Acoustic mixing of droplets is a promising way to implement biosensors that combine high speed and minimal reagent consumption. To date, this type of droplet mixing is driven by a volume force resulting from the absorption of high-frequency acoustic waves in the bulk of the fluid. Here, we show that the speed of these sensors is limited by the slow advection of analyte to the sensor surface due to the formation of a hydrodynamic boundary layer. We eliminate this hydrodynamic boundary layer by using much lower ultrasonic frequencies to excite the droplet, which drives a Rayleigh streaming that behaves essentially like a slip velocity. Three-dimensional simulations show that this provides a threefold speedup compared to Eckart streaming. Experimentally, we shorten a SARS-CoV-2 antibody immunoassay from 20 min to 40 s.

Keywords: Rayleigh acoustic streaming, efficient mixing, immunoassay

I. INTRODUCTION

Surface biosensors integrated in microfluidic devices enable the fast detection of molecules in small liquid samples. However, they face a trade-off between detection speed and sample waste. Indeed, the detection of dilute molecules by surface biosensors proceeds in three steps: the mass transfer of analyte from the bulk of the sample to the sensor surface, the adsorption of the analyte on the sensor surface (which can be made specific using antibody, DNA hybridization, molecular imprints) and the detection of the adsorbed analyte (by fluorescence, surface plasmon resonance (SPR), field-effect transistor...). Most often, mass transfer is the slowest step of all, and therefore determines the detection speed of the sensor [1–3]. This slow mass-transfer is mainly due to the formation of a concentration boundary layer that prevents the analytes from the bulk to reach the sensor. The thickness of this boundary layer can be decreased by introducing convection (mixing). However, for the simplest devices such as a biosensor embedded in a microchannel, increasing the flow rate also increases the waste of analyte [1] and the resulting shear may also damage the sensor [3].

The mixing of sessile droplets is a promising strategy to address this trade-off between detection speed and waste. On the one hand, convection ensures a negligible boundary layer thickness (fast mass transfer) and on the other hand, the batch-mode mixing ensures recycling of the outgoing fluid. Such droplets can be mixed using electrowetting actuation, electro-osmosis, Marangoni effect or acoustic streaming (AS), with the latter featuring high flow speed, insensitivity to fluid composition and viscosity, excellent biocompatibility and the ability to mix pinned droplets (which is not possible for electrowetting).

Acoustic streaming results from the transfer of pseudo-momentum from an acoustic wave to the fluid, which can happen through two mechanisms [4, 5]: due to the viscous attenuation of the wave in the bulk (Eckart streaming [6]) and due to the ultrasonic shear near the droplet boundaries (Rayleigh streaming) [7–9]. Eckart streaming is easily generated using high-frequency surface acoustic waves (SAW) ultrasonic transducers (interdigitated transducers) and behaves like a volume force that can mix droplets [10] and, therefore, accelerate surface bioassays such as DNA hybridization assays [11], immunoassays [12], and SPR detection [13]. However, while Eckart streaming can reduce the thickness of the concentration boundary layer by stirring the droplet, the no-slip boundary condition at the solid-liquid interface creates a slow-moving hydrodynamic boundary layer near the sensor surface. As a result, the mixing improvement is limited by the slow convection near the solid wall [14, 15].

Unlike Eckart streaming, Rayleigh streaming behaves like a slip velocity that reaches an asymptotic value within a few micrometers from the surface [7–9]. In this regard, it obliterates the hydrodynamic boundary layer near the sensor, and therefore ensures a faster detection. In most studies, Rayleigh streaming is created by oscillating sharp edges [16–18] or microbubbles [19–21], and therefore the slip boundary condition is limited to the surface of the edges and bubbles. Instead, when con-
Considering surface biosensing applications, the slip velocity should occur on the sensor to maximize the detection speed. Although this type of full-device Rayleigh streaming has been achieved in microfluidic channels with hard-boundaries [22], we are not aware of studies where it would have been applied to droplets in order to accelerate surface biosensing.

In this paper, we develop a microfluidic chip to expedite surface bioassays by mixing droplets without contact. Unlike previous studies based on Eckart streaming, using Rayleigh streaming eliminates the hydrodynamic boundary layer at the bottom of the droplet and accelerates analyte mass transfer to the sensor, which shortens the detection time. We first use finite element simulation to show that Rayleigh streaming is faster than Eckart streaming at equal flow velocity in the droplet. We then use this Rayleigh streaming to shorten immunoassays from 20 min to just 40 s.

II. METHODS

Our experiment (Fig. 1(a)) aims to detect the presence of SARS-CoV-2 antibodies (thereafter referred as the analyte). The biosensor is first coated with SARS-CoV-2 spike proteins (the antigen). The analyte then binds with the recombinant spike protein from SARS-CoV-2, and is subsequently detected with a secondary antibody (FITC * Goat anti-mouse IgG). The mass transfer of species to the surface can be accelerated by mixing the droplet with acoustic streaming (Fig. 1(b)). A piezoelectric disc excited by an alternating voltage transmits its vibration to the droplet by the mean of a glass slide. Nonlinear interactions between the fluid and the solid then result in a steady flow in the droplet: the Rayleigh acoustic streaming. Eventually, the droplet is rinsed and the adsorbed species are observed by fluorescence microscopy.

