Electronic Supplementary Information

Acoustofluidics for simultaneous droplet transport and centrifugation facilitating ultrasensitive biomarker detection

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S1 Measured reflection coefficient S_{11} of the SIDT.

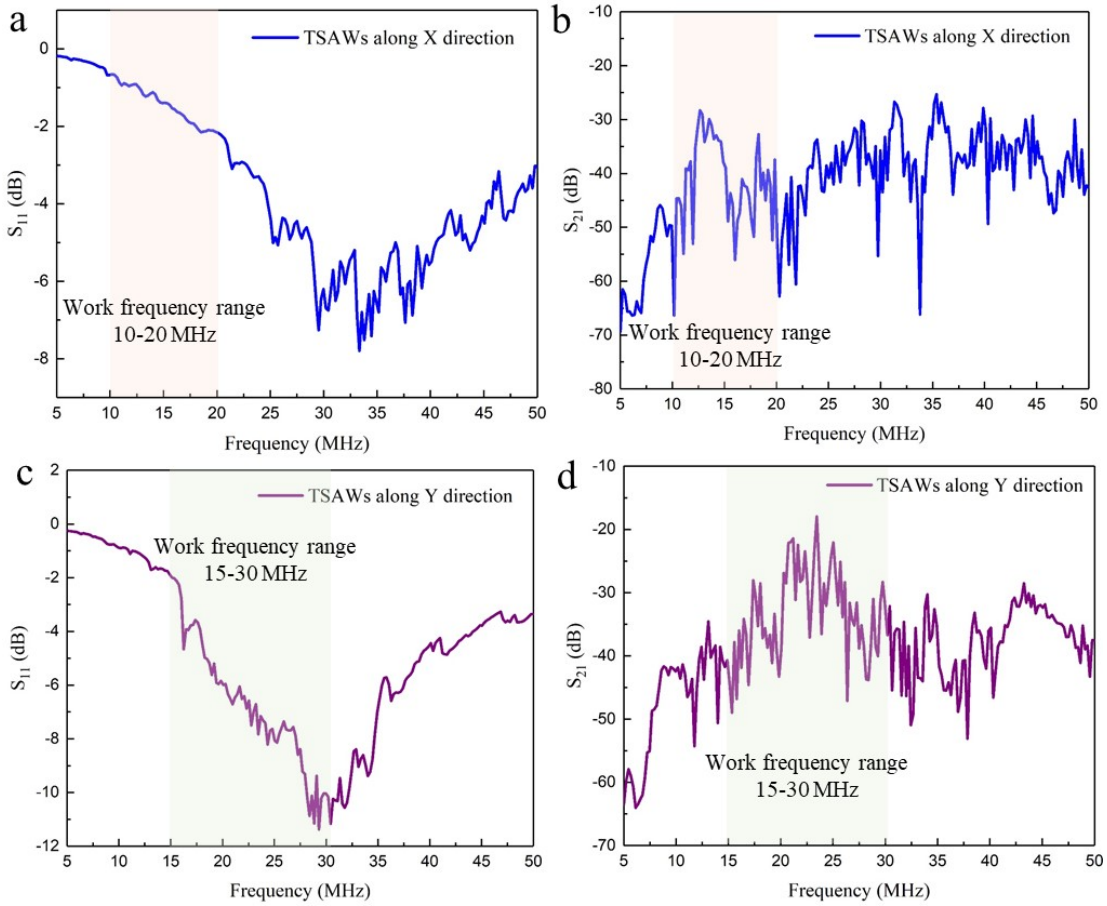


Fig. S1 Measured reflection coefficient S11 of the SIDTs shows the working frequency in the X and Y directions ranges from 10 to 20 MHz and from 15 to 30 MHz, respectively.

S2 Contact angle measurement of sample droplets.

