Inertial microfluidics combined with selective cell lysis for high throughput Separation of nucleated cells from whole blood

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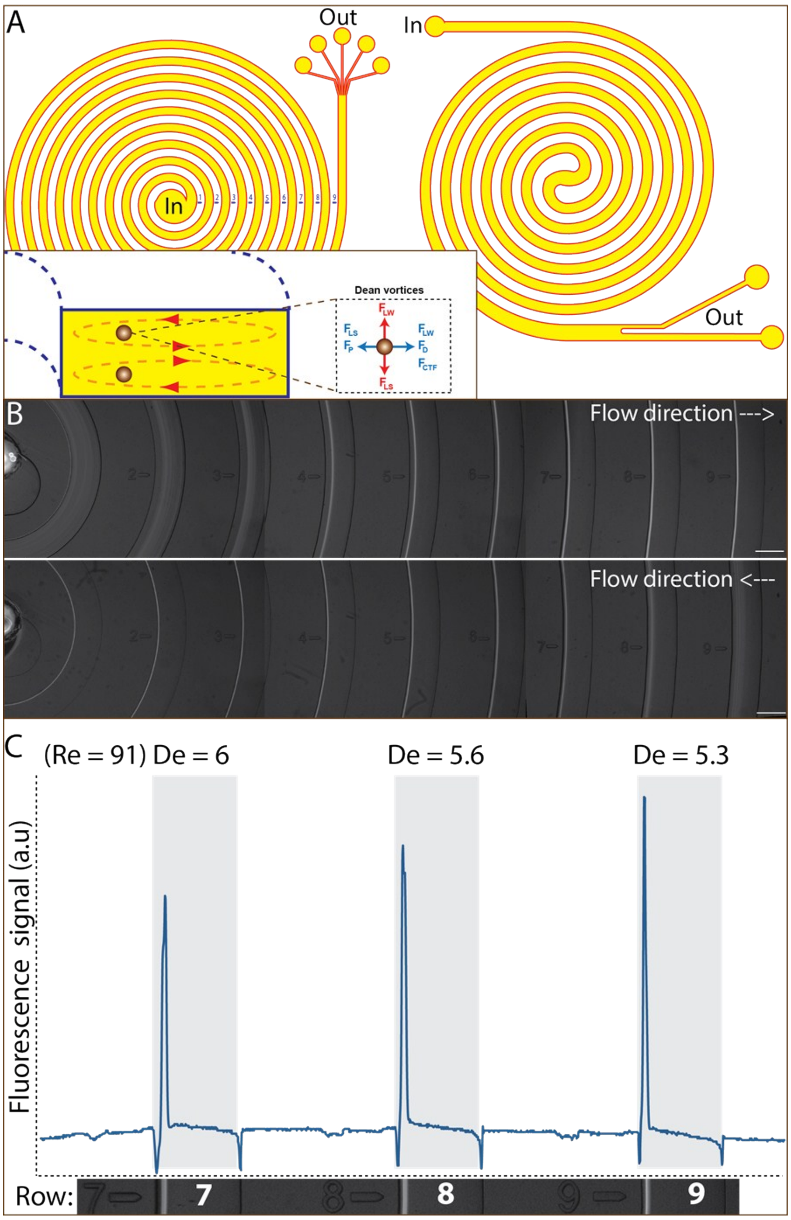
Supplementary data

