

## Surface acoustic wave-driven pumpless flow for sperm rheotaxis analysis

Junyang Gai<sup>1</sup>, Citsabehsan Devendran<sup>1</sup>, Adrian Neild<sup>1,\*</sup>, and Reza Nosrati<sup>1,\*</sup>

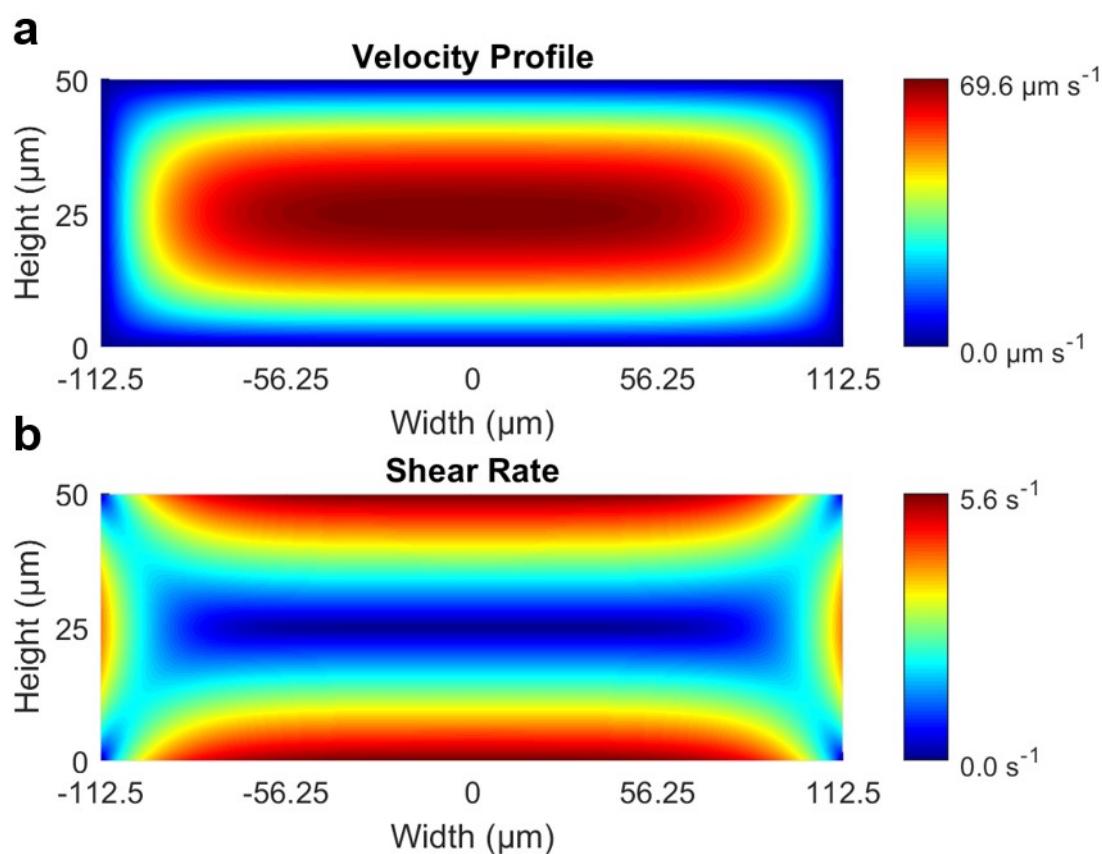
<sup>1</sup> Department of Mechanical and Aerospace Engineering, Monash University, Clayton, Victoria 3800, Australia

\* Corresponding authors:

