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

Electronic supplementary information (ESI) for:

Versatile hybrid acoustic micromixer with demonstration of circulating cell-free DNA extraction from sub-ml plasma samples

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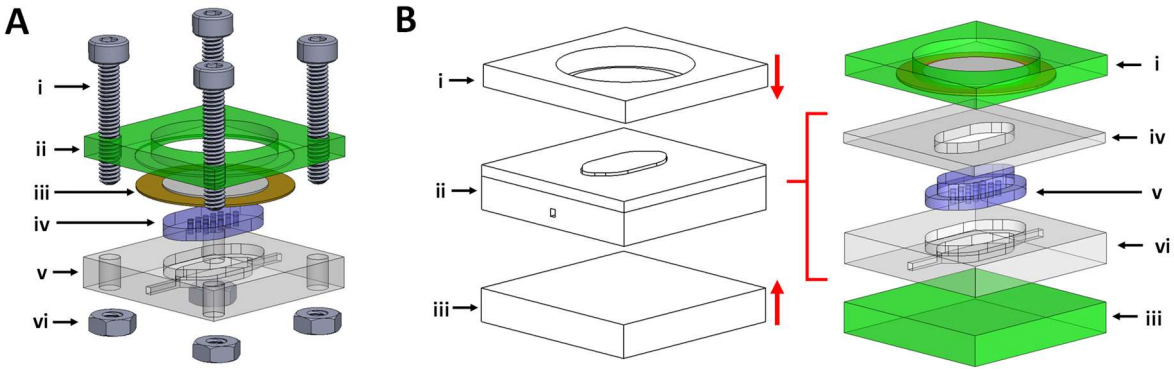

Fig. S1. A) Experimental version of the hybrid concept in exploded view: i) Mounting screws, ii) fitting frame for the piezoelectric, iii) piezoelectric disc, iv) slab, v) substrate and vi) mounting nuts. B) Disposable version of the hybrid concept in exploded view: i) fitting frame with a glued piezoelectric disc, ii) monolithic disposable chip integrating the micromixer, iii) base, iv) auxiliary chip layer to compress the slab and provide sealing, v) slab, vi) substrate. The red arrows indicate that the chip is compressed in between layers i and iii to provide intimate contact between the piezoelectric disc and the slab.

