A High-throughput, Inertial-microfluidic, Digital, Radiofrequency-encoded Array (HIDRA) Parallel Flow Cytometer

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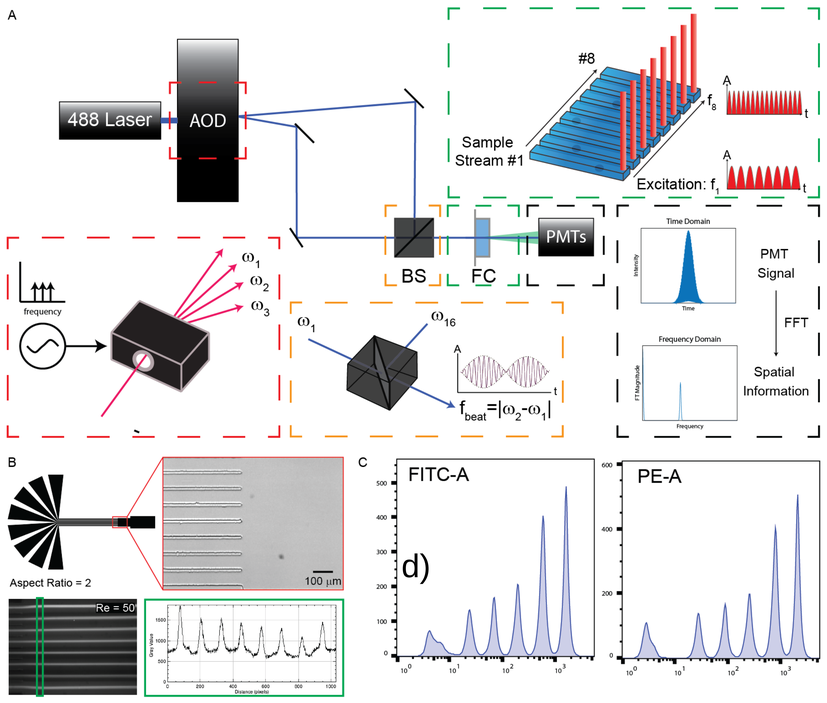
Introduction

Flow cytometry is a powerful biological tool, allowing multiparameter, single-cell measurements of biochemical markers. Although conventional flow cytometers are capable of high-throughput, their sample throughput is limited by serial processing. Here, we demonstrate a high-sample-throughput, inertial-microfluidic, digital radiofrequency-encoded array (HIDRA) parallel flow cytometer that is capable of simultaneous interrogation of eight samples through Fluorescence Imaging using Radiofrequency-tagged Emission (FIRE) and inertial microfluidic focusing. We perform validation with 6-peak fluorescent calibration beads and demonstrate compatibility with conventional biological assays. With this system, we adapt a research tool for high throughput screening, enabling the study of drug-cell interactions at the single cell level.

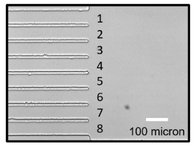
System Schematic and Validation

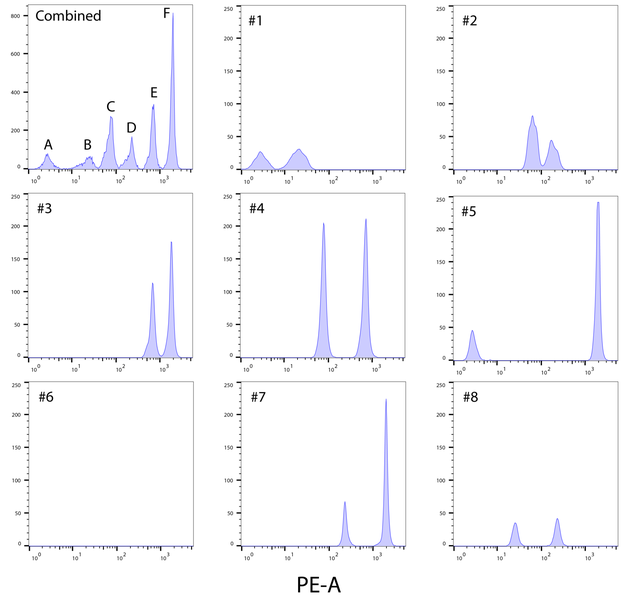


A) Optical system. A 488 nm laser passes through an acousto-optic deflector (AOD), generating frequency-shifted beams which are combined in an interferometer to generate amplitude-modulated excitation beams. Each excitation beam illuminates a separate fluid stream in the flow cell. Fluorescent emission and scattered light are measured with photomultiplier tubes. *Red inset*: An AOD produces 16 beams. *Orange inset*: Beams are interfered with a beam splitter producing 8 beams, each modulated at a unique frequency. *Green inset*: Beams illuminate eight sample streams. *Black inset*: Example raw data. The fast Fourier transform (FFT) determines which beam an event originated from. Conventional flow cytometry measurements are performed as normal. B) Microfluidic device. Eight channels inertially focus particles then merge into an interrogation region where samples are illuminated. *Clockwise*: Device schematic, brightfield image of interrogation region, fluorescent streak images of Jurkat cells stained with Calcein Red-Orange (sample throughput = 960 μl/min), and line plot of fluorescence intensity vs. distance corresponding to green region. Inertial focusing localizes cells to channel midlines. C) Fluorescence sensitivity was measured using calibration beads. All 6 peaks were detected in the FITC and PE channels MESF of <700 and <300 respectively.

