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2.09 High-Resolution CT for Small-Animal Imaging Research

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Glossary

Micro-CT The preclinical equivalent of CT largely used to study small-animal models of human disease.

Microfocus (x-ray source) A special type of x-ray source having focal spot size < 100 μm ,

particularly used for micro-CT imaging at high magnification.

Preclinical imaging A type of imaging performed on living animals for research purpose.

2.09.1 Introduction

High-resolution computed tomography (CT) or micro-CT is the preclinical equivalent of CT and is largely used to study small-animal models of human disease. The translation of clinical imaging methods from human to rodents presents scaling challenges for all modalities including micro-CT. For example, acquiring CT images of the mouse heart with organ definition and temporal resolution comparable to that which can be achieved in humans requires an increase in volumetric spatial resolution by a factor of about 3000 and a tenfold increase in temporal resolution (Badea et al., 2008a). During

the last decade, micro-CT has introduced viable alternatives to autoradiography and histological techniques used by basic scientists. Micro-CT has served well in disease detection and monitoring, in morphological and functional phenotyping, and in testing potential therapies and the safety of new drugs. Furthermore, micro-CT represents a test bed for the development and evaluation of novel x-ray imaging approaches. This chapter focuses on a general description of micro-CT instrumentation and shows examples of its applications in preclinical research.

A micro-CT scanner is based on the same underlying physical principles as a clinical CT scanner, but it is designed for

Figure 1 The two design geometries for micro-CT are based on rotating gantry (a) or rotating specimen (b). The effect of penumbra blurring: for the same focal spot size (fs) of the x-ray source, the penumbra blurring b is larger when the object is closer to the source (c) than when the object is closer to the detector (d). sod, source–object distance; odd, object–detector distance; sdd, source-to-detector distance.

higher-resolution imaging. It typically produces 3D tomographic data proportional to the mean linear attenuation coefficient at microscopic resolution (voxel size $\leq 100 \mu\text{m}^3$) by taking a few hundreds of 2D cone beam projections from multiple angles around the animal (Holdsworth and Thornton, 2002). The multiple projections are combined using a reconstruction method.

A typical micro-CT scanner uses an x-ray source and a high-resolution digital x-ray detector (Paulus et al., 2000, 2001), with two possible design geometries: (1) rotating gantry (x-ray source and detector) or (2) rotating specimen (see Figure 1(a) and 1(b)). The majority of current commercial systems for *in vivo* scanning use the rotating gantry geometry, that is, they are scaled versions of the clinical CT scanners (Figure 1(a)). Using this design, the x-ray source and detector, which are mounted on a gantry, rotate around a central axis, while the animal lies stationary on a table positioned between the x-ray tube and detector. For most of these systems, the source-to-object distance (sod) is less or comparable to the object-to-detector distance (odd). A geometric representation of these distances is shown in Figure 1(c) and 1(d). Positioning the specimen closer to the x-ray source and farther from the detector allows for an increase in magnification, defined as $M = 1 + \frac{\text{odd}}{\text{sod}}$, which in turn allows for an increase in resolution (provided that the system is based on the use of a small focal spot x-ray source in order to compensate for the penumbra blurring effect). The term *penumbra blurring* represents the unsharpness produced by the finite focal spot size of the x-ray source. As will be further explained in Section 2.09.4, the resolution limit due to penumbral blurring (b) in the projection plane is related to the focal spot diameter (fs), odd, and sod by eqn [1]:

$$b = \frac{\text{odd}}{\text{sod}} \text{fs} \quad [1]$$

$$= (M - 1) \text{fs}$$

Figure 1 illustrates what happens when the scanning is performed with the object closer to the source (Figure 1(c)) or to the detector (Figure 1(d) of Chapter 1.10, Figure 2). According to eqn [1], if odd and sod are similar, as in most of the rotating gantry systems, the penumbra blurring b becomes comparable or larger than the size of the focal spot and could compromise the spatial resolution, if the system uses a large focal spot tube.

For systems with a rotating specimen (Figure 1(b)), the x-ray source and detector are stationary. Such system design (mostly used for *ex vivo* imaging) is more flexible in changing the magnification by changing the position of the specimen. The specimen could also be placed closer to the detector, that is, $\text{odd} < \text{sod}$, resulting in the reduction of penumbra blurring to less than the focal spot size. In this configuration, larger focal spot tubes that provide greater x-ray fluence, and therefore enable shorter exposure times, can be used. Despite the benefits of the rotating specimen design, this type of system has not gained popularity for *in vivo* imaging primarily because the animal must be mounted in a vertical position. Combining the benefits of an object magnification and rotating gantry design, commercial systems with $\text{odd} < \text{sod}$ ($M = 1.3$) have also become available (Du et al., 2007; Ross et al., 2006). Such systems allow rapid scanning, albeit at the price of somewhat reduced spatial resolution.

2.09.2 Fundamentals of Micro-CT Design

2.09.2.1 x-Ray Detectors

As in any other x-ray imaging system, the x-ray detector is a key component of a micro-CT scanner. Differently from clinical CT, where stacked 1D arrays of detection elements on a cylindrical surface are used, the majority of micro-CT systems to date employ digital flat-surface 2D detectors leading to a cone beam scanning geometry. Apart from a single case known in the literature of high-resolution CT reconstruction of a rat kidney from the digitization of multiple film radiographs (Kujoory et al., 1980), early prototype micro-CT systems employed x-ray image intensifiers read by charged coupled devices (CCDs) (Boone et al., 1993; Feldkamp et al., 1989; Holdsworth et al., 1993). Later in the mid-1990s, combined detection systems made up of scintillator screens coupled to CCDs via fiber-optic bundles, with various demagnifying ratios, became the standard for micro-CT imaging (Goertzen et al., 2004; Paulus et al., 2000). More recently, the advances in CMOS technology led to the production of large-area detectors with high frame rates, which are the most widely used systems for *in vivo* small-animal imaging (Kalender and Kyriakou, 2007; Lee et al., 2003). Cooled CCDs are still in use, especially in applications involving low x-ray fluences, due to their very low dark noise with respect to flat-panel CMOS detectors. Also

reported in the literature are studies on the use of direct conversion detectors coupled to thin-film transistor (TFT) arrays (Goertzen et al., 2004) and of single-pixel or small-area detectors operating in photon-counting mode (Panetta et al., 2007; Paulus et al., 2000; Roessl et al., 2011) for small-animal micro-CT.

The performance parameters that must be taken into account for the selection of an x-ray detector for micro-CT may be different to those considered for other imaging systems. Among the key factors affecting the overall performance of the tomographic system are (1) spatial resolution, (2) detection efficiency, (3) dark noise, (4) stability, (5) sensitive area, (6) dynamic range, and (7) readout speed. Many of the parameters in the preceding text are dependent on each other, so it is important to know how they can influence the performance of a micro-CT system in order to select the optimal detector in the design process.

2.09.2.1.1 Spatial resolution

The main goal in micro-CT imaging is in obtaining a high-spatial resolution, so one may believe that the most desirable feature of an x-ray detector for micro-CT is the highest possible resolving power. This is in part true, but as shown later in Section 2.09.4, the influence of the detector resolving power on the overall resolving power of the micro-CT system is progressively less important for increasing values of M . For tabletop specimen micro-CT and nano-CT scanners working at magnification $M > 10$, the resolving power of the tomographic system is dominated by the penumbra effect. Conversely, most systems for *in vivo* imaging work with low-to-medium magnifications in the range $1.1 < M < 2$, and hence the spatial resolution of reconstructed images strongly depends on the resolving power of the detector. The spatial frequency at 10% of the presampling modulation transfer function (MTF) of most indirect detectors used for *in vivo* micro-CT imaging varies in the range of 5–10 lp mm⁻¹, depending on the scintillator type and thickness. For such detectors, the pixel size falls in the range of 50–120 μ m. Mainly thallium-doped cesium iodide (CsI:Tl) and gadolinium oxysulfide are used as scintillators for this kind of application. CsI:Tl screens with thickness of about 150–200 μ m are shown to be a good compromise between efficiency and spatial resolution because of columnar-grown structure that reduces the spread of the visible light that is generated at the site of interaction of the x-ray photon. On the other hand, the high atomic number of gadolinium allows drastic reduction in the thickness of the scintillator layer (of a factor of 3 or more) and thus the light spreading while keeping comparable detection efficiency with respect to CsI. Detectors that do not use scintillator (i.e., direct conversion detectors) mostly based on amorphous selenium (a-Se) (Goertzen et al., 2004) or amorphous silicon (a-Si) (Yang et al., 2010) on top of TFT arrays show excellent performance in terms of resolving power, with MTFs very close to the ideal sinc-shaped function for the detector sampling pitch. Similar spatial resolution performances have been observed for direct conversion detectors operating in photon-counting mode (Belcarì et al., 2007; Panetta et al., 2007).

