

## Thresholding Approach for Automatic Detection of *Pseudomonas aeruginosa* Biofilms from Fluorescence in situ Hybridization Images

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**Abstract :** *Pseudomonas aeruginosa* is an opportunistic pathogen that forms surface-associated microbial communities (biofilms) on artificial implant devices and on human tissue. Biofilm infections are difficult to treat with antibiotics, in part, because the bacteria in biofilms are physiologically heterogeneous. One measure of biological heterogeneity in a population of cells is to quantify the cellular concentrations of ribosomes, which can be probed with fluorescently labeled nucleic acids. The fluorescent signal intensity following fluorescence in situ hybridization (FISH) analysis correlates to the cellular level of ribosomes. The goals here are to provide computationally and statistically robust approaches to automatically quantify cellular heterogeneity in biofilms from a large library of epifluorescent microscopy FISH images. In this work, the initial steps were developed toward these goals by developing an automated biofilm detection approach for use with FISH images. The approach allows rapid identification of biofilm regions from FISH images that are counterstained with fluorescent dyes. This methodology provides advances over other computational methods, allowing subtraction of spurious signals and non-biological fluorescent substrata. This method will be a robust and user-friendly approach which will enable users to semi-automatically detect biofilm boundaries and extract intensity values from fluorescent images for quantitative analysis of biofilm heterogeneity.

**Keywords :** image informatics, *Pseudomonas aeruginosa*, biofilm, FISH, computer vision, data visualization

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