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TITLE:

Assembly and Characterization of an External Driver for the Generation of Sub-Kilohertz Oscillatory Flow in Microchannels.

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KEYWORDS:
oscillatory, pulsatile flow, microfluidics, audible frequency, microchannel

SUMMARY:
The protocol demonstrates a convenient method to produce harmonic oscillatory flow from 10–1000 Hz in microchannels. This is performed by interfacing a computer-controlled speaker diaphragm to the microchannel in a modular manner.

ABSTRACT:
Microfluidic technology has become a standard tool in chemical and biological laboratories for both analysis and synthesis. The injection of liquid samples, such as chemical reagents and cell cultures, is predominantly accomplished through steady flows that are typically driven by syringe pumps, gravity, or capillary forces. The use of complementary oscillatory flows is seldom considered in applications despite its numerous advantages as recently demonstrated in the literature. The significant technical barrier to the implementation of oscillatory flows in microchannels is likely responsible for the lack of its widespread adoption. Advanced commercial syringe pumps that can produce oscillatory flow, are often more expensive and only work for frequencies less than 1 Hz. Here, the assembly and operation of a low-cost, plug-and-play type speaker-based apparatus that generates oscillatory flow in microchannels is demonstrated. High-fidelity harmonic oscillatory flows with frequencies ranging from 10–1000 Hz can be achieved along with independent amplitude control. Amplitudes ranging from 10–600 µm can be achieved throughout the entire range of operation, including amplitudes > 1 mm at the resonant frequency, in a typical microchannel. Although the oscillation frequency is determined by the speaker, we illustrate that the oscillation amplitude is sensitive to fluid properties and channel geometry. Specifically, the oscillation amplitude decreases with increasing channel circuit length and liquid viscosity, and in contrast, the amplitude increases with increasing speaker tube thickness and length. Additionally, the apparatus requires no prior features to be designed on the microchannel and is easily detachable. It can be used simultaneously with a steady flow

created by a syringe pump to generate pulsatile flows.

INTRODUCTION:

The precise control of liquid flow rate in microchannels is crucial for lab-on-a-chip applications such as droplet production and encapsulation[1], mixing[2,3], and the sorting and manipulation of suspended particles[4–7]. The predominantly used method for flow control is a syringe pump that produces highly controlled steady flows dispensing either a fixed volume of liquid or a fixed volumetric flow rate, often limited to entirely unidirectional flow. Alternative strategies for producing unidirectional flow include using gravitational head[8], capillary forces[9], or electro-osmotic flow[10]. Programmable syringe pumps allow for a time-dependent bidirectional control of flow rates and dispensed volumes but are limited to response times greater than 1 s due to the mechanical inertia of the syringe pump.

Flow control at shorter time scales unlocks a plethora[6,11–15] of otherwise inaccessible possibilities due to qualitative changes in flow physics. The most practical means of harnessing this varied flow physics is through acoustic waves or oscillatory flows with time periods ranging from 10^{-1}–10^{-9} s or 10^{1} –10^{9} Hz. The higher end of this frequency range is accessed using bulk acoustic wave (BAW; 100 kHz–10 MHz) and surface acoustic wave (SAW; 10 MHz–1 GHz) devices. In a typical BAW device, the entire substrate and the fluid column are vibrated by applying a voltage signal across a bonded piezoelectric. This enables relatively high throughputs but also results in heating at higher amplitudes. In SAW devices, however, the solid-liquid interface is oscillated by applying voltage to a pair of interdigitated electrodes patterned on a piezoelectric substrate. Due to the very short wavelengths (1 µm–100 µm) particles as small as 300 nm can be precisely manipulated by the pressure wave generated in SAW devices. Despite the ability to manipulate small particles, SAW methods are limited to local particle manipulation since the wave rapidly attenuates with distance from the source.

At the 1–100 kHz frequency range, oscillatory flows are usually generated using piezo-elements that are bonded to a polydimethylsiloxane (PDMS) microchannel above a designed cavity[16,17]. The PDMS membrane above the patterned cavity behaves like a vibrating membrane or drum that pressurizes the fluid within the channel. At this frequency range, the wavelength is larger than the channel size, but the oscillation velocity amplitudes are small. The most useful phenomenon in this frequency regime is the generation of acoustic/viscous streaming flows, which are rectified steady flows caused due to non-linearity inherent in the flow of liquids with inertia[18]. The steady streaming flows typically manifest as high-speed counter-rotating vortices in the vicinity of obstacles, sharp corners, or micro-bubbles. These vortices are useful for mixing[19,20] and separating 10 µm sized particles from the flow stream[21].

For frequencies in the range of 10–1000 Hz, both the velocity of the oscillatory component and its associated steady viscous streaming are considerable in magnitude and useful. Strong oscillatory flows in this frequency range can be used for inertial focusing[22], facilitate droplet generation[23], and can generate flow conditions (Womersley numbers) that mimic blood flow for *in vitro* studies. On the other hand, streaming flows are useful for mixing, particle trapping, and manipulation. Oscillatory flow in this range of frequencies can also be accomplished using a piezo-element bonded to the device as described above[23]. A significant hurdle to implementing oscillatory flows through a bonded piezo element is that it requires features to be designed beforehand. Furthermore, the bonded speaker elements are not detachable, and a new element must be bonded to each device[24]. However, such devices present the advantage of being compact. An alternative method is using an electromechanical relay valve[20]. These valves require pneumatic pressure sources and custom control software for operation and therefore increase the technical barrier to testing and implementation. Nevertheless, such devices enable the application of set pressure amplitude and frequency.

In this article, the construction, operation, and characterization of a user-friendly method to generate oscillatory flows in the frequency range of 10–1000 Hz in microchannels is described. The method offers numerous advantages such as cost-effective assembly, ease of operation, and ready to interface with standard microfluidic channels and accessories such as syringe pumps and tubing. Additionally, compared to previous similar approaches[25], the method offers the user selective and independent control of oscillation frequencies and amplitudes, including the modulation between sinusoidal and non-sinusoidal waveforms. These features allow users to easily deploy oscillatory flows and, therefore, facilitate widespread adoption into a broad range of currently existing microfluidic technologies and applications in the fields of biology and chemistry.

PROTOCOL:

1. Rapid prototype mold design and fabrication

1.1. Open AutoCAD on a PC. Select File on the taskbar, then select **Open** and browse to and click on a three-dimensional (3D) model file of the channel mold having .dxf or .dwg extension.

1.2. Select the entire model by clicking and dragging a box around it. Export the design as a stereolithography file with. stl extension.

1.3. Upload the file to a high precision resin stereolithographic (SLA) printer. Pour the resin into the resin chamber and initiate printing. Produce the mold with the smallest z-axis steps.

1.4. Wait for the automatic part printing to be completed.

NOTE: Molds with features as small as 0.1 mm can be fabricated in this way.

1.5. After removing the part from the resin, agitate it in isopropanol for 5 min to remove any remaining resin.

1.6. Dry the mold with air or nitrogen gas for 2 min.

NOTE: Conventional microfluidic mold fabrications with silicon wafers and photolithography with any SU8 or KMPR photoresists can also be used to produce a mold with smaller features.

2. PDMS microchannel fabrication

2.1. Place the mold on a sheet of aluminum foil. To ease the delamination of PDMS, spray coat the mold with silicone mold release in 1 or 2 passes.

2.2. Pour PDMS resin and cross-linker into a disposable cup in the ratio of 10:1 by weight and mix with a disposable spoon.

2.3. Pour the resulting mixture onto the mold to produce a film of required thickness. To prevent large channel wall deformation, maintain PDMS thickness of more than 5 mm or 3–4 times the maximum feature thickness.

2.4. Place the mold with poured PDMS into the degas chamber and close the lid. Ensure that the O-ring hermetically seals the chamber.

2.5. Close the exhaust valve and turn on the vacuum rough pump to initiate degassing.

2.6 Degas the poured mixture in a vacuum pump for over 4-6 cycles with each cycle lasting approximately 5 min. Manually remove any remaining bubbles (in corners and trenches) using a fine wire.

2.7 Set oven temperature to 80 °C and allow it to preheat. Place the mixture in the oven at 80 °C for 2 h to cure.

2.8 Remove the cured mold from the oven and leave it at room temperature for 10 min to cool.

2.9 Using a scalpel, carefully cut out the edges of the mold. For optimal delamination, use a syringe to inject isopropanol in between the mold and the cured PDMS.

2.10 Peel the cured PDMS off from the mold and cut it into individual devices with a razor blade. The size of each device must range between 10 mm x 10 mm to 30 mm x 70 mm to be bonded with the glass slide.

