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Molecular Imaging in Small Animals—Roles for Micro-CT

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Abstract X-ray micro-CT is currently used primarily to generate 3D images of micro-architecture (and the function that can be deduced from it) and the regional distribution of administered radiopaque indicators, within intact rodent organs or biopsies from large animals and humans. Current use of X-ray micro-CT can be extended in three ways to increase the quantitative imaging of molecular transport and accumulation within such specimens. (1) By use of heavy elements, other than the usual iodine, attached to molecules of interest or to surrogates for those molecules. The accumulation of the indicator in the physiological compartments, and the transport to and from such compartments, can be quantitated from the imaged spatial distribution of these contrast agents. (2) The high spatial resolution of conventional X-ray attenuation-based CT images can be used to improve the quantitative nature of radionuclide-based tomographic images (SPECT & PET) by providing correction for attenuation of the emitted gamma rays and the accurate delineation of physiological spaces known to selectively accumulate those indicators. Similarly, other imaging modalities which also localize functions in 2D images (such as histological sections subsequently obtained from the same specimen), can provide a synergistic combination with CT-based 3D microstructure. (3) By increasing the sensitivity and specificity of X-ray CT image contrast by use of methods such as: K-edge subtraction imaging, X-ray fluorescence imaging, imaging of the various types of scattered X-ray and the consequences of the change in the speed of X-rays through different tissues, such as refraction and phase shift. These other methods of X-ray imaging can increase contrast by more than an order of magnitude over that due to conventionally-used attenuation of X-ray. To fully exploit their potentials, much development of radiopaque indicators, scanner hardware and image reconstruction and analysis software will be needed. J. Cell. Biochem. Suppl. 39: 116–124, 2002.

Key words: X-ray; PET; SPECT; cell labeling; transport; contrast; indicators

INTRODUCTION

Imaging involves quantitation and discrimination of various materials at selected regions within the body. It is the arrangement of these regional values into a planar array that constitutes an image of those characteristics within that plane within the body. Up until recently, the predominant application of X-ray CT imaging has been the detailed description of anatomic structures and their 3D spatial interrelationships within the body. Additional applications are currently being explored to extend the utility of X-ray micro-CT imaging into the field of molecular imaging.

The Stimuli for Molecular Imaging

Since the mid 1990s, there has been a rapid increase in the need to screen small animals for drug discovery and genomics purposes [Service, 1999]. This need is seen to be well met by use of special-purpose microimaging methods because it has the potential for accurate measurement of organ anatomy and the spatio-temporal distribution of function within the organs in a minimally invasive manner. The minimally invasive aspect is required when longitudinal studies, which are statistically preferable to cross-sectional studies, are desired and in genomics it means that once the genetically expressed modification of interest is identified, the (now) valuable animal is left intact for more detailed study, use as a disease model and/or reproduction.

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The Current Microimaging Repertoire

There are currently a number of imaging methods that are used for study of small animals. This article will focus on X-ray micro-CT but other methods (each using a different energy "probe," and therefore with their particular advantages and limitations) include high resolution Nuclear Magnetic Resonance (MRI) [Smith et al., 1998], Ultrasound (US) [Turnbull and Foster, 2002], single-photon gamma rays (SPECT) [Weber and Ivanovic, 1999], positron-annihilation gamma rays (PET) [Chatziioannou et al., 2001] and Bioluminescence (BLI) [Rice et al., 2001]. The particular advantage of X-ray micro-CT imaging is that it can generate a 3D image of an intact volume greater than 1 cm^3 in size at spatial resolutions down to micrometers.

Special Challenges and Opportunities

Scaling. The volume of mammals' internal organs scale with their body weight [Calder, 1984]. Hence, if we wish to merely scale the current clinical images (with CT image voxel, or 3D pixel, dimensions of the order of $(1mm)^3$) to a mouse, we need to have a scanner that has voxels of the order of $(100\ \mu m)^3$ for a 25 g adult mouse or $(30\ \mu)^3$ for a neonatal mouse. However, a mouse's Basic Functional Units (BFU—the smallest aggregation of diverse cells within an organ that functions like the organ, such as an hepatic lobule or a nephron, are of the order of $(100\ \mu m)^3$) and are approximately the same size in humans and mice. Thus, if 'clinical' images scaled to the animal are desired, it would not be necessary to scale below the size of a BFU. However, with a voxel size of less than $(50\ \mu m)^3$ it is possible to also obtain information not achievable in clinical scanners, such as the number, size, and packing of the BFUs. With voxel sizes less than $(10\ \mu m)^3$ even cellular dimensions, such as osteoclast erosions in the surface of bone [Ritman et al., 1998], and with voxel's less than $(1\ \mu m)^3$, subcellular dimensions can be imaged [Stampanoni et al., 2002].