2.09.2.1.2 Image noise

As in any other CT system, the image noise in micro-CT images for a given dose and scanning geometry is mainly determined by the efficiency of the x-ray detector. The detective quantum

efficiency (DQE) does not seem to be a good predictor of the image noise level in micro-CT images. For example, not only direct conversion detectors show DQE at zero frequency as high as 60%, which is roughly twice that of most scintillator-based detectors, but they also produce tomographic images with higher noise levels (Goertzen et al., 2004; Panetta et al., 2007). This can be explained by considering that direct conversion detectors usually have higher values of MTF at Nyquist frequency, leading to a higher transfer of high-spatial-frequency quantum noise to the final images. In other words, the blurring introduced by scintillators in indirect conversion detectors acts as a low-pass filter that wipes out some of the noise from the reconstructed images, at the cost of a reduced high-contrast spatial resolution. For the reason in the preceding text, image noise in tomographic systems should only be compared after having adequately smoothed the data in order to match the MTFs of the systems under comparison. In this sense, there is no direct evidence in the literature of the superiority of direct conversion detectors with respect to scintillator-based detectors for small-animal imaging. For applications characterized by high x-ray fluences, such as *in vivo* small-animal imaging, the quantum noise due to the fluctuation in x-ray counting statistics dominates over the thermal noise. CMOS detectors are highly indicated for such applications, especially because of their higher readout speed with respect to most CCDs, thus allowing faster scanning. For very high-resolution micro-CT and nano-CT, working with very low-power x-ray sources and long source-to-detector distances, the x-ray fluence on the detector is so low that the integrated signal due to thermal noise during the (very long) acquisition time can be comparable or higher than the signal due to the x-rays. In such cases, CMOS detectors do not show good performance because of their higher dark signal compared to CCDs. Cooled CCDs and intensified CCDs are better suited for this application (Jorgensen et al., 1998; Sasov and Van Dyck, 1998).

2.09.2.1.3 Stability

A tomographic acquisition involves hundreds of consecutive x-ray projection of the same object at different angles. The overall quality of the reconstructed tomographic image requires that both the x-ray beam and the detector response remain stable over the duration of the entire acquisition. If this condition is not met, independent correction of the raw projection data should be made for each angle (or, at least, several times during the same scan), which could lead to an unacceptable increase of the scanning time. Flat-panel imagers with a-Si TFT readout can have pronounced image lag and ghosting that can considerably affect the quality of tomographic images if not corrected (Bloomquist et al., 2006; Siewerdsen and Jaffray, 1999). Non-negligible drifts of the dark signal over time have been observed for the same type of detectors.

2.09.2.1.4 Sensitive area

As mentioned in the previous sections, most micro-CT scanners for *in vivo* imaging of small animals work with low-magnification factors. This is due in part to field-of-view (FoV) considerations; in fact, it is easy to show that, for given width and height of the selected detector, the FoV size of a cone beam CT system (both axial and transaxial) varies as $1/M$. Whole-body imaging of mice or rats is feasible with a single,

circular orbit scan by using flat-panel detectors with transverse size of 5–10 cm and axial size of 10–20 cm. CCD-, CMOS-, or TFT-based detectors of such size or even larger are widely available on the market from several manufacturers. An advantage of CMOS and TFT detectors over CCDs is that these latter are commonly used with demagnifying fiber-optic tapers to increase the sensitive area, thus leading to geometric distortions that must be accurately corrected prior to image reconstruction to avoid artifacts (Lee et al., 2003).

2.09.2.1.5 Dynamic range

As shown by Grodzins (1983), optimal x-ray energies for imaging of small samples are considerably lower than those used for clinical imaging. Consequently, the photoelectric effect ($\propto Z^4/E^3$) dominates over Compton scattering ($\propto Z/E$) as the main interaction mechanism in the attenuation of the x-ray beam. This results in wide range of exposure levels across the detector area, even for small-sized samples, which calls for detectors with high dynamic range. Clinical CT scanners usually employ bow tie-shaped filters to compensate for such high differences of exposure levels; there is no evidence on the use of similar filters in the field of small-animal micro-CT, although there is some initial study on the use of bow tie filters in clinical flat-panel-based cone beam CT (Lee et al., 2003).

2.09.2.1.6 Readout speed

The need for high-speed x-ray detectors in micro-CT imaging of small animals is motivated by (1) the reduction in scan time per animal, thus reducing the time window of observation of biological processes that can vary in time, especially when using contrast agents with fast kinetics, and (2) the need to increase the imaging throughput. Furthermore, continuous rotational acquisition with high frame rates (>20 fps) is required for retrospectively gated respiratory and cardiac micro-CT imaging.

2.09.2.2 x-Ray Sources

The choice of the x-ray source can strongly affect the resulting performance of the resulting micro-CT system. Due to the tradeoff between focal spot size and maximum filament current, most x-ray tubes with minifocus or microfocus have very low photon output compared to the high-power tubes used in clinical scanners. This fact, together with the often lower quantum efficiency of the x-ray detectors and the very narrow solid angle with which the source sees each detector pixel in systems working at high magnification, leads to a dramatic increase in the average scan time required in micro-CT with respect to clinical CT to obtain similar image noise levels.

Among the different types of x-ray sources for micro-CT found in the literature, the most used are microfocus, fixed tungsten anode tubes operating in continuous mode, with voltages in the range of 20–100 kV and anode current in the range of 50–1000 μ A. *In vivo* micro-CT systems working at low magnification often use pulsed x-ray sources with higher power and wider focal spots, in the range of 0.3–0.6 mm. The use of pulsed x-ray sources is especially important for prospective ECG gating (Badea et al., 2008b). This type of imaging can be done also with sources operating in continuous mode by using external shutters with open time windows >30 ms; nevertheless, the total number of photons emitted in each pulse is very

low in this second case, and the x-ray transmission through the closed shutter must be taken into account for proper raw data calibration. More recently, Cao et al. have investigated the use of a compact field emission microfocus x-ray source based on carbon nanotube (Cao et al., 2009, 2010). In this type of source, the metal filament cathode is replaced by a field emission cathode that is capable of emitting electrons at room temperature with voltage-controlled output current (Yue et al., 2002). The authors obtained stable emission of x-rays at 50 kV and 1 mA, with pulse length down to 20 ms and with a focal spot size of 100 μ m. Despite the improvement of a factor of 5–6 in focal spot size with respect to standard pulsed x-ray sources, the overall performance of the resulting micro-CT scanner in terms of spatial resolution (5 lp mm⁻¹ at 10% MTF) is comparable to that of other systems for *in vivo* imaging because of the low magnification used.

For *ex vivo* imaging of small biological samples at very high magnification, the need for very small focus is more stringent because it represents the main limiting factor of the spatial resolution. Reflection anode x-ray tubes with focal spots in the range of 5–20 μ m are available on the market. Because of the very high power density that such narrow electron beams can release on the target surface, the anode current must be kept at very low values to prevent its melting (Grider et al., 1986). For this reason, maximum anode currents in most microfocus x-ray tubes are in the order of 100–200 μ A, with maximum power <10 W. A well-known empirical formula for determining the maximum power of a microfocus source for the most common target materials is

$$P_{\max} = 1.4w^{0.88} \quad [2]$$

where $P_{\max}$ is the maximum power in watts and w is the focal spot size in micron (Flynn et al., 1994). Other drawbacks of reflection-type x-ray sources with thick target include a relatively narrow aperture of the cone beam (in the range of 30°–60°) and a limited capability of reducing the focus-to-object distance below few millimeters. Yet, the nonuniformity of the beam intensity due to the heel effect must be taken into account. Open-type sources with thin-target transmission anode overcome these limitations, providing radiation beams with angular aperture up to 140°–160° with the possibility of putting the object virtually in contact with the focus. The spatial resolution obtainable with this type of source can be less than 1 μ m, which is in the same order of magnitude as those obtainable with synchrotron radiation. Nevertheless, synchrotron-based micro-CT and nano-CT still remain unbeaten in terms of contrast resolution and signal-to-noise ratio over laboratory-level micro- and nano-CT systems because of the possibility of having adjustable monochromatic radiation and thus avoiding beam hardening artifacts (Brunke et al., 2008; Ritman et al., 1997; Stolz et al., 2011).

2.09.2.3 Choice of the Scanning Geometry

2.09.2.3.1 FoV and voxel size

The size and shape of FoV of a micro-CT scanner in cone beam geometry with in-axis detector depend on several variables such as the width and height of the x-ray detector, the magnification, and the source-to-detector distance. Simple

geometric calculations can be used to derive the relationship between the quantities in the preceding text. Depending on the design goal, such equations can be used to appropriately select the x-ray detector and to optimize the scanner geometry to match the size of a given specimen (it being rabbit, rat, mouse, zebra fish, insect, or a small sample on the tip of a needle), by maximizing the spatial resolution (i.e., maximizing the magnification) or by maximizing the scanner sensitivity (i.e., by minimizing the source–detector distance).

The geometry of the micro-CT scanner is shown in **Figure 2**. The figure shows the typical diamond shape of the FoV of a CBCT scanner, given by the intersection of all pyramid-shaped radiation beams at all projection angles of the tomographic acquisition. The case shown in **Figure 2** also highlights a typical situation that may arise at low magnification, where three different FoV radii are distinguished: the ‘virtual’ radius, R_{vir} ; the maximum radius, R_{max} ; and the object radius, R_{obj} . The virtual FoV has a radius that is only dependent on the transaxial size of the detector, but because of the presence of the detector itself, an object having a radius equal than R_{vir} would not fit physically into the imaging system. The maximum radius of an object that can fit in the imaging system without mechanical interference with the detector is R_{max} , but it is always advisable to allow some margin (dependent on the animal being imaged) that leads to $R_{\text{obj}} = R_{\text{max}} - D_{\text{min}}$, where

D_{min} is the minimum distance between the object and the detector plane. It must be observed that for most flat-panel x-ray detector, D_{min} is also constrained by the mechanical envelope that protects the scintillator from external light and scratches. Some definitions used throughout the next section are reported in the succeeding text:

sdd Source-to-detector distance.

aor Axis of rotation. Conventionally, aor coincides with the z-axis.

sod Source-to-object (or source-to-aor) distance.

