3D Imaging of Microbial Biofilms: Integration of Synchrotron Imaging and an Interactive Visualization Interface

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Abstract— **Understanding the structure of microbial biofilms and other complex microbial communities is now possible through x-ray microtomography imaging. Feature detection and image processing for this type of data focuses on efficiently identifying and segmenting biofilm biomass in the datasets. These datasets are very large and segmentation often requires manual interventions due to low contrast between objects and high noise levels. New software is required for the effectual interpretation and analysis of such data. This work specifies the evolution and ability to analyze and visualize high resolution x-ray microtomography datasets. Major functionalities include read/write with multiple popular file formats, down-sampling large datasets to generate quick-views on low-power computers, image processing, and generating high quality output images and videos. These capabilities have been wrapped into a new interactive software toolkit, BiofilmViewer. A major focus of our work is to facilitate data transfer and to utilize the capabilities of existing powerful visualization and analytical tools including MATLAB, ImageJ, Paraview, Chimera, Vaa3D, Cell Profiler, Icy, BioImageXD, and Drishti.**

I. Introduction

Studying biofilms presents a new approach to learning more about the microbiology of problems that affect a plethora of applications from industry to public health issues [1]. Biofilm infections can result in different diseases and adverse medical conditions. Microbial biofilms that affect the human body share characteristics with those found in other natural environments [1]. Examples include periodontitis, cystic fibrosis pneumonia, infection of catheters, and the colonization of prosthetic joints.

X-ray microtomography can produce high-resolution 3D images, but obtaining adequate contrast to visualize nearest-to-native state hydrated biofilms is extremely difficult, as the absorption coefficient of hydrated biofilm is similar to that of water. Synchrotron-based x-ray microtomography has been used to render high-resolution quantifiers of the gross spatial distribution within biofilms in permeable media [2], [3]. In these studies, the spatial stratification of metabolic activity suggested that understanding the 3D internal structure within biofilms may be essential to provide insight into how biofilms interact with substrates and influence larger, pore-scale processes [2], [4]. The facultatively anaerobic bacterium, *Shewanella oneidensis* strain MR-1 (MR-1), is capable of dissimilatory metal reduction and forms thick biofilms in the same growth condition. Therefore, understanding the internal structure of MR-1 biofilms will offer an improved understanding of how Fe(III)-reducing microorganisms stimulate redox reactions in their local subsurface environments [2], [5].

Understanding the 3D internal structure of biofilms can yield insight into how biofilms cause disease and successfully colonize medical devices. However, new basic capabilities for enhanced structural characterization of biofilms are required. This project addresses this need by developing fundamental capabilities for enhanced visualization and analysis of biofilms. The incorporated features include: quantitative analysis; qualitative visualization; manual, aided, and automated image analysis and segmentation; extensive preprocessing; rendering multidimensional images; and analysis of whole stack of images or individual biological substances in the images.

There are several toolkits that are already available for the visualization and analysis of biological images, including commercial packages Imaris (Bitplane, CT, USA), MetaMorph (Molecular Devices, CA, USA), Volocity (Perkin Elmer, MA, USA), ImagePro Plus (Media Cybernetics), SlideBook (Intelligent Imaging Innovations, Gottingen, Germany), Avizo (VSG, MA, USA), Amira (VSG, MA, USA), and open-source packages ImageJ [6], [7], [8], CellProfiler [9], Vaa3D [10], BioImageXD [11], Icy [12], Chimera [13], and Drishti [14]. The software packages differ in their proposed application areas, usability, availability of the source code, accepted file formats and cost. For example, Icy has unique capabilities for cell tracking and segmentation, Fiji aids analysis of microscopic images, and both Vaa3D and BioimageXD are excellent for neurobiological applications, taking advantage of the VTK and ITK 3D visualization capabilities [10].

