Controlled microfluidic droplet acoustoinjection on one chip

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Abstract
We present an all-in-one acoustofluidics device for controlled acoustic field-mediated injection of surfactant stabilized water-in-oil droplets. The microfluidic channels and interdigitated transducer (IDT) channels are produced on the same master wafer and cast within one PDMS slab, making our acoustofluidics device simple to construct while retaining the same height for all channels. The IDTs with a curved, serpentine, paired and focusing geometry are easily embedded into the PDMS slab by filling the IDT channels with low melting point metal alloy. In this article, we propose the working mechanism of our embedded IDTs, which we call acoustoinjection, and carry out a precise characterization by laser doppler vibrometry (LDV) and infrared imaging to describe the injection of droplets within microfluidic channels. Although we observe that the device has acoustic resonance in the MHz frequency domain, we show that it operates most efficiently for acoustoinjection in the kHz frequency domain. In this frequency domain, our acoustofluidics device generates a pressure wave that causes destabilization of the surfactant-supported droplet interface enabling the injection of aqueous solution into the water-phase of the droplet with minimum heat generation. We show droplet injection for different surfactant concentrations, droplet passing speeds, and injection rates with
high accuracy. This integrated device has the potential to serve as an alternative to electric field mediated picoinjection technologies by acoustic field-mediated and non-harmful manipulation of droplets with bio-content.

**Integrated Acoustoinjection Device**

**TOC:** Acoustic field-mediated microfluidic droplet injection device. One-chip device consisting of a PDMS slab with T-channels for the water-in-oil droplets, injection fluid, and channels for the interdigitated transducers (IDTs), bound to an optical grade piezoelectric lithium niobate (LiNbO₃) substrate. The focal point of the acoustic field, produced as a function of the geometry of the serpentine IDT finger-pairs, is aligned to the T-junction of the microfluidic channels where manipulation of the passing droplets takes place. At the T-Junction, the surfactant-supported droplet interface is destabilized by the acoustic field and facilitates injection of the orthogonal fluid into the passing droplets.
1 Introduction

Microfluidic techniques precisely miniaturize macroscale experiments by decreasing volumes of reagents and enhancing spatio-temporal control over reactions. Implementing droplet-based microfluidics [1, 2] enables automation of bioassays [3-7] with high-throughput analysis and data precision in self-contained miniature reactors. Surfactant-stabilized droplets and their contents can further be manipulated by external physical stimuli in a variety of ways by integrating functional microfluidic units into device construction. For example, implementing electric field generation into microfluidic units enables tasks such as: sorting and selection of specific water-in-oil droplet populations [8-10]; releasing droplet content [11, 12]; initiating reactions by merging droplets with different cargos [13, 14]; or injecting additional fluids directly into the aqueous phase of the droplets [15, 16]. Apart from sorting in the microfluidic units described above, droplet manipulations including picoinjection may be achieved by electrocoalescence. [15, 17] Specifically, a T-junction microfluidic injection device can be combined with an electric field generating microfluidic unit that induces a thin-film instability at the water-oil interface as droplets pass the orthogonal injection channel. This interface destabilization leads to pore formation in the surfactant membrane and enables picolitre volumes of aqueous reagents from the perpendicular junction to be injected into the aqueous core of passing droplets.

Surface acoustic waves (SAWs) are an alternative source of external physical stimuli for water-in-oil droplet manipulations. SAWs are formed when an electronic waveform, usually in the MHz frequency domain, is transduced into mechanical energy through metal electrodes deposited onto a piezoelectric substrate. The metal electrodes are commonly deposited with a geometry that creates interdigitated transducers (IDTs) to broadcast the electronic waveform along finger-pairs that convert the oscillating signal into mechanical vibrations across the substrate. Typically, the substrate thickness and distance between the finger-pairs determines the resonance frequency. Devices that generate SAWs are designed to function with minimal energy losses by propagating the SAW across substrate surfaces exposed to air. [18]. SAWs applied to an air-to-fluid interface refract into the fluid leading to compressional waves that enable manipulation of droplets via local interface deformations [19]. The refraction of SAWs at such interfaces is usually associated with strong losses in the waveform amplitude, which has been exploited when, for example, heat generation is a desired function [20]. Among other characteristics important for optimizing the manipulation of fluids with sound, the acoustic streaming velocity is influenced by the applied waveform frequency and amplitude as well as the type and geometry of the IDT producing the signal.

