Acoustofluidic centrifuge for nanoparticle enrichment and assortment

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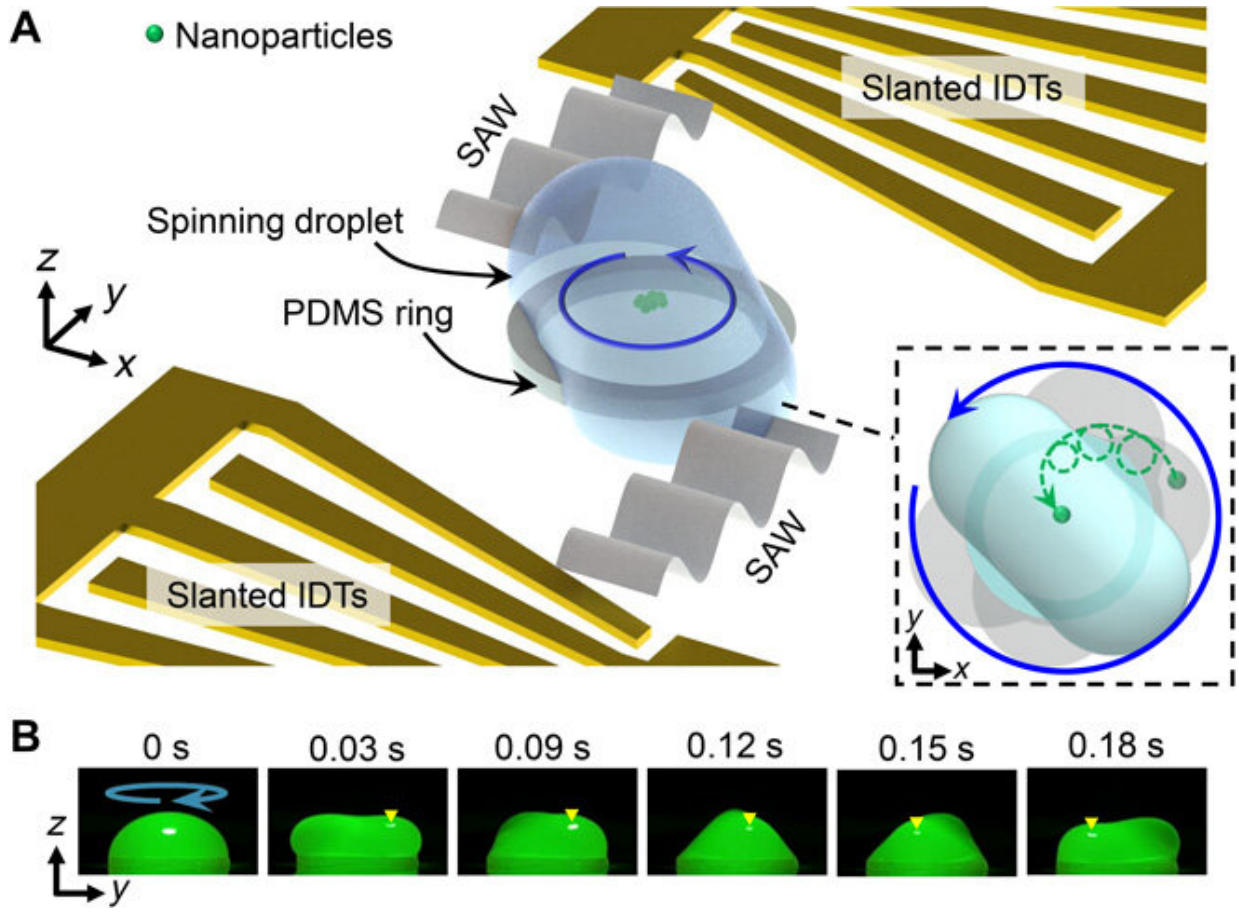


