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Acoustic Assisted Hydrodynamic Focusing for High-Resolution Imaging in Conventional Flow Cytometers

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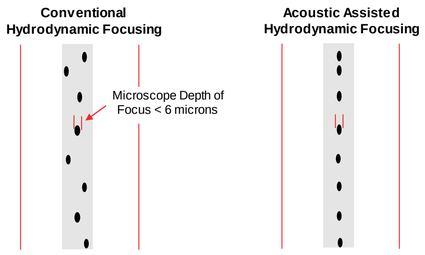
Introduction

The basic physics of imaging cells at flow cytometry speeds of meters per second presents difficult challenges. Among these is the requirement for precise positioning of particles within the very short depth of focus of an imaging system. For instance, a 20x objective with a 0.45 NA lens and 405 nm illumination has a depth of focus of approximately 5.6 microns. In a conventional flow cytometer, the hydrodynamic sample core typically has a diameter exceeding this value. As a result, many images will be out-of-focus, especially for higher sample input rates where core diameters can be many times the depth of focus of the imaging system. Additionally, the lateral position of the particle can vary across this core, requiring larger frame sizes to capture the particle images. The research presented here evaluates addressing the depth of focus challenges in a flow cytometer by using acoustic focusing to finely position particles within the depth of focus of the imaging system. Experiments are run with particles traveling from 4 m/s to 8m/s and hydrodynamic core sizes ranging from a few microns to over 58 microns. The data presented here demonstrates that the combination of acoustic focusing and hydrodynamic focusing dramatically increases the yield of in-focus images (from less than 50% to up to 100%) for particles traveling in sample cores exceeding many times the depth of focus.

Figure 1. Particles within hydrodynamic core



Particles in a hydrodynamically focused flow system (left) are distributed throughout the core stream. For most flow cytometers the core diameter is larger than the depth of focus. Acoustic focusing (right) is used to further align the particles within the core stream to contain them within the depth of focus.

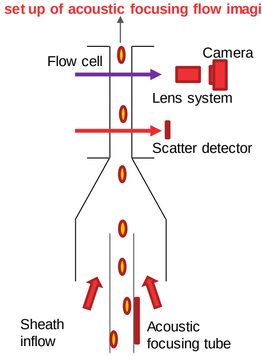
MATERIALS AND METHODS

An acoustic focusing device is constructed from a stainless-steel capillary of inner diameter 340 microns. The capillary is excited with a PZT element to drive a coupled resonance of the tube/cavity combination. The signal is produced with a function generator operating in a range of frequencies around 2.2 MHz.

The fluidics system is comprised of two pumps: a rotary gear pump to deliver sheath fluid and a syringe pump to inject sample. To control the size of the hydrodynamic core, adjustments are made to the volumetric sheath and volumetric sample delivery.

Image data is collected by a microscope lens system comprised of a 0.45 NA objective (20x), tube lens, and a sCMOS camera. The system has a theoretical depth of focus of 5.6 microns. Image illumination is done with a 405 nm laser. To trigger the camera a forward scatter signal was collected from the combination of a 488 nm laser and a photo diode. The sample contains 15 μm latex beads to simulate typical cell sizes. A diagram of the system is shown in Fig. 2.

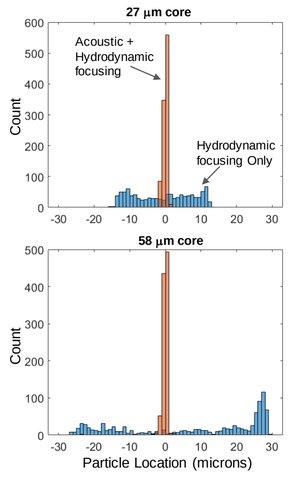
Figure 2. Experiment set up of acoustic focusing flow imaging system



The sample flows up through an acoustically driven tube where particles are aligned along the axis. The sample stream is then injected into a sheath stream. Particles travel up through a scatter detector system that sends a delayed trigger to the camera.

