

**Supplementary Information for manuscript titled:**

**Microrheology and spatial heterogeneity of *Staphylococcus aureus* biofilms modulated by hydrodynamic shear and biofilm-degrading enzymes**

J.W. Hart<sup>1</sup>, T.A. Waigh<sup>1,2\*</sup>, J.L. Lu<sup>1</sup>, I.S. Roberts<sup>3\*</sup>

<sup>1</sup>School of Physics and Astronomy, Schuster Building, The University of Manchester, Oxford Road, Manchester M13 9PL, UK.

<sup>2</sup>Photon Science Institute, The University of Manchester, Oxford Road, Manchester M13 9PL, UK.

<sup>3</sup>Faculty of Life Sciences, Michael Smith Building, The University of Manchester, Dover Street, Manchester M13 9PL, UK.

\*To whom all correspondence should be made, [t.a.waigh@manchester.ac.uk](mailto:t.a.waigh@manchester.ac.uk), [i.s.roberts@manchester.ac.uk](mailto:i.s.roberts@manchester.ac.uk)

Number of pages: 5

Number of supplementary figures: 5

The supplementary information contains four figures with additional details to convey the complete dataset for all biofilm studies.

Firstly, Figure S1 shows the bacteria counts corresponding to the same experiments. No significant differences in bacteria population are observed for biofilms grown under flow, but fewer bacteria were observed in the stationary biofilm at greater heights.

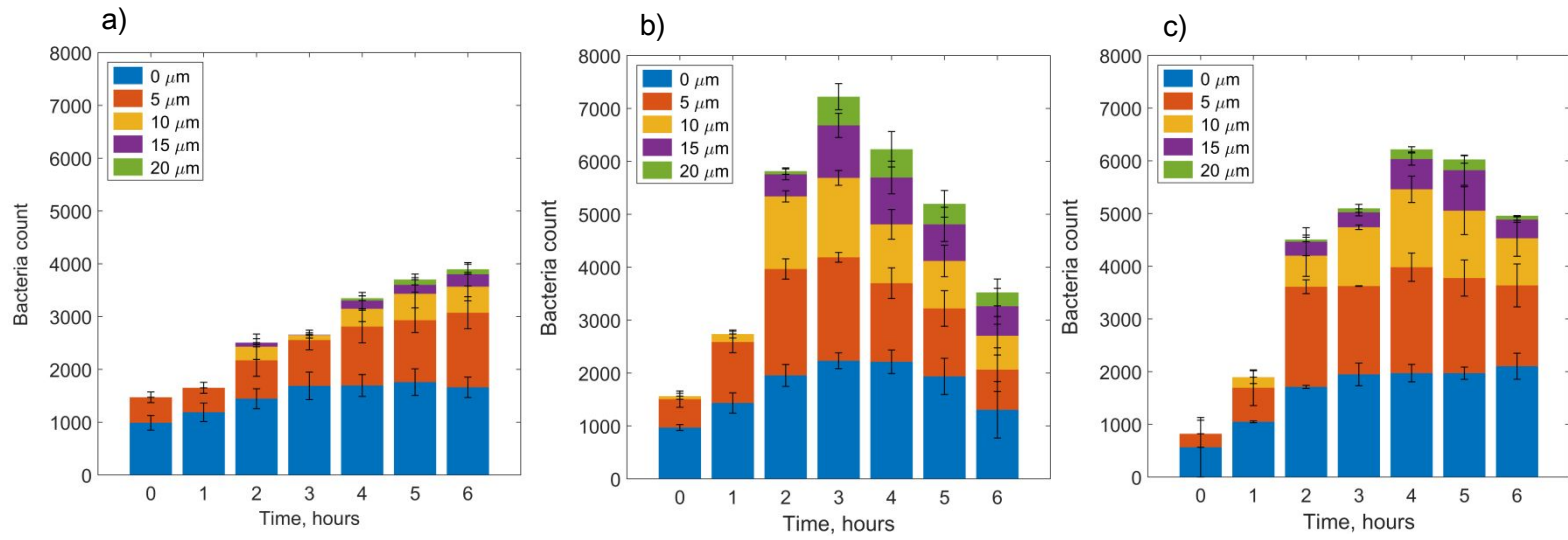
Figure S2 shows the identified bacteria radii across all experimental time points, heights (indicated by colour) and hydrodynamic regimes. As the maximum variation is on the order of 6%, discrepancies in the bacteria size cannot alone explain the changes in creep compliance observed.

