

## Abstract

This application note provides updated guidelines for choosing CT acquisition settings in a variety of CT imaging applications for imaging in mice. These guidelines should be treated as a starting point and adjusted accordingly for specific applications.

## Introduction

X-ray computed tomography (CT) is an established imaging technique where 2D X-ray projection images acquired around the axis of the subject are computationally reconstructed into a 3D volume image, enabling 3D visualization of the subject features. Contrast is obtained from the difference in attenuation of X-rays by different types of tissue. Commonly visualized biological tissues/components using CT include bone, soft tissue, fat, lungs, water, and air. Additionally, use of contrast agents, especially for vascular imaging applications, aids in CT visualization. Optimal visualization of each of these components requires fine tuning of the various acquisition parameters available to the CT user, which include:

- X-ray tube voltage
- X-ray tube current
- X-ray detector exposure time
- X-ray filter
- Number of projections
- Scanning mode

The InSyTe FLECT/CT is equipped with a CT subsystem that is placed inline with the FLECT subsystem. In addition to providing anatomic reference for FLECT, the CT subsystem was designed for use as a standalone CT system. Specifications of the CT subsystem:

<b>X-ray tube voltage range</b>	30-50 kVp
<b>X-ray tube maximum current</b>	1000 $\mu$ A (1 mA)
<b>X-ray filters</b>	Al 1 mm, Al 2 mm, Sn, Mo
<b>Scanning modes</b>	Continuous, Step & Shoot

Optimization of these parameters is necessary for optimal imaging of tissues of interest. In this application note, acquisition setting recommendations are presented for different types of imaging applications. These include soft tissue in vivo imaging, contrast enhanced imaging, specimen imaging, and fast imaging. These settings are given as a starting point guideline and are intended to be adjusted for specific applications.

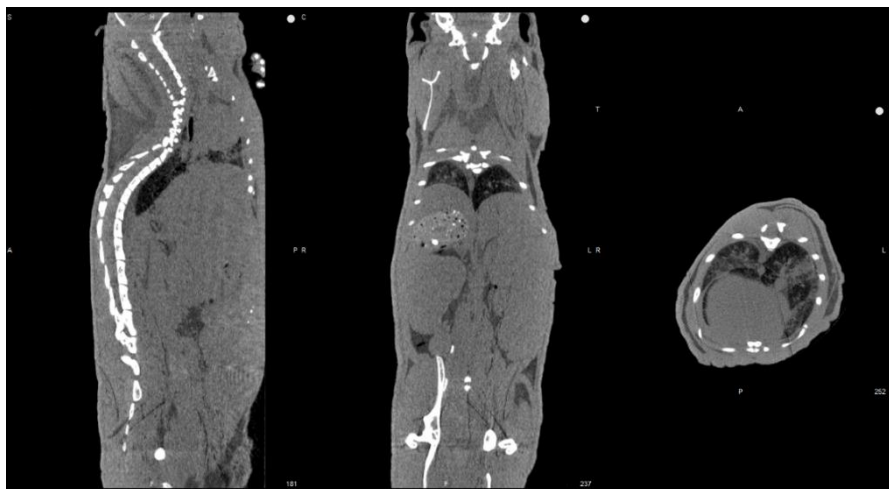
## Soft tissue in vivo imaging

The X-ray tube energy range on the InSyTe FLECT/CT is optimized for soft tissue imaging in small animals. CT images acquired can be used to provide anatomic reference for FLECT scans and to be used in final fused FLECT and CT images.

The X-ray tube voltage should be carefully considered based on the mouse mass and may require trial and error to optimize, as the tube voltage determines the X-ray energy spectrum (Bremsstrahlung), which affects soft tissue contrast. Typical settings that give excellent contrast between bone and soft tissue are:

Parameter	Setting
X-ray tube voltage	<25 g mouse: 30 kV 25-30g mouse: 30-35 kV 30-35g mouse: 35-40 kV >35g mouse: 40-45 kV Rats: 45-50 kV
X-ray tube current × detector exposure time (mAs)	0.1 mAs
>> Example X-ray tube current	1000 $\mu$ A
>> Example X-ray detector exposure time	100 ms
X-ray filter	None
Number of projections	360
Scanning mode	Continuous
Reconstruction voxel size	75 $\mu$ m

