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

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Introduction and Background

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 and machine learning approaches to detect and segment biofilm regions that were studied by fluorescence in situ hybridization (FISH).

Motivation for Our Analysis

Software packages currently available to analyze microscopic images such as Napari are not ideal for 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 are required.

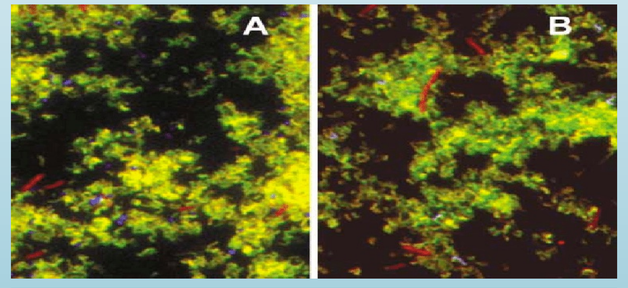


Fig.1. FISH technique used for detection of RNA and DNA
Ref: Community Proteomics of a Natural Microbial Biofilm (R.Blake,2005)

Data

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.
The data shown in table below was generated at Dr. Michael Franklin's lab in the department of microbiology and immunology at Montana State University, Bozeman, MT.

STRAINS	DAY 2			DAY 6			DAY 10		
	R1	R2	R3	R1	R2	R3	R1	R2	R3
PAO1	297	211	196	158	201	159	239	130	140
HPF	283	257	204	466	172	90	159	113	168
HPFc	200	256	146	248	127	179	174	117	327
relAspoT	268	272	201	189	165	150	77	189	150

Visualizing Our Biofilm Image Data

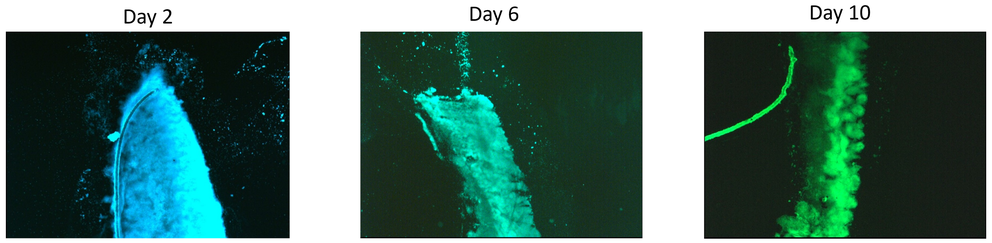


Fig. 2. PAO1 WT RGB Images

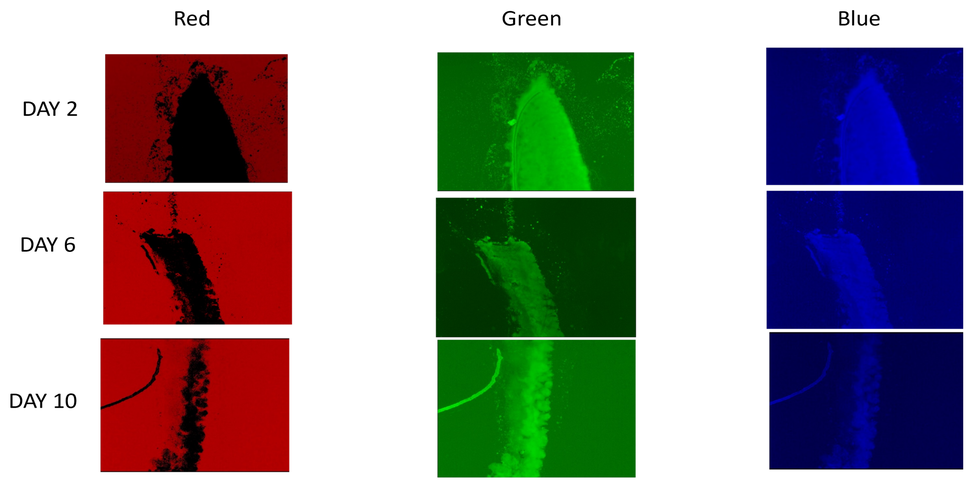
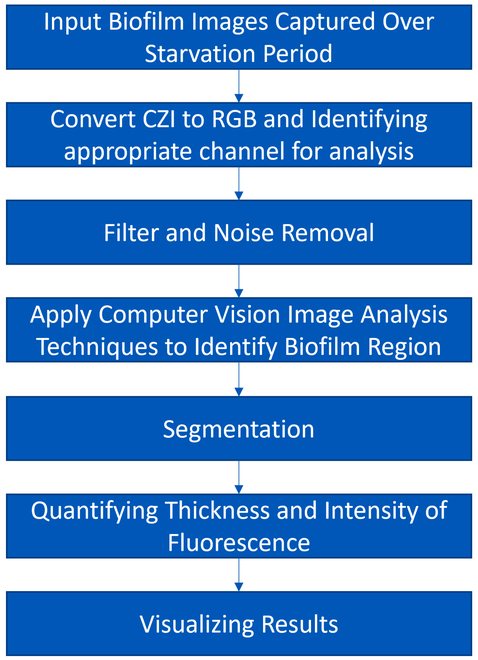


