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John Essick, *Editor*
Department of Physics, Reed College, Portland, OR 97202

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Acoustic trapping in the undergraduate laboratory

Andrea Boskovic,[a] Kate M. Jones,[b] Alejandra Velasquez,[c] Isabel P. Hardy,[d] Maya L. Bulos,[e] and Ashley R. Carter[f]
Department of Physics and Astronomy, Amherst College, Amherst, Massachusetts 01002

Martin Wiklund[g]
Department of Applied Physics, KTH-Royal Institute of Technology, Stockholm 100 44, Sweden

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Acoustic trapping is used in modern biophysics laboratories to study cell adhesion or aggregation, to sort particles, or to build model tissues. Here, we create an acoustic trapping setup in liquid for an undergraduate instructional laboratory that is low-cost, easy to build, and produces results in a 1-hour laboratory period. In this setup, we use a glass slide, cover slip, and double-sided tape to make the sample chamber. A piezo-electric transducer connected to a function generator serves as the acoustic source. We use this setup to measure the node spacing (millimeters) and the acoustic trap force (picoNewtons). We anticipate that the simplicity of the experimental setup, the tractability of the theoretical equations, and the richness of the research topics on the subject will lead to an undergraduate laboratory with many interesting student projects.
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I. INTRODUCTION

Acoustic levitation in air was first observed by August Kundt in 1866.[1] In this landmark experiment, Kundt placed a small amount of fine powder along the bottom inner length of an air-filled tube and attached a metal rod at one end of the tube. He then created a vibration in the metal rod to transmit a longitudinal acoustic wave into the tube and adjusted the tube's length. Kundt observed that when the standing wave was present, the powder within the horizontal tube would rise up from the bottom of the tube into a series of vertical lines. The powder would not only levitate, but be trapped in the line, creating a striking pattern within the tube that he called a "dust figure." This experiment, later dubbed Kundt's tube, is still used to demonstrate standing waves and acoustic trapping.

Today, acoustic traps are used in many biophysical or biomedical applications. This is because acoustic traps are (i) biocompatible and will not damage cells, (ii) can trap many different kinds of objects, (iii) are low-cost with a simple design and build, and (iv) have a compact design that is particularly amenable to manipulate cells or particles.[2–8] Acoustic traps can be used to rapidly differentiate cells by their motility,[9] to pattern cells and build model tissues,[5] or to interact cells with each other to study cell-cell interactions.[2] Likewise, acoustic traps can facilitate particle aggregation,[10] sort particles based on size or material properties,[11] or create enriched or dilute regions of particles at a particular location.[12] Here, our goal is to create a simple acoustic trap that can be used in the undergraduate instructional laboratory.

In today's acoustic traps, the source of the acoustic wave is often a piezo-electric transducer, which consists of a material that changes thickness with voltage.[13] When an ac voltage is applied to the transducer, the transducer oscillates in thickness, producing an acoustic wave in its surrounding medium (e.g., air and water). Often piezo-electric transducers have frequencies f in the MHz range,[14] which produce wavelengths λ in water that are in the millimeter range ($\lambda = v/f$, where the velocity of sound in water v is 1500 m/s). In addition, piezo transducers have acoustic energy densities U/V of 10–100 J/m^3, which produce forces on the order of pN.[14] Forces on this scale are perfect for trapping micron-sized objects in water such as polystyrene beads or cells. Finally, the chamber used in acoustic trapping experiments is often a fabricated chamber that has a perfectly tuned spacing to produce standing waves.[15]

Though others have described acoustic levitation demonstrations,[16–19] here we would like to be able to create an acoustic trap in liquid for undergraduates. This task presented several challenges. First, we needed to create a simple, low-cost sample chamber. Typically, much care is taken in fabricating a sample chamber so that it has an exact spacing and so that its geometry and material are conducive to coupling of the acoustic source.[8,15] Second, we needed an

acoustic source that would create an acoustic wave powerful enough to trap particles in liquid. Often piezo transducers for acoustic traps have amplifiers that can be fairly complex and expensive (>$10,000).[20,21] Finally, we needed to couple the transducer to the sample chamber. Typically, transducers are fabricated into the sample chamber at well-defined distances and orientations.[14,15]

