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Development of a microfluidic device with integrated high frequency ultrasound probe for particle characterization

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***Abstract*—A microfluidic flow device incorporating a 200 MHz ultrasound probe has been developed to rapidly characterize micron-sized particles. The device hydrodynamically focuses a particle stream under the ultrasound transducer, where pulse-echo ultrasound is used to probe the passing particles one by one. When the ultrasound wavelength is similar to the particle size, the scattered wave depends strongly on the particle size, and the sound speed and density of the particle and surrounding fluid. Each particle type and size has a unique acoustic signature from which it can be identified. To demonstrate this, polystyrene microbeads with two different sizes were used, 6 or 10 μm. Each particle produced an ultrasound signal, and it was identified as either 6 or 10 μm according to unique features in the ultrasound power spectrum. The ultrasound spectral features agreed with those measured from stationary beads using an acoustic microscope, and also to theoretical predictions. These results show for the first time, a new high-speed method of characterizing micron-sized particles using sound waves with applications towards classifying biological cells.**

Keywords— Microfluidics, particle characterization, ultrasound

I. Introduction

The field of microfluidics has been in development over the last two decades, with the goal of miniaturizing complex analytical techniques [1]. Due to their small size and simple construction, microfluidic systems are also attractive for focusing particles into a narrow stream for rapid analysis [2]. In these flow focusing systems, the particle suspension is infused at a slower rate than the surrounding sheath fluid, which hydrodynamically focuses the particles to the stream center.

Rapid analysis of particles is important in many fields. The number, size and their distributions are commonly used parameters for classifying particles such as counting cells in a blood sample, sizing microspheres, developing nanoparticles and identifying cell types. Using microfluidic devices for these purposes can reduce the overall size, complexity and cost of the instrument.

Microfluidic devices have been developed for particle and cell characterization and/or sorting [3], trapping [4], particle development [5], and coating particles [6]. Particle analysis has also been demonstrated in microfluidics, by using fluorescence [3], electrical impedance [7] or light scattering [8]. However, these methods rely on external additives, or specific assumptions about the particle that can limit their usefulness or sensitivity. Sound waves provide some advantages to other analysis methods as they are sensitive to the size, morphology and internal structure of particles. When particles are insonified, the reflected sound waves are altered such that each type of particle has its own unique acoustic signature; these can be compared to theoretical models [9], [10] or an existing database from which the particle size and type can be deduced.

Previously we had shown good agreement to theory with micron-sized beads and cells suspended in water when insonified with ultrasound in the 40-60 MHz range using ultrasound imaging devices [11], [12]. Probes in this frequency range have focal depths up to several centimeters, slow pulse repetition rates and relatively poor sensitivity for particles and cells that have diameters 15 μm or less (length scales in the micron range). This limits the probes' usability in compact, fast moving environments such as microfluidic devices. For better results, ultrasound frequencies over 200 MHz should be used. The 200 MHz transducer used in this study has a focal depth of 500 μm and can be driven up to MHz pulse repetition rates, enabling rapid characterization of cells or particles.

We present for the first time, a microfluidic device with an integrated ultrasound probe for particle characterization. Measurements using 6 and 10 μm beads demonstrate the proof of concept method. Future applications include counting, sizing and identifying cell types in a fluid medium such as blood.

II. Methods

A. Acoustic Microscope Measurements

Single stationary 6 and 10 μm beads (Polysciences, USA) were scanned in an acoustic microscope to measure a baseline signal. The acoustic microscope is an inverted optical

microscope with an ultrasound transducer attached that can scan the sample from above (fig. 1A). The beads were deposited onto a 1% agar gel on top of a glass substrate with water used as the coupling fluid. The agar has properties similar to water, and prevents back-reflections from the substrate interfering with the acquired signal. A total of 10 ultrasound signals from the center of different beads were recorded (fig. 1B). The 200 MHz transducer had a 500 µm focal length, 60° aperture, f# = 1 and 42% bandwidth (Kibero GmbH, Germany). Measurements were made using a pulse repetition frequency of 125 kHz, and 40 points were averaged to increase the signal to noise ratio (SNR). Signals were sampled at 4 GS/s using an Acqiris DC252 digitizer (Agilent, USA), amplified by a 40 dB low noise amplifier (Miteq, USA) and then saved to a computer. The signals were post-processed offline where the power spectrum was calculated. The acoustic microscope is described further in [13].