Operating mechanism of the acoustofluidic centrifuge platform. (A) Illustration of the acoustofluidic centrifuge system. The droplet is placed on a PDMS ring that confines the fluid boundary and is located between two slanted IDTs. As the SAWs propagate into the droplet, the liquid-air interface is deformed by the acoustic radiation pressure, and the droplet starts to spin. Particles inside the droplet will follow helical trajectories (inset) under the influence of both induced

vortex streaming and the spinning droplet. (B) A sequence of images showing the side view of a 30-μl rotating droplet. The SAW is activated at 0 s. The sequence shows that as the droplet starts spinning, it stretches out to a concave ellipsoid shape, as illustrated in (A). Yellow arrow indicates the reference position that rotates along with the spinning droplet. Credit: Science Advances, doi: 10.1126/sciadv.abc0467

Liquid droplets have recently gained renewed attention as a simplified model for a variety of fascinating physical phenomena at the scale of the cell nucleus to stellar black holes. In a new report now published in *Science Advances*, Yuyang Gu and a team of scientists in the U.S. presented an acoustofluidic centrifugation technique that used the entanglement of acoustic wave actuation and the spin of a fluidic droplet to accomplish nanoparticle enrichment and separation. They combined acoustic scanning and droplet spinning methods to achieve rapid nanoparticle concentrations and size-based separation with a resolution sufficient to identify and isolate exosome sub-populations.

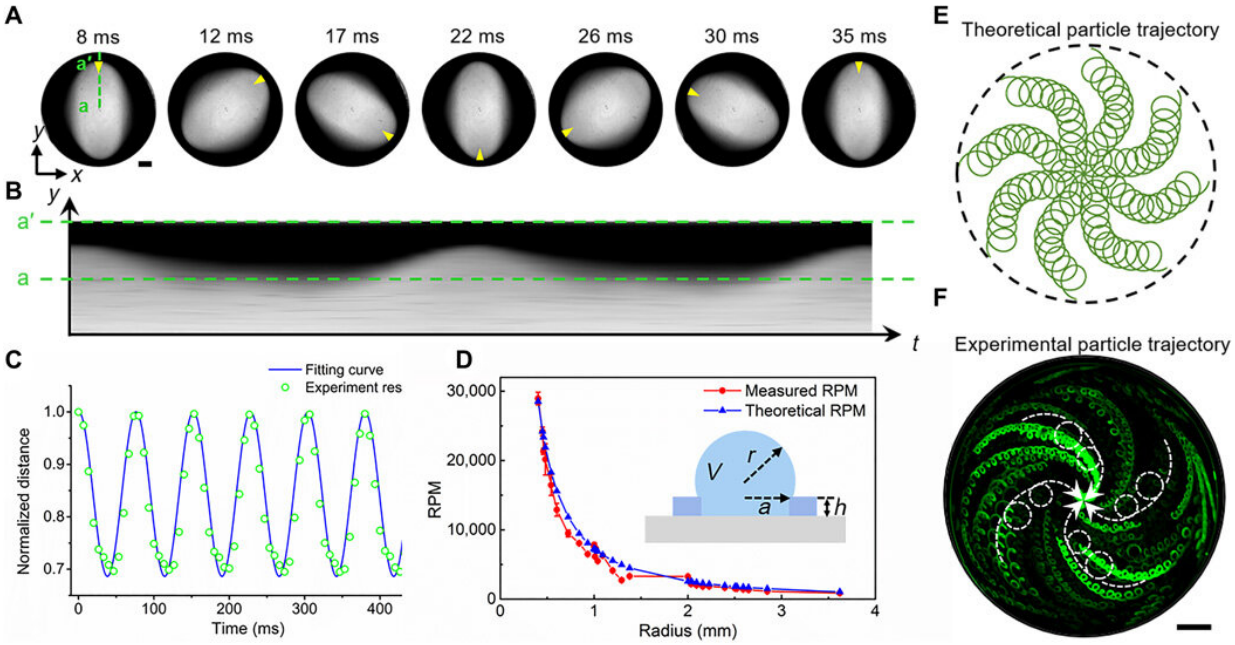
Exosomes are nanoscale extracellular vesicles that can carry molecular cargo from cell to cell and are therefore a powerful vector/vehicle in biomedical research for drug delivery and biomolecular discovery applications. The team characterized the mechanisms underlying the process both numerically and experimentally, alongside the ability to process biological samples within the devices. The acoustofluidic centrifuge method overcame existing limits of nanoscale bioparticle manipulation across multidisciplinary fields of biology, chemistry, engineering, materials science and medicine.

