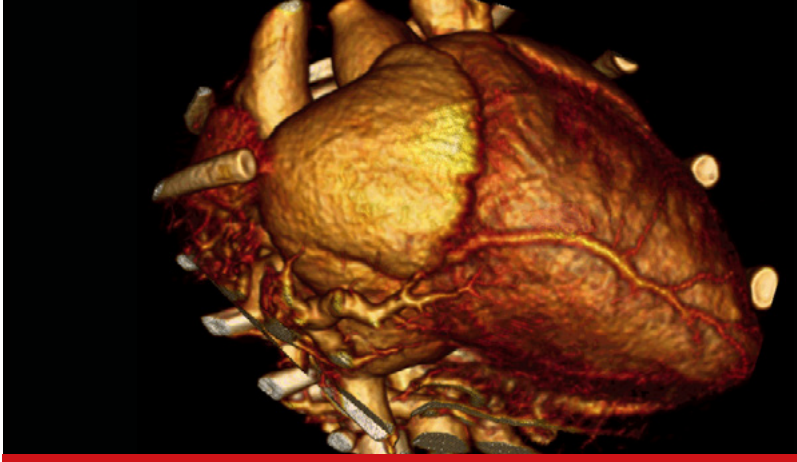

TECHNICAL NOTE

In vivo 4D dual gated cardiac CT

4D cardiac-respiratory gated CT of the mouse heart

Ruud M. Ramakers[1,2,3], David A. Rendon[1,3], Danielle Franke[4], Anne Kirchherr[4], Freek J. Beekman[1,2,3], and Andreas Briel[4]
[1] MILabs B.V., Utrecht, the Netherlands
[2] Delft University of Technology (TU Delft), Delft, the Netherlands
[3] University Medical Center Utrecht (UMCU), Utrecht, the Netherlands
[4] nanoPET Pharma GmbH, Berlin, Germany

Abstract

In vivo cardiac imaging in mouse models has shown to facilitate the study of human cardiovascular diseases, as well as the development of potential treatment strategies[1]. However, due to the small size of the mouse heart as well as the animal's high cardiac and respiratory rates, murine cardiac imaging is rather challenging and requires recognition of both spatial and temporal scales. Despite these challenges, we herein report a feasible method using the U-CT^{UHR} µCT system combined with the CT contrast agent ExiTron^{TM} nano 12000 to effectively visualize the beating mouse heart *in vivo*.

Introduction

The introduction of µCT has enabled substantial improvements in the anatomical imaging of small animals since its high spatial resolution enables non-invasive assessment of morphological changes in small structures. Besides its high spatial resolution, further advantages of this imaging modality include its relatively low cost and short scan time. However, due to its relatively poor soft-tissue contrast, the use of contrast agents is required to improve visualization of these structures. Since clinical CT contrast agents are rapidly cleared from the blood pool of small animals, these agents are not optimal for small animal imaging[2,3]. As a result, µCT studies are generally combined with contrast agents, commonly nanoparticulate in nature, which avoid rapid clearance and persist in the blood pool for a prolonged period of time. ExiTron^{TM} nano 12000 (Viscover^{TM}, *nanoPET Pharma* GmbH, Berlin, Germany) is an innovative alkaline earth metal-based nanoparticulate contrast agent specifically formulated for preclinical CT. It has a prolonged blood half-life of approx. 4 h in mice and, due to its high metal content, provides exceptionally high contrast at a low injection volume. Owing to the high cardiac rate (~600 beats/min) and rapid respiratory rate (~300 breaths/min) of small animals, cardiac µCT requires not only the application of blood pool contrast agents but also of reconstruction methods that allow significant reduction of motion artifacts. The U-CT^{UHR} µCT system (*MILabs* B.V., Utrecht, the Netherlands) provides simultaneous cardiac and respiratory motion compensation resulting in superior dual gated images. The device combines high resolution, high speed and low radiation dose making it ideal for routine CT imaging of small animals, especially for imaging of the heart and lungs. Herein, we provide a µCT method using the U-CT^{UHR} system with intrinsic cardiac-respiratory gating combined with ExiTron nano 12000 to obtain real-time, high-resolution images of the beating mouse heart *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 animal by inhalation of isoflurane in an air-oxygen mixture vaporized at concentrations of up to 4% in the induction phase and 1-2% for the maintenance of anesthesia.