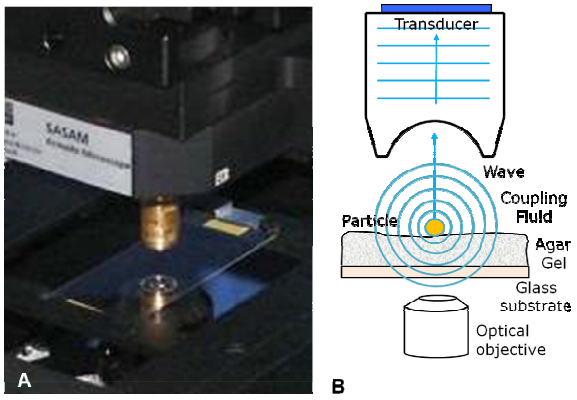

Figure 1: The acoustic microscope. (A) The transducer scans the sample from above, while the sample is viewed optically from below. (B) A schematic showing how the ultrasound waves are recorded in the acoustic microscope (Fig. 1A courtesy of Robert Lemor, Kibero GmbH).

B. *Flow Device*

A microfluidic device was designed and built using layers of bonded acrylic with laser-cut channels (fig. 2). Three layers were cut; the middle layer contained channels that have an approximate 0.8 mm width and height. These channel dimensions were required due to the focal length of the transducer. Solid layers of acrylic were bonded on each side to enclose the channel. Syringe pumps (Elite 11, Harvard Apparatus, USA) were used to infuse a particle solution into the center channel, and water into the outer two channels. The particle solution contained either 6 or 10 µm beads, or a mixture of both (Polysciences, USA). Black ink was added to the solution to provide visual contrast. The microfluidic device was positioned on top of an optical microscope, and the transducer attached to the top of the flow device. The optical microscope was used to examine the flow patterns and ensure the stream was aligned with the transducer (fig. 3). The sheath fluid flow rate was 0.1 mL/min per channel, the particle flow rate was 1 µL/min and the stream was insonified using a pulse repetition rate of 2 kHz. The results were compared to the Faran analytical model [9][10]. All measurements were made at room temperature.

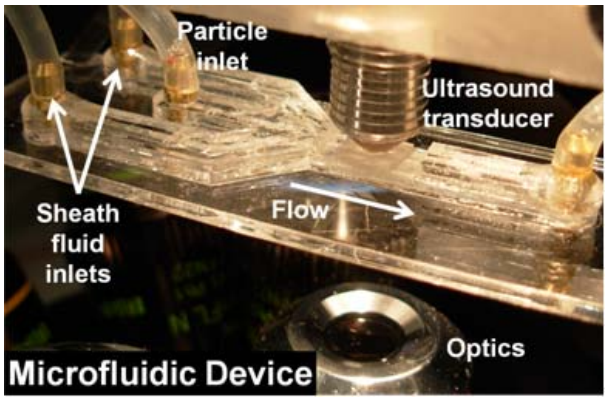

Figure 2: The microfluidic device positioned on top of the optical microscope. The transducer is attached from above, and the particle stream can be viewed from below.

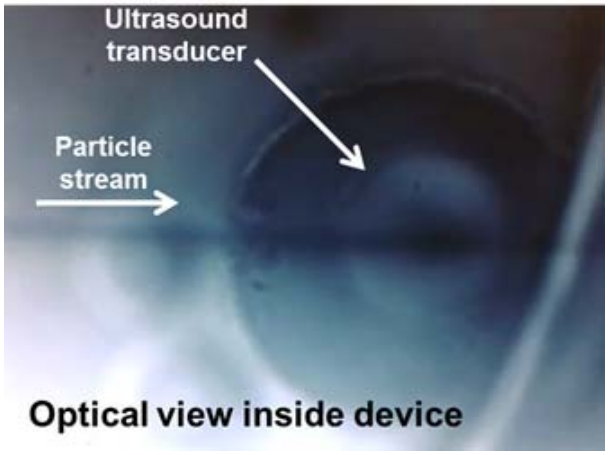

Figure 3: An optical view inside the microscope. The particle stream (dyed black for visibility) flowed underneath the transducer. Individual beads were not visible due to the fast flow rate and the limited CCD capture rate.

