



Imaging of Vascular Anatomy in C57Bl/6 Mice

This case study provides guidance and recommendations for conducting vascular imaging studies in normal mice with Fenestra VC. You should note that other examples of the capabilities of Fenestra and a selection of additional case studies are available on the MediLumine website at

<http://www.medilumine.com/resources>



Fenestra® VC provides flexible, long-lasting contrast enhancement for a wide range of computed tomography imaging applications, including vascular and hepatobiliary anatomy and function.

Animal Model

Strain

C57Bl/6 (31 to 36 g males).

Model

Normal mice

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APPLICATIION NOTE

Animal Preparation

Anesthesia

Mice were anesthetized with an intraperitoneal injection of a mixture of ketamine (80 mg/kg body weight) and xylazine (5 mg/kg body weight), which afforded 45 to 60 minutes of anesthesia. Anesthesia was maintained with quarter dose increments during the duration of the study.

Administration

Fenestra® VC was injected intravenously into the lateral tail vein of anesthetized mice at a dose of 0.4 ml per 20 g body weight over a period of 30 to 60 seconds. A 1 ml disposable syringe fitted with a 30-gauge needle was used to inject the contrast agent. Prior to injection, the tail vein was immersed in warm water for 30 to 60 seconds to increase blood flow to the tail and dilate the vessels.

NOTE: Refer to the MediLumine's Publication 'Optimal Usage of Fenestra Nanoemulsions' for recommendations and detailed instructions related to dosage, animal preparation, and administration at <http://www.medilumine.com/resources/user-guides>.

Image Acquisition

Equipment

ImTek MicroCAT II microCT scanner.

Animal Placement

Anesthetized mice were placed on the imaging table in the prone position. Use of gas anesthesia and its associated tubing for gas supply required the animals to be positioned with their tails toward the gantry. The desired body region was selected from the scout view as the anatomic landmark for image acquisition.

Settings

The settings selected for this medium resolution contrast-enhanced study were as follows:

X-Ray Camera

Parameter	Setting
Serial CCD Length	2048
Parallel CCD Length	3072
Serial Bin Factor	2
Parallel Bin Factor	2
Exposure Time	750 ms
Warp Correction	Yes
Defect Map Correction	No

X-Ray Tube

Parameter	Setting
X-Ray Voltage	80.0 kVp
Anode Current	500.0 A

CT Scan

Parameter	Setting
Rotating Stage Start Position	0.000 degrees
Bed Axis Position	313.863 mm
Bed Height	43.026 mm
Detector Position	0.000 mm
Total Rotation	360 degrees
Number of Rotation Steps	520
Number of Axial Bed Steps	0
Number of Detector Steps	0
Number of Acquired	20
Projection Display Period	1
Raw Data	Written to File
Real Time Reconstruction	No
Total Scan Time	11.07 min

Scanner Geometry Setup

Parameter	Setting
Source to Detector Distance	309.600 mm
Source to Center Distance	256.200 mm
Physical Detector Pitch	32.700 mm
Detector Array Height	2048 elements
Detector Array Width	3072 elements
Center Offset	4.7 unbinned detectors

Images were obtained immediately after administration (T = 0) with subsequent scans acquired at 15, 30, 60, 120, 180, and 240 minutes post-injection. Beginning at T = 0, vascular contrast rapidly increased to a level that was sustained at all time points for the remainder of the study. The liver showed a slight increase in CT density due to its significant vascular supply, while the highly vascular structure of the spleen resulted in a high level of contrast enhancement. After the four-hour time point, vascular contrast enhancement declined gradually as Fenestra VC underwent hepatobiliary elimination.

Data Reconstruction and Visualization

Data Reconstruction

Machine-based reconstruction does not allow for down-sampling of projections prior to reconstruction, as does software-based reconstruction programs. However, down-sampling was unnecessary for the selected resolution in this study.

NOTE: Reconstructed image files can be stored as CT or ATT files, which can be exported to a 3D visualization application such as Visage Imaging's Amira software for viewing as axial, coronal, and sagittal images, in addition to a number of other image representations.