We overcame these challenges to create a simple experimental setup for undergraduates that could be employed in a one-hour lab period. We use sample chambers made from double-sided tape, microscope slides, and glass cover slips to create microfluidic channels. These sample chambers are cheap (<$1), simple to assemble, and can be updated by students to incorporate multiple channels or varying designs. We use a piezo-electric transducer for an acoustic source and are able to create a strong enough acoustic wave using a standard function generator found in the undergraduate laboratory. Finally, we found that placing the piezo transducer on top of the glass cover slip with a thin layer of microscopy oil was enough to couple the acoustic waves into the chamber. With this system, we were able to measure the spacing of the nodes of the acoustic trap and the force of the acoustic trap, quantities on the order of mm and pN, respectively. This project gives students an opportunity in the undergraduate laboratory to design and build acoustic traps similar to the ones being used in modern research laboratories.[5]

II. EXPERIMENTAL MATERIALS AND METHODS

A. Sample

The sample chamber is made by attaching a rectangular, glass cover slip (Fisher, 12–544-B, $40 \times 22\,\text{mm}^2$, thickness of 0.16–0.19 mm) to a rectangular, glass microscope slide (Corning, 294 775 × 25, $75 \times 25\,\text{mm}^2$, thickness of 0.96–1.06 mm glass). This type of the sample chamber is often used in optical trapping experiments.[22,23] Attachment is achieved by cutting a 12.7-mm-wide (90-μm-thick) piece of double-sided tape (Scotch) into two equal strips. The double-sided tape is placed between the glass cover slip and microscope slide. This tape acts as a spacer, creating a microfluidic channel that holds about 20 μl of liquid. While the tape can be placed to create multiple channels or different pathways, here we use the tape to create a single channel with a straight path. The edges of the cover slip are sealed to the microscope slide with 5-min Epoxy (Devcon, 20445) to prevent disassembly of the sample chamber. The entrance and exit of the channel is open. Thus, the cavity consists of two open air ends with a solution in between.

The solution in the sample chamber is a solution of polystyrene beads (Invitrogen, C37253, 4% w/v, CML Latex, 3.9-μm-diameter) in deionized water. These beads are large enough to see under a microscope with a 40× objective. Beads of at least 2–3 μm in diameter are known to undergo acoustic trapping with a 2 MHz acoustic source without the influence of acoustic streaming.[7] To create the bead solution, we dilute the stock solution by a factor of 3 for experiments without a microscope. Specifically, we add 20 μl of bead stock to 40 μl of de-ionized water. For experiments with a microscope, we dilute by a factor of 100 (1 μl of bead stock to 99 μl of deionized water) so that we can resolve individual beads in the field of view. We pipette 20 μl of the bead solution onto the edge of the sample chamber and wait for the solution to enter the chamber via capillary action. To exchange solutions, a pipette tip attached to a vacuum is necessary to remove the solution from the chamber and create a flow to deposit a new solution. Once the solution is in the chamber, we seal the sample chamber at both the channel entrance and exit using nail polish or epoxy in order to limit evaporation. An air layer should be left between the solution and the nail polish or epoxy.

B. Acoustic source

The acoustic source in our setup is a piezo-electric transducer [American Piezo, 10-mm-diameter disc, 840 material, lead zirconate titanate (PZT), 0.8–1.5 MHz resonance frequency, wrap-around electrode]. A transducer with similar material properties from Meggitt Ferroperm (PZ26) was also used. We choose the 840 (or PZ26) material due to its use in high-power ultrasonic applications. We solder wires to the positive and negative electrodes of the piezo transducer using lead-based solder and a soldering iron below the Curie temperature (325 °C for the 840 material) of the transducer. At the other end of the wires, we solder a female BNC connector, which allows easy attachment to the function generator.

