**Data-driven modeling of heterogeneous viscoelastic biofilms**

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**Abstract**

Biofilms are typically heterogeneous in morphology, structure, and composition, resulting in non-uniform mechanical properties. The distribution of mechanical properties, in turn, determines the biofilm mechanical behavior, such as deformation and detachment. Most past studies neglected heterogeneity of biofilms. In this study, an image-based modeling approach was developed to transform two-dimensional optical coherence tomography biofilm images to a pixel-scale non-Newtonian viscosity map of the biofilm. The spatial distribution of non-Newtonian viscosity was applied in an established Oldroyd-B constitutive model and implemented using the phase-field continuum approach for the deformation and stress analysis. The heterogeneous model was able to predict deformations and stresses more accurately than a homogenous one. This is the first time, to the best of our knowledge, that an image-based approach is used to map the mechanical heterogeneity of biofilms for computational studies. It provides an efficient method to characterize biofilm mechanical behavior.

**Keywords:** Heterogeneous biofilm, data-driven modeling, phase-field model, viscoelastic Oldroyd-B model, optical coherence tomography

**1. Introduction**

Biofilms are complex, viscoelastic materials consisting of microorganisms and extracellular polymeric substances (EPS) (Flemming & Wingender, 2010; Hall-Stoodley et al., 2004). When subject to hydrodynamic forces, biofilms can deform and detach, potentially affecting the biofilm porosity, density, mass transfer characteristics, and microbial activity (Laspidou & Aravas, 2007; Liu & Tay, 2001; Picioreanu et al., 2018; Van Loosdrecht et al., 2002).

The type and extent of biofilm deformation and detachment are governed by the biofilm’s morphology and mechanical properties. Biofilms usually exhibit heterogeneity in both morphology and mechanical properties at micro (micron), meso (mm), and macro (cm) scales, which complicates the understanding of biofilm behavior. Pores and channels surrounding cell clusters increase the mass transfer efficiency of nutrients (i.e., oxygen transfer) (Dirk de Beer, 1993). Voids in membrane-aerated biofilms may provide space for predators (Aybar et al., 2019; Kim et al., 2020). *Pseudomonas aeruginosa* biofilms with highly structural heterogeneity due to the over-expression of alginate were significantly more resistant to antimicrobial stresses (Hentzer et al., 2001). Shen et al. (2015) found that irregularities in biofilm structure could impact local hydrodynamics, leading to detachment.

Only a few studies have addressed the structural and mechanical heterogeneity of biofilms. In recent years, microscale techniques, including atomic force microscopy (AFM) and microrheological techniques, have been developed and applied in biofilm studies (Cao et al., 2016; Galy et al., 2012; Pavissich et al., 2021; Volle et al., 2008). However, these methods are limited. For example, some techniques only assess certain regions of the biofilm, e.g., the outer biofilm, or only provide an average value for the entire biofilm. Many require removing the biofilm from its native environment or deforming it, potentially changing its properties. Also, there is a lack of models that can incorporate a heterogeneous distribution of mechanical properties. Hence, it is critical to develop a method to assess mechanical heterogeneity and use these parameters in a model.

Image-based modeling that considers spatial distribution of mechanical properties is a promising approach emerging in various fields, including the medical and material sciences (Brooks & Grigsby, 2013; Chin et al., 2017; Y. Li et al., 2020; Matouš et al., 2017; Zarei et al., 2017; Zhao et al., 2019). For example, Chen et al. (2010) related the Young’s modulus of bone to the bone density, which was assumed to linearly relate with the Hounsfield unit of computed tomography (CT) images. The concentration of chemical constituents in soil samples was spatially correlated with the grayscale intensity of X-ray images in work of Hapca et al. (2015). Lee et al. (2011) and Gillman & Matouš (2014) used microtomographic data to compute mechanical and thermal properties of particulate systems. Yushu et al. (2017) used sharp volumetric billboards (SVB) to study complex 3D Ni/Al high energy ball-milled composites. Gillman et al. (2017) developed microstructure statistics–property relations of silver paste interconnects using the combination of focused ion beam (FIB) milling and scanning electron microscopy (SEM) imaging. Ramos & Matouš (2018) developed experimental procedures for linking microstructure to void growth response in particulate composites using X-ray micro-computer tomography. Yushu & Matouš (2020) developed image-based multiscale multigrid solver and reduced order model using SVB from experimental data.

