Supplementary Materials for

Simultaneous Morphology, Motility and Fragmentation Analysis of Live Individual Sperm Cells for Male Fertility Evaluation

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Figs. S1 to S5
Movies S1 to S2 – Captions

Other Supplementary Materials for this manuscript include the following:

Movies S1 to S2

Fig. S1

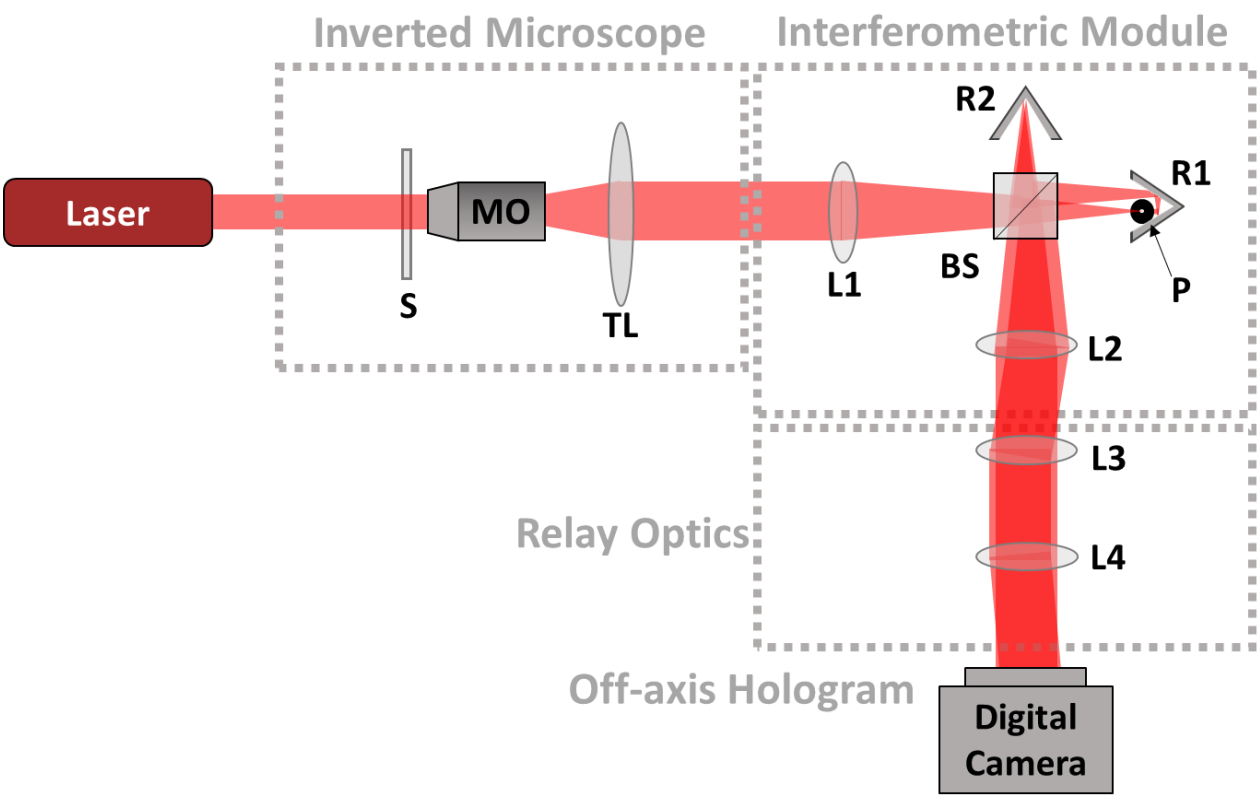
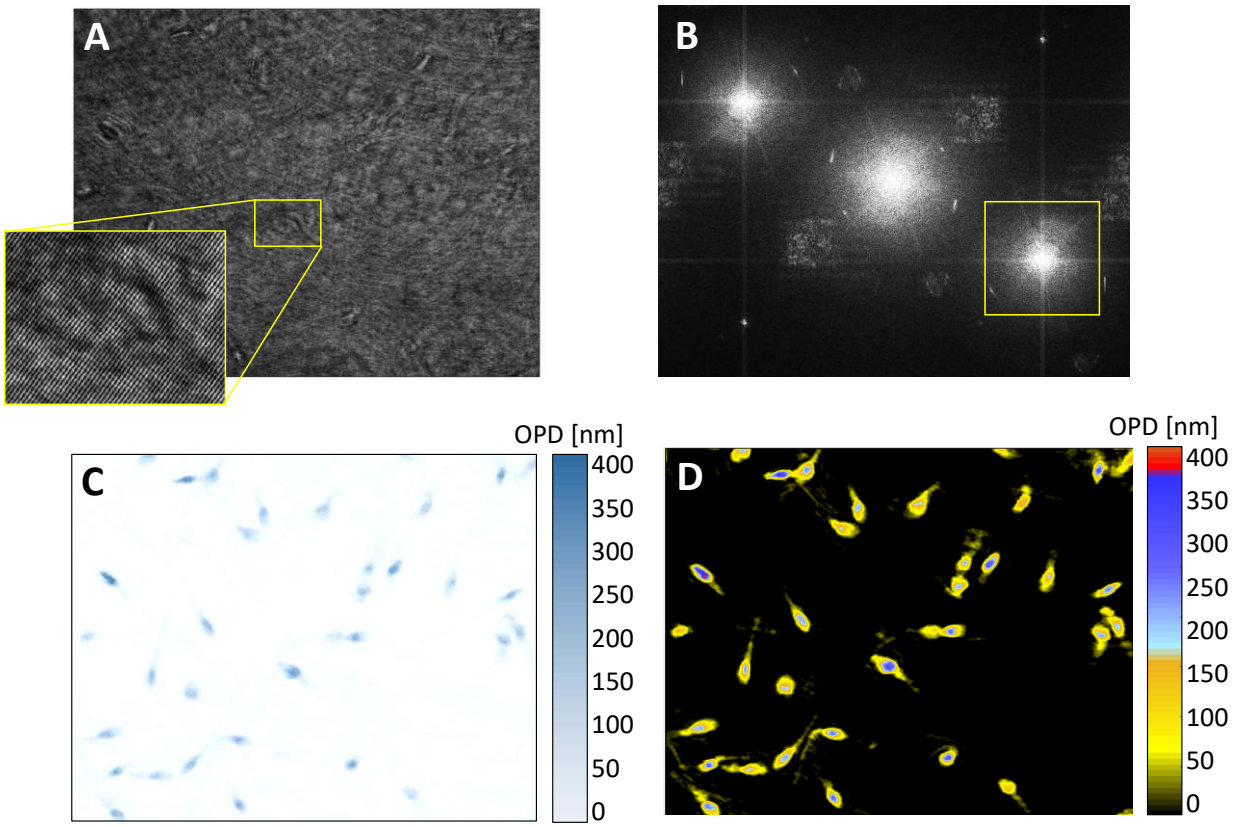