Two types of IDT manufacturing approaches have been used to date: (1) patterning IDTs directly onto piezoelectric substrates exposed to air, commonly lithium niobate wafers; or (2) embedding conductive metal liquids into channels shaped in the form of IDTs within PDMS-based microfluidic devices constructed on a piezoelectric substrate. SAW-producing microfluidic units with patterned IDTs open to the air have already been established for several droplet-based microfluidics applications such as droplet merging [19, 21, 22], production [23, 24], injection [24], splitting [25] and sorting [26, 27]. In contrast, embedded IDTs have so far been implemented for droplet production [28] and micro-mixing within a single aqueous droplet [29, 30]. In the case of a continuous aqueous flow, the embedded IDTs were used for the separation of cells [31] and mixing of two separated liquid flows [32].

Both IDT types require high input frequencies in the MHz domain which can lead to the development of heat in the device. In particular, the thermal contribution in operating microfluidic SAW devices has been shown to evaporate fluids in digital microfluidics and has negative impacts on the rheological properties of suspension media, in vitro protein stability, and cell viability [33, 34]. For example, many systems that operate in the MHz frequency domain restrict exposure time of biological samples to the acoustic field [35] or implement
complicated feedback temperature control strategies including Peltier stages [36] to mitigate thermal contributions. Other works attempt to exploit device heating by using the phenomena in applications such as continuous flow polymerase chain reactions [20] or to deliberately kill cells [24]. A simple, integrated droplet-based acoustofluidics device that is mechanistically characterized and acoustically manipulates droplets with physiologically relevant conditions has yet to be shown.

In this article, we present an acoustofluidic device with PDMS-embedded IDTs for controlled injection of water-in-oil droplets, operating in the kHz frequency domain and therefore mitigating thermal side-effects in droplet manipulations. We design a one-chip acoustofluidic PDMS-device consisting of curved serpentine-paired channels for embedding liquid metal IDTs with a focusing geometry. The mechanical actuation from our embedded IDTs is pin-pointed to the T-junction of the microfluidic channels where we demonstrate an acoustically-mediated droplet injection strategy that we call *acoustoinjection*. First, we investigate the working principle underlying acoustoinjection by correlating IDT quality with function. We perform laser doppler vibrometry (LDV) measurements to physically determine the optimal resonant frequencies of our devices and visualize the acoustic field when working under high- or low- frequencies. Using infrared imaging, we highlight minimal thermal contributions when operating the devices at kHz frequencies. It is important to note that the minimal heating of the PDMS slab during device operation is advantageous when considering future applications that require stable conditions for handling complex fluids or biological samples. Finally, we demonstrate controlled acoustoinjection of surfactant stabilized water-in-oil droplets with the acoustic field produced by our IDTs. We evaluate different droplet passing rates with variable surfactant concentrations and measure the total amount of injected droplets, finding a high reproducibility and reliability for our devices. Given the simple production, operation, and consistency of our integrated acoustofluidics device, we expect that any lab with microfluidics capabilities could adopt droplet acoustoinjection technology into their toolboxes.

2 Results and Discussion

2.1 Structure and theoretical harmonic wavelength of the acoustoinjection device

The device comprises an optical-grade piezoelectric lithium niobate (127.68° Y-cut LiNbO₃) substrate containing microfluidic flow channels as well as focused, serpentine, and embedded IDTs integrated into one PDMS slab. **Fig. 1A-C** shows our design schematics and explains the functions of the inlets and outlets, the spacing of the IDTs, and the channel dimensions.

The device uses an IDT with a curved serpentine geometry where each finger pair reduces in radii until a particular aperture diameter is achieved. Our unique design enables focusing of the harmonic wavelength precisely to the injection nozzle of the flow channels where we inject passing droplets, as we depict in the illustration of **Fig. 1D** and show by brightfield microscopy in **Fig.1E**. The harmonic wavelength is described by Equation 1:

\[
    f_0 = \frac{v_0}{\lambda} \tag{1}
\]