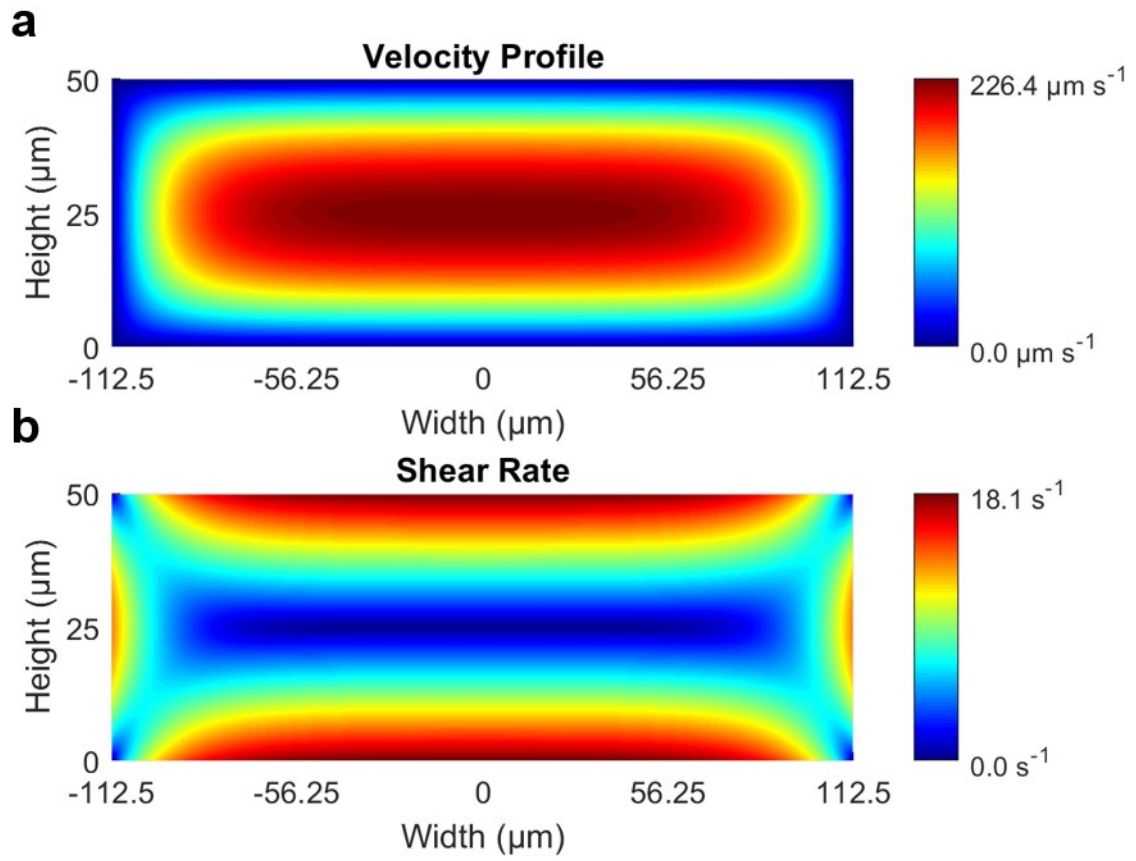
Department of Mechanical and Aerospace Engineering, Monash University, Clayton, Victoria 3800, Australia

Emails: [Reza.Nosrati@monash.edu](mailto:Reza.Nosrati@monash.edu) (R.N.); [Adrian.Neild@monash.edu](mailto:Adrian.Neild@monash.edu) (A.N.)

