

A 3D-PRINTED OPTICAL READER FOR COST-EFFICIENT ENUMERATION OF CD4 CELLS FOR POINT-OF-CARE DIAGNOSTICS OF HIV IN RESOURCE-POOR SETTINGS

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ABSTRACT

Here we present a low cost, 3D printed optical reader which is compatible with a disposable, finger actuated chip which can be used for rapid CD4+ enumeration. This portable and widely autonomous device provides the next step in a workflow which can meet the WHO ASSURED requirements for assessment of HIV status in resource-poor settings. The reader is composed of 3D printed parts where the microfluidic chip can be moved along the optical axis of the objective lens for focusing using a screw adapted from a masonry bolt. Performance of the reader shows good agreement with measurements made using a conventional inverted microscope.

KEYWORDS: 3D Printing, Optical Measurement, Point of Care, HIV, CD4 Enumeration

INTRODUCTION

Since its identification and widespread emergence in the early 1980s, HIV (human immunodeficiency virus) infection and the associated pathology of AIDS (acquired immunodeficiency syndrome) remain an ongoing global pandemic disease. It is estimated that over 35 million people were living with HIV in 2012, with total deaths since the emergence of the disease of around 36 million. The delivery effective diagnostic testing at endemic regions which are typically characterised by poor infrastructure still remains the primary roadblock to treatment [1, 2]. Slow treatment and poor infrastructure means often patients will not receive their status results; this ‘loss to follow up’ greatly exacerbates this problem [3].

A number of groups have made progress towards developing disposable point-of-care CD4 enumeration platforms which meet the gold-standard WHO ASSURED criteria for POC diagnostics [3-6]. Recently, Glynn *et al.* [7] introduced a microfluidic based approach which provided a semi-quantitative CD4+ counts on a low-cost, disposable chip. Flow is driven through finger actuation of a flexible reservoir and the target cells are separated by magnetophoresis. CD4+ numbers can be estimated by reading packed cell height from graduated hatch marks (Fig 1). Sample preparation takes within approximately 15 minutes and the entire test can be completed within ~30 seconds from sample application. The chip uses stable, IVD grade magnetic beads for cell capture.

EXPERIMENTAL

Here we present a cost-efficient, 3D printed (Dimension μ Print Plus) optical reader which is compatible with the above described, finger actuated microfluidic chips. The reader is composed of a chassis to which a modular lens holder and diffusion plate holder are attached. This chassis also features a rail onto which a moveable chip holder is mounted. The compacted bead height can be read from the chip by eye-balling through the objective lens. The optical path is backed by diffusion glass such that ambient light enhances contrast. A screw driven system, adapted from a low-cost masonry bolt, positions the chip along the rail in order to focus the packed cells and the hatch marks (Fig. 2). The reader is modular in design; the optical lens can be swapped out for a larger lens. Similarly, replacing the diffusion grating with an LED can permit to project the shadow of the scale / packed cells onto the wall of a darkened room. In parts and components, the entire reader costs less than €10.

Prior to testing, microfluidic chips were manufactured and primed using a ‘running buffer’ composed of PBS (Phosphate Buffered Saline pH 7.4) as outlined in [7]. BSA (Bovine Serum Albumin, 0.1% w/v and EDTA (Ethylenediaminetetraacetic acid, 1 mM). Serial dilutions of 4.5- μ m diameter polystyrene superparamagnetic beads (CD14 tagged beads from Dynabeads® T4 Quant Kit, Life Technologies) were created at the dilutions (Fig. 3).

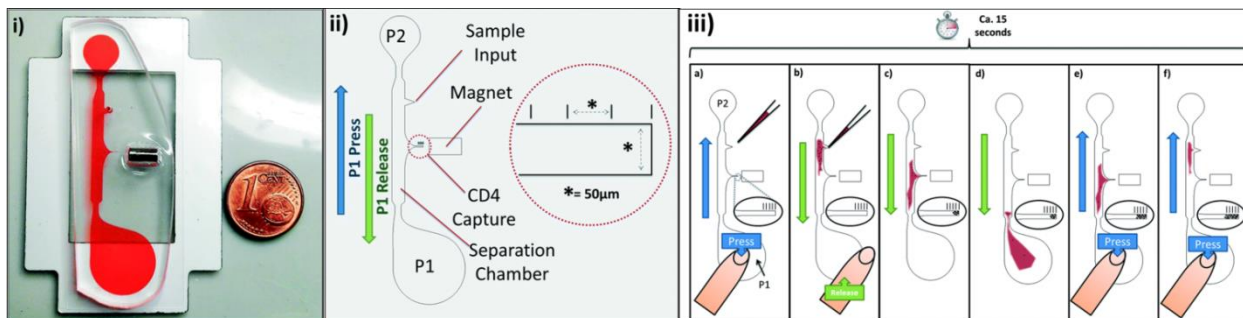


Figure 1 – The finger-actuated CD4 Enumeration Chip (i) The chip filled with dyed water to highlight its geometry. (ii) Schematic of chip and (iii) chip operation. Figure is adapted from [7] by permission of The Royal Society of Chemistry (RSC).

