

Journal of Visualized Experiments

Fabrication, operation and flow visualization in surface-acoustic-wave-driven acoustic-counterflow microfluidics
--Manuscript Draft--

Manuscript Number:	JoVE50524R2
Article Type:	Invited Methods Article - JoVE Produced Video
Corresponding Author:	Marco Cecchini, Ph.D. NEST, Istituto Nanoscienze-CNR and Scuola Normale Superiore Pisa, Pisa ITALY
First Author:	Marco Travagliati
Order of Authors:	Marco Travagliati
	Richard Shilton
	Fabio Beltram
	Marco Cecchini, Ph.D.

Fabrication, operation and flow visualization in surface-acoustic-wave-driven acoustic-counterflow microfluidics.

Authors: Marco Travaglini^{1,2}, Richie Shilton¹, Fabio Beltram² and Marco Cecchini^{2,*}

1. Center for Nanotechnology Innovation @ NEST, Istituto Italiano di Tecnologia, Piazza San Silvestro 12, I-56127 Pisa, Italy

2. NEST, Scuola Normale Superiore and Istituto Nanoscienze-CNR, Piazza San Silvestro 12, I-56127 Pisa, Italy

Corresponding author email: marco.cecchini@nano.cnr.it

Keywords: surface acoustic wave, microfluidics, flow visualization, acoustofluidics, MEMS, STICS, PIV, microfabrication, acoustics, particle dynamics

Short Abstract:

In this video we first describe fabrication and operation procedures of a surface acoustic wave (SAW) acoustic counterflow device. We then demonstrate an experimental setup that allows for both qualitative flow visualization and quantitative analysis of complex flows within the SAW pumping device.

Abstract:

Surface acoustic waves (SAWs) can be used to drive liquids in portable microfluidic chips via the acoustic counterflow phenomenon. In this video we present the fabrication protocol for a multi layer SAW acoustic counterflow device. The device is fabricated starting from a lithium niobate (LN) substrate onto which two interdigital transducers (IDTs) and appropriate markers are patterned. A polydimethylsiloxane (PDMS) channel cast on an SU8 master mold is finally bonded on the patterned substrate. Following the fabrication procedure, we show the techniques that allow the characterization and operation of the acoustic counterflow device in order to pump fluids through the PDMS channels grid. We finally present the procedure to visualize liquid flow in the channels. The protocol is used to show on-chip fluid pumping under different flow regimes such as laminar flow and more complicated dynamics characterized by vortices and particle accumulation domains.

Introduction:

One of the continued challenges facing the microfluidic community is the need to have an efficient pumping mechanism that can be miniaturized for integration into truly portable micro-total-analysis systems (μ TAS's). Standard macroscopic pumping systems simply fail to provide the portability required for μ TAS's, owing to the unfavorable scaling of the volumetric flow rates as the channel size decrease down to the micron range or below. On the contrary SAWs have gained increasing interest as fluid actuation mechanisms and appear as a promising avenue for the solution of some of these problems^{1,2}.

SAWs were shown to provide a very efficient mechanism of energy transport into fluids³. When a SAW propagates onto a piezoelectric substrate, e.g. lithium niobate (LN), the wave will be

radiated into any fluid in its path at an angle known as the Rayleigh angle $\Theta_R = \sin^{-1}(c_f/c_s)$, owing to the mismatch of sound velocities in the substrate, c_s , and the fluid c_f . This leakage of radiation into the fluid gives rise to a pressure wave which drives *acoustic streaming* in the fluid. Depending on the device geometry and power applied to the device, this mechanism was shown to actuate a wide variety of on-chip processes, such as fluid mixing, particle sorting, atomization, and pumping^{4,1}. Despite the simplicity and effectiveness of actuating microfluids with SAW, there is only a small number of SAW driven microfluidic pumping mechanisms that have been demonstrated to date. The first demonstrated simple translation of free droplets placed in the SAW propagation path on a piezoelectric substrate³. This novel method generated much interest in using SAWs as a microfluidic actuation method, however there was still a need for fluids to be driven through enclosed channels—a more difficult task. Tan *et al.* demonstrated pumping within a microchannel which was laser ablated directly into the piezoelectric substrate, and by geometric modification with respect to the channel and IDT dimensions, was able to demonstrate both uniform and mixing flows⁵. Glass *et al.* recently demonstrated a method of moving fluids through microchannels and microfluidic components by combining SAW actuated rotations with centrifugal microfluidics, as a demonstration of true miniaturization of the popular *Lab-on-a-CD* concept^{6,7}. However, the only fully enclosed SAW driven pumping mechanism that has been demonstrated remains to be Cecchini *et al.*'s *SAW-driven acoustic counterflow*⁸—the focus of this video. It exploits the atomization and coalescence of a fluid to pump it through a closed channel in the direction *opposing* the propagation direction of the acoustic wave. This system can give rise to surprisingly complex flows within a microchannel. Moreover, depending on the device geometry it can provide a range of flow schemes, from laminar flows to more complex regimes characterized by vortices and particle-accumulation domains. The ability to easily influence the flow characteristics within the device shows opportunities for advanced on-chip particle manipulation.

In this protocol we wish to clarify the main aspects of practical SAW-based microfluidics: device fabrication, experimental operation, and flow visualization. While we are explicitly describing these procedures for the fabrication and operation of SAW-driven acoustic counterflow devices, these sections can easily be modified for their application to a range of SAW-driven microfluidic regimes.

Protocol:

1. Device fabrication

1.1 Design two photomasks, the first for patterning the surface acoustic wave (SAW) layer, and the second for the polydimethylsiloxane (PDMS) microchannel mold.

1.1.1 The first photomask has a pair of opposing interdigital transducers (IDTs)—also known as a SAW delay line—and markers for channel alignment and spatial reference during microscopy. In our standard device we have single-electrode IDTs with a finger width $p = 10\ \mu\text{m}$, aperture of $750\ \mu\text{m}$, and 25 straight finger pairs. The resulting IDT generates SAWs with a wavelength $\lambda = 4p = 40\ \mu\text{m}$ corresponding to an operating frequency $f_o = c_{\text{SAW}}/\lambda \approx 100\ \text{MHz}$ on $128^\circ\ \text{YX}$ lithium niobate (LN). Each IDT width should be above two times the width of the microchannel to

reduce any misalignment effects while bonding the layers. IDT design parameters are discussed comprehensively in several books⁹⁻¹¹. We remark that only one IDT (placed at the channel outlet) is necessary to drive the fluid into the channel in acoustic-counterflow, but patterning a full delay line assists in device testing.