where \( f_0 \) is the excitation frequency, \( v_0 \) is the velocity of the SAW, and \( \lambda \) is the harmonic wavelength. Given our design of one serpentine electrode finger distance in 100 µm and a SAW velocity of 3980 m/s for our 250 µm thick LiNbO₃ wafer, a harmonic excitation frequency of 39.8 MHz is calculated.
Fig. 1 Droplet-based microfluidic acoustoinjection device. (A) Technical drawing of the device with the numbers of the legend indicating the inlets and outlets. Preformed droplets are injected into the droplet inlet channel and droplet spacing is controlled by introducing an additional separation oil inlet. The injection area and channels for the low melting point alloy forming the IDTs are marked with the red square (scale bar: 3 mm) and expanded for more detail in (B). The geometry of the IDTs as interlaced finger pairs is aligned to a focal point where the manipulation of the droplets takes place. The IDTs consist of 2 individual channels arranged with curves and corners in mirrored yet offset alignment to achieve the final pattern; each channel is 25 µm wide and has 25 µm spacing between each channel. The respective side, either positive or negative current, has 10 finger pairs amounting to 20 finger pairs in total over an area of 2000 µm and a channel height of 30 µm. (C) Enlargement of the injection area of the device with the channel width of 30 µm. (D) Illustration of the manipulation area of the acoustoinjection device. At the T-Junction the surfactant-stabilized interface of the passing droplets (pink) is disrupted by the acoustic field, enabling the injection of the fluid phase of the injection channel (purple). (E) Bright-field image of the acoustic field-mediated injection into a surfactant stabilized water-in-oil droplet. Scale bar: 60 µm.

2.1 Mechanism of dual frequency range actuation and acoustoinjection

When operating our acoustoinjection device for the first time, we do not observe acoustic manipulations of water-in-oil droplets at the calculated harmonic excitation frequency (39.8 MHz). During subsequent empirical testing using frequency sweeps across a broad range of frequency domains, we surprisingly observe droplet manipulations in the audible frequency domain (kHz ranges), three orders of magnitude lower than the calculated resonant frequency. We then set out to investigate why manipulation in the kHz range is possible and aim to understand the influence of PDMS, channel geometries, and the embedding of the IDTs in our unique design.
We conjecture that the mechanism of acoustoinjection in the kHz frequency domain is caused by the transmission of acoustic energy at the interfaces between PDMS, the liquid-metal IDTs, and liquids within the flow channels. The acoustic transmissivity at the LiNbO$_3$-wafer to PDMS interface is related to both the density, $\rho$, and speed of sound, $c$, in the components resulting in the acoustic impedance: $\rho_1 c_1 / \rho_2 c_2$. As an example, the interface between water and air acts as a nearly perfect mirror, with a transmissivity of only a few hundredths of a percent, especially in the case of high frequencies (>500 Hz) due to the large difference in density and speed of sound. To overcome this effect, transmission of an acoustic pressure wave in a medium of similar density and speed of sound is required. Since PDMS has a speed of sound of 1070 m s$^{-1}$ and a density of 0.965 g cm$^{-3}$, similar properties to water (1500 m s$^{-1}$ and 1 g cm$^{-3}$), a transmissivity of around 68% of the initial intensity at the interface between both is expected at the injection spot. Therefore, embedding IDTs with the same height as the flow channels within same PDMS slab enables efficient transmission of acoustic pressure waves from the IDT aperture towards the focal region where we observe an impact on the passing droplet. Further, we assume that embedding the IDTs into PDMS decreases the eigenfrequency of the entire system.

### 2.2 Characterization of the acoustofluidic device

Visualization of the acoustic fields produced by our devices at the empirically determined kHz frequency domain as well as at the calculated resonance frequency is essential to understand the physical functioning of our devices. The corresponding characteristics of the acoustic fields, such as field form, mean displacements caused by the transduced oscillations, and thermal contributions or losses thereof are also fundamental to understanding the discrepancy of our system with previously understood principles. To corroborate our proposed mechanism, we perform laser-doppler-vibrometry (LDV), Fig.2, and infrared imaging measurements, Fig.3, providing both visual and physical understanding of the underlying external stimuli affecting droplet acoustoinjection.

