Supporting Information for:

Continuous-flow cytomorphological staining and analysis

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Electronic Supplementary Material (ESI) for Lab on a Chip

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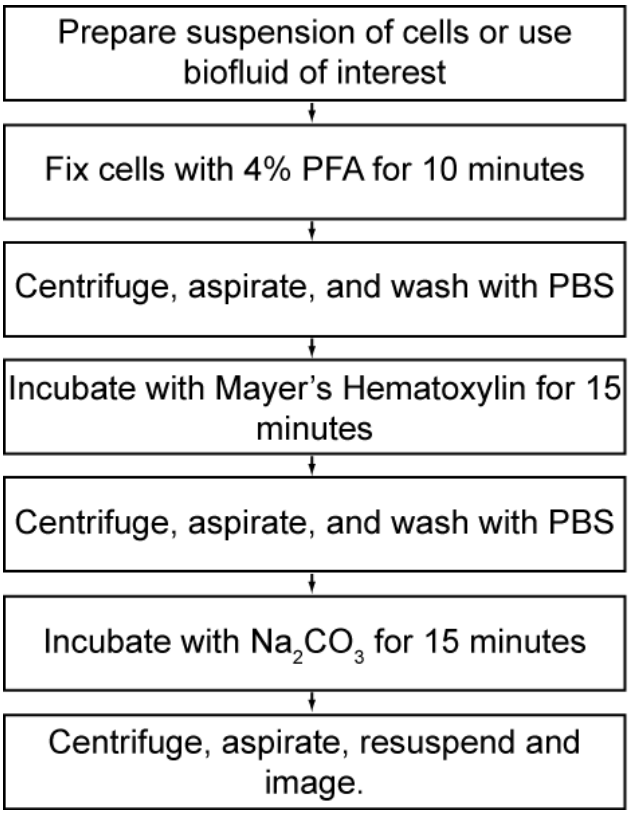
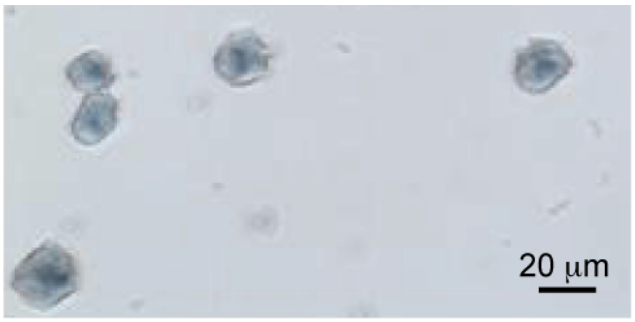
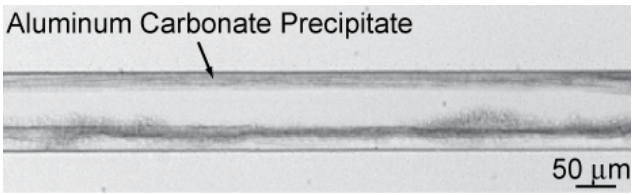
Supporting Figures:



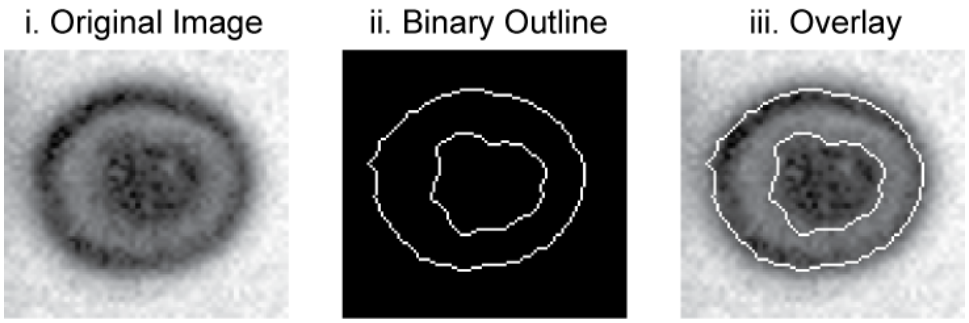
SI Figure 1. Manual colorimetric staining in suspension. Colorimetic staining in suspension may be conducted off-chip by incubating cells in staining solution in a microcentrifuge tube, with aspiration and manual fluid exchange steps being conducted with the aid of a benchtop centrifuge. Modifications from traditional Hematoxylin and Eosin (H&E) staining include longer incubation periods, substitution of Eosin counterstain with sodium carbonate bluing solution, and removal of ethanol dehydration steps. However, the need for interspersed wash steps to facilitate fluid exchange (hematoxylin precipitates out of solution at high pH) can prove problematic when working with samples with low cellularity due to increased risk of cell loss.



SI Figure 2. Color images of suspension stained MCF7 cells. Images of stained cells taken under 40x magnification using a 3-Mpx QImaging Go-3 color CMOS camera at an exposure time of 1.63 ms. Images were captured with cells pipetted on a slide after colorimetric staining, as the QImaging Go-3 camera cannot image rapidly enough for high-throughput in-flow image capture.



SI Figure 3. Microchannel fouling upon interaction of hematoxylin and sodium carbonate. The acetic acid (used to segregate hematoxylin and sodium carbonate solutions in flow) flow was stopped during operation of the device. This resulted in immediate precipitation within the device as the hematoxylin solution could now interact with the sodium carbonate. Fouling occurs at the interface of the two solutions, preventing inertial transfer of cells. Mayer's Hematoxylin is a low pH solution where hematein ions form a chelate with an Al^{3+} cation.[1] However, when combined with high pH solutions, such as a bluing agent in staining (e.g., sodium carbonate) the aluminum cations participate in a side reaction with the anion in the basic solution, here carbonate. This forms an aluminum carbonate precipitate.



SI Figure 4. Image analysis and review. The Q-Path program is applied to the captured images of cells in flow. The original image (i), the binary outline (ii), and the overlay (iii) are presented for review by the program. This could prove valuable to a cytopathologist who wishes to analyze images directly.

Supporting Video Captions:

SI Video 1. Cell transfer across streams. Cells enter the main channel at either the top or bottom. As cells migrate downstream, lift forces cause them to migrate towards the center of the channel, into the central sodium carbonate solution stream.

SI Video 2. Rotation of cells in the microfluidic channel provides additional perspectives for imaging. As cells travel through the channel, they are observed to rotate in the direction of flow. Capturing images at a rate of 20,000 fps provides unique angular views. In this video, we demonstrate this feature ensures a perspective of the cell revealing the process of mitosis is provided.

Supporting References:

1. S. N. Meloan and H. Puchtler, *Journal of Histotechnology*, 1987, **4**, 257–261.