

# MATCHING AND COMPARING OBJECTS IN A SERIAL CYTOMETER

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## ABSTRACT

Flow cytometers are indispensable for clinical studies yet are limited by inherent uncertainties. We have developed an optofluidic device capable of multiple measurements along a microfluidic channel, whereby many of the uncertainty components can be systematically evaluated and reduced. This study addresses the challenge associated with identifying and tracking the order of objects throughout their time of transit. An algorithm was developed to characterize objects as they travel. We discuss methods of testing the efficacy of this algorithm, and other tools that allow us to gain more information than is provided by a conventional cytometer.

**KEYWORDS:** Flow Cytometry, Microfluidics, Optofluidics, Reproducibility

## INTRODUCTION

Traditional flow cytometers can measure thousands of cells per second, but the intensity of the fluorescent biomarkers on cells is only measured once and used to calculate scalar values such as integrated area.<sup>1</sup> Thus, cytometers have limited ability to characterize biomarker distributions with high precision, which reduces their capacity to discriminate small changes within a population and to distinguish rare objects. Our group is developing an optofluidic cytometer that increases measurement resolution and precision by repeating measurements four times along the flow path: two axially symmetric waveguides collect fluorescence at each of two measurement regions separated along a microchannel. In order to aggregate the replicate measurements, an algorithm was created in MATLAB (MathWorks) to perform the piecewise temporal alignment across all four data channels. The algorithm evaluates the likelihood of object ordering based on time-of-flight and shape of the fluorescence signal, which includes high-temporal resolution of the intensity as the particle moves through the measurement region. We discuss challenges associated with objects passing one another, scenarios with multiple particles in the measurement region, such as doublets, and the unique opportunities that our high-resolution approach provides in extracting detailed information from objects, which would normally be impossible with other approaches. We also discuss metrics to assess the efficacy of matching and doublet separation.

## 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.) The manufacturing process for these devices has been detailed in a previous publication.<sup>2</sup> Briefly, two poly(dimethylsiloxane) (PDMS) layers with lithographed microchannels were aligned and bonded. The two measurement regions each included one waveguide which transmitted laser light and two symmetrically angled waveguides that collected emitted fluorescence from objects (15  $\mu\text{m}$  diameter microspheres, Bangs Lab FSDG009) passing the laser (Fig. 1A). Fluorescence emission was measured on a photomultiplier tube (Hamamatsu H11903-20) and digitized (National Instruments PCIe-6374).

## RESULTS AND DISCUSSION

Fluorescent microspheres passing through two measurement regions (each with a fluorescence collector angled upstream and downstream of the excitation) created signals as shown in Fig. 1. Calibration of our serial cytometer requires that standard objects be compared between regions to establish a scale factor for normalization. Importantly, this exercise requires unambiguous object matching from one region to another. Object matching was strongly dependent on a consistent particle velocity, which we maintained to within 0.2 % coefficient of variation (CV) using a combination of inertial and hydrodynamic focusing. Coincident events (doublets) impose additional challenges of identification and decoupling. Because our system records an object's intensity both in time and from different perspectives at two different regions (Fig. 1B), a number of methods were tested in MATLAB, including idealized peak fitting, to facilitate individual object resolution and ordering (Fig. 1D, E).

To test algorithm efficiency, a number of *in silico* simulations were performed. Experimental data were lowpass filtered, normalized, temporally aligned, and averaged to form an idealized signal. We then modeled distributions of variables such as particle velocity, time between particles, white noise, and particle intensities from these data.

Lastly, idealized signals were scaled, time-shifted, and had noise added to them to match the modeled distributions of those components, thereby creating simulated data equivalent to that of a mock bead population. Overall, when analyzing artificial signals, our algorithms correctly determined the identity of synthetic events across replicate measurement channels with 100 % tracking efficiency. In extracting integrated fluorescence area from the artificial signals, our algorithms demonstrated a precision of  $\pm 0.04\%$  ( $\pm 1$  standard deviation) compared to the synthetic ground truth. Using hydrodynamic focusing to position particles in the center of the channel, with event rates near what is typically achieved by commercial cytometers (1000 events per second), over 10 % of objects were predicted to pass each other between regions and  $\approx 25\%$  could manifest as doublets (Fig. 1F). Adjusting hydrodynamic focusing to align particles to a single inertial focusing node reduced the velocity distribution to 0.2 % CV, leading to a 10-fold improvement in passing probability and 2-fold improvement in doublet likelihood.

