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Ultrasonic Scattering Measurements of a Live Single Cell at 86 MHz

Changyang Lee,

Department of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089, USA

Hayong Jung,

Department of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089, USA

Kwok Ho Lam,

Department of Electrical Engineering, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong

Changhan Yoon, and

Department of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089, USA

K. Kirk Shung

Department of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089, USA

Changyang Lee: changyal@usc.edu

Abstract

Cell separation and sorting techniques have been employed biomedical applications such as cancer diagnosis and cell gene expression analysis. The capability to accurately measure ultrasonic scattering properties from cells is crucial in making an ultrasonic cell sorter a reality if ultrasound scattering is to be used as the sensing mechanism as well. To assess the performance of sensing and identifying live single cells with high-frequency ultrasound, an 86-MHz lithium niobate press-focused single-element acoustic transducer was used in a high-frequency ultrasound scattering measurement system that was custom designed and developed for minimizing noise and allowing better mobility. Peak-to-peak echo amplitude, integrated backscatter (IB) coefficient, spectral parameters including spectral slope and intercept, and midband fit from spectral analysis of the backscattered echoes were measured and calculated from a live single cell of two different types on an agar surface: leukemia cells (K562 cells) and red blood cells (RBCs). The amplitudes of echo signals from K562 cells and RBCs were 48.25 ± 11.98 mV_{pp} and 56.97 ± 7.53 mV_{pp}, respectively. The IB coefficient was -89.39 ± 2.44 dB for K562 cells and -89.00 ± 1.19 dB for RBCs. The spectral slope and intercept were 0.30 ± 0.19 dB/MHz and -56.07 ± 17.17 dB, respectively, for K562 cells and 0.78 ± 0.092 dB/MHz and -98.18 ± 8.80 dB, respectively, for RBCs. Midband fits of K562 cells and RBCs were -31.02 ± 3.04 dB and -33.51 ± 1.55 dB, respectively. Acoustic cellular discrimination via these parameters was tested by Student's *t*-test. Their values, except for the IB value, showed statistically significant difference ($p < 0.001$). This paper reports for the first time that ultrasonic scattering measurements can be made on a live single

cell with a highly focused high-frequency ultrasound microbeam at 86 MHz. These results also suggest the feasibility of ultrasonic scattering as a sensing mechanism in the development of ultrasonic cell sorters.

I. Introduction

Cell separation and sorting techniques have been employed in such biomedical applications as cancer diagnosis and cell gene expression analysis, and most frequently are based on differences of specific cellular properties such as density [1], charge [2], specific immunologic targets [3], dielectric properties [4], and size [5]. Currently, the fluorescence activated cell sorter (FACS), which works by attaching fluorescent molecules to the target cells and sorting by injecting electric charges to the cells, has been popular for these applications because of its high throughput and accurate sorting performance. Although the instrument is widely used for single cell-based rare cell isolation, including circulating tumor cells (CTCs), it is rather bulky and complicated, and needs multiple cellular manipulation steps and hours of sample pretreatment time by well-trained users. Microfluidic technologies using soft lithography have been investigated to overcome these drawbacks because of their advantages in high throughput, high sensitivity, and low cost.

The combination of high-frequency ultrasound microbeams and microfluidic channels has been reported as one of the possible alternatives to replace FACS. Its feasibility as a single-cell sorter has been demonstrated by size-based single droplet sorting by analyzing scattered echo signals [6], [7] with advantages in noncontact sensing and sorting, being a closed system for bio-hazardous samples, being simpler in instrument structure, and requiring no labeling.

In this paper, the capability of making ultrasonic scattering measurements at the single-cell level is discussed. The capability to accurately measure ultrasonic scattering from cells is crucial if ultrasound scattering is to be used as the sensing mechanism in a cell sorter.

