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

Electronic Supplementary Information

Selection of High-quality Sperm with Thousands of Parallel Channels

Mohammad Simchi,[a] Jason Riordon,[a] Jae Bem You,[a] Yihe Wang,[a] Sa Xiao,[a] Alexander Lagunov,[b] Thomas Hannam,[b] Keith Jarvi,[c] Reza Nosrati,*[d] and David Sinton*[a]

*Emails: reza.nosrati@monash.edu, sinton@mie.utoronto.ca

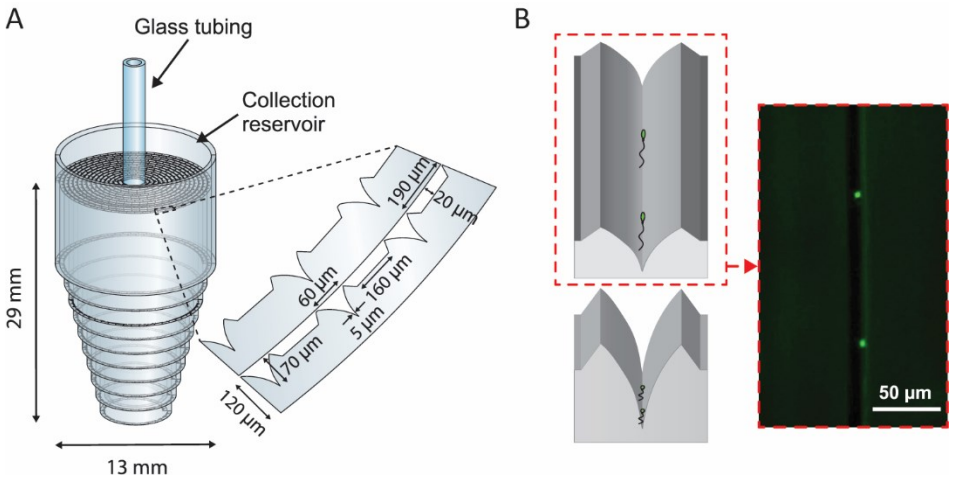


Fig. S1 (A) Schematic view of the 3D device with more than 6,500 channels (triangular and trapezoidal). (B) A fluorescent image of sperm swimming at the sharp corner of a triangular channel. The image is taken from sperm swimming in a separate 2D device consists of one layer of the patterned PET film that allowed a better visualization, as the cylindrical shape of the 3D device did not allow a perfect visualization of sperm movement.

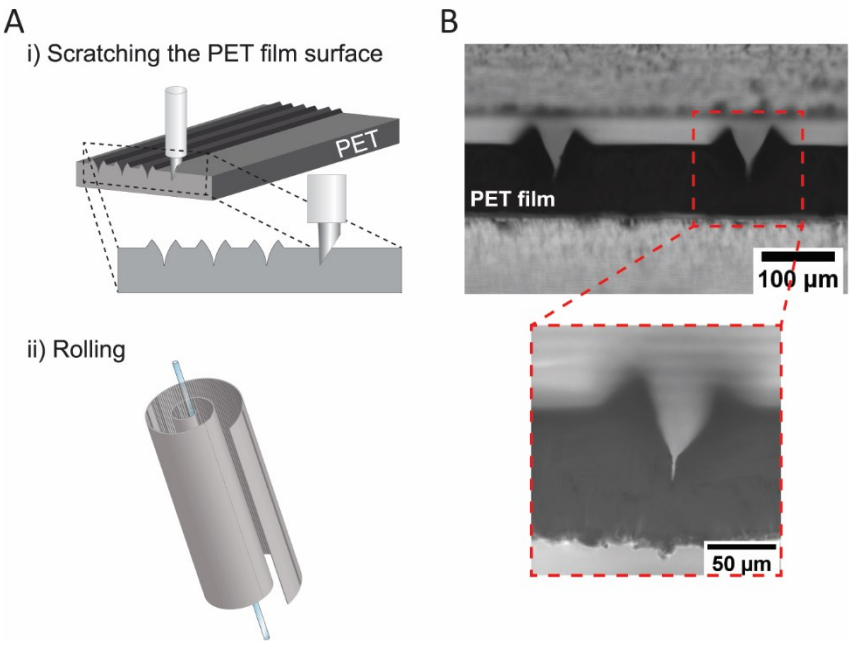


Fig. S2 (A) Schematic overview of the roll-up fabrication process: (i) polyethylene terephthalate (PET) film was patterned using a cutting plotter, then, (ii) rolled around a glass rod. (B) Cross-section microscope images of v-groove channels.

