APPLICATION NOTE

Contrast-enhanced imaging of vasculature and soft tissues using the Quantum MicroCT system.

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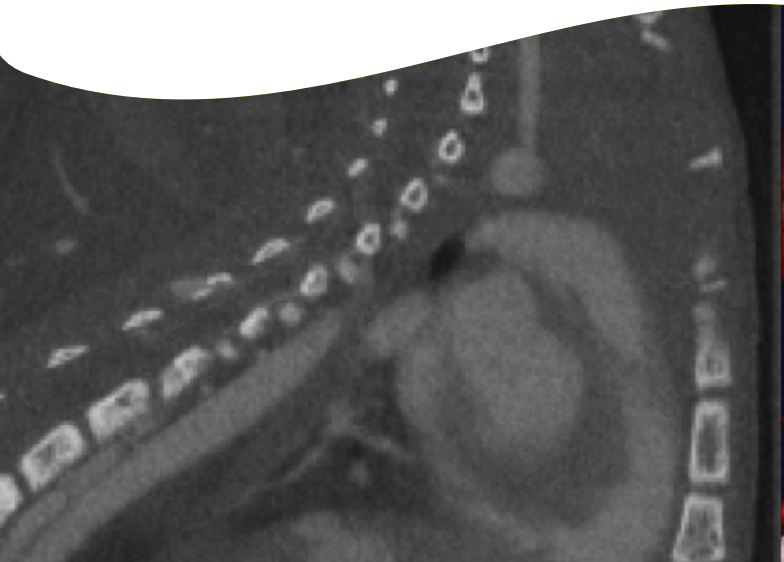
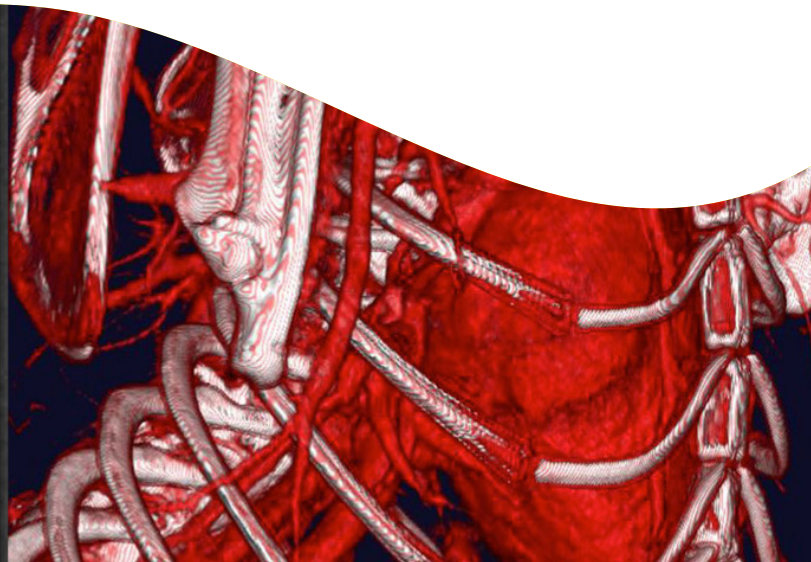
Abstract

The Quantum GX x-ray microCT imaging system represents the latest advances in image resolution, acquisition speed, and data visualization. This versatile preclinical imaging system is proven for a broad-range of applications including, but not limited to, pulmonary disease, cardiovascular disease, diabetes, orthopedics, cancer, and dentistry.

X-ray CT imaging is commonly used for skeletal imaging as bones are densely mineralized tissues with excellent x-ray attenuation properties. In contrast, soft, less dense tissues often prove to be challenging to image due to their lack of sufficient tissue density. Soft tissues such as muscle, blood vessels and internal organs share similar x-ray attenuation characteristics and are not distinguishable under typical CT settings. In order to introduce density that would improve soft tissue contrast, several contrast agents have been developed for use in clinical and preclinical settings. For a contrast agent, the type of target tissues and applications depend on its biodistribution and pharmacodynamic properties in the living subject. For example, contrast agents with a long circulation half-life are suitable for vessel imaging in blood-rich organs, such as the heart, liver and kidneys. Alternatively, as the agent is removed from the bloodstream and excreted in organs such as the liver, spleen and kidneys, sufficient image contrast can be established in these organs for visualizing their inner structures. This application note outlines the use of iodine-based and nanoparticle contrast agents for imaging soft tissues and vasculature in various organs. Furthermore, it demonstrates the advantages of multimodality imaging using the Quantum GX microCT and PET/CT to visualize the response of a tumor to an anti-vascular therapy. The Quantum GX microCT provides qualitative information about changes in tumor vasculature, while the PET/CT system provides functional assessment of the drug's impact on glucose metabolism in the tumor.





Contrast agents used for CT imaging

Computed Tomography (CT) technology is based on the differential attenuation and diffraction of x-rays between different tissues. As bones have the highest density in the body, CT imaging is frequently used for structural and skeletal imaging. On the other hand, soft tissues and most internal organs do not possess sufficient density for direct CT imaging. To address this issue, several contrast agents are available that enhance the soft tissue density for x-ray and CT imaging purposes. Table 1 lists the contrast agents currently available for preclinical or clinical CT imaging applications. Generally, these agents can be grouped into two categories based on the key chemicals used to enhance tissue density: (1) the iodine-based compounds; or (2) metal-containing nanoparticles. Iodine is the heaviest halide that can be used in a variety of compounds, such as iopamidol, iohexol, iopromide, and iodixanol[1]. These small molecule iodinated contrast agents are commonly used in the clinic for x-ray angiography and CT imaging. To improve their pharmacodynamics for vascular imaging, these dense, iodinated molecules can be incorporated into larger colloidal lipid micelles that provide better stability and prolong their circulation time in the bloodstream[2]. Contrast agents based on nanoparticles with metal cores have been widely used for preclinical imaging of small laboratory animals.[2] As metal is intrinsically dense with regard to x-ray attenuation, this type of agent produces better vascular contrast as well as a longer imaging window for microCT imaging in comparison with iodinated agents.

Table 1. CT contrast agents and their applications.

Contrast agent	Agent type	Application	Comment
Iodine-based			
Isovue®	Clinical	Excellent for imaging kidney/bladder; Requires continuous infusion for vascular imaging	Iopamidol-based
Omnipaque			Iohexol-based
Visipaque™			Iodixanol-based
ExiTron™ U	Preclinical	Kidney/bladder/vasculature	Iopromide-based
ExiTron™ V			Iodixanol-based
OptiPrep™			Iodixanol-based density gradient medium
eXIA	Preclinical	Vasculature/liver/spleen/brown adipose tissue	An aqueous colloidal polydisperse contrast agent with longer circulation time
Fenestra®	Preclinical	Vasculature/liver	Polyiodinated triglyceride lipid emulsion
Nanoparticle			
ExiTron™ nano6000 or 12000	Preclinical	Vasculature/liver/spleen ExiTron nano12000 has double concentration	Alkaline earth metal-based nanoparticles
AuroVist	Preclinical	Vasculature	Gold nanoparticles

Iodine-based contrast agents for kidney imaging

Iodinated contrast agents are used in the clinic to assess kidney function and detect blockages in the urinary tract. Ingeneral, these iodinated agents are comprised of small iodinated molecules with short circulating half-life in the bloodstream. Commonly used iodine-containing contrast molecules include iopamidol, iohexol, iopromide and iodixanol[1]. After systemic delivery, they are quickly removed from the bloodstream by the kidneys. As the agents are excreted via the renal pathway, they produce excellent tissue contrast in the kidneys and urinary tract.[3] Figure 1 illustrates the kidney images acquired using ExiTron V, an iodixanol based preclinical contrast agent. Kidney contrast can beestablished within 10 minutes after a single bolus injection ofthe contrast agent via the tail vein (100 µL, as recommended by the manufacturer). The 2D slice views showed the agent was concentrated in the kidneys with high contrast being seen in the ureters. The cortex and medulla showed lessintense contrast signals.

