Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information for

An affordable 3D-printed positioner fixture improves the resolution of conventional milling for easy prototyping of acrylic microfluidic devices

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1. Diagram of the control and sensor monitoring system

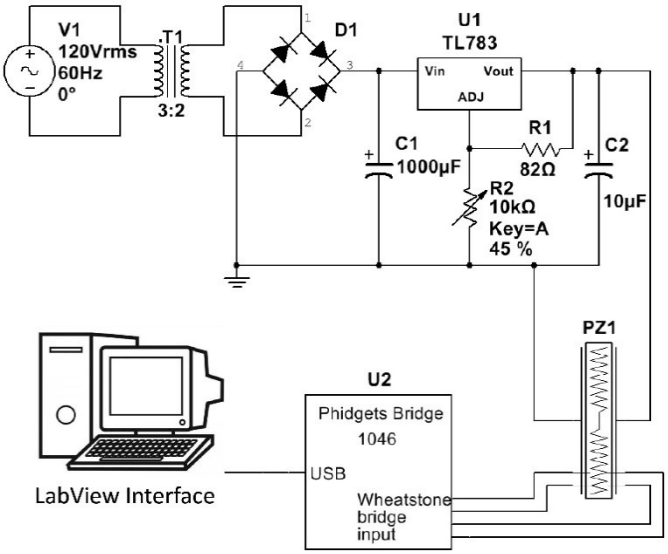

Fig S1. Diagram of the control and sensor monitoring system of the piezoelectric actuators (U1 voltage regulator, U2 Wheatstone bridge reader, PZ1 piezoelectric actuator).

2. Manufacturing of the immunoassay chip

2.1 Micromilling

The fabrication of the immunoassay device starts off by cutting out two PMMA (ME303018, Goodfellow, USA) slabs of 1 x 2.5 cm in the milling machine (MDX-40A, Roland AG, Germany). Then, one slab is placed on the piezoelectric platform with double sided adhesive tape (Tuk, 404, Mexico). The X and Y machine origin are set to match the lower right corner of the slab. The Z origin is set to 50-100µm below the surface of the slab.

The next step consists in leveling the PMMA surface to the new Z origin established in the previous step, **Figure S3a**. This step ensures a precise positioning of the chip relative to the machine. To avoid losing this calibration step due to inaccurate movements produced by the machine's proprietary software (as a home return), we performed the subsequent steps with a numerical control code (G code).

To mill each weir filter (**Figure S3b**), the drill bit is moved to location where the weir filter will be without moving the Z axis. Next, the spindle is set at 15000 rpm, the piezoelectric platform is raised to 5um and the microchannel carved out by moving the Y axis to the end of the weir filter. This process is repeated for each weir filter.

Finally, the 100 µm depth channels are machined with the milling machine's proprietary software (Dr.Engrave) according to the design shown in **Figure S2**. A 200 µm drill bit (Kyocera, 1600-0080L012) is used for the channels (**Figure S3c**) while a 0.8 mm drill bit (Kyocera, 1600-0320L048, USA) is used for drilling access holes (**Figure S3d**). Next, the chip is removed from the milling machine, washed with isopropanol and dried with nitrogen.

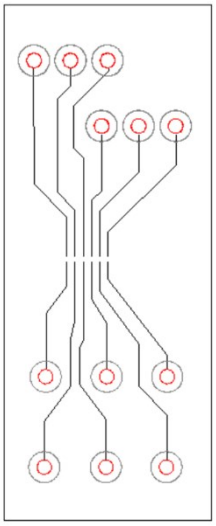

Figure S2. Design of the microfluidic immunoassay chip that is used in the proprietary software for the last two steps.

2.2 Bonding protocol

The bonding of the two acrylic layers is achieved using a solvent method. First, 1 ml of chloroform is deposited in a 100 mm Petri dish and left still for 5 min to saturate its interior with chloroform vapor, **Figure S3e**. Then, using double-sided adhesive tape (Tuk, 404, Mexico) the acrylic pieces are glued to the lid of the petri dish and exposed to the chloroform vapor for 1 min. It is essential to leave a gap of 7.5 mm between the surface of the acrylic and the solvent surface. Next, the pieces are incubated for 5 min in an atmosphere without chloroform. Finally, the treated pieces are pressed together at 250 psi and 90°C for 10 min using a home-made mechanical press, **Figure S3f**. (section 2.3 Mechanical press).

