

1 **Development and Characterisation of Acoustofluidic Devices Using Detachable**
2 **Electrodes Made from PCB**

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28 **Abstract:**

29 Acoustofluidics has been increasingly applied in biology, medicine and chemistry due to its
30 versatility in manipulating fluids, cells and nano-/micro-particles. In this paper, we develop a novel
31 and simple technology to fabricate a surface acoustic wave (SAW)-based acoustofluidic device by
32 clamping electrodes made using a printed circuit board (PCB) with a piezoelectric substrate. The
33 PCB-based SAW (PCB-SAW) device is systematically characterised and benchmarked with a
34 SAW device made using the conventional photolithography process with the same specifications.
35 Microparticle manipulations such as streaming in droplets and patterning in microchannels were
36 demonstrated in the PCB-SAW device. In addition, the PCB-SAW device was applied as an
37 acoustic tweezer to pattern lung cancer cells to form three or four traces inside the microchannel
38 in a controllable manner. Cell viability of ~97% was achieved after acoustic manipulation using
39 the PCB-SAW device, which proved its ability as a suitable tool for acoustophoretic applications.

40 **Introduction**

41 Acoustophoresis is a technique well-known for actuating and manipulating micro¹-/nano^{2,3}-
42 particles using acoustic waves. Its applications have been demonstrated in a wide-range of
43 biomedical applications such as separating blood cells and platelets⁴, separating circulating tumour
44 cells from whole blood^{5,6}, isolating exosomes^{7,8}, washing⁹ and coating of cells¹⁰, handling liquid
45 ¹¹ and versatile manipulations of micro-objects¹²⁻²⁰, alongside the continuous development of
46 acoustophoretic theories²¹⁻²⁴ and simulations²⁵⁻²⁷. Acoustophoretic devices using either bulk
47 acoustic waves (BAWs)^{4,28} or surface acoustic waves (SAWs) produce an acoustic pressure
48 gradient and streaming within a fluid, thus achieving the capability of actuating micro-/nano-
49 particles inside. SAW-based devices have been intensively investigated in manipulating biological
50 cells because of their versatility and being less-dependent on the acoustic properties of the
51 microchannel material when compared to those made using BAWs²⁸.

52 SAW devices are generally fabricated by patterning an interdigitated transducer (IDT)¹⁷ onto
53 piezoelectric substrate such as lithium niobate (LiNbO₃). IDTs typically have two comb-shaped
54 arrays of metallic electrodes, which are driven by radio frequency (RF) signals to produce SAWs.
55 The fabrication process of SAW devices typically employs photolithography, which includes the
56 following steps: 1. mask design and manufacturing; 2. spin-coating photoresist onto LiNbO₃; 3.
57 mask aligning for patterning with UV light; 4. metal layer deposition; 5. lift-off process to form
58 the IDT¹⁷. The brittle²⁹ bulk LiNbO₃ substrate is vulnerable during manufacturing and operation.
59 Furthermore, the SAW device made by photolithography is a one-off component, any modification
60 will require to go through the entire aforementioned manufacturing processes again. It is also
61 difficult to repair a damaged substrate (i.e., scratches). The facilities and skills required for making
62 IDTs limit the use of SAW devices. To address these issues and simplify the process, the creation
63 of shear-SAWs has been demonstrated on the surface of LiNbO₃ by stacking aluminum foil strips
64 onto the substrate³⁰. IDTs have also been created by pouring low-melting point metal into an IDT
65 mold made by PDMS³¹. Superstrates have also been implemented on the conventional SAW
66 devices to allow their reuse for different applications³².

67 Interdigital electrodes (IDEs), with a similar pattern as that in SAW devices, have also been
68 fabricated on printed circuit boards (PCBs). They have been applied in various applications such
69 as moisture sensing³³, water level measurements³⁴, electro wetting³⁵, biosensing³⁶ and even cell
70 manipulation³⁷. A standard PCB laminate consists of a layer of thin copper foil and an insulating

71 layer typically laminated together with glass reinforced epoxy resin (FR4). Further choices for
72 core materials are commercially available such as PET (Polyethylene terephthalate), flexible
73 polyimide or Teflon. This allows versatility based on different applications. The fabrication of
74 IDEs on the PCB by metallisation of the copper layer is routinely employed within the industry
75 with a wide variety of gold or silver electroplating processes commercially available.