A. Materials and Chemicals

Fluorescence microparticles were purchased from Huge Biotechnology (China), 2019-nCoV (SARS-CoV-2) Spike (S) Protein (His-Avi) was purchased from Yazyme Biomedical Technology Co. (CG202-01; China), SARS-CoV-2 (2019-nCoV) Spike Neutralizing Antibody was purchased from Sino Biological (40591-MM43; China), FITC * Goat Anti-Mouse IgG (H+L)) was provided by Beyotime Biotechnology (A0568; China), 3-APTES toluene solution was provided by Aladdin Bio-Chem Technology (A107147; China), Toluene and Methanol were provided by Sinopharm (10022818 and 10014108, respectively; China). All chemicals and reagents were used as received without further purification.

B. Surface chemistry

The glass slides are first functionalized with 3-aminopropyl triethoxysilane (3-APTES) to enable the binding of proteins. The functionalized substrate is then used either to measure the mass transfer of fluorescent antibodies (FITC * Goat Anti-Mouse IgG (H+L)) or to perform an immunoassay.

1. Preparation of APTES-functionalized glass slides

A clean glass slide is first activated (hydroxilated) by treating it for 3 min with an air plasma. The glass slide is then silanized by immersion in a 20 mM 3-APTES toluene solution overnight. Finally, the substrate is rinsed with toluene and methanol to remove the unbounded 3-APTES.

2. Mass transfer assay

A 0.2 mg/mL FITC * Goat Anti-Mouse IgG (H+L) solution is directly incubated on the APTES-functionalized substrate (Section II B 1). After a predetermined duration, the slide is rinsed thoroughly with DI water and observed by fluorescence microscopy. The exposure time is 307 ms.

3. Fluorescent immunoassay

The experiment can be divided in three steps: the substrate functionalization with APTES, priming with 2019-nCov antigen and the analyte detection itself. When acoustic streaming is used, only the analyte detection is accelerated.

a. Substrate priming

The assay begins with a priming step where 0.1 mg/mL 2019-nCoV (SARS-CoV-2) Spike (S) Protein (His-Avi) are incubated for 30 minutes at 25 \(^\circ\)C on the APTES-functionalized glass substrate (Section II B 1). Accordingly, the amount of spike protein used for each 2 \(\mu\)L droplet is 0.2 \(\mu\)g. In order to prevent non-specific adsorption of other proteins on remaining APTES sites, the substrate is then immersed in a solution of 5 % skim milk diluted with 0.5 % Tween Phosphate Buffer Saline (PBST) for 30 minutes at 25 \(^\circ\)C.

b. Analyte detection

Before the assay, the optimal antibody concentration for comparison between diffusion and acoustic mixing is determined by a pure diffusion titer assay described in SI. We find that the fluorescent immunoassay can detect antibody concentrations ranging from 2 nM to 60 nM of SARS-CoV-2 (2019-nCoV) Spike Neutralizing Antibody. A concentration of 28.5 nM was selected for our experiments. Primary antibody specificity, secondary antibody specificity and blank comparison results are shown in SI.
FIG. 1. Accelerated SARS-CoV-2 antibody detection immunoassay. (a) Main steps of the immunoassay: spike proteins of the virus are deposited on a treated glass substrate, then skim milk is added to prevent non-specific adsorption. The analyte (primary antibody) can bind to the antigen is remains on the surface despite washing. Finally, the analyte is detected by a secondary fluorescent antibody. During the latest two stages, the mass transfer of antibodies to the sensor surface can be accelerated by mixing the liquid. (b) Method for the ultrasonic mixing: a droplet is deposited on the biosensor. When the transducer is turned on, the ultrasonic agitation of the glass is transmitted to the liquid, which triggers a steady acoustic streaming. After experiment, the glass slide is washed and the substrate fluorescence is measured by microscopy.

In our experiments, the target analyte is suspended in a 2 µL droplet. Acoustic streaming mixing is active during the whole immunoassay. A solution of SARS-CoV-2 (2019-nCoV) Spike Neutralizing Antibody is first incubated on the substrate, then thoroughly rinsed with deionized (DI) water. The secondary antibody (0.2 mg/mL FITC * Goat Anti-Mouse IgG (H+L)) is then incubated on the substrate and rinsed thoroughly with DI water. Overall, 0.4 µg of secondary antibody is used for each droplet. To evaluate the amount of adsorbed antibody, fluorescence microscopy images of the sensor are recorded, and the average fluorescence intensity is computed with ImageJ.

FIG. 2. Acoustic streaming velocity fields. (a) Main difference between Eckart and Rayleigh streaming. Rayleigh streaming velocity is nonzero even within a few micrometers from the biosensor surface, whereas Eckart streaming features a hydrodynamic boundary layer due to the no-slip boundary condition on the sensor surface. (b) Experimental acoustic streaming field. To facilitate visualization, yellow and green arrows represent upward and downward velocities, respectively.