Physics and technology. Although scaling down the voxel size involves machining the scanner components down to a smaller size (which has technological implications for precision tolerances and more efficient component packing) it also means that different aspects of the well understood physics underlying CT scanning must be accommodated. Thus if the X-ray photon-energy used is less than 25 keV, the X-ray interaction with matter is predominated by the photo-electric effect whereas in clinical scanners, in which the photon energy generally exceeds 50 keV, it is predominantly Compton scatter [Dowseth et al., 1998]. A desirable feature of the low photon energy is that the X-ray attenuation is up to two decades higher than it is in clinical CT images, thereby allowing better discrimination of soft tissue types. Another consequence is, however, that if maximum signal-to-noise ratio is to be achieved, the X-ray photon energy must be adjusted to match the object diameter [Grodzins, 1983] and the radiation should be essentially monochromatic if excessive imaging artifacts such as X-ray beam hardening are to be minimized. This has the implication that individual, scaled, scanners must be used for individual mice because the simultaneous scanning of multiple mice within a clinical scanner will result in suboptimal image signal-to-noise and resolution. Analogous physics aspects (e.g., high rates of change of magnetic field in MRI, high frequency ultrasound in B-mode US, the fixed distance between positron emission and its annihilation in PET, and the limitations of collimation of gamma rays in SPECT) must be considered in the other imaging modalities when they are used at high spatial resolution in the small, intact, animal.

X-RAY MICRO-CT AS THE PRIMARY METHODOLOGY

Capabilities of X-Ray CT Imaging

X-ray micro-CT for biomedial applications was first developed for the study of cancellous bone microarchitecture [Feldkamp et al., 1984]. These systems used bench-top X-ray sources with small (less than 100 μm) diameter focal spots because this was necessary when the inherent cone beam divergence of the X-ray beam from that focal spot was used to magnify the projected X-ray shadow onto a large-area X-ray imager. The problem with this approach is that the intensity in the X-ray image is limited by the intense heating of the small focal spot, which could readily melt the anode material. However, when synchrotron radiation was used [Margaritondo, 1988], which is very intense, more rapid scanning is possible [Kinney et al., 1995]. Some cyclic motions such as due to the heart beat can be accommodated by acquiring the necessary scan data incrementally at the

same phase of the cardiac cycle of sequential heart beats. However, non cyclic transient events, such as the progressive accumulation or washout of a contrast agent from a physiological space, cannot be imaged by such a gated scanning method. The transient process can, however, be literally snap-frozen within the tissue of interest and that frozen specimen can then be scanned while frozen [Kantor et al., 2002].

Essentially, all current X-ray micro-CT scanners use the attenuation of the X-ray by tissues as the signal for generating the X-ray images. This means that the contrast signal is closely related to the electron density [Rutherford et al., 1976]. At the X-ray exposures tolerated by living tissues, this means that the signal-to-noise in the CT image is adequate only for differentiating air, fatty tissue (e.g., brain white matter), non fatty tissue (e.g., muscle, brain grey matter), and bone (within bone several layers at different stages of mineralization can be distinguished). In some tissues there are heavy elements (e.g., iodine in the thyroid, iron in hemoglobin and in the hemachromatotic liver) at concentrations which just reach a level at which pathological increases, or decreases, can be detected by change in CT image contrast [Riederer and Mistretta, 1977; Dilmanian, 1992].

The discrimination of different physiological spaces can be achieved by selectively opacifying those spaces with administered contrast agents. The most commonly used is intravascularly injected iodine-labelled carbohydrates which selectively opacify the blood within the blood vessel lumens [Paroz et al., 1987]. As some of this contrast agent leaks through the vascular endothelium (especially if it is impaired by reduced oxygen levels or because it is newly formed, as is often the case in malignancies) and remains in the extravascular space for up to several minutes, the extravascular space and endothelial permeability can also be estimated from the images [Ritman, 1994]. As these contrast agents are generally preferentially excreted through the kidney, the opacification of the nephrons (which occurs over many minutes) can be used to quantitate several aspects of renal function as well [Lerman et al., 1999].