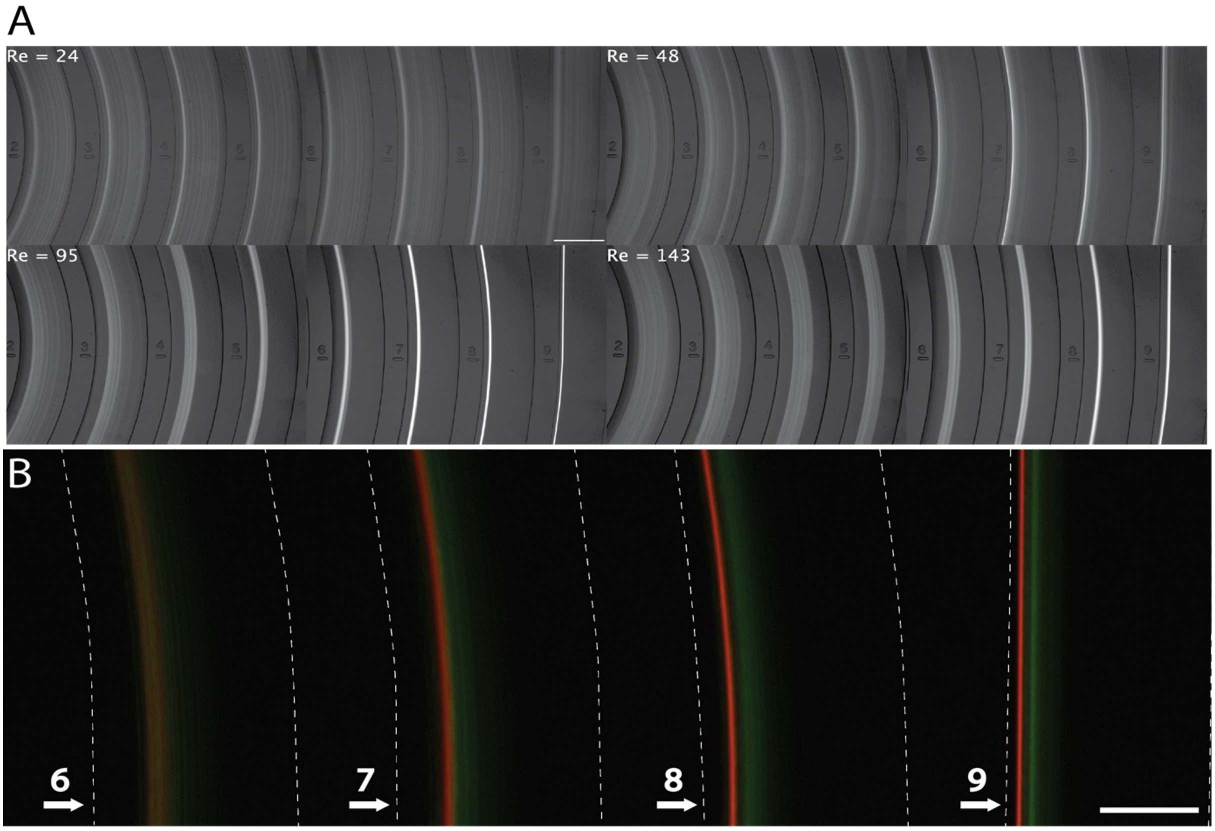
Inertial microfluidics (S1-S2)

Inertial focusing of micro-particles in spiral microdevices: Particles and cells flowing through curved spiral microchannels experience two major forces; namely, lift forces (FL) due to inertial flow, and Dean forces (FD) resulting from Dean flow due to curvature. Depending on the relative magnitude of FL and FD acting on a particle, focusing close to the inner wall (dominant lift) or mixing (dominant Dean force) can occur[64]. Experimentally, we designed different spiral devices (Supplementary Fig. S1A) to study the particle focusing in flow through curved channels. As can be seen in Supplementary Fig. S1B, particles can be focused independent of the direction of flow. The particles are initially unfocused due to insufficient FL and FD and start to get focused with increased channel length. For the inward flow direction (Fig.1B lower panel), the particles start to focus already after the first couple of rows (row 8 and 9), indicative of sufficient lift forces developed due to the length the particles have travelled. It is noticeable that once the particles reach the equilibrium position close to the inner wall, they remain focused throughout the remaining channel length independent of the flow direction. Supplementary Fig.S1C shows details of the fluorescence intensity profile for the last three rows (7-9). The particles are increasingly focused with increased channel length of the spiral, indicating that focusing is primary dictated by FL, while FD primarily acts to speed up the focusing by acting on particles to quickly find their lateral equilibrium position.

We have previously shown that a single turn is enough to focus particles in flows through low aspect ratio (up to 1:10) channels, provided enough lift is developed prior to the curved region[65]. When the aspect ratio was decreased further, it was not possible to obtain particle focusing using the single curved channel65. However, as can be seen in Supplementary Fig. 2, spiral geometry (a height of 50 µm and width of 1 mm) is able to focus particles despite an extremely low aspect ratio (1:20). As can be seen in Supplementary Fig. S2A, three distinct flow regimes are obtained in a flow through spiral channels: at a low flow rate, there will not be enough FL and FD developed to focus particles. When the flow rate is increased, FL and FD start to interact with the particles, which start to focus at equilibrium positions. Finally, if the flow rate is further increased, the particles start to be pushed outward and defocus. This is because FD increases faster than FL with increased flow rate leading to a shift in dominance (FL< FD) resulting in defocusing behaviour and the particles start to mix. To evaluate the capability of the spiral to focus different particle sizes to different streams, 10 µm and 7 µm particles were mixed and flowed through the spiral (Supplementary Fig. S2B). At row 6 of the spiral, both particle sizes are unfocused and displaced away from the inner wall. As the spiral length increases (row 7), the larger particles start to focus and migrate closer to the inner wall while the smaller particles lag behind and remain largely unfocused. Finally, both particles are differentially focused at distinct lateral positions (rows 8 and 9) where the smaller 7µm particles are further away from the inner wall compared to the 10µm particles. Our observation is well in agreement with the predicted increased contribution from lift forces to the larger particles. Hence, by harnessing the interplay between the two dominant forces, FL and FD, it is possible not only to focus particles above a certain size cut-off, but also to differentially focus and separate particles of different sizes.