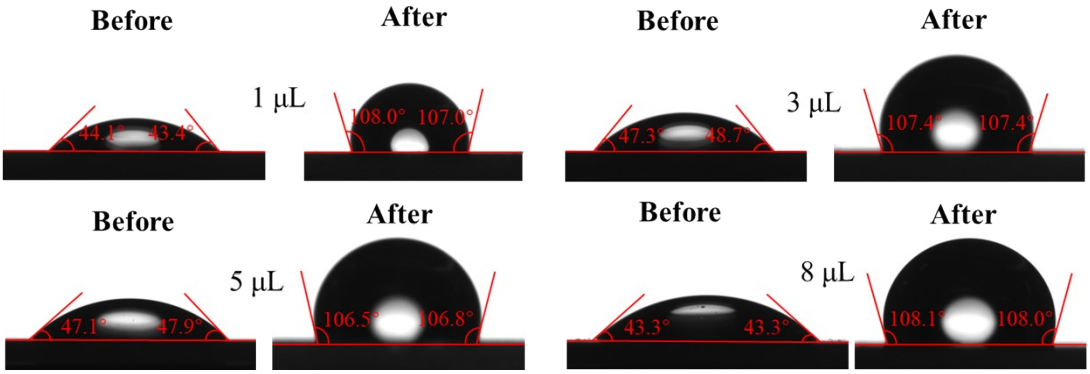


Fig. S2 Measured contact angle of different volumes of deionized water droplets before and after surface treatment.

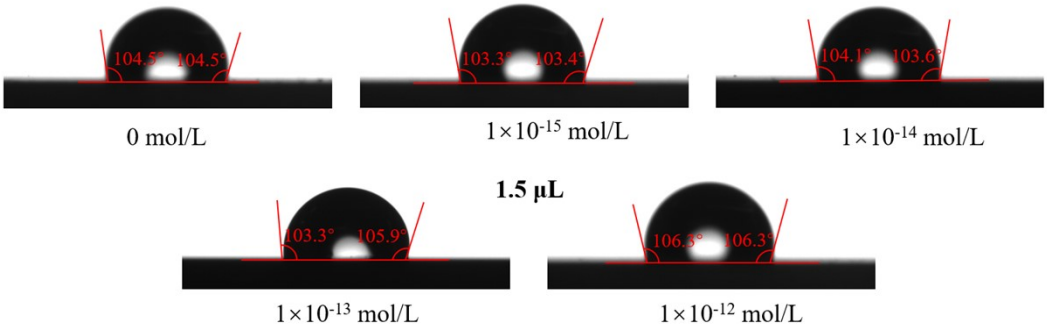
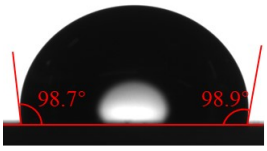


Fig. S3 Measured contact angle of sample droplets of 1.5 µL containing different concentrations of targets (miRNA biomarkers).



15 µL

Fig. S4 Measured contact angle of a 15 µL sample droplet containing a mixture.

S3 Details on the miRNA detection.

S3.1 The principle of ISAR for miRNA detection.

The stem of the designed hairpin-DNA (hDNA) is labeled with BHQ1 and Fam as molecular beacons probe. There was no Fam fluorescence signal while the hDNA was at the hairpin structure. The hDNA is designed to be complementary with miRNA-21 targets. When there is a positive sample, the miRNA-21 could open the hDNA due to Watson-Crick base pairing. Then, the hairpin structure of hDNA is destroyed to produce Fam fluorescence. Meanwhile, the primer could hybridize with the stem of the opened hDNA and extend with the help of Klenow polymerase, replacing and setting free the miRNA-21. By cyclic reaction, the ISAR transforms one target miRNA into an abundance of opened molecular beacons hDNA probe, which amplifies the Fam fluorescence signal at constant temperature and effectively bypasses the requirement of the thermal cycler. More miRNA targets could accelerate ISAR to destroy more hDNA and produce more dsDNAs for multiple magnifications of the Fam fluorescence signal.

S3.2 The preparation process for miRNA detection.

(1) The sequence is set and placed at -20 ℃.

(2) Take **hDNA**, **primer**, and **target** according to the requirements on the wall of the tube to add the corresponding volume of ddH_2O configured into 100 µmol/L (100 µM) solution.

(3) Dissolve 10 µL of liquid from the original **hDNA** tube into 90 µL of **1×PBS** (10 µM), heating both tubes for 10 minutes using a metal bath at 95 ℃, and then cool down stepwise to ambient temperature for use.

(4) The primer and target to take 10 µL liquid dissolved into 90 µL of **1×PBS** configured to 0 µM.

