ABSTRACT
A simple and low-cost strategy to generate and control internal flows in a sessile droplet for particle and cell agglomeration in droplets is presented. The agglomeration is achieved by structured internal flows within the sessile droplet driven by an oscillating rigid substrate under certain vibration modes. In this work, oscillation of the substrate is experimentally measured, and its corresponding flow patterns are documented. The particles or cells in the sessile droplet are subsequently tracked using the different flow patterns with different flow intensities. The agglomeration capabilities and their dependence on flow characteristics are experimentally verified.

KEYWORDS
Sessile droplet, mode shape, internal flow, particle agglomeration

INTRODUCTION
Using sessile droplets provides a shift from conventional continuous flow techniques such as microfluidic channels, yet offers similar benefits: low sample consumption, high throughput, automation, and, most importantly, flexibility and versatility [1]. As a powerful tool, the use of an internal flow within a sessile droplet has drawn considerable attention in microfluidic applications due to more functionalities, such as mixing [2], cell agglomeration [3], and particle separation [4]. However, it is usually difficult to generate strong circulating flows in small volumes due to low Reynolds numbers [5].

Various methods have been developed to generate internal flows in sessile droplets with external energy input. For example, the electro-wetting-on-dielectric (EWOD) method uses an alternating electric field to induce droplet oscillation and accompanying flows [6]. Surface acoustic waves (SAW) are actuated on a piezoelectric disk to produce acoustic streaming [7]. Others have used magnetic particles in the droplet to control the flows with an external magnetic field [8]. Low-frequency vertical oscillation of the droplet substrate can also be utilized to cause flows driven by the gas/liquid interfaces vibrating at resonance frequencies [9]. However, these methods have some limitations in their applications, i.e., liquid to be actuated in the sessile droplet must contact special materials (electric conductive, piezoelectric, or magnetic). With vibrating droplets, though no specific materials are required, precise control of droplet volume and driving frequency is required.

In this research, we present a low-cost approach to generating and controlling flows within sessile droplets using an oscillating substrate. The substrate is actuated to give rotational oscillation with a non-uniformly distributed vertical vibrating velocity that induces an internal flow in an attached droplet. Our experiment verifies the correlation between various internal flow patterns and corresponding vibrating velocities distribution of the substrate. The capability for particle and cell agglomeration is verified with experiments.

METHODS
In the present experiment, a sessile droplet with a volume of 100 μL is confined in a recessed retainer 8 mm in diameter and 0.5 mm thick made of polydimethylsiloxane (PDMS). The PDMS pad is cast in a mold using the doctor blade method. Then a biopsy punch is used to make a circular hole on the pad to form the droplet retainer. The retainer is then attached to a glass substrate 0.5 mm thick, which is a rigid, oscillating structure. To enable different mode shapes of the substrate, the glass substrate is attached to the tip of a long polymethyl methacrylate (PMMA) cantilever beam that assumes various vibrational modes when driven at various frequencies.

The experimental setup is schematically shown in Figure 1. With the glass substrate attached at the tip, the PMMA cantilever beam is actuated by an electromagnetic shaker attached near the beam’s anchor. Vertical oscillation of the substrate is measured with a Laser Doppler Vibrometer (LDV). The laser beam is focused on the upper surface of the glass substrate and the mode shape of the substrate is recorded by scanning certain areas of the substrate: the rectangles along the length of the cantilever beam, and the circles on the different droplet retainers. The retainer is placed at one of three locations on the substrate indicated in Figure 1 as near, intermediate, and far.

![Figure 1: Schematic of the experimental setup](image)

The flow patterns in a sessile droplet are characterized using the particle image velocimetry (PIV) method. The PIV test is done while operating with a 100 μL volume of deionized water containing 15 μm polystyrene (PS) particles. The solution is injected into the retainer to form a droplet and the movement of particles is captured with a high-speed camera located under the glass substrate while the cantilever beam is actuated to vibrate. The particle agglomeration test is conducted using the same PS particle solution while the cell agglomeration experiment uses human pancreatic cancer cells Panc-1 suspension in 1X Phosphate-buffered saline (PBS) pH 7.4 solution.

