Supplementary Online Materials for:

Crossing Microfluidic Streamlines to Lyse, Label and Wash Cells

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1. Supplementary Online Figures

Figure S1: Cross-section Scanning Electron Micrograph (SEM) of typical post array directly after silicon deep reactive ion etching (DRIE) and before remaining photoresist was removed from the top of each post. The DRIE etching was optimized to achieve vertical sidewalls and to minimize scalloping from the cyclic etch process.

Figure S2: False color imaging details for Figure 2b(main text). A) Red channel: Average of 20 video frames from Video M3, showing CD41-PE jet with buffer below and washing buffer above. B) Green Channel: Sum of 4 frames of platelet movement after subtracting the averaged dye flow image given in (A) from each individual frame. (C) Merge: False color composite image to highlight the movement of freshly labeled supercritical platelets relative to the the labeling stream and into the wash buffer stream.

2. Supplementary Online Videos

Video M1: Motion of 3.0 micron fluorescent beads (red) tracking across hydrodynamic jet of 0.5 micron fluorescent beads (green).

Video M2: Motion of 3.0 micron fluorescent beads (red) escaping a stream of Rhodamine 6G dye (green). The beads are "washed" as they travels from the region the region of high dye concentration in the lower half of the array into the top half containing no dye.

Video M3: Stream of freshly labeled platelets exiting a jet of PE-CD41 surface labeling dye into a stream of washing buffer.

Video M4: Video of spheroplasted, GFP expressing *E. coli* crossing into an adjacent, co-flowing lysis solution (the two streams are delineated by a tracer jet of GFP) and lysing. After lysis, the GFP in the cell follows the flow through the array.

Video M5: Video of spheroplasted, GFP expressing *E. coli* with additional chromosome labeling lysing after passing into a stream of lysis solution. Following lysis the expressed GFP follows the flow while the larger chromosome continues along the bumping trajectory.

3. Supplementary Online Text

Part 1: Device Fabrication (Extended)

Post arrays, inlet/outlet microchannels and reservoirs were fabricated in silicon wafers using single-layer photolithography. (PDMS replica molding of silicon master can also be used.) Photomasks (Photosciences, Torrence, CA, USA) were designed using L-Edit (Tanner Research, Monrovia, CA, USA). Etch masks were formed on silicon wafers using standard photolithography (Karl Suss, MA6) with AZ 5214 photoresist (AZ Electronic Materials, Branchburg, NJ, USA) and DI:MIF 312 (1:1) developer. Samples were deep etched using an STS ASE Multiplex tool (Newport, UK) for Deep Reactive Ion Etching (DRIE) with an optimized recipe that ensured vertical sidewalls and low sidewall scalloping (~100nm peak to peak). Scanning Electron Microscopy (SEM) (LEO 1550) was used to characterize the etching recipes and measure array parameters such as post size, spacing and gap. The array parameters of the devices presented here are as follows:

Devices showing Bead Manipulation: (Figures 1A and B)

Array Angle: 11.3°
Post Pitch: 11 um
Gap between Posts: 4 um
Etch Depth: 18 um

Device for Platelet Label and Wash (Figure 2)

Array Angle: 5.7°
Post Pitch: 20 um
Gap: 5 um
Etch Depth: 12 um

Device for *E. Coli* lysing and chromosome separation (Figure 3 and 4)

Array Angle: 5.7°
Post Pitch: 8 um
Gap: 1.4 um
Etch Depth: 9.8 um

Individual devices were cleaved from the main wafer and prepared for through-wafer access hole drilling (PrepStart, Danville Engineering, San Ramon CA) with protective layers of photoresist and tape. Devices were then thoroughly cleaned with acetone, methanol and isopropanol and dried with with a N_2 gun. Devices were sealed with thin layer of non-plasma treated PDMS on a glass coverslip backplane, allowing for repeated use of individual devices.

Finished devices were mounted in a reusable acrylic jig with inlet holes aligned to o-rings and macro-reservoirs (~ 50uL capacity) in the jig. A thin steel manifold with PDMS spacers was use to clamp the chip in place and complete the o-ring seal to the fluid reservoirs. Devices were wet after mounting in the jig by loading the inlet wells with filtered and degassed buffer solutions. Inlet reservoirs were left open to atmosphere to facilitate cell and bead loading. Outlet reservoirs were connected to an external vacuum pump; fluid flow speed was controlled by this differential air pressure. Pressure was varied in the range of 1 psi - 12 psi using a bleed valve to achieve a range of possible particle speeds in each of the various devices from 50-500 um/s. Running buffer for fluorescent microspheres (Duke Scientific, Fremont, CA, USA) was Ultrapure DI water containing 2 g/l pluronic F-108 (BASF, Florham Park, New Jersey, USA).

