HIGH-THROUGHPUT IMAGING FLOW CYTOMETRY AT 10,000 CELLS/SECOND

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Fluorescence imaging flow cytometry (FIFC) is an emerging method for analyzing a population of cells with high accuracy over conventional flow cytometry by virtue of its imaging capability, but its cell throughput (~1,000 cells/s) is much lower than that of standard non-imaging flow cytometers due to the use of a CCD image sensor having a limited pixel data rate (up to ~100 MHz), making it difficult to analyze a large population of cells [1]. To overcome this limitation, we present two different approaches of high-throughput imaging flow cytometry, both of which reach a throughput of 10,000 cells/sec. The first approach is to use a high-speed single-pixel photodetector for image acquisition (Fig. 1). Specifically, we employed frequency-division-multiplexed confocal fluorescence microscopy to FIFC, enabling an effective pixel data rate of 800 MHz [2]. With this approach (confocal FIFC), we obtained images of mouse lymphocytes (the bright-field and the fluorescence of the nucleus stained by SYTO16) in a 1-m/s flow (corresponding to a throughput of 10,000 cells/s) (Fig. 2), demonstrating the feasibility of the approach. The second approach is to use a high-speed camera such as a scientific CMOS (sCMOS) camera for the image acquisition (Fig. 1). Since the high-throughput FIFC essentially suffers from the low imaging sensitivity due to the shorter exposure time, we developed a mirror-embedded microfluidic chip that enabled efficient excitation of fluorescent probes by a light-sheet excitation beam on the chip. With this approach (light-sheet FIFC), we obtained fluorescence images of MCF-7 cells (the cytoplasm stained by CellTracker Red and the nucleus stained by SYTO16) in a 1-m/s flow using a sCMOS camera having a pixel data rate of 572 MHz (Fig. 2).

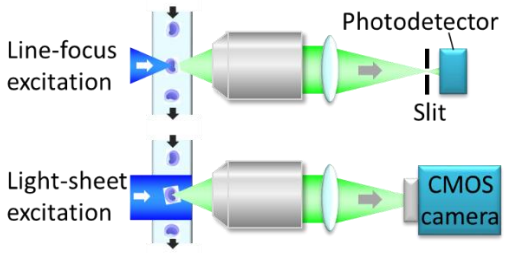

Fig. 1: Schematics of the confocal FIFC (top) and the light-sheet FIFC (bottom).

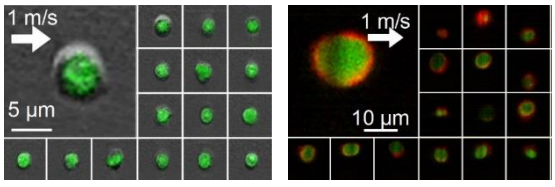

Fig. 2: Images of lymphocytes obtained by the confocal FIFC (left) and MCF-7 cells (right) obtained by the light-sheet FIFC..

[1] N. S. Barteneva and I. A. Vorobjev eds., *Imaging Flow Cytometry* (Springer, 2016)
[2] H. Mikami, *et al*., Optica (2018), in press.