Supplementary Information (SI) for Lab on a Chip.

Electronic Supplementary Information

Microfluidic-Assisted Sperm Sorter: A High-Throughput Sperm Selection Device

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Content:

S1: Simulation results of to set up the flow field inside the channel for sperm cell rheotaxis

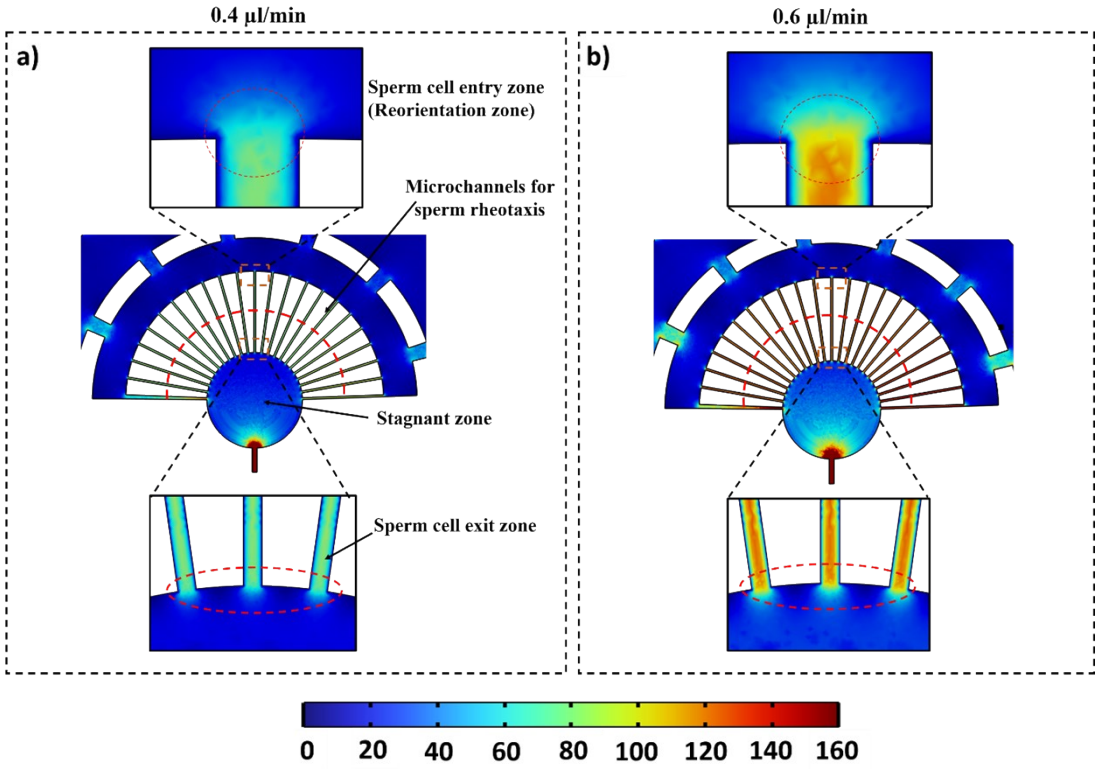

Fig S1. Velocity contour at different flow rate: a) 0.4µl/min, b)0.6 µl/min

