LASER-SCANNING FLOW CYTOMETER WITH A THREE-DIMENSIONAL MICROFLUIDIC CHIP

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ABSTRACT

We have developed a new flow cytometer utilizing a three-dimensional (3D) microfluidic disposable plastic chip and real-time multi-spectral deconvolution analysis. The chip has microfluidic channels where sample particles flow down in the center of each channel. Three laser diodes (LDs) are used, of which the laser beams are scanned with a polygonal mirror, and only the centers of the microfluidic channels are irradiated with pulsed flashes. Emitted fluorescence light is separated in spectral distribution with a grating and is detected with a 32-arrayed photomultiplier tube (PMT). The spectral signal is deconvoluted with reference spectra of all fluorescent components.

KEYWORDS: Scanning Flow Cytometer, Microfluidic Chip, Three-Dimensional Laminar Flow, Spectral Deconvolution

INTRODUCTION

In conventional flow cytometers, only one glass-made flow cell is usually loaded in equipment and biomolecules such as cells are successively analyzed. However once replacing it with a plastic chip, a lot of advantages are expected such as design flexibility, disposability, low cost, low contamination risk, functionalization, and so on. Multiple flow channels can be easily designed on a plastic chip and it permits the flow speed to decrease and the applied pressure to be lower. That is gentle for living cells. One issue in using microfluidic chips is how to drive the sample flow into the center of the microfluidic channel to keep the sample flow speed constant. That could be settled with three-dimensional sample focusing with a novel orthogonal flipped channel.

On the other hand, an increased demand for simultaneously measuring more fluorescence colors and improving fluorescent signal quality to examine biomolecular expression in cells is driving flow cytometry technologies [1-2]. Whereas it is another issue how to compensate the fluorescence levels when the number of fluorescence colors is large. No more manual compensation could be done by an operator and accuracy of analysis would become lower. That could be also solved with spectral detection of the fluorescence with a PMT array and deconvolution into all the spectrum components.

EXPERIMENT AND RESULT

We used the structure shown in Fig.1(a) to stabilize the position and the velocity of the cells in the microfluidic channel (W100μm x D100μm). The stream of cells is hydrodynamically focused by two sheaths in the microfluidic channel. After the first sheath, cells in the channel are distributed perpendicular to the chip surface. The channel is redirected at point A 90 degrees horizontally, then again at point B 90 de-

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grees from horizontal to vertical direction and simultaneously 90 degrees from vertical to horizontal direction, and then the cells are distributed parallel to the chip surface. Then the channel is focused by the second sheath and the stream of cells is focused in the center of the channel. Figure 1(b) shows a 3D hydrodynamically focusing when a rhodamine B dye was used as a sample. The sample stream was experimentally confirmed to be focused three dimensionally, and controlled by the sheath flow. Then, as is shown in Fig.2, we fabricated a microfluidic chip by mechanical process, which performs 3D focused streams in ten channels. The sample stream in each ten channel was observed to be focused three dimensionally.
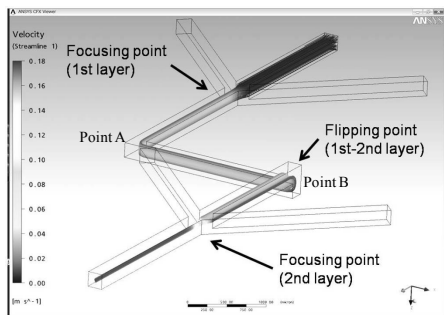

Figure 1(a). Schematic of the three-dimensional hydrodynamically focusing design that was simulated.
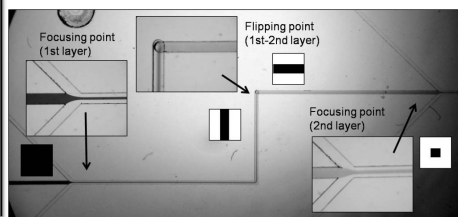
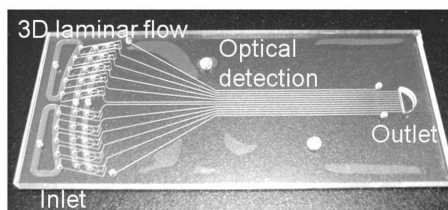Figure 1(b). 3D observation of flow using a rhodamine B dye solution.

