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Optical tracking and detection of immunomagnetically selected and aligned cells

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We have developed a platform for cell analysis based on immunomagnetic selection and magnetic alignment of cells in combination with an epi-illumination tracking and detection system. Whole blood was labeled with ferromagnetic nanoparticles and fluorescent probes, and placed in a magnetic field in a chamber. Cells labeled with ferromagnetic nanoparticles moved upward and aligned along ferromagnetic lines deposited by lithographic techniques on an optically transparent surface of the chamber. An epi-illumination system using a 635 nm laser diode as a light source scanned the lines and measured signals obtained from the aligned cells. The cell counts per unit of blood volume obtained with the system correlated well with those obtained from the counts from a standard hematology analyzer and flow cytometer. The cell analysis platform is significantly less complex and more sensitive than current cell analysis equipment and provides additional functionality through its ability to subject the cells to repeated and varied analyses while they remain in a natural environment (i.e., whole blood).

Keywords: cytometry, cell analysis, compact disk player, ferrofluids, ferromagnetic nanoparticles

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Evaluation of the cellular composition of blood is a critical assessment for determining a patient's health and diagnosing disease. For routine examination, the differentiation of cells based on the size and the shape of the nucleus, cytoplasm, granules, and nucleoli using a microscope has been replaced by automated cell counters. These devices use impedance, light scatter, and/or absorption measurements of cells passing with high speed through a measurement orifice[1,2]. Although the automated cell counters give fast and accurate results for the most common cell types, in cases with an abnormal cell distribution one has to rely on visual examination of blood cells in smears and/or the immunophenotyping of the cells by flow cytometry. The latter has been the technique of choice for differentiating types of leukemia and monitoring the immunological status of patients.

A drawback of flow systems in general is that individual cells cannot be viewed or examined after the analysis. This disadvantage is overcome in systems in which the cells are deposited on a microscopic slide[3] or disk[4] and scanned by a light source. However, as with flow cytometry, blood still needs extensive preparation before it can be analyzed. Another method of analysis is volumetric capillary cytometry, in which whole blood can be analyzed inside a capillary by scanning a laser across the capillary[5]. With this method, cells can be revisited for further analysis, and the blood does not need extensive preparation. The drawbacks of this system are that the cells of interest are hidden under a layer of randomly distributed red blood cells, and a larger laser spot is used to scan the capillary resulting in a high background, poor signal-to-noise ratio.

Here we present a cell analysis platform that uses a compact disk (CD) tracking and detection system, which differentiates and quantifies fluorescently labeled cells that are immunomagnetically selected and aligned in whole blood. The system is more sensitive than a flow cytometer, the analysis speed is not dependent on cell concentration, and the identified cells can be reexamined with or without changing the medium in which they reside.

Results

Principles of the cell analysis platform. Figure 1 shows a schematic representation of the cell analysis platform. A blood aliquot is incubated with ferromagnetic nanoparticles labeled with monoclonal antibodies. Fluorescence probes are added to differentiate further among the different cell types. During incubation, the cells that express the target surface antigen become covered with ferromagnetic particles and fluorescently stained. The blood mixture is put in a special cell presentation chamber placed between two angular-shaped magnets. The upper wall of the cell chamber is optically transparent, but has ferromagnetic lines of nickel (Ni) deposited by lithography. The spacing between these lines is approximately the diameter of one white blood cell. The external magnets force the magnetically labeled cells upward to the top of the chamber. When they reach the top of the chamber, they become subject to a high local gradient induced by the Ni lines and align between them. The cells that are not magnetically labeled slowly move down under the influence of gravity. In this way, the cells labeled by the immunomagnetic particles are well aligned at the upper surface of the chamber (Fig. 2).

The cells' regular and linear distribution on the chamber surface allows them to be efficiently identified and counted. For this, light in the shape of an ellipse from a laser diode is focused on the chamber surface through an objective lens from an ordinary CD player. By moving the cell chamber in the y-direction, the cells pass under the laser one after the other. To maintain optimum illumination and provide position information, a feedback system was developed that keeps the laser focused on a line of cells while the chamber itself is moved. The fluorescent light emitted by the cells in between the Ni