As highlighted above, the mapping between imaging information and material parameters is well-establish field. However, the use of image analysis to map the heterogeneous mechanical properties and subsequent modeling of heterogeneous biofilms has not been done before, to the best of our knowledge. In this study, we established a quantitative mechanical property map that links pixel-scale biofilm heterogeneity, based on optical coherence tomography (OCT) signal intensity, and macroscopic mechanical behavior. Next, this property map is used in continuum simulations to predict the velocity and stress profiles in the biofilm.

In recent years, OCT has been increasingly used in biofilm studies as a tool for the visualization of biofilm structure. OCT can non-invasively provide mesoscale information of the biofilm (Wagner & Horn, 2017). The OCT signal detects objects based on light scattering (Huang et al., 1991). OCT has been used to study biofilm thickness, roughness, and other morphological characteristics (Aybar et al., 2019; Shen et al., 2016; Wagner et al., 2010). Biofilm geometry and deformation can be extracted from OCT images, and mechanical properties inferred by combining those with biofilm deformation models (Blauert et al., 2015; M. Li et al., 2020; Picioreanu et al., 2018). Also, Hou et al. (2019) showed that the signal intensity of OCT images were linearly correlated with cell density. Since the mechanical properties of biofilms are largely dependent on cell density (Kwok et al., 1998; Van Loosdrecht et al., 2002), OCT images can be a useful tool to map effectively biofilm mechanical properties.

In this study, a 2D map of biofilm mechanical properties (i.e., the non-Newtonian viscosity, , in the Oldroyd‐B constitutive model) was developed based on the grayscale intensities of an OCT image at the pixel scale. This link was established assuming that biofilm mechanical properties were proportional to the grayscale values. We also employed a continuum phase-field (PF) model, together with incompressible Navier-Stokes equations, and incorporated this heterogeneity map for real-time deformation simulations under fluid flow. The model included biofilm non-Newtonian behavior, extending our previous work on homogeneous biofilms (M. Li et al., 2020). Navier-Stokes equations and PF models have been applied to biofilm studies (Lindley et al., 2012; Tierra et al., 2015; Zhang et al., 2008b, 2008a) for its computational advantages and ability to simulate large deformations and detachment. However, uniform biofilm mechanical properties were considered in all prior PF research.

The objectives of this study were to (1) develop a method to assess biofilm deformation with the consideration of morphological and mechanical heterogeneities using a data-driven approach; and (2) compare the impact of morphological and material heterogeneity on biofilm deformations and stress. This study sheds light on the impacts of morphological and mechanical heterogeneity on biofilm behavior and provides potential methods to improve the biofilm control and mitigation strategies.

**2. Materials and Methods**

The biofilm was cultured in a flow cell, and mechanical properties of biofilm were determined by a map between OCT images and Weibull distribution of the material data. Real time biofilm deformation under fluid flow, also monitored by OCT, was saved as a series of images with identical dimensions and resolution. An image processing algorithm was developed and applied to the images to determine biofilm boundaries over time and establish a map of the non-Newtonian viscosity in the Oldroyd-B model based on grayscale intensities of the OCT image. The map of mechanical properties and initial biofilm boundary were used as model inputs. The incompressible Navier-Stokes equations with the phase-field model from our previous work (M. Li et al., 2020) were then employed for the biofilm deformation analysis. Two types of biofilms were analyzed in the simulation: a biofilm with uniform, averaged mechanical properties obtained from a rheometer, and biofilm with spatial distribution of non-Newtonian viscosity.

