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Comparison of 2D SURE and 3D CT imaging of cortical vessels in a rat kidney

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Abstract*—SUper-Resolution ultrasound imaging using the Erythrocytes (SURE) can visualize blood vessels at about 25 to 50 µm resolution, but validation is required to assess how accurately the vasculature and its morphology are represented. Previous work compared ultrasound imaging to maximum intensity projections (MIP) of micro-CT volumes at an insufficient voxel size of 22.6 µm. Here, a 5 µm voxel size micro-CT volume of a cortical region in an excised rat kidney was acquired to test the hypothesis that blood vessels down to 50 µm in diameter are detected with SURE imaging. For the micro-CT volume, the blood vessels were segmented by an intensity threshold, and local thickness estimates were computed using expanding spheres within the segmentation mask. An affine registration between the SURE image and the micro-CT volume was manually defined, and a MIP of the micro-CT volume across 2 mm from the ultrasound imaging plane was computed for vessels with diameter estimates greater than 50 µm. The SURE image depicts 12 cortical radial vessels with high intensities and 9 dimmer cortical radial vessels. Notably, the 12 high intensity vessels are also depicted in the micro-CT projection, but 5 cortical radial vessels and 2 arcuate blood vessels are only visible in the SURE image. Of the faintly depicted cortical radial vessels, 5 vessels are not readily matched to vessels in the micro-CT projection. On the other hand, the micro-CT projection contains 6 cortical radial vessel segments and 2 arcuate blood vessels not depicted in the SURE image. These discrepancies in the comparison may arise from the challenging registration of the ultrasound focus beam and the micro-CT volume that is complicated further by the different states of the imaged tissue. Despite these challenges, SURE depicts most vessels at least down to 50 µm and even resolves parallel vessels not visible in the micro-CT projection, thus highlighting its clinical potential.

***Index Terms*—Super resolution imaging, Flow imaging, Ultrasound imaging, Micro computed tomography imaging, Imaging validation**

I. Introduction

ULTRASOUND imaging of renal blood vessels has proven to be an important tool for assessing kidney function and detecting vascular abnormalities [1–3]. One recent advancement in this field is SUper Resolution ultrasound imaging using Erythrocytes (SURE), which has shown promising results in visualizing blood vessels at resolutions ranging from 25 to 50 µm [4, 5]. However, it is essential to subject SURE imaging to rigorous validation to assess how accurately and reliably it depicts blood vessels and their morphology.

Previous work compared ultrasound images with maximum intensity projections (MIP) of micro-CT volumes [5]. However, these efforts were limited by the use of micro-CT volumes with an insufficient voxel size of 22.6 µm, which cannot reliably work as a baseline for the comparison because the SURE images have a similar resolution [5].

This study addresses this limitation by acquiring a high-resolution 5 µm voxel size micro-CT volume of a blood vessel network in a cortical region in an excised rat kidney. The use of a finer voxel size enables testing the hypothesis that blood vessels down to 50 µm in diameter can be accurately detected using SURE imaging. This is because smaller vessels can be specifically removed from the volume through diameter-based segmentation and because multiple voxels will constitute the remaining blood vessels. Hence, this finer resolution micro-CT volume will provide a more robust validation of the SURE technique, yielding insights into its ability to visualize intricate blood vessel networks in great detail.

The significance of this research lies in the validation of SURE imaging. The ability to reliably visualize blood vessels with non-invasive ultrasound with only a few seconds of data holds immense potential for various applications, including evaluating vascular changes in disease [2], and intra-operative imaging of vessels [6].

This paper presents the methodology used to acquire the high-resolution micro-CT image, the comparison of SURE imaging results with the micro-CT data, and the implications of the findings. By addressing the validation of SURE imaging and demonstrating its ability to detect small blood vessels accurately, this study contributes to the growing field of super-resolution ultrasound imaging and expands its potential applications in biomedical research and clinical practice.

II. Methods

This section explains the animal study setup, the image acquisition and analysis procedures.

In brief, a SURE image and a micro-CT volume were acquired of the same rat kidney at about equal resolution along with an additional micro-CT volume of a selected region of interest (ROI) with a voxel size of 5 µm. The images were co-registered and the blood vessels in the ROI were visually compared between the SURE image and a micro-CT projection image.

