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

Electronic Supplementary Information (ESI) for

Behaviour responses of zebrafish with sound stimuli in microfluidics

By

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This supporting information file includes:

1. Supplementary Figures S1 and S3
2. Legend for Supplementary Videos S1 to S3

1. Supplementary Figures

Figure S1

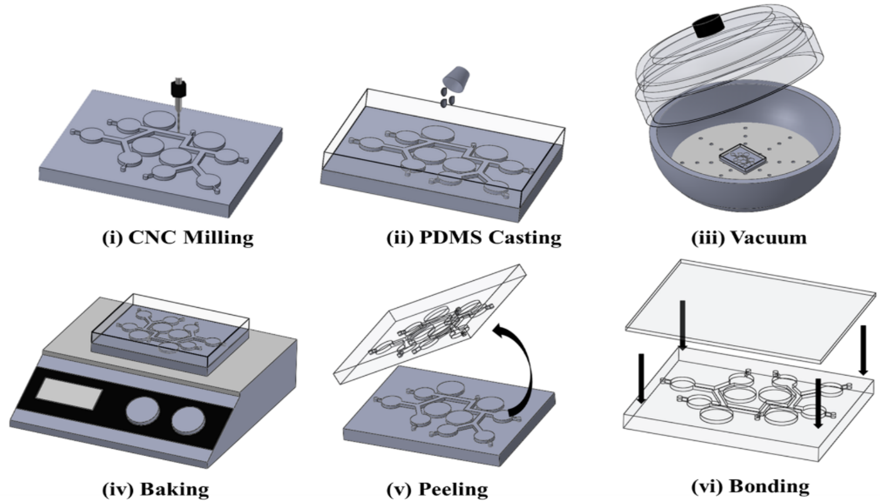

Fig. S1 Schematic illustration of various stages (from i to vi) involved in the fabrication of the proposed microfluidic chip. (i) The design outline of the chip was engraved on an acrylic substrate using the CNC micromachining technique. (ii) A mixture of PDMS and curing agent in a ratio (10:1) was evenly mixed and poured for casting the microfluidic chip. (iii) Degassing process of the casted microfluidic chip in a vacuum chamber. (iv) The fabricated microfluidic chip was then incubated on a hot plate for curing. (v) The PDMS microfluidic chip channel was separated from the mold by peeling, and the inlet & outlet sections were punched through a biopsy puncher. (vi) In order to avoid fluid leakage, the microfluidic chip was finally bonded with another PDMS layer through an oxygen plasma process.

Figure S2

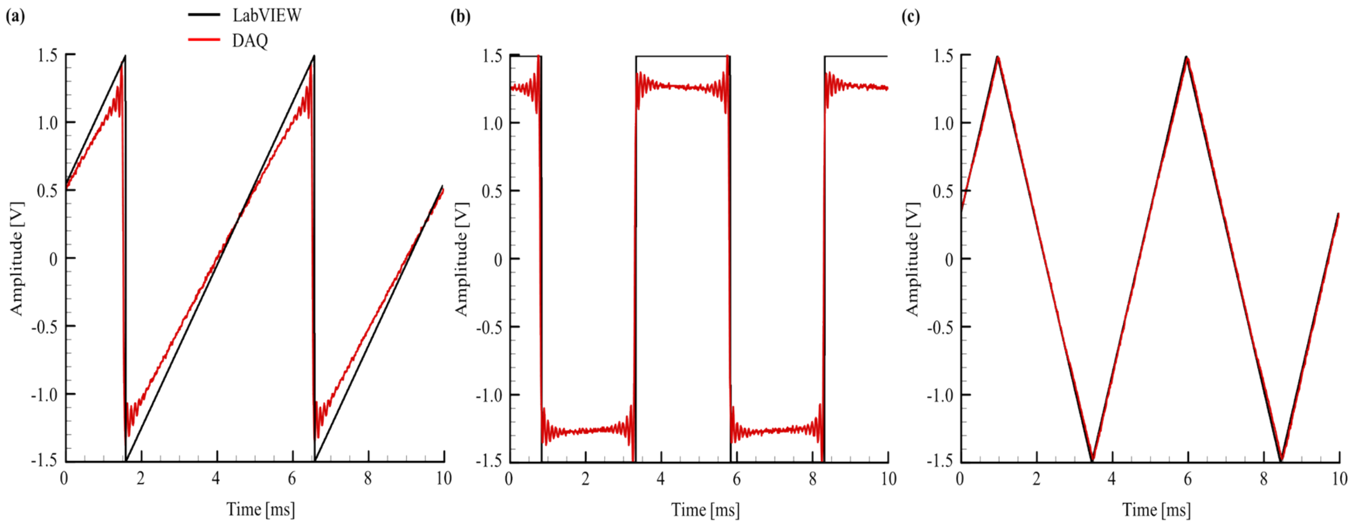

Fig. S2 Comparison of the time-domain signals simulated by Labview and measured by DAC (data acquisition device) in three representative waveforms including the sawtooth wave (a), the square wave (b). and the triangle wave (c). The difference between the Labview and DAC methods was significantly less in the triangular waveform test. Therefore, this waveform was employed to the transducers for generating significant sound pressure level in the present study.

