Supplementary Material

High Efficiency Sperm Enrichment from Forensic Mock Samples in Bubble-based Acoustic Filtration Devices for Short Tandem Repeat (STR) Analysis

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1. Bubble-based Acoustic Streaming Using CFD Simulation

In order to gain insights into the flow profiles of the acoustic streaming due to the bubble oscillations in our device, computational fluid dynamics (CFD) simulations are undertaken using COMSOL 5.6 (Comsol AB, Burlington, MA) to harness the acoustic streaming effect. A two-dimensional (2D) finite element model is constructed, in accordance with the thermoacoustics, laminar flow module. The parameters used in the simulation are listed in **Table S1**. This module is governed by the Navier-Stokes equations and applies a no-slip boundary condition, with the assumption of incompressible flow. Iterative optimization of the channel design is pursued by modifying the geometric variables.

Table S1: The parameters used in the COMSOL simulation.

Parameter name	Parameter value
Width of membrane, W_m	200[um]
Pore, p	7[um]
Bubble radius, r	250[um], 25[um]
Study frequency, f_0	15.5[kHz], 155[kHz]
Ambient temperature, T_0	298.15[K]
Ambient pressure, P_0	101.33[kPa]
Speed of sound in water, c_0	1495.3[m/s]
Wall displacement, d_0	100[nm]
Particle radius, a	15[um]
Particle density, *rho*	1050[kg/m^3]

The simulation results, as illustrated in **FIG. S1**, narrate the distinct particle trajectories for the designs outlined in **Table 1**, each influenced by an oscillating bubble meniscus that generates flow streaming. This visualization allows for an evaluation of flow uniformity and the likelihood of flow separation in each design scenario.

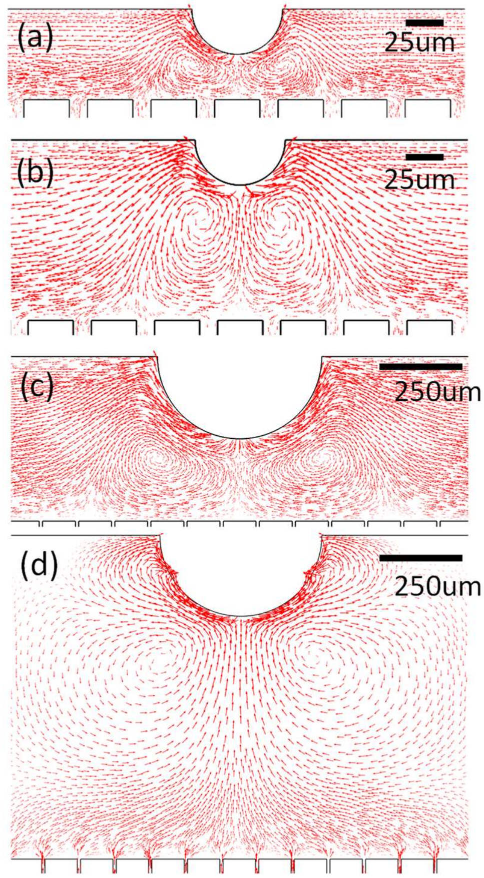

FIG. S1: CFD simulation illustrating the in-plane vortex as a result of acoustically-excited bubbles from the x-y plane (top view) with (a) Design 1 (b) Design 2 (c) Design 3, and (d) Design 4.

Design 1 (W_f=50μm, W_{br}=50μm) shows potential uniformity issues and a high risk of flow separation due to densely packed streamlines and significant velocity changes. Design 2 (W_f=100μm, W_{br}=50μm) offers some improvement with more even flow, though challenges persist with flow separation. A substantial enhancement is observed in Design 3 (W_f=500μm, W_{br}=500μm), where smoother flow and reduced separation risks are evident. The most significant improvement is seen in Design 4 (W_f=1000μm, W_{br}=500μm), which facilitates the most uniform flow and the least likelihood of flow separation. Across these designs, it becomes apparent that wider channels tend to minimize the disruptive effects of oscillations, maintaining more stable and uniform flow compared to narrower configurations.

2. Grayscale Intensity Analysis

We utilized image analysis technique to quantitatively analyze the acoustic streaming effect on fouling removal. The region of interest (ROI, shown in yellow) in **FIG. S2** was defined based on the highest y-value of the connected pixels in the binary image as the upper boundary, while the lower boundary of the ROI was set as the top of the filter. The average grayscale intensity within the selected ROI was used to quantify particle transport during cross-flow filtration. The Grayscale Intensity Normalization was calculated using the following formula:

$$Grayscale\ Intensity\ Normalization\ (a.u.) = \frac{G_{background} - G_{cleaning}}{G_{background} - G_{foulingformat}} \quad \text{(S.1)}$$

When $G_{cleaning}$ = $G_{foulingformat}$, the maximum value is 1. When $G_{cleaning}$ = $G_{background}$, the minimum value is 0.

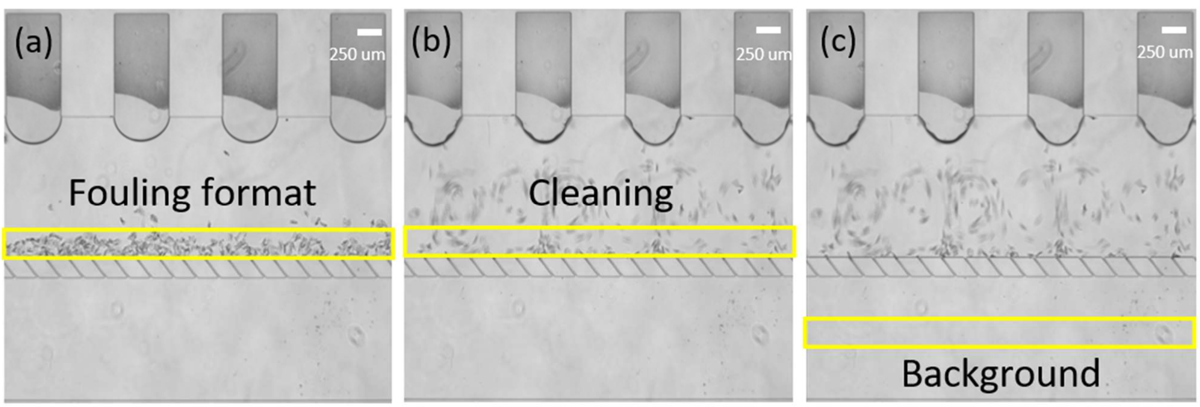

FIG. S2: Grayscale intensity sample regions and process. (a) The position of the region of interest (ROI) during fouling formation. (b) The position of the ROI during fouling cleaning process. (c) The location of the background ROI with minimal changes before and after the process.