A. Image acquisition

The left kidney of a 40-week-old Zucker Diabetic rat was exposed through surgical incision through the abdominal wall. The kidney was ultrasound scanned using a 10 MHz GE L8-18iD probe (GE Health-care, Chicago, IL, USA) connected to a Vantage 256^{TM} (Verasonics, Kirkland, WA, USA) research scanner with a frame rate of 208 Hz. Data to generate the SURE image were acquired for 5 s resulting in 2000 frames. The SURE image was generated as previously described [4, 5]. After the ultrasound scanning, Microfil intravascular contrast agent (MV122, Flow Tech Inc., Carver, MA) was infused through the renal artery and the kidney was excised. The tissue was fixated in formalin and embedded in paraffin. An ROI was selected in the SURE image to include one vessel network from an arcuate blood vessel branch point. The micro-CT scans were conducted 123 days after excision using a Zeiss Xradia 410 Versa (Carl Zeiss Microscopy GmbH, Jena, Germany). The full kidney was scanned with a large-field-of-view LE2 filter at 40 keV with 4 s exposure at an isotropic voxel size of 22.6 µm, and the scan of the ROI was acquired with a X-LE2 filter at 40 keV with 40 s exposure at an isotropic voxel size of 5 µm. The experiments were performed in accordance with protocols approved by the Danish Animal Experiments Inspectorate under the Ministry of Environment and Food (license number 2020-15-0201-00547 issued on June 4th 2020).

B. Image analysis

The slice thickness of the SURE image was set to 2 mm, and the SURE and micro-CT volumes were co-registered with the ITK-SNAP software tool [7]. The registration of the ROI micro-CT scan and the SURE scan was obtained by registering the SURE image to the full kidney micro-CT volume, and registering the ROI and full kidney micro-CT scans. In the micro-CT volume, the blood vessels were segmented with an intensity threshold and local thickness estimates [8] were computed for the vessel segmentation. Only vessels with an estimated diameter greater than 50 µm were included in a maximum intensity projection (MIP) onto the ultrasound imaging plane. The blood vessels and their morphology were visually compared between the SURE image and the segmented MIP of the 5 µm micro-CT volume.

III. Results

Considering only the cortical region, there are 21 cortical radial vessel endpoints in the SURE image which are used to label most of the blood vessels (see labels 1-12 and nine triangle labels in Fig. 1). Of the 21 labelled blood vessels, the SURE image depicts 12 blood vessels with high intensities and 9 dimmer vessels (see Fig. 1). Notably, the 12 high intensity vessels are also depicted in the micro-CT projection and the vessel shapes appear similar.

Labels 1-9 show cortical radial blood vessels that are encompassed by a vessel network from an arcuate blood vessel branch point (see Fig. 1). Similarly labels 10-12 indicate another vessel network, but its arcuate blood vessel branch point is not visible (see Fig. 1). The yellow markings on the micro-CT projection indicate arcuate vein/artery pairs to the left and to the right of the branch point, and the markings also indicate cortical radial vessels that go upwards from the branch point and intersect in the micro-CT projection (see Figs. 1 and 2). The overall network shape is comparable between the SURE image and the micro-CT projection.

However, there are a few discrepancies where vessels are only visible in one of the images. In the micro-CT projection, labels a-h points to vessels that are not depicted in the SURE image (see Fig. 2). Specifically, labels a-f in the micro-CT projection highlight cortical radial vessels that are not shown in the SURE image, whereas labels g and h point to arcuate blood vessels closer to the branch point that are only shown in the micro-CT projection (see Fig. 2). Notably, label b in the micro-CT projection points to a cortical radial vessel parallel to the vessel labelled 6, but the parallel vessel is not shown in the SURE image (see vessel label 6 in Fig. 1). In addition, the region between labels 8 and 9 in the SURE image contains disorganised detections where the micro-CT projection contains blood vessels that overlap each other.

On the other hand, there are also vessels that are only depicted in the SURE image. Labels a-g points to vessels that are not depicted in the micro-CT projection (see Fig. 1. Labels a-c points to cortical radial vessels that are faintly represented in the SURE image, and label d shows a high intensity vessel detection. Neither of these vessels are part of the micro-CT projection (see labels a-d in Fig. 1). Labels e and f in the SURE image points to two arcuate vessel segments that are parallel to the arcuate vessels with yellow markings in the micro-CT projection (see Fig. 1). However, in the micro-CT projection the those arcuate vessels do not appear as separable parallel vessels (see yellow markings in Fig. 2).

Lastly, label g in the SURE image points to a region where two separate cortical radial vessels are depicted even though their separate vessel trajectories are not readily found in the SURE image (see Fig. 1). Indeed there are separate vessels in that region, but the micro-CT projection does not visualise the separation clearly (see yellow markings of cortical radials in Fig. 2).