The beads were then processed as shown in Figure 1 [7]. Subsequent to bead processing, the user noted the reading on the graduated scale by interrogating the chip with the handheld optical reader. The microfluidic chip was then imaged using a laboratory microscope. Next, the user took the reading on the graduated scale on the image which was independently confirmed by a second experimenter. Where the first and second experimenter disagreed the reading from the second experimenter was used. Note that a strict ‘round up from highest point’ policy is followed to avoid experimenter bias. Therefore, the packed bead height shown in Figure 3(a) was read as ‘3’ and the height in Figure 3(b) was read as ‘6’. Measurement data ($n = 4$ chips) is shown in Figure 3(c).

RESULTS AND DISCUSSION

The performance of the reader has been characterized using paramagnetic beads over a series of dilutions (Fig 3(c)). These dilutions are chosen as, loaded on-chip, they provide similar bead packing heights to those expected from the healthy / unhealthy range of CD4+ cell counts. As shown in Figure 3, the chip reader shows good agreement with a standard, commercial laboratory microscope though the chip reader show a slight bias towards underestimating the cell count compared to the microscope readings.

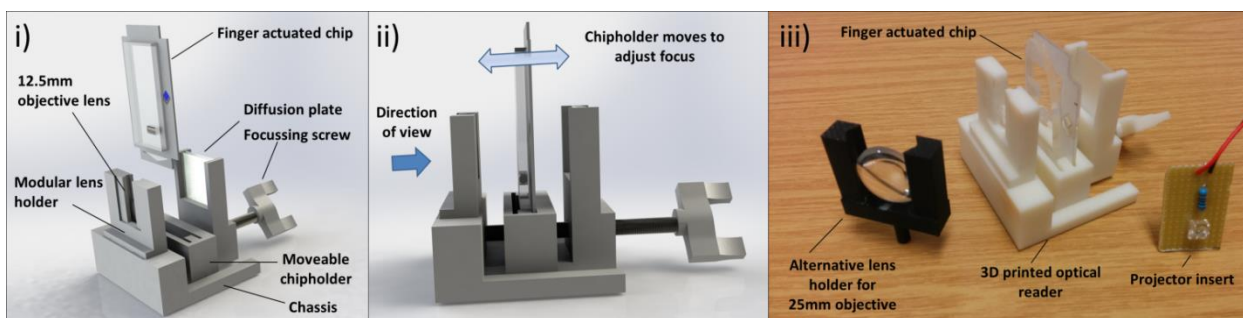


Figure 2 – The Optical Reader for CD4 Cell Enumeration (i) The low-cost reader is 3D-printed from 4 separate parts. Additional parts are a threaded screw, objective lens and diffusion plate. The finger actuated chip is inserted into a moveable chip holder. Its chip location relative to the objective lens, and thus the focus, can be finely adjusted by turning the focussing screw. (ii) The chip is read by looking through the objective. The packed height of CD4+ cells and bead conjugate can be estimated from graduated hatch marks. Alternatively, the hatching can be calibrated as ‘treat’ or ‘no treat’ based on clinical guidelines. (iii) Image of the 3D printed optical reader. Also in the image is an alternative lens holder for larger objectives. Additionally, a ‘Projector insert’, a powerful, low cost LED powered by a 3V battery, can be placed into the reader in place of the diffusion plate. In this case, the shadow of the packed cells and graduated hatch marks can be projected against a wall or floor.

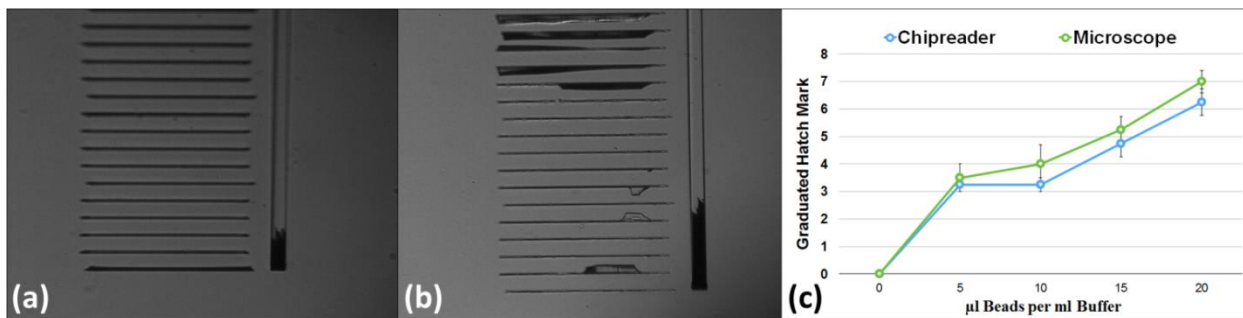


Figure 3 – Comparison of Handheld Chip reader to a laboratory microscope (a) Bead capture from low (5μ) concentration of paramagnetic beads in running buffer (and is read as '3'). (b) shows high ($15\mu\text{l}$) concentrations of same (read as '6'). (c) Packed bead heights measured using the optical reader and inverted microscope ($n = 4$).

CONCLUSIONS

The development of this low-cost reader provides the next step in a workflow which can meet the ASSURED requirements. As a next step, this reader will be characterized using whole blood which has been artificially depleted of CD4+ cells to the clinically relevant range. Furthermore, the chip can also be mounted directly onto a smartphone to obtain CD4+ counts based on both packed cell height and packed cell area by implementing proper image processing algorithms. This combination provides a simple 'treat / no-treat' answer using a workflow well suitable for point-of-care applications.

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