A HIGH-THROUGHPUT DETERMINISTIC LATERAL DISPLACEMENT DEVICE FOR RAPID AND SENSITIVE FIELD-DIAGNOSIS OF SLEEPING SICKNESS

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ABSTRACT

We present a simple and rapid microfluidic device capable of extracting and concentrating the parasite causing the fatal disease sleeping sickness (SS) from blood. The device is based on deterministic lateral displacement (DLD) and constructed with a single inlet with flow induced by an ordinary syringe. The simplicity is crucial as the device is intended for use in the resource depraved areas where the disease is endemic. With only one inlet an intricate design with multiple depths has been utilized to create a cell free stream from the blood plasma into which the parasites are forced and subsequently collected in a detection region. In order to maximize the sample volume up to 10 device layers were stacked on top of each other which resulted in a throughput of ~10 µL/min. This allowed for an approximate time per test of below 15 min.

KEYWORDS

High-throughput, multilayered, shape sorting, point-of-care, deterministic lateral displacement, separation science.

INTRODUCTION

SS is a disease caused by the protozoan parasite *Trypanosoma brucei* with the tsetse-fly as vector, injecting the parasite into the blood stream of a human. The parasites proliferate in the hemolymphatic system during stage 1 infection. In stage 2 of the disease the trypanosomes invade the central nervous system, causing increasing neurological dysfunction, including severe sleeping disorder which has contributed to the name of the disease. [1] We have previously shown a device capable of separation of parasites and blood cells. [2] In the current work, throughput has been increased greatly by optimization of the flow rates and stacking multiple devices achieving the first stacked separation in a DLD device. Furthermore, cells that exit the device to the trypanosome outlet are captured in a sieving structure which allows for easy verification of the result. Due to the flow being induced by a simple syringe and the fact that separation in a DLD device is based on passive hydrodynamic interactions; no power source is needed for the analysis. Consequently, this device has a clear potential of being of use in the remote areas where the disease is endemic. [3]

Today, advanced machines for accurate and relatively quick diagnosis of SS exist but their bulky sizes and requirement of electricity makes them hard to put into field use. Instead, manual microscopic examinations of blood smears is often used, which is very time consuming with poor detection limit due to a parasitemia down to 500 per mL. [3] This stands in stark contrast to the blood cell concentration, mainly red blood cells (RBCs) at ~5.10^9 per mL blood.

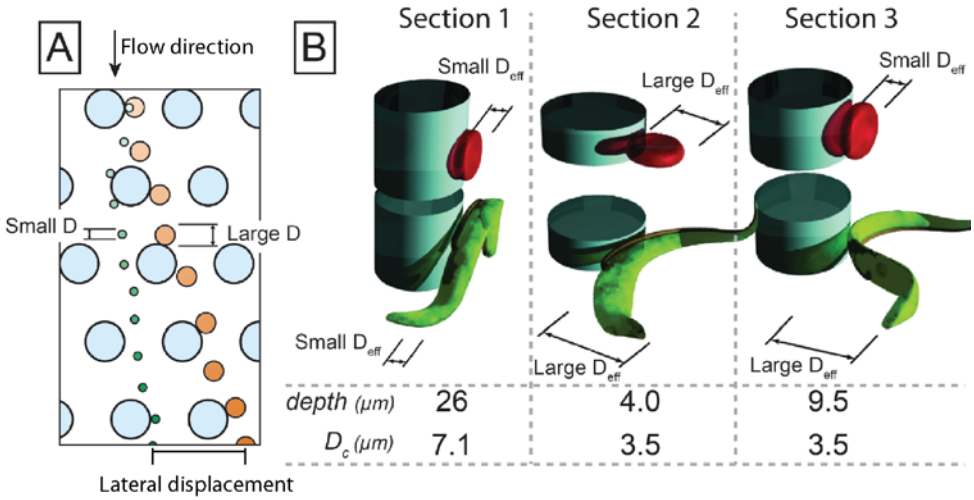

Figure 1. (A) Illustration of size-based separation in a DLD device where the radii of spherical particles determine if the particle is displaced or not. In this specific array the radius of the orange particle is larger than the critical radius of the array while the green particles radius is below the critical radius. (B) In the device presented herein we control the orientation of the particles in order to control what dimension while be measured in the device. Three sections are used, the first is deep which act, together with the shear field of the fluid, to minimize the size of these non-spherical particles. The second section is very shallow, acting to maximize the particles effective sizes and consequently they are easily displaced. In the final section, the depth is increased to being above the diameter of the RBCs which minimizes their effective size. The rotation of the longer trypanosomes is however still hindered to some degree and will still have an effective size larger than the critical radius of this section.

