A microfluidic scanning flow cytometer with superior signal-to-noise-ratio for label-free characterization of small particles

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Abstract. Single-cell analysis without immune-specific labelling is essential across research fields, but conventional flow cytometers (FCMs) struggle with label-free analysis. We introduce a novel microfluidic scanning flow cytometer (μSFC) designed for label-free analysis within a simple microfluidic chip. Our system outperforms traditional FCMs for label-free analysis but its signal-to-noise ratio (SNR) limits the minimum detectable size. We present three modifications to enhance SNR and improve the smallest detectable particle size: additional neutral optical density filtering, a lower noise-equivalent-power photoreceiver, and laser spot size reduction. These improvements enable reliable characterization of particles as small as 3 μm. Experimental results validate the correlation between angular profile oscillations and particle size. While reliable detection down to 1 μm is achieved, further refinement is needed. The simplicity and low setup of the μSFC make it promising for integration into multi-parametric single-cell analysis systems, facilitating comprehensive cellular characterization for diagnostic and point-of-care applications.

1 μSFC: A Microfluidic scanning flow cytometer

The analysis of biological samples at the single-cell level has become essential for many research fields. In particular, the label-free analysis of cells, i.e. their characterization without any immune-specific labelling, holds significant potential for diagnostic and point-of-care (POC) applications [1]. Flow cytometry represents the gold standard for single cell analysis, but conventional flow cytometers (FCM) heavily rely on fluorescent labels and have poor performance when used for label-free analysis [2]. Scanning flow cytometers (SFC) are a class of FCM which can achieve better label-free performance

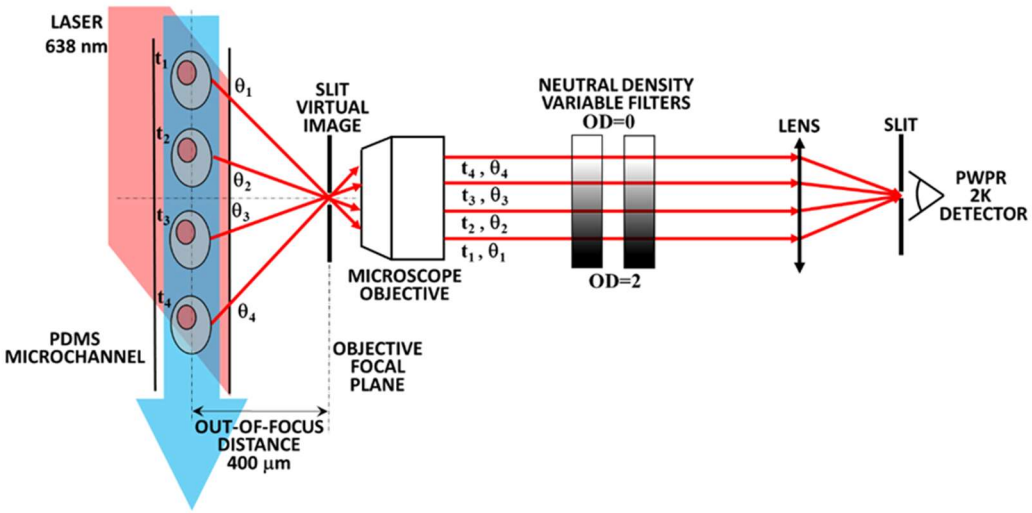

Fig. 1. Schematic drawing of the microfluidic scanning flow cytometry set the system. A cylindrical telescope (not shown) expands the excitation laser beam which is focused by a cylindrical lens (not shown) with a 40° angle onto the microfluidic channel, which lies on a plane 400 μm apart from the focal plane of a microscope objective. Light scattered by flowing particles is collected by the objective and focused by a lens onto a slit placed in front of a low-noise detector. The virtual image of the slit selects a different subset of scattering angles at each position along particle trajectories. Two filters with linearly variable optical density located in the back-focal plane of the objective reduce the dynamic range and increase the signal-to-noise-ratio of the measurement.

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by measuring the light scattered by particles at different angles [3], but require custom components which make them unsuitable for POC applications.

