

## Precise CD4 T Cell Counting Using Red Diode Laser Excitation: for Richer, for Poorer

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### ABSTRACT

**Background:** Measuring CD4 T cell counts at low cost is relevant in dealing with the HIV epidemic throughout the developing world. The recently introduced novel concepts in gating strategies and sample stabilization facilitate affordable immunophenotyping by flow cytometry but the impact of these developments is still limited by the high cost and the antiquated complicated design of the currently available flow cytometers.

**Methods:** Diode lasers emitting 10-15 mW at 635 nm are one tenth the size and cost, and require one thousandth the power of an equivalent 488 nm argon ion laser. We used the available 635 nm diode-based flow cytometers, including PA-II (13), Luminex 100 (14), SuperMot (15) and FACSCalibur (17) to investigate whether these instruments can generate reliable CD4 counts when used with allophycocyanin (APC) and cyanin-5 (Cy5) labelled CD4 antibodies.

**Results:** We document the feasibility of obtaining leucocyte differential counts using orthogonal side scatter (SSC) without the need for forward scatter (FSC). Accurate CD4% values among lymphocytes and leucocytes can be obtained by primary CD4 gating using a single CD4 monoclonal antibody, conjugated to APC or Cy5. Double immunofluorescence (IF) staining with CD4-APC (FL1) and CD45-APC-Cy7 (FL2) introduce pan-leucogating for a convenient assessment of absolute CD4 counts on double platforms. We demonstrate that small flow cytometers with laser diodes are capable of delivering absolute CD4 T cells counts with a precision similar to the performance of the current state-of-the-art 'single-platform' instruments (e.g. the Cytoron Absolute;  $R^2=0.961$ ). In this respect, they appear to be superior to the non-flow CD4 counting techniques.

**Conclusions:** Accurate CD4 counts can be generated at minimal cost on red diode laser operated flow cytometers while retaining the potential for high throughput capacity without compromising precision. With further improvements in volumetric technology and clinical software, these cytometers may develop into prototypes for a new generation of inexpensive battery-operated laboratory hardware that combines cellular phenotyping and bead-based multiplexing technology for HIV serology

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Flow cytometers have reached a high level of sophistication, with the most complex instruments used in research employing three lasers for illumination and capable of discriminating as many as 11 fluorochromes (1). This task cannot be accomplished without the use of fluorescent labels emitting in the far red and near infrared spectral regions (2). It has been known for some time (3-5) that such labels can be excited by low-power light sources such as helium-neon (He-Ne) and diode lasers, which have been made available as supplements to the 488 nm argon

ion lasers in benchtop flow cytometers. Such apparatus, however, remains considerably heavier, more power-hungry, and higher in cost than would be practical for clinical use in resource-poor environments (and perhaps in some areas of the developed world in which power failures are becoming increasingly common).

Measuring CD4 T cell counts under adverse conditions is particularly relevant in dealing with the HIV epidemic throughout the developing world, since both the appropriate use of anti-retroviral therapy (ART) and the success of clinical vaccination procedures are critically dependent upon the availability of CD4 counts (6, 7). In this paper we demonstrate that newly developed gating strategies for accurate T cell counting (8, 9) in combination with the new generation of flow cytometers which use a red

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