1.1.2 The second has a simple microchannel structure to be aligned along the SAW delay line, with a microchamber to form the channel inlet. In our typical devices, the channels have a width $w = 300 \mu\text{m}$ and a length of 5 mm. As a general rule, the channel width should be at least 10λ to avoid diffraction effects during SAW propagation in the microchannel, however in our testing we found that a width of $\sim 7\lambda$ would not significantly affect SAW propagation within the channel.

1.2 Begin with an LN wafer and cleave a 2 cm by 2 cm sample. In order to perform transmission microscopy it is necessary to use a double side polished wafer. Note that LN is a standard for its biocompatibility and the SAW polarization and high piezoelectric coupling coefficient along the major axis, however other piezoelectric materials may be used with appropriate design considerations.

1.3 Clean the substrate by rinsing it in acetone, 2-propanol and drying with a nitrogen gun.

1.4 Spin coat the sample with Shipley S1818 at 4000 rpm for 1 minute.

1.5 Soft bake at 90°C for 1 minute on a hot plate.

1.6 Align the sample with the SAW layer mask using a mask aligner and expose it to UV light with a 55 mJ/cm^2 . Care should be taken to align the IDT direction along the major axis of the LN substrate.

1.7 Rinse the sample in Microposit MF319 developer for 30 seconds to remove the unexposed photoresist.

1.8 Stop the development by rinsing the sample in deionized water and dry it with a nitrogen gun.

1.9 Deposit a 10-nm-thick titanium adhesion layer followed by 100-nm-thick gold layer by thermal evaporation.

1.10 Perform *lift-off* by sonicating the sample in acetone, then rinse it in 2-propanol and dry with a nitrogen gun.

1.11 Silanize the device surface to make it hydrophobic in the microchannel area¹².

1.11.1 Mask the microchamber area with AR-N-4340 negative tone photoresist by optical lithography according to the manufacturer's datasheet.

1.11.2 Activate the sample surface with a 2 minute oxygen plasma (Gambetti Kenologia Srl, Colibri) of 0.14 mbar pressure and 100 W power giving a bias voltage of approximately 450 V.

1.11.3 Mix 35 mL Hexadecane, 15 mL carbon tetrachloride (CCl_4), and 20 μL Octadecyltrichlorosilane (OTS) into a beaker inside a fume hood. Place the device in the solution, and leave covered for two hours.

1.11.4 Rinse the device with 2-propanol and dry it with a nitrogen gun.

1.11.5 Check that the contact angle of water on the surface is above 90° . If the contact angle is insufficient, clean the sample and re-perform the steps in 1.11.

1.11.6 Remove the residual resist on the sample by rinsing in acetone, 2-propanol and drying with a nitrogen gun.

1.12 Mount the sample on a printed circuit board with radio frequency waveguides and standard coaxial connectors (RF-PCB), and then put acoustic absorber (First Contact polymer) on the sample edges and connect the IDT by wire bonding or using pogo connectors.

1.13 A master mold of the channel layer is patterned with SU-8 onto a small piece of Silicon (Si) wafer using standard optical photolithography. SU-8 type and photolithography recipe will be dependent on the final PDMS internal channel height required.

1.14 Cast PDMS on the mold

1.14.1 Mix PDMS with a curing agent at a ratio of 10:1.

1.14.2 Centrifuge the PDMS for 2 min at 1320 rcf for degassing.

1.14.3 Pour the PDMS gently onto the SU-8 mold in a petri dish to a total PDMS height on the order of 1 mm. The open Petri dish can be placed in a vacuum desiccator for approximately 30 minutes in order to degas the PDMS further.

1.14.4 Once degassed, cure PDMS by heating to 80°C for one hour in an oven. Note that baking time and temperature can affect the mechanical properties of PDMS.

1.15 Prepare the solid PDMS layer

1.15.1 Cut around the channel using a surgical blade, being careful not to damage the SU8 master, and peel it off.

1.15.2 Replica edges are then refined and straightened using a razor blade leaving at least 2 mm clearance on lateral side of the channel and no clearance (cut right through) at the channel outlet.

1.15.3 Punch a hole in the microchamber using a Harris Unicore puncher to form the fluid-loading inlet.

1.16 Bond the PDMS channel with the LN substrate by simple conformal bonding. In this way the bond will hold throughout the fluid testing stage while remaining reversible.

1.16.1 Both surfaces are cleaned prior to joining by blowing away any excess debris with compressed nitrogen air. It is critical when joining the pieces to align the channel with the major axis of the LN according to the patterned alignment marks.

1.17 The complete device schematic is shown in Fig. 1. Store completed devices in a clean environment until use.

Note: It is important that all fabrication steps are carried out in a clean room environment to avoid contamination of the device before use.

Note: Any of the optical lithography steps may be replaced by the user preferred methods.

Note: The silanization procedure may be substituted for a preferred hydrophobic coating method¹³.

2. RF device testing

2.1 Calibrate the network or spectrum analyzer with an open/short waveguide on your RF-PCB

2.2 Connect the SAW delay line to the ports of a spectrum analyzer and measure the scattering matrix of the device. The transmission for a pair of single-electrode transducers will resemble the absolute value of a sinc function centered at the operating frequency of the IDT. In the reflection spectrum a dip (minimum) is observed at the same frequency⁹⁻¹¹. In our devices at 100 MHz operating frequency along the major axis typical values are -15 dB for S_{11} and S_{22} and -10 dB for S_{12} (without PDMS channels).

3. Microfluidics and particle flow dynamics visualization experiment and analysis

3.1 Place the sample under a microscope. The specific optical setup depends on the SAW microfluidics phenomena to be observed. For example, a simple reflection microscope equipped with a 4x objective and a 30 fps video camera will be suitable to study fluid filling dynamics. To investigate more complex microparticle dynamics it may be necessary to use a microscope equipped with a 20x objective and a 100 fps or higher video camera. It is important that both the objective and frame rate are high enough to capture spatially and temporally any important flow features.

3.2 Connect the IDT in front of the channel outlet to an RF signal generator and operate it at the resonant frequency observed in the scattering matrix measurements. The typical operating power in acoustic-counterflow experiments is 20 dBm. If necessary, use a high-power UHF amplifier. Acoustic-streaming and atomization phenomena are observed without acoustic counterflow while running the device at lower power: typically acoustic-streaming recirculation begins at 0 dBm and atomization occurs above 14 dBm.