C. Generation of acoustic streaming

Rayleigh acoustic streaming is powered by the shearing motion of acoustic waves at the interface between a solid and a fluid. In our experiments, the acoustic power is provided by a piezoelectric transducer glued to the glass slide, and located several centimeters away from the droplet. The acoustic vibrations are then transmitted through the solid to the liquid, where they accumulate due to acoustic resonance.

a. Electroacoustic setup As shown in Fig. 1(b), a lead zirconate titanate (PZT) piezoelectric disc is glued to the edge of a 50 × 50 × 1.8 mm glass slide using a cyanoacrylates glue. To enhance repeatability, the disc
was aligned with the glass slide using a 3D-printed template and pressed using a binder clip. The disc is powered by an AG1021 (T&C) radiofrequency power amplifier connected to a signal generator (2207B, Picoscope). The excitation frequency was set to 800 kHz and the power output from the amplifier regulated to 2 W. During vibrometry experiments, the AG1021 was replaced with a LZY-22+ amplifier (Minicircuits) because the AG1021 could not be transported to the vibrometer. The signal generator voltage amplitude was set to 2 V, and the transmitted power was measured to be 4.63 W, 4.80 and 4.95 W at 800, 830 and 860 kHz, respectively.

b. Laser Doppler vibrometry. The vibration amplitude of the droplet is measured by a Laser Doppler Vibrometer (LDV) [23]. The laser hits the apex of the droplet and bounces back through a 40× microscope lens. Thanks to its large numerical aperture, such high-magnification lens can collect light from many directions, which makes it relatively insensitive to small misalignment errors between the droplet vibrating surface and the vibrometer. The droplet vibration amplitude (120 nm) being comparable to the laser wavelength (633 nm), the perturbation approach described in Royer et al. [23] was not suitable. The non-perturbative method is described in SI. The PZT was controlled by amplifier Minicircuits LZY-22+ and Picoscope 2207B (2 V). During vibrometry characterization, the droplet profile was photographed by an auxiliary side-looking microscope.

c. Acoustic mixing assay. During acoustic mixing assays, a 2 μL droplet is placed on the glass substrate (Fig. 1). The droplet composition varies depending on the assay and is given in the corresponding method section (immunoassay/laser vibrometry/particle tracking). To enhance repeatability, the droplet contact line is immobilized using a gold ring patterned by photolithography and made hydrophobic using 1-dodecanethiol. In immunoassays, the gold ring was replaced with a silicone ring made of polydimethylsiloxane. Once the droplet is in position, the transducer is activated and the droplet content is mixed using acoustic waves for the entire duration of the incubation step. Once the assay is completed, the surface is rinsed with DI water and observed using fluorescence microscopy.

d. General Defocusing Particle Tracking (GDPT). In order to visualize the acoustic streaming, the droplet is seeded with 2 μm fluorescent particles (name?, brand?). An f=200.0 mm cylindrical lens (LJ1653RM-A, Thorlabs) is placed above the microscope objective to introduce a strong astigmatism that allows to calculate the out-of-plane location (z) of the particles based on their shape of their deformed image [24–27] (see supplementary information for a detailed example). These calculations of the particle position and subsequent reconstruction of the particle trajectories across multiple video frames are done using the Matlab implementation of the general defocus particle tracking (GDPT) method [25].

D. Simulation of acoustic streaming and mass transfer

Compared to surface acoustic wave (SAW) driven Eckart streaming [25, 26], Rayleigh streaming in sessile droplets has received a limited attention. Peng et al. [31] have used a perturbation method to compute the acoustic streaming in two dimensions. In their paper, they resolve the viscoacoustic boundary layer to calculate the acoustic forcing. This requires a very fine mesh in order to capture all the details of this boundary layer [32] and therefore this method is limited to two-dimensional models due to computer memory limitations. Here, we use the equivalent slip velocity as given by Eq. (1) to simulate acoustic streaming in three dimensions. For a given radial standing acoustic velocity field \( \tilde{v}_r(r, t) \), Nyborg provides an expression of the slip velocity \( \tilde{v}_{slip} \) in the axisymmetric incompressible case \( \text{[8]} \).

The simulations are implemented with Comsol multiphysics using two-dimensional axisymmetric finite element models, using laminar flow (creeping flow) and mass transfer of dilute species modules. First, the acoustic field in the droplet is estimated to the LDV calibration (see SI), and the associated velocity field \( \nabla \tilde{v}_r(r) \) is input in the model. The acoustic streaming is then computed from Eq. (1) using a stationary solver for the laminar flow equations. Next, this streaming field is used as input for a time-dependent convective mass-transfer and reaction problem. In the latter, antibody adsorption is simulated as a fast irreversible reaction. In first approximation, a typical antibody diffusivity is taken as \( 4 \times 10^{-11} \text{ m}^2/\text{s} \) [33].