(5) Take 10 µL target (10 µM prepared above) tenfold gradient ddH_2O diluted into four groups of 1 pM, 100 fM,10 fM, and 1 fM.

(6) The above table five groups of experiments each tube volume is 20 uL, 37 ℃ reaction 1 h.

(7) Target and mixture solution volumes should be extracted in equal proportions (1:10).

Table S1 The volume of the targets and analytes.

Amplification reactions triggered by different concentrations of target	1	2 (1 fM)	3 (10 fM)	4 (100 fM)	5 (1 pM)
Target (miRNA)	0 (uL)	2(uL)	2(uL)	2(uL)	2(uL)
hDNA (10uM)	5	5	5	5	5
primer (10uM)	5	5	5	5	5
PBS(1x)	7	5	5	5	5
klenow polymerase	1	1	1	1	1
Dntp (10mM)	2	2	2	2	2
Klenow buffer (10x)	2	2	2	2	2

S4 Continuously evaporation monitoring of biological sample droplet.

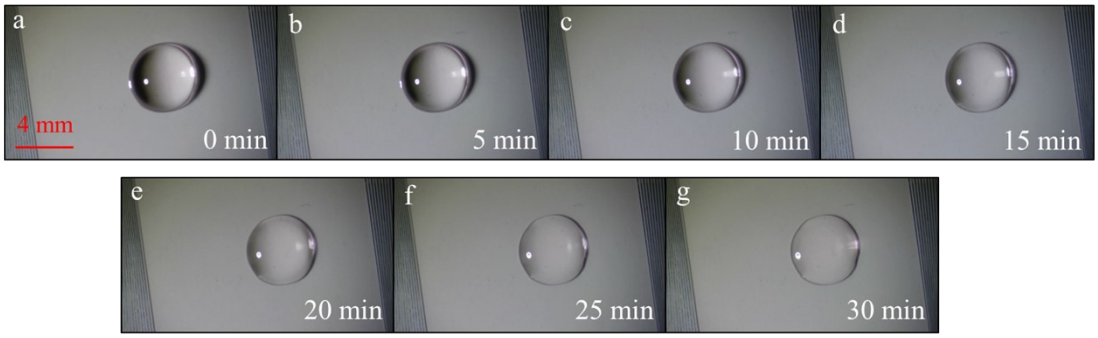


Fig. S5 Continuous monitoring of biological sample droplet volume (initial 16.5 μL) over a period of 30 min. The area of the droplet did not change significantly. Still, the droplet height had visibly decreased, indicative of a reduction in droplet volume, which does not affect fluorescence detection in any case.

S5 Droplet temperature change during the acoustic manipulation (mimicking the process of miRNA detection)

Experimental steps and results for measuring droplet temperature (N≥3):

(1) The starting room temperature of the droplet was about 23.7℃, which then increased to 27.76℃ after TSAW treatment for 40 s.

(2) Turning off the TSAW for 5 min, the temperature decreases back to room temperature. Then turning on the TSAW treatment for about 20 s, the temperature increases again.

(3) Step (2) was then repeated six times at an interval of 5 min (i.e., a total time of 30 min. The minimum and maximum temperatures always stayed within the range between 23.56 ℃ and 27.76℃.

As shown in Fig. S6, we have characterized the temperature change on the droplet during acoustic manipulation (mimicking the process of miRNA detection).

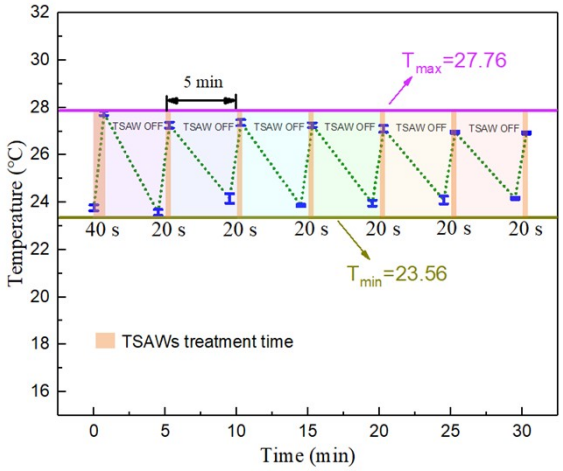


Fig. S6 Temperature changes on the droplet during acoustic manipulation (mimicking the process of miRNA detection).