76 In this work, we demonstrated a novel SAW device fabrication technique done by mechanically
77 clamping IDEs on the PCB to a LiNbO₃ wafer. This PCB-based SAW (PCB-SAW) device was
78 characterised and benchmarked against an IDT with the same specifications made using the
79 conventional photolithography process. The PCB-SAW device was used as an acoustic tweezer to
80 actuate and pattern both polystyrene microspheres and cancer cells. The proof-of-concept
81 demonstrated that the simple mechanical clamping technique could be applied as an alternative to
82 the conventional photolithography, by transferring the photolithography effort in fabricating SAW
83 devices to the mature PCB manufacturing industry.

84 **Methods and materials**

85 **Design and working mechanism**

86 The schematic illustration of the PCB-SAW device shown in Figs. 1a and 1b consists of six
87 components: a base plate, a LiNbO₃ wafer, a PCB with a pair of patterned IDEs, a clamp, a pressure
88 ring and a microchannel. The base plate supports the LiNbO₃ wafer and is bolted with the clamp
89 to apply clamping force between the PCB and the LiNbO₃ wafer via the pressure ring. Once a
90 proper clamping force is applied by fastening the four screws on the clamp, the pair of IDEs make
91 good contacts to the LiNbO₃ wafer to couple RF signals that generate counter-propagating SAWs
92 to form standing SAWs (SSAWs) between the two IDEs. The microchannel is bonded to the
93 LiNbO₃ wafer at the middle between the two IDEs for handling fluid samples. Depending on the
94 size of microparticles and other application parameters, the SAW wavelength can be customised
95 by producing PCBs with alternative IDEs specifications.

96

97 **PCB-SAW fabrication and experimental setup**

98 The PCB was designed using the Eagle software (Autodesk, US) and manufactured externally
99 (circuitfly.com). All the design files are accessible as supporting files of this work. The PCB design
100 had a conventional IDT pattern for SAW devices with the wavelength of 200 μm, corresponding

101 to Rayleigh mode frequency of 19.9 MHz. This is based on that the speed of sound in the LiNbO₃
102 is 3,980 m/s. Each IDT consists of 40 pairs of 10 mm (aperture size) long finger electrodes. The
103 manufactured single-sided PCB is shown in Fig. 2a. The thickness of the PCB laminate is 1.6 mm
104 with the IDE layer thickness of 34.8 μm of copper. The IDEs and the buses are exposed without
105 pasted solder mask. The PCB dimensions are 10 cm (L) × 10 cm (W) with a milled open window
106 of 3.5 cm (L) × 1.5 cm (W) at the centre for accommodating the microchannel. Alignment markers
107 (holes and lines) are present on the PCB to help align the microchannel and the LiNbO₃ wafer. A
108 microscope was used to check the IDE manufacturing quality (Fig. S1). Two coaxial cables were
109 soldered to the bus pads at the edges of the PCB for signal transmissions.

110 Before the assembly process, both the PCB IDEs and a 3-inch, 500-μm thick, 128-deg-rotated
111 Y-cut X-propagation LiNbO₃ wafer were thoroughly cleaned using isopropyl alcohol (IPA) and
112 inspected under a microscope. The pressure ring, clamp, and base plate were printed using a 3D
113 printer (Ultimaker 2+ extended, Utrecht). The exterior dimensions of the PCB-SAW device are
114 120 mm (L)×120 mm (W)×30 mm (H). Additionally, two localised pressers for focusing clamping
115 force onto IDE region and a round holder for supporting the LiNbO₃ wafer, were also printed.

116 The assembly process is shown in Fig. 2b. The LiNbO₃ wafer was placed onto the round holder
117 and its reference flat edge was aligned to be parallel with IDEs to ensure that the SAW generation
118 was in the direction of the X direction of the LiNbO₃. The PCB was then placed on the LiNbO₃
119 wafer with the IDEs facing down. The clamp was mounted to the PCB and bolted to the base plate
120 by slightly fastening the four screws. The pressure ring was screwed into the clamp to provide
121 localised force to the PCB via the localised pressers and then the four screws were fully tightened.
122 There was an observation window on the base plate for light transmission during microscopic
123 measurement. A PDMS microchannel with the channel dimensions of 200 μm (L) × 100 μm (W)
124 × 60 μm (H) was bonded to the LiNbO₃ wafer using plasma treatment before the above assembly.
125 Tubing was connected to the inlet and the outlet of the single channel. Fig. 2c shows the assembled
126 PCB-SAW device.

