Electronic Supplementary Material (ESI) for Lab on a Chip.
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# Electronic Supplementary Information

# Rapid, Low-Cost and Instrument-Free CD4+ Cell Counting for HIV Diagnostics in Resource-Poor Settings

Macdara Glynn, David J. Kinahan and Jens Ducrée

Biomedical Diagnostics Institute, National Centre for Sensor Research,
School of Physical Sciences, Dublin City University, Ireland

## S.1 - Sample Preparation

Bead Samples

Mixed samples were prepared by combining the indicated number of 10-µm magnetic (PS-MAG-S1791) and 11-µm non-magnetic FITC particles (MF-FITC-PARTICLES) - both from Microparticles GmbH, Germany.

Blood Sampling and Preparation

Blood was extracted directly from healthy donors *via* finger-prick using 1.5-mm sterile lancets (BD Biosciences, NJ, USA). To prevent coagulation, 60-mM EDTA solution was immediately added to the sample, resulting in a final concentration of 6 mM EDTA. The whole blood / EDTA mixture was then further mixed 1:3 with blood dilution buffer (100 mM PBS, pH 7.4 / 0.1 mM EDTA). Blood was isolated and prepared fresh, directly before experimental use. All cell culture reagents were obtained from Sigma-Aldrich, MO, USA. HL60 cells (DSMZ, Braunschweig, Germany) were cultured in 75-$cm^2$ flasks in RPMI 1640 media, with 10% un-inactivated foetal bovine serum (FBS), 100 U / ml penicillin and 100 µg / ml streptomycin. Cultures were maintained at 37°C with 5% $CO_2$. Where indicated, live HL60 cells were stained with NucBlue™ Live Cell Stain (Life Technologies, CA, USA) according to manufactures instructions.

There are obvious biosafety restrictions inherent in using HIV infected blood as experimental material. Therefore we replicated the CD4-based cellular nature of healthy and infected blood by mixing normal, CD4+ populated blood with blood artificially depleted of all CD4+ cells. Extracted and diluted whole blood (prepared as described above) were split into two 380-µl aliquots A and B. White blood cells were stained in both aliquots by adding 4',6-diamidino-2-phenylindole (DAPI) at a final concentration of 10 µg / ml. Although the majority of CD4 expressing cells in whole blood are the T-helper cells of interest, monocytes can also express low levels of CD4. Hence, in these experiments monocytes were first removed from both aliquots by adding 30 µl Dynabead™ CD14 (Life Technologies) and incubated under rotation at room temperature for 5 minutes. Monocyte-bound beads were removed from the sample by positioning the tube alongside a NdFeB magnet

(Supermagnete, Germany) for 30 seconds and removal of the sample to a new tube, taking care not to carry over any of the immobilised monocyte-bound magnetic beads.

At this point, aliquot A is set aside and regarded as the "CD4-positive" or "native" blood sample. Aliquot B is further processed to remove all CD4+ cells. This is carried out identically to the protocol for CD14 removal, but using Dynabead™ CD4 beads. After elimination of both CD14 and CD4 cells from aliquot B, this sample is regarded as "CD4-depleted". CD4 cells are enumerated in both aliquots using the T4 Quant Kit (Life Technologies), and native and CD4-depleted samples are then mixed to generate experimental samples representing 1600, 1000, 500 and 0 CD4+ cells / µl of whole blood (Fig. S1). These samples are tested on our diagnostic chip. Where indicated, experimental samples were also prepared by mixing CD4-depleted blood with known concentrations of HL60 cells pre-stained with NucBlue™.

