

INTEGRATION OF CENTRIFUGO-MAGNETOPHORESIS AND BRIGHT-FIELD BASED T-CELL ENUMERATION FOR HIV DIAGNOSTICS IN RESOURCE-POOR SETTINGS

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ABSTRACT

Using a lab-on-a-disc platform integrating 2-dimensional cell separation and subsequent enumeration by both a semi-quantitative and a fully quantitative “digital” method, we present a CD4+ cell counting solution from low sample volumes of blood for HIV diagnostics. Depending on the cell population from blood that is bound to magnetic beads, the system will operate in one of two modes: “Positive” or “Negative”. Enumeration of the CD4+ cells is then carried out without the use of fluorescent markers or any other staining methods, lending the system to low costs and complexity – hallmarks of a viable diagnostic test for deployment in low-income countries where HIV is endemic.

KEYWORDS: Lab-on-a-Disc, HIV Diagnostics, Cell Counting, Magnetophoresis

INTRODUCTION

Advances in anti-retroviral therapies (ART), as well as charity initiatives making such therapy available to low-income countries, have resulted in HIV/AIDS becoming a largely chronic disease rather than an acute and terminal infection. Yet, in endemic regions with high populations, diagnosis remains a bottleneck as accurate tests are laborious involving costly equipment and high-level operator skills. Point-of-Care (POC) testing helps alleviate the bottleneck, but can be expensive and complex themselves [1]. The here presented work demonstrates that CD4+ count (the gold standard of “Treat / No Treat” HIV diagnostics) can be achieved without need of cell staining or cumbersome blood processing. Furthermore, the mechanical actuation is comparable to the simple, low-cost spindle motor of a conventional CD-player, coupled with a simple bright-field microscope. Combining two technologies of cellular magnetophoresis and scale-matched geometrical cell barriers (“V-cups”) [2] previously developed in the group, cell counts can be either semi or fully quantitative. Magnetophoresis is a dual-force particle separation strategy allowing cells of interest that have been bound to magnetic beads to be isolated from a background population that is travelling under the impact of a centrifugal field. The V-cup strategy then further allows isolated cells to be enumerated as they are distributed in a sharply peaked, single-occupancy distribution over discrete loci that can be visually examined. The application of such technology allows high efficiency of isolation, while providing the user with quantitative data at the point-of-care.

THEORY

In our dual-force particle separation strategy [3], all suspended cells migrate radially in stopped-flow under the sheer impact of the rotationally induced centrifugal force. Magnetically labeled cells are furthermore deflected by the perpendicularly oriented field of on-disc magnets to a designated capture locus (Fig. 1). For isolation and enumeration of CD4+ cells from whole blood, we here implement a positive as well as a negative selection strategy.

