Supplementary Information to manuscript B927012D

Confocal Raman microspectroscopy as a quick and powerful analysis tool to assess the mitochondrial status in human spermatozoa

Konrad Meister, Diedrich A. Schmidt, Erik Bründermann, and Martina Havenith

Lehrstuhl für Physikalische Chemie II, Ruhr-Universität Bochum

Universitätsstr. 150, D-44801 Bochum, Germany

Depth-profiling of human spermatozoa:

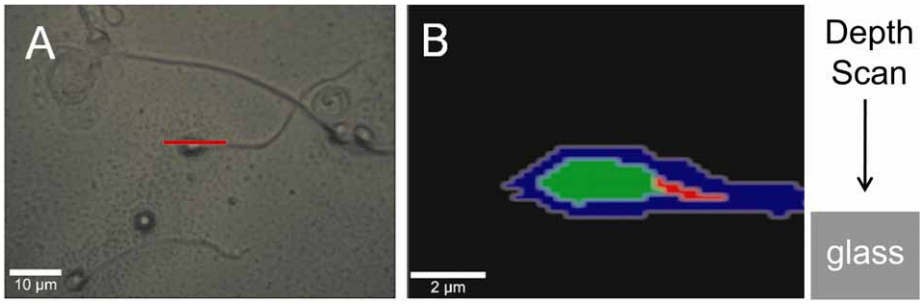

Fig. S1 Panel A shows a bright field optical image (top view) of human sperm cells with a scale bar of 10 µm. Panel B shows the chemical map constructed from Raman measurements taken on a single cell in vertical direction (side view). The chemical image is based upon a k-means cluster analysis from 400-3400 cm^{-1}. The nucleus (green) and middle piece (red) can be identified. The blue cluster corresponds to the overall image of the cell. The red scale bar indicates the horizontal scan positions of the vertical scans.

Fig. S1 shows the optical image of human sperm cells and the corresponding Raman image in a vertical cross-section. The chemical map represents a depth slice through the cell. Confocal Raman microspectroscopy allows lateral and vertical scan measurements in the x,y, and z-direction, respectively. A three dimensional image of a single cell can be measured.

Raman map of another human spermatozoon with higher spatial resolution:

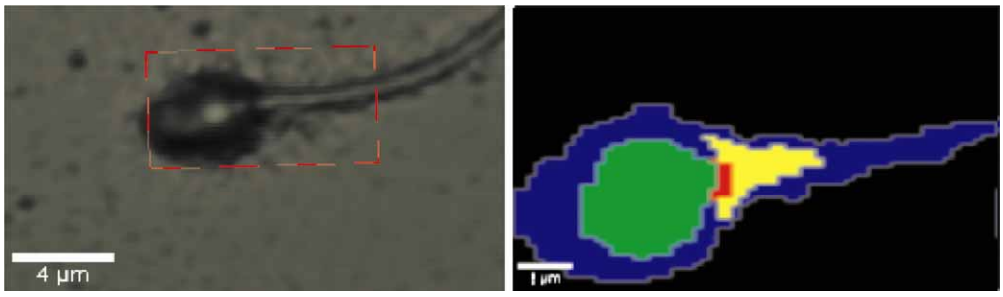

Fig. S2 Panel A shows a bright field optical image of a human sperm cell with a scale bar of 4 µm. Panel B shows the chemical map constructed from Raman measurements taken on a single cell within the red square as indicated in Panel A. The chemical image is based upon a k-means cluster analysis from 400-3400 cm^{-1}. The nucleus (green) and neck (red) and the middle piece (yellow) can be identified.

Additional raw-data Raman mapping of human spermatozoa:

