TECHNICAL NOTE

Blood vessel imaging with µCT

High-resolution *in vivo* µCT of the mouse vasculature

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Abstract

In recent years, µCT has emerged as a highly useful imaging modality for vascular applications. Herein we present a µCT method using the X-CUBE system combined with the contrast agent ExiTron™ nano 12000 to visualize the mouse vasculature *in vivo* at high acquisition speed whilst using a low radiation dose. The approach serves as a tool for the *in vivo* monitoring of the progression of diseases with vascular involvement such as cancer, and aids in the evaluation of changes in pathology through therapeutic intervention.

Introduction

In vivo visualization of changes in the vasculature of small animals, associated with various diseases such as cancer, is critical to the development of novel therapies. Over the past few years, largely due to its high spatial resolution, µCT has emerged as a highly useful imaging modality for vascular applications. However, the CT contrast resolution of vessels, as well as that of other soft tissue, is intrinsically low. Thus, vascular µCT requires the use of blood pool contrast agents (also known as intravascular contrast agents), which persist in the blood for a prolonged period of time, thereby providing significant contrast between the vessels and surrounding soft tissue. ExiTron™ nano 12000 (Viscover™, *nanoPET Pharma* GmbH, Berlin, Germany) is an innovative blood pool contrast agent specifically designed and developed for *in vivo* preclinical CT. It has a long blood half-life and provides excellent contrast of blood vessels in small animals at a low injection volume. Owing to the small size of the vessels in small animals, vascular imaging with µCT requires not only the application of a blood pool contrast agent but also of a µCT scanner that provides images with a very high spatial resolution and supports accurate 3D analysis of the vascular network. These features allow visualization of the physiological arrangement of the entire vascular network of the animal in three dimensions at a high-resolution scale, enabling improved evaluation of pathological changes in small vessels, crucial to the understanding of diseases and the development of effective treatments. The X-CUBE system (*MOLECUBES* NV, Ghent, Belgium) is a high-speed, low dose, benchtop µCT scanner that enables whole-body scanning of mice and rats. It is extremely easy to operate and delivers high resolution 3D images at low radiation dose, making it well-suited for routine CT imaging of small animals. The scanner can be used individually or in combination with the γ-CUBE (SPECT) or β-CUBE (PET) to provide multimodal imaging, making the system extremely versatile. Herein we provide a µCT method using the X-CUBE system combined with the contrast agent ExiTron nano 12000 to provide rapid and detailed visualization of the mouse vasculature *in vivo*.

Protocol

All animal work in this study was approved by the local committee on animal welfare and was in accordance with the guidelines issued by the Federation of European Laboratory Animal Science Associations (FELASA). Please seek institutional animal care and use committee approval before commencing this work.

1. Animal Preparation

1.1. Anesthetize the mouse by inhalation of isoflurane in an air-oxygen mixture vaporized at concentrations of 3-4% in the induction phase and 1-2% for the maintenance of anesthesia.
1.2. Place the animal in the animal bed coupled to the docking station.
1.3. Fix the animal into position using the fleece system provided.
1.4. Place a catheter in the lateral tail vein of the mouse so that the contrast agent can be injected when the mouse is positioned within the scanner.
1.5. Cover the bed with the bed cap.
1.6. Decouple the animal bed from the docking station and couple it to the bed stage on the X-CUBE system (Fig. 1).
1.7. Monitor the animal's vital functions (Fig. 2) and, if necessary, adjust the level of anesthesia.

Figure 1: The X-CUBE system showing its small footprint as well as intuitive and user-friendly design.

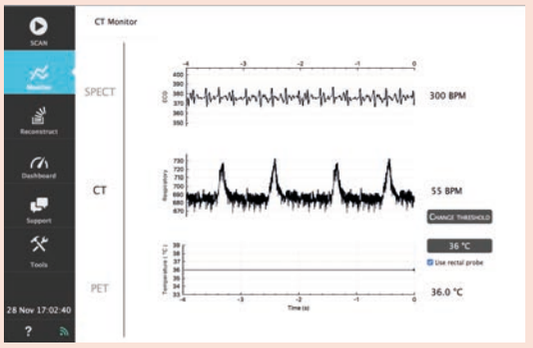

Figure 2: Screenshot of the monitoring function of the X-CUBE graphical user interface for monitoring of the animal's vital functions.

