

Citation for published version:

Mikhaylov, R, Wu, F, Wang, H, Clayton, A, Sun, C, Xie, Z, Liang, D, Dong, Y, Yuan, F, Moschou, D, Wu, Z, Shen, MH, Yang, J, Fu, Y, Yang, Z, Burton, C, Errington, RJ, Wiltshire, M & Yang, X 2020, 'Development and characterisation of acoustofluidic devices using detachable electrodes made from PCB', *Lab on a Chip*, vol. 20, no. 10, pp. 1807-1814. <https://doi.org/10.1039/c9lc01192g>

DOI:

[10.1039/c9lc01192g](https://doi.org/10.1039/c9lc01192g)

Publication date:

2020

Document Version

Peer reviewed version

[Link to publication](#)

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~~Cost-effective technique for prototyping a~~ **Acoustofluidic SAW devices for droplet, microparticle and cancer cell manipulations using via PCB-based interdigitated electrodes on lithium niobate substrates**

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Abstract:

Acoustofluidic research has been intensively growing because of the versatility and biocompatibility of acoustofluidic devices in manipulating cells and nano-/micro-particles. Lowering the cost and facility requirement in manufacturing surface acoustic wave (SAW)-based devices can significantly help more researchers enter ~~to~~ this area or ~~allow~~ ~~enable~~ ~~more~~ ~~variety~~ ~~diversification~~ ~~in~~ new applications. We present herein a novel and simple technology using the advantage of printed circuit board (PCB) manufacturing (PCB) to integrate with piezoelectric substrate such as lithium niobate to produce ~~more~~ cost-effective but ~~equally high~~ quality SAW devices. Systematic characterisation ~~of~~ the PCB-based SAW devices (PCBSAW) has been performed revealing similar capability and functionality were achieved comparing with conventional SAW devices made by photolithography. The PCBSAW was used for actuating microparticles within droplets and microchannels, which showed good agreement with the simulation in creating particle streaming and alignment, respectively. Acoustic tweezer has ~~also~~ been realised by the PCBSAW in manipulating human lung cancer cells to form patterns inside the microchannel. The cell viability test informed the PCBSAW had high biocompatibility to be potentially applied as a cost-effective and low-requirement tool in diverse acoustofluidic investigations.

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Introduction

Acoustophoresis is a method well-known for actuating and manipulating micro¹-/ nano^{2,3}-particles using acoustic waves. It has been demonstrated to widely apply in biomedical applications such as separating circulating tumour cells from whole blood, enriching and isolating exosomes, separating blood cells, washing and coating cells, and manipulating cells, with continuous development of the acoustophoretic theories¹²⁻¹⁵ and simulations¹⁶⁻¹⁹. Acoustophoretic devices use either bulk acoustic waves (BAWs)²⁰⁻²⁸ or surface acoustic waves (SAWs) to produce acoustic pressure for actuating particles. SAW-based devices have been intensively investigated in manipulating cells because they are less dependent on the acoustic properties of the material for making microchannel.

The majority of SAW devices use a lithium niobate (LiNbO₃) wafer as the substrate for interdigitated transducer (IDT) deposition. The device fabrication process is using photolithography which includes the following steps required the use of cleanroom: 1. Mask manufacturing, 2. Spin-coating LiNbO₃ with photoresist, 3. Mask aligning for patterning with UV, 4. Double metal layer deposition. 5. Lift-off to form the IDT. In the UK, the average cost of fabricating a pair of IDTs working at MHz frequencies to produce standing SAW (SSAW) on a 2-inch LiNbO₃ wafer is at least £1,000 including all the chemicals, consumables, labors, and the usage of various facilities such as spinner, mask aligner, evaporator or sputter and wet chemistry. Although modelling and simulation are now very capable of guiding the design of acoustophoretic devices including the parameter of the SAW device, such as electrode finger period, pitch, number and width of fingers, and space between two IDTs, it's inevitable to manufacture multiple devices to achieve repeatability and solve particle application by trial and error.

In addition, LiNbO₃ wafers are very brittle material which could be easily damaged during manufacturing and operation. The SAW device made by photolithography is a one-off component, any modification to the electrodes requires going through the entire aforementioned manufacturing process and paying the costs. The vulnerability of conventional manufacturing of SAW devices, i.e. lengthy fabrication time, demand of cleanroom facilities, and cost, is the bottleneck of fast and cost-effective prototyping SAW devices for exploring much wider applications in biomedical research.