A major limitation of X-ray is its ionizing effect and this can result in immediate radiation damage (largely mediated by superoxides and free radicals) and in the long term by genetic damage [Dowseth et al., 1998]. As the signal-to-noise of X-ray attenuation imaging depends largely on the number of X-ray photons detected per voxel of the 3D image (i.e., the X-ray exposure has to increase with increasing spatial resolution [Chesler et al., 1977]), its sensitivity, therefore, is ultimately limited by its potential for radiation damage.

Capabilities of Small Animal X-Ray Micro-CT

Much has, and continues to be, published about the scope in applications and the resolution of micro-CT based imaging of small mammals and biopsy-sized specimens from larger animals and humans. The micro-anatomic information in these images has been used to define the phenotypes of a large range of genetically-based pathophysiological states such as polycystic disease in the liver and kidneys, osteopetrosis of bone, and high aerobic capacity of skeletal muscles. The breadth of this application of micro-CT continues to be advanced by increasing spatial, temporal, and contrast resolution by incremental technological improvements in the X-ray sources (especially reduced focal spot size, increased heat loading capacity, narrower bandwidth of photon energies—i.e., quasi monochromatic), X-ray imaging detectors (especially larger arrays of smaller detector elements and more efficient coupling of the X-ray-to-charge conversion process), and implementation of helical scanning for increasing the length of an intact specimen imaged and use of multiple X-ray sources and their corresponding detector arrays to decrease the scan duration. Considerable activity is also involved in improving the algorithms used for doing the tomographic reconstruction—either to reduce the approximations used in algorithms such as used for cone beam reconstruction (an inevitable consequence of use of a small focal spot X-ray source), to overcome CT image artifacts resulting from incomplete scanning data sets—such as by local reconstruction [Ritman et al., 1997], or to increase spatial resolution beyond what would normally be expected for the scanner's detector element size [Faridani and Ritman, 2002] (Fig. 1).

Radiopaque Indicators of Physiological Spaces and Processes

The most commonly used clinical contrast agents are based on iodine or barium. Iodine is