According to the literature[1,2], both the hDNA probe and miRNA are highly temperature-stable, and the used enzyme is inactivated only when the temperature is higher than 65℃. **Therefore, the thermal effect of the OTAT does not affect the detection sensitivity of miRNA biomarkers.**

S6 Comparison of droplet microfluidic technologies for miRNA detection.

Table S2 A comprehensive comparison of recently developed droplet microfluidic technologies for miRNA detection.

Methods or design	Turnaround time	Detection sensitivity	Limit of detection	Linear range	Year
Water-in-oil droplets with continuous-flow microfluidic[3]	＞40-60 min	Low	1 nM	/	2018
Renewable superwettable miRNA biochip[4]	100 min	Average	88 pM	0.1~50 nM	2018
Microfluidic multi-color fluorescence droplets[5]	90 min	Average	36~39 pM	0.1 nM~1 μM	2019
Fluorescence and SERS dual-response droplet microfluidic[6]	Several hours	Average	10 pM	10 pM~0.1 μM	2022
This work (OTAT)	**30 min**	**High**	**0.1 pM**	**/**	**2023**

S7 Proposed protective measures against sample droplet evaporation

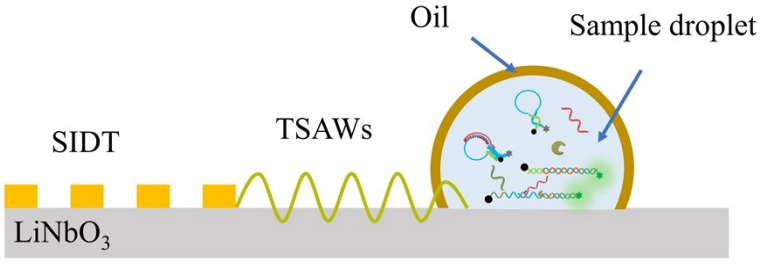


Fig. S7 Schematic diagram of proposed protective measures against sample droplet evaporation.

S8 Discussion on the capabilities of OTAT for multi-step operations

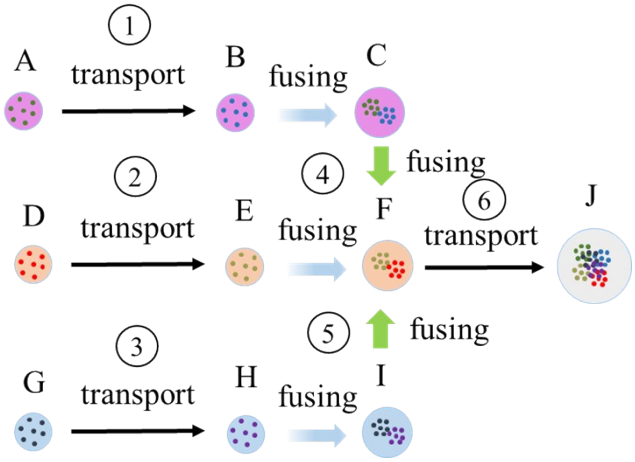


Fig. S8 Schematic diagram of multi-step manipulation of multiple droplets.

As demonstrated in Fig.6 and 7, the OTAT performs a wide range of droplet manipulation capabilities, such as unidirectional transportation, multi-direction transportation, round-trip transport, tilt angle movement, and multi-droplet transportation and fusion. In addition to microRNA detection, many bioassays require the complex multi-step manipulation capabilities of microfluidic devices, such as high throughput and multiple sample reactions. As schematized in Fig. S8, multi-step manipulation of multiple droplets is divided into several steps, including 2D transportation and 2D fusion for multiple droplets. Interestingly, these steps have already been validated by the developed OTAT. The other capabilities of OTAT for simultaneous μ-droplet centrifugation contribute to improving the detection accuracy and sensitivity of analytes. Therefore, the proposed OTAT with multifunctional complex droplet manipulation capability is expected to be widely used in point-of-care disease diagnosis. To accurately transport droplets to designated areas for any droplet in each step, we could quickly adjust the excitation frequency to correct the droplet transport trajectory for a single SIDT. Alternatively, we also could modify the droplet trajectory through the interaction of multiple SIDTs.

References:

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