Micro-CT in small animal and specimen imaging

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Laboratory systems for microscopic computed tomography (micro-CT) have recently evolved from specialized prototype tools to become essential components of many research laboratories. The availability of commercial systems with almost microscopic resolution and the capability to image live animals has opened up entirely new applications for micro-CT in laboratory investigation. This review describes the technical aspects of micro-CT and highlights some current research applications. Micro-CT has the potential to replace serial histology as the reference standard in many *in vitro* studies, and provides a practical approach to obtain quantitative information during some longitudinal investigations *in vivo*.

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Computed tomography (CT) has revolutionized clinical diagnostic practice since its introduction in the early 1970s [1]. Until recently, readily available CT equipment was not useful during laboratory investigations of small rodents, mainly because of limitations in spatial resolution. For example, the 1 mm^3 volume elements (voxels), commonly obtained in humans with clinical CT scanners, would be approximately equivalent to $(50\ \mu m)^3$ voxels in rodents. Recent technical advances (such as the availability of megapixel charge-coupled device detectors and significant increases in computer memory and speed) have made it practical to obtain such high-resolution CT images of small-specimens and -animals during research investigations. For the purposes of this review article, we define micro-CT systems as those capable of volumetric CT analysis with isotropic voxel spacing of <100 μm. Note that this level of spatial resolution enables the study of sample volumes of <1 nL. We limit our discussion to systems that are based on conventional X-ray tube sources, rather than synchrotron-based systems, which are capable of much higher resolution [2,3].

Methods

Several micro-CT systems are now commercially available and most of these share some design features (Fig. 1). A typical laboratory micro-CT scanner will consist of a tungsten-anode X-ray tube with a relatively small focal spot (~10 μm), coupled to a high-resolution X-ray detector system (~50 μm pixel spacing). Note that a small X-ray focal spot must be used with X-ray imaging systems that incorporate geometric magnification, to minimize penumbral blurring. To produce a truly 3D dataset using CT, X-ray projection views are acquired at hundreds of equally spaced angular positions around the object of interest. These views are then used to reconstruct a CT image, typically using a convolution back-projection approach (implemented in 3D) similar to that described by Feldkamp *et al.* [4,5]. Although CT performed in such a 'cone-beam' geometry does not provide an exact solution, the solution is an acceptable approximation in the case of relatively small cone angles (typically <10° cone half angle) [6,7].

Several systems that follow this basic design have been described over the past few years. The earliest reported systems used X-ray image intensifiers (XRIIs) as the detector [8–10], although this approach limits spatial resolution unless a micro-focus X-ray tube is used. The use of high-resolution solid-state detectors enables a significant increase in spatial resolution, coupled with a reduction in the overall size of the system [11]. 3D images can be constructed either by combining contiguous single-slice images, each of which is acquired in a fan-beam configuration [12], or by using the cone-beam approach described above [13]. The cone-beam approach is favoured in applications where imaging-time is a factor, as it makes much more efficient use of the available X-ray fluence.

In any micro-CT system, several factors affect spatial resolution. These factors include inherent resolution of the X-ray detector, geometric magnification, focal spot size, stability of the rotation mechanism, and the filtering algorithm used during CT reconstruction [9]. It is important to note that spatial resolution is not determined solely by the spacing of volume elements within the image (which might be considered the nominal resolution); in fact, this spacing is typically designed to be smaller than the true resolving power of the CT device.

The general type of micro-CT system described above has been implemented in two configurations, for either *in vitro* imaging (where the specimen rotates) or *in vivo* scanning (where the X-ray system rotates). Several recent reviews have provided a comprehensive overview of laboratory and commercial micro-CT systems that are currently in use [14–16]. In the following section, we briefly compare the general features of *in vitro* and *in vivo* scanning systems. Representative micro-CT images used to illustrate

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current techniques have been obtained with commercially available equipment (Models MS-8 and RS-9; Enhanced Vision Systems, London, Canada).