2.11 Make a hole of 1.0–3.0 mm diameter at the inlet and outlet using a biopsy punch.

2.12 Turn on the handheld radio frequency (RF) plasma generator. To activate the glass slide, steadily pass the wire electrode over a clean dry glass slide multiple times for 2 min. Maintain a wire to glass gap of approximately 5 mm. Place the device side of the cured PDMS in contact with the activated glass slide and then place in an 80 °C oven for 2 h.

2.13 Cut polyethylene inlet and outlet tubing to the required length and insert them into the inlet and outlet holes.

2.14 To prevent tube detachment during operation, apply silicone sealant on the contact surface and let cure for 2 h to secure the tubing.

3 Oscillatory driver assembly

3.1 Clamp the alligator clip ends of a pair of alligator-to-pin wires to the terminals of a speaker. Here a 15 W speaker with an 8 cm cone was used although other speakers can also be used.

3.2 Place the aux controller chip on an insulating component. Insert the pin ends into the screw sockets of the aux controller chip and firmly tighten with a screwdriver to ensure connectivity.

3.3 Connect one end of an aux cable to the controller chip and the other end to an aux port on a computer or smartphone.

3.4 Connect a 12 V direct current (DC) adapter to the power supply. Power the controller chip on by connecting the coaxial end of the DC adapter to the power socket.

3.5 Using an internet browser, navigate to an online tone generator website (e.g., https://www.szynalski.com/tone-generator/).

3.6 Type in the desired frequency (5–1200 Hz) in the online application. Scroll the volume bar to the required amount (e.g., 100%).

3.7 Click on the **Wave-Type Generator** symbol and select the desired waveform (sine, square, triangle, sawtooth). Note the default is a sine waveform. Press **Play** to actuate the speaker.

4 Adapter assembly

NOTE: The complete speaker-to-tube adapter assembly is illustrated by the schematic in **Figure 1**.

4.1 Tape the speaker (**Figure 1**(I)) and the controller chip onto the 3D printed speaker mount (**Figure 1**(II)) (see *speakermount.stl* in **Supplementary File 1**) for positioning on the microscope stage.

4.2 Place the 3D printed adapter (**Figure 1**(III)) (see *speakertubeadapter.stl* in **Supplementary File 2**) concentrically on the speaker cone.

4.3 Tape down the speaker mount to prevent it from moving when the speaker is in operation.

4.4 Apply silicone sealant generously along the edges of the adapter and let cure for 2 h.

4.5 Cut a 200 μL micro-pipette tip approximately 2 cm from its narrow end and dispose the wider half of the tip. The narrow conical end will serve as a wedge seal for reversible attachment.

4.6 Connect the polyethylene tubing (**Figure 1**(V)) to the microchannel (**Figure 1**(VI)) outlet by first threading through the micro-pipette tip (**Figure 1**(IV)), and then through the adapter's coaxial end and finally out through the side.

4.7 Firmly wedge the narrow end of the pipette tip into the adapter's coaxial end to create a detachable tight seal.

5 Operation of the experimental setup for oscillatory flows in microchannels

5.1 Add tracer particles into a vial of 22% weight/weight (w/w) glycerol solution to produce a neutrally buoyant suspension with a volume fraction of 0.01%–0.1% polystyrene in liquid at 20 °C. Mix vigorously by shaking to produce a homogenous suspension.

5.2 Load a 1 mL inlet syringe with 1 mL of sample. Mount and fasten the loaded syringe onto an automatic syringe pump. Insert the syringe needle into the inlet tubing of the device to create a watertight seal.

5.3 Ensure the outlet tube is routed through the adapter assembly and into a reservoir (see previous section on adapter assembly).

5.4 Turn on the syringe pump. Using the touch screen, select the syringe type as **Becton-Dickinson 1 mL**. Then, select **Infuse**. Then select the required flow rate (0–1 mL/min) or flow volume (< 1 mL).

5.5 Initiate the steady flow using the syringe pump. Wait until sufficient volume of fluid has flowed and the outlet tube is filled with liquid up to the speaker.

NOTE: The oscillatory amplitude for a given setting will not vary with steady transport flow if the outlet tube is primed.

5.6 Select a required frequency, amplitude, and waveform in the tone generator application as described in step 3.5 and press **Play** to generate oscillatory flow inside the microchannel.

6 Observation and amplitude measurement

6.1 Mount the device on the microscope. Set up the optical configuration by selecting an objective lens with a magnification between 10x and 40x adjusting the focal plane and positioning the stage.

6.2 To obtain measurements in a well-defined focal plane, ensure that the depth of field of the objective lens is smaller than the channel depth by a factor of 5 or more.

6.3 To observe the oscillatory flow, use a high-speed camera with a frame rate of at least twice the oscillation frequency as calculated using the Nyquist sampling theorem. For a practically useful resolution of the waveform, measure at least 10 points per time period using a framerate > 10 times that of the oscillation frequency.

6.4 Alternatively, to observe only the rectified or long-time effects of pulsatile flows, perform stroboscopic imaging by setting the observation frequency to any perfect divisor of the oscillation frequency.

6.5 For both direct and stroboscopic imaging, use a camera equipped with a global shutter to avoid the jello-effect. In either case, keep the exposure time considerably smaller than the oscillation time period (by a factor of 10 or more) to prevent streaking.

6.6 To measure the oscillation amplitude without a high-speed camera, record at a framerate maintained close to but not equal to the stroboscopic frame rate (e.g., 49 frames/s for a 50 Hz signal). This results in a highly slowed-down oscillation from which the amplitude can be accurately measured.

6.7 Observe and record the amplitude measurements.

REPRESENTATIVE RESULTS:

To illustrate the capability and performance of the above setup, representative results of oscillatory flow in a simple linear microchannel with a square cross-section are presented. The width and height of the channel are 110 µm and its length is 5 cm. First, we describe the motion of spherical polystyrene tracer particles and how these can be used to check the fidelity of the oscillatory signal as well as the range of oscillation amplitudes achievable. We then discuss the effect of specific fluid properties or microfluidic materials on oscillation amplitude. Finally, we illustrate the capability for non-sinusoidal waveforms.

For comparison, we define the reference case by the following fluid properties, channel geometry, and microfluidic materials. The working liquid is deionized water (μ = 1.00 mPa.s) with 0.01% volume fraction of tracer particles which have diameter, d = 1 µm and density, ρ = 1.20 kg/m^3. The corresponding particle response time, given by $\rho d^2/18\mu$, is 70 ns which is far less than the corresponding oscillatory time scales (1–100 ms). The particles are observed at the channel mid-height with a 10x objective and a depth of focus of 10 µm. The microfluidic tube has diameters 1.27 mm x 0.76 mm (outer x inner) and an outlet tube length of 12 cm that is held 5 cm above the channel level.

The tracked displacements of tracer particles at the channel midplane for different oscillation frequencies are shown in **Figure 2**. A harmonic signal is observed for all of the oscillation frequencies shown, which are 100 Hz, 200 Hz, 400 Hz, and 800 Hz. The imaging frame rate was greater than or equal to 20 times the oscillation frequency. The amplitude (speaker volume) setting was maintained constant across the different oscillation frequencies. For the frequencies 100 Hz, 200 Hz, 400 Hz, and 800 Hz, the corresponding amplitudes are approximately 125 µm, 100 µm, 25 µm, and 10 µm, respectively.

The tracked displacement of particles is also used to determine the fidelity of the harmonic motion and the range of oscillation amplitudes, a critical step in the calibration process. The fidelity of the harmonic displacement of particles at different oscillation frequencies and amplitudes is illustrated using the Fourier spectra and shown in **Figure 3A**. For frequencies of 50 Hz, 200 Hz and 400 Hz respectively, three different amplitudes characterized by the potential difference in the aux cable (or amplifier input voltage) are considered. The settings are named low (30%, 1.5 V, yellow), intermediate (60%, 3 V, orange), and high (90%, 4.5 V, red). Here, the percentage represents the magnitude of the volume setting with respect to the maximum speaker volume, or corresponding voltage of 5 V. The Fourier spectra of particle displacement at oscillation frequencies of 50 Hz, 200 Hz, and 800 Hz are shown in **Figure 3A** for three different amplifier input voltages (1.5 V, 3 V, 4.5 V) corresponding to yellow, orange and red colors respectively. The primary peak of the spectrum corresponds exactly to the applied frequency for all volume settings. The primary peak is > 10 times the secondary peaks, even at the highest amplitude.