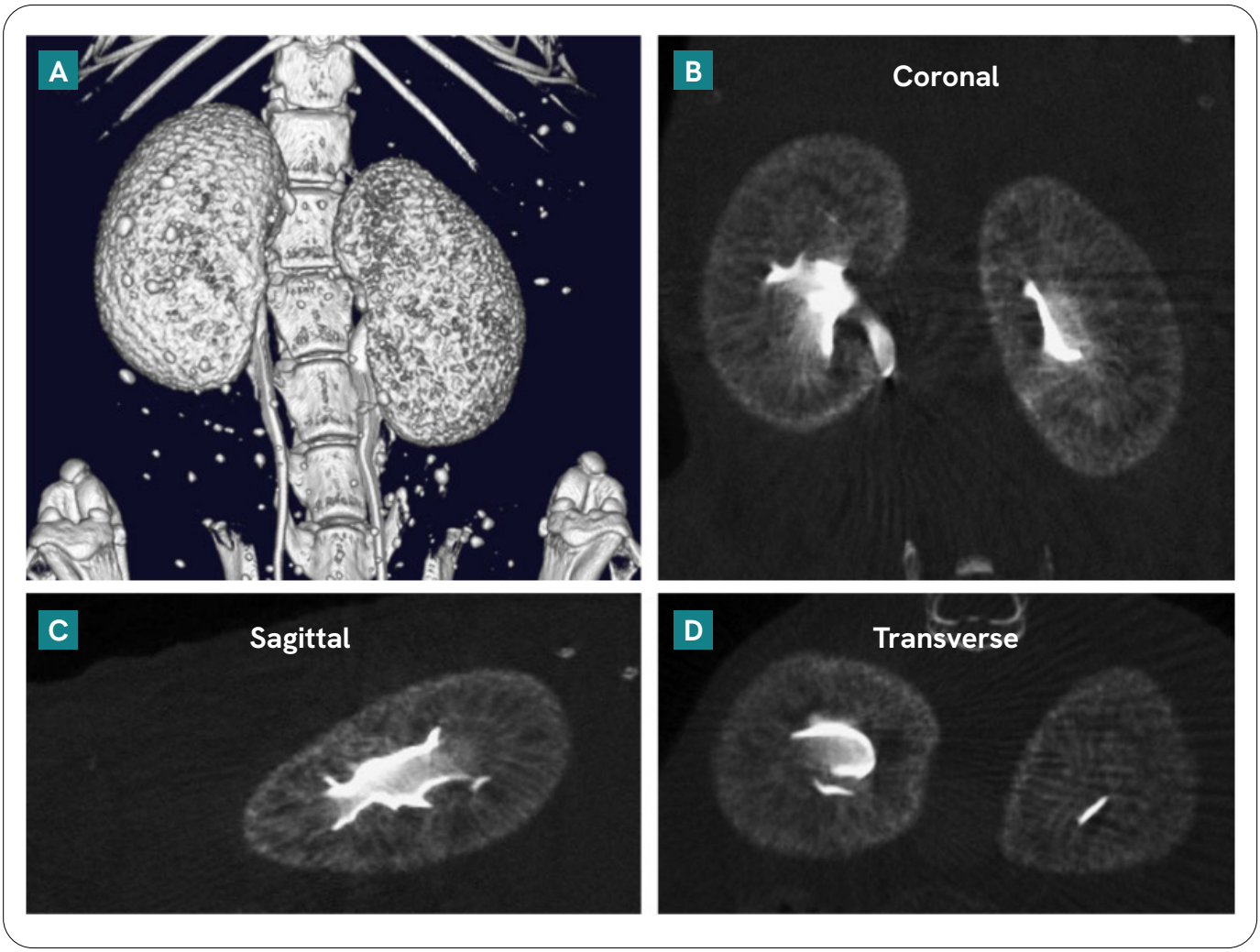


Figure 1. Kidney microCT imaging using an iodinated contrast agent, ExiTron V. This commercially available imaging agent contains iodixanol and is formulated for preclinical use. After a single bolus i.v. injection of the agent at the dose recommended by the manufacturer (100 µL), the agent is excreted via the renal pathway to establish increased image contrast in the kidneys. (A) 3D tomography imaging of the kidneys acquired 10 min after ExiTron V injection (FOV = 36 mm, standard two min). The coronal (B), sagittal (C) and transverse (D) views show strong image contrast in the central ureter regions of kidneys.

Dynamic imaging of mouse kidneys using OptiPrep iodinated density gradient medium

Iodixanol is an iodinate molecule with high density and low viscosity, making it useful both as a contrast agent, and as a medium for gradient centrifugation in subcellular organelle isolation. In particular, OptiPrep (Sigma-Aldrich) is an isosmotic gradient medium which contains a concentrated preparation of 60% (w/v) iodixanol in water (density = 1.32 g/ml).[4] It comes as a sterile, endotoxin-tested preparation that is nonionic, metabolically inert and non-toxic to cells. The medium is commercially available and is a good alternative to other preformulated iodinated agents, providing greater dose flexibility and cost effectiveness for preclinical imaging applications. To determine the optimal time for kidney contrast imaging using OptiPrep, a diluted solution was prepared in PBS and injected i.v. into mice at an iodixanol dose of 1.8 g/kg. Kidney microCT imaging was performed at 4, 15, 30, 45, 60 and 120 min post injection (standard two min, FOV = 36 mm). Kidney images were observed similar to the those obtained using the ExiTron V contrast agent as kidney contrast was established within minutes after contrast agent injection (Figure 2). The kidney signals gradually improved and it was determined that the optimal time for iodixanol kidney imaging was 30-60 min after injection. In this example, peak kidney contrast was observed at 45 min. Images acquired after one hour showed poor contrast, suggesting that contrast agent blood levels were likely too low to deliver contrast to the kidneys. Since iodixanol is also the contrast ingredient in Visipaque (clinical) and ExiTron V (preclinical), we believe these two agents should share a similar imaging profile with OptiPrep.

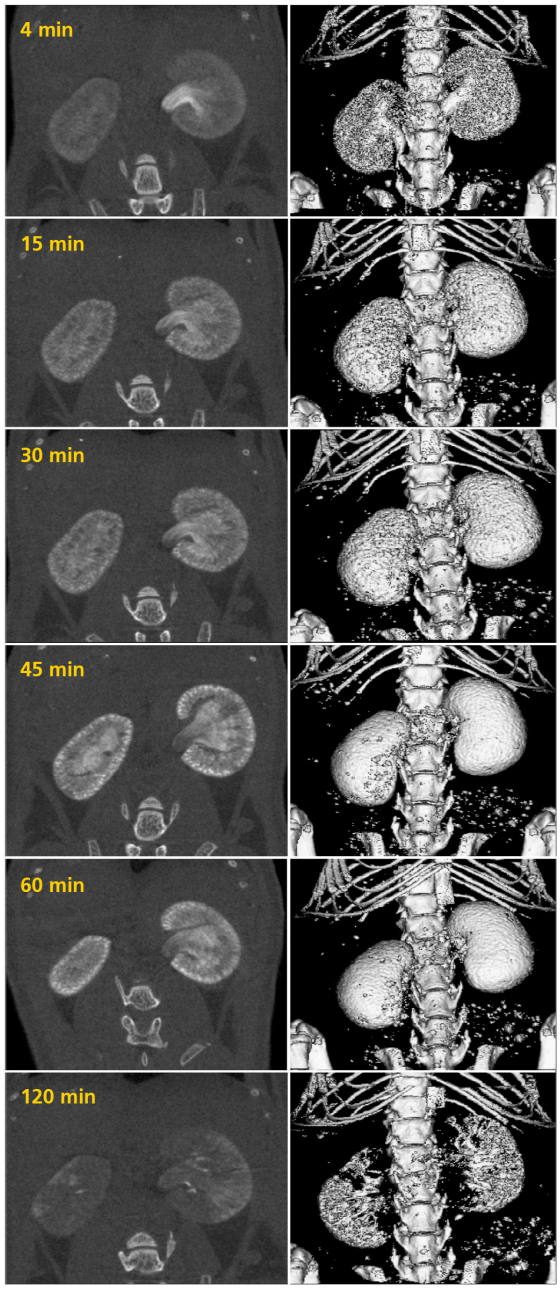


Figure 2. Kinetic imaging of the kidney using the iodixanol-based OptiPrep gradient medium. OptiPrep, a concentrated solution of iodixanol optimized for gradient centrifugation, can be used for preclinical contrast imaging of the kidneys. In this example, a solution was prepared by diluting OptiPrep in PBS which was injected i.v. into a C57BL/6 mouse at a dose of 1.8 g/kg iodixanol. Serial Quantum GX microCT images (FOV = 36 mm, standard 2 min) were acquired at indicated time points post injection. The coronal slice views (left) and 3D tomography images (right) of the kidneys are shown. Similar to ExiTron V, OptiPrep establishes kidney contrast as early as four min after injection. The optimal imaging time for kidney imaging is 30-60 min post injection.

OptiPrep/Iodixanol dose-finding study for kidney contrast imaging

Optimal odixanol dose for kidney contrast imaging was then determined using the Quantum GX (Figure 3). Mice received an i.v. injection of 0.3, 0.6, 1.2, 1.8 or 2.4 g/kg iodixanol (prepared from OptiPrep) via the tail vein and were the imaged using the Quantum GX imaging system (standard two min, FOV = 36 mm). Mice receiving no contrast agent (0 g/kg) were also imaged as controls. For this set of studies, all animals were imaged 30 min after contrast injection, allowing sufficient time to establish kidney contrast.

In the absence of the contrast agent, control mice showed no contrast in the kidneys. By viewing the 2D coronal slices, the outlines of the kidneys were not visible except for the lower parts of kidneys where the organs are surrounded by less dense peritoneal fluid. At 0.3 and 0.6 g/kg of iodixanol, minimal contrast was observed in the kidneys, insufficient for good imaging. On the other hand, doses above 1.2 g/ kg are sufficient to generate good contrast in the kidneys. Iodixanol doses higher than 1.8 g/kg not only make the inner structures visible but also produce high quality 3D tomography images of the organs.

This dose ranging study led to two important findings.

1. A dose of 1.2 g/kg is sufficient for increased kidney contrast and co-localization studies.
2. A dose of 1.8 g/kg can be used to delineate the internal anatomical features of the kidney.

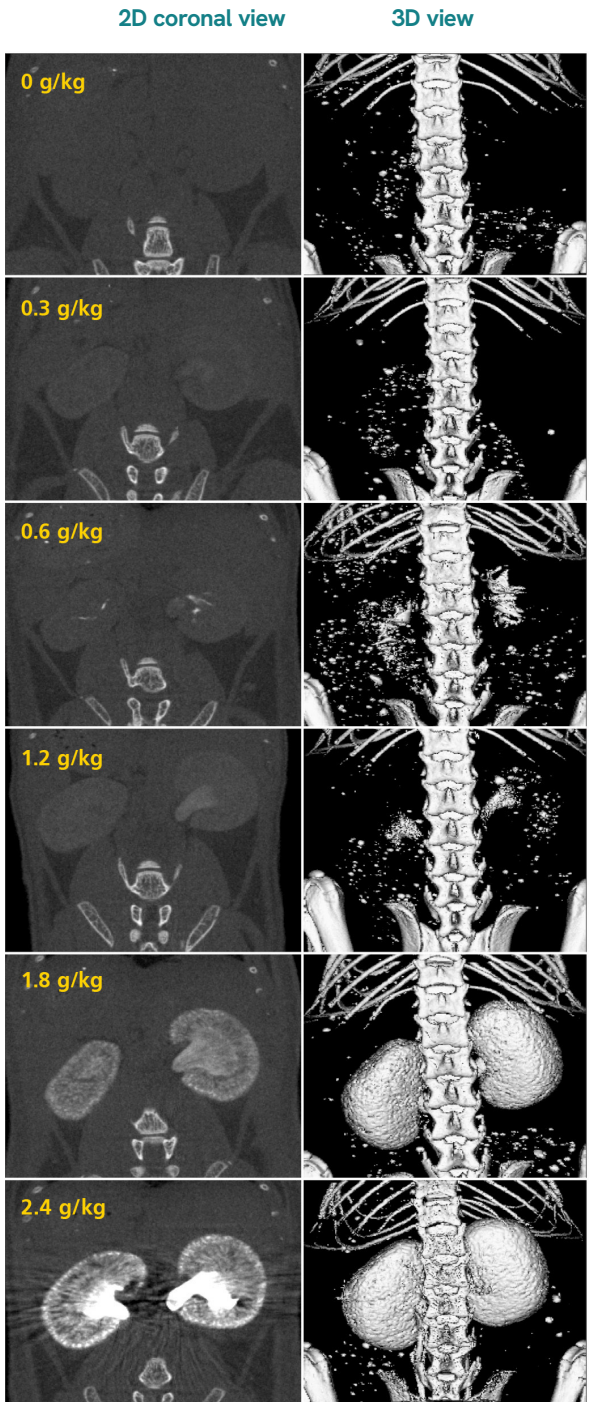


Figure 3. Dose titration study of an iodixanol contrast agent, OptiPrep, for kidney imaging. OptiPrep was injected i.v. into C57BL/6 mice at various doses, ranging from 0.3 to 2.4 g/kg. Thirty minutes after injection, contrast imaging of the kidneys was performed for each animal using the Quantum GX microCT system (FOV = 36 mm, standard two min). Figure demonstrates coronal slice views (left) and 3D tomography images (right) of the kidneys.