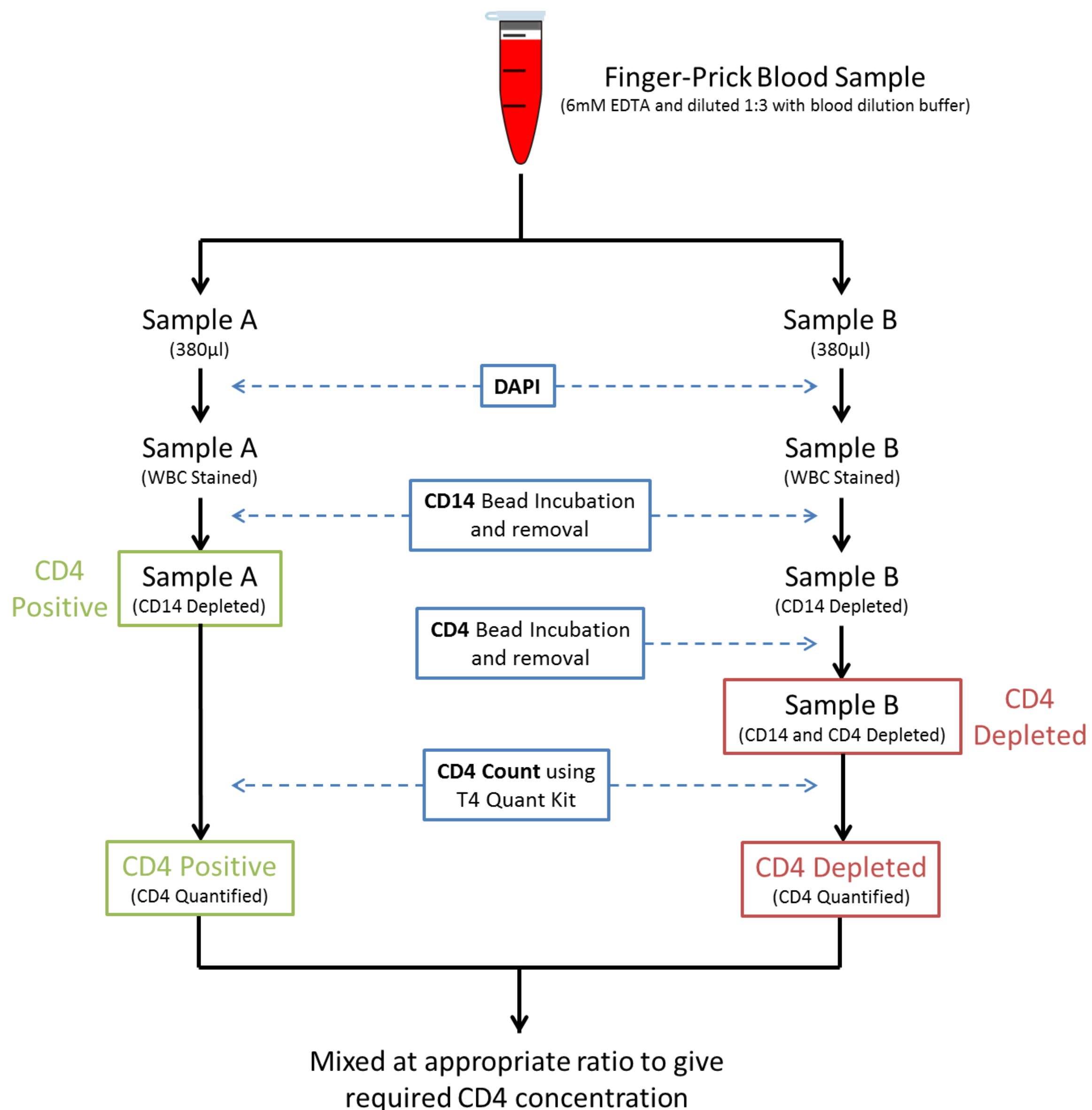



**Figure S1:** ***Preparation of blood samples containing specific numbers of native CD4 cells.***

For the diagnostic tests, 3 µl of Dynabead™ CD4 is added to 85 µl of experimental sample in a 2-ml Eppendorf tube, resulting in a Dynabead™ CD4 concentration of ca. $5.1 \times 10^4$ beads per µl. The sample was rotated at room temperature for 3 minutes, which may be done by hand. Following this incubation, 4 µl of the sample is applied to the input of the chip as described in Fig. 2 of the primary text.

## S.2 - Optional Fluorescent Imaging and Quantification of Signal

For fluorescence-based quantification, we employ DAPI staining of WBCs, or the NucBlue™ staining of spiked HL60 cells – both of which are detected using a UV-excitation filter set on the microscope (Ex = 370 nm, Em = 460 nm). Relative levels of fluorescent signal between samples were calculated as follows using the ImageJ software package (NIH, MD, USA). For a given experiment, the overall cell coverage of the capture chamber across a number of chips was observed, and the chamber containing the largest zone of cell-occupied area was selected. A region-of-interest (ROI) was generated using a brightfield image that encompassed all cells present in the selected chamber. An identically sized and shaped ROI was then applied to all capture chambers within the experiment. The same ROIs were subsequently utilized for the corresponding fluorescent images for each chamber and the raw integrated density values (RawIntDen) within the ROIs were measured. An identically shaped ROI was also generated in an area of the chambers not containing any bioparticles to quantify the background signal to be subtracted from the cell-containing ROIs.

## S.3 – Supplementary Experimental Results

Native CD4+ Cell Analysis from Whole Blood

Blood samples with adjustable numbers of target CD4+ cells were prepared as described in S.1 of ESI. Experiments measuring levels of the cell / bead pack in the capture chamber were repeated n = 4 times, and the upper level of the pack was measured against the integrated scale bar. If the pack level reached a point between two notches, the higher of the notches was reported. Figure 5 of the primary text shows representative images of the results with statistical analysis. Figure S2 below shows all 16 images used to generate the statistics.

