On-chip flow cytometry: where is it now and where is it going?

"*There are increasing needs for portable, low-cost and maintenance-free flow cytometry platforms for disease diagnosis and monitoring in resource-limited environments, such as developing countries and first-line clinical offices.*"

KEYWORDS: FACS ■ flow cytometry ■ fluorescence-activated cell sorting ■ microfabrication ■ microfluidics

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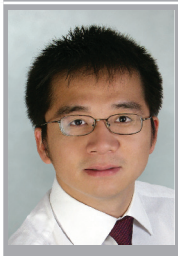

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Flow cytometry is a powerful, high-throughput, single-cell characterization and sorting tool, which has a unique capability to provide fast and quantitative analyses of individual cells and physical separation of cells of particular interest from other cells [1]. In flow cytometry, the cell analyses are performed by passing a narrow stream of cells through a focused laser beam at a rate of thousands of cells per second. Information regarding the size, type and content of cells can subsequently be derived through the analyses of the excited fluorescence emission or scattered light arising from each individual cell. In recent years, flow cytometry has rapidly become an indispensable instrument for many clinical diagnostics, ranging from routine blood tests to diagnoses of lethal diseases such as leukemia, respiratory infection and HIV/AIDS. Flow cytometry can screen multiple parameters and can be used for immunophenotyping. This has allowed diagnosis of diseases associated with B lymphocytes [1] and progress in the Human Immunology Project [2]. HIV is also typically monitored using flow cytometry [1]. Additionally, cell-sorting techniques have enabled flow cytometry to become a key tool for studying circulating tumor cells, which have potential in screening for and monitoring cancer [3,4].

With its extraordinary single-cell analysis and sorting capabilities, flow cytometry has been used extensively in numerous fields such as molecular biology, pathology, immunology, plant biology and marine biology. Despite their significant impact, current commercial flow cytometry systems have the following drawbacks: high cost, large instrumentation size and the constant requirement of highly trained personnel for maintenance (the latter two resulting from its complex system configuration). A typical flow cytometer with cell-sorting function costs US$200,000–1,000,000. Most cell analysis/sorting tests are typically performed at well-funded, centralized share-facility laboratories and hospitals due to the economic reasons. There are increasing needs for portable, low-cost and maintenance-free flow cytometry platforms for disease diagnosis and monitoring in resource-limited environments, such as developing countries and first-line clinical offices. In this editorial, we will discuss the current efforts on microfluidic-based miniaturized flow cytometry, so-called 'flow-cytometry-on-a-chip', and our perspectives for future developments.

Overview of flow cytometry

A typical flow cytometry system includes three major components [5–7]: a fluidic subsystem to three-dimensionally focus the stream of biological cells; an optical subsystem to detect fluorescence emission and scattered light arising from individual cells; and a cell-sorting subsystem to separate cells of interest from other cells. The stunning capability of flow cytometry is enabled with the seamless integration of these three individual modules.

The fluidic subsystem of flow cytometry carries an inner sample flow (cell suspension) and an outer sheath flow (water or buffer). The outer sheath flow is injected at a much higher flow rate than the inner sample flow to create the so-called 'hydrodynamic focusing' to position cells in the core of the flow. This configuration ensures that cells are passing through the laser beam one at a time and at the same speed. The alignment of the hydrodynamically focused cell stream with the laser ensures identical interrogation conditions for each individual cell, to improve the fidelity and accuracy of the

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detection. The fluidic subsystem will also require a fluid-pumping mechanism that transports a sample of cells through all of the other subsystems. Traditionally, flow cytometers used pressure-based pumps; however, more recently, peristaltic pumps and syringe pumps have been utilized. The fluidic subsystem is crucial as it increases the consistency and reliability of the detected signals.

> "*It might be necessary to change the approaches applied to microfluidic flow cytometry.*"

The optical subsystem of flow cytometry uses a series of lenses and mirrors to align the interrogation laser beam with the cell stream and to perform multiparametric optical measurements for different light signals from individual cells including forward scattering, side scattering and multiple fluorescence emissions. Optics are typically the most expensive components in the flow cytometer due to expensive materials, precision machining, polishing, alignment and coupling. These optical components are sensitive to impact, shock and vibrations.

The cell-sorting subsystem is essentially a mechanism to deflect the hydrodynamically focused cells into different collectors. Sorting is not available on all flow cytometers as it is typically a research tool, only necessary when cells require postprocessing.