127 To study the reliability of the assembly, the PCB-SAW device was thoroughly characterised by
128 using *s*-parameters and power transmission test. Details of the electrical characterisation can be
129 found in the Supplementary Information, in which the working frequency is identified and
130 matching networks (MNs) are recommended to couple the power amplifier and the PCB-SAW
131 device to maximise power transmission.

132

133 **PCB-SAW test with droplet actuation**

134 The device is purely integrated by mechanical clamping and the contact quality between the
135 PCB and the LiNbO₃ wafer is associated with the clamping force produced by the bolt torque (Fig.
136 2b). A droplet actuation test was performed to investigate the relationship between the clamping
137 force and SAW generation indicated by droplet movement. The LiNbO₃ substrate was coated with
138 a hydrophobic substance CYTOP™ (AGC Chemicals Europe), which was done by evenly
139 distributing across the LiNbO₃ substrate³⁸.

140 For the clamping test, the clamping force between the LiNbO₃ substrate and the PCB IDEs was
141 increased by adjusting the torque of the M5 screw torque on top of the localised pressers. A digital
142 torque screwdriver (5-50 cNm, Adema, Taiwan) with a digital display was used to apply and read
143 the torque. The torque was converted to clamping force by $F = \frac{T}{cD}$, where F , c , D and T
144 correspond to clamping force (N), coefficient of friction, screw diameter (m) and torque (Nm),
145 respectively. The standard value c for unlubricated steel is equal to 0.2. The readability of the
146 digital torque screwdriver was 0.05 Nm, which allowed a minimum reading of the clamping force
147 of 50 N. The VNA was used to monitor the real-time S_{11} while fastening the bolt. During each
148 assembly, the clamping was adjusted so that the same minimum S_{11} value was achieved. This
149 process facilitated establishing a correlation between the S_{11} and the clamping force, which allowed
150 the use of S_{11} rather than the clamping force to guide the assembly.

151 Under each measured torque, a 1- μ L water droplet was pipetted onto the LiNbO₃ substrate 5
152 mm away from the first finger electrode. Then an input power of 1.26 W was applied to the PCB-
153 SAW device to actuate the droplet. The slight location variance of droplet initial positions in each
154 test is insignificant as the SAW attenuation in the LiNbO₃ substrate is negligible³⁹. Even though
155 the droplet was placed in nearly identical location before actuation, a variation in speed of droplet
156 transportation can be observed on both the devices. We hypothesise that this could have been
157 caused by slightly uneven CYTOP coating, coating deterioration, slight contact angle variance,
158 droplet volume variation, or a combination of these factors. Therefore, the droplet actuation by
159 SAW was repeated five times before changing to another clamping force. A camera was used to
160 capture the droplet moving and a calibrated software Tracker (www.compadre.org/osp/) was
161 applied off-line to analyse the droplet velocity for indicating SAW amplitude. The captured droplet
162 videos were analysed frame by frame using the leading edge of the droplet, as the reference to

163 determine the displacement of the same droplet. Any two consecutive frames could produce one
164 velocity using the displacement multiplied by the framerate. Five consecutive frames after the
165 droplet moved were used to get four velocities, which were averaged to get the mean droplet
166 velocity. The pixel size and the frame rate of the camera system were 10 μm and 60 fps,
167 respectively, resulting in a velocity resolution of 0.6 mm/s, which was sufficient for capturing
168 droplet movement.

169 To benchmark the performance of the PCB-SAW device with the SAW device made by
170 standard photolithography¹, another IDT made by the same LiNbO₃ substrate using the
171 conventional photolithography process in cleanroom was prepared using the identical geometry as
172 the PCB-SAW device. The cleanroom-made IDT (CR IDT) was also coated with CYTOP™ for
173 the droplet test.

174

175 **Sample preparations**

176 To demonstrate the PCB-SAW device capability in manipulating microparticles within droplets,
177 a 3-4 μL glycerol droplet (3 mm in diameter) was prepared on the LiNbO₃ substrate and 20 μm
178 polystyrene microspheres were pipetted into the glycerol droplet (concentration of $\sim 18,000 / \mu\text{L}$).
179 An input power of 0.2 W was used for manipulating the microspheres.

