Detecting kidney fibrosis using H-scan

Jihye Baek
Department of Electrical and Computer Engineering
University of Rochester
Rochester, NY, USA
jbaek7@ur.rochester.edu

Eno Hysi
Division of Nephrology, Department of Medicine
St. Michael's Hospital, Unity Health
Toronto and University of Toronto
Toronto, ON, Canada
eno.hysi@unityhealth.to

Xiaolin He
Division of Nephrology, Department of Medicine
St. Michael's Hospital, Unity Health
Toronto and University of Toronto
Toronto, ON, Canada
xiaolin.he@unityhealth.to

Darren A. Yuen
Division of Nephrology, Department of Medicine
St. Michael's Hospital, Unity Health
Toronto and University of Toronto
Toronto, ON, Canada
darren.yuen@mail.utoronto.ca

Michael C. Kolios
Division of Nephrology, Department of Medicine
St. Michael's Hospital, Unity Health
Toronto and University of Toronto
Toronto, ON, Canada
mkolios@ryerson.ca

Kevin J. Parker
Department of Electrical and Computer Engineering
University of Rochester
Rochester, NY, USA
kevin.parker@rochester.edu

***Abstract*— Kidney fibrosis plays a significant role in determining the outcome of kidney transplantation. Accurate measurement of kidney fibrosis is thus important not only in the diagnosis of chronic kidney disease but also in the assessment of donor grafts. Currently, biopsy is only procedure used to assess kidney fibrosis. Thus, the development of non-invasive and accurate imaging tools would be advantageous. This study aims to demonstrate the ability of H-scan to quantify kidney fibrosis.**

We performed an *ex vivo* murine study using the unilateral ureteral obstruction (UUO) model to induce renal fibrosis. 15 mice in total were used in this study. 10 mice underwent UUO surgery on the left ureter, with the right kidneys serving as controls. The kidneys were extracted on day 7 (n = 5) and day 14 (n = 5). The other 5 healthy mice served as sham controls, and their kidneys were also extracted. Ultrasound imaging of the extracted kidneys was performed using a 15 MHz center frequency transducer. After imaging, the kidneys were stained with picrosirius red to quantify fibrosis levels. H-scan was applied to the whole kidneys. The percentage of lower frequency scattering encoded as red (% red) was used as the H-scan quantification, and these results were compared to the histology fibrosis score.

We demonstrated that H-scan imaging provided differentiated color images for normal and fibrotic kidneys. Fibrotic kidneys tended to contain more red, indicating increased collagen fiber arising during the buildup of kidney fibrosis. Furthermore, quantification of fibrosis using the H-scan and histology was compared, showing a strong correlation of R = 0.99. Overall, we demonstrated that the H-scan can accurately detect the buildup of kidney fibrosis, suggesting its potential use for renal fibrosis assessment as an alternative to biopsy.

Keywords—H-scan, Ultrasound, Tissue characterization, Kidney fibrosis, Fibrosis assessment, Kidney transplantation

I. Introduction

Fibrosis of the kidneys is a hallmark of end-stage renal disease in chronic kidney disease (CKD) patients. It also plays a significant role in kidney transplants. Typically, donations that contain fibrosis have poorer outcomes and shorter longevity. The ability to quantify the degree of kidney fibrosis can have a significant clinical impact as it allows nephrologists to predict the outcome of a graft and better allocate the scarcely available donations. Currently, biopsy remains the only means of measuring renal fibrosis, although it has drawbacks related to the sampling volume, extraction site, and bleeding risk.

The development of a non-invasive, accurate, and safe tool to measure renal fibrosis is needed, but, there is currently no standard imaging modality for this [1]. Recent studies for non-invasive assessment of fibrosis have been performed, using imaging modalities such as ultrasound (US) and magnetic resonance (MR) elastography [2], MR imaging utilizing agents [3], and photoacoustic (PA) imaging [4]. US and MR elastography are not accurate enough to evaluate renal fibrosis [2]. Utilizing contrast agents targeted to fibrosis has limitations, including nephrotoxicity. Further, PA imaging requires the use of a laser, limiting the penetration depth to only cortical regions and *ex vivo* examinations.

