Electronic Supplementary Material (ESI) for Lab on a Chip.

Lab on a Chip

Supplementary Information

Title: Label-free 1D Microfluidic Dipstick Counting Of Microbial Colonies And Bacteriophage Plaques

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Supplementary Methods

Phage Stock Preparation

For phage K bacteriophage amplification, inoculate with a single colony of *S. aureus* into a sterile BHI broth and place in incubator at 37 ºC, 150 RPM. This overnight culture was used to inoculate a new sterile BHI broth flask with an initial 0.05 OD_{600}. Typically, this was achieved with 1 ml added overnight to 99 ml of BHI broth and placed this in the Incubator at 37 ºC, 150 RPM. When the OD_{600} reached 0.2 (approximately 90-120 minutes), the bacteria were inoculated with phage K at a multiplicity of infection (MOI) of 0.1. After 4 hours and the OD_{600} started to decrease, it was centrifuged at 4500 x g for 10 minutes. The supernatant was filtered using a 0.45 µm filter and stored at 4°C until use.

For T2 bacteriophage amplification, inoculate with a single colony of *E. coli* into a sterile LB broth and place in incubator at 37 ºC, 150 RPM. This overnight culture was used to inoculate a new sterile LB broth flask with an initial 0.05 OD_{600}. Typically, this was achieved with 1 ml added overnight to 99 ml of LB broth and placed this in the Incubator at 37 ºC, 150 RPM. When the OD_{600} reached 0.2 (approximately 120-150 minutes), the bacteria were inoculated with T2 phage at an MOI of 0.1. After 4-5 hours and the OD_{600} started to decrease, it was centrifuged at 4500 x g for 10 minutes. The supernatant was filtered using a 0.45 µm filter and stored at 4°C until use.

Double Agar Layer Method (DAL)

For bacteriophage enumeration, 10 mL of BHI top agar (BHI broth with 0.5% agar) was mixed with 5 mL of salt solution (400mM $MgCl^2$ and 100mM $CaCl^2$) for *S. aureus* and LB top agar (LB with 0.5% agar) for *E. coli* were prepared. This was kept in a water bath at 60°C for 1 hour. 10 µl of host bacteria at $4x10^8$ CFU/mL and 10µl of bacteriophage were added to this tube, which was left to cool but still molten. The mix was poured into a Petri dish and left to dry. These procedures were repeated for serial dilutions of bacteriophage samples and incubated for 24 h at 37 °C and plaques counted (Figure S1).