All of the open-source packages were bound by the limits of computational power. Only Drishti was able to directly open the dataset used in this paper without having to use an external

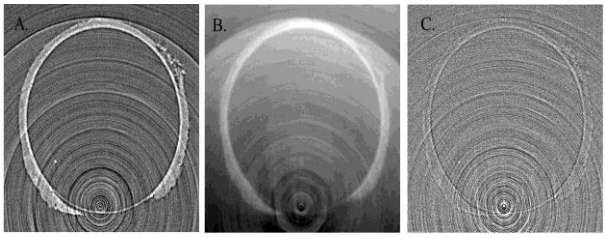

Figure 1. Differential phase contrast images of Osmium-stained, hydrated biofilms, in absorption (A), phase contrast (B) and scatter contrast (C). The MR-1 biofilm sample used in this paper was grown on the surface of a porous hollow fiber and inspected using high resolution x-ray microtomography (0.74 μm/pixel sampling) and with a Talbot interferometer (2.00 μm period fringe pattern). The sample was reconstructed in the HDF4 format.

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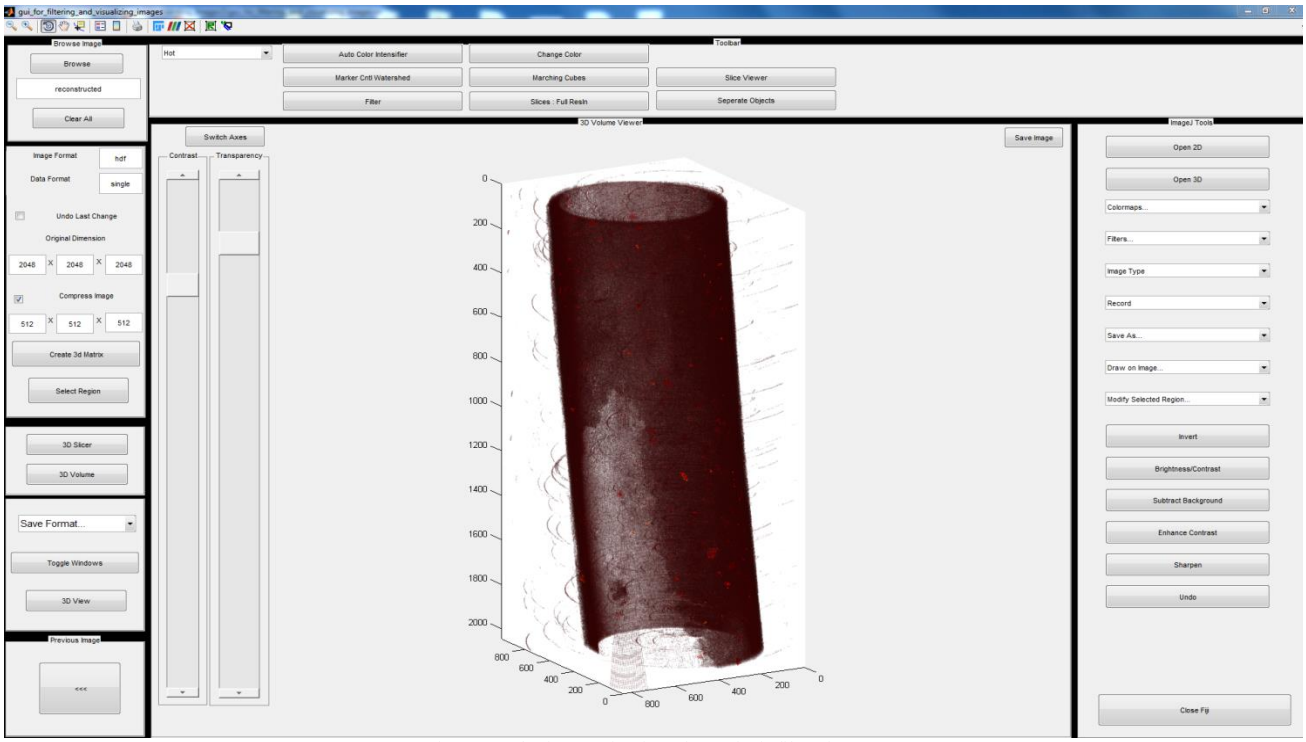

Figure 2. The interactive interface of BiofilmViewer.

plugin for format conversion. This resulted in the scientists initially visualizing and analyzing the dataset in 2D slices (Fig. 1). In addition, the datasets obtained can be in the form of 2D slices or a single 3D object. Hence, it is necessary to be able to break the 3D object into slices in order to be able to visualize it efficiently.