Figure S3 shows the characteristic creep compliance at a reference time of 10 ms over the complete experiment time course of 6 hours for the hydrodynamic shear stresses (no flow, 1 mPa and 10 mPa).

Figure S4 shows the rescaled Ripley-*K* function for a radius of 10  $\mu\text{m}$  for all time points and flow regimes, indicating the increase in spatial heterogeneity as a function of height.

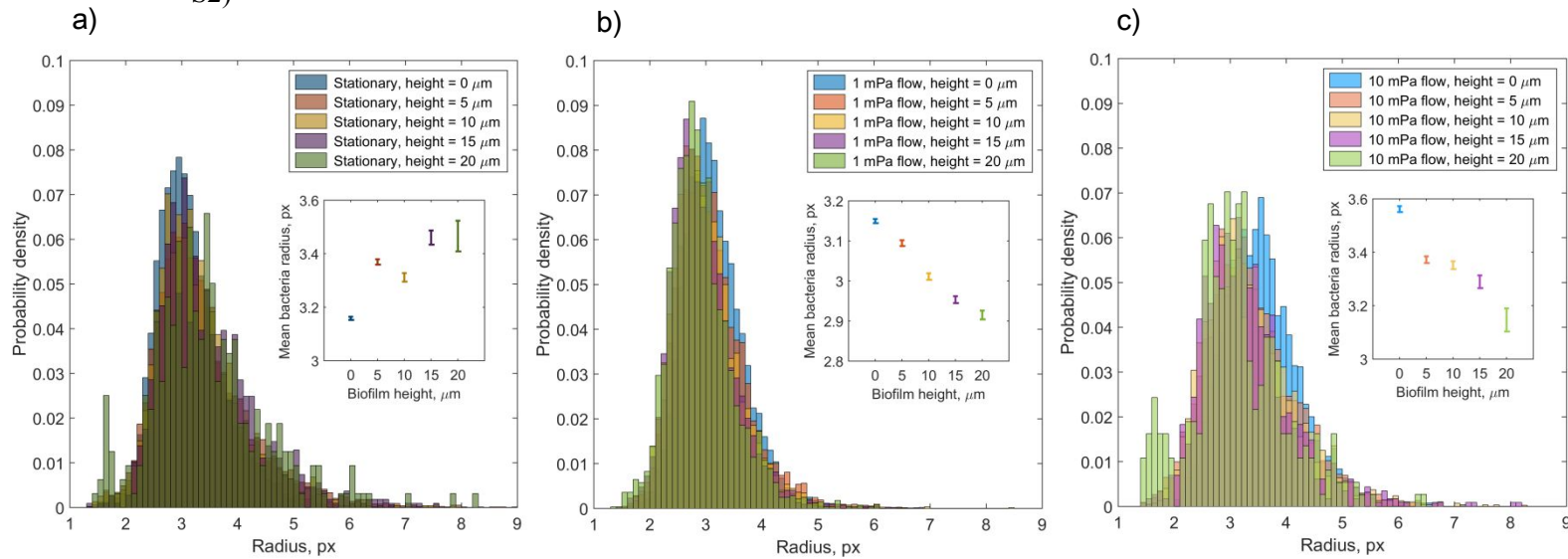
Figure S5 conveys the biofilm characteristic creep compliances at a shear rate of 1 mPa when subject to 60  $\mu\text{g/mL}$  of Proteinase-K and 100  $\mu\text{g/mL}$  of DNase-1. A large increase in creep is seen for all time points in the biofilm treated with Proteinase K, however DNase-1 made little impact (compare with Figure S3b).

S1)



