Novel Morphometric Image Analysis of Chronic Kidney Disease STNx Model Generates Quantitative and Continuous Scores for the Evaluation of Fibrosis

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Introduction

Kidney fibrosis is characterized by excessive production and deposition of collagens and other extracellular matrix proteins primarily in the organ interstitium, and is a prominent histological finding in chronic kidney disease (CKD). Here, we used a novel image analysis platform (FibroNest™) to quantify fibrosis and generate a normalized continuous Phenotypic Fibrosis Composite Score (Ph-FCS) in a murine model of CKD characterized by structural damage and impairment of renal function.

Objective

In the Subtotal Nephrectomy (STNx) model, traditional staining methods of tissue fibrosis show sensitivity limitations to detect differential patterns of collagen deposition between sham and STNx mice. The objective of this study was to test the ability of the innovative FibroNest™ platform to quantify and score the severity of fibrosis in this CKD model.

Materials and Methods

Kidney fibrosis was induced in 129S6 mice by subtotal (5/6) nephrectomy (STNx, n=10) and sham operated mice were used as controls (n=6). Kidney function was assessed at 12-weeks post surgery: Glomerular filtration rate (GFR) was measured by transdermal detection of FITC-Sinistrin, urea nitrogen was measured in plasma, and urine albumin and creatinine were used to calculate the albumin to creatinine ratio (uACR). Remnant kidneys were formalin fixed and paraffin embedded, 8µM sections (unstained) were imaged by the Genesis200© Two-Photon/Second Harmonic Generation (SHG) microscope at 20X to detect fibrillar collagen, and subsequently stained with PicroSirius Red (total Collagen) and digitally scanned at 20X.

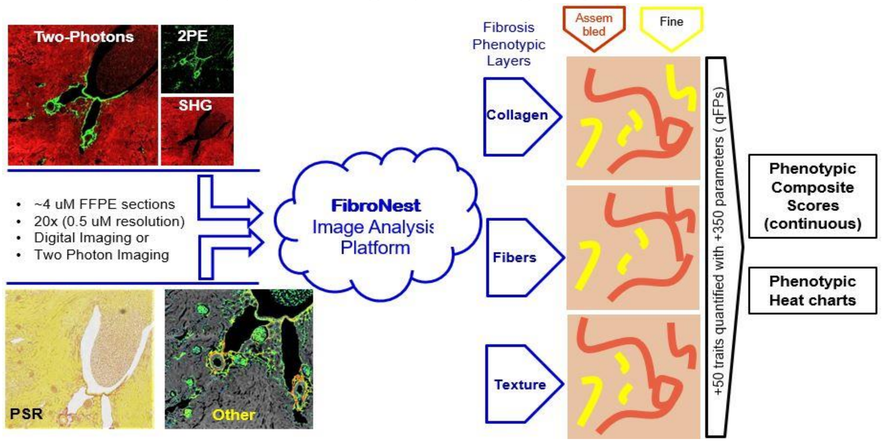

Figure 1: A summary of the FibroNest™ phenotypic Image analysis method and related workflow. FibroNest can handle +250 image formats as well as multiple fibrosis stains. Images can be uploaded worldwide

FibroNest™ image analysis software was used to assess 350+ quantifiable fibrosis parameters (qFPs), based on collagen content, structure, morphometry and texture. The resulting selected qFPs were combined to form a phenotypic fibrosis composite score (Ph-FCS), which was calculated for cortex, medulla and whole kidney sections.