Ultrasonic scattering in an inhomogeneous medium has been analyzed theoretically for many years. Ultrasound scattering from a sphere of varying composition has been theoretically modeled and analyzed, including solid [8], fluid [9], elastic [10], and rigid [11] spheres. Ultrasound scattering from suspended OCI-AML-5 cells and PC-3 cells (less than $10\,000\text{ cells/cm}^3$) has been experimentally measured and results were compared with theoretical results from elastic and fluid spheres at 19, 40, and 55 MHz [12]. The study showed that the backscatter responses of OCI-AML-5 cells and PC-3 cells were better modeled as elastic spheres and fluid spheres, respectively, because of their difference in nucleus-to-cell-volume ratio. Falou *et al.* reported theoretical and experimental backscattering results on elastic and fluid spheres from a micrometer-sized polystyrene object and single OCI-AML-5 cell at 25 and 55 MHz, and these results showed that backscattering from a single OCI-AML-5 cell can be modeled as an elastic and a fluid sphere [13]. The results showed a good agreement between theoretical and experimental results. The authors validated that ultrasound backscatter response of a live single cell could be modeled as a single fluid sphere. O'Brien's group reported ultrasound backscattering characterization of solid tumors in mice by three scattering models including a two

concentric sphere model based on fluid spheres with experimental measurement [14], and demonstrated that the backscatter coefficient of a Chinese Hamster ovary (CHO) cell pellet between volume density 10% and 30% can be estimated by a two concentric sphere model based on fluid spheres [15]. O'Brien *et al.* also studied backscattering and attenuation of cell pellets and tumors *ex vivo* to provide a better understanding of the scattering process [16]. These studies have provided fundamental knowledge on the theoretical and experimental ultrasonic scattering processes of a cell.

High-frequency ultrasound backscattering measurements used in the present experiments have been validated on single lipid droplets previously. The experimentally measured integrated backscatter was found to be in good agreement with theoretically calculated values from the T-matrix method [17], suggesting that this approach may be employed as a means for quantitatively measuring ultrasonic backscattering from a single microparticle. High-frequency ultrasound was subsequently employed for microdroplet sensing and sorting in a microfluidic device [7].

This paper reports for the first time experimental results from scattering measurements carried out on a live single cell with a highly focused high-frequency ultrasound microbeam at 86 MHz. These results show that it is possible to characterize cells—in this case two different live cells, namely leukemia cells (K562 cells) and red blood cells (RBCs)—by ultrasound spectral analysis of back-scattered echoes from these cells. Highly focused single-element high-frequency ultrasound microbeams were shown to be capable of distinguishing human red blood cells from human leukemia cells in these experiments. The potential of cell sensing with highly focused ultrasound microbeam is clearly demonstrated.

II. Materials and Methods

A. Live Cells Preparation

Human leukemia cells (K-562 cell line, ATCC CCL-243) were cultured in Dulbecco's modified eagle medium (DMEM; GIBCO, Invitrogen, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin–neomycin (PSN; GIBCO, Invitrogen) in an incubator supplied with 5% CO₂ and set at 37°C. Human RBCs obtained from a volunteer were prepared and stored in a mixed solution of Alsever's solution (Sigma-Aldrich, St. Louis, MO, USA) for its anticoagulant and blood preservative properties due to isotonic and balanced salt solution and phosphate-buffered saline (PBS) for cell sensing experiments with high-frequency ultrasound.

B. High-Frequency Ultrasound System

A high-frequency ultrasound scattering measurement system was custom designed and built for minimizing noise and allowing better mobility as part of an ultrasound cell sorting system which requires several modules such as power amplifier (PA), pre-amplifier, and protection circuits. The PA is used to drive the ultrasonic transducers and the pre-amplifier is used to amplify the echo signal to allow detection. As the operating frequency of the transducer increases, the PA design becomes more challenging because of the more demanding specifications, including higher noise level and wider bandwidth of the