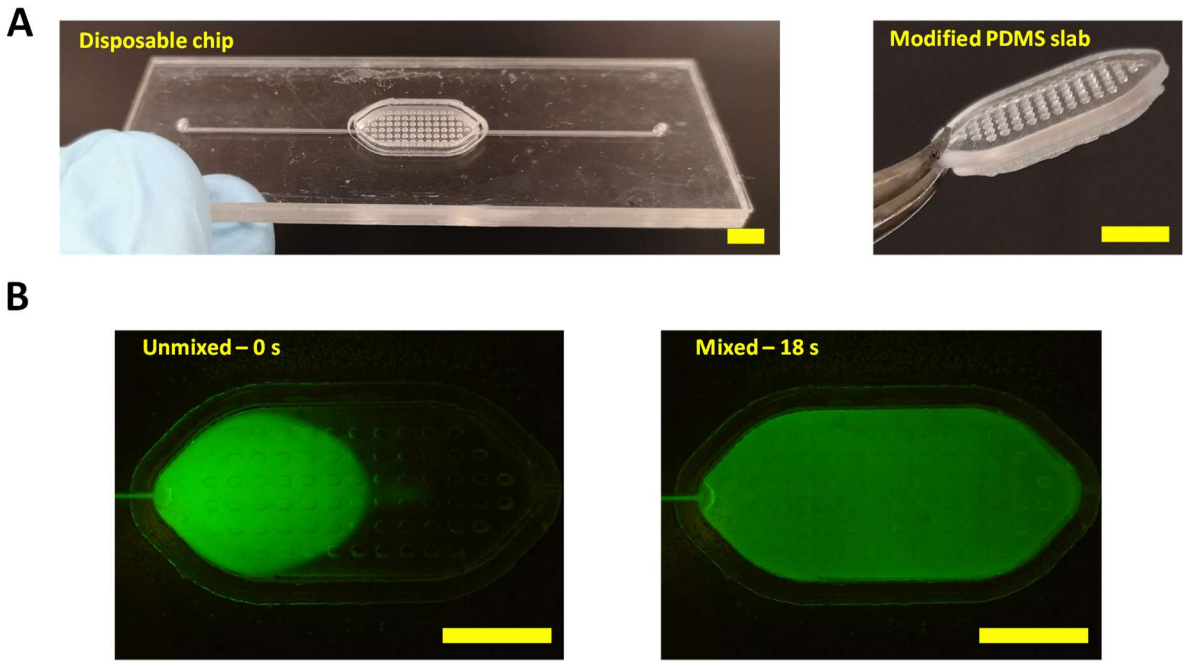

Fig. S2. A) Photographs of a PMMA micromixer chip and the modified PDMS slab using the disposable configuration. B) Micrographs of a 200 µl mixing chamber under static flow conditions before and after mixing using the disposable configuration. Scale bars are 4 mm.