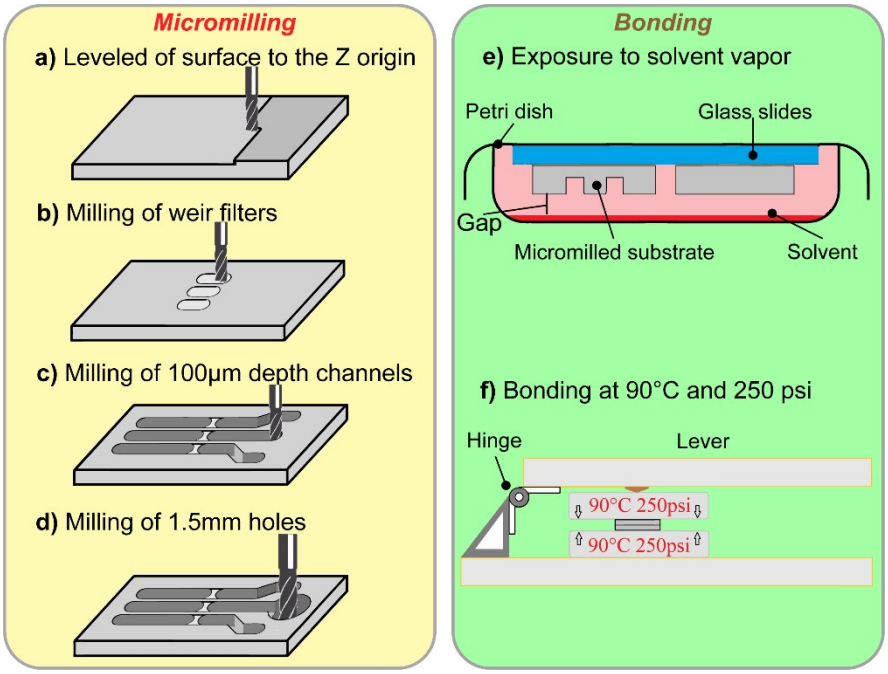

Figure S3. Schematic representation of the immunoassay chip manufacturing steps.

2.3 Mechanical press

The mechanical press is based on a lever with an amplification factor of 1:10. To reach 17.5 Kgf/cm^2 (250 psi) of pressure on the chip; we have to apply a force of 43.75 Kgf on an area of 2.5cm^2, this translates into a mass of 4.37 kg on the lever. To control the temperature, we used a pair of 200W heaters (DBK, PCT heaters HP06-2/20-240) and a PID controller (Autonics, TC4Y-N4R) with a K type thermocouple.

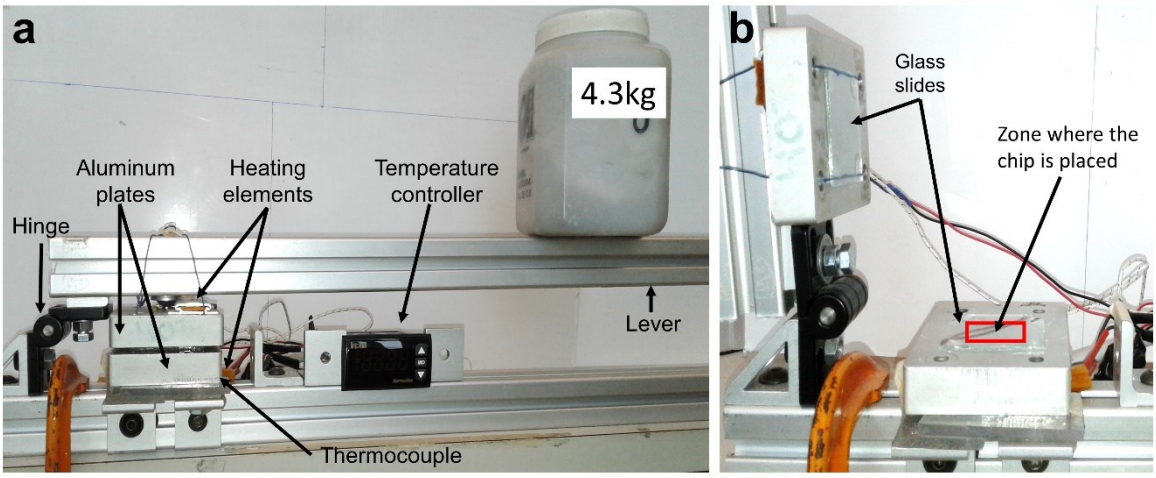

Figure S4. (a) Photographs of the mechanical press and (b) close-up to the bonding plates.

3. Review of advantages and disadvantages of different microfabrication methods

1-10um microfabrication technology	Resolution	Equipment cost	Time	comments
Soft lithography	>submicron range	> 15k $ without mold fabrication equipment[4]	0.5-1hr to replicate a mold	Expensive[5] molds made by lithography, slow prototyping
High-end CNC	>submicron range[6]	>100k $[7]	5-30 min	Channels[5] limited by drill bit size
Hot embossing	>submicron range	>20k $	10-30min to replicate a mold	Mold is expensive to fabricate, slow prototyping[8]
microinjection	>submicron range	>50k $	10-30 seg	Long prototyping time[8]
High-resolution 3D printing	>submicron range	In development[9]	5-10 hr	Limited to small pieces[10], low biocompatibility[2]
Laser-cut	>micron range	<10k $	0.5-1hr	X, Y plane low resolution, cuts of >100µm[3]
CNC with our fixture	>micron range	<10k $	5-30 min	Channels[5] limited by drill bit size

Table S1. Comparison of other fast prototyping techniques for microfluidic devices

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