Supplementary materials for

Navigation and selection of spermatozoa in a radial flow microfluidic device

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Note 1: Modeling of sperm navigation with various propulsive velocities in radial flow

We ran the simulations for the same radial flow strength but considering sperm cells with various propulsive velocities (v_p) ranging from 0 μm s^{-1} (considered as dead cell or debris) to 100 μm s^{-1} (considered as progressively motile bovine sperm). Dead sperm and cells with motility less than 60 μm s^{-1} are swept away by the flow to the outer boundary (**Figure S1 a-f**).

Sperm with v_s of 60 μm s^{-1} showed a notable navigation pattern (**Figure S1 g**). They first are swept away by the flow but gradually reorient themselves and start swimming toward the center (**Figure S1 h**). Obviously, this procedure slows down their navigation compared to the higher flow rate. Cells with propulsive velocity faster than 80 μm s^{-1} showed rotary rheotaxis; due to local flow conditions, sperm start to rotate while progressively moving toward the center.

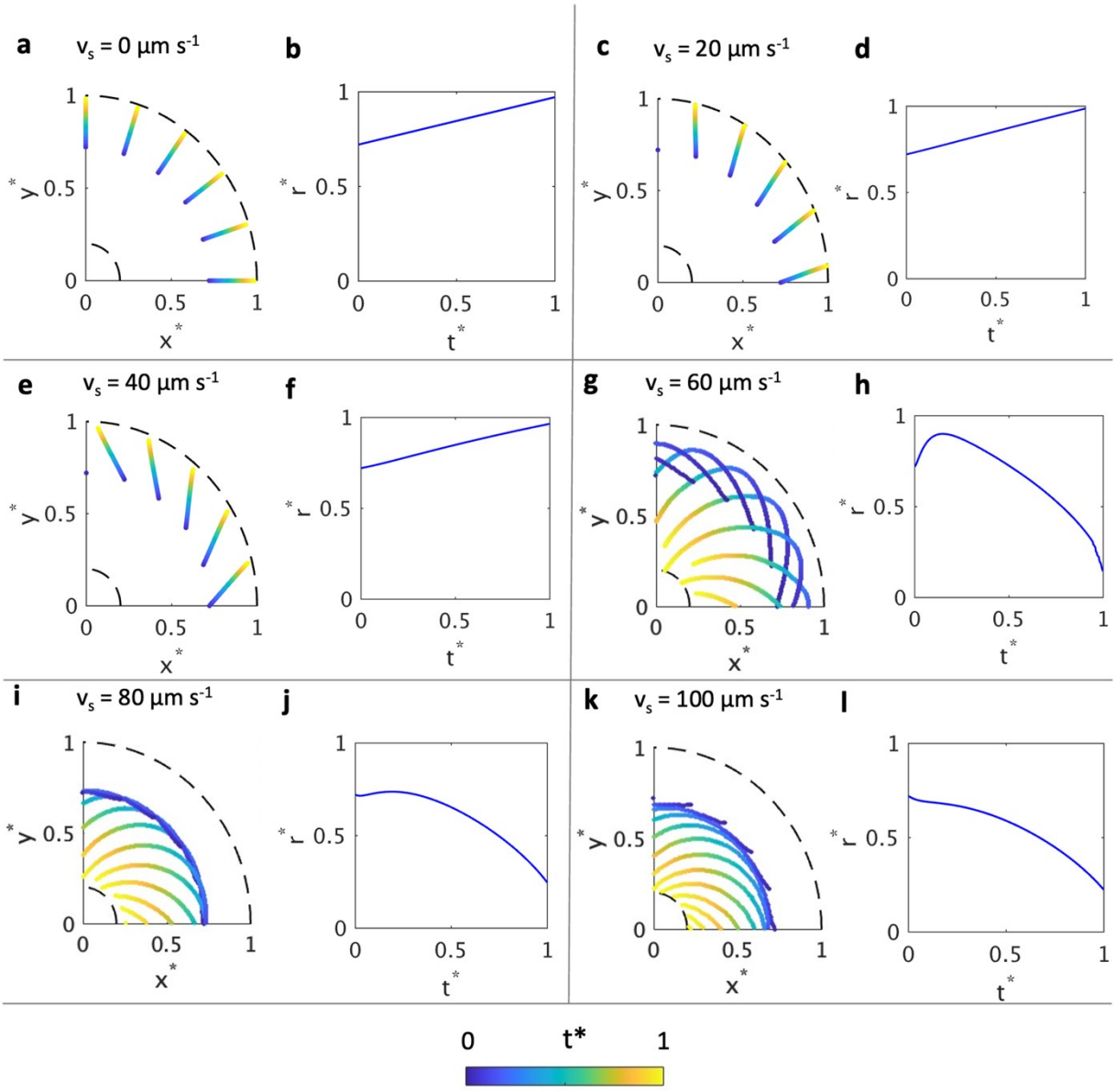