3.3 Load 60 μ L of fluid into the microchamber with a micropipette. Fluid will passively diffuse into the microchamber. If necessary, gently push on the microchamber surface in order to favor the microchamber filling.

3.3.1 In order to visualize the flow it is necessary to add microbeads to the fluid. Note that in order to avoid particle clustering, sonicate the particle suspension prior to the experiments. To avoid particle adhesion on the substrate apply a 0 dBm signal to the device while loading.

3.4 Start recording the video through the microscope and increase the operating power in order to observe acoustic counterflow. Different flow schemes will be determined by input power, chip design and particle diameter.

3.4.1 In order to qualitatively capture the dynamics, fluid flow has to be recorded in proximity of the meniscus and inlet at different stages of channel filling using markers as a spatial reference.

3.4.2 In order to perform quantitative measurement of particle dynamics by micro particle image velocimetry (μ PIV)^{14,15} or spatial temporal image correlation spectroscopy (STICS)^{16,17}, fluid flow has to be recorded in the point of interest with a fixed field of view for at least 100 frames at a frame rate imposed by the particle dynamics.

3.5 Analyze the video with image processing software. The choice of the software to be used depends on the phenomena of interest. For example, to quantify the size distribution of atomized droplets, spatial periodicity of particle accumulation, or manual tracking of diluted particles, simple freeware image analysis software such as Fiji is suitable¹⁸; whereas in order to obtain streamlines and velocity field measurements, customized μ PIV¹⁹ or STICS²⁰ code is required. In our analysis customized STICS code is written in MATLAB, however a preferred alternative coding language may be equally acceptable.

Representative Results

Figure 2 shows representative results of device RF testing which were taken prior to bonding the LN layer to the microchannel layer: typical S_{11} and S_{12} spectra are reported in panel a) and b) respectively. The depth of the valley at central frequency in S_{11} spectrum is related to the efficiency of conversion of RF power in SAW mechanical power. Hence, for a fixed number of IDT finger pairs, a reduction in the valley minimum will result in a reduction of the power required to operate the device. At the frequency of this minimum the device will most efficiently generate the acoustic wave to actuate the fluid pumping, and therefore is the point at which we choose to operate the device. In our devices at 100 MHz operating frequency along the major axis typical values are below -10 dB for S_{11} . Values above -10 dBm may signify a damaged or shorted transducer which, if working, will require increased input power. This value can be reduced by matching the IDT impedance, using an external matching network, or by IDT design⁹⁻¹¹. The maximum of the S_{12} spectrum is both related to the efficiency of conversion of RF power and SAW mechanical power by the IDTs and the attenuation of SAW along the delay line. Reduction of this value (typically around -10 dBm in our devices) can stem from defects in IDTs (observed also by a reduction of the dip magnitude in the S_{11} spectrum), misalignment of the SAW delay line, or cracks.

Figure 3 shows four different characteristic flow patterns observed using 500-nm latex beads. Each panel shows particle streamlines resulting from STICS. Analysis was performed on a 2 second recording at 100 fps obtained by optical transmission microscopy. The detailed

dynamics results from the balance between the two dominant forces acting on the particles: drag force and acoustic radiation force^{21,22}. The drag force has two components in acoustic counterflow: one results from mass transport due to channel filling, the other results from the dissipation of acoustic energy in the fluid arising in a recirculation known as acoustic streaming. Both acoustic streaming and acoustic radiation force decay as the pressure wave in water attenuates. Panels a) and b) show two different results at the channel inlet. In panel a) two symmetrical vortices are observed due to the acoustic-streaming phenomena at the beginning of the acoustic-counterflow channel filling. After some time when the channel is partially filled, panel b) shows laminar flow due to suppression of acoustofluidic effects at the inlet by the advancing fluid front. Panel c) and panel d) show two different situations in the proximity of the meniscus when the channel is partially filled. In panel c) particles are observed accumulating in lines and moving at the same speed as the meniscus. This is the representative case in which particle dynamics is dominated by the acoustic radiation force. The representative dynamics of the dominance of drag force and acoustic streaming effects is shown in panel d) in which particles follow two vortices and accumulate only in bands within 300 μm from the meniscus, close to the substrate surface.

Tables and Figures:

Name	Company	Catalog Number	Comments
Double side polished 128° YX lithium niobate wafer	Crystal Technology, LLC	n/a	
Silicon wafer	Siegert Wafers	n/a	We use <100>
IDT Optical lithography mask with alignment marks (positive)	Any vendor	n/a	
Channel Optical lithography mask (negative)	Any vendor	n/a	
Positive photoresist	Shipley	S1818	
Positive photoresist developer	Microposit	MF319	
Negative tone photoresist	Allresist	AR-N-4340	
Negative tone photoresist developer	Allresist	AR 300-475	
SU8 thick negative tone photoresist	Microchem	SU-8 2000 Series	
SU8 thick negative tone photoresist developer	Microchem	SU-8 developer	
Hexadecane	Sigma-Aldrich	H6703	

Carbon tetrachloride (CCl ₄)	Sigma-Aldrich	107344	
Octadecyltrichlorosilane (OTS)	Sigma-Aldrich	104817	
Acetone CMOS grade	Sigma-Aldrich	40289	
2-propanol CMOS grade	Sigma-Aldrich	40301	
Titanium	Any vendor	99.9 % purity	
Gold	Any vendor	99.9 % purity	
PDMS	Dow Corning	Sylgard® 184 silicone elastomer kit with curing agent	
Petri dish	Any vendor		
5 mm ID Harris Uni-Core multi-purpose coring tool	Sigma-Aldrich	Z708895	Any diameter greater than 2 mm is suitable
Acoustic absorber	Photonic Cleaning Technologies	First Contact regular kit	
RF-PCB	Any vendor		
Spinner	Laurell technologies corporation	WS-400-6NPP	Any spinner can be used
UV Mask aligner	Karl Suss	MJB 4	Any aligner can be used
Thermal evaporator	Kurt J. Lesker	Nano 38	Any thermal, e-beam evaporator or sputtering system can be used
Oxygen plasma asher	Gambetti Kenologia Srl	Colibri	Any plasma asher or RIE machine can be used
Centrifuge	Eppendorf	5810 R	Any centrifuge can be used
Wire bonder	Kulicke & Soffa	4523AD	Any wire bonder can be used if the PCB is used without pogo connectors
Contact Angle Meter	KSV	CAM 101	Any contact angle meter can be used
Spectrum analyzer	Anristu	56100A	Any spectrum or network analyzer can be used
RF signal generator	Anristu	MG3694A	Any RF signal generator can be used
RF high power amplifier	Mini Circuits	ZHL-5W-1	Any RF high power amplifier can be used
Microbeads suspension	Sigma-Aldrich	L3280	Depending on the experimental

			purpose different suspension of different diameter and different material properties can be used
Optical microscope	Nikon	Ti-Eclipse	Any optical microscope with spatial resolution satisfying experimental purposes can be used
Video camera	Basler	A602-f	Any video camera that has enough frame rate and sensitivity satisfying experimental purposes can be used
Camera acquisition software	Advanced technologies	Motion Box	Any software enabling high and controlled frame rate acquisition can be used