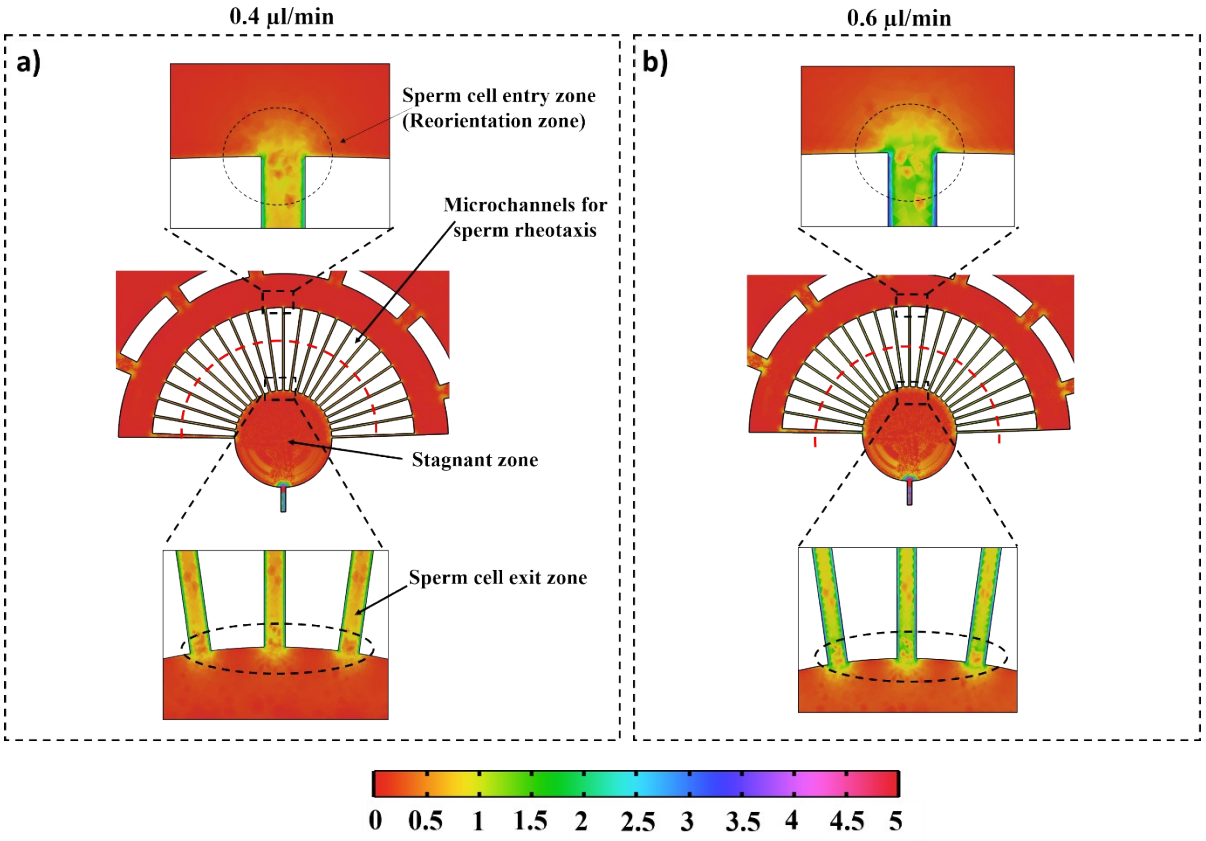

Fig S2. Shear rate contour at different flow rate: a) 0.4µl/min, b) 0.6 µl/min

S2. Kinematic Parameters of sperm cell motion.

The different parameters associated with the sperm cell motion is shown in Fig. S1.[1,2]

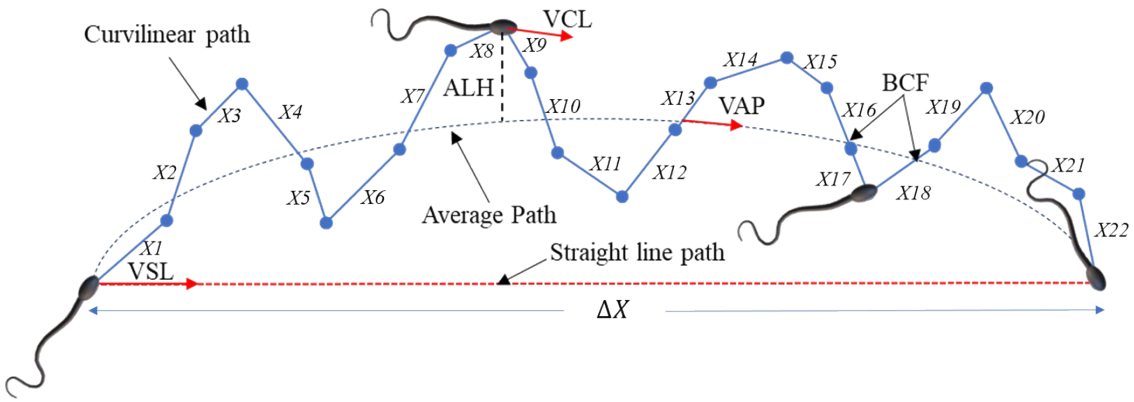

Figure S3. Kinematic parameters of sperm trajectory (VSL, VCL, VAP, and ALH) were tracked using ImageJ.