The acoustofluidic centrifuge system

Materials scientists aim to manipulate nanoparticles for a variety of

biomedical and biochemical applications including gene or drug delivery , bioassays, diagnostics and catalytic reactions. It is therefore necessary to perform the steps of nanoparticle concentration or separation for applications of nanostructures across multidisciplinary fields. Acoustofluidics aim to combine acoustics and microfluidics for a simplistic device design. In this work, Gu et al. presented an acoustofluidic centrifuge system to acoustically manipulate particles with sizes down to a few nanometers. The method allowed various functions including nanoparticle concentration, separation and transport.

The basic system contained a pair of slanted interdigital transducers (IDTs) and a circular polydimethylsiloxane (PDMS) ring to encapsulate a portion of the droplet and define its shape. The team generated surface acoustic waves (SAWs) to initiate droplet spinning motion. The process allowed Stokes drift along a circular closed path to transfer momentum of the fluid to notably increase the inner streaming velocity and shear rate within the droplet by many folds. According to numerical simulations, the acoustic waves could rotate a liquid droplet with a variable sample volume to influence nanoparticles of various sizes residing within the droplet. The team expect to translate the work at the micro-/nanoscale to simplify the process of transfection to automate vesicle cargo loading and to accelerate liquid biopsies.

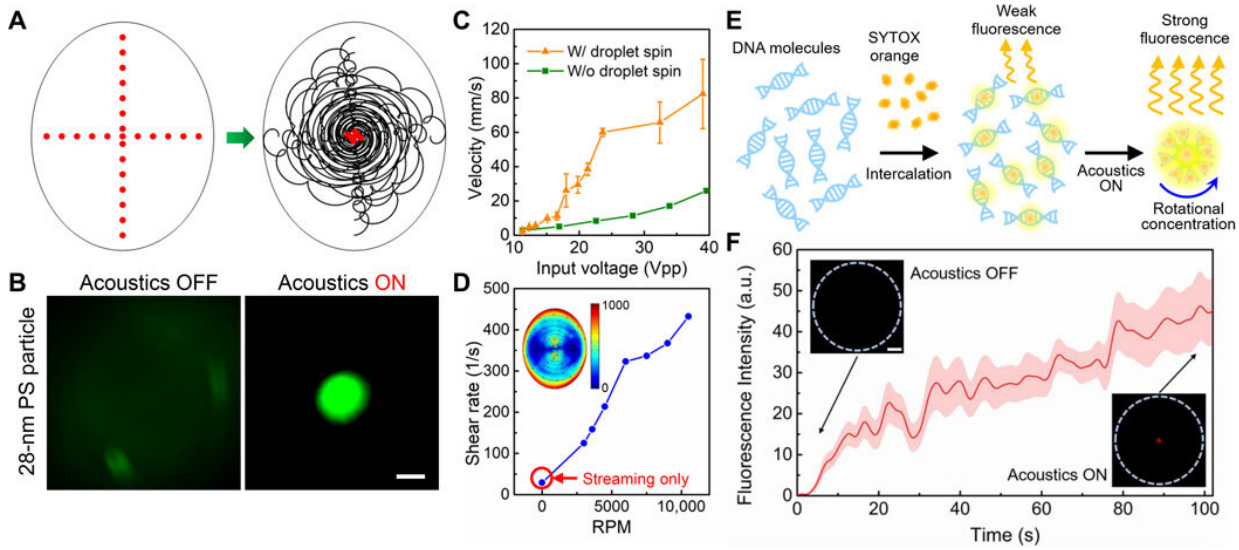


Characterization of droplet spin and particle movement in the acoustofluidic centrifuge device. (A) A sequence of images showing the top view of a spinning droplet under a microscope. (B) Corresponding time sequence of stacked images along the line a-a′, which shows the periodic spin of the ellipsoid droplet. (C) The instantaneous velocity at a point on the spinning droplet can be extracted from this normalized fit of the distance change versus time (B). (D) Theoretical and experimental droplet rotation speed [rotations per minute (RPM)] versus the change in droplet radius. The volume (V) of the droplet refers to the volume above the PDMS ring. (E) Theoretically calculated and (F) experimentally observed particle trajectories showing the dual rotation modes; particles trace a helical path as they approach the center of the droplet while also rotating around their local axes. Scale bar, 500 μm. Credit: Science Advances, doi: 10.1126/sciadv.abc0467

The working principle of the device

Gu et al. placed a droplet on a PDMS ring to confine the fluid boundary and located it between two slanted interdigital transducers (IDTs). They

then applied an electrical signal to the slanted IDTs to generate two traveling surface acoustic waves to propagate along the substrate from two opposing directions to enter the droplet. The process deformed the liquid-air interface as a result of acoustic radiation pressure and the droplets started to spin. The particles inside the droplet followed helical trajectories due to the influence of induced vortex streaming and droplet spinning motions. The scientists obtained a sequence of images to show the side-view of a 30 μL rotating droplet. They calculated the rotational speed of the spinning droplet using a Fourier transform of the waveform and extracted the droplet speed from the waveform and compared the spin rate to classical droplet oscillation dynamics.