1.2. Place the mouse on a warm surface and warm the tail to dilate the veins and enhance their visibility.

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 (5 µL/g body weight) of the contrast agent using a sterile low dead space syringe equipped with a sterile needle (27G–30G).

2.4. Inject the agent slowly into the lateral tail vein of the mouse and note the time of injection. Alternatively 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.

Note: In the case of weak animals with severe cardiac pathologies, a saline flush (25 µL) is recommended after injection of the contrast agent.

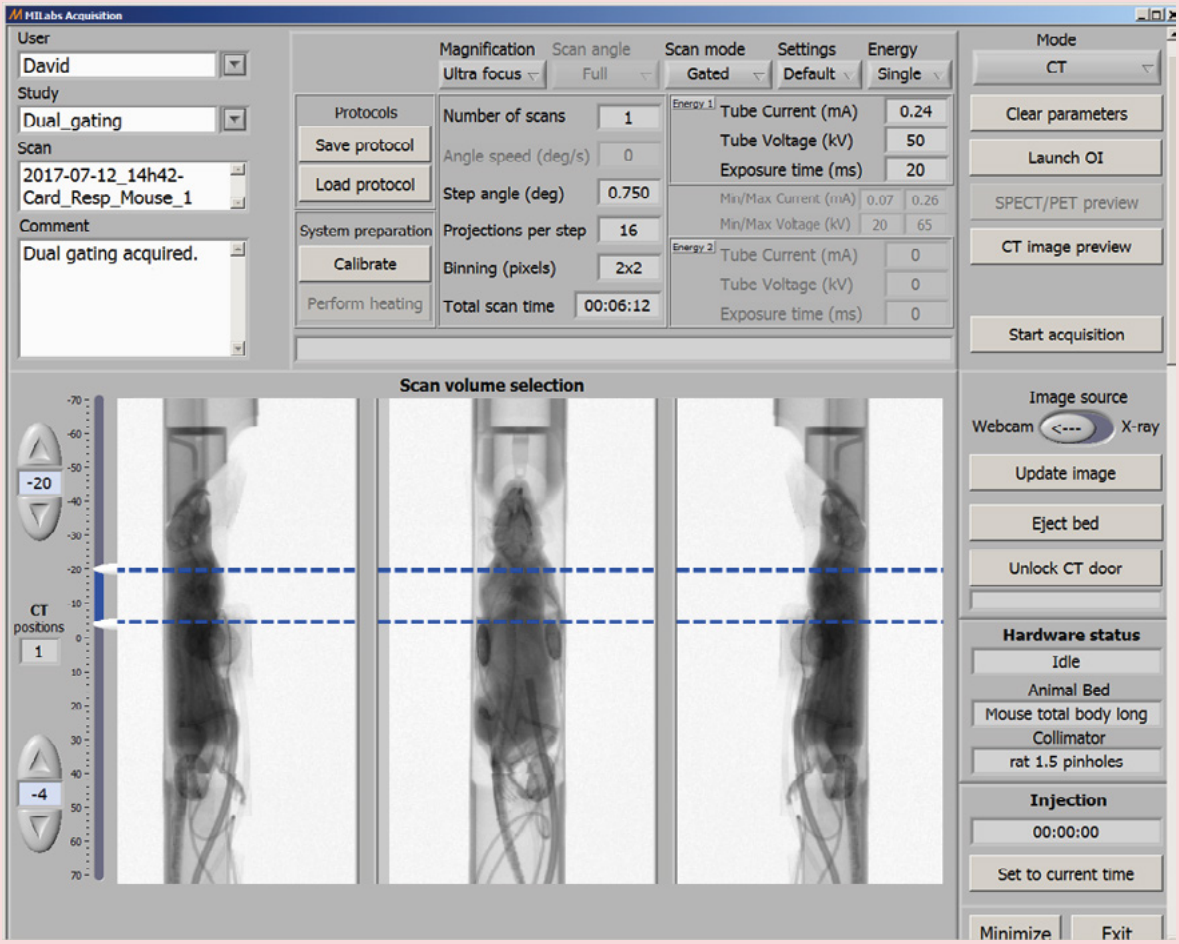

Figure 1: Screenshot of the U-CT^{UHR} control software window illustrating the selected scan acquisition settings and the selected FOV (blue lines).