LDV enables visualization of the acoustic field shape, for example pressure waves vs standing waves, by measuring the magnitude of acoustically-induced displacements propagating through the system. We obtain LDV measurements and present representative heat-maps that represent the mean displacements measured within the region of interest on the devices. In the kHz frequency domain at 1 V$_{pp}$ applied voltage, we observe a bulk acoustic pressure wave with mean displacements in the nanometer range (Fig. 2A and B) as a function of the amplitude (0.5 V$_{pp}$ and 1.5 V$_{pp}$ not shown). As can be seen in Video S1, an alternating deflection of the respective finger pairs causes a pressure wave across the injection nozzle region of our acoustofluidic device. Table S1 lists the respective values for the displacements across the entire set of operating parameters investigated. In contrast to this, we do indeed observe a focused SAW propagating through the system (Video S2) when operating the device near the calculated resonance and across a range of MHz frequencies and amplitudes. The acoustic wave focuses to the injection nozzle region of our device with a notable decrease in the mean displacement (Fig. 2C and D, Table S1). We expect that the discrepancy between our physical observations in the audible frequency range in contrast with the calculated resonance frequency is caused by embedding the IDTs with matched channel heights into the same PDMS slab. For example, other researchers have observed significant damping of SAWs when directly overlying PDMS onto hard metal IDTs patterned on LiNbO$_3$ substrates [20, 37].
Fig. 2 Laser doppler vibrometry of the acoustofluidics device from two frequency domains at time points that attribute to different amplitudes (1 Amplitude: $T_0 = 180^\circ$. LDV displacement in the range: 0 $\rightarrow$ 180). (A) and (B) present microscopy-based LDV measurements obtained while operating the device at 10 kHz and 1 V$_{p-p}$. The form of the acoustic field, visualized by the heat map overlay of the mean displacement field, demonstrates a pressure wave with displacements in the nanometer range. (C) and (D) present the device operating at 36 MHz and 1 V$_{p-p}$ with the mean displacement mapping showing the generation of a focusing SAW with an order of magnitude lower displacement. Scale bars: 100 µm.

We consider whether the observed phenomena of relatively large mean displacements produced by the acoustic pressure wave (kHz) vs reduction in the mean displacement of the SAW (MHz) propagating through our devices is due to damping or thermal contributions. We use infrared imaging to visualize thermal excitations using the same conditions measured by LDV, to determine the characteristics of the physical properties. When the device is operated in the pressure wave mode at 10 kHz and 1 V$_{p-p}$, we observe minimal increases in thermal energy (Fig. 3, A). In comparison, at 36 MHz and 1 V$_{p-p}$, the parameter combination where our device generates SAWs, the temperature in the device drastically increases up to 323 K (Fig. 3 B).

These data validate our proposed working mechanism, that being, in our system the density of the PDMS and speed of sound leads to the refraction of MHz-generated SAWs into the PDMS layer, thereby developing heat. The diminished displacement coupled to loss of acoustic energy as heat in the MHz frequency domain prevents SAWs from destabilizing the surfactant shell of water-in-oil droplets as they pass the injection nozzle and therefore no acoustoinjection occurs. On the other hand, the acoustic pressure wave triggered when operating our device in the kHz frequency domain actuates acoustoinjection. This acoustic pressure field maintains most of the acoustic energy, enables transmission of acoustic energy into the droplets, and thereby causes the surfactant layer at the water-oil interface of the passing
droplet to be destabilized and injected with an aqueous phase from the injection channel. Because we observe the most efficient and highest mean displacements at 10 kHz (Table S1), we use this optimized parameter as the standard for the further characterization of the devices.

![Image](image_url)

**Fig. 3** Infrared Images of the acoustoinjection device after operating for 6 sec. at two frequencies of interest. (A) The frequency is set to 10 kHz and the input voltage to 1 V$_{p-p}$. The thermal increase is very low. (B) The frequency is set to 36 MHz and the input voltage to 1 V$_{p-p}$. In the high frequency range there is a drastic thermal increase up to 30 K in the region of the IDT. Scale bars: 2 mm.

In comparison to other techniques, the fabrication of our devices is rather straightforward and does not require complicated mask alignments nor extraneous equipment. To gauge the quality of our integrated acoustofluidics devices, we construct 3 individual devices and evaluate them further by assessing the output power and the fidelity of the IDTs by micro-CT imaging. We perform an amplitude calibration across a range of input voltages for each of 3 devices at 10 kHz (Fig. S2) and compare it to the signal directly output by the waveform generator, demonstrating excellent agreement regardless of the IDT fidelity. Comparing this amplitude calibration with micro-CT images of the three devices (Fig. S2, Device 1-3), it is clear that IDT quality has no influence on acoustic field actuation.

### 2.1 Controlled acoustoinjection of water-in-oil droplets

We characterize our concept of droplet acoustoinjection in the kHz frequency domain by testing different droplet passing rates with variable surfactant concentrations and determine the total amount of injected droplets. Because we observe the highest displacement at 10 kHz for several input voltages and devices, independent of the IDT quality (Table S1), we use this frequency for the remainder of our investigations.

