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Surface acoustic wave concentration of microparticle and nanoparticle suspensions

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Abstract

A rapid particle concentration method in a sessile droplet has been developed using asymmetric surface acoustic wave (SAW) propagation on a substrate upon which the droplet is placed. The SAW device consisted of a 0.75-mm thick, 127.68 YX-axis-rotated cut $LiNbO_3$ as a substrate. An interdigital transducer electrode (IDT) with 25 straight finger pairs in a simple repeating pattern, 12 mm aperture, and a wavelength of $\lambda = 44$ μm was patterned on the substrate. The IDT was then driven with a sinusoidal signal at the resonance frequency 8.611 MHz. To investigate the effect of particle type and size on the concentration process, several types of particles were used in this study, including fluorescent particles (1 μm), polystyrene microspheres (3, 6, 20, and 45 μm), and living yeast cells (10-20 μm). Different RF powers were applied ranging from 120 to 510 mW. The concentration processes occurs within two to twenty seconds, depending on the particle size, type and input radio frequency (RF) power, much faster than currently available particle concentration mechanisms due to the large convective velocities achieved using the SAW device.

Introduction

The study of surface acoustic waves (SAW) dates from Rayleigh's original exposition in 1885 [15], and are most commonly encountered by humans in earthquakes as *ground roll* [2]. Such waves are common to diverse fields of physics, from studying the geological characteristics of the moon [10] to how spiders locate their prey [20]. Engineering application of such waves appeared [5] in telecommunications and signal processing soon after a convenient method for forming such waves atop piezoelectric materials was discovered by White and Voltmer in 1965 [23]; microdevices using SAW pervade consumer and military technology with an average of four in every mobile phone produced [5].

In contrast, microfluidics has a relatively short history, but also has an extraordinarily promising future, especially for medical and biological applications [24]. The transport of fluids on the micro and nano scales is a particularly thorny problem, with the effects of viscosity and laminar flow conditions consipiring against simply shrinking larger devices down to these scales with the expectation of having something useful [19]. Even today, despite the variety of methods explored by researchers, most "microfluidic" systems are still the size of a desktop computer. Electrowetting and electrophoresis are two of the most prevalent technologies in applied microfluidics [1], and offer much improved performance in comparison to passive diffusive processes. Further, they are convenient for digital droplet microfluidics, where the complexity of channel fabrication is traded for the problems of evaporation and surface-tension-driven effects in individual nanoliter-order droplets atop a surface [8]. The advantages include an ability to reconfigure or tailor the device for new applications, the sheer simplicity of the approach, and ease of loading and unloading fluids. Yet for mixing, transport and concentration of those droplets they are a challenge to construct, requiring complex electrode patterns and fluids or particles within those fluids amenable to manipulation. Worse, for biomedical applications, these technologies tend to modify the charge on particles within the fluid, affecting chemical bonding, cellular membranes, opening ion channels, and causing damage through bubble formation [7, 4].

Alternatives do exist, and one promising approach is the use of very high-frequency acoustic waves to perform all of the tasks common in droplet micro/nanofluidics—moving, splitting, combining, and pinning droplets—along with new processes[12, 13, 21] enabled via acoustic forces on objects within the droplets. Rotation of a droplet to perform mixing, for example, is shown in Fig. 1. Though one can use bulk Love and other such waves to perform manipulation and sensing [22], such waves are transmitted throughout the substrate holding the droplet, and so are subject to losses and diffraction from mounting. Surface acoustic waves are isolated to within 4–5 λ of the surface upon which they are generated, and so the dispersion of such waves is usually far less than bulk waves—the reason these waves are so popular in electronics applications. Wixforth [18, 25] and Renaudin [16] have made significant advances in the use of SAW for droplet microfluidics.

