Micro-Computed Tomography of the Lungs and Pulmonary-Vascular System

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Three-dimensional imaging of the intact lung and its vasculature is essential if the hierarchical and volumetric aspects of its structures and functions are to be quantitated. Although this is possible with clinical multislice helical CT scanners, the spatial resolution does not scale down adequately for small rodents for which cubic voxel dimensions of 50–100 μm are required. Micro-computed tomography (micro-CT) provides the necessary spatial resolution of 3D images of the intact thoracic contents. Micro-CT can provide higher resolution so that basic micro-architectural structures, such as alveoli, can be individually visualized and quantitated. Dynamic events, such as the respiratory and cardiac cycles, can be imaged at multiple time points throughout a representative cycle by coordinating the scan sequence (i.e., gating) to the cycle phase of a sequence of cycles. Fusion of the micro-CT image data with other image data, such as micro-SPECT or histology, can enhance the information content beyond the mainly structural information provided by micro-CT. Conventional attenuation-based X-ray imaging can involve significant X-ray exposures at high spatial resolutions, and this could affect the phenotype (e.g., via interstitial fibrosis) and genotype (e.g., via mutation), so its use in longitudinal studies using micro-CT may be limited in some cases. However, because of recent developments in which the phase shift or refraction of X-rays rather than attenuation is used, the X-ray exposure may be significantly reduced.

Keywords: microarchitecture; pulmonary hypertension; alveoli; airways

Micro-computed tomography (micro-CT) is a rapidly developing imaging capability that has been described in detail elsewhere (1). This development is driven in large measure by the fact that because mice are rapidly becoming the experimental animals of choice for many research endeavors, there is motivation to scale clinical imaging capabilities down to the mouse level. There are several aspects to the scaling of the spatial and temporal resolution and volume scanned (2). One is to scale so as to generate a transaxial tomographic image equivalent to a clinical scan. Thus, with clinical multislice helical scanning, CT of the thorax generates 3D images that are approximately 300 to 400 mm in transverse diameter and are made up of voxels (a 3D picture-element or pixel) $\sim 1 \text{ mm}^3$ in volume. A “mini” CT scanner that is scaled to a proportional voxel resolution in a mouse (with a 20 mm thoracic diameter) would involve voxels $\sim 75 \mu\text{m}$ in diameter. The mouse’s lungs have a combined volume of about 1.3 cm^3 ; therefore, in the mouse a 3D volume of at least 2 cm^3 must be imaged to capture the entire lung. How-

ever, the mouse heart and respiratory rate are much higher than in humans, so cardio-respiratory dynamic processes are much more difficult to image in mice than in humans (3) because the micro-CT scan durations are generally limited by the rate of X-ray production.

If microscopic structures are to be imaged with micro-CT, image resolution needs to be higher than that required for “mini” CT. For instance, because murine alveolar diameter is $\sim 40 \mu\text{m}$ and alveolar membranes are $< 10 \mu\text{m}$ thick, adequate imaging of these structures requires voxels of the order of $5 \mu\text{m}$. Despite this high resolution, the entire volume of the lung must be imaged because some diseases start in small localized regions; the entire lung is searched so the initiation site is not missed (4).

Use of mice often involves characterizing the phenotype of a genetic variant and/or sequentially after a disease process to establish its natural history and/or response to treatment. X-ray CT involves radiation exposures that increase approximately as the cube of the resolution (5) (i.e., halving a cubic voxel’s side length requires an eightfold increase of radiation). This is of some concern because the radiation may alter the phenotypic expression or result in neoplasia. The X-ray dose experienced by a mouse in a typical micro-CT scanner is $< 1 \text{ Gy}$ (100 rad) (6), which represents approximately 5% of the dose that kills 50% of mice within 30 d of exposure. Hence, multiple scans used to capture dynamic events and/or to follow disease progression over a period of months could result in significant cumulative radiation exposures.

Despite these issues that must be considered when planning use of micro-CT scanning in mice or other small animals, the attractions of this approach are that multiple cubic centimeters of intact organ can be imaged at near-microscopic resolution and that structural continuity within vascular and bronchial trees is retained, something that cannot readily be achieved with conventional microscopic imaging and associated image analysis methods. In cases where the CT images cannot provide the cellular or molecular information desired, subsequent histologic and/or radionuclide image analysis of the scanned specimen can be spatially registered to the micro-CT images, which provide synergistic information. Such an approach requires that the specimen be prepared for the CT study without destroying the capability of the subsequent cellular/molecular methods to be used.

