

In vivo Imaging of Kidney using Fluorescent Markers with 2 Photon Microscopy

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Renal function is a combination of glomerular filtration of the blood and tubular modification of the filtrate. It is hard to appreciate this dynamic process from the typical 2 dimensional images of fixed tissue. There are three components to this process, a) renal blood flow, b) glomerular filtration and c) tubular re-absorption and secretion. These normal features can now be directly evaluated as well as pathological processes in rodent kidney such as: altered filtration properties, glomerulosclerosis, tubule endocytosis, exocytosis, intracellular trafficking, apoptosis, increased endothelial permeability, vasoconstriction, epithelial membrane blebbing and mitochondrial function. These processes are viewed in near real time since there are a number of excellent fluorescent markers that can be used *in vivo*, in combination with 2 photon microscopy (1). The anesthetized rat can be placed directly on the microscope with the kidney exteriorized (Figure 1). Renal tubules are responsible for metabolism and clearing of many different drugs, some of which are toxic to the kidney. For example, Gentamicin, a nephrotoxic antibiotic can be directly visualized as it is filtered, passing through the proximal then distal tubules and finally through the collecting ducts (Figure 2) however some of the gentamycin is endocytosed by the proximal tubule, where it reaches toxic levels (2). Using fluorescent dyes also allows an evaluation of blood flow including the determination of flow rate by evaluating the transit time for RBCs to pass through capillaries and for the determination of plasma clearance rate (Figure 3) by following the disappearance of FITC inulin from the blood (3). Details of *in vivo* renal imaging methodology have been clearly described so that any lab with 2 photon microscopy capability can perform these types of studies (4).

References

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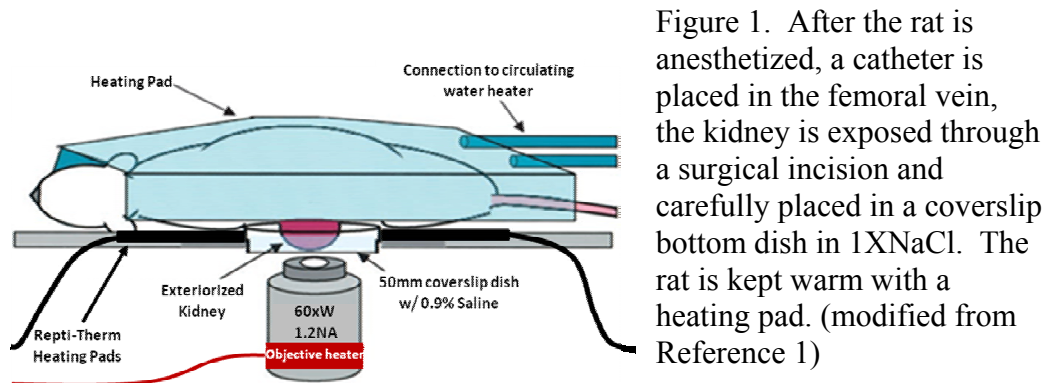


Figure 1. After the rat is anesthetized, a catheter is placed in the femoral vein, the kidney is exposed through a surgical incision and carefully placed in a coverslip bottom dish in 1XNaCl. The rat is kept warm with a heating pad. (modified from Reference 1)

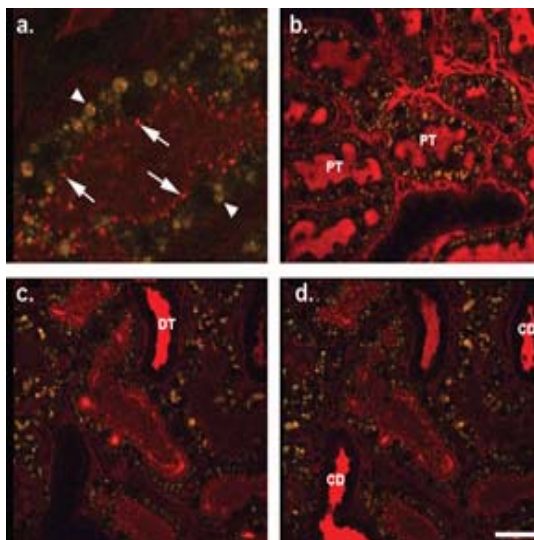


Figure 2. Using a timed series of images (b.-d.) from an intact rat given Texas-red gentamicin i.v., the gentamicin was found in proximal tubules (PT in b.) at 1 min. and then seen in distal tubules (DT in c.) at 3 minutes and shortly thereafter in the collecting ducts (CD in d.). However, some Texas-red gentamicin was seen in the endosomes of the proximal tubule (arrows in a.). The retention of gentamicin in PTs initiates in nephrotoxic affect on this segment. Scale bar = 40 um (modified from reference 1.)

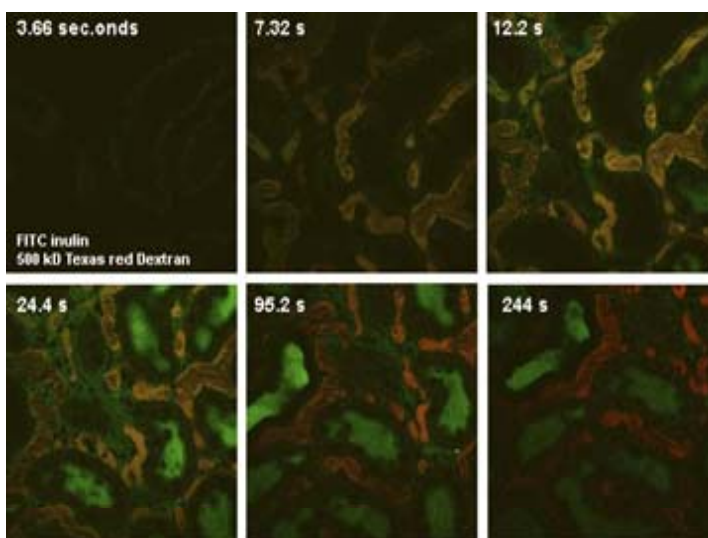


Figure 3. A timed series of images from an intact rat given FITC-inulin (filtered) and Texas-red 500kD dextran (not filtered). The green and red are initially together in blood vessels, but as the inulin is filtered (and is seen in the renal tubules, green) it disappears from the blood leaving the Texas-red in the vessels. The disappearance of the FITC from the blood can be used to determine the clearance rate (GFR). (modified from reference 3)