However, the manipulation of micro and nanoparticles within these droplets represent an important application of any such technology for biomedical applications, and curiously this aspect remains unexplored for droplet structures. Though the process dates from the study of acoustic particle agglomeration by Kundt [9] eleven years prior to Rayleigh's SAW publication, only recently have they found use in microfluidics [14, 12, 13, 21]. In this work we describe the discovery of an ability to perform concentration and dispersion of micro and nanoparticles inside sessile droplets set atop a lithium niobate substrate via SAW passed into the droplet in an asymmetric fashion. After discussing the reasons for the concentration process, the method is used to concentrate live yeast to illustrate its possible application to live cells while avoiding lysis and to counter the typical perception that ultrasound is generally damaging to cells [3].

Method

The SAW is generated on a 0.75-mm thick, 127.68 Y-X-axis-rotated cut, X-propagating $LiNbO_3$ (Lithium Niobate or LN, Roditi, London UK) single crystal substrate using a simple interdigital electrode [23] custom-fabricated onto the surface at the MicroNanophysics Research Laboratory. The wavelength of the excited SAW is defined by the geometry of the IDT, and here 25 straight finger pairs in a simple repeating pattern, a 12 mm aperture, and a wavelength of $\lambda = 440$ μm were used to give a fundamental resonance frequency of 8.611 MHz. Different RF powers were used to study the effect of input power on the concentration process.

The droplet was deposited directly on the LN surface, which is mildly hydrophyllic. No droplet translation was induced from acoustic streaming since the input RF power was below the threshold defined by the power necessary to release the contact

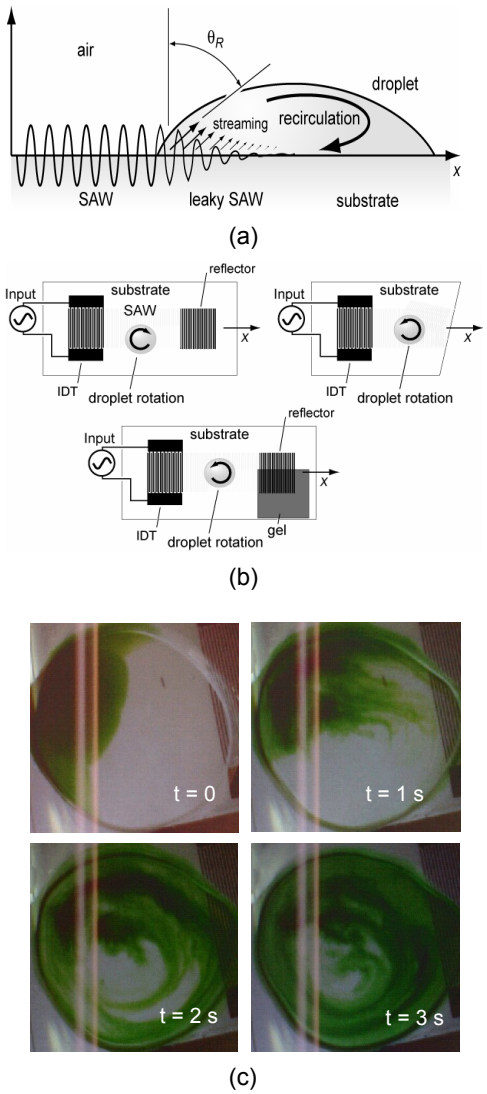

Figure 1: Surface acoustic waves are attenuated upon (a) contact with a fluid droplet; the energy is reradiated into the fluid at the Rayleigh angle θ_R as a compressive wave. Without (b) asymmetric delivery or absorption of the acoustic radiation into the droplet there can be no (c) rotation and subsequent mixing of the droplet. Here, the droplet was placed to one side of the SAW causing rapid mixing of the water-glycerin (green) mixture.

line pinning present at the edge of the droplet. A micro-pipette was used to deliver the droplets onto the substrate surface, and after each run the remains of the droplet and particles was removed from the surface in preparation for the next run.