High-resolution contrast imaging of inner kidney structures using sub-volume reconstruction

After optimal time and dose were determined for kidney contrast imaging, the Quantum GX was used to visualize inner kidney structures. The Quantum GX is capable of "zooming in" on the kidney region and enhancing imaging quality by performing "sub-volume reconstruction." To demonstrate this unique feature, a C57BL/6 mouse was injected i.v. with 1.8 g/kg of iodixanol (OptiPrep) and imaged 30 minutes later with a standard two minute scan (FOV 36 mm), generating a 72 µm voxel image. Figure 4 shows enhanced image quality as a result of subvolume reconstructions. Voxel size was improved to 20 µm in the subvolume reconstruction and pixel size was improved to 4.5 µm in the slice subreconstruction. The three layers of kidney, e.g. cortex, medulla and ureter, are clearly visible.

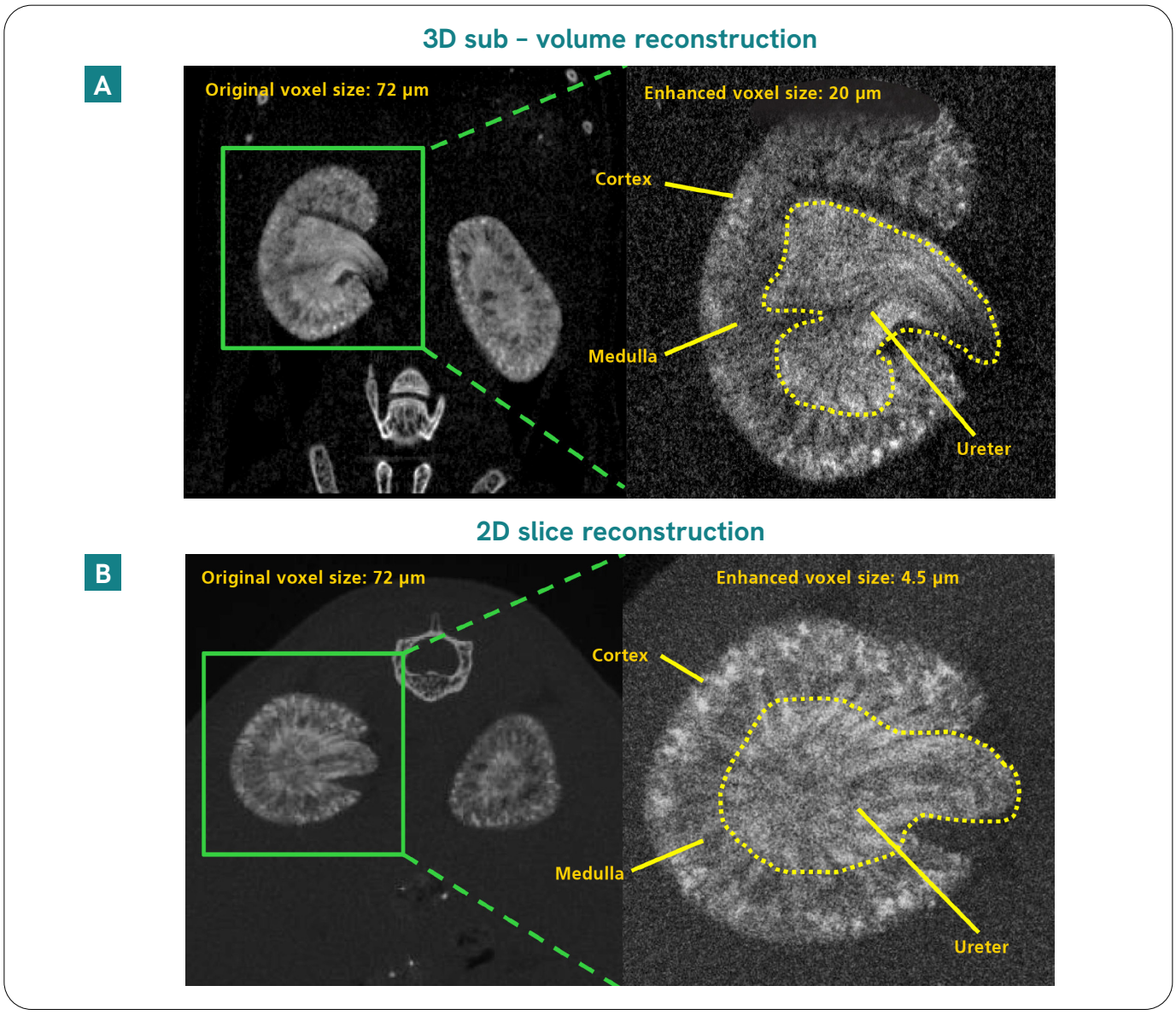


Figure 4. High-resolution contrast imaging of kidney structures. The image quality of the original acquisition can be further enhanced by sub-volume reconstruction in order to visualize structures within the kidneys. This figure demonstrates microCT imaging originally acquired from a mouse injected i.v. with 1.8 g/kg of OptiPrep. (A) Original coronal image (left) acquired using standard mode (FOV = 36 µm, two min). This setting by default produces an image reconstruction with a voxel size of 72 µm. By selecting a region of interest corresponding to the kidney (green box), we can use the Quantum GX software to perform a 3D sub-volume reconstruction on just this region to reduce the voxel size to 20 µm (right). (B) Transverse slice of kidneys (left) subjected to slice (2D) sub-region reconstruction that further reduced the pixel size down to 4.5 µm. Enhanced images provided sufficient resolution to identify inner kidney structures such as cortex, medulla and ureter.

3D Tomographic segmentation of the inner kidney structures

In addition to generating 2D structural images of the kidneys, the Quantum GX microCT system can produce high-quality 3D substructure images of the kidneys. As previously discussed, mice injected with a high dose of iodixanol (2.4 g/kg) showed intense contrast signals in the kidneys. In particular, the ureters produced the highest contrast since the contrast agent was excreted and concentrated in the urine. The density difference between the ureter and the less dense cortex and medulla allows 3D object identification and segmentation of the ureters (Figure 5). For kidney contrast imaging, the Quantum GX acquisition software generated high-quality 3D contrast images that depict detailed structural features in the organ (Figure 5A). The presence of dense contrast in the ureter can also be easily seen using the 2D slice views of kidneys (Figure 5B).

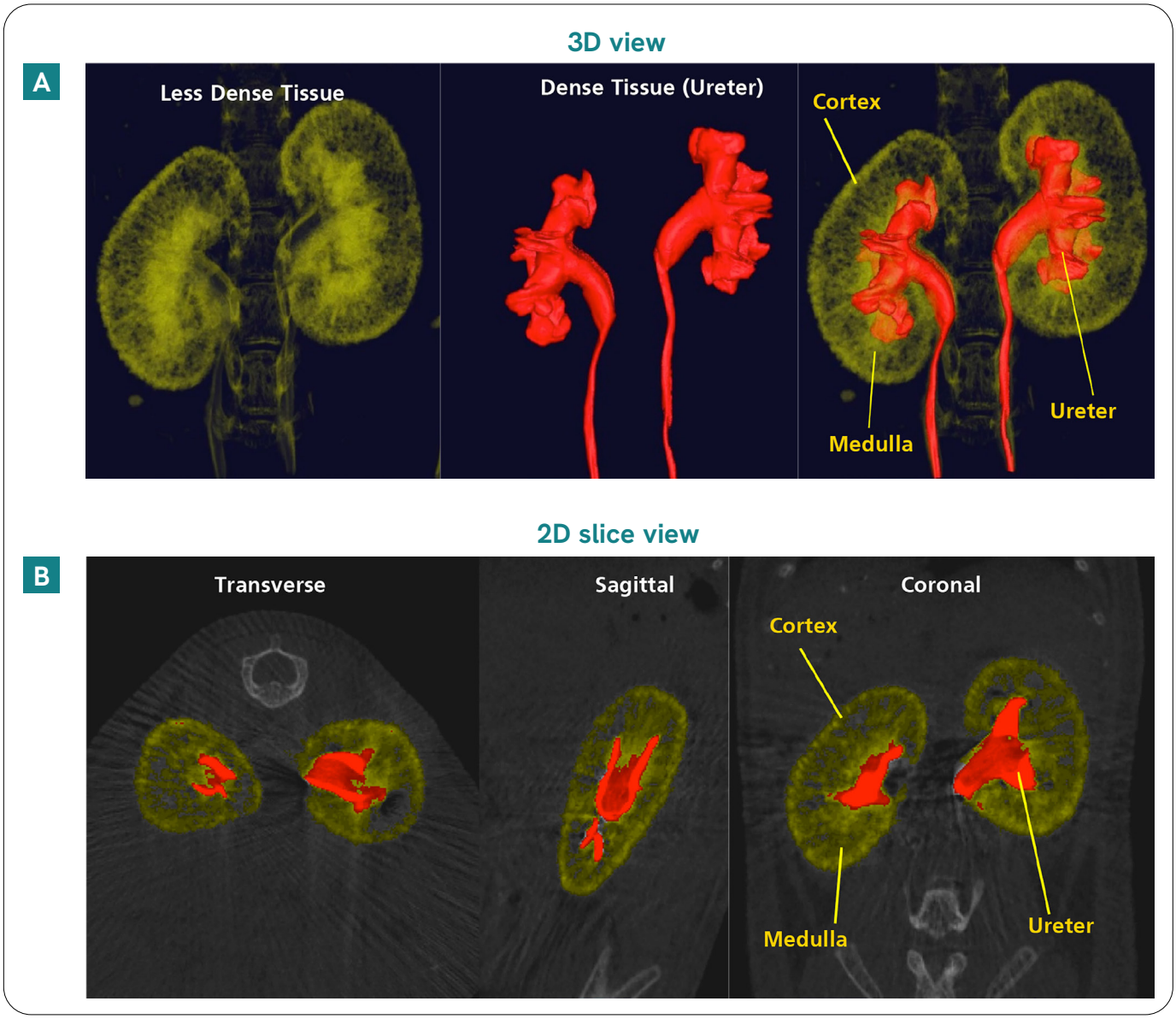


Figure 5. Structural imaging of the kidneys with an iodixanol contrast agent. 3D structural imaging of mouse kidneys was performed using the Quantum GX. In order to establish density difference in the kidney tissues, 2.4 g/kg of OptiPrep was injected i.v. into a C57BL/6 mouse. Thirty minutes after injection, kidney images were acquired using the standard two min setting with a 36 mm FOV. (A) Using the Quantum GX software image, we generated the 3D tomography and found that the cortex and medulla (yellow) have a lower contrast density in comparison to the ureter (red). (B) Corresponding 2D slice views of the kidney tomography image.