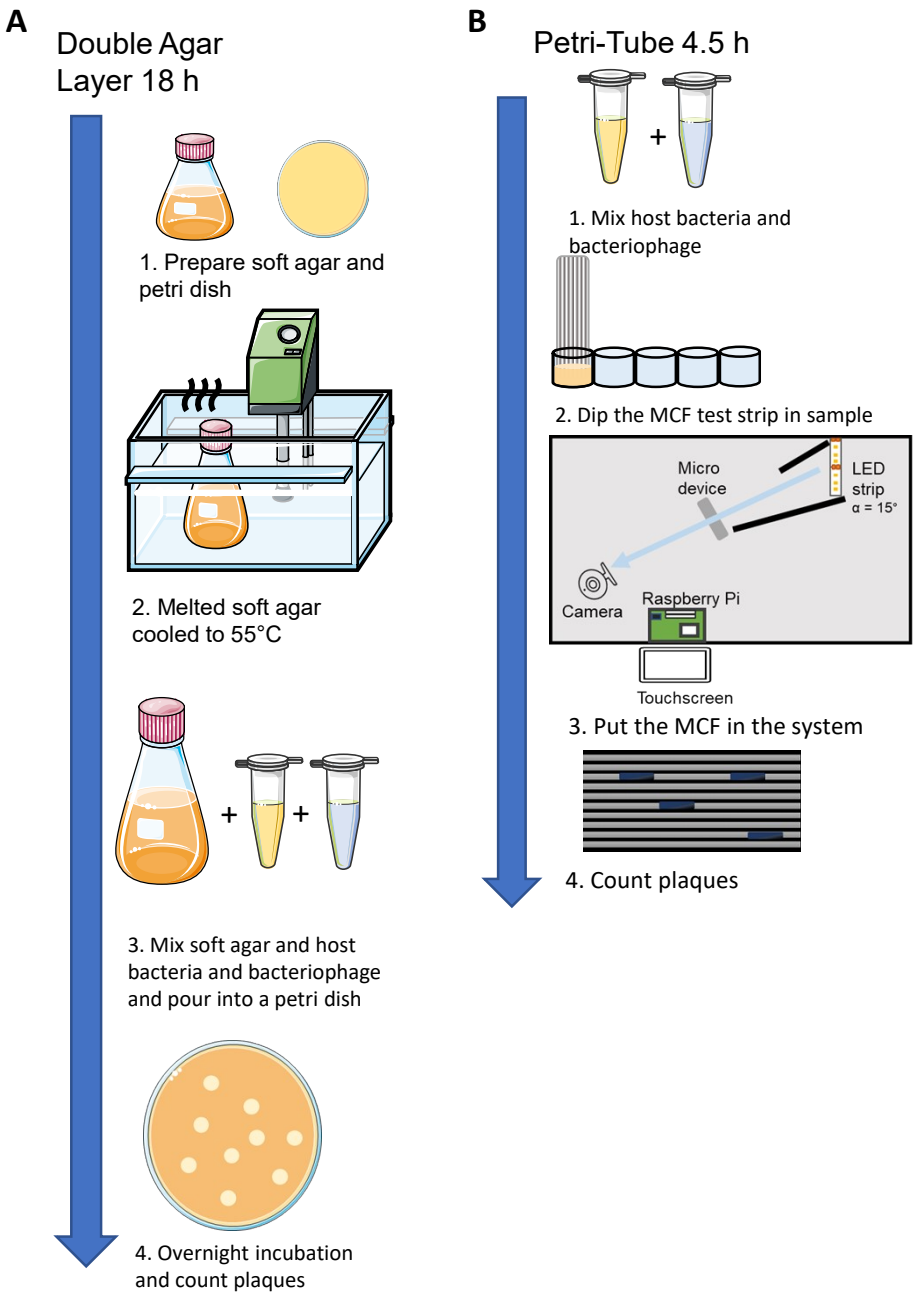

Figure S1. Conventional petri dish double agar layer (DAL) method comparison with liquid microcapillary film (MCF) method.

Plate Spread Method

An aliquot of a ~8.0 × 10^8 CFU/mL *E. coli* stock (ATCC 25922 and B strain) is divided into 3 titrations (250, 500, and 1000 CFU/mL), and 100 µL of each titration is plated in LB agar plates, resulting in expected CFU counts of 25, 50, and 100, respectively. After a night, 37 °C incubation, the number of colonies from each titration was counted and recorded directly on the plate. After repeating the same experiment for 3 titrations in triplicate, the counted number of colonies is plotted against the expected number of colonies, with error bars showing +/- 1 standard deviation in both axes- all error bars are plotted but in some cases are too small to distinguish.

Supplementary Results

1D individual non-motile bacterial colony formation

MCFs were dipped in *E. coli B* samples of different concentrations and photographed with time-lapse imaging every 10 min in the darkfield system. Colony formation was observed after 4 hours, and colony formations could be counted after 5.5 hours. Since the formed colonies did not have the ability to move, they remained in a limited area and allowed to be counted. During growth, using the point where it started to grow as the origin, its growth is not distributed over the entire capillary (Figure S2). The full video is shown in S1 Video.fileformat.