**2.1 Biofilm growth, deformation experiments and rheometry tests**

A pure culture biofilm consisting of *P. aeruginosa* PAO1 (ATCC 15692) was grown in a flow cell with dimensions 5×5×150mm (width×height×length), following M. Li et al. (2020). The biofilm growth medium was based on 100 mg/L of acetate as an electron donor (see complete list of growth medium in Aybar et al., 2019). Gentamicin sulfate (15 μg/mL, Sigma-Aldrich, USA) was added to maintain axenic conditions. After 5 days of growth, biofilm growth on the glass wall was observed. Deformation under controlled fluid flow was recorded by OCT (Ganymede II; Thorlabs GmbH, Lübeck, Germany) (more details provided below). The effluent was collected in a graduated cylinder to determine the exact flow rate. The same piece of biofilm was sampled and transferred to a shear rheometer (Discovery HR-2 Hybrid; TA Instruments, IL) to determine the average mechanical properties. The detailed experimental set up was described in our previous work (M. Li et al., 2020) and can be found in the Supplementary Information (SI). The rheometer data is shown in Fig. S1, and the experimental averaged and were obtained by data fitting to the Maxwell model (Table 1) (see detailed information in the SI).

**2.2 Image pre-processing for boundary extraction**

Real-time image sequence with the information of biofilm deformation was acquired using the OCT. In this study, the biofilm boundary with detailed geometrical information was extracted from two-dimensional (2D) OCT images and implemented in the modeling as biofilm geometry. The OCT had a 930 nm center wavelength white light beam and a Thorlabs LSM03 objective scan lens, providing 4.1 µm axial resolution and 4.5 mm imaging depth in water. The OCT software (ThorImage OCT 4.1) was used with the following settings: 1.33 refractive index, 30 kHz image frequency, 101 dB sensitivity.

The image sequence was acquired using OCT under the following conditions: (i) dimensions (width × length): 4.8 × 3 mm2; (ii) resolution: 2304 × 1478 pixels; (iii) pixel size: 2.08 µm; (iv) time step: 0.1 s. The total acquisition time was 8s. The image sequence was then cropped to the region of interest (ROI), containing the biofilm structure, with a resolution of 756 × 620 pixels.

To extract biofilm boundaries, the following steps were performed: (i) conversion from grayscale images to binary images; (ii) void filling to make the biofilm a solid white object; (iii) de-noising; (iv) biofilm boundary extraction based on binary values (Fig.1). The image processing steps were developed based on previous work (Gillman et al., 2013; Ramos & Matouš, 2018). A detailed description of image pre-processing is provided in the SI.

**2.3 Two-dimensional map of biofilm mechanical properties**

With the selected biofilm boundaries from the previous step, we analyzed the histogram of grayscale intensities (Fig. 2a). In the OCT image, grayscale intensity of 0 (black) indicates soft biofilms that has a same signal intensity as water (the solvent), while grayscale intensity of 255 (white) indicates stiff bacterial colonies. A small background area within ROI, but outside of the biofilm structure was selected to construct a histogram of the background fluid (Fig. 2b). The grayscale values of the background fluid were in the range of 0 and 40. Therefore, we set the threshold of grayscale intensity as 40. Any pixel value that is lower than this threshold was replaced by *Im*=40 in the image. The processed OCT image has a range of grayscale intensity between 40 and 255.

The Weibull function, which has been used to represent material data in a wide range of fields (Andersons et al., 2002; Cañigueral et al., 2009; Trujillo et al., 2014), was applied to describe the distribution of non-Newtonian viscosity in the biofilm. The 3-parameter cumulative distribution function is shown as follows:

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| --- | --- |
|  | (1) |

where *Y* is the grayscale intensity ranging from 40 to 255, *Yin* is the threshold of grayscale intensity which is 40, and *p1* and *p2* are scale and shape parameters, respectively.

In previous studies (Galy et al., 2012; Pavissich et al., 2021), biofilm mechanical properties, including Young’s modulus and viscosity, differed up by as much as two orders of magnitude within the same biofilm. Therefore, in this work we assumed the non-Newtonian viscosity varied between 100 -1100 Pa⋅s. Because a region that contains matter with the grayscale pixel value of 40 has the same refractive index of water, we assumed this region had water-filled voids or undetected extracellular polymeric substances (EPS) (Wagner & Horn, 2017). Therefore, we selected Pa⋅s for the water-filled/undetected EPS regions with grayscale intensity of 40. Next, the maximum value of the non-Newtonian viscosity was chosen as Pa⋅s. To fit the Weibull distribution in Eq. (2), we considered an average, Pa⋅s, value that was obtained from the rheometer test described in Section 2.1. This average was assigned to mean grayscale intensity value of *Y*=81. By fitting fixed points , and keeping the range of as 100-1100 Pa⋅s, *p1* and *p2* were obtained (*p1* = 1.2, *p2* = 1). The fitted Weibull function is shown in Fig. 3, and was implemented in the model with an image intensity map which is described in Section 2.5.