Multimodal imaging of the kidneys with CT and fluorescent contrast agents.

The high-quality 3D tomography images produced by the Quantum GX microCT system can also be used for image co-registration with other Revvity *in vivo* imaging platforms using a universal shuttle system for multimodal imaging across the CT (Quantum GX), optical (IVIS® Spectrum) and PET imaging systems. Multimodal imaging enables researchers to simultaneously visualize several biological activities in the same animal subjects. Figure 6 provides an example of dual modal imaging in the same kidneys using the CT contrast iodixanol (Figure 6A) and a fluorescent contrast agent, IVISense™ Folate Receptor 680, which is excreted via the renal pathway (Figure 6B). The Quantum GX provides detailed contrast CT images of the kidneys (Figure 6C) while specific distribution of the fluorescent agent in the kidneys can be visualized using the IVIS Spectrum system indicating the agent was concentrated in the ureter region for excretion.

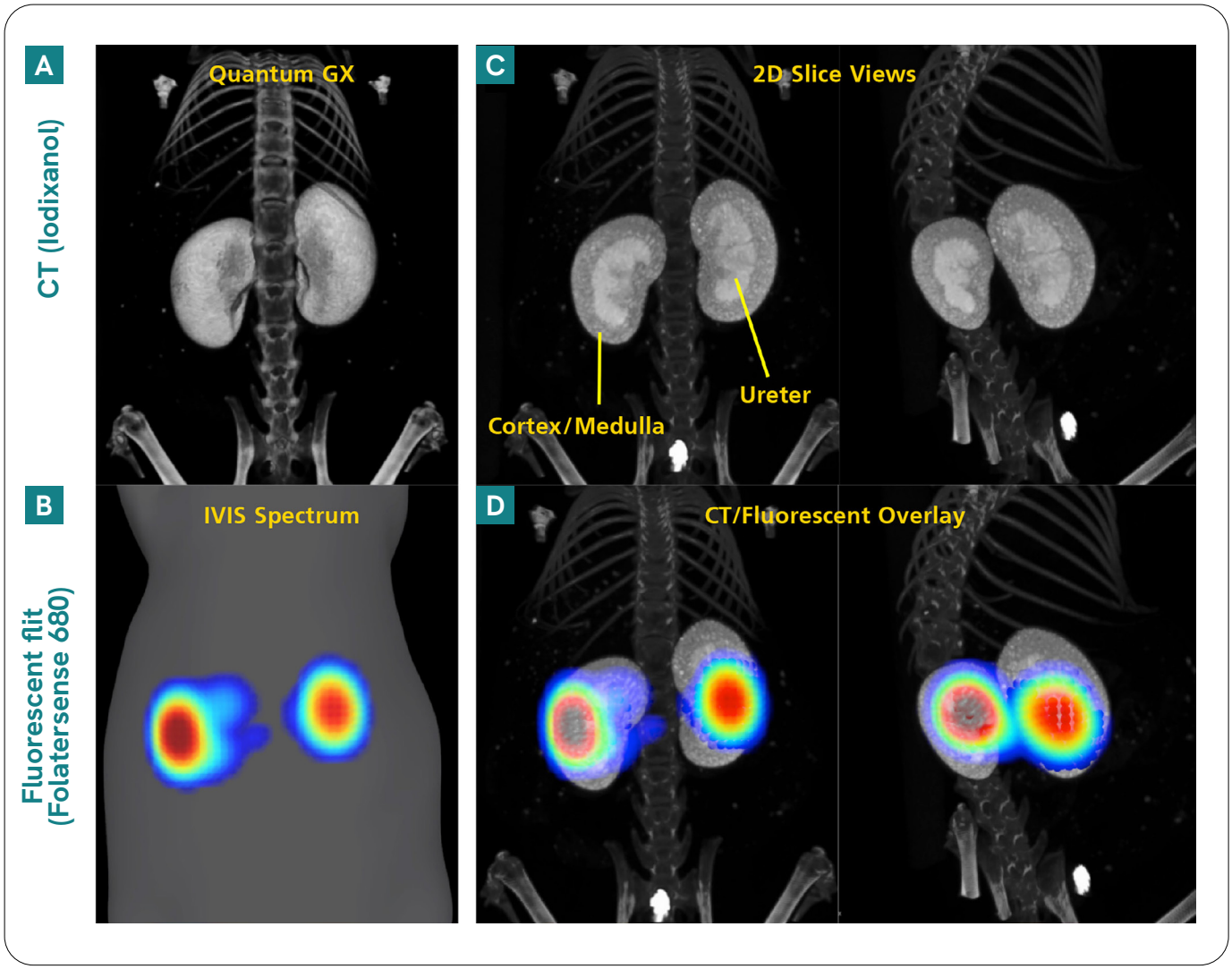


Figure 6. Multimodal contrast imaging of the kidneys using the iodixanol and IVISense Folate Receptor 680 agents. A nude mouse was injected i.v. with 2 nmol of IVISense Folate Receptor 680 four hours prior to establishing fluorsecent contrast in the kidneys. 30 minutes prior to imaging the mouse received 2.4 g/kg of iodixanol i.v. (A) CT tomography image of the kidneys. (B) IVIS Spectrum fluorescent tomography imaging (FLIT) of IVISense Folate Receptor 680 in the kidneys. (C) 2D slice views of the microCT kidney images. (D) microCT and IVIS Spectrum image overlay of kidneys.

Nanoparticle-based contrast agents for heart and vascular imaging

The key to successful cardiovascular and vessel contrast imaging is to maintain sufficient contrast level in the bloodstream during image acquisition. Commonly used clinical iodinated contrast agents are small iodine-containing molecules (MW 700-1500) with a relative short half-life in circulation and thus require constant infusion in order to maintain sufficient contrast levels for imaging. This approach is challenging in mice due to the limited fluid volumes that can be infused. Additional complications arise when taking into account the health status of mice used for cardiovascular applications, such as myocardial infarction and atherosclerosis. The fast removal of iodine-containing agents from the blood is due to their small size, lower than the glomerular filtration cutoff which is about 6 nm[5]. To improve blood circulation time, iodinated molecules can be encapsulated into larger micelles or colloid particles.

Such incorporation also increases the density of the micelles for better x-ray absorption. Another approach to increase particle density is the use of metal-based nanoparticles. As metals are intrinsically good for x-ray absorption, nanoparticles with metal cores generate great vascular contrast. Typical metals used in the particle for preclinical imaging include barium and gold. The particles can be made larger than 6 nm in diameter (typically >100 nm), preventing their passage through the glomerular filtration in the kidneys. In addition, the particle surface can be modified to increase stability and circulating half-life in the bloodstream. In comparison to iodinated contrast agents, nanoparticles produce better vascular contrast imaging of the blood rich organs, such as the heart, liver and kidneys (Figure 7A-C). In addition, nanoparticles provide sufficient density for visualizing fine tumor vasculature (Figure 7D).