C. Determining the resonance frequency of the piezo transducer

To create acoustic standing waves, we need to apply a voltage at the resonance frequency of the piezo transducer. This resonance frequency depends on the composition, shape, and volume of the transducer.[15]

To measure the resonance frequency (Fig. 1), we first connect a function generator (Agilent, 33220A) to an oscilloscope (Tektronix, TDS2024C) and the piezo transducer. Second, we output a square wave on the function generator with the maximum peak-to-peak voltage (12 V) and tune the frequency of the wave to the nominal resonance frequency. If the piezo transducer is not connected or if the function generator is off-resonance, a square wave forms on the

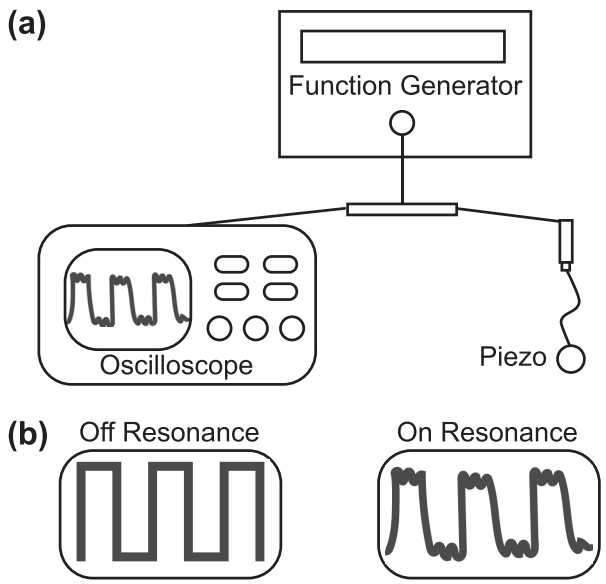

Fig. 1. Setting up the transducer. (a) A function generator is connected to an oscilloscope and a piezo transducer. (b) When the frequency of the square wave output by the function generator is off-resonance, the oscilloscope displays a square wave. When the frequency is on-resonance, the oscilloscope displays a square wave with feedback from the transducer.

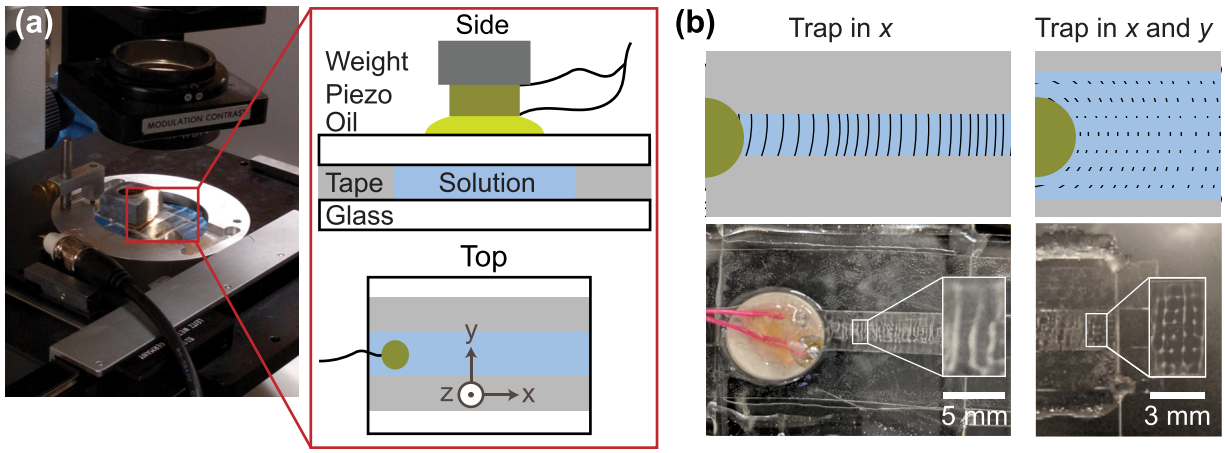

Fig. 2. Measuring the node spacing. (a) Cartoon of the sample chamber and picture of the chamber in the microscope. The chamber is created by attaching a glass cover slip to a microscope slide with double-sided tape. The piezo transducer is coupled to the chamber using a weight and a thin layer of oil. The transducer is placed at one edge of the sample chamber to create an acoustic trap in the lateral dimensions, channel length x and channel width y. There is no trapping in z due to the thickness of the chamber. Solution is 4-μm-diameter beads in water. (b) If the spacing between the tape is less than $\sim$1 cm (left), a trap only forms in the x-dimension, creating a series of lines. If the spacing between the tape is larger than $\sim$1 cm (right), a trap forms in both the x- and y-dimensions, creating a series of dots. *Zoom in*. Pattern has millimeter spacing. Scale bar gives length of image.

oscilloscope. However, when the transducer is connected and the function generator is on-resonance, the transducer outputs significant feedback. This is because when you excite the piezo transducer with a square wave, it oscillates at the fundamental frequency and all of the odd harmonics. These harmonics show up on the oscilloscope as smaller-amplitude, higher-frequency sinusoidal waves on top of the square wave.

