A microfluidic cytometer exploiting a spatially modulated fluorescence signal to simultaneously measure fluorescent intensity and size on a single cell level

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Abstract

For conventional flow cytometry, cell properties are derived from their response on short laser pulse excitations of about 10μs. The cell excitation within such devices is realized by a narrow laser spot causing high alignment effort and complex imaging optics which makes miniaturization of the apparatus difficult. We present a method, which replaces the single pulse by an extended excitation area resulting in a time modulated signal for every cell in the order of milliseconds. Cells in a microfluidic channel pass through an excitation area and the fluorescence signal is modulated as the cells pass by a spatially patterned shadow mask on top of the channel. In this way the detector records a fluorescent signal from each cell which is modulated in time according to the binary information of the pattern. Furthermore, simultaneous multiple cell passages can be resolved down to a distance of one cell diameter using standard correlation techniques. The enlarged excitation area mitigates the need to precisely align the excitation laser spot and the cell stream. With this technique significantly more fluorescent light of each cell is collected in comparison to conventional flow cytometry and offers an improved sensitivity. The spatial pattern of the shadow mask is the key feature which enables the precise determination of a cells position in the fluid stream whithout optical components such as lenses. As the technique does not require optical imaging it is perfectly suited for miniaturization and implementation in microfluidic systems. Conventional flow cytometry uses scattered excitation light to gain integral information on cell morphology. In contrast we describe a method to measure the intensity and cell size directly from the fluorescence signal. As a consequence the detector for the scatter signal is rendered unnecessary, resulting in reduced system complexity. In summary, we present a new approach, which overcomes alignment issues, has no need for imaging optics and requires a single fluorescence detector which is capable of measuring fluorescence intensity and cell size. It enables the development of a simplified and miniaturized flow cytometer.

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