Fluorescent particles were used to monitor the concentration process via fluorescence microscopy. The concentration process was recorded via high-speed video (Olympus iSpeed camera, Tokyo) used in conjunction with a reflection fluorescent stereomicroscope (Olympus BXFM, Tokyo, Japan). Wideband blue light was applied to excite the fluorescent particles and a fluorescence mirror unit (U-MWB2) with a DM500 dichroic mirror was used to filter the fluorescent signal from the illumination. The quality and speed of concentration was determined using centre-normalized pixel intensity analysis (NPI) of individual image frames extracted from the video. Acoustic streaming velocities and the particle velocities were estimated using Diatrack 3.01 (Semasopht, North Epping, Australia).

Fluoresbrite 1 μm polystyrene (PS) microspheres with an initial concentration of 4.55×10^{10} particles/mℓ in deionized water were diluted to obtain a concentration of 4.55×10^{6} particles/mℓ, and then dispersed using high-power 2 MHz ultrasound before use. Plain PS microspheres, 3, 10, 20 and 45 μm in diameter (Polysciences, Inc., USA) were selected to study the effect of particle size on the behaviour of the SAW device; all were diluted to provide a consistent sample concentration of 4.99×10^{5} particles/mℓ in deionized water. Living yeast cells were used to demonstrate the concentration of bioparticles. In assessing the manipulation of cells, stock cultures of yeast cells were grown and maintained on standard agar consisting of 1% yeast extract, 0.5% neutralized bacteriological peptone, and 1% glucose solidified with 1.5% agar (w/v). All media were autoclaved immediately after preparation at 121 degrees C and 15 psi for 15 min. Yeast cells were grown aerobically to the required cell density at room temperature. The viability of the cells was investigated using methylene violet stain which only penetrates dead yeast cells.

Results

Figure 2 shows the behaviour of 1-μm fluorescent PS particles as they are convected within the bulk of the drop by SAW-induced acoustic streaming as individual image frames acquired by high speed microscopy at 60 fps. The NPI decreases rapidly toward an asymptotic value of 0.2 in just 10 s, indicating the concentration process is extremely rapid, and the images of the process indicate the particles in the droplet collect at the centre (and bottom in images not shown here). Defining the time taken for concentration as the period over which the NPI decreases from a value of 1 to a constant value (0.2 in this case), one can then quantify the particle concentration process by plotting the NPI with respect to time. The rate of concentration depends on the RF power input into the device and specifically the amplitude of the SAW propagating across the substrate. Figure 3 indicates that the rate of particle concentration, as described the temporal variation in the NPI, is enhanced as the input power is increased to a critical value of approximately 300 mW. Beyond this point, the concentration efficiency begins to decrease, and there is a sharp decrease upon passing about 500 mW. At these power levels there is virtually no particle concentration, similar to the situation when low input power ($\leq$120 mW) is used. The decrease in particle concentration at high power is attributed to the redispersion of the aggregated particles. The particle dispersion at high input power is associated with large acoustic streaming velocities which oppose the dominant particle aggregation mechanism. At low power, the behaviour is similar, but the reason is entirely different: the convection velocity is insufficient to drive particle aggregation.

Indeed, the concentration process is a balance between the flow in the droplet induced via acoustic streaming [17] and the process of shear-induced migration [11] responsible for the inward propagation of the particles towards the center of the droplet over time. Though the former is entirely expected given the nature of leaky SAW used to drive the motion in the fluid droplet, the latter is the conclusion of the results shown in Fig. 4. As the size of the PS particles are changed, the time required to perform the collection scales with $1/a^2$, where a represents the size of the particles, a distinct characteristic of shear-induced migratory flow. Note that particles at less than 3 μm must agglomerate to larger sizes via a local acoustically-driven process prior to shear migration, and so the trend is displaced from 3 μm downwards, and may be observed down to 20 nm (not shown). The concentration process may be defined in terms of a *concen-*