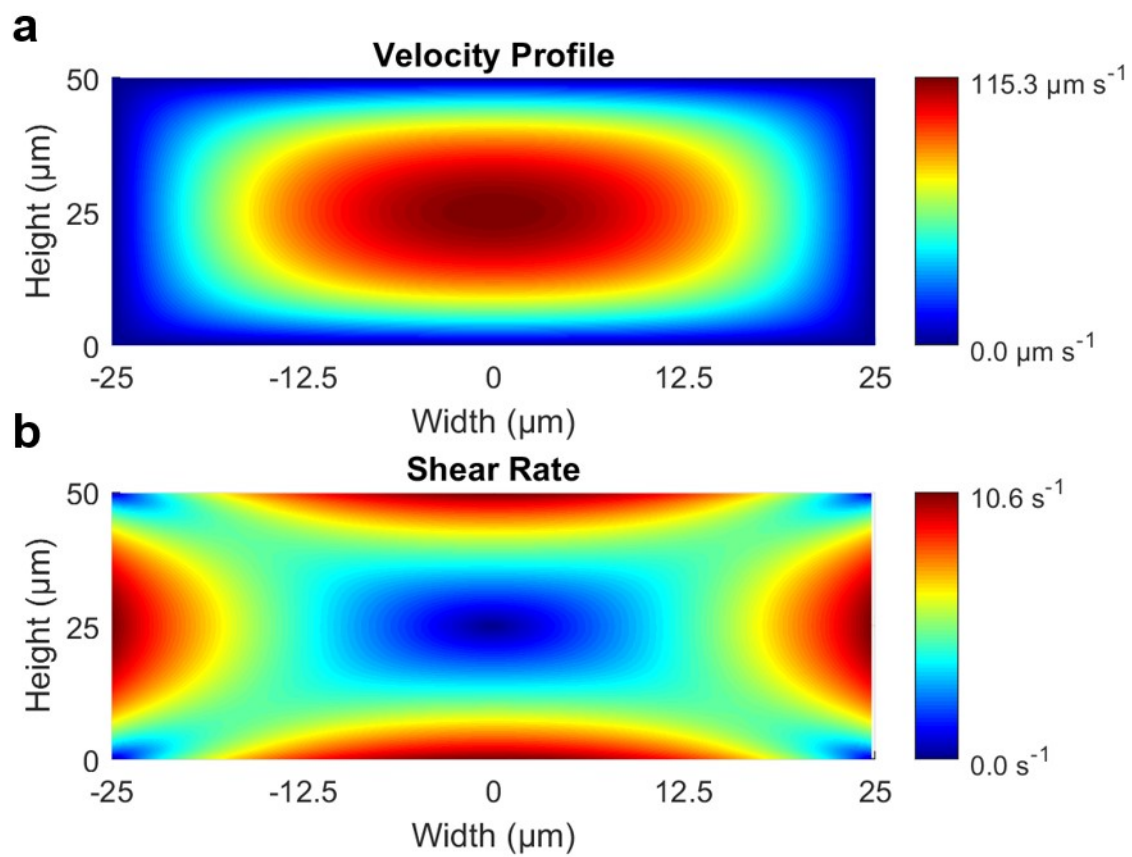
Phone numbers: +61 3 990 53627; +61 3 990 54655



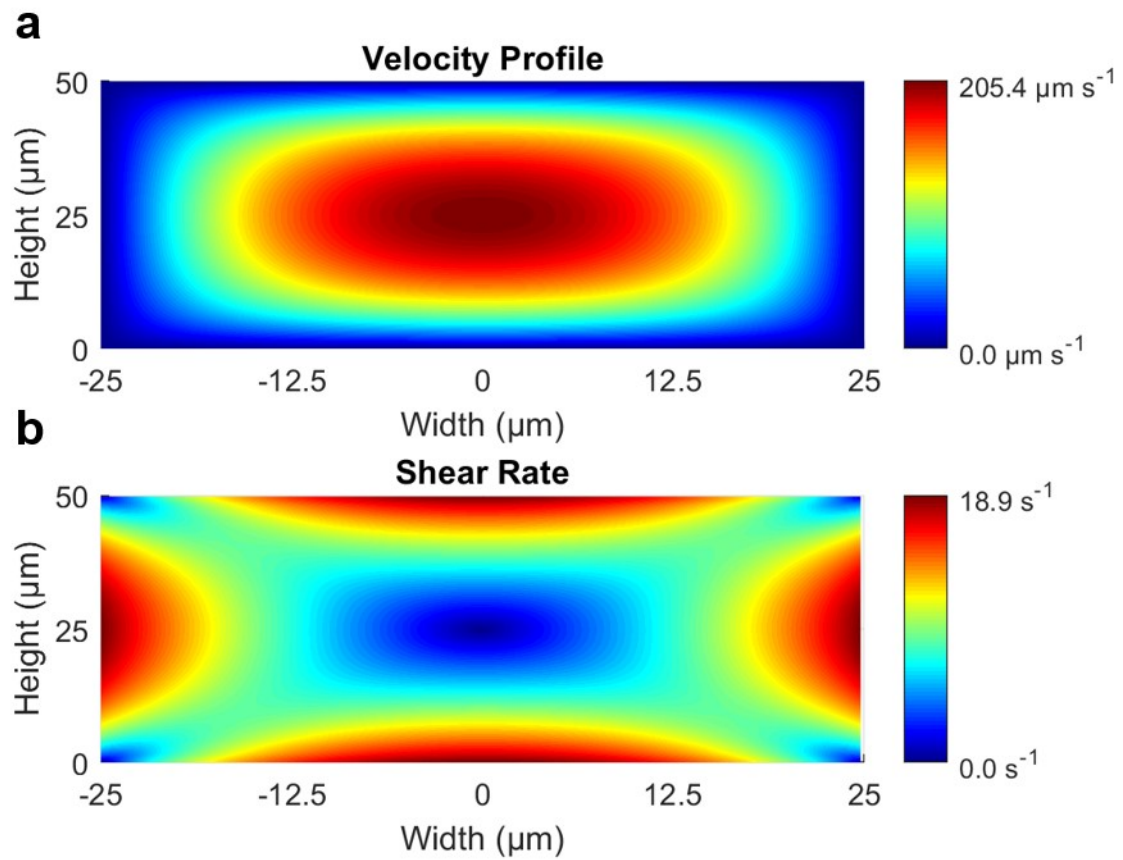
**Figure S1.** (a) Flow velocity and (b) shear rate profiles in the cross-sectional area of 225 μm microchannels at the average velocity of 40 μm s<sup>-1</sup>.