The cost and price of flow cytometers is no surprise when one considers the complexity of the subsystems mentioned above. Therefore, in order to reduce the size and cost of a flow cytometry system, innovations must emerge to:

- Reduce the size and cost of each subsystem;
- Reduce the consumption of sheath fluid;
- Simplify the fabrication of the flow cell;
- Reduce the number and complexity of optical components;
- Simplify optical alignment;
- Simplify the components for cell sorting;
- Integrate the subsystems.

Current progress on microfluidic-based, on-chip flow cytometry

The majority of research towards the miniaturization of flow cytometry has been on disposable microfluidic flow cells, which reduce the cost of manufacturing, reduce the amount of sheath flow consumed and allow integration with other microfluidic elements for processing of samples. The key requirement of a microfluidic flow cell is the focusing of the cells, which has been accomplished via hydrodynamics [8], acoustics [9,10] and dielectrophoresis [11].

Innovations in optics are important for reducing the size and cost of flow cytometers. A device from Honeywell Laboratories (MN, USA) relaxed the optical alignment process by using an array of lasers and detectors; however, this process requires a calibration step each time a new microfluidic chip is installed [12]. Optical alignment in microfluidic flow cytometers has been relaxed by aligning optical fibers using fiber insertion channels [13] or waveguides [14]. Fabricating all components on a single chip could drastically reduce the size and cost of the device. Such systems have been produced using integrated, complementary metal oxide semiconductor compatible lasers, microfluidic channels integrated with diode lasers on a gallium arsenide substrate and SU8 microfluidics on silicone [15]. These feats of integration are excellent examples illuminating the possibility of miniaturized flow cytometry.

> "*The current challenge … is to integrate these systems and realize fully integrated on-chip flow cytometry.*"

Many on-chip microfluidic pumps have been designed; however, it remains difficult to integrate such pumping systems. Typical integrated fluidic pumps are realized using compressed air systems [12,16], which are usually rather bulky. Cell sorting has been performed using valves, optoelectronic tweezers, electrical charging, magnetic tagging, optical tweezers [17], dielectrophoresis [18] and acoustics [19,20]. Several of these systems have been integrated with detection and feedback systems to produce activated cell sorting. For a portable flow cytometry system, the sorting methods should easily integrate with the system and consume little power.

Future perspective

A number of innovative technologies have been integrated with microfluidics to create on-chip flow cytometry systems; however, the ability of such techniques to truly reduce the size and cost of the overall flow cytometry system remains unproven. Many so-called on-chip methods rely on large, expensive external support systems. For instance, on-chip hydrodynamic

focusing requires several high-precision syringe pumps; optical sorting requires high-powered external lasers; and compressed-air-based valves for pumping and sorting require an external compressed air source. Commercial systems based on these on-chip techniques may not have significant advantages over existing systems in terms of size and cost. Systems using acoustics or dielectrophoresis have shown great potential for focusing and sorting cells without the need for large external systems; however, the performance (e.g., throughput) of such systems needs to be further characterized and improved.

It might be necessary to change the approaches applied to microfluidic flow cytometry. One approach to consider would be to use on-chip systems to reduce the cost (through microfabrication) and increase the performance of flow cytometry systems (through new innovations), but disregard the idea that these systems will reduce the size of the device. An alternate approach would be to focus on low-cost, miniaturized systems with performance slightly lower than existing commercial systems. Such systems could be considered 'smart cytometers' analogous to a smart phone, a miniaturized portable computer with limited functionality. Finally, reducing the size and cost of the external supporting systems needed to facilitate on-chip flow cytometry, and integrating such systems on-chip, could also lead to an overall cheaper and smaller system. Multidisciplinary teams would be needed to facilitate such technical innovations and integration. In our opinion, future research and development of miniaturized flow cytometry systems should consider the following aspects:

- Reducing the need for external supporting systems;
- Methods for reduced consumption of sheath fluids;
- Methods for integrating optics and pumping systems on-chip;
- Simpler, more effective, and more compact cell-sorting mechanisms;
- A focus on one main advantage, such as decreasing cost, decreasing size or increasing performance, but not necessarily all in one system.

In summary, thus far microfluidic-based approaches have addressed most of the critical systems necessary to create on-chip flow cytometry. The current challenge left to researchers in this area is to integrate these systems and realize fully integrated on-chip flow cytometry. The current work in this field is moving in this direction and increased innovations in microfluidic technology could make such a dream a reality.

Financial & competing interests disclosure

MI Lapsley is currently employed as a product application scientist for a medical diagnostic company called QBC Diagnostics. L Wang and TJ Huang are cofounders for Ascent Bio-Nano Technologies, Inc., a start-up company specializing in miniaturized flow cytometry. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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