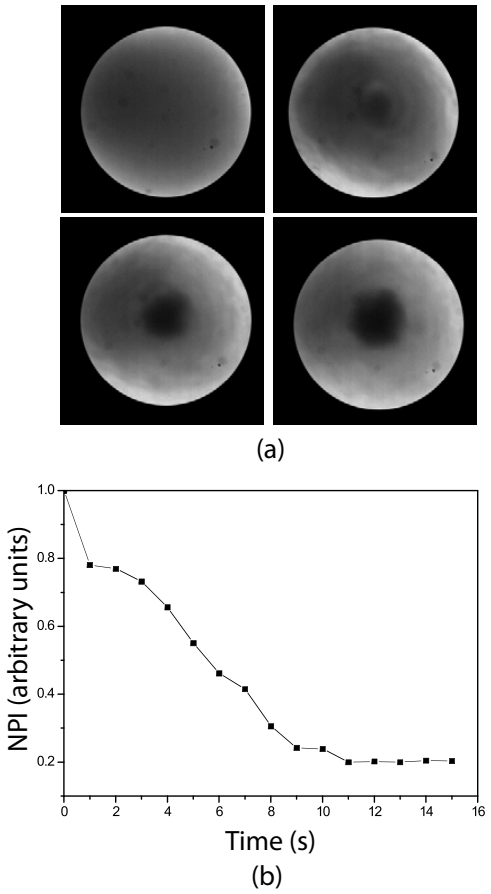

Figure 2: The (a) concentration of 1 μm PS particles in a sessile droplet is rapid as indicated by the (b) reduction of the normalized pixel intensity of the video image in ten seconds, corresponding to what is (a) observed with the eye in the images.

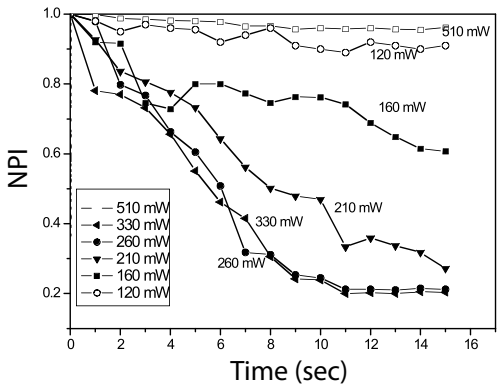

Figure 3: Concentration of particles with respect to time, as indicated by the NPI for input power levels from 120 to 510 mW.

tration factor, χ, which represents the ratio of acoustic streaming velocity induced in the fluid to the shear-induced migration velocity of the particles. Figure 5 plots the change in the particle concentration at the centre of the droplet, proportional to the inverse of the NPI, with respect to the concentration factor. Above a factor $\chi \sim \chi_c = 2$, the particles always concentrate, while below that factor, the concentration process fails.

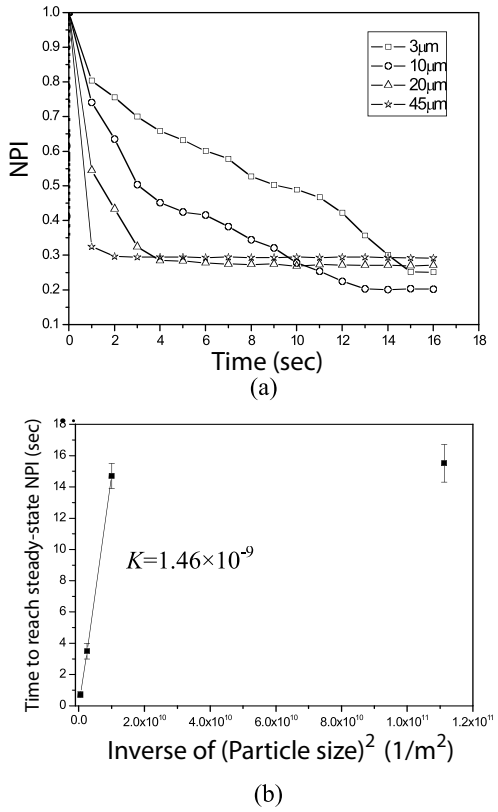

Figure 4: (a) Collection of particles (NPI) versus time for several different PS particle sizes. Note that (b) plotting the time of collection versus $1/a^2$ gives a linear relationship, except for PS particles less than about 3 μm in diameter.