Parameter	Setting
Number of Voxels in Volume	512x512 (transaxial) 768 (axial)
Voxel Size	100 μ M (transaxial) 150 μ M (axial)
Reconstruction Filter	Shepp-Logan
Reconstruction Algorithm	Fledkamp cone-beam

Data Visualization

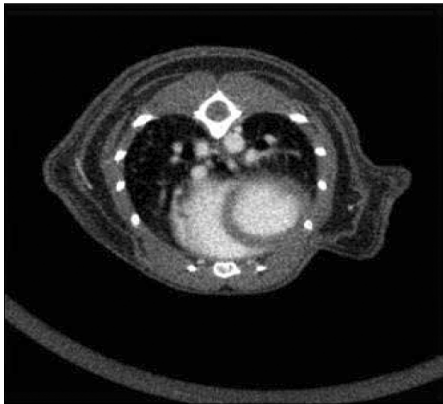
Data is routinely imported from reconstruction programs as raw CT image data or as bitmaps windowed to a vascular contrast setting. Data can be viewed in Amira using the Standard Display format with simultaneous display of the axial, coronal, and sagittal images, or as 3D isosurface images that can be manipulated to view anatomic structures with or without orthoslice display of 1, 2, or all 3 of the planar slices. The isosurface image can also be cropped to eliminate extraneous data and saved as an Amira map file, which can accelerate isosurface viewing and save storage space.

Representative Images

Representative images from studies conducted in CT-26 tumor-bearing BALB/c male mice using Fenestra LC, and that were obtained with MicroCAT II scanner, are provided in the figures below.



Figure 1. Non-contrast coronal scan of a male mouse. Poor soft tissue contrast is evident in the thoracic and abdominal cavities. The bright spots observed in the intestines were caused by minerals in the rodent chow that attenuated X-ray energy.



a.



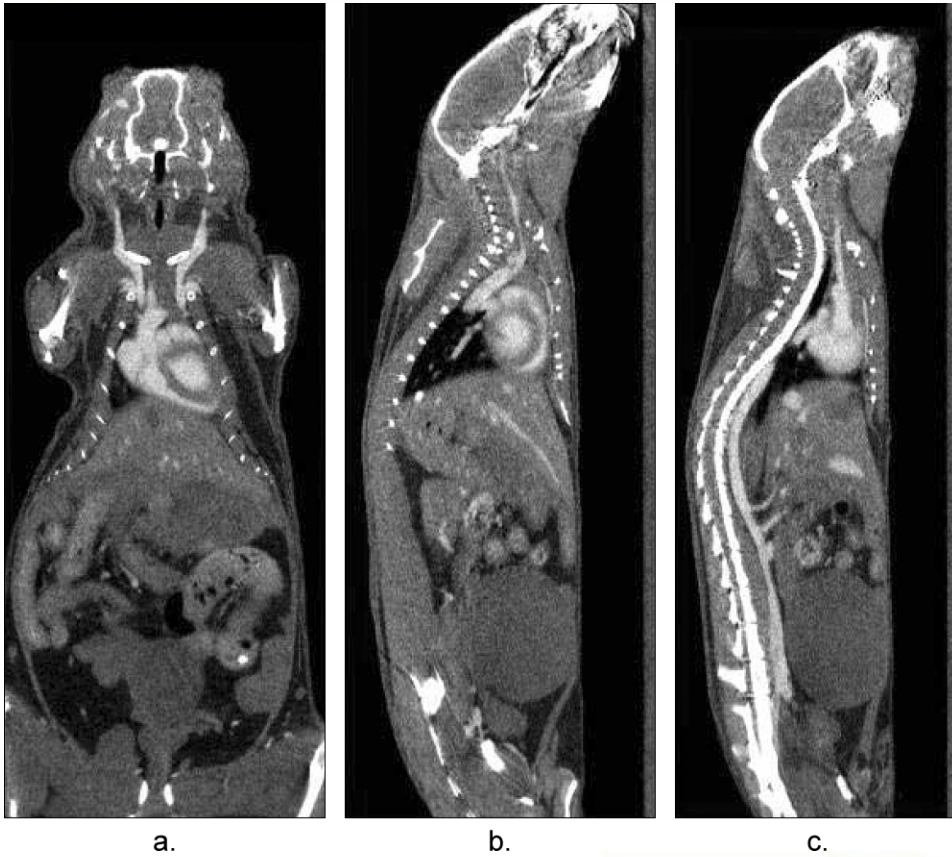
b.