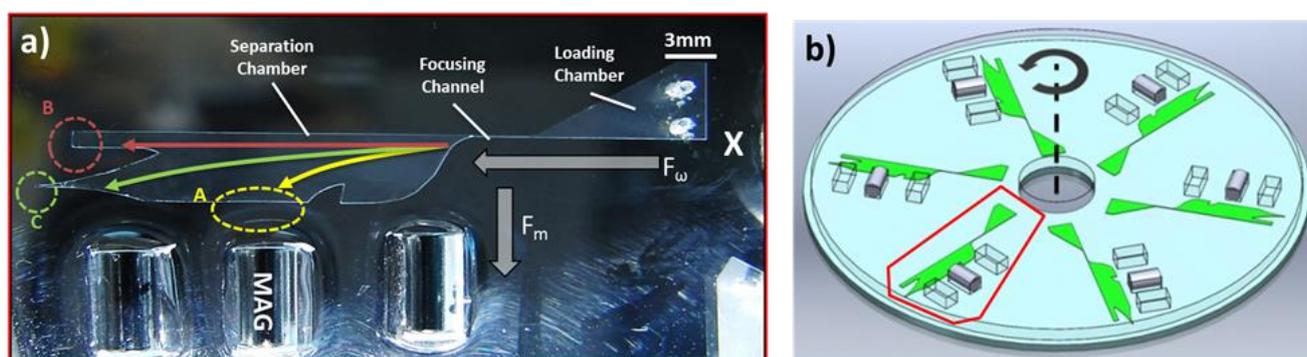


Figure 1: a) Centrifugo-magnetophoresis chamber imaged during a test run. The center of rotation is marked with X. After exiting the focusing channel, unbound magnetic beads are directed to the “gutter” at position A, non-magnetic cells (including RBCs) maintain the radial trajectory to B, while bead-bound cells experience both the centrifugal and lateral magnetic force to follow the moderately curved trajectory indicated by the green arrow to C. The direction of the centrifugal force is indicated by the arrow marked F_{ω} , and the (main) direction of the magnetic force is indicated by the arrow marked F_m . An image of a full disc accommodating six separation modules is shown in (b), with the region represented by (a) delineated by a red outline. Although the Positive Mode chamber is pictured here, the Negative Mode operates on identical principles.

For positive-selection (Fig. 2), beads specifically targeted to the CD4+ cells (“T-cells”) mediate the centrifugo-magnetic routing towards a narrow capture structure while undeflected RBCs and non-CD4+ cells are directed to waste (Fig. 2b). In this mode, the level of packing of beads/CD4+ cells indicates the initial concentration in the starting sample. Conversely, in negative-selection mode (Fig. 3), a cocktail of magnetic beads directed to a number of antigens (CD8, CD14, CD16, CD19, CD36, CD56, CD123 and CD235a) are incubated with the white blood cells isolated from whole blood. These beads will bind non-CD4+ cells, hence, in a WBC sample only the CD4+ cells will remain unbound and thus travel in the straight (radial) direction; all other cells will be deflected by the perpendicular magnetic force towards the chamber wall. As the so isolated CD4+ cells are not magnetically tagged, the formation of macro-cellular clusters such as those in the positive-selection strategy is ruled out. We can therefore enumerate these free CD4+ cells on a “digital”, i.e. one-by-one basis, in a V-cup array which was integrated in the radial sedimentation path following the centrifugo-magnetophoresis module. This array comprises of 7410 structures, each specifically sized to accommodate one CD4+ cell at maximum.

EXPERIMENTAL

Whole blood was isolated from healthy volunteers *via* finger-prick lancet (as this is the preferred method of sampling in resource-poor regions), and, in the case of the positive mode, HL60 cells stained with Hoechst 33342 were spiked into the samples to confirm the specificity of CD4+ cell isolation. For the negative mode data shown here, native CD4+ cells were isolated on-chip after the RBCs were lysed using a hypotonic buffer (Pharm Lyse, BD Biosciences) and the WBCs analyzed for CD4+ cell concentration. For both modes, 200- μ l samples were incubated with appropriate magnetic beads at room temperature for 10 minutes before a 4- μ l aliquot was applied to the chip. Chips were spun at 10 Hz for 45 minutes, and analyzed using bright-field and UV-fluorescent microscopy. Note, fluorescence was only required for confirmation of CD4+ isolation, it would not be necessary for a POC instrument.

