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Backscattering Measurement From a Single Microdroplet

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Abstract

Backscattering measurements for acoustically trapped lipid droplets were undertaken by employing a P(VDF-TrFE) broadband transducer of f -number = 1, with a bandwidth of 112%. The wide bandwidth allowed the transmission of the 45 MHz trapping signal and the 15 MHz sensing signal using the same transducer. Tone bursts at 45 MHz were first transmitted by the transducer to hold a single droplet at the focus (or the center of the trap) and separate it from its neighboring droplets by translating the transducer perpendicularly to the beam axis. Subsequently, 15 MHz probing pulses were sent to the trapped droplet and the backscattered RF echo signal received by the same transducer. The measured beam width at 15 MHz was measured to be 120 μm . The integrated backscatter (IB) coefficient of an individual droplet was determined within the 6-dB bandwidth of the transmit pulse by normalizing the power spectrum of the RF signal to the reference spectrum obtained from a flat reflector. The mean IB coefficient for droplets with a 64 μm average diameter (denoted as cluster A) was -107 dB, whereas it was -93 dB for 90- μm droplets (cluster B). The standard deviation was 0.9 dB for each cluster. The experimental values were then compared with those computed with the T-matrix method and a good agreement was found: the difference was as small as 1 dB for both clusters. These results suggest that this approach might be useful as a means for measuring ultrasonic backscattering from a single microparticle, and illustrate the potential of acoustic sensing for cell sorting.

I. Introduction

Ultrasound scattering from biological tissues has been extensively studied because ultrasonic images are formed from echoes backscattered from these tissues, and a variety of methods have been developed to measure their backscattering characteristics [1]–[3]. Both narrowband [4] and wideband [5] substitution methods have been employed to quantitatively estimate the backscattering properties by measuring the ratio between the magnitudes of RF signals from a reflector of known reflectivity and a sample volume of interest in a tissue. Scattering measurements have recently been extended to higher frequencies and performed on collections or pellets of a variety of cells. The results suggested that the backscattering properties may be correlated to the cellular structures and pathological states of those cells, including mitosis [6], necrosis [7], and apoptosis [8].

A method [9] has been proposed to deduce the backscattering from single polystyrene beads and human acute myeloid leukemia cells (OCI-AML-5, mean diameter of 13.4 μm). It was found that air bubbles were introduced by stirring vials that contained the particle suspension, which led to inaccurate backscattering transfer functions. More recently, the bubble problem was addressed by the same researchers [10]. In that study, a micropipette was utilized to directly aspirate a single object from the agglomerates, while simultaneously monitoring the process with optical guidance. Practical issues including cell damage and clogging at the pipette tip, however, still remained unsolved.

This paper presents an approach that allows the measurement of the integrated backscatter coefficient of a single microparticle which is isolated by a two-dimensional acoustic trap. Lipid droplets were used as target particles, and focused sound beams at two different frequencies enabled acoustic characterization of a trapped droplet without mechanical contact. A P[VDF-TrFE] (or PVDF copolymer, Ktech Corporation, Albuquerque, NM) transducer was fabricated to achieve both backscattering measurement and droplet trapping in the Rayleigh and the Mie regimes. By taking advantage of the transducer's broad bandwidth, both functions were able to be implemented with a single source. The integrated backscatter (IB) coefficients were measured to demonstrate the feasibility of identifying particles of different physical property, e.g., size. The coefficients were determined as a function of droplet radius and compared with the theoretical prediction using the T-matrix method developed by Waterman [11]. The potential application of a cell sorter using the proposed approach was discussed along with a comparison with other acoustic techniques.

II. Methods

A. Fabrication of Broadband Transducer

Previous investigations have been carried out to show that acoustic traps could be formed in the Mie regime (mean particle diameter $D > \text{wavelength } \lambda_1$) [12], [13].