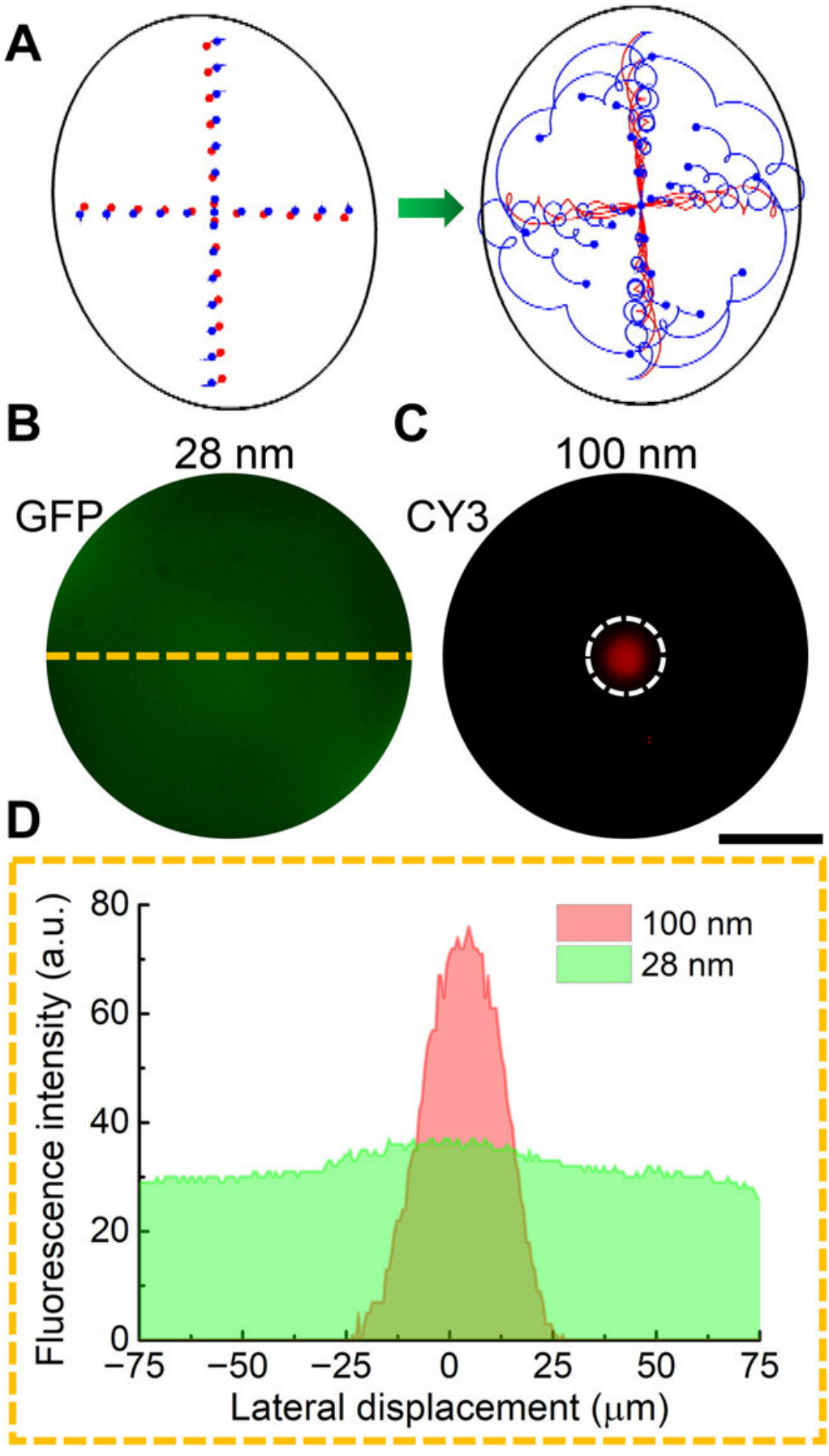


Rapid nanoparticle enrichment via acoustofluidic centrifuge. (A) Numerically simulated particle trajectory within a spinning droplet. As the droplet starts to spin, the particles that were initially randomly distributed inside the droplet (left) follow a helical trajectory until concentrated at the middle of the droplet (right). (B) Fluorescence images before (left) and after (right) the acoustic field is turned on, which shows the enrichment of 28-nm PS particles. Scale bar, 50 μm. (C) Streaming velocity with (experimental result) and without (simulation result) droplet spinning. (D) Plot of the calculated average shear rate inside the droplet

versus speed. The shear rate increases with a higher spinning speed and rises to several times higher than the shear rate when there is no rotating droplet (streaming only). (E) Flowchart showing the process of DNA enrichment and fluorescent signal enhancement in a spinning droplet. (F) Plot of the measured DNA fluorescence intensity versus time in the spinning droplet. Insets: Fluorescence images before and after signal enhancement. Scale bar, 50 μm. a.u., arbitrary units. Credit: Science Advances, doi: 10.1126/sciadv.abc0467

The kinetics of the droplets and nanoparticles within the device

The team then studied the droplet spin and particle movement in the acoustofluidic centrifuge device using a sequence of images. The particles showed dual rotation modes—tracing a helical path when approaching the center of the droplet while also rotating around their local axes. They used a range of frequencies to excite the spin of the droplets. As the applied power increased, the droplet maintained its equilibrium shape and then started to experience small oscillations until the acoustic power reached a threshold value, at which point the droplet entered stable spinning. Previous studies showed how SAWs (surface acoustic waves) induced acoustic streaming vortices inside a droplet, therefore, the team analyzed the motion of particles inside the spinning droplet. During the experiments, the nanoparticles moved along helical trajectories corresponding to a Stokes drift effect. They monitored the movement of 1 μm particles with a fast camera and analyzed the videos using particle tracking velocimetry to observe the helical-shaped trajectories that the particles followed. With each rotation of the droplet, the particles made one local rotation while simultaneously moving closer to the global center of the droplet along its helical path. In this way, the process pushed the particles inward to concentrate nanoparticles to the droplet center.