The finishing of evaporation or sputtering realises IDTs on the LiNbO₃ which are two interlocking comb-shaped arrays of metallic electrodes. These electrodes are connected to a radio frequency (RF) source to produce alternating current (AC) on the LiNbO₃, which develops an alternating electric field that is translated into the mechanical SAW by the piezoelectric effect. Similar interdigital electrodes (IDEs) without piezoelectric substrate have been widely created on a printed circuit board (PCB) and applied in applications such as moisture sensing³², water level measurements³³, electro wetting³⁴, biosensing, and even cell manipulation³⁵. A standard PCB laminate consists of a layer of thin copper foil and an insulating layer typically laminated together with glass reinforced epoxy resin pre-impregnated (FR4); further options for core materials are commercially available such as PET, flexible polyimide or teflon. The fabrication of IDTs on the PCB is done by a wet etching process to remove the unwanted copper leaving only the desired patterns. Further metallization of the copper layer is routinely employed within the industry, with a wide variety of gold or silver electroplating processes commercially available to designers.

The PCB manufacturing is a highly mature process with relatively low cost comparing with using cleanrooms to fabricate customised electrodes. Such electrodes can be driven by RF source and produce alternating electric field. In this work, we pilot to introduce the use of PCB IDEs to integrate with LiNbO₃ wafer to produce SAW devices (PCBSAW), in an effort to gradually

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[migrate from traditional cleanroom processing and render SAW devices amendable to cost-effective mass production](#). Requiring no cleanroom but only simple mechanical assembly process, we have achieved the manufacturing of PCBSAW devices working at 20 MHz and demonstrated their capability similar to the cleanroom made acoustofluidic devices on manipulation microparticles and cells. The advantages of the PCBSAW technique include low-cost, fast-recovery, interchangeability of piezoelectric materials, no-cleanroom required and robustness, which have the potential to lower the entry requirement of acoustofluidic experimentation and keep the cost of device fabrication minimal.

Methods and materials

Design and working mechanism

Our design of the PCBSAW is shown cross-sectionally in Fig. 1a, consisting six components: a base plate, a LiNbO₃ wafer, a PCB [hosting the](#) patterned ~~with~~ IDEs, a clamp, a pressure ring and a microchannel. The base plate supports the above LiNbO₃ wafer, the metal IDEs side on the PCB contact the LiNbO₃ wafer with a pressure ring screwed in the clamp holding the PCB in place. Radio frequency (RF) signals applied to the IDEs will be converted to mechanical waves at the electrode regions and propagating along the wafer to form SSAW. The microchannel is bonded to the LiNbO₃ wafer at the middle between the two IDEs for handling fluid samples. Depending on the wavelength and the microchannel, the size of the PCBSAW can be customised. For our application using PCBSAW as an acoustic tweezer, the dimension is 120 mm(L)×120 mm(W)×30 mm(H) as the 3D model shown in Fig. 1b. The pressure applied to the PCB can be adjusted by the distance the pressure ring screwing into the clamp. Once a proper pressure applied, the IDEs make good contact to the surface of the LiNbO₃ wafer, which effectively works as the directly patterned electrodes on the wafer.

Particles in either droplets or microchannel have been demonstrated to be actuated by SSAW. Briefly, SAWs are in the form of Rayleigh waves³⁶ restricted to a depth of few wavelengths to the surface and decay exponentially³⁷ into the LiNbO₃. If a droplet is placed on the path of a SAW (Fig. 2a), the SAW will leak into the droplet with a Rayleigh angle (θ_R), which will induce streaming flow field within the droplet while the wave rapidly decaying³⁸. Using a microchannel allows versatile manipulation of microparticles (Fig. 2b). By using two IDTs that are counter-propagating SAW towards each other, the interference of these two SAWs can be translated through superposition into the SSAW. Due to the “static” nature of the SSAW, pressure nodes (PN), i.e. low-pressure zone, and pressure anti-nodes (AN), i.e. high-pressure zone, are distributed along the propagation. Particles in the acoustofluidic field experience two forces, an acoustic radiation force (\mathbf{F}^{rad}), exerted by the acoustic wave scattering at the particle-liquid interfaces, and a viscous drag force (\mathbf{F}^{drag}), generated by acoustic-induced streaming flow (\mathbf{v}_{str}) due to viscous attenuation of the acoustic wave¹⁷. Under the influence of the \mathbf{F}^{rad} a particle tends to migrate towards the nearest PN and stay trapped there, while under \mathbf{F}^{drag} it tends to be continuously driven by the streaming and circular movement could be formed. It has been well described the two forces as