Figure 2. 3D microfluidic chip with multi-channels.

With the multi-channel microfluidic chip, laser beams are scanned over the channels and all the sample streams are analyzed simultaneously. A schematic view of the optical setup of the new cytometer is shown in Fig.3. Three LD beams of 405nm, 473nm, 658nm wavelength are scanned at 20 kHz with a 24-face polygonal mirror in the speed of 50,000rpm. Then the parallel microfluidic channels that sample particles are passing through are exposed by the three LD beams with a 7mm-field objective lens. The beam spot sizes are approximately 50μm and aligned at about 100μm intervals to minimize cross-talk signals. The LD beams are quickly flashed and only the centers of the 100μm-wide channels are excited so as not to pick up the scattering signal from the channel edges, so the side scattering signal from a single particle is easily detected. Fluorescence emission is collected through a 0.4NA objective lens, broken into a spectrum by a blazed grating, and quantified by a 32-arrayed PMT. The spectral range is from 400nm to 800nm. A 10μm cell would be

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scanned and averaged from 4 to 33 times, for example, depending on the stream velocity.

As for measuring a lot of fluorescence colors, we adopted a grating for spectral separation and a 32-arrayed PMT for detection. Through deconvolution of detected fluorescence into composed fluorescence spectra, the ratio of the fluorescence molecules can be analyzed without any compensating operation. Using multi-wavelength excitation source enables to increase fluorescence color variation. The data acquisition-system employs low-noise 16-bit analog-digital converters (ADCs) and the obtained spectral and scattering data are calculated at high speed with a Field Programmable Gate Array (FPGA) to deconvolute overlapping emission spectra. Then all the fluorescence intensity levels can be obtained without compensation process. In order to confirm FPGA calculation, calibration particles for flow cytometry (Ultra Rainbow: Bangs Laboratories, Inc.) were used instead of cells. Using five fluorescent component dyes such as UV, Yellow, Nile Red, Purple and Sky Blue, and other two background factors such as amplifier offset and system self-emission, real-time deconvolution was applied to the spectrum of the calibration particles. Figure 4 shows the Ultra Rainbow spectrum before deconvolution and the spectrum of all the used dyes plus amplifier offset and system self-emission. As a result, the reconstructed spectrum was in good agreement with the original fluorescent spectrum of Ultra Rainbow.
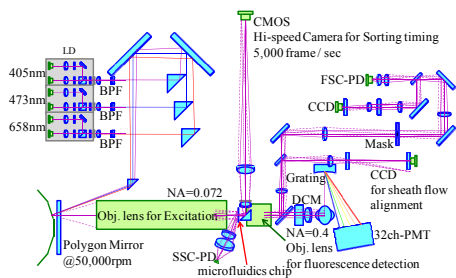

Figure 3. Schematic view of the optical setup for a laser scanning multi-spectral flow cytometry.
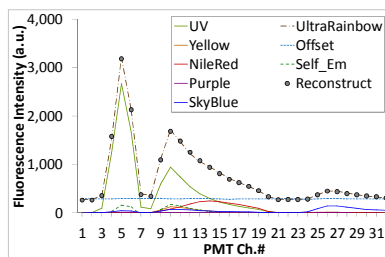

Figure 4. The spectrum of an Ultra Rainbow calibration particle and the reconstructed one and other component spectra.

CONCLUSION

Three dimensional microfluidic chip enabled sample stream to stabilize in the center of the channel. Sample streams in a multi-channel microfluidic chip were irradiated with scanning laser pulses and the detected fluorescence spectrum was separated into all the component spectra by automatic deconvolution and original spectrum was confirmed to be reconstructed with high accuracy.

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