Imaging Hepatic CT26 Tumors in BALB/c Mice as a Model of Metastatic Liver Cancer

This case study provides guidance and recommendations for visualizing hepatic focal lesions and anatomy with Fenestra LC. 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

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

Animal Model

Strain

Balb/c mice (20 to 21 g males) bearing solitary hepatic CT-26 tumors.

Model

A solitary CT-26 tumor (adenocarcinoma of colonic origin) was created in the liver of each of three anesthetized male Balb/c mice by direct injection of 5 ´ 105 cells in 50 ml PBS into the exposed medial lobe of the liver through a 30-gauge needle. Stasis was achieved with light pressure at the site of needle insertion. The laparoscopic incision was closed with surgical staples, which were removed prior to imaging. Tumors reached a size of 2 to 3mm in diameter within five days of implantation.

APPLICATION NOTE

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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 LC 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 publication 'Optimal Usage of Fenestra Contrast Agents' for recommendations and detailed instructions related to dosage, animal preparation, and administration.

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Image Acquisition

Equipment

ImTek (now Siemens) MicroCAT II microCT scanner.

Animal Placement

Anesthetized mice were placed on the imaging table in the prone position with heads oriented into 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 $^{.00}$A

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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 .00m
Detector Array Height	2048 elements
Detector Array Width	3072 elements
Center Offset	4.7 unbinned detectors

Images were obtained immediately after administration of Fenestra LC (T = 0) with subsequent scans at 30, 60, 120, 180, and 240 minutes post-injection. At T = 0 and T = 30 minutes vessels typically have higher contrast levels than the liver, but by T = 60 the liver should display similar levels of contrast enhancement. At later time points the vessels usually become hypodense relative to the liver, which will usually remain enhanced up to 6 hours post-injection. Hepatic tumors normally display very little or no contrast enhancement. Hepatobiliary elimination of Fenestra causes liver contrast values to return to baseline levels within 18 to 24 hours after administration.

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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	512 $\frac{3}{2}$512 (transaxial) $\frac{3}{2}$768 (axial)
Voxel Size	100 $^{.00}$m (transaxial) $\frac{3}{2}$150 $^{.00}$m
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.

Using Amira's image capture feature, planar and 3D images can be captured for presentations or publication purposes, while movies can be created for fly-through of 3D image data sets.

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Representative Images

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

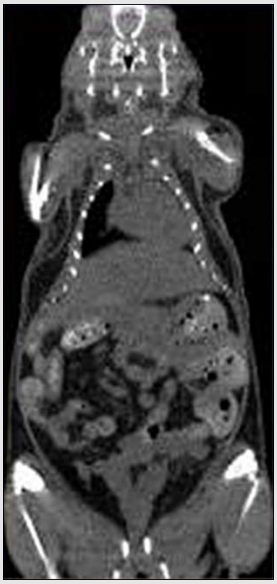

Figure 1.

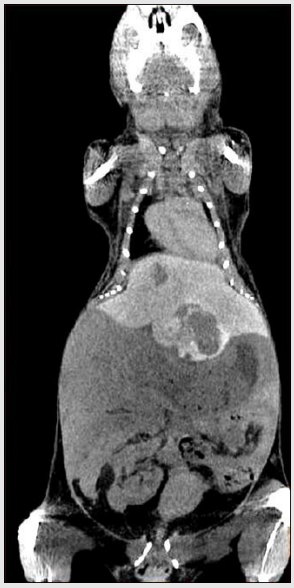

Figure 2.

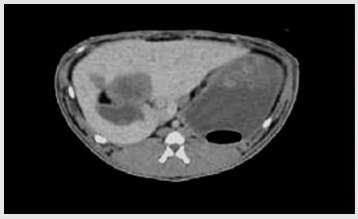

Figure 3.

Figure 4.

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.*

Figures 2 to 4. *Coronal view (figure 2), transverse view (figure 3), and 3D reconstruction (figure 4) of male Balb/c mouse with hepatic CT-26 adenocarcinoma 2 hr after IV injection of Fenestra LC. At this time point the vascular system is still enhanced as evidenced by the lack of hepatic vascular detail and isodensity of the ventricles of the heart. The hypodense gall bladder near the dome of the liver is readily visualized in the coronal view. The CT-26 tumor is observed at the lower edge of the liver with a narrow rim of enhanced liver below the tumor and was colorized (purple) in the 3D reconstruction. The absence of artifacts in the lower abdomen is a result of the mouse having been fed a soft, non-chow diet for 48 hours prior to imaging.*

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