RESULTS AND DISCUSSION
The frequency response of the entire substrate with three retainers atop it is measured using a frequency chirp signal at frequencies from 0.15 to 2.0 kHz with a voltage of 0.1 V, as shown in Figure 2(a). The magnitude refers to the averaged peak vibrating velocity of each scan point distributed in the scan area. The peaks in frequency response curves correspond to several resonance frequencies of the cantilever beam in different orders of vibrational
mode. Note that the peak height at the intermediate retainer at 1.27 kHz is clearly lower than that at the near and far retainers. This is most likely induced by a nodal line (where the vibrating amplitude is zero along the line) crossing the intermediate retainer. This hypothesis is verified by the mode shape results, as shown in Figure 2(a) insert and Figure 2(b), which correspond to the vibrating velocity distributions across the substrate and three droplet retainer positions, respectively. The colors ranging from dark blue to dark red indicate vibrating velocities from 0 to 0.3 mm/s. This frequency, having a nodal line across the intermediate retainer (shown as the dark blue area in Figure 2(b), where zero vibrating amplitude is detected), corresponds to one of the resonance peaks, which enables the substrate to have a seesaw-like rotational oscillation with the nodal line as the axis of rotation.

To have a better vision of vibrating velocity changes with time, the velocity distribution along the diameter of the retainer that is aligned with the cantilever longitudinal direction is measured with the LDV and plotted in Figure 3(a), 3(c), and 3(e), corresponding to the three different droplet retainers. The curves in each plot, ranging from blue to red, indicate various times within half an oscillation cycle. For all the three positions of the retainers, the glass substrate shows an almost rigid plane oscillation in that the velocities measured along the diameter are shown as straight lines. Thus, the movement of the glass substrate is considered a rotational oscillation rather than a flexible vibration. For the far and near retainers, the bottom substrate shows an oblique oscillation where the two ends have different amplitudes. For the intermediate retainer, the substrate shows a rotational oscillation, like a seesaw. Trajectories representing flow directions of the PS particles are calculated with the standard deviation of frames captured by the high-speed camera taken over 0.1 s using ImageJ software. They are shown in Figure 3(b), 3(d), and 3(f), for the three different retainers. Flow directions are distinguished using the original video and are labeled with red arrows. For droplets in the near (Figure 3(b)) and far (Figure 3(d)) retainers, there are two symmetrical vortices, and the flow direction in the center is aligned with the vertical vibrating velocity gradient of the substrate oscillation, from higher to lower velocities. For the intermediate retainer (Figure 3(f)), there is a nodal line where the vibrating velocity is zero and the number of vortices becomes four. Similarly, the flows on the left and right sides aim toward the nodal line and then go along with the line, after gathering in the center.

Figure 2: (a) Measured frequency response of the entire glass substrate and each droplet retainer, insert is the peak velocity distribution of the substrate. (b) mode shape of each retainer. The mode shape is obtained at 1.27 kHz.

Figure 3: (a), (c), and (e) are measured vibrating velocity distributions along the diameter of the droplet retainer within a half period vibrating at 1.27 kHz. (b), (d), and (f) are flow patterns at different retainers obtained using the same driving frequency. The red arrows indicate the flow direction obtained from the video.

Figure 4: Measured peak velocity distributions of the intermediate droplet retainer (on the top) and flow patterns at different driving frequencies (in the bottom). The red arrows indicate flow direction.
The results in Figure 3 indicate that the internal flow is generated in the sessile droplet once the substrate is actuated into a seesaw-like rotational oscillation. The flow direction will follow the vertical vibrating velocity gradient of the substrate, from higher velocity areas to lower ones. To assess the generality of this phenomenon, the correlations of vertical vibrating velocity gradients with flow patterns are further experimentally described under different driving frequencies using the intermediate droplet retainer, as shown in Figure 4. Regardless of driving frequency, the particle flow direction within the droplet is always in the same direction as that of the vertical vibrational velocity gradient of the oscillating substrate surface. Thereby, this substrate rotational oscillation strategy can provide highly controllable internal flow patterns in a sessile droplet that can potentially be used in various microfluidic applications.