Figure 3: Screenshots of the scan function of the X-CUBE graphical user interface illustrating **A.** the entered study details and **B.** the selected acquistion settings.

2. Contrast Agent Injection

2.1. Vortex the ExiTron nano 12000 vial to ensure thorough mixing.

2.2. Disinfect the septum with 70% ethanol. Let septum dry.

2.3. Withdraw the required volume (10 µL/g body weight) of the contrast agent using a sterile low dead space syringe equipped with a sterile needle (27G-30G).

2.4. Using an infusion pump, inject the contrast agent at a rate of 150 µL/min followed by a saline flush, and note the time of injection.

3. CT Imaging

3.1. Using the graphical user interface, enter the details of the imaging study (Fig. 3A).
Note: These details will be automatically saved in the DICOM[1] header.

3.2. Set the scan acquisition settings (Fig. 3B) by selecting the high-resolution multi-rotation protocol with a single-bed position (FOV 70 mm x 40 mm).
Note: In this preset mode, the settings are automatically as follows:
- *2 continuous gantry rotations with 720 projections/rotation*
- *Tube current: 440 µA*
- *Tube voltage: 50 kV*
- *Radiation dose: 169 mGy*
- *Total scan time: 00:02:18*

3.3. Position the bed in the X-CUBE system.
Note: The animal bed can be pushed only until halfway within the scanner and will move into the scanner automatically when the scan is initiated.

3.4. Initiate the CT scan within one hour after injection of the contrast agent.
Note: The blood half-life of ExiTron nano 12000 is approx. 4 h in mice. To obtain high vessel contrast, the CT scan should be initiated as soon as possible after contrast agent injection.

3.5. Following the scanning process, remove the animal bed from the X-CUBE system and allow the mouse to recover from anesthesia in a recovery box.

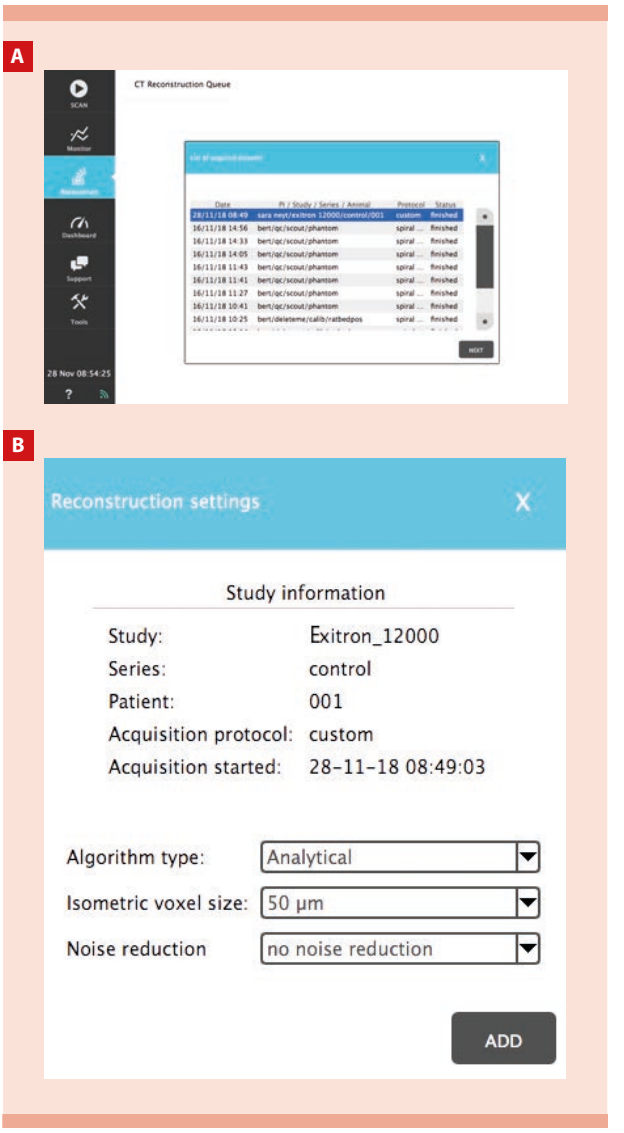

Figure 4: Screenshots of the reconstruction function of the X-CUBE graphical user interface ilustrating **A.** the selected dataset for subsequent reconstruction and **B.** the reconstruction settings for selection of the reconstruction parameters.