H_{det} Height of the active area of the detector (parallel to the aor).

W_{det} Width of the active area of the detector (perpendicular to the aor).

M Magnification, $M = \text{sdd}/\text{sod}$.

D_{min} Minimum distance between the scanned object and the detector plane during the rotational scanning.

Figure 2 The FoV of a micro-CT scanner working in cone beam geometry (gray-shaded volume) is given by the intersection of all the pyramid-shaped radiation beams coming from all the projection angles. The dependence of its size and shape on the geometric parameters of the scanner can be derived from simple geometric calculations (see text for details).

R_{obj} Max. radius of the scanned object, taking into account D_{min} . This is the final FoV radius considered as the design target.

R_{max} Max. radius of the FoV allowed by the scanner geometry. It must be $R_{\text{max}} > R_{\text{obj}}$.

R_{vir} Radius of the virtual FoV. At low magnification and for large W_{det} it is possible to have $R_{\text{vir}} > R_{\text{max}}$.

$H_{\text{obj},1}$ Max. height of the FoV, along the aor.

$H_{\text{obj},2}$ Height of the FoV at $R = R_{\text{obj}}$, that is, height of the cylindrical part of the FoV. $H_{\text{obj},1} > H_{\text{obj},2}$.

α_{tube} Maximum angular aperture of the x-ray cone beam.

α_{ax} Axial angular aperture (along the aor) of the cone beam as seen by the detector. It must be $\alpha_{\text{ax}} < \alpha_{\text{tube}}$.

α_{tra} Transaxial angular aperture (perpendicular to the aor) of the cone beam as seen by the detector. It must be $\alpha_{\text{tra}} < \alpha_{\text{tube}}$.

It is easy to show that the following equations apply to the FoV on the central transaxial plane:

$$\begin{cases} R_{\text{vir}} = \frac{\text{sod}}{\sqrt{1 + 4 \left(\frac{\text{sdd}}{W_{\text{det}}} \right)^2}} = \text{sod} \cdot \sin \left(\frac{\alpha_{\text{tra}}}{2} \right) \\ R_{\text{vir}} \geq R_{\text{max}} \geq R_{\text{obj}} \\ R_{\text{max}} = R_{\text{vir}} \Leftrightarrow \text{sdd} - \text{sod} > R_{\text{vir}} \end{cases} \quad [3]$$

Given the radius R_{obj} of the FoV in the transaxial plane, the maximum and minimum longitudinal lengths (denoted as $H_{\text{obj},1}$ and $H_{\text{obj},2}$) are given by

$$\begin{cases} H_{\text{obj},1} = H_{\text{det}} \frac{\text{sod}}{\text{sdd}} = \frac{H_{\text{det}}}{M} \\ H_{\text{obj},2} = H_{\text{det}} \frac{\text{sod} - R_{\text{obj}}}{\text{sdd}} \end{cases} \quad [4]$$

Conversely, if the design target is the longitudinal size of the FoV, we can compute the distances between the different components by solving the equations in the preceding text with respect to sdd and sod, thus obtaining

$$\begin{cases} \text{sod} = R_{\text{obj}} + \frac{2R_{\text{obj}} + D_{\text{min}}}{\frac{H_{\text{det}}}{H_{\text{obj},2}} - 1} \\ \text{sdd} = (2R_{\text{obj}} + D_{\text{min}}) \left(1 + \frac{1}{\frac{H_{\text{det}}}{H_{\text{obj},2}} - 1} \right) \end{cases} \quad [5]$$

Once the system geometry has been set up, the 'natural' voxel size Δx is just obtained by dividing the detector pitch Δu by the magnification M (Kak and Slaney, 1988):

$$\Delta x = \frac{\Delta u}{M} \quad [6]$$

It is worth noting that the equation in the preceding text does not pose a constraint on the choice of the reconstruction grid sampling step, as both finer and coarser voxels can be obtained by interpolating or rebinning the projection data, respectively. Of course, reconstructing on a finer grid will not lead to an improvement of the spatial resolution of the tomographic system that is only dependent on the presampling MTF of the detector and on the x-ray focal spot size, for a given M . On the other hand, rebinning of the projection data will produce smoother images at the cost of a reduced spatial resolution.

2.09.2.3.2 Sensitivity and temporal resolution

The photon fluence at a given point of the space coming from an x-ray tube follows with good approximation an inverse square law. In other words, neglecting the contribution of scattered radiation from the object the number of x-ray photons per unit surface and in the unit time on the detector plane along the cone beam axis is proportional to $1/\text{sdd}^2$. Hence, by halving the sdd, we can gather on the detector roughly the same number of photons in a quarter of the time. From the considerations earlier, it can be argued that the system's relative sensitivity can be improved by minimizing the source-to-detector distance. This improvement can be translated in an improved of the temporal resolution of the micro-CT scanner: the smaller the sdd, the shorter will be the integration time per projection in the tomographic acquisition required to detect an adequate number of photons on each detector element.

The lower limit of the sdd in a micro-CT scanner for small animals not only is set by the physical dimension of the object under study but also depends on the angular aperture of the cone beam produced by the x-ray source. In fact, eqns [3] and [4] are valid as long as the detector is fully covered by the radiation beam. Denoting by D_{rad} the diameter of the beam cross section at the detection plane, it must be (see Figure 3)

Figure 3 In order to compute the effective size of the FoV using eqns [3] and [4], the cone beam cross section on the projection plane fully covers the x-ray detector. The minimum value of sdd is obtained when D_{rad} is equal to the diagonal of the detector.

$$D_{\text{rad}} \geq \sqrt{W_{\text{det}}^2 + H_{\text{det}}^2} \quad (6)$$

so that the minimum sdd for which the detector is fully irradiated by the x-ray beam is

$$\text{sdd}_{\text{min}} = \frac{\sqrt{W_{\text{det}}^2 + H_{\text{det}}^2}}{2 \tan\left(\frac{\alpha_{\text{tube}}}{2}\right)} \quad (7)$$

where α_{tube} is the angular aperture of the cone beam. In practice, the unused radiation (overbeam) falling outside the active area of the detector is shielded by primary collimators at the exit window of the x-ray tube, thus reducing the unnecessary radiation exposure to the animal as well as part of the scattered radiation that may reduce the image quality (IQ).

2.09.2.4 Data Workflow

A typical experimental setup for micro-CT imaging is depicted in Figure 4. The image refers to the rotating specimen configuration, but the data workflow is very similar to the case of rotating gantry scanners. A personal computer (PC) is used to control the x-ray tube, to configure and grab data from x-ray detector, and to drive the rotating stage. The raw projection data are stored in the PC, where they must be preprocessed prior to image reconstruction. The preprocessing consists in (a) the subtraction of the offset due to the dark current, denoted in the preceding text by I^{dark} , and (b) a gain correction for the nonuniformity of the detector response measured by flat-field images, I^{flat} . The preprocessed (corrected) projection image, I' , is obtained from the uncorrected image I using the following equation:

$$I'_{ij} = I'_0 \frac{I_{ij} - \langle I_{ij}^{\text{dark}} \rangle}{\langle I_{ij}^{\text{flat}} \rangle - \langle I_{ij}^{\text{dark}} \rangle} \quad (8)$$

for each detector pixel ij , with $i=1, \dots, M$ and $j=1, \dots, N$. The operator $\langle \rangle$ denotes pixel-wise averaging of images, and the constant I'_0 is a normalization factor given by the mean value of all the pixels of the average dark-subtracted flat-field image:

$$I'_0 = \frac{1}{MN} \sum_{i=1}^M \sum_{j=1}^N \langle I_{ij}^{\text{flat}} - \langle I_{ij}^{\text{dark}} \rangle \rangle \quad (9)$$

The acquisition of I^{dark} and I^{flat} should be performed periodically in order to ensure proper IQ and to reduce artifacts. Subsequently, the corrected projection images of each projection angle are log-transformed to give a set of line integrals of the object function μ :

$$P_{ij} = -\ln \frac{I'_{ij}}{I'_0} \quad (10)$$

The set of log-transformed projection images, also referred to as the divergent beam x-ray transform of μ (Hamaker et al., 1980; Natterer, 2001), can be sent as input of the tomographic reconstruction algorithm as explained in the succeeding text in Section 2.09.3. This is generally done on a dedicated workstation because of the high computational complexity of this task. As an example, the reconstruction of a volume of 1024^3 voxels at 16-bit per voxel (2 GB) from 720 projections of 1024^2 pixels (1.4 GB) will require an amount of 3.4 GB of data that must fit into the random access memory of the workstation in order to perform the reconstruction in reasonable times. The corrected projection data can also be analyzed prior to reconstruction in order to accurately measure the misalignment parameter of the system, as explained in the succeeding text in Section 2.09.4. These geometric parameters are then incorporated into the reconstruction algorithm to produce tomographic images free from misalignment artifacts.

