Nondestructive Diagnosis of Kidney Cancer on 18-gauge Core Needle Renal Biopsy Using Dual-color Fluorescence Structured Illumination Microscopy

James Liu, Mei Wang, David Tulman, Sree H. Mandava, Katherine N. Elfer, Andrew Gabrielson, Weil Lai, Caleb Abshire, Andrew B. Sholl, J. Quincy Brown, and Benjamin R. Lee

OBJECTIVE To present a novel imaging technique used for rapid, nondestructive histological assessment of renal neoplasias using a dual-component fluorescence stain and structured illumination microscopy (SIM).

MATERIALS AND METHODS After Institutional Review Board approval, 65 total biopsies were obtained from 19 patients undergoing partial or radical nephrectomy. Biopsies were stained with a dual-component fluorescent, and optically sectioned SIM images were obtained from the surface of the intact biopsies. Specimens were subsequently fixed and analyzed using hematoxylin and eosin (H&E) histopathologic methods and compared with SIM images. A single, board-certified pathologist blinded to specimens reviewed all SIM images and H&E slides, and determined the presence or absence of neoplasias. Results of blinded diagnosis of SIM were validated against traditional pathology.

RESULTS Of the 19 patients, 15 underwent robotic partial nephrectomies and 4 underwent laparoscopic nephrectomies. Indications included clinical suspicion of renal cell carcinoma. In total, 65 biopsy specimens were available for review. Twenty-one specimens were determined to be neoplastic on H&E, whereas 41 represented benign renal tissue. The final sensitivity and specificity of our study were 79.2% and 95.1%, respectively.

CONCLUSION SIM is a promising technology for rapid, near-patient, ex vivo renal biopsy assessment. By improving the ability to rapidly assess sufficiency of biopsy specimens and enabling immediate diagnostic capability, SIM aids in more effective biopsy performance, tissue triage, and patient counseling regarding management options. Additionally, because tissue is preserved, effective utilization of downstream diagnostic tests and molecular assessments are possible. UROLOGY ■■: ■■–■■, 2016. © 2016 Elsevier Inc.

The role of percutaneous renal-core biopsy analysis of localized small renal masses (<4 cm) has continuously evolved in recent years. Historically, renal biopsy has been overshadowed by traditional imaging analysis like computed tomography and magnetic resonance imaging in surgical management guidance.[1] However, imaging modalities have demonstrated limited accuracy in characterization and prognosis of localized small renal masses when compared to histopathological analysis of sectioned specimens.[2] Cross-sectional imaging is effective in detecting the presence of a small renal mass but is unable to distinguish malignancy from a benign tumor (ie, oncocytoma) in a majority of these cases.[3] Additionally, with the rise of nephron-sparing surgery and point-of-procedure histopathology assessment, there is a resurgence of interest in renal biopsy in the clinical management of patients with small renal tumors.[4] Significant interest has developed in the comprehensive molecular evaluation of renal cell carcinoma, yet translation of these efforts to change patient management has yet to be fully defined. Overall, 78.7% of renal

Financial Disclosure: *The authors declare that they have no relevant financial interests.* **Funding Support:** *NIH NCI R21CA159936 and NIH NCI R33CA196457. Institutional funding came from the Tulane University School of Science and Engineering. Katherine N. Elfer—National Science Foundation Graduate Research Fellowship Program. David Tulman—National Science Foundation IGERT Fellowship (DGE-1144646).*

From the Department of Urology, Tulane University School of Medicine, New Orleans, LA; the Department of Biomedical Engineering, Tulane University, New Orleans, LA; the Department of Pathology and Laboratory Medicine, Tulane University School of Medicine, New Orleans, LA; and the Division of Urology, University of Arizona College of Medicine, Tucson, AZ

Address correspondence to: Benjamin R. Lee, M.D., Division of Urology, University of Arizona College of Medicine, Tucson, AZ 85724. E-mail: brlee@surgery.arizona.edu

Submitted: May 20, 2016, accepted (with revisions): August 27, 2016

tumors present with a genomic alteration considered clinically relevant.[5]

Renal core biopsy analysis is the gold standard for histological analysis of renal masses; however, there are numerous shortcomings in its preparation. The process of fixation or freezing, sectioning and staining with hematoxylin and eosin (H&E) destroys the integrity of the specimen and evaluation by a pathologist is typically performed at least 24 hours after specimen acquisition. Touch preparations can be useful; however, without a dedicated cytopathologist to perform on-site assessment, this method will require additional time and cost, without concomitant improvement in efficacy. Unfortunately, this time-consuming approach becomes logistically difficult to obtain point-of-procedure histopathology. Likewise, the limited tissue collected on core biopsy makes frozen section virtually impossible as a method for rapid diagnosis. Sufficiency of tissue from biopsy is also a reoccurring obstacle. Consequently, the current renal biopsy protocol is a lengthy, time-consuming process where patients sometimes are unable to obtain a definitive diagnosis and must return for additional biopsy procedures to further characterize the lesion.

