Electronic Supplementary Information (ESI) for:

A thin-reflector microfluidic resonator for continuous-flow concentration of microorganisms: a new approach to water quality analysis using acoustofluidics

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S1 Bacteria culturing and sample preparation

S1.1 *Escherichia coli*

E. coli is a Gram-negative rod-shaped bacterium, approximately 2.0 μm long and 0.5 μm in diameter,[2] with an average cell volume of 0.6–0.7 μm^3 which is equivalent to a spherical particle of 1.05-1.10 μm diameter.

Bacteria were routinely cultured on sterile lysogeny broth (LB) agar plates (Bio-Rad Laboratories, Inc., USA) and were incubated at 37°C and 5% CO_2 in air with 95% humidity. Sample preparation was carried out by picking a single colony of *E. coli K12* from the culture plate by using a sterile inoculation loop (1 μL, Fisher Scientific Ltd., USA), and immersing the loop into a centrifuge tube (50 ml, Fisher Scientific Ltd., USA) filled with 30 ml of LB broth (Sigma Aldrich Corporation, USA). The bacterial culture was subsequently incubated for ~14 hr in an incubator.

For bacteria detection and counting, *E. coli* were fluorescently stained with a bacterial viability kit (LIVE/DEAD® *Bac*Light™, Invitrogen Corporation, USA), a rapid staining method for direct enumeration of viable and total bacteria in drinking water.[3] Briefly, the bacterial culture was centrifuged at 3000 rpm, for 3 min at 20°C. The supernatant was aspirated with a micropipette and replaced with a solution of 5 mM SYTO9 in Phospate Buffered Saline (PBS) (2:1000 by volume). Bacteria were incubated at 37°C for 25 min, followed by centrifugation and washing with fresh PBS. The washed bacterial pellet was resuspended in PBS at a concentration of ~2×10^4 CFU/ml. SYTO9 stained the nucleic acid of all bacteria in the suspension, and produced a green fluorescence.

S1.2 *Staphylococcus epidermidis*

S. epidermidis is a Gram-positive spherical-shaped bacterium (referred to as coccus). The average diameter is in the range 0.5–1.5 μm,[4] with an average cell volume of 0.52 μm^3.

Sample preparation was carried out as follows. Firstly, a 15 ml capacity falcon tube containing 8 ml of Tryptic Soy Broth (TSB) (Sigma Aldrich, UK) was inoculated with a single colony of *S. epidermidis ATCC 12228*, and grown over night (~14 hr) at 37°C.

As with *E. coli*, bacteria were fluorescently stained with using a cell viability kit (LIVE/DEAD® *Bac*Light™, Invitrogen Corporation, USA). For this purpose, the bacterial culture was centrifuged at 3000 rpm, for 3 min at 20°C. The supernatant was aspirated with a micropipette and replaced with a solution of 5 mM SYTO9 in Phospate Buffered Saline (PBS) (2:1000 by volume). Bacteria were incubated at 37°C for 25 min, followed by centrifugation and washing with fresh PBS. The washed bacterial pellet was resuspended in PBS at a concentration of ~2×10^4 CFU/ml.

S2 Device Priming

In order to prevent bubbles formation during device priming, sequential flushing cycles of 100% CO_2 and ultrapure water (Milli-Q, EMD Millipore Corporation, USA) were performed. After each cycle, any remaining CO_2 dissolves in the water. Multiple cycles are required to ensure that any original gases are eliminated. On average 3 and 6 cycles were required for HW and TR resonators, respectively. 100% CO_2 was manually injected into the device using a 20 ml plastic syringe (Terumo Corporation, Japan) at an estimated average injection pressure and flow rate of ~11 kPa and ~0.75 ml/s, respectively. In order to ensure that all parts of the device were flushed with CO_2, each individual outlet line was sequentially opened whilst the others remained closed.

S3 Stabilisation of the reflector layer in TR devices

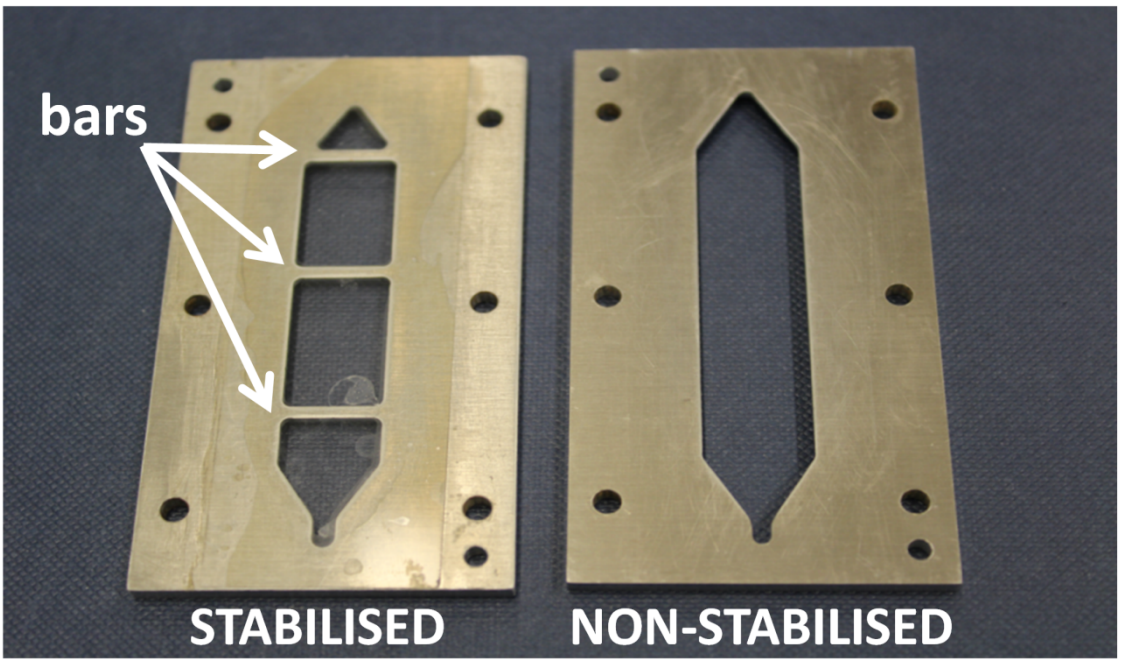

Fig. S3 Stabilisation of the reflector layer in TR devices. In order to counteract potential bending of the reflector layer in TR devices, three transversal bars (white arrow) were added to the metal frame (left). Bars were ~1.5 mm wide, and crossed the entire width of the fluidic chamber. A bars-free metal frame is reported on the right, which corresponded to a non-stabilised reflector layer. Circular holes have been milled in the frame, allowing for alignment with the other layers of the device.

References

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