**OPTICAL SUPER-RESOLUTION MICROSCOPY OF THE KIDNEY – A PRACTICAL GUIDE**

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**ABSTRACT:** In the diagnosis research of renal impairments, immunofluorescence microscopy is routinely used. However, to investigate the fine subcellular structure of the kidney’s glomerular filtration barrier, the resolution of this technique is insufficient. In contrast, the high resolution afforded by electron microscopy comes at the cost of poor preservation of immunogenic epitopes. Furthermore, antibody penetration and acquisition throughput are limited. Some of these drawbacks can be overcome with Super-Resolution (SR) microscopy methods. So far, the SR techniques Single Molecule Localization Microscopy (SMLM), Stimulated Emission Depletion (STED) microscopy, Structured Illumination Microscopy (SIM), and Expansion Microscopy (ExM) have been reported in studies of the kidney [1]. However, preservation methods and labelling strategies varied widely in these investigations. In this work [2], all four techniques were performed and critically compared (Figure 1) on kidney slices obtained from mouse tissue, treated with the most commonly used preservation technique: formalin-fixed, paraffin-embedded (FFPE). The strengths, weaknesses and practicalities of each method are discussed to enable users of super-resolution microscopy in renal research make an informed decision on the best choice of technique. The methods discussed enable the efficient investigation of biopsies stored in kidney banks around the world.

![Figure 1: Comparison of nephrin imaging with different conventional and SR microscopy techniques. Immunolabelling of nephrin enables visualization of the structure of specialized epithelium cells called podocytes, which are essential for filtering the blood.](image)