Electronic Supplementary Material (ESI) for Nanoscale.


# Supporting Information

## Nanomechanical sensors for direct and rapid characterization of sperm motility based on nanoscale vibrations

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## Supplementary Material and Methods

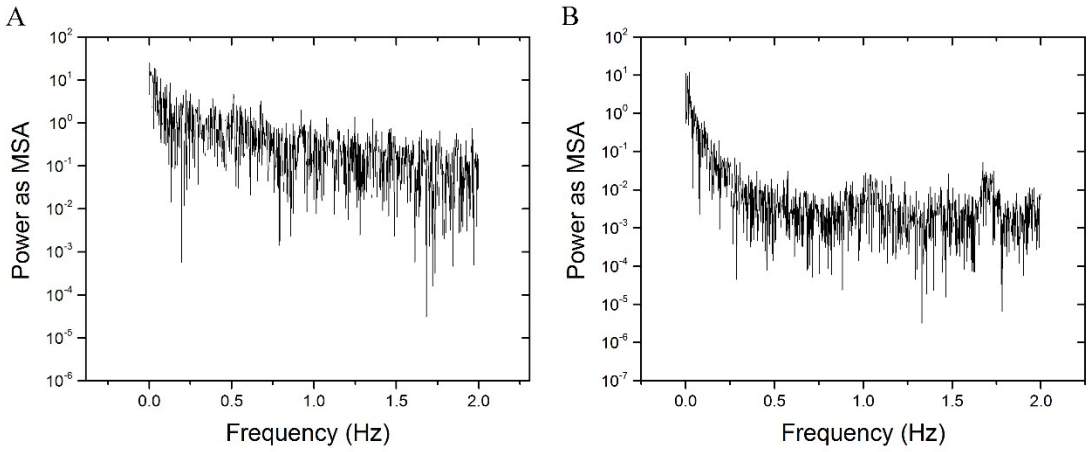
### Microscope observation of alcohol efficacy upon sperm motility

The effect of alcohol upon sperm motility was directly observed using a conventional optical microscope (Cinv Optical Instrumensts CO., LTD, Suzhou, China). Fertilization medium (200 μL) containing sperms (~$1\times10^7$ cells) was added to a well of a 96-well polystyrene microtiter plate. Movements of the sperm in the microplate well were recorded by the optical microscope (Movie S4). Then, 22 μL of alcohol was added into the well, to produce an alcohol concentration of approximately 10%. Ten minutes after the addition of alcohol, sperm movements were re-recorded (Movie S5).

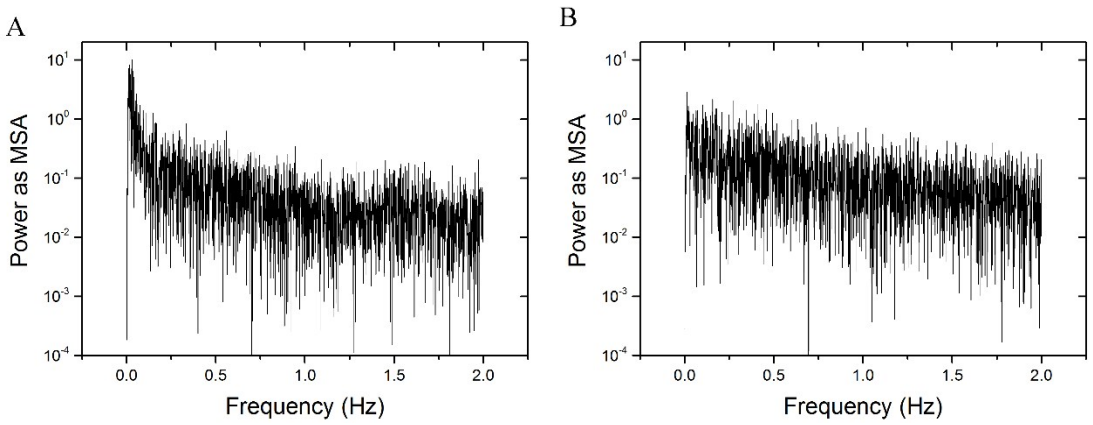
### Optical characterization of the alcohol efficacy upon attached sperm on the cantilever