In vitro imaging

Micro-CT systems designed for specimen imaging and *in vitro* applications are typically optimized for spatial resolution to obtain an image that approaches histological microscopy as closely as possible [17]. This constraint typically requires the use of a micro-focal spot X-ray tube, combined with high geometric magnification (typically 2–4×). The advantage of this approach is that it can be applied easily to intact specimens, providing images that retain information about 3D connectivity, topology and micro-architecture, with spatial resolution of 15–50 μm, over a field of view of 15–50 mm. For many applications, *in vitro* micro-CT can be used as a cost-effective adjunct (or replacement) for serial histology. As the specimen remains intact, the use of micro-CT does not preclude subsequent histological analyses and, in fact, micro-CT images can be registered with histology to provide comparative data [18].

In vivo imaging

The basic principles described above for *in vitro* imaging also apply to *in vivo* applications for micro-CT. However, imaging of live rodents places additional design constraints on the system, the first of which is the requirement for a rotating gantry system that holds the imaging equipment (X-ray source and 2D X-ray detector). In addition, systems designed for live animal imaging are usually optimized for slightly reduced spatial resolution, typically with 50–100 μm voxel spacing. This is because image noise is proportional to $(\Delta x)^{-2}$ (for isotropic voxel spacing Δx) if X-ray exposure to the animal is held constant [9]. Thus, extremely high-resolution imaging might necessitate unacceptably high whole-body X-ray doses for live animals. Relaxation of the resolution requirements for *in vivo* imaging enables the use of lower geometric magnifications and larger X-ray tube focal spot size, facilitating higher tube current and shorter exposures (typically <10 min). Such a relatively short acquisition interval makes it possible to acquire high-quality images in anaesthetized rats and mice [15,19], provided that appropriate gating techniques are used to eliminate artifacts caused by cardiac and respiratory motion.

Data handling and analysis

It is important to bear in mind that current micro-CT techniques can routinely deliver volumetric images containing $>10^9$ voxels, equivalent to several gigabytes of data. This quantity of information places an extra burden on data handling, analysis and storage systems. Micro-CT images are optimally viewed and analysed on computers with large amounts of random-access memory (RAM), fast processors and dedicated graphics cards. Specialized stereological analysis software might be required to derive meaningful parameters from high-resolution 3D data; this type of software has been described for the analysis of vascular [20] and trabecular [21] micro-architecture.

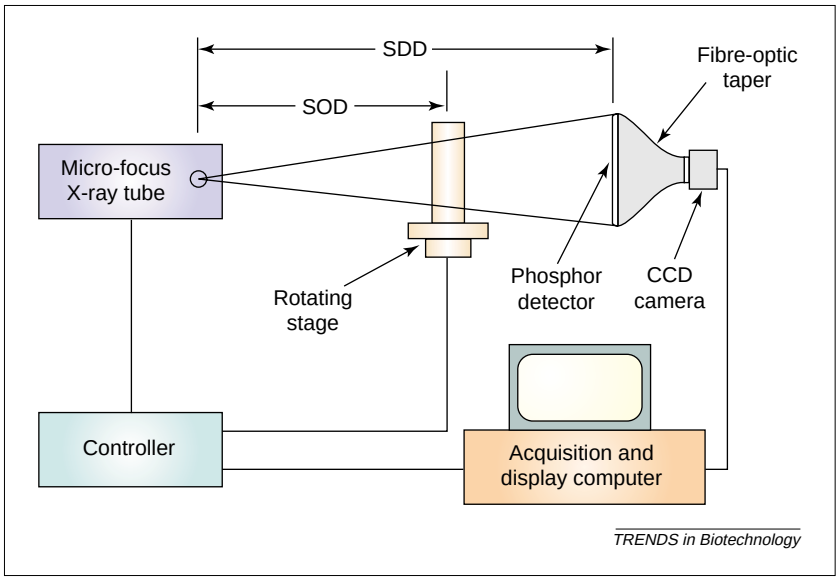

Figure 1. An *in vitro* microscopic computed tomography (micro-CT) scanner

A specimen, mounted on a rotating stage, is positioned between an X-ray source and detector. The source-to-object distance (SOD) and source-to-detector distance (SDD) are selected to provide the appropriate amount of geometric magnification. Typically, the SDD is ~20 cm, and the SOD ranges between 7 and 18 cm. X-ray projections are acquired by a phosphor detector, coupled to a CCD camera by a fibre-optic taper, which reduces the size of the image. During acquisition, the computer controls the X-ray tube and specimen stage, obtaining X-ray projections at hundreds of angular positions.