Figure 4 Schematic drawing illustrating the main components of a typical experimental setup for rotating specimen benchtop micro-CT imaging. The raw projection images are grabbed and stored in a PC and then are preprocessed to correct for dark signal (offset correction) and detector nonuniformity (gain correction). Finally, the preprocessed projection images are sent as input to a reconstruction program that computes the volumetric image.

2.09.3 Reconstruction Algorithms

Any CT image reconstruction solves an inverse problem and essentially involves the mapping, via a reconstruction algorithm, of cone beam projection data into a 3D matrix corresponding to the tomographic representation of the specimen (Paulus et al., 2001). There are two major types of algorithms: (1) filtered backprojection (FBP)-based algorithms and (2) iterative algorithms (Kak and Slaney, 1988). In the category of iterative algorithms, we also encounter statistical algorithms. The most commonly used reconstruction algorithms in CT and micro-CT are based on FBP. In FBP, the acquired 2D cone beam projections are filtered, using a convolution kernel that reduces the blurring inherent to the backprojection process, and then are backprojected (i.e., smeared back) through the object space at the appropriate angle to generate an image. While first developed for 2D imaging (parallel or fan beam), convolution backprojection was adapted by Feldkamp et al. (1984) to compensate for the cone beam geometry. This method forms the basis for the reconstruction algorithms used in most current micro-CT systems. While Feldkamp's algorithm is considered to be an approximation, since the circular sampling trajectory does not satisfy Tuy's data sufficiency condition (Tuy, 1983), the IQ of the reconstructed images is acceptable if the cone angle is less than about 10° (Holdsworth and Thornton, 2002), except for very unusual object configurations (Defrise and Clack, 1995).

When the number of projections is limited, iterative and statistical reconstruction algorithms can provide better IQ than FBP. Reduction in the number of views translates directly to reduced radiation dose to the animal. Furthermore, dynamic imaging with micro-CT, such as in cardiopulmonary and perfusion applications, often requires the use of scanning protocols that challenge the constraints of traditional reconstruction algorithms based on FBP, and violation of these constraints can result in artifacts in the reconstructed data. Iterative algorithms, such as algebraic reconstruction technique (ART) (Gordon et al., 1970), present an alternative to FBP with the potential for overcoming its limitations (Kak and Slaney, 1988). With such algorithms, the reconstructed volume is refined by repeatedly comparing simulated reprojections from the reconstructed volume with the original projections.

Artifacts from FBP reconstruction with an irregular angular distributed set of projections are often manifested as long thin streaks. Total variation minimization (TV) has been recognized for its purported ability to suppress streaks while leaving boundaries between homogeneous regions intact (Rudin et al., 1992). The TV minimization can be interleaved with

iterative reconstruction algorithms such as simultaneous algebraic reconstruction technique ART (SART) by performing a TV minimization step at each iteration. To illustrate the power of TV-based reconstruction, we present its application in 4D cardiac micro-CT. As described later in this chapter, in 4D cardiac micro-CT, sampling can be performed with prospective or retrospective cardiorespiratory gating. Although faster than prospective gating (PG), retrospective cardiorespiratory-gating results in an irregular angular distribution of projections and when used with analytical reconstruction algorithms, such as FBP, it creates streaking artifacts. Figure 5 compares IQ of FBP (a) and SART-TV (b) reconstructions when only 95 projections with an irregular angular distribution were used (Song et al., 2007). Note the reduction in the streaks with SART-TV.

Statistical image reconstruction is another class of iterative reconstruction algorithms, first not only introduced for transmission imaging in nuclear medicine but also applied for CT reconstruction (Elbakri and Fessler, 2002). In essence, these algorithms treat the reconstruction as a statistical estimation problem and have the advantage that they can take into account the Poisson model noise in projection data. This results in lower noise levels with statistical reconstruction for the same data when compared with FBP reconstruction.

One major limitation of iterative reconstruction algorithms is their running time. To overcome this limitation, the graphics processing unit (GPU) has been recognized as an alternative computer architecture with potential for accelerating CT reconstruction (Xu and Mueller, 2007). Contemporary GPUs include several hundred processor cores capable of performing the same function on multiple elements of an array simultaneously. This parallel architecture is ideal for several steps of CT reconstruction algorithms, in which the same memory and arithmetic operations can be performed at multiple pixels or voxels in parallel. The development of programming tools that enable general-purpose programming on the GPU, such as the Compute Unified Device Architecture (CUDA) (NVIDIA, Santa Clara, CA), has facilitated the acceleration of CT reconstruction programs, presenting the opportunity to explore more complex algorithms. Micro-CT is already benefiting from GPU-based implementation for iterative and statistical image reconstruction (Jakab et al., 2009; Johnston et al., 2010; Zhao et al., 2007).

2.09.4 Image Quality

This section focuses on some aspects of tomographic IQ assessment that are specific to micro-CT imaging. Figuring out how the choice of the components and of the geometry affects the

Figure 5 Comparison of image quality of FBP (a) and SART-TV (b) reconstructions, when only 95 projections with an irregular angular distribution were used (Song et al., 2007) for a 4D micro-CT application involving retrospective gating.

high-contrast spatial resolution could help on the good design of a micro-CT scanner depending on the desired application. The fundamental limits of spatial resolution achievable with a proper scanner design can only be obtained after an accurate estimation of the even small geometric misalignments of the system. Furthermore, the physiological movement of living animals due to respiration and cardiac cycle will further hamper the resulting spatial resolution without the use of specific cardiorespiratory-gated acquisition protocols and reconstruction algorithms.

2.09.4.1 High-Contrast Spatial Resolution

One of the most relevant figures of merit of performance in micro-CT imaging is the high-contrast spatial resolution, defined as the minimum distance that two-point objects must have in order to be distinguished as separated objects. In this section, we will first show how the in-plane spatial resolution of a micro-CT scanner can be estimated based upon the knowledge of its active components and on the geometry of the system. In the present discussion, we will assume that the system is shift-invariant, so that we can neglect the variations of the system response throughout the FoV. At the first order, the overall MTF of the tomographic system of a given geometry depends on three components: (1) the penumbra effect due to the finite size of the x-ray focal spot, (2) the spatial resolution of the detector array, and (3) the reconstruction algorithm (Holdsworth et al., 1993):

$$\text{MTF}_{\text{tot}}(f) = \text{MTF}_{\text{foc}}(f) \cdot \text{MTF}_{\text{det}}(f) \cdot \text{MTF}_{\text{alg}}(f) \quad [11]$$

where f denotes the spatial frequency. If the angular sampling is properly done, it is safe to assume that the total MTF does not depend on a particular direction in the transaxial plane, that is, we can assume that the system's point spread function is rotationally invariant. Let us denote by $\text{LSF}_{\text{tot}}(x)$ the normalized line spread function of the micro-CT scanner in the transaxial plane at an arbitrary orientation; from the linear system's theory, we obtain

$$\text{MTF}_{\text{tot}}(f) = \left| \mathfrak{F}_{1D}\{\text{LSF}_{\text{tot}}(x)\} \right| \quad [12]$$

where $\mathfrak{F}_{1D}$ denotes 1D Fourier transform. Let us now assume that the 2D point spread function $\text{PSF}_{\text{tot}}(x, y)$ in the transaxial plane is a rotationally symmetrical Gaussian function with standard deviation σ_{tot} :

$$\text{PSF}_{\text{tot}}(x, y) = g_{2D}(x, y; \sigma_{\text{tot}}) \quad [13]$$

The corresponding LSF can be obtained by integrating the 2D PSF along an arbitrary direction in the x - y plane; from the properties of the Gaussian function, it can be shown that the integration of a 2D symmetrical Gaussian function along an arbitrary direction yields a 1D Gaussian function with the same width. This property has a practical consequence because it allows us to measure the system's LSF and thus the MTF, by integrating the system's PSF image along an arbitrary direction. In CT, it is easy to realize a physical measurement of the 2D PSF on a given plane by scanning an object with negligible cross-sectional size (e.g., a tungsten wire) placed perpendicularly to the plane of interest (Kwan et al., 2007; Mettievier and Russo, 2011). A drawback of this method is that the cross-sectional

diameter of the wire must be negligible with respect to the width of the system's PSF; if the diameter of the wire cannot be neglected, the measured PSF must be deconvolved for the finite aperture of the object. This can be done conveniently in the frequency space dividing the resulting MTF by the central profile of the 2D Fourier transform of a disk with the same diameter of the wire (Nickoloff, 1988).