This set of acquisition parameters provides a balance between imaging speed and acquisition of sufficient data for soft tissue contrast. The recommended 75  $\mu$ m reconstruction voxel size will result in a large file size (75 mm FOV, > 1 GB reconstructed image). This is an important consideration since large CT image file sizes can be difficult and time consuming to manipulate in VivoQuant. A workaround for this is to use a larger reconstruction voxel, such as 100 or 150  $\mu$ m, to reduce the file size at the expense of resolution.

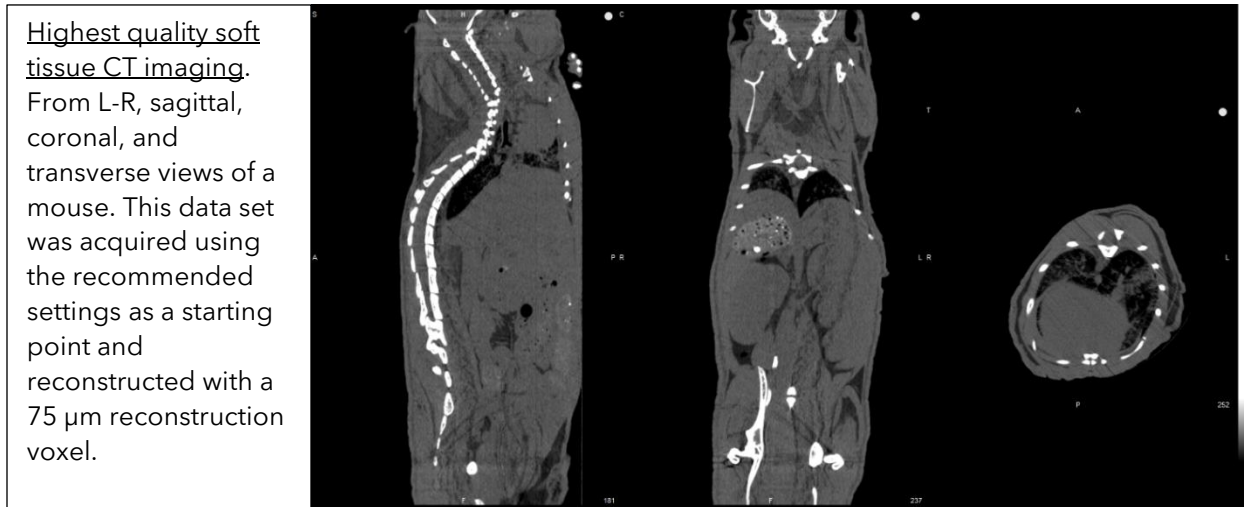


Soft Tissue CT imaging. From L-R, sagittal, coronal, and transverse views of a mouse. This data set was acquired with the recommended settings listed above and reconstructed with a 75  $\mu$ m reconstruction voxel.

For highest quality soft tissue imaging, additional projections can be acquired to enhance image clarity and resolution. Note that the scan times will be long due to the increased projection angles (720) and that the X-ray dosage on the mouse will be high:

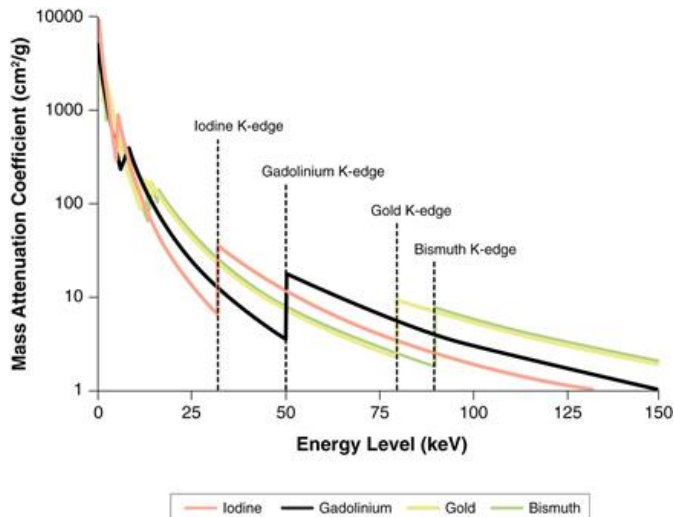
Parameter	Setting
X-ray tube voltage	<25 g mouse: 30 kV 25-30g mouse: 30-35 kV 30-35g mouse: 35-40 kV >35g mouse: 40-45 kV Rats: 45-50 kV
X-ray tube current × detector exposure time (mAs)	0.25 mAs
>> Example X-ray tube current	1000 $\mu$ A
>> Example X-ray detector exposure time	250 ms
X-ray filter	Al 1 mm or Al 2 mm
Number of projections	720
Scanning mode	Continuous
Reconstruction voxel size	75 $\mu$ m