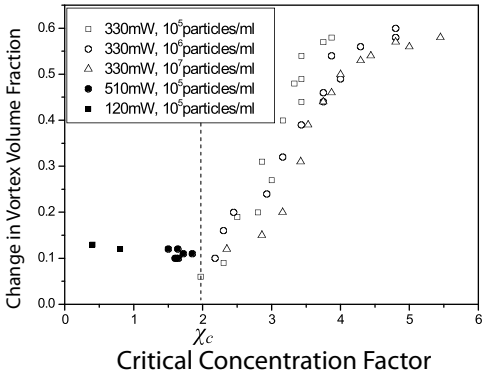

Figure 5: The concentration process may be described via a *critical concentration factor* representing the ratio of the acoustic streaming velocity [17] induced in the fluid to the shear-induced migration velocity [11] of the particles towards the center of the droplet.

The concentration of live yeast is illustrated in Fig. 6, where the yeast cells remain alive post-concentration according to the violet dye evaluation process.

Conclusions

To our knowledge, there is no method available for particle concentration in the tens of seconds that the system reported in this work describes. The importance of concentration, especially

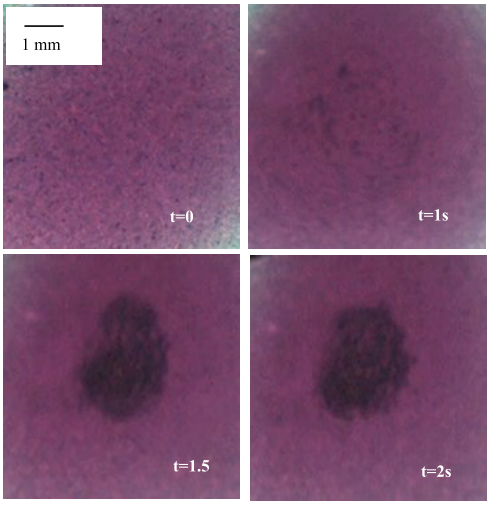

Figure 6: The rapid concentration of live yeast cells—a 1 mm mass is formed at the center of the droplet in 2 seconds.

on the micro and nano scales, cannot be underestimated. Not only do molecular binding and related chemical processes take longer as the scale is reduced due to diffusion, in most devices sensing is an important part of the process and is dependent on the time required to provide a detectable amount of binding [6]. Further, in applications of such technology to pathogen and dangerous chemical detection, an enhancement of the sensitivity and speed of sensing through concentration and rapid movement of the target molecules or cells would represent a key advancement. We hope that this approach provides a viable solution to the problems of diffusive sensing processes.

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References

[1] Beebe, D., Mensing, G. and Walker, G., Physics and applications of microfluidics in biology, *Annual Review of Biomedical Engineering*, **4**, 2002, 261–286.

[2] Byerly, P., Earthquake Mechanisms, *Science*, **131**, 1960, 1493–1496.

[3] Ciaravino, V., Miller, M. and Carstensen, E., Pressure-Mediated Reduction of Ultrasonically Induced Cell Lysis, *Radiation Research*, **88**, 1981, 209–213.

[4] Eijkel, J. and Berg, A., Nanofluidics: what is it and what can we expect from it?, *Microfluidics and Nanofluidics*, **1**, 2005, 249–267.

[5] Hashimoto, K., *Surface Acoustic Wave Devices in Telecommunications: Modelling and Simulation*, Springer, 2000.