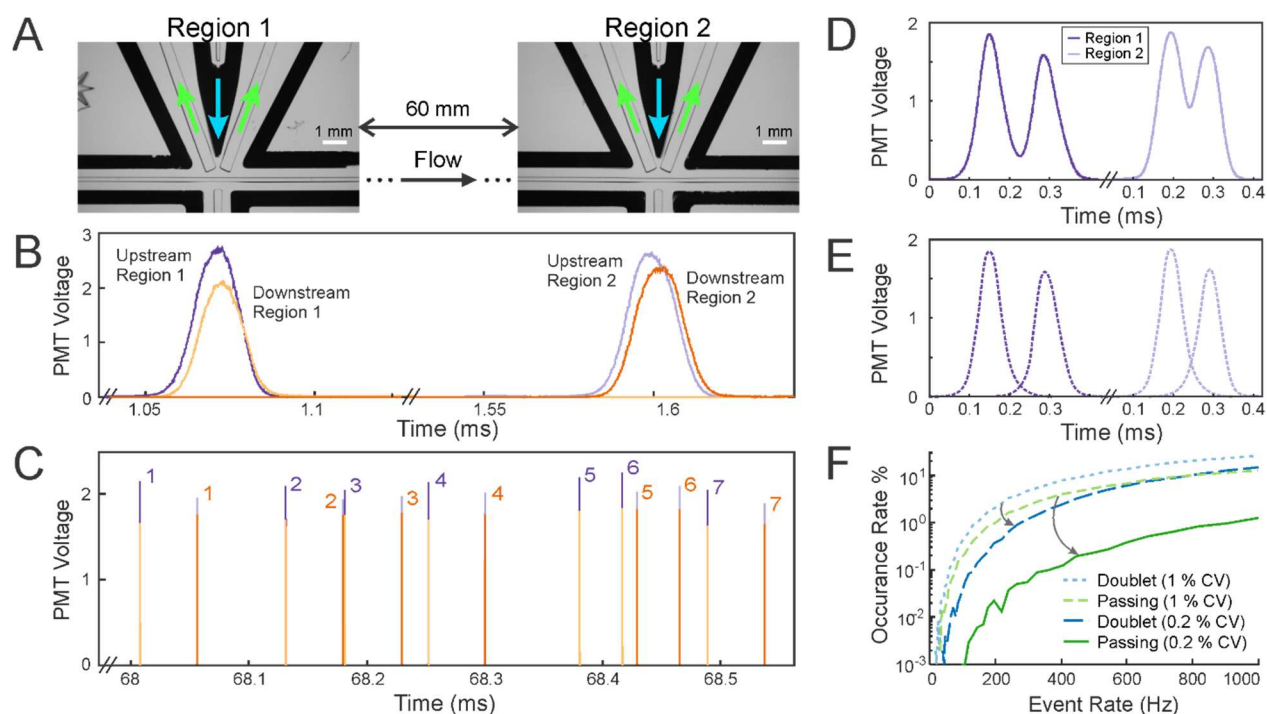


Figure 1: (A) Microscopy images of the 2 measurement regions in the microfluidic flow cytometer. Cyan: laser excitation; Green: fluorescence emission. (B) Representative fluorescence peaks as a bead travels through the 2 measurement regions. (C) Representative bead matching from time-of-flight and shape analysis. Numbers show tracking of each bead's data at region 1 (purple) and region 2 (orange). (D) Example of measured doublet and (E) simulated separation of each bead from the signal in both regions. (F) Simulation results comparing average rates of objects entering the device and the occurrence of passing and doublet events before (1 % velocity CV) and after (0.2 % velocity CV) improvement in flow focusing using combination of hydrodynamic and inertial focusing.

## CONCLUSION

By providing multiple high-resolution measurements of objects in flow, our serial flow cytometer enables characterization of particles while promising improved discrimination of aberrant signal events. Importantly, we have demonstrated the need for an effective tracking and fitting algorithm for any microfluidic system that implements repeated measurement of objects along a flow path. Work is ongoing to extract additional measurement features from the upstream and downstream projections on the same object, including how these measurements can be used to extract the particle's size, shape, and fluorophore distribution.

## REFERENCES

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