ultrasound system. Another issue is that the commercial equipment is quite bulky, so long coaxial cables must be used for integration with other components, causing a cable loading effect on the high-frequency transducer performance which must be minimized. The block diagram of this low-noise and wideband portable all-in-one system that was developed for this application is illustrated in Fig. 1(a). Fig. 1(b) shows the high-frequency ultrasound system, consisting of a power amplifier, a pre-amplifier, and protection circuits. The power amplifier has a gain of about 40 dB in the range of 40 to 120 MHz and lower noise amplitude (up to 20 mV_{pp}) than common commercial power amplifier's noise amplitude (>100 mV_{pp}). The pre-amplifier gain is ~30 dB and the noise figure is less than 3 dB. The diode-based protective expander and limiter were built for protecting the power amplifier from low-voltage echo signals and the pre-amplifier from the high-voltage signals of the power amplifier, respectively. A function generator (AFG3251, Tektronix, Beaverton, OR, USA) was used to send a sinusoidal burst signal to the system.

C. High-Frequency Ultrasound Transducer

An 86-MHz lithium niobate press-focused single-element acoustic transducer was designed with a Krimholtz, Leedom, and Matthaei (KLM) model and fabricated [18]. Depending on the material properties of lithium niobate single crystal, the optimized thickness of the active element was 34 μm with an aperture size of 2.0 mm. The first acoustic matching layer was a $\lambda/4$ -thick (3 μm) silver epoxy made from a mixture of silver particles (2 to 3 μm diameter) and electrical insulating epoxy was cast onto the negative side of the chrome / gold sputtered element. A very lossy (attenuation of 112 dB/mm at 30 MHz) conductive silver epoxy (E-Solder 3022, Von Roll Isola Inc., New Haven, CT, USA) was served as a backing layer, which was cast onto the positive side of the sample. The acoustic stack was then press-focused at a focal length of 1.5 mm so that the f-number of the device was as small as 0.75. A 3.2 μm -thick parylene layer (PDS2010 Labcoater; Specialty Coating Systems Inc., Indianapolis, IN, USA) was vapor-deposited on the front face of the transducer to serve as the second acoustic matching layer and as a protective layer. The transducer was finally assembled in an SMA connector for further experiments. The performance of the transducer was measured by a pulse-echo method from a quartz plate reflector with a pulser/receiver (Model 5900PR, Panametrics-NDT, Waltham, MA, USA) in 0 dB amplifier gain because reliable commercial hydrophone measurement over 60 MHz is not available. The center frequency of the single-element transducer was found to be 86 MHz and the -6-dB bandwidth was ~37%, as shown in Fig. 2. The theoretical axial resolution and lateral resolution, i.e., the -6-dB beam width at focus in water, were calculated to be ~23 μm and ~13 μm , respectively.

D. Ultrasonic Spectrum Analysis

Parameters extracted from ultrasonic spectrum analysis of echoes from tissues in the frequency domain have been shown to yield additional information for clinical diagnosis [19]–[27]. Calibration procedures in the analysis can reduce system artifacts and noise, and allow quantitative measurements of backscattered signals [28]. These parameters likewise can be applied to single microparticle sensing because they are related to particulate acoustic and physical properties. Three types of ultrasonic spectral parameters were employed for

microparticle discrimination in these experiments. Peak-to-peak amplitude of the echo signal was measured as well.

1. Integrated backscatter (IB) coefficients, in decibel units, use a frequency-domain averaging technique and can reduce the effects of frequency-dependent fluctuations resulting from inhomogeneous scattering distributions in tissues [19]. The IB coefficient is defined as the ratio of the frequency average of the backscattered energy from a scattering volume over the bandwidth of the signal to the one from a flat quartz or glass reflector [20]. In this paper, the reference signal of the 86-MHz transducer was measured from a flat quartz reflector.

$$IB = 10 * \log_{10} \left(\frac{1}{2\Delta f} \int_{f_c - \Delta f}^{f_c + \Delta f} \frac{|V(f)|^2}{|R(f)|^2} df \right),$$

where $R(f)$ and $V(f)$ are the spectra of the reference and sample signals, respectively. The symbol f_c denotes the center frequency signal and Δf is the bandwidth of $R(f)$. In this study, the -6 -dB bandwidth (69 MHz to 98 MHz) was used to calculate the IB coefficient, spectral slope, and spectral intercept.

