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ACUTE KIDNEY INJURY – EXPERIMENTAL

FP174 INTRAVITAL MULTIPHOTON KIDNEY IMAGING USING A CUSTOM DESIGNED MICROSCOPE AND EXTENDED WAVELENGTH EXCITATION LASER

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Introduction and Aims: Intravital multiphoton microscopy is a powerful tool to investigate structure and function in the intact kidney at high resolution, but several issues have constrained its usage to date. Imaging depth is limited to the outer cortex with standard femtosecond excitation laser sources (680-1050 nm) due to strong excitation light scattering, whilst fluorescent probes with emission maxima in the green can be masked by auto-fluorescence (AF) signals. Moreover, recording of dynamic events in vivo can be complicated by movement artefacts. Thus, there is a need for new approaches to overcome these practical hurdles to fully realise the potential of intravital imaging in investigating kidney physiology and disease mechanisms. Newly available extended wavelength excitation lasers can work in the far red range where the scattering is less pronounced thereby offering the possibility to image deeper into tissues and spectrally away from AF signals.
Methods: We have constructed a custom built multiphoton microscope for in vivo kidney imaging, coupled to an extended wavelength excitation laser (680-1300nm). The microscope is modularly designed rendering it flexible for adaption e.g. concerning objectives and detectors. C57Bl/6N mice were used for experiments and were anaesthetised with inhaled isoflurane. All procedures were conducted in accordance with Swiss Cantonal regulations. A jugular vein catheter was used for application of dyes. Kidneys were externalized via a lateral flank incision. Animals were placed on a stage specially designed to reduce transmission of respiratory movements, and kidneys were embedded in agarose to further dampen movements. Fluid-phase and receptor-mediated endocytosis in proximal tubules (PTs) were imaged using fluorescent-labelled small dextrans and/or proteins. Lysosomes and mitochondria were labelled with established probes (Lysotracker and Rhodamine 123). Acute kidney injury (AKI) was induced with sodium maleate (600 mg/kg).
Results: We gained several distinct advantages with this novel set-up. First, we could excite far red probes such as Albumin-Alexa 647, away from masking AF signals, thus increasing the signal to background ratio. Second, with far red probes we were able to image deeper into the kidney than with comparable blue/green/red dyes, enhancing the visualisation of key structures such as glomeruli. Third, we could perform label-free imaging of structures such as collagen and lipid vesicles using second and third harmonic generation signals, respectively. Fourth, we could acquire 4 signals simultaneously (blue, green, red, far red), and could thus image multiple different structures within cells (including endosomes, lysosomes and mitochondria). Fifth, due to enhanced stability of the imaged organ, we could acquire images over prolonged periods of time (up to 5 hours), and discovered that the intracellular environment in PT cells is highly dynamic, with rapid and directed movement of organelles. Real-time changes in organelle function during AKI were also imaged following injections of maleate.
Conclusions: In summary, using a custom-designed multiphoton microscope and extended wavelength excitation, we have made significant improvements to intravital kidney imaging that will enhance the capabilities of this technique in the future investigation of renal physiology and pathophysiology.