

## SECOND HARMONIC MICROSCOPY TO QUANTIFY RENAL INTERSTITIAL FIBROSIS AND ARTERIAL REMODELING

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We used second harmonic (SHG) microscopy to investigate the three-dimensional heterogeneous accumulation of fibrillar collagen during fibrotic pathologies [1, 2]. We showed that SHG is obtained specifically from fibrillar collagens in tissues, which results in an intrinsically small background in the images and enables sensitive measurements. We proposed fibrosis scores based on the volume density of voxels exhibiting significant SHG signal or alternatively on the SHG averaged intensity. We also developed image segmentation algorithms in order to calculate SHG scores in various morphological regions. For that purpose, we visualized the tissue morphology using endogenous 2-photon excited fluorescence signals excited simultaneously with SHG signals.

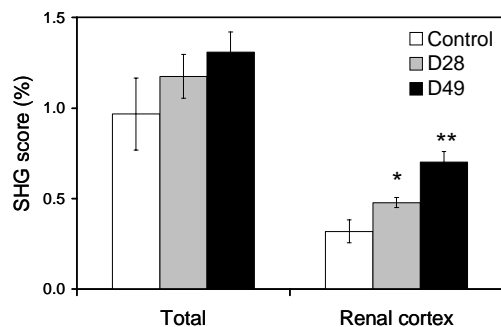


Figure 1: Mean value of the SHG density scores calculated in the whole kidney image or in cortical areas after automated segmentation for control mice versus AngII-infused mice for 28 or 49 days. Cortical scores show smaller values but larger differences upon AngII-induced hypertension (\*  $P < 0.05$  versus control, \*\*  $P < 0.01$  versus control;  $n = 6-8$  per condition)

We focused on a murine model of hypertensive renal fibrosis induced by Angiotensin II (AngII) to gain insight into the mechanisms whereby hypertensive conditions lead to renal fibrosis [3]. Automated segmentation and scoring procedures proved sensible and reproducible to evidence interstitial fibrosis located in the renal cortex: SHG scores measured in the outer cortical region successfully sorted out control and AngII-infused mice (see figure 1). We also evidenced constriction of the arcuate arteries in AngII-infused mice compared to control mice. These results showed that SHG microscopy provides complementary information compared to histological staining and physiological parameters such as systolic blood pressure and albuminuria. We furthermore investigated how cross-linking of collagen by transglutaminase 2 (TG2) may influence tissue remodeling, and compared fibrosis progression in wild type and TG2 deficient mice. SHG scores measured in the renal cortex accordingly showed reduced fibrosis progression upon AngII-infusion in TG2 deficient mice.

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