2. Spectral slope, in units of decibels per megahertz, and spectral intercept, in decibels, [21], [22] utilize the regression line of the spectral band. Spectral slope is the slope of the regression line, which is computed from the calibrated power spectrum. The calibrated power spectrum is plotted by RF spectrum from sample echo over calibration spectrum of echo signal of a flat reflector. Spectral intercept can be calculated by the extrapolation of the regression line to zero frequency.
3. Midband fit, in decibels, is defined as the value of the regression line at the center frequency of the analyzed spectral band [24]–[26]. The midband fit value is directly related to IB coefficient [27].

Fig. 3 shows how the spectral slope, intercept, and midband fit were calculated.

E. Equipment Setting

To assess the performance of sensing and identify live single cells with this approach, a tissue-mimicking phantom, an agar substrate of 500 μm thickness, was fabricated in a 30-mm-diameter culture dish as a measurement platform because the acoustic impedance of agar substrate (1.50 to 1.55 MRayls) is similar to that of water (1.48 MRayls) [29].

The phantom materials were manufactured with gelatin or agar gel and graphite powder. Agar gel powder (Agar A360–500, Fisher Scientific, Pittsburgh, PA, USA) was hydrated with deionized water and n-propanol. The solution was stirred slowly to avoid clumps during hydration, and then placed in a vacuum (66 to 73 cmHg) for a few minutes to degas the solution. After this process, it was stirred to avoid formation of a thin film on surface and heated on a hotplate to around 70°C in a water bath to make the solution transparent by dispersing colloids, and to release gasses. After reaching a temperature over 70°C, the mixture was heated directly on the hotplate until the temperature reached 90°C with vigorous stirring to prevent burning of the agar solution at the bottom of beaker. Then, the

mixture was degassed for short sessions to draw trapped air bubbles to the surface. After the gel solution had been liquefied by these processes, graphite powder (silicon dioxide, Sigma-Aldrich) of particle size between 0.5 μm and 10 μm was added to increase scattering and absorption, as in typical tissue-mimicking phantoms. However, the graphite powder was not added to the transparent window for optical microscopy in this case. The phantom material was then rotated to be homogeneously mixed in the solution, and heated to prevent gelation. In the last process, the mixture was cooled in the water bath until around 45°C to allow cross-linking while slowly stirring, and then the solution was poured into the mold to produce phantom materials. The designed phantom measurement platform was acoustically and optically transparent. The echo signal from surface of the fabricated phantom was collected for measuring thickness using a commercial pulser/receiver (5900PR, Panametrics). as shown in Fig. 4. The phantom thickness was $\sim 680 \mu\text{m}$.

The cultured K562 cells and RBCs were suspended in a mixture of Alsever's solution and PBS, and then placed on the top of an optically transparent agar substrate in the 30-mm culture dish, where cells were monitored by an inverted microscope (IX-71, Olympus, Tokyo, Japan). The ultrasound transducer was located at the focal distance from the agar surface, using the echo signal from the pulse–echo measurement method. The beam center of the ultrasound transducer was placed in the center of the microscope's field of view. The transducer was then used to interrogate the cell from above, driven in a sinusoidal burst mode whose peak-to-peak voltage amplitude was as high as 40 V. A single-cycle pulse was applied by a custom-made high-frequency ultrasound system and a function generator (AFG3251, Tektronix, Beaverton, OR, USA). The pulse repetition frequency was set to 200 Hz. Prior to the cell sensing experiment, a pulse–echo test was performed to ensure that the distance between ultrasound transducer and the phantom surface was the focal length of ultrasound transducer. The microscope stage was manually moved to place a single cell near the center of ultrasound beam in the focal plane. A single cell was then randomly targeted within the microscope's field of view. After a single cell was located at the ultrasound beam center, the position of the stage was fixed to reduce signal distortion by moving the stage, and then 8 echo signals from a single live cell were collected and averaged. Results were acquired from 50 cells to allow statistical analysis. Fig. 5 shows the experimental setting and a schematic diagram. Fig. 6 shows an optical micrograph of the mixed samples of K562 cells and RBCs. Note that the beam center is indicated by a pink circle at the screen's center.