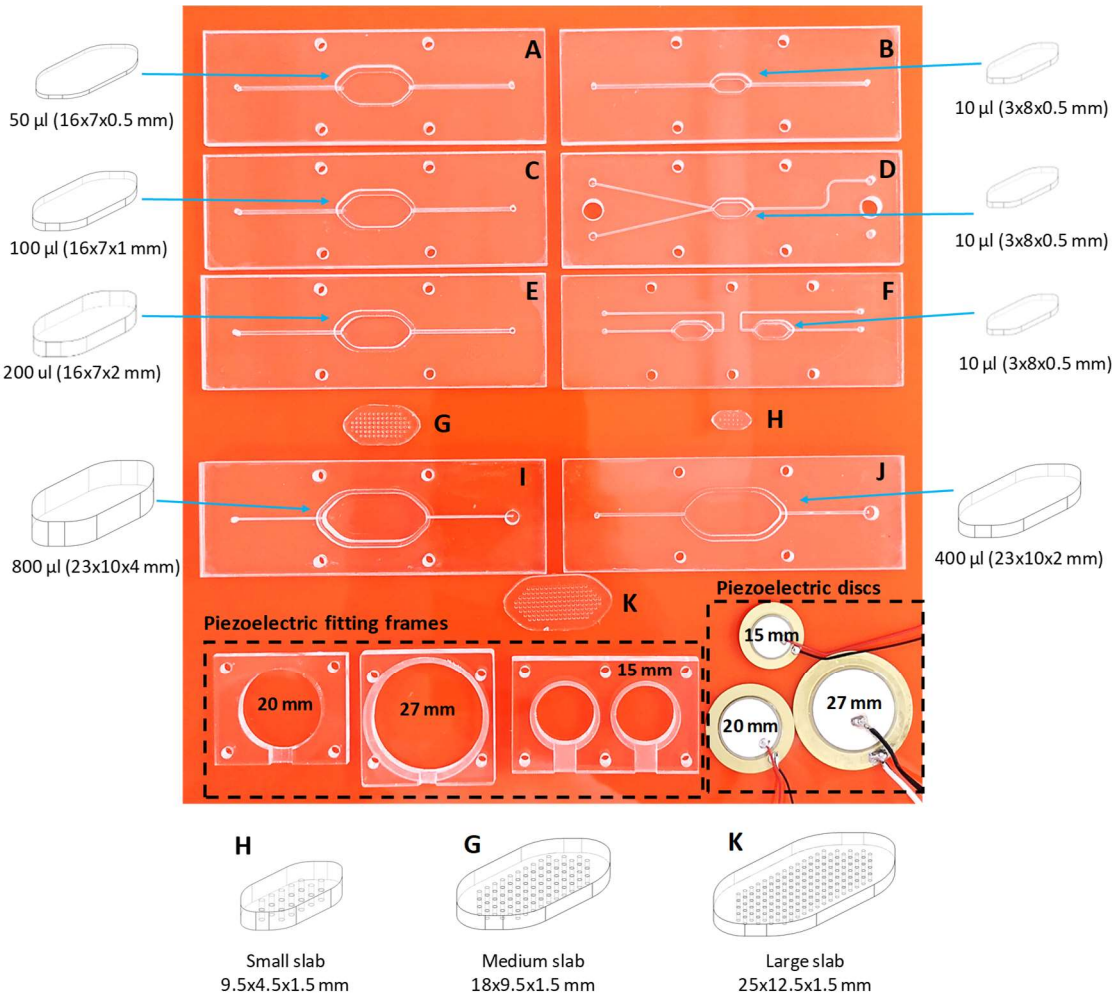

Fig. S3. Photograph showing the PMMA microfluidic chips, the PDMS slabs, the fitting frames and the piezoelectric discs used for the experimental characterisation. A) 50 µl static-flow micromixer, B) 10 µl static-flow micromixer, C) 100 µl static-flow micromixer, D) Continuous-flow micromixer (mixing chamber 10 µl), E) 200 µl static-flow micromixer, F) chip for evaluation of the capability to individually-address different mixing modules within the same chip (mixing chamber 10 µl), G) medium slab, H) small slab, I) 800 µl static-flow micromixer, J) 400 µl static-flow micromixer, K) large slab. All the chambers and slabs dimensions are expressed as maximum length, maximum width and height. All the chips are 25.4 x 76.2 mm in size.

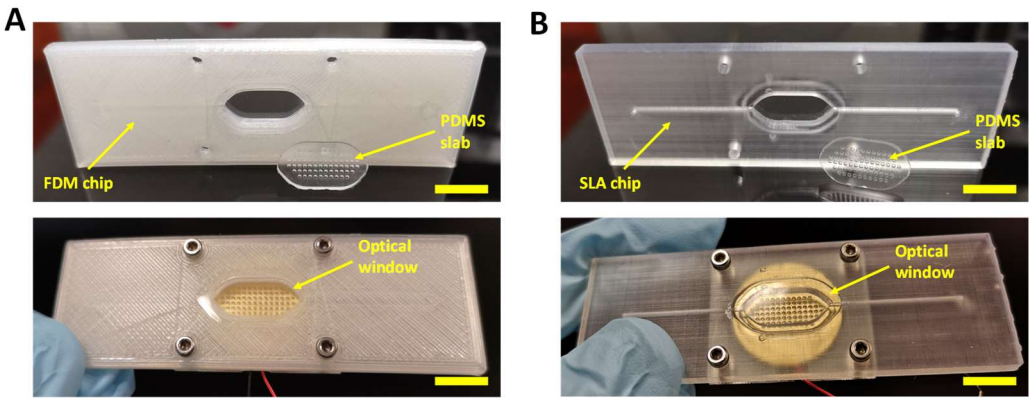

Fig. S4. A) Micromixer chip fabricated using FDM-3D printing technology. B) Micromixer chip fabricated using SLA-3D printing technology The PDMS slab is the same for both chips (medium slab). Scale bars are 10 mm.

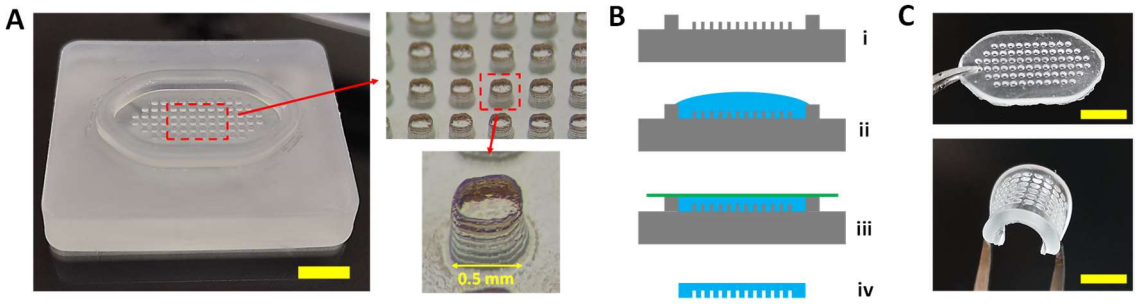

Fig. S5. A) Photograph of a SLA 3D-printed mould and micrographs showing the detail of the pillars. B) Process for the fabrication of the PDMS slabs: i) clean 3D printed mould, ii) casting of the PDMS mixture, iii) addition of PMMA layer to flatten the top surface, iv) peeled PDMS slab. C) Photographs of a fabricated PDMS slab. Scale bars are 5 mm.

