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Characterizing Fibrosis in Mouse Kidney using Auto Fluorescence Flim and SHG in UUO Model

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Chronic kidney disease (CKD) arises from a diverse list of renal injuries. Renal fibrosis is considered to be the final common pathway for most forms of CKD and involves glomerular sclerosis and/or interstitial fibrosis. Because of that there is great interest in identifying renal fibrosis in the early stages of CKD to prevent progression. Unilateral ureteral obstruction (UUO) is a well-described model of CKD and renal fibrosis. Kidney injury and fibrosis usually are assessed by histology. Histological methods include Picro-Sirius Red staining, Masson's Trichrome staining or immunohistochemistry for collagen isoforms. Quantitation of fibrosis using histologic techniques may be difficult due to variability in staining and pathologist scoring. The goal of this study is to compare histologic measures of renal fibrosis to Fluorescence Lifetime Imaging (FLIM) and Second Harmonic Generation (SHG) techniques in our deep tissue imaging microscope called DIVER. Male C57Bl6 mice were subjected to UUO of right kidney. At 21 days, both kidneys were collected, fixed, and paraffin-embedded. The uninjured left kidney was used as a control. Serial sections of both kidneys were analyzed by Picro-Sirius Red staining or FLIM with SHG. Quantification of a whole kidney section from the Picro-Sirius Red staining showed $34.32 \pm 0.99\%$ area of fibrosis in the left kidney compare to $5.55 \pm 1.07\%$ in the control kidney. Using the Phasor approach to FLIM, comparisons between the two kidneys show that the auto fluorescence lifetime signature give rise to two well separate phasor clusters. Quantification of ten different fields of view for each kidney from SHG suggests the presence of more collagen I in the diseased kidneys ($17.44 \pm 4.21\%$ compared to $2.59 \pm 1.98\%$ control kidney). Finally the combined FLIM and SHG images let us create a criterion to separate fibrosis directly from the microscope images.