Table 1: Materials

Figure 1: Top view (a) and isometric view (b) of the completed counterflow device (not to scale). The device is constructed from two layers; the lower comprised of gold patterned IDTs on LN, and the upper of the PDMS micro channel. The RF signal is applied to the left IDT, and the corresponding SAW will propagate to the right. The fluid will flow from the circular fluid inlet on the right towards the left IDT. Typical chip dimensions are 25 mm x 10 mm x 0.5 mm for the SAW layer, and 10 mm x 5 mm x 4 mm for the PDMS layer. Feature dimensions are given in point 1 of the protocol.

Figure 2: Typical S-parameters for a SAW-counterflow device. The resonance frequency in the spectra (a) S_{11} and (b) S_{12} can be seen at 95 MHz.

Figure 3: Four different characteristic flow patterns observed using 500-nm latex beads within the acoustic counterflow channel. The streamlines shown in each panel result from the STICS analysis of 2-second recordings at 100 fps with optical transmission microscopy, and are overlaid onto the final frame of each video. The channel inlet can be seen at (a) time $t = 0$, when the channel begins to fill, and at a (b) later time after the channel is partially filled. The leading edge of the meniscus can be seen for the case of (c) laminar flow with particle accumulation lines, and (d) more complex vortical flow; the scheme being determined by the device geometry. The flow patterns were obtained on a typical device operated at 20 dBm. Flow rates for these experiments were on the order of 1-10 nL/s through the channel, while the mean flow velocity in the vortices could be as high as 1 mm/s.

Discussion:

One of the greatest challenges faced by the microfluidic community is the realization of an actuation platform for truly portable point-of-care devices. Among the proposed integrated micropumps²³, those based on surface acoustic waves (SAWs) are particularly attractive due to their associated capabilities in fluid mixing, atomization and particle concentration and separation⁴. In this paper we have demonstrated how to fabricate and operate a lab-on-chip

device in which fluid is steered in a closed PDMS microchannel by integrated on-chip SAW actuators as first described by Cecchini et al.⁸.

Concerning the device fabrication as illustrated in the procedure above, it is very important to maintain cleanliness at every point of the fabrication protocol otherwise imperfections in the IDTs, microchannel shape, and surface wettability may arise. Imperfections in the IDTs can lead to an increase of the required operating power or even ineffective transduction of the SAW. Attention must be given to microchannel fabrication. A flat clean surface is needed for microscopy. Defects in microchannel edges can cause meniscus pinning and reduce both channel filling velocity and chip reliability. These defects can also nucleate bubbles which alter the flow characteristics and may disable the fluid pumping altogether. Caution must be taken in surface functionalization. If the channel walls consisting of the substrate bottom interface and PDMS lateral and top surfaces are overall hydrophilic, capillary driven filling prevents SAW active pumping. Conversely, if the substrate surface is too hydrophobic, droplets atomized out of the meniscus would not coalesce effectively, preventing channel filling. Inhomogeneity in the substrate functionalization hence leads to unreliable channel filling dynamics with pinning points and capillarity driven regions.

Concerning flow visualization and particle dynamics studies, the particle diameter is critical to the resulting observed dynamics. Particles are subjected both to drag force (due to fluid flow) and acoustic radiation force (due to direct momentum transfer from the pressure waves in the fluid). While drag force is proportional to particle radius, the acoustic radiation force is proportional to particle volume. The drag force will dominate the particle dynamics as the particle diameter is reduced, and the particles will therefore follow the fluid flow more closely. In this way we can obtain an accurate visualization of the fluid flow by choosing an appropriately small particle diameter with respect to the device design. Note that particles of the same diameter could either reproduce the fluid streamlines accurately, or conversely be dominated by the acoustic radiation force, depending on the device geometry. Depending on the size of the beads and the visualization technique, the optics required may change. Particle concentration depends also on the experimental purpose: in the case of μ PIV low particle concentration is preferred^{14,24}, but large particle concentration allows for better statistic and qualitatively visualized streamlines in single images. The particle solution should be monodisperse and without clusters for both qualitative and quantitative understanding of the particle velocity fields.

Much effort was also devoted to understand the behavior of micro-sized particles²⁵ in view of sorting applications in biological samples. In order to perform fundamental sorting studies with beads, particle and channel functionalization are of paramount importance in order to avoid particle adhesion and channel clogging.

In this video we showed how to fabricate and operate SAW-driven acoustic counterflow devices in which fluids are driven on-chip in closed PDMS microchannel grids. Particular attention was been devoted to the visualization of the particle dynamics that is at the basis of acoustophoretic sorting applications.

Acknowledgements: Authors have no one to acknowledge.

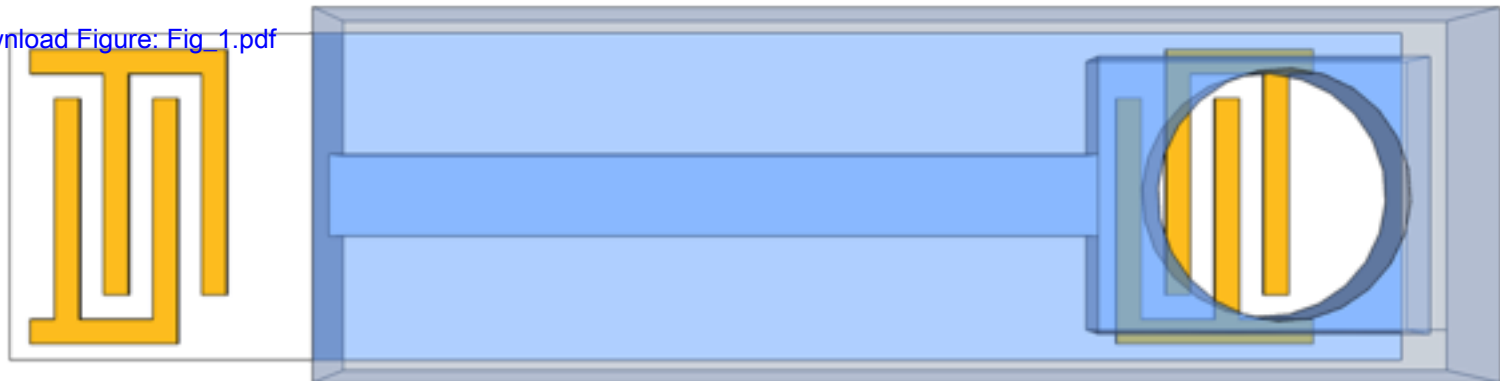
Disclosures: Authors have nothing to disclose.