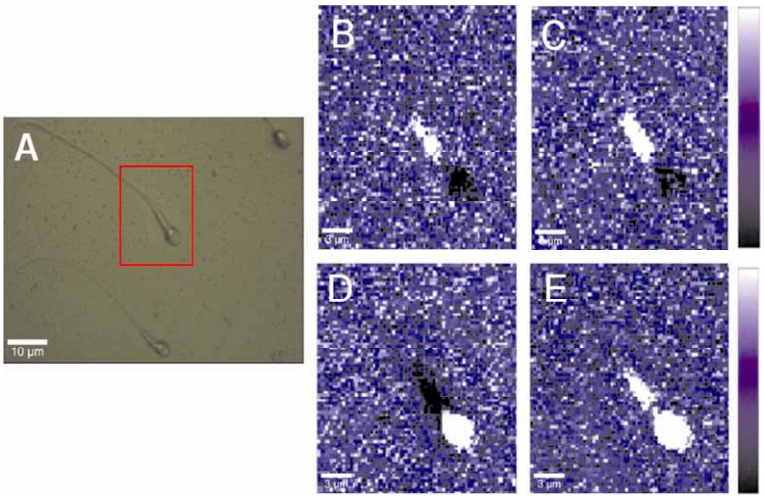

Fig. S3 A: Optical image of human sperm cells. Panel B, C, D, and E show the integration of raw data over the Raman bands at (1602 ± 2) cm-1, (751 ± 2) cm-1, (788± 2) cm-1, and (1575 ± 2) cm-1. The scale bar for the Raman measurement images is 3 µm.

Fig. S3 shows Raman images which result from the integration over characteristic Raman bands at (751 ± 2) cm^{-1} (C), (788± 2) cm^{-1}(D), and (1575 ± 2) cm^{-1} (E). A forth band at (1602± 2) cm^{-1} (B) was chosen to investigate whether this band corresponds to mitochondrial structures. Using this spectral information allows mapping the distribution of this band with high resolution and shows that the 1602 cm^{-1} band is found within the mitochondria-containing middle piece. However, a superposition with another band around 1585 cm^{-1} which has an influence on the intensity distribution can not be completely excluded.

Average nucleus spectra from k-means cluster analysis for different UV exposure times:

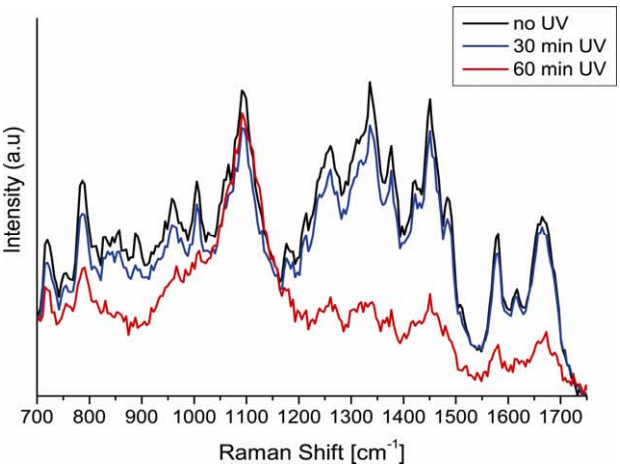

Fig.S4 Raman spectra derived from the k-means cluster analysis of the nucleus cluster after 0 min, 30 min and 60 minutes UVA exposure.

In Figure S4 the Raman spectra taken within the nucleus are shown. Due to the UV exposure and the cellular damage the overall intensity of the Raman bands decreases.

Determining the signal-to-noise ratio for different integration times:

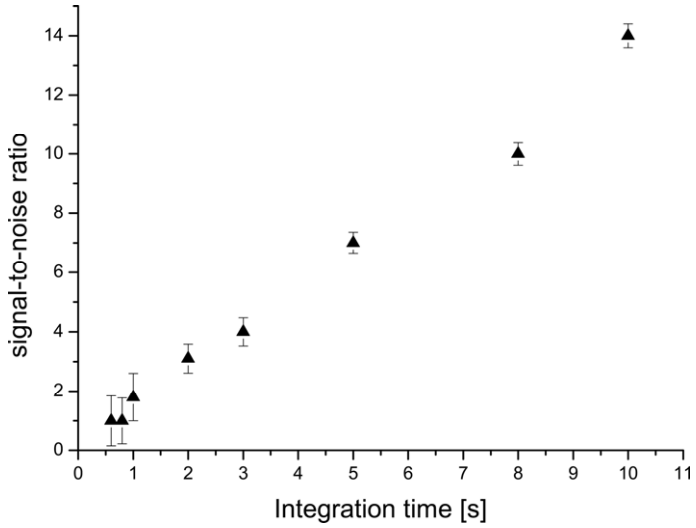

Fig. S5 Decreasing signal-to-noise ratio of the 788 cm-1 band as a function of total integration time for a single pixel.

Fast diagnostic methods require a short integration and measurement time per pixel. Fig. S5 shows a time series for the signal-to-noise ratio of the 788 cm^{-1} Raman band in reference to the noise background of the CCD detector. The minimum integration time can be as low as 1 s which can be used to assess the mitochondrial status from raw spectral data without using clustering methods.