Supplementary Information

3D Printed Imaging Platform for Portable Cell Counting

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3D Printed Assembly

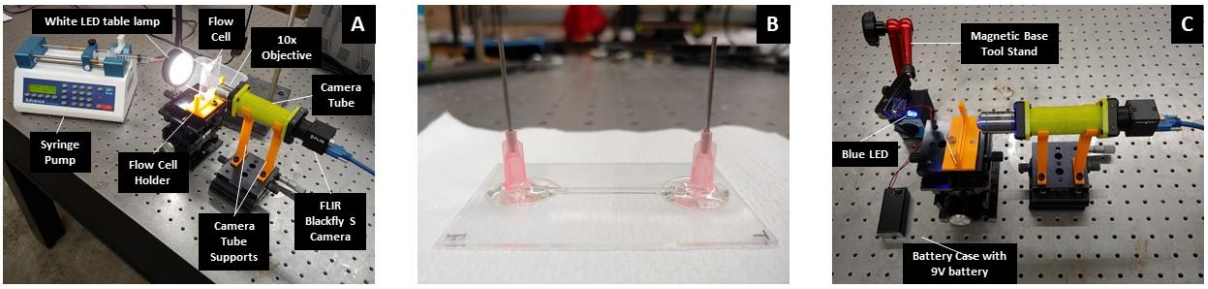

Figure S1 – Images of the 3D-printed imaging platform (3DPIP) set-up. A) The 3DPIP used for particle counting with all of its components. B) The flow cell that consists of a capillary tube and syringe injection ports. C) The static imaging configuration used for fluorescence microscopy.

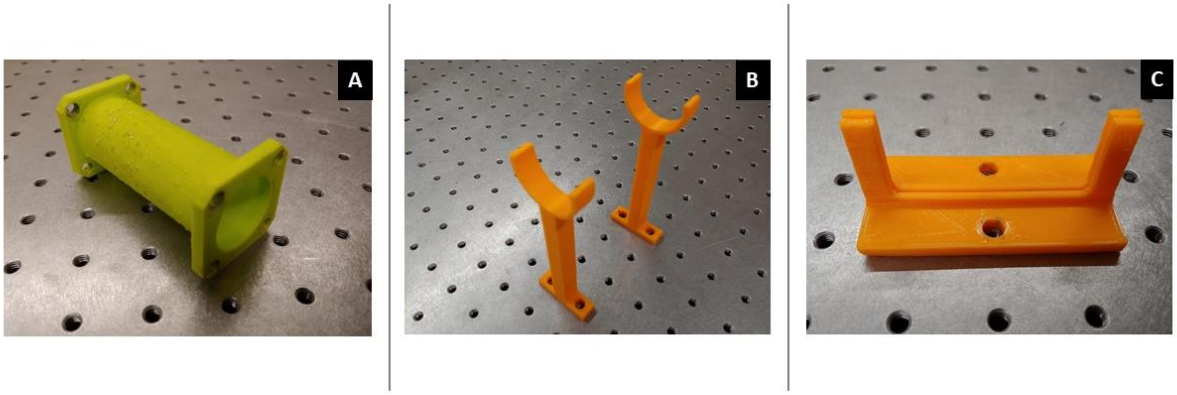

Figure S2 – 3D-printed assembly: A) Camera Tube. B) Camera Tube Supports. C) Flow Cell Holder.

Table S1 – Dimensions of the 3D printed components shown in Figure S2

Part Name	Length/Height	Width	Diameter
Flow Cell Holder	76.21 mm	35.01 mm	-
Camera Tube	86.2 mm	-	30.47 mm
Camera Tube Support	91.44 mm	63.5 mm	-

Table S2 –Results of particle counting using the 3DPIP device at 1 mL/h flow rate.

1 mL/hour	Experiment 1 Count	Experiment 2 Count	Experiment 3 Count	Average Count
3000 particles/mL	4440	4512	4344	4432
1000 particles/mL	1646	1620	1722	1663
333 particles /mL	504	480	492	492
111 particles /mL	108	144	120	124

Table S3 – Results of particle counting using commercial particle counter at 1 mL/h flow rate.

1 mL/hour	Experiment 1 Count	Experiment 2 Count	Experiment 3 Count	Average Count
3000 particles/mL	4146	4718	3834	4233
1000 particles/mL	1716	1952	1548	1739
333 particles/mL	496	466	480	481
111 particles/mL	160	116	104	127

Table S4 – Results of particle counting using the 3DPIP device at different flow rates.