3.6. Using the reconstruction function of the graphical user interface, select the obtained dataset from the list (Fig. 4A).

3.7. Set the reconstruction settings by selecting the analytical reconstruction algorithm, a voxel size of 50 µm and no noise reduction (Fig. 4B).

3.8. Reconstruct the dataset to obtain high resolution images with a matrix size of 800 x 800 x 800 (Fig. 5).

3.9. Perform automated volume rendering using image processing software (e.g. Horos[2]) to obtain 3D volume-rendered images (Fig. 6).

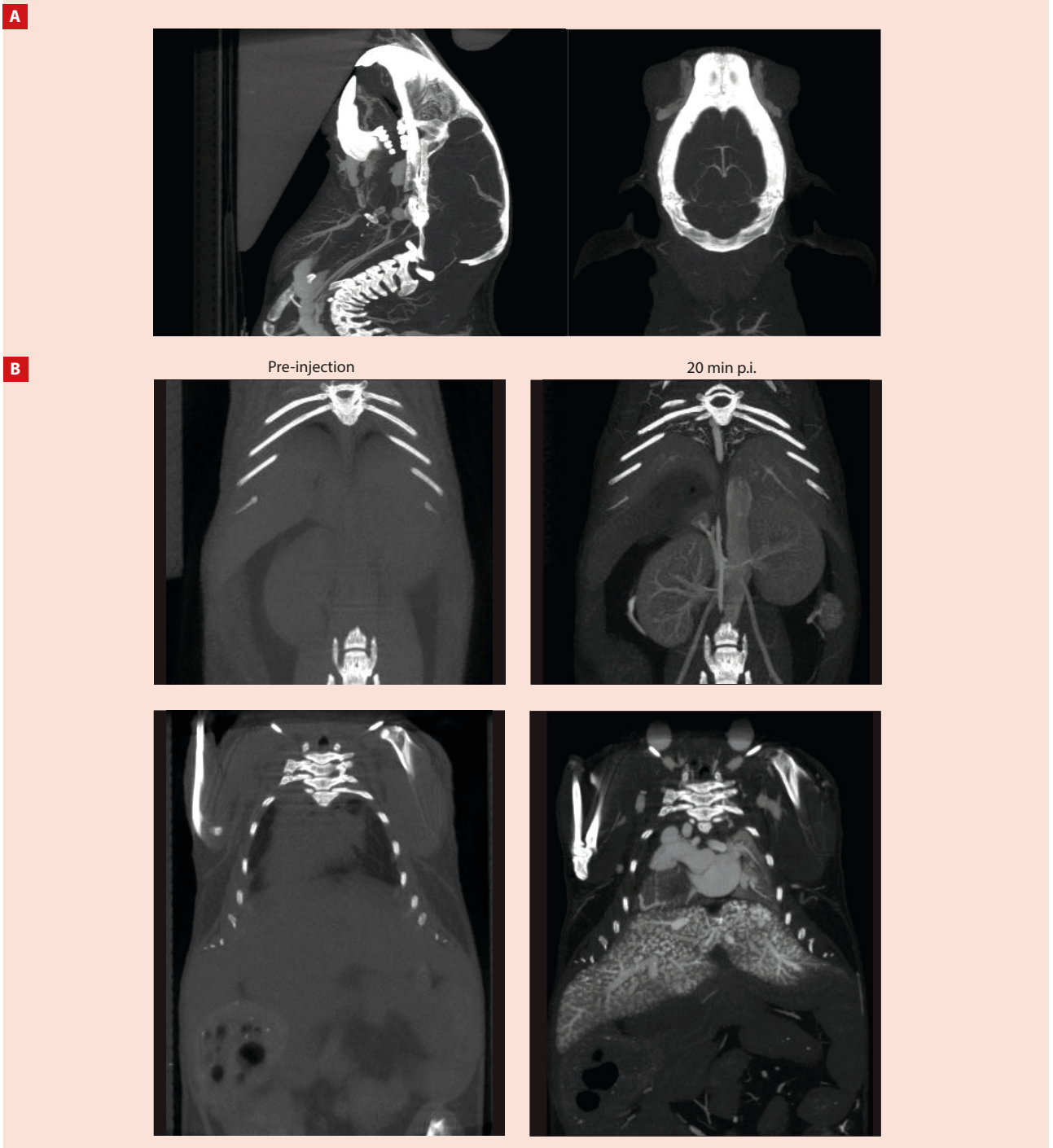

Figure 5: *In vivo* CT images of the healthy mouse obtained over a total scan time of 2 min using the X-CUBE system.
A. Sagittal (left) and coronal (right) CT images of the head and neck at 30 min post injection of ExiTron nano 12000 clearly showing the associated network of blood vessels.
B. Sagittal images of the abdomen before and 20 min after injection of ExiTron nano 12000. The images acquired post injection show highly-contrasted blood vessels in the kidneys (upper panel) and liver (lower panel)

