

MORPHOLOGY-BASED SORTING -BLOOD CELLS AND PARASITES-

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ABSTRACT

Morphology represents a hitherto unexploited source of specificity in microfluidic particle separation and may serve as the basis for label-free particle fractionation. There is a wealth of morphological changes in blood cells due to a wide range of clinical conditions, diseases, medication and other factors. Also, blood-borne parasites differ in morphology from blood cells.

We present the use of Deterministic Lateral Displacement to create a chip-based, label-free diagnostic tool, capable of harvesting some of the wealth of information locked away in red blood cell morphology. We also use the device to separate the parasites that cause sleeping sickness from blood.

KEYWORDS: Deterministic lateral displacement, morphology, trypanosomiasis, erythrocytes, separation.

INTRODUCTION & THEORY

Deterministic lateral displacement (DLD) is a powerful separation technique with high-resolution [1] and a simple principle of operation by which particles such as RBCs (red blood cells), white blood cells and platelets are sorted by size [2]. The technique is based on the flow of particles through an ordered array of micrometer-sized obstacles. The particles follow trajectories through the array so that particles with diameters less than the critical diameter, D_c , move with the flow and larger particles move along the direction defined by the array.

We have previously introduced the concept of morphological sorting by controlling the orientation of normal disc shaped RBCs in DLD devices to choose which dimension they are sorted by [3]. Our current work takes this concept further and shows how DLD devices can be optimized by tuning the depth to sort a range of RBC morphologies and also how foreign bodies, such as parasites, can be separated from blood.

All DLD devices reported so far in the literature have been fabricated to depths that are greater than the diameters of the largest particles being separated. In such deep devices non-spherical particles tend to rotate such that they present the smallest possible dimension to the array. When the depth of a DLD device approaches the largest dimension of a non-spherical particle this rotation is impeded and the effective size of the particle increases. Some particle systems are difficult to separate in traditional, deep devices due to this effect. Our approach is that we tailor the depth of the device in order to accentuate morphological differences and improve the separation.

We have made devices and carried out experiments using both polystyrene beads as a model system and RBCs of varying morphologies. To test our devices ability to detect morphological differences we change the size and morphology of RBCs by varying the osmolarity of the suspending solution. We also use the technique to separate trypanosomes from RBCs, a separation that suffers from poor resolution in standard, deep devices.

Trypanosoma brucei causes human african trypanosomiasis or sleeping sickness as it is otherwise known [4]. Diagnosis is based on finding and counting parasites in blood. Existing methods are either expensive, time consuming or not optimized for field work [5].

All work with the pathogenic *T. brucei* should be carried out in level 3 biosafety laboratories. In order to perform a proof of principle in our own microfluidics lab we work with a model system (*Trypanosoma cyclops*, a parasite of the Macaque monkey, *Macaca*, found in Southeast Asia) that is harmless to humans while displaying all the interesting mechanical characteristics of pathogenic trypanosomes. Despite small differences in size between *T. cyclops* and *T. brucei*, in both cases it is the difference in morphology between the parasites and the blood cells that our device leverages for separation.

EXPERIMENTAL

Our sorting devices were designed with a range of critical diameters $D_c=[3-9]\mu\text{m}$, (*Fig. 1. A*) separating samples into 14 size fractions at $0.5\mu\text{m}$ intervals and $160\mu\text{m}$ lateral displacement for each half micrometer increase in effective diameter. They were fabricated in PDMS using standard soft-lithography protocols, mounted in an inverted microscope and imaged using a CCD camera. Devices were treated with 0.2% PLL(20)-g[3.5]-PEG(2) in order to stop non-specific cell adhesion to the walls. The particles were driven by fluid flow through an applied pressure difference across the device. (*Fig. 1. A and B*).

Freshly drawn blood was diluted around 20 times in autoMACS™ running buffer (Miltenyi Biotech, Auburn, CA) the osmolarity of which was varied (*Fig. 2. A*). The effects of morphological changes were then studied by controlling the particle orientations by varying the depths of the devices (*Fig. 2. B*).

T. cyclops were cultured in Cunningham's medium [6] with 20% Fetal Calf Serum (FCS, Sigma-Aldrich, St. Louis, MO: F2442) at 28°C under which conditions the parasites thrived.