III. RESULTS AND DISCUSSION

A. Rayleigh acoustic streaming in droplets

We first optimize acoustic excitation to maximize the acoustic streaming velocity. For water droplets on glass, the velocity of the acoustic streaming is proportional to the ultrasonic energy density in the liquid. In high-frequency Eckart streaming experiments, the acoustic energy is stored in whispering gallery modes that drive a fast acoustic streaming [30]. Provided that the acoustic wavelength is much shorter than the droplet size, these modes are insensitive to the ultrasonic frequency and the exact geometry of the droplet. However, at low frequencies characteristic of Rayleigh streaming, the acoustic wavelength becomes comparable to the droplet and energy can only be stored in discrete frequency modes.
FIG. 3. Measurements of the droplet surface vibrations by Laser Doppler Vibrometry (LDV). (a) Experimental profiles of the droplet at $t = 174$ s, 184 s and 202 s. (b) Vibration amplitude over time with excitation frequencies of 800 kHz, 830 kHz and 860 kHz.

FIG. 4. Numerical simulation of acoustic mixing in droplets. (a,b) Hydrodynamic flow streamlines (left) and analyte concentration profile (right) at $t = 10$ min in Rayleigh (a) and Eckart (b) mixing. The thickness of the streamlines is proportional to the flow velocity. (c) Radial distribution of adsorbed analyte ($\Gamma$) on the biosensor surface at different time points. To locate stagnation points, the slip velocity (Eq. (1)) is given by the right axis. (d) Total amount of adsorbed analyte ($n$) on the sensor surface over time for various mass transfer methods. The dashed line indicates half the amount of analyte initially available.

Among those modes, Eq. (1) shows that only those having a velocity $V_r$ tangential to the solid walls can drive a strong Rayleigh streaming. In the appendix, we show that the fundamental spherical breathing mode can efficiently excite Rayleigh streaming. At acoustic resonance, the acoustic field intensity peaks, which leads to the strongest acoustic streaming. However, due to evaporation, the droplet volume and contact angle change over time, and so does the resonance frequency. The experimental droplet profiles at three different stages of evap-
failing to hit exactly the resonance frequency.

cies are most likely due to the experimental excitation of 78 nm based on vibrometer data (see SI). Discrepan-
tained from simulations of a droplet vibration amplitude
µ simulation. The experimental average velocity is 10
poloidal flow compares qualitatively well to our numeri-
the computed velocity field is shown in Fig. 2 (b). The
perspective of 2
µ

ging the frequency.

s. Conversely, it is conceivable that a droplet could be
maintained resonant at all times by dynamically adjust-
ing the excitation amplitude.

FIG. 5. (a) Total amount of adsorbed analyte \( n \) on the
sensor surface over time for various droplet surface vibration
amplitude \( u_0 \). The dashed line indicates half the amount
of analyte initially available. Inset: scaling of the adsorption
characteristic time \( \tau_{1/2} \) depending on the droplet vibration
amplitude. (b) Effect of the droplet surface vibration ampli-
tude \( u_0 \) on the radial distribution of adsorbed analyte \( \Gamma \) on
the biosensor surface after 2 h. To locate stagnation points,
the slip velocity \( \text{Eq. } \) is given by the right axis.

oration are shown as dotted lines in Fig. 3 (a), in which
the red profile is the initial state of the droplet. Fig. 3
(b) shows the experimental vibration amplitude of the
droplet apex over time for a given excitation frequency.
All the curves feature a bell-curve that indicates the onset
of acoustic resonance. For instance, a droplet excited
at 800 kHz becomes resonant after 174 s of evaporation,
whereas a droplet excited at 860 kHz resonates after 202
s. Conversely, it is conceivable that a droplet could be
maintained resonant at all times by dynamically adjusting
the frequency.

In order to visualize the acoustic streaming, the three-
dimensional trajectory of 2 \( \mu \)m fluorescent particles dis-
persed in the droplet is reconstructed by GDPT and the
computed velocity field is shown in Fig. 2 (b). The
poloidal flow compares qualitatively well to our numeri-
sical simulation. The experimental average velocity is 10
\( \mu \)m/s, which is of similar magnitude to the 16.0 \( \mu \)m/s ob-
tained from simulations of a droplet vibration amplitude
of 78 nm based on vibrometer data (see SI). Discrepan-
cies are most likely due to the experimental excitation failing to hit exactly the resonance frequency.

