High-throughput flow alignment of barcoded hydrogel microparticles

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Supplemental Information

Materials and Methods

Soft Lithography and Device Fabrication:
Master molds for the devices were created by spin-coating a clean silicon wafer with negative photoresist (SU-8 25, MicroChem). High-resolution photomasks (10,000 dpi, CAD Art Services) were then used to selectively expose the coated wafers to UV light, thus creating the desired patterns. Following treatment with SU-8 developer (MicroChem), the wafers were flood exposed to UV light and baked. A profilometer (Dektak) was used to determine the heights of features located on the left, right, and central portions of the wafer. The wafers were then treated with a fluorosilane ((tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane, United Chemical Technologies, Inc.) under vacuum for 60 min. PDMS pre-polymers (10 parts base, 1 part curing agent) were poured over the molds to a depth of 5 mm and allowed to cure in an oven at 65^{o}C for 12 h. Individual channel designs were cut from the mold with a scalpel. Inlet and outlet holes were punched with blunt 15-gauge luer stub adapters (Clay Adams). The devices were then rinsed with water and ethanol, dried with Ar gas, placed channel-side down on PDMS-coated slides, and baked in an oven at 65^{o}C for 5 h.

Atomic Force Microscopy Measurements:
Atomic force microscopy (AFM, Agilent Technology) was incorporated within an optical microscope (IX 81, Olympus) to enable positioning of AFM cantilevered probes above particle samples. Calibration of AFM cantilevers of nominal spring constant k = 0.01 nN nm^{-1} and probe radius R = 25 nm (Veeco) was conducted. Briefly, inverse optical lever sensitivity [nm V^{-1}] (InvOLS) was measured from deflection-displacement curves recorded on rigid glass substrates. For each measurement of elastic moduli, at least 25 replicate indentations were acquired to maximum depths of 20 nm. Acquired probe deflection-displacement responses were converted to force-depth responses using measured spring constants and InvOLS (Scanning Probe Imaging Processor, Image Metrology). Elastic moduli, E, were calculated by applying a modified Hertzian model of spherical contact to the loading segment of the force-depth response with the scientific computing software Igor Pro (Wavemetrics).

Particle Synthesis:
It should be noted that differences in cross-linking density between the DA20 and DA30 formulations led to a slight size disparity between the particles that were synthesized from the two prepolymer solutions. For a collection of 10 particles of each composition with AR =3.44, DA20 particles were 4% longer. DA30 particles had dimensions that were nearly identical to those expected from the transparency mask dimensions, implying that the DA20 particles were larger due to swelling effects.

Theory and Simulation

Lubrication Approximation:
To better understand the hydrodynamic forces acting on the particles, a lubrication approximation was developed to describe the bypass fluid flow in the side gaps in the narrow detection portions of the channel. Based on the negligible deformation of particles in the optimized design, the particles are hereafter assumed to be rigid bodies. Building off previous observations of deformable red blood cells in rectangular channels, [28, 29] the bypass flowrate in the larger side gaps is assumed to be much greater than the flowrate through the small height gaps, leading to the neglect of any three-dimensional flow effects in the side gaps (*i.e.*, flow only in the *xy*-plane). The side gap is essentially a long, narrow channel, and as a result, the flow within it can be regarded as nearly unidirectional (*x*-direction) and dominated by viscous stresses. For the lubrication approximation to be valid in this situation, both geometric and dynamic requirements must be met.[31] Using the physical setup depicted in the schematic in Fig. 1c and assuming a constant volumetric flowrate in the detection region of the channel, the geometric requirement becomes

$$\frac{|h_0 - h_1|}{L\cos\alpha} = \frac{L\sin\alpha}{L\cos\alpha} = \tan\alpha << 1 \qquad \text{(Eqn. S1)}$$

and the dynamic requirement becomes

$$\left(\frac{u(x)h(x)}{\nu}\right)\left(\frac{|h_0-h_1|}{L\cos\alpha}\right)=\frac{q}{\nu}\tan\alpha<<1 \quad \text{(Eqn. S2)}$$