The selection of the Al filter provides general filtering of the X-ray beam and reduces the delivered X-ray dosage. Depending on the tube voltage selected, however, it may be necessary to use the 2 mm Al filter to avoid saturating the X-ray detector. Selection of the 75  $\mu$ m reconstruction voxel and 720 projection angles are recommended for best image quality.



## Contrast enhanced imaging

Since the X-ray tube energy range on the InSyTe FLECT/CT is optimized for soft tissue imaging in small animals, contrast agents, such as iodine and gold nanoparticles, work very well with the low energy X-rays produced by the InSyTe. This is due to the high attenuation of low energy X-rays by these contrast agents.



Contrast enhanced CT imaging. The plot shows the typical K-edge attenuation energies for different CT contrast agents.

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CT settings used for soft tissue imaging also work very well for contrast agents. Typical settings are (from above):

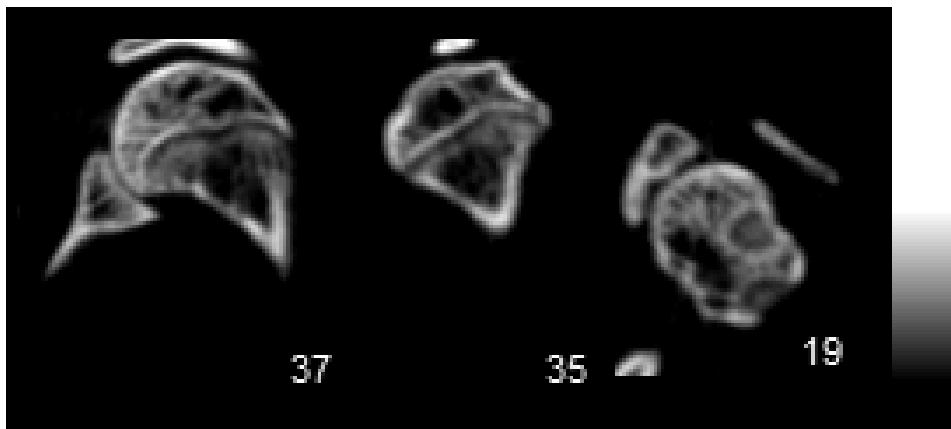
Parameter	Setting
X-ray tube voltage	<25 g mouse: 30 kV 25-30g mouse: 30-35 kV 30-35g mouse: 35-40 kV >35g mouse: 40-45 kV Rats: 45-50 kV
X-ray tube current × detector exposure time (mAs)	0.1 mAs
>> Example X-ray tube current	1000 $\mu$ A
>> Example X-ray detector exposure time	100 ms
X-ray filter	None
Number of projections	360
Scanning mode	Continuous
Reconstruction voxel size	75 $\mu$ m

## Specimen imaging

This type of imaging is intended for high resolution imaging applications, such as imaging of bone specimens. These settings are not recommended for live animals as the X-ray dosage is very high and should only be limited to specimens.