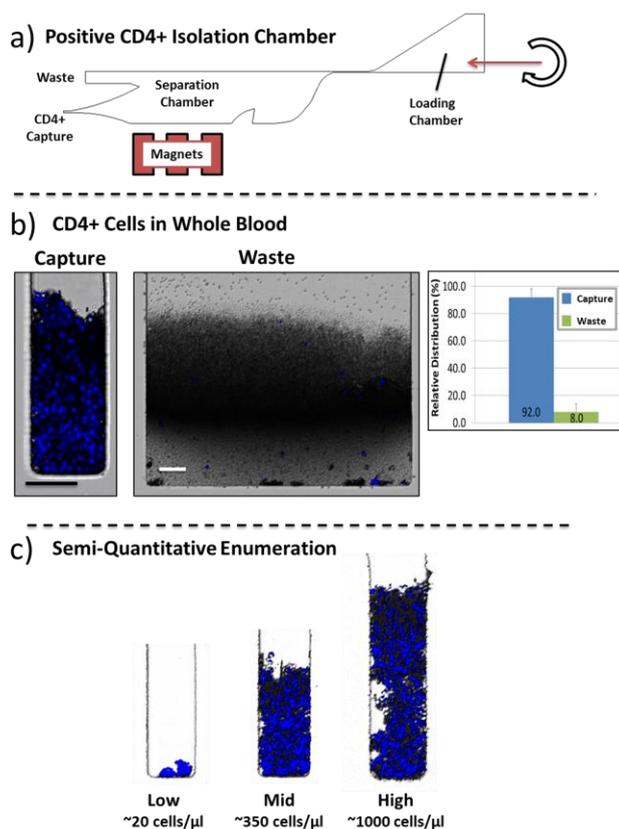


Figure 2: Semi-quantitative enumeration in Positive mode. a) Design of the positive isolation chamber. b) Following incubation with CD4+ magnetic beads, HL60 spiked whole blood was introduced to the Positive Isolation chamber and spun at 10 Hz for 45 minutes. Pixel analysis of capture and waste chambers indicates isolation efficiency. c) Semi-quantitative enumeration was achieved by spiking known numbers of CD4+ cells to CD4+ depleted whole blood. Examination of the packing in the capture chamber allowed estimation of the CD4+ count above and below a medically relevant threshold.

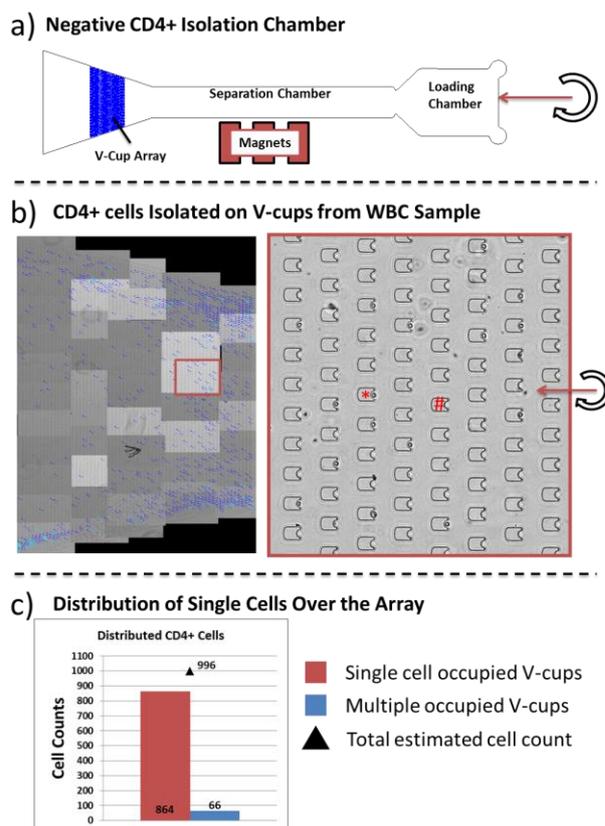


Figure 3: Fully quantitative enumeration in Negative mode. a) Design of the negative isolation chamber. b) WBCs were incubated with negative selection beads and introduced to the disc. After spinning, the V-cup array was imaged to show cell distribution over the array. Single- and multiple-occupancy cups are marked with dark and light blue tags, respectively. The expanded view of the red box shows capture of single cells: an occupied (*) and non-occupied (#) cup are indicated. c) Occupied cups were enumerated by image analysis and the total CD4+ cells was obtained by counting of occupied cups.

RESULTS AND DISCUSSION

Using the Positive Isolation mode to direct CD4⁺ cells to a capture chamber, the current system demonstrated $92 \pm 5.6\%$ efficiency of CD4⁺ cell capture from whole blood (Fig. 2b), with only $0.6 \pm 0.8\%$ capture of non-CD4⁺ cells (i.e. false positives). Moreover, by examining the level of packed cells/beads in the capture area, an estimation of the cell count can be inferred. This semi-quantitative readout is sufficient to identify whether blood samples from HIV patients are in the healthy range (ca. $1200 \text{ cells } \mu\text{l}^{-1}$) or below a level ($350 \text{ cells } \mu\text{l}^{-1}$) requiring medical intervention (Fig. 2c). For the purposes of rapid diagnostics where a simple “treat / no-treat” decision is required, such an easily interpreted method is attractive.