180 To demonstrate the PCB-SAW device in manipulating microparticles inside the microchannel,
181 10 μm polystyrene microspheres were mixed with a custom media at a volume ratio of 1:2.7. The
182 custom media consisted of glycerol and phosphate-buffered saline (PBS) with a volume ratio of
183 1:4.4, which was made to prevent particle deposition. Before sample introduction, the
184 microchannel was flushed with bovine serum albumin (BSA) solution (water:BSA = 100:1, mass
185 ratio) for 20 min at a flow rate of 20 $\mu\text{L}/\text{min}$. The input power in this experiment was 0.5 W.

186 For cell manipulation, A5499 human non-small-cell lung carcinoma (NSCLC) cell lines were
187 grown in Dulbecco's modified eagle media and supplemented with L-Glutamine (200 mM at 1:100
188 dilution, Gibco), Penicillin/Streptomycin (10,000 U/mL at 1:100 dilution, Gibco), and 10% foetal
189 bovine serum (FBS) in 75-cm³ cell culture flasks until their density reached $1 \times 10^7 / \text{mL}$. The cells
190 were harvested from the plastic surface by trypsinisation, and then concentrated by centrifugation
191 (3500 rpm, 5 min) to $2 \times 10^7 / \text{mL}$. The input power was set to be ~ 1 W in the experiment.

192

193 **Viability test**

194 There were three sample groups for viability test: (1) SAW-on Group, in which the NSCLC cells
195 were continuously run through the PCB-SAW device for 5 minutes under the input power of ~1
196 W and flow rate of 20 $\mu\text{L}/\text{min}$. (2) SAW-off Group, in which the cells were running through the
197 PCB-SAW device at the same flow rate and duration without applying SAW. (3) Control Group,
198 in which the cells were kept in a steady tube on an ice bath for the same period of time.

199 Acridine orange (AO, 30 $\mu\text{g}/\text{mL}$) and di-amino-phenyl-indole (DAPI, 100 $\mu\text{g}/\text{mL}$) were mixed
200 at the volume ratio of 3:10 to prepare an AO-DAPI solution for cell staining. For both SAW-on
201 and SAW-off Group, 100- μL sample in total was collected after 5 minutes, of which three 10- μL
202 samples were taken out to mix with the AO-DAPI solution at the volume ratio of 5:1 to stain the
203 cells. The three stained samples were then pipetted into three cell chambers on a cell counter slide
204 for viability analysis using a cell counter (NucleoCounter® NC-3000™). For the Control Group,
205 the same amount of the sample was taken for staining and viability test. All the tests were repeated
206 three times.

207 **Results and discussion**

208 **Characterisation of the PCB-SAW device**

209 The average width and spacing of the finger electrodes on the PCB were measured to be 38.7
210 μm and 61.1 μm , respectively (Fig. S1), which led to a SAW wavelength of 199.6 μm . The MNs
211 were designed for the PCB-SAW device, which managed to reduce the device's reflection
212 coefficients to -18.4 dB and -21.4 dB (Fig. S2c) and improve the transmission coefficients to -11.9
213 dB (Fig. S2e).

214 Under the unique clamping bonding of the PCB-SAW device, Fig. 3a shows the $|S_{11}|$ and droplet
215 velocity against the clamping force. Despite large variance of SAW amplitude indicated by the
216 droplet velocity, the optimal clamping force of 50 N produced the minimum S_{11} of -46 dB and the
217 maximum average droplet velocity of 24.4 mm/s. Further increase in the clamping force to the
218 PCB-SAW device decreased the $|S_{11}|$, the SAW amplitude and its variance. The reduction of the
219 droplet velocity at a higher clamping force could be a result of over compressing the piezoelectric
220 material, thus resulting in reduced SAW amplitudes or higher power reflection. The use of the
221 MNs improved the sensitivity of the S_{11} reading, which allowed to easily achieve an optimal
222 clamping assembly by reading the real-time S_{11} spectrum.

223 Once the optimal state of the PCB-SAW device was achieved by applying the clamping force
224 of 50 N, the S_{11} spectrum was compared with that of the CR IDT with the same specifications as
225 shown in Fig. 3b. It can be observed that the minimum S_{11} for both the devices had a difference of
226 ~ 0.21 MHz, which could be caused by the errors in the PCB manufacture and the parasitic
227 capacitance and inductance introduced by the MN circuits.

