SERIAL MICROFLUIDIC CYTOMETRY WITH INERTIAL AND HYDRODYNAMIC FLOW FOCUSING

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ABSTRACT

Microfluidics are increasingly used to develop flow cytometers with novel functionalities. Although various approaches exist to control particle positioning within microfluidics, the magnitude of and mechanisms leading to measurement uncertainties that arise from particle positioning within microfluidic channels remains an open question. Here, we report a novel combination of 3D-hydrodynamic and inertial focusing and demonstrate particle confinement, velocimetry, and quadruple replicate fluorescence measurements in a serial cytometer. Compared to inertial focusing alone, this combined focusing approach demonstrated lower particle velocity coefficient of variation (CV) (0.3 %), particle population fluorescence CV (9.4 %), and replicate measurement CVs (1.4 %).

KEYWORDS: Flow Cytometry, Microfluidics, Optofluidics, Reproducibility

INTRODUCTION

Flow cytometry is a primary tool for characterizing heterogeneous cell populations. Nonetheless, traditional cytometers are limited to a single measurement event per object and thus cannot assess the measurement uncertainty of each object. Recently, we reported an optofluidic serial cytometer that was capable of quadruple fluorescence measurements but was limited in throughput by particle velocities, which were below 1 mm s^{-1} [1]. The current work improves the consistency of the laser induced fluorescence by adding laser collimators and a combined inertial (IF) and 3D hydrodynamic focuser (3DHDF) to the design. By focusing to a single inertial node, serial measurements were recorded with a precision of 1.4 % at particle velocities above 0.3 m s^{-1}.

EXPERIMENTAL

(DISCLAIMER: Identification of commercial products does not imply recommendation or endorsement by NIST. The materials and equipment used may not necessarily be best for purpose.) Two-layer microfluidic devices were fabricated from poly(dimethylsiloxane) using soft lithography as reported [1], [2]. Combined IF&3DHDF utilized flow rates of (1, 4, 4, 10, and 40) µL min^{-1} for the sample stream, lower sheath, upper sheath, left sheath, and right sheath, respectively (See Figs. 1, 2A). The IF only control consisted of 59 µL min^{-1} sample flow. Four-channel digitized intensity spectra from photomultiplier tubes (Hamamatsu H11903-20) were recorded at a temporal resolution of 500 ns. Green fluorescent microspheres (15 µm diameter) were used for all experiments.

RESULTS AND DISCUSSION

The microfluidic serial cytometer performed four measurements of particle fluorescence: two replicate measurements per excitation, reproduced at two laser excitation regions spaced 1.2 cm apart (Fig. 1). When particles flowed at a particle-based Reynolds number $Re_p \approx 1.3$ within the 40 µm wide × 80 µm tall channels, they focused to two spatial nodes with maximum fluorescent intensities displaced approximately 9 µm from the centerline (Figs. 2B,C). This phenomenon is consistent with inertial focusing (IF) effects at similar aspect ratios and Re_p [3], and could lead to measurement variation due to varying the geometry between the laser, particle, and detector. To eliminate one inertial node and accelerate inertial equilibrium, particles were introduced at $Re_p \ll 1$ and then surrounded by a 3D water sheath biased 1:4 towards one side of the channel at the onset of $Re_p \approx 1.3$ flow (Fig. 2A). As characterized by microscopy image analysis, the focusing approach isolated microbeads to one node with a purity of 100 %, and variations in their positions were near the resolution limit of 0.23 µm per camera pixel (Fig. 2C).

Serial fluorescence and velocimetry measurements were obtained and compared between IF&3DHDF or Re_p-matched IF conditions to characterize the effect of the focusing approach. Measurements were recorded from 1536 green fluorescent microspheres over 3.9 minutes under IF&3DHDF and from 1475 particles over 2.4 minutes under IF. The four serial measurements for each particle were matched on the basis of anticipated time-of-flight, providing an analysis yield exceeding 97 % and 87 % for IF&3DHDF and IF conditions, respectively. Coefficient of variations (CVs) of integrated fluorescence intensity measurements generally decreased compared to the IF control (Fig. 3A). Particle velocity CVs decreased from 0.7 % to 0.3 % for IF and IF&3DHDF, respectively. After combining all 4 replicates, the population CV was 9.4 % with a precision (median of the replicate CV) of 1.4 % (Fig. 3B).