where $u(x)$ is the mean velocity at a given x position in the channel, υ is the kinematic viscosity of the fluid, and q is equivalent to $u(x)h(x)$. Upon entrance into the detection region of the optimized channel, the observed angle of deflection, α, for a typical particle is between 0^o and 5^o, thereby giving a **maximum** value of 0.087 for tan α and satisfying the geometric requirement. Meanwhile, based on a typical volumetric flowrate per width, q, of 2 x 10^{-5} m^2 s^{-1} and a kinematic viscosity, υ, of 1 x 10^{-5} m^2 s^{-1} for the PTET carrier fluid, the q/υ prefactor in Eqn. S2 has a value of 2 and thus the **maximum** value of the central expression in Eqn. S2 is 0.174. These values suggest that the use of the lubrication approximation is indeed valid within this side gap region.

A reference frame moving with a particle with a center of mass located at the center of the channel was adopted to simplify calculations (Fig. 1c). Considering only the lower half of the channel and assuming the particle has a constant velocity, U_w, in the +x-direction, the no-slip boundary conditions are:

$$v_x(x,0)=-U_w \quad \text{(Eqn. S3)}$$

$$v_x(x,h(x))=0 \quad \text{(Eqn. S4)}$$

where

$$h(x)=h_0+\left(\frac{h_1-h_0}{L\cos\alpha}\right)x. \quad \text{(Eqn. S5)}$$

The lubrication approximation reduces the Navier-Stokes equation to

$$\frac{\partial^2 v_x}{\partial y^2}=\frac{1}{\mu}\frac{dP}{dx} \quad \text{(Eqn. S6)}$$

where P is solely a function of x. As discussed elsewhere, [31] the distinction between dynamic pressure, P, and the regular pressure, p, is quantitatively insignificant within thin layers of fluid that have large pressure variations. Integrating twice with the boundary conditions gives

$$v_x(x,y)=\frac{y}{2\mu}\frac{dP}{dx}(y-h(x))+U_w\left(\frac{y}{h(x)}-1\right). \quad \text{(Eqn. S7)}$$

When this expression is used to evaluate the mean velocity in the channel, it is found that

$$u(x)=-\frac{dP}{dx}\frac{h(x)^2}{12\mu}-\frac{U_w}{2}. \quad \text{(Eqn. S8)}$$

Upon rearrangement, this provides:

$$\frac{dP}{dx}=-\frac{12\mu q}{h(x)^3}-\frac{6\mu U_w}{h(x)^2}. \quad \text{(Eqn. S9)}$$

When this expression for dP/dx is used in the expression for the velocity in the x-direction, the following result is obtained:

$$v_x(x,y)=-\frac{y}{h(x)^2}\left(\frac{6q}{h(x)}+3U_w\right)(y-h(x))+U_w\left(\frac{y}{h(x)}-1\right). \quad \text{(Eqn. S10)}$$

In order to determine the force (per unit width) which the fluid exerts on the surface of the particle, it is necessary to calculate the stress vector, **s**:

$$\mathbf{s}=\mathbf{n}\cdot\boldsymbol{\sigma} \quad \text{(Eqn. S11)}$$

$$\mathbf{n}(x)=\frac{1}{g}\left(\frac{dh}{dx}\mathbf{e}_x-\mathbf{e}_y\right) \quad \text{(Eqn. S12)}$$

$$g(x)=\left[\left(\frac{dh}{dx}\right)^2+1\right]^{1/2} \quad \text{(Eqn. S13)}$$

where **n** is a vector normal to the particle surface, **σ** is the stress tensor, $g(x)$ is a normalization factor, and $\mathbf{e}_x$ and $\mathbf{e}_y$ are the unit normal vectors in the *x*- and *y*-directions, respectively. The nonzero components of the stress vector are then given by

$$s_x=\frac{1}{g}\left(\frac{dh}{dx}\sigma_{xx}-\sigma_{yx}\right)=\frac{1}{g}\left[\frac{dh}{dx}(-P+\tau_{xx})-\tau_{yx}\right] \quad \text{(Eqn. S14)}$$

$$s_y=\frac{1}{g}\left(\frac{dh}{dx}\sigma_{xy}-\sigma_{yy}\right)=\frac{1}{g}\left[\frac{dh}{dx}\tau_{xy}-(-P+\tau_{yy})\right] \quad \text{(Eqn. S15)}$$