In this study, to assess renal fibrosis, we used the conventional B-mode US procedure and then analyzed backscattered signals utilizing acquired radiofrequency (RF) data. H-scan analysis was performed by extracting frequency-dependent information from the RF data. This enabled the quantification of fibrosis buildup in kidney. To evaluate the accuracy, the H-scan output and histology fibrosis scores were compared.

II. Methods

A. Kidney fibrosis mouse model

A total of 15 C57BL/6 mice (Charles River Laboratories, Wilmington, MA, USA) were investigated. Fibrosis was induced only on the left kidneys in 10 mice using the unilateral ureteral obstruction (UUO) model [5], whereas the right kidneys were not injured, serving as control (healthy and non-fibrotic). The fibrotic kidneys were extracted on day 7 (n = 5) and day 14 (n = 5) following the UUO surgeries. In addition, n = 5 healthy mice served as sham controls, as their ureters were not obstructed. The sham surgery kidneys on the left and right sides were also extracted on day 0. In total, 15 mice with 30 kidneys were investigated to assess fibrosis buildup.

The extracted kidney specimens were scanned with ultrasound and immediately following the scanning, the specimens underwent histological examinations. The kidneys were stained with picrosirius red (PSR, Millipore Sigma, Burlington, MA, USA) to quantify fibrosis levels by visualizing collagen content.

B. Ultrasound data acquisition

The VevoLAZR-X imaging system (FUJIFILM, VisualSonics, Inc., Toronto, CA) utilized to image the kidneys was equipped with a 15 MHz center frequency linear array transducer with 256 elements. During the scanning, kidney

2022 IEEE International Ultrasonics Symposium (IUS) | 978-1-6654-6657-8/22/$31.00 ©2022 IEEE | DOI: 10.1109/IUS54386.2022.9957217

specimens were placed in 4°C phosphate buffered saline, and the largest cross section of each kidney was scanned while acquiring the delay-and-sum beamformed RF data. The H-scan methodology was applied to the RF data of the whole kidneys to estimate fibrosis levels. The whole kidney area was manually contoured.

C. H-scan analysis

The H-scan approach [6] makes use of matched Gaussian filters to estimate frequency components present in each image. The H-scan process is shown in Fig 1. Since attenuation causes frequency downshifts over depths, attenuation correction [6] with an attenuation coefficient of 0.5 dB/MHz/cm was performed on the acquired RF data. Utilizing the attenuation-corrected RF data, we estimated frequency components for all samples in the axial direction and all scanlines in the lateral direction within the manually contoured kidney boundary. For each estimation, 256 Gaussian bandpass filters with peak frequencies from 3.5 MHz to 11.5 MHz were applied in the frequency domain. Each filter was programmed to output a convolved image in the time domain, which highlights a peak frequency component of the corresponding Gaussian filter. Among the 256 convolved images, a Gaussian filter index having the maximum intensity for each pixel was selected. The selected indices become H-scan color levels ranging from 1 to 256. The lower and higher frequency components correspond to the lower and higher color levels, respectively. The H-scan color levels from 1 to 256 are color coded from red to blue, respectively, as shown in the color bar in Fig. 1. The red (lower frequency components) and blue (higher frequency components) colors can visualize larger and smaller US scatterers, respectively. To quantify the H-scan imaging, the percentage of red pixels (% red) was calculated:

$$\%\ \text{red} = \frac{\text{Number of pixels encoded in red}}{\text{Total number of pixels}} \times 100\% \quad (1)$$

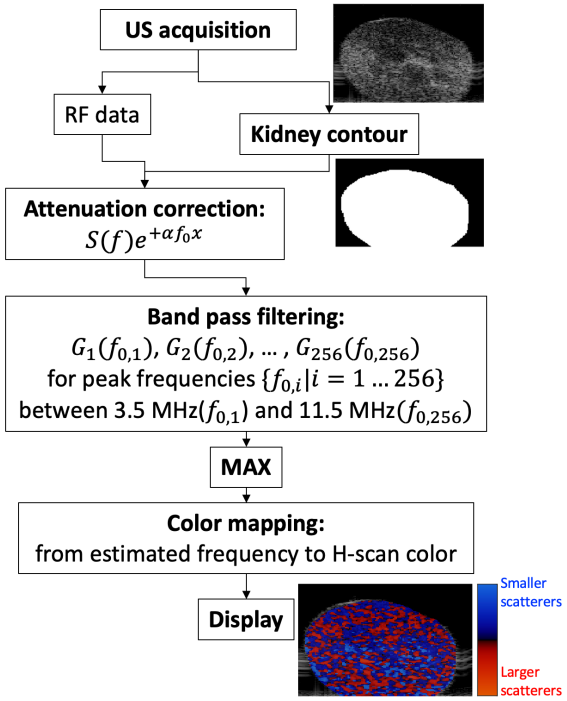

Fig. 1. H-scan process flow chart.

where the pixels encoded in red only include pixels having H-scan color levels < 128. Therefore, % red was used as the H-scan metric to assess the level of renal fibrosis.