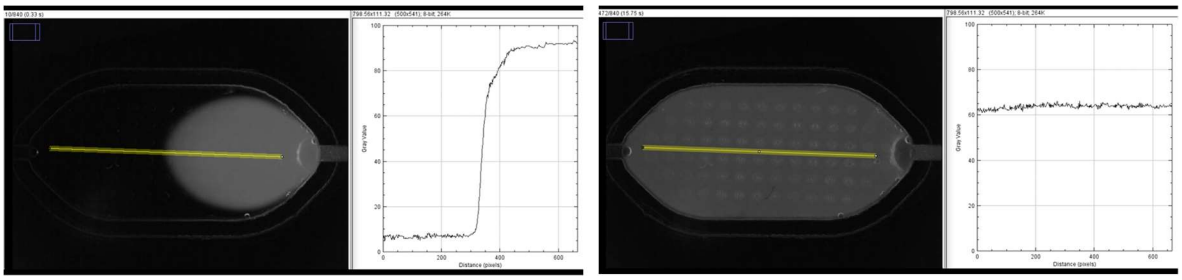

Fig. S6. Mixing evaluation using image analysis from the supporting video 2 before (left) and after mixing (right). The cut line (averaged width 20 px) is slightly tilted to avoid the interference of the bubbles with the image analysis.

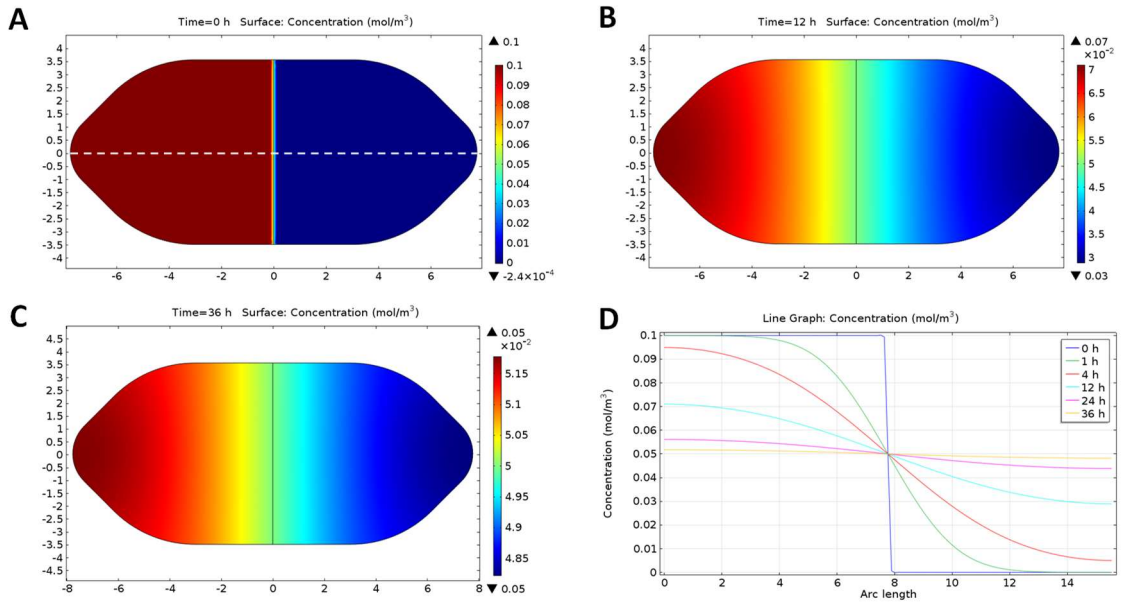

Fig. S7. 2D finite element simulation of a 200 µl chamber performed using the transport of diluted species module in COMSOL Multiphysics 5.0 as a time-dependent study. Mesh: physics-controlled mesh, extremely fine element size. Fluorescein diffusion coefficient: $4.2e^{-10}$[m^2/s]. Fluorescein initial concentration: 0.1 mol/m^3. A) Initial conditions (time = 0h) where half of the chamber is filled with the diffusing molecule (red colour). B) Surface plot of the concentration distribution after 12 h. C) Surface plot of the concentration distribution after 36 h. D) Concentration profile across the dashed white cut line shown in Fig. S7A for different times. All size dimensions are in mm.