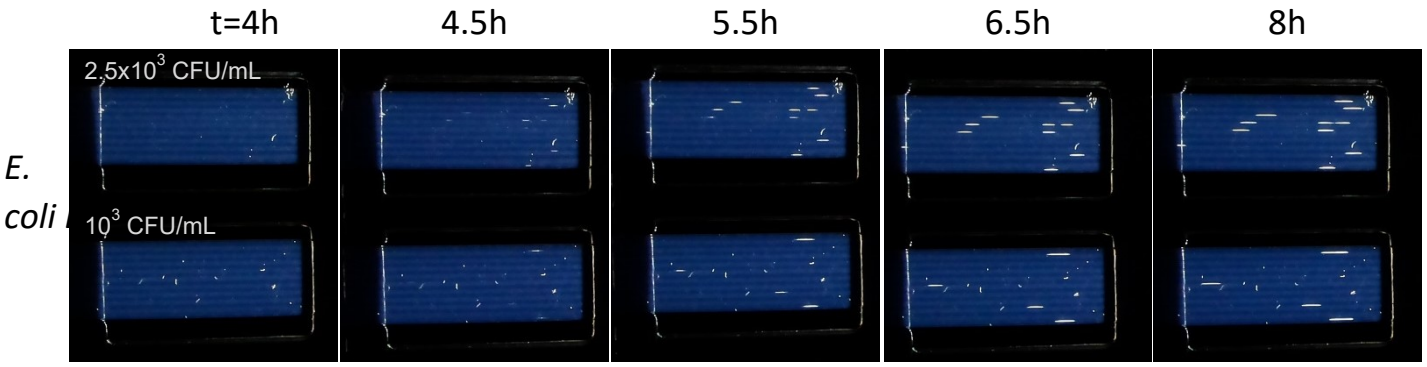

Figure S2. Diagram illustrating various timepoints and endpoint to accompany video of individual bacteria counting in 1 dimensional microcapillaries by time-lapse imaging. First MCF test strip was dipped into suspension of 2.5x10^3 CFU/mL *E. coli* B strain, second MCF test strip with 10^3 CFU/mL.

1D individual motile bacterial colony formation

To understand how motile *E. coli* 25922 grew as a single colony in MCF, different concentrations of both broth and soft agar were prepared and MCFs were dipped into these samples. Prepared MCFs were photographed with time-lapse imaging every 10 minutes in the darkfield system. Colonies started to grow after 4 hours in this motile bacterium like *E. coli* 25922. However, the bacteria growing at one point spread throughout the whole capillary thanks to their motility. Soft agar was also not sufficient to restrict the movement of this bacteria (Figure S3). The full video is shown in S2 Video.fileformat.

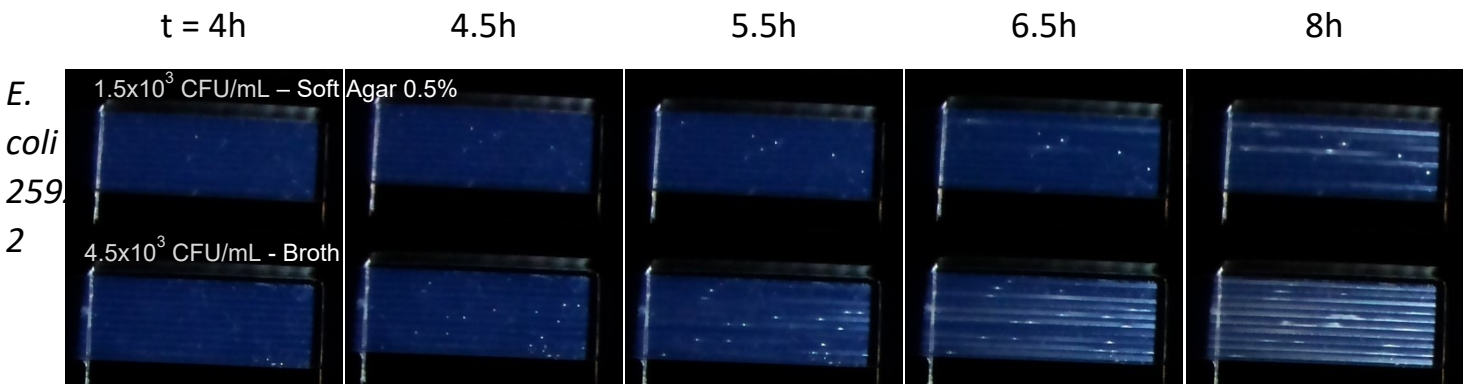

Figure S3. Motile *E. coli* 25922 growth in soft agar vs liquid broth in MCF. Starting inoculum at 1.5 x10^3 CFU/mL in soft agar and 4.5x10^3 CFU/mL in liquid media.