978-0-9798064-5-2/µTAS 2012/$20©12CBMS-0001

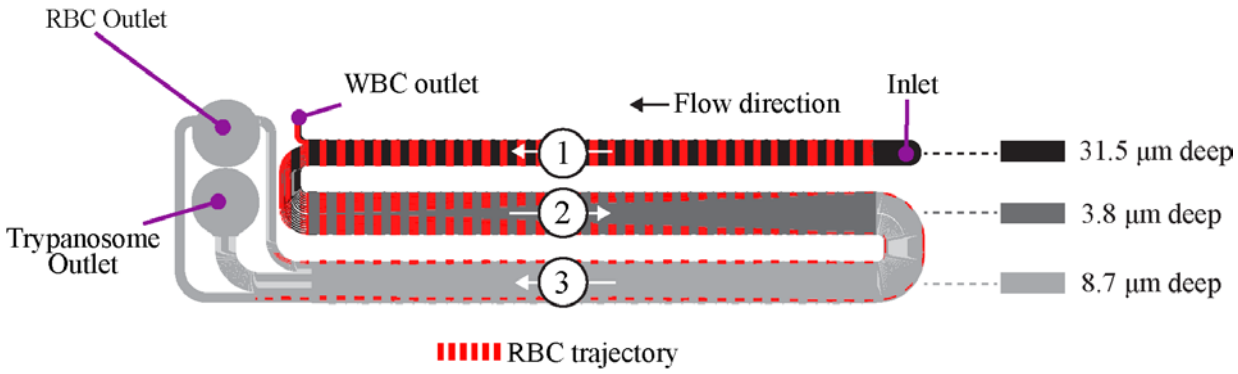

Figure 2. Illustration of the DLD device used herein. Three different sections are used, each with its specific purpose and depth. In the deep section 1 the WBC and other cells too large to enter the preceding sections are sorted out. The very shallow section 2 maximizes the effective size of all the cells making it easy to laterally displace them to the sides of the channel, opening up for a cell-free stream in the center. Section 3 is deeper than the diameter of the RBCs acting to minimize the effective size of the RBCs while the longer trypanosomes continue to be displaced, this time towards the center trypanosome outlet

THEORY

Our method is based on DLD, first demonstrated for size-based sorting [4] and recently shown by our group to have the potential for shape [5] and deformability-based [6] sorting. In short, DLD consist of an ordered array of obstacles. The laminar flow together with interactions with the array forces particles into specific trajectories through the device. Small particles follow the overall flow direction while larger particles continuously interact with the array constantly displacing them in the same direction. (Figure 1A) The smallest size at which a particle will be displaced is known as the critical radius. For non-spherical particles the orientation will have an impact on its effective size, *i.e.* the radius a spherical cell which follows the same trajectory would have.

By decreasing the channel depth we can control the orientation of non-spherical cells. The device consists of three different sections, each with its own specific task. (Figure 1B and 2) Section 1 is deep which means that the shear field of the fluid act to minimize the non-spherical cells effective sizes. Here, the large spherical white blood cells (WBCs) and other cells large enough to clog the preceding sections are sorted out. Section 2 is shallow in order to maximize the effective size of all particles as their rotation is hindered. Consequently the particles can easily be laterally displaced towards the sides of the channel, opening up a cell-free stream in the center. In section 3 the depth is optimized so that only the larger trypanosomes are displaced, this time towards the cell-free center stream where they exit the device to the trypanosome outlet and subsequently immobilized in a sieving structure for easy read-out.

EXPERIMENTAL

The device was constructed in PDMS by soft lithography. The master for replica molding was constructed in three separate cycles of UV-lithography of SU8, where each layer corresponds to the specific depth in each section. In order to maximize the throughput, and consequently minimize the time of analysis, up to ten devices were stacked on top of each other with precise microscopic alignment. (Figure 3B) Pressure series were carried out in order to see at what flow rates the separation starts to decrease. These, first part of the measurements, were carried out with human blood spiked with a to-human nontoxic relative to the *Trypanosoma brucei* parasite, namely the *Trypanosoma cyclops.* After these initial tests, further analyses were performed with the *Trypanosoma brucei parasite* in order to verify our findings. In all of the experiments presented herein the blood was diluted 5 times while the trypanosomes where kept at a high concentration (~100/µL) to acquire good statistics of the device performance.

RESULTS AND DISCUSSION

The cell sorting carried out at different flow rates show that there is a point above which the separation efficiency starts to decline. (Figure 4A) Due to the deformability of the cells, high flow rates will act to decrease their effective size. For RBCs this effect stems from section 2, where high flow rates act to disperse a fraction of the cells, which is interesting in itself, instead of focusing them along the side of the channel. For trypanosomes the decrease in separation efficiency is also a result of avoiding being laterally displaced in section 3. Due to the huge fraction of RBCs in the samples, a high specificity is needed. Consequently, the tests with *Trypanosoma brucei* where carried out at 0.6 µL/(min·layer) to achieve the lowest fraction of RBCs possible in the trypanosome outlet. (Figure 4B) The resulting fraction of cells exiting through the center outlet is 98.4% of the trypanosomes and 96.1% of the RBCs. This implies that *T. brucei* has a larger effective size than our model organism, *T. cyclops*, resulting in higher sensitivity. The few parasites which exit to the RBC outlet can be compensated for by simply analyzing a marginally larger volume. With Poisson statistics we need to analyze 145 µL to have a 1 in a million risk for a false positive, which currently would take less than 25 minutes. With further optimization of the device design we hope to achieve even higher flow rates and consequently lower the time of analysis.