Considering the shapes of the detected blood vessels, most vessels are depicted with comparable morphological traits between the SURE image and the micro-CT projection. However, there are also discrepancies in the shapes of some of the detected blood vessels. For instance, between labels 7 and 8 (see label c in Fig. 2) the SURE image depicts a vessel close by the vessel labelled with 7, whereas the micro-CT projection shows that the two vessels are further apart. Between the cortical radial vessels labelled with 9 and 10, the SURE image depicts a vessel with a bifurcation (see label c in Fig. 1). However, in the micro-CT projection that area contains several interleaved blood vessels, and the aforementioned bifurcation

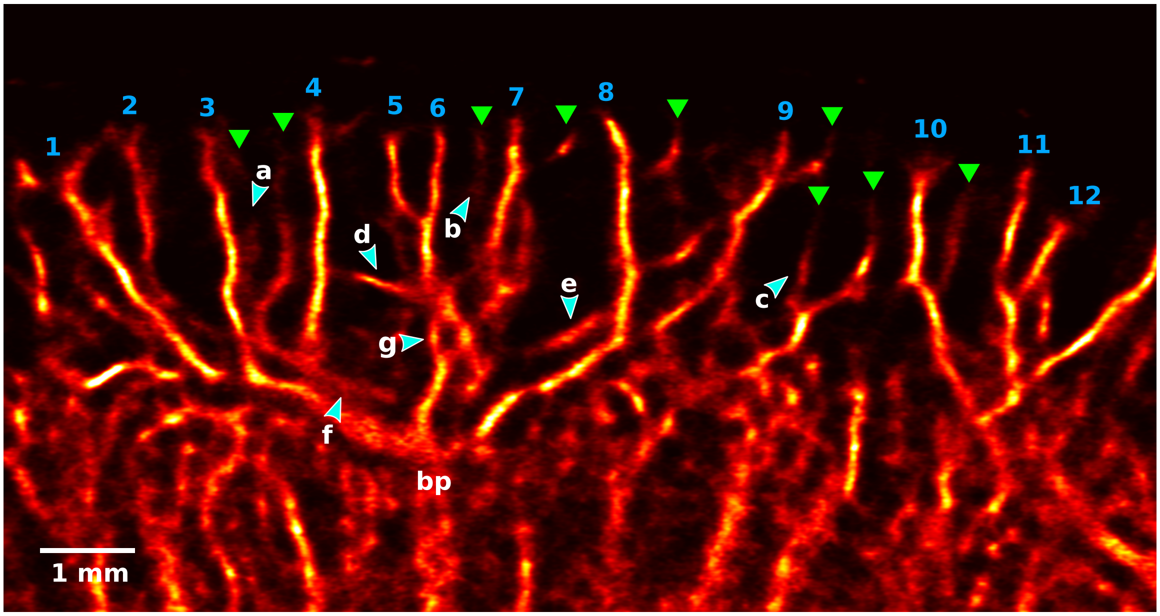

Fig. 1. SURE image annotated with blue numbers for high-intensity cortical radial vessels and green triangles for low-intensity cortical radial vessels. Labelled cyan arrows points to blood vessels that are not visible in the micro-CT projection. These include faintly represented vessels (labels a-c) and high intensity vessel detections (labels d-g). The latter includes arcuate vessels (labels e and f) that are not depicted in the micro-CT projection. The arcuate blood vessel branch point that is part of the blood vessel network with vessels 1-9 is annotated with bp.