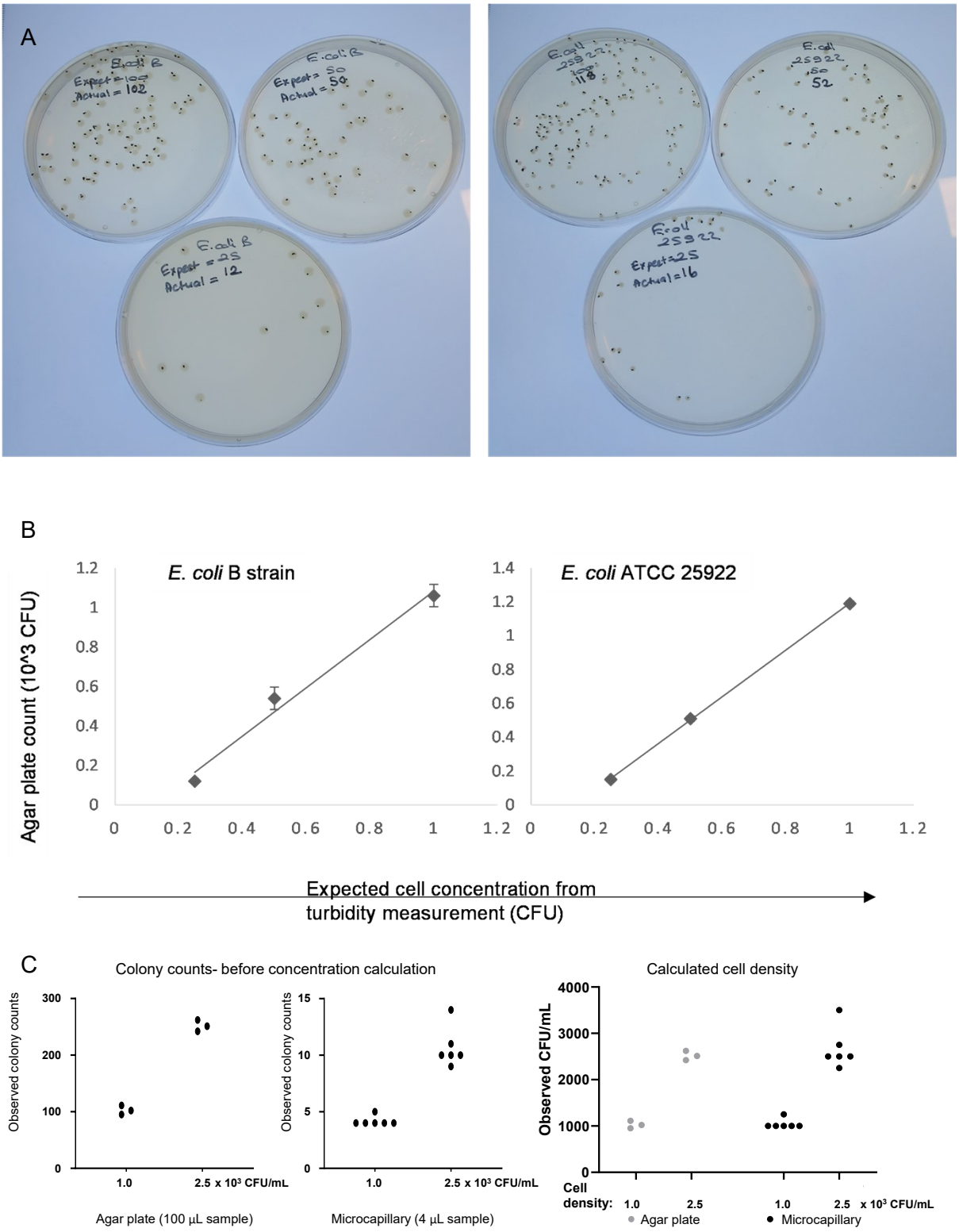

Figure S4. Measurement of *E. coli* Cell Concentration by Plating and Microcapillary Counts. **A)** The turbidity of an aliquot of a ~8.0 × 10^8 CFU/mL *E. coli* culture (ATCC 25922 and B strain) was used to calculate expected cell concentrations and diluted into 3 suspensions (250, 500, and 1000 CFU/mL), and 100 µL of each plated onto LB agar plates, expected to give expected CFU counts of 25, 50, and 100, respectively for these 3 plates. Images illustrate manual colony counting for replicate plates. **B)** the number of colonies from each was manually counted and recorded directly on the plate. After repeating the same

experiment in triplicate, the counted number of colonies was plotted against the expected number of colonies based on turbidity, with error bars showing +/- 1 standard deviation in both axes of the triplicate experiments- all error bars are plotted but in some cases are too small to distinguish. **C)** To explore the repeatability of colony counting in microcapillaries a set of 6 repeat measurements were taken (each comprising 4 µL total volume per MCF strip within 10 capillaries) and compared with triplicate agar plate counts (plating 100 µL). Raw colony counts for the two methods were plotted (left) and calculated cell densities (right) and in all cases all individual points are shown to illustrate the distribution of counts.