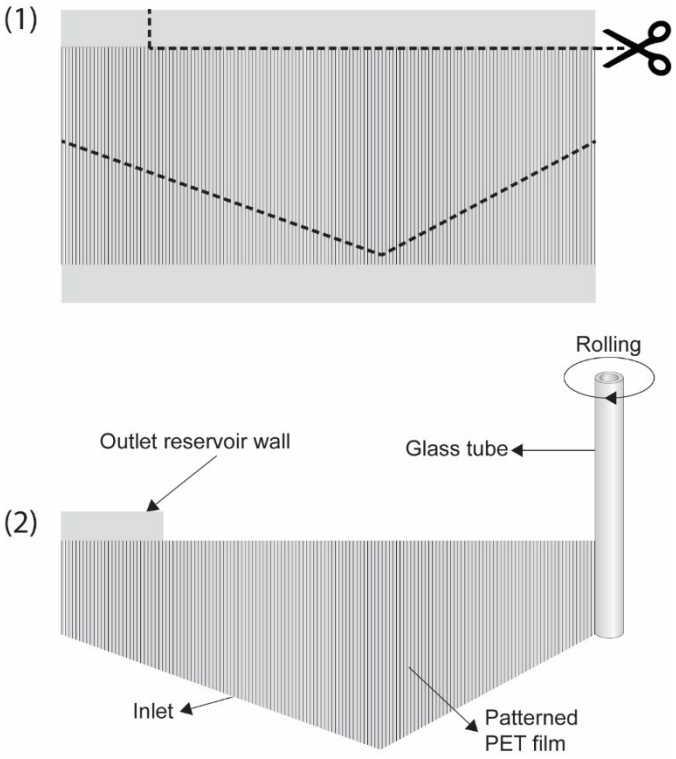


Fig. S3 After patterning the surface of the PET film, (1) the side edges of the patterned film were cut prior to the rolling to (2) make microchannels with different lengths. In this way, after rolling up the film around the glass tube, the stepped feature of the inlet and the wall of the collection reservoir were formed.

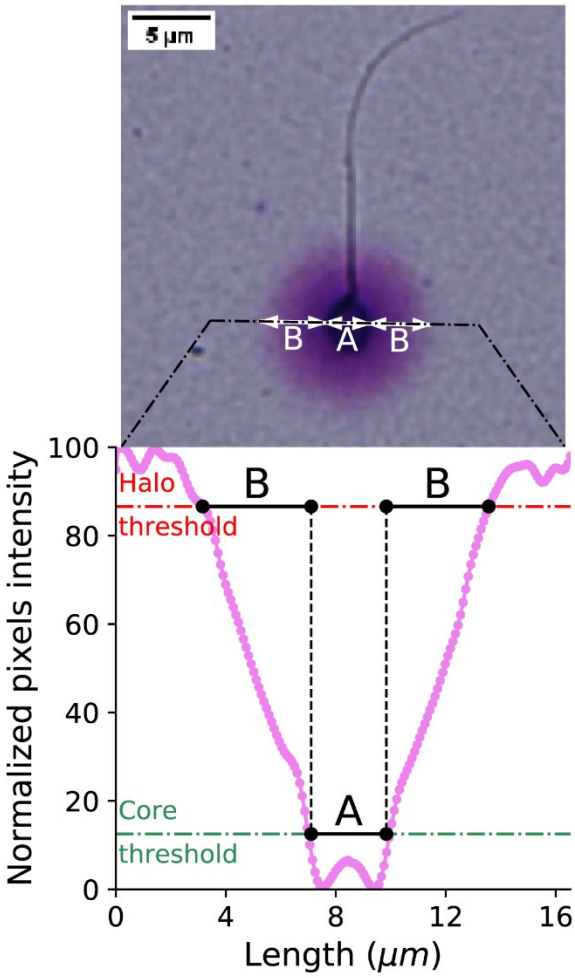


Fig. S4 The developed python code method for Sperm Chromatin Dispersion (SCD) assay analysis. The software looks at the normalized intensity of pixels at a perpendicular line to the sperm head direction. Based on two thresholds (halo and core threshold), sperm minor diameter and the DNA halo width are found.