For an amplifier input voltage of 5 V, the amplitude of the speaker cone displacement has a maximum value of 5 mm and remains a constant for frequencies up to 50 Hz and then decreases approximately quadratically for frequencies above 50 Hz (e.g., 1.5 mm at 100 Hz). The particle oscillation amplitude in the liquid is proportional to the power transduced given by the product of the speaker cone amplitude and the oscillation frequency. We therefore expect that the oscillatory amplitude is maximum near the speaker resonant frequency and decreases for frequencies on either side of it for a fixed amplifier input voltage. Further, we may also expect that the oscillatory amplitude of the fluid varies linearly with the amplifier input voltage and its value cannot exceed that of the speaker cone amplitude.

These expectations are confirmed in a plot of oscillation amplitude *versus* frequency shown in **Figure 3B**. For all speaker volume settings, the characteristic curve has a resonant peak, which occurs at approximately 180 Hz, beyond which the amplitude decreases with increasing frequency. The curves at different voltages appear identical except for vertical translations in log-scale implying that the oscillatory amplitude varies linearly with voltage. Finally, the maximum amplitude is less than 1.5 mm even at the resonant frequency of 5 V. Nevertheless, a volume setting can be selected such that oscillation amplitudes of > 100 µm can be achieved over the entire operational frequency range.

Next, select example cases are presented on the effect of the liquid viscosity, the tube diameter, and tube length on the oscillatory amplitude over the range of operational frequencies with respect to the reference case described above. For these experiments, the driver amplitude (speaker volume) is maintained constant at the intermediate level and only one setup parameter is modified at a time while the remaining parameters are identical to the reference control case (diamond symbols). The corresponding results for oscillation amplitude *versus* frequency are shown in **Figure 4**. When the viscosity of the working liquid is increased by changing to a 25% glycerol solution (μ = 1.81 mPa.s) the amplitude decreases by a factor of nearly 2 over the range of operating frequencies (square symbols). This suggests that, in general, increasing the liquid viscosity compared to that of deionized water would result in a similar characteristic amplitude *versus* frequency curve with a constant factor decrease in the amplitude. When the microfluidic tubing diameter for the same material (polyethylene) is increased to 2.41 mm x 1.67 mm, the amplitude increases compared to the reference case by a factor between 1.5–3 depending on the frequency (circle symbols). The increase is larger at high frequencies and smaller at low frequencies, indicating the resonant frequency has increased. When the tube length for the same material (polyethylene) is increased to 24 cm (by a factor of 2), the amplitude increases significantly near the resonant frequency but remains unchanged from the reference control case at very low and very high frequencies (triangle symbols).

In addition to the sinusoidal waveforms discussed above, non-sinusoidal waveforms are also demonstrated. Particle displacement tracks for square, triangle, and sawtooth waveforms are shown in **Figure 5A**. Here, the amplitude setting is intermediate (60% of maximum), the driving frequency is 100 Hz, and particles are observed at 4000 frames/s. As expected, very sharp changes in position associated with square and sawtooth waveforms are not possible in real systems with a finite response time. For this speaker system, the response time may be estimated to be 0.5 ms. Nonetheless, the Fourier spectra of these waveforms are observed to be in good agreement with the ideal spectra, at least up to the third harmonic as shown in **Figure 5B**.

FIGURE AND TABLE LEGENDS:

Figure 1. A schematic to illustrate the apparatus design and assembly. The critical components are (I) speaker, (II) speaker mount, (III) speaker-to-tube adapter, (IV) pipette-tip wedge seal, (V) polyethylene tubing, and (VI) PDMS microchannel.

Figure 2. Examples of particle displacement during oscillatory flow. Representative particle tracks during sinusoidal waveform input at different frequencies were obtained using high-speed imaging.

Figure 3. Analysis of particle displacement for signal fidelity and amplitude range. (**A**) Fourier spectrum analysis of sinusoidal oscillations at different oscillation frequencies and amplitudes, or speaker volumes. (**B**) The characteristic curve of the oscillation amplitude *versus* frequency at three different speaker volume settings.

Figure 4. Effects of tube length, tube diameter, and liquid viscosity on oscillatory amplitude. When compared to the reference case, an increase in tube length or tube diameter will lead to an increase in oscillation amplitude over the range of operational frequencies. An increase in viscosity, however, decreases the oscillation amplitude.

Figure 5. Examples of non-sinusoidal waveforms. (**A**) Particle displacements for square, triangular, and sawtooth waveforms at an oscillation frequency of 100 Hz. (**B**) The corresponding Fourier spectra for non-sinusoidal particle displacements.

Supplementary File 1. Stereolithography file to produce a 3D printed speaker mount referred to in Figure 1 (II).

Supplementary File 2. Stereolithography file to produce a 3D printed speaker tube adapter referred to in Figure 1 (III).

DISCUSSION:

We have demonstrated the assembly (see protocol critical steps 3 and 4) and operation (see protocol critical steps 5 and 6) of an external speaker-based apparatus for the generation of oscillatory flow with frequencies in the range of 10 to 1000 Hz in microfluidic devices. Particle tracking of suspended tracer particles is required to determine the fidelity of the harmonic motion as well as for calibrating the range of oscillation amplitudes achievable over the range of operating frequencies. The amplitude-frequency curve for a given volume setting depends primarily on the characteristics of the speaker, which cannot be changed (see discussion of speaker characteristics in representative results for **Figure 3A,B**). However, for a particular channel design, the oscillatory amplitude can be modified and tuned by appropriately modifying the tubing properties, the liquid viscosity, or combinations thereof. For example, we show in **Figure 4** that a larger tube diameter or longer tube length can increase the magnitude of the oscillatory amplitude for the same volume setting. Increasing viscosity, however, decreases the range of oscillatory amplitudes, providing users with a range of amplitudes, extending from 10 µm to 1 mm.

The significant advantage of this method is its ease of assembly, implementation, and operation. The entire cost of the oscillatory driver is less than $60 and its assembly will only take approximately 2 h once the parts are purchased (see **Table of Materials**). Unlike alternative methods for generating oscillatory flow in microfluidic devices[25], this method imposes virtually no design constraints and ensures minimal lead time to implementation. Despite its simplicity, our method allows the user surprisingly precise control of oscillation amplitudes while maintaining the fidelity of both sinusoidal and non-sinusoidal oscillatory waveforms. The technique also generates harmonic motion over a frequency range of two orders in magnitude. Lastly, this technique can be used together with a steady flow component generated by standard microfluidic flow controllers, such as syringe pumps or pressure generators, to generate a high-frequency pulsatile flow. As previously demonstrated[22,28], the oscillatory amplitude and frequency are not affected by the presence of a steady transport flow when the steady flow velocity is small compared to the oscillatory flow velocity. This method is therefore ideal for a research laboratory setting.

A corresponding limitation of the method is that the amplitude cannot be set at the desired value. It must be measured and calibrated to the amplitude for a given microfluidic channel. It is currently not scalable and thus not immediately suitable for industrial applications. Further development of this apparatus would involve the design of a simple diaphragm that can be bonded to and actuated by the speaker to permit larger amplitudes and minimize the dependence on the tubing and microfluidic channel.

Overall, this work provides a low-cost, robust, and customizable approach for generating oscillatory flows in microfluidic channels in a relatively unexplored frequency range. This technique has been shown to be useful for the microrheology of Newtonian[26] and non-Newtonian[27] liquids, enhanced mixing at the microscale[28], and inertial focusing in channels of reduced length[22]. The approach outlined in this work provides an accessible and adaptable methodology to generate purely oscillatory flows, or pulsatile flows when combined with a steady flow from a syringe pump. As a result, this convenient technique can enable the implementation of oscillatory flows into existing research and industrial at the microscale.

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DISCLOSURES:
The authors have nothing to disclose.

