Electronic Supplementary Material (ESI) for Lab on a Chip


# Rapid Selection of Sperm with High DNA Integrity

# Electronic Supplementary Information

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The Electronic Supplementary Information contains two videos (separate files) showing: (1) real time movie of human sperm in a 9 mm microfluidic device, live sperm swim from the inlet toward the outlet using boundary following navigation; (2) real time movie showing bull sperm selection and semen purification in a 7.5 mm device, live and dead sperm are stained fluorescently as green and red, respectively. Here, live sperm (green) swim from the inlet toward the outlet while debris and dead sperm (red) remain at the inlet. Three figures are provided below showing (S1) experimental procedure with the device, (S2) representative sperm chromatin structure assays (SCSAs) for 10 min human sperm experiment with 7.5 mm device, (S3) percentage ratio of inlet to outlet concentration. One table is provided below showing details of DFI and HDS results.

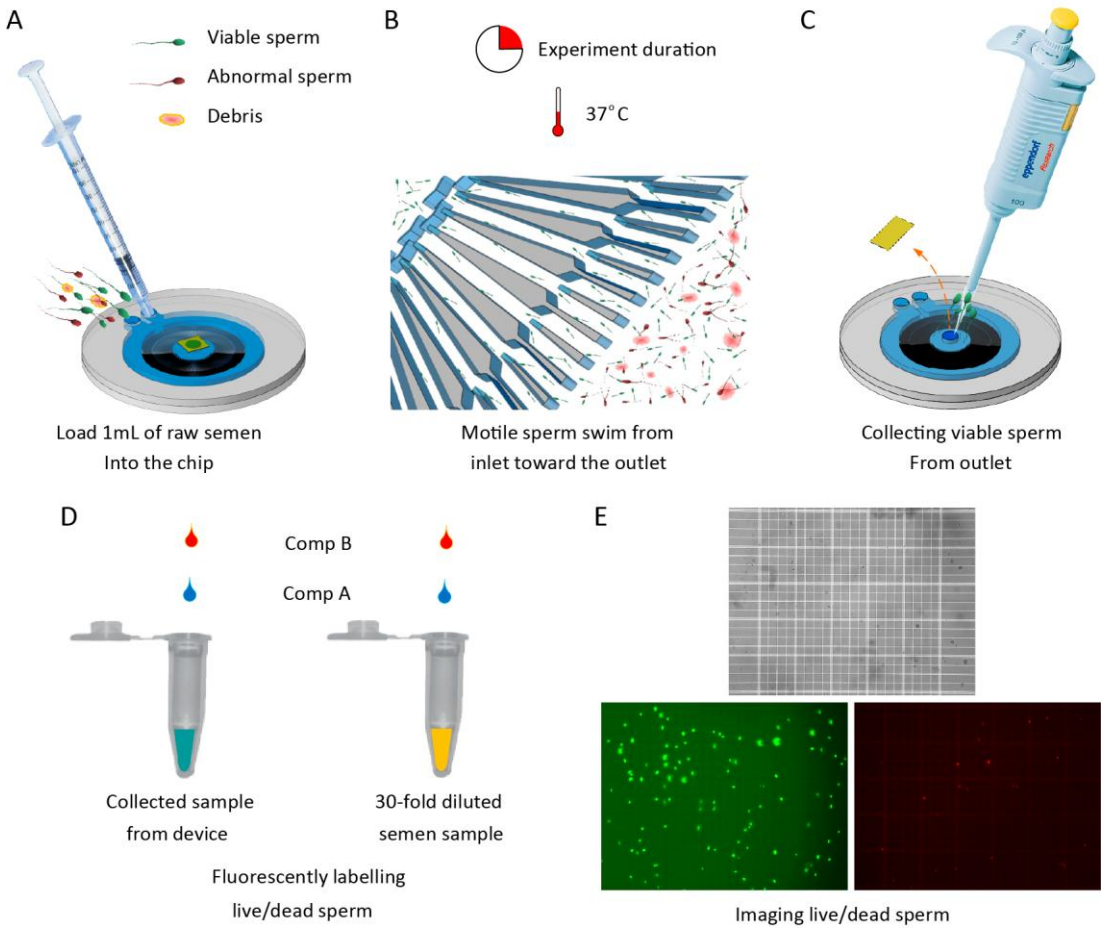


**Fig. S1** Experimental procedure for live/dead sperm labelling after sperm isolation. (A) A plastic syringe was used to inject 1mL of raw semen into the device at physiological temperature. (B) Motile sperm swam from the inlet towards the outlet during the experiment. Non-motile sperm and debris stayed in the injection ring due to no-flow environment inside the device. (C) After removing the temporary layer, motile sperm were collected from the outlet. (D) 10 min after the experiment, 0.6 µL of 50-fold Component A(SYBR 14 dye) were added to the 100 µL of the collected sample and to a 30-fold dilution of the initially used semen sample. After 10 min, this step is repeated with 0.6 µL of Component B (propidium iodide). (E) Images are acquired by fluorescent microscopy to evaluate sperm concentration and motility.