5 mL/hour	Experiment 1 Count	Experiment 2 Count	Experiment 3 Count	Average Count	Expected Count	% Error
3000 particles/mL	23601	23963	23106	23557	22160	+5.93
1000 particles/mL	8835	8722	9245	8934	8315	+7.44
333 particles/mL	2366	2101	2616	2361	2460	-4.02
111 particles/mL	565	573	584	574	620	-7.42

9 mL/hour	Experiment 1 Count	Experiment 2 Count	Experiment 3 Count	Average Count	Expected Count	% Error
3000 particles/mL	45269	45828	44499	45199	39888	+13.31
1000 particles/mL	16949	16773	17648	17123	14967	+14.41
333 particles/mL	3962	3755	3906	3874	4428	-12.50
111 particles/mL	896	957	943	932	1116	-16.49

13 mL/hour	Experiment 1 Count	Experiment 2 Count	Experiment 3 Count	Average Count	Expected Count	% Error
3000 particles/mL	68629	69582	67399	68537	57616	+18.95
1000 particles/mL	25714	25379	26688	25927	21619	+19.93
333 particles/mL	7951	7640	7792	7794	6396	+21.86
111 particles/mL	1152	1321	1209	1227	1612	-23.86

Theory

The equation of motion for a spherical particle entrained in a vertically oriented flow is described by Eq. S1,

$$\frac{du_p}{dt} = -6\pi\mu a\left(u_p - u_f\right) - F_g + F_b \quad \text{(S1)}$$

where, u_p is the particle velocity, t is time, μ is the fluid viscosity, a is particle radius, u_f is the fluid velocity, F_g is the gravitational force acting on the particle and F_b is the particle buoyancy force. At steady state, Eq. S1 is described by Eq. S2,

$$0 = -6\pi\mu a\left(u_p - u_f\right) - F_g + F_b \quad \text{(S2)}$$

which, can be re-arranged to solve for the particle velocity as a function of fluid velocity and body forces. This expression is given by Eq. S3,

$$u_p = u_f + \frac{\left(F_g - F_b\right)}{6\pi\mu a} \quad \text{(S3)}$$

which, can be simplified by recognizing that $u_s = \left(F_b - F_g\right)/6\pi\mu a$ is the Stokes settling velocity.[1]

The fluid velocity may be found by solving the creeping flow momentum (Eq. S4) and continuity (Eq. S5) equations[2],

$$-\nabla P + f_b + \mu\nabla^2 u_f = 0 \quad \text{(S4)}$$

$$\nabla \cdot u_f = 0 \quad \text{(S5)}$$

where, P is the applied pressure and f_b is a body force acting on the fluid per unit volume. The solution to these equations for a square geometry is given by Eq. S6[3],

$$u_f(x,y) = 4\frac{Q}{w^2}\sum_{n=1,3,5,\ldots}^{\infty}\sum_{m=1,3,5,\ldots}^{\infty}\frac{sin(n\pi x/w)sin(m\pi y/w)}{nm(n^2+m^2)} \qquad \text{(S6)}$$

where, Q is the fluid flow rate and w is the inner width of the square capillary used in these experiments. In this model, Q/w^2 represents the average fluid velocity in the capillary, and the maximum fluid velocity of a square geometry is $u_{max} \approx 1.79u_{f,avg}$. Thus, by combining Eqs. S3 and S6, it is possible to see that the particle velocity should depend on position. However, given the nature of this experiment setup, the particle position can only be seen in x and z directions. This limits the ability to determine how far into the sample (y direction) the particle actually is. It is possible to get around this issue by averaging Eq. S3 with position, as described by Eq. S7,

$$u_{p,avg} = u_{f,avg} - u_s \qquad \text{(S7)}$$

The average fluid velocity is proportional to Q/w^2, thus Eq. S7 can be expressed as a function of flow rate, described by Eq. S8,

$$u_{p,avg} = kQ/w^2 - u_s \qquad \text{(S8)}$$

where, k is a constant of proportionality that depends of capillary geometry.

Bacteria Count Calculations

For each concentration, 2 µL of bacteria stock solution was placed in between two 50 mm by 75 mm glass slides. The total number of bacteria cells (TNBC) was calculated as described by Eq. S9,

$$\text{TNBC in 2µL of Sample} = \text{TNBC on Glass Slide} = \left(\frac{\text{Area Occupied by Sample on Glass Slide}}{\text{Area of a Single Sample Image}}\right) \times \text{Average Number of Bacteria Cells in a Single Sample Image} \qquad \text{(S9)}$$

Therefore, for 10^3 cells/mL sample,

$$\text{TNBC in 2µL of Sample} = \left(\frac{440.987\ mm^2}{0.225\ mm^2}\right) \times 10 = 1.9599 \times 10^4$$

$$\text{TNBC in 1 mL of Sample} = 1.9599 \times 10^4 \times 500 = 9.7997 \times 10^6$$

Table S5 - Bacteria cell count per image.

