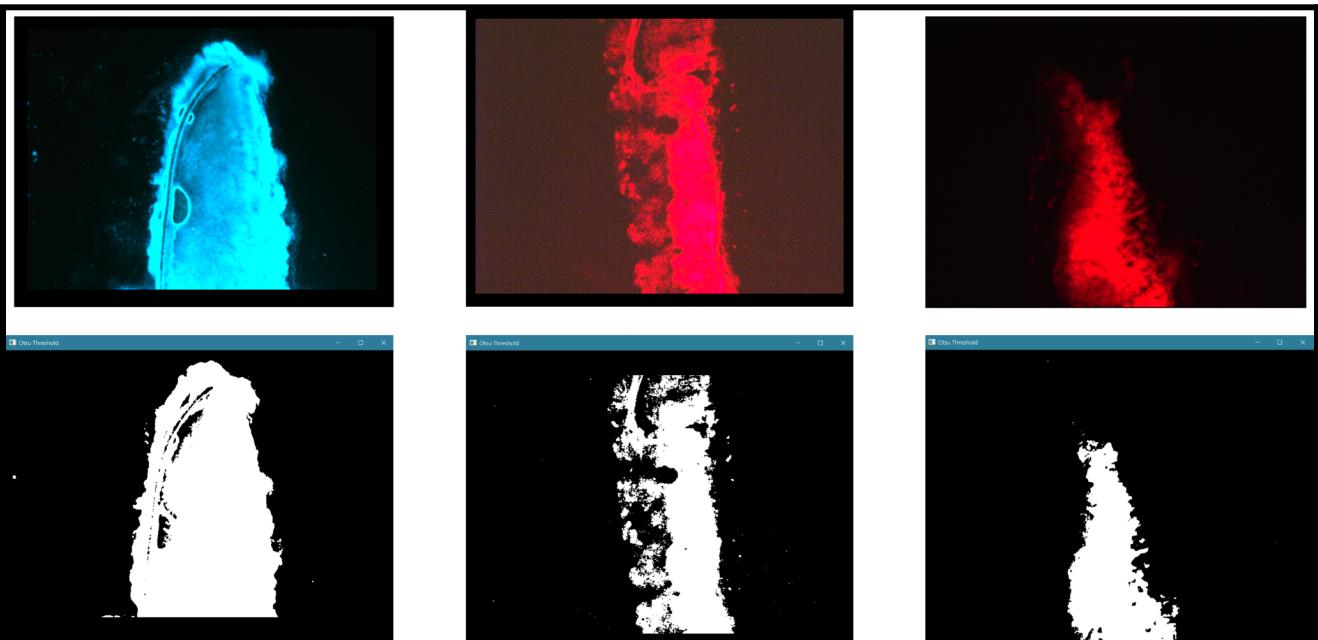


Fig. 4. Day 2, Day 6, Day 10 Images processed through Otsu Algorithm Thresholding

- ii. Biofilm edge identification using Canny edge detection algorithm
- iii. Customized thresholding approach

The customized thresholding approach is derived from the usage of multiple image analysis techniques. In the case of **Fig 5**, the biofilm image is first pre-processed with Otsu Thresholding, and then Canny edge detection is applied. The top row is without using Otsu pre-processed images and rather using raw RGB images from Napari software.

B. Rotate and clip image to obtain final biofilm region

3. Transform results to corresponding TRITC image
4. Quantify thickness of boundary and intensity

Results and Conclusion

Currently we make use of existing tools to generate RGB images from CZI files and split the RGB to their respective Red, Green, and Blue channels and collect metadata information provided in the CZI file. After identifying the appropriate channel to use downstream analysis described in the methodology section, the aspects of computer vision techniques such as thresholding and edge detection can be used on the biofilm images for further analysis.

Using the Otsu algorithm thresholding as described in 2.A.i, we can see the method to produce clear biofilm boundaries using image

analysis with acceptable results. Having a binary scale, the boundary will be that which the black is directly contrasted with the white in the image.

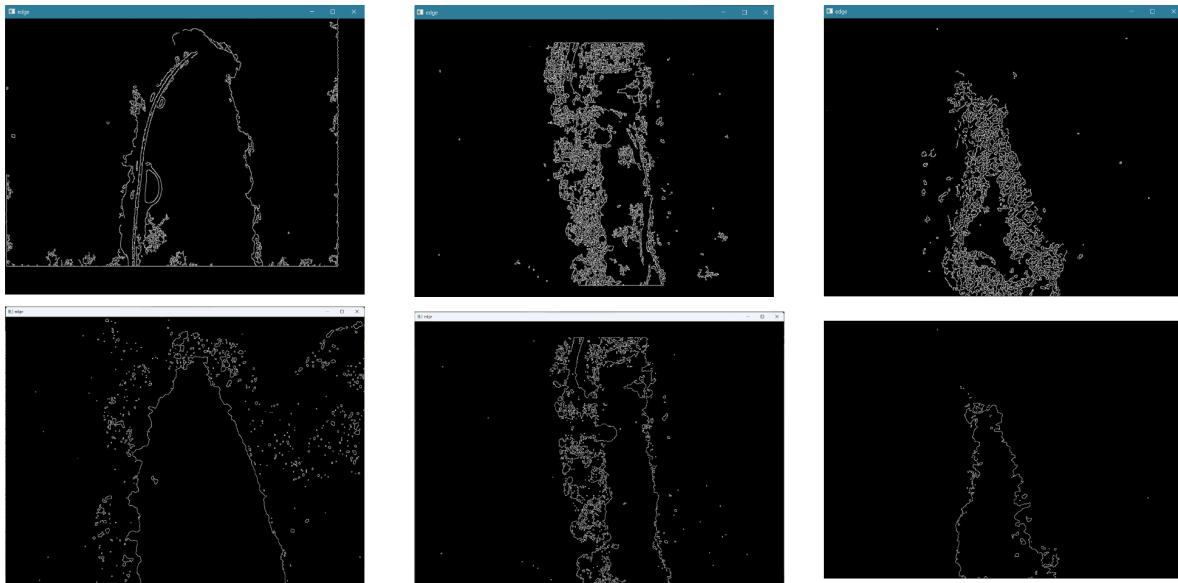


Fig. 5. Results of Canny Edge Detection Algorithm from images without pre-processing and Otsu Thresholding

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