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Figure 1

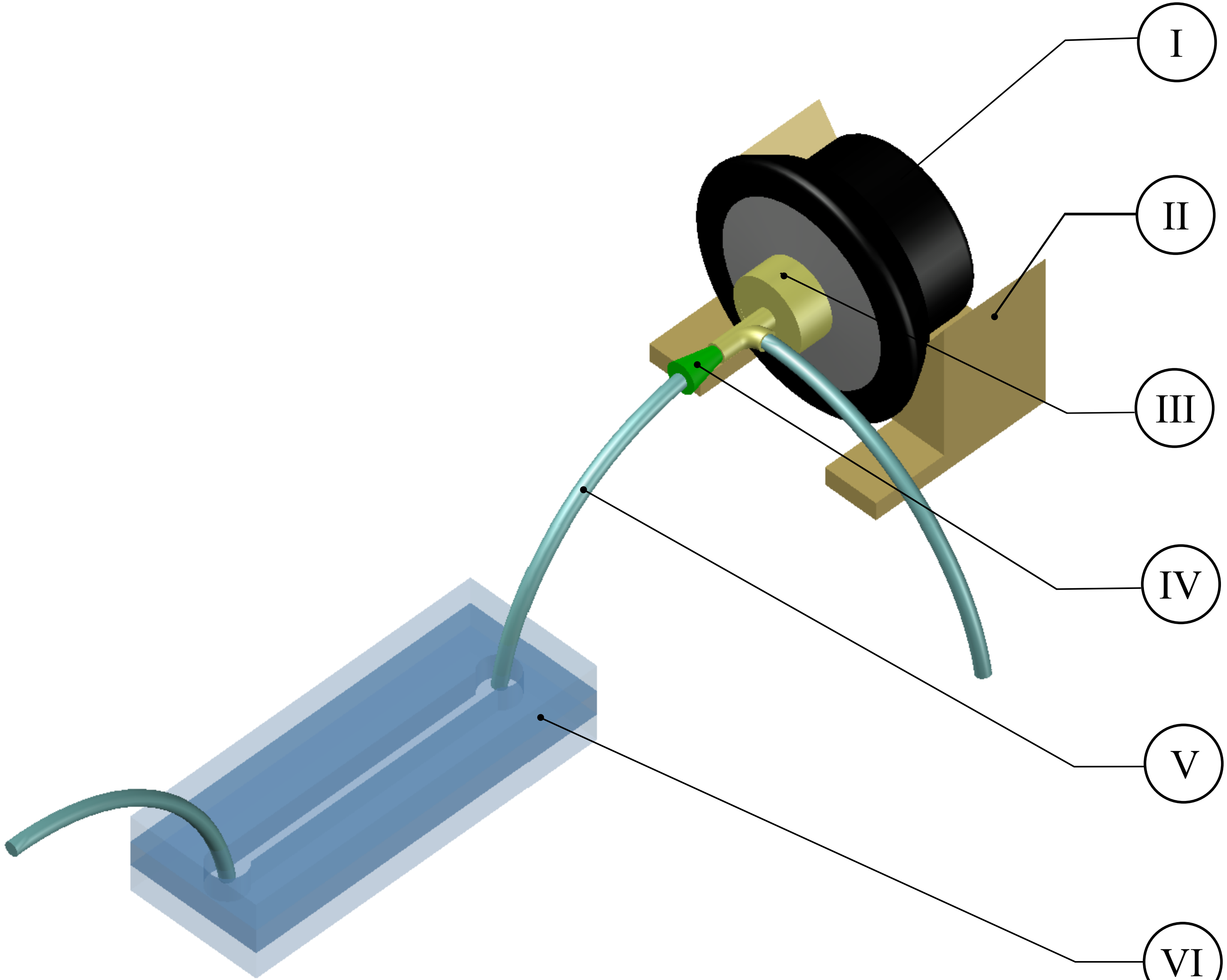


Figure 2

Click here to access/download;Figure;JoVE_resub_Fig_02.pdf

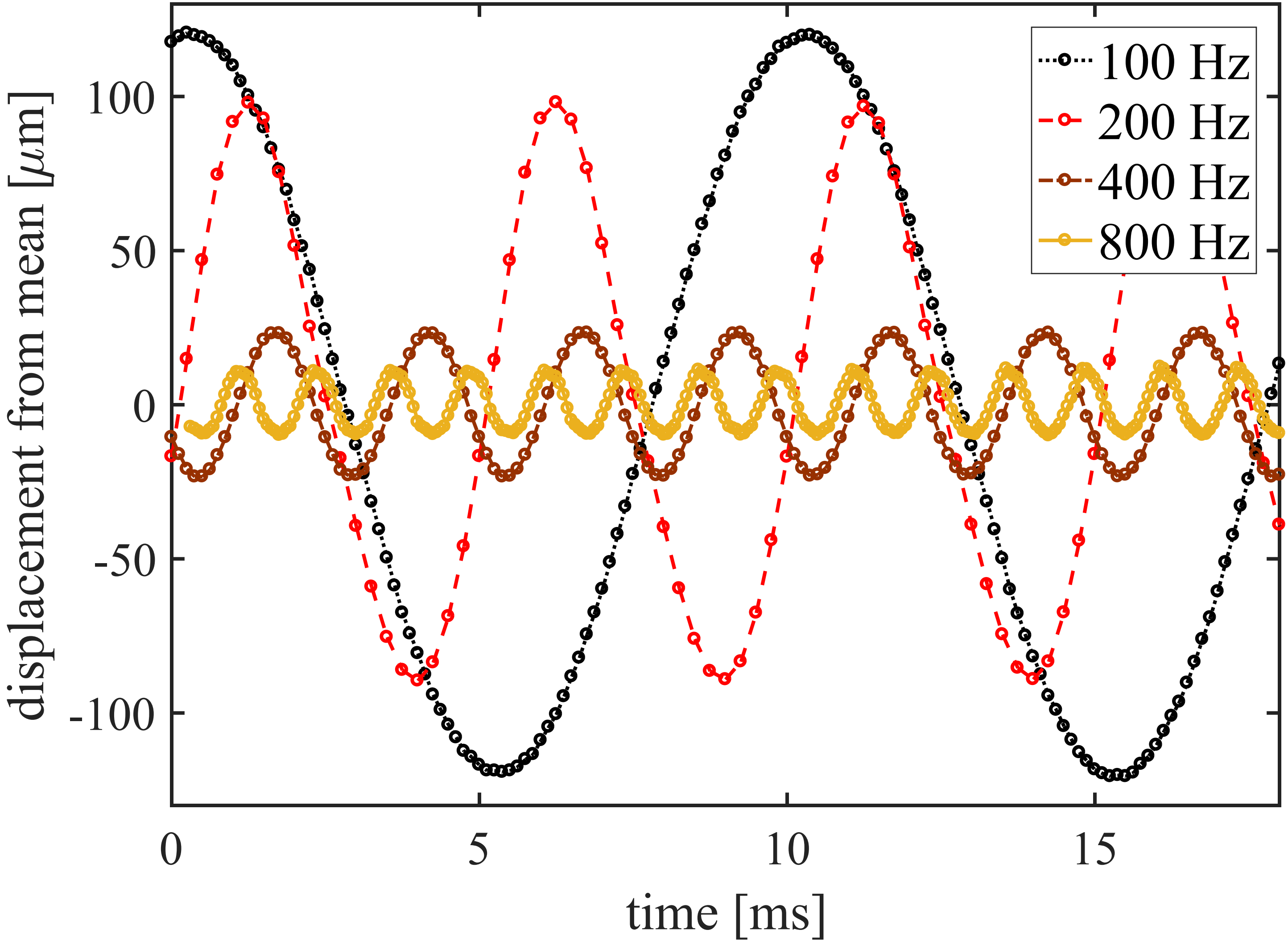
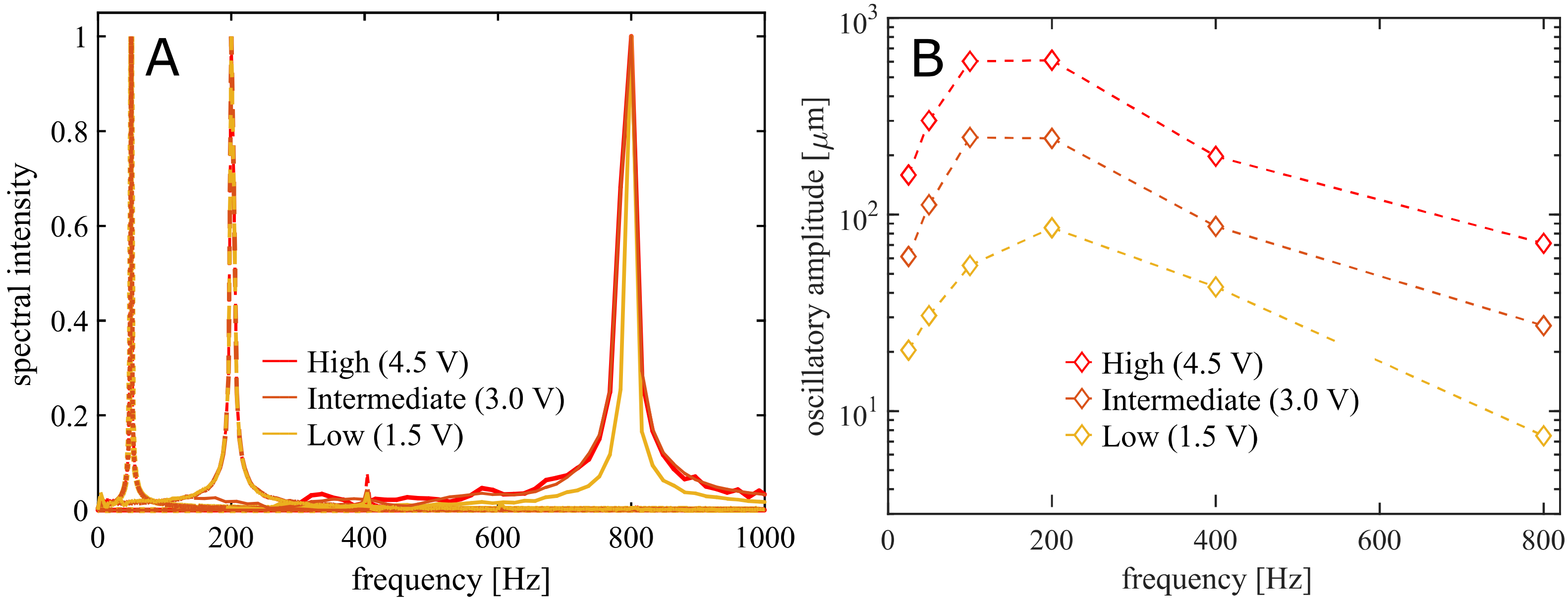
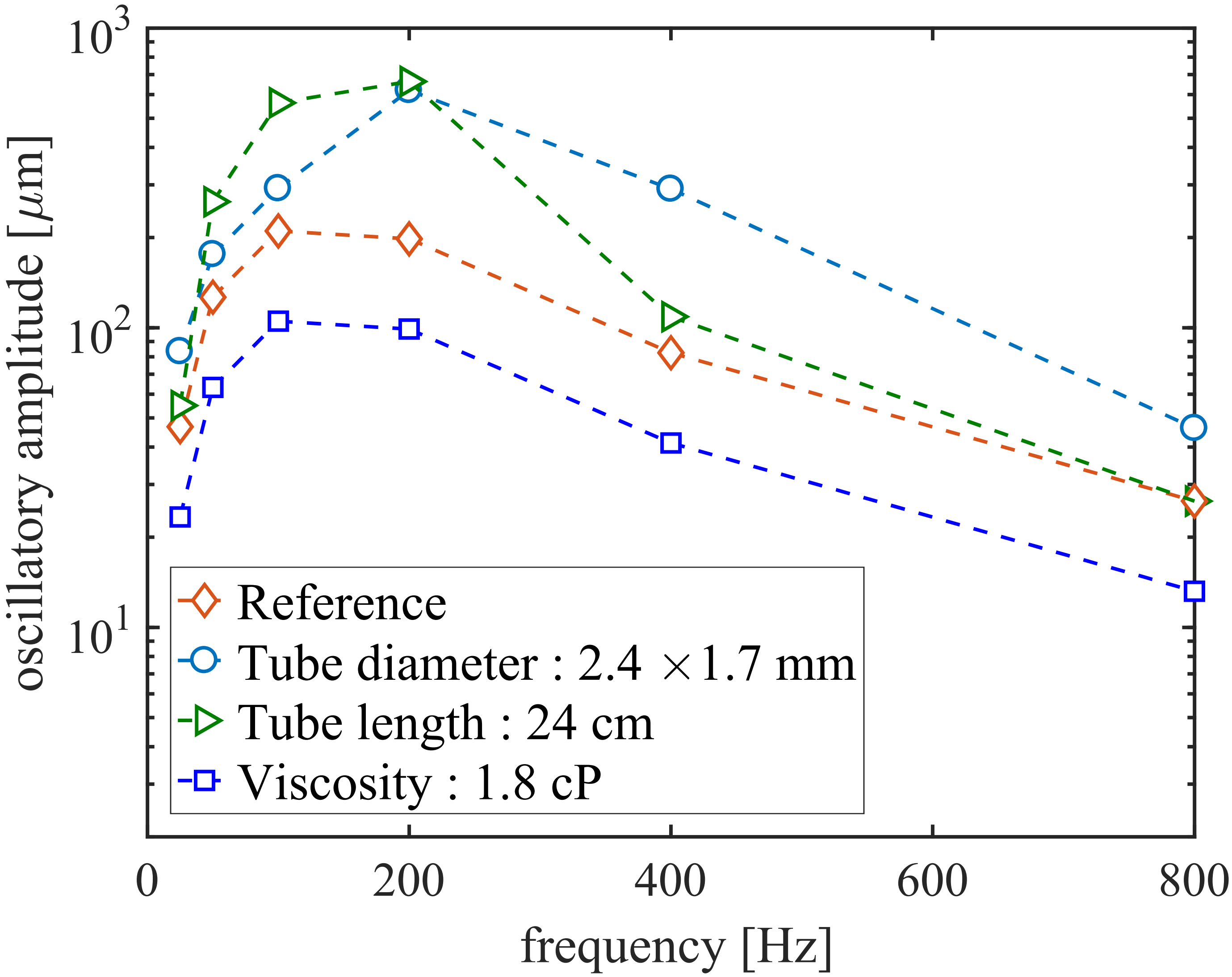


