

A Droplet-Based Microfluidic Impedance Flow Cytometer for Detection and Quantification of Microplastics in Water

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Standard photolithography was used to create the electrode patterns. The mask made in AutoCAD 2022, and CAD/Art Services, Inc. printed it (Figure S1). First, a glass wafer with gold sputtered on top was coated with a layer of positive photoresist, S1813 (The Dow Chemical Company), using a spin coater (Laurell Technologies). The substrate was then placed in a mask aligner (OAI model 804), where it came into direct contact with a photolithography mask and was exposed to UV light for 12 seconds (150 mJ/cm²). Afterwards, the chip was submerged in a photoresist developer (MF-319 developer, Kayaku Advanced Materials, Inc.) to remove any remaining unexposed photoresists. Then, the excess gold and chromium were etched away using gold and chromium etchants (Sigma-Aldrich). Next, a photoresist remover (MICROPOSIT® REMOVER1165, Kayaku Advanced Materials, Inc.) was used to get rid of any leftover traces of the photoresist. The chip was then cleaned by rinsing it with DI water and drying it with compressed air. For channel fabrication, initially, a 3-inch silicon wafer (WaferPro) was spin-coated with SU-8 3025 in a two-step coating process. First, the substrate was spun at 500 rpm for 10 seconds and then at 1800 rpm for 45 seconds to achieve a height of 40 µm. The wafer was then soft-baked for 15 minutes at 95 degrees Celsius. The next step was to expose the wafer to UV light beneath a photolithography mask (CAD/Art Services) for 16 seconds (212 mJ/cm²), to crosslink the SU8 to the desired pattern on the chip. The size of the channel was 60×40 µm.

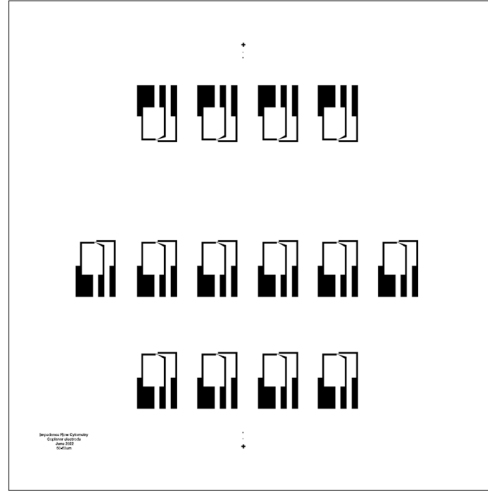


Figure S1. The photomask that was used for electrode fabrication.

Table S1-The full factorial design of the experiment.

EXPERIMENT	EXP 01	EXP 02	EXP 03	EXP 04	EXP 05	EXP 06	EXP 07	EXP 08
SIZE	3 μm	0.5 μm	1 μm	3 μm	3 μm	0.5 μm	1 μm	6 μm
CONCENTRATION	0.10%	0.05%	0.10%	0.20%	0.20%	0.10%	0.20%	0.20%
EXPERIMENT	EXP 09	EXP 10	EXP 11	EXP 12	EXP 13	EXP 14	EXP 15	EXP 16
SIZE	0.5 μm	6 μm	1 μm	6 μm	0.5 μm	3 μm	6 μm	3 μm
CONCENTRATION	0.20%	0.10%	0.05%	0.20%	0.20%	0.10%	0.10%	0.05%
EXPERIMENT	EXP 17	EXP 18	EXP 19	EXP 20	EXP 21	EXP 22	EXP 23	EXP 24
SIZE	6 μm	1 μm	1 μm	0.5 μm	1 μm	6 μm	0.5 μm	3 μm
CONCENTRATION	0.05%	0.05%	0.20%	0.05%	0.10%	0.05%	0.10%	0.05%

Each droplet produces two peaks in the signal read by the lock-in amplifier. The output signal is at its baseline before a droplet enters the electric field (state 1 in Figure S2). The first peak appears as soon as the droplet enters the detecting zone. The signal reaches its maximum when the droplet completely covers the initial gap between the excitation and sensing electrodes (state 2 in Figure S2). When a droplet enters the second gap between the excitation and sensing electrodes, a signal with the opposite sign of the first peak is produced (state 3 in Figure S2). As a result, the second peak will be about the same height as the first, but in the opposite direction. The prominence of the positive peak was extracted for each frequency component.