a. **Straight line velocity (VSL) or Progressive velocity**: The straight-line velocity represents the net distance traveled by the sperm head along the straight path connecting its first and last tracked positions within a given time interval, shown in eq. S1. It quantifies the overall progression efficiency of the sperm.

$$VSL = \frac{\Delta X}{\Delta t} \tag{S1}$$

Where ΔX is the straight line distance between the initial and final position of the sperm head and Δt is the time taken by the sperm to move from the start point to the end point.

b. **Curvilinear velocity (VCL):** VCL represents the total distance travelled by the sperm head along its actual trajectory. It is calculated by summing the displacement of the sperm head positions over equal time intervals and dividing by the total time between the first and last points of the traced path, shown in eq. S2. This parameter reflects the true speed of the sperm along its curvilinear (non-linear) motion.

$$VCL = \frac{\sum_{i=1}^{n} X_i}{\Delta t} = \frac{X_1 + X_2 + \ldots + X_n}{\Delta t} \tag{S2}$$

Xi is the distance between two positions traced by the sperm head.

c. **Average velocity or smooth path velocity (VAP):** VAP represents the velocity of a sperm cell along its smoothed trajectory, calculated using a five-point moving average given in eq. S3 and eq. S4. To obtain the smooth path shown in *Fig. S1*, the positions of five consecutive points were averaged at each acquisition frame, and this averaged point was taken as the corresponding sperm position on the trajectory. This approach effectively filters out high-frequency lateral head displacements, providing a more accurate representation of the sperm's average progression along its path.

$$VAP_{(i)} = \frac{\sum_{j=1}^{5} VCL(i+j)}{5} \tag{S3}$$

$$VAP = \sum_{i=1}^{n} VAP_{(i)} \tag{S4}$$

d. Beat frequency (Hz): The frequency of flagella beats is calculated by counting the number of waves initiated every second. The flagella traces were inspected, and the wave's start was identified as the first picture displaying the flagella bend, which was then propagated. The number of initiations was counted manually for 50 sperm, and the average beat frequency was determined.

e. **Amplitude of lateral head displacement (ALH):** It is the maximum displacement of the sperm head about the average path or smooth path (i.e., Track width) shown in Fig S1.

f. **Linearity (LIN):**

Linearity quantifies how closely the sperm's actual trajectory follows a straight path. It is defined as the ratio of straight-line velocity (VSL) to curvilinear velocity (VCL), representing the degree to which the sperm moves directly toward its destination rather than following a curved path shown in eq.S5.

$$LIN = \frac{VSL}{VCL} \tag{S5}$$

Higher LIN values indicate more linear (progressive) motion, while lower values correspond to highly curved or irregular trajectories.

g. **Straightness (STR):**

Straightness describes the alignment of the sperm's average path relative to the straight line connecting its starting and ending points given by eq. S6. It is calculated as the ratio of straight-line velocity (VSL) to average path velocity (VAP).

$$STR = \frac{VSL}{VAP} \tag{S6}$$

A higher STR value indicates that the sperm moves along a path that is closely aligned with the straight direction of travel, suggesting efficient forward progression.

h. **Wobble (WOB):**

Wobble quantifies the oscillatory nature of sperm movement by relating the average path velocity (VAP) to the curvilinear velocity (VCL) given by eq. S7. It provides an estimate of how much the sperm head deviates laterally around its average trajectory.

$$WOB = \frac{VAP}{VCL} \quad \text{(S7)}$$

Higher WOB values reflect smoother, more stable swimming paths, whereas lower values indicate pronounced lateral head displacement or erratic motion.

S3: DNA fragmentation assessment images

The DNA fragmentation index is calculated using eq. S8

$$DFI\ \% = \frac{Number\ of\ sperm\ cells\ with\ fragmented\ DNA}{Total\ number\ of\ sperm\ cell\ analyzed} \quad \text{(S8)}$$