A rigorous derivation of the analytical form of the various components of the MTF is reported in the literature (Holdsworth et al., 1993; Yester and Barnes, 1977). In the typical case of micro-CT systems employing indirect conversion detectors (i.e., for the case in which the spatial resolution of the detector is not limited by the sampling pitch), the three blurring components can be modeled with good approximation with Gaussian functions (Paulus et al., 2000) with widths equal to σ_{foc} , σ_{det} , and σ_{alg} , respectively. From the convolution theorem, it follows from eqns [11] and [12] that the width of the system's LSF is given by

$$\sigma_{\text{tot}} = \sqrt{\sigma_{\text{foc}}^2 + \sigma_{\text{det}}^2 + \sigma_{\text{alg}}^2} \quad [14]$$

In turn, the three blurring components depend on the geometric magnification so that (Belcari et al., 2007; Paulus et al., 2000)

$$\sigma_{\text{tot}} = \sqrt{\left[\left(1 - \frac{1}{M} \right) \sigma'_{\text{foc}} \right]^2 + \left(\frac{1}{M} \sigma'_{\text{det}} \right)^2 + \left(\frac{1}{M} \sigma'_{\text{alg}} \right)^2} \quad [15]$$

where σ'_{foc} and σ'_{det} represent the width of the x-ray focal spot and of the detector LSF on the detection plane, respectively. These quantities have to be measured experimentally, while the algorithm component σ'_{alg} can be estimated theoretically upon the following consideration. The backprojection involves linear interpolation of filtered data, which is equivalent to a convolution with a triangle function FWHM equal to the detector sampling pitch Δu . If no further apodization window is used (i.e., standard ramp filter is used), the linear interpolation is the only source of blurring attributable to the reconstruction algorithm. It is easy to show that the focal spot contribution in eqn [15] is equal to b/M , that is, it is obtained by scaling the geometric unsharpness of the penumbra effect (eqn [1]) by the geometric magnification. Because eqn [15] has been derived on the assumption that all blurring components are Gaussian-shaped, for the sake of simplicity, we approximate the triangle to a Gaussian with standard deviation equal to

$$\sigma'_{\text{alg}} = \frac{\Delta u}{2.355} \quad [16]$$

Equation [15] can be used for the in-plane spatial resolution of a micro-CT scanner from the performance of its components and its geometry, and it has been validated experimentally over a range of magnifications relevant for *in vivo* imaging (Belcari et al., 2007). It is interesting to study the behavior of the spatial resolution as a function of M , for typical values of σ'_{foc} , σ'_{det} , and σ'_{alg} . The graphs in Figures 6 and 7 show this dependence in terms of the two most used figures of merit for spatial resolution, that is, the full width at half maximum, w_{FWHM} ,

$$w_{\text{FWHM}} = 2\sqrt{2 \ln 2} \sigma_{\text{tot}} \simeq 2.355 \sigma_{\text{tot}} \quad [17]$$

and the spatial frequency at 10% of the MTF, $f_{10\%}$,

Figure 6 In-plane theoretical spatial resolution expressed as the spatial frequency at 10% MTF (left) and FWHM of the PSF (right), as a function of the geometric magnification for several values of σ'_{det} . The focal spot size and the detector pitch are kept fixed at $w_{\text{foc}} = 50 \mu\text{m}$ (FWHM) and $\Delta u = 50 \mu\text{m}$. At high magnification, the spatial resolution converges toward a plateau as it is limited by the finite size of the x-ray focal spot.

Figure 7 Same as **Figure 5**, for several values of w_{foc} . The detector resolution and the sampling pitch are both kept fixed at $\sigma'_{\text{det}} = 50 \mu\text{m}$ and $\Delta u = 50 \mu\text{m}$. At low magnification, the spatial resolution of the tomographic system is limited by the spatial resolution of the x-ray detector.

$$f_{10\%} = \frac{\sqrt{2 \ln 10}}{2\pi\sigma_{\text{tot}}} \simeq \frac{0.342}{\sigma_{\text{tot}}} \quad [18]$$

As expected, and as mentioned earlier in this chapter, the intrinsic spatial resolution of the detector is progressively less important for increasing values of the magnification. In fact, for $M \gg 1$, the object is more and more close to the x-ray focal spot, the size of which will dominate the overall image unsharpness. On the other hand, for $M \simeq 1$, the object is nearly in contact with the detector so that the penumbra effect almost vanishes. In this case, only the combined contribution of the detector resolution and of the reconstruction algorithm influences the overall spatial resolution. Using eqn [15], it is possible to estimate the minimum value of σ_{tot} (and hence the optimal spatial resolution for the selected components) as a function of M :

$$\sigma_{\text{tot, min}} = \frac{\sigma'_{\text{foc}}}{\sqrt{1 + \frac{\sigma'^2_{\text{foc}}}{\sigma'^2_{\text{det}} + \sigma'^2_{\text{alg}}}}}, \text{ for } M = 1 + \frac{\sigma'^2_{\text{det}} + \sigma'^2_{\text{alg}}}{\sigma'^2_{\text{foc}}} \quad (19)$$

2.09.4.2 Misalignment Artifacts

The modeling of the spatial resolution obtained in the previous subsection applies only in the ideal case of a perfect alignment of all the system components. This is normally achievable in clinical scanners, but it is nearly impossible in micro-CT where the allowed mechanical tolerances are on the same order of magnitude of the thermal drifts of the components (Sasov et al., 2008). An uncorrected displacement of the axis of rotation (aor) of a tenth of the detector pitch may result in a

significant image blurring and consequently in a loss of image spatial resolution. Displacements are continuously added in the system by many factors, including mechanical instability, vibrations, and thermal drifts of mechanical parts. Many methods have been proposed for the measurement of the misalignment parameters from the analysis of the acquisition data in cone beam geometry (Beque et al., 2003; Defrise et al., 2008; Noo et al., 2000; von Smekal et al., 2004; Yang et al., 2006). Such parameters can be embedded into the reconstruction process in order to produce misalignment-free reconstructed images (Karolczak et al., 2001).

The most used method to date for the geometric calibration of cone beam scanners is the one proposed by Noo and successively refined by Bequé and Defrise. This method makes use of a phantom consisting of two or more small spherical objects (at least three in the modification of Bequé); each object traces an ellipse on the detector plane during the tomographic acquisition, and hence, the geometry of the tomographic system can be determined by the analysis of all the ellipses.

Despite their accuracy, a drawback of the calibration methods based on special phantom is that they need dedicated acquisition sessions to be applied. Many commercial systems for micro-CT imaging now provide zoom-in capability and variable geometry. In such case, nearly continuous calibration of the system's geometry should be necessary to guarantee the stability of the image spatial resolution. For this purpose, methods of self-calibration have been developed that attempt to estimate a subset of the geometric parameters of the system from the projection data of a generic object (Panetta et al., 2008) or by postreconstruction optimization of image-based metrics of image sharpness (Wicklein et al., 2012).

2.09.4.3 Cardiorespiratory Gating in Small-Animal Micro-CT

To minimize the influence of physiological motion, cardiorespiratory-gating strategies play an important role during *in vivo* scanning. 4D micro-CT used in cardiopulmonary studies typically employs either PG or retrospective gating (RG). In PG, acquisition is triggered by the coincidence of a selected respiratory phase and a selected cardiac phase. This produces a set of projections with a constant angular step, resulting in reconstructed images that are free of streaking artifacts. However, because of the time spent waiting for the coincidence of cardiac and respiratory events, the scan time can take as long as 1 h to cover 10 different phases of the cardiac cycle (Badea et al., 2004).

In RG, the projection images are acquired at a rapid and constant rate without waiting for cardiac and respiratory coincidence. Respiratory and ECG signals are monitored and saved in synchrony with the acquisition of the projections. Post-sampling using these signals, the projections are retrospectively sorted into different subsets corresponding to different cardiac and respiratory phases. With this protocol, the scan time can be shortened to 50 s, when using a slip-ring gantry (Drangova et al., 2007). However, the irregular angular distribution causes streaking artifacts in the FBP-based reconstructed images. As shown by Figure 7, superior results are possible using iterative algorithms such as SART-TV (Song et al., 2007). Recently, a new strategy called fast prospective gating (FPG) has been introduced (Guo et al., 2012), which combines the regular

angular distribution of PG with the fast scan time of RG. In FPG, multiple projections are acquired at the same angle, corresponding to all cardiac or respiratory phases to be reconstructed, before the cradle is rotated to the next angle. FPG requires on-the-fly computation of the triggering events, which are delayed from the peaks of the respiratory or cardiac signals.

In terms of implementation, most of the proposed methods involve extrinsic cardiorespiratory gating, in which the cardiac and respiratory signals are acquired with dedicated monitoring devices, that is, ECG leads and a pneumatic respiratory pillow. In addition to these extrinsic gating techniques, an intrinsic image-based gating approach without any external devices has been developed initially for a clinical cone beam spiral CT scanner (Kachelriess et al., 2002). In intrinsic gating, a post-processing algorithm evaluates the center of mass of certain regions of interest within each projection to detect the respiratory and cardiac motion (Bartling et al., 2008; Dinkel et al., 2008; Sawall et al., 2011).

2.09.5 Applications of Small-Animal Micro-CT

2.09.5.1 Bone Imaging

Micro-CT has been used to investigate the structure and density of rodent bone since its very beginning (Feldkamp et al., 1989; Kinney et al., 1995), due to its high spatial resolution and high contrast in imaging mineralized tissues. In fact, the study of bone architecture and density drove the early developments of micro-CT systems (Rueggsegger et al., 1996). When applied to *in vitro* studies, the spatial resolution of dedicated benchtop systems approaches that provided by synchrotron sources (Boyd et al., 2006; Chappard et al., 2006). For *in vivo* applications, the acquisition protocol is constrained by dose to the animal (Ford et al., 2003), leading to a reduction in spatial resolution. Nonetheless, a nominal spatial resolution of about 50 μm has been shown to be effective in following live rats during studies of osteoarthritis (Appleton et al., 2007; McErlain et al., 2008) and bone remodeling (Cowan et al., 2007).