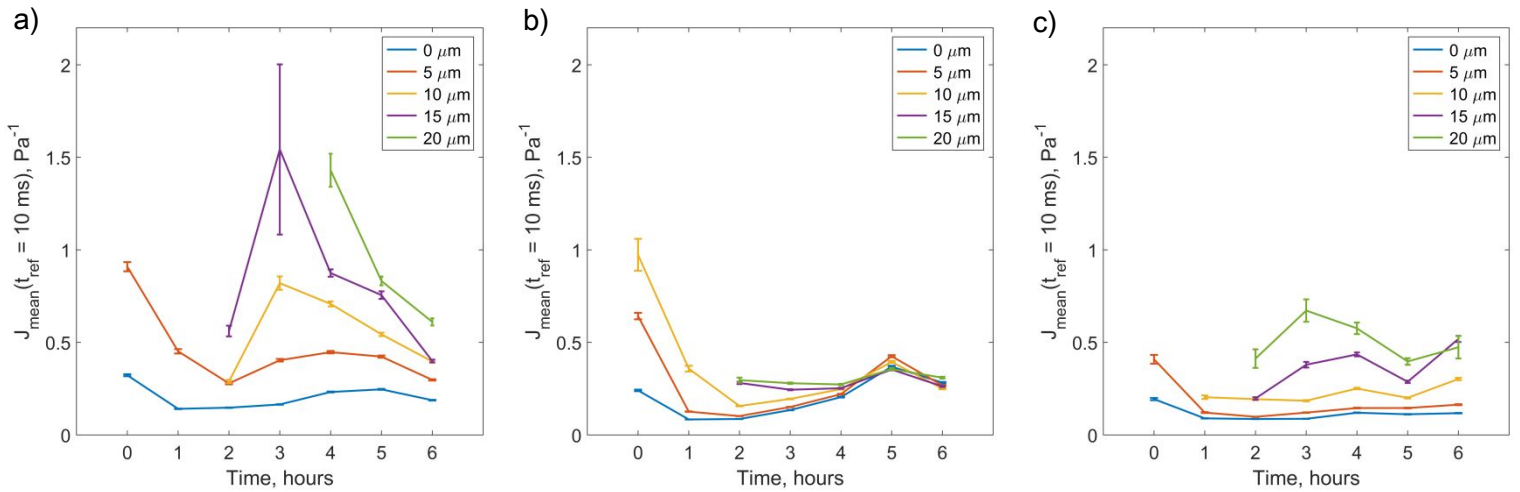
**Figure S1.** The number of bacteria found in a biofilm as a function of time at a series of shear rates, namely a) 0, b) 1 mPa and c) 10 mPa. Different colours represent height above the attachment surface in the flow cell. Similar proportions of bacteria were found at all shear rates. Within errors, bacteria proliferation was approximately the same for the 1 mPa and 10 mPa, but was reduced for static biofilms.

S2)



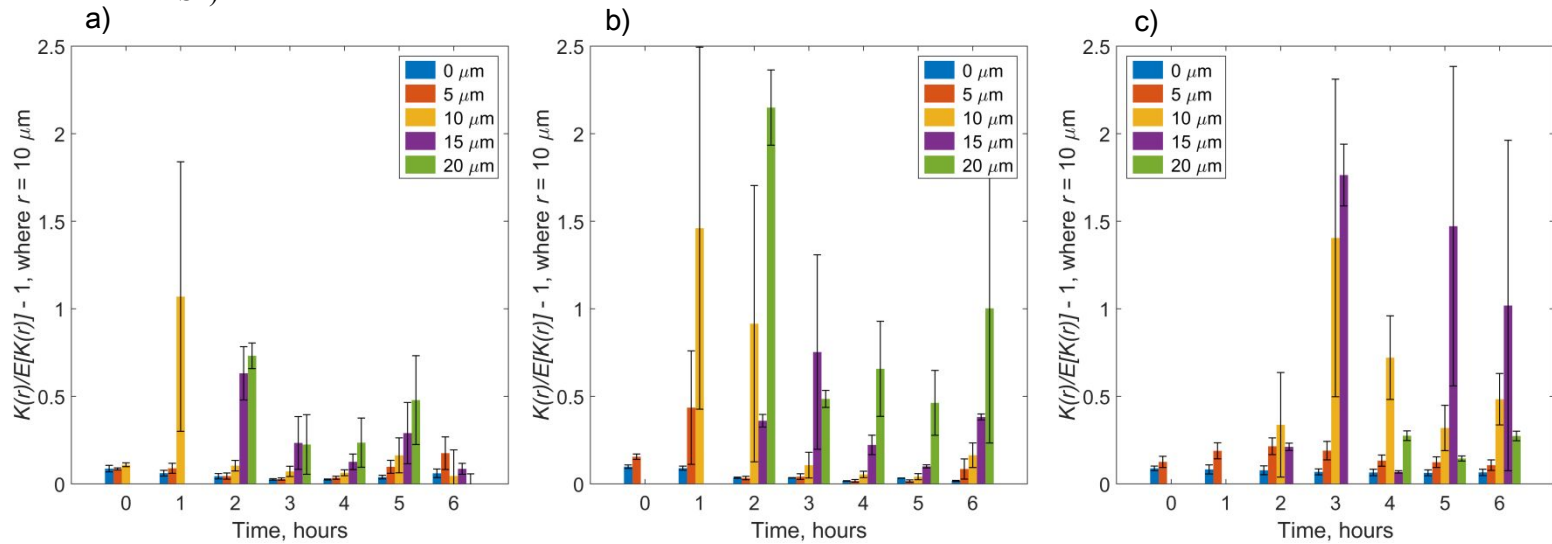
**Figure S2.** Identified bacteria radii distributions across all experiments at all times and heights (indicated by colour) and hydrodynamic shear regimes, namely a) stationary, b) 1 mPa and c) 10 mPa. The insets in each graph show the mean (and standard error) bacteria radius corresponding to a given height within the biofilm. As can be seen, the maximum variation in radius size is ~6%, indicating no significant contribution to the creep compliances calculated using Equation 2.

