Chronic kidney disease (CKD) currently affects approximately 23 million people in the United States alone. As such, scientists are actively researching ways to treat and slow the progression of such ailments. The primary means of studying the onset and progression of CKD and other renal disorders is to measure the glomerular filtration rate (GFR). Current methods of measuring kidney function involve the observation of creatinine and inulin clearance. Progression of CKD presents with a reduction in GFR, as measured by reduced creatinine and inulin clearance rates. Studies utilizing nonradioactive iodine X-ray contrast media have more recently been used in order to measure renal insufficiency. Iodinated contrast agents are a reliable measure of GFR through a bolus injection followed by blood sampling. This technique is used both in laboratory rats and in the clinic because the rate of contrast agent clearance can be measured reproducibly. Furthermore, administration of iodinated X-ray contrast media (iodixanol) produces no major changes in glomerular filtration rate and is a safe diagnostic tool in patients with kidney functions ranging from normal to end-stage renal disease.

We hypothesized that the rate of renal clearance could be measured through the use of a bolus injection of X-ray contrast agent followed by non-invasive, time-lapse planar X-ray imaging. Indeed, previous studies have shown that contrast-enhanced X-ray computed tomography (CT) presents as an effective method for determining GFR in both dogs and laboratory mice. However, CT methods typically apply high doses of radiation and are much slower during data acquisition—both factors a result of the need to acquire hundreds of images for reconstruction. Here we show proof of a principle for a technique that utilizes planar X-ray time-lapse imaging to measure GFR dynamics with a much lower radiation dose than that applied by CT.

Since iodinated compounds have well known X-ray absorption properties, they can be directly visualized by X-ray imaging as they pass from systemic circulation to the kidneys and then to the bladder. The detection of the contrast agent can be further enhanced through X-ray differential imaging. This method applies a post-image processing algorithm that subtracts the pre-contrast injection image from the subsequent images. Thus, we are able to minimize contrast from bone and soft tissue while boosting the relative contrast of the injected iodinated contrast agent. This serves to enhance visualization of the contrast agent and also permits better quantification given that only absorbance of the X-ray contrast agent is measured. This study utilized a commercially available multimodal imaging system, the In-Vivo MS FX PRO, which includes a high-resolution planar X-ray (>25 lp/mm resolution) to image the clearance of the iodinated X-ray contrast agent Visipaque™ (GE Healthcare Ltd., Piscataway, NJ, USA, iodixanol) through a live mouse. Effective visualization and quantification of X-ray contrast media clearance in digital images could present as an alternative method for measuring GFR as an indicator of CKD onset and progression and also hold utility for phenotyping and drug efficacy studies.

Materials and Methods
A male mouse (age = 10 wk, BALB/cAnNTac, Taconic Farms, Germantown, NY, USA) was used in this study. The mouse was anesthetized by isoflurane (1.5% flow rate), kept under at 1.5% via a nosecone setup, and restrained on a clear bottom animal tray with clear tape.

An initial X-ray image was captured using an In-Vivo MS FX PRO. Capture settings were as follows: 60 sec exposure, 2 x 2 binning, f-stop 2.8, field of view (FOV) 100 mm, kVp = 35, 0.4 mm aluminum X-ray filter, illumination corrected.
This image acted as the pre-injection image, which was used to calculate X-ray difference images.

After acquisition of a pre-injection image, the animal tray was removed from the system with the mouse still restrained to enable future reproduction of the mouse’s initial position. While restrained on the tray, the mouse was administered a single bolus injection of 100 μL of Visipaque, an iodinated X-ray contrast agent with an iodine content equivalent of 320 mgI/mL (32 mgI) intravenously by way of the lateral tail vein. (Note: This process could alternatively be accomplished through tail vein catheterization.) The animal tray with the restrained mouse was placed back inside the imaging system and subsequent time-lapse X-ray images were taken to observe contrast agent clearance. Images were taken from 3 min to 40 min post-injection of contrast agent.

MI software was used to generate X-ray difference images. This was accomplished using the Image Math subtraction formula Z = X – Y, with the initial pre-injection image used as Y, and each of the subsequent experimental post-injection images served as X.

The raw X-ray images (without image subtraction) were exported as unscaled 16-bit TIFF files, and X-ray difference images were exported as 16-bit TIFF files scaled to display min/max for image preparation in Image J. Images were contrasted to show renal structures of interest, such as the kidneys, ureters, and bladder. A montage of both raw X-ray images and X-ray difference images at various time points was created using Image J.

**Results/Discussion**

A Sequential time-lapse imaging resulted in effective visualization of renal structures (see Figure 1). Post-injection, the kidneys can be observed in the raw X-ray image and become more prominent as Visipaque filters to the kidneys. At 6 min post-injection, the ureters (approximately 0.4 mm in diameter as measured by the MI software) can be clearly visualized. As the Visipaque contrast agent continues to clear, the calyces and renal pelvis of the kidneys are starkly contrasted. Further clearance of contrast agent allows for bladder visualization. Bladder expansion, as a result of greater Visipaque-containing urine volume, can also be visually observed and measured quantitatively in MI software.

The optical signal was precisely converted to X-ray density units, and quantification of Visipaque clearance was accomplished through region of interest (ROI) analysis in MI software. ROI analysis of the X-ray subtraction images in density units are shown in Figure 2.

ROIs were tightly drawn around renal structures of interest at the 40 min time point, and the same position ROIs were applied to all subtraction images using the ROI template feature in MI, thus tracking the changes in X-ray density over time at each respective site.

Quantification of the X-ray difference images, converted to X-ray density units, provides a metric for flow measurement of X-ray contrast agent through the renal system. Images show (Figure 1) that the calyces and renal pelvis of the kidneys are initially detected in the time course, which is noted by a marked increase in density, as shown in Figure 2. These structures appear to maintain their contrast throughout with decreases noted near the end of the time course. The entirety of the kidneys lack contrast initially but accumulate X-ray density over time and then diminish at later time points, albeit with less overall change than the calyces. Analysis of the bladder, however, may provide the most effective way to measure GFR. Initially, the concentration of Visipaque in the bladder is very low and increases gradually over time, as demonstrated by a strong linear fit (R² = 0.9574) of X-ray density unit increase in the bladder over time.

![Figure 1](image_url)

Raw X-ray images (left panel) and subtraction images (right panel) of interest during time course. Top row (l to r): pre-Visipaque injection, 3 min post-injection (PI), 6 min PI; middle row (l to r): 10 min PI, 17 min PI, 26 min PI; bottom row (l to r): 30 min PI, 36 min PI, 40 min PI.
References


Conclusion
Through the use of the In-Vivo MS FX PRO digital imaging system, one can putatively visualize and quantify renal clearance utilizing an iodinated contrast agent. X-ray subtraction imaging can be performed to further enhance contrast of renal structures of interest by minimizing innate soft tissue. Dynamic X-ray imaging of such pathways could potentially be useful in the diagnosis and monitoring of chronic kidney disease and other renal disorders that affect glomerular filtration rate, demonstrated by altered rates of contrast agent clearance from the kidneys and bladder accumulation. The present study is given as proof of the principle that the high-X-ray (>25 lp/mm) of the In-Vivo MS FX PRO can effectively image renal clearance of Visipaque in a pre-clinical setting. Features such as conversion to X-ray density units, facile time-lapse acquisition software, and X-ray subtraction imaging further enhance both visualization and quantification of such a dynamic process.