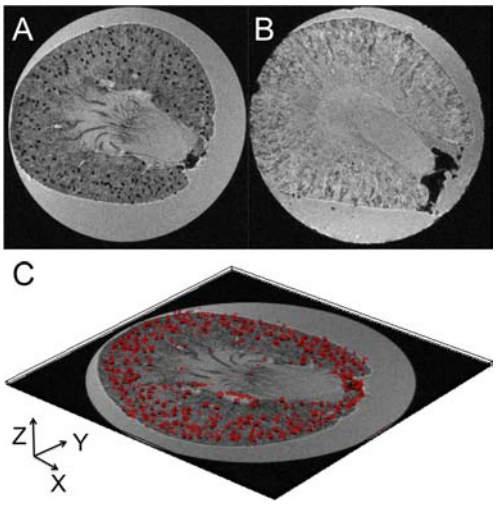
Accurate Mouse Kidney Morphology with Glomerulus-Targeted Contrast Agents

Edwin Baldelomar[1], Scott Beeman[2], Luise Cullen-McEwen[3], Jennifer R Charlton[4], John F. Bertram[3], and Kevin M. Bennett[5]

[1]*Department of Physics, University of Hawai'i at Manoa, Honolulu, Hawaii, United States,* [2]*Radiology, Washington University School of Medicine, St Louis, Missouri, United States,* [3]*Department of Anatomy and Developmental Biology, Monash University, Melbourne, Victoria, Australia,* [4]*University of Virginia Medical Center, Charlottesville, Virginia, United States,* [5]*Department of Biology, University of Hawai'i at Manoa, Honolulu, Hawaii, United States*

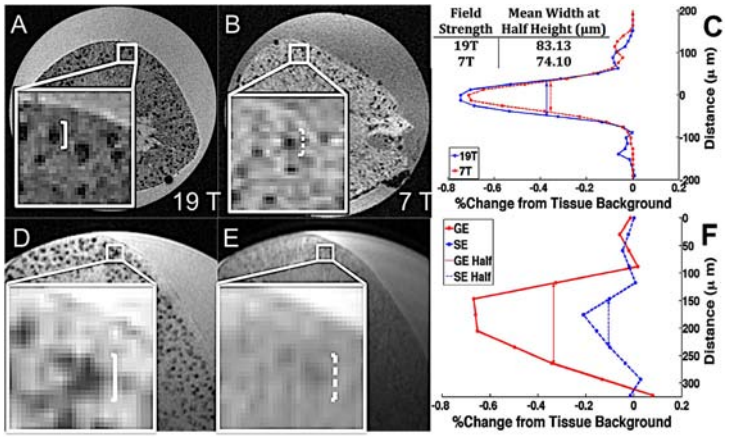
Introduction: Kidney structure and function are tightly coupled. Nephron endowment is strongly linked to susceptibility to chronic kidney (CKD) and cardiovascular diseases. (1, 2) Nephron endowment can be measured in intact kidneys using MRI with cationized ferritin (CF) as a superparamagnetic, glomerulus- specific contrast agent. (3, 4) Understanding the link between functional number (Nglom) and volume (Vglom) in mouse models is important to understanding causes of CKD and to establishing techniques to assess and improve transplant viability. Here we use CF as an MRI contrast agent to measure Nglom and Vglom. We optimized measurements of Vglom by considering field strength and static susceptibility due to CF labeling.

Figure 1 – 3D GE-MRI of CF (*A*) and NF (*B*) labeled mouse kidneys. (*C*) 3D reconstruction of identified glomeruli in a single image slice.