The ability to apply micro-CT for longitudinal studies in orthopedic applications has also been demonstrated in the study of bone architecture changes over time (David et al., 2003; Waarsing et al., 2005), where parameters such as volumetric bone mineral density, bone volume ratio, bone surface ratio, and trabecular thickness are reported. A comprehensive review of the morphometric parameters that can be extracted from bone micro-CT images has been published recently (Bouxsein et al., 2010). Such micro-CT bone structural parameters were used to show significant effects due to VEGF (Li et al., 2009) and stem cell (Lee et al., 2009) treatments in bone healing or to investigate trabecular bone loss and cortical thickening in total body irradiation and bone marrow transplantation in mice (Dumas et al., 2009). In osteoporosis research, micro-CT data of the proximal tibiae in small-animal models allow the study of the progression of disease after ovariectomy (Laib et al., 2001) or immobilization (Laib et al., 2000). Similar quantification has been performed also on human biopsy specimen from iliac crest bone to assess the effect of therapy, as the injection of human parathyroid hormone (Dempster et al., 2001). In bone regeneration studies, the calvarial defect model in rats has been used extensively

with micro-CT imaging, which can provide quantitative information on bone mineral content and bone fractional area (Umoh et al., 2009). Also in the field of regenerative medicine, micro-CT plays a fundamental role in the accurate determination of the three-dimensional structure of engineered bone scaffolds, with particular emphasis on porosity and connectivity (Ho and Huttmacher, 2006; Lin et al., 2005). Finally, the use of finite element methods applied on computerized meshes generated from segmented bone images allows the nondestructive evaluation of the mechanical properties of bones and scaffolds, with a considerable improvement in the understanding of bone biomechanical properties and a reduction in the number of sacrificed animals (Borah et al., 2001).

Some applications, such as studies of skeletal development (Guldborg et al., 2004), may accept lower resolution but require larger FoV, that is, imaging the whole mouse or rat using the larger field of view offered by flat-panel-based systems (Bartling et al., 2007a,b; Drangova et al., 2007; Missbach-Guentner et al., 2007). As an example of bone imaging using micro-CT in Figure 8, we present images from a longitudinal study of bone healing in a model of large lesion in rat femur. Due to the lesion site and extension, the *in vivo* imaging required the acquisition of the whole lower abdomen at a relatively large voxel size (80 μm in this case) and low dose, allowing the quantification of healing progression with different therapies by measuring bone volume and cortical thickness at the lesion site through the time. The same femur has also been imaged at high resolution after animal sacrifice and organ excision at a voxel size of 18 μm , allowing the quantification of the callus porosity. This type of imaging helps in the assessment of the efficacies of different therapeutic approaches in bone pathologies involving a significant loss of substance or in subjects with production deficit of the bone callus. In particular, there is high interest in the development of novel biomimetic materials with osteogenic properties, which can also be associated with cellular therapies (Granero-Molto et al., 2008; Kokubo et al., 2003).

The study of cancellous bone requires higher resolution and is often performed on excised bones *ex vivo*. This is due to the

small thickness of trabeculae in small-animal cancellous bone, which can be as thin as 30–50 μm (Martin-Badosa et al., 2003). To avoid quantification errors due to the partial volume effect, voxel size in the range of 1–20 μm is used for this type of application. As a rule of thumb, avoiding partial volume artifacts requires that the minimum thickness of the measured object should be at least two times the imaging system's FWHM spatial resolution. The quantification of the morphometric parameters is performed on a binary image obtained by segmenting the original micro-CT volume. In most cases, due to the high contrast between bone marrow and muscle, simple threshold-based segmentation is sufficient for both cancellous and cortical bone segmentation on micro-CT images of adequate spatial resolution; in some cases, the original images must be corrected for nonuniformity in order to apply reliably a single global threshold. In all cases, care must be taken in the selection of the threshold value as the result of the quantification can depend sensibly on this quantity (Ding et al., 1999). Several automatic methods have been developed for the robust selection of the threshold value, mostly based on the analysis of the histogram of the original image or volume of interest (Buie et al., 2007). Figure 9 shows an example of *ex vivo* study of cancellous bone on a mouse vertebra, acquired at a voxel size of 11 μm .

2.09.5.2 Lung Imaging

Helped by the inherent contrast provided at air/tissue interfaces, CT is a powerful modality for lung imaging. However, pulmonary micro-CT imaging in small animals has been challenging, due to the small size and rapid respiratory motion. As shown by Ritman (2005), a micro-CT voxel size on the order of 75 μm is required to provide anatomic resolution in the mouse comparable to that obtained in CT for humans. Nonetheless, micro-CT can be used successfully to study various lung disease models, such as emphysema and fibrosis. An emphysema model was created in mice by using intratracheal instillation of pancreatic elastase and imaged with micro-CT (Postnov et al., 2005). Emphysemic regions have shown lower HU

Figure 8 Longitudinal study of bone healing in a model of large lesion in rat femur. In order to reduce the radiation dose, the images acquired *in vivo* are reconstructed with a relatively large voxel size (80 μm). After euthanasia, the femurs are excised and placed in test tubes, thus allowing longer acquisition times and higher magnification and smaller voxel size (18 μm in this case).

Figure 9 Quantitative 3D morphometry on cancellous bone is usually performed *ex vivo* using high-resolution rotating specimen micro-CT. The image earlier shows a vertebra of a mouse reconstructed at isotropic voxel size of 11 μm .

values than the normal lung, while the lung volume in these diseased animals was increased. Fibrosis models have also been created in mice, by instillation of bleomycin, and studied longitudinally with micro-CT during diseases progression (Shofer et al., 2007). The fibrotic lung regions are identified in micro-CT images by the resulting increase in HU. Furthermore, some studies have also shown that lung compliance can be measured in small animals using micro-CT, by implementing breath-holding over a range of specified pressures and calculating lung volumes from the corresponding images to produce a pressure–volume curve (Guerrero et al., 2006; Shofer et al., 2007). Micro-CT imaging-based compliance measurements were validated against the gold standard provided by quasistatic compliance measurements using a commercially available small-animal ventilator (Shofer et al., 2007). The loss of pulmonary compliance was investigated in a study of irradiated mice (Guerrero et al., 2007). Micro-CT imaging of lung tumors is generally performed without the administration of a contrast agent. However, in some lung disease models, the use of nanoparticle-based blood pool contrast agents may be beneficial, since it may allow the assessment of the vascular density of the lung or even vascular permeability of tumors via enhanced permeability and retention (EPR) (Badea et al., 2012). *In vivo* micro-CT also provides the capability for contrast-enhanced imaging of the rodent lung using stable xenon gas as an inhaled contrast agent. This procedure has been described in a study with adult rats (Lam et al., 2007).

2.09.5.3 Cardiac Imaging

Micro-CT has been used to visualize the 3D coronary circulation *ex vivo* in the young and aged rodent heart (Sangaralingham et al., 2012). Alternatively, rapid 3D phenotyping of cardiovascular development in mouse embryos has been achieved by *ex vivo* micro-CT with iodine staining (Degenhardt et al., 2010). *In vivo* micro-CT-based cardiac

morphological and functional imaging in mice and rats has been reported using both PG and RG approaches (Badea et al., 2005, 2007, 2008c; Bartling et al., 2007a,b; Detombe et al., 2008; Drangova et al., 2007; Nahrendorf et al., 2007; Song et al., 2007). Using both approaches, quantitative estimates can be made for ejection fraction, stroke volume, and cardiac output on the basis of left ventricle (LV) volume measurements in the 4D (3D + time) micro-CT data sets.

Cardiac micro-CT requires the use of contrast agents to be able to discriminate between the myocardium and the blood. In most of the proposed studies, blood pool contrast agents have been used; although with faster scanning available with the flat-panel-based systems (Bartling et al., 2007a,b; Drangova et al., 2007), this may be optional. For example, recently, a method for imaging cardiac perfusion in the mouse has been proposed using a scan protocol that consists of repetitive injections of conventional low-molecular-weight contrast media within several consecutive scans (Sawall et al., 2012). However, most of the cardiac micro-CT studies use a blood pool contrast agent based on which 4D cardiac micro-CT image is used to measure cardiac function, via ejection fraction and cardiac output. The accuracy of these measurements was studied as a function of the volume of contrast agent and the number of projections used for reconstruction (Badea et al., 2008c). Reduction of the volume of contrast agent reduces hypervolemia effects on the hemodynamics, while the reduction in the number of projections translates directly into reduced radiation dose. It was determined that if 5% error in LV volumetric estimation is acceptable, as suggested for human cardiac CT (Haraokawa et al., 2004), then sufficient cardiac micro-CT IQ could be provided with approximately 126 projections and with a contrast dose of 0.01 ml Fenestra VC per gram body weight. With other formulations of blood pool contrast agents that contain higher iodine concentration, the total volume injected may be further reduced. Other contrast agent formulations, such as eXIA 160, an aqueous colloidal

polydisperse contrast agent with a high iodine concentration (160 mg I per milliliter), have also been proposed for cardiac micro-CT, since it creates strong contrast between the blood and the tissue with a low injection volume of only 0.005 per gram in a mouse (Detombe et al., 2012). Notably, this contrast agent was also taken up by the myocardium and provided continued enhancement when it was eliminated from the blood.