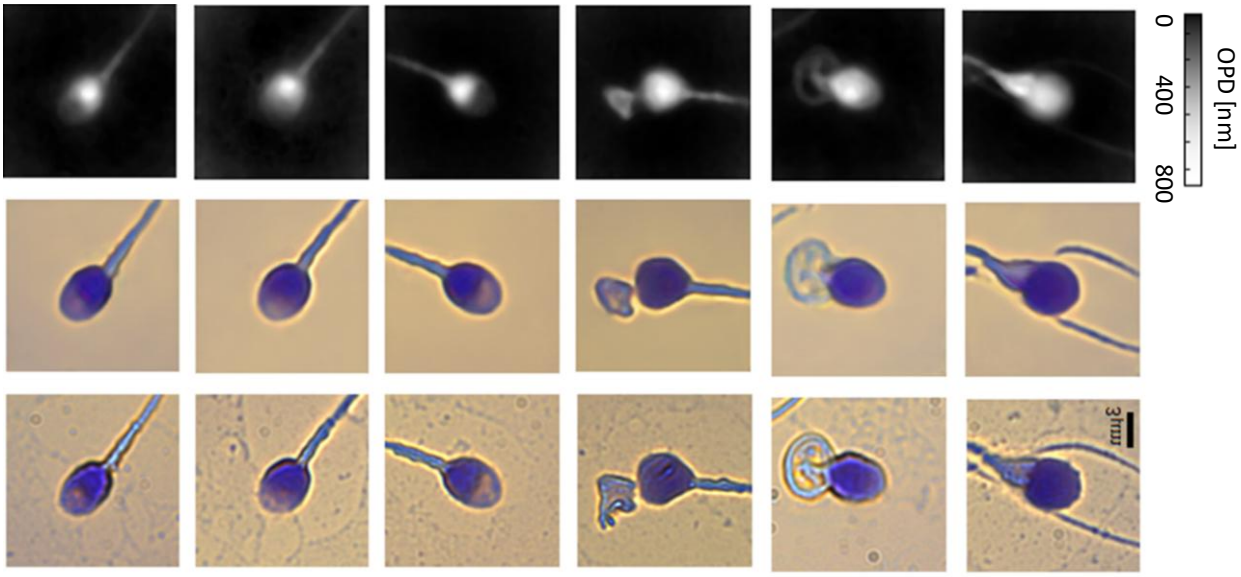
Diagram of internal design of the optical system. S, sample; MO, microscope objective; TL, tube lens; L1, L2, L3, L4, lenses; BS, beam splitter; RR1, RR2, retro-reflector mirrors; P, Pinhole.