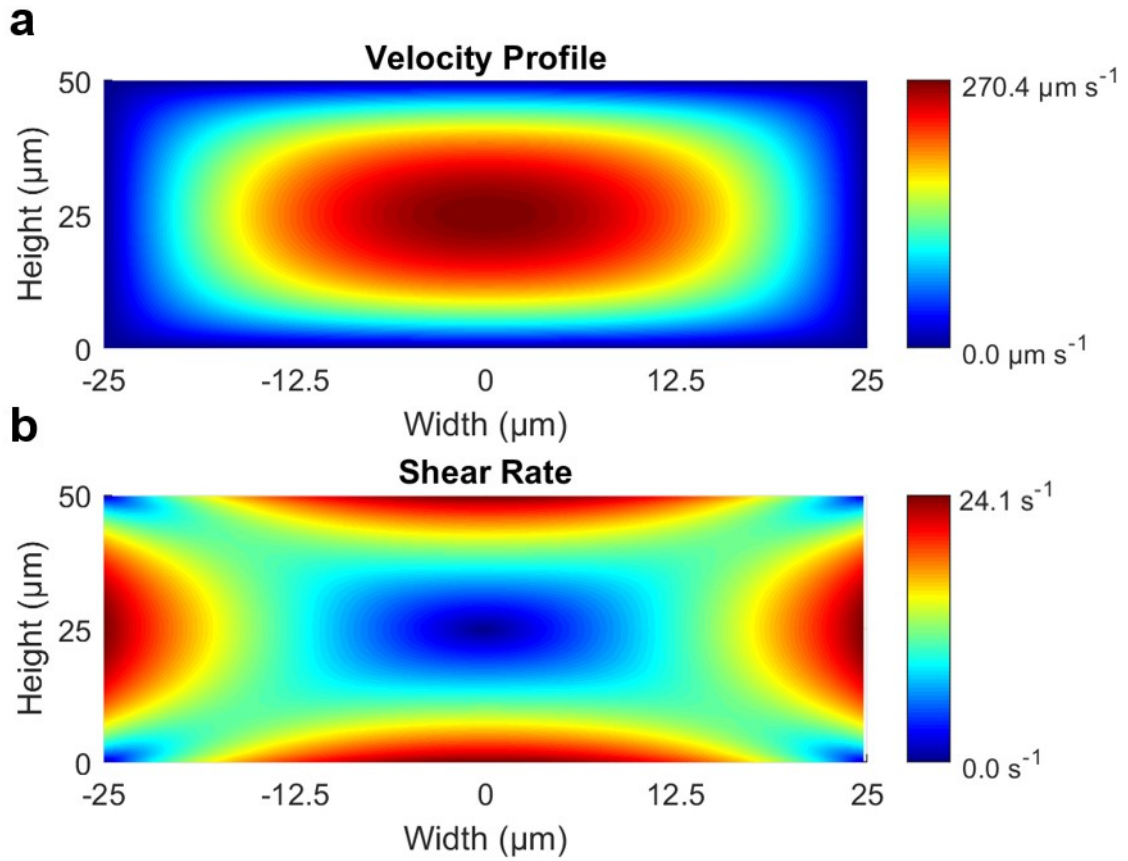
**Figure S2.** (a) Flow velocity and (b) shear rate profiles in the cross-sectional area of 225  $\mu\text{m}$  microchannels at the average velocity of  $130\mu\text{m s}^{-1}$ .