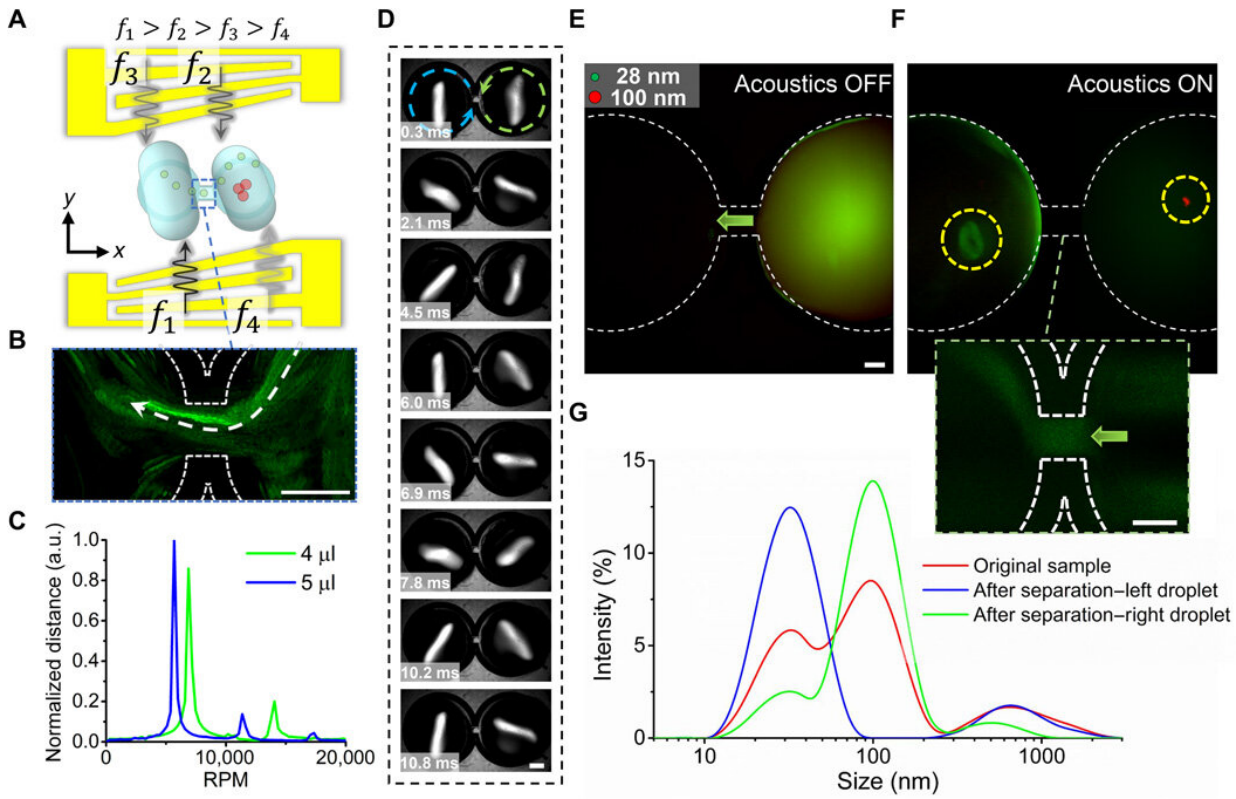

A
B
28 nm
GFP
C
100 nm
CY3
D
Fluorescence intensity (a.u.)
80
60
40
20
0
100 nm
28 nm
−75
−50
−25
0
25
50
75
Lateral displacement (μm)

Differential nanoparticle concentration via acoustofluidic centrifuge. (A) Numerical simulation results showing the difference in nanoparticle trajectories for particles with sizes of 100 nm (red) and 28 nm (blue). While the 100-nm particles become concentrated in the center of the spinning droplet, the 28-nm particles follow a helical trajectory but remain randomly distributed throughout the droplet. GFP, green fluorescent protein. (B, C) Microscope images showing the experimental result of particle separation with 100- (C) and 28-nm (B) particles. Scale bar, 100 μm. (D) Fluorescence intensity along the axis of the droplet showing the concentration effect on the 100-nm particles. Credit: Science Advances, doi: 10.1126/sciadv.abc0467

