

# Quantitative Assessment of Renal $T_2^*$ Heterogeneity with Minkowski Functionals for the Detection of Ischemia/Reperfusion Injury

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**Target audience:** MR scientists, imaging scientists, clinicians and clinical scientists with an interest in kidney injury.

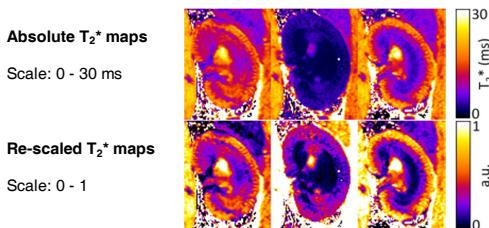
**Introduction and Purpose:** Two million per year is the estimated worldwide death toll of AKI,<sup>1</sup> with renal ischemia-reperfusion (I/R) being one of the major causes.<sup>2</sup> AKI diagnosis still largely relies on serum creatinine, which is a poor marker because of its low absolute and temporal sensitivities. Imbalance between renal tissue oxygen demand and supply is a pivotal element in the pathophysiologic cascade of I/R injury.<sup>3,4</sup> We have demonstrated the feasibility of fast continuous parametric mapping of renal  $T_2^*$ , which is known to be blood oxygenation level dependent, to monitor I/R in rats.<sup>5</sup> Here, renal  $T_2^*$  values during I/R could be related to the individual baseline values prior to the injury. In patients experiencing AKI, however, individual reference values are usually not available. Even if available, comparison of absolute values of renal  $T_2^*$  is barely feasible, because absolute values depend on magnetic field strength,  $B_0$  homogeneity, voxel size, etc. Also quantitative analysis is commonly based upon regions-of-interest, which are prone to operator bias. Recently, image heterogeneity analysis by 2D Minkowski functionals (MF) has been applied to examine MR imaging based treatment response of tumors.<sup>6,7</sup> MFs are not biased by overall image intensities or scale and do not require any assumptions about image content.<sup>8,9</sup> By applying the MF approach to renal  $T_2^*$  data we investigated whether the quantitative analysis of renal  $T_2^*$  heterogeneity – while disregarding differences in absolute  $T_2^*$  – allows a differentiation between healthy naïve kidneys, ischemic kidneys, and kidneys during subsequent reperfusion.

**Methods:** *Animal model:* 7 anesthetized male Lewis rats, aged 2-3 months, weighing 250–300g underwent renal I/R inside a 9.4T MR system (Bruker Biospin, Germany).<sup>5</sup>  $T_2^*$  was continuously monitored with a temporal resolution of ~3 min. Ischemia was induced by closing a remotely controlled hydraulic occluder around the renal artery and vein for 45 minutes, subsequent release of the occlusion led to reperfusion.<sup>5</sup> Interruption and restoration of renal blood flow was confirmed by time-of-flight MR angiography. *MR imaging:* Experiments were carried out with a birdcage RF resonator for transmission in conjunction with a four channel receive RF coil array (Bruker Biospin, Germany) customized for rats.  $T_2$ -weighted pilot scans and local  $B_0$  shimming that uses a voxel tailored to the kidney were performed first. For  $T_2^*$  mapping a respiratory gated multi gradient echo (MGE) sequence (TR = 50 ms, number of echoes = 10, first TE = 1.43 ms, echo spacing = 2.14 ms, averages = 4) was employed with a total acquisition time of approx. 1 min 20 s. A coronal oblique image slice was acquired with a spatial in plane resolution of (226x445)  $\mu\text{m}^2$  and a slice thickness of 1.4 mm.  $T_2^*$  maps (Fig.1) were calculated using MATLAB (Mathworks, USA). *Heterogeneity analysis:* Following manual segmentation of the kidneys, the bias of differences in the absolute overall  $T_2^*$  was removed by re-scaling 0.01 to 0.99 percent of the intensities in each map to the range [0,1]. The maps were then thresholded in 10 steps to create binary images (Fig.2). For the visible pixels Minkowski functionals were calculated using MATLAB, yielding the area, perimeter, and genus as a function of the threshold,<sup>8,9</sup> based on counting the number of pixels, edges and vertices.