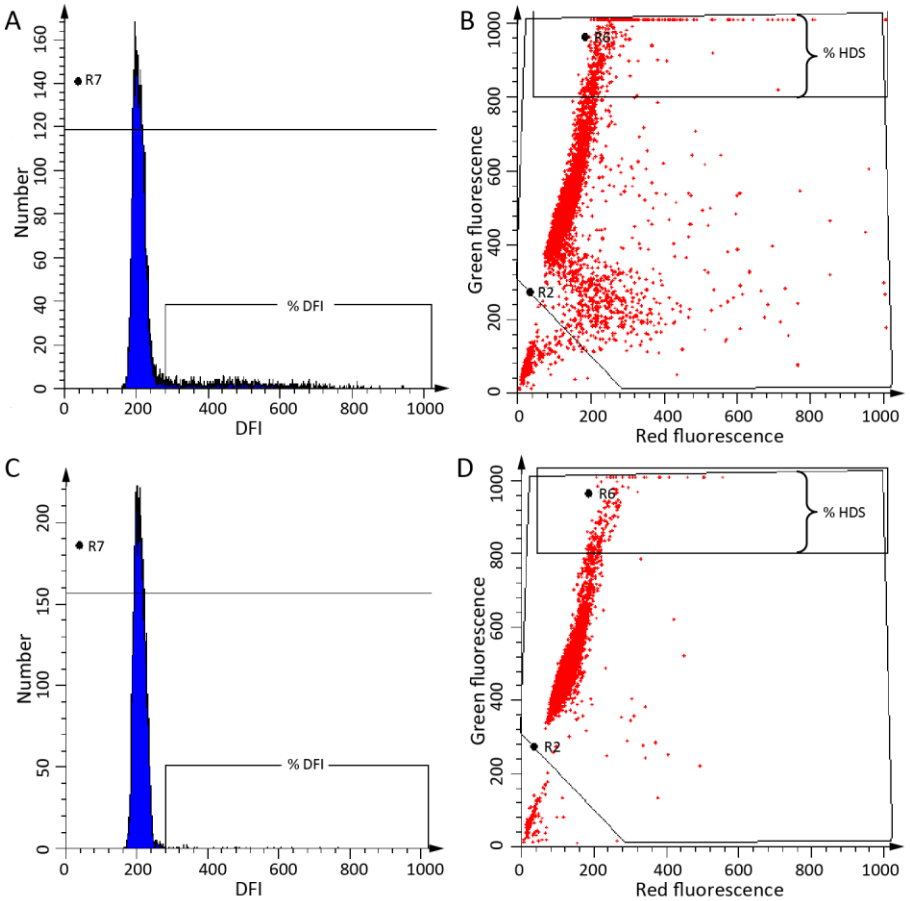


**Fig. S2** Representative sperm chromatin structure assays (SCSAs) for 10 min human sperm experiment with 7.5 mm device. (A) A human sperm chromatin structure assay histogram indicating the population of sperm with DNA fragmentation (%DFI) for raw semen. (B) A human sperm chromatin structure assay cytogram indicating the population of sperm with high DNA stainability (%HDS) for raw semen. (C) and (D) show analogous assay results for selected sperm collected from the device. Green fluorescence indicates very low levels of DNA fragmentation and red fluorescence indicates high levels of DNA fragmentation.

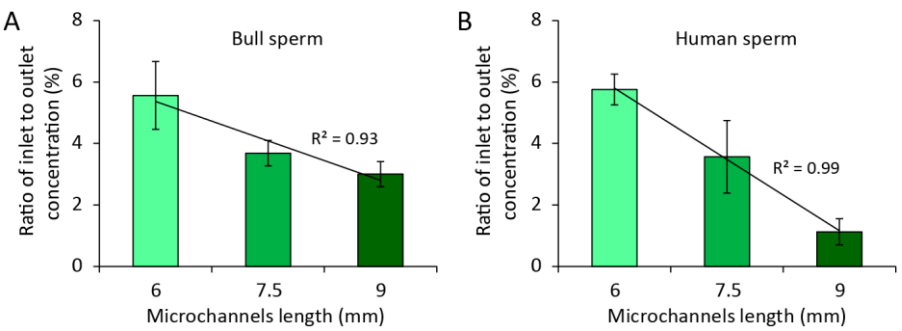


**Fig. S3** Percentage ratio of inlet to outlet concentration. Percentage ratio of inlet to outlet concentration for both bull (A) and human (B) sperm (n≥4) for 6 mm, 7.5 mm, and 9 mm device runs. It is noteworthy that the concentrations show a consistent trend in both human and bull sperm tests. Due to the 10-fold difference inlet and outlet sample volume, however, it is important not to confuse the concentration ratio plotted with selection rate (i.e. count of sperm selected versus count of sperm injected).

**Table S1** DFI and HDS results (n=4).

| Parameters (n=4) | 10 min | | 15 min | | 20 min | |
|---|---|---|---|---|---|---|
| | Raw | Outlet | Raw | Outlet | Raw | Outlet |
| Concentration×$10^6$/mL | | | | | | |
| Mean ± s.e | 118±18.9 | 4.12±1.47 | 107±22.7 | 4.91±1.81 | 117±24.4 | 6.05±1.97 |
| (range) | (185-71) | (8.05-1.19) | (177-58) | (7.9-0.23) | (185-71) | (9.6-2.8) |
| % DFI | | | | | | |
| Mean ± s.e | 10.9±2.54 | 3.37±2.28 | 7.29±1.46 | 1.26±0.49 | 9.41±2.69 | 1.78±1 |
| (range) | (16.68-2.67) | (10.18-0.55) | (12.37-3.83) | (2.24-0.64) | (14.18-2.67) | (3.89-0.44) |
| % HDS | | | | | | |
| Mean ± s.e | 4.21±0.77 | 0.86±0.51 | 5.22±0.44 | 0.62±0.3 | 3.58±0.59 | 0.34±0.14 |
| (range) | (6.72-1.85) | (2.37-0.19) | (6.64-4.27) | (1.19-0.15) | (4.42-1.85) | (0.61-0.13) |