Results – Imaging and Image Analysis

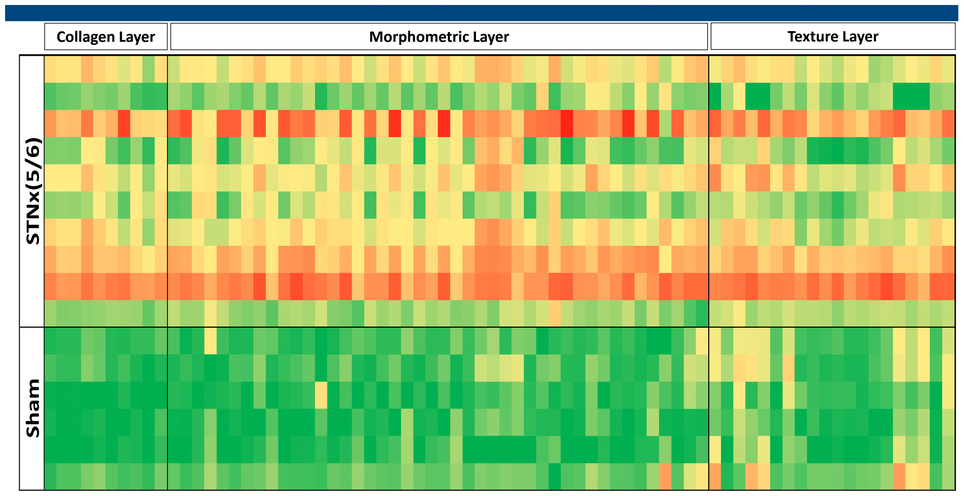

Figure 2: The Phenotypic Map visualizes the severity and progression (Green|Low > Red|High severity) of each fibrotic trait