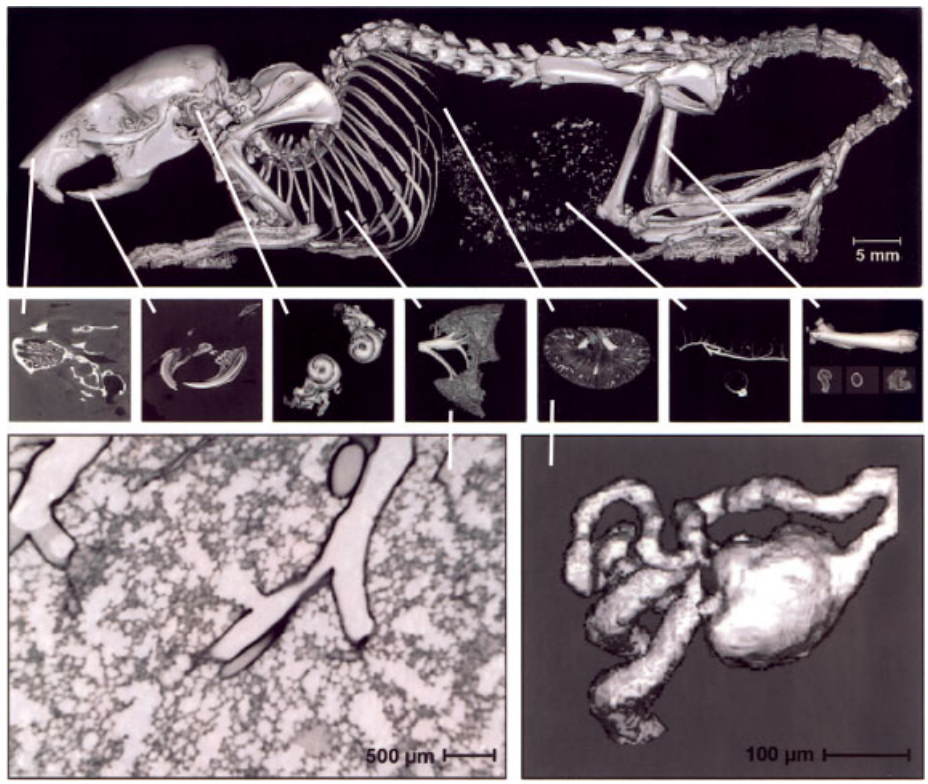

Fig. 1. Multi-resolution micro-CT images of a mouse. **Upper panel**—volume-rendered image thresholded to show just the skeleton at 20 µm voxel resolution; **mid panels**—selected organs displayed at same voxel resolution and at various thresholdings to show aspects of the soft tissues; **left lower panel**—basic functional units (lung terminal bronchiole and its alveoli) at 5 µm voxel resolution; **right lower panel**—glomerulus and tubules at 2 µm voxel resolution. (Reproduced with permission from [Ritman et al., 1998])

attractive because it is readily attached to sugars, which are tolerated at relatively high concentrations in blood and barium because it forms insoluble salts and therefore useful for opacifying the gut or airway. Other elements such as bromine for bowel [Gonzalez et al., 1986] and xenon [Fenster, 1978] for aeration of the lung and for cerebral perfusion are also used on occasion—primarily in research settings. The relatively low sensitivity of X-ray attenuation imaging to these agents requires that relatively high concentrations are needed for quantitation of local concentrations of the contrast agent in small regions of interest. Clinical CT scanners need at least 10 mg iodine per cm^3. This brings with it the problem of the physiological perturbation caused by the high concentration of the indicator as well as the perturbation caused by the physical characteristics of the volume, viscosity, and specific gravity of the injected contrast agent [Paroz et al., 1987].

For micro-CT it is also possible to use heavy elements which replace, or are physiologically treated as, the normal elements. An example is the use of strontium (Fig. 2) as a surrogate for calcium in bone deposition [Ritman et al., 1998]. In this case a short duration of strontium loading, repeated at, say, a month's interval, will provide two, separated, narrow bands of strontium laid down in the newly deposited calcium in bone. This can be used to quantitate local bone growth or resorption and relate it to local 3D microarchitecture (which can be used, for instance, to compute local mechanical stress and strain under loading). Another method is use of osmium tetroxide staining because in preferentially 'stains' structures high in lipid content, such as cell walls, in high resolution micro-CT images (Fig. 3).

X-Ray Micro-CT as a Complementary Methodology

No one imaging modality can provide all the information about microstructure, function, and molecular processes in one image. Indeed scanners within each modality need special

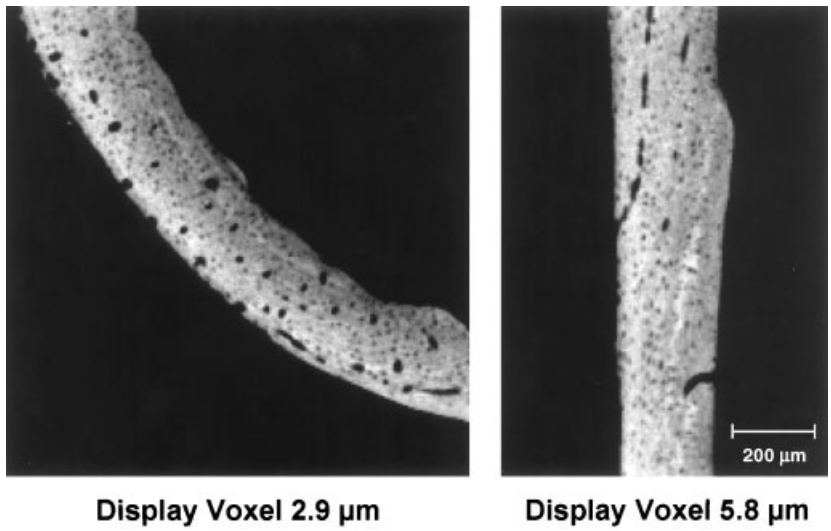

Fig. 2. The **left panel** shows a micro-CT image (5.8 µm cubic voxels) partial transverse section of the intact rat tibia and the **right panel** shows a longitudinal section through that same bone cortex. Note the narrow bright ''line'' within the bone—the location of strontium accumulation. The spacing between that ''line'' and the surface of the bone indicates the new deposition of bone since the intrapertoneal injection of a bolus of 250 mg/kg of strontium chloride solution 48 h before sacrifice. (Reproduced with permission from [Ritman et al., 1998])

emphasis of a particular physics-aspect in order to optimize the imaging of a particular aspect of organ microstructure and/or function. Consequently, no one imager is likely to provide even the full range of the possible applications that are possible for any one imaging modality. Examples are the commitment that needs to be made to discrete spectral energies available for an X-ray CT scanner, to the size of the coils of the MRI, to the crystals (frequency) of the US scanner, and to the gamma ray energy and radionuclide half-life of the radionuclides used for PET and SPECT imaging.

