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-CTUHR μCT system and the contrast agent ExiTron nano 12000, exceptionally high quality in vivo imaging 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.

**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-CTUHR μCT system and the contrast agent ExiTron nano 12000, exceptionally high quality in vivo imaging 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**

4. Zaragoza, C. et al. (2011) 4D cardiac-respiratory gated CT images of the healthy mouse heart obtained over a total scan time of ~6 min using the U-CTUHR μCT system at ~300 breaths/min post-injection of ExiTron nano 12000. The time-averaged 4D video of the beating mouse heart can be found at www.milabs.com/image-gallery/In-vivo-4D-CT.

**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. 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-CTUHR μCT system combined with the CT contrast agent ExiTron 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 currently CT contrast agents are rapidly cleared from the blood pool of small animals, these agents are not optimal for small animal imaging. 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. Despite these challenges, we herein report a feasible method using the U-CTUHR μCT system combined with the CT contrast agent ExiTron nano 12000 to effectively visualize 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 set 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 maintained at concentrations of up to 4% in the induction phase and 1-2% for the maintenance of anesthesia.

1.2. Place the mouse in supine position on the scanner.

1.3. Maintain anesthesia using the gas cone of the μCT scanner and allow for full recovery from anesthesia.

2. Contrast Agent Injection

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

2.2. Dissolve the system with 70% ethanol. Let septum dry.

2.3. Withdraw the required volume (5µL body weight) of the contrast agent using a sterile low dead space syringe equipped with a sterile needle (25G-32G).

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.

3. CT Imaging

3.1. Place the mouse in supine position on the temperature-regulated animal bed of the μCT nanomorphology and imaging system (μCT).

3.2. Maintain anesthesia using the gas cone of the animal bed (1-1.5% 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 μCT nanomorphology and imaging 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 intervals: 1.2-2.5 kVp
- Number of scans: 1
- Step angle: 0.75°
- Projection per scan: 16
- Binning: 2x2
- Total scan time: 00:06:12

3.7. Acquire CT local 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 areas of the heart lie outside the region of the heart, 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 contrast agent injection 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. 4h 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.

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 15 µm and 40 µm, depending on the desired image quality.

4.3. Select the anatomical region for reconstruction by designing the first and last transverse slices (blue lines), as well as the previous and next slices (pink lines).

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, Ferretis SARL, Bernex, Switzerland).

Note: This step can also be performed using any other image processing software.
Protocol

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

1. Animal Preparation

1.1. Anesthetize the animal by inhalation of isoflurane in an air-oxygen mixture saturated with concentrations of up to 4% in the induction phase and 1-2% for the maintenance of anesthesia. 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.

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

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

1.4. 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 dorsomedial and ventrolateral as far as possible so that during imaging the ECG leads are outside the cardiac field of view (FOV).

1.5. Adjust the threshold for both the respiratory signal and the cardiac ECG signal for correct triggering.

1.6. Set the scan acquisition settings by selecting the following parameters from the drop-down menus of the U-CTUHR central software window (Fig. 1):
- Acquisition window: 10
- Magnification: Ultra focus
- Scan mode: Gated
- Number of scans: 1
- Tube current: 0.24 mA
- Tube voltage: 50 kV
- Step angle: 0.750°
- Projections per step: 16
- Step time: 20 ms
- Total scan time: 00:06:12
- Energy: Single

2. Contract Agent Injection

2.1. Anesthetize the animal by inhalation of isoflurane in an air-oxygen mixture saturated with concentrations of up to 4% in the induction phase and 1-2% for the maintenance of anesthesia.

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.

3. CT Imaging

3.1. Place the mouse in supine position on the temperature-regulated animal bed of the U-CTUHR 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. Acquire CT scout view of the animal.

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

3.6. Select the anatomical region for reconstruction by positioning of the FOV (blue lines) so that it encompasses the entire heart. If any ECG leads lie outside the region of the heart, adjust the level of anesthesia. If necessary, adjust the animal’s paws so that the ECG leads lie outside the region of the heart.