Figure S1. Mathematical modeling of sperm with various intrinsic propulsive velocities of 0 µm s^{-1} or dead sperm(a and b), 20 µm s^{-1} (c and d), 40 µm s^{-1}(e and f), 60 µm s^{-1}(g and h), 80 µm s^{-1}(i and j), 100 µm s^{-1}(k and l), identical radial flow equivalent to $\gamma = 3\ \gamma_c$. The shape of r* versus t* plot describes the behavior of microswimmer in the flow field. Time and dimensions are normalized with respect to their maximum values for enhanced visualization. Sperm location at each time step is color-coded indicating the time progression.

<u>Note 2</u>: Design of sperm unidirectional navigation (SUN) chip

Design specifications of each section of SUN chip is provided in **Fig. S2**. Regions of the chip are: (1) Inlet port where sperm are prevented from entering the washing medium stream through excess shear rate and modulated sperm-barrier interaction (**Fig. S2a**), (2) Capture and reorienting barriers with a gap size of 100 µm and apex angle of 45˚(**Fig. S2b**), (3) Second ring of capture and reorienting barriers with extended tail for enhanced capture of sperm from bulk semen (**Fig. S2c**), and (4) Outlet funnel where the shear rate gradient provides the last chance for sperm to reorient toward flow origin and remain in the chip before being washed out by flow (**Fig. S2d**).

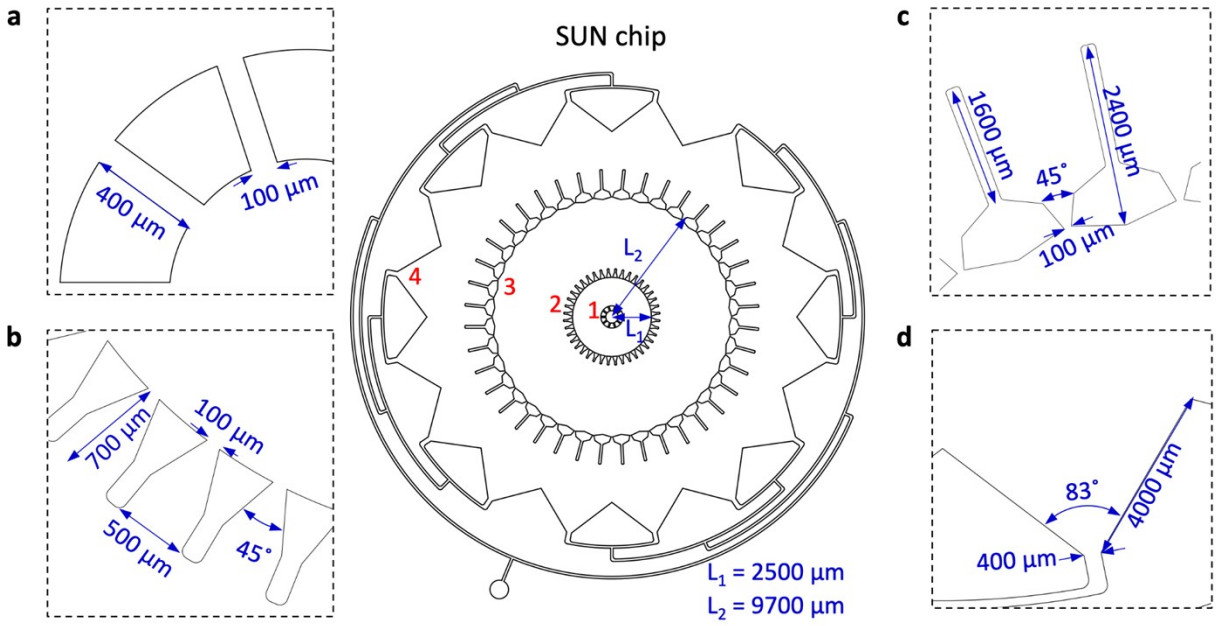

Fig. S2 Design of SUN chip and dimensions of each region: (a) region 1, (b) region 2, (c) regions 3, and (d) region 4.