Properties of capillary colonies

We examined colony length for *E. coli* B and *S. aureus* as a 1-dimensional measure of colony growth in liquid media. Photographs were selected at 6 regular time points from the full time-lapse image stack, including the first time that the colony became clearly visible. Graphs of the mean colony lengths of 6 time points of different colonies were plotted against on the mean light scattering intensity (Figures S5-6A). Then, the light scatter intensity of 9 different bacteria was examined for 6 different time points for both organisms. Graphs prepared without normalization showed much variation for each colony (Figure S6).

Growth kinetics were normalized to define a single fixed rectangular area of interest that completely contained the colony at the final stage of growth, and then calculate the grayscale intensity with that area. At this stage, it was observed that the two organisms drew very different growth patterns from each other. While *E. coli* started to rapidly multiply, it lost its growth rate at some point and continued to expand more slowly in the capillary. On the contrary, *S. aureus* started at a slower growth rate but maintained this rate throughout the entire period (Figure S7). Here, taking advantage of Monod's kinetics, which correlates microbial growth rates in an aqueous medium with a limiting nutrient concentration, we can say that while *E. coli* showed a rapid growth regime, it quickly consumed the nutrient concentrations in the environment and thus the growth rate of the bacteria was limited.

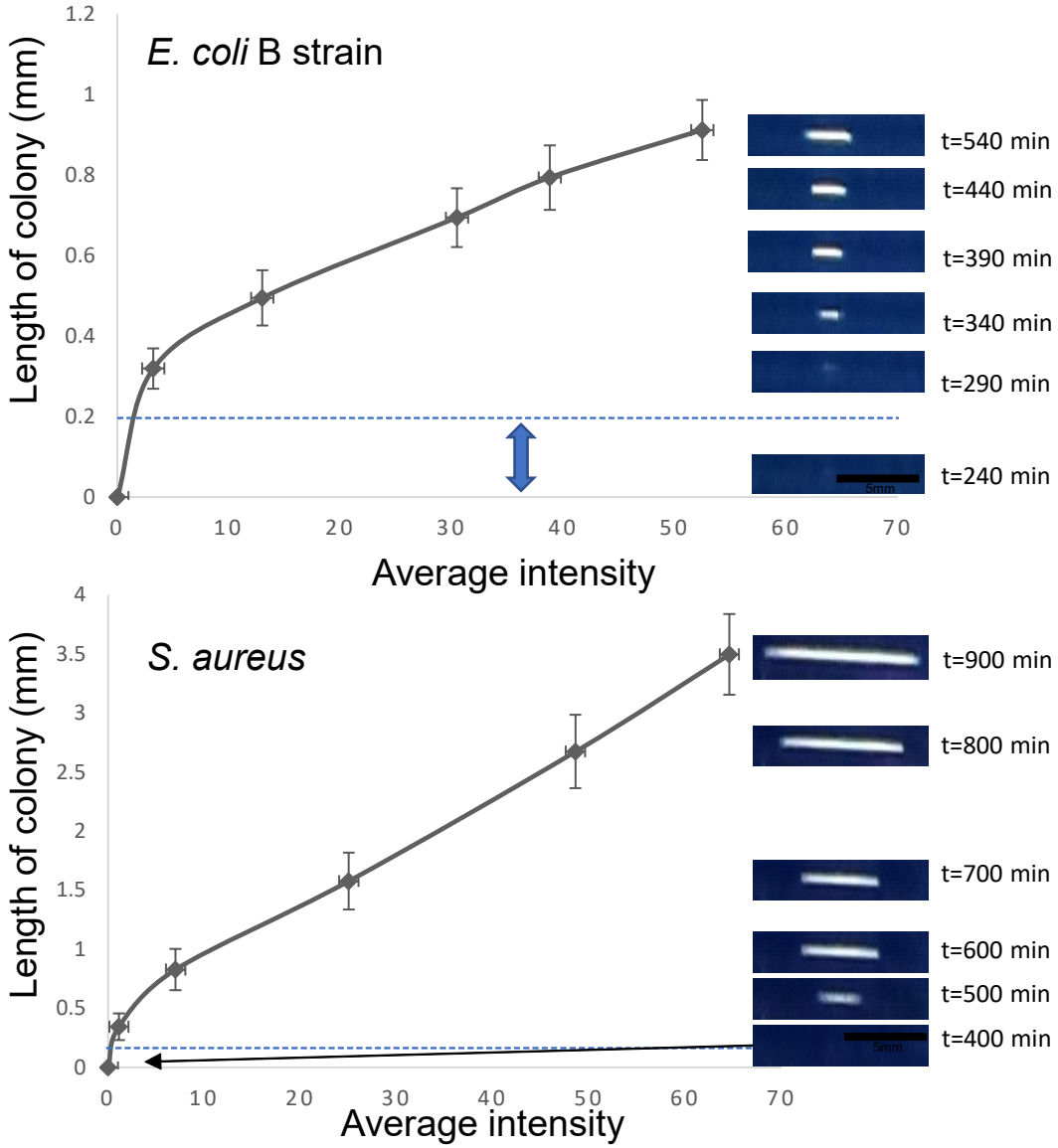

Figure S5. Properties of capillary colonies. Colony length and light scattering intensity were measured for 9 *E. coli* (top) and *S. aureus* (lower) colonies at the 6 timepoints indicated in figure 3, and images of one example colony displayed at each time. Mean length versus integrated intensity of the 9 colonies are plotted, with error bars indicating ±1 standard deviation.