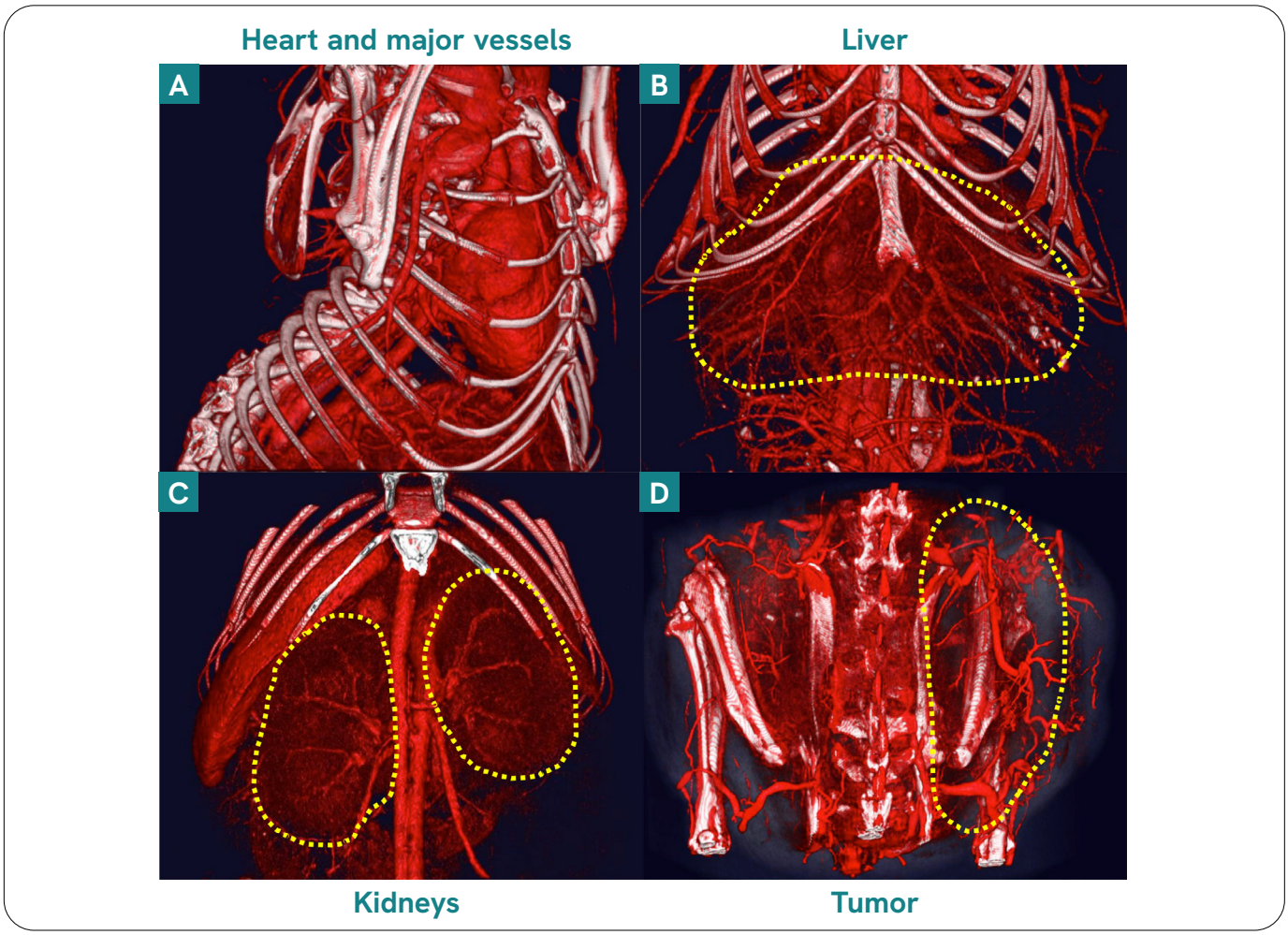


Figure 7. Vascular imaging of various organs using a nanoparticle-based agent. (A) Heart and major vessels in the chest; (B) liver; (C) kidneys; and (D) subcutaneous 4T1 tumor. Yellow dashed lines indicate the organ regions. Contrast was established using the ExiTron nano6000 nanoparticle contrast agent.

Contrast imaging of the heart with cardiac gating

Cardiovascular imaging is one of the major preclinical microCT applications in which applying gating may be critical. As the mouse has a resting heart rate in the range of 450-750 bpm, cardiac gating is of great importance to minimize motion artifacts in heart structures which are in constant, rapid motion. Retrospective cardiac gating using the Quantum GX microCT system can facilitate accurate visualization of the heart at the diastolic or systolic stage in the cardiac cycle. The process involves image acquisition in the fast mode that allows for a rapid camera response time in order to accommodate the rapid motion of heart. After image acquisition, the software automatically applies a retrospective gating algorithm to the acquired raw image data to identify the corresponding diastolic and systolic frames. The image frames are then sorted and reconstructed separately into diastolic or systolic 3D voxel images. The sorting process is achieved using the acquisition software, and does not require physical EKG wiring to the animal during acquisition. Figure 8 shows the diastolic and systolic heart images of a normal mouse injected i.v. with the nanoparticle contrast agent, ExiTron nano6000. The images clearly depict the changes in the heart chambers between diastole and systole stages. One of the most striking differences is the enlarged left ventricle (LV) during the diastole phase of cardiac cycle.

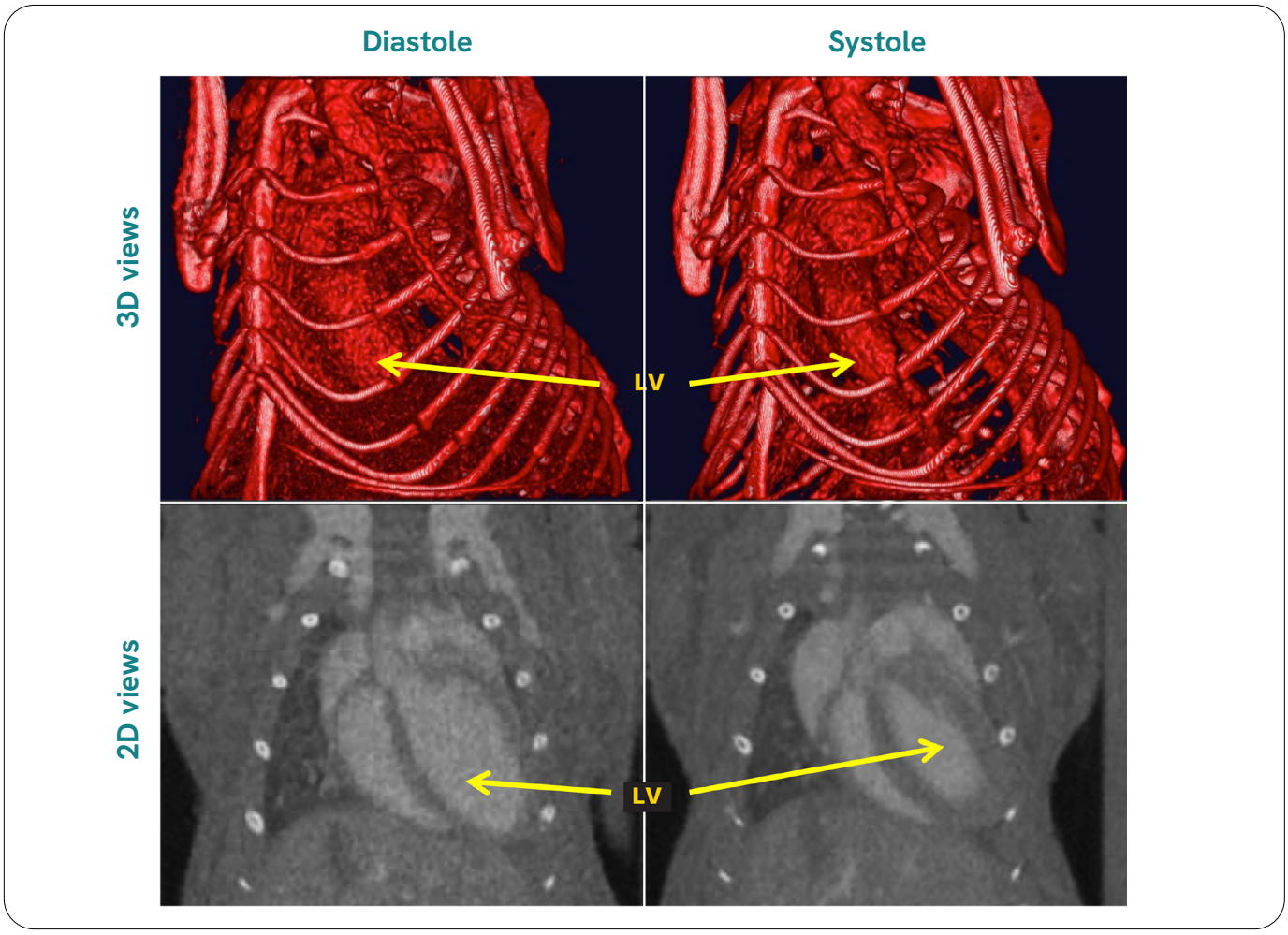


Figure 8. Cardiac gated imaging of the heart using a nanoparticle-based contrast agent. After i.v. injection of ExiTron nano6000 contrast agent (100 µl), the mouse heart was imaged using the Quantum GX. Image acquisition was performed with a camera setting for retrospective cardiac gating (FOV = 36 mm, Fast camera mode, four min scan). The gated images clearly show anatomical changes in the heart chambers between the diastole and systole phases of the cardiac cycle. The left ventricle (LV, yellow arrows) shows the most dramatic changes. (Top) 3D tomography view of the heart, (Bottom) 2D slice views of the hearts, where the heart chambers are clearly visible with the contrast agent.

Gated vs. Non-gated cardiac contrast imaging

As the heart beats, its valves and muscle walls are constantly in rapid motion. This poses a challenge for accurate visualization of the inner structures of the heart. To demonstrate the importance of cardiac gating on structural accuracy, heart microCT images were acquired and compared under gated or non-gated camera settings. Since the Quantum GX uses retrospective gating to reconstruct the diastolic and systolic images of the heart, the original image data is acquired using a fast camera mode in order to accurately capture the phases during the constant motion of the heart beat. Although the fast mode has a lower theoretical voxel resolution in comparison with the standard and high resolution modes, the final gated images are sharper and have better contrast than the other non-gated images. Figure 9 illustrates a mouse imaged under three different camera settings: (A) Gated fast mode, (B) nongated standard mode, and (C) non-gated high-resolution mode. Line intensity profiles across the heart were then analyzed at the same position for the three settings.
The comparisons show that cardiac gating provides sharper and more distinguishable downward spikes corresponding to the chamber boundaries and muscle walls in the heart (Figure 9). Although the high-resolution mode produced an overall smoother image, it contained noticeable motion artifacts within heart chambers due to rapid motion. Importantly, proper gating is necessary for accurate structural measurement of the heart as we will discuss in the following sections.