References:

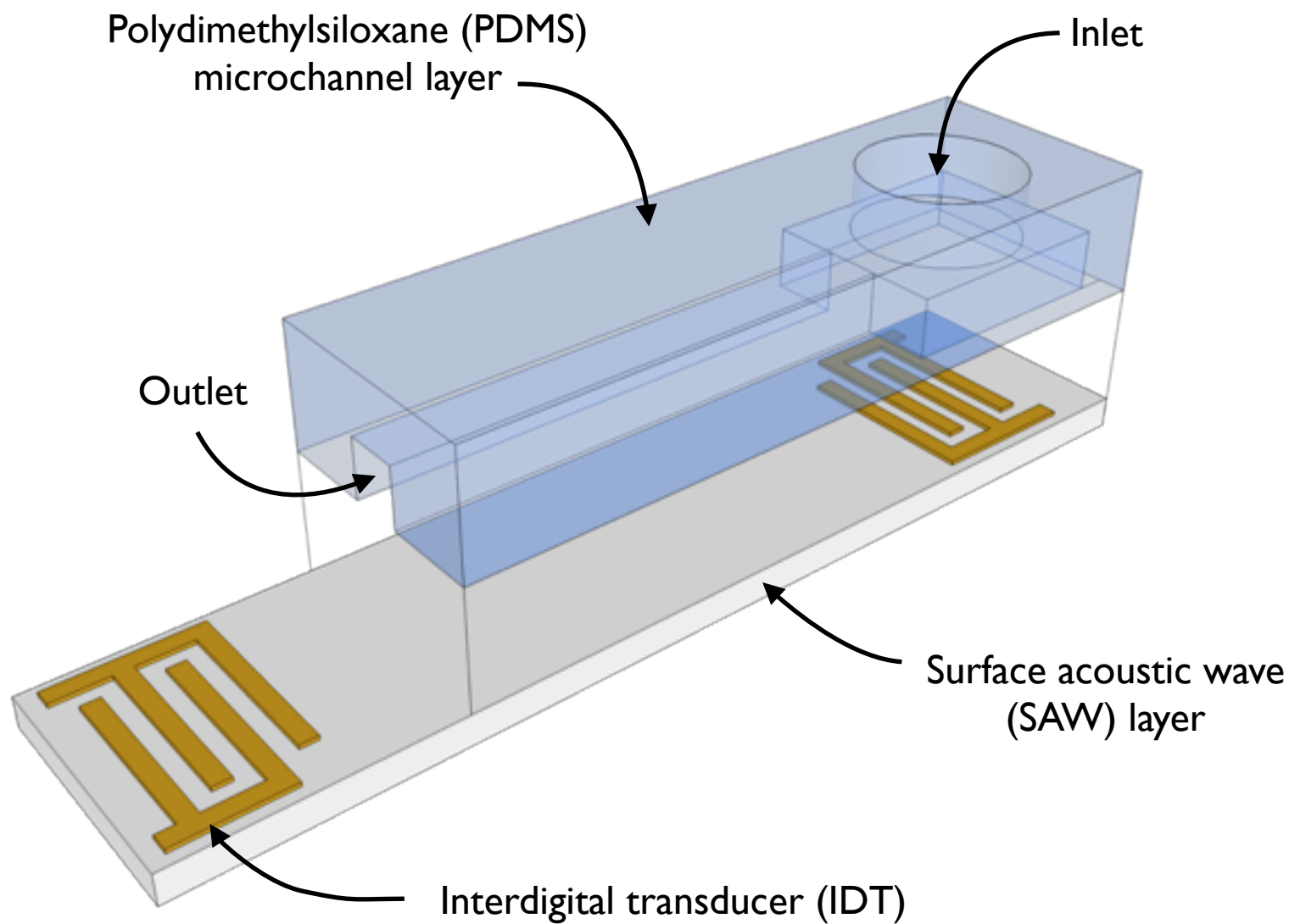
1. Masini, L., Cecchini, M., Girardo, S., Cingolani, R., Pisignano, D., and Beltram, F. Surface-acoustic-wave counterflow micropumps for on-chip liquid motion control in two-dimensional microchannel arrays. *Lab on a Chip*, **10**(15), 1997-2000 (2010)
2. Travagliati, M., De Simoni, G., Lazzarini, CM., Piazza, V., Beltram, F., and Cecchini, M. Interaction-free, automatic, on-chip fluid routing by surface acoustic waves. *Lab on a Chip*, **12**(15), 2621–2624 (2012)
3. Wixforth, A. Acoustically driven planar microfluidics. *Superlattices and Microstructures*, **33**(5), 389-396 (2003).
4. Friend, J., & Yeo, L. Y. Microscale acoustofluidics: Microfluidics driven via acoustics and ultrasonics. *Reviews of Modern Physics*, **83**(2), 647 (2011).
5. Tan, M. K., Yeo L. Y., & Friend, J. R. Rapid fluid flow and mixing induced in microchannels using surface acoustic waves. *Europhysics Letters*, **87**, 47003 (2009)
6. Glass, N., Shilton, R., Chan, P., Friend, J., & Yeo, L., Miniaturised Lab-on-a-Disc (miniLOAD), *SMALL, Small* **8**(12), 1880-1880 (2012)
7. Madou, M.J., & Kellogg, G.J. LabCD: a centrifuge-based microfluidic platform for diagnostics. *Proceedings of SPIE*, 3259, 80 (1998)
8. Cecchini, M., Girardo, S., Pisignano, D., Cingolani, R., & Beltram, F. Acoustic-counterflow microfluidics by surface acoustic waves. *Applied Physics Letters*, **92**(10), 104103-104103 (2008).
9. Campbell, C. *Surface acoustic wave devices for mobile and wireless communications* (Vol. 1). San Diego; Toronto: Academic Press (1998).
10. Hashimoto, K. Y. *Surface acoustic wave devices in telecommunications: modelling and simulation*. Springer (2000).
11. Royer, D., & Dieulesaint, E. *Elastic Waves in Solids II: Generation, Acousto-Optic Interaction, Applications* (Vol. 2). Springer (2000).
12. Renaudin, A., Sozanski, J. P., Verbeke, B., Zhang, V., Tabourier, P., & Druon, C. Monitoring SAW-actuated microdroplets in view of biological applications. *Sensors and Actuators B: Chemical*, **138**(1), 374-382 (2009).
13. Glass, N. R., Tjeung, R., Chan, P., Yeo, L. Y., & Friend, J. R. Organosilane deposition for microfluidic applications. *Biomicrofluidics*, **5**(3), 036501 (2011).

