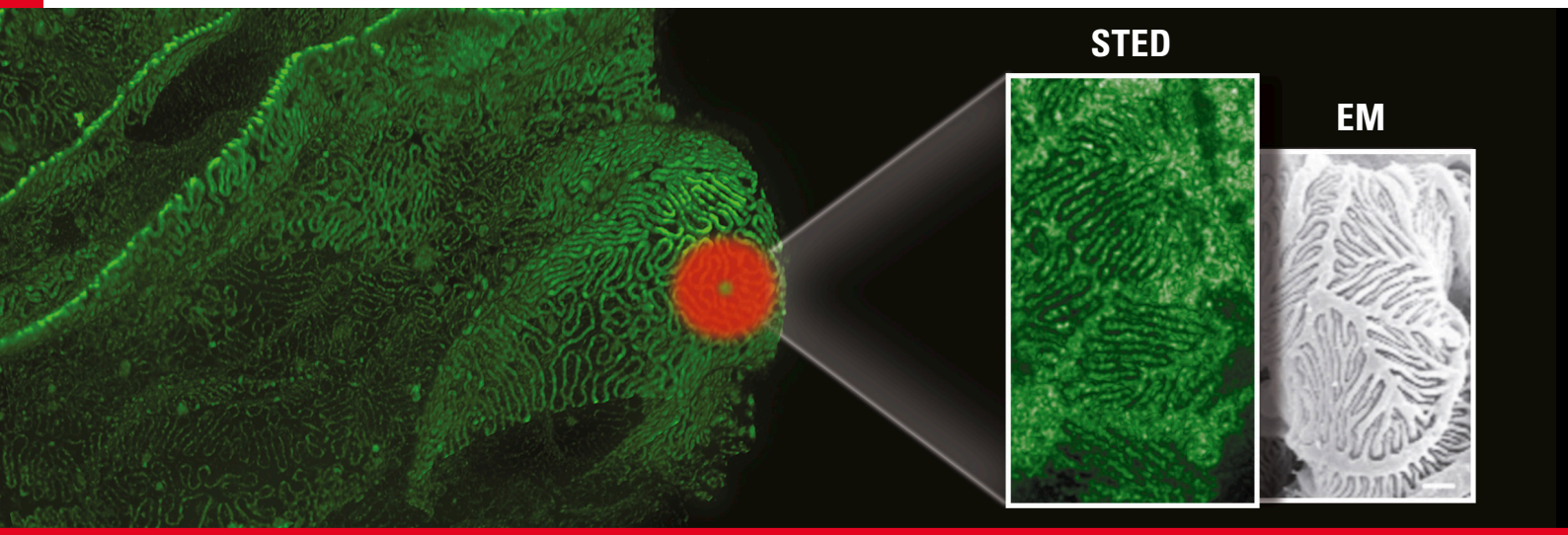
From Eye to Insight

Leica MICROSYSTEMS



Kidney Research: Visualize ultrastructural details by deep 3D Nanoscopy

Resolve nanoscopic structures in cleared tissues deeper than 150 µm

Key player in kidney diseases is the podocyte cell layer in the glomerular filtration barrier. Alterations in the architecture of the podocytes and the basement membrane are linked to the onset of renal failure.

Standard electron microscopy techniques based on elaborated protocols identify glomerular diseases based on two-dimensional information. The combination of optical clearing and 3D STED (Stimulated Emission Depletion) Nanoscopy with the Leica TCS SP8 STED 3X opens a new avenue for imaging the kidney at unprecedented three-dimensional detail in light microscopy.

Even deeper than 150 µm within the tissue the ultrastructural features of the specimen come to light enabled by the new STED WHITE glycerol objective lens. Deep nanoscopy is achieved not only at 23°C, but also under live cell imaging conditions at 37°C with a comfortable working distance of 300 µm.

Forget the hassle of physical sectioning. Enjoy spectral freedom and keep up with the growing palette of fluorescent biomarkers. Be at the forefront of clinical research.

Typical fields of research

- Renal disease
- Glomerulopathies
- Clinical research
- Pathology research
- Pharmaceutical research

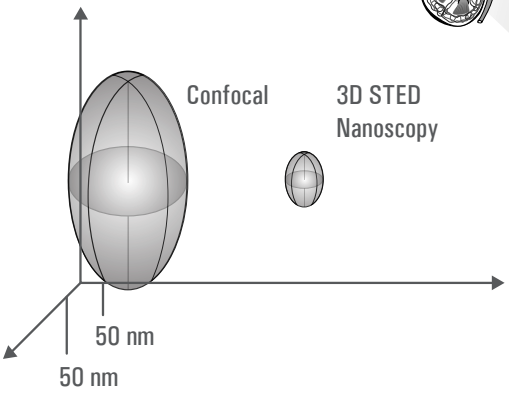
References

- Blom and Widengren., Chem. Rev., 2017, 117 (11)
- Randles et al., Sci. Rep., 2016, 6
- Unnersjö-Jess et al., Kidney Int., 2015, 89 (1)

TCS SP8 STED

*Panel above: Cleared kidney sample revealing the podocyte cell layer; detailed view podocytes by STED and electron microscopy (EM).
Cleared kidney sample courtesy of David Unnersjö-Jess, KTH, Stockholm, Sweden.
Scanning EM image of kidney podocytes courtesy: Center for Microscopy and Image Analysis, Univ. of Zurich, Dr. Urs Ziegler.

3D STED Nanoscopy workflow for cleared kidney samples



Representation of the sampling volume in 3D (point spread function); a smaller sampling volume results in a better optical resolution.

Sample Preparation
Dissect and fix kidney samples.
Embed the sample in hydrogel solution.

Tissue Sectioning
Cut kidney slices (thickness up to 500 µm) using a microtome.

Optical Clearing
Transfer kidney sections to clearing solution. Optical clearing increases antibody and fluorophore penetration and staining quality.

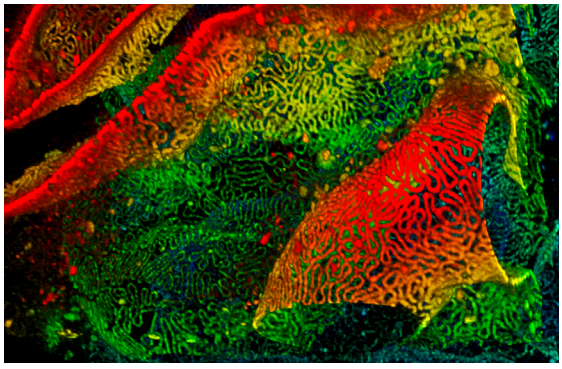
Fluorescence Immunolabeling
Stain kidney sections using immunolabeling protocols and fluorophores suitable for STED with excellent signal-to-noise ratio in cleared samples.

Sample Mounting
Mount samples in fructose with matching refractive index to image with high penetration depth.

3D Nanoscopy with Smart STED Wizard
Resolve the ultrastructure of the glomerular filtration barrier in deeper planes. The Smart STED Wizard allows an easy optimization of the experiments and ensures the best STED performance.

Visualization
Inspect the 3D STED images with the LAS X 3D visualization tool - easy and straightforward.

Leica provides:
The combination of optical clearing and 3D STED Nanoscopy to access structural details far beyond the diffraction limit.



Representative kidney section labeled for Nephrin. 20µm stack depth coded by color. Courtesy of David Unnersjö-Jess, KTH, Stockholm, Sweden

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