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A practical new way to measure kidney fibrosis

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Abstract

Proper histological measurement of kidney fibrosis is essentially important in both clinical pathology and basic research using animal models of chronic kidney disease (CKD). However, standard histology techniques and their blind evaluation are cumbersome. Ranjit et al. applied an advanced optical microscopy technique for hassle-free, unbiased, and highly sensitive characterization of kidney fibrosis and tested it in a classic model of chronic kidney disease in mice. This commentary emphasizes the advantages and future promise of this new approach.

A variety of optical imaging techniques, including multiphoton microscopy (MPM), have been applied to study kidney structure and function in both live and fixed tissues.[1] The recent proliferation of femtosecond pulse lasers have made it possible to use many MPM modalities routinely in most biomedical research centers for contrast-enhanced imaging using exogenous fluorophores or MPM imaging of intrinsic signals within the native tissue. The latter approach has the practical advantage of being label-free and includes MP excitation of intrinsic (auto) fluorescence (mainly originating from reduced nicotinamide adenine dinucleotide phosphate, flavins within cells, and/or mitochondria) and second-harmonic generation (SHG). Fibrillar collagen (collagen I and III) in the extracellular matrix is a classic example for optically anisotropic molecules with non-centrosymmetric structures that are capable of generating strong SHG signals.[2] On the basis of these biophysical features, the matrix and/or cell composition of kidney tissue can be evaluated by the SHG/autofluorescence ratio. During the process of interstitial fibrosis, which is a predictor of chronic kidney disease (CKD) progression, the SHG/autofluorescence ratio continuously increases as renal cells are depleted and replaced by the extracellular matrix (fibrillar collagen).

In this issue, Ranjit *et al.*[3] (2016) reports on the combined application of SHG and fluorescence lifetime imaging (FLIM) of tissue autofluorescence as a practical new way of measuring kidney fibrosis in fixed tissue sections. They used their deep tissue-imaging Deep Imaging Via Enhanced-Photon Recovery (DIVER) microscope (Laboratory of Fluorescence Dynamics [LFD], University of California, Irvine, CA) with high-sensitivity, forward-propagating SHG and a recently developed phasor approach to autofluorescence FLIM[4] (see

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DISCLOSURE

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the technique and instrument in Supplementary Figure 5 in the article by Ranjit *et al.*)[3] The main novelty in the current work is the first-time application of FLIM for characterizing kidney fibrosis, with the SHG/FLIM ratiometric measurement providing additional specificity and sensitivity compared with previous, mainly SHG-based similar work.[5,6] This new technical advance and approach for the detection and quantitative analysis of the extent of renal fibrosis was tested in the classic experimental renal fibrosis model of unilateral ureter obstruction, and its sensitivity and accuracy was compared with gold standard histology techniques. The authors found several advantages and benefits of their new SHG-FLIM method, which is a label-free approach, and therefore, it will simplify the sample preparation process.[3] Also, it uses ratiometric SHG/FLIM imaging, which results in highly sensitive and quantitative measurements of renal fibrosis, because collagen SHG increases, but autofluorescence FLIM decreases, during fibrosis progression. The technique eliminates the need for operator intervention (bias) and allows for automation. In comparison with Masson's trichrome and Picrosirius Red methods, this new approach was approximately 3 times more sensitive for fibrosis detection.[3] Another advantage was the ability to perform 3-dimensional reconstruction from a thicker tissue slice.[3]

This smart new approach by Ranjit *et al.* is expected to make the renal pathologists' life much easier. Also, this technical advance is available at the perfect time, when other parallel significant advances in optical microscopy can further maximize its use. The extended infrared range of commercial 1300-nm lasers now allows for label-free live tissue imaging with third-harmonic generation (THG), which has been proposed for the detection and measurement of lipids in various tissues.[7] Simultaneous quantitative imaging of characteristic fibrotic proteins (collagen) and lipids would provide more insights into the pathobiology of the tubulo-interstitium in CKD. In addition, using a combination with recently developed and highly popular tissue clearing techniques (such as CLARITY), quantitative imaging of tissue fibrosis in the entire intact kidney would become possible in 3 dimensions. This would provide additional detail on focal fibrotic patterns, as was shown recently.[8] Also, future studies will likely apply and test these techniques for live animal imaging. Tracking the development and progression of the fibrosis process in the same animal and tissue region over time, as shown recently for tracking individual cell types,[9] would further expand the capabilities of this SHG-FLIM—based approach. However, besides the many significant benefits of the new technique, 1 potential weakness of the approach is its dependence on expensive instrumentation, MP lasers, FLIM, and microscopy equipment. Access to advanced imaging core facilities and continuous equipment maintenance will be required.

In summary, this new technique represents a significant advance in kidney research, because it provides for the more sensitive, accurate, fast, and automated quantitation of renal tissue fibrosis compared with existing histological standards. Also, it has a great potential for future developments, for tracking fibrosis and CKD progression noninvasively in the intact, living kidney in 3 dimensions both in basic research, and clinical pathology and diagnostics.

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