where **τ** is the viscous stress tensor. Because the particle is assumed to be a rigid body, both of the normal viscous stresses are zero. Moreover, in the nearly unidirectional flow in the gap, the normalization factor, g, can be approximated as unity, and the $\partial v_x/\partial y$ term will be much larger than the $\partial v_y/\partial x$ term in the calculation of the remaining viscous stress component ($\tau_{xy}=\tau_{yx}$). Thus it is found that

$$s_x=-\frac{dh}{dx}P-\mu\frac{\partial v_x}{\partial y} \quad \text{(Eqn. S16)}$$

$$s_y=\mu\frac{dh}{dx}\frac{\partial v_x}{\partial y}+P. \quad \text{(Eqn. S17)}$$

In calculating the *y* component of the stress vector, it is permissible to neglect the shear stress term. The ratio of the pressure to the shear stress is ~ $L\cos\alpha/|h_1-h_0| = \cot\alpha$, and since α is not usually more than 5° in the detection region, it is estimated that the pressure contribution will always be at least ten times greater than the shear stress contribution. The resulting expression is simply

$$s_y=P. \quad \text{(Eqn. S18)}$$

For calculation of the drag and lift forces (per unit width) involved in orientation and alignment, these stresses can then be integrated along the surface of the particle using the following equations:

$$F_x=-\int_{x1}^{x2}\left(\frac{dh}{dx}P+\mu\frac{\partial v_x}{\partial y}\Big|_{y=h(x)}\right)dx \quad \text{(Eqn. S19)}$$

$$F_y=\int_{x1}^{x2}P\,dx. \quad \text{(Eqn. S20)}$$

For the curves plotted in Fig. 6a, the stress expressions derived above were used to calculate the torque that the gap flow exerted on particles in the detection region. The pressure field was calculated by integrating Eqn. S9 and using boundary values that matched the bulk flow ahead of and behind the particle.[31] Torque (per unit width), **G**, was calculated about the central point on the trailing edge of the particle, $\mathbf{r_0}$ (Fig. 6b), using numerical integration in MATLAB and the following equation:

$$\mathbf{G}=\int_{x1}^{x2}(\mathbf{r}-\mathbf{r_0})\times\mathbf{s}(\mathbf{n})\,dx. \quad \text{(Eqn. S21)}$$

The torque was calculated about this point because high-speed videos of poorly aligned particles in the upstream portion of the detection region were observed to most often rotate about this point in their movement into a properly-aligned orientation. For all calculations with this lubrication approximation, the effect of the upper gap was modeled in an analogous fashion to that just described for the lower gap and the contributions summed for the determination of the total force and torque. The primary difference between the two situations is that the lower gap involved a contraction while the upper gap involved a symmetric expansion.

Details of COMSOL Simulations:

COMSOL Multiphysics' Incompressible Navier Stokes module was used to model the 2-D fluid flow in the microfluidic devices for particle focusing. For all simulations, a stationary nonlinear solver was used with the Direct (UMFPACK) linear system solver. Relative tolerance for the solver was at least 1.0 x 10^{-4} for all converged flow profiles, and high mesh densities were used to increase resolution in areas of particular interest. Based on a "full-device" simulation without particles present, it was determined that devices with w_d = 125 µm and N = 4 had a mean fluid velocity of 6.79 m s^{-1} at the point of particle measurement in the detection region. This full-device simulation specified no-slip boundary conditions at all walls of the channel except the inlet port (normal pressure condition set at 9 psi) and the outlet port (neutral condition set). With a channel Reynolds number defined as $Re_c = UD_h/\upsilon$ (U is mean flow velocity, D_h is hydraulic diameter of channel, υ is kinematic viscosity of fluid), these simulations demonstrate that $Re_c \approx 40$ for typical detection-zone geometries.