Applications

Bone imaging

The research application that drove the early development of micro-CT is the study of bone architecture and density during preclinical investigations of osteoporosis and osteoarthritis in appropriate animal models [22–24]. Micro-CT is especially well-suited for applications involving measurements of bone density, owing to the high signal contrast between bone and soft tissue, so it is not surprising that the first applications of this technique have been in bone studies [25]. Several groups have applied micro-CT techniques to non-destructive evaluation of trabecular bone structure [26,27] and micro-CT has been shown to be an accurate tool to precisely measure changes in bone stereology, volume and microarchitecture [28]. Micro-CT has also been used to assess the 3D architecture of trabecular bone in an experimental rat model for tumour-induced osteolytic defects [29]. Recently, micro-CT has been used as the basis for micro-finite element models, enabling calculation of the elastic properties of trabecular bone *in vitro* and *in vivo* [30]. Because it is non-destructive, micro-CT can also be used in conjunction with measurements of strain under compression in intact samples [31]. Micro-CT applications are not limited to preclinical investigations; the same techniques can be applied to microstructural analysis of excised human bone specimens [32]. Figure 2

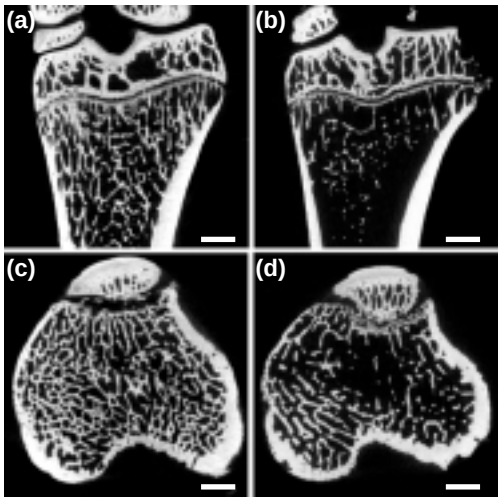

Figure 2. Cross-sectional microscopic computed tomography (micro-CT) images of excised rat limbs

Images were obtained from sham-operated **(a,c)** and ovariectomized **(b,d)** Sprague–Dawley rats (80 kVp, 0.5 mm added Al filtration, 14 μm isotropic voxel spacing). Micro-CT data facilitates measurement of changes in bone density and micro-architecture, such as the significant loss of trabecular bone in the ovariectomized animals (b,d). (Specimens courtesy of A.B. Hodsman, P.H. Watson and L.J. Fraher, Lawson Research Institute, St Joseph's Health Centre, London, Canada.) Scale bars, 1 mm.

illustrates an application of micro-CT techniques in osteoporosis research. These images were acquired in excised specimens from sham-operated (Fig. 2a,c) and ovariectomized (Fig. 2b,d) rats using an *in vitro* micro-CT system. 3D data such as these can be easily reformatted into coronal, sagittal or transverse viewing planes to investigate changes in bone-mineral content and bone architecture.

Phenotype evaluation

Significant advances in the development of transgenic and knockout animal models of human disease have made whole-animal imaging an important new application for micro-CT. In many studies of genetically altered animals, investigators require a non-destructive, 3D technique to characterize the phenotype of the animal. This approach has been used successfully in investigations of skeletal structure in transgenic mice [33] and in knockout mice [34,35]. It is also possible to use micro-CT as a quick and effective method for assessing phenotypic parameters of soft tissue, such as muscle thickness or fat, in populations of mice [36], although body composition can also be evaluated effectively with magnetic resonance (MR) techniques [37]. Figure 3 illustrates micro-CT image data acquired post-mortem in an intact normal mouse, demonstrating the type of image data that micro-CT can provide for the evaluation of the phenotype of whole animals.

