Optofluidic flow cytometer with in-plane spherical mirror for signals enhancement

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Abstract. Statistical analysis of properties of single microparticles, such as cells, bacteria or plastic slivers, has attracted increasing interest in recent years. In this field flow cytometry is considered the gold standard technique, but commercially available instruments are bulky, expensive, and not suitable for use in Point-of-Care (PoC) testing. Microfluidic flow cytometers, on the other hand, are small, cheap and can be used for on-site analysis. However, in order to detect small particles, they require complex geometries and the aid of external optical components. To overcome these limitations here we present an opto-fluidic flow cytometer with an integrated 3D in-plane spherical mirror for enhanced optical signal collection. As result the signal-to-noise ratio is increased by a factor of 6, enabling the detection of particle sizes down to 1.5µm. The proposed optofluidic detection scheme allows the simultaneous collection of particles fluorescence and scattering - using a single optical fiber - which is crucial to easily distinguish particle populations with different optical properties. The devices have been fully characterized using fluorescent polystyrene beads of different sizes. As a proof of concept for potential real-world applications, signals from fluorescent HEK cells and *Escherichia coli* bacteria were analyzed.

1 Introduction

The ability to discern the composition of a fluid and thus real-time monitoring of elements that may pose harm to humans is subjects of intense interest. Drinking water contaminated by bacteria, viruses, and parasites, if not readily identified, can cause a range of health problems. In fact, it's widely recognized that diseases stemming from *Escherichia coli (E. coli)* in water causes hundreds of thousands of illnesses annually[1], and is estimated to be the second leading cause of death in children under five years of age[2]. At the same time, the ability to analyze single cells up to large numbers within body fluids allows a wide range of scientific investigations to be carried out, including studying the effects of the same marker or pollutant on their physical and chemical properties. In addition, huge amounts of plastic are known to enter the oceans every year, and as they decompose, even very small debris (microplastics) can be harmful to wildlife, and humans. To date, the effects on cells of ingesting these microplastics, have not yet been studied in detail[3].

Flow cytometry is a commonly used powerful and quantitative method for analyzing the properties of individual elements of a population mixed in a given fluid. It has a wide range of applications, including the diagnosis of blood cancers, DNA sequencing and the cells detection, to the extent that it is considered as the gold standard for the statistical characterization of the biochemical and biophysical properties of individual cells/particles. It is complementary to imaging; although it does not provide the same level of cell detail, it offers several unique advantages, allowing multi-parametric, rapid and semi-quantitative analysis of cell populations (even heterogeneous ones) at the single cell level. Since the early days of cell counting, efforts have been made to automate the process to achieve high throughput while maintaining accuracy and providing user-friendly interfaces. In this case, the fluid to be analyzed (and the particles it contains) is placed in a specific area of the instrument and illuminated by a focused laser beam. The fluorescence and scattered light are collected at specific discrete angles and sent to detectors, which convert it into electrical signals for analysis. The amplitude and shape of these electrical signals provide important information about the particle and/or cell. Scattering, due to light deflected from the original direction of propagation of the incident light, can be divided into forward scattering (FSC) and side scattering (SSC), and it provides information about the structure and morphology of the particle. In particular, FSC (low angle of beam deflection) is typically related to the size of the particle, although in a very approximate way as the relationship proves to be unfortunately not monotonic for every application, while

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side SSC (high angle of beam deflection) mainly contains information about the granularity/morphology and internal complexity of the micro-object / cell[4]. Fluorescence is proportional to the amount of fluorophore that enters or attaches externally to the particle, and it can be used to distinguish between different cell populations and even to identify dead or membrane-deficient cells[5]. However, although there are currently instrument able of analysing up to 14 parameters simultaneously commercial flow cytometers present several disadvantages: (i) misalignment of the small focal point of the probing laser with respect to the flowing micrometric object during the run time; (ii) lack of flexibility in rearranging the measurement chamber, which can affect the analysis. Additionally, these laboratory instruments are expensive, complex to use and bulky, handle relatively large (mL) sample volumes and require trained operators for operating and for maintenance. These factors make commercial flow cytometers difficult to use outside laboratories and strongly limit their use in remote and resource-poor areas, making them not suitable for developing countries or for portable point-of-care diagnostics (PoC).

To overcome all these problems, significant efforts have been made to miniaturize benchtop flow cytometers into microfluidic systems. Besides miniaturization, a microfluidic platform typically offers integration, automation, and parallelization of (bio-)chemical processes. As a result, microfluidic flow cytometers have many advantages, the most typical being low fluid consumption (μL-nL), high throughput, improved process control, increased sensitivity and compactness by integrating of many functionalities in a single Lab on a Chip (LoC) of a few mm^2. Improving the accuracy of optofluidic flow cytometers, and so the minimum detectable particle size at high throughput, two main critical factors need to be addressed: the precise control of the position in 3D of the microparticles in the flow and a high signal-to-noise ratio of measurement. To meet these requirements, we report here the fabrication and characterization of a new optofluidic flow cytometer that exploits the integration of an innovative and user-friendly 3D flow cell with integrated in-plane 3D spherical micro-mirror, on the same glass platform, to increase S/N by several units. For smaller particle focusing, a new geometry[6] is designed to allow hydrodynamic focusing using only two inlets, exploiting a simple and fast manufacturing process. The same fabrication tool was used to simultaneously integrate the optical element into the chip avoiding misalignments. The motivation for selecting this smart in-plane optical element is to ensure the collection of useful signals from a wider, non-discrete solid angle, while reducing background noise by placing it as close as possible to the sensing core components. As a result, the obtained signal increase makes it possible to detect flowing particles down to the size of 1.5 microns (including bacteria).

2 Experimental Studies

To fabricate our optofluidic LoC, we used a femtosecond light induced chemical etching (FLICE) fabrication technique. It is a well-known direct writing manufacturing for rapid prototyping of 3D structures in a fused silica substrate[7]. The same tool is used to spherical micro optics fabrication. Therefore, in order to improve the optical reflectivity optical polishing was performed using a CO2 laser. In this way a final roughness below 5 nm was achieved, giving an optical quality to the surface. Finally, the curved surface of the scaffolding to be working as a spherical mirror was coated with a metal-based ink (silver nanoparticles, ANP SilverJet DGP 40LT-15C). The device was tested by individually identifying polystyrene beads of different sizes and morphology from their mixture, fluorescent HEK293T cells and the presence of bacteria in water. In our LoC, scattering and fluorescence are collected from the same fiber output: this simplifies the geometry and facilitates the processing of the two optical signals using simple and widely available integrated electro-optical tools. This makes the device robust and compact, with valuable advantages in terms of portability, sensitivity, and ease of use.

3 Conclusion

A remarkable feature of this device is its high throughput in detecting of cells and bacteria - the latter impossible to detect without the integration of the spherical mirror - at over 4,000 events per second without the need for expensive high frame rate vision systems or time-consuming post-processing analysis.

The possibilities offered by this innovative platform, in particular the easy integration of the optical element with microfluidic technology help to bridge the gap between the macroscopic world and chip-based analysis, paving the way for automated, portable and high-throughput applications capable of detecting small particles below the micron-size, analyzing their different properties at the same time. The S/N enhancement achieved (over 6 times) can be seen as a first step towards using these devices for more challenging applications, such as the detection of microplastics in water samples or the detection of Circulating Tumor Cells in biological samples.

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