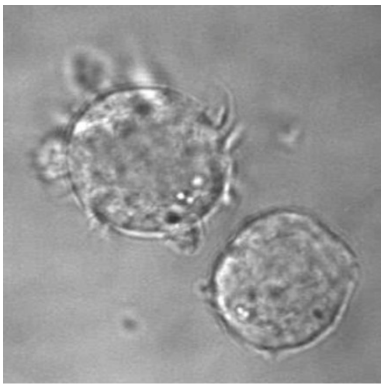


Supplementary Figure S1: Inertial focusing in flow through spiral microchannels. (A) Spiral CAD designs used in this study. Inset: Cross-section of the channel showing the presence of Dean vortices and how the dominant forces acting on a particle forcing particles to focus close to the inner wall of the curved channel. Among the lift forces, the vertical lift forces dominate and will tend to focus particles vertically on top and bottom while the Dean forces then move participles towards the lateral focusing position. (B) 10 μ m particles flowing through a spiral channel from the centre outwards (upper panel) and inwards towards the centre (lower panel) are focused close to the inner wall. The particles initially unfocused are successively being focused after some rows and remain focused throughout the remaining of the channel. The channel dimensions are as follows: width and height 500mm x 50μm; Flow conditions: Re = 91 (corresponding volumetric flow rate: 1 mL/min). Scale bar: 500 μm. (C) Intensity profile of the three last spiral channels (row 7-9) for Re=91.

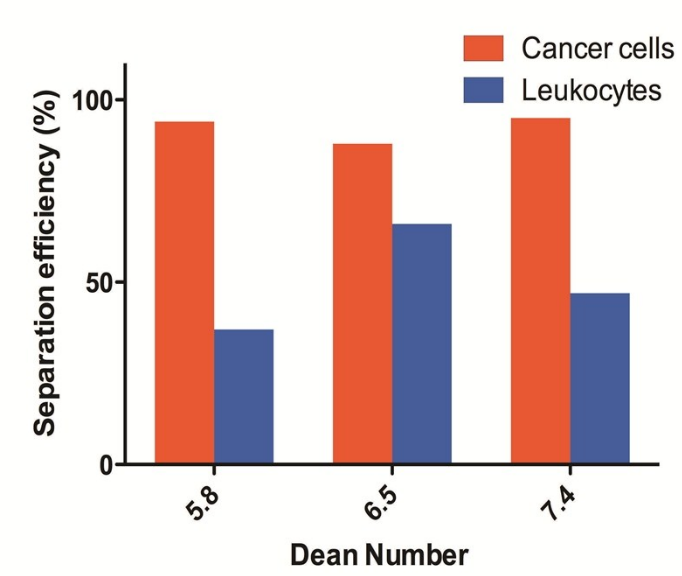


Supplementary Figure S2. Differential inertial focusing. (A) Particle behavior as a function of Re in flow through curved 9 channels. The 10 μm particles are initially unfocused at low flow rate (Re=24), and start to focus with increased flow rate (Re=48 and 95). When the flow is increased even more (Re=143), particles start to defocus due to increased Dean forces. (B) Differential inertial focusing of 10 (red) and 7 (green) μ m particles flowing through a 1000 μ m channel width geometry at Re = 119 (flow rate of 2.5 mL/min). The particles, initially unfocussed in row 6 are gradually focused and gradually to focus with increased length to finally differentially focus at different lateral positions in row 9. Scale bar: 1000 μm.

Supplementary video S-1



Supplementary video S-1: Cancer cell lines swelling upon exposure to hypotonic solution.



Supplementary Fig.S3: Separation of spiked cancer cells from whole blood diluted to 2.5% on chip at different flow rates. A yield of 95% was achieved for the cancer cells while 63% of the leukocytes could be depleted to outlet 2 at De= 5.8.