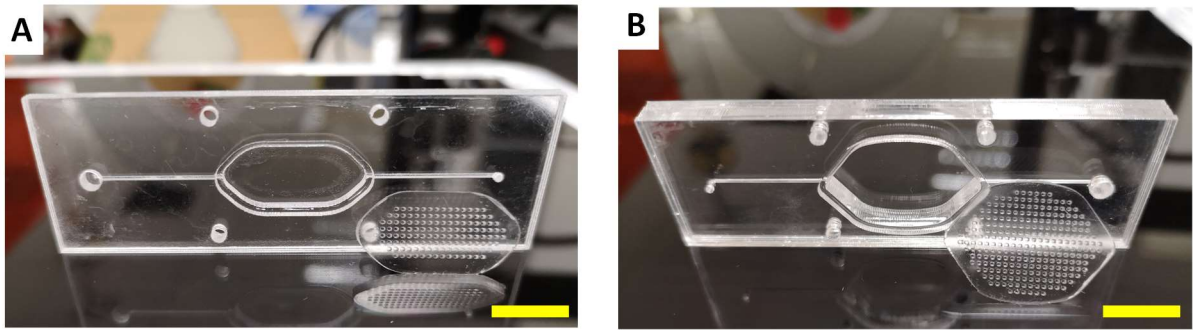

Fig. S8. Photographs of the PMMA chips and corresponding PDMS slabs used for the cfDNA extraction experiments. A) 255 µl chip and B) 1100 µl chip. Scale bars are 10 mm.

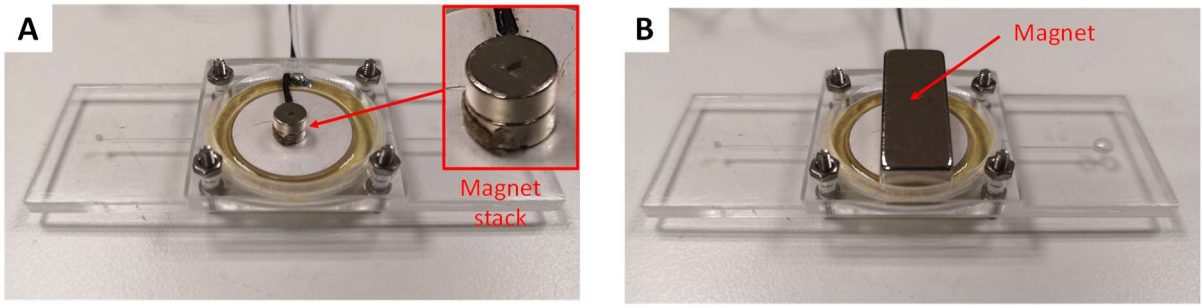

Fig. S9. Magnetic capture set-up for A) the beads disaggregation experiments and B) cfDNA capture experiments.

	Chip extraction	Bench
Sample	Plasma 100 µl / 500 µl	Plasma 100 µl / 500 µl
Lysis/Binding	150 µl L/B solution + 5 µl beads 590 µl L/B solution + 10 µl beads	150 µl L/B solution + 5 µl beads 590 µl L/B solution + 10 µl beads
	Mix for 10 min	Shake for 10 min
	Collect with magnet for 5 min	Centrifuge and collect on magnet for 5 min
	Remove supernatant	Remove supernatant
Wash 1	Resuspend in 500 µl / 1100 µl Wash solution	Resuspend in 500 µl / 1100 µl Wash solution
	Mix for 30 s	Vortex for 30 s
	Collect with magnet for 2 min	Centrifuge and collect on magnet for 2 min
	Remove supernatant	Remove supernatant
Wash 2 & 3	Resuspend in 500 µl / 1100 µl 80% ethanol	Resuspend in 500 µl / 1100 µl 80% ethanol
	Mix for 30 s	Vortex for 30 s
	Collect with magnet for 2 min	Centrifuge and collect on magnet for 1 min
	Remove supernatant	Remove supernatant
	Repeat ethanol wash and collect beads in eppendorf	Repeat ethanol wash
	Airdry 10-15 min	Airdry 10-15 min
Elution	Add 20 µl of Elution Solution	Add 20 µl of Elution Solution
	Shake for 5 min	Shake for 5 min
	Collect beads on magnet for 2 min	Collect beads on magnet for 2 min
	Collect elution with cfDNA	Collect elution with cfDNA