In addition to a particular scanner's limitations, there are the inherent limitations of the imaging modality. However, such deficiencies

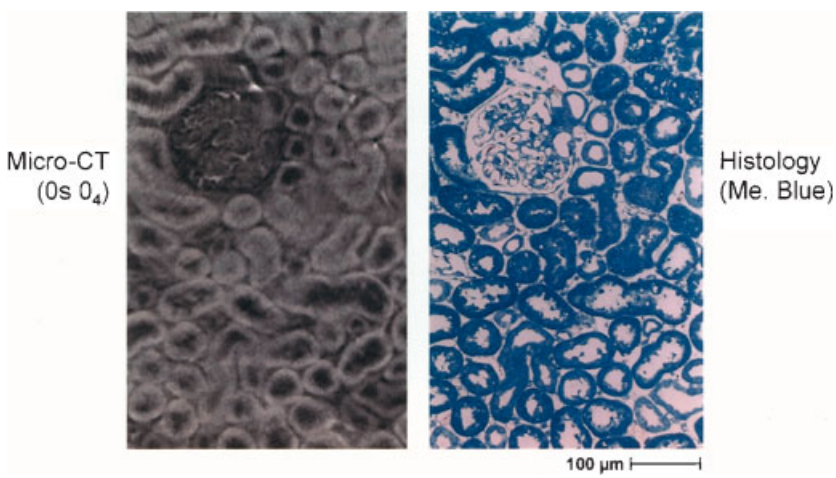

Fig. 3. **Left panel** shows a micro-CT image (1.0 µm cubic voxels) and the corresponding, subsequently acquired, histological section stained with methylene blue (**right panel**) of the cortex of a rat kidney. The tissue was stained with 1% osmium tetroxide and embedded in plastic prior to having its micro-CT scan. After the micro-CT scan, 1 µm thick histological sections were prepared. The sections show glomerulus and tubular components. The micro-CT scan was done at the synchrotron Brookhaven National Laboratories. (Courtesy of Dr. Michael D. Bentley, Minnesota State University, Mankato MN)

can often be overcome by combined use of another imaging modality which can provide information that greatly increases the sensitivity and/or specificity of either method. A necessary aspect of this synergistic use of multiple modalities is that accurate spatial registration of the 3D anatomy is achieved, such as illustrated in Figure 3. Ideally, this is accomplished by simultaneously operating the two modalities which are incorporated in the same scanner so that the geometric scaling and location, as well as the timing of the scans, are identical. This presents a challenging technological problem which, if not fully solved, can in part be overcome by software registration algorithms which warp, rotate, and/or translate an image within the 3D image, for optimal matching.

SPECT (4) and PET (5). SPECT (4) and PET (5) are radionuclide-based imaging methods whose great strength is the very high specificity and sensitivity of the images. This sensitivity allows use of pico-molar concentrations of the radionuclide-labelled biologically-active molecules and of labeling of relatively sparcely distributed specific cells. Unfortunately, the methods are limited by poor spatial resolution (PET more so than SPECT) and the poor photon statistics that can be achieved within a reasonable time interval (SPECT more than PET). However, X-ray CT can provide the high spatial resolution distribution of X-ray attenuation coefficients which can be used to improve the accuracy of the radionuclide imaging methods by enabling correction for the gamma ray attenuation through the tissues. Similarly, if there is a priori knowledge as to what physiological space the radionuclide is likely to accumulate in, then radionuclide activity can be constrained to that space (as determined from the X-ray CT image data) so that the concentration of that radionuclide can be more accurately estimated. Conversely, the location of the radionuclide accumulation relative to detailed X-ray CT-based 3D micro-anatomic features could provide the link between the accumulation (or lack thereof) of a selected molecule and the local function in that region.