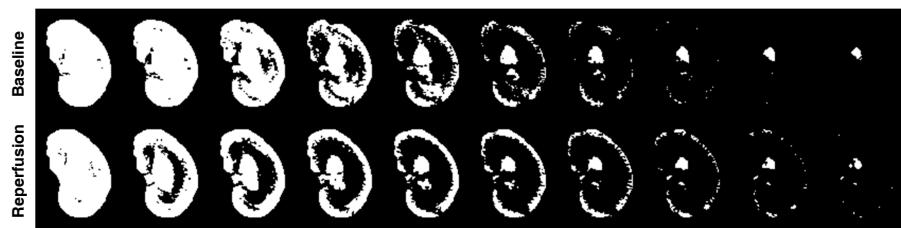
**Results:** Absolute and rescaled renal  $T_2^*$  maps obtained for baseline, ischemia and 100 min reperfusion are shown in Fig. 1. Binary images deduced from thresholding of baseline and 100 min reperfusion  $T_2^*$  maps are demonstrated in Fig. 2. Minkowski functionals comparing renal  $T_2^*$  at baseline, 45 min ischemia, and 100 min reperfusion are shown in Figure 3. The analyses of renal  $T_2^*$  heterogeneity supports differentiation between healthy naïve kidneys, ischemic kidneys, and kidneys after 100 min reperfusion. t-tests were performed at one threshold selected for each comparison. Significant differences to baseline were observed in the area and perimeter at a threshold of 0.2 (p<0.01). A further differentiation between ischemia and reperfusion was possible when using the area obtained for a threshold value of 0.6 (p<0.05).

**Discussion and Conclusion:** This study shows that analysis of renal  $T_2^*$  heterogeneity alone, i.e. disregarding differences in absolute  $T_2^*$ , allows a distinction between healthy kidneys, ischemic kidneys, and kidneys during subsequent reperfusion. Although in this experimental model the differences in absolute  $T_2^*$  were large and sufficient to distinguish the different phases of I/R,<sup>5</sup> renal  $T_2^*$  heterogeneity analysis using MF is a promising new technique. MF analysis holds the potential to identify and assess renal pathophysiological scenarios independently of baseline  $T_2^*$  references, magnetic field strength,  $B_0$  homogeneity, and choice of regions-of-interest. MF analysis within a given renal layer may provide an even more detailed insight and higher sensitivity.

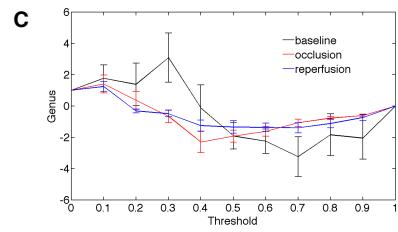
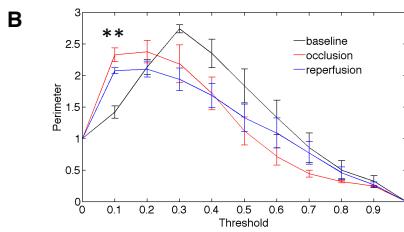
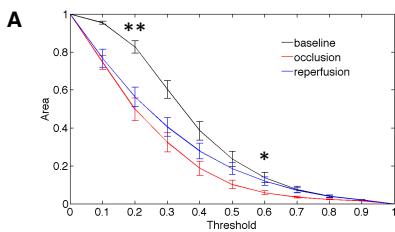
**References:** [1] Chawla LS, et al. (2012) Kidney Int 82:516. [2] Lameire N, et al. (2008) Lancet 372:1863. [3] Schrier RW, et al (2004) J Clin Invest 114:5. [4] Bonventre JV, Yang L (2011) J Clin Invest 121:4210. [5] Pohlmann A, et al. (2013) PLoS ONE 8(2) e57411. [6] Larkin TJ, et al (2013) doi:10.1002/mrm.24644. [7] Canuto HC, et al (2009) MRM 61(5):1218. [8] Michielsen K, et al (2001) Physics Reports 347:461. [9] Legland D, et al (2007) Image Anal Stereol 26:83.



**Figure 1:**  $T_2^*$  maps of a rat kidney at baseline (left), after 45 min ischemia (middle), and after 100 min reperfusion (right).



**Figure 2:**  $T_2^*$  maps were thresholded in 10 steps (increasing from left to right) and the Minkowski functionals were calculated as a function of threshold. Examples for baseline (top) and 100 min reperfusion (bottom) are shown.



**Figure 3:** Minkowski functionals (mean $\pm$ SEM, n=7) were calculated as a function of threshold: area (A), perimeter (B), and genus (C), comparing baseline (black), 45 min ischemia (red), and 100 min reperfusion (blue). Renal  $T_2^*$  heterogeneity analyses allowed a distinction between naïve kidneys and pathophysiological kidneys during ischemia and reperfusion. Each t-test was performed at only one threshold (to avoid multiple-comparisons): a significantly lower area (at threshold 0.2) and higher perimeter (at threshold 0.1) distinguished ischemia and reperfusion from baseline (\*\* p<0.01). Further differentiation between ischemia and reperfusion was possible on the basis of the area being significantly smaller for occlusion compared to reperfusion at threshold 0.6 (\* p<0.05).