Fig. 3. Images taken from **Fig.4** split into 3 channels (Red, Green, Blue)

Methodology



1. **Image Preprocessing**
   i. Conversion from Microscopic files
   ii. Identify Split RGB Channels
2. **Image Analysis**
   i. Apply Computer Vision Techniques
   ii. Find Optimal Thresholding Algorithm
   iii. Biofilm Edge Detection Algorithms
3. **Combine Techniques for Optimal Results**
4. **Quantify Thickness and Fluorescence Intensity**

Applying Computer Vision Techniques

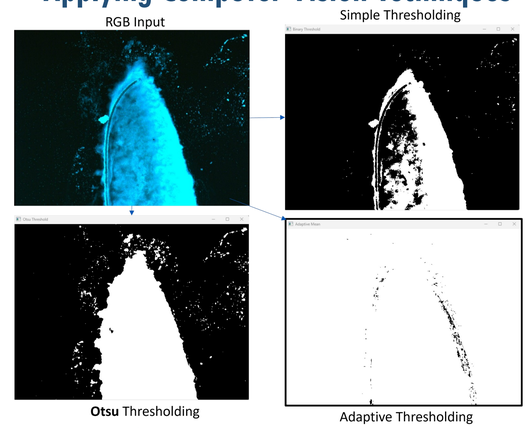


Fig. 4. Day 2 Thresholding Results

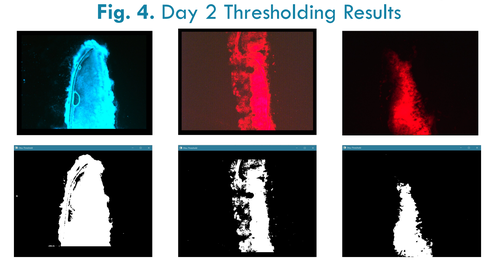


Fig. 5. Day 2, Day 6, Day 10 Images processed through Otsu Algorithm Thresholding

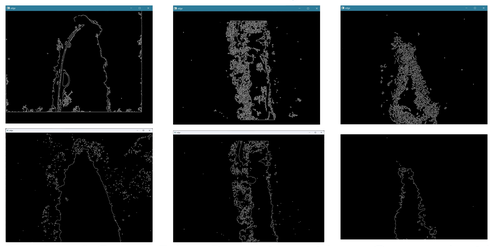


Fig. 6. Results of Canny Edge Detection without Otsu and with Otsu Thresholding

Results and Conclusion

- Effective automated approaches that do not have to be individually parametrized, rather combined
- Developed segmentation processes and images with clear boundaries
- Fluorescent Intensity from split **green and blue channel** to quantify cellular concentration and boundaries with **red channel**

References

1. Akiyama et al. 2017. Resuscitation of Pseudomonas aeruginosa from dormancy requires hibernation promoting factor (PA4463) for ribosome preservation. PNAS 114:3204-3209.
2. Otsu N. 1979. A Threshold Selection Method from Gray-Level Histograms, p 62-66, IEEE Transactions on Systems, Man, and Cybernetics, vol 9. IEEE.
3. Canny J. 1986. A Computational Approach to Edge Detection, IEEE Transactions on Systems and Cybernetics, vol 9. IEEE.
4. FIJI: https://fiji.sc/; Napari: https://napari.org/; ImageJ: https://imagej.nih.gov/ij/; CZIFILE: https://pypi.org/project/czifile/

NIH National Institute of Allergy and Infectious Diseases
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