However, backscattering measurements could be more easily made in the Rayleigh ($D < \lambda_2$) regime. To satisfy these requirements simultaneously, a broadband transducer capable of generating sound waves at two distinct, widely separated frequencies, f_1 and f_2 , was designed and built. Because the mean droplet diameter D was either 64 or 90 μm , $f_1 = 45$ MHz ($\lambda_1 = 33$ μm) and $f_2 = 15$ MHz ($\lambda_2 = 100$ μm) were selected as the operating frequencies for trapping and backscattering experiments, respectively.

A 25- μm -thick copolymer with a diameter of 5.6 mm was mounted on a backing layer of silver-loaded epoxy (5.9 MRayls, E-solder 3022, VonRoll Isola, New Haven, CT). No matching layer was cast on its front surface because the copolymer had relatively low acoustic impedance that matched well with a fluid medium (or water in this case). The

geometric focus of the transducer was formed at 5.1 mm by press-focusing the aperture with a chrome/steel ball of the same radius of curvature. The center frequency was found at 24 MHz, and the -6 -dB bandwidth was 112%. Measured pulse echo responses for the transducer are displayed in Fig. 1; they were obtained by time-averaging 50 R F echoes. The beam widths produced by the transducer at 15 and 45 MHz were measured to be 120 and 40 μm by a needle hydrophone (HPM04/01, Precision Acoustics, Dorchester, UK).

B. Droplet Preparation

Droplet-based microfluidic channels were built from poly(dimethyl) siloxane (PDMS) to synthesize fluid spheres with conventional soft lithography techniques [14]. This type of device has frequently been used to form monodispersed particles in the nanometer to micrometer size range [15]. The channel was formed by bonding PDMS to glass via oxygen plasma treatments. This oxidization process made the channel hydrophilic after vapor deposition of tridecafluorochlorosilane (SIT8174.0, Gelest Inc., Morrisville, PA), which was crucial to produce water-in-oil emulsion and allow complete wetting of the wall by aqueous solutions. Oleic acid (viscosity 27.64 mPa and interfacial tension 15.6 dyne/cm, Fisher Scientific, Pittsburgh, PA) was then used as a dispersed phase, and purified deionized water served as a continuous phase. As two liquid phases merged at a shear junction, oleic acid was sheared into droplets by the aqueous phase that consisted of a 5 wt% mixture of Pluronic F-68 (Sigma-Aldrich Co., St. Louis, MO) and ultra-pure water (Millipore, Billerica, MA). Pluronic F-68 was necessary to stabilize droplets during storage and transport. An expanding nozzle at the flow-focusing junction led to a local shear maximum that ensured repeatable break up of the fluid stream [16]. The resultant droplets were formed at a rate of 50 counts/sec and collected in a reservoir to restore its spherical shape. The droplet size was controlled by changing the flow rates of each solution and later measured using commercial software (Infinity Analyze, Lumenera, Ottawa, Canada). As the rate of oil phase increased, larger droplets were constructed. The typical rate used for this study varied from 0.5 to 1 $\mu\text{L}/\text{min}$.

C. Particle Entrapment and Isolation

Fig. 2 illustrates an experimental layout for the proposed method, including two sub-systems: acoustic trapping [17], [18] and backscattering measurement. Note that the droplets were in contact with a thin Mylar membrane and the transducer was placed normal to the membrane at the focus. To form an acoustic trap at the focus, 45-MHz tone bursts from an arbitrary waveform generator (AFG 3251, Tektronix, Beaverton, OR) were first input to the transducer, followed by a 50-dB power amplifier (325LA, ENI, Rochester, NY). The excitation amplitude was set to 32 V_{pp} and the duty factor was 6.7%. The transducer was mounted on a programmable motorized positioner (LMG26 T50MM, OptoSigma, Santa Ana, CA) and the droplet motion was observed with a stereomicroscope (SMZ1500, Nikon, Tokyo, Japan). During the experiment, the axial distance between the transducer and the cluster was increased to break a droplet cluster into individual droplets. After being brought back to the focal length, the transducer was aimed at a single droplet to place it in the acoustic trap. By moving the trap center perpendicularly to the beam axis, the trapped droplet was directed away from the cluster, typically over a distance of hundreds of micrometers. This allowed enough separation to minimize unwanted echoes from the adjacent droplets. The peak pressure applied by the 45-MHz trapping beam was less than 2 MPa, where the acoustic power emitted from the transducer was 3 mW. The corresponding thermal and mechanical indexes were estimated to be 0.6 and 0.3, respectively.

