Contrast enhanced vessel imaging using microCT

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Short Abstract:

Contrast enhanced small animal vessel imaging by microCT is a rapid, cost-effective and high-throughput technique for serial *in situ* examination for tumor development, for analyzing the network of blood vessels that nourish them, and for following the response of tumors to preclinical therapeutic intervention(s).

Long Abstract: (400 words maximum)

Microscopic computed tomography (microCT) offers high-resolution volumetric imaging of the anatomy of living small animals. However, the contrast between different soft tissues and body fluids is inherently poor in micro-CT images. Under these circumstances, visualization of blood vessels becomes a nearly impossible task. To overcome this and to improve the visualization of blood vessels exogenous contrast agents can be used. Herein, we present a methodology for visualizing the vascular network in a rodent model. By using a long-acting iodinated triglyceride blood-pool contrast agent, Fenestra VC™, we optimized image acquisition parameters and volume-rendering techniques for finding blood vessels in live animals. This technique was successfully employed in examining the intermediate and large vessels of complex spontaneous tumors (*e.g.*, alveolar rhabdomyosarcomas) in transgenic mice. Our findings suggest that, to achieve a superior contrast between bone and soft tissue from vessel, multiple-frames (at least 5-8/ frames per view), and 360–720 views (for a full 360º rotation) acquisitions were mandatory. We have also demonstrated the use of a two-dimensional transfer function (where voxel color and opacity was assigned in

proportion to CT value and gradient magnitude), in visualizing the anatomy and highlighting the structure of interest, the blood vessel network. This promising work lays a foundation for the qualitative and quantitative assessment of anti-angiogenesis preclinical studies using transgenic or xenograft tumor-bearing mice.

Text:

All animal procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Texas Health Science Center at San Antonio.

2.1) Animal Injections

Live mice should be injected with 0.4 mL/25 g of a 50-mg iodine/mL (particle diameter: 150-nm) iodinated triglyceride bloodpool contrast agent (Fenestra VC™; Advanced Research Technologies (ART) Inc. Montreal, Canada) via the distal tail vein using a 27- or 30-gauge needle.

2.2) Animal preparation

After 10 minutes of injection, the animals should be prepared for microCT imaging. The animals can be imaged by keeping, 2% isoflurane in 100% oxygen at 2.5 liters per minute. Body temperature should be maintained at 37ºC by a heat pads. To minimize movement artifacts and stability, mice can be placed in a custom-built, commercially available multi-modality chamber (Numira Biosciences, Salt Lake City, UT) with provision for air and exhaust.

2.3) MicroCT imaging

Mice then should be scanned on a microCT unit capable to perform live animal scans. The following imaging protocol can be used as a guideline to help determine the appropriate scan parameters for any microCT units.

The animals used in this experiment were scanned at 93 µm resolution on a volumetric CT scanner GE eXplore Locus (GE Healthcare, London, Ontario). This volumetric scanner uses a 3500 × 1750 CCD detector for Feldkamp cone-beam reconstruction. The platform independent parameters of current, voltage and exposure time were kept constant at 450 µA, 80 kVP and 100 ms, respectively. The standard parameters of exposure time, frames per view and number of views can be varied and could be 100 ms, 5-8, and 360-720, respectively with total scan time of approximately 25-40 minutes. Images were reconstructed with the manufacturer's proprietary EVSBeam software.

2.4) Image rendering

The reconstructed microCT data can be used for advanced isosurface, one-dimensional, or two-dimensional transfer function rendering Images using ImageVis3D™ image processing software (ImageVis3D, http://www.sci.utah.edu/cibc/software).

Representative Results

Figure 3B (right panel) from the enclosed manuscript.

Discussion

The foremost goal of this technique of contrast enhanced vessel imaging using microCT is to provide optimal method for generating high quality datasets for analysis of vascular networks in live mice. For microCT imaging, better signal-to-noise ratio of soft tissue is achieved by longer scan times with increased numbers of views and number of frames per view.

Vessel identification is critically dependent upon accurate soft tissue identification and differentiation from bone. Fenestra VC contains fine particles of iodinated triglyceride which attribute the property of being radio-opaque and a density which is different form soft tissue and bone. This makes it convenient to focus on the blood vessels in a microCT data.

A 2-dimensional transfer function even further improves the distinction between vessel contrast and bone during rendering. In case of two-dimensional transfer function image renderings, colors and opacities are assigned to the ray sample based on the transfer function, which in turn relies on CT value and gradient magnitude. Transfer functions can be manually adjusted based on guidance provided by CT value histograms to highlight areas/objects of interest.

This technique could very well be used for qualitative and quantitative assessment of anti-angiogenesis preclinical studies using transgenic or xenograft mice. In future, advances in CT technology can easily make vessel imaging in transgenic or xenograft mouse models easier, faster, and more accurate over time. Better resolutions, will also allow visualization of fine structures like capillaries.