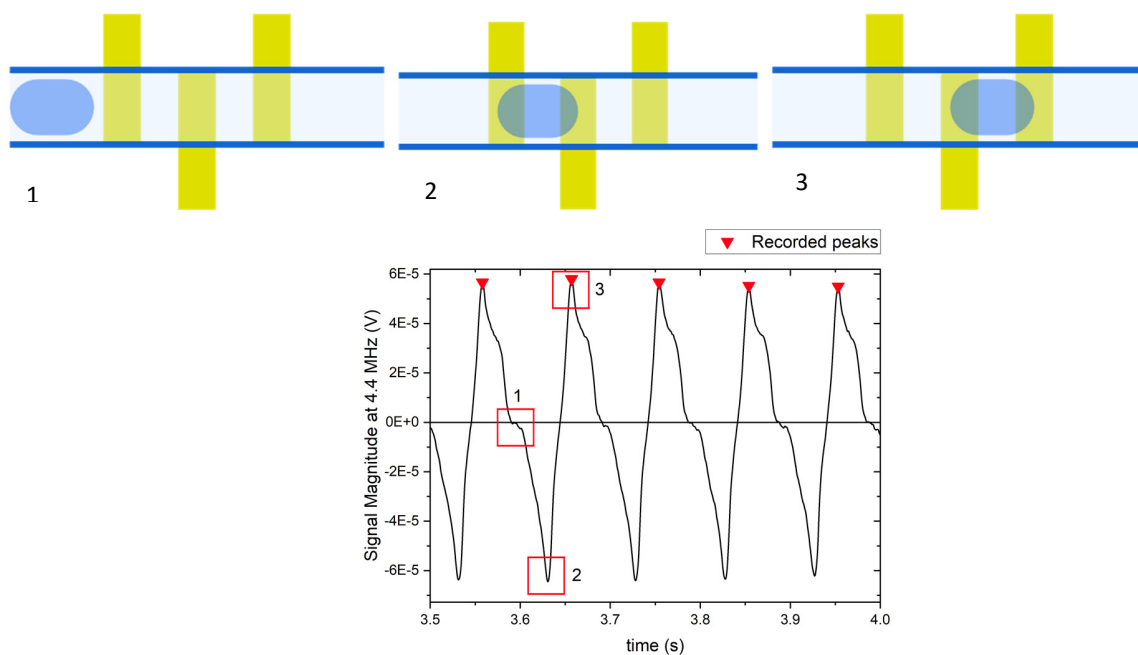
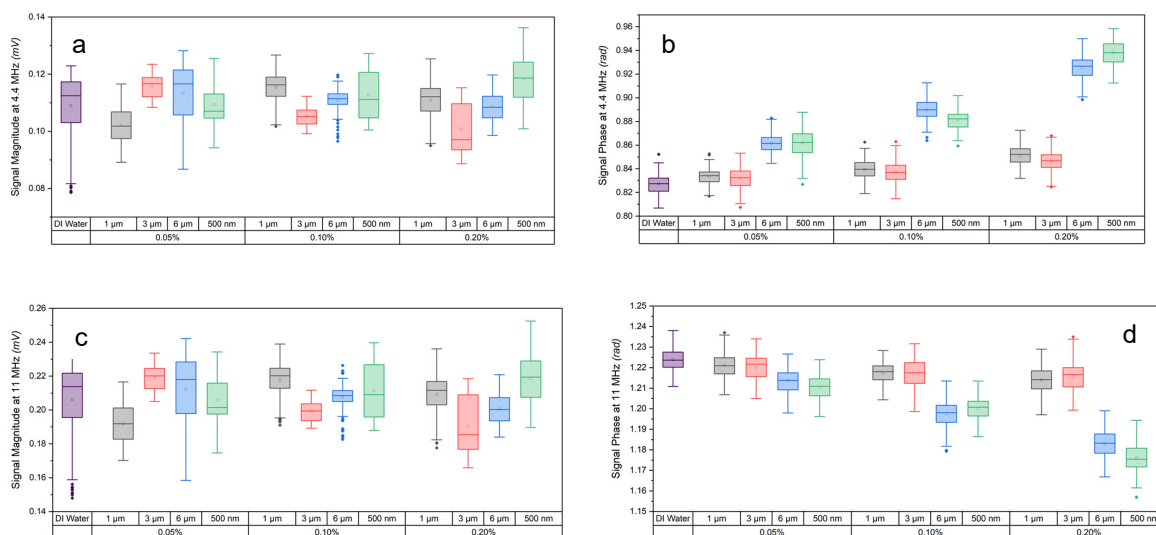


Figure S2. The corresponding signal measured for frequency of 4.4 MHz.

The box plots of all the data were then shown at each frequency to visually compare the data statistics for each size and concentration (Figures 3Sa-f).



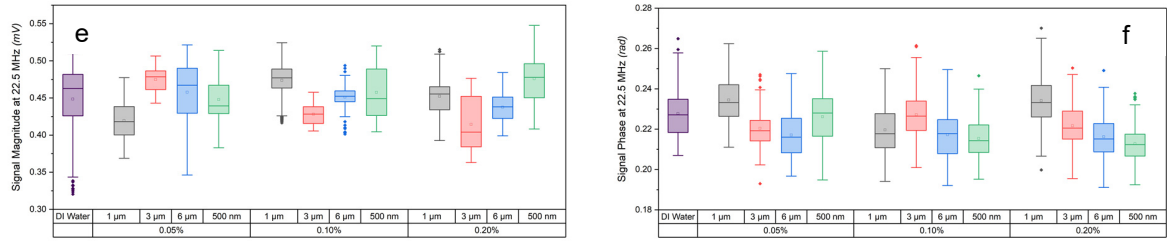


Figure S3. The box and whisker plot of the measurements at 4.4 MHz, a) The signal magnitude, b) The signal Phase, The box and whisker plot of the measurements at 11 MHz, c) The signal magnitude, d) The signal Phase, The box and whisker plot of the measurements at 22.5 MHz, e) The signal magnitude, f) The signal phase.