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Implementation of Tissue Clearing Protocols for Ex-vivo Analysis of Renal Tissue

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OTC techniques have been of ground-breaking importance for the 3D imaging of whole organs and whole-organism and have disclosed detailed information about intact biological systems at cellular and subcellular level. In several studies the use of OTC for visualization, mapping and quantification of tissue biopsies, has proven to be crucial for identifying pathological features. Since OTC protocols are often based on time-consuming and cumbersome procedures and require expensive or toxic reagents, there is a genuine need for further optimization. Moreover, the large amount of information deriving from 3D optical imaging demands a deep expertise for data handling and the data analysis.
In this study, we optimized existing OTC techniques by focusing on the following major aspects: reduction of throughput time for sample preparation, implementation of staining strategies, improving of tissue handling, obtainment of suitable data for morphometric analysis of the kidney. By optimizing existing methods, we were able to visualize and analyse renal structures that play a key role in the kidney function such as peritubular capillaries, glomeruli and podocytes. Interestingly, by selecting new fluorescent biomarkers, we provide novel solutions to stain the whole renal vasculature as an alternative to expensive and time-consuming options. In addition, we replaced some shortcomings in previously described tissue clearing methods by faster and more easy-to-perform protocols. However, the future application of these techniques for the assessment of pathophysiological conditions will require additional optimisation in terms of microscope systems innovation and automated strategies for processing 3D data in order to accomplish a more accurate and less time consuming image acquisition and analysis. Yet, the optimization of clearing technologies that was carried out in this project offers novel insights on the application of these techniques in kidney research and opens new avenues for advancing the diagnostic field, by reducing the complexity of the currently available procedures.