Parameter	Setting
X-ray tube voltage	50 kVp
X-ray tube current × detector exposure time (mAs)	0.5 mAs
>> Example X-ray tube current	1000 $\mu$ A
>> Example X-ray detector exposure time	500 ms
X-ray filter	Al 2 mm
Number of projections	720
Scanning mode	Continuous or Step & Shoot
Reconstruction voxel size	50 $\mu$ m (or smaller)

The Al 2 mm filter reduces beam hardening effects in the final reconstructed image. Use of 720 projection angles will result in a long scan time. Additionally, the reconstruction voxel size of 50  $\mu$ m will result in a very large reconstructed file size (1-2 GB). An even smaller reconstruction voxel size of 37.5  $\mu$ m or 25  $\mu$ m can also be used, however, cannot be performed in Fluoroview and requires restricting the reconstructed field of view in the reconstruction software. This procedure, described in an application note, requires a custom set up of the reconstruction software and is only recommended for advanced users.



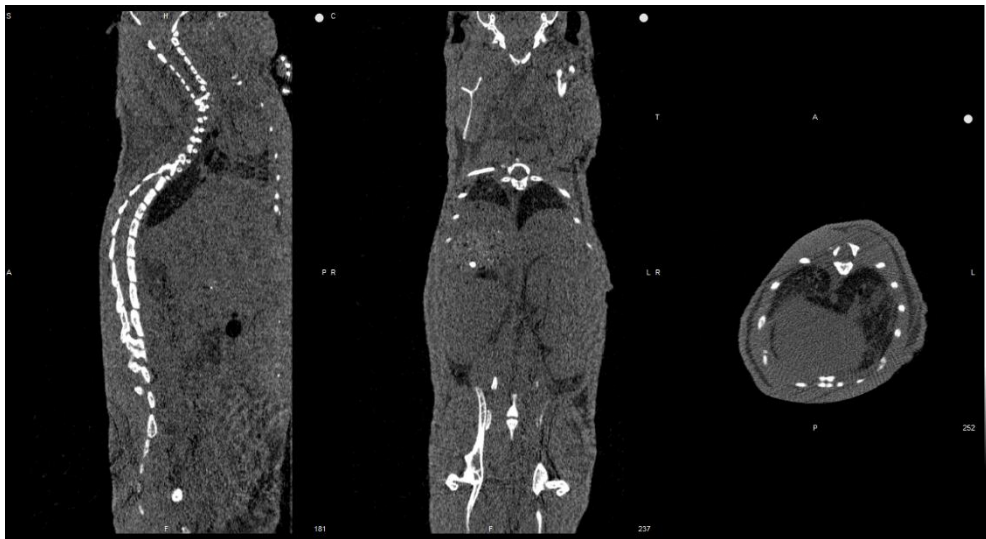
Resolution focused CT imaging. From L-R, sagittal, coronal, and transverse views of the mouse humerus. CT data of the entire mouse was acquired with the recommended settings listed above and reconstructed with a 50  $\mu$ m reconstruction voxel. Images were then cropped to a region of interest comprising the humerus.

## Low dose imaging

This set of CT acquisition parameters will enable a 75 mm FOV (the entire area of mouse) to be scanned in < 2 minutes while also minimizing X-ray dosage.

Parameter	Setting
X-ray tube voltage	30 kVp
X-ray tube current × detector exposure time (mAs)	0.02 mAs
>> Example X-ray tube current	500 $\mu$ A
>> Example X-ray detector exposure time	40 ms
X-ray filter	None
Number of projections	180
Scanning mode	Continuous
Reconstruction voxel size	75 $\mu$ m

Image quality is sacrificed for low dosage, as the short exposure time enables sufficient acquisition of scattered X-ray photons, but results in low quality contrast. This type of acquisition sequence is useful for applications where a fast CT scan is necessary, or for those applications where the CT is meant only for anatomic reference for reconstructed FLECT images. Scans can also be performed with 45 projections for an extremely fast scan (75 mm FOV, < 1 min), however, there is very poor contrast between bone and soft tissue. The X-ray tube voltage can also be increased to increase signal-to-noise, though the software will warn the user if the X-ray detector is saturated.



Low dose CT imaging. From L-R, sagittal, coronal, and transverse views of a mouse. This data set was acquired with the recommended settings listed above and reconstructed with a 75  $\mu$ m reconstruction voxel.