When a single live cell on the substrate surface was moved to the focal position of ultrasound beam, eight segment-averaged echo signals were collected by an oscilloscope (WaveRunner 104MXi, LeCroy, Santa Clara, CA, USA) and analyzed by custom developed LabVIEW program. 50 cells of each cell group were measured without other signal processing, including filtering and windowing. Fig. 7 shows the representative measured echo signals and their frequency responses from a single live cell at the beam center in the focal plane of transducer, a K562 cell in Figs. 7(a) and 7(b) and an RBC in Figs. 7(c) and 7(d). The representative normalized calibrated spectra of a K562 cell and an RBC are shown in Figs. 7(e) and 7(f), respectively.

III. Results and Discussion

Amplitudes of measured backscattered signals from K562 cells and RBCs are shown in Fig. 8(a). The amplitudes of echo signals from K562 cells and RBCs at 86 MHz were 48.25 ± 11.98 mV_{pp} and 56.97 ± 7.53 mV_{pp}, respectively. The IB coefficients of the two different types of cells on the agar surface were -89.39 ± 2.44 dB for K562 cells and -89.00 ± 1.19 dB for RBCs, as shown in Fig. 8(b). The spectral slope in Fig. 8(c) and intercept in Fig. 8(d) were 0.30 ± 0.19 dB/MHz and -56.07 ± 17.17 dB, respectively, for K562 cells and 0.78 ± 0.092 dB/MHz and -98.18 ± 8.80 dB, respectively, for RBCs. Note that these graphs are illustrated with standard deviation whiskers. Midband fits of K562 cells and RBCs were -31.02 ± 3.04 dB and -33.51 ± 1.55 dB, respectively. Their values showed statistical difference between the two graphs ($p < 0.001$). A midband fit graph is not presented in Fig. 8. Acoustic cellular discrimination via these parameters was tested. The spectral parameters, including amplitude, except for the IB value, showed statistically significant differences by Student's *t*-test. These results suggest that cell sensing and sorting decisions can be made with these parameters in real time with high throughput.

Because the cells were placed on the agar surface, the collected scattering signals from a single live cell included the reflected signal from the agar surface. To minimize influence of the reflection signal, an agar phantom without graphite powder was selected because the echo amplitude from an agar surface is minimized in this way because its acoustic impedance is very similar to that of water. Although the echo from the agar surface may influence the echo from a cell, its amplitude is much lower and its effect should be negligible. This assumption was validated by calculating the backscatter parameters after subtracting out the agar surface echo and comparing to those with surface echo. The results are similar. Therefore, it is safe to conclude that the effect of agar surface echo on the scattering results estimated for cells is negligible.

K562 cells and RBCs have different volumes and sizes, as well as different physical properties. Despite these differences, the difference between the echo signals was found to be statistically significant ($p < 0.001$), although the IB values were similar and could not discriminate those cells. The reason for this may be that IB denotes an averaged value over the spectral bandwidth. In the scattering regime where $ka > 1$, the slope rapidly decreases as scatterer size increases, whereas intercept increases rapidly [27]. The spectral slope of smaller particles should be larger than that of larger particles and the spectral intercept is the opposite. In this study, K562 cells having a size of 13 to 20 μm are bigger than RBCs having a size of 7 to 8 μm . As expected, the spectral slope of K562 cells was smaller than that of RBCs and the spectral intercept of K562 cells was larger than that RBCs. These spectral parameters also have relationships with interior structures in tissue characterization, as previous researchers have shown [19]–[27]. However, the relationship between ultrasound spectral parameters and microstructure at the single micro-object scale is not clear in high frequency. Further research is required for cell sensing or discrimination.