III. Results and Discussion

Before measuring the signals from flowing beads, stationary beads were examined in the acoustic microscope. The ultrasound backscattered signals from ten different stationary 6 and 10 µm beads were measured as a baseline signal. Representative time domain signals from the 6 and 10 µm beads are shown in fig. 4. The signals have similar features which may make it difficult to rapidly differentiate them using algorithms. The beads can be better identified by examining the power spectrum. Fig. 5 shows the power spectra from 10 beads from 6 µm beads (left) and 10 µm beads (right) overlaid on top of each other. The Faran analytical solution describing the scattered ultrasound waves from solid spheres was solved for the known bead parameters (2350 m/s sound speed, 1050 kg/m^3 density) and plotted over the measured signals. The location of the minima and maxima between the analytical solution and measured beads are in good agreement, and good consistency from bead to bead was observed.

The flow was device attached to the optical microscope as shown in fig. 1A. This system enabled optical viewing from the bottom, and alignment of the ultrasound transducer to the passing particles. The stream size could be adjusted by varying the relative flow rates of the sheath and particle inlets. Streams as narrow as 15 μm were achieved.

A solution of 6 μm beads was added to the particle inlet syringe tube. The sheath flow and particle flow were adjusted so that the particles would flow in a narrow stream under the transducer. The particle solution was dyed black for visibility as the CCD camera was unable to capture fast events (fig. 3). Ultrasound pulse-echo signals were recorded from beads passing under the transducer using a pulse repetition rate of 2 kHz with no signal averaging. Fig. 6 shows the ultrasound power spectrum from 10 different 6 μm beads, along with a comparison to the Faran analytical solution. Good agreement between the flowing beads, the stationary beads (using the acoustic microscope) and the Faran analytical model was observed. Variations in the amplitude of the flowing particles were greater than the stationary particles, which is likely due the position of the beads flowing under the transducer. In the stationary measurements, the ultrasound transducer focus can be positioned directly over the center of the bead. In the flow measurements, the bead may be outside of the optimum transducer focus. Our measurements indicate that beads outside of the focus still exhibit the expected power spectra, but with reduced amplitude. The same procedure was repeated for a 10 μm bead sample with similar results (fig. 6).

The robustness of the particle characterization method was examined by using a solution of 6 and 10 μm beads in two different concentrations: 1) 66% 6 μm and 33% 10 μm beads, and 2) 40% 6 μm and 60% 10 μm beads. The concentration was measured using a haemocytometer. Using the flow device, the bead concentration was found to be 88% 6 μm and 12% 10 μm (scenario 1), and 78% 6 μm and 22% 10 μm (scenario 2) by comparing each power spectrum to theory (table I). A total of 500 events were used for the calculation, which took several minutes to acquire. In both cases, the measured concentration of the 10 μm beads was 36% lower than the expected values. During the measurements, the number of events/minute measured decreased over time. The events/minute increased again once the particle solution was mixed and the measurement restarted. This is likely due to particle sedimentation; since the 10 μm beads are heavier than the 6 μm beads, they would settle faster. The 10 μm beads would also encounter more resistance through the 30 cm long tube from the pump to the flow device. After the measurements, we found a buildup of particles along the bottom of the tube. Future implementations will use a vertically orientated tube and device to prevent biased concentration measurements.

TABLE I. MEASURED PARTICLE CONENTRATIONS

	Flow-measured Concentration		Actual Concentration		Difference in 10 μm bead measurements
	6 μm	10 μm	6 μm	10 μm	
Solution 1	88%	12%	66%	33%	36%
Solution 2	78%	22%	40%	60%	37%

IV. CONCLUSION

A new type of particle characterization method has been developed using sound waves. These results show for the first time, an ultra-high frequency ultrasound probe integrated into a microfluidic device for the rapid characterization of particles. Beads were used in this proof of concept study as they are strongly echogenic, homogeneous, have known properties with tight size distributions and can be compared to analytical solutions. Future applications will be classifying biological cells in a sample.

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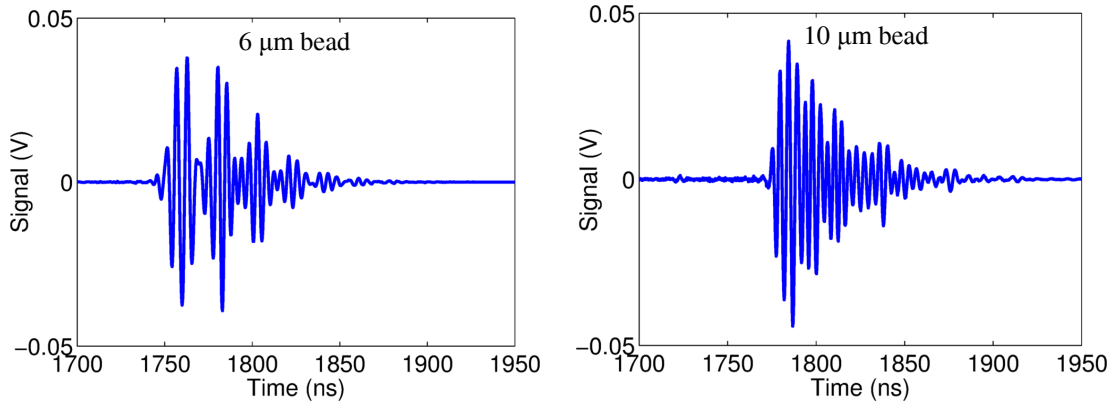