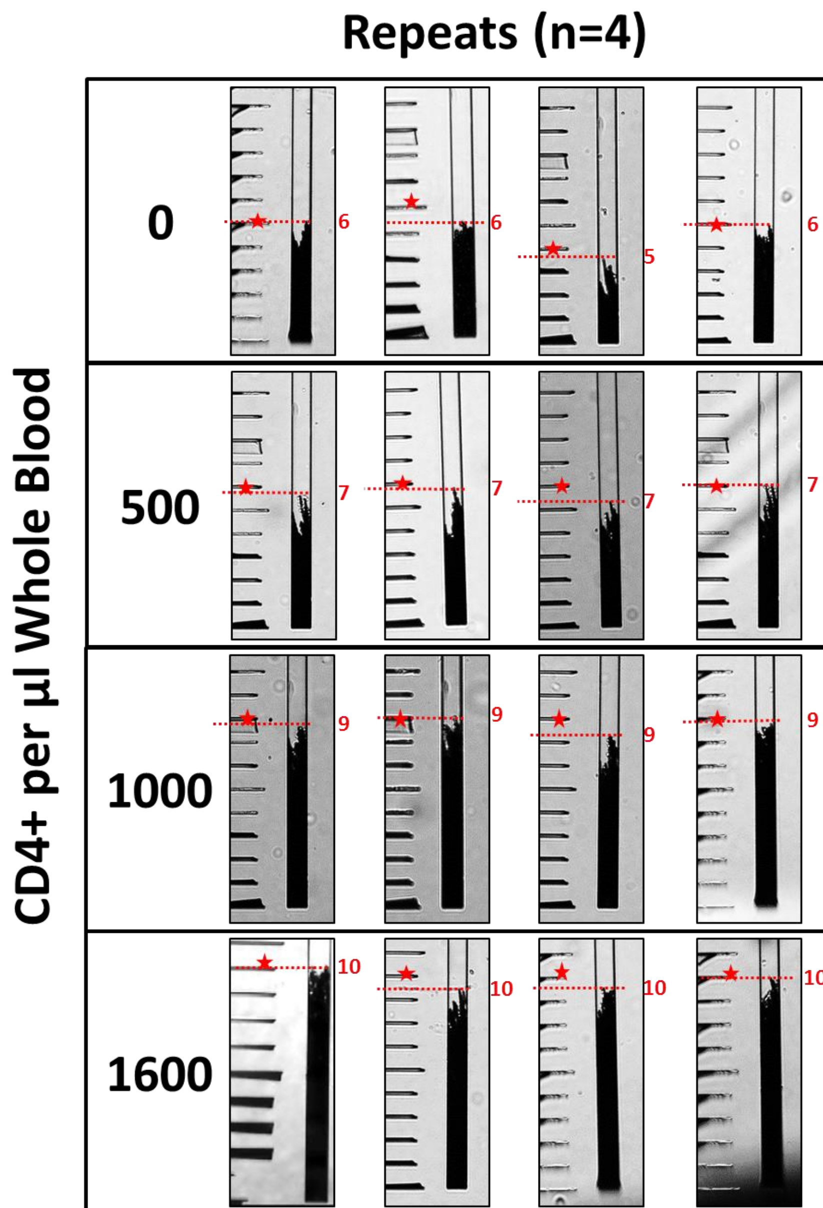
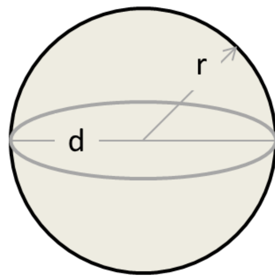
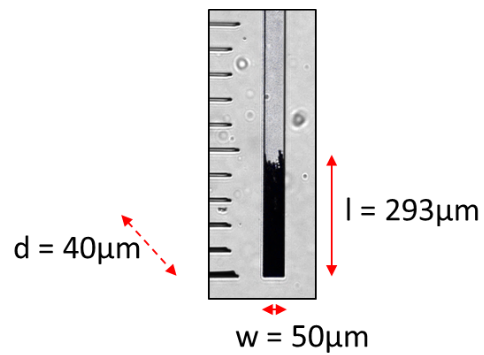



**Figure S2:** ***Native CD4+ Cell Analysis from Whole Blood.*** A red hatched line represents the upper level of the pack, a red star represents the corresponding notch used as the metric, and the number in red is the label for this notch.

## S.4 – Packing Volume Analysis




| CD4 Dynabead Volume | |
|---|---|
| Diameter (d) | 4.5 µm |
| Radius (r) | 2.25 µm |
| **Single Bead Volume** | **$4.8 \times 10^{-5}$ nl** |
| # Beads on Chip | 204545 |
| **Total Bead Volume** | **9.8 nl** |

| Dynabead Pack Volume | |
|---|---|
| Length (l) | 293 µm |
| Width (w) | 50 µm |
| Depth (d) | 40 µm |
| **Total Pack Volume** | **0.586 nl** |

**Figure S3:** ***Comparison of Predicted and Measured Bead Volumes.*** The volume of a single CD4 Dynabead is calculated and multiplied by the estimated number of beads present on chip (Left). The packing volume of CD4 Dynabeads following processing of the 0 CD4+ cells in whole blood (Fig. 5 in primary text) is calculated based on the dimensions of the capture chamber and the height of the bead packing.