HA-coated cantilever was placed in a well of the 96-well polystyrene microtiter plate, and then Fertilization medium (200 μL) containing sperms (~$1\times10^7$ cells) was added to the well. After exposure in the sperm solution for approximately one hour with a binding a sufficient number of sperm, 2 μL of alcohol was added into the well, to produce an alcohol concentration of approximately 1%. The 96-well polystyrene microtiter plate was placed under an optical microscope (Cinv Optical Instrumensts CO., LTD, Suzhou, China). A microscopic video of 30 seconds was collected (Movie S6). After 40 minutes of the addition of the alcohol, another microscopic video of 30 seconds was collected (Movie S7). Comparing these Videos disclosed that the motility

of sperm on the cantilever was reduced and the number of sperm attached to the sensor remains constant during the experiment.

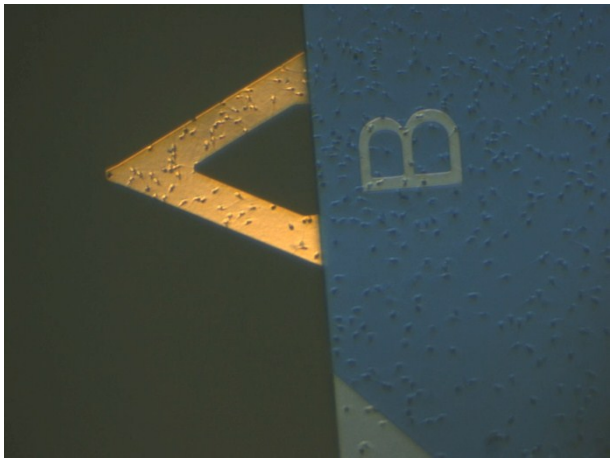



**Supplementary Figure 1. Spectral analysis of the fluctuation curve for sperm treated with alcohol.** (A) Power spectrum of the fluctuation curve in the step prior to the injection of alcohol (step: "Fertilization medium") in Figure 3c. (B) Power spectrum of the fluctuation curve in the step after the injection of alcohol (step: "Alcohol 10%") in Figure 3c.

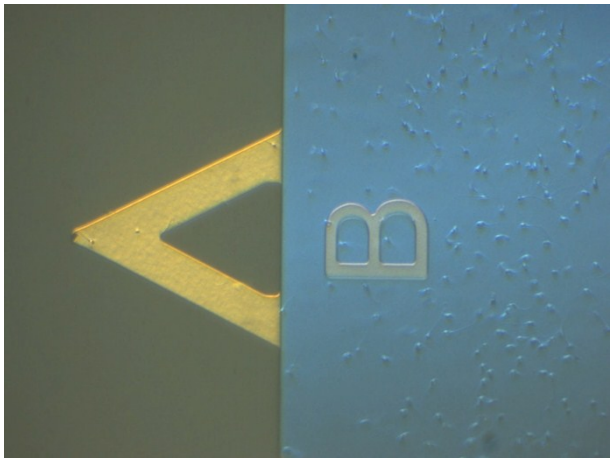



**Supplementary Figure 2. Spectral analysis of the fluctuation curve for sperms treated with SperMagic.** (A) Power spectrum of the fluctuation curve in the step prior to the injection of SperMagic (step: “Fertilization medium”) in Figure 4b. (B) Power spectrum of the fluctuation curve in the step prior to the injection of SperMagic (step: “SperMagic”) in Figure 4b. The concentration of the injected SperMagic was 0.25%.

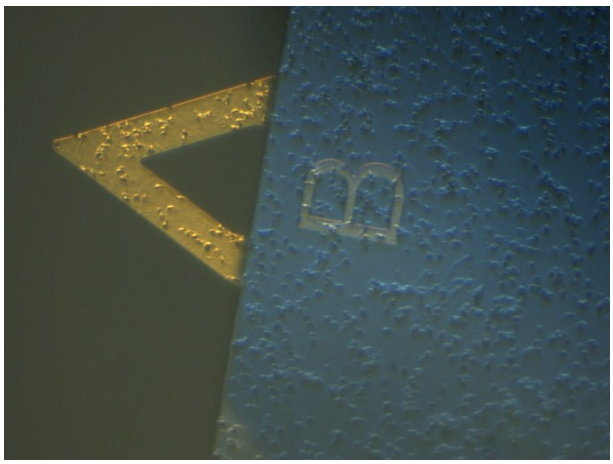
**Still Image for Movies and Movie Captions**