Ultrasound scattering for $ka < 1$ can be predicted by Rayleigh scattering theory, where k is the wave number and a is the radius. Under the condition $ka < 1$, ultrasound backscattering is not affected by the shape of scatterers [30]. Savery and Cloutier reported that ultrasound

backscattering behavior of RBCs as a function of frequency is very similar to Rayleigh scattering below 87 MHz with analytical and semi-analytical models study [31]. However, they show that the theoretically derived and experimentally measured angular dependence of scattering started exhibiting a slight discrepancy at $ka = 0.6$ and reached 10 dB at 87 MHz, where $ka = 1$ for RBCs. In this paper, although the RBCs have shape of biconcave discs, they were treated as spheres of the same volume. The ultrasound spectral parameters using 86 MHz for RBCs were calculated as a sphere of same volume.

Box plots with 5%–95% whiskers were employed in Fig. 9 to show the distribution of ultrasonic spectral parameters. Outliers are represented by solid dots and middle lines in boxes indicate median value of each group data set ($n = 50$). Dot-plots in Fig. 10 show two ultrasound spectral parameters simultaneously. In these graphs, each dot indicates the ultrasonic spectral parameter of a sample cell. Voltage value in Figs. 10(a)–10(c) are shown in the range from 0.03 to 0.08 V_{pp}, whereas IB values are from –94 to –83 dB. Clustered dots in Fig. 10(a) by voltage and IB values are not clearly separated. However, K562 cells and RBCs can be clearly identified as two groups using a two-dimensional dot-plot of voltage and slope or voltage and Y-intercept, as illustrated in Fig. 10(b)(c). Slope and Y-intercept plots with IB in Figs. 10(d) and 10(e) allow two groups to be separated as well. Compared to other plots, Fig. 10(f) shows a better and clearer grouping of the two cell types. In Fig. 11, the correlation of three types of ultrasound spectral parameters is shown with two separate groups.

The biological effect of highly focused high-frequency ultrasound beam is of critical concern when live cells are exposed to ultrasound. More studies will need to be carried out to examine the effect of ultrasound microbeams on cells, where damages may be caused by heating and high pressure if the energy level is sufficiently high. Mechanical index (MI) and thermal index (TI) have been employed for many years to evaluate the exposure levels of conventional medical ultrasound in the frequency range from 2 to 20 MHz because of their quantitative nature in estimating the risk of adverse ultrasonic effects caused by the non-thermal and thermal mechanisms. However, there are still no general guidelines to consider the safety of high-frequency ultrasound. Because at higher frequencies the beam is more focused and the energy more concentrated in a smaller area, heating and other bioeffects may result, causing cell damage during sensing or sorting. In addition, analytical error may result from damaged cells.

A FACS labels different cells with different fluorescent molecules. This is a time-consuming process. An ultrasound cell sorter, on the other hand, is label free and is capable of cell sensing by merely adopting several ultrasound backscattering parameters, for instance from spectral analyses discussed in this paper, and angular scattering. It was shown previously that the spectral analysis, although rather complicated, can be easily carried out in real time [6], [7]. By combining fast ultrasound sensing capability with ultrasound cell sorting, it is envisioned that an ultrasonic cell sorter may become an alternative to FACS with more developments in the future.

It should be noted here that this is only the first step in the development of ultrasonic cell sorters. More in-depth study on a large number of cells with a full spectrum of statistical analyses will be needed to demonstrate its feasibility.

The concentration of circulating tumor cells (CTCs) is typically extremely low, in the order of 5~100 cells per milliliter of blood [32], compared with RBCs and white blood cells (WBCs). Although CTCs are not fully understood, the potential clinical value of CTCs has long been recognized in the early diagnosis of cancer which allows treatment of metastatic spread [33]. Huang *et al.* showed that microfluidic devices can be employed for probing CTCs [34]. Integration of high-frequency ultrasound scattering and a microfluidic device may allow for sensing and sorting of CTCs for the diagnosis of tumor metastasis.