B. Acceleration of mass transfer by Rayleigh
acoustic streaming

Having validated the numerical method, we use our
model to compare the mass transfer performance of pure
diffusion, Eckart streaming and Rayleigh streaming. For
the sake of comparison, the average velocity of Eckart
streaming is set to the same value as Rayleigh streaming
(16.0 \( \mu \)m/s). The resulting velocity fields and concentra-
tion profiles after 5 min are shown in Figure 4(a,b).
The concentration in the pure diffusion case is shown
in SI). Concentration distribution in droplets in each
case after 5 min indicates that the analyte is mainly
depleted near the solid in the case of Eckart streaming
diffusion, whereas Rayleigh streaming has a much better uniformity. Simulations also reveal that the spatial
distribution of adsorbed analyte (adsorption profile)
evolves over time Figure 4(c). The adsorption is mainly
concentrated at the stagnation point at the center of the
droplet, where streaming intensity is minimum. This is
similar to the case of Rayleigh-streaming-enhanced heat
transfer between two plates held at constant tempera-
ture where heat transfer primarily occurs at the acoustic
velocity nodes [34]. However, the adsorption minimum
is located at another stagnation point. We believe that
there is almost no analyte adsorbed there because the
inward poloidal flow ensures that all analyte is adsorbed
before reaching this second stagnation point. In order
to compare the mixing performance of Eckart acoustic
streaming, Rayleigh acoustic streaming and pure diffusion
Fig. 4(d), we define \( \tau_{1/2} \) as the time it takes to
transport half the analyte to the solid surface. Diffu-
sion takes \( \tau_{1/2} = 2108 \text{ s (35 min) , SAW induced Eckart
streaming shortens this process to } \tau_{1/2} = 929 \text{ s (≈ 15
min) } \) and Rayleigh streaming takes \( \tau_{1/2} = 275 \text{ s (≈ 5
min). Therefore, Rayleigh streaming is 3 times faster than
Eckart mixing.}

Finally, we use the simulations to evaluate the effect
of the excitation amplitude on \( \tau_{1/2} \) Fig. 3(a). For small
oscillation amplitude \( (≤ 50 \text{ nm) , we obtain a linear rela-
tionship, similar to Vainshtein et al. [34]. At high ampli-
tude, the adsorption kinetic differs from the high Peclet
asymptotic regime of Vainshtein et al. [34]. In their work,
adsorption is mainly concentrated at the hydrodynamic
nodes, whereas in the case of the droplet the streaming
stagnation points are more ambiguous and can either be
the location of maxima or minima of adsorption.

Next, we evaluate experimentally the acceleration of
protein mass transfer to the surface. A fluorescent anti-
body (FITC * Goat Anti-Mouse lgG (H+L)) able to bind
to silanized glass is used to assess mass transfer enhance-
dment due to acoustic streaming. The intensity of fluo-
rescence after different conditions of incubation is shown
in Fig. 6. A positive and negative control samples
are prepared to bracket the pixel intensity value between
the noise floor and the maximum expected intensity. The
positive control (Fig. 6(a)) is the fluorescence obtained
after immersing the slide in the antibody solution for 2
FIG. 6. Fluorescence microscopy images after incubation in different conditions (a) 0.2 mg/mL FITC * Goat Anti-Mouse lgG (H+L) after two hours of diffusion at 37 °C, (b) deionized (DI) water after 2 min of Rayleigh acoustic streaming (AS), (c) 0.2 mg/mL FITC * Goat Anti-Mouse lgG (H+L) after 2 min of Rayleigh acoustic streaming (d) 0.2 mg/mL FITC * Goat Anti-Mouse lgG (H+L) after 5 min of diffusion.

FIG. 7. Comparison of adsorption kinetics of 0.2 mg/mL FITC * Goat Anti-Mouse lgG (H+L) with and without acoustic mixing. The limit of detection is obtained from DI water.

h at 37 °C. The negative control (Fig. 6(b)) is the fluorescence image of a pure deionized (DI) water sample after 2 min of acoustic streaming. Finally, the fluorescence level after 2 min of antibody adsorption with and without streaming are shown in Fig. 6(c,d), respectively. The droplet without acoustic mixing looks similar to the negative control (DI water), whereas the droplet that was acoustically mixed looks like the positive control, indicating a saturated adsorption.

The variation of fluorescence intensity over time is shown in Fig. 7 for pure diffusion and acoustic streaming cases. In the case of pure diffusion, the fluorescence intensity increases steadily, but after 20 minutes the droplet is completely evaporated and yet the fluorescence intensity remains weaker than the positive control (2 h, 37 °C). In contrast, acoustically mixed droplets reach the saturation value within 30 s (fastest sample) to 2 min (slowest sample), the dispersion being due to the sensitive interplay between droplet volume and resonance frequency (Fig. 3).

C. Acceleration of SARS-CoV-2 detection with acoustic streaming

Droplet-based assays are known to reduce reagent use. For surface bioassays, the important metric is the surface concentration of each species, and therefore the total amount of reagent needed is proportional to the surface area of the droplet bottom or, in microplates, of the well bottom. The droplet used in this paper are 2 mm diameter, which is twice smaller than the well of a 96-well plate (5 mm) recommended for such assays. While antigen and secondary antibody concentration were not optimized, we note that 1.5 ng of SARS-CoV-2 Spike Neutralizing Antibody are required for this assay whereas 96-well plate protocols typically recommend 30 to 60 ng per well [35].