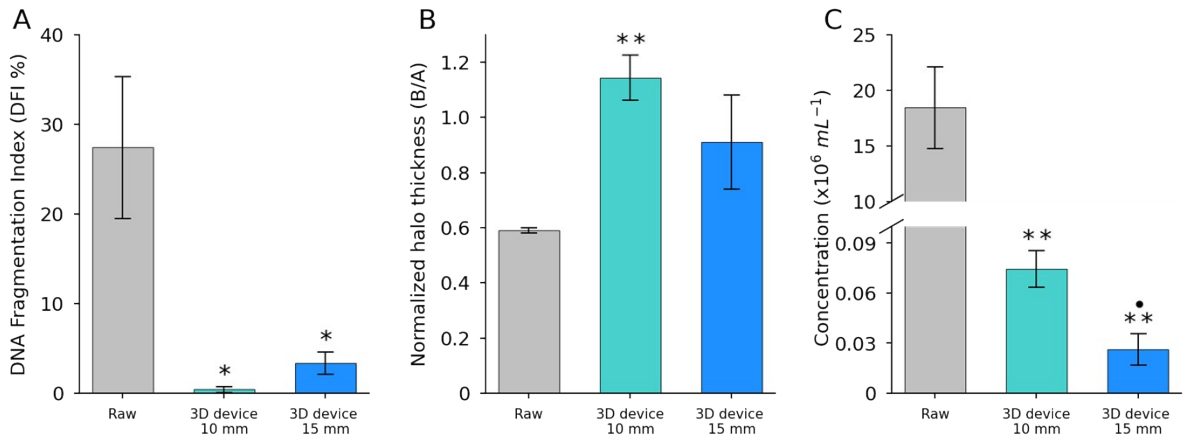


Fig. S5 Sperm selection performance of the device with 10 mm and 15 mm microchannels length. (A) Percentage of DNA fragmentation index (%DFI), (B) Normalized DNA halo thickness, and (C) concentration of raw and selected sperm samples. Error bars represent the sampling error of measurements (3 different donor samples tested). *p<0.05 compared to the Raw, **p<0.01 compared to the Raw, •p<0.05 compared to the 3D device – 10 mm. Differences between treatments were tested using one-way analysis of variance (ANOVA).