[6] Hatch, A., Kamholz, A., Hawkins, K., Munson, M., Schilling, E., Weigl, B. and Yager, P., A rapid diffusion immunoassay in a T-sensor, *Nature Biotechnology*, **19**, 2001, 461–465.

[7] Irimia, D. and Toner, M., Cell handling using microstructured membranes, *Lab Chip*, **6**, 2006, 345–352.

[8] Joanicot, M. and Ajdari, A., Applied physics: Droplet control for microfluidics, 2005.

[9] Kundt, A. and Lehmann, O., Longitudinal vibrations and acoustic figures in cylindrical columns of liquids, *Annalen der Physik und Chemie (Poggendorff's Annalen)*, **53**.

[10] Latham, G., Ewing, M., Dorman, J., Press, F., Toksoz, N., Sutton, G., Meissner, R., Duennebier, F., Nakamura, Y., Kovach, R. et al., Seismic Data from Man-Made Impacts on the Moon, *Science*, **170**, 1970, 620.

[11] Leighton, D. and Acrivos, A., The shear-induced migration of particles in concentrated suspensions, *Journal of Fluid Mechanics*, **181**, 1987, 415–439.

[12] Li, H., Friend, J. and Yeo, L., Surface acoustic wave concentration of particle and bioparticle suspensions, *Biomedical Microdevices*.

[13] Li, H., Friend, J. R. and Yeo, L. Y., A scaffold cell seeding method driven by surface acoustic waves, *Biomaterials*, **9**, 2007, 647–656.

[14] Nilsson, A., Petersson, F., Jönsson, H. and Laurell, T., Acoustic control of suspended particles in micro fluidic chips, *Lab Chip*, **4**, 2004, 131–135.

[15] Rayleigh, L., On Waves Propagated along the Plane Surface of an Elastic Solid, *Proceedings of the London Mathematical Society*, **1**, 1885, 4.

[16] Renaudin, A., Tabourier, P., Zhang, V., Camart, J. and Druon, C., SAW nanopump for handling droplets in view of biological applications, *Sensors and Actuators B*, **113**, 2006, 389–397.

[17] Rife, J., Bell, M., Horwitz, J., Kabler, M., Auyeung, R. and Kim, W., Miniature valveless ultrasonic pumps and mixers, *Sensors and Actuators A: Physical*, **86**, 2000, 135–140.

[18] Sritharan, K., Strobl, C., Schneider, M., Wixforth, A. and Guttenberg, Z., Acoustic mixing at low Reynold's [sic] numbers, *Applied Physics Letters*, **88**, 2006, 054102.

[19] Stone, H., Stroock, A. and Ajdari, A., Engineering flows in small devices—microfluidics toward a lab-on-a-chip, *Annual Review of Fluid Mechanics*, **36**, 2004, 381–411.

[20] Stürzl, W., Kempter, R. and van Hemmen, J., Theory of Arachnid Prey Localization, *Physical Review Letters*, **84**, 2000, 5668–5671.

[21] Tan, W. M., Friend, J. and Yeo, L., Microparticle collection and concentration via a miniature surface acoustic wave device, *Lab on a Chip*, **7**, 2007, 618–625.

[22] Varadan, V. and Varadan, V., Microsensors, microelectromechanical systems (MEMS), and electronics for smart structures and systems, *Smart Mater. Struct*, **9**, 2000, 953–972.

[23] White, R. and Voltmer, F., Direct piezoelectric coupling to surface elastic waves, *Applied Physics Letters*, **7**, 1965, 314.

[24] Whitesides, G., The origins and the future of microfluidics, *Nature*, **442**, 2006, 368–373.

[25] Wixforth, A., Strobl, C., Gauer, C., Toegl, A., Scriba, J. and v. Guttenberg, Z., Acoustic manipulation of small droplets, *Analytical and Bioanalytical Chemistry*, **379**, 2004, 982–991.