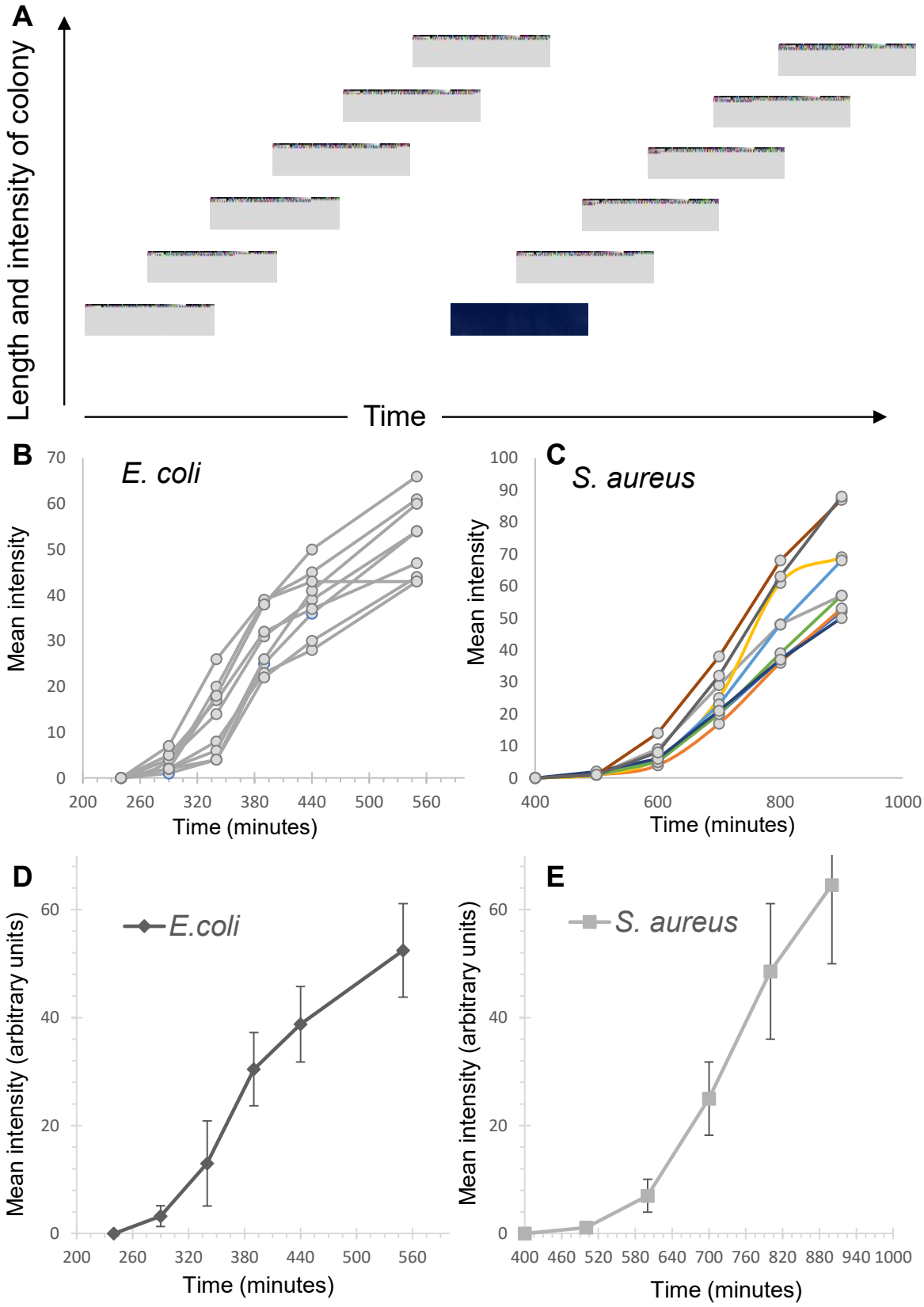

Figure S6. Colony intensity estimating initial growth. Each colony was identified, and a box used to select that contained the whole colony at a late time-point, and the same box

highlighted for all measured timepoints; images in A show example for colonies for each organism, increasing in length and intensity as the colony grows. B-D Mean light scattering intensity were measured for 9 individual *E. coli* (B, D) and *S. aureus* (C, E) colonies at the 6 timepoints indicated in figure 3. B, C individual mean intensities for all colonies plotted to illustrate spread in intensity. D, E Means for the 9 colonies plotted with error bars indicating ±1 standard deviation.