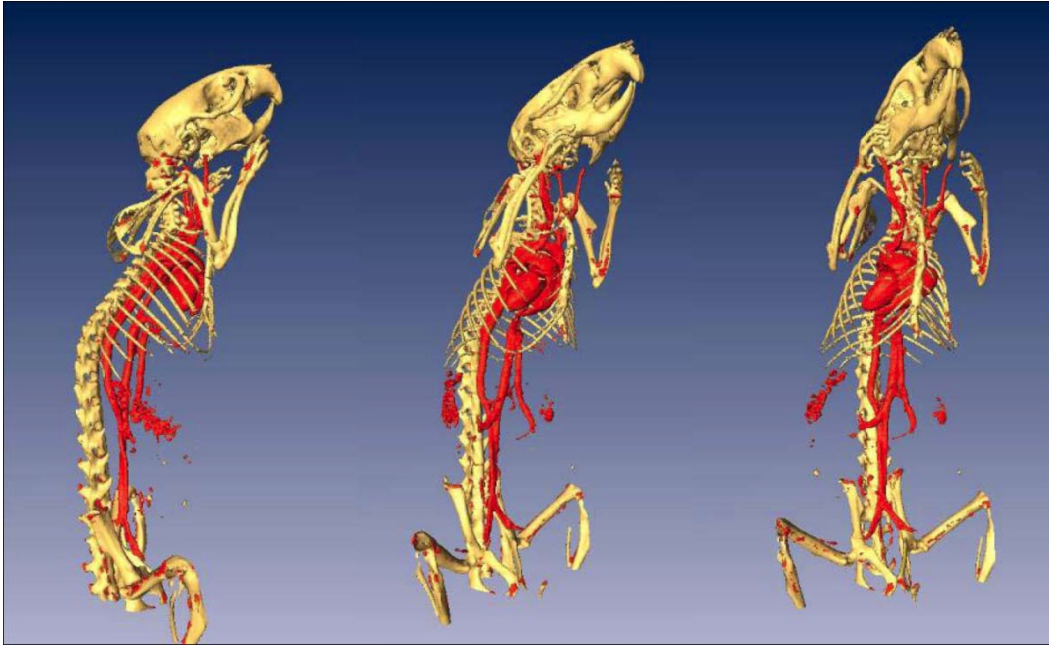
c.

Figures 2. Axial, coronal, and sagittal views of a male mouse 15 minutes after IV injection of Fenestra VC. a. The axial image depicts both ventricles of the heart, as well as several major vessels in the thoracic cavity. b. The coronal image shows the inferior vena cava, the renal veins, additional renal

vasculature, as well as several veins lower in the abdomen. The spleen (seen adjacent to the left kidney) is slightly enhanced due to its high degree of vascularity. c. In the sagittal view, the inferior vena cava and a short segment of the portal vein can be seen below the dome of the liver, while the heart, aorta and several other vessels can be seen in the thoracic region.