We recently proposed a novel microfluidic scanning flow cytometer (μSFC) to measure the angle-resolved scattering profile of cells within a simple polydimethylsiloxane microfluidic chip [4]. The optical apparatus of the system (Fig.1) was formed by a microscope objective (40x) which collected the scattered light and a lens to focus the light onto a slit placed in front of a photoreceiver. The microfluidic channel was 400 μm out-of-focus allowing different scattering angles to reach the detector at different times. A filter with linearly variable optical density was placed in the back-focal plane of the objective, leading to a reduction in the dynamic range of the signal and higher signal-to-noise-ratio. The μSFC outperformed conventional FCM for label-free characterization of polymeric particles, and its applicability on a biological target was demonstrated. Nevertheless, the smallest detectable particle is dictated by the SNR of the system and was ~9 μm.

2 Strategies for improved signal-to-noise-ratio.

Here, we demonstrate that it's possible to significantly increase the SNR of the μSFC and thus decrease the smallest detectable particle size with three setup modifications. First, an additional linearly variable optical density filter is added in series with the previous one, resulting in further signal linearization and dynamic range reduction. Second the OE-200-SI photoreceiver (Femto) is replaced with a PWPR-2K photoreceiver (Femto) with lower noise-equivalent-power (8 vs 240 fW/√Hz). Lastly, a cylindrical telescope is used to achieve a 3x expansion of the excitation laser, reducing the size of the focused spot down to 30 μm and thus reducing the background scattering noise. The new configuration was tested on polymeric beads with different size and refractive index (Tbl.1), and was able to reliably characterize polymeric particles with diameters 3 μm, previously below the limit of detection (Fig.2).

The frequency of the oscillations in the angular profile, f_θ, obtained using a Fast Fourier Transform of the signal correlates with particle size. A linear regression can be used to correlate measured f_θ with nominal beads diameters, resulting in an R^2=0.99 for the best fit, and an R^2=0.95 for the theoretical Mie model (Fig.3). This system can also detect beads with diameter 1 μm, but f_θ is too low to be reliably estimated with the current angular window.

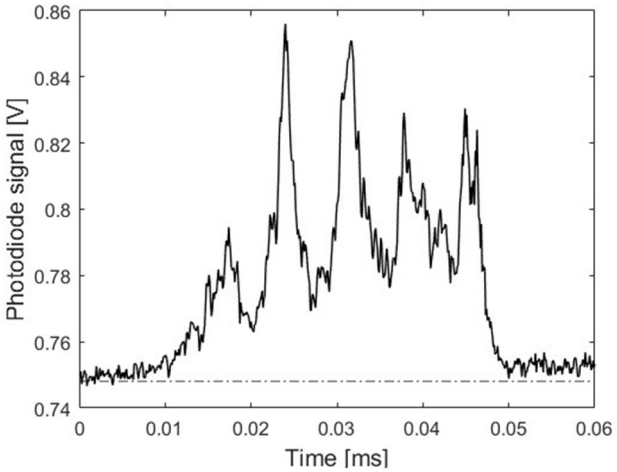

Fig. 2. Angle-resolved scattering signal for a 3 μm Polystyrene bead.

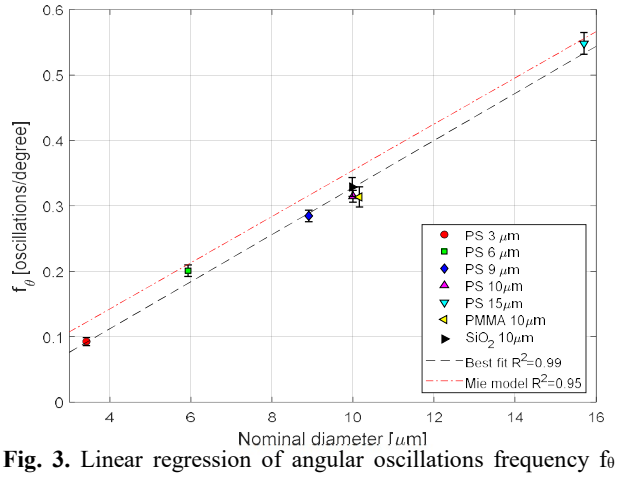

Fig. 3. Linear regression of angular oscillations frequency f_θ with nominal beads diameter. Markers represent distribution means and error-bars represent one standard deviation. Black dashed line indicate the best-fit linear regression (R^2=0.99, a=0.036, b=-0.318). The red dotted line represents the regression with the theoretical Mie model (R^2=0.95).

3 Conclusions.

Thanks to its simplicity and low setup requirements, the μSFC holds potential for integration within other single-cell analysis systems to achieve a multi-parametric cell analysis, facilitating comprehensive cellular characterization. This advancement holds significant potential for various diagnostic and POC applications in biomedicine and beyond.

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