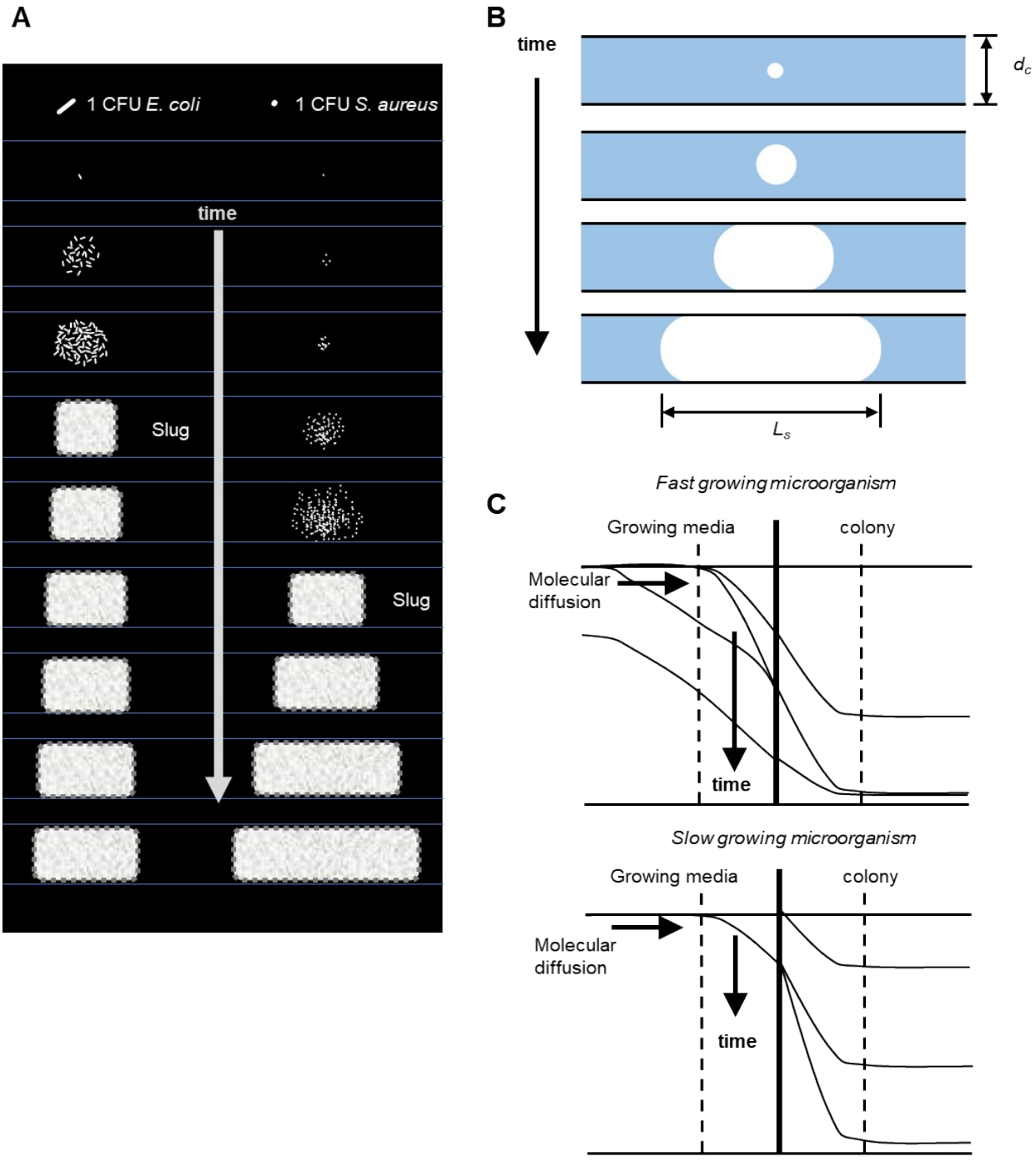

Figure S7. Representative growth chart of fast and slow growing colonies**.** A) Capillary growth chart for *E. coli* and *S. aureus*. When the size of the colony reaches the microcapillary diameter, the 1-dimensional growth of the colony becomes limited by the diffusion of carbon source, glucose. B) 1-dimensional expansion of length of colony 'slug', L_S in a microcapillary with inner diameter d_c. C) Predicted diffusion plot for a fast-growing

organism. With a fast-growing microorganism such as *E. coli*, the glucose concentration at the interface is depleted, forcing the colony to grow at lower concentration of glucose, therefore showing reduced growth rate