3. CT Imaging

3.1. Place the mouse in supine position on the temperature-regulated animal bed of the U-CT^{UHR} µCT scanner.

3.2. Maintain anesthesia using the gas cone of the animal bed (1-2 % isoflurane).

3.3. Attach the respiration pad to the animal.

3.4. Attach the three ECG leads to the paws, one on each of the front paws and one on either of the back paws. Place the front paws downwards and stretched out as far as possible so that during imaging the ECG leads are outside the cardiac field of view (FOV).

3.5. Adjust the thresholds for both the respiratory signal and the cardiac ECG signal for correct triggering.

3.6. Set the scan acquisition settings by selecting the following parameters from the drop-down menus of the U-CT^{UHR} control software window (Fig. 1):
- Magnification: Ultra focus
- Scan mode: Gated
- Settings: Default
- Energy: Single

Note: In the default-setting mode, the settings are automatically as follows:
- *Tube current: 0.24 mA*
- *Tube voltage: 50 kV*
- *Exposure time: 20 ms*
- *Number of scans: 1*
- *Step angle: 0.750°*
- *Projections per step: 16*
- *Binning: 2x2 pixels*
- *Total scan time: 00:06:12*

3.7. Acquire a CT scout view of the animal.

3.8. Locate the heart and adjust the FOV (Fig. 1, blue lines) so that it encompasses the entire heart. If necessary, adjust the animal's paws so that the ECG leads lie outside the region of the heart.

3.9. Start the data acquisition within one hour after injection of the contrast agent and monitor the animal's vital functions throughout. If necessary, adjust the level of anesthesia.
Note: The blood half-life of ExiTron nano 12000 is approx. 4 h in mice. To obtain high vessel contrast, data acquisition should be initiated as soon as possible after contrast agent injection.

3.10. After data acquisition, remove the animal from the µCT scanner and allow for full recovery from anesthesia in a recovery box.

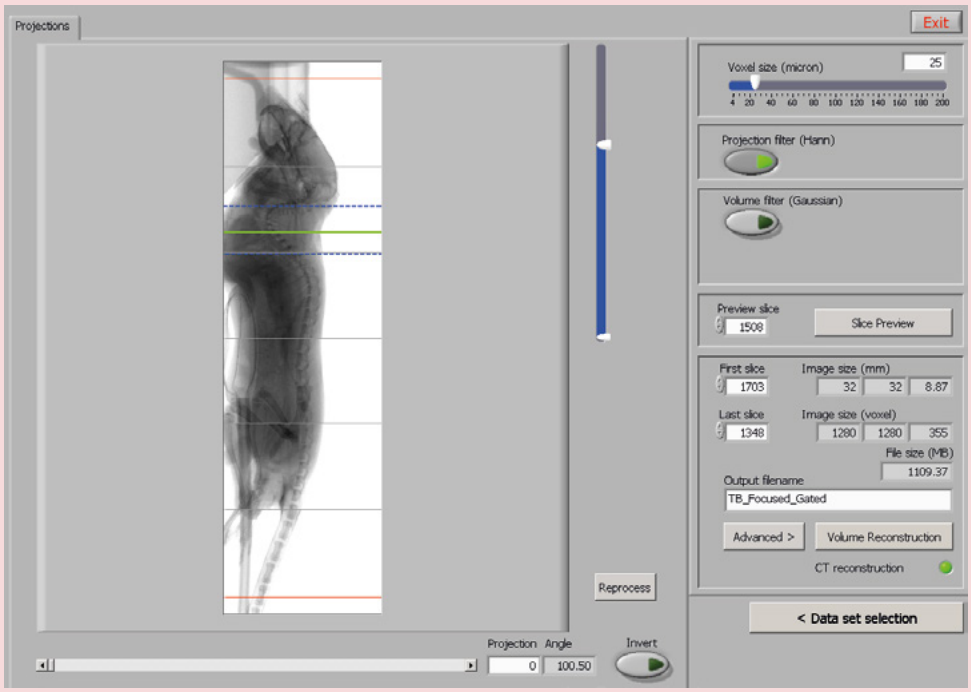

Figure 2: Screenshot of the default reconstruction settings for selection of the voxel size, anatomical region for reconstruction and subsequent gating parameters (advanced settings).