Rapid enrichment of nanoparticles

Using numerical and experimental investigations, the team showed how nanoparticles could be rapidly concentrated within the spinning droplet with particle sizes as small as 28 nm in diameter. Rapid concentration of nanoparticles could also facilitate the detection of fluorescently tagged biospecimen such as DNA molecules, which Gu et al. demonstrated in this work. The team used a fluorescent dye to detect DNA samples within the droplet, and generated an acoustic signal for droplet spin. They achieved signal amplification and enhanced signal detection based on the concentration of DNA in the sample. Aside from the rapid enrichment of nanoparticles, the system also differentially concentrated nanoparticles of varying sizes. For example, the interplay of acoustic parameters including frequency and amplitude, and the droplet dimensions generated different particle trajectories within the same droplet. However, the time scale and migration speed to reach a specific position varied for particles within the same droplet. For instance, when nanoparticles of two different sizes were contained within a spinning droplet, the larger particles experienced higher acoustic radiation forces

and smaller effects from Brownian motion.



Particle separation and transport via a dual-droplet acoustofluidic centrifuge. (A) Schematic of the dual-droplet acoustofluidic centrifuge. This dual-droplet functionality is achieved using binary frequency shift keying, which involves sequentially shifting between two frequencies for each IDT. With a high shifting frequency, two droplets can be rotated simultaneously. The two droplets are connected by a microchannel, which serves as the passage for particle transport. Here, the specific frequencies are 15.3 MHz (f4), 15.7 MHz (f3), 20.3 MHz (f2), and 21.7 MHz (f1), with a shifting frequency of 100 kHz. (B) A composite image showing the particle trajectory through the center channel. (C) The Fourier transform of the waveform plot of a fixed point on the droplet as it spins, indicating the peak rotational frequency of the two droplets with different volumes. (D) Image sequence showing the top view of dual-droplet acoustofluidic centrifuge. Fluorescence images (E) before and (F) after the acoustic signal is turned on, showing the nanoparticle separation and transport

from one droplet to another. Inset: Fluorescence image of the middle channel indicating the particle transport process. (G) Particle size distribution comparison between the pre- and postseparation samples. The original sample, which was placed into the right droplet, has two peaks at 28 and 100 nm. After separation, most of the 28-nm particles have been separated and have been transported to the left droplet, which has only one peak at 28 nm. Scale bars, 200 μm. Credit: Science Advances, doi: 10.1126/sciadv.abc0467

Dual-droplet acoustofluidic centrifuge

A single-droplet device could also adversely affect the purity of subsets of nanoparticles contained within them during the processes of differential concentration and retrieval; therefore, Gu et al. developed a dual-droplet based acoustofluidic centrifuge for practical nanoparticle separation. Using the device, they excited two pairs of surface acoustic waves (SAWs) to propagate asymmetrically across the flanks of the two droplets to cause simultaneous spins to generate two acoustic beams via a single interdigital transducer. The team used a frequency shift keying to switch between two different excitation frequencies and excitation locations, with practical applications for exosome subpopulation separation. The method allowed rapid fractionalization of exosome samples into different subpopulations for measurements via nanoparticle tracking analysis.

In this way, Yuyang Gu and colleagues developed and demonstrated an acoustofluidic centrifuge platform to efficiently and rapidly enrich or separate nanoscale bioparticles. This platform can substantially simplify the speed of sample processing, detection and reagent reactions across various applications including point-of-care diagnostics, bioassays and biomedicine.

More information: 1. Gu Y. et al. Acoustofluidic centrifuge for

nanoparticle enrichment and separation, *Science Advances*, DOI: 10.1126/sciadv.abc0467

2. Zhang P. et al. Ultrasensitive detection of circulating exosomes with a 3D-nanopatterned microfluidic chip, *Nature Biomedical Engineering*, doi: doi.org/10.1038/s41551-019-0356-9

3. Lee H. et al. Molecularly self-assembled nucleic acid nanoparticles for targeted in vivo siRNA delivery. *Nature Nanotechnology*, doi.org/10.1038/nnano.2012.73