Spermiogenesis in 3D: Pitfalls and Challenges

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In recent years, there has been a large breakthrough in imaging and data processing methods. One of them, volume imaging, presents a powerful tool to visualize (sub)cellular structure in its tissue context and enable us to explore its spatiotemporal changes. Moreover, using serial sections and 2D microscopy can lead to loss of information and incomplete knowledge of dynamics of(sub)cellular processes, e.g. spermiogenesis. So far, an atlas of sperm development in 3D is still missing even though the stages of spermiogenesis are well-known and described in testicular sections. In our research, we utilized the CLARITY clearing technique which provides great optical properties and mechanical support to our samples. We utilized existing protocols to detect individual stages of spermiogenesis in individual seminiferous tubules (iSTs) under transmitting light. Next, we detected and analysed DAPI (nuclei) and PNA (acrosomes) signals in iSTs using confocal microscopy (LSM 880).

Even though we obtained optically transparent and well-stained samples, we faced serious issues during the data processing. We employed commercially available Imaris software to render and statistically evaluate surface-rendered nuclei from individual stages of spermiogenesis. Leading information for the surface creation is the signal intensity. Therefore, nucleoli are more likely to be rendered than the whole nucleus as their staining is more intense. This results in an artificially increased number of nuclei in the sample. Secondly, iST contains various cells with different sizes and shapes of the nucleus (15 µm vs 5 µm, round shape vs oval). This poses a challenge for proper filter settings to capture all nuclei as individual objects.
In the presented work, we focused on optimizing surface rendering in Imaris software, so it is suitable to describe spermiogenesis. This approach can lead to new insights into mechanisms behind physiological and pathological spermiogenesis and help us understand male infertility. Moreover, we compared the surface rendering abilities of Imaris with various software using diverse algorithms to visualize individual steps of spermiogenesis in 3D.

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