D. Producing an acoustic trap

To produce an acoustic trap (Fig. 2), we adhere the piezoelectric transducer to the top of the cover slip and use a coupling liquid, which removes any air gaps between the cover slip and the transducer. A suitable coupling liquid might be glycerol, but here we use a thin layer of microscope immersion oil (Nikon, Type F). The thin layer of coupling liquid and reduced thickness of the cover slip improve coupling of the acoustic wave. The wires should be soldered to the transducer so that the transducer lies flat on the glass cover slip. Coupling with immersion oil as opposed to epoxy allows for transducers to be reused and avoids air bubbles.

We then viewed the bead solution in the sample chamber while operating the function generator at the resonance frequency. Here, we used a square wave, though a sine wave could be used as well. It is also possible to use a clock generator to drive the transducer for low power applications.[21] Turning on the function generator turns on the acoustic trap. When the acoustic trap is on, the beads that are not at the trap location begin to move toward the node. For 4-μm-diameter polystyrene beads in water at 1.5 MHz, the beads move toward the trap location at the pressure nodes. Other experimental parameters could create a trap location at the velocity nodes.[7,13] After a few seconds or minutes, we can see lines of beads forming inside of the sample chamber by eye. This is similar to the "dust figures" in the Kundt's Tube demonstration. It is important to conduct these experiments at or near the resonance of the transducer to produce a strong enough acoustic wave for trapping.

Sometimes we saw trapping in more than one dimension.[24,25] If the sample chamber had a channel width (a spacing between the tape) that was less than $\sim$1 cm, a standing wave formed along the channel length (x-dimension), but not along the channel width (y-dimension). If the channel width was greater than $\sim$1 cm, there was a standing wave in both the x- and y-dimensions. A larger channel width makes it easier to fulfill the condition for a standing wave. Specifically, there is a given width of the resonance peak in the frequency spectrum. If the channel width is larger, then it is more likely that the channel width will be a half-wavelength multiple of one of the frequencies in the resonance peak. Trapping in two dimensions causes the pattern to look like a series of dots.

Sometimes we did not see trapping. One issue is that the length of the channel may not be ideal for the creation of a standing wave. To fix this problem, we moved the transducer slightly or changed the frequency of the function generator slightly. Another issue occurs if the sample chamber has been sitting out too long. In that case, the beads collect at the bottom of the chamber due to gravity. Storing the chamber in an inverted position with the cover-slip-down (and then flipping the chamber over before use) solves this problem. Another solution to this problem is to use beads that are smaller in diameter. These beads will have a smaller gravitational force and will take longer (days) to collect at the bottom of the chamber. However, we note that beads less than 2 μm in diameter will be influenced by acoustic streaming (a second order effect that produces circulation) if a 2 MHz source is used.[7]

E. Measuring node spacing

To measure the spacing between nodes in a one-dimensional acoustic trap, we use two different methods. In both methods, we measured the distance between multiple lines of trapped beads and divided by the number of lines to acquire the node spacing. For example, if the distance between the first line and the fifth line is 3 mm, then the spacing is $3\,\text{mm}/4 = 0.75\,\text{mm}$. In the first method, we use a ruler to measure the node spacing. In the second method, we took a picture of the sample chamber and measured the node spacing in ImageJ in pixels. The conversion back to

millimeters was determined by measuring the width of the 22-mm-wide cover slip in pixels. We report values with the second method.

We repeated the node spacing measurements for three or more sample chambers and recorded the mean as the measurement and the standard deviation as the uncertainty. We plot the standard deviation rather than the standard error of the mean to give a sense of the variation. Some student groups had much more reproducible measurements with uncertainties of ∼0.05 mm in their node spacing, while other groups had uncertainties of 0.25 mm.

When measuring the node spacing, we note that complete evaporation of the liquid will occur after several minutes, especially in an unsealed channel. Once the liquid has completely evaporated, the beads cannot move, and the pattern of lines is fixed. This may be useful since the pattern of lines is easier to see. (The refractive index change between air and polystyrene is higher than the refractive index change between water and polystyrene.) However, the evaporation process does cause smearing of the pattern, so measurements should be taken when the liquid is present.