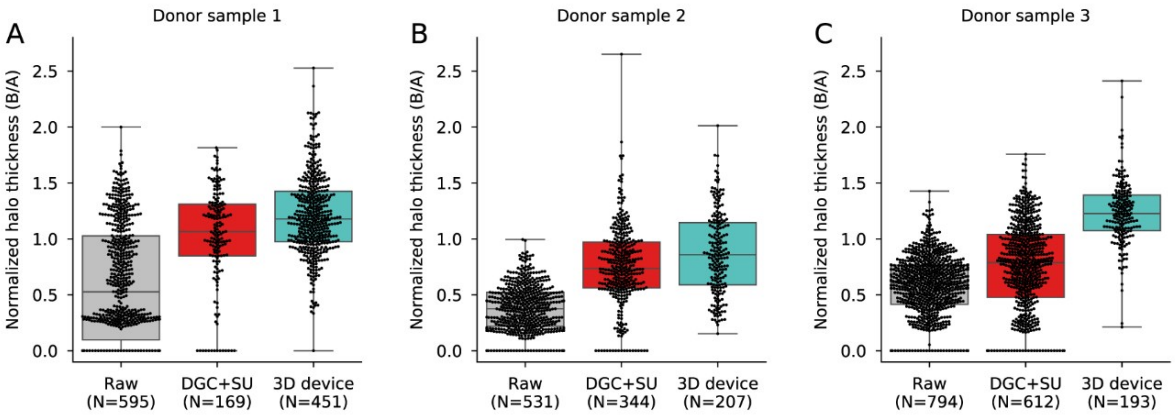
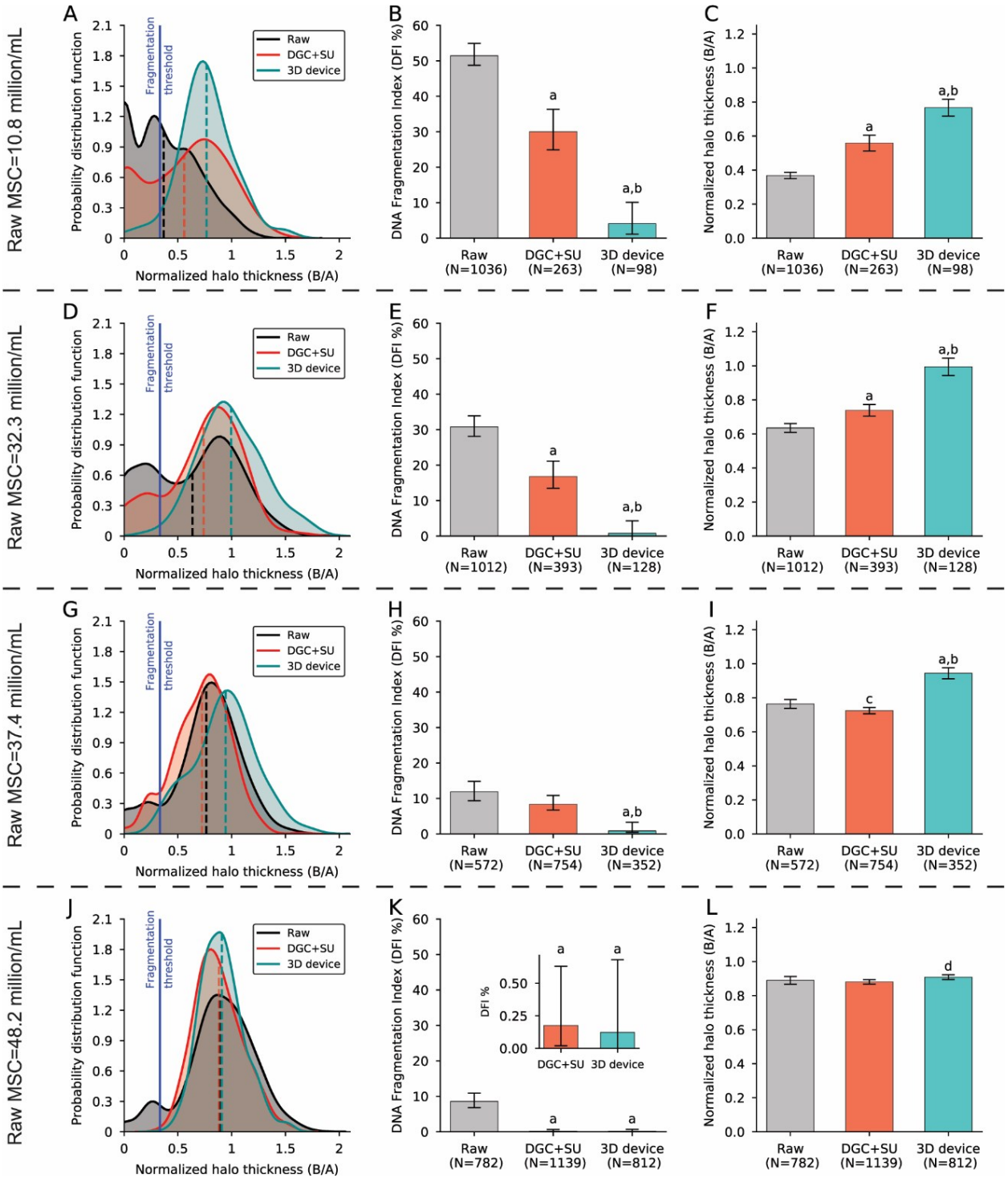


Fig. S6 SCD assay results for the donor samples experiments. Box plot demonstrates the first quartile, median, and third quartile values as three horizontal lines of the box. The whiskers show the lowest and the largest data points.


Raw MSC=10.8 million/mL
Raw MSC=32.3 million/mL
Raw MSC=37.4 million/mL
Raw MSC=48.2 million/mL
A
B
C
D
E
F
G
H
I
J
K
L
Probability distribution function
Normalized halo thickness (B/A)
Fragmentation threshold
Raw
DGC+SU
3D device
DNA Fragmentation Index (DFI %)
Normalized halo thickness (B/A)
DFI %
Raw (N=1036)
DGC+SU (N=263)
3D device (N=98)
Raw (N=1012)
DGC+SU (N=393)
3D device (N=128)
Raw (N=572)
DGC+SU (N=754)
3D device (N=352)
Raw (N=782)
DGC+SU (N=1139)
3D device (N=812)