We find that acoustoinjection of droplets take place for fluorosurfactant concentrations of 1, 3 and 5 wt% (Fig. 4). The most efficient and reproducible injection occurs with 3 wt% fluorosurfactant concentration (Fig. 4 pink squares and an example for acoustic pressure field-mediated injection in Video S3), displaying a balance between interfacial tension and impact of the acoustic field on destabilizing the surfactant layer to permit droplet injection. At 1 wt% fluorosurfactant concentration (Fig. 4 blue circles), some droplets after injection also coalesce with each other as an effect of the higher interfacial tension and the associated lower stability of the water-in-oil droplet. With increasing surfactant concentrations (Fig. 4 green triangles), the droplet stability increases and the average change in the droplet aspect ratio, taken as the length of the droplet in the channel by the width of the channel, decreases. Moreover, it seems
that a higher input amplitude, 1 vs 3 V_p-p, leads to a slightly higher change in the droplet aspect ratio, suggesting that more fluid is injected. This trend substantiates the values of the LDV mean displacement measurements (Table S1), where a higher input amplitude leads to a larger mean displacement, in turn demonstrating that higher input amplitudes lead to stronger droplet destabilization. The difference between individual aspect ratio values can be explained when interrogating brightfield images of the analyzed high-speed-camera videos (Fig. S3). These images demonstrate that the volume injected into a droplet depends on the size of the drop formed at the injection nozzle. The formation of this droplet is affected by the Laplace pressure [15, 38] and may be responsible for small variations in the injection volume between individual droplets within the same injected droplet population.

**Fig. 4** Impact of surfactant concentration on droplet destabilization during acoustoinjection. Frequency distribution of the change in droplet aspect ratio (length of the droplet divided by the channel width from before and after acoustoinjection) of droplets acoustoinjected with 1 wt% Fluorosurfactant (blue circles), 3 wt% Fluorosurfactant (pink squares), and 5 wt% Fluorosurfactant (green triangles) at 10 kHz and two input amplitudes, 1 vs 3 V_p-p. Error bars represent the coefficient of variations for the percent change in droplet aspect ratio (n = 36 droplets sized for each, individual population).

For a closer look at the correlation between amplitude and injection pressure rate, we measure the variation of droplet injection as a function of the change in droplet aspect ratio, influence of three input amplitudes, and two different inlet pressure rates for the injection fluid across three different devices (Fig. 5A) with all droplets initially stabilized by 3 wt% fluorosurfactant in the oil phase. The general trend of increasing droplet aspect ratio with increasing input amplitude is confirmed where a higher pressure at the injection nozzle increases the volume of injected fluid into the passing droplet (Fig. 5b, top panel vs bottom panel). The reproducibility of the change in the droplet aspect ratio between the different devices seems to be more stable for 135 mbar pressure rate at the injection nozzle. Described a different way, the variability in aspect ratio of each droplet in comparison to the population of analyzed droplets for each condition is higher at 120 mbar pressure rate in comparison to 135
mbar. This behavior shows the influence of the pre-drop formation at the injection nozzle as a function of the applied pressure. A higher coefficient of variation, or CV determined as the ratio of the standard deviation to the mean and presented by the diameter of the points in Fig. 5A, indicates a less constant pre-drop formation. The error could be minimized by increasing the pressure at the injection nozzle so that the formation of the pre-drop happens faster or before the pre-drop is cut off by the continuous oil phase. Additionally, the CV could be minimized by a feedback control that adjusts the droplet passing rate at the injection nozzle so that the droplet passing and the formation of the pre-drop is synchronized. A final alternative for minimizing the error in injection is to optimize the design of the injection nozzle, for example by reducing the width of the nozzle.

**Fig. 5** Stability of acoustoinjection. **(A)** Image analysis results of the percentage change in droplet aspect ratio for \( n = 36 \) droplets as a function of amplitude/injection pressure rate combinations. The diameter of each bubble on the chart corresponds to the coefficient of variation (CV), representing the dispersion to which the injection of a single droplet increased its aspect ratio in comparison to the entirety of injected droplets. Further, the bubble chart demonstrates the percentage change in droplet aspect ratio when the injection is actuated at 10 kHz across 3 different devices shown by: Device 1 yellow bubbles with diagonal dot pattern; Device 2 blue bubbles with horizontal line pattern; and Device 3 red bubbles without a pattern. The injection pressure rates are: top panel 120 or bottom panel 135 mbar as indicated across a range of input amplitudes. **(B)** Bright-field images of droplets before and after passing the T-Junction at various input amplitudes at each injection pressure of 120 mbar, top panel, and 135, bottom panel, for the indicated applied voltages at 10 kHz. Scale bars: 50 µm.