F. Imaging acoustic trapping with a microscope

To image acoustic trapping of individual beads, we use a light microscope (Leitz, Labovert FS, 40× objective in air) and a megapixel camera (Thorlabs, DCC1545M, 5 ms exposure, 15 Hz, 60 nm per pixel). When the trap is turned on, beads not at the trap location will feel a force toward the trap location. We use a microscope to record this bead movement.[26] Samples were placed in the microscope sample holder such that the piezo transducer was not in the way of the imaging plane. We use ImageJ to extract individual bead positions from the video and form tracks.

To measure the bead velocity during acoustic trapping, we measure the average velocity of three individual beads in the same video, and then calculate the mean of the measurements and the standard deviation. To measure the average velocity of an individual bead, we measured the positional difference of the bead in the last frame vs the first frame, and then divided by the time between the first and last frame.

III. OBSERVING ACOUSTIC TRAPS AND MEASURING THE NODE SPACING

Acoustic traps can be one-, two-, or three-dimensional.[27] In the one-dimensional acoustic trap, acoustic waves are confined to a long channel or pipe, say in the x-dimension. These waves propagate along the channel or pipe before being reflected at the end. The interference of these waves produces a standing wave in the channel, if the channel length is a multiple of half the wavelength. Once the standing wave forms, particles will start to collect at the nodes and become trapped. For 4-μm-diameter polystyrene beads in water at 1.5 MHz, the particles are trapped at the pressure nodes.[7,13] The spacing d of the nodes is half a wavelength and is the same whether the end of the channel is open or closed. Given that the wavelength changes in different media, we give the relation for the node spacing in terms of the frequency f of the acoustic wave and the speed of sound v in the medium such that

$$d = \frac{v}{2f}. \tag{1}$$

Using our experimental setup, we can view these one-dimensional acoustic traps by eye and measure the spacing of the nodes. Specifically, we make a sample chamber and flow through a solution of beads in water. We place the piezo-electric transducer on top of the sample chamber and connect it to a function generator and oscilloscope (Fig. 2). We set the function generator to apply the resonance frequency to the piezo transducer (voltage = 12 V peak-to-peak, frequency = 0.8–1.5 MHz). Then, when we view the sample chamber from the top, we see lines of beads! These are beads that are trapped in the nodes of the standing wave in one dimension, but are free to move in the other two dimensions. We note that the lines of trapped beads may not be straight. One reason could be that the piezo transducer causes evaporation of the liquid, which smears the pattern. Another reason could be that some trapping in the y-dimension is occurring, leading to uneven lines.

We can also create a two-dimensional acoustic trap with our setup. To do this, we create a wide (>1 cm) channel and turn on the function generator to create the acoustic wave. We see that the beads are trapped in pressure nodes along the length of the channel (x-dimension) but also along the width of the channel (y-dimension). When the sample chamber is viewed from above, these nodes appear as a series of spaced-out dots, each containing trapped beads (Fig. 2).

To characterize the acoustic trap, we measure the spacing of the nodes where the trapped particles collect and compare this to what we would expect from Eq. (1). For example, at a driving frequency of 1.5 MHz and a speed of sound in water of 1500 m/s, we would expect a spacing of 0.5 mm. We can measure the spacing for the lines in the one-dimensional trap or the spacing for the points in the two-dimensional trap. Both are produced by the same acoustic wave in the same medium and will therefore have the same node spacing. To keep things simple, we just measure the line spacing in the one-dimensional trap. We measure 0.52 ± 0.03 mm at a frequency of 1.5 MHz, which agrees with our theoretical expectation within uncertainty.

We repeat this measurement for different transducers and plot the node spacing along with the theoretical expectation (Fig. 3). For all measurements, we find there is good agreement with the theory to within the error bars (the standard deviation). Some student groups (data at 1 and 1.2 MHz in Fig. 3) will have larger error bars than other student groups (data at 0.8 and 1.5 MHz in Fig. 3). Be sure that students have sealed the chambers to prevent evaporation and that students store the chambers in an inverted position to prevent the beads from aggregating at the bottom of the chamber due to gravity.