**2.4 Model Governing Equations**

The modeling work used in this study has been previously described in detail (M. Li et al., 2020) and is briefly summarized here. In this model, the water phase (solvent) and biofilm were considered as two incompressible, immiscible components of a single fluid. The phase-field variable is defined such that the relative volume fractions of solvent () and biofilm () are and , respectively. The density of fluids is:

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|  | (2) |

where represents the density of a biofilm and represents the density of a solvent.

The governing Cahn-Hillard equation for phase-field model is coupled with the incompressible Navier-Stokes equation in this study. The viscoelastic Oldroyd-B equation is applied to consider the biofilm nonlinear viscous behavior. The system of equations is given as follows:

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| --- | --- |
|  | (3) |
|  | (4) |
|  | (5) |
|  | (6) |

where is the fluid velocity [m/s], γ is the mobility [m3⋅s/kg], is the mixing energy density [N], is the interface thickness parameter [m], and is the chemical potential. ***τ*** is the extra (i.e., viscous) stress tensor, denotes the upper-convected time derivative (Oldroyd derivative) of the stress tensor, λ= is the biofilm elastic relaxation time [s]. is the biofilm shear modulus [Pa], is dynamic viscosity of the biofilm [Pa∙s], and **d** is the symmetric part of the velocity gradient, . The mixing energy density is defined as:, where is surface tension coefficient [N/m]. The mobility is defined as:, where is the mobility coefficient [m⋅s/kg].

In equation (4), the Cauchy stress tensor is split into two parts:

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|  | (7) |

where ***T*** is the deviatoric part of the stress tensor, is the pressure [Pa], and **1** is the second-order identity tensor. In equation (6), the Oldroyd-B equation has the following definitions:

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|  | (8) |
|  | (9) |

where is dynamic viscosity of the solvent [Pa∙s].

**2.5 Boundary condition, initial condition, and numerical implementation**

The 2D continuum biofilm model was implemented using COMSOL Multiphysics (COMSOL v5.4; COMSOL Inc., Burlington, MA) using the mixed finite element method. In this work, we use finite elements for velocity and pressure to produce a stable numerical solution. The dimension of simulated flow cell was 5 mm × 7 mm (height × length). The velocity is (Fig. 4a). The averaged bulk velocity measured from effluent flow was ramped up linearly from 0 to within 0.1 s interval. We set  for the inlet boundary. The boundary conditions for the extra stress were ,,, where . The initial condition for the velocity and the extra stress were set as and in the whole domain.

The biofilm was modeled first as a homogeneous material, then as a heterogeneous one to understand the effect of spatial heterogeneity in non-Newtonian viscosity. The biofilm boundary extracted from the OCT image (Section 2.2) was used as the biofilm geometry in the models (Fig. 5a). For the homogeneous biofilm example, the averaged relaxation time, , and non-Newtonian viscosity averaged Pa⋅s, were obtained from the rheometer experiment listed in Table 1. In the heterogeneous biofilm example, was represented as the Weibull function from Section 2.3, and we kept the same mean . The Weibull function is use to map grayscale values in Fig. 5a to produce a viscosity map, . The spatial distribution of , which is used in the computational mode (i.e., Eq (7)), is shown in Figure 5b, ranging from 100 to 1100 Pa·s.

To overcome the computational difficulties caused by irregular biofilm geometries, heterogeneous material data, and fluid incompressibility, we used streamline and crosswind diffusion schemes under Laminar Flow physics in COMSOL software. Finally, we applied the same boundary and initial conditions that we reported in our previous work (M. Li et al., 2020). To select a proper mesh density, we performed mesh convergence tests and selected a maximum mesh size of 100 μm for this study (see SI). Next, we compared the values of velocity in the two simulations to experimental data by implementing a point probe in the middle of the flow cell domain. Figures 4b & 4c show a window of interest for both cases (5mm H × 4mm L). The computed velocity (i.e., in the center of the simulation window) compared well to the experimental flow velocity of m/s.