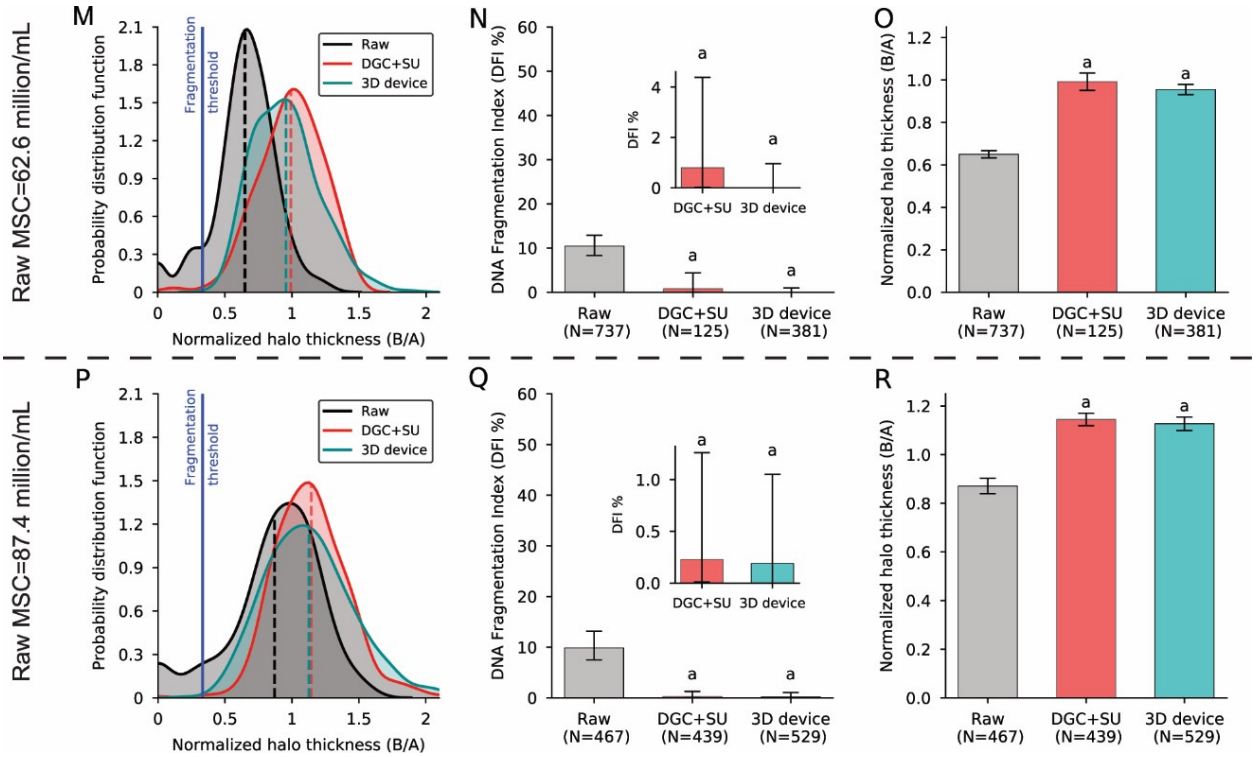


Fig. S7 SCD assay results for the clinical side-by-side tests using patient samples. The motile sperm concentration (MSC) of each raw patient sample is mentioned in the left side of each row. (A, D, G, J, M, P) The estimated probability distribution function of normalized DNA halo thickness of sperm selected from patient samples. The fragmentation threshold defined by the SCD kit (B/A≤1/3) is demonstrated as a solid blue line. The dashed lines represent the average B/A value of the correspondent sample. (B, E, H, K, N, Q) DNA fragmentation index and (C, F, I, L, O, R) average normalized DNA halo thickness of raw and selected sperm. Error bars represent 95% confidence interval. a p<0.0001 compared to raw; b p<0.0001 compared to DGC+SU; c p<0.02 compared to raw; d p<0.005 compared to DGC+SU.