IV. Conclusion

Previous studies have reported acoustic devices using acoustic radiation force for single-cell based sorting which integrate the advantages of microfluidic channel and highly focused high-frequency ultrasound microbeams [7]. The advantages of these devices are their simpler structure and higher sensitivity for sensing, and their capability of identifying and sorting of flowing particles in a microchannel with minimal time delay between the sensing and sorting steps. This work evaluated the sensing and characterization aspects of ultrasonic sorting through the use of two different types of live cells, leukemia (K562) and red blood cells. These results demonstrated the feasibility of ultrasonic scattering as a sensing mechanism in the development of ultrasonic cell sorters. In the course of this effort, it was also shown that it is possible to measure ultrasonic scattering from a single cell.

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Biographies

Changyang Lee received the B.S. degree in mechanical engineering from Kookmin University, Seoul, Korea, in 2002 and the M.S. degree in mechanical engineering from Korea University, Seoul, Korea, in 2004. He worked in the Biomedical Science Center at the Korea Institute of Science and Technology from 2002 to 2008 as a researcher. He had received the Ph.D. degree in biomedical engineering at the University of Southern California under the direction of Prof. K. Kirk Shung in 2014. He is currently a postdoctoral research

associate in the National Institutes of Health Resource Center for Medical Ultrasonic Transducer Technology at the University of Southern California, Los Angeles, CA. His research interests include biomedical applications using high-frequency ultrasound and cell mechanobiology.

Hayong Jung received his B.S and M.S degrees in electrical engineering from Kwangwoon University, South Korea, in 2007 and 2009, respectively. He received his M.S degree in electrical engineering from the University of Southern California (USC), Los Angeles, CA, in 2013. He is currently a Ph.D. candidate in the Department of Biomedical Engineering at USC. He is conducting his research in the National Institutes of Health Ultrasonic Transducer Resource Center (UTRC) on the design of high-frequency ultrasound imaging systems and applications under the direction of Dr. K. Kirk Shung.

Kwok Ho Lam received the M.Phil. and Ph.D. degrees in applied physics from The Hong Kong Polytechnic University (HKPolyU). During his graduate studies, he worked on the processing and characterization of piezoelectric materials and their applications in sensors and actuators. Dr. Lam was a visiting scholar at Stanford University in 2007. From 2007 to 2009, he received the HKPolyU-funded Postdoctoral Research Fellowship and worked on the applications of smart devices in civil engineering structures at HKPolyU. Dr. Lam became a Research Associate at the National Institutes of Health Resource Center on Medical Ultrasonic Transducer Technology in the Department of Biomedical Engineering of the University of Southern California (USC) from 2011 to 2013. Dr. Lam is currently an Assistant Professor in the Department of Electrical Engineering at HKPolyU. His research interests include multifunctional smart materials, smart sensor and actuator technology, ultrasound transducer technology, condition and structural health monitoring, and biomedical applications of ultrasonic transducers.

Changhan Yoon received his M.S. and Ph.D. degrees in electronic engineering in 2009 and 2013, respectively, from Sogang University, Seoul, Korea. He is currently a postdoctoral research associate in the National Institutes of Health Resource Center for Medical Ultrasonic Transducer Technology at the University of Southern California, Los Angeles, CA. His main research interests include medical ultrasound and photoacoustic imaging systems, clinical applications, and ultrasound microbeams.

K. Kirk Shung received a Ph.D. degree in electrical engineering from the University of Washington, Seattle, WA, in 1975. He was a faculty member at Penn State University, University Park, PA, until 2002, when he moved to the Department of Biomedical Engineering, University of Southern California, Los Angeles, CA, as a professor. He was named a dean's professor in biomedical engineering in 2013. He has been the director of the National Institutes of Health Resource Center on Medical Ultrasonic Transducer Technology since 1997.

Dr. Shung is a life fellow of IEEE and a fellow of the Acoustical Society of America and the American Institute of Ultrasound in Medicine. He is a founding fellow of American Institute of Medical and Biological Engineering. He received the IEEE Engineering in Medicine and Biology Society Early Career Award in 1985, and was the coauthor of a paper that received the best paper award for the *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control* (UFFC) in 2000. He was selected as the distinguished lecturer for the IEEE UFFC society for 2002. In 2010 and 2011, he received the Holmes Pioneer Award in Basic Science from American Institute of Ultrasound in Medicine and the academic career achievement award from the IEEE Engineering in Medicine and Biology Society. He is the recipient of the 2016 IEEE Biomedical Engineering Award.