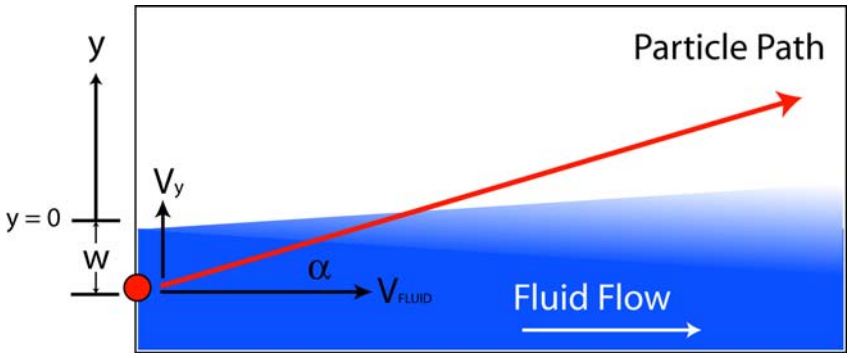
Part 2: Concentration Reductions with Washing

Washing is something of a nebulous term. It implies a reduction in concentration, but how much can vary from process to process. Therefore, we would like to provide a guideline for how far a particle should be removed from a reagent stream to achieve a desired level of washing. This will assist in designing devices that have many treatment streams by giving a quick estimate for how far apart they should be placed to limit cross contamination. It will also help if the particle needs to be collected or moved to another section of the chip free of chemicals from previous treatments.

Assume initially a semi-infinite concentration profile, with concentration C_o for $y < 0$ and 0 for $y > 0$ and that the lateral diffusion of the reagent (in the y direction) is governed by the diffusion equation.

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial y^2}$$

$$C(y, t=0) = \begin{Bmatrix} C_o, & y < 0 \\ 0, & y > 0 \end{Bmatrix}$$



This model ignores the broadening of the lower edge of the reagent stream to simplify the derivation. This will produce a conservative estimate for the spacing because it effectively extends the lower edge of the stream to infinity and gives an infinite supply of reagent particles. This results in the concentration at $y = 0$ being pinned at $C_o/2$ instead of slowly decreasing in the case where the broadening of both edges is considered and there are a finite number of reagent particles. This assumption also changes the meaning of the stream width w to a parameter that takes into account how long it takes the target particle to get to $y = 0$. If the particle initially starts at the bottom edge of the stream, then w is just the stream width. However, w can also take into account if the particle has some additional displacement from the bottom edge of the reagent stream, or, if the array parameters are changed so the particle follows the streamline mode to increase the residence time, the additional time it takes the particle to get to the edge of the

stream. This extra distance/time will result in greater stream broadening and require the particle to be displaced farther to achieve the same degree of washing.

Particles in the bumping mode of the array are laterally displaced at a constant velocity equal to the overall fluid velocity multiplied by tangent of the array angle α.

$$v_y = tan(\alpha)\ v_{fluid}$$

Assuming the particle is initially a distance *w* from the edge of the stream, its position with respect to time is then

$$y = v_y t - w$$

The diffusion equation with the given initial conditions has a solution (normalized to C_o)

$$\frac{C(y,t)}{C_o} = \frac{1}{2}(1 - \text{erf}\,(\frac{y}{\sqrt{4Dt}}))$$

For variables y and t, there exist curves of constant concentration such that the argument of the error function equals a constant, referred to as φ. Some φ for various concentration reductions are presented in the table below.

$\frac{C(y,t)}{C_o}$	$\varphi = \frac{y}{\sqrt{4Dt}}$
10^{-1}	0.906
10^{-2}	1.645
10^{-3}	2.185
10^{-4}	2.630
10^{-5}	3.016
10^{-6}	3.361
10^{-7}	3.676
10^{-8}	3.968
10^{-9}	4.241

So, for a given concentration reduction,

$$\varphi = \frac{y}{\sqrt{4Dt}}$$

Substituting in for the time from the above equation ($t = \frac{y + w}{v_y}$) gives

$$y^2 = 4\varphi^2 Dt = 4\varphi^2 \frac{D}{v_y}(y+w) \rightarrow y^2 - 4\varphi^2 \frac{D}{v_y} y - 4\varphi^2 \frac{D}{v_y} w = 0$$