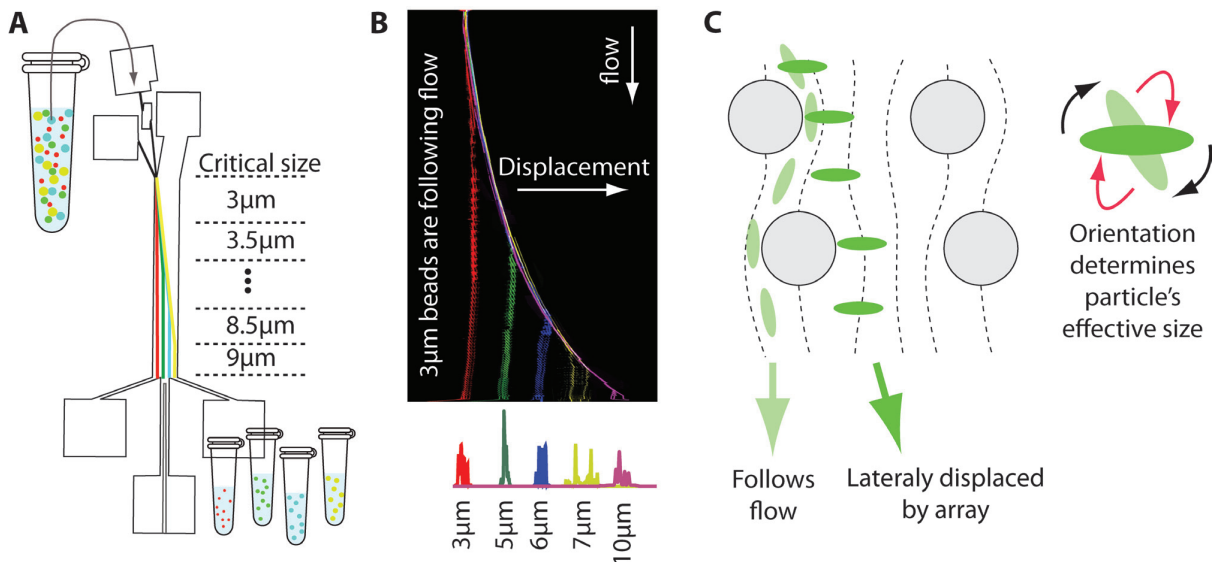


Figure 1: **A.** Device overview. **B.** Five sizes of polystyrene spheres are separated in order to calibrate the device. For spheres only the diameter is important. **C.** For non-spherical particles, effective size depends on particle orientation.

RESULTS AND DISCUSSION

As the osmolarity of the buffer was changed the morphology of the RBCs also changed, (Fig. 2. A). In a hypoosmotic buffer (low salt concentration) the concentration of stomatocytes was greatly increased. In hyperosmotic conditions many of the RBCs become echinocytes. We were able to resolve sub-populations of RBCs with specific morphologies such as echinocytes and stomatocytes (Fig. 3. A and B).

An example of how morphology comes into play as device depth is changed can be seen in Fig. 3. A. In a deep device discocytes rotate and have a smaller effective size ($\sim 2\mu\text{m}$) than echinocytes, essentially corresponding to their thickness. In a device that is shallower than the diameter of the disc of the discocytes, these RBCs are unable to rotate and behave instead as if they are larger than the echinocytes, with a size corresponding to their overall diameter ($\sim 8\mu\text{m}$). As well as being a good model system in which various morphologies can be created, the distributions of morphological subpopulations of RBCs may have clinical relevance for various types of anaemia.

Fig. 3 C and D show the separation of trypanosomes from RBCs. In a deep device the RBCs and trypanosomes rotate and present their smallest dimensions to the array. The smallest dimensions are essentially identical, which causes the RBCs and the trypanosomes to move along the same trajectory. As the device depth is decreased the effective size of the parasites, which are much longer ($\sim 30\mu\text{m}$) than RBCs, starts to increase more rapidly than for the RBCs. Eventually, at a depth of $12\mu\text{m}$ almost full separation occurs. For clinical use the cutoff size can then be chosen to be between the discocyte and echinocyte peaks to maximize collection efficiency or to be slightly above the echinocyte peak for maximum purity of the enriched trypanosomes.

To summarize we have demonstrated separation of particles with respect to morphological features that we are able to choose by controlling the orientation of the particles as they pass through a DLD device. Future work will explore other more versatile means of controlling the orientation as well as increasing the throughput of the parasite sorter to make it clinically relevant.