**2.6 Image post-processing for skeleton extraction**

It is difficult to quantify biofilm deformation based on a fixed 2D section of a 3D biofilm. The experimental biofilm experienced some movement in and out of the OCT focal plane. Therefore, the 2D biofilm boundary may have changed slightly over time in experimental results. To reduce the impact of these small changes, the skeleton of the biofilm was extracted from both experimental and simulated data for the deformation analysis. The skeletonization reduces the biofilm to a centerline, preserving the biofilm topology and reducing the impact of small changes along the biofilm boundary (Saha et al., 2017).

We used the medial axis transform algorithm (Kerschnitzki et al., 2013; Lee et al., 1994) to extract the biofilm skeleton (Fig. 6a; detailed information in SI). To analyze and compare the biofilm deformation, we evenly selected 13 markers on the main skeleton from the top to bottom (Fig. 6b). The movement of the marker points was tracked by image analysis with a linear assignment problem framework (Jaqaman et al., 2008). This was implemented using the TrackMate Plugin in Fiji (Tinevez et al., 2017) (see SI).

The relative error norm was used to compare the deformation of the homogeneous and heterogeneous models. This is based on the following discrete equation:

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|  | (11) |

where and are displacement from simulations and experiment, respectively. Here we sum over 13 selected maker points introduced above.

**3. Results and Discussion**

The non-deformed biofilm boundary extracted from OCT image was used as the model geometry which retained all the detailed irregularities. To analyze the accuracy of our novel data-driven computational scheme, two types of simulations were performed: a) biofilm with homogeneous mechanical properties, and b) biofilm with heterogeneous mechanical properties.

**3.1 Comparison of experimental deformations to model predictions**

The experimental biofilm deformation data was compared to the simulations for both the homogeneous and heterogeneous biofilms. The fully developed laminar flow with a Reynolds number (Re) of Re = 4 was applied as the inlet boundary velocity. The time-dependent simulation was performed for 8 s, as the experimental flow was observed for the same time. The boundaries of experimental and simulated biofilm are shown in Fig. 7(a). Fig. 7(a) shows that biofilm experiences large motions especially at the top of the structure. In general, the experimental deformation is larger to one obtained from the computational simulations. As mentioned above, different OCT planes can come in and out of focus during the time dependent analysis. This can account for some of the local discrepancies.

To further compare the experimental and modeled deformation, a skeletonization was performed to describe the aggregate motion of the biofilm structure. Furthermore, one marker (P1) on the tip of biofilm skeleton (Fig. 6b) was selected for the comparison of biofilm deformation over time (Fig. 7b). The black line with dots indicates the experimental measurement. Fig. 7b shows that the deformation increased for the first 2 seconds and then stabilized. This near steady-state deformation fluctuated in a range of 69-250 μm, with an average deformation of 172 μm (t=2s - 8s). The large variations from point to point in the experimental data indicate the difficulty in tracking biofilm physical behavior during the experiment, since the biofilm moved in and out of the OCT focal plane.

For the simulation results of the biofilm tip, the near steady-state deformation of heterogenous model had better accuracy (13% error) than the homogeneous one (42% error). With a gradual increase of deformation for all cases, the simulated deformation reached to a near steady-state state after 3 seconds in both homogeneous and heterogeneous studies.

A more comprehensive comparison of the overall deformation was analyzed by considering the relative discrete error of biofilm deformation over 13 marker points along the biofilm main skeleton. The positions of points are shown in Fig. 6b with red markers. The relative error of the deformation is plotted in Fig. 7c. In general, heterogeneous simulation led to a higher accuracy of the biofilm deformation prediction over the whole simulation time.

Based on the deformation results of the biofilm tip and the discrete error norm over the entire main skeleton, we can conclude that the heterogeneous model with the image map of the non-Newtonian viscosity as the input can provide a more accurate prediction of biofilm deformation.

To interpret the biofilm deformation, we also plotted velocity profiles within the biofilm domain in both simulations (Fig. 8). The magnitude and direction of the velocity vector indicate the direction of the movement. Both simulations show a velocity distribution including the smaller velocity near the biofilm bottom and larger velocity near the biofilm tip, which is consistent with the applied velocity profile of the entire domain.