Vascular imaging

Unlike bony anatomy, blood vessels provide very little inherent contrast for CT imaging. The implementation of novel vascular contrast agents has resulted in several new applications for micro-CT in the evaluation of microvascular anatomy [38]. *In vitro* imaging of vascular specimens is made possible by casting the vasculature *in situ* with a silicon-based compound (Microfil MV-122, Flow Tech, Carver, MA, USA) containing lead chromate [11]. This approach has been used to investigate the vasculature of the rat kidney [39,40], heart [41] and liver [42]. It has also been used to study the microvasculature of larger animal models, such as pigs, through the investigation of excised specimens [43,44]. Figure 4 illustrates the level of detail that can be achieved in the vasculature of a rat, using contrast-enhanced micro-CT imaging.

Limitations

There are numerous exciting applications for micro-CT in laboratory investigations; however, some fundamental

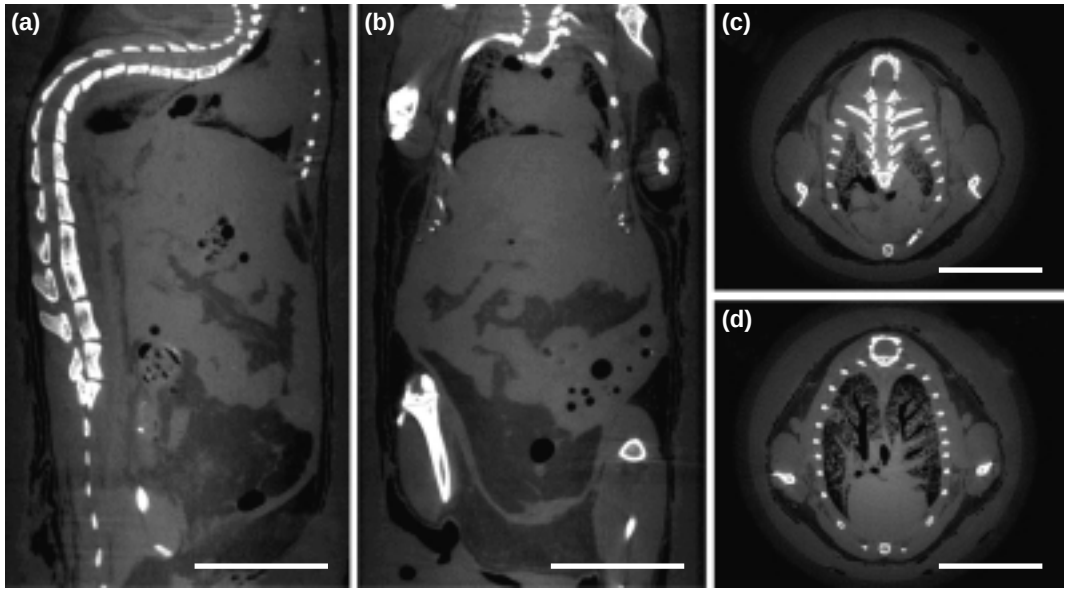

Figure 3. Cross-sectional microscopic computed tomography (micro-CT) images of the torso of an intact normal mouse

The mouse was obtained post-mortem with a rotating-gantry system (80 kVp, 0.5 mm added Al filtration). Multiplanar reformatted images of the volumetric data have been generated to provide **(a)** sagittal and **(b)** coronal views with 100 μm isotropic voxel spacing. Also shown are transverse sections through the cervical spine **(c)** and lung **(d)**, reconstructed with 50 μm isotropic spacing. Note that, in these images, bright objects are high density (bone) and black represents air voids within the animal. Scale bars, 1 cm.