$$\mathbf{F}^{\text{rad}} = -\left(\frac{\pi\rho_p^2V_p\beta_f}{2\lambda}\right)\phi(\rho, \beta) \mathbf{sin}(2kx) \quad (1)$$

$$\phi(\rho, \beta) = \frac{5\rho_p - 2\rho_f}{2\rho_p + \rho_f} - \frac{\beta_p}{\beta_f} \quad (2)$$

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where F^{rad} , ϕ , p_0 , V_p , β_p , β_f , λ , x , ρ_p and ρ_f are the acoustic radiation force, acoustic contrast factor, acoustic pressure, particle volume, particle compressibility, fluid compressibility, SAW wavelength, distance from the nearest PN, particle density, and fluid density, respectively. k is the wave number defined by $k = 2\pi/\lambda$. The acoustic contrast factor $\phi(\rho, \beta)$ described in Eq.2 informs the direction of particle movement: a positive sign indicates its tendency to move towards the PN while a negative sign indicates towards the AN.

The drag force F^{drag} is proportional to the particle relative velocity ($v_{str} - u$), which is the difference between the particle velocity u and the streaming velocity v_{str} , as given by²⁷:

$$F^{drag} = 6\pi\mu a(v_{str} - u) \quad (4)$$

where μ and a are the fluid viscosity and radius of the particle, respectively.

As F^{rad} is proportional to the volume of the particles (a^3) and the F^{drag} is proportional to the radius (a), large particles' movement is dominated by F^{rad} and thus they are driven towards the PN.

One can observe that the acoustic pressure p_0 in Eq.1 determines the magnitude of the force, which is defined by

$$p_0 = \sqrt{\frac{\alpha P_i \rho_s c_s}{A_w}} \quad (3)$$

where α , P_i , ρ_s , c_s and A_w are the power conversion efficiency, input power, the density and speed of sound of the substrate, and the area under the influence of the IDTs, respectively.

PCBSAW fabrication and experimental setup

The PCB was designed in Eagle software (Autodesk, US) and the [relevant](#) Gerber file was sent to Circuitfly (www.circuitfly.com) for manufacturing. A conventional IDE pattern was used with a designed wavelength of 200 μm , and the finger width and spacing both equal to 50 μm . The working frequency was estimated to be 20 MHz given the wavelength and the speed of sound in the LiNbO₃ wafer. The IDT consists of 40 pairs of 10-mm long fingers. As shown in Fig. 3a, the thickness of the PCB laminate was 1.6 mm with the copper traces layer 34.8 μm (1 oz) thick coated with gold. No solder mask was added. The PCB dimension is 10 cm (L) \times 10 cm (W) with a milled window of 35 cm (L) \times 15 cm (W) at the center. Alignment markers (holes and lines) were made on the PCB to help with the LiNbO₃ alignment. A microscope was used to measure the IDE manufacturing quality. The mean finger width and spacing were found to be 38.7 \pm 6.2 μm (mean \pm SD) and 61.1 \pm 6.8 μm , respectively, which gave a period of 199.6 μm (Fig. 3b). The PCB IDE manufacturing [had considerable featured](#) high accuracy [te](#) as the period was very close to the designed [wavelength-dimension](#) of 200 μm . The smaller width than the spacing was due to the difficulty in controlling the development time of the electrodes. After the microscope measurement, two coaxial cables were soldered to the gold pads at the edges of the PCB IDE for signal transmission.

Both the PCB IDE and a 3-inch 500- μm thick Y-cut 128° LiNbO₃ wafer were thoroughly cleaned using isopropyl alcohol (IPA) and inspected under the microscope for dust. The assembly schematic is shown in Fig. 3c, all the mechanical components were printed by a 3D printer (Ultimaker 2+, Utrecht). The wafer was placed onto a round holder where there is a flat edge used for aligning the LiNbO₃ reference flat. The PCB was then placed on the LiNbO₃ with the IDE facing down. Alignment markers on the PCB were used to ensure the IDEs were parallel with the reference flat on the LiNbO₃. Then, the pressure ring was mounted to the PCB and fastened on the base plate using four screws. There was an observation window on the base plate for light

transmission during microscopic measurement. The PDMS microchannel with the dimension of $200\ \mu\text{m}(\text{L}) \times 100\ \mu\text{m}(\text{W}) \times 60\ \mu\text{m}(\text{H})$ with a single inlet and outlet tubing was bonded to the LiNbO_3 using plasma treatment. Fig. 3d shows the assembled PCBSAW device.