D. Integrated Backscatter (IB) Coefficient

The transducer then transmitted 15-MHz single-cycle sinusoidal pulses and the backscattered echoes from the isolated droplet were received by the same transducer. The

pulse repetition frequency (PRF) was 200 Hz. The RF echoes were amplified by a receiver (Model 5910R, Panametrics, Waltham, MA), and sampled at the rate of 10 GHz by an 8-bit digital oscilloscope (104MXi, LeCroy Corp., Chestnut Ridge, NY). The digitized data were exported to the computer via a LabView interface (National Instruments, Austin, TX) for postprocessing. The IB coefficient was defined as the ratio of backscattered energy from a scatterer volume to the one from a flat quartz reflector [19]:

$$IB = \frac{\int_{f_c - \Delta f}^{f_c + \Delta f} \frac{|V(f)|^2}{|R(f)|^2} df}{\frac{\int_{\tau - \Delta \tau}^{\tau + \Delta \tau} |v(t)|^2 dt}{\int_{\tau - \Delta \tau}^{\tau + \Delta \tau} |r(t)|^2 dt}}, \quad (1)$$

where $v(t)$ is a received A-line from the scatterer and $r(t)$ is a reference signal from the reflector, whereas $V(f)$ and $R(f)$ are their Fourier transforms. Fifty samples were time-averaged for each signal. τ and $\Delta \tau$ correspond to the center of the echo and its half-width in time, respectively. f_c and Δf are the resonant frequency of $v(t)$ and its halfwidth bandwidth. The 15-MHz received RF echoes from the reflector were used as a reference signal $r(t)$ as plotted in Fig. 3. The instrument dependence of power spectrum measurements was factored out by normalizing $|V(f)|^2$ to the reference spectrum $|R(f)|^2$ with known reflectivity.

E. Theoretical IB Calculation: T-Matrix Analysis

Let $H(f)$ be the backscatter transfer function at a frequency f , and note that the IB coefficient was approximated as the frequency-averaged $H(f)$ over the bandwidth of a backscattered signal [20], [21]. The T-matrix formulation for a sphere was used to compare with its experimental results. T_{mv} , an indexed element in the matrix, was defined as the ratio of scattering field coefficients Q_{mv}^s to incident field coefficients Q_{mv}^i , and written as [22]

$$T_{mv} = \frac{Q_{mv}^s}{Q_{mv}^i}, \quad (2)$$

$$Q_{mv}^s = -\frac{ik_w}{4\pi} \int_0^\pi r^2 \sin \theta \left[\varepsilon_m \frac{2m+1}{2m!} i^v (2v+1) \right] P_m(\cos \theta) P_v(\cos \theta) \times \left[\frac{\rho_p}{\rho_w} k_w j_m'(k_w r) j_v(k_p r) - k_p j_m(k_w r) j_v'(k_p r) \right] d\theta, \quad (3)$$

$$Q_{mv}^i = -\frac{ik_w}{4\pi} \int_0^\pi r^2 \sin \theta \left[\varepsilon_m \frac{2m+1}{2m!} i^v (2v+1) \right] P_m(\cos \theta) P_v(\cos \theta) \times \left[\frac{\rho_p}{\rho_w} k_w h_m^{(1)'}(k_w r) h_v^{(1)}(k_p r) - k_p h_m^{(1)}(k_w r) h_v^{(1)'}(k_p r) \right] d\theta, \quad (4)$$

where k_w and k_p are the ultrasonic wave numbers; ρ_w and ρ_p are the mass densities in the water (1000 kg/m³) and a sphere (950 kg/m³), respectively; j_m and j_m' are the spherical Bessel function of the first kind and its derivative; h_m and h_m' are the spherical Hankel function of the first kind and its derivative; m and v are the orders of the spherical functions as well as the Legendre polynomials $P_{m,v}(\cos \theta)$; θ is the angle of the incident beam; and r is the radius of the sphere. Note that the mean value of r was 32 or 45 μ m in this paper. $\varepsilon_m = 1$ for $m = 0$ and $\varepsilon_m = 2$ for $m > 0$. For simulation, the maximum integer m was set to 20. Because of the orthogonal property of Legendre polynomials, T_{mv} was simplified to