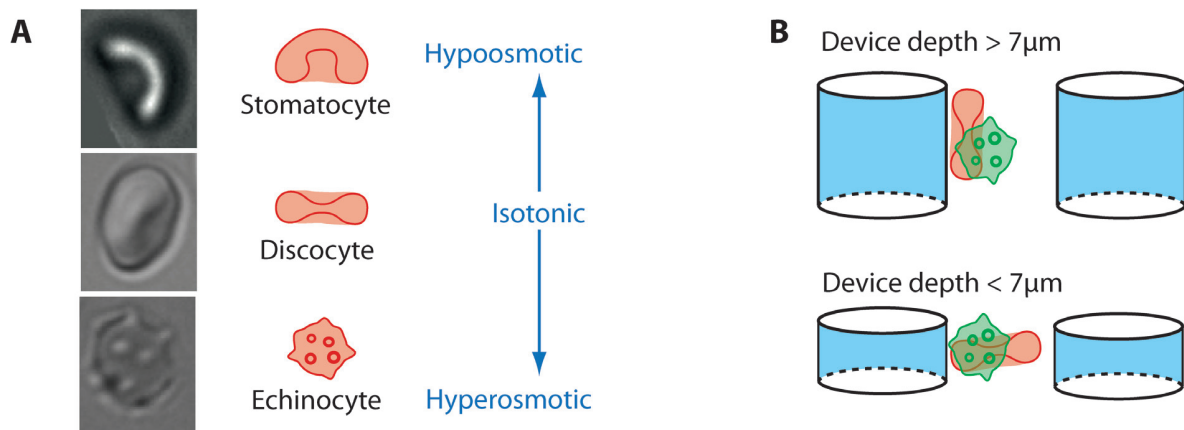


Figure 2: **A.** RBCs can adopt a variety of morphologies as a function of buffer osmolarity. **B.** In a deep device, particles are oriented by shear forces such that they present their smallest dimension to the posts. The thickness $\sim 2\mu\text{m}$ of the discocyte then determines its trajectory and it appears smaller than the echinocyte. In a shallow device its overall diameter $\sim 7\mu\text{m}$ determines its trajectory and it appears larger than the echinocyte.

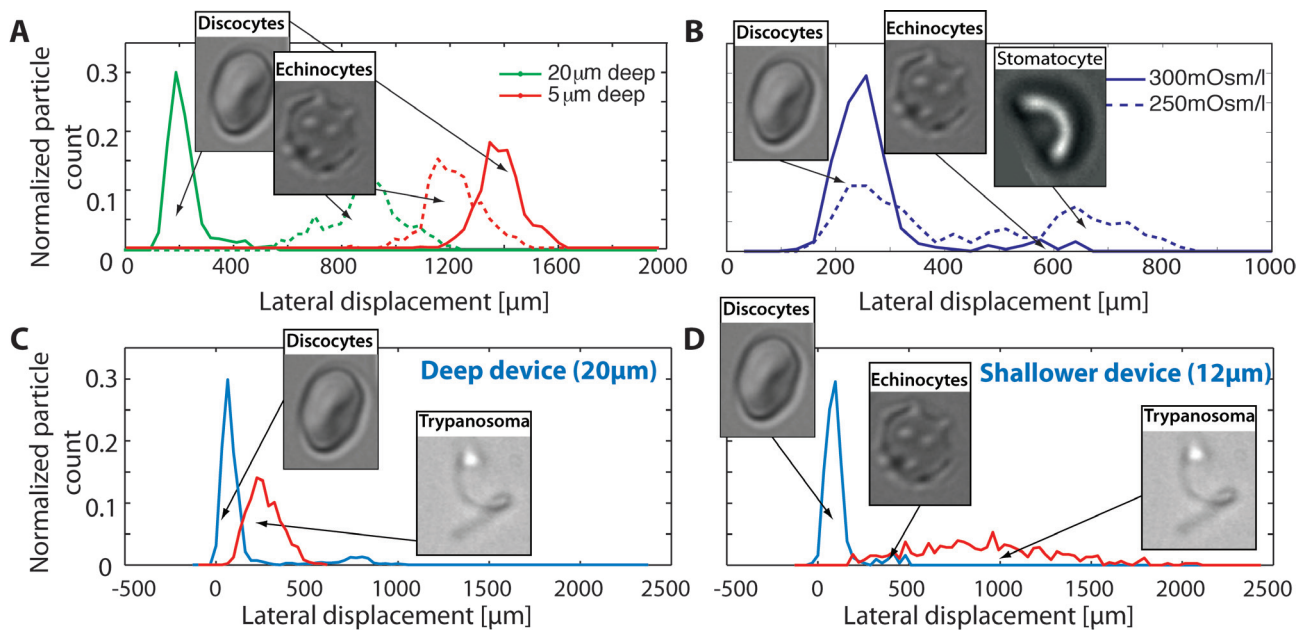


Figure 3: **A.** At 300mOsm/l RBCs are discocyte (solid lines). At 390mOsm/l the RBCs become echinocyte (dashed lines). The resultant shift highlights the effect described in Fig. 2 **B.** In an isotonic buffer discocytes and echinocytes are separated. Normal blood contains ~1% echinocytes. At 250mOsm/l ~half of the RBCs become stomatocyte and can also be separated from discocytes. **C.** In a 20µm deep device separation between RBCs and trypanosomes is poor. **D.** In a shallower device (12µm) the effective size of trypanosomes increases but not that of RBC and separation is greatly improved. The broad distribution reflects the distribution in parasite size, shape and orientation in the device.

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