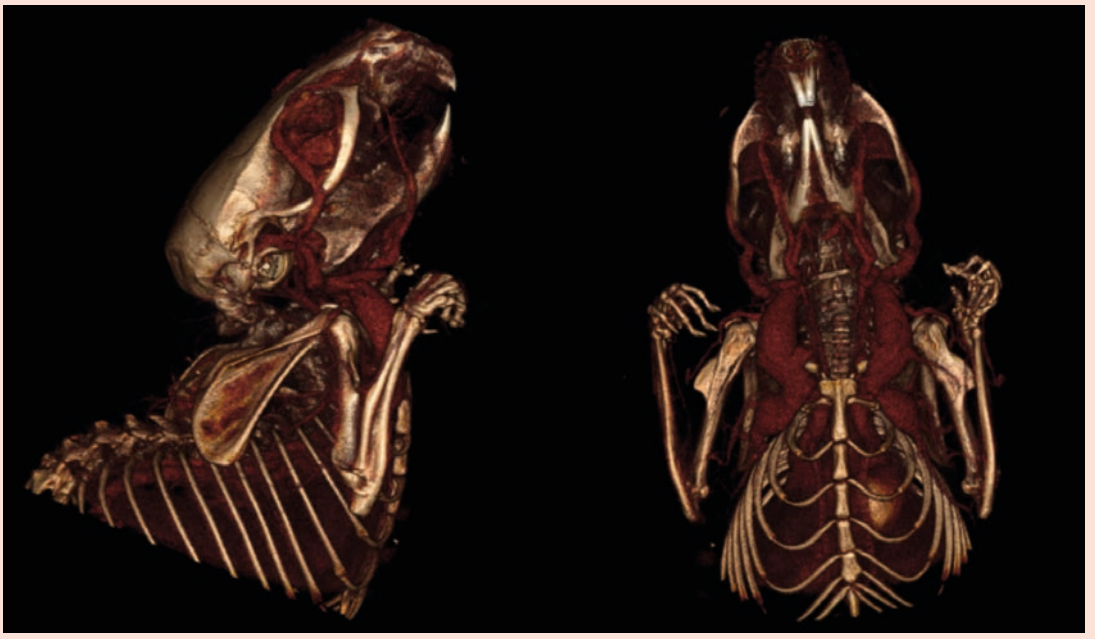

Figure 6: *In vivo* 3D volume-rendered CT images of the upper part of the healthy mouse obtained over a total scan time of 2 min using the X-CUBE system at 30 min post injection of ExiTron nano 12000. The vasculature can be seen in great detail in both lateral view (left) and frontal inferior view (right).

Conclusion

We herein provide a µCT method using the X-CUBE system and ExiTron nano 12000 to effectively perform *in vivo* vessel imaging in small animals. The method allows for fast and easy scanning at a low radiation dose and yet results in high resolution CT images with strongly enhanced vessel contrast. Thus, the approach is highly suited for routine examination of vascular structures and serves as a tool for the evaluation of therapeutic strategies in pathologies with vascular involvement.

References

1. NEMA PS3 / ISO 12052, Digital Imaging and Communications in Medicine (DICOM) Standard, National Electrical Manufacturers Association, Rosslyn, VA, USA (available free at http://medical.nema.org/).
2. Horos is a free and open source code software (FOSS) program that is distributed free of charge under the LGPL license at Horosproject.org and sponsored by Nimble Co LLC d/b/a Purview in Annapolis, MD USA.

MOLECUBES
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At MOLECUBES NV we provide three separate benchtop imagers or "CUBES". The γ-CUBE (SPECT), β-CUBE (PET) and X-CUBE (CT) are perfectly suited for *in vivo* multimodal whole body rat and mouse imaging. User-friendliness and expert servicing with no compromise on image quality is what we stand for.

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