The same module was also utilized for the study of hydrodynamic forces on particles in the detection region of the channel (Fig. 6). Using pressure estimates from the full-device simulation, flow profiles were solved for a 1.5 psi drop across a 700-µm long detection zone with w_d = 125µm that contained a single particle with various values of θ. No-slip boundary conditions were set at the two side walls, a normal pressure condition was set at the inlet, and a neutral condition was set at the outlet. In addition, a normal flow velocity condition was set at the boundary of the particle to match the typical velocity of the particles in the detection region (50 cm s^{-1}). To determine the forces acting on the particle surface, the post-processing feature of COMSOL was used to export data on drag and lift forces at each point of the line segments used to construct the particle. This data was then combined with Eqn. S21 to numerically calculate the torque with a MATLAB script.

Results and Discussion

Gradually Tapering Channel Designs:

Additional flow trials were conducted in two channel designs that did **not** use sheath streams (N = 0). These designs featured only a central channel (of the same length as the central channel in A, B, and C) that ***gradually*** tapered to a final width of either 100 µm (D) or 150 µm (E). The final width persisted for 2.6 mm in D and 3.4 mm in E. Bifunctional particles with 7-µm column spacing were used for these trials. At throughputs of only ~20 particles s^{-1}, success rates were lower (83% for D, 97% for E) than those in channels with side streams and abrupt contractions. A throughput of ~40 particles s^{-1} in E led to a 92% rate of success. Analysis of the upstream behavior in these simple taper channels revealed a disordered flow of tumbling particles, as well as particles that slowly traveled along the walls of the channel (a behavior that was seen earlier in single-focus devices). While many of these particles were able to eventually adopt a proper orientation further downstream, the chaotic upstream tendencies led to a flow pattern and velocity distribution in the detection zone that exhibited more variability than those of designs with sheath flow. Indeed, for comparable throughputs, the standard deviation of particle velocity in D was 40% greater than in A and the deviation in E was 47% greater than in C. In many instances, consecutive particles in the detection zone in D and E were touching one another, with some even wedging a small portion of their probe region under the code region traveling ahead of it. These observations indicate poor conditioning of the particles in the upstream region and underscore the importance of side streams for the reliable establishment of well-ordered, single-file particle flows.

Video Captions

Video S1: This video shows three bifunctional particles (235 × 65 × 35 µm) passing through a detection zone with w_d = 125 µm at velocities between 46 and 50 cm s^{-1}. The particles flow through probe-first with the proper orientation required for a one-dimensional line scan analysis. The dark streams along the walls correspond to the dyed sheath flow that is used to focus the particle-bearing stream. This video was captured with a 10x objective at a frame rate of 10,000 frames s^{-1}. The video has been slowed by a factor of 333 for playback. Particle loading density for the trial was 17.5 particles µl^{-1}. Size: 1.24 MB

Video S2: This video shows three bifunctional particles (235 × 65 × 35 µm) at the final focus junction of a device with w_d = 125 µm and N = 4. The dyed sheath stream can be seen on the edges of the channels. Although the first two particles approach the entrance to the detection region with improper alignment and poor positioning, they are both guided away from a collision with the walls by the sheath flow. The more flexible probe region of the particles can be seen temporarily deforming at the juncture, as the velocity in the channel abruptly increases and the particles move into a more proper alignment. This video was captured with a 10x objective at a frame rate of 8,888 frames s^{-1}. The video has been slowed by a factor of 300 for playback. Particle loading density for the trial was 15 particles µl^{-1}. Size: 4.49 MB

Video S3: This video shows eight bifunctional particles (235 × 65 × 35 µm) passing through the second (left), third (center), and fourth (right) focus junctions of a device with w_d = 125 µm and N = 4. Improvements in position/alignment and increases in particle velocity can be observed as the particles move further down the channel. The behavior of the sixth particle illustrates the tendency of code-first particles to resist adopting a lengthwise orientation prior to the detection region. It is the only particle that does not settle into a well-behaved trajectory along the centerline by the third focus junction. The dark sheath stream can be seen emerging from the side channels and traveling in a thin film along the walls of the main channel. The dark, horizontal band visible on the left side of the video is an artifact of the high-speed camera and is not related to the sheath flow. This video was captured with a 4x objective at a frame rate of 5625 frames s^{-1}. The video has been slowed by a factor of 200 for playback. Particle loading density for the trial was 17.5 particles μl^{-1}. Size: 10.10 MB