228 Benchmarking the PCB-SAW device at the optimal state with the CR IDT in terms of actuating
229 droplets under a range of input powers is shown in Fig. 3c. The CR IDT showed higher efficiency
230 in converting the input power to SAW comparing with the PCB-SAW device. This is reasonable
231 as the electrodes for the PCB-SAW device were mechanically clamped onto the piezoelectric
232 substrate resulting in an imperfect signal coupling. This issue can be easily compensated by
233 doubling input power to the PCB-SAW device. For example, operating the CR IDT at ~ 0.6 W
234 drives the droplet velocity of 20 mm/s, which can be achieved by the PCB-SAW device working
235 at ~ 1.2 W.

236

237 **Manipulation of microparticles**

238 SAW devices have been previously demonstrated in manipulating microparticles within
239 droplets for sample mixing^{38,40}. On the PCB-SAW device, a droplet sample containing polystyrene
240 microspheres was placed at the centre between the two IDTs (Fig. 4a). When one of the IDTs was
241 activated, a streaming pattern with two major vortices was observed (Fig. 4b, Video S1), which
242 was in good agreement with the pattern formed on conventional SAW devices⁴⁰. When both the
243 IDTs were activated, a four-vortex streaming pattern was generated (Fig. 4c, Video S2), which
244 again agreed with that produced on conventional SAW devices³⁸. Each of the IDT in the tests was
245 driven by an input power of 0.2 W.

246 Further tests using the PCB-SAW device as an acoustic tweezer were performed by introducing
247 polystyrene microspheres into the PDMS microchannel. The acoustofluidic model of the PCB-
248 SAW device was developed to study acoustic pressure distribution and predict the microparticle
249 trajectories as shown in Fig. S5, which was adopted from conventional SAW device modelling^{25,26}.

250 A polystyrene microsphere sample was injected into the microchannel. After an evenly
251 dispersed pattern was formed within the microchannel (Fig. 5a), RF signals with the same phase
252 ($\Delta\phi=0^\circ$) were applied to both IDTs to produce SSAWs with the PNs located at the centre and near
253 the two walls, which trapped microspheres to form three aggregation traces as shown in Fig. 5b

254 and Video S3. By applying a 180° phase difference ($\Delta\varphi=180^\circ$) to the RF signal driving one of the
255 IDTs, ANs were formed at the centre and near the two walls, resulting in four microsphere traces
256 as shown in Fig. 5c and Video S4. Both these cases show good agreements with the simulation
257 results (Fig. S5).

258

259 **Manipulation of cancer cells**

260 To validate the manipulation of cells and test the biocompatibility, the PCB-SAW device was
261 filled by the NSCLC cell sample (Fig. 6a) and repeated the same operation for microspheres.
262 Applying RF signals with $\Delta\varphi=0^\circ$ and $\Delta\varphi=180^\circ$ to the two IDTs resulted in the formation of three-
263 cell column (Fig. 6b, Video S5) and four-cell column (Fig. 6c, Video S6), respectively. The results
264 demonstrated that the PCB-SAW device can be used as an acoustic tweezer to manipulate and re-
265 position cells controlled by changing RF signal phase.

266 The ability of the PCB-SAW device in preserving cell viability was tested using three sample
267 groups, including Control, SAW-off and SAW-on. The results shown in Fig. 6d denote the
268 viabilities of $98.2\pm 0.8\%$ (average \pm SD), $97.6\pm 1.2\%$ and $96.9\pm 0.6\%$, respectively. The analysis of
269 variance showed no significant differences among these three groups ($p = 0.166$).

270 **Conclusion**

271 In this paper, we demonstrated that the novel PCB-SAW device is capable of performing all
272 the functions realised using the standard SAW devices. The PCB-SAW has the main advantages
273 of easy fabrication and low-skill entry requirement. The systematic characterisation to the PCB-
274 SAW device and the comparison with the standard SAW device confirm the new technique has
275 similar ability in actuating droplets. The PCB-SAW device can also be used as an acoustic tweezer
276 to pattern microspheres and cells in a controllable manner, while maintaining high cellular
277 viability.

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