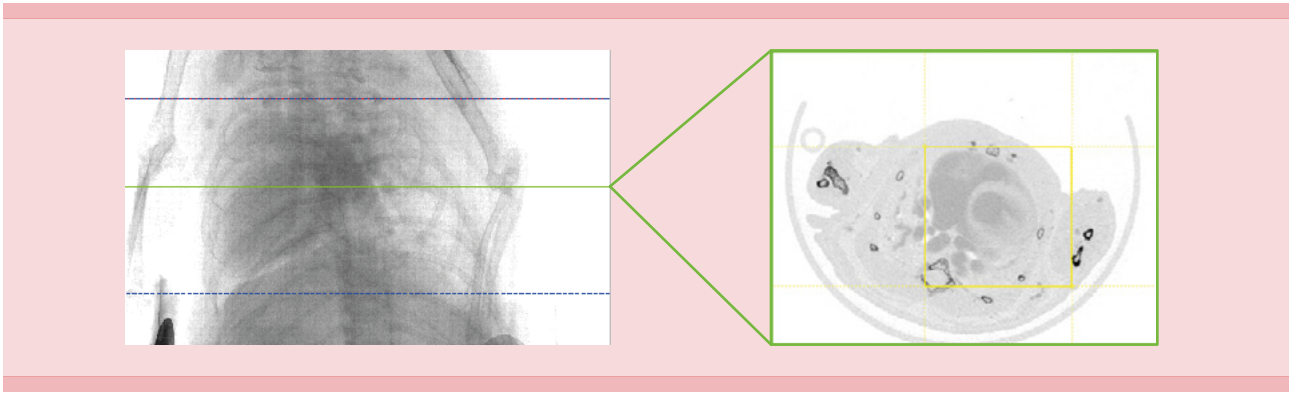

Figure 3: Preview CT images showing the selected anatomical region for reconstruction via positioning of the FOV (blue lines) in the coronal image (left) and the yellow bounding box in a transverse image (green box).

4. Image Processing

4.1. For image processing, load the obtained dataset on the reconstruction PC.
Note: After imaging is completed, the acquired data is automatically transferred to the reconstruction PC.

4.2. Using the default reconstruction settings (Fig. 2), select a voxel size between 10 µm and 40 µm, depending on the desired image quality.

4.3. Select the anatomical region for reconstruction by designating the first and last transverse slices (blue lines) as well as the preview slice (green line).

4.4. Using the slice preview, position the yellow bounding box on the transverse image (Fig. 3).
Note: Only the image data within these areas will be used for reconstruction.

4.5. Using the advanced settings (Fig. 2), select 16 cardiac phases and a single respiratory phase (Fig. 4).

4.6. Perform volume reconstruction (Fig. 2) to obtain the dual gated CT images.

4.7. For further processing of the reconstructed images (Fig. 5) use image processing software (e.g. OsiriX, Pixmeo SARL, Bernex, Switzerland).
Note: This step can also be performed using any other image processing software.

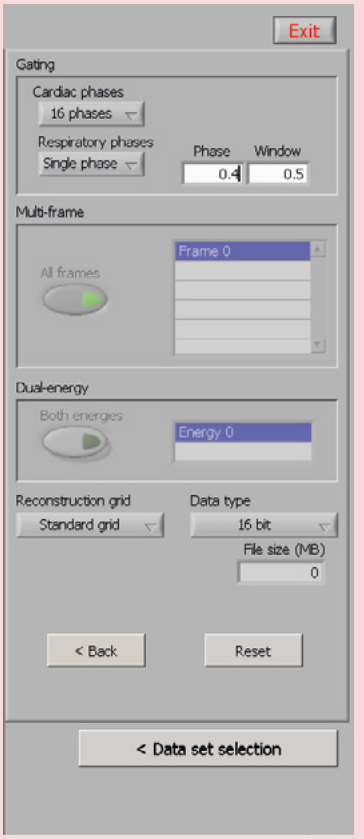

Figure 4: Screenshot of the advanced settings for selection of the gating parameters.