Sample preparation

The PCBSAW device was used to test on manipulation of droplets, microparticles and cancer cells. A5499 human NSCLC (non-small cell lung) cell line was grown in DMEM (Dulbecco's modified eagle media) and supplemented with L-Glutamine (200mM at 1:100 dilution, Gibco), Penicillin/Streptomycin (10,000 U/ml at 1:100 dilution, Gibco), and 10% foetal bovine serum (FBS) in 75cm^3 cell culture flasks (Greiner) (TMEG, Cardiff University) until their density was 1×10^7 cells/ml. The cells were harvested from the plastic surface by trypsinization, and were concentrated by centrifugation (3500 rpm, 5 min), to 2×10^7 cells/ml.

Results

Characterisation of the PCBSAW device

Due to the unique "bonding" mechanism of the PCBSAW device, it's crucial to understand the bonding quality and the capability in producing SAW. Since the direct measure of the bonding between the PCB IDE and LiNbO_3 wafer is unpractical, we used the electrical parameters to characterise the device. Such measurement is also essentially helpful to the assembly process. The two IDTs on the PCBSAW were considered to form a two-port network as shown in Fig. 4a, whose scattering parameters (*s*-parameters) were measured by a vector network analyzer (VNA, E5061B ENA, Keysight), including S_{11} , S_{21} , S_{12} , and S_{22} with the definition given in Fig. 4a. Of which, S_{11} and S_{22} measure the signal reflection from the left and right IDTs, respectively. S_{12} and S_{21} measure the signal transmission from one IDT to another. It's desired to have a dip at working frequency on S_{11} and S_{22} measurements which indicate both IDTs are well converting electrical energy to SAWs. The VNA was monitoring the real-time *s*-parameters during the assembly. Once the PCB began to contact the LiNbO_3 wafer, the S_{11} showed a small dip around 20 MHz which indicated the emergence of the working frequency. While tightening the four screws on the PCBSAW device, the PCB was continuously compressed onto the LiNbO_3 wafer resulting improved contact between the gold fingers and the piezoelectric material. The dip on the S_{11} was lowering during the tightening process while the peak pattern of the dip kept unchanged.

Since the LiNbO_3 wafer is brittle, an excessive pressure applied by the screw tightening could easily damage the wafer. After a number of attempts ~~with-at~~ the cost of ~~broken~~ LiNbO_3 wafers, the best S_{11} and S_{22} are plotted by the black curves in Fig. 4b, which are -2.7 dB and -1.7 dB, respectively, corresponding to the working frequency of 19.7662 MHz and 19.8377 MHz, respectively. Comparing with cleanroom fabricated IDTs, these values are not low enough for efficient signal conversion. To optimise the PCBSAW device, L matching networks (MN) were designed and added to match the impedance of both IDTs to approximate $50\ \Omega$ (Fig. 4c). When MN were in place, all the *s*-parameters became highly sensitive to applied pressure on the PCB, which could act as indication to guide tightening the four screws. In addition, MN made the working frequencies easier to observe and distinguish as the remeasure of S_{11} and S_{22} shown by the red curve in Fig. 4b, which are significantly improved to -18.4 dB and -21.4 dB, respectively. Other parameters such as S_{12} , S_{21} , and Smith Chart, before and after adding the MNs, were also measured using the VNA with the results shown in Fig. 4d and 4e. All the parameters are improved by adding the MNs.

To prove the repeatability of the assembly of the PCBSAW device and the consistency of the device properties, as well as with the addition of the MN, the PCBSAW device was assembled and disassembled 23 times with all the above parameters registered in each assembly. The results for the mean working frequencies and s -parameters with and without the MN are shown in Fig. 5. A slight increase of the mean frequency identified by the s -parameters are noted when the MN is implemented (Fig. 5a), with $S_{21/12}$ informing a smaller frequency coverage. Fig. 5b provide the evidence of the reduction of signal reflection by using the MN and Fig. 5c shows a better signal transmission from one IDT to another using MN is used. Given the consistency and quality of the characterisation, the PCBSAW technique can be used to fabricate IDTs as the photolithography done in cleanroom, with the flexibility of changing piezoelectric materials and recovering functional devices on demand.