METHODS

All CT scanners follow a basic approach that involves a point-source X-ray source, a large array X-ray detector system, and a mechanism for rotating the specimen relative to the X-ray source/detector scanner (i.e., rotating the specimen or the scanner system) (7). With micro-CT, several significant modifications that are not valid for use in clinical CT scanners can be used to advantage. One of these is the use of “contact” X-ray imaging, where the specimen is held close to the fluorescing crystalline plate (which converts the X-ray image to a light image) detector array so that optical magnification of the X-ray can be achieved to provide the increased spatial resolution (8). This approach does not require a microscopic X-ray focal spot; therefore, a higher flux of X-ray can be generated. It also has the advantage

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that the geometry is less of an issue for the tomographic image reconstruction process. The usual approach involves X-ray cone-beam magnification where the specimen is held close to the focal spot and some distance from the detector array so that the X-ray beam divergence supplies the image magnification, as permitted by the penumbral effect of a small focal spot and the reconstruction algorithm's ability to cope with the cone-beam angle. The other is that lower-energy X-ray photons (e.g., 20 keV) can be used to provide increased soft tissue contrast (9) but with the disadvantage that this can generate more severe beam-hardening artifacts (i.e., the X-ray beam becomes less attenuating as it progresses through the specimen), which results in incorrect gray scale values in the CT image. Consequently, as close to monochromatic radiation as possible should be used, and this can be achieved with an appropriate X-ray source anode material and a metal foil filter. Where cardio-respiratory motion may blur the image if anesthetized animals are scanned, higher-energy photons (e.g., 80 keV) can be used to provide the required image data more quickly, albeit at the expense of reduced soft tissue contrast. The lung has inherent high tissue contrast; therefore, the higher X-ray photon energies are generally acceptable.

Because the respirations can be arrested, end-inspiration and end-expiration states can be maintained for the duration of exposure needed for each angle of view (i.e., the respiratory cycle is "gated" to the scanner capability provided the duration of recording each angle of view is of the order of 1 s). The heart rate being of the order of 5 or more heart cycles per second would tend to blur out the cardiogenic distension of arteries and the cardiac ventricles to the longest duration phase of the cardiac cycle (i.e., diastole) (10, 11).

Some transient processes are not cyclic (e.g., a transient bolus of intravascular contrast agent or an inhaled bolus of xenon gas) and cannot be imaged by gated scanning. One approach that can be of use is cryostatic micro-CT in which the *in vivo* organ is quickly harvested and snap frozen at a selected time point within the transient process (12). The frozen specimen can be scanned, at leisure, while it is frozen. The added advantage of this method is that the specimen can be used for sophisticated histo-immuno-chemical histology or biochemical analysis (13) because the tissue is not altered by the usual fixation processes, such as immersion in formalin.

An important component of micro-CT imaging is the contribution provided by the choice of contrast agent. Contrast agents can be selected to give prominence to specific physiologic spaces.

Examples are the blood vessel lumen via iodine-based nanoparticles (14, 15) *in vivo* or lead chromate-doped silicon polymers (e.g., Microfil; Flow Tech, Inc., Carver, MA) postmortem (16). The *in vivo* extravascular space can be delineated by nonionic contrast agents that pass through the microvascular endothelium (17) and the airway epithelial surface by tantalum powder inhalation (18). Unfortunately, it is explosive when aerosolized, whereas an alternative, tantalum oxide, is not (19). Some tissue components (e.g., cell membranes) that are selectively "stained" by osmium tetroxide postmortem can be used to provide some information about the cells (20). The combination of micro-SPECT with micro-CT also makes for synergistic imaging (21).

RESULTS

The main strength of micro-CT is the quantitative characterization of 3D anatomy in the case of the lung the vasculature and the airways. Figure 1 is a 3D micro-CT image of a mouse pulmonary circulation within the intact thorax and shows the virtual "dissection" of the opacified pulmonary arterial tree down to the microvascular domain.