Examples of 4D micro-CT in F344 rats acquired using cardiorespiratory PG and reconstructed at voxel size of 88 μm are shown in Figure 10. The cardiac function analysis based on 4D micro-CT is performed with Vitrea software (Vital Images, Inc., Minnetonka, MN). 4D micro-CT data are segmented; LV lumen, endocardium, and epicardium are traced using a fast semiautomatic approach (a,b). Some advanced heart visualization in diastole/systole is shown in (c,d). LV end-diastolic/end-systolic volumes are shown in (e). Bull's eye plots display LV wall thickness, wall motion, and regional ejection fractions. Global cardiac functional measures such as cardiac output, stroke volume, and ejection fraction are shown in the inserted table. These cardiac measures require laborious segmentation of the murine heart in 4D, which can be faster achieved via registration (Clark et al., 2012a).

Cardiac micro-CT can also be used for weekly imaging following a sham or coronary ligation surgery to monitor LV remodeling postinfarction (Detombe et al., 2008). Assessment of cardiac function and infarct size was also demonstrated using delayed contrast enhancement by combining the blood pool contrast agent Fenestra VC and a low-molecular-weight contrast agent (Nahrendorf et al., 2007).

Cardiac micro-CT shows considerable promise as a tool for routine structural and functional phenotyping of transgenic

and knockout mouse models, as was demonstrated by Badea et al. who imaged transgenic muscle LIM protein-null mice to compare the micro-CT measurements with M-mode echocardiography in a model of heart failure (Badea et al., 2007). Cardiac micro-CT has also been used for the evaluation of drug effects, such as those shown in a study on dobutamine-induced cardiac stress (Badea et al., 2011a) in rats.

As with all *in vivo* micro-CT, the dose delivered to the animal must be considered. When PG-based cardiac micro-CT was initially proposed, it involved long scan times and a radiation dose higher than 1 Gy. With retrospective gating (RG), the entrance exposure was <0.3 Gy to reconstruct scans covering the entire cardiac cycle (Drangova et al., 2007). More recent 4D micro-CT methods using superior image reconstruction algorithms have achieved a dose close to 0.2 Gy (Armitage et al., 2012; Badea et al., 2011b,c; Johnston et al., 2012; Sawall et al., 2011).

2.09.5.4 Cancer Imaging

Micro-CT has proven to be useful in tumor detection, progression, and imaging tumor angiogenesis. In terms of detecting lung tumors, for example, micro-CT has been used to detect lung nodules with a minimum volume of 0.63 mm^3 (Cody et al., 2005), when using a ventilator and a PG triggering approach.

Cancer imaging with micro-CT is intimately related to the use of contrast agents. Dynamic micro-CT has also been applied to the direct measure of perfusion in tumors following the injection of conventional contrast agent (Kan et al., 2005; Phongkitkarun et al., 2004). The acquisition of perfusion information requires rapid scanning and has only become

Figure 10 Cardiac function analysis (F344 rat) based on 4D micro-CT using Vitrea software. 4D data are segmented; left ventricle (LV) lumen, endocardium, and epicardium are traced using a fast semiautomatic approach (a,b). Advanced heart visualization in diastole/systole (c,d). LV end-diastolic/end-systolic volumes are computed and measures are derived (e). (f) Bull's eye plots display LV wall thickness, wall motion, and regional ejection fraction.

possible with the introduction of slip-ring-based micro-CT scanners capable of acquiring 3D image data sets once per second for periods of a minute or longer (Du et al., 2007). To achieve adequate coverage in the longitudinal direction, the rapid scans often require a reduction in spatial resolution in the longitudinal direction. CT perfusion measurements enable the quantitative evaluation of functional parameters, such as blood flow, blood volume, and permeability–surface area product – a measure of the leakage rate of contrast from the capillaries into the interstitial space. But due to their rapid clearance in small animals, conventional low-molecular-weight contrast agents are not widely used for micro-CT. Instead, large-molecular-weight or blood pool contrast agents are preferentially used for cancer imaging with micro-CT. We present next a number of examples of cancer imaging using large-molecular-weight or nanoparticle-based contrast agents.

Using Fenestra VC, Kindlmann et al. (2005) examined the intermediate and large vessels of alveolar rhabdomyosarcomas in a transgenic mouse model. The investigators suggested that micro-CT with Fenestra VC had the best potential for defining vessel diameter, tortuosity, and density for anti-angiogenesis models, but to visualize tumor capillaries, it was necessary to develop instruments that had a higher resolution than those used in their study.

Micro-CT imaging studies were performed in nude mice bearing STC-1 tumors 15 and 30 days after grafting (Almajdub et al., 2007). Animals were injected with Fenestra LC and imaged at 2 and 4 h after the injection. The liver and spleen were clearly differentiated after administration of the contrast agent, reaching mean contrast-to-noise ratios of >2.0 for the liver and ~ 10 for the spleen at the different imaging times. In the spleen, quantification of the tumors with Fenestra LC was not precise, whereas in the liver, tumors (0.3–1.5 mm) were detectable at day 30. Significant amounts of the contrast media were observed to persist in the animals for up to 15 days after a single injection, allowing quantitative follow-up of tumors in both the liver and the spleen, without additional injection of contrast.

Liver tumor volumes have also been quantified *in vivo* using Fenestra VC mice injected with murine B16F1 melanoma cells; in this case, Fenestra VC was used instead of Fenestra LC in order to remove the ambiguity between unopacified liver vessels and tumors in the liver by selecting a time point post-contrast injection (8 h) (Graham et al., 2008). Because many liver metastases grow on the surface of the liver, a combined strategy of Fenestra VC plus an intraperitoneal injection of extracellular contrast agent was shown to improve the precision and accuracy of tumor volume measurement and enables the quantitation of total tumor burden (Graham et al., 2008). Small lesions like liver metastases have also been imaged using a nanoparticle alkaline earth metal-based contrast agent (Viscover ExiTron nano) developed for small-animal liver CT imaging (Boll et al., 2011).

The advances in synthesizing nanoparticle contrast agents (Hainfeld et al., 2006; Jackson et al., 2011; Mukundan et al., 2006; Rabin et al., 2006) have enabled high-resolution rodent studies using micro-CT. As imaging probes, these nanoparticle-based contrast agents have also opened the door for molecular CT imaging using various targeting strategies (Chanda et al., 2011; Cormode et al., 2010; Ghann et al., 2012; Popovtzer

et al., 2008; Reuveni, 2011; Wyss et al., 2009). A liposomal-iodinated (Lip-I) contrast agent has been used to image the tumor microenvironment with exquisite detail (Ghaghada et al., 2011; Moding et al., 2012). The same Lip-I has enabled to differentiate between fast- and slow-growing primary lung tumors in mice (Badea et al., 2012) based on its differential accumulation over time caused by the EPR effect (Maeda, 2001). A natural extension of these studies is to introduce multiple probes that incorporate different high-Z materials (e.g., iodine, gold, and gadolinium) to allow colocalization studies using multienergy photon-counting detectors (Schlomka et al., 2008).

As an example, we show how two types of nanoparticle contrast agents based on Au and I are used for vascular imaging with dual-energy micro-CT of a soft tissue sarcoma (see Figure 11). To decompose micro-CT data containing a mixture of the two nanoprobe into quantitative, 3D maps of the concentrations of each element, a postreconstruction decomposition method was applied (Badea et al., 2011b,c; Clark et al., 2012b). For each voxel, the decomposition entailed the solution to a system of equations with two unknowns representing the concentrations of I and Au. The protocol first involved the injection of the AuNP followed by an immediate dual-energy micro-CT scan (0 h). At this early imaging time point, the AuNPs are intravascular and enable the visualization of the 3D vascular tree and computation of the fractional blood volume. Three days later (i.e., at 72 h) post-AuNP injection, Lip-I nanoparticles were injected. A new dual-energy micro-CT scan allowed the visualization of both the architecture of vessels and the extravasation associated with their permeability. The passive accumulations of AuNP are due to an EPR effect.

2.09.5.5 Multimodality Imaging Using Micro-CT

Another way in which micro-CT can be used in the study of both function and anatomy is by combining it with other imaging modalities, such as positron emission tomography or single-photon emission tomography (PET or SPECT; see also Chapters 1.06, 1.07, and 1.04 as well as Chapter 1.10).

Figure 11 Dual-energy micro-CT is used to evaluate both blood volume and permeability for a primary sarcoma tumor in a genetically engineered mouse using gold (AuNP)- and iodine (Lip-I)-based nanoparticles at two imaging time points (i.e., 0 and 72 h post-AuNP injection).