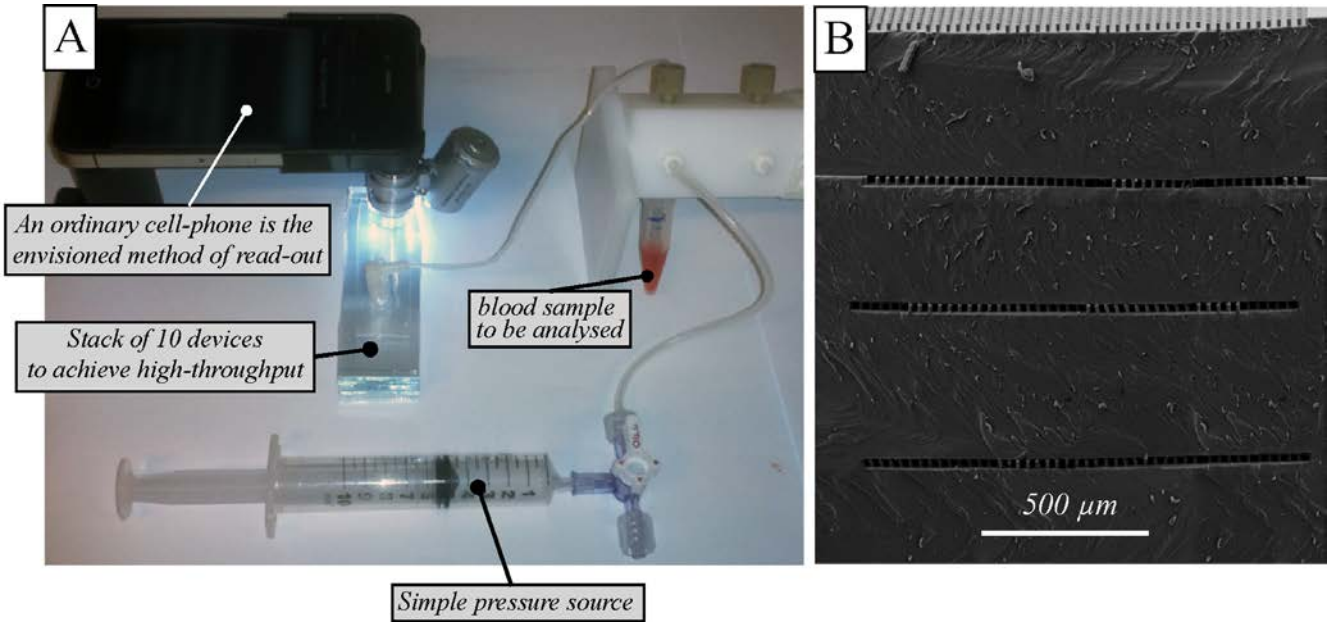

Figure 3 (A) Image of the entire setup with a simple syringe driving the sample through the 10 layers of the device. Here, a normal cell-phone is being used; this is the envisioned method of read out in the future during field-operation. (B) An SEM-micrograph of the top four layers in a stack of ten devices which efficiently increases the throughput without increasing the device size.

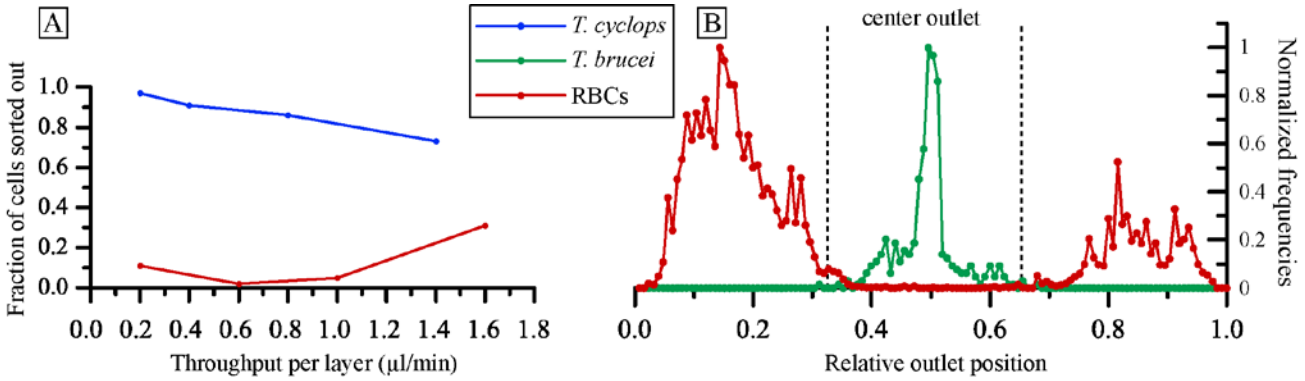

Figure 4 (A) Graph showing what fraction of the different cell types that exit through the center outlet to the detection region. It can be seen that too high flow rates act to decrease the separation efficiency. (B) The distribution of T. brucei and RBCs at the end of the device when running the device at 0.6 μL/(min·layer). Cells between the dotted lines exit to the detection region.

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