Prepared concentration (CFU/mL)	Count 1 in 2 µL (cells/image)	Count 2 in 2 µL (cells/image)	Count 3 in 2 µL (cells/image)	Average Count (cells/image)
4.8 x10^3	10	11	9	10 ± 1.0
4.8 x 10^4	41	39	40	40 ± 1.0
4.8 x 10^5	146	150	155	150 ± 4.5
4.8 x 10^6	250	241	259	250 ± 9.0

Bacteria Count Validation

For each concentration, 200 µL of 10 µm polystyrene beads sample was dried on a 50 mm by 75 mm glass slide. The total number of particles was calculated as described by Eq. S10,

$$\text{Total Number of Particles in 200 µL of Sample} = \text{Total Number of Particles on Glass Slide}$$
$$= \left(\frac{\text{Area Occupied by Sample on Glass Slide}}{\text{Area of a Single Sample Image}}\right)$$
$$\times \text{Average Number of Particles in a Single Sample Image} \qquad (S10)$$

Therefore, for 111 particles/mL sample, with microscope,

$$\text{Total Number of Particles in 200 µL of Sample} = \left(\frac{588.134\ mm^2}{1.63\ mm^2}\right) \times 0.0667 = 24.0665$$

$$\text{Total Number of Particles in 1 mL of Sample} = 24.0665 \times 5 = 120.2728$$

Therefore, for 111 particles/mL sample, with 3DPIP,

$$\text{Total Number of Particles in 200 µL of Sample} = \left(\frac{588.134\ mm^2}{1.79\ mm^2}\right) \times 0.0667 = 21.9153$$

$$\text{Total Number of Particles in 1 mL of Sample} = 21.9153 \times 5 = 109.5769$$

Table S6 - Particle count per image using optical microscope.

Particles/mL	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	Avg. ct.	Avg. ct./mL
111	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0.0667	120.2728
333	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0.2	360.8184
1000	2	1	1	1	0	2	1	1	0	1	1	0	1	0	1	0.8667	1563.6065
3000	3	3	3	2	2	3	2	2	3	3	2	1	3	2	2	2.4	4329.8208

Table S7 - Particle count per image using the 3DPIP.

Particles/mL	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	Avg. ct.	Avg. ct./mL
111	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0.0667	109.5769
333	0	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0.2667	438.1434
1000	1	2	1	1	0	1	0	1	0	1	2	1	2	0	1	0.9334	1533.4197
3000	3	3	3	3	2	3	3	2	3	2	3	3	2	2	2	2.6	4271.3642

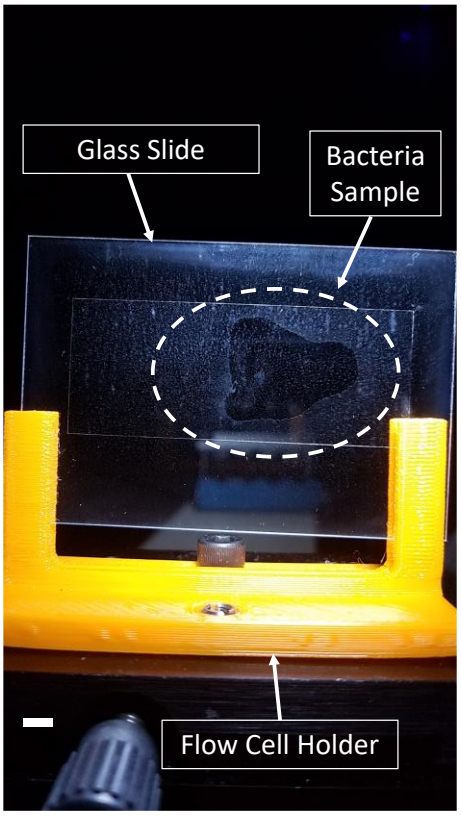

Figure S3 – Image of the bacteria counting experiment mounted to the flow cell holder with 10^2 CFU/mL. Scale bar is 5 mm.

References

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(4) Thielicke, W.; Stamhuis, E. J. PIVlab – Towards User-Friendly, Affordable and Accurate Digital Particle Image Velocimetry in MATLAB,. *Journal of Open Research Software* **2014**, *2* (1), 30.
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