Although very sensitive, the nuclear imaging methods are limited by poor spatial resolution, which becomes an issue when imaging mice. Micro-CT is therefore useful in providing anatomical reference and also enables corrections of γ -ray attenuations through tissue. Multimodality can be best performed by combining both imaging modalities (micro-PET/micro-CT or micro-SPECT/micro-CT) in one scanner (sometimes referred to as hardware image fusion) and performing simultaneous or sequential data acquisitions (Goertzen et al., 2002; Kastis et al., 2004; Liang et al., 2007). Even in case of hardware fusion, a rigid registration must be applied to match the reference frames of the different acquisition systems (Jan et al., 2005). An advantage of simultaneous acquisition is to cancel out the temporal gap between the two scans, so that the animal is in the same position in both images and no further (nonrigid) registration procedures are required. Nevertheless, most of the multimodal preclinical scanners to date provide sequential scanning of the anatomical and functional images, mainly due to severe constraints on the system hardware limiting the IQ of both modalities in case of simultaneous acquisition (Townsend, 2008). Simultaneous data acquisition will be still of interest in the future for the hardware fusion of different functional modalities such as PET and SPECT (Bartoli et al., 2007) or PET and MRI (Judenhofer et al., 2008) because of the unique possibility of adding temporal correlation to the spatially coregistered volumes. Differently from clinical multimodal imaging, in small-animal imaging, it is possible in some degree to perform sequential anatomical and functional image acquisition with two separate scanners (referred to as software fusion), as long as those scanners are equipped with compatible mechanics and interchangeable animal beds (Chow et al., 2006; Vanhove et al., 2009). In this latter case,

attention must be paid on not perturbing the animal on the common bed or imaging chamber if it is not tightly immobilized.

2.09.5.5.1 Attenuation correction in emission tomography

Micro-CT images are used in hybrid small-animal micro-PET/CT scanner to perform the attenuation correction of micro-PET images. The data processing workflow for micro-CT attenuation correction (micro-CTAC) is very similar to that used for correction of PET data from CT images in combined clinical PET/CT scanners (Figure 12). For this purpose, the voxel values are first scaled to the corresponding attenuation coefficient of 511 keV γ -photons using a bilinear function (Chow et al., 2005); then, the micro-CT volume is blurred with a 3D Gaussian kernel to match the resolution of the micro-PET image. Finally, the blurred μ -map is forward projected to generate the attenuation correction factors (ACF) for the PET sinograms. The ACF sinograms are simply applied to the corresponding original PET sinograms by element-wise multiplication. It is very important that the micro-PET and micro-CT volumes are correctly registered before generating ACF sinograms; otherwise, severe artifacts may arise on PET images especially near to interfaces between structures with highly different x-ray attenuation coefficient, such as skin–air or myocardium–lung interfaces (Kennedy et al., 2009). The effect of CTAC is shown in Figure 13 for micro-PET images of the NEMA NU4-2001 IQ phantom, acquired with the YAP-(S)PET II scanner (ISE s.r.l., Vecchiano, Italy) (Del Guerra et al., 2006); the μ -map was generated from micro-CT volumetric data of the same phantom acquired with the x-ray Animal Tomograph (Xalt) prototype scanner (Panetta et al., 2012). The two scanners are equipped with compatible mechanics for bed positioning,

Figure 12 Similar to the case of clinical PET/CT, micro-CT images are used in hybrid small-animal micro-PET/CT scanner to perform the attenuation correction of micro-PET images. For this purpose, the voxel values are first scaled to the corresponding attenuation coefficient of 511 keV γ -photons, and then, the micro-CT volume is blurred to the resolution of the micro-PET image. Finally, the blurred μ -map is forward projected to generate the attenuation correction factors (ACF) for the PET sinograms.

Figure 13 The effect of CT attenuation correction (CTAC) of micro-PET images (YAP-(S)PET II, ISE s.r.l., Vecchiano, Italy) from a micro-CT derived μ -map. After CTAC, the apparent nonzero activity in the empty and water-filled holes (top row) and the cupping effect (bottom row) are correctly removed.

allowing for software fusion of micro-CT and micro-PET images. After CTAC, the apparent nonzero activity in the empty and water-filled holes (top row) and the cupping effect (bottom row) are correctly removed. More important, the CTAC procedure corrects for the underestimation of the reconstructed activity inside the object, which can be in the order of 30–60% for mice and rats.

Less straightforward is the attenuation correction of micro-SPECT or pinhole SPECT data from micro-CT-derived μ -maps. In this case, the attenuation of γ -photons does depend on the actual location where the radioactive decay takes place, information that is not available in the projection space of the emission data. For this reason, the most used method for AC in SPECT simplifies the problem by considering a uniform attenuation coefficient through the entire object, thus avoiding the use of transmission data (Chang, 1978). More accurate correction can be obtained by including the information of the micro-CT-derived coregistered μ -map into the system matrix of the maximum-likelihood reconstruction algorithm (Hwang and Hasegawa, 2005; Vanhove et al., 2009). It is worth noting that the absence of attenuation correction in SPECT can lead to a higher underestimation and nonuniformity of the reconstructed activity because of the lower energy of the emitted γ -photons (140 keV for ^{99m}Tc , 27–36 keV for ^{125}I , and 159 keV for ^{123}I). Nevertheless, due to relative lower degree of quantification error attributable to the absence of attenuation correction in small-animal PET and SPECT with respect to the corresponding clinical examinations, several small-animal nuclear imaging studies are still performed without correction for γ -photon attenuation (Hwang et al., 2006; Vanhove et al., 2009).

Apart from just an anatomical reference, micro-CT can provide complementary vascular information in tumor studies where metabolic micro-PET imaging is also performed. To illustrate this point, Figure 14 shows both micro-CT and micro-PET images in a mouse with a mammary 4T1 tumor. A

liposomal blood pool contrast agent (Lip-I) was injected 3 days prior to imaging. ^{18}F -FDG was injected (dose 0.019 mCi g^{-1}) 30 min preimaging. A hybrid micro-PET/micro-CT Siemens Inveon MM scanner was used for image acquisition. Note the accumulation of the liposomes in liver (blue arrow), spleen (red arrow), and tumor (green ellipse) in the micro-CT images. The tumor presents high inhomogeneities in enhancement. Interestingly, the hypoenhanced necrotic center of the tumor in micro-CT (green arrow) lacks also enhancement in the micro-PET image, indicating the lack of metabolism.

The use of multipinhole collimators has resulted in micro-SPECT systems exhibiting submillimeter resolution, which are well suited for *in vivo* studies of rats and mice (Beekman et al., 2005). It is typically advantageous to combine such a micro-SPECT system with micro-CT, in order to provide high-resolution coregistered images of anatomy (Ritman, 2002; Tai et al., 2008). Figure 15 illustrates the capability to perform coregistered cardiac imaging with micro-SPECT and micro-CT in a mouse with the U-SPECT-II/CT system (MILabs, Utrecht, the Netherlands). This hybrid system is fitted with an ultra-high-resolution 0.35 mm multipinhole collimator. The anesthetized mouse was injected with 185 MBq of ^{99m}Tc -tetrofosmin (GE Healthcare, Arlington Heights, IL) via a tail vein catheter. Although optional, 0.008 ml per gram mouse of a liposomal-iodinated blood pool contrast agent has been injected prior to imaging to allow a better delineation of the myocardium (also shown in the micro-CT image). The micro-SPECT data were acquired over 30 min (3 frames, 10 min per frame) in list mode and reconstructed using the pixel-based ordered subset expectation maximization (POSEM) iterative reconstruction algorithm (6 iterations, 16 subsets, and 0.125 mm voxel size). Both micro-CT and micro-SPECT images were viewed using PMOD v.3.3 biomedical image quantification software (PMOD Technologies Ltd., Zurich, Switzerland). Note how the micro-SPECT signal reflects myocardial perfusion information.

Figure 14 Micro-CT-only (a,b) and combined micro-PET/micro-CT images (c,d) in axial (a,c) and coronal (b,d) orientations in a mouse at 3 days after the injection of a liposomal contrast agent. Note the accumulation of the liposomes in the liver (blue arrow), spleen (red arrow), and tumor (green ellipse). The tumor presents high inhomogeneities in enhancement. The hypo-enhanced necrotic center of the tumor in micro-CT (green arrow) lacks also enhancement in the micro-PET image.

Figure 15 A micro-SPECT image (b) can be overlaid on micro-CT(a) as in (c). The signal in SPECT image reflects myocardial perfusion.

2.09.6 Conclusions

Micro-CT can provide a reliable platform for small-animal imaging that is complementary to other small-animal methods and can be used not only for morphological but also for more challenging functional imaging. While an exhaustive comparison of micro-CT to other small-animal imaging techniques is beyond the scope of this review, some broad generalizations are possible. Micro-CT methods are generally faster and less expensive than micro-MRI, micro-SPECT, and micro-PET. The resolving power of micro-CT is certainly greater than micro-PET and usually higher than that of micro-MRI. But the sensitivity of x-ray methods to the presence of specialized probes falls far below other imaging methods. New generation of ultrasound systems can provide higher spatial resolution than x-ray methods, are less expensive, and do not suffer from the problems related to dose. But in 3D or 4D, micro-CT can provide considerably more accurate and precise measures of volumes than ultrasound. Radiation dose and the limited

contrast available in x-ray methods can be a limitation; at the same time, newer contrast agents and novel new acquisition and reconstruction strategies show extraordinary promise. These developments, and exciting new opportunities in molecular imaging, will surely continue to expand the applications for micro-CT in the small animal.

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