This work seeks to develop the capability to facilitate off-site analysis, to visualize the large datasets on laptops with low computational power, to utilize existing toolkits efficiently, and to provide scientists with file format flexibility. This paper presents a toolkit that allows scientists to quickly process and visualize datasets while comparing, communicating and utilizing the capabilities of other existing powerful visualization and analytic tools.

II. METHOD

A. Software overview

Software called BiofilmViewer, which includes a graphical user interface (GUI), has been implemented to aid visualization and analysis of x-ray microtomography data (Fig. 2). The tool is easily expandable and is equipped with function pipelines to analyze and visualize this type of data. Importantly, this tool can be executed on lower end computers as well as on those with high computational capability. There is no conversion of the data format required by the user to visualize the data. BiofilmViewer was written in MATLAB R2011b (Mathworks, Inc., Natick, MA, USA) and compiled using the MATLAB® Compiler™.

B. Main functionalities

The currently accepted file formats in BiofilmViewer include tiff, hdf4/hdf5, st, rec, jpeg, gif, png and bmp. File format, bit-size type, and overall size of the dataset are displayed for the folder to be analyzed. Users can choose to start reading in the data at any slice number and also specify the total number of slices to be analyzed. There are options to down-sample the dataset along the x, y or z direction to generate a quick-view of the dataset and then generate a subset of the region of interest (ROI) at full resolution depending on the computational limitations. For example, a dataset of size 2048^3 can be broken down into 512^2 X 1024 by sampling every 4^{th} pixel along the x- and y-axis and every 2^{nd} pixel along the z-axis. The axis limits can be switched to display the location of ROI within the original dataset.

BiofilmViewer is equipped with functionalities that include: contrast control; adjusting angle of rotation; transparency levels; manipulation of 3D slices and volumes; saving videos/images; accessing various filters; selecting ROI(s) from compressed datasets; generating images; recording movies for the selected section at full data resolution; isosurface generation using marching-cubes at a user-selected threshold; marker-controlled watershed algorithms to separate connected objects; and automatic labeling of components which allow the user to visualize the different objects separately and perform measurements (area, volume, centroid etc.) on the 3D stacks. Users can select a region in either the 2D or 3D view for defining the background or for subtracting the range of intensity values within the selected region from the entire dataset.

C. Access to third-party software

The main advantages of MATLAB are its matrix manipulations, intermediate plotting capabilities, rapid prototyping, and visualization of algorithms. However, MATLAB is not the optimal software for visualizing and rendering very large datasets. To address this, we have been working towards developing efficient data transfer methods between BiofilmViewer and other third-party software. This provides the user with the ability to apply functions from multiple software packages.