Fig. S2



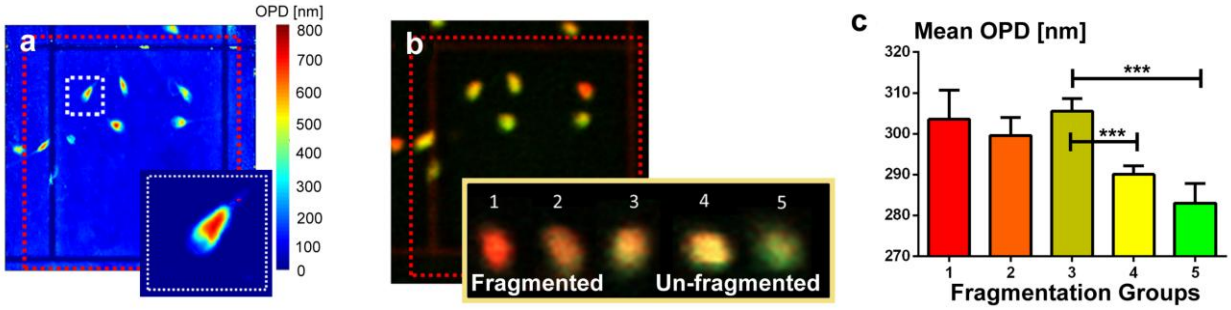
Dynamic quantitative phase imaging of rapidly swimming sperm cells. (A) Off-axis hologram acquired by the digital camera without chemical staining in the full frame rate of the camera. **(B)** Spatial power spectrum, with the selected cross-correlation term marked by a yellow square. **(C)** The stain-free OPD (optical thickness) profile obtained by processing the cropped cross-correlation term. **(D)** Parametric coloring of the OPD profile, resembling HEMA chemical staining. See dynamics in Supplementary Movie S1.

Fig. S3



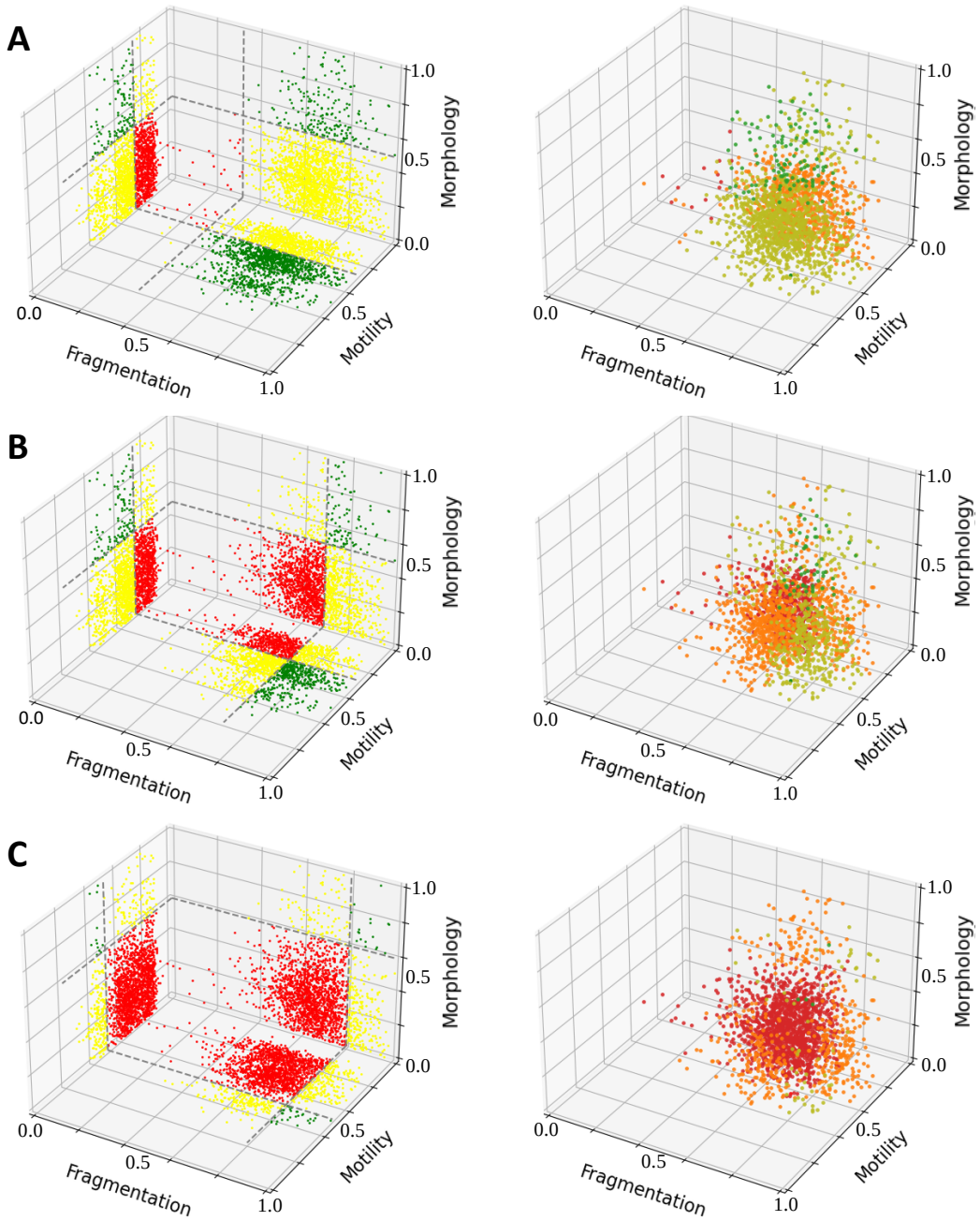
Morphological virtual staining of individual sperm cells by deep learning. We used the stain-free quantitative phase profiles of the cells as inputs to a neural network that performed the virtual-staining mapping, after being trained with the coinciding chemical staining images. The first row shows the quantitative phase images extracted from the single holograms. The second row shows the coinciding virtual stained images, generated by the network. The third row shows the coinciding bright-field chemically stained images of the same sperm cells that the network did not see; yet it could present the cells as if they were stained, as shown in the second row, based only on the stain-free quantitative phase images shown in the first row. The first three columns show normal morphology cells, and the last three columns show pathological cells.