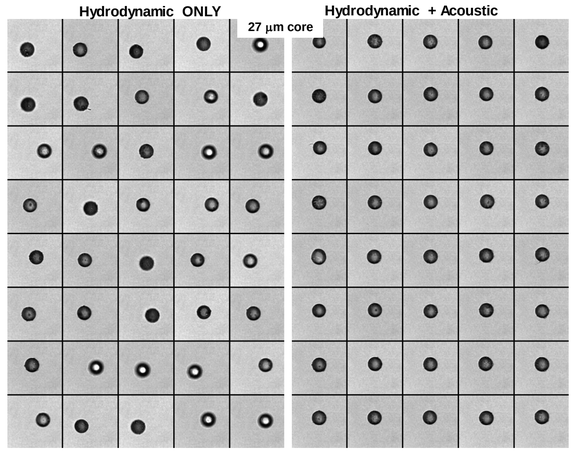
RESULTS

Figure 3. Particle spread within core stream for hydrodynamic focusing only and the combination of acoustic plus hydrodynamic focusing

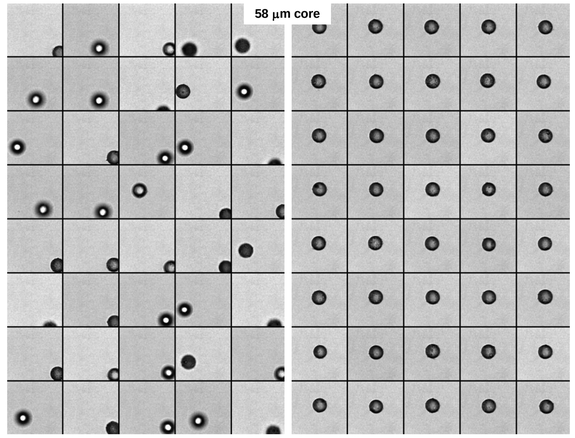


Measurements of Particle Location of 15 μm latex spheres traveling within the core stream of a hydrodynamically focused system. The figure displays results for core stream diameters of 17 μm and 58 μm. A value of zero corresponds to particles traveling along the central axis of the core stream. For hydrodynamic focusing only, the particles are spread across the core diameter. The addition of acoustic focusing positions the particles well within the core stream with a spread of only a few microns. For the imaging system described in this research, the 58 μm diameter core is more than 10x's its depth of focus.

Figure 4. Images obtained from particles traveling in a hydrodynamic core.

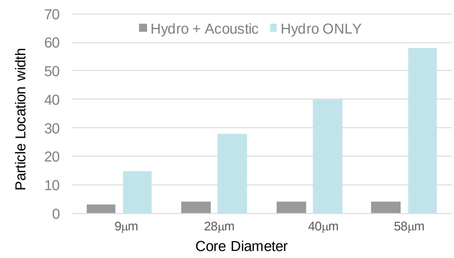


Hydrodynamic focusing with a core size of 27 μm results in particles traveling through different focus planes of the imaging system creating many out-of-focus images (above left). The addition of acoustic focusing brings all the particles into focus by finely placing them within the depth of focus of the imaging system (above right).



Increasing the core size to 58 microns results in more out of focus images and introduces positional changes within the image frame in the hydrodynamically focused system. The particle's spatial position within the image varies considerably due to velocity variations across the core (left). Adding acoustic focusing aligns the particles so that they are within the plane of focus as well as traveling along the same velocity streamline (right).

Figure 5. Particle location as a function of core size



Range of particle locations for different core stream diameters. For hydrodynamic focusing only, in most cases, the particles are distributed across the core and their spread is thus approximately equal to the core size. Adding acoustic focusing limits the particle spread to < 5 microns.

CONCLUSIONS

Flow cytometry core stream diameters are typically greater than the depth of focus of microscope imaging systems. As a result, imaging cells within a hydrodynamically focused core presents many optical challenges. The research presented here shows that acoustic focusing can further align the particles within the core stream. It is shown that acoustic assisted hydrodynamic focusing can confine the particles to a region less than 5 microns in diameter within a 58 micron diameter core stream. Data comparing hydrodynamic focusing ALONE against acoustic assisted hydrodynamic focusing demonstrates a significant increase in yield of in-focus images in the acoustically focused system. Additionally, it is shown that positional variations of the particle within the core stream correlate to positional variations of the particles within the image frame due to particle velocity variations associated with Laminar flow profiles in small channels.

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TRADEMARKS/LICENSING

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