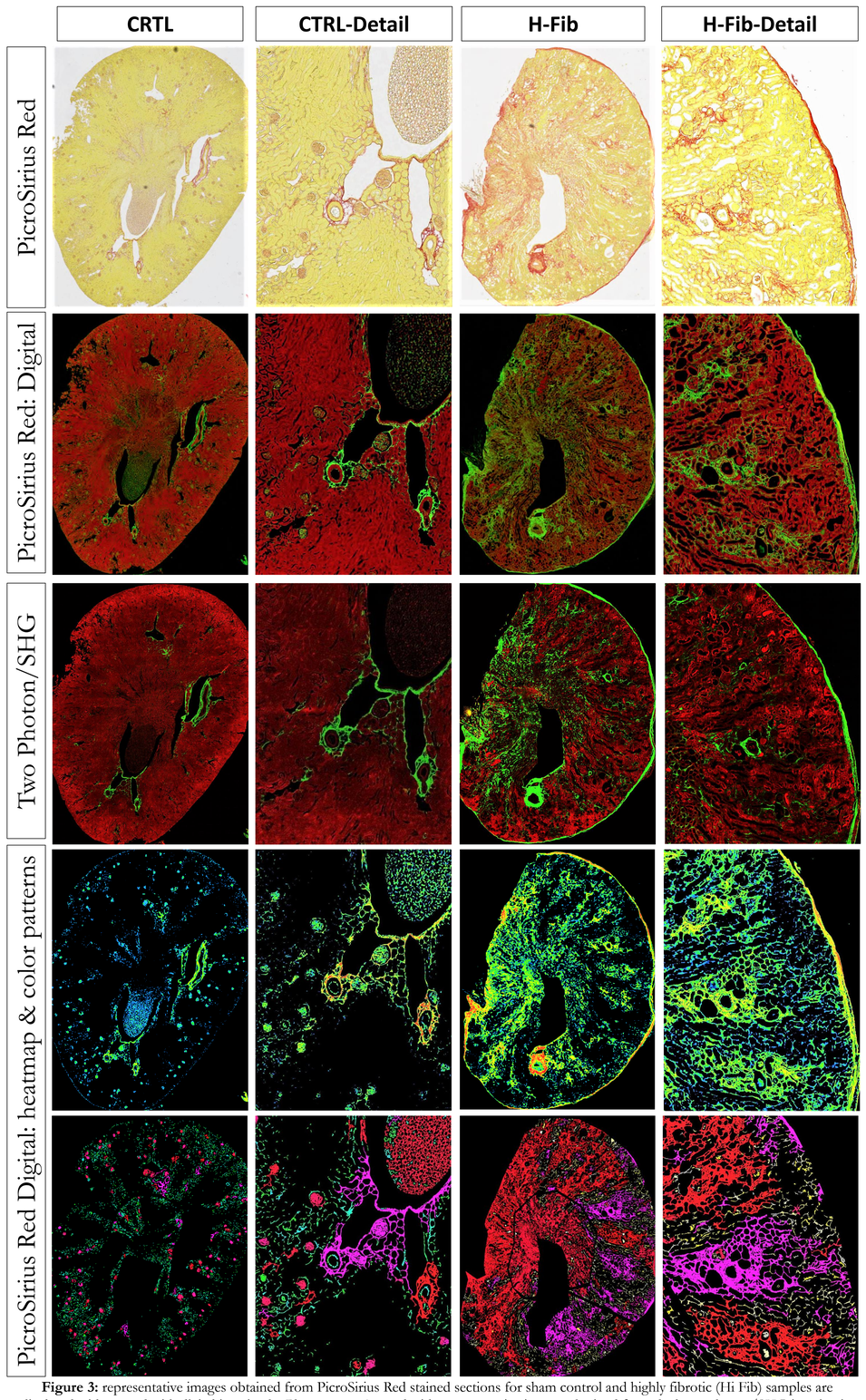

Figure 3: representative images obtained from PicroSirius Red stained sections for sham control and highly fibrotic (Hi Fib) samples are displayed without and with digital imaging by Pharmanest. A panel with representative images obtained from dual two photon/SHG imaging is presented as well. Digital images of stained sections are also presented in the form of heatmaps and color-patterns, where the sophisticated color deconvolution identifies the most collagen-enriched areas of the kidney.

Results – Quantification

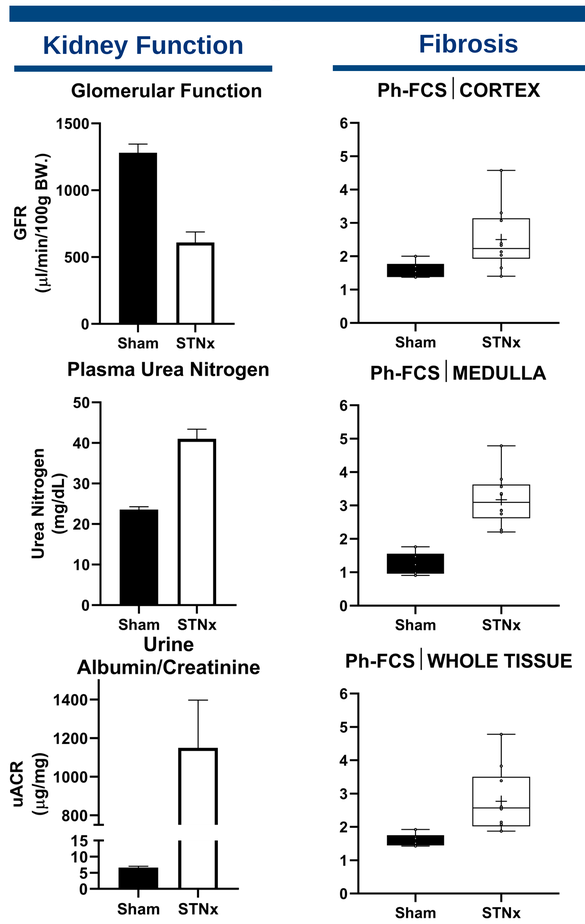

Figure 4: In-life measures of kidney function (GFR, Urea Nitrogen and uACR) were significantly different between sham and STNx groups. The Ph-FCS was significantly higher in STNx compared to control when assessed in the cortex, medulla and whole tissue section.

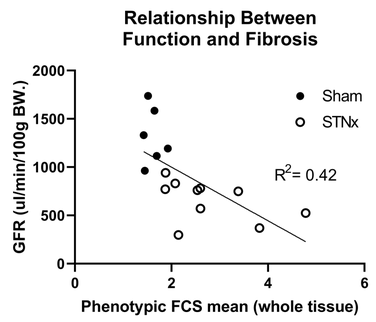

Figure 5: There was a significant correlation between kidney function as measured by glomerular filtration rate (GFR) and whole tissue Phenotypic fibrosis composite score.

Conclusion

The PharmaNest platform offers a sensitive method for fibrosis detection and quantification. The Phenotypic continuous Fibrosis Composite Score (Ph-FCS) calculated with the FibroNest™ software correlates with the disease severity in the STNx model and is an effective multiparameter scoring endpoint for fibrosis studies. Additional evaluation of disease progression, interglomerular fibrosis and response to an anti-fibrotic therapy will further validate the FCS as a key endpoint in the discovery of novel CKD therapeutics.

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