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In Situ Volume Imaging of Cleared Mice Seminiferous Tubules Opens New Window to Study Spermatogenic Process in 3D

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Abstract : Studying the tissue structure and histogenesis in the natural, 3D context is challenging but highly beneficial process. Contrary to classical approach of the physical tissue sectioning and subsequent imaging, it enables to study the relationships of individual cellular and histological structures in their native context. Recent developments in the tissue clearing approaches and microscopic volume imaging/data processing enable the application of these methods also in the areas of developmental and reproductive biology. Here, using the CLARITY tissue procedure and 3D confocal volume imaging we optimized the protocol for clearing, staining and imaging of the mice seminiferous tubules isolated from the testes without cardiac perfusion procedure. Our approach enables the high magnification and fine resolution axial imaging of the whole diameter of the seminiferous tubules with possible unlimited lateral length imaging. Hence, the large continuous pieces of the seminiferous tubule can be scanned and digitally reconstructed for the study of the single tubule seminiferous stages using nuclear dyes. Furthermore, the application of the antibodies and various molecular dyes can be used for molecular labeling of individual cellular and subcellular structures and resulting 3D images can highly increase our understanding of the spatiotemporal aspects of the seminiferous tubules development and sperm ultrastructure formation. Finally, our newly developed algorithms for 3D data processing enable the massive parallel processing of the large amount of individual cell and tissue fluorescent signatures and building the robust spermatogenic models under physiological and pathological conditions.

Keywords : CLARITY, spermatogenesis, testis, tissue clearing, volume imaging
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