3.7. Acquire 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 any ECG leads lie outside the region of the heart, adjust the level of anesthesia.

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 approximately 4 h in mice.

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. Acquire CT scout view of the animal.

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 a voxel size between 10 µm and 40 µm, depending on the desired image quality.

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

4.7. For further processing of the reconstructed images (Fig. 3) use image processing software (e.g. OsiriX, thermometer SAB, Bernex, Switzerland). Note: This step can also be performed using any other image processing software.
Protocol

All animal work in this study was approved by the local committee on animal welfare and was in accordance with the guidelines set out 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 at a concentration 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.

1.3. Withdraw the required volume (25 µL) of the contrast agent using a sterile low dead space syringe equipped with a sterile needle (27G–30G).

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

1.5. Set the scan acquisition settings by selecting the following parameters from the drop-down menus of the U-CTUHR control software window (Fig. 1):

- Magnification: Ultra focus
- Scan mode: Gated
- Energy: Single
- Settings: Default
- Scan mode: Gated

2. Contract 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 (15 µL 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.

3. CT Imaging

3.1. Place the mouse in supine position on the temperature-regulated animal bed of the μCT scanner.

3.2. Maintain anesthesia using the gas cone of the anesthesia unit and allow for full recovery from anesthesia in a recovery box.

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-CTUHR control software window (Fig. 1):

- Number of scans: 1
- Tube voltage: 50 kV
- Tube current: 0.24 mA
- Step angle: 0.750°
- Projections per step: 16
- Binning: 2x2 pixels
- Total scan time: 00:06:12
- Exposition time: 20 ms
- Energy: Single
- Tube current: 0.2 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 any of the ECG leads lie outside the region of the heart, adjust the animal’s position so that the ECG leads lie outside the region of the heart.

3.9. Start the data acquisition within one hour after contrast agent injection. Note: In the default-setting mode, the settings are automatically as follows:

- Total scan time: 00:06:12
- Binning: 2x2 pixels
- Projections per step: 16
- Step angle: 0.750°
- Number of scans: 1
- Tube voltage: 50 kV
- Tube current: 0.24 mA
- Exposition time: 20 ms
- Energy: Single
- Tube current: 0.2 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

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

4. Image Processing

4.1. For image processing, load the obtained dataset on the reconstruction PC.

4.2. Using the default reconstruction settings (Fig. 2), select a voxel size between 15 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 as well as the previous (green line) and next (blue line) slice.

4.4. Using the slice preview, position the yellow bounding box on the transverse image (Fig. 5).

4.5. 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 other image processing software.
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-CTUHR μCT system and the contrast agent ExiTron nano 12000, exceptionally high quality in vivo imaging 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 diseases, enabling detection of pathophysiological changes thereby allowing for disease diagnosis as well as monitoring of therapeutic interventions.

**Conclusion**

In vivo cardiac imaging in mice has shown to facilitate the study of human cardiovascular diseases, as well as the development of potential treatment strategies. Nonetheless, 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-CTUHR μCT system combined with the CT contrast agent ExiTron-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 classical CT contrast agents are rapidly cleared from the blood pool of small animals, these agents are not optimal for small animal imaging. 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. These contrast agents are not optimal for small animal imaging. 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. These contrast agents are not optimal for small animal imaging, 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 diseases, enabling detection of pathophysiological changes thereby allowing for disease diagnosis as well as monitoring of therapeutic interventions.

**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. Nonetheless, 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-CTUHR μCT system combined with the CT contrast agent ExiTron-nano 12000 to effectively visualize the beating mouse heart in vivo.
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 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 pathological changes thereby allowing for disease diagnosis as well as monitoring of therapeutic interventions.

References

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. However, due to the small size of the mouse heart as well as the animal’s high cardiac and respiratory rates, mouse 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 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 noninvasive 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. 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 nano 12000 (Viscover 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 its high cardiac rate (~300 breaths/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 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. Hence, we provide a μCT method using the U-CT μCT system with intrinsic cardiac-respiratory gating combined with ExiTron nano 12000 to obtain real-time, high-resolution images of the beating mouse heart.