Figures 3. Coronal and sagittal scans of a male mouse obtained 3 hr after IV administration of Fenestra VC. a. Coronal image shows Fenestra VC continued to produce vascular contrast 3 hr after injection, clearly delineating the ventricles and ventricular wall, the aorta, and the carotid arteries in the neck. Small vessels are clearly observed in the liver, which is only slightly enhanced due to the initial elimination of the contrast agent, although the gall bladder has yet to enhance as a result of excretion of the metabolized agent. b. This sagittal view shows a major vessel diagonally traversing the liver with a number of smaller vessels seen in cross section. The heart and the aorta are readily visualized, as is another vessel, which appears to bifurcate just below the base of the skull. c. Sagittal image shows the descending aorta and several other arteries in the abdomen. The heart, aorta, and several additional vessels are seen in the thorax and neck of the mouse. Chow-induced artifacts are evident in the intestines in all three images.



Figures 4. Volumetric representations of vascular enhancement from scans of a male mouse 3 hr after IV administration of Fenestra VC. Fenestra VC produces prolonged vascular contrast enhancement throughout the entire body from a single peripheral injection.

The custom parameters selected for this study were as follows:

Parameter	Setting
Number of Samples	512
Number of Projections	3072
Integration Time	200

You should note that changes in these parameters can influence both total image acquisition time and image quality. The average time for image acquisition for vascular imaging with Fenestra VC was approximately eight minutes for acquiring the scout view and the 250 step study with the vivaCT 40 scanner. To save time, you can “pre-calibrate” the scanner so that the scans begin as soon as the animal is inserted.

Images were obtained immediately after administration, T=0, with subsequent scans acquired at several time points. Beginning at T=0, vascular contrast rapidly increased to a level that was sustained for the remainder of the study. The liver showed a slight increase in CT density due to its significant vascular supply, while the highly vascular structure of the spleen resulted in a high level of contrast enhancement in that organ. Beyond the 6-hour time point, vascular contrast enhancement declined gradually as Fenestra VC began to undergo hepatobiliary elimination. You should note that the gray scale may have to be adjusted to display low contrast regions.

Data Reconstruction and Visualization

Data Reconstruction

Parameter	Setting
Number of Voxels in Volume	1024 x 1024 or User-defined values (Custom Protocol)
Voxel Size	FOV divided by the number of samples
Reconstruction Filter	None
Reconstruction Algorithm	Cone-beam

Data Visualization

Acquired images can be visualized in the evaluation window. You should note that low contrast images may be viewed better with a black background than the standard blue. You can toggle between backgrounds by clicking on the scalar on the right side of the window or by choosing the LUT option and then selecting the correct LUT and optimizing the contrast.

In addition to exporting AIM and ISQ files, images can also be exported as TIFF files. You should note that AIM files can be converted to the DICOM file format for export. Any data visualization program capable of reading and displaying TIFF or DICOM file formats can be used to display the data.

Representative Images

Representative images from studies conducted in normal mice with Fenestra VC, and that were obtained with SCANCO Medical's vivaCT 40 scanner, are provided in the figures below.

Pre-contrast Exam

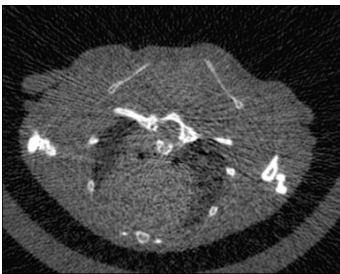


Figure 1. Non-contrast axial scan of a mouse. Poor soft tissue contrast is evident in the thoracic cavity and heart chambers.

Fenestra VC Exam



Figure 2. Axial views of a mouse 2 hours after IV injection of Fenestra VC. Images a and b depict both ventricles of the heart, as well as several major vessels in the thoracic cavity. Image c also shows both cardiac chambers, as well as additional thoracic vasculature near the bottom of the heart.

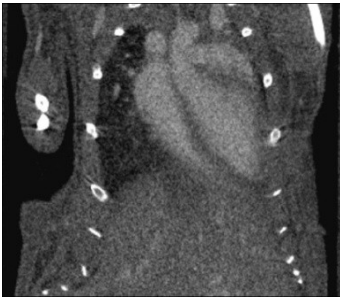


Figure 3. Coronal view of a mouse 2 hours after IV injection of Fenestra VC. The image shows both cardiac chambers, as well as small hepatic vessels