A significant difference in velocity distribution, however, was observed between the heterogeneous and homogeneous simulations. The velocity profile of the homogeneous biofilm suggested a cantilever beam like behavior (Fig. 8a). The velocity vectors gradually increased in magnitude and forced the biofilm to bend to right. The large motions and initial tilted profile of the biofilm resulted in the velocity vectors pointing almost downwards at the head (i.e. top) of the biofilm structure.

For the heterogeneous biofilm, the velocities were oriented upwards near the biofilm bottom due to the weak connection of the biofilm base (see viscosity distribution in Fig. 5b). The middle portion of the biofilm was more rigid compared with the other area (Fig. 5b). Therefore, the velocity had horizontal and slight upward direction and stretching existed in the biofilm middle area.

These differences in velocity profiles reveal importance of material heterogeneity on mechanical behavior, even with a similar overall deformation. Since the structure of biofilm is complex, a simplified cantilever beam geometry may not be suitable for biofilm studies. The complex motion of the biofilm can be better predicted with the consideration of material heterogeneity. The simulated results illustrated that our heterogeneous model could achieve both greater quantitative accuracy and a reasonable prediction of deformation.

**3.2 Comparison of pressure and extra stress in heterogeneous and homogeneous simulations**

Deformation is not the only factor that affects the biomechanical functions of the biofilm. The overall stress as well as the pressure distribution within the biofilm matrix are also affected. Knowledge of the stress in the biofilm matrix is important as the biofilm detachment and tearing are substantially influence by it. First, we analyzed and compared the pressure for both heterogeneous and homogeneous models (Fig. 9 a&b). The pressure for both simulations displayed entirely different distributions within the biofilm domain.

The pressure distribution in the homogenous biofilm indicates that the biofilm behaves like a cantilever beam (Fig. 9b) as mentioned above. Negative pressure on the left side of the biofilm indicated an extension, whereas the positive pressure on the right side of the biofilm indicated a compression. Moreover, this positive/negative pressure pattern is present in the whole biofilm structure from the base to head. The complex biofilm morphology led to an uneven pressure distribution over the biofilm domain with pressure concentrations close to irregular boundary.

The pressure distribution in the heterogeneous case exhibits a markedly different character. The base (i.e., bottom) of the structure is weak (i.e., void-filled area) and pressure is tensile across the whole structure as the biofilm is pulled upwards. The middle of the biofilm moves almost horizontally (see Fig. 5b) and pressure is compressive and fairly constant. The head (i.e., top) of the biofilm structure exhibits large positive (i.e., compressive) pressure. We attribute this behavior to a large material heterogeneity. The weak (i.e., void-filled area) top part of the head is compressed to much stiffer bottom part of the head leading to large compressive pressure.

We also plot the magnitude of the extra stress tensor in biofilms (Fig. 9 c&d) for both homogeneous and heterogeneous cases. We note that the heterogeneity of non-Newtonian viscosity is directly related to the extra stress tensor. Figures 9c & 9d show the irregular distribution of the magnitude of the extra stress tensor especially close to the boundary. In the homogeneous case, the magnitude of the extra stress tensor was higher on average (see lighter blue color in the whole biofilm structure). This is reasonable because the average in the homogeneous model is larger than one used for weaker areas of the heterogeneous biofilm. The marked difference in the extra stress can be observed at the base of the biofilm, where heterogeneous model predicts large stress concentrations. These concentrations are well correlated with the heterogeneity distribution in and are due to the weaker structures connected to rigid structures (see Fig. 5b).

Based on our simulations, the predicted biofilm pressure and stress were significantly different even though similar biofilm deformation was obtained in both cases. Therefore, we can conclude that the mechanical property map provided a more detailed pressure and stress results. Moreover, with the average non-Newtonian viscosity, the biofilm behaved like an imperfect cantilever beam which bent under the flow. In reality, the biofilm is a soft viscoelastic matrix. More rigid particles, such as clusters of cells, are present within the soft EPS matrix. This microstructure of biofilms can largely alter the pressure and stress.

