LIVE-CELL MULTI-COLOUR 3D SIM OF HUMAN SPERMATOZOA

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The spermatozoon (or sperm cell) is the reproductive cell in males, contributing about half the genetic material to offspring. Although a range of morphological variations exist among different spermatozoa, healthy and fertile cells have a characteristic shape comprising a DNA containing head (~ 3 µm wide and 5 µm long), and an about 50 µm long tail vigorously propelling the head forwards. The propeller is powered by closely packed mitochondria in the neck piece, connecting the head and tail. The morphology and motility of sperm are good indicators of fertility, although much remains to be understood about sperm morphometry´s potential in diagnosis and sperm selection for improving outcomes of fertility treatment[1].

While the most common male fertility criterion has been considered to be the swimming speed of sperm cells, this could be correlated with sperm morphology to better understand the swimming mechanics. Considering the importance of energy to the vigorously propelling tail, it seems reasonable to expect an association between sperm motility and morphology of its mitochondria, the energy releasing organelle. In spermatozoa, mitochondria are exceptionally small (~ 0.1 µm diameter) and densely packed, making them a challenging target for optical microscopy. Optical nanoscopy of living sperm cells is complicated because of their small size, fast motion, and the toxicity of commonly used fluorescent labels. Keeping these considerations in mind, we opted for structured illumination microscopy (SIM) for imaging living spermatozoa. Since common labelling techniques like immunolabelling and genetic introduction of fluorescent fusion proteins are impractical when studying living human sperm cells, we tested many of the remaining alternatives (e.g. MitoTracker, JC-1, NAO, PicoGreen and CellMask) with emphasis on mitochondria, DNA and cell membrane of spermatozoa. Some probes (like MitoTracker and CellMask) worked fine for 3D SIM, while others (e.g. NAO) were less suitable for 3D SIM, but worked well for conventional deconvolution microscopy.

In this work, we provide an overview of sub-cellular anatomy of living human spermatozoa obtained using 3D SIM, and the protocols for sample preparation. To the best of our knowledge, this is the first study of living spermatozoa using optical nanoscopy.

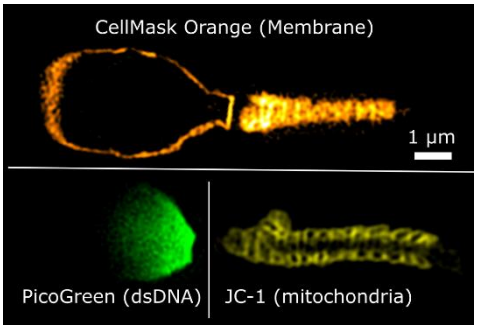

Figure 1: Living human Spermatozoon imaged using 3D SIM. Images are single z-slices.

1. García-Vázquez, F.A., et al., *Importance of sperm morphology during sperm transport and fertilization in mammals.* Asian Journal of Andrology, 2016. **18**(6): p. 844-850.