Fig. S4



Stain-free DNA fragmentation measurements, used as the database of the deep learning classifier. (A), Sperm OPD map of sperm cells obtained without staining. **(B),** The same sperm cells, later chemically stained by acridine orange, indicating the sperm cells with fragmented DNA by the emitted color. **(C),** Mean OPD of different DNA fragmentation groups (*** p value < 0.001). A database of pairs of images, example of which are shown in **(A)** and **(B)**, have been used for training the deep neural networks that can classify live cells by their fragmentation group without chemical staining and for DNA-fragmentation virtual staining.

Fig. S5



Cell classification for Donor 5 using different thresholds. Left: Projections on the 2-D planes. Green cells pass both criteria on the plane, yellow cells pass only one criterion, and red cells pass none. Right: 3-D

locations of each cell. Green cells pass all criteria and are qualified as good sperm cells, olive cells pass only two out of the three criteria, orange cells pass only one and red cells fail in all. **(A)** Morphology, motility and fragmentation thresholds of 0.5, 0.29 and 0.3, respectively. **(B)** Thresholds of 0.5, 0.29, 0.7. respectively. **(C)** Thresholds of 0.6, 0.5, 0.8, respectively. See dynamic threshold changing in Supplementary Movie S2.

Movie S1

Demonstration of the dynamic OPD profile of live sperm cells, as well as tracking and feature extraction. This video shows the following scenarios in order; The dynamic OPD profile while displaying the tracked cell IDs. After a certain cell is chosen for inspection, the ID display is turned off, the motility features for the chosen cell are shown. Next, after dismissing the cell and moving to another field of view, another cell is chosen and investigated. There, DNA fragmentation features are first shown while also showing the acridine-orange virtually stained cell, followed by morphological features while also showing the morphologically virtually stained cell.

Movie S2

Cell classification for Donor 5 using dynamic thresholds. Left: Projections on the 2-D planes. Green cells pass both criteria on the plane, yellow cells pass only one criterion, and red cells pass none. Right: 3-D locations of each cell. Green cells pass all criteria and are qualified as good sperm cells, olive cells pass only two out of the three criteria, orange cells pass only one and red cells fail in all. The video shows which cells should be chosen for various thresholds selected, while in the beginning just the WHO2010 morphology threshold is changed, then the DNA fragmentation threshold, and finally the motility threshold.