To understand the optimal working frequency for the PCBSAW to efficiently convert RF signals to SAWs, all the frequency readouts from the above s -parameters measurement were tested individually in the following power transmission tests. Fig. 6a shows the setup of the power transmission test, a bi-directional coupler was used to couple the RF signal to the MN and PCBSAW device. The coupler allowed the incident and reflected powers to be monitored separately by connecting two power meters (PM_1 and PM_2) to the incident and reverse ports, respectively. A third meter (PM_3) was connected to the opposite IDT to measure the transmitted power. Fig. 6b shows the transmitted power using the working frequency defined by s -parameters in the case with and without MNs. Addition of MNs improved the transmitted power in all cases, working on the frequency defined by the S_{21} or S_{12} with MN, i.e. 19.792 MHz, had the higher transmitted power than that of S_{22} or S_{11} . One can use this frequency to operate the IDT to efficiently produce SAWs. Additionally, using the same setup the transmission was measured by tuning the RF frequency around the working frequency. As shown in Fig. 6c, readings from the left or right IDT are close to the VNA measurement, which confirms the maximum power transmission taken place at 19.792 MHz.

Typically, using the frequency identified by the S_{21} or S_{12} with MN as the working frequency of the PCBSAW device allows optimal power conversion. When imperfect assembly happened causing poor contact between the IDEs and LiNbO_3 wafer, or the IDEs / LiNbO_3 wafer is poorly cleaned, the frequency defined by the S_{21} or S_{12} with MN no longer produces maximum transmitted power. As a rule of thumb, the PCBSAW working frequency can be tuned ± 0.1 MHz around the frequency identified by S_{21} or S_{12} with MN to figure out the optimal frequency for efficient SAW generation.

Manipulation of microparticles

To demonstrate the PCBSAW can produce SAW using the optimal frequency obtained from the above measurement to actuate microparticles in droplets, sample consisting of water and sized 20 μm polystyrene microspheres was pipetted ($\sim 3\text{-}4 \mu\text{l}$, 3mm in diameter, concentration is $\sim 2,000 / \mu\text{l}$) onto the PCBSAW as shown in Fig. 7a. The slot vertically located in the inset is the window on the base plate for microscope lighting. When one of the IDTs was activated (19.955 MHz, input power 23 dBm), a streaming pattern was observed (Fig. 7b, Video S1, Supporting Information) which agreed with the simulation given in ³⁸. When both IDTs activated, a four-roll streaming patterns appeared in also good agreement with ³⁹ (Fig. 7c, Video S2, Supporting Information). The droplet test confirmed the PCBSAW device was able to produce SAWs from both IDTs and generate similar magnitude of SAWs to actuate particles as the device made by photolithography.

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Further test cooperating with the PDMS microchannel was performed to demonstrate the PCBSAW device able to work as the acoustic tweezer. To predict the distribution of acoustic pressure and particle trajectories corresponding to the applied signals, COMSOL was used to model the XZ cross-section of the microchannel of the PCBSAW device. Fig. 8a and 8b show the distribution of the acoustic pressure when the PN and AN located at the middle of the microchannel, respectively. The acoustic pressure is a time-averaged result, meaning that the instantaneous amplitude of AN is constantly interchanging between positive and negative values. Particle trajectories corresponding to both conditions are given in Fig. 8c and 8d, respectively. The first case indicates three aggregations on the plane, two of which are on the walls. The second case creates a more complex aggregation on the plane. It is worth noting that the aggregation at the middle (yellow dot) is difficult to trap microparticles due to the occurrence of force imbalance, i.e. $\sum F_x \neq 0$, thus microparticles tend to migrate towards more stable PN locations resulting absent microparticles at the middle. In addition, as the two aggregations marked by black arrows are very close, one can anticipate to see a 'thick' particle traces merging the two aggregations.