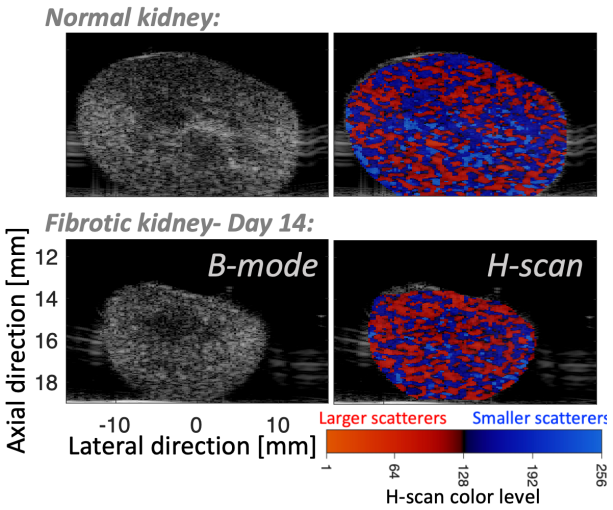

Fig. 2. Representative B-mode and H-scan images from normal and fibrotic kidneys. By day 14 following UUO surgery, the fibrotic kidney contains a larger proportion of red (less blue) compared to the normal kidney.

III. Results and Discussion

Fig. 2 shows representative B-mode and H-scan images from normal and fibrotic kidneys 14 days following UUO surgery. The fibrotic kidney contains more red pixels as fibrosis was induced for 14 days. The H-scan visualization provides a robust tool for identifying the low and high-frequency scattering components arising during the buildup of kidney fibrosis. Thus, it is hypothesized that the collagen fibers deposited during fibrosis increase effective scatterer diameter, increasing % red, i.e. low-frequency components.

The H-scan % red and histology fibrosis score are compared in Fig. 3, demonstrating a very strong correlation of $R = 0.99$ where R is Pearson's linear correlation coefficient. The % red increased as collagen content increased due to fibrosis buildup.

This study demonstrated the potential use of the H-scan format for kidney fibrosis assessment. The study was

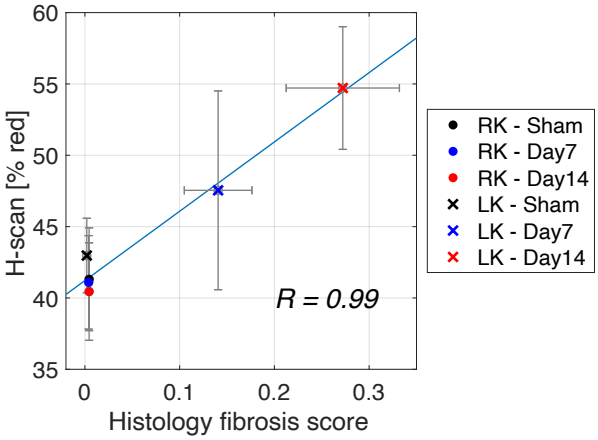

Fig. 3. Comparison between the H-scan-derived fibrosis score (% red) and histology score. The correlation between the H-scan and histology is $R = 0.99$ (RK: right kidney, LK: left kidney)

performed solely on *ex vivo* animal kidneys, therefore further evaluation in animals and humans *in vivo* is required. However, the H-scan analysis has been applied to *in vivo* animal and human studies for tissue characterization [7-14]. No challenges or limitations were observed when H-scan was extended to *in vivo* studies. The H-scan is capable of identifying tissue characteristics if (1) RF data for targets are acquirable during conventional B-mode scans and (2) adequate attenuation correction can be performed before applying the H-scan. Therefore, we anticipate that further *in vivo* studies would confirm our findings on the ability of the H-scan to accurately quantify renal fibrosis.