Bioluminescent imaging. Bioluminescent imaging [Wu et al., 2001] is a very powerful methodology in that light-emitting proteins or fluorescent molecules can be attached to molecules of interest so that their transport to, and accumulation in, physiological spaces can be detected and timed. However, this method is severely limited by the strong scattering of light within tissues so that the localization and concentration are poorly quantitated. It is likely that high spatial resolution X-ray micro-CT can be used to improve the localization, and thereby the fluorescence concentration quantitation. By modeling the diffusion of light through highly scattering tissues such as the liver and the transport through tissues with light "guiding" channels, such as those containing cerebrospinal fluid in the brain or air in airways or relatively clear tissues in the thin alveolar walls of the lung [Wang et al., 1997]. The ability to compute the pathway of the light passage through the tissue can be used to inversely solve for the internal distribution of the bioluminescent molecule accumulations from the detected surface distribution of the light brightness.

Histological techniques. Histological techniques, with their broad spectrum of stains and immunochemical methods, can provide much useful information about molecular and cellular processes. A weakness of the histological method is that it is destructive and provides isotropic 3D microstructural information only with considerable difficulty. As illustrated in Figure 3, X-ray micro-CT can provide the 3D micro-anatomy which can be used to register the histological sections obtained at postmortem or in an organ biopsy. This could greatly extend the information content beyond that of both methods taken alone.

Molecular analysis methods. Molecular analysis methods, such as immunohistochemistry or mRNA quantitation, require fresh specimens and generally involve destruction of the specimen. Respectively, these methods provide either sparcely sampled or values averaged over an entire tissue sample so that local concentration distributions cannot be easily determined. Some tissue-analysis methods can tolerate fixation whereas others cannot. In the former, the 3D microanatomy obtained via micro-CT can be used to determine the fraction of the tissue sample that has, and/or the likely location where, the molecule of interest accumulates so that a more meaningful comparison between animals can be made. The latter case can be dealt with by doing a micro-CT scan of the freshly frozen specimen, while frozen, so that the specimen can subsequently be destroyed for molecular analysis without loss of the 3D micro-anatomic information [Maran et al., 2003]. Here, too, the average value of the molecular

concentration in the tissue sample can be corrected for the physiological space's volume. As illustrated in Figure 4, the 3D micro-CT images can be used to direct where the "biopsy" within the specimen should be obtained so as to enhance the likelihood that the concentration of the molecule of interest is highest in or, conversely, how that concentration is related to, microarchitectural features.

FUTURE DEVELOPMENTS OF X-RAY MICRO-CT

Medical imaging has developed rapidly since the late sixties. This is, in part, due to the enabling introduction of compact, powerful digital computational capabilities, and of large X-ray imaging arrays of small detector elements. With the recent onset of the interest in molecular and small-animal imaging methods, we can expect continued rapid development of improved small-animal and molecular-imaging methods. For micro-CT, there will be continued incremental improvements of the technology of the scanners and there are several potential physics aspects that are likely to be explored for the purposes of broadening the spectrum of contrast mechanisms that can be utilized. In addition, new contrast media will also continue to be explored, especially as indicators of specific physiological spaces (including selected cells such as is already the case with clinical therapeutics containing heavy metals such as platinum). All these methods have been shown to be feasible in very controlled experimental situations, primarily with use of synchrotron radiation [Margaritondo, 1988]. Much technological development is needed to make such approaches suitable for "routine," bench-top CT, imaging use. The following five physics principles are being explored for the purposes of extending the contrast range and sensitivity.