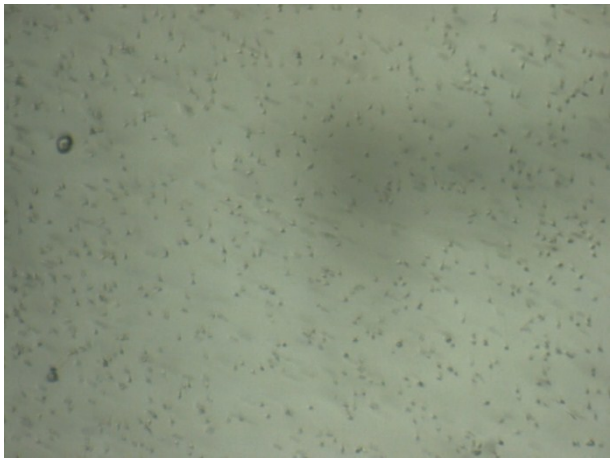
Movie S1: Microcantilever with living sperm adsorbed on its surface.



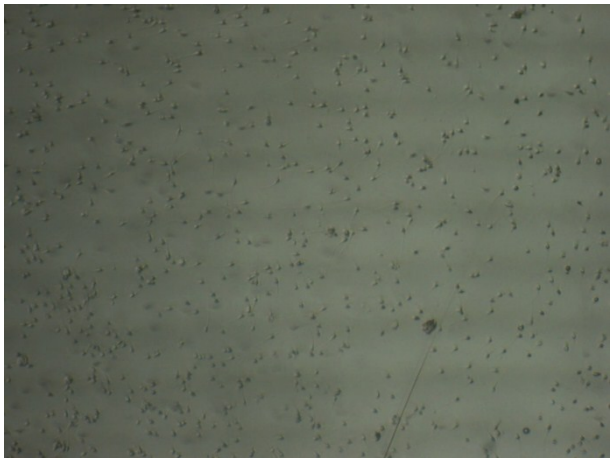
Movie S2: Microcantilever without sperm on its surface.



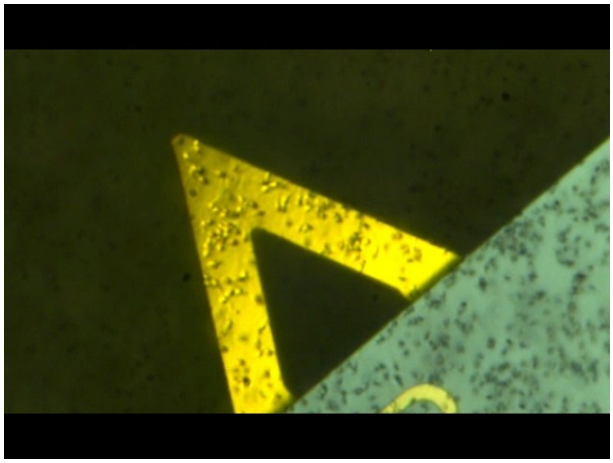
Movie S3: Microcantilever with dead sperm on its surface.



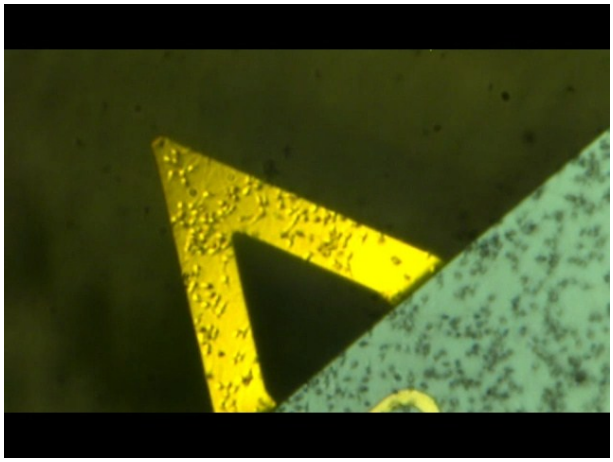
Movie S4: Microscope observation of movements of the sperm.



Movie S5: Microscope observation of movements of the sperm after treated with alcohol.



Movie S6: Microcantilever with living sperm adsorbed on its surface before treated with alcohol.



Movie S7: Microcantilever with living sperm adsorbed on its surface after treated with alcohol.