The PCBSAW was experimentally tested to actuate microparticles within the microchannel. 10- μ m polystyrene microspheres ($\phi > 0$) mixed with a custom media to 1:2.7 ratio (vol:vol). The custom media consisted of 1:4.4 glycerol to phosphate-buffered saline (PBS), which was made to stop particle deposition. Before applying the microspheres, the microchannel was flushed with bovine serum albumin (BSA) solution (water:BSA = 100:1, mass ratio) for 20 min at a flowrate of 20 μ L/min. Microsphere sample was injected using a syringe into the microchannel, an evenly dispersed pattern was formed (Fig. 8e). Once microspheres still, both PCB IDTs were activated (19.884 MHz, 27 dBm), and three particle traces immediately formed inside the microchannel as shown in Fig. 8f (Video S3, Supporting Information), which agreed with the simulation when PN located at the middle of the microchannel. By shifting the phase of the RF signal sent to one of the IDTs by 180°, the SSAW was rearranged with the AN located at the middle of the microchannel resulting redistribution of the microspheres as shown in Fig. 8g (Video S4, Supporting Information). The four traces with two 'thick' lines also well agreed with the simulation.

Cell manipulation

To validate the manipulation of cells and biocompatibility, the PCBSAW device was used to conduct the following cell actuation and cell viability tests. Lung cancer cell sample was maintained on ice in suspension for the experiment. The cells were injected into the microchannel in culture medium by the syringe. When an evenly still distributed pattern was formed as shown in Fig. 9a, PCBSAW was powered by 30 dBm to produce SSAWs. The cells were immediately driven by the acoustic pressure and forming three cell traces as shown in Fig. 9b (Video S5, Supporting Information), which corresponded to the case that PN located at the middle of the microchannel. Shifting the phase of one IDT by 180°, the AN was set at the middle of the microchannel, which allows the redistribution of the cancer cells to form four traces as shown in Fig. 9c (Video S6, Supporting Information). As predicted by the simulation (Fig. 8d), the aggregation at the middle was unstable allowing cells to escape from the center towards nearest PNs. 'Thick' cell traces were noted due to the proximity of the two aggregation in this scenario.

The viability of cells was tested on the lung cancer cells running through the PCBSAW device for 5 min with (SAW ON group) and without (SAW OFF group) SAW applied. A control group was used in which cells were staying constantly in ice bath for the same period. A power of 32 dBm was used to drive the PCBSAW device and a flowrate of 20 μ L/min was used. The microchannel was thoroughly flushed to remove any cells between repetition tests. 40- μ L sample collected from the PCBSAW outlet was mixed with 2 μ L acridine orange (30 μ g/ml) and

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diaminophenylindole (100 µg/ml) for staining cells for testing the viability. The stained sample was pipetted into cell counter slide and analysed by a cell counter (NucleoCounter® NC-3000™). The viability test results are shown in Fig. 9d, with the viability of 97.98%, 97.58% and 96.94 for the control, SAW OFF and SAW ON, respectively.

Conclusion

We present herein a cost-effective technique using mature PCB manufacturing to develop acoustofluidic devices. The overall cost of fabricating the PCBSAW device was approximately £300, which was significantly lower than manufacturing similar devices using cleanroom facilities. The PCBSAW is an on-demand process and able to quickly recover any broken device, [as opposed to the existing SAW devices, where a new patterned LiNbO₃ device needs to be fabricated](#). All the materials for constructing the PCBSAW device can be either 3D printed or mechanically engineered. The fabrication requires only a screwdriver to complete.

Thorough characterisation to this new technique, including repeatability and stability, informed the PCBSAW is able to achieve efficient electromechanical energy conversion and quality SAW production. The working frequency was precisely defined by using *s*-parameters such as S_{12} and S_{21} , which allows optimally operating the PCBSAW device. Droplets, microparticles and cells were all tested using the PCBSAW device resulting similar performance of conventional acoustofluidic devices and in good agreement with simulations. The cell viability confirmed the PCBSAW has good biocompatibility in actuating cells.

The potential of the PCBSAW technique is [the rapid fast prototyping of SAW devices with considerably low costs for the first time](#). Different types of piezoelectric substrates can be easily switched on the device to select the best material without the need of patterning IDEs [on them](#); this allows recycling piezoelectric substrates and diversity [in testing](#) in acoustofluidic research. [Future work, aiming to reduce further the cost of the presented device, includes inkjet printing of piezoelectric material on top of the PCBSAW IDTs, thus minimizing further the major cost implication of the presented device, i.e. LiNbO₃ substrates.](#)

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