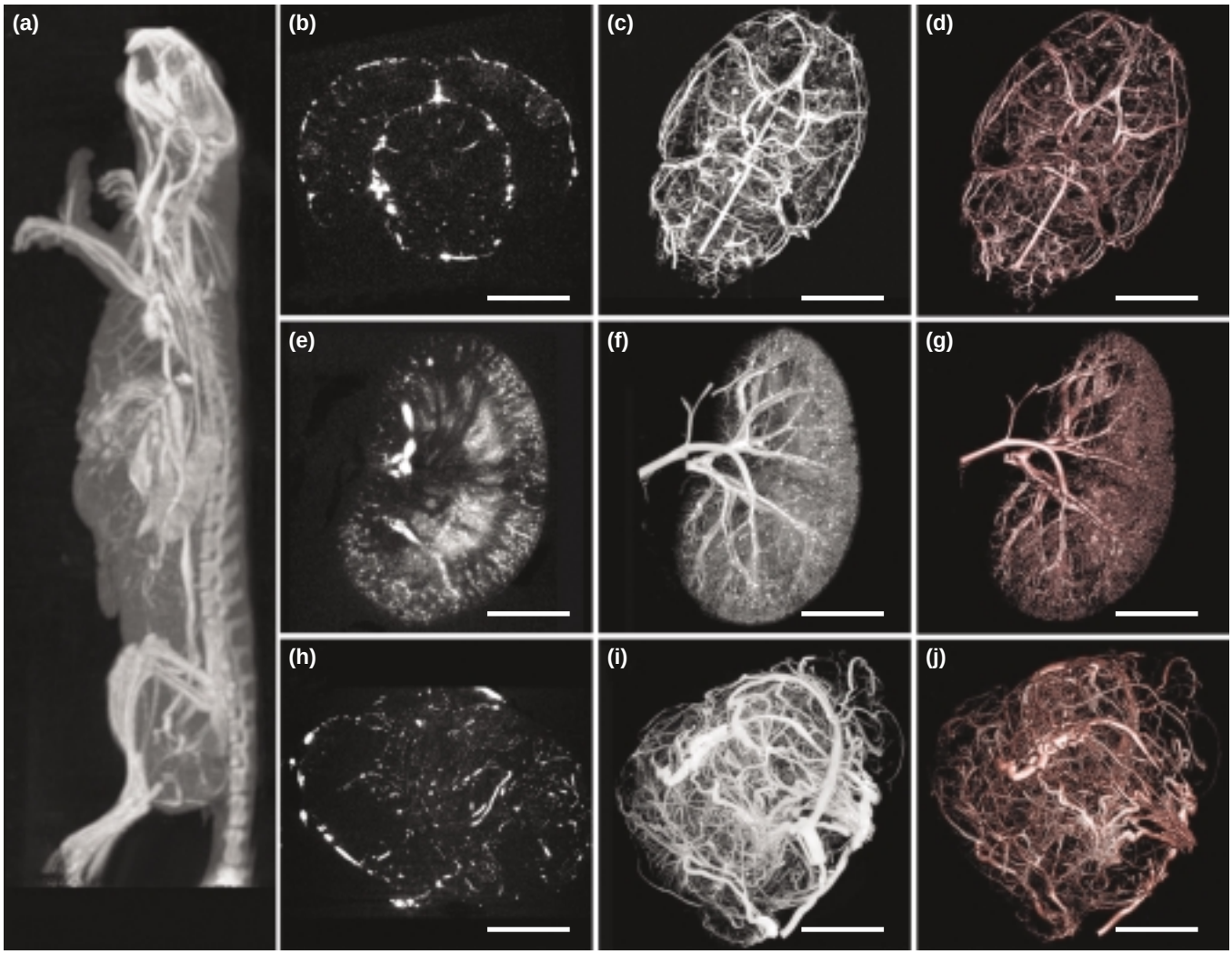

Figure 4. Demonstration of the capabilities of microscopic computed tomography (micro-CT) in vascular imaging