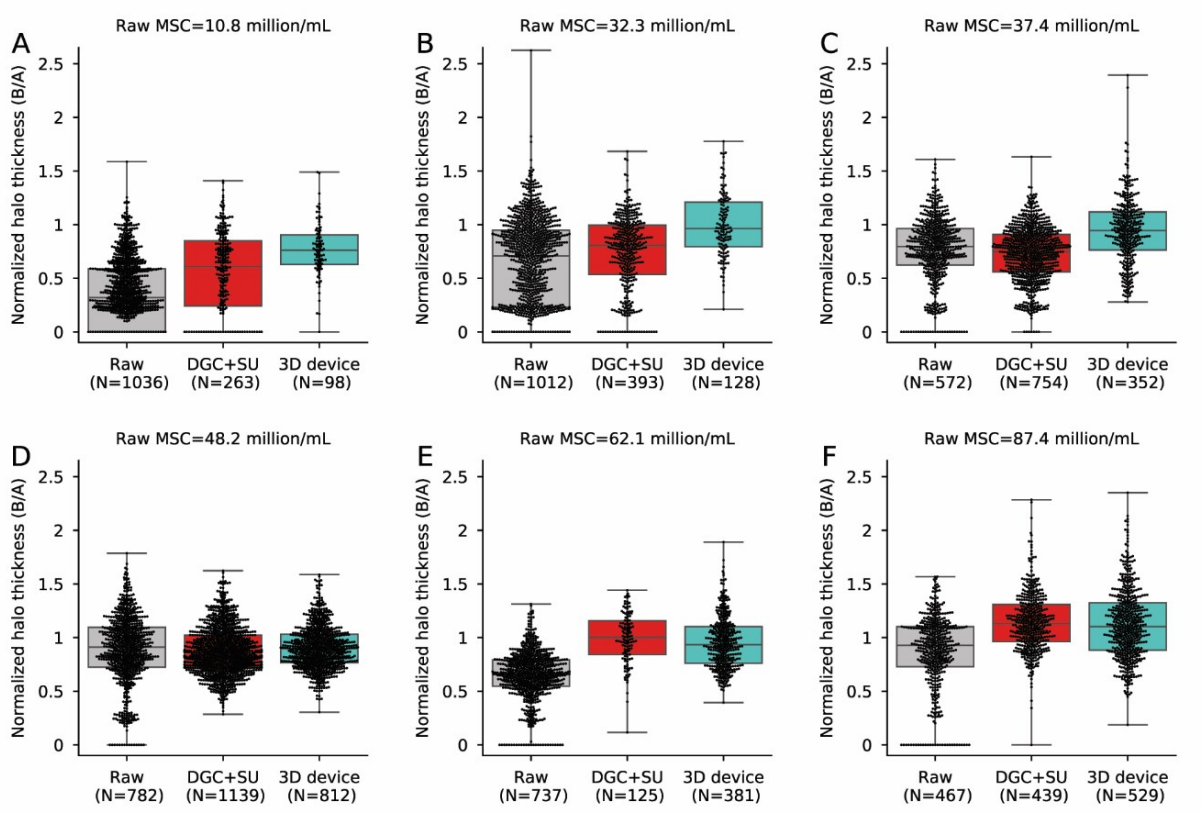


Fig. S8 SCD assay results for the side-by-side clinical testing. Box plot demonstrates the first quartile, median, and third quartile values as three horizontal lines of the box. The whiskers show the lowest and the largest data points. The motile sperm concentration (MSC) of each raw patient sample is mentioned in the title of each figure.

Table S1. Characteristics of infertility patient samples.

Patient #	1	2	3	4	5	6
Concentration ($\times 10^6$ mL^{-1})	27	95	70.5	102.5	107	190
Initial volume (mL)	1.2	1.2	4	3	1.07	1.5
Motility (%)	40	34	53	47	58	46
Motile sperm concentration ($\times 10^6$ mL^{-1})	10.8	32.3	37.4	48.2	62.1	87.4

Table S2. Statistical power and Cohen's effect size for %DFI results of clinical tests using N=6 different patient samples.

Group 1		Group 2		Cohen's d	power
Mean	SD	Mean	SD		
Raw		DGC+SU		0.82	0.25
20.5	15.76	9.4	10.98		
Raw		3D Device		1.28	0.52
20.5	15.76	1.05	1.42		
DGC+SU		3D Device		1.22	0.48
9.4	10.98	1.05	1.42		

Table S3. Statistical power and Cohen's effect size (d) for average B/A results of clinical tests using N=6 different patient samples.

Group 1		Group 2		Cohen's d	power
Mean	SD	Mean	SD		
Raw		DGC+SU		-0.78	0.23
0.7	0.17	0.84	0.19		
Raw		3D Device		-1.75	0.78
0.7	0.17	0.95	0.11		
DGC+SU		3D Device		-0.72	0.20
0.84	0.19	0.95	0.11		

Video S1 (separate file). Sperm swimming at the sharp corner of a triangular microchannel. Sperm is highly confined to a single dimension in the sharp corner and swim in a near-straight line along the corner.

Video S2 (separate file). Sperm motion in the sharp corner of triangular microchannels. Sperm are stained with SYBR 14. The movie is sped up 4x.

Video S3 (separate file). Sperm guidance within the sharp corner of a triangular microchannel. Sperm is stained with SYBR 14. The movie is sped up 4x.

Video S4 (separate file). Sperm selected usign the 3D device.