14. Wereley, S. T., & Meinhart, C. D. Recent advances in micro-particle image velocimetry. *Annual Review of Fluid Mechanics*, **42**, 557-576 (2010).
15. Augustsson, P., Barnkob, R., Wereley, S. T., Bruus, H., & Laurell, T. Automated and temperature-controlled micro-PIV measurements enabling long-term-stable microchannel acoustophoresis characterization. *Lab on a Chip*, **11**(24), 4152-4164 (2011).
16. Hebert, B., Costantino, S., & Wiseman, P. W. Spatiotemporal image correlation spectroscopy (STICS) theory, verification, and application to protein velocity mapping in living CHO cells. *Biophysical journal*, **88**(5), 3601 (2005).
17. Rossow, M., Mantulin, W. W., & Gratton, E. Spatiotemporal image correlation spectroscopy measurements of flow demonstrated in microfluidic channels. *Journal of biomedical optics*, **14**(2), 024014-024014 (2009).
18. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J-Y., White, D. J., Hartenstein, V., Eliceiri, K., & Cardona, A. Fiji: an open-source platform for biological-image analysis. *Nature Methods*, **9**(7), 676-682 (2012).
19. <http://www.oceanwave.jp/software/mpiv/>
20. <http://wiseman-group.mcgill.ca/software.php>
21. Rogers, P. R., Friend, J. R., & Yeo, L. Y. Exploitation of surface acoustic waves to drive size-dependent microparticle concentration within a droplet. *Lab on a Chip*, **10**(21), 2979-2985 (2010).
22. Muller, Peter Barkholt and Barnkob, Rune and Jensen, Mads Jakob Herring and Bruus, Henrik A numerical study of microparticle acoustophoresis driven by acoustic radiation forces and streaming-induced drag forces. *Lab on a Chip*, **12**(22), 4617-4627 (2012).
23. Luo, J. K., Fu, Y. Q., Li, Y., Du, X. Y., Flewitt, A. J., Walton, A. J., & Milne, W. I. Moving-part-free microfluidic systems for lab-on-a-chip. *Journal of Micromechanics and Microengineering*, **19**(5), 054001 (2009).
24. Lindken, R., Rossi, M., Große, S., & Westerweel, J. Micro-particle image velocimetry (μ PIV): recent developments, applications, and guidelines. *Lab on a Chip*, **9**(17), 2551-2567 (2009).
25. Gedge, M., & Hill, M. Acoustofluidics 17: Theory and applications of surface acoustic wave devices for particle manipulation. *Lab on a Chip*, **12**(17), 2998-3007 (2012).

(a)



(b)