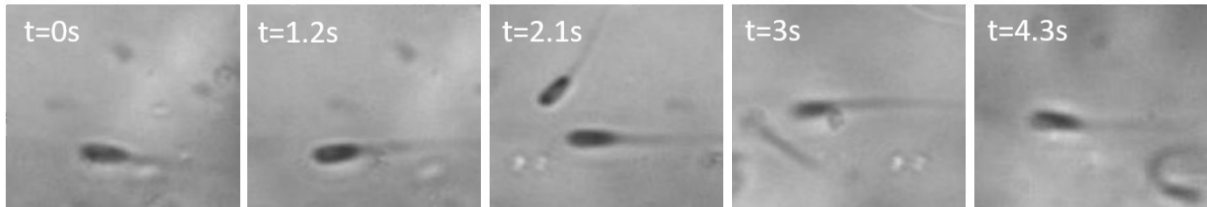
**Figure S3.** (a) Flow velocity and (b) shear rate profiles in the cross-sectional area of 50  $\mu\text{m}$  microchannels at the average velocity of 55  $\mu\text{m s}^{-1}$ .



**Figure S4.** (a) Flow velocity and (b) shear rate profiles in the cross-sectional area of 50  $\mu\text{m}$  microchannels at the average velocity of  $98\mu\text{m s}^{-1}$ .



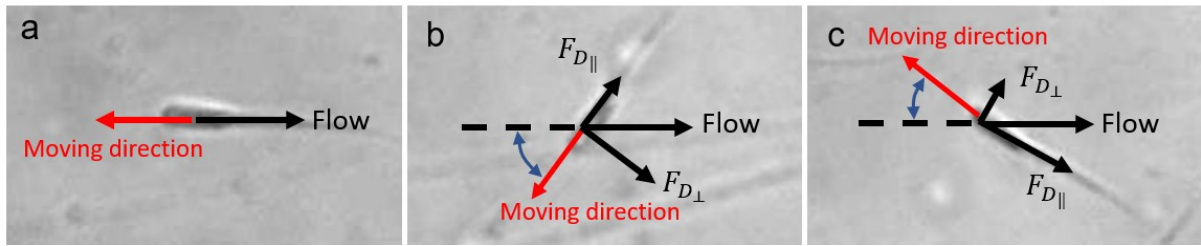
**Figure S5.** (a) Flow velocity and (b) shear rate profiles in the cross-sectional area of 50  $\mu\text{m}$  microchannels at the average velocity of 128  $\mu\text{m s}^{-1}$ .