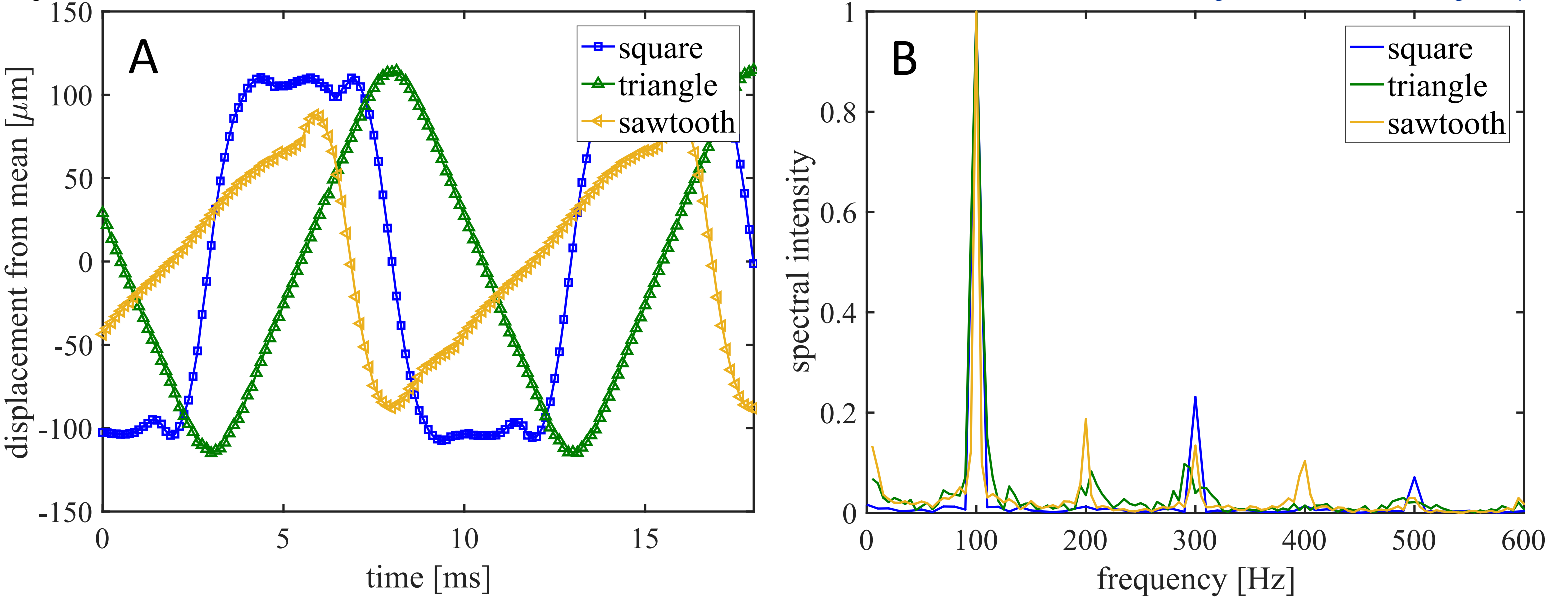
Figure 3


A
spectral intensity
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
frequency [Hz]
High (4.5 V)
Intermediate (3.0 V)
Low (1.5 V)
B
oscillatory amplitude [μm]
10^3
10^2
10^1
0
200
400
600
800
frequency [Hz]
High (4.5 V)
Intermediate (3.0 V)
Low (1.5 V)



oscillatory amplitude [μm]
10^3
10^2
10^1
0
200
400
600
800
frequency [Hz]
Reference
Tube diameter : 2.4 ×1.7 mm
Tube length : 24 cm
Viscosity : 1.8 cP

Figure 5


A
square
triangle
sawtooth
displacement from mean [μm]
150
100
50
0
-50
-100
-150
0
5
10
15
time [ms]
B
square
triangle
sawtooth
spectral intensity
1
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
frequency [Hz]

Click here to access/download
Table of Materials
JoVE_Materials.xls

Editorial comments:

Editorial Changes

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

>>> Completed.