Note 3: Operation of SUN chip

The operation of sperm unidirectional navigation (SUN) chip consists of three simple steps: 1) filling the chip with raw semen sample, 2) washing the sample with medium to remove the dead cells and debris and maintaining motile sperm inside the chip using a syringe pump, and 3) Extracting the remaining sperm inside the chip by a pipette (**Figure S3a**). To quantify the washing step, we simulated the process by using dyed water representing the semen sample and washing the chip with water. We consequently captured images from chip every 1 s; and the results showed highly distributed flow inside the chip resulted from the radial design and flow distributors(**Figure S3b**).

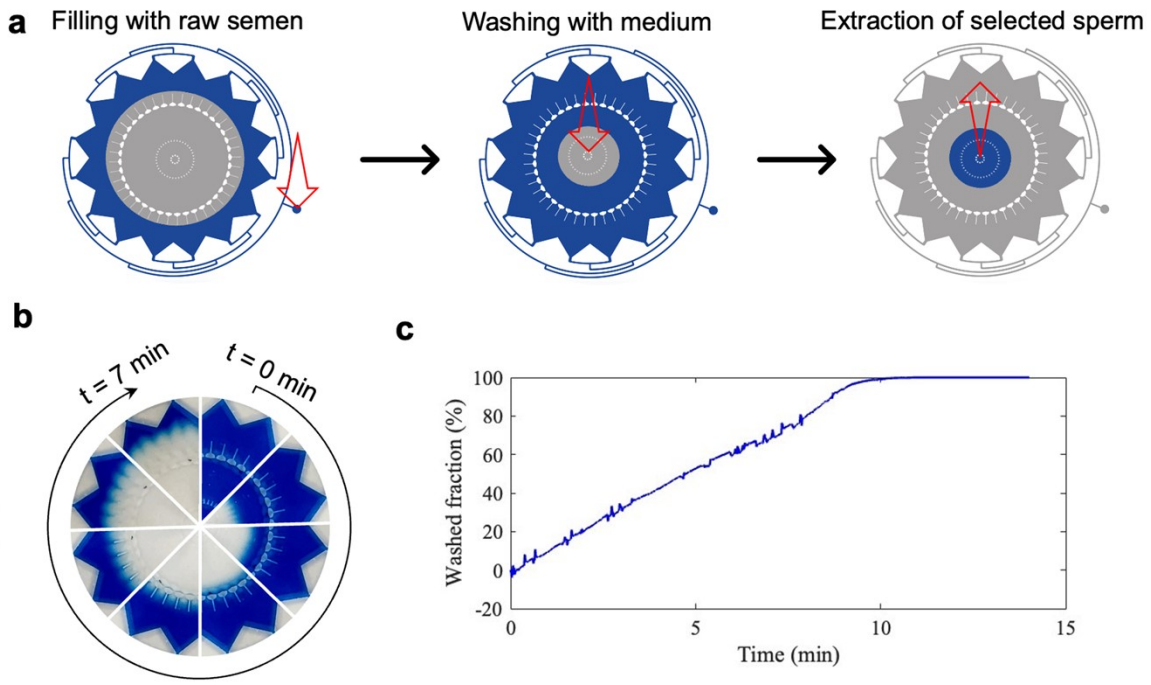

Figure S3. Operation of sperm unidirectional navigation chip. (a) operational steps of sperm selection using sperm unidirectional navigation chip. (b) washing time lapse snapshots of chip under flow rate Q = 1000 µlhr^{-1}. (c) quantification of washing step by pixel intensity analysis. The washing is accomplished after approximately 10 minutes.

We quantified the washing step by measuring the average pixel intensity of each frame and estimating the washed fraction by [1- (average intensity-initial average intensity)/(initial average intensity)]*100 using a MATLAB code. By plotting the washed fraction over time for a washing with flow rate of 1 mL hr^{-1}, the washing dynamics is identified (**Figure S3c**). Complete washing was achieved by approximately 9 min of medium flow corresponding to a dispensed volume of 150 μL. In the case of a real semen sample, due to its particulate nature because of proteins and other bioparticles presence, we assumed a total flow dispensed equal to 300 μL (correction factor of 2 compared to dyed water experiment).