Application of a Novel Software for Analysis and Visualization of Biofilms on Implant Materials

Adrian Turcu, AB[1,2,4]; Ellis Berns, ScB[1,2,4]; Caitlin Barrett[2,4]; Carole Spake, MS[1,2,4]; Jacqueline Leong, BA[1,2,4]; Christopher Born, MD[1,3,4]; Dioscaris Garcia, PhD[1,3,4]

1. Warren Alpert Medical School of Brown University, Providence RI
2. Brown University, Providence, RI
3. Department of Orthopaedic Surgery, Warren Alpert Medical School of Brown University, Providence, RI
4. Weiss Center for Orthopaedic Trauma Research, Rhode Island Hospital, Providence, RI

BACKGROUND

Understanding how infection develops following surgery is of paramount importance for orthopaedic surgeons. Infection is particularly problematic in arthroplasty and fracture implants, where colonization by opportunistic pathogens can occur. These organisms form biofilms, dense colonies of bacteria largely resistant to antibiotics and the immune system. Research to formulate materials and irrigation methods that reduce or remove biofilm formation on implants is being carried out[1, 2], but no reproducible platform to visualize and quantify biofilm formation is available. Confocal laser scanning microscopy has proved very useful in imaging biofilms, but a consistent and accurate computational tool is needed to allow for improved analysis of the biofilm image stacks generated by this method. Knowing the precise coverage present and being able to visualize the biofilm more clearly in three dimensions can improve understanding of biofilms and how to best treat them.

OBJECTIVE

The objective of this project is to develop a novel software with the ability to rapidly analyze and create visualizations for confocal microscope images of biofilms on implant materials.

METHODS

- Computer program was written in Python to analyze images of biofilm slices generated by confocal microscopy.
- Tested on 97 different experimental trials.
- Bacteria was cultured on polyether ether ketone (PEEK) implant material. For the sample trial shown, *Pseudomonas aeruginosa* was used. The *P. aeruginosa* biofilms were irrigated with a series of solutions to test their efficacy.
- Biofilms were stained with SyproRuby, TOTO-1, and Concanavalin A dyes to reveal protein, extracellular DNA, and exopolysaccharide, respectively.
- The biofilm was imaged and segmented into vertical slices using a confocal laser scanning microscope (Olympus FV-1000 MPE Multiphoton Microscope).
- Z-stacks of these images were created, split into individual images, and sorted into appropriate folders for analysis.
- The Python program searched through every pixel in each image, storing the coordinates and counts of all pixels exceeding the threshold intensity of 100 (for 16-bit images)
- Coverages and VisPy graphs were output for each component.

RESULTS

Table 1. Coverage values for irrigation solutions in the sample experiment

	Pixels Covered	% Pixels Covered	% eDNA	% Protein	% Exopolysaccharide
Control	3564205	24.93	53.12	39.69	7.19
PBS	1961529	14.23	48.44	46.71	4.85
Phenol	1083176	16.36	97.18	2.32	0.5
EDTA	1950619	19.82	47.32	46.03	6.65
1:1 EDTA-phenol	719147	12.77	56.27	33.49	10.23

Figure 1. *P. aeruginosa* biofilm grown on PEEK (control)

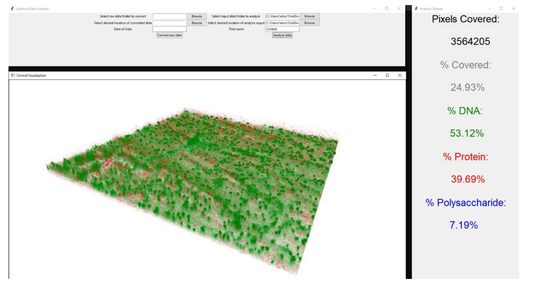


Figure 2. *P. aeruginosa* biofilm grown on PEEK and irrigated with PBS

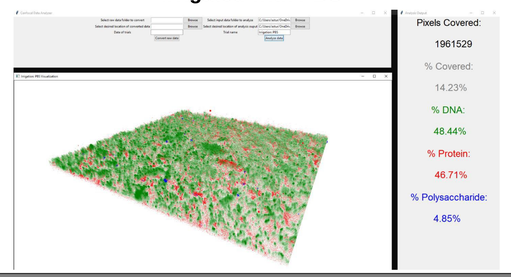


Figure 3. *P. aeruginosa* biofilm grown on PEEK and irrigated with 2.5% phenol

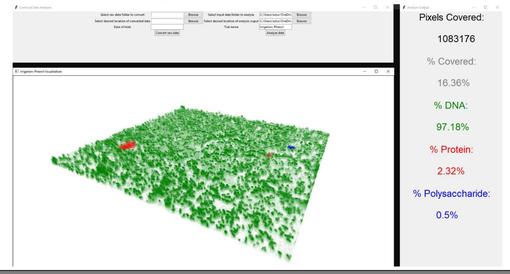


Figure 4. *P. aeruginosa* biofilm grown on PEEK and irrigated with 2.5 mM EDTA

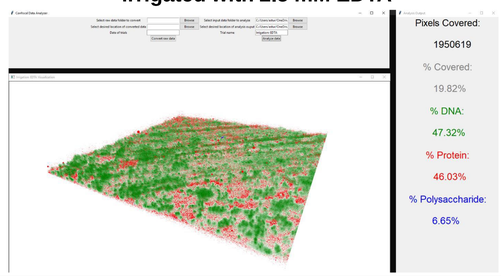
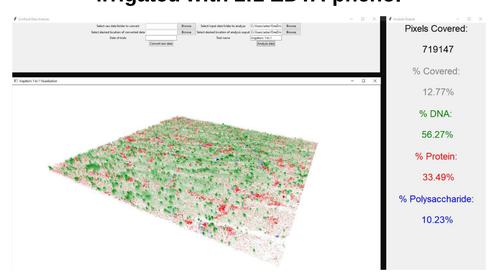


Figure 5. *P. aeruginosa* biofilm grown on PEEK and irrigated with 1:1 EDTA-phenol



CONCLUSIONS

- Program successfully generated coverage values and visualizations for the different *P. aeruginosa* biofilms.
- For this sample trial, the control was found to have the most bacterial coverage whereas the 1:1 EDTA-phenol solution had the greatest success in reducing biofilm.
- Different irrigation solutions were found to have very different impacts on the levels of eDNA, protein, and exopolysaccharide.
- With a pixel intensity threshold of 100 (16-bit), code generally finished running in 30 seconds to 2 minutes (increased with stack size and bacterial coverage).
- Time complexity of code is polynomial, namely *O(xyz)* where *x* and *y* are the number of pixels in the x and y dimensions, and *z* is the size of the image stack.
- The program worked as intended for all 97 trials that were tested and has potential to be a key analytical took for biofilm assessment and infection diagnostics.

FUTURE DIRECTIONS

- Develop new methods for thresholding that are more accurate and mathematically robust.
- Expand from biofilm analysis to clinical diagnosis of infections in orthopaedics.

REFERENCES

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