2. For in-text formatting, corresponding reference numbers should appear as numbered superscripts.

>>> Completed.

3. Please provide suitable citations for the following lines: 83-90.

>>> Completed. The updated version is now: **"**A significant hurdle to implementing oscillatory flows through a bonded piezo-element is that it requires features to be designed beforehand. Furthermore, the bonded speaker elements are not detachable, and a new element must be bonded to each device[18]. However, such devices present the advantage of being compact. An alternative method is using an electromechanical relay valve[14]. These valves require pneumatic pressure sources and custom control software for operation and therefore increasing the technical barrier to testing and implementation. Nevertheless, such devices enable the application of a set pressure amplitude and frequency.**"**

4. Please define all abbreviations upon first use. For example, PDMS, 3D, RF, etc.

>>> Completed

5. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Any text that cannot be written in the imperative tense may be added as a "Note."

>>> Completed

6. The Protocol should contain only action items that direct the reader to do something.

>>> Completed

7. Please use SI units as much as possible and abbreviate all units: L, mL, µL, cm, kg, etc. Use h, min, s, for an hour, minute, second.

>>> Completed

8. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed?

Step 1.1: Which of the methods mentioned here was used for mold fabrication? Please describe the steps associated. Alternatively, add references to published material specifying how to perform the protocol action. Please remember that our scripts are directly derived from the protocol text. Please include all actions associated with each step.

>>> Completed. We have added an additional section on how to fabricate an SLA based mold.
Step 1.4: What is the thickness of the film? Alternatively, how is the suitable thickness determined?
>>> Completed. We have added: "Pour the resulting mixture onto the mold to produce a film of required thickness. To prevent large channel wall deformation, poured PDMS must be thicker than 5 mm or 3-4 times the maximum feature thickness."
Step 1.5: How was degassing done? Please provide all associated steps.
>>> Completed. We have added: "Place the mold with poured PDMS into the degas chamber and close the lid. Ensure that the O-ring hermetically seals the chamber. Close exhaust valve and turn on the vacuum rough pump to initiate degassing. Degas the poured mixture in a vacuum pump over 4-6 cycles with each cycle lasting approximately 5 minutes. Remove any remaining bubbles (in corners and trenches) manually with a fine wire."
Step 1.7: Please specify the device shape and dimensions.
>>> Completed. We have added: "Note that the size of each device must range between 10 mm x 10 mm to 30 mm x 70 mm to be bonded with the glass slide."

Step 2.5: What are the desired frequency, amplitude, and waveform values?
>>> Completed. We have added: **"**Actuate the speaker by typing in a desired frequency (5 – 1200 Hz), scrolling the amplitude, clicking on the desired waveform (sine, square or sawtooth) from the dropdown box by typing in the frequency online signal generator application. Use earplugs or other ear protection if using frequencies greater than 400 Hz.**"**

Step 4.1: What is the concentration used?
>>> Completed. The updated version reads: "Add tracer particles into a vial of liquid sample to produce a suspension with volume fraction 0.01 - 0.1 % polystyrene in liquid."

Step 4.2: What is the flow rate used here?
>>> Completed. The updated version reads: "Mount the loaded syringe onto an automatic syringe pump. Set the syringe volume and the required flow rate (0 – 1 ml/min) or flow volume (< 1 ml)."

Step 4.5: How were the flow parameters set? Please describe in brief.
>>> Completed. We have added: **"**Mount the loaded syringe onto an automatic syringe pump and set a required flow rate (0 – 1 ml/min) or flow volume (< 1 ml).**"**

Step 5.2: Please provide all the microscope settings used. If this step needs to be filmed, please make sure to provide all the details such as "click this", "select that", "observe this", etc. Please mention all the steps that are necessary to execute the action item. Please provide details so a reader may replicate your analysis including buttons clicked, inputs, screenshots, etc. Please keep in mind that software steps without a graphical user interface (GUI) cannot be filmed.

>>> Completed. We have added: "Using an internet browser, navigate to an online tone generator website (eg: https://www.szynalski.com/tone-generator/).
Type in the desired frequency (5 – 1200 Hz) in the online application.

Scroll the volume bar to the required amount (eg. 100%).

Click on the wavetype generator symbol and select the desired waveform (sine, square, triangle, sawtooth). Note the default is sine.

Press play to actuate the speaker."

9. Please remove the embedded figure(s) from the manuscript.
>>> Completed.
10. Please also include the critical steps within the protocol in the Discussion along with suitable citations.
>>> Completed. The protocol is self-contained and explained as clear as possible in the newly revised manuscript. Therefore, we believe that there is no need to mention any specific or critical steps in the discussion.
11. Please include an Acknowledgements section, containing any acknowledgments and all funding sources for this work.
>>> Completed. We have acknowledged Rapid prototyping lab at the University of Illinois. There is no funding to acknowledge.
12. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al.
>>> Completed.

<u>Reviewers' comments:</u>

Reviewer #1:

Manuscript Summary:

This submission to JoVE by Vishwanathan and Juarez presents a method to generate oscillatory flow in PDMS microchannels using a speaker. The frequency and the waveform can be controlled, resulting in oscillatory flows in the microchannel. One advantage of this method is that it does not require an embedded actuator in the PDMS chip and could thus be used with different experimental setups.

Overall, I found the approach interesting and worth communicating to the scientific community. The resulting article and video should be interesting to different groups who want to generate such flows.

We thank the reviewer for their thoughtful comments and overall positive review of this manuscript and experimental approach. We have addressed all reviewer comments below and we believe that the updated version of the manuscript is now acceptable for publication in JoVE.

Major concerns:

None

Minor concerns:

- abstract: another method to produce a steady flow is through a pressure-driven system. This should be mentioned, and perhaps the limitation also discussed. Currently, most (commercial: Fluigent, Elveflow) pressure-driven systems can only reach oscillation frequency of the order of the hertz

We have updated the abstract and added the following sentence: “Advanced commercial syringe pumps (eg: Elveflow &Fluigent) that can produce oscillatory flow, are often more expensive and only work for frequencies less than a Hertz.”

- "Flow control at shorter time scales unlock a plethora of otherwise inaccessible possibilities due to qualitative changes in flow physics": the authors may want to add a few papers/reviews on why pulsatile flow are interesting

We have added references recent literature (papers and reviews) to justify this statement.

- The authors mention that "At the 1 - 100 kHz frequency range, oscillatory flows are usually

generated using piezo-elements that are bonded to a Polydimethylsiloxane (PDMS) micro-channel above a designed cavity". This sentence is not correct since some studies have used a similar approach to generate signals of a few tens of hertz [see, e.g., Geschiere, Sam D., et al. "Slow growth of the Rayleigh-Plateau instability in aqueous two-phase systems." Biomicrofluidics 6.2 (2012): 022007]

We are aware of this and acknowledge this in the very next paragraph about oscillatory flows in 1 – 1000 Hz range that piezo element can also be used for low frequencies but it requires bonding. We have also added the reference to the mentioned manuscript. "For frequencies in the range of 10 – 1000 Hz, both the velocity of the oscillatory component, and its associated steady viscous streaming are considerable in magnitude and useful. Strong oscillatory flows in this frequency range can be used for inertial focusing[22], facilitate droplet generation[23] and can generate flow conditions (Womersley numbers) that mimic blood flow for in-vitro studies. On the other hand, streaming flows are useful for mixing, particle trapping and manipulation. Oscillatory flow in this range of frequencies can also be accomplished using a piezo-element bonded to the device as described above[23]. A significant hurdle to implementing oscillatory flows through a bonded piezo-element is that it requires features to be designed beforehand. Furthermore, the bonded speaker elements are not detachable, and a new element must be bonded to each device[24]. However, such devices present the advantage of being compact."

- The approach described here seems very similar to the one used in the generation of all aqueous emulsions [see e.g., Sauret, Alban, and Ho Cheung Shum. "Forced generation of simple and double emulsions in all-aqueous systems." Applied Physics Letters 100.15 (2012): 154106], although it seems that the present system offers more control. This should be discussed to highlight the novelty of the method and why it's different.

We agree that there are similarities with the mentioned manuscript but that our method does indeed offer more control and has been characterized in more detail. We have added the following statements and the reference to the mentioned article in the introduction and discussion sections, respectively.

"Additionally, compared to previously similar approaches[25], our method offers the user selective and independent control of oscillation frequencies and amplitudes, including the modulation between sinusoidal and non-sinusoidal waveforms."