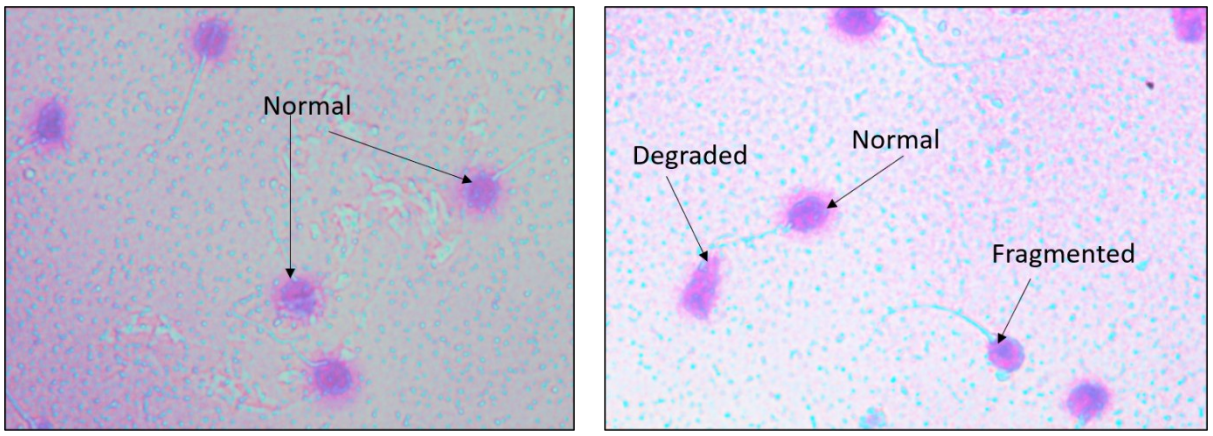

Fig S4: Images showing the result of sperm chromatin dispersion assay.

Sperm without halos and small halos are sperm with damaged DNA structures. Sperm with medium and large halos have a healthy DNA structure[3].

S4: Supplementary table

Table S1: Sample concentration used to perform the separation study

Sample	Sample concentration (millions per ml)
1	68.5
2	60.62
3	80.54
4	55.57

5	62.35
Total	**65.52±8.57**

Table S2: Concentration of separated sperm ($\times 10^6$ mL^{-1}) at different flow rates. Values represents mean ± standard deviation for three replicates (n=3) for each sample

Sample No.	**No Flow**	**0.2 µL min^{-1} (Mean ± SD, n=3)**	**0.4 µL min^{-1} (Mean ± SD, n=3)**	**0.6 µL min^{-1} (Mean ± SD, n=3)**
1	0.43 ± 0.04	2.7 ± 0.1	1.7 ± 0.2	00.5 ± 0.3
2	0.63 ± 0.06	2.8 ± 0.3	1.3 ± 0.2	0.4 ± 0.1
3	0.7 ± 0.05	3.4 ± 0.3	1.6 ± 0.3	1.2 ± 0.2
4	0.55 ± 0.04	2.0 ± 0.2	0.9 ± 0.2	0.4 ± 0.1
5	0.61 ± 0.03	2.2 ± 0.4	1.2 ± 0.2	1.0 ± 0.2
Mean ± SD	**0.58 ± 0.1**	**2.6 ± 0.5**	**1.3 ± 0.3**	**0.7 ± 0.3**

Table S3: Comparison of sperm motility (%) for raw, swim-up, and MASS device samples.

Sample No.	**Raw (Mean)**	**Swim-up (Mean ± SD, n=3)**	**No flow (Mean ± SD, n=3)**	**MASS device (Mean ± SD, n=3)**
1	43.5	78.00 ± 1.32	88.00 ± 3.51	99.50 ± 0.50
2	48.6	78.83 ± 1.61	91.3 ± 2.51	98.00 ± 0.50
3	52.4	79.67 ± 1.26	95 ± 2	98.50 ± 1.32
4	58.4	80.00 ± 2.00	91.3 ± 3.78	98.83 ± 0.58
5	44.6	79.33 ± 1.76	92.33 ± 1.76	99.33 ± 0.58
Mean ± SD	**49.5 ± 6.3**	**79.17 ± 0.79**	**91.67 ± 2.4**	**98.83 ± 0.61**

Table S4: Isolation efficiency (%) of sperm cells at different flow rates.