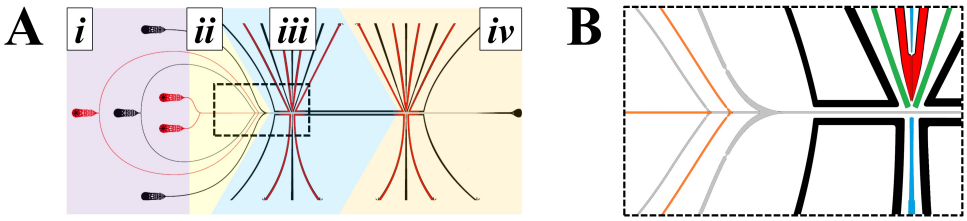

Figure 1: Microfluidic serial flow cytometer schematic. A) Device overview: i, inlets and particle traps; ii, flow focusing region; iii, measurement region 1; iv measurement region 2. B) Expanded view from the dashed area from A). Orange and gray: fluidic microchannels; black: light blocks; blue: waveguides; green: detection waveguides; red: light collimator.

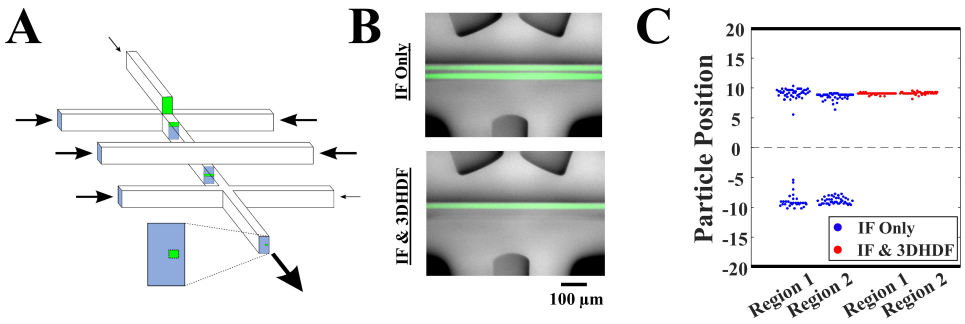

Figure 2: Inertial focusing (IF) and 3D hydrodynamic focusing (3DHDF) results. A) Principle of IF&3DHDF approach. B) Widefield composite microscopy images of single-particle streamlines. Green: particle fluorescence; Grayscale: bright-field. C) Distribution of particle positions. Dashed line: channel centerline; Solid lines: channel edges.

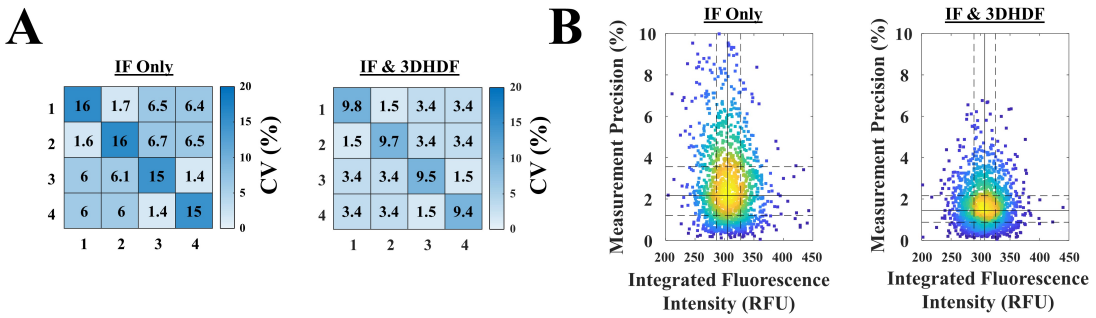

Figure 3: Integrated fluorescence area measurement precision. A) Heatmaps showing the pairwise comparison of CVs of integrated fluorescence intensities for the four replicated measurements. Diagonal: CVs of fluorescence intensities within one replicate. B) Scatter plots of measurements with associated measurement precision. Each point represents the mean fluorescence measurement from four replicates (x-axis) and the CV of those replicates (y-axis). Points are colored from blue to yellow in increasing density. Solid lines: median for each dimension; dashed lines: upper and lower quartiles.

CONCLUSION

Particle focusing methods and operating parameters can influence both the position and velocity of particles in flow cytometers, which impacts measurement repeatability. A serial cytometer provides a novel approach to directly observe measurement variations, enabling dissection of individual contributors to system uncertainty, thus elucidating paths towards maximizing measurement precision. In the future, the functionality of the serial cytometer can be extended by adding multicolor fluorescence and scattered light measurements. In addition, a systems control approach could be developed to adjust operating conditions on-the-fly to maximize precision over time.

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