We demonstrate the acceleration of a bioassay to measure the level of SARS-CoV-2 specific antibody (SARS-CoV-2 Spike Neutralizing Antibody) in a liquid sample (here 0.5% Tween Phosphate Buffer Saline (PBST)). The SARS-CoV-2 specific antibody (primary antibody) specifically binds to an antigen on the surface (2019-nCoV Spike(S) Protein (His-Avi)). This primary antibody is then detected by a fluorescent secondary antibody (FITC * Goat Anti-Mouse lgG (H+L))). There-
fore, this experiment involves two incubation steps. For the sake of simplicity, both steps have the same duration. The fluorescence levels depending on the incubation method (diffusion or acoustic mixing) are shown in Fig. 8. Accordingly, 20 s of acoustic mixing yields fluorescence levels comparable to 10 min of diffusion. This shows that Rayleigh streaming is a highly efficient method to accelerate surface bioassays. It is also instructive to compare Rayleigh streaming results to previous immunoassays using Eckart mixing \[12, 36\] that needed 5 to 10 min of mixing (or “sample concentration”) to complete. Even though these assays did not use the exact same experimental conditions (see Table I), the speed up offered by mixing Eckart streaming \[12, 36\] that needed 5 to 10 min of Rayleigh streaming results to previous immunoassays using diffusion. This shows that method (diffusion or acoustic mixing) are shown in Fig. 8.

In first approximation, the air-liquid interface can be considered as infinitely hard \( \delta = 0 \), and the liquid-solid interface as infinitely soft \( \delta = 0 \), where the acoustic vibration velocity field \( \tilde{v} \) is given by the linearized Euler equation \( \rho_0 \partial_t \tilde{v} = -\nabla \tilde{p} \). Therefore, writing \( R \) the droplet radius, the acoustic field must satisfy \( \tilde{v}(k_0) |_{r=R} = 0 \). This yields a discrete set of allowed values for \( k \), the largest one being for \( \ell = 0 \): \( k R = \pi \). Setting \( \ell = 0 \) immediately sets \( m = 0 \), and therefore \( \tilde{v} = p_0 j_0(k_0) \). It is straightforward to show that \( \partial_z \tilde{p} \big|_{z=0} = -\frac{1}{c} \frac{\partial}{\partial \vartheta} \tilde{p} \big|_{\vartheta=\pi/2} = 0 \). Therefore \( \tilde{p} = p_0 j_0(k_0) \) is solution of the acoustic problem.

The value of \( p_0 \) is obtained from the LDV measurements. At the top of the droplet, \( \tilde{v}_{LDV} = \tilde{v}_e |_{z=R} = \tilde{v}_e \big|_{\vartheta=0} = -\frac{1}{\rho_0} \frac{\partial}{\partial \vartheta} \int \tilde{p} dt \big|_{\theta=0, \vartheta=R} \) this yields \( \tilde{v}_{LDV} = \frac{k}{\rho_0} p_0 j_1(k_0) \sin(\omega t) \). The droplet vibration amplitude at a power of 4.63 W is measured to be 120 nm. The streaming experiments are carried out at 2 W. Since the droplet and substrate are essentially linear (acoustic streaming is a very small perturbation), the displacement scales quadratically with the electrical power, therefore the displacement at 2 W is taken as 78 nm.

Appendix B: Generalization of Nyborg formula for axisymmetric acoustic streaming

Nyborg calculation is valid for standing waves at solid-fluid interfaces and yields an expression for an effective slip velocity near walls exposed to acoustic waves at a resolution between the viscoacoustic boundary layer thickness and the acoustic wavelength \[8\]. For standing waves, the pressure may be written \( \tilde{p} = \tilde{P}(\vartheta) \cos(\omega t) \), and consequently the velocity reads \( \tilde{v} = \tilde{V}(\vartheta) \sin(\omega t) \). In the following, the linearized conservation of mass and the linearized conservation of momentum will be useful:

\[
\partial_t \tilde{v} + c_0^2 \rho_0 \nabla \cdot \tilde{v} \, \tilde{v} \, \nabla \cdot \tilde{V} = \omega \tilde{P} \tag{B1}
\]

\[
\rho_0 \partial_t \tilde{v} = -\nabla \tilde{p} \, \tilde{v} \text{ reads } \quad \rho_0 \omega \tilde{V} = -\nabla \tilde{P} \tag{B2}
\]

For a flat hard surface (purely tangential acoustic velocity field), Nyborg slip reads:

\[
\tilde{V}_{slip} = -\left( \frac{1}{4 \omega} \right) \left[ Q + 2 \tilde{V} \nabla \cdot \tilde{V} - \partial_z \tilde{V}_z \right], \tag{B3}
\]

with \( \tilde{Q} = \tilde{V} \cdot \nabla \tilde{V} \). Near the surface, the velocity field is purely radial, which yields \( Q_r = \frac{1}{2} \partial_r V_r \).