Sample No.	No Flow	0.2 µL min⁻¹ (Mean ± SD, n=3)	0.4 µL min⁻¹ (Mean ± SD, n=3)	0.6 µL min⁻¹ (Mean ± SD, n=3)
1	1.6 ± 0.1	8.3 ± 0.2	5.4 ± 0.7	1.6 ± 0.7
2	2.7 ± 0.3	9.5 ± 0.9	4.5 ± 0.8	2.0 ± 1.2
3	2.5 ± 0.2	8.1 ± 0.6	3.7 ± 0.8	2.9 ± 0.5
4	2.3 ± 0.2	6.9 ± 0.7	2.9 ± 0.3	2.5 ± 0.8
5	3.1 ± 10.1	7.9 ± 1.6	4.4 ± 0.8	3.5 ± 0.7
Mean ± SD	**2.4 ± 0.5**	**8.1 ± 1.0**	**4.2 ± 0.9**	**2.5 ± 0.7**

Table S5: Comparison of DNA Fragmentation Index (DFI, %) for raw, swim-up, and MASS device samples

Sample No.	Raw (Mean ± SD, n=3)	Swim-up (Mean ± SD, n=3)	No flow (Mean ± SD, n=3)	MASS device (Mean ± SD, n=3)
1	9.0 ± 0.5	7.2 ± 1.3	3.6 ± 0.5	3.2 ± 0.6
2	15.7 ± 0.6	10.3 ± 1.3	3.9 ± 0.4	3.5 ± 1.0
3	19.0 ± 0.5	14.5 ± 2.2	3.6 ± 0.2	2.7 ± 0.3
4	27.8 ± 2.6	6.0 ± 1.5	3.7 ± 0.2	2.3 ± 0.6
5	23.3 ± 1.4	17.8 ± 2.5	3.6 ± 0.4	2.5 ± 1.8
Mean ± SD	**19 ± 7.2**	**11.2 ± 4.6**	**3.7 ± 0.1**	**2.84 ± 0.46**

Table S6: Comparison of Sperm Viability (%) for raw, swim-up, and MASS device samples

Sample No.	Raw (Mean ± SD, n=3)	Swim-up (Mean ± SD, n=3)	No flow (Mean ± SD, n=3)	MASS device (Mean ± SD, n=3)

1	34.0 ± 6.6	69.7 ± 1.5	94.83 ± 3.1	96.5 ± 3.5
2	45.7 ± 2.3	75.3 ± 2.5	95.5 ± 2.5	98.7 ± 2.3
3	59.0 ± 3.0	81.0 ± 2.0	96.8 ± 2.9	98.8 ± 0.8
4	51.3 ± 2.1	88.0 ± 3.0	98.2 ± 4.2	99.7 ± 0.3
5	72.0 ± 11.4	92.3 ± 3.0	96.2 ± 3.0	97.5 ± 1.7
Mean ± SD	**52.4 ± 14.1**	**81.3 ± 8.5**	**96.3 ± 1.3**	**98.2 ± 1.3**

S5: Videos related to experimentation of MASS device (Supplementary movies)

Movie S1: Sperm cells in a radial microchannel during the no-flow (control) condition.

Movie S2: Motile sperm cells at the outlet of the radial microchannel during no flow condition.

Movie S3: Rheotaxis in the radial microchannel at 0.2µl/min.

Movie S4: Reorientation and alignment of the sperm cells against the flow at the entry of the microchannel.

Movie S5: Motile sperm cells entering the collection chamber through the microchannel by performing rheotaxis at 0.2µl/min.

Movie S6: Motile sperm cells inside the collection chamber after 20 minutes near the microchannel arrays.

Movie S7: Motile sperm cells in the collection chamber, captured using a 4× objective lens.

Movie S8: Accumulation of motile sperm cells in the collection chamber after 5 minutes at 0.2µl/min. (A cyan-blue filter was applied using ImageJ software for enhanced visualization.)

Movie S9: Accumulation of motile sperm cells in the collection chamber at the end of 20 minutes under a flow rate of 0.2 µl/min.

Movie S10: Sperm cells in the raw semen storage zone

References:

1. E. Sloter, E. T. Schmid, F. Marchetti, B. Eskenazi, J. Nath and J. A. Wyrobek, *Hum. Reprod.*, 2006, **21**(11), 2868–2875.
2. T. S. Mortimer, D. Schoevaert, A. M. Swan and D. Mortimer, *Hum. Reprod.*, 1997, **12**(5), 1006–1012.
3. S. Sharma, M. Alamgir Kabir and W. Asghar, *Analyst*, 2022, **147**, 1589–1597.