AFFORDABLE HIV MONITORING USING AUTOMATED FLUORESCENCE MICROSCOPE

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Summary
We have developed an affordable battery operated instrument for CD4 and CD8 enumeration. Target cells are immunomagnetically selected and immunofluorescently labeled. Counting of target cells takes place in an easy-to-handle automated image cytometer. The whole method is dedicated to monitor HIV of both adults and children in resource-poor settings.

Method
Reagents, which contain CD3 conjugated magnetic particles and CD4-PE and CD8-PerCP immunofluorescent labeling, are added to whole blood. After 15 minutes of reagents incubation the sample chamber is inserted into a magnetic holder (Magnest™). CD3⁺CD4⁺ and CD3⁺CD8⁺ populations are brought to an imaging surface. Excited by LEDs the emission of CD4 and CD8 immunofluorescent labeled cells is filtered respectively by PE and PerCP emission filters and captured by a CCD camera. The image consists of a dark background with high intensity events for PE and PerCP labeled cells. Cells are selected and counted based on intensity and size using dedicated image analysis software.

Instrumentation
The image cytometer is in principle an automated fluorescence microscope. It incorporates a small 650MHz Single Board Computer and 8.4” TFT-LCD display, four high power 493nm 3 Watt LEDs for excitation, a 10x achromatic and planar objective, 550nm SP excitation filters, 595nm and 695nm BP filter for collecting respectively PE and PerCP emission. The images are captured using a 16-bit CCD camera. Enumeration of cells is done automatically using a software shell that includes image analysis. The number of cells per µl is displayed within less than 1 minute after starting the measurement and results are stored on hard disk.

Results and conclusion
CD4 counts obtained by our method show good correlation (R=0.97) compared with flow cytometer results for specimens of 63 HIV+ patients. The instrument is battery operated, automated, portable, easy-to-handle and can be produced for only €3500 Euro (single unit price), which is far less than conventional cell enumerating instruments and therefore, being very suitable for resource-poor settings.

References