Ex vivo imaging of fresh tissue specimens stained with nondestructive fluorescent dyes is an attractive alternative to conventional biopsy modalities.[6] Previous studies done by our group have demonstrated the success of the ex vivo imaging modality, structured illumination microscopy (SIM), for rapid high-resolution diagnostic imaging of prostate biopsies.[7,8] SIM can be performed within minutes of tissue acquisition and produces images that closely recapitulate tissue histology visualized on traditional H&E stained slides.[7] Likewise, by imaging fresh tissue we avoid the need for tissue processing (eg, fixation or freezing and sectioning), thereby preserving tissue for downstream definitive H&E analysis as well as any medically necessary additional diagnostic testing.

In this manuscript we demonstrate the potential of SIM for diagnosis of fresh, unfixed, and unsectioned 18-gauge core needle biopsies for accurate, nondestructive diagnosis of fluorescently stained renal biopsies in point-of-procedure time frames. We demonstrate that SIM can provide images with sufficient contrast and detail to allow delineation of benign vs neoplastic renal tumors while streamlining clinical workflow.

MATERIALS AND METHODS

Intact core needle biopsies (n = 65) from 19 patients receiving either robotic partial nephrectomy or laparoscopic radical nephrectomy were obtained under an Institutional Review Board protocol. Biopsies were taken ex vivo using a standard 18-gauge core-needle biopsy technique (Bard Monopty, Bard, Tempe, AZ) from the renal neoplasm and adjacent benign renal parenchyma immediately after being removed from patient cavity. The biopsies were placed in saline and promptly mounted and scanned in the imaging suite within a half hour. The biopsies were then formalin fixed and prepared using standard H&E.

The tissue staining and imaging protocol was adapted from prior work performed by Elfer et al.[9] Tissue specimens were prepared after being submerged in phosphate-buffered saline (PBS) postcollection. Biopsies were submerged in Eosin Y solution 2% v/v prepared in 80% ethanol (E4009, Sigma-Aldrich, St. Louis, MO) for 10 seconds and then rinsed with PBS to remove excess stain. Excess fluid was removed from the biopsy with laboratory tissue and then submerged in 50 μM DRAQ5 in PBS for 3 minutes (Biostatus, Shepshed, UK). In some cases, Eosin Y solution was reintroduced after the DRAQ5 step to increase the intensity of eosin staining. Biopsies were then patted with laboratory tissue to remove excess fluid and placed on slides for VR-SIM imaging (Fig. 1).

The custom SIM has been described in detail previously.[7,8] In this work, the system was modified to enable imaging of the dual-component stain, specifically by adding a 630 nm light-emitting diode (LED) for DRAQ5 excitation (UHP-MIc-LED-630, Prizmatix, Southfield, MI) in addition to the 475 nm LED for eosin excitation (Thorlabs, Newton, NJ). The two LEDS were combined using a dichroic beam combiner (Prizmatix). A ferroelectric spatial light modulator (3DM, Forth Dimension Displays, Fife, UK) was used to project patterns for structured illumination microscopy onto the sample through a 10× 0.45 NA Plan Apo objective lens (Nikon, Tokyo, Japan) in epi-illumination

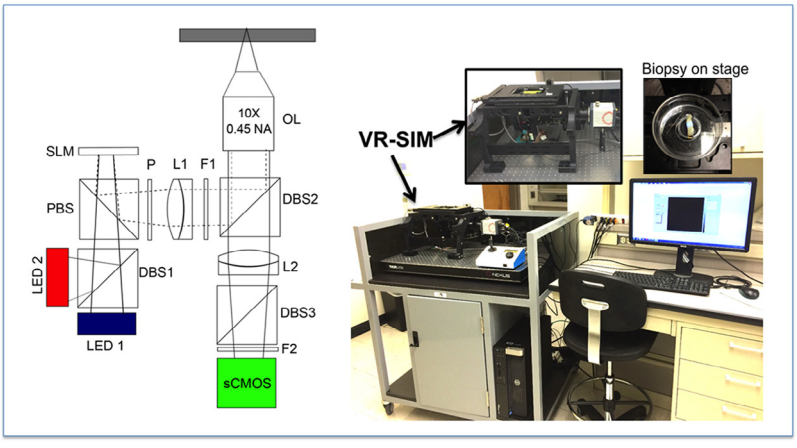

Figure 1. Schematic and photograph of structured illumination microscopy (SIM) setup. SLM, spatial light modulator; PBS, phosphate-buffered saline. (Color version available online.)