Eq. (B1) yields \( \nabla \cdot \tilde{V} = -\frac{\omega}{\rho_0 c_0^2} \tilde{P} \). In \[8\], this term was eliminated by the incompressible assumption. Using Eq. (B2), the vertical component is recast as:

\[
-\partial_z \tilde{V}_z = \frac{1}{\rho_0 \omega} \partial_z \tilde{P} = \frac{1}{\rho_0 \omega} \left[ \Delta \tilde{P} - \frac{1}{r} \partial_r \partial_r \tilde{P} \right] = \rho_0 \omega \tilde{V}_z.
\]

**IV. CONCLUSION**

Accelerating fluid mixing in droplet is a key problem to improve biosensors speed without compromising on reagent consumption. In this paper, we propose to use Rayleigh acoustic streaming to bypass the hydrodynamic boundary layer that has long limited SAW-based droplet mixing. Our simulations and experiments unveil the three-dimensional hydrodynamic flow in the droplet. The simulations also reveal the adsorption profile of the analyte in the droplet and the scaling of the adoption with the acoustic excitation amplitude. Experimentally, we demonstrate that Rayleigh streaming can speed up SARS-CoV-2 Spike Neutralizing Antibody detection by up to 30 times, and reduce detection time below 20 s. The application of acoustic streaming in immunoassays provides the possibility for rapid detection of emergent pathogens.

**ACKNOWLEDGMENTS**

This work was supported by the National Natural Science Foundation of China with Grant Nos. 12040478 and 61874033; State Key Lab of ASIC and System, Fudan University Nos. 2021MS001, 2021MS002, and 2020KF006; Science and Technology Commission of Shanghai Municipal No. 22QA1400900 and No. 22WZ2502200. This project was supported by the Ministry of Science and Technology of China (Grant No. 2021YFC0634400, 2022YFE0114700), G4 funding from Institut Pasteur, Fondation Merieux and Chinese Academy of Sciences to G.W., and the International Affairs Department of the Institut Pasteur of Paris.
TABLE 1. Comparison of adsorption speed up between Eckart and Rayleigh mixing.

| Method  | Droplet volume (µL) | fluorescent species | acoustic duration | speedup Ref. |
|---------|---------------------|---------------------|-------------------|--------------|
| Eckart  | “microliter”        | 2 µm beads          | 5 min 40 s        | NA [12]      |
| Eckart  | 20                  | fluorescent carcinoembryonic antibody | 10 min           | 12 [36]      |
| Rayleigh| 2                   | SARS-CoV-2 Spike Neutralizing Antibody | 20 s             | 30 This work (Exp) |
| Eckart  | 2                   | NA                  | 15 min            | 2.3 This work (Sim) |
| Rayleigh| 2                   | NA                  | 5 min             | 7.6 This work (Sim) |

Combining all the terms in Eq. (B3), we find that \( \frac{k^2 \tilde{P}}{P_{\text{pow}}} \) and \( \nabla \cdot \tilde{V} \) that were omitted in the original calculation [8] cancel each-other. All the other terms combined yield Eq. (1) in accordance with [8].

\[
\frac{1}{\omega} \left[ -k^2 \tilde{P} - \frac{1}{2} \partial_r r \partial_r \tilde{P} \right].
\]

The \( k^2 \tilde{P} \) was eliminated by the incompressible assumption in [8].