$$T_{mm} = - \frac{\frac{\rho_p}{\rho_w} k_w j'_m(k_w r) j_m(k_p r) - k_p j'_m(k_p r) j_m(k_w r)}{\frac{\rho_p}{\rho_w} k_w h_m^{(1)'}(k_w r) h_m^{(1)}(k_p r) - k_p h_m^{(1)}(k_w r) h_m^{(1)'}(k_p r)}. \quad (5)$$

As a result, $H(f)$ was calculated as

$$H(f) = \pi \left[\frac{2}{k_w} \left| \sum_{m=0}^{\infty} (-1)^m (2m+1) T_{mm} \right| \right]^2. \quad (6)$$

The experimental IB coefficients of droplets are compared with these analytical values of $H(f)$ in the following section.

III. Results

The average droplet sizes used in the experiment were 64 and 90 μm in diameter (or equivalently, 32 and 45 μm in radius), as denoted by clusters A and B in Fig. 4.

Representative echoes and their frequency responses from these droplets are given in Fig. 5. The first signal to the left (in a dashed box) was produced by the trapped droplet, and the second echo came from the Mylar membrane. Figs. 5(a) and 5(c) display time-averaged signals of $s_1(t)$ and $s_2(t)$, originated from each cluster. The interference between the trapped and the neighboring droplets became negligible by separating them by more than a couple of beam widths, 250 μm . The Hanning window function was applied to select the signal from the trapped droplet. Frequency spectra for such gated signals, $S_1(f)$ and $S_2(f)$ are plotted in Figs. 5(b) and 5(d). For both cases, multiple resonance peaks were found but within the system bandwidth there was only one, at 15 and 17 MHz, respectively.

Peak-to-peak voltages of the received echoes were as high as 7 mV. Although their amplitude was low, the difference was clearly seen between backscattered and reference signals as shown in Figs. 3 and 5. In case of bulk tissues, the SNRs for a tissue-mimicking phantom and a human liver were 1.74 ± 0.03 and 1.49 ± 0.11 , as reported in [23]. Oosterveld *et al.* obtained 2.0 ± 0.05 for similar liver samples [24]. In contrast, the measured SNRs in this paper were 1.1 ± 0.05 for cluster A and 1.3 ± 0.06 for cluster B. The result was in a reasonable range, given that the measurement was done for single weak scatterers rather than a bulk specimen. IB coefficients corresponding to 66 individual droplets were then estimated by (1). As shown in Fig. 6, the mean and the standard deviation were -107 and 0.9 dB for cluster A; they were -93 and 0.9 dB for cluster B. The difference between the two clusters was nearly 14 dB on average. Hence, IB values for the larger cluster were greater than those for the smaller one, as expected from the Rayleigh scattering.

The experimental IB coefficients were further compared with those from the T-matrix formulation. The estimated values (solid line) were in agreement with the measured data (circles). In particular, the mean experimental and the theoretical IB coefficients were, respectively, -107 and -106 dB for droplets in cluster A, and they were -93 and -94 dB for cluster B. The difference was as small as 1 dB for both cases, implying that the IB coefficient can be used as a metric by which particles of distinct sizes can be sieved. The tangential slope of the theoretical curve was observed to deviate slightly from those of the experimental data for clusters A and B. The theoretical and measured slopes were, respectively, 1.2 and 0.6 dB/ μm for cluster A, and 0.6 and 0.2 dB/ μm for cluster B. The

cause for this discrepancy is unclear. It may be due to 1) the limited diameter range within which the slope was estimated, or 2) experimental error in the backscattering measurements.