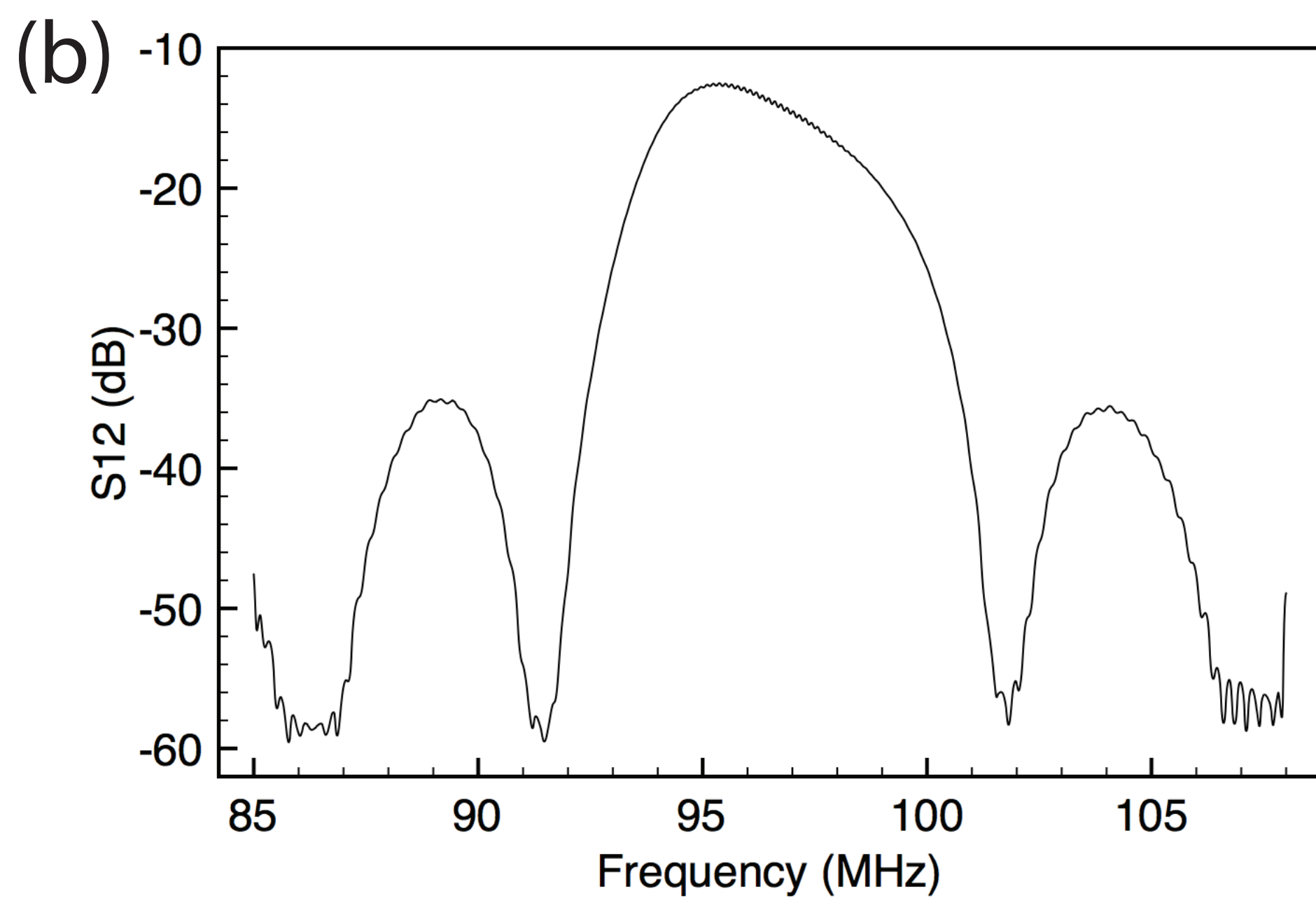
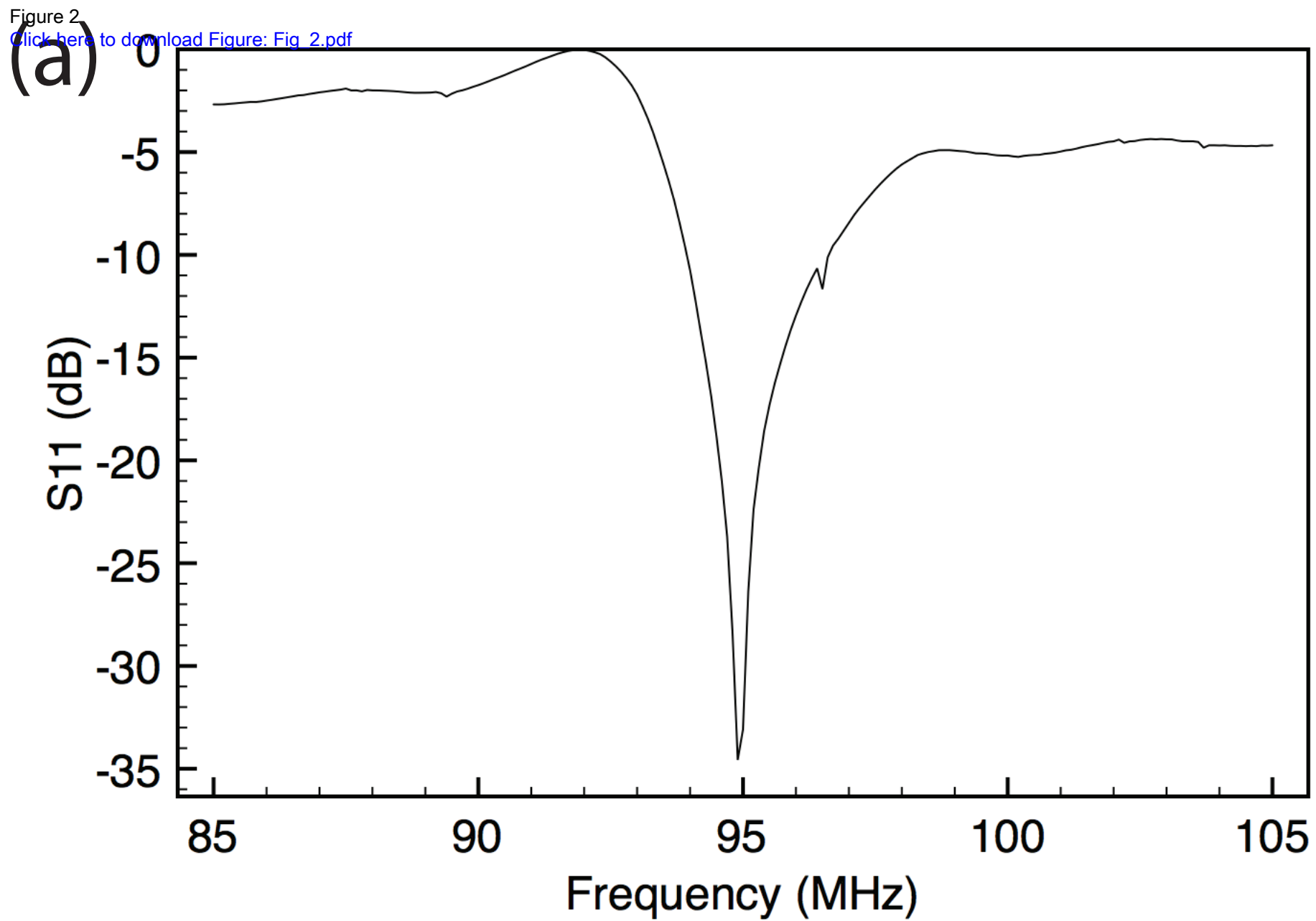
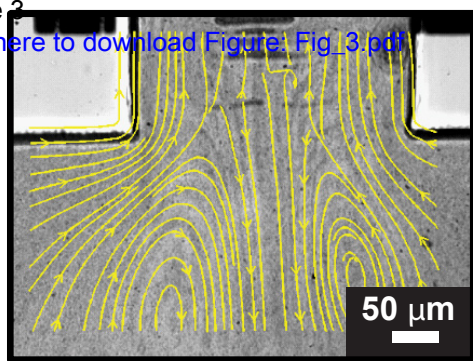


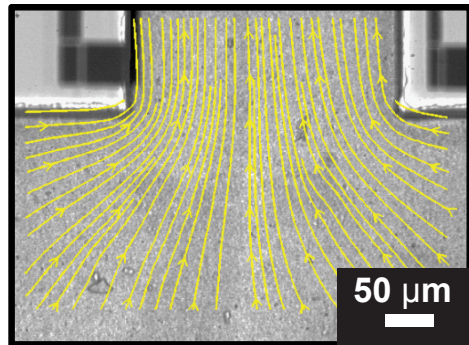
Figure 2

[Click here to download Figure_Fig_3.pdf](#)

(a)

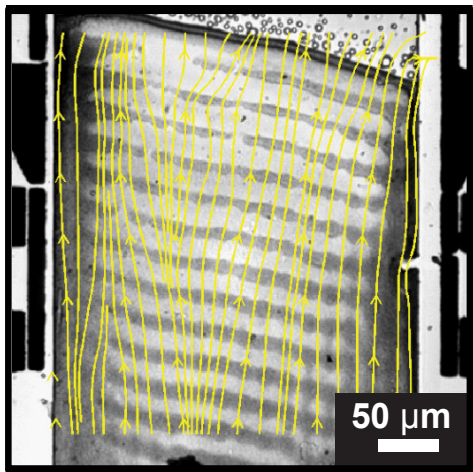


(b)