Methods: *MRI*- Six Male C57BL/6 mice were given 5.75mg/100g of CF in 1.5-hour intervals. Controls received native ferritin (NF). Three received two retro-orbital injections and three (n=3) received three IV injections of CF. After sacrifice, left kidneys were resected and imaged with gradient echo (GE) MRI. Three of the mice (Group A) were imaged with a Varian 19T NMR/MRI (TE/TR = 8/60 ms; 34x34x54 μm^3). The other three mice (Group B) were imaged with a Bruker 7T/30 MRI (TE/TR = 20/40 ms; 41x41x39 μm^3). One male Sprague-Dawley rat was given IV CF (same dose) and a second rat received no injection. *Image processing/validation* –Previous custom developed software measured apparent Nglom (aNglom) and Vglom (aVglom) from MRI (1). Resected kidneys were either acid macerated or received dissector/fractionator stereology for validation (5, 6). We developed a correction factor of aVglom base on measurement of local susceptibility artifact from CF by comparing spin echo (SE) and GE MRI of CF-labeled and naive rat and mice kidneys. We further investigated the dependence of this correction factor on field strength. We then applied this correction to the mouse aVglom and compared to stereology. For these scans, we used: (*i.*) Mouse - GE 3D 19T vs. 7T (*ii.*) Rat - 2D GE vs. SE labeled. (*iii.*) Rat - SE labeled vs. unlabeled. 2D parameters: SE-TE/TR = 10.878/3200 ms, and GE-TE/TR = 20/54.62 ms.

Figure 2 – Correcting for CF susceptibility. Line profiles in MRI of glomeruli in rats (*A,B – Varied field; D, E – SE vs GE on same location). C*, Mean line profile width ratio of 19T:7T was 1.12. *F*, Example of overlapping line profiles. Mean width ratio of all widths was .7207 ± .1580.



Results: CF-labeled glomeruli were visible as dark spots in the cortex in MRI of mice, not present in controls (Fig 1a-b). The software identified individual glomeruli in 3D (Fig 1c). Mean aNglom values for 19T(MRI/Stereology) were 12,500±154/11,390±157, and for 7T(MRI/Histology) were 12738±234/12267±2275. Group A uncorrected mean aVglom was 1.709 x 10^{-4} mm^3. Group B uncorrected mean aVglom was 27% larger than mean stereological volumes of 2.145 and 1.657 x 10^{-4} mm^3. *Susceptibility Correction:* (*i.*) Mean profile widths was 12% higher at 19T vs. 7T (Fig. 2 a-c) (*ii.*) GE and SE images on same labeled kidney of line profiles had a mean width ratio, SE:GE, of .7207 ± .1580. (Fig. 2 d-f) (*iii.*) SE of unlabeled kidney shows bright glomerular structure whose mean line profiles match rat glomerular diameter (7). Mean widths of unlabeled SE images were 20% larger than in CF labeled images. From this we determined a correction factor for aVglom. Corrected mean aVglom for 19 T, initially 2.145x10^{-4} mm^3, was 1.661 x 10^{-4} mm^3 after correction, compared to stereology-based mean estimate of 1.657 x 10^{-4} mm^3. Mean corrected aVglom for 7T was 1.477 x 10^{-4} mm^3.

Conclusion: MRI-based measurements of aNglom are consistent with histology. Resolution plays a large role in glomerular location in the voxel map, and will change the size of the apparent glomerulus in MRI. Higher resolution will produce higher voxel coverage per glomerulus and increase mean aVglom and lower resolution will decrease aVglom, which was seen at 19 and 7T. MRI after CF labeling can be used to accurately measure kidney glomerular number and volume in experimental mice.

References: (1) Brenner, et al. Am J Hypertens. 1:335, 1988. (2) Hoy, et al. Curr Op. Nephrol Hypertens. 17:258, 2008. (3) Beeman, et al. Am. Journ. Phys. Renal Phys. 300. 2011. (4) Heilman, et al. Neph. Dial. Trans. 100, 2012 (5) Bonvalet, et al. Kidney Int. 1:391, 1972. (6) Bertram, et al. Cell Tiss. Res. 270:37, 1992. (7) Smith, et al. Oxf. Univ. Press. 1951.