# Measuring whole kidney nephron endowment using MRI

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**Introduction:** The goal of this work was to measure nephron endowment in the whole kidney using MRI. The nephron is the major functional unit of the kidney, directly filtering components of the blood plasma both by size and charge and regulating pH and ion concentrations in the blood. Changes in the number of nephrons has been linked to a number of renal and systemic diseases (1, 2). A tool for non-invasive measurement of nephron number would thus serve as a useful diagnostic and predictive tool in animal studies and potentially in the clinic. Recently, intravenous injections of the iron binding protein ferritin modified with cationic amine groups (3) have been used to detect individual nephrons both *in vivo* and *ex vivo* with MRI (4). Here we develop and validate a robust molecular imaging and image processing technique based on systemic injection of cationic ferritin (CF) to produce an accurate count of individual nephrons with 3D MRI. This work makes it possible to assess whole kidney nephron endowment to study susceptibility to systemic renal diseases in animals and humans.

**Methods**: *Synthesis of Cationic Ferritin*: Cationic ferritin (CF) was synthesized by conjugating horse spleen ferritin (Sigma Aldrich, St Louis) to N, *N* - Dimethyl-1,3-propanediamine (DMPA) using 1-Ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), according to Danon et.al. (3). *In Vitro Preparation/Imaging*: Male Sprague-Dawley rats were given 3 bolus doses for a total of 5.75 mg/100g of CF (n=6) or native ferritin (NF) (n=6) with 1.5 hours between injections. The perfused left kidneys were imaged in glutaraldehyde on a Varian 19T NMR using a 3D GRE sequence with TE/TR = 7/40 ms and a resolution of 62x62x78 μm. *Post-processing*: Nephrons were counted in the 3D MRI dataset using custom software written in Matlab (The Mathworks, Inc.). Maximum spatial signal gradients in the original volume were calculated first to extract any dramatic spatial changing areas in the volume. Regional minima were located in these areas and regions considered to be nephrons were defined based on morphological thresholds. A 'guided' watershed algorithm was used to distinguish individual nephrons where signal overlap of multiple nephrons might occur. *Histology*: We performed two histological techniques to validate the MRI-based counts of nephrons: 1) acid maceration and counting and 2) sterology. Acid maceration was based on techniques established in the literature (5). Kidneys were cut into 1 $mm^3$ pieces immediately after resection and incubated in 5 ml of 6 N HCl for 1.5 hours. Incubated tissue was crushed and strained until homogenous. The solution was then brought up to 30 ml with deionized water. Nephrons in solution were counted in a counting chamber (1 $mm^2$ scored 35 mm culture dish, Nunclon delta). The total number of nephrons for each kidney was calculated based on the average number of nephrons per area. Stereology was performed on two of the kidneys in which serial sections of the left (imaged) kidneys were taken and individual nephrons across serial sections were compared and counted. Sections were chosen and processed based on the dissector/fractionator method, where the total number of nephrons is estimated based on the number of overlapping nephrons in serial sections identified in coupled light microscopes (6).

**Results and conclusions:** 3D gradient echo images of CF treated kidneys revealed successful labeling individual nephrons at 19T (fig. 1A), while NF failed to label nephrons (fig 1C). To assess the ability of our algorithm to specifically isolate CF-labeled nephrons, regions defined by the algorithm as nephrons were colored in CF (fig. 1B) and NF (fig. 1D) treated kidney volumes. Counting of labeled nephrons from 3D MRI datasets yielded 32,263±2,967 nephrons (n=6). The same counting algorithm yielded 932±578 nephrons from 3D MRI datasets of unlabeled control (NF) kidneys (n=6). Acid maceration counting of contralateral kidneys yielded 30,585±2,053 nephrons. The Dissector/fractionator stereology yielded counts of 35,421 and 34,504, compared to MRI-based counts in the same kidneys of 29,088 and 31,339 nephrons, respectively. Counting results are reported in Table 1. It should be noted that false positives counted in the control kidneys suggest that we have a counting error approximately one thousand extra nephrons per kidney, or ~3% error. We conclude that the MRI-based technique is capable of isolating individual nephrons in 3D and yields comparable results to both histological methods with the advantage of maintaining the entire organ. This result, along with previous in-vivo detection of nephrons with MRI, suggests the possibility of counting nephrons in animals and humans. There were systematic differences between MRI-based nephron counts and histological counts, with MRI-based counts obtaining ~ 5% more nephrons than acid maceration, and ~5% fewer than stereology. We note that both of the histological techniques for measuring nephron endowment are based on extrapolations of a limited number of counts to the entire kidney. Future work will be focused on estimating nephron endowment and function in-vivo. To the best of our knowledge, this is the first report of a technique to directly count every nephron in the whole kidney.

**Table 1** – The number of nephrons counted using the MRI technique are comparable to both the acid maceration and stereology techniques.

| Kidney | Imaging count | Maceration | Stereology |
|---|---|---|---|
| A | 29,088 | 27,504 | 34,504 |
| B | 29,533 | 31,190 | - |
| C | 31,339 | 28,944 | 35,421 |
| D | 32,880 | 31,075 | - |
| E | 37,118 | 33,321 | - |
| F | 33,599 | 31,478 | - |
| avg±std dev | 32,263±2,967 | 30,585±2,053 | 34,963 |

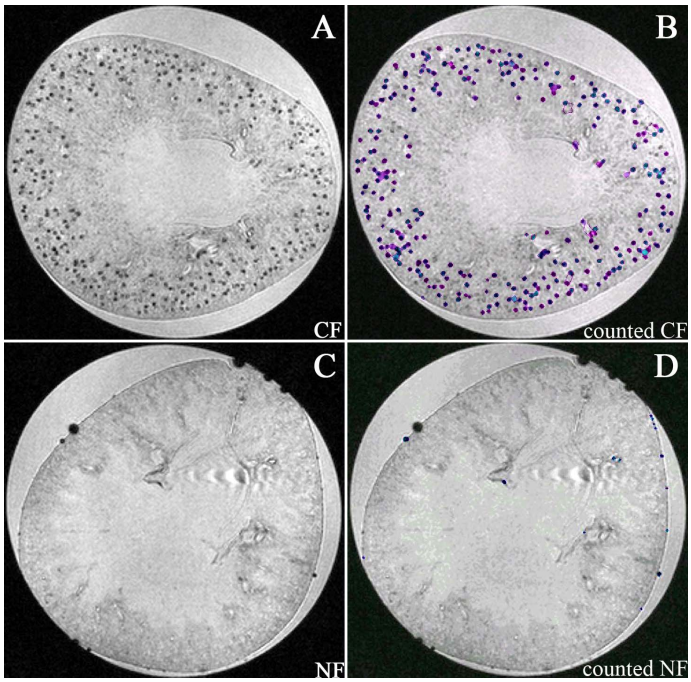


**Figure 1** – Individual nephrons are identified by dark spots in the CF-labeled kidney (A). No nephrons are visible in the NF control kidney (C). Regions defined by the computational 3D counting algorithm as nephrons are colored in kidneys (2D slices shown from a 3D MRI dataset) after CF (B) and NF (D) injections and perfusion.

**References**: (1) Olivetti G et al. Kidney Int. 17(4), 1980. (2) Brenner BM et al. Am J Hypertension 1(4), 1988. (3) Danon et al. J Ultrastr Res. 38(5-6), 1972. (4) Bennett KM et al. Mag Res in Med. 60(3), 2008. (5) Godley, L et al, G&D 1996 (6) Sterio, D et al. J Microsc, 134, 1984