Video Article

Fabrication, Operation and Flow Visualization in Surface-acoustic-wave-driven Acoustic-counterflow Microfluidics

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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 multilayered 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 channel 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.

Video Link

The video component of this article can be found at <http://www.jove.com/video/50524/>

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 decreases 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^{1,4}. Despite the simplicity and effectiveness of actuating microfluids with SAW, there are only a small number of SAW driven microfluidic pumping mechanisms that have been demonstrated to date. The first demonstration was the 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 that was laser ablated directly into the piezoelectric substrate. By geometric modification with respect to the channel and IDT dimensions, they were 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. Design two photomasks, the first for patterning the surface acoustic wave (SAW) layer, and the second for the polydimethylsiloxane (PDMS) microchannel mold.
 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 books^{9–11}. 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.
 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 $5\ \text{mm}$. 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.
2. Begin with an LN wafer and cleave a $2\ \text{cm}$ by $2\ \text{cm}$ 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.
3. Clean the substrate by rinsing it in acetone, 2-propanol and drying with a nitrogen gun.
4. Spin coat the sample with Shipley S1818 at $4,000\ \text{rpm}$ for $1\ \text{min}$.
5. Soft bake at 90°C for $1\ \text{min}$ on a hot plate.
6. Align the sample with the SAW layer mask using a mask aligner and expose it to UV light with a $55\ \text{mJ}/\text{cm}^2$. Care should be taken to align the IDT direction along the major axis of the LN substrate.
7. Rinse the sample in Microposit MF319 developer for $30\ \text{sec}$ to remove the unexposed photoresist.
8. Stop the development by rinsing the sample in deionized water and dry it with a nitrogen gun.
9. Deposit a 10-nm -thick titanium adhesion layer followed by 100-nm -thick gold layer by thermal evaporation.
10. Perform *lift-off* by sonicating the sample in acetone, then rinse it in 2-propanol and dry with a nitrogen gun.
11. Silanize the device surface to make it hydrophobic in the microchannel area¹².
 1. Mask the microchamber area with AR-N-4340 negative tone photoresist by optical lithography according to the manufacturer's datasheet.
 2. Activate the sample surface with a $2\ \text{min}$ oxygen plasma (Gambetti Kenologia Srl, Colibri) of $0.14\ \text{mbar}$ pressure and $100\ \text{W}$ power giving a bias voltage of approximately $450\ \text{V}$.
 3. Mix $35\ \text{ml}$ hexadecane, $15\ \text{ml}$ carbon tetrachloride (CCl_4), and $20\ \mu\text{l}$ octadecyltrichlorosilane (OTS) into a beaker inside a fume hood. Place the device in the solution, and leave covered for two hours.
 4. Rinse the device with 2-propanol and dry it with a nitrogen gun.
 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.
 6. Remove the residual resist on the sample by rinsing in acetone, 2-propanol and drying with a nitrogen gun.
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.
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.
14. Cast PDMS on the mold
 1. Mix PDMS with a curing agent at a ratio of $10:1$.
 2. Centrifuge the PDMS for $2\ \text{min}$ at $1,320\ \times g$ for degassing.
 3. Pour the PDMS gently onto the SU-8 mold in a Petri dish to a total PDMS height on the order of $1\ \text{mm}$. The open Petri dish can be placed in a vacuum desiccator for approximately $30\ \text{min}$ in order to degas the PDMS further.
 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.
15. Prepare the solid PDMS layer
 1. Cut around the channel using a surgical blade, being careful not to damage the SU8 master, and peel it off.
 2. Replica edges are then refined and straightened using a razor blade leaving at least $2\ \text{mm}$ clearance on lateral side of the channel and no clearance (cut right through) at the channel outlet.
 3. Punch a hole in the microchamber using a Harris Unicore puncher to form the fluid-loading inlet.
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.

- 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.

17. The complete device schematic is shown in **Figure 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

- Calibrate the network or spectrum analyzer with an open/short waveguide on your RF-PCB.
- 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 S₁₁ and S₂₂ and -10 dB for S₁₂ (without PDMS channels).

3. Microfluidics and Particle Flow Dynamics Visualization Experiment and Analysis

- 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 any spatially and temporally important flow features.
- 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.
- 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.
 - 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.
- 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.
 - 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.
 - 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.
- 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 mPIV¹⁹ 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 S₁₁ and S₁₂ spectra are reported in panel **a**) and **b**) respectively. The depth of the valley at central frequency in S₁₁ 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 S₁₁. 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 S₁₂ 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 S₁₁ 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-sec 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.

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 microchannel. 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 step 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. [Click here to view larger figure.](#)

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 (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/sec.

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 mPIV 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 understanding 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 devoted to the visualization of the particle dynamics that is at the basis of acoustophoretic sorting applications.

Disclosures

Authors have nothing to disclose.

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References

- Masini, L., Cecchini, M., Girardo, S., Cingolani, R., Pisignano, D., & 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).
- Travaglini, M., De Simoni, G., Lazzarini, CM., Piazza, V., Beltram, F., & Cecchini, M. Interaction-free, automatic, on-chip fluid routing by surface acoustic waves. *Lab on a Chip*. **12** (15), 2621-2624 (2012).
- Wixforth, A. Acoustically driven planar microfluidics. *Superlattices and Microstructures*, **33** (5), 389-396 (2003).
- Friend, J. & Yeo, L.Y. Microscale acoustofluidics: Microfluidics driven via acoustics and ultrasonics. *Reviews of Modern Physics*, **83** (2), 647 (2011).
- 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).
- Glass, N., Shilton, R., Chan, P., Friend, J., & Yeo, L., Miniaturised Lab-on-a-Disc (miniLOAD), *SMALL, Small*. **8** (12), 1880-1880 (2012).
- Madou, M.J., & Kellogg, G.J. LabCD: a centrifuge-based microfluidic platform for diagnostics. *Proceedings of SPIE*. **3259**, 80 (1998).
- 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).
- Campbell, C. *Surface acoustic wave devices for mobile and wireless communications.*, Vol. 1, Academic Press, San Diego, Toronto, (1998).
- Hashimoto, K.Y. *Surface acoustic wave devices in telecommunications: modelling and simulation.*, Springer, (2000).
- Royer, D. & Dieulesaint, E. *Elastic Waves in Solids II: Generation, Acousto-Optic Interaction, Applications.*, Vol. 2, Springer, (2000).
- 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).
- Glass, N.R., Tjeung, R., Chan, P., Yeo, L.Y., & Friend, J.R. Organosilane deposition for microfluidic applications. *Biomicrofluidics*. **5** (3), 036501 (2011).
- Wereley, S.T. & Meinhardt, C.D. Recent advances in micro-particle image velocimetry. *Annual Review of Fluid Mechanics*. **42**, 557-576 (2010).
- 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).
- 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).
- 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).
- 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. mpiv - MATLAB PIV Toolbox, <http://www.oceanwave.jp/softwares/mpiv/>, (2012).
20. The Wiseman Research Group, <http://wiseman-group.mcgill.ca/software.php>, (2012).
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, P.B. & Barnkob, R., and Jensen, M.J.H., & Bruus, H. 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).