Using the most stable surfactant concentration (3 wt%) as optimized for our acoustoinjection approach, we analyze the injection efficiency of various droplet passing rates.
With the respective inlet pressure rates, 14 to 164 droplets per second passed the microfluidic channel and we achieve 100% injection (Table S2), further supporting the reliability of our acoustoinjection device. In contrast, injection experiments in the MHz frequency domain show no acoustoinjection of passing droplets, which is a finding that perfectly aligns with our proposed mechanism (Section 2.1). Interestingly, because the droplets that we inject are 50-80 µm diameter, they can be excited by their respective eigenfrequency in the ultrasonic frequency range of 1-100 kHz. This finding is supported by the field [38, 39] but has never before been demonstrated, as it is here, within an integrated PDMS based acoustofluidics device. This leads us to emphasize the potential implications of interface stabilizing surfactants on the efficiency of acoustoinjection, as our proposed mechanism of injection is based on interface destabilization.

3 Conclusions

In this study, we present a one-chip microfluidic device for the controlled acoustoinjection of water-in-oil droplets at kHz frequencies. Our focused, embedded-IDTs are in the same PDMS slab as the microfluidic channels for droplet manipulation, making our device simple to manufacture and operate.

Our device functions in the kHz frequency domain in contrast with the theoretically predicted MHz domain which is characteristic for the generation of SAWs. We therefore precisely analyze the working principle and quality of our embedded-IDTs and define a mechanism for our observed acoustoinjection in the kHz frequency domain on our one chip device. By LDV measurements, we characterize the resonance frequencies and the characteristics of the acoustic fields when operating our acoustofluidic devices in the MHz and kHz frequency ranges with various input amplitudes. In the MHz domain, we observe a precisely focused SAW. However, infrared camera measurements clearly demonstrate that in the MHz frequency domain, much of the energy required for the droplet manipulation is lost as heat. When operating in the kHz frequency domain, our one-chip device transmits an acoustic pressure wave, with minimal thermal loses, causing destabilization of water-in-oil droplet interfaces and therefore enabling controlled injection of water-in-oil droplets on one, integrated chip. We characterize the substrate displacement, or actual mechanical propagation, of the produced acoustic fields to be the most efficient at a frequency of 10 kHz. The comparison of input/output power measurements of several devices on different substrates together with micro-CT images of the quality of the individual IDTs supports that our devices work reproducibly in the kHz frequency domain regardless of the quality of the IDTs. Finally, we use the newly developed device for the injection of water-in-oil droplets in the kHz frequency domain. We evaluate the injection efficiency for different surfactant concentrations, droplet passing speeds, injection rates, applied frequencies and amplitudes. With these experiments, we find perfect conditions in which 100% of our passing droplets are injected at droplet passing rates up to 160 Hz, with the potential to progress to even higher passing speeds.

We show that in our system, density and speed of sound of the PDMS leads to the diffraction of some fraction of the acoustic wave into the PDMS layer whereby heat is developed. The acoustic pressure wave that propagates when operating our device in the kHz frequency domain maintains most of its mechanical power and enables the coupling of acoustic energy into the surfactant layer between the water droplet and continuous oil phase. This acoustic field mediated surfactant destabilization causes injection of droplets passing a T-junction with profound precision. Finally, modifications in the design of the injection nozzle could improve the accuracy of the injected volume into each droplet. Our IDT design and operation in the kHz frequency domain could be applied in other droplet manipulation strategies like droplet merging [13, 14] and content release [11, 12]. All in all, our acoustoinjection device
is a cost-effective, reliable, and gentle option for microfluidic applications in which future biological content within water-in-oil droplets is not negatively impacted by thermal energy.