Table S1. CfDNA extraction on-chip and manual workflow comparison

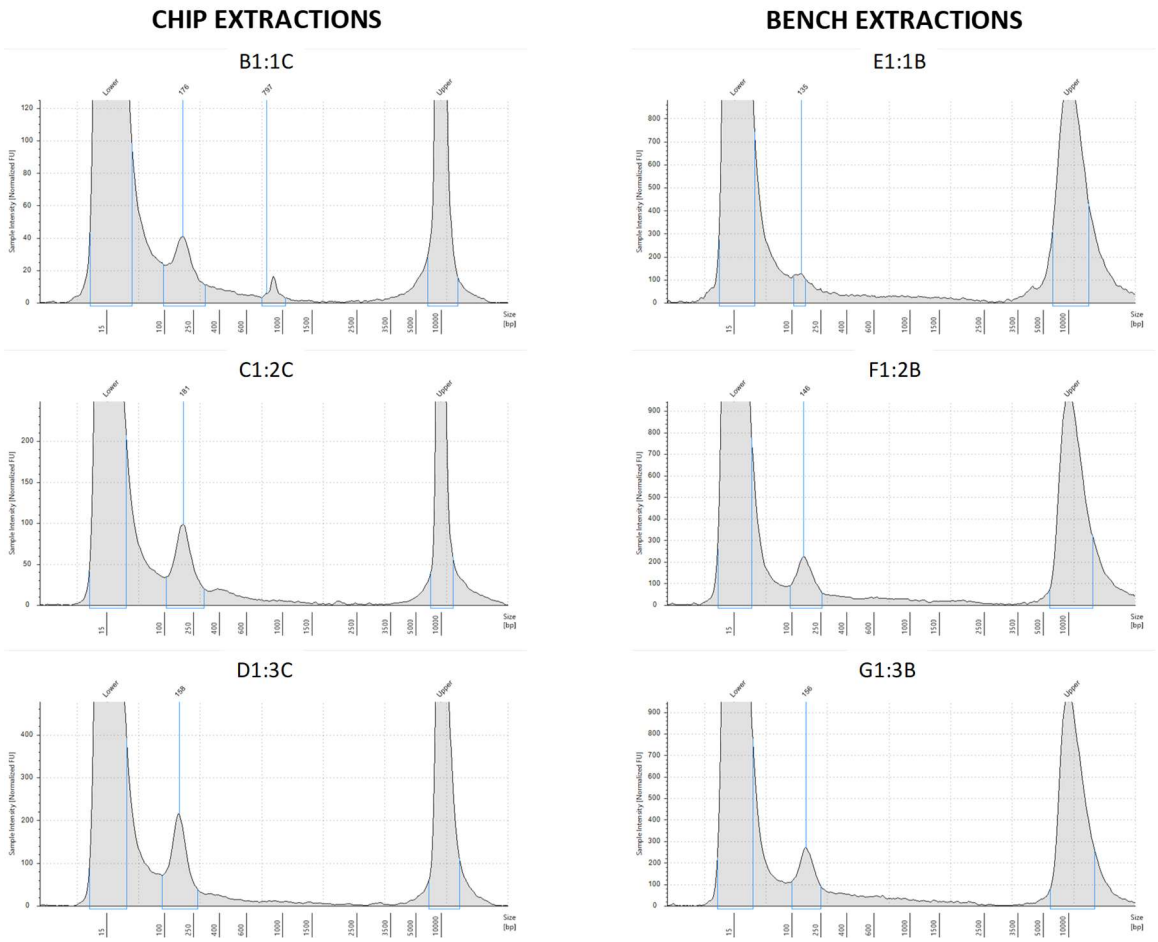

Fig. S10. Electropherograms of cfDNA from Agilent 4200 TapeStation HS D5000 ScreenTape System, extracted from 500 µl of plasma. Only the 500 µl plasma samples were run since the cfDNA concentration extracted from the controls of the 100 µl of plasma sample was on the limit of detection of our preferred assay. Electropherograms B1-D1, cfDNA extracted on-chip; electropherograms E1-G1, cfDNA extracted manually on bench.

SUPPORTING VIDEOS

- **Supporting video 1.** Video of a 100 µl mixing chamber loaded with fluorescent microparticles showing the microstreaming effect produced by the oscillating bubbles when the piezoelectric is turned ON.
- **Supporting video 2.** Video of a 200 µl mixing chamber (PMMA) showing the mixing effect and its corresponding image analysis from the cutline shown in the chamber under static flow conditions.
- **Supporting video 3.** Video of a mixing chamber under continuous flow conditions showing the mixing effect when the piezoelectric is turned ON and OFF. The total flow rate is 200 µl/min.
- **Supporting video 4.** Video demonstrating the capability of the module to individually address two different micromixers integrated within the same monolithic chip.
- **Supporting video 5.** Video of a 200 µl mixing chamber integrated into a chip fabricated with Fused Deposition Modelling (FDM) 3D printing technology showing the mixing effect under static flow conditions.
- **Supporting video 6.** Video of a 255 µl mixing chamber showing the magnetic beads disaggregation and mixing effects after the piezoelectric is turned ON.