**4. Conclusions**

In this study, we developed image-based (data-driven) modeling protocols for heterogeneous distribution of non-Newtonian viscosity and implemented this heterogeneous map into Navier-Stokes /phase-field model. The average non-Newtonian viscosity value was obtained from rheometer tests. Deformation was tracked under OCT. The image processing was performed on the sequential images to (1) extract the actual biofilm geometry for modeling, (2) develop a 2D map of non-Newtonian viscosity of biofilm, and (3) extract the biofilm skeleton for deformation comparison between experimental and modeling results. The results show that the deformation prediction was improved by considering the heterogeneity of material properties. A more detailed pressure and extra stress distributions can also be obtained from heterogeneous biofilm simulation. With the consideration of heterogeneity of the non-Newtonian viscosity, image-based model can achieve both quantitative accuracy and reasonable prediction on pressure, stress, and movements. This data-driven model can be applied for future studies of biofilm mechanical behavior. By considering models with more realistic distribution of mechanical properties, improved biofilm control strategies may be possible. More accurate statistical 2D or 3D property maps that consider more OCT planes and assimilate more experimental data should be included in the future work.

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**Table 1**. Parameters used in the model

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Value** | **Units** | **Description** | **Source** |
|  | 8×10-4 | m/s | Averaged flow velocity for biofilm test | Experiment |
| χ | 1×10-9 | m⋅s/kg | Mobility parameter | from Zhang et al. (2008b) |
|  | 1×10-2 | N/m | Surface tension coefficient | from Koza et al. (2009) |
|  | 2.5×10-5 | m | Interface thickness parameter | from Yue et al. (2006) |
|  | 1000 | kg/m3 | Solvent density | Value at 20oC |
|  | 0.001 | Pa∙s | Viscosity of solvent | Value at 20oC |
|  | 1000 | kg/m3 | Biofilm density | Assumed (Sevillano et al., 2008; Tsezos & Benedek, 1980) |
|  | 699 | Pa⋅s | Averaged viscosity of the biofilm | Experiment |
|  | 0.4 | s | Averaged relaxation time of the biofilm | Experiment |

**Figure Legends**

**Figure 1.** The ROI with biofilm structure during the image characterization process (time = 0). (a) Raw grayscale image, (b) binary image with a threshold of 0.3, (c) binary image after hole filling and noise removal, (d) binary image after opening operation, following by a second-time hole filling.

**Figure 2**. Histograms of grayscale intensity for biofilm structure (a) and background (b). The selected biofilm and background grayscale area is shown as a subplot of (a) and (b), respectively.

**Figure 3**. Weibull function representing non-Newtonian viscosity with corresponding grayscale intensities. The red starred marker represents an averaged viscosity value of Pa⋅s measured from the rheometer test and is associated with the average grayscale intensity.

**Figure 4.** Schematic of experimental setup, modeling coordinate system (a) and simulated magnitude of velocity for homogeneous biofilm (b) and heterogeneous biofilm (c) at t=8s.

**Figure 5**. The map of biofilm non-Newtonian viscosity . (a) 2D OCT biofilm image with extracted boundary highlighted yellow. The unrelated data were filtered out. (b) The 2D spatial distribution of non-Newtonian viscosity that implemented in the heterogeneous biofilm simulation.

**Figure 6**. (a) A skeleton of stagnant biofilm (time=0). The skeleton was highlighted with a 21-pixel length yellow curve. (b) The positions of 13 tracking markers along the main skeleton of biofilms. P1 shows the first tracking point on the skeleton tip. Unrelated data were filtered out.

**Figure 7.** The comparison of biofilm boundaries and displacements of the skeleton and point P1. Flow was from left to right. (a) The comparison of biofilm boundaries. Black line: biofilm contour at t=0 (in experiment and computational model); red line: biofilm contour at t=8 s (in experiment); blue line: biofilm contour at t=8 s (in homogeneous simulation); green line: biofilm contour at t=8 s (in heterogeneous simulation). Unrelated data were filtered out. (b) The deformation of marker point P1 over time for the biofilm in experiment, homogeneous simulation, and heterogeneous simulation. (c) The relative deformation error over time in the heterogeneous simulation and homogeneous simulation for all 13 marker points along the skeleton. See Figure 6 for location of skeleton points, including P1.

**Figure 8**. The simulated biofilm velocity **v** at t=8 for homogeneous simulation (a) and heterogeneous simulation (b).

**Figure 9**. Simulated pressure and magnitude of the biofilm extra stress tensor at t=8 for homogeneous simulation (a&c) and heterogeneous simulation (b&d).