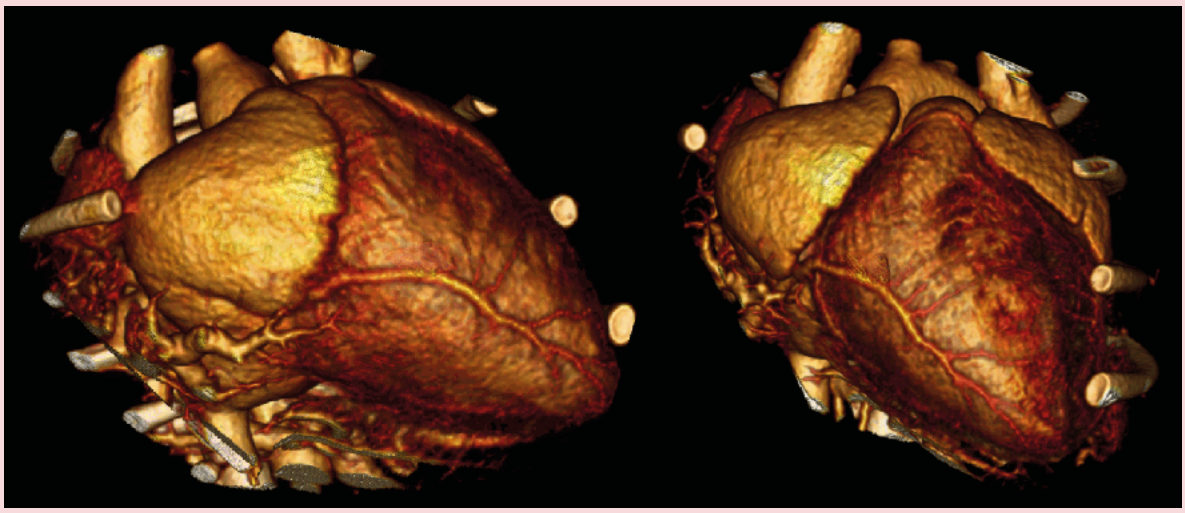

Figure 5: Cardiac-respiratory gated *in vivo* CT images of the healthy mouse heart obtained over a total scan time of ~6 min using the U-CT^{UHR} µCT system at 30 min post injection of ExiTron nano 12000. The time-resolved 4D video of the beating mouse heart can be found at *www.milabs.com/image-gallery/#PreclinicalCTGallery.*

Conclusion

We herein provide a 4D cardiac-respiratory gated CT imaging method to effectively perform *in vivo* cardiac imaging in mice. By a combination of the U-CT^{UHR} µCT system and the contrast agent ExiTron nano 12000, exceptionally high quality *in vivo* CT images of the beating mouse heart can be obtained at high speed and at low radiation dose. Thus, the implemented technique allows rapid evaluation of important cardiac structural and functional parameters *in vivo* without the need for invasive procedures. This method can be further extended to animal models of cardiovascular disease, enabling detection of pathophysiological changes thereby allowing for disease diagnosis as well as monitoring of therapeutic interventions.

References

1. Zaragoza, C. *et al.* (2011) Animal models of cardiovascular diseases. J Biomed Biotechnol. 2011: 497841.
2. Ritman, E.L. (2011) Current Status of Developments and Applications of Micro-CT. Annual Review of Biomedical Engineering. 13: 531–552.
3. Clark, D.P. *et al.* (2014) Micro-CT of rodents: State-of-the-art and future perspectives. Physica Medica. 30: 619–634.

MILabs
Making Molecular Imaging Clear

***MILabs* B.V.**
Heidelberglaan 100 STR 4.105
NL 3584 CX Utrecht, the Netherlands
Phone: +31 88 756 5343
Fax: +31 88 756 0094
Email: info@milabs.com

MILabs products are used in more than 65 countries and are supported by a global network of distributors and factory trained support engineers outside Europe and North America.

Please contact *MILabs* directly for more information about your local *MILabs* business partner.

viscover
powered by
nanoPET

***nanoPET Pharma* GmbH**
Robert-Koch-Platz 4
10115 Berlin, Germany
Phone: +49 30 890 49 74 - 0
Fax: +49 30 890 49 74 - 99
Email: imaging@viscover.berlin

nanoPET Pharma GmbH provides products and services worldwide. Visit **www.viscover.berlin** to find your nearest sales contact.