[1] T. Gervais and K. F. Jensen, Mass transport and surface reactions in microfluidic systems, Chemical engineering science 61, 1102 (2006).
[2] R. Hansen, H. Bruus, T. H. Callisen, and O. Hassetsger, Transient convection, diffusion, and adsorption in surface-based biosensors, Langmuir 28, 7557 (2012).
[3] I. Pereiro, A. Fomitcheva-Khartchenko, and G. V. Kaigala, Shake it or shrink it: Mass transport and kinetics in surface bioassays using agitation and microfluidics, Analytical Chemistry 92, 10187 (2020).
[4] J. Vanneste and O. Bühler, Streaming by leaky surface acoustic waves, Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences 467, 1779 (2011).
[5] S. Sadhal, Acoustofluidics 15: Streaming with sound waves interacting with solid particles, Lab on a Chip 12, 2600 (2012).
[6] C. Eckart, Vortices and streams caused by sound waves, Physical review 73, 68 (1948).
[7] L. Rayleigh, On the circulation of air observed in kundt’s tubes, and on some allied acoustical problems, Philosophical Transactions of the Royal Society of London 175, 1 (1884).
[8] W. L. Nyborg, Acoustic streaming near a boundary, The Journal of the Acoustical Society of America 30, 329 (1958).
[9] J. S. Bach and H. Brus, Theory of pressure acoustics with viscous boundary layers and streaming in curved elastic cavities, The Journal of the Acoustical Society of America 144, 766 (2018).
[10] R. Shilton, M. K. Tan, L. Y. Yeo, and J. R. Friend, Particle concentration and mixing in microdrops driven by focused surface acoustic waves, Journal of Applied Physics 104, 014910 (2008).
[11] A. Wixforth, Acoustically driven planar microfluidics, Superlattices and Microstructures 33, 389 (2003).
[12] Y. Bourquin, J. Reboud, R. Wilson, Y. Zhang, and J. M. Cooper, Integrated immunoassay using tuneable surface acoustic waves and lensfree detection, Lab on a Chip 11, 2725 (2011).
[13] A. Renaudin, V. Chabot, E. Grondin, V. Aimez, and P. G. Charette, Integrated active mixing and biosensing using surface acoustic waves (saw) and surface plasmon resonance (spr) on a common substrate, Lab on a Chip 10, 111 (2010).
[14] H. Salman and P. Haynes, A numerical study of passive scalar evolution in peripheral regions, Physics of Fluids 19, 067101 (2007).
[15] V. Lebedev and K. Turitsyn, Passive scalar evolution in peripheral regions, Physical Review E 69, 036301 (2004).
[16] X. Li, J. Huffman, N. Ranganathan, Z. He, and P. Li, Acoustofluidic enzyme-linked immunoabsorbent assay (elisa) platform enabled by coupled acoustic streaming, Analytica Chimica Acta 1079, 129 (2019).
[17] C. Zhang, P. Brunet, L. Royon, and X. Guo, Mixing intensification using sound-driven micromixer with sharp edges, Chemical Engineering Journal 410, 128252 (2021).
[18] M. R. Rasouli and M. Tabrizian, An ultra-rapid acoustic micromixer for synthesis of organic nanoparticles, Lab on a Chip 19, 3316 (2019).
[19] H. Chen, C. Chen, S. Bai, Y. Gao, G. Metcalfe, W. Cheng, and Y. Zhu, Multiplexed detection of cancer biomarkers using a microfluidic platform integrating single bead trapping and acoustic mixing techniques, Nanoscale 10, 20196 (2018).
[20] L. Meng, X. Liu, Y. Wang, W. Zhang, W. Zhou, F. Cai, F. Li, J. Wu, L. Xu, L. Niu, et al., Sonoporation of cells by a parallel stable cavitation microbubble array, Advanced Science 6, 1900557 (2019).
[21] A. Marin, M. Rossi, B. Rallabandi, C. Wang, S. Hilgenfeldt, and C. J. Kähler, Three-dimensional phenomena in microbubble acoustic streaming, Physical Review Applied 3, 041001 (2015).
[22] M. Bengtsson and T. Laurell, Ultrasonic agitation in microchannels, Analytical and bioanalytical chemistry 378, 1716 (2004).
[23] D. Royer and E. Dieulesaint, Optical probing of the mechanical impulse response of a transducer, Applied physics letters 49, 1056 (1986).
[24] P. B. Muller, M. Rossi, A. Marin, R. Barnkob, P. Augustsson, T. Laurell, C. J. Kähler, and H. Bruus, Ultrasound-induced acoustophoretic motion of microparticles in three dimensions, Physical Review E 88, 023006 (2013).
[25] R. Barnkob, C. J. Kähler, and M. Rossi, General defocusing particle tracking, Lab on a Chip 15, 3556 (2015).
[26] R. Barnkob and M. Rossi, Defocustracker: A modular toolbox for defocusing-based, single-camera, 3d particle
tracking, arXiv preprint arXiv:2102.03056 (2021).

[27] M. Rossi, A. Marin, and C. J. Kähler, Interfacial flows in sessile evaporating droplets of mineral water, Physical Review E 100, 033103 (2019).

[28] S. Shikawa, Y. Matsui, and T. Ueda, Study on saw streaming and its application to fluid devices, Japanese journal of applied physics 29, 137 (1990).

[29] R. V. Raghavan, J. R. Friend, and L. Y. Yeo, Particle concentration via acoustically driven microcentrifugation: micropiv flow visualization and numerical modelling studies, Microfluidics and Nanofluidics 8, 73 (2010).

[30] A. Riaud, M. Baudoin, O. Bou Matar, J.-L. Thomas, and P. Brunet, On the influence of viscosity and caustics on acoustic streaming in sessile droplets: an experimental and a numerical study with a cost-effective method, Journal of Fluid Mechanics 821, 384 (2017).

[31] T. Peng, L. Li, M. Zhou, and F. Jiang, Concentration of microparticles using flexural acoustic wave in sessile droplets, Sensors 22, 1269 (2022).

[32] P. B. Muller, R. Barnkob, M. J. H. Jensen, and H. Bruus, A numerical study of microparticle acoustophoresis driven by acoustic radiation forces and streaming-induced drag forces, Lab on a Chip 12, 4617 (2012).

[33] B. Pokrić and Z. Pučar, The two-cross immunodiffusion technique: diffusion coefficients and precipitating titers of igg in human serum and rabbit serum antibodies, Analytical biochemistry 93, 103 (1979).

[34] P. Vainshtein, M. Fichman, and C. Gutfinger, Acoustic enhancement of heat transfer between two parallel plates, International Journal of Heat and Mass Transfer 38, 1893 (1995).

[35] Microplates for enzyme linked immunosorbent assays (elisa), https://www.dutscher.com/data/pdf_guides/en/Guide_de_selection_ELISA_Microlon_Greiner_Bio-One.pdf, accessed: 2022-08-31.

[36] J. Liu, S. Li, and V. R. Bhethanabotla, Integrating metal-enhanced fluorescence and surface acoustic waves for sensitive and rapid quantification of cancer biomarkers from real matrices, ACS sensors 3, 222 (2018).