Figure 4: Stationary bead measurements: The measured ultrasound time domain signals from a stationary 6 µm (left) and a 10 µm (right) polystyrene bead using the SASAM acoustic microscope.

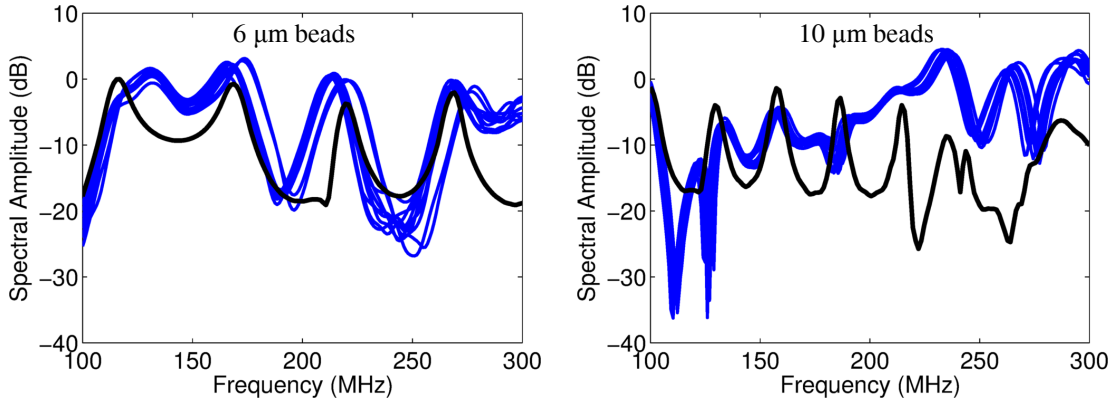

Figure 5: Stationary bead measurements: The power spectrum of 10 signals (blue) compared to the Faran scatteirng model (black) for the 6 µm (left) and 10 µm (right) beads measured using the SASAM acoustic microscope. Distinctive features are observed that can easily differentiate the two bead sizes.

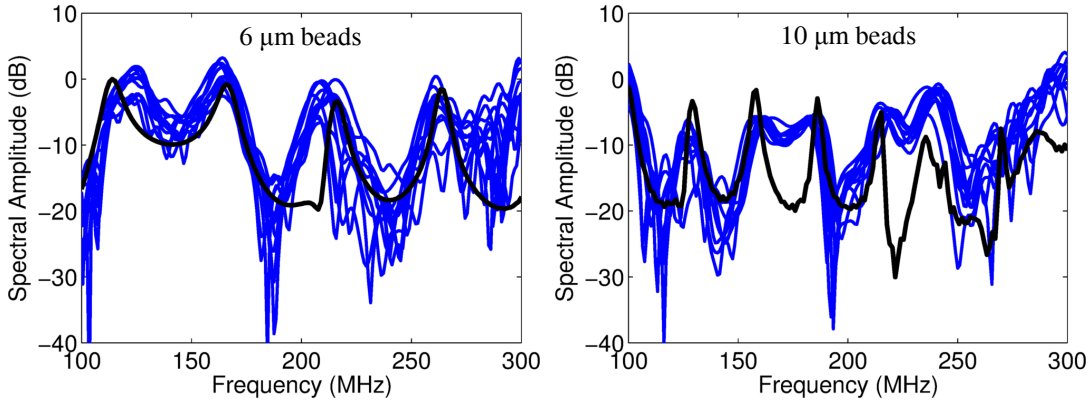

Figure 6: Flowing bead measurements: The power spectrum of 10 signals (blue) compared to the Faran scattering model (black) for the 6 µm (left) and 10 µm (right) beads measured using the flow system. The SNR is not as good as the beads measured in the acoustic microscope, and some shifts are observed that can be attributed to measurements of beads outside of the transducer focal region.