and linear expansion rate of the colony. With a slow growing microorganism such as *S. aureus*, there is sufficient time for glucose to diffuse to from the bulk of the microcapillary to the liquid-colony interface, therefore enabling growth of the colony closer to maximum growth rate, sustaining longer linear expansion along the microcapillary.

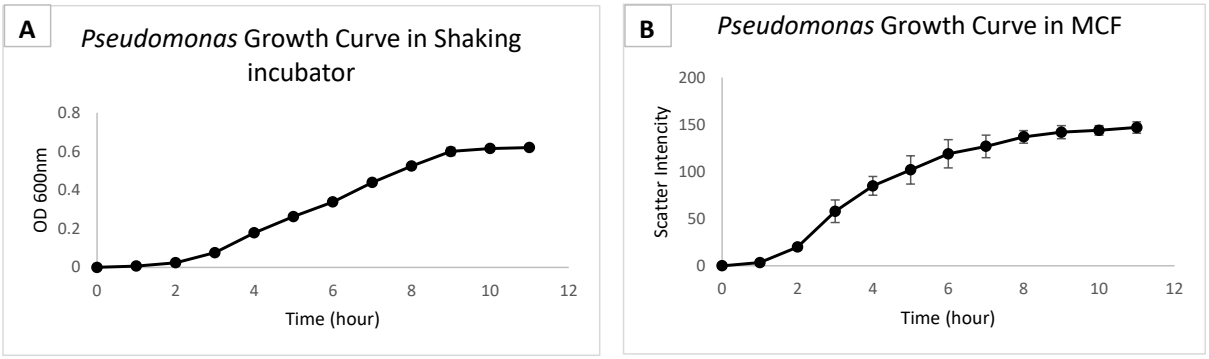

Figure S8. *Pseudomonas* growth curves were measured in parallel using cuvette (A) in spectrophotometer vs within MCF (B) and comparing cell growth kinetics in flask vs within 20 replicate microcapillaries. All error bars are plotted and indicate the standard deviation, in some cases this is smaller than the symbol size.