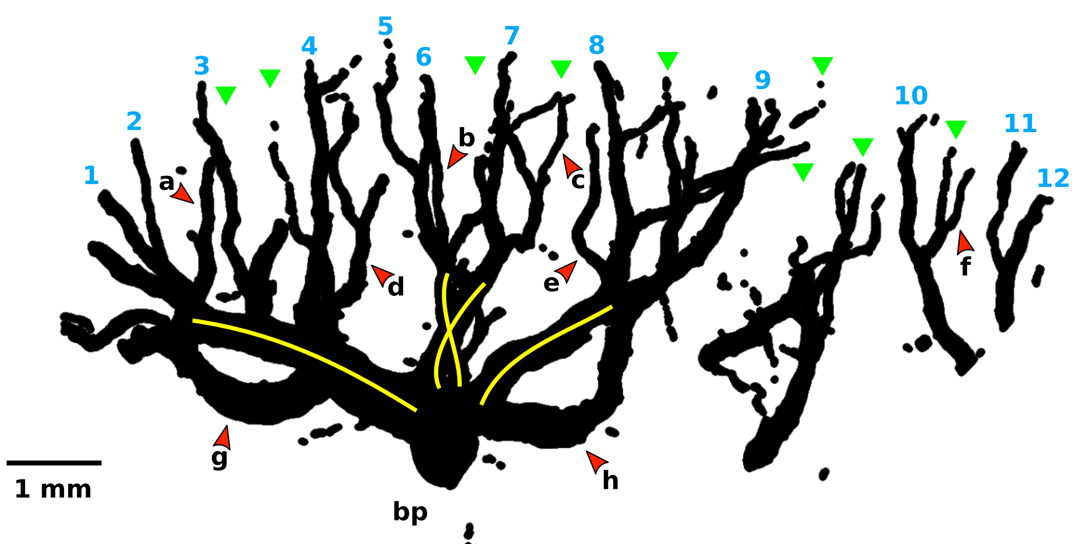

Fig. 2. Micro-CT maximum intensity projection for vessel diameter estimates > 50 µm. The projection is annotated with blue numbers and green triangles to mark correspondences between the SURE image and the micro-CT projection. The red arrows point to blood vessels that are visible in the micro-CT projection, but not in the SURE image. Yellow markings highlight blood vessels that go from the arcuate blood vessel branch point (bp) to the left, right and upwards where the latter vessels are inseparable in the micro-CT projection.

is not readily visible (see Fig. 2).

IV. Discussion

This qualitative evaluation covers a single example and does not present a statistical analysis of the performance of SURE imaging. However, the visual resemblance between the SURE image and the micro-CT projection highlights its clinical potential, and further quantitative assessments of vessel detection and shape representation should be considered to substantiate the validation of SURE imaging.

The micro-CT volume with a voxel size of 5 µm enables a detailed vessel characterisation in the excised kidney, but when the overlapping micro-CT volume is projected onto the ultrasound imaging plane the finer details may be lost. This could be because errors in the co-registration cause an offset in the projection perspective relative to the ultrasound imaging plane. It is also possible that changes in shape or relative position of the blood vessels are introduced between the two states of the tissue for either scan. This may lead to cases where a vessel is only visible in the SURE image because a smaller vessel is either hidden behind or in front of another vessel relative to the perspective of the micro-CT projection.

Importantly, the micro-CT projection only includes blood vessels with an estimated diameter of more than 50 µm in the micro-CT volume. Therefore, all the vessels that are only depicted in the SURE image may have a smaller estimated diameter than the threshold. These include the parallel vessels that may be depictions of arcuate veins and arteries in close proximity to each other where the artery is not included in the micro-CT projection because of the diameter constraint. However, most of the blood vessel endpoints appear similar in width and position in the SURE image and in the micro-CT projection. So, if the micro-CT size constraint causes parallel vessels to be excluded from the projection, the SURE image may not represent vessel diameters consistently because the parallel vessels appear similar to other vessels in the network (e.g. compare the width of labels e and f to label 2 in Fig. 1). On that note, it is important to acknowledge that neither SURE nor micro-CT are expected to render accurate representations of the natural diameter of the blood vessels in the kidney. Specifically for the micro-CT scan which was subject to the size constraint, the preparation of the kidney and possibly also the storage will have affected the size of the entire kidney and hence the vessels. This means that the diameter threshold at 50 µm for the micro-CT projection cannot be easily translated into natural blood vessel sizes.

The intensities in the SURE image depend on a non-linear signal accumulation from blood vessels that is not equally weighted in 3D [4, 5]. Therefore, the faintly depicted vessels may lie in the outer region of the ultrasound focus area and give less signal relative to other vessels. By assuming an ultrasound slice thickness of 2 mm across the entire image, the non-linearity of the SURE image in 3D was not accounted for in this analysis. Therefore, the overlapping micro-CT volume may be exaggerated and include more vessels than were part of the ultrasound data. Omitting vessels that may be hidden in the micro-CT projection as discussed (i.e. labels e-g in Fig. 1), there are eight instances of vessels only included in the micro-CT projection (i.e. labels a-h in Fig. 2) compared to four vessel segments only in visible the SURE image (i.e. labels a-d in Fig. 1) which may indicate that the assumed ultrasound slice thickness cause an exaggerated overlapping region with the micro-CT volume.

Since all cortical radial vessels depicted with high intensities in the SURE image were also included in the micro-CT projection, it is possible that the vessels only visible in the micro-CT projection were simply not part of the ultrasound data.

V. Conclusion

Despite the challenges with tissue preservation and multi-modal, 2D-to-3D image registration and analysis, SURE depicts most vessels at least down to 50 µm in diameter, and even resolves parallel vessels not visible in the micro-CT projection. In addition, the SURE image shows most blood vessels true to both their shape and relative position compared to the micro-CT projection.

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