Introduction: The human kidney contains between 400,000 and 2,000,000 nephrons, each contributing to bulk macromolecular filtration and fluid retention or excretion. Kidney damage often occurs with hypertension, diabetes, and a range of autoimmune diseases, leading eventually to dialysis or transplant. Changes in GFR and levels of serum markers such as creatinine are often not observed before the development of severe or end stage renal disease. Nephrons are thought to provide compensatory hyperfiltration when there is a loss of bulk glomerular filtration, making this loss clinically undetectable. To better develop drugs to treat kidney and systemic disease, and to understand the physiological mechanisms of kidney damage during disease development, it is critical to develop noninvasive techniques to measure whole kidney function at the level of the single nephron. Studies using micropuncture of superficial glomeruli have revealed heterogeneity in single nephron GFR in rats, though these studies are spatially limited and are impractical for clinical applications such as transplant evaluation and in vivo diagnostics. Recent advances in MR have also been used to show individual glomerular function within small cortical volumes. Here we investigated the combined use of two MRI contrast agents, cationic ferritin (CF) and gd-DTPA, to measure local macromolecular uptake and glomerular filtration rates in 3D MRI at voxel resolution in isolated, perfused rat kidneys under physiological conditions. CF is a glomerulus-specific, superparamagnetic nanoparticle, and gd-DTPA is eliminated through the tubule. CF uptake rate was measured dynamically as a marker for macromolecular filtration, and Gd-DTPA passage was used as a marker for local (voxel) glomerular filtration.

Methods: All experiments were approved by an Institutional Animal Care Use Committee. Three male Sprague-Dawley Rats were anesthetized and transcardially perfused with saline. Left kidneys were extracted from the animal with the renal artery (RA) and ureter intact. A catheter was placed through the RA toward the kidney and sutured to prevent back flow. The kidney was secured on a custom holder to prevent motion. To accurately mimic physiological conditions, we perfused with Krebs-Ringer bicarbonate solution (Sigma-Aldrich) with 7.5 mg/100mL of Fraction-V BSA (Roche) and a bubbling infusion of CO2/O2 (95%/5%). Temperature was maintained at 37°C. A perfusion pump (Harvard Apparatus) was used to perfuse the perfusate media at a constant flow of 5mL/min throughout all experiments. 120 mL of cationized ferritin (CF), homogeneously mixed with the Krebs-Ringer solution (0.071 mg/mL of CF), was infused into the system at time(t) equal to 12 min. After CF infusion, Krebs-Ringer solution was re-infused to wash.. At t=12min after re-infusion, a bolus of 0.25mmol Gd-DTPA (Magnevist) in 10mL of Krebs-Ringer solution was infused into the perfusion system. Kidneys were imaged during the perfusion with a Bruker 7T30 MRI (Billeraica, MA, USA) using either a T1 or T2* weighted, 3D gradient recalled echo with partial acquisition and the following imaging parameters: T1s: TE/TR = 10ms/60ms, resolution = 104 x 188 x 234 μm3; T1w: TE/TR = 3.18ms/19.01ms, resolution = 104 x 188 x 234 μm3. A T1 weighted image was acquired for 5 time points during the Gd-DTPA infusion. A T1*-weighted sequence was used for all other images acquired. Time-courses were created with consecutively acquired 3D data sets (2 min temporal resolution). Voxel time courses were then fitted to a bi-exponential model: $S(t) = C + L \cdot t + k \cdot \left(e^{-\alpha t} + e^{-\beta t}\right)$. Here $C$ = Y-Intercept, $L$ = Linear Factor, $k$ = Multiplication Constant, $t_0$ = Response Time, $\alpha$ = Absorption Rate, $\beta$ = Elimination Rate. Both CF and Gd-DTPA absorption and elimination were modeled this way. Glomeruli were segmented in the images by difference in signal intensity from beginning to end of perfusion. A filtration rate of each voxel was determined from the elimination rate of Gd-DTPA multiplied by a fixed unit amount of fluid flowing through the voxel, assuming uniform distribution of total inflowing volume.

Results: CF was visible accumulating in glomeruli as dark spots in images of the kidney 8 minutes after the beginning of perfusion (Fig 1 & 3). Coefficients of the parameters were mapped in each voxel. Spatial maps revealed heterogeneous uptake rates of both CF and Gd-DTPA in the kidney (Fig 2-A,B). Glomeruli were distinguished by uptake of CF from the rest of the kidney after segmentation, and a distribution of CF accumulation rates was visible (Fig 2-C). We used the location of the glomeruli in 3D and the timecourse of CF labeling to estimate uptake rate of CF in each glomerulus. Glomeruli were readily distinguished from the surrounding tissue in histograms of CF uptake rate (Fig 4 - insert). Voxels with identified glomeruli had an uptake rate of (0.0-0.3/s), while surrounding tissue had a bi-modal, higher rate of uptake. Voxel filtration rates of Gd-DTPA in the same glomeruli, identified by the time course after Gd-DTPA perfusion, were 5 μL/min on average, with a bi-modal distribution (Fig 5). Since the lumen of the tubule would occupy ~2% of a voxel this size our voxel estimates of local GFR agree with previous estimates of single-nephron GFR of ~50 μL/min.

Discussion: CF and gd-DTPA were used in conjunction to map glomerular filtration in the isolated, perfused rat kidney in 3D. The heterogenous distribution of macromolecular uptake and glomerular filtration warrant investigation, and may be important for detection of a range of kidney diseases. Further work is required to validate the MRI-based estimates of glomerular filtration, and to understand the source of bi-modal glomerular filtration. Recent improvements in contrast agent relaxivity and image acquisition should make these types of measurements practical in vivo. To our knowledge this is the first report of whole-kidney estimates of glomerular filtration mapped at the scale of the single nephron.

Fig 1. Ex- Vivo GRE-MRI of Rat Kidney labeled with CF, showing individual glomeruli. Fig 2. (A) Kidney with (B) map of CF uptake rate. (C) CF accumulation (Uptake/elimination) highlights glomeruli. Fig 3. MRI at during CF uptake (t1-t3), followed by Gd-DTPA (t5). Fig 4. Comparison of CF uptake rates for glomerular and non-glomerular areas. Insert shows example voxel time courses, centered around a glomerulus. Fig 5. Bi-modal voxel distribution of Gd-DTPA filtration rate through the identified glomeruli and map of Gd-DTPA filtration rate.