In this case, an intact Sprague–Dawley rat has been sacrificed and perfused with Microfil MV-122 contrast agent, facilitating the visualization of bones and vessels throughout the animal. **(a)** A low-resolution maximum-intensity reprojection through a clinical CT image of the intact rat. The animal was part of an investigation of a mammary fat pad tumour model, and had developed a 1.5 cm tumour. After sacrifice, excised organs were scanned in a micro-CT scanner (80 kVp, 0.5 mm Al, 27 μm isotropic voxel spacing), illustrating the ability to visualize small arterioles in the brain **(b–d)** and kidney **(e–g)**, and abnormal vessels within the tumour **(h–j)**. The volumetric data can be viewed with any type of post-processing algorithm; illustrated here are multiplanar reformatted images (b,e,h), maximum intensity projections (a,c,f,i), and volume renderings (d,g,j). One of the main advantages of this technique is the ability to investigate the 3D connectivity of the vascular tree within each organ. (Data courtesy of L. Friesen-Waldner and B.K. Rutt, Robarts Research Institute, London, Canada.) Scale bars, 0.5 cm.

limitations must be borne in mind. Foremost is the inherent use of ionizing radiation, which results in potential risks to operating staff and research animals. The compact design of small animal micro-CT scanners facilitates X-ray shielding, such that there should be essentially no exposure to ionizing radiation for the operator. Of course, exposure to the animal is unavoidable, and could approach the lethal dose for small rodents (~6 Gy) in scans that combine both high-resolution and low-noise. The impact of this dose of ionizing radiation must be considered during any longitudinal experiment with live animals. Additional technical limitations include the fact that CT reconstruction normally requires the acquisition of an X-ray projection of the entire object, not a truncated portion [45]. This is true even if reconstruction of only a smaller sub-field of view (FOV) is desired. This constraint forces the use of sufficiently large FOVs, with a correspondingly large number of X-ray detectors.

There are also several known artifacts in CT imaging, each of which can interfere with quantitative analysis [46]. One of the most serious of these artifacts is associated with images of extremely dense objects, such as bone, metal and concentrated contrast media. Images of these objects can contain streaks with erroneous signal values, although techniques exist to compensate for these errors [47]. In any case, soft-tissue contrast is inherently poor with X-ray CT, and the development of additional contrast agents might be required to discern some anatomical features *in vivo*.

Alternative imaging techniques

Micro-CT is only one of several available techniques for micro-imaging; alternative approaches include MR microscopy, micro positron-emission tomography (PET) and ultrasound microscopy, all of which have been described in previous reviews [48–50]. MR microscopy provides the advantages of excellent soft-tissue contrast and spatial resolution ranging from 50–100 μm [51,52]. MR microscopy has been applied to the study of bone [53] and cartilage [54], and in the determination of anatomic phenotype in small animals [55]. Micro-PET systems can provide information on both the anatomy and function of living organisms, using positron-emitting labels that attach to specific molecular targets [50,56]. Micro-PET is practically limited in spatial resolution to ~1.5–2 mm, owing to a combination of intrinsic factors and the finite range of the positron [57]. Ultrasound microscopy, implemented with high-frequency (40–60 MHz) transducers, can also provide 50 μm resolution, with the added benefit of real-time imaging capabilities [49]. Ultrasound microscopy is particularly well-suited to the investigation of mouse embryonic development [58] and image-guided manipulation *in vivo* [59]. Each of the alternative techniques described here provides unique information that is often complementary with other approaches, including micro-CT.

Concluding remarks

Micro-CT has become a mature technique that can augment or replace histological analysis in many research applications, with a major advantage being that it does not interfere with any additional anatomical or mechanical study on fresh or fixed tissue. The major application of micro-CT to date has been in quantifying the density and architecture of bone, but there are rapidly growing applications in vascular studies and in the characterization of the phenotype of transgenic and knockout animal models during preclinical investigations. Micro-CT will probably become a standard tool in many laboratories in the future, owing to the quantitative 3D data that it provides. Micro-CT is also cost-effective in comparison to serial histological analysis because it often requires little sample preparation and relatively simple data post-processing. Future technical advances will probably include much faster 3D scanning techniques and the development of new contrast techniques to provide detailed images of the vasculature of live animals.

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