S3)



**Figure S3.** The characteristic creep compliance at a single time interval (10 ms) plotted as a function of time for the entirety of the experiment (6 hours) with a) no flow, b) 1 mPa shear stress and c) 10 mPa shear stress. Heights in 5  $\mu\text{m}$  intervals are shown as different coloured lines. The biofilms are seen to be softer the further they are away from the surface of attachment in both no flow and 10 mPa conditions. Under flow conditions the bacteria appear to be more securely fastened to the surfaces and the compliances are lower (harder, with larger shear moduli).

S4)



**Figure S4.** Rescaled Ripley  $K$ -function for all time points (6 hours) for biofilms three different hydrodynamic growth regimes, namely a) no flow, b) 1 mPa shear stress and c) 10 mPa shear stress. The significant increase in the values of the rescaled Ripley- $K$  as a function of height (represented by different colours in 5  $\mu\text{m}$  intervals) indicates spatial clustering vertically during all time points of the biofilm growth.

S5)

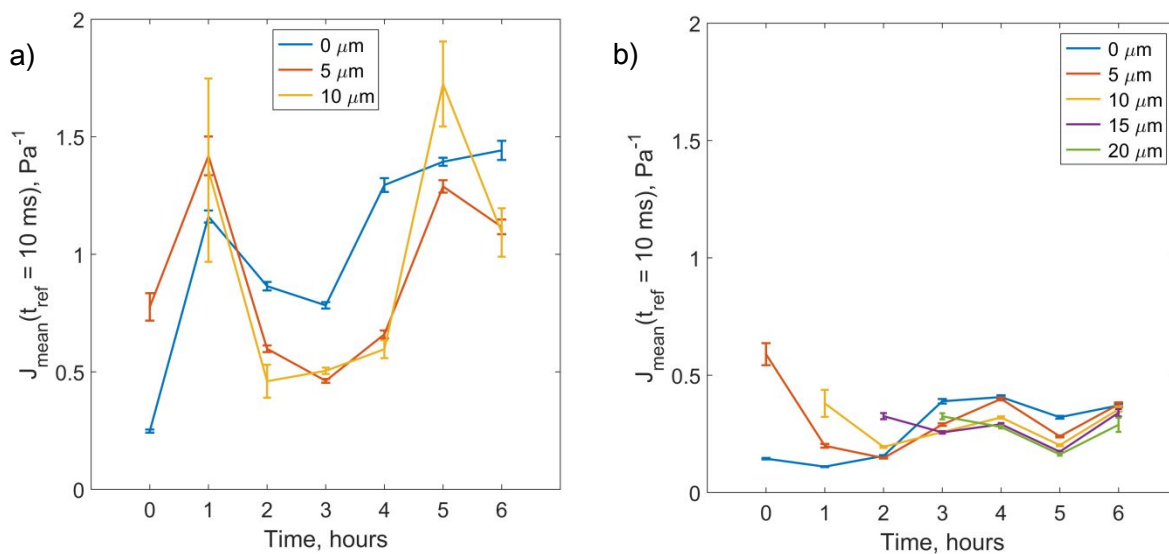


Figure S5. Full time course results over 6 hours for biofilm characteristic creep compliances at a reference time interval of 10 ms when in a) a 60  $\mu\text{g/mL}$  solution of Proteinase K and b) a 100  $\mu\text{g/mL}$  solution of DNase-1. Height increments of 5  $\mu\text{m}$  away from the flow cell surface are represented as different colours.