Dr. Shung has published more than 500 papers and book chapters. He is the author of the textbook *Principles of Medical Imaging*, published by Academic Press in 1992, and two editions of the textbook *Diagnostic Ultrasound: Imaging and Blood Flow Measurements*, published by CRC Press in 2005 and 2015. Dr. Shung is currently serving as an associate

editor of the *IEEE Transactions on Biomedical and Engineering*, the *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control*, and *Medical Physics*. Dr. Shung's research interest is in ultrasonic transducers, high-frequency ultrasonic imaging, ultrasound microbeams, and ultrasonic scattering in tissues.

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Fig. 1.

Block diagram of the custom-designed ultrasonic scattering measurement system. (a)

Designed module as parts for all-in-one high-frequency ultrasound system. (b) The developed system has a power amplifier of ~40 dB gain in the range 40 to 120 MHz, a pre-amplifier of ~30 dB gain and low noise figure (<3 dB), and diode-based protective circuits: an expander for protecting the pre-amplifier from high-voltage driving signals and a limiter for protecting the power amplifier from low-voltage echo signals.

(a)

(b)

Fig. 2.

(a) Pulse-echo signal from flat quartz and its spectrum from the fabricated high-frequency single-element ultrasound transducer. (b) Fabricated LiNbO_3 high-frequency single-element ultrasound transducer. Note that the echo signal was used as the reference signal for analyzing ultrasonic spectral parameters.

Fig. 3.

Calculation of ultrasonic spectral parameters. (a) RF spectrum from sample (solid line) and flat quartz reference spectrum for calibration procedure from a flat reflector (dashed line). (b) Normalized calibrated spectrum (solid line) calculated by RF spectrum over quartz reference spectrum in spectral bandwidth of reference spectrum and linear regression line (dashed line). Spectral slope (slope of regression line) and intercept (INT) at zero frequency by extrapolation, and midband fit (MBF) at center frequency, f_0 .

Fig. 4.

A pulse–echo signal from agar surface for location of ultrasound transducer. The echo signal from the agar surface (indicated in the red box) measured using a commercial pulser/receiver (5900PR, Panametrics, USA). Measured agar phantom thickness was $\sim 680\ \mu\text{m}$.

Fig. 5.
(a) Photograph and (b) schematic diagram of the experimental configuration.

Fig. 6. Leukemia cells (K562) and red blood cells. The 10-µm bead was placed on the agar surface for comparing cell size. The pink circle and its center indicate the ultrasound beam and its center.

Fig. 7.

Measured echo signals and ultrasonic spectrum analysis from single live cell in the beam center on the focal plane of the transducer: [(a) and (b)] K562 cell and [(c) and (d)] RBC. The normalized calibrated spectra of (e) a K562 cell and (f) an RBC for calculating ultrasound spectral parameters are also shown.

Fig. 8.

Ultrasonic spectral analysis of scattered echo signals from 50 samples of a live single RBC and K562 cell. (a) Amplitude of echo signals of a K562 cell and an RBC, (b) Integrated backscatter coefficient in decibels of a K562 cell and an RBC. (c) Spectral slope in decibels per megahertz of a K562 cell and an RBC. (d) Spectral intercept in decibels of a K562 cell and an RBC. **** $p < 0.0001$; ns = no statistically significant difference between conditions ($p > 0.05$).

Fig. 9.

Box plots with 5%–95% whiskers (echo amplitudes, IB coefficients, spectral slope, and intercept). Outliers were presented on each dot and the middle line of each box is the median of the group.

Fig. 10.

Relations between parameters (echo amplitudes, IB coefficients, spectral slope, and intercept).

Fig. 11.
Correlations of ultrasonic scattering parameters.