4 Materials and Methods

4.1 Device production

The microfluidic acoustoinjection device is designed with computer-aided design (CAD) software, QCAD-pro (Ribbonssoft, Switzerland) and transferred onto a photoresist-layered silicon wafer (master wafer) with a micro Pattern Generator µPG 101 (Heidelberg Instruments, Germany). Fig. 1 and S1 show a precise sketch of our designs and explain the constructions. As previously described [12], for the production of the master wafer, negative photoresist (SU8-3025, MicroChem, USA) is spin-coated (Laurell Technologies Corp., USA) onto a silicon wafer at 2650 rpm to achieve a uniform coating of 30 µm thickness. The wafer is then placed on a hot plate for a 5 min soft bake at 65 °C, then ramped slowly to 95 °C and held for 15 mins. The CAD design is exposed onto the photoresist with the writing mode II setting of the micro Pattern Generator. The output power of the laser is set to 50 mW with a pixel pulse duration of 20 %. For the post exposure bake, the wafer is placed on a hot plate for 1 min at 65 °C and then ramped and held at 95 °C for 5 min. The unexposed parts of the resist are removed with mr-DEV 600 (MicroChemicals, Germany). The following hard bake is carried out in an oven at 150 °C for 15 min. To fabricate the microfluidic device, Polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning, USA) is mixed at a 9:1 (w/w) ratio and poured over the master wafer, degassed for several minutes in a desiccator, and cross-linked for 2 h at 65 °C in an oven. After hardening, the PDMS slab is peeled off of the wafer and a biopsy punch is used (World Precision Instruments, USA) to punch holes for the fluid inlets/outlets (0.5 mm) and electrodes (1.0 mm). Prior to the attachment of the PDMS to a 2 inch, double-side polished, lithium niobate (LiNbO3) piezoelectric crystal (128° Y-cut, 0.25 mm, Precision Micro-Optics, USA), both materials are activated using an oxygen plasma (PVA TePla 100, PVA TePla, Germany; 0.45 mbar, 200 W, 20 sec). After activation, the PDMS slab is pressed on the lithium niobate substrate and heated for at least 1 h at 65 °C. The IDT channels are directly integrated into the microfluidic design and subsequently filled with liquid metal. For this, the microfluidic device is heated to 80 °C on a hot plate and a low melting-point alloy (In0.51Bi0.325Sn0.165, Indium Corporation of America, USA) is molten injected into the IDT channels. Electric wires are connected to the melted solder and fixed with UV hardening glue (Loctite 352, Henkel, Germany) after allowing the molten solder to cool down and harden.

4.2 Electronic setup

Because our device operates across a broad-spectrum frequency range that includes both kHz and MHz frequencies, we use two different electronic set-ups to create and control the electronic sine waveforms applied to the embedded IDTs. In the range of 1-30 kHz, we create the sine waveform using an Arbitrary Function Generator (AFG 31000, Tektronix, Germany) across a range of applied peak-to-peak power that is then amplified by factor of 100 using a High Voltage Amplifier (TREK Model 2210, Acal BFi, Germany). In the MHz range, we create the sine waveform using an Arbitrary Function Generator (AFG1062, Tektronix, Germany) with the same range of applied peak-to-peak power, amplified by a custom-built amplifier comprising a Mini-Circuits Model ZHL-32A+ op-amp powered by an RS-PRO RS-3005D Digital Control DC Power Supply. All electronic connections are established using standard BNC cords, connectors, and test clip adapters from Thorlabs Inc.

4.3 Device characterization
4.3.1 Laser doppler vibrometry (LDV)

We use laser doppler vibrometry (LDV) to evaluate the contribution of acoustic energy to surface topography and dynamic motion as well as to visualize the operation of our acoustofluidics device. Our devices are evaluated using a full-field scanning microscope-based vibrometer (MSA-600 X/U, Polytec GmbH, Germany) and software (PSV, Polytec GmbH, Germany) that enables us to measure sub-nanometer displacement of our PDMS-embedded IDT’s as a function of modulation across frequency and amplitude parameters. To record acoustic displacement maps, we use an opaque device that is suspended on an observation stage such that only the edges of the SAW wafer are in contact with the mount. We then obtain scans of at least one full IDT finger-pair along with the region where the T-junction of our microfluidics channels reside using combinations of frequency and applied voltages. These bandwidth scans allowed us to identify potential resonance frequencies of our devices (10 kHz, 20 kHz, 30 kHz, 5 MHz, 21 MHz, and 36 MHz) at a range of applied voltages (0.5, 1, and 1.5 \( V_{pp} \)).

4.3.2 Infrared imaging

The infrared image measurements are taken with an IRCAM EQUUS 327k M (IRCAM, Germany) infrared camera at a wavelength of 3.7 \( \mu \text{m} \) – 5 \( \mu \text{m} \), a 640 x 512 px resolution, a 15 \( \mu \text{m} \) Pitch size and a NETD < 20 mK. The thermographic images are recorded with a 100 Hz Framerate.

4.3.3 IDT amplitude calibration and influence of different devices

Three devices were built to measure the impact of PDMS slab positioning and IDT quality on the piezoelectric effect propagating through the LiNbO\(_3\) substrate. A sine-wave of variable amplitude between 0.5 – 5 V at 10 kHz was fed to the amplifier and the resulting peak-to-peak voltages were measured across the IDTs. Measurements were performed using an oscilloscope (Voltcraft DSO-1062D 2 Channel Digital Storage, Germany) using a standard BNC cord connected to a 100x probe.