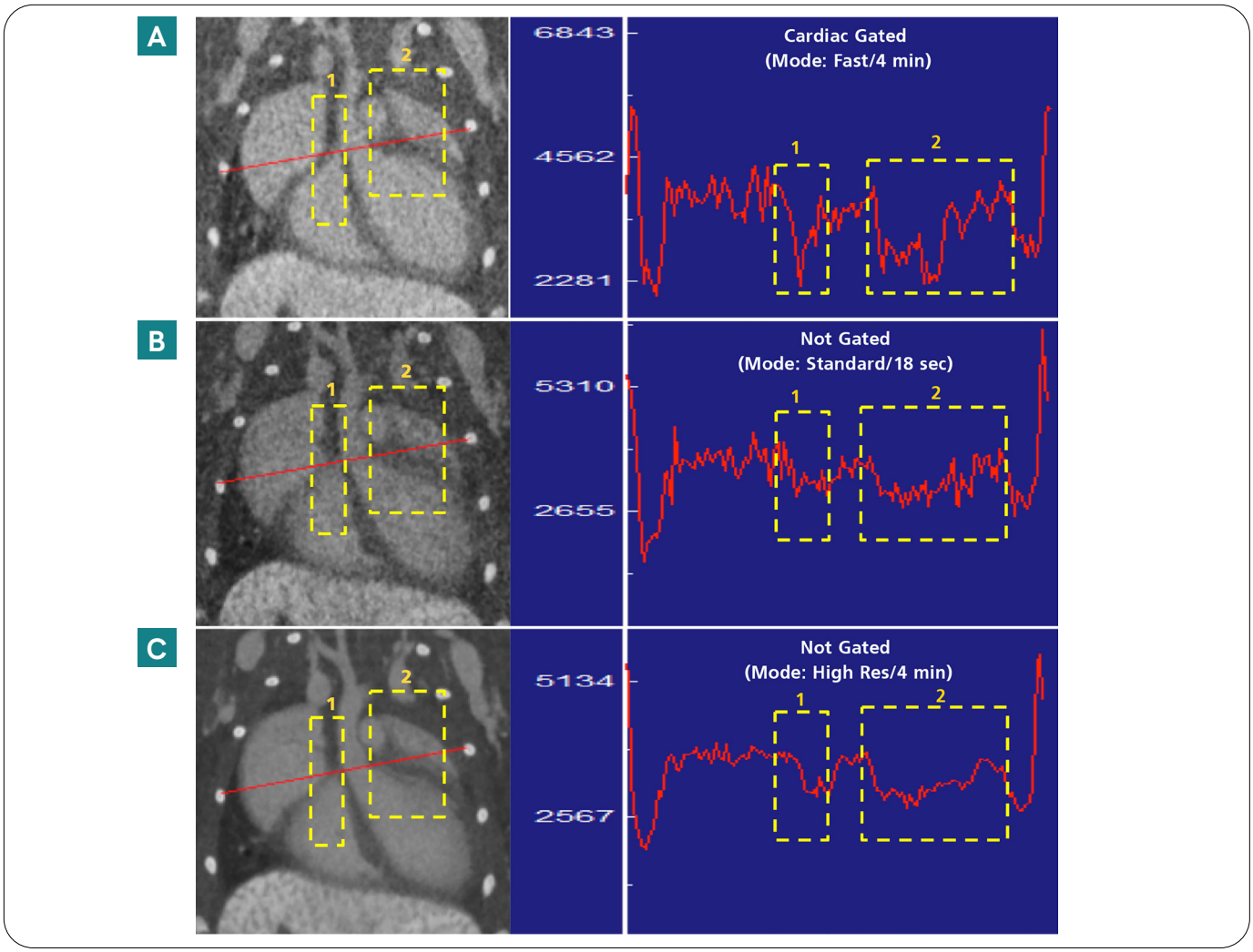


Figure 9. Cardiac gating improves image sharpness. Heart contrast images acquired with (A) or without cardiac gating (B and C) and viewed using the standard microCT software. The camera setting are (A) Cardiac gated; fast mode for four min acquisition, (B) Not gated; standard mode for 18 sec, and (C) Not gated; high resolution for four min. For comparison, line intensity profiles were calculated at the same position in the heart for all three settings. Boxed areas 1 and 2 indicate the regions the showed significant motion-related imaging artifacts.

Contrast microCT imaging of mouse cardiac hypertrophy

To assess the capabilities of structural and anatomical analysis of the heart on the Quantum GX microCT system, transgenic Gαq40 mouse (JAX stock #012460) that develops spontaneous cardiac hypertrophy6 was used. Expression of Gαq is regulated by an α-myosin heavy chain (Myh6) promoter, which causes specific overexpression of Gαq in heart tissue. Gαq overexpression results in cardiac hypertrophy, increased heart weight/size, and increased cardiomyocyte size, which severely compromises systolic cardiac function, ultimately resulting in overt cardiac failure. Both wild-type (WT) control and Gαq40 mice were injected i.v. with ExiTron nano6000 and imaged with cardiac gating for better structural accuracy as described in the previous section. In this study, diastolic images of both mice for better comparison were used. Figure 10 summarizes the Quantum GX cardiac imaging results in 3D tomography (top panels) and in 2D slices (coronal, sagittal and transverse). The images clearly show striking heart enlargement in the Gαq40 transgenic animals.

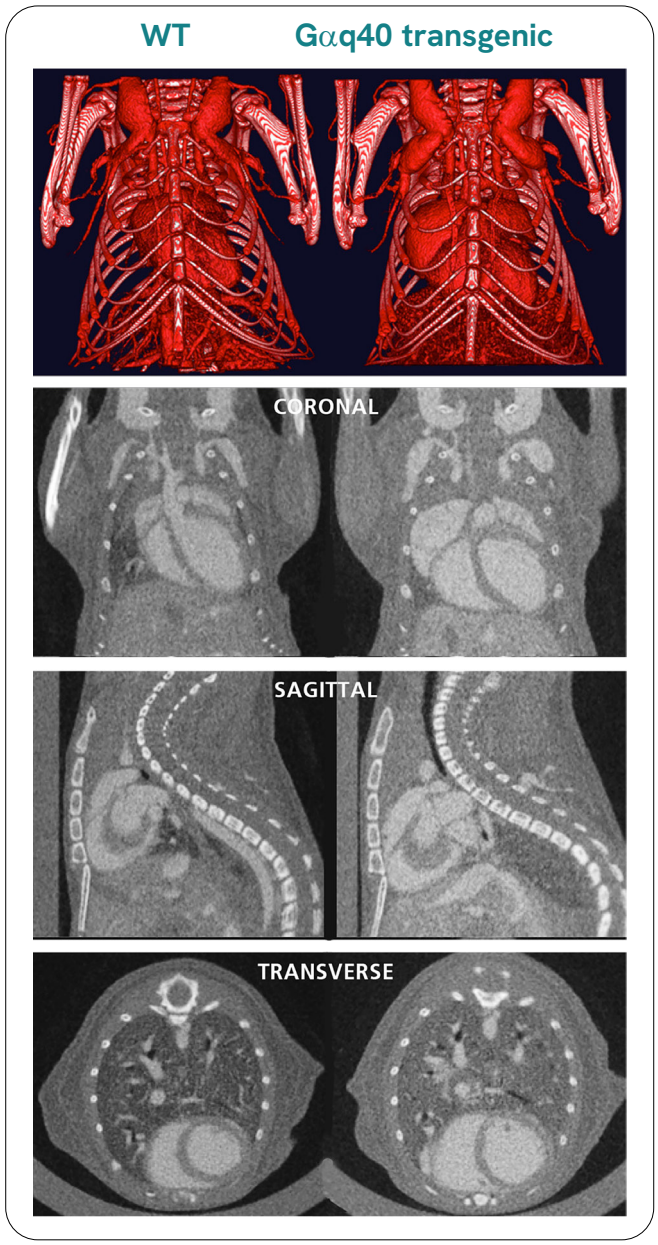


Figure 10. Contrast imaging of a transgenic mouse with cardiac hypertrophy. The Gαq40 transgenic mice, FVB/N-Tg(Myh6- Gnaq)40wd/J, develop spontaneous cardiac hypertrophy which can be visualized using the Quantum GX system. For chamber visualization, ExiTron nano6000 was injected i.v. into the animals and imaged immediately. Compared to the wild-type (WT) mouse, the 3D tomography images of Gαq40 mice clearly show abnormal heart enlargement in the transgenic animals. The 2D slice views revealed larger chambers inside the transgenic heart. The images were acquired using the cardiac gating setting (Fast mode four min, FOV = 36 mm), and only the diastolic images were shown.

Visual identification and measurement of the mouse heart chambers

As shown in Figure 10, the combined use of a contrast agent and cardiac gating makes it possible to visualize the individual heart chambers of the mouse. The abnormal heart enlargement can be clearly seen in the Gαq40 mice using the Quantum GX microCT system. To illustrate the impact of Gαq over-expression on heart development, standard microCT software was used to review the 2D coronal slices and identify all four chambers in both control and Gαq40 hearts (Figure 11A, top panels). Four different colors were used to mask the chambers; right atrium in blue, right ventricle in yellow, left atrium in pink, and left ventricle in green. Corresponding transverse slices also showed hypertrophy in the atria (Figure 11A, Transverse 1) and ventricles (Figure 11A, Transverse 2). For volume calculation, each chamber can be individual masked by manually scanning through the entire heart (Figure 11B). In comparison to the control heart, the Gαq40 transgenic mice show hypertrophy in all four chambers and especially in the atria.

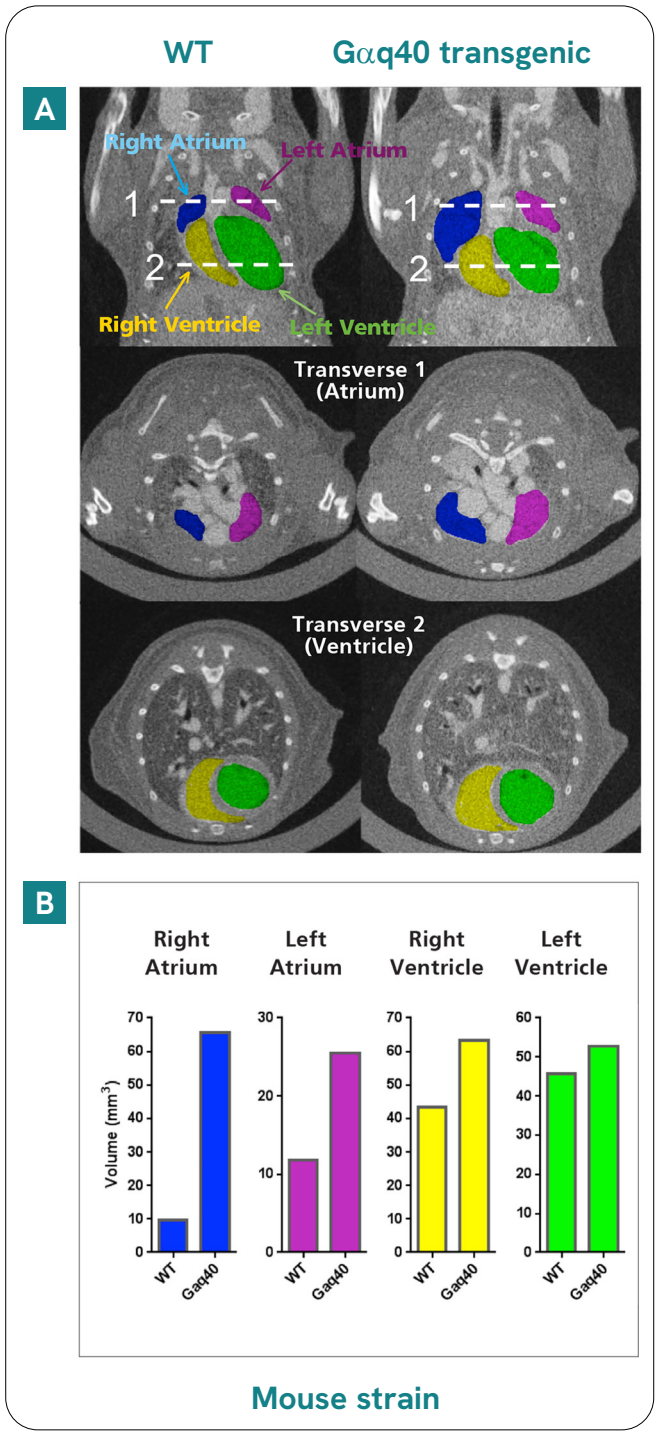


Figure 11. Quantitative analysis of heart chamber volume using cardiac gated contrast imaging. (A) Using standard microCT software, each heart chamber in WT and Gαq40 mice can be identified. The top two panels show the coronal views of normal (WT) and Gaq40 hearts, different colors are used to mask the four heart chambers (left/right ventricles and left/right atria). Dashed lines show the two positions of cross sections for transverse views (lower panels). Cross section 1 shows the transverse view of the atriums and cross section 2 shows the ventricles. (B) Once the chambers were identified, the volume was calculated.