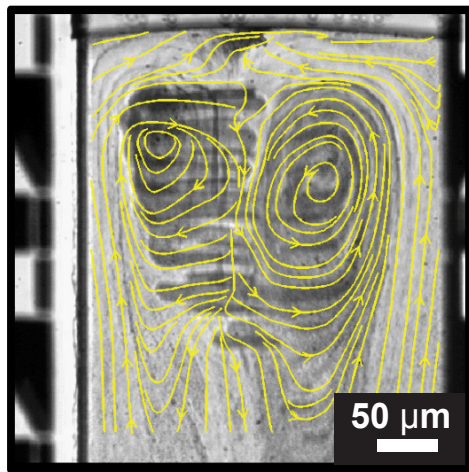


↑
flow direction

(c)



(d)



↑
flow direction

*Table of Reagents/ Materials Used

[Click here to download Table of Reagents/ Materials Used: JoVE_Materials.xlsx](#)

Name	Company	Catalog Number
Double side polished 128° YX lithium niobate wafer	Crystal Technology, LLC	n/a
Silicon wafer	Siegert Wafers	n/a
IDT Optical lithography mask with alignment marks (positive)	Any vendor	n/a
Channel Optical lithography mask (negative)	Any vendor	n/a
Positive photoresist	Shipley	S1818
Positive photoresist developer	Microposit	MF319
Negative tone photoresist	Allresist	AR-N-4340
Negative tone photoresist developer	Allresist	AR 300-475
SU8 thick negative tone photoresist	Microchem	SU-8 2000 Series
SU8 thick negative tone photoresist developer	Microchem	SU-8 developer
Hexadecane	Sigma-Aldrich	H6703
Carbon tetrachloride (CCl ₄)	Sigma-Aldrich	107344
Octadecyltrichlorosilane (OTS)	Sigma-Aldrich	104817
Acetone CMOS grade	Sigma-Aldrich	40289
2-propanol CMOS grade	Sigma-Aldrich	40301
Titanium	Any vendor	99.9 % purity
Gold	Any vendor	99.9 % purity
PDMS	Dow Corning	Sylgard® 184 silicone elastomer kit with curing agent
Petri dish	Any vendor	
5 mm ID Harris Uni-Core multi-purpose coring tool	Sigma-Aldrich	Z708895
Acoustic absorber	Photonic Cleaning Technologies	First Contact regular kit
RF-PCB	Any vendor	
Spinner	Laurell technologies corporation	WS-400-6NPP
UV Mask aligner	Karl Suss	MJB 4
Thermal evaporator	Kurt J. Lesker	Nano 38

Oxygen plasma asher	Gambetti Kenologia Srl	Colibrì
Centrifuge	Eppendorf	5810 R
Wire bonder	Kulicke & Soffa	4523AD
Contact Angle Meter	KSV	CAM 101
Spectrum analyzer	Anristu	56100A
RF signal generator	Anristu	MG3694A
RF high power amplifier	Mini Circuits	ZHL-5W-1
Microbeads suspension	Sigma-Aldrich	L3280
Optical microscope	Nikon	Ti-Eclipse
Video camera	Basler	A602-f
Camera acquisition software	Advanced technologies	Motion Box

[illegible]

Any plasma asher or RIE machine can be used

Any centrifuge can be used

Any wire bonder can be used if the PCB is used without
pogo connectors

Any contact angle meter can be used

Any spectrum or network analyzer can be used

Any RF signal generator can be used

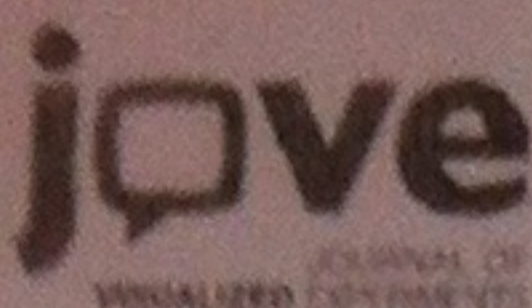
Any RF high power amplifier can be used

Depending on the experimental purpose different
suspension of different diameter and different material
properties can be used

Any optical microscope with spatial resolution satisfying
experimental purposes can be used

Any video camera that has enough frame rate and
sensitivity satisfying experimental purposes can be used

Any software enabling high and controlled frame rate
acquisition can be used



17 Sellers Street
Cambridge, MA 02139
tel. +1.617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article:

Fabrication, Operation and Flow Visualization in Surface Acoustic Wave Acoustic Cocurrent Flow Mixing Devices.

Author(s):

TRAVAGLIA, SHILTON, BEGRAM AND CECCHINI

Item 1 (check one box): The Author elects to have the Materials be made available (as described at <http://www.jove.com/publish>) via: ☒ Standard Access ☐ Open Access

Item 2 (check one box):

- ☒ The Author is NOT a United States government employee.
- ☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.
- ☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: <http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode>; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJoVE Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments*; "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. **Grant of Rights in Article.** In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4 and 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. **Retention of Rights in Article.** Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the

ARTICLE AND VIDEO LICENSE AGREEMENT

Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict

shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including,

ARTICLE AND VIDEO LICENSE AGREEMENT

without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or

damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US\$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to be one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

AUTHOR:

Name:

MAREO CECCHINI

Department:

NEST, ISTITUTO NANOSCIENZE-CNR AND SCUOLA NORMALE SUPERIORE

Institution:

NEST, ISTITUTO NANOSCIENZE-CNR AND SCUOLA NORMALE SUPERIORE

Article Title:

Fabrication, Operation and Flow Visualization in Surface Acoustic Wave Acoustic Counterflow Microfluidics

Signature:

Mareo Cecchini

Date:

10/11/2012

Please submit a signed and dated copy of this license by one of the following three methods:

- 1) Upload a scanned copy as a PDF to the JoVE submission site upon manuscript submission (preferred);
- 2) Fax the document to +1.866.381.2236; or
- 3) Mail the document to JoVE / Attn: JoVE Editorial / 17 Sellers St / Cambridge, MA 02139

For questions, please email editorial@jove.com or call +1.617.945.9051.

MS # (internal use):

Dear JoVE,

the "acknowledgements" section was added in our manuscript, as required.

Best Regards,
Marco Cecchini