Figure S3

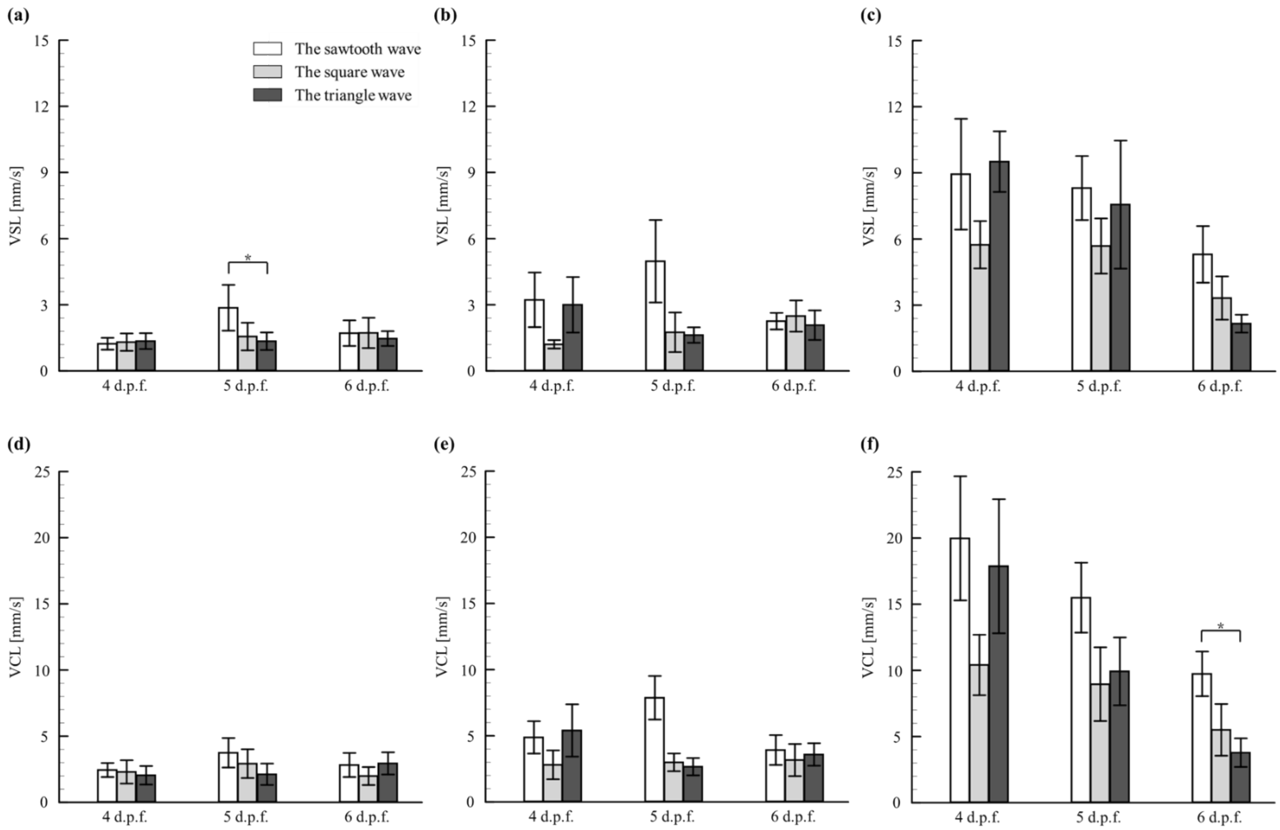

Fig. S3 Sound stimuli with different waveforms and frequencies to induce distinct swimming behaviors in zebrafish larvae as a result of escape response. (a), (b), and (c) corresponds to the straight-line velocity (VSL) at 200, 600, and 1200 Hz, respectively. (d), (e), and (f) corresponds to the curvilinear velocity (VCL) at 200, 600, and 1200 Hz, respectively. The data represented as mean ± SD (N=20) in quantifying the swimming behaviour are obtained from three independent experiments. The statistical significance of larvae swimming parameters corresponding to the frequency was further determined using the one way ANOVA Scheffe method, $^*P \leq 0.05$.

2. Description of Supplementary Videos

Video S1: Larval motion under the influence of acoustic stimuli. To elicit the stimuli avoidance behavior, the transducer placed at the top-right corner was energized (ON). Consequently, there was a startle zone developed around the transducer. As a result, the C-start reflex was initiated as one of the prominent avoidance behaviors. Eventually, the larvae were observed to change their flow path and successfully reach the target section. Such an experimental paradigm was accentuated to test the spatial memory features of zebrafish larvae under acoustic stimulation.

Video S2: Representation of innate C-start reflex. Zebrafish larvae for 4 d.p.f. were placed in the chamber near the transducer inside the microfluidic platform. To demonstrate the C-start reflex, the transducer was operated at 1200 Hz. Eventually, the mentioned escape behavior was initiated at 8 ms and attained the maximum C-bend at 12 ms. In order to assist the reader in perceiving the clear escape behavior, the movie shown here was recorded in slow-motion mode.

Video S3. Demonstration of conditioning session in the long path. Transducers employed in the microfluidic platform were manipulated to establish the accusation of spatial memory under the complex environment (long path). Furthermore, the target section was adequately chosen to quantify the spatial memory features under the long path.