Contrast microCT imaging of tumor vasculature using nanoparticles

Since angiogenesis, i.e. generation of new blood vessels, is critical for tumor development and growth, we tested if nanoparticle contrast imaging can be used to assess treatment responsiveness in terms of vascular function. The dense metal core and prolonged circulating time make nanoparticles ideal for imaging tumor vasculature. The Quantum GX system was used to visualize changes in tumor vessels after the treatment with a potent vascular disruption agent, combretastatin A4 (CA4)[7]. Nude mice bearing 4T1 tumors on the flank were treated with 50 mg/kg of CA4. Six hours after the treatment, ExiTron nano6000 contrast agent was injected i.v. and microCT imaging was performed of the lower abdomen including the tumor region (Figure 12). Compared with the untreated control tumor, the vessels in the CA4-treated mouse tumor were thinner, more sparse and less connective, suggesting the treatment greatly inhibited vascular function and affected tissue perfusion in general.

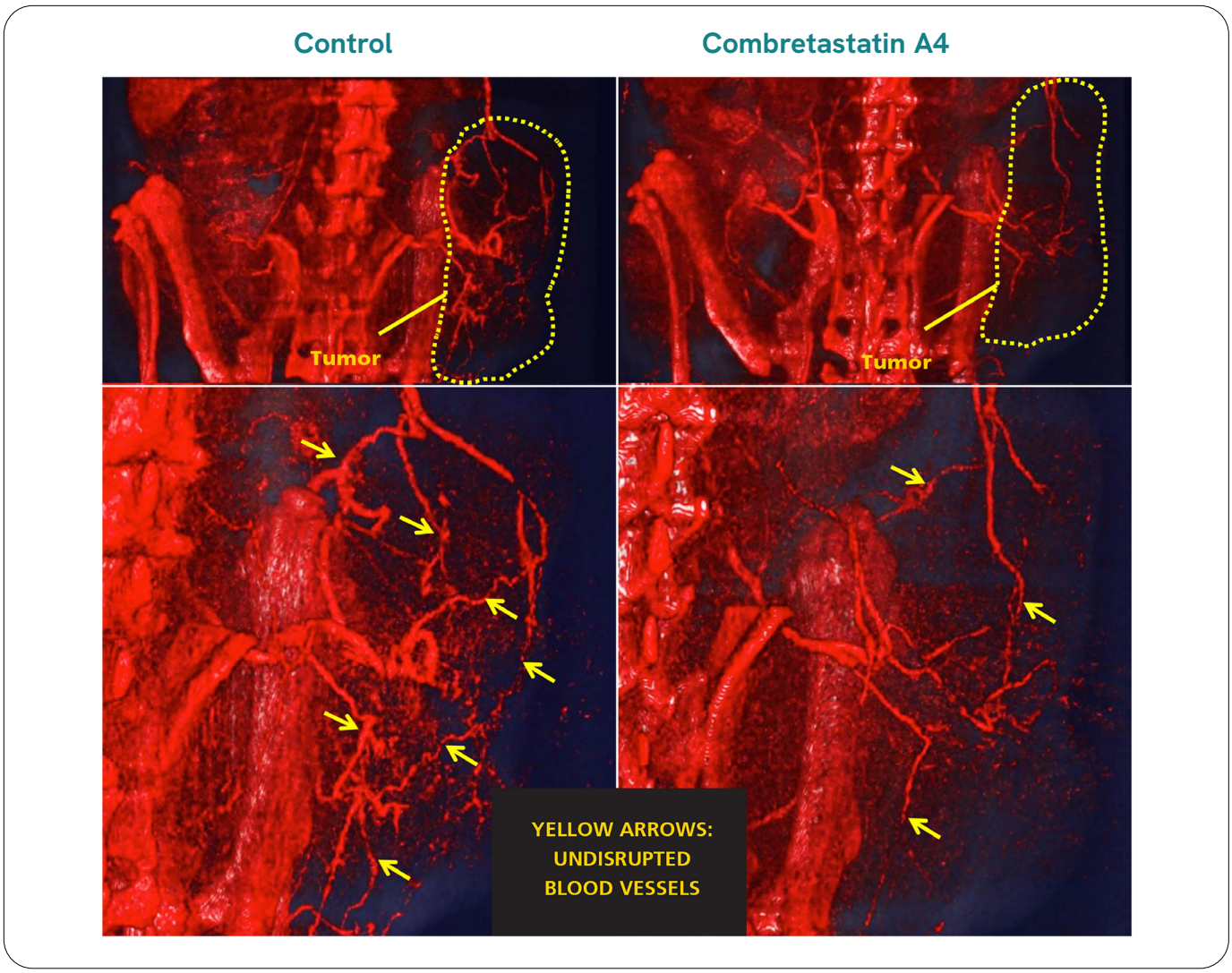


Figure 12. Vascular imaging of tumors treated with a vascular disruption agent. The 4T1 tumor-bearing mice were either untreated (control) or treated with CA4 (50mg/kg, i.p.), an effective vascular disruption agent for tumor treatment. Six hours after the CA4 treatment, ExiTron nano6000 nanoparticle contrast agent was injected i.v. (100 µl) in control and tumor animals and imaged using the Quantum GX to visualize the drug's anti-vascular effects in tumors. Top panels show the vascular contrast images of the general pelvic areas (including the tumor region). Bottom panels show the corresponding enlarged tumor images. The yellow dotted lines indicate the border of the tumors. The CA4 treatment clearly suppressed vascular burden in the tumor, as the treated mouse had fewer and less dense vessels in the tumor.

Vascular disruption agent treatment greatly decreases glucose uptake in the tumor

To validate whether the loss of vascular function induced by combretastatin A4 also causes reduced metabolic activity in the tumor, ^{18}F-FDG microPET imaging of the tumors was performed using a PET/CT imaging system 24 hours after treatment. ^{18}F-FDG is a glucose analog often used to assess tumor glycolytic activity. As illustrated in Figure 13, the treatment not only suppressed vascular function but also greatly reduced glucose metabolism in the tumor. Quantitative analysis of the PET data showed the CA4 treatment suppressed approximately 90% of glucose uptake in the tumor. Importantly, this study demonstrates the advantage of using multimodal imaging to simultaneously investigate diverse biological activities. In this example, microCT imaging provides information about tumor vascular burden, while PET imaging provides complementary functional assessment of tumor metabolic activities in response to an anti-cancer treatment.

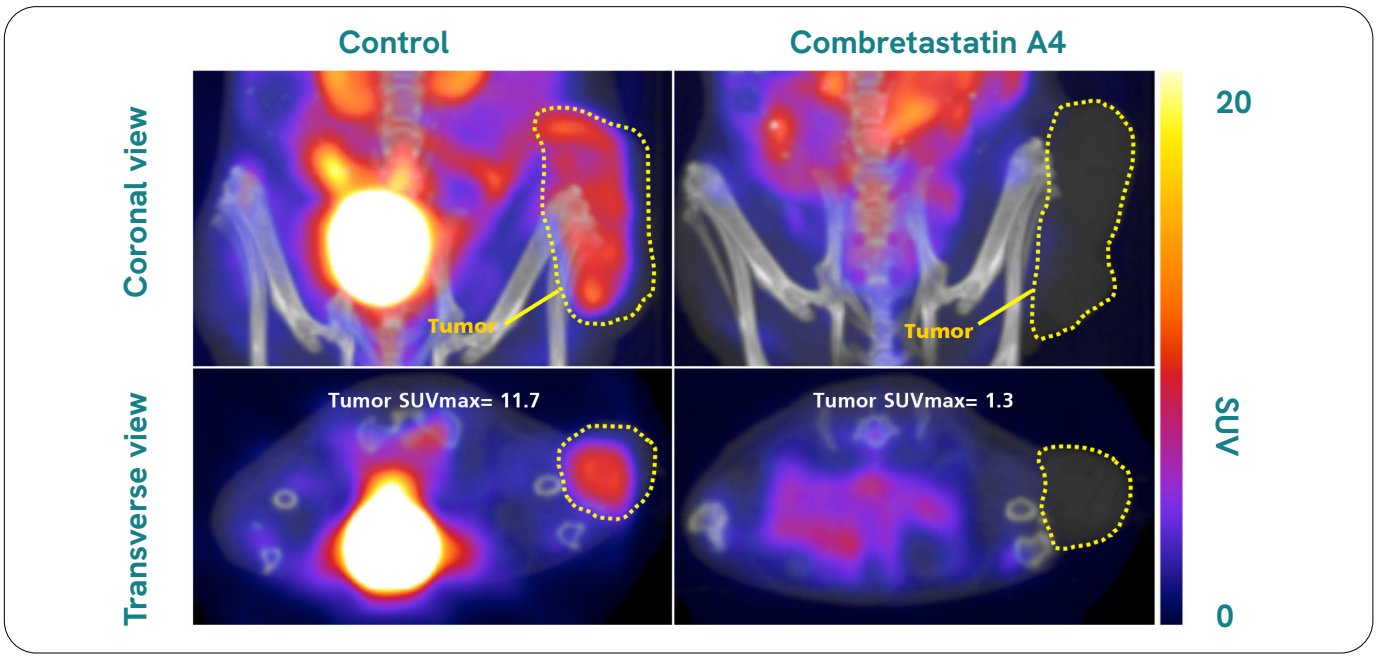


Figure 13. ^{18}F-FDG PET imaging showed reduced glucose uptake in response to the combretastatin A4 treatment. To validate the Quantum GX microCT imaging results as shown in Figure 12, we performed ^{18}F-FDG PET imaging to determine glucose uptake activities in the 4T1 tumors. The day after the CA4 treatment, mice were injected with 50 µCi of ^{18}F-FDG, a radioactive glucose tracer. The animals were then incubated under isoflurane anesthesia for one hour prior to PET imaging using the PET/CT scanner. The yellow dotted lines indicate tumor regions and quantitative analysis shows approximately a 9-fold reduction in glucose uptake in the treated tumor.