4.3.4 Micro-CT

The wires of the IDTs in PDMS from the three devices we use to calibrate are cut and then the samples are used for x-ray micro-CT (SKYSCAN 1272, Bruker, Germany). To obtain a tomographic image, a source current of 100 \( \mu \text{A} \) and a source voltage of 100 kV with a 0.11 \( \mu \text{m} \) copper foil as filter is applied on the cathode. An isotropic voxel size of 4 \( \mu \text{m} \) and a total field view of 16.128 x 10.752 mm was set and 1800 sequential projections on a 360° rotation are collected with a respective exposure time of 2414 ms. The radiographic projections are automatically post-corrected for alignment, ring artifacts and beam hardening using the Software NRecon from Bruker. The visual reconstruction is taken using the software CTVox supplied by Bruker.

4.4 Droplet microfluidics and acoustoinjection

4.4.1 Production of water-in-oil droplets

Surfactant stabilized water-in-oil droplets are produced in the flow-focusing junction of the droplet production device (Fig. S1). The continuous phase comprises 1, 3 and 5 wt% perfluoropolyether-polyethylene glycol (PFPE-PEG) block-copolymer fluorosurfactant (Ran Biotechnologies, Inc., USA) dissolved in fluorinated oil (HFE-7500, 3M, USA). The dispersed
phase is pure Milli-Q water. Both phases are injected into the production device with syringe pumps (11 PicoPlus Elite, Harvard Apparatus, USA). The fluids are loaded into 1 mL syringes (Omnifix®-F, B.Braun, Germany) and connected to the device by a cannula (Sterican®, 0.4 x 20 mm², BL/LB, B.Braun, Germany), and PTFE-tubing (0.4 x 0.9 mm, Bola, Germany). The flow rates for the oil and aqueous phases are set to 400 and 200 µl/hr, respectively and the produced droplets are collected in an Eppendorf tube (Eppendorf, Germany).

### 4.4.2 Acoustoinjection of water-in-oil droplets

The previously produced droplets are reinjected into the microfluidic acoustoinjection device by a pneumatic flow controller (MFCSTM-EZ, Fluigent, Germany). The droplet inlet pressures are varied between 100 and 500 mbar, while the distance between the droplets is adjusted by adding the corresponding continuous phase into a separate inlet channel under certain pressures. For better visualization, black ink is used as droplet injection fluid. The pressure in the injection fluid channel is adjusted to the pressures of the droplet channel to ensure contact between the passing droplets and the injection fluid. For the determination of the injection with different input amplitudes and surfactant concentrations (Fig. 4), the inlet pressure is 300 mbar at the droplet inlet channel, 295 mbar at the separation fluid inlet channel and 120 mbar at the injection fluid inlet channel. The exact pressure rates for the injection efficiency experiments under certain droplet passing speeds can be found in Table S2. Droplet injection is observed with an inverted microscope (Olympus IX71, Olympus, Japan) and high-speed camera videos (Phantom v2511, Vision Research, USA) are taken for further analysis of the injection process.

### 4.4.3 Quantification and analysis of acoustoinjected water-in-oil droplets

The acoustoinjection of microfluidic droplets is quantified using ImageJ analysis of high-speed camera videos from triplicate experiments. To investigate the impact of surfactant concentration on acoustoinjection, droplets are injected with actuation at 10 kHz, a low and high amplitude within the linear range of power, and 1, 3 and 5 wt% surfactant concentrations. The aspect ratios of droplets (n = 36) are measured before and after passing the injection region of the device to determine the percentage change in the aspect ratio of the injected populations. The percent change in droplet aspect ratios as a function of surfactant concentration are plotted using a frequency distribution plot with the coefficient of variation represented by error bars in MATLAB (Fig. 4). This same measurement approach is repeated to investigate the impact of the inlet pressure rate for the injection fluid onto the pre-drop formation and therefore the injected volume into each passing droplet. The experiments were performed at 10 kHz frequency, input amplitudes of 0.5 V_{pp}, 1.0 V_{pp} and 1.5 V_{pp} with 120 mbar and 135 mbar pressure at the fluid injection nozzle. The aspect ratios of droplets (n = 36) are measured before and after passing the injection region of the device to determine the percentage change in the aspect ratio of the injected population. The data are plotted using bubble chart in MATLAB to depict the injection fidelity of each device with the diameter of each point representing the coefficient of variation (Fig. 5). For the determination of the injection efficiency, three high-speed-camera videos of 8 to 50 sequential passing droplets each are analyzed by counting the number of injected droplets.

### Conflicts of interest

There are no conflicts to declare
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