Dividing by w^2 gives the equation in dimensionless form

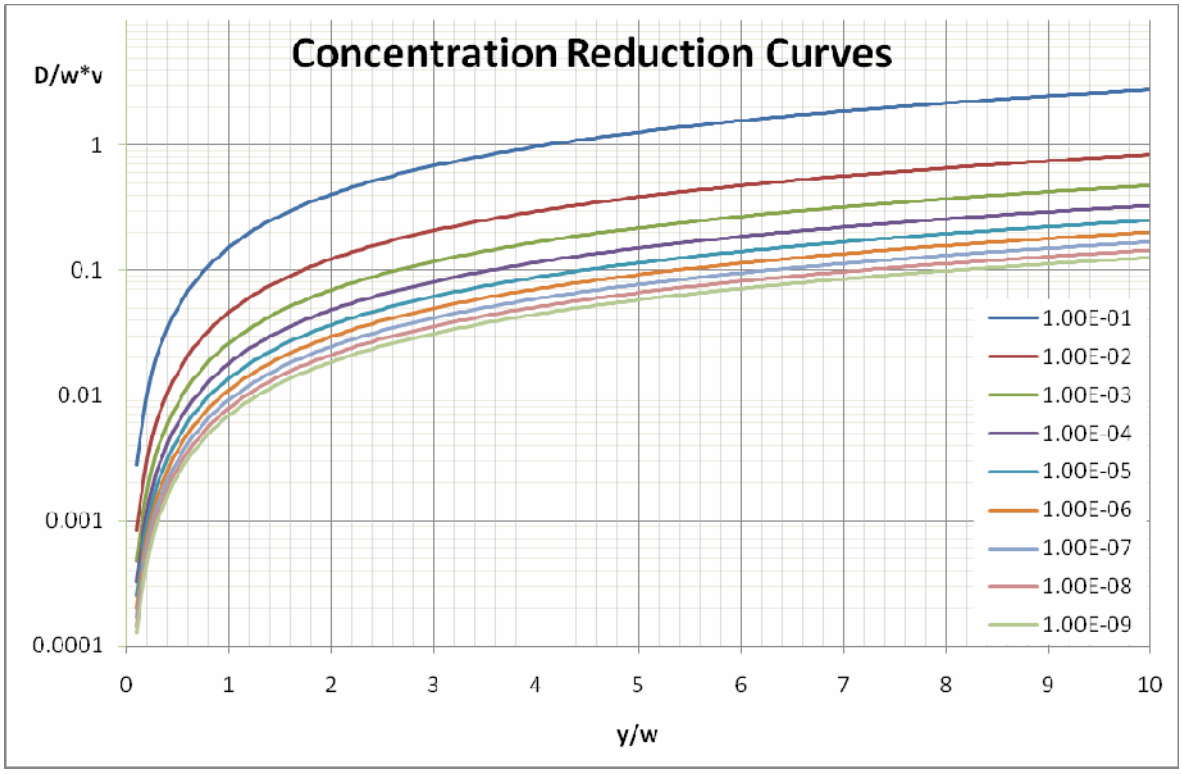
$$\frac{y^2}{w^2} - 4\varphi^2 \frac{D}{wv_y}\frac{y}{w} - 4\varphi^2 \frac{D}{wv_y} = 0$$

Let $m = \frac{y}{w}$ and n $= \frac{D}{wv_y}$ and solve for n

$$m^2 - 4\varphi^2 mn - 4\varphi^2 m = 0$$

$$n = \frac{1}{4\varphi^2}\frac{m^2}{1+m}$$

From this relationship, we can plot curves for each φ listed above. For given parameters D, w, and v, the spacing y, normalized to w, can be found for any of the concentration reductions just by looking at the graph.



As an example, consider the bead washing demonstration given in Fig 1C of the main text and assume a width of 50 um, diffusion constant 300 um^2/s (actual 280 um^2/s), and overall fluid flow

about 300 um/s (actual 312 um/s). For an array angle of 12°, this gives a v_y of 60 um/s. This gives a ratio of D/wv_y of 1/10. Looking at the graph, we can see that if we were to displace a particle 100 microns away from the edge of the stream, the concentration of dye would decrease by more than 100 times. Displacing it 200 microns away would decrease the dye concentration by more than 10000 times.

Alternatively, we could solve for *m* in the equations above and (discarding the negative solution) substitute in the appropriate parameters.

$$m = 2\left(n\varphi^2 + \sqrt{n\varphi^2(1+n\varphi^2)}\right) \quad \rightarrow \quad \frac{y}{w} = 2\left(\frac{D\varphi^2}{wv_y} + \sqrt{\frac{D\varphi^2}{wv_y}\left(1+\frac{D\varphi^2}{wv_y}\right)}\right)$$

Using this method, we get separations of 85.5 um and 177.5 um for 100 and 10000 times concentration reduction, respectively.