Phagocytic uptake of nanoparticle contrast agents for liver and spleen imaging

In the previous sections, the use of ExiTron nanoparticles for vascular contrast imaging was discussed. Interestingly, the same nanoparticle agent can also be used for imaging the liver and spleen due to its preferred biodistribution to these organs. Nanoparticle contrast imaging can be used to identify small hepatic metastases and leukemic splenomegaly in animal cancer models[8]. Due to the lack of intrinsic density differences, soft tissues and organs in the peritoneal cavity can be difficult to distinguish by microCT imaging. After i.v. injection into the animal, the nanoparticles initially circulate in the bloodstream to provide sufficient contrast for vascular imaging as previously described. However, the nanoparticles are generally taken up by cells with phagocytic capability[5]. These professional phagocytic cells include the Kupffer cells in the liver and the macrophages in the red pulp of spleen (Figure 14A).
The specific uptake enables us to perform contrast imaging of the liver and spleen, as both are indistinguishable without contrast (Figure 14B). Of note, the particle can remain stable in these cells for an extended period of time; a single nanoparticle injection can provide sufficient tissue contrast in the liver and spleen for several weeks (>4 weeks).

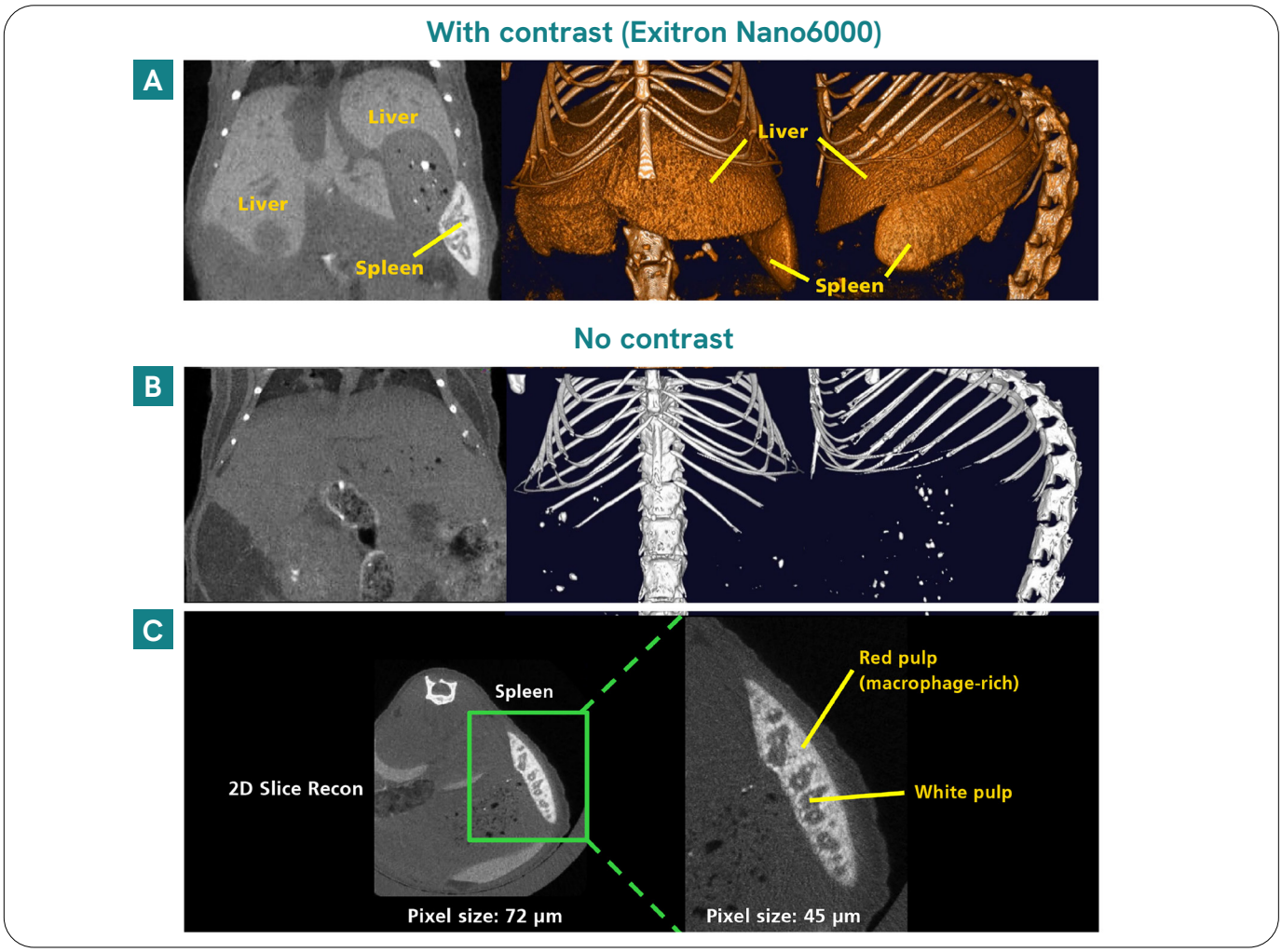


Figure 14. Contrast imaging of the liver and spleen using a nanoparticle-based contrast agent. Nanoparticle-based ExiTron contrast agents can be used for long-term visualization the liver and spleen. Within hours, specific uptake in these organs produces strong image contrast which remains detectable for several weeks. (A) The mouse received i.v. injection of ExiTron nano6000 (100 µl) to establish contrast in the liver and spleen for Quantum GX microCT imaging. The image was taken three weeks after injection, and the liver and spleen are clearly visible. (B) Without any contrast agent, it is difficult to identify the liver and spleen. The left panel is the 2D coronal view and the right panels are the 3D tomography views. (C) Sub-region reconstruction of the transverse slice revealed detailed sub-structures in the spleen. The nanoparticle contrast preferably present in the macrophage-rich red pulp .

Conclusions

This application note provides Quantum GX users with an overview of contrast agents and their potential use for vascular and soft tissue imaging. Iodinated contrast agents are small molecules, generally considered safe for clinical use, and these agents are rapidly cleared via the renal pathway. Iodinated agents can be used for vascular imaging only when the agent is at sufficiently high levels in circulation, often requiring continuous infusion. On the other hand, nanoparticle-based agents are not approved for human use, likely due to prolonged retention in the liver and spleen that may raise toxicity and safety concerns. Nevertheless, nanoparticles show long circulation half-lives, making them much better for generating CT contrast in vasculature for their dense metal cores. The Revvity Quantum GX microCT system is well suited for cardiovascular imaging and visualizing vasculature in the liver, kidney and tumor. The sub-volume reconstruction feature enhances image quality and makes it possible to visualize the inner sub-structures of the kidney and spleen with high resolution. Furthermore, the use of contrast imaging to perform volumetric measurement of heart chambers in normal or diseased animals was well demonstrated. Table 2 provides a comparative summary of applications possible for these two types of contrast agents in *in vivo* microCT imaging.

References

1. Pasternak, J.J., Williamson, E.E. 2012. Clinical Parmacology, use, and adverse reactions of iodinated contrast agents: a primer for the non-radiologist. Mayo Clinic Proceedings 87(4):390-402.
2. Cormode, D.P., Naha, P.C., Fayad, Z.A. 2014. Nano-particle contrast agents for computed tomography: a focus on micelles. Contrast Media Molecular Imaging 9(10):37-52.
3. Katzberg, R.W. 1997. Urography into the 21st century: new contrast media, renal handling, imaging characteristics, and nephrotoxicity. Radiology 204(2):297-312.
4. Li, X., Donowitz, M. 2008. Fractionation of subcellular membrane vesicles of epithelial and nonepithelial cells by OptiPrep density gradient ultracentrifugation. Methods in Molecular Biology 440:97-110.
5. Longmire, M., Choyke, P.L., Kobayashi, H. 2008. Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats. Nanomedicine (Lond) 2(5):703-717.
6. D'Angelo, D.D., Sakata, Y., Lorenz, J.N., Boivin, G.P., Walsh, R.A., Liggett, S.B., Dorn, GW 2nd. 1997. Transgenic Galphaq overexpression induces cardiac contractile failure in mice. Proc Natl Acad Sci U.S.A. 94(15):8121-6.
7. Nagajah, G., Remick, S.C. 2010. Combretastatin A4 phosphate: a novel vascular disrupting agent. Future Oncology 6(8):1219-28.
8. Ashton, J.R., West, J.L., Badea, C.T. 2015. *In vivo* small animal micro-CT using nanoparticle contrast agents. Front. Pharmacol. doi:10.3389/fphar.2015.00256.

Table 2. Iodinated vs. nanoparticle contrast agents.

	Iodine-based contrast	Nanoparticles-based contrast
Design	Small iodinated molecules; M.W. 700-1500	Nanoparticles with metal cores; typical size ~100 nm
Clinical relevance	Many clinical agents are iodinated molecules which canalso be used pre-clinically	Currently metal-cored nanoparticles are only used in pre-clinical settings
Blood clearance	Fast; a single bolus injection can be cleared from circulation within 10 minutes	Slower; Circulating nanoparticles can still be imaged one hr after injection
Route of clearance	Glomerular filtration in the kidneys	Phagocytic uptake by Kupffer cells and macrophages
Area of application		
Kidney	+++ (General structural)	++ (Blood vessels)
Heart	+ (Requires infusion)	+++ (No infusion required; Imaged within one hr)
Liver	-	+++ (Contrast established within one hr after injection, and can last for weeks)
Spleen	-	+++ (Image more than one hr after injection, and contrast lasts for weeks)
Tumor	-	++ (No infusion required; Imaged within one hr)

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