"Unlike alternative methods for generating oscillatory flow in microfluidic devices[25], this method imposes virtually no design constraints and ensures minimal lead time to implementation. Despite its simplicity, our method allows the user surprisingly precise control of oscillation amplitudes while maintaining the fidelity of both sinusoidal and non-sinusoidal oscillatory waveforms.”

- The protocol for the preparation of the PDMS microchannel is very standard (and one could likely use a different SU8 if needed)

We agree with the reviewer and have removed references to SU8 and have written the protocol for 3D printed SLA molds.

- The assembly and description are clear and well written

We thank the reviewer for this comment.

- Perhaps, to help other groups measure the amplitude of the fluctuation, the equation to obtain the particle response time (line 196) could be given as it may be one quantity that others will have to estimate

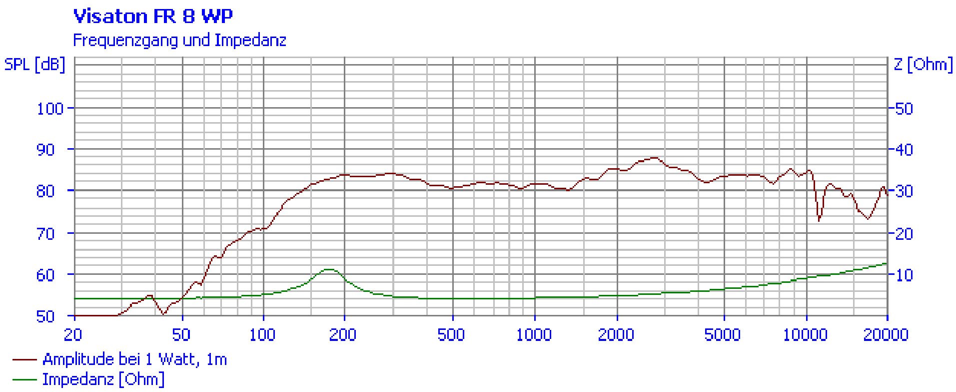
For clarity and to assist other groups, we have made the edit “The working liquid is deionized water (µ = 1.00 mPa.s) with 0.01% volume fraction of tracer particles which have diameter d = 1 µm and density of \rho = 1.20 kg/m^3. The corresponding particle response time given by \rho*d^2/18µ = 70 ns which is far less than the corresponding oscillatory time scales (1 – 100 ms).”

- Could some clarification be given on how the speaker volume relates to the amplitude of the pulsation of the speaker to tube adapter?

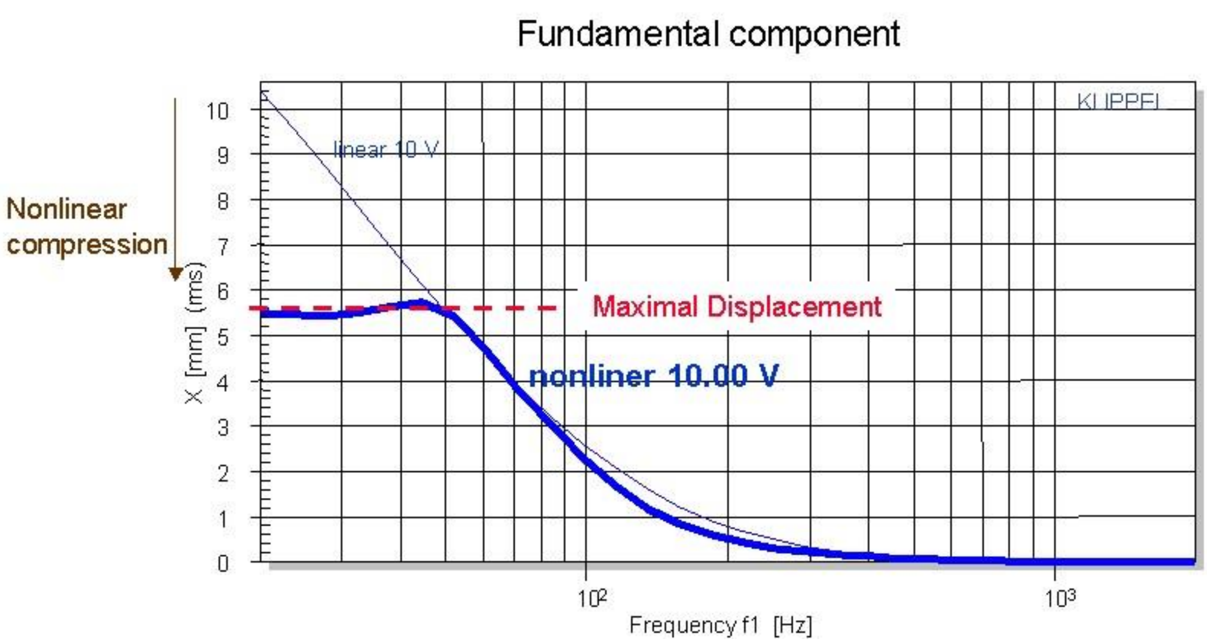
This precise relationship is complicated and ultimately not very relevant to the operator which is why we avoid it in the text. However, we briefly summarize it in this response:
The voltage in the aux cable (computer/phone output) varies linearly with volume and has a maximum value of 5 V Vpp at 100% volume for all frequencies. The power amplifier takes this as input and gives a max output power of 15 W at 5 V for all frequencies.

The speaker impedance is 4 Ohm across most of the range and rises to 12 Ohm at the resonant frequency of around 180 Hz. This information is obtained from a manufacturer datasheet.

(http://www.loudspeakerdatabase.com/VISATON/FR8WP-4/VISATON_FR8WP-4.pdf).



The tube oscillation amplitude is equal to that of the speaker cone. The actual excursion distance or displacement of the speaker cone was not precisely measured for all frequencies. However, we have seen that at 100 % volume, it remains constant at 5 mm for frequencies below 50 Hz and decreases approximately quadratically with frequency beyond this (eg 1.5 mm at 100 Hz). Something similar is seen in this graph taken from Voice Coil Displacement (klippel.de):



- Figure 3(b): could more information be given instead of "high", "intermediate", "low"? In practice, how should one choose a speaker to implement this?

As mentioned above, at 100 % volume the aux cable voltage which is the input to the amplifier is 5 Vpp. To implement low, medium and high one can use 4.5 V, 3.0 V, 1.5 V Vpp respectively as input to the amplifier. These details have been added to the manuscript and to Figure 3 B.

Overall, I believe that the method developed here will lead to an interesting video that other researchers could then use

Reviewer #2:

Manuscript Summary:
This manuscript presents a convenient and novel methodology to produce harmonic flow that oscillate between 10 and 1000 Hz in microfluidic devices. The proposed idea is based on interfacing a computer-controlled speaker diaphragm to the microfluidics inlet tubing. Overall, the authors propose a low-cost but robust and adjustable method for generating flows that oscillate in microfluidic channels within a frequency range that is not explored as other ranges.

The author have shown that the proposed methodology is useful for the study of New- tonian and non-Newtonian liquids microrheology, but also for important applications as microscopic enhanced mixing. The method proposed also allows for pulsatile flows when combined with a steady flow from a syringe pump: this is discussed to have a potential as a convenient technique to implement such flows also in existing research and industrial systems.

The relevance of this novel methodology is clear to me. The method is low cost and easy to assembly. The draft is easy to read and well written; the figures are all of high quality and clear to understand. I recommend to accept this draft for publication in the Journal of Visualized Experiments.

We thank the reviewer for their support for publication and for their comments. We have addressed all reviewer comments and updated the manuscript accordingly. We believe that the current version has improved in clarity and acceptable for publication in JoVE.

Major Concerns:
none

Minor Concerns:
*** I recommend to add a sentence (or a couple of sentences) to motivate better the scientific relevance of the frequency range explored here (10-1000 Hz).**

We appreciate the comment, and have added "For frequencies in the range of 10 – 1000 Hz, both the velocity of the oscillatory component, and its associated steady viscous streaming are considerable in magnitude and useful. Strong oscillatory flows in this frequency range can be used for inertial focusing[22], facilitate droplet generation[23] and can generate flow conditions (Womersley numbers) that mimic blood flow for in-vitro studies. On the other hand, streaming flows are useful for mixing, particle trapping and manipulation."

*** line 103. Why SU8-2075? I would refer here to general SU8, for other application with thinner or thicker microfluidic devices other SU8 could be used (as SU8-2010 or SU8-2025).**

We agree with the reviewer. We have removed references to SU8 and have written the protocol for 3D printed SLA molds. We also acknowledge that any SU8 or KMPR molds can also be used. "1.6 Note that conventional microfluidic mold fabrications with silicon wafers and photolithography with any SU8 or KMPR photoresists can also be used to produce a mold with smaller features."