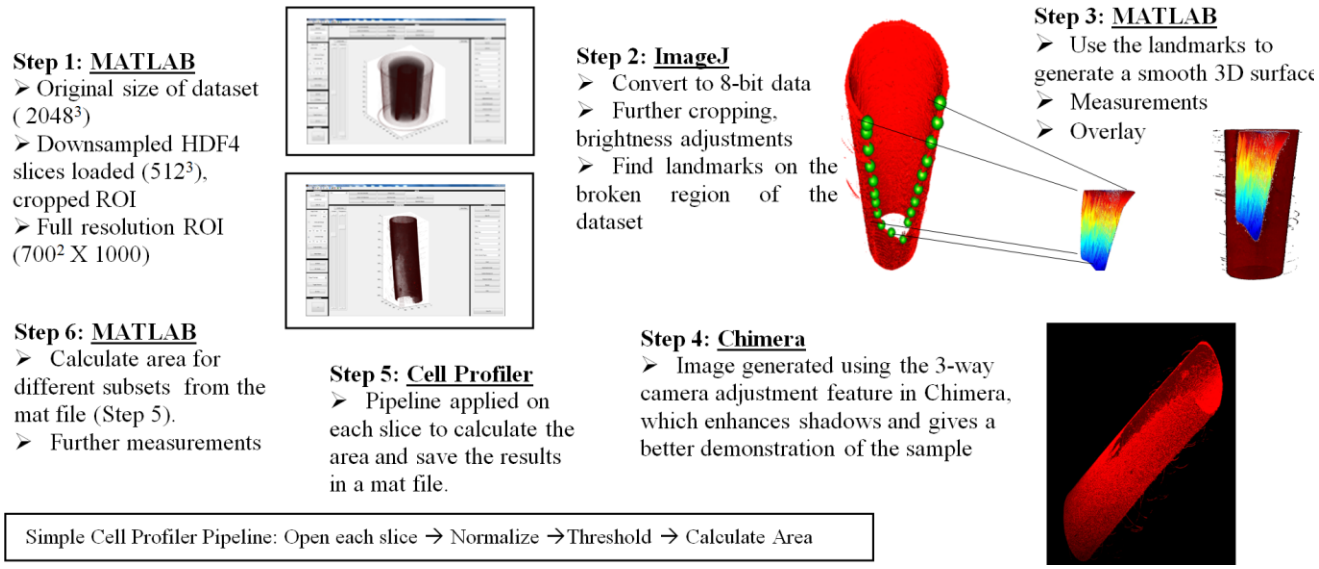

Figure 3. Example application of BiofilmViewer

BiofilmViewer with ImageJ/Fiji: ImageJ is a Java-based open-source multi-platform interface that allows a range of plug-ins and macro extensibility for image processing [6], analytic and visualization tasks [7], [8]. It has good viewers and browsers for multidimensional stacks, volume rendering and surface rendering. Imaris, Vaa3D, Icy and Cell Profiler have been able to utilize some of the functionalities from both MATLAB and ImageJ. MIJ (Biomedical Imaging Group, Ecole Polytechnique Federale de Lausanne, Switzerland) is a Java package for bi-directional communication and data exchange from MATLAB to ImageJ/Fiji [9]. This provides access to all functions/plug-ins from MATLAB and ImageJ/Fiji, with direct data transfer between the two platforms. We incorporated the essential features from MATLAB and ImageJ into BiofilmViewer. For example, the data format manipulations in BiofilmViewer are performed in MATLAB, while image bit-type conversions and generating/saving images/videos are accomplished using ImageJ.

BiofilmViewer with Paraview: Paraview performs interactive visualization of extreme scale data and uses a client/server model along with parallel processing. Paraview allows for quantitative probing and arbitrary processing of data sets with filters such as subsetting, contouring and clipping, along with the ability to generate animations and publication quality views. Paraview is extremely useful especially when data volumes are large and flexibility is required to focus on important features. Views and particular viewing angles can be saved and reused so that multiple data sets can be visualized in the same way for exact comparison. Paraview is able to utilize multiple cores and processors to improve the speed of visualization. BiofilmViewer can directly load 3D objects into the Paraview interface.

BiofilmViewer with Cell Profiler: Cell Profiler allows image processing and analysis of 2D slices using pipelines defined by the user and generates results as .mat files that can be directly accessed within MATLAB. BiofilmViewer can also communicate with Cell Profiler through ImageJ using a combination of the MIJ toolkit and the RunImageJ function embedded in Cell Profiler. Once the correct processing parameters have been determined for sub region(s), the pipeline can be automatically run on the entire dataset.