Next, the internal flow in the sessile droplet is utilized for particle and cell agglomeration. A 100 μL deionized water droplet containing 15 μm PS particles is injected into the near, intermediate, and far droplet retainers, successively, and allowed to stand still for 60 s to let gravity drive the particles approaching the bottom. Then the substrate is actuated into rotational oscillation at a driving frequency of 1.27 kHz with a driving voltage of 0.3 V. Meanwhile, the trajectories of PS particles are captured with the high-speed camera located under the substrate. The results are shown in Figure 5, where the three columns correspond to the different retainers. Before oscillation is applied, the particles are uniformly distributed on the bottom of the glass substrate. After the substrate oscillates for 60 s, the particles have accumulated into several regions: for the near and far retainers, the particles are concentrated at two points, as indicated with red circles. These two points are the centers of the two vortices observed in the flow pattern shown in Figure 3(b) and 3(f). For the intermediate retainer, since there exist four vortices (Figure 3(d)), the particle agglomerated pattern shows four gathering points. The particle movement trajectory within 60 s is plotted by calculating the standard deviation of the entire agglomeration process, as shown in the bottom row of Figure 5. It is very clear that PS particles are driven to accumulate in specific locations. By using different flow patterns controlled by substrate rotational oscillation, particles in a sessile droplet can form controlled agglomeration patterns with different numbers of gathering regions.

Further, particle agglomeration of different substrate oscillation intensities was recorded. In this case, the substrate was actuated into 1.27 kHz oscillation using different driving voltages, and the far droplet retainer was used. The results are demonstrated...
in Figure 6 in which the five columns correspond to five different driving levels, while the three rows represent various times during substrate oscillation. As the driving voltage increases from 0.1 to 0.5 V, it is observed that the 15 μm PS particles cannot be agglomerated when a low oscillation intensity is used (0.1 V). The distribution of particles is almost unchanged in this driving voltage. Increasing the driving input triggers the particle agglomeration (0.2 V) and the particle accumulation speed and efficiency are enhanced at a higher input level (0.3 V). However, further increasing oscillation intensity (0.4 V) applies an even faster particle gathering speed but induces a smaller number of accumulated particles. This is due to the strong internal flows that tend to redispere the particles back into the solution. The red circles in Figure 6 indicate accumulated particles. The internal flows can not only accumulate particles in sessile droplets but can also break the agglomerated patterns.

Finally, cell agglomeration capability is evaluated using human pancreatic cancer cells (Panc-1 suspension in 1X PBS solution) in the far droplet retainer. The driving frequency and amplitude are 1.27 kHz and 0.3 V, respectively. Optical images of cell suspension at different times are shown in Figure 7. Different from the results in the PS particle agglomeration experiment, the Panc-1 cells turn to form a single line at the location where the two vortices converge (labeled with a red oval). This is most likely to be induced by the size of the cells. Since the cell suspension contains a large number of aggregates that consist of several attached cells, their overall sizes are much larger than that of the 15 μm PS particles. Due to the difference in drag force attributed to the difference in size, the agglomeration pattern of the Panc-1 cell shows different results.

Figure 7: Panc-1 cell agglomeration results at the far droplet retainer with a driving frequency of 1.27 kHz.

CONCLUSION

This paper presents a simple and low-cost strategy to produce and control internal flows in a sessile droplet. This strategy is achieved by actuating a rigid substrate into a rotational oscillation on which a sessile droplet is located. Oscillation with certain vertical vibrating velocity distributions establishes circulation patterns in the sessile droplets. Particle image velocimetry results indicate that the oscillation-induced internal flows form different numbers of symmetrical vortices. Flow patterns are determined by the mode shape of substrate oscillation. The flow direction is the same as the vertical vibrating velocity gradient of the substrate surface. Observations from particle and cell movement also demonstrate that the oscillation-induced internal flows can be employed for particle or cell agglomeration. By adjusting the pattern and intensity of the internal flows, particles can accumulate in different areas and can even be redispersed back to the bulk of the droplet.

ACKNOWLEDGEMENTS

 Portions of this work were conducted in the Minnesota Nano Center, which is supported by the National Science Foundation through the National Nano Coordinated Infrastructure Network (NNCI) under Award Number ECCS-2025124. Funding for this project was partially provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR).

REFERENCES


CONTACT
*Tianhong Cui, tel: +1-612-626-1636; cuixx006@umn.edu