Using the Negative Isolation mode, native CD4⁺ cells were isolated from (non-spiked) whole blood on the system. The size of the capture area of the individual V-cups was scale-matched to the expected size of T-cells, and led to a 93.3% single-occupancy distribution of the CD4⁺ cells. Of the V-cups that showed multiple occupancy, the most cells that were present at a single cup was 2. Enumerating the occupied V-cups allowed an estimation of the number of CD4⁺ cells present in the sample as shown in Fig. 3c. Given that the specificity of the negative isolation system is derived upstream *via* the removal of non-CD4⁺ cells, the operator can be confident that the cells detected only using bright-field microscopy at the V-cup array have a high probability of being CD4⁺ cells. To clarify this, a further experiment was done in which the native CD4⁺ cells were depleted from whole blood, and Hoechst-stained HL60 were spiked to the depleted blood. Following negative isolation, 94% of the cells captured on the V-cup array were shown to be the spiked cells (Fig. 4).

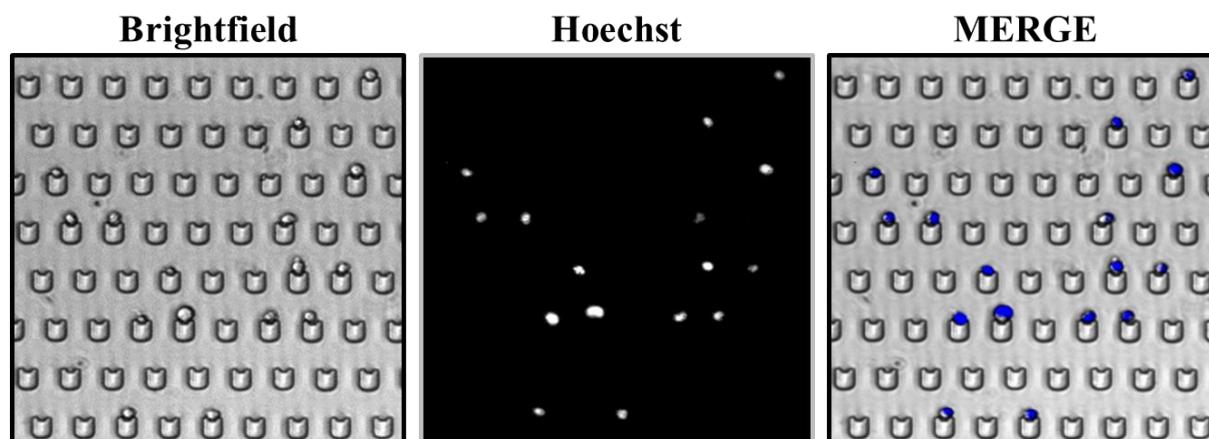


Figure 4: Whole blood was depleted of CD4⁺ cells, and Hoechst-stained, CD4-positive HL60 cells were added to the depleted blood. Following isolation via negative mode and distribution of the recovered cells on the V-cup array, the arrayed cells were imaged by bright-field and fluorescent microscopy to identify the spiked HL60 cells. 94% of the cells imaged in bright-field also stained positive for Hoechst, indicating a high degree of CD4⁺ cell isolation.

CONCLUSION

We here present two strategies based on centrifugo-magnetophoresis on a lab-on-a-disc platform for the rapid diagnostic estimation of CD4⁺ cell concentration from a finger-prick derived sample of whole blood. Using the dual-force system described, the cells can be extracted in either positive or negative mode; wherein the positive mode has the advantage of using whole blood as an input sample, the negative mode still requires off-chip RBC lysis. However, the negative mode does provide a fully quantifiable method by individually imaging each cell isolated in the chip. The authors propose that both systems could be operated on a single platform, where positive mode could be deployed for general screening to identify patients on the high and low end of the medically relevant spectrum of CD4⁺ concentration, but patients presenting with counts at the threshold for treatment could be further tested using the negative mode on the same device.

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