The IB values obtained in this work were distributed approximately between -90 and -110 dB, lower than those previously reported from a single-cell backscattering experiment [10], -60 to 90 dB. Because the previous experiment was undertaken at higher frequencies, the scattering became more dominant than at the 15 MHz frequency adopted here. No sharp resonance peaks in the backscattering spectrum, which are typical to the elastic wave scattering, were observed in either study. The difference in the IB level might also be attributed to the elastic characteristics of the cell's nucleus or cytoplasm [25]. Because of its complex structures, not all cells can be exactly approximated by simple fluid sphere models including the T-matrix and Anderson's model [26]. Good agreement between theoretical and experimental backscattered data, however, has been demonstrated by Anderson's model, for cells like OCI-AML-5, PC-3 prostate carcinoma cells [27], and sea urchin oocytes [28], whose nuclei are relatively small compared with their cell bodies.

These results thus suggest the potential use of the proposed method as a cell-sorting scheme. Cell separation techniques with high throughput have recently become important in lab-on-a-chip systems, and simple and reliable fractionation in such systems is increasingly needed. Both analysis and sorting procedures can be accomplished by integrating a broadband focused transducer with a microfluidic channel. Suppose that mixed cells are flowing along the center of a channel and two lateral sheath flows are guiding the center flow. Acoustic characterization like that proposed here, i.e., quantitative backscattering measurement from all cells streaming through the channel is carried out. If a targeted streaming cell is identified, it can be readily diverted by a trapping beam into another channel. In the proposed scheme, trapping and sensing beams are emitted from the same transducer. Depending on the measured IB coefficient, the transducer can be electronically switched to a trapping mode to collect the scanned cell in another area. For practical purposes, higher frequency than reported in this paper should be employed to meet the criteria for the Mie and the Rayleigh regimes, because the size of most cells ranges from 1 to a few tens of micrometers.

IV. Conclusion

In this paper, a method in which an acoustic trap was formed to isolate a droplet from adjacent ones was reported to show that it might be utilized to perform backscattering measurements on a single microdroplet. Integrated backscatter coefficients were experimentally obtained as a function of droplet radius. The measured results were in agreement with the theoretical values based on the T-matrix formulation, suggesting that this proposed approach might be suitable as a means for making ultrasound scattering measurements of an individual micrometer-sized object. The results merely indicate that the IB coefficient may be used as a useful index to distinguish one particle from the others based on several physical properties of the particle, including size and acoustic impedance. This work was performed as an initial attempt to realize ultrasonic cell sorting in which both cell sensing and sorting are carried out by sound beams.

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Fig. 1. Pulse-echo response curves in (a) time and (b) frequency domains.

Fig. 2.

Diagram for integrated backscatter measurement of single droplet. A 45-MHz burst signal was used to acoustically hold a droplet in degassed deionized (DI) water and to further isolate it from the neighboring droplets. A 15-MHz pulse was then transmitted to the droplet, and the backscattered RF echo was received by the same transducer.

Fig. 3.

Reference signal from a flat reflector: (a) a time-averaged signal of $r(t)$ (number of samples = 50) and (b) its frequency response. A resonant peak was found at 15 MHz and its -6 -dB bandwidth ranges from 8 to 21 MHz.

Fig. 4. Size distribution of trapped droplets: Clusters A and B indicated 64 and 90 μm droplets in mean diameter ($r = 32$ and $45 \mu\text{m}$), whose standard deviations were 4 and $6 \mu\text{m}$, respectively. The number of droplets in each cluster was 33.

Fig. 5.

Examples of received echoes from two droplets of different size: (a) and (b) represent a time-averaged signal of $s_1(t)$ and the frequency response $S_1(f)$ for a 64- μm droplet. (c) and (d) correspond to $s_2(t)$ and $S_2(f)$ for a 90- μm droplet. Multiple resonant peaks were found within the bandwidth for both droplets. Maximum resonances occurred at 15 and 17 MHz, respectively.

Fig. 6.

Comparison between IB measurements and theoretical T-matrix values in the Rayleigh and the Mie scatterings. Two clusters A and B were separated by 14 dB on average. The mean and the standard deviation of the coefficients were -107 and 0.9 dB for cluster A, and -93 and 0.9 dB for cluster B.