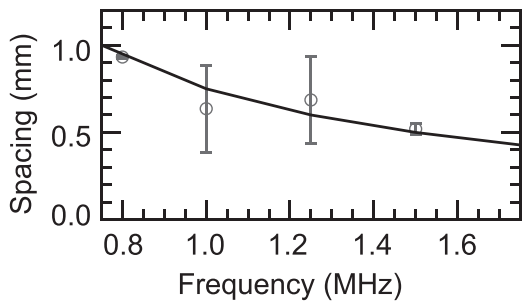

Fig. 3. Spacing between the lines (gray circles) follows the theoretical curve for the spacing between the nodes of the acoustic wave (black line). Error bars represent the standard deviation.

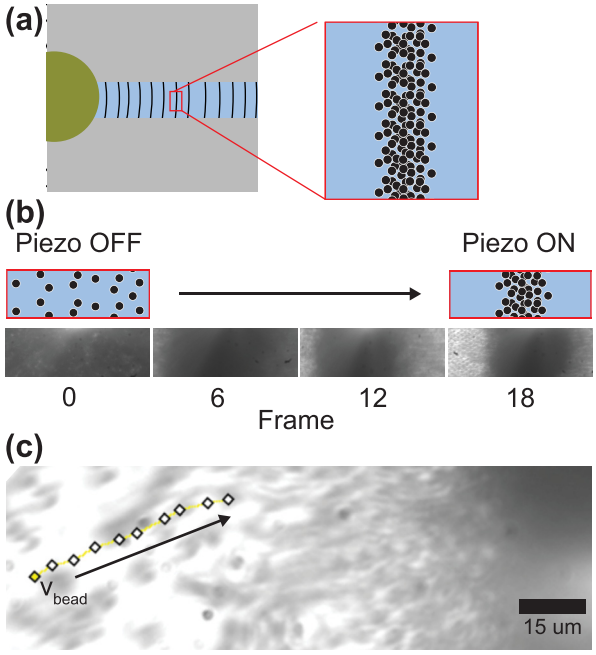

Fig. 4. Measuring the acoustic force. (a) Cartoon of the field of view within the microscope. The acoustic trap creates a line of many beads on top of one another and less beads at the periphery. (b) Frames 0–18 in a 15 Hz video of the formation of the acoustic trap. Function generator is turned on at frame 0. Edges of the frame become more sparse as beads move toward the center. (c) We track the centroid location (yellow diamonds) of an individual bead in ten frames and overlay the track onto the last frame. We use this track to calculate the velocity of the bead, v_{bead}, to be 43 ± 7 μm/s.

IV. VIEWING TRAPPED PARTICLES AND MEASURING THE ACOUSTIC TRAP FORCE

If you have access to a microscope, then the acoustic trap becomes even more impressive! With the microscope, students can view the particles collecting in the acoustic trap (Fig. 4). Here, we use a microscope with a 40× objective and place the sample chamber in the microscope. At first, when we view the sample and the acoustic trap is off, we just see a field of view inhabited by a lot of individual beads [Fig. 4(b), frame 0]. When we turn on the function generator to create the acoustic trap, in about a second we see all of the beads move toward the node, creating dark regions of trapped beads and bright regions where there are no beads [Fig. 4(b), frame 18]. Turning off the function generator allows the beads to spread out again, and the excitement of turning on the acoustic trap can be repeated, much to the delight of the students!

Tracking beads in the sample chamber using a microscope allows for measurements of the acoustic trap force. This is because a micron-sized bead in water is heavily damped, with the acoustic force on the bead equal to the drag force on the bead. The drag force F_{drag} on a bead of radius a in a medium with a viscosity of η (1 mPa s for water) and a bead velocity of v_{bead} is given by Stokes's law,[28]

$$F_{\text{drag}} = 6\pi\eta a v_{\text{bead}}. \tag{2}$$

Thus, once we measure the velocity of a bead moving toward the trap center, we will be able to find the acoustic force using the drag force. To measure the velocity of a bead moving toward the trap, we take a video of individual beads within the sample chamber and record their motion. Specifically, we measure the bead centroid in each frame for 10 frames and plot the bead track [Fig. 4(c)]. The average velocity for the bead is the distance between the position in the last frame minus the first frame over the time between the first and last frame (0.66 s). We repeat this for three different beads and find a mean average velocity of 43 μm/s and a standard deviation of 7 μm/s. This corresponds to an acoustic force of 1.6 ± 0.3 pN. Of course, this is the force on the bead at about 50 μm from the node. The highest force should be a quarter of the wavelength (250 μm at 1.5 MHz in water) away from the node.