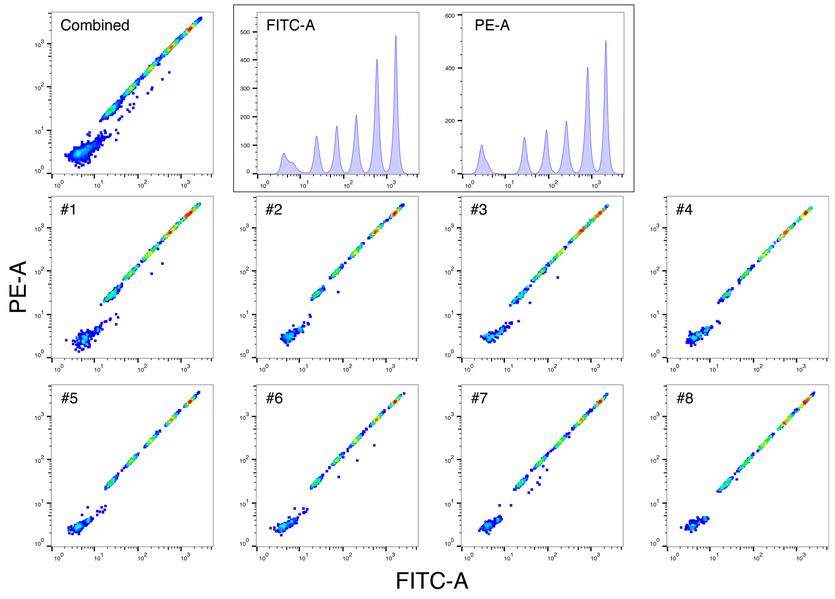
System Performance

#	Bead	Bead
1	A	B
2	C	D
3	E	F
4	C	E
5	A	F
6	none	none
7	D	F
8	B	D





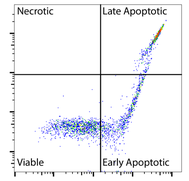
Eight unique samples were generated by choosing combinations of two bead intensities from the six-peak calibration beads used to characterize the system. All samples were run and interrogated simultaneously and events were assigned automatically using frequency-domain analysis. The upper left histogram is the aggregated data from all eight channels. Flow channel 6 was not spiked with any beads.

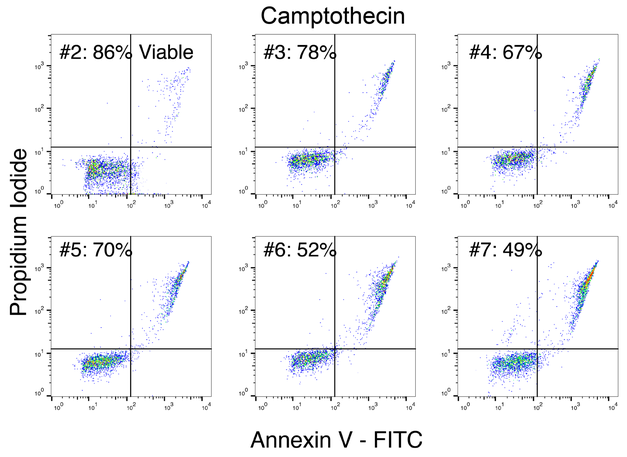


Six peak calibration beads were infused into all 8 sample inlets. After detection of sample stream of origin for each event and application of a multiplicative equalization factor to each channel, all 6 peaks are visible in both FITC and PE channels for all 8 sample streams. The upper left plot shows combined FITC and PE data from all 8 channels.

Results

#	Camptothecin
1, 8	Compensation
2	0 μM
3	0.01 μM
4	0.1 μM
5	1 μM
6	10 μM
7	100 μM





Jurkat cells were treated with varying concentrations of camptothecin for 24 hours. The cells were then labeled with Annexin V, a marker of early apoptosis, and with propidium iodide (PI), a cell membrane impermeable nucleic acid stain that serves as an indicator of cell death. *Upper left*: Legend of treatment conditions. Samples 1 and 8 were designed as experiments to compensate for spectral overlap of FITC and PI. *Lower left*: Gating strategy. Cells that are FITC and PI negative (FITC-/PI-) are considered viable. Cells that are FITC+/PI-, FITC+/PI+, or FITC-/PI+ are considered early apoptotic, late apoptotic, or necrotic, respectively. *Right*: Results from 6-point IC_{50} experiment. Increasing concentrations of camptothecin is correlated with increasing degrees of apoptosis. All 6 samples and 2 compensation experiments were measured simultaneously at a total volumetric sample throughput of 960 microliters per minute.

Conclusions

We have demonstrated a parallel flow cytometry system that utilizes optical and fluidic multiplexing to simultaneously measure 8 samples with a single fluidic and optical system. The instrument represents a significant cost savings and complexity reduction compared to running multiple flow cytometers. Additionally, the instrument does not require a complex, large field of view optical system. Rather, it uses a laser and PMTs, standard flow cytometer components, to perform measurements. The components of this system not traditionally found in flow cytometers represent a small price increase and minimal increase in complexity. The instrument exceeds the volumetric sample throughput of commercially available flow cytometers, sidestepping the need to trade sample throughput for sample volume. Additionally, the ability to run time sensitive experiments such as an IC_{50} in parallel removes the need to account for measurement time, reducing the experimental complexity.

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