BiofilmViewer with Chimera: Chimera was primarily designed for the visualization and processing of molecular structures, including sequential alignments, trajectories, supra-molecular assemblies, docking results, and density maps. It provides different 3D display options including contour surfaces, meshes and volumetric display, and the option to interactively adjust the thresholds. Maps can be colored, sliced, and segmented. Markers can be placed and structures can be traced. At present, there is no direct data transfer between BiofilmViewer and Chimera. However, a dataset written in MRC format can be read, modified, and saved by both BiofilmViewer and Chimera.

BiofilmViewer with BioImageXD: BioImageXD is a multi-purpose processing software toolkit for bio-imaging that can be used from basic visualization of temporal image stacks with multiple channels to complex 3D rendering of multiple channels at once. It is based on an advanced motion tracking algorithm and numerical analyses. At present, there is no direct data transfer between BiofilmViewer and BioImageXD. Datasets written in MRC format are compatible with the BiofilmViewer and BioImageXD software packages.

BiofilmViewer with Vaa3D: Vaa3D is a versatile multi-dimensional visualization and analysis software for bioimages and surface objects. It provides powerful algorithms for registration, segmentation, tracing and analysis of large-scale multi-dimensional datasets. BiofilmViewer interacts with Vaa3D utilizing the I/O ImageJ and MATLAB toolboxes available with Vaa3D.

BiofilmViewer with Drishti: Drishti has been developed primarily for visualizing tomography and electron-microscopy data. It provides the Drishti_Importer tool to convert data files into its own proprietary format which can then be visualized and processed in Drishti_Renderer. Drishti software is dependent on the contrast of samples within the dataset. BiofilmViewer allows the user to save the dataset in a raw format that can be imported by Drishti.

BiofilmViewer with Icy: Icy is open source software for bio-imaging based on the bioimaging libraries VTK and ITK. The

following libraries are integrated within Icy: BioFormat, Substance, LOCI, VTK, Flamingo, Jama, Flanagan, JFreeChart, JXL, JMF, JTransforms, JEval, SwingX, Phys2D, and Xuggler. BiofilmViewer interacts with Icy through ImageJ [11].

Software	Direct Data Transfer	File Format Save Data
ImageJ/Fiji	√	
Paraview	√	
Cell Profiler	√	
Chimera		mrc
BioImageXD		mrc
Vaa3D	√	
Drishti		raw
Icy	√	
Amira		am

Table 1. Modes of data transfer between BiofilmViewer and other software. File formats used for software that do not have direct data transfer are also displayed.

III. Results and Discussion

An example framework application of BiofilmViewer with third-party software is shown in Fig. 3. When presented with different image analysis and visualization tools, it can be challenging for the user to select the most appropriate software to use. BiofilmViewer provides a platform for testing different toolkits without concern for data format and size. BiofilmViewer software allows scientists to identify the most useful functions and to then construct automatic pipelines that incorporate unique features from different tools.

We plan to further simplify the communication steps between the different software. Currently, direct data transfer and functionality access is only possible between BiofilmViewer and ImageJ, Paraview, Icy, Vaa3D, and Cell Profiler (Table 1). For the other software, there is still the extra step of saving and loading the data, although the users can start-up the third-party software interface directly from BiofilmViewer. Improving the command line functions for these programs will streamline BiofilmViewer pipelines and minimize the learning curve for users. In addition, this software is being incorporated into a multi-modal integration framework for integrated structural and chemical imaging studies [5], [15], [16].

We have described the construction of BiofilmViewer for the analysis of large x-ray microtomography datasets. BiofilmViewer is a unique software package in multiple ways: (1) it generates quick visualizations of big data volumes at lower resolution, or high resolution visualizations of smaller ROI(s); (2) it can utilize existing open source software for visualization and analysis on high-performance computing systems; (3) it allows internal conversion and transfer of most commonly used file formats between the embedded third party software; (4) it uses MATLAB as the development engine, which provides the platform for developing quick modules.

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