To check if our force measurement seems reasonable, we can estimate the acoustic trap force using the properties of the acoustic wave. The acoustic trap force F_{ac} is the force exerted by the trap on the particle. This force is dependent on the acoustophoretic contrast factor Φ, which depends on the compressibility ratio of the bead $\tilde{\kappa}$ and the density ratio of the bead to water $\tilde{\rho}$.[29] The acoustic trap force also depends on the wavenumber k of the acoustic wave, the radius of the bead a, and the acoustic energy density E_{ac}. Specifically, the acoustic force is given by the equation[29]

$$F_{ac} = 4\pi\Phi(\tilde{\kappa}, \tilde{\rho}) k a^3 E_{ac} \sin(2kz). \tag{3}$$

In this equation, the acoustophoretic contrast factor for a one-dimensional planar standing wave is[29]

$$\Phi(\tilde{k}, \tilde{\rho}) = \frac{1}{3}\left[\frac{5\tilde{\rho} - 2}{2\tilde{\rho} + 1} - \tilde{k}\right]. \tag{4}$$

To evaluate the force, we use a density ratio of 1.04 and a compressibility ratio of 0.44 for polystyrene,[30] which produces an acoustophoretic contrast factor of 0.20. The wavenumber for 1.5 MHz is $2\pi f/v$, which is 6280 m^{-1}. The bead radius is 2 μm, the acoustic energy density is 10–100 J/m^3,[29] and the maximum value of the sine function is 1. Given these values, we can use Eq. (3) to estimate the maximum acoustic trap force. We calculate a theoretical, maximum acoustic force of 1–10 pN. If the bead is 10 μm from the node, then we have $\sin(10/250 \times \pi/2) = 6\%$. Thus, the force should be about $1/0.06 = 16$ times higher at the maximum. Since our experimental measurements are at about 50 μm from the pressure node, the theoretical force should be 0.3–3 pN, which agrees with our experimentally determined value of 1.6 ± 0.3 pN.

V. CONCLUSION

Here, we describe a low-cost and simple way to produce acoustic traps in liquid in a 1-hour undergraduate laboratory. Using this method, we can produce one- or two-dimensional acoustic traps of micron-sized beads and measure the spacing (order of mm) of the nodes in the standing acoustic wave. The addition of a microscope allows for viewing of individual beads during trapping and the measurement of the acoustic force (order of pN).

Given the flexibility of the experimental setup and the simplicity of the acoustic theory, we believe that this lab will be particularly amenable to "plug-and-play" laboratories[31] and student projects.[32] Projects engage students on all of the recommended learning outcomes for the experimental laboratory,[33] including modeling, designing experiments, analyzing data, and developing technical skills. Student projects

can include measuring acoustic trapping in different media or with a different piezo transducer. Students can explore different channel geometries or materials, including changing the reflection coefficient of the chamber walls (changing from double-sided tape to another material with higher or lower acoustic impedance). Students can also trap beads of different sizes, produce acoustic streams of smaller-diameter beads,[34] or perhaps trap only one bead size and add flow to the system to create a particle sorter. A very interesting potential student project is the trapping of bacteria or cells, though students may need to overcome the heating ($\sim$5 °C) produced with this setup, if they intend to do longer term experiments (>1 h). Thus, we anticipate a rich laboratory experience for undergraduates without the high cost of a research-grade system.

In addition, bringing acoustic trapping into the undergraduate instructional physics or biophysics laboratory gives students insight into the biophysics research topics happening today. As interdisciplinary and biomedical applications become more prevalent, students will need more laboratory experiences in these growing fields.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

[a]ORCID: 0009-0008-3461-7371.
[b]ORCID: 0009-0002-9921-8669.
[c]ORCID: 0009-0001-5474-8669.
[d]ORCID: 0009-0005-1751-4184.
[e]ORCID: 0009-0001-9002-4270.
[f]Electronic mail: acarter@amherst.edu, ORCID: 0000-0001-7513-4845.
[g]ORCID: 0000-0002-3247-1945.

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