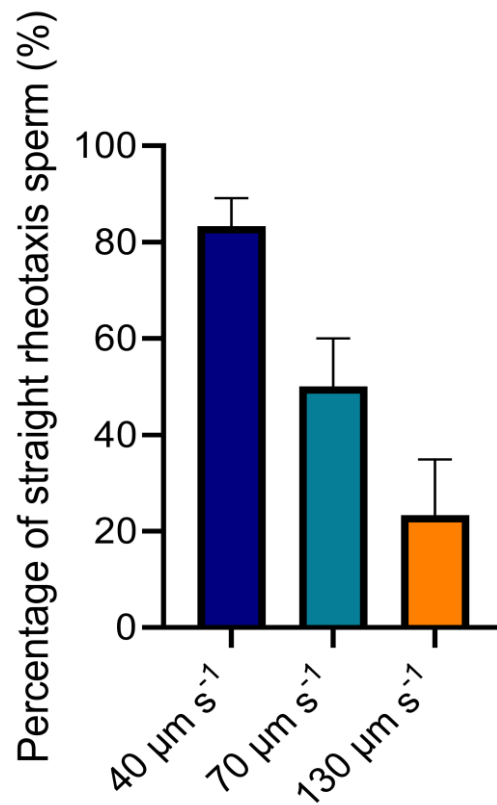
**Figure S6.** Representative time-lapsed images of sperm oriented parallel to the flow.



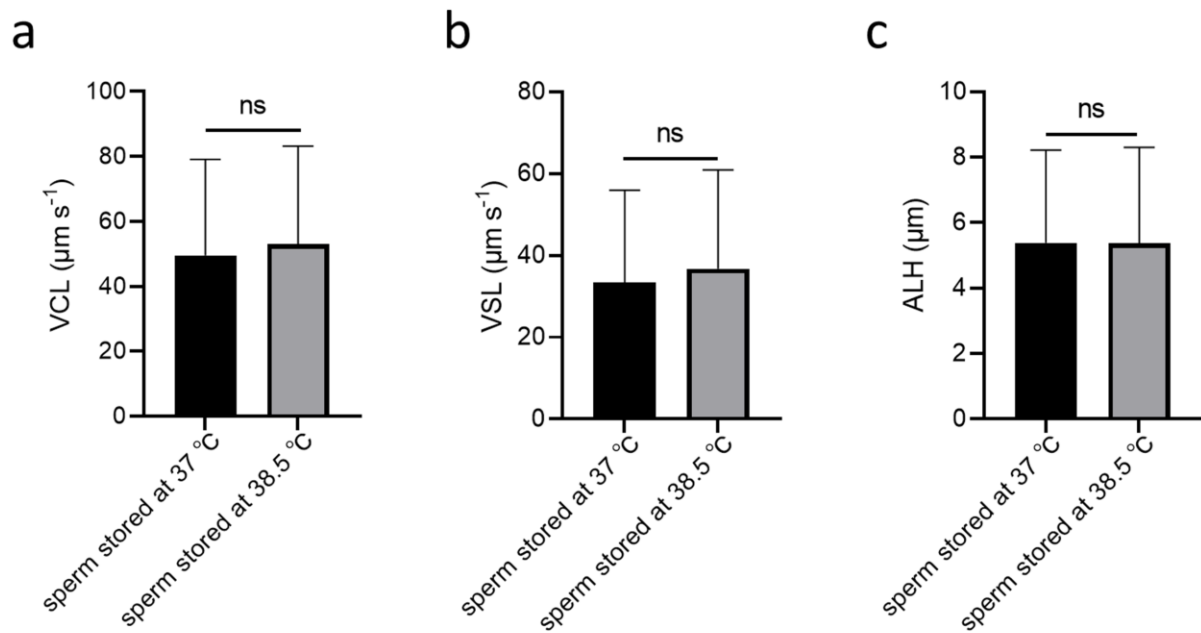
**Figure S7.** Representative time-lapsed images of sperm oriented tilted to the flow.