*** line 127. I suggest that the authors clarify whether the power of the speaker (here 15 W) is or not a key parameter for the method?**

We have now clarified this and added: "3.1 Clamp the alligator clip ends of a pair of alligator-to-pin wires to the terminals of a speaker. Note that we use a 15 W speaker with an 8 cm cone for demonstration purposes although other speakers can also be used."

*** lines 170-172. I suggest that the authors clarify why the observation frequency must be larger than 10 times that of oscillation?**

We have clarified this further. "To observe oscillatory flow, use a high-speed camera with a frame rate of at least twice the oscillation frequency due to the Nyquist sampling theorem. Note that a practically useful resolution of the waveform often needs at least 10 points per period for which the observation framerate must be > 10 times that of the oscillation frequency"

*** I suggest to indicate in a setup figure where the catted micro-pipette tip is (may be figure 1).**

We have updated the schematic shown in Figure 1 to include the micro-pipette tip wedge seal.

*** lines 193-200. I suggest that the authors clarify how important it is to observe, in this configuration, the moving particles through a thin field of view?**

We have added: "To obtain measurements in a well-defined focal plane ensure that the depth of field of the objective lens is smaller than the channel depth by a factor of 5 or more."

Reviewer #3:

Manuscript Summary:

The authors provided the assembly and operation of a low-cost, plug-and-play type speaker-based apparatus that generates oscillatory flow in microchannels. They found that the precise amplitude of oscillation is dependent on fluid properties and channel geometry.

We thank the reviewer for their comments and suggestions. We have addressed all of the reviewer's comments and modified the manuscript accordingly. The manuscript has improved in clarity and focused on the oscillatory flow component, which is the main contribution and focus of this manuscript and experimental approach.

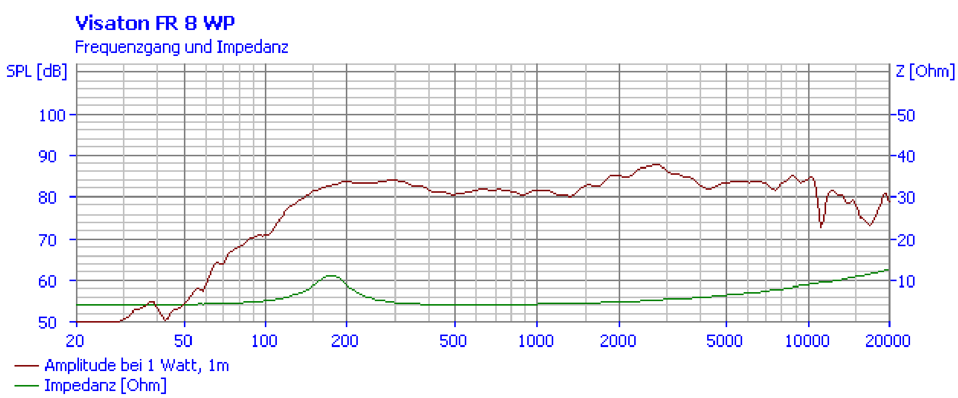
Major Concerns:

1. Even though "mixing" and "separation" have been considered in "Introduction" section, the authors need to briefly explain micomixing and particles separation using updated review papers, such as: https://doi.org/10.1016/j.cep.2019.107771 and https://doi.org/10.1016/j.cep.2020.107984.

We thank the reviewer for pointing this out and have now added more references to the introduction, including the papers mentioned by the reviewer.

2. What are the characteristics of speaker volume? for example, Fig. 3B.

The impedance and intensity characteristics for a 4 Ohm 15 W speaker with an 8 cm cone are given in the plot below taken from a manufacturer's datasheet when operated at 1 W power. The peak-to-peak voltage supplied to the speaker by our apparatus can be estimated as: 0.08 x Volume % (8 V for 100 % volume). (http://www.loudspeakerdatabase.com/VISATON/FR8WP-4/VISATON_FR8WP-4.pdf).



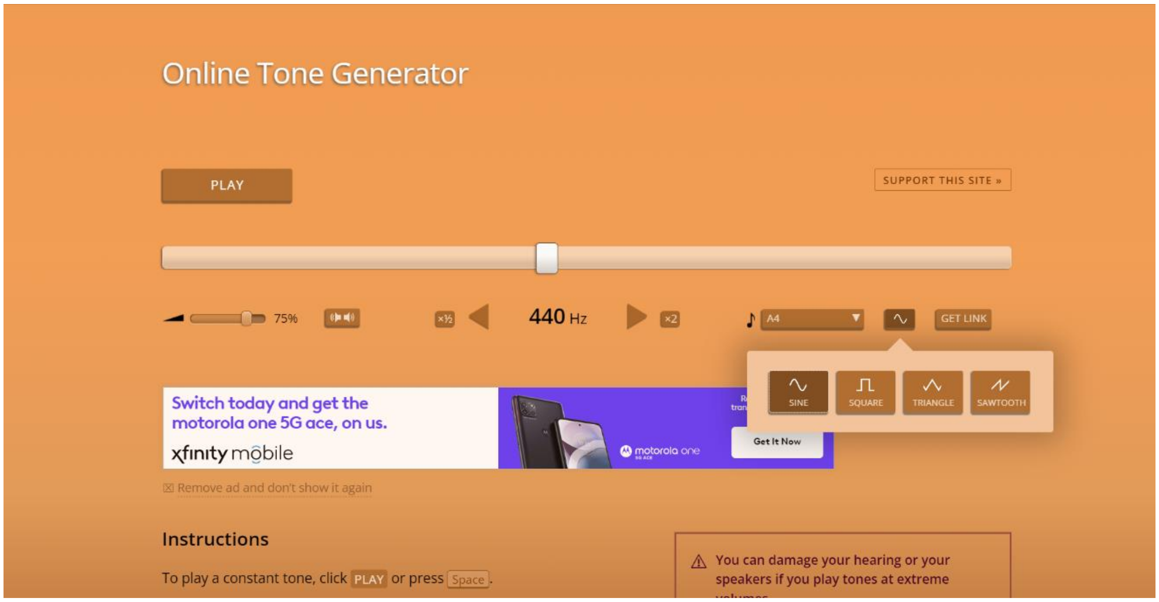
3. Fig. 4: you examined the effect of tube length, tube diameter and liquid viscosity on oscillatory amplitude. To evaluate the impact of as parameter, you need to use different values of the parameter. In this figure, the value of tube length, tube diameter and liquid viscosity is assumed to be constant.

We agree that we did not completely study the effects of varying the parameters, I.e., we did not measure oscillation amplitude for 10 different liquid viscosities. Instead, we present example cases of how oscillation amplitude changes compared to the "reference" case that was described (Fig 1) and fully characterized (Fig 2 and Fig 3). We present example cases of varying only one parameter at a time and spanning the oscillation frequency from 10 to 800 Hz. We believe that this is still useful, for example, if the user were to increase the dynamic viscosity of the liquid, then they should expect the measured oscillation amplitudes to decrease relative to their "reference" case. It would be up to the reader to perform the measurement of oscillation amplitude as a function of *N* different liquid viscosities.

In attempts to clarify this, we have removed the use of "examined" in the results section and replaced with "select example cases are presented on the effect of ... with respect to the reference case".

4. How did you generate square, triangular and sawtooth waveforms?

The square, triangle and sawtooth waveforms can easily be generated using online tone generator applications such as https://www.szynalski.com/tone-generator/. We have clarified it step by step in the revised manuscript.



5. What is the effect of inlet flow rate?

The inlet flow rate has no effect on the oscillatory flow amplitude provided the tube is primed with liquid. We have added a point “5.7 Ensure that the outlet tube is primed with liquid. Note that the oscillatory amplitude for a given setting will not vary with steady transport flow if the outlet tube is primed.”

6. In the presence of sheath flow, please explain the oscillatory flow characteristics?

We have not characterized the oscillatory amplitude in complex flow configurations like sheath flow and this would be out of the scope of this fabrication manuscript. However, we have seen that it can be used for oscillation induced mixing in flow focusing configuration of miscible liquids (see Vishwanathan & Juarez, *Microfluidics and Nanofluidics* 2020: https://doi.org/10.1007/s10404-020-02373-z) . We have not tried it for droplet generation with two aqueous immiscible phases. We do not expect any change to the oscillatory amplitude compared to a case where only a sheath fluid is used (I.e only continuous phase) without the discrete phase. This is because the oscillatory amplitude depends only on the liquid viscosity but does not depend appreciably on the flow rate.

Minor Concerns:

1. Fig. 4: 2.4 by 1.7 mm is tube diameter!

We have modified Figure 4 and changed the caption as the reviewer suggests.

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