IV. Conclusion

These findings suggest that the H-scan format can accurately detect the buildup of kidney fibrosis, providing potential avenues for detecting this detrimental condition in patients with CKD.

Acknowledgment

This work was supported by the following grants awarded to D.A.Y and M.C.K: Collaborative Health Research Project grant co-founded by the Canadian Institutes of Health Research (CIHR) and the Natural Sciences and Engineering Research Council of Canada, a Banting and Best Diabetes Centre Pilot and Feasibility grant, a Canadian Society of Transplantation and Canadian National Transplant Research Program Basic Research Catalyst grant, and funds from the St. Michael's Hospital Foundation. E.H. is supported by a Kidney Research Scientist Core Education and National Training (KRESCENT) Program postdoctoral fellowship (cosponsored by the Kidney Foundation of Canada, the Canadian Society of Nephrology, and CIHR) and a Banting Fellowship from awarded from the CIHR.

References

[1] G. Leung, A. Kirpalani, S. G. Szeto *et al.*, "Could MRI be used to image kidney fibrosis? A review of recent advances and remaining barriers," *Clinical Journal of the American Society of Nephrology,* vol. 12, no. 6, pp. 1019-1028, 2017.

[2] N. Grenier, P. Merville, and C. Combe, "Radiologic imaging of the renal parenchyma structure and function," *Nature Reviews Nephrology,* vol. 12, no. 6, pp. 348-359, 2016.

[3] Q. Sun, M. Baues, B. M. Klinkhammer *et al.*, "Elastin imaging enables noninvasive staging and treatment monitoring of kidney fibrosis," *Science translational medicine,* vol. 11, no. 486, pp. eaat4865, 2019.

[4] E. Hysi, X. He, M. N. Fadhel *et al.*, "Photoacoustic imaging of kidney fibrosis for assessing pretransplant organ quality," *JCI insight,* vol. 5, no. 10, 2020.

[5] S. G. Szeto, M. Narimatsu, M. Lu *et al.*, "YAP/TAZ are mechanoregulators of TGF-β-Smad signaling and renal fibrogenesis," *Journal of the American Society of Nephrology,* vol. 27, no. 10, pp. 3117-3128, 2016.

[6] K. J. Parker, and J. Baek, "Fine-tuning the H-scan for discriminating changes in tissue scatterers," *Biomedical Physics & Engineering Express,* vol. 6, no. 4, Jul, 2020.

[7] J. Baek, R. Ahmed, J. Ye *et al.*, "H-Scan, Shear Wave and Bioluminescent Assessment of the Progression of Pancreatic Cancer Metastases in the Liver," *Ultrasound in Medicine and Biology,* vol. 46, no. 12, pp. 3369-3378, Dec, 2020.

[8] J. Baek, L. Basavarajappa, K. Hoyt *et al.*, "Disease-specific imaging utilizing support vector machine classification of H-scan parameters: assessment of steatosis in a rat model," *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control,* vol. 69, no. 2, pp. 720-731, 2021.

[9] J. Baek, and K. J. Parker, "H-scan trajectories indicate the progression of specific diseases," *Medical Physics*, Aug 3, 2021.

[10] J. Baek, and K. J. Parker, "Disease-specific imaging with H-scan trajectories and support vector machine to visualize the progression of liver diseases." pp. 1-4.

[11] J. Baek, S. S. Poul, L. Basavarajappa *et al.*, "Clusters of ultrasound scattering parameters for the classification of steatotic and normal livers," *Ultrasound in Medicine & Biology,* vol. 47, no. 10, pp. 3014-3027, 2021.

[12] J. Baek, S. S. Poul, T. A. Swanson *et al.*, "Scattering Signatures of Normal Versus Abnormal Livers with Support Vector Machine Classification," *Ultrasound in Medicine and Biology,* vol. 46, no. 12, pp. 3379-3392, Dec, 2020.

[13] J. Baek, S. S. Qin, P. A. Prieto *et al.*, "H-scan imaging and quantitative measurement to distinguish melanoma metastasis." pp. 1-4.

[14] J. Baek, A. M. O'Connell, and K. J. Parker, "Improving the diagnosis of breast cancer based on biophysical ultrasound features utilizing machine learning," *arXiv preprint arXiv:2207.06560*, 2022.