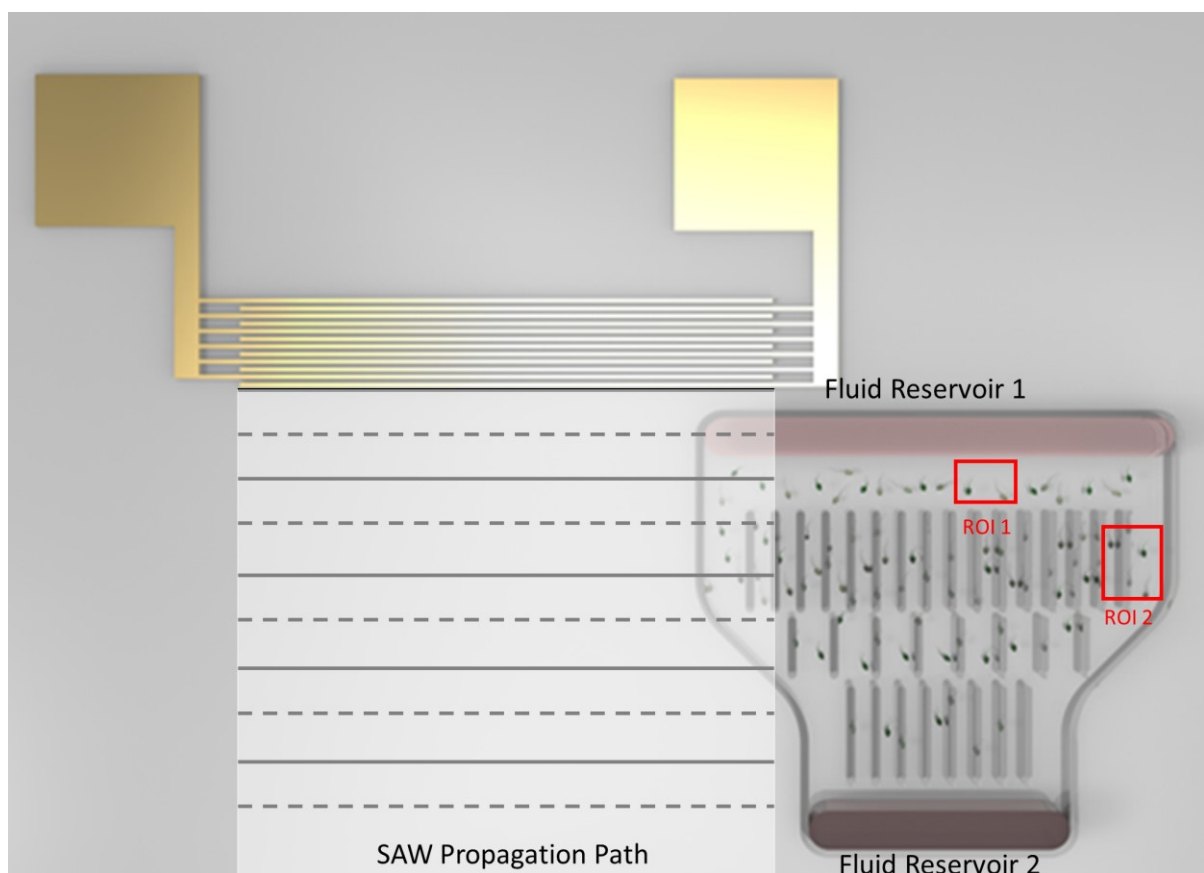
**Figure S8.** Representative (a) straight rheotaxis sperm (b) left-turning rheotaxis sperm, and (c) right-turning rheotaxis sperm alignment direction with respect to the flow direction. Black arrow, red arrow, and blue arrow specify flow direction, sperm moving direction, and sperm orientation angle.



**Figure S9.** Percentage of sperm oriented parallel to the flow in 225- $\mu\text{m}$ -wide microchannels in a flow field with an average velocity of (a) 40  $\mu\text{m s}^{-1}$ , (b) 70  $\mu\text{m s}^{-1}$  and (c) 130  $\mu\text{m s}^{-1}$ .



**Figure S10.** Comparison of (a) Curvilinear velocity (VCL), (b) Average path velocity (VAP) and (c) Lateral head displacement (ALH) of bull sperm samples cultured at 37 °C and 38.5 °C. All data represented as mean  $\pm$  s.d from three independent experiments performed using different samples. *p*-values were determined by unpaired t test with welch's corrections.



**Figure S11.** Relative position of the microfluidic channel and SAW propagation path.





**Figure S12.** Acoustic streaming in the droplet (a) entirely present on SAW propagation path, (b) partially present on SAW propagation path, and (c) absent on SAW propagation path.