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

High-DNA integrity sperm selection using rheotaxis and boundary following behavior in a microfluidic chip

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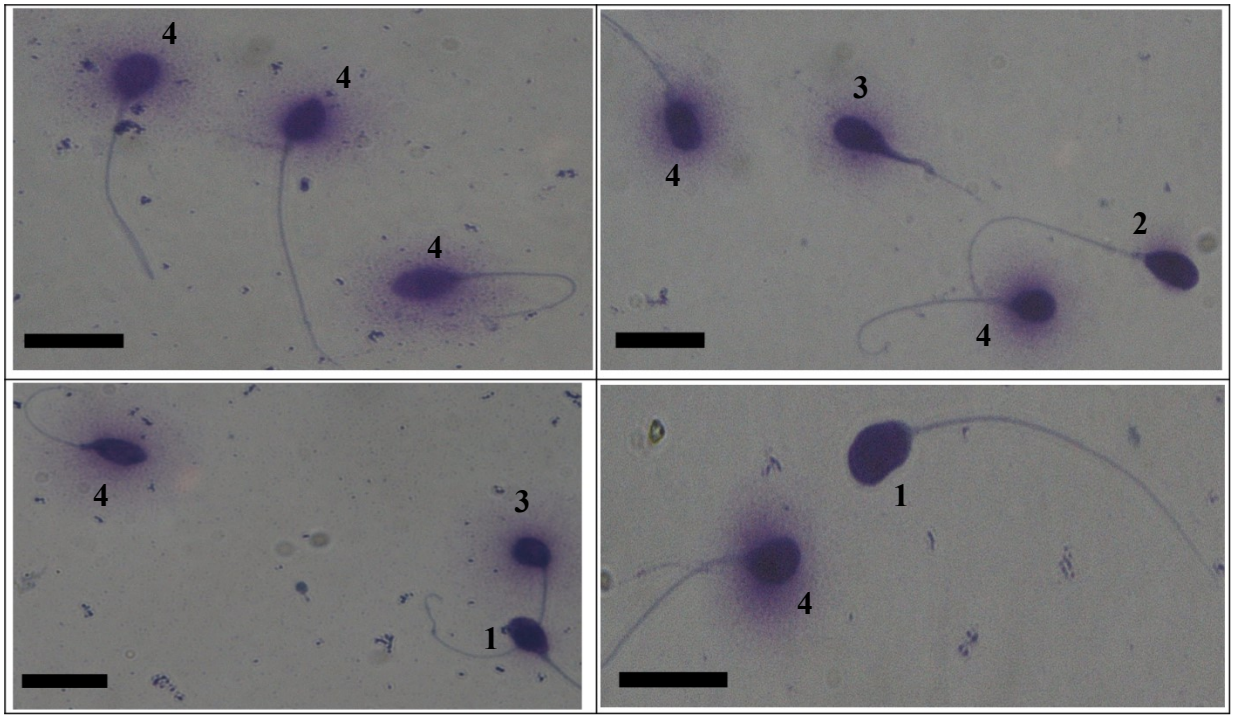

Figure S1. Representative images of sperm chromatin dispersion test indicating 1: abnormal cell with no halo, 2: abnormal cell with small halo, 3: normal cell with medium halo, and 4: normal cell with large halo. Scale bars, 10 μm.

Table S1. Comparison of the device performance with previous rheotaxis and conventional clinical methods for sperm selection in terms of count, volume, separation time, motility, DNA improvement, ability to separate highly motile sperm and having a separation reservoir.

Method	Count	Volume (µl)	Time (min)	Motility	Advantage 1	Advantage 2	Advantage 3
					DNA improvement	Separation Reservoir	Selecting highly motile cells
Present work (rheotaxis)	13280-18880	27	20	96%	80%	+	+
Zaferani *et al.*[1] (rheotaxis)	1200	NA	5	100%	NA	-	-
Wu et al.[2] (rheotaxis)	200×10^6	1000	15	93%	NA	-	+
Sarbandi *et al.*[3] (rheotaxis)	9200	2.5	20	100%	NA	-	+
Sharma *et al.*[4] (rheotaxis)	11200 – 79200	80	60	99.47%	84 %	+	-
L.H. Ng et al.[5] (swim-up)	2640000	500	80	89%	47%	+	-
L.H. Ng et al.[5] (DGC)	4050000	500	30	58%	11%	+	-

References

1 M. Zaferani, S. H. Cheong and A. Abbaspourrad, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, 115, 8272–8277.

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4 S. Sharma, M. A. Kabir and W. Asghar, *Analyst*, 2022, **147**, 1589–1597.

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Movie S1. Trajectories of the motile sperm undergoing rheotaxis and showing boundary-following behavior, and the ones which are not motile enough to overcome the flow.

Movie S2. Sperm separation and calculating the separation rate at the inlet concentration of 100 million sperm/ml

Movie S3. Sperm separation and calculating the separation rate at the inlet concentration of 150 million sperm/ml

Movie S4. Sperm separation and calculating the separation rate in the flow rate of 0.2 $\mu l/min$

Movie S5. Sperm separation and calculating the separation rate in the flow rate of 0.8 $\mu l/min$

Movie S6. Separated motile sperm in the reservoir of the chip.