Figure 2 is a maximum-intensity projection of the 3D micro-CT image of a rat lung, one from a control rat and the other from a rat with pulmonary hypertension induced by intraperitoneal injections of monocrotaline (22). The reduction in lumen diameter, especially in the distal vessels, is obvious to visual inspection. The hemodynamic implications (e.g., resistance to flow) of this pattern of vascular narrowing can be deduced by performing a fluid dynamics analysis (e.g., using Poiseuille's law) of the quantitated 3D dimensions of the vascular tree's 3D image (23).

Although mice are of particular interest, biopsies or postmortem tissue specimens taken from large animals can also be scanned to provide useful data not readily available by conventional methods. Figure 3 (p. 501) is a display of the 3D micro-CT image of an $\sim 2 \text{ cm}^3$ segment of porcine lung after injection of Microfil contrast agent into the aorta. This shows the network of opacified bronchial arteries surrounding the air-filled bronchi. To illustrate the power of micro-CT in its application to examining pathophysiologic processes, Figure 4 shows the ability of micro-CT to quantitate the effect of endotoxin on alveolar membrane edema (24).

Micro-CT of the dynamics of pulmonary artery branching geometry, in combination with high-resolution dynamic projection imaging, has provided new information about the dynamics of pulmonary arterial anatomy and transit time characteristics of pulmonary blood flow along the pulmonary arterial tree (25, 26).

Figure 1. Left panel: Volume-rendered display of a 3D micro-CT image of the rib cage and pulmonary vessels (injected with radiopaque Microfil) of the thorax of a dead mouse. Middle panel: Display of the segmented pulmonary arteries and veins after the surrounding ribs have been selectively removed from the 3D image. Right panel: The central pulmonary artery is displayed after pruning of its branches performed by image analysis software. The insert shows detail down to the 20- μm voxel limit of resolution.

Figure 2. Computer-generated projection display of the pulmonary arterial tree within isolated rat lungs. *Left panel* is the image of the pulmonary artery in a control rat. *Right panel* shows a pulmonary artery from a rat with pulmonary hypertension due to monocrotaline injection.

DISCUSSION

The current state of the art in micro-CT imaging is providing much useful experimental data. However, its limitations for *in vivo* scanning in terms of scan speed, radiation exposure, and soft-tissue contrast differentiation remain major challenges. There have been several attempts to speed up the scan by the use of multiple X-ray sources (thereby providing more angles of view at essentially the same time), the use of higher-intensity X-ray sources to reduce the exposure time per angle of view, and by attempting to eliminate physical rotation of the X-ray source about the horizontal specimen by electronic movement of the focal spot. For all these advances, the fundamental problem that remains is that with conventional attenuation-based

X-ray imaging, the signal is the reduction in transmitted X-ray photons. To have a signal (i.e., change in attenuation) that adequately exceeds the photon noise (the statistical noise due to the quantum nature of the X-ray photons $\approx \sqrt{N}$, where N is the number of photons per resolution unit), at least 10^4 – 10^5 photons have to be exposed to the region of interest per resolution unit (27).

A methodology that shows some promise toward practical use is X-ray imaging, which is based on the consequences of refraction of X-rays (resulting from the slightly different velocity of X-ray photons through different materials, essentially the same as the refraction of light) within the tissues (28, 29). This has been shown to increase the density resolution of soft tissues tenfold or more over current attenuation-based imaging, but it also has the potential for reduced radiation exposure because the signal is the phase shift or refraction of individual photons rather than the reduction in numbers of photons. This approach is not practical for routine experimental use, but it seems to be a promising approach. This method should be particularly appropriate for use in the lung because the optical properties of the air-filled, thin-walled alveoli should have a specific “signature” impact on the phase image in proportion to the alveolar wall thickness and/or alveolar diameter (30).

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Figure 4. Typical gray-scale inverted micro-CT images of a rat lung isolated after intravenous infusion of barium sulfate-gelatin thymol or normal saline. *Panel A* was obtained after infusion of lipopolysaccharide endotoxin. *Panel B* was obtained from the control group (without endotoxin infusion). Capillaries are filled with barium sulfate-gelatin thymol. Note the interstitial edema causing the swollen connective tissue space and the “unsharp” capillaries. *Panel B* is a similar micro-CT image of normal lung after treatment with normal saline. Section thickness = 6 μ m; X-ray voltage = 60 kV. (Reproduced with permission from Reference 24.)

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