

Electronic Supplementary Information

Live sperm trap microarray for high throughput imaging and analysis

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Estimation on the diffusion time of acridine orange (A.O.) molecule

The diffusion time (t) was estimated using the following relationship:

$$t \approx \frac{x^2}{D}$$

where x is the mean distance traveled by the diffusing molecule and D is the diffusion coefficient of the molecule. Here, x was set to 50 μm and D was set to $4.2 \times 10^{-6} \text{ cm}^2/\text{s}$ in bulk water at 20 $^\circ\text{C}$.

Table S1. Diffusion coefficients for various cell staining dyes [1]

Dye	D [cm^2/s]	T [$^\circ\text{C}$]
Alexa 488	414	25
Alexa 546	341	-
eGFP	95	25
Fluorescein	436	25
Rhodamine 6G	426	25

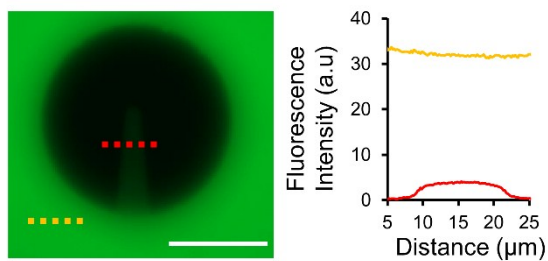


Fig. S1 Fluorescence image of a trap and the intensity measured inside (red) and outside (yellow) of trap. The difference in intensity demonstrates that trap height is much lower than the main pillar height

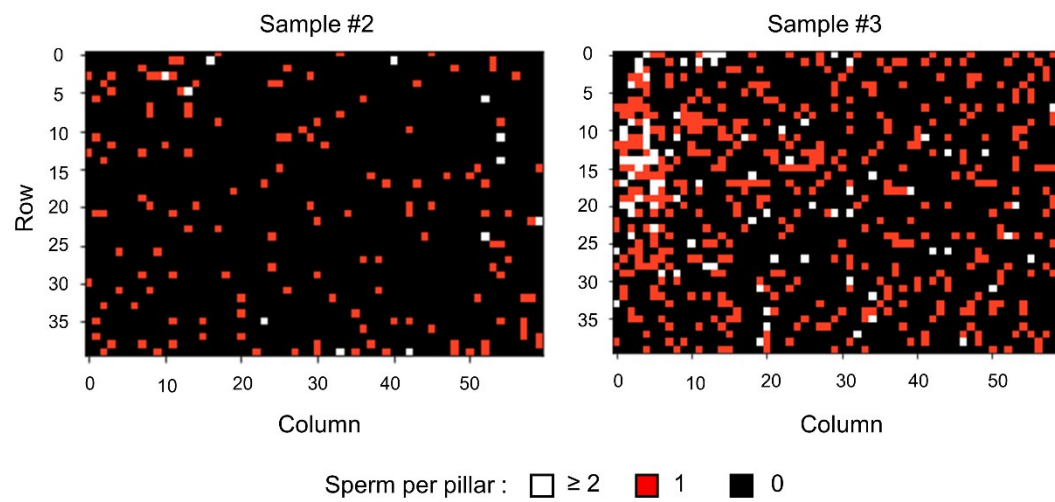


Fig. S2 Mapping of pillars with sperm for trial #2 and trial #3

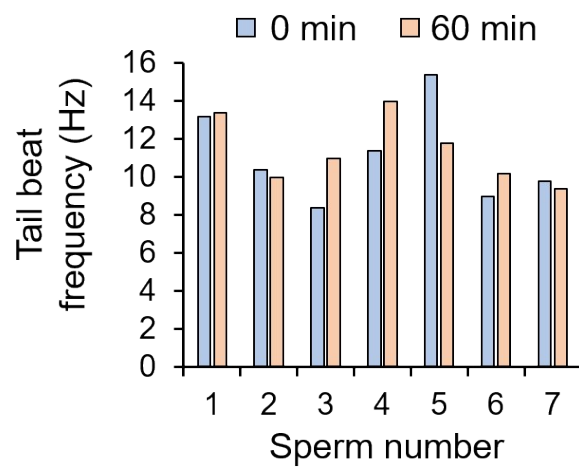


Fig. S3 Tail beating frequency of 7 individual sperms at 0 min (blue) and at 60 min (orange) after trapping

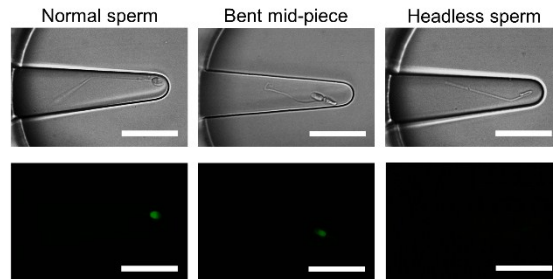


Fig. S4 Brightfield (top) and fluorescence (bottom) of normal sperm, sperm with bent mid-piece and headless sperm

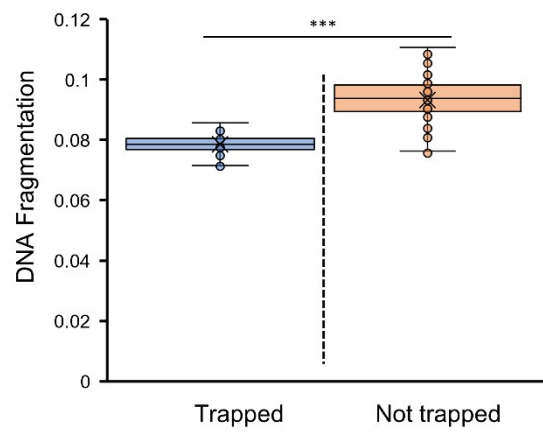


Fig. S5 DNA fragmentation index of trapped and not-trapped sperm (n = 48 for both) *** $p < 0.001$;

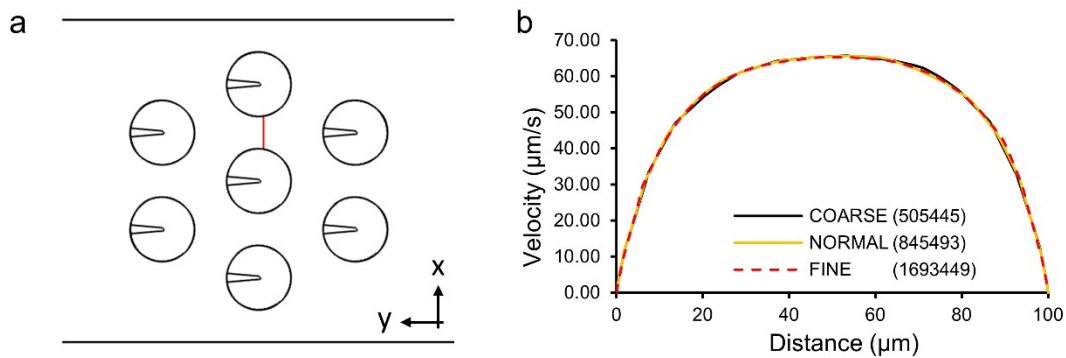


Fig. S6 a) Location where the velocity was measured for mesh independence study b) Mesh independence study on the velocity of liquid inside the trap microarray. Predefined COARSE (mesh size = 505445), NORMAL (mesh size = 845493) and FINE (mesh size = 1693449) were used;

Movie S1. Tail beating of sperm with bent mid-piece in the trap

Movie S2 ~ 4. Tail beating of headless sperms in the trap

References

1. Z. Petrášek, P. Schwille, *Biophys. J.* 2008, **94**, 1437.