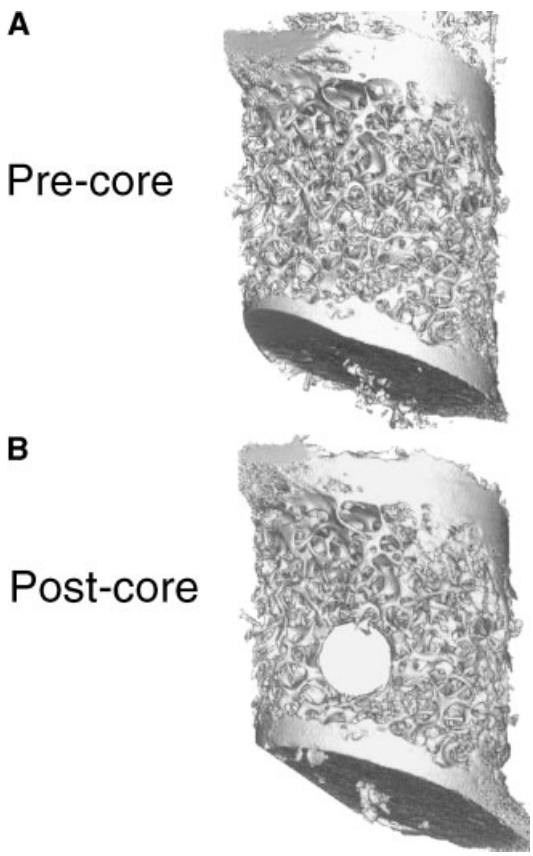

Fig. 4. Gradient shading display of a 3D cryogenic μ-CT image of a human iliac crest biopsy reconstructed using 18 μm cubic voxels. **A**: Pre-core; and **B**: Post-core. The core was 2 mm in diameter and extended through the biopsy and was used for mRNA analysis, which could then be referred to the specific micro-anatomic structures known to be in the core. (Modified and reproduced with permission from [Maran et al., 2003])

K-edge subtraction. K-edge subtraction has been explored for medical CT in the past [Riederer and Mistretta, 1977]. The method is based on the fact that every element has a well defined, up to 10-fold, sharply defined transition of X-ray attenuation value. This characteristic can be utilized by making a CT image at an X-ray photon energy just below and again just above this transition photon energy. The small difference between the local X-ray attenuation values in these two images essentially eliminates the tissues which have very slowly changing attenuation coefficient at this photon energy, thereby leaving the image of just the specific element that was selected for imaging. A concentration of 15 ng iodine/mm^3 has detected with this method. A considerable difficulty has been the generation of the tuned, narrow spectral bandwidth, X-ray photon energy needed for this method. Synchrotron imaging can overcome this difficulty, but routine access is currently very limited for large-scale applications, although this logistic bottleneck is being reduced by several new synchrotrons that have recently been, or almost to be, added at several international locations.

X-ray phase delay. X-ray phase delay [Beckman et al., 1997] can also be used for X-ray imaging. X-rays travel at different velocities through different tissues so that as an X-ray beam passes through a particular tissue a phase delay (or advance), relative to an X-ray not

passing through that tissue develops. Phase differences can be represented by interference patterns in the X-ray intensities, which in turn can be mathematically converted to actual phase delay. This method provides a tissue contrast much like that of MRI and is at least an order of magnitude higher than that achieved with attenuation-based X-ray imaging. This method is impractical in a clinical situation because of the very large number of phase shifts that occur along the X-ray beam as it passes through many centimeters of tissue, but for small animals or their in vitro organs, this method is reasonably well suited, especially when subtle differences in tissue characteristics, such as occur in nerve tissue, are of interest.

X-ray scatter. X-ray scatter [Westmore et al., 1997; Wernick et al., 2002], at both small angle and large angle (to the direction of the X-ray beam) contains much information about lower atomic number elements and to some extent the molecules along the X-ray beam path. Attenuation-based CT is very limited in terms of differenting the low atomic weight elements, let alone discriminating organic molecules. However, a difficulty with scatter imaging is the inefficient mechanics currently available for measuring, and localizing, the source of the scattered radiation.

X-ray diffraction. X-ray diffraction [Harding et al., 1987] is akin to powder spectroscopy in that the chemical bonds diffract the X-rays such that the diffracted X-ray photon energy and angle at which it emerges from the X-ray beam can be used to solve for the local concentration of certain chemical bonds. This is about as close as X-ray CT can get to direct molecular imaging, at least in terms of detecting the presence of certain chemical bonds. This method, too, is currently inefficient in terms of measuring the deflected X-rays energy from the specimen.

X-ray fluorescence. X-ray fluorescence [Takeda et al., 1996] occurs when an electron, that has been knocked out of its atomic orbit by an X-ray photon, is replaced by an electron from another orbit which results in a secondary X-ray photon being generated with a characteristic energy. This characteristic energy is the signature of a specific element. Hence, the concentration and location of that element can be accurately derived. The concentration of iodine down to 60 ng/mm^3 having been detected with fluorescence CT. A problem with this method is that the energy of those fluorescent X-ray photons is less than that of the illuminating X-rays and hence are generally quite severely attenuated and are difficult to distinguish from photons which have been scattered by other physics mechanisms.

CONCLUSIONS

Attenuation-based X-ray CT micro-imaging has its primary strengths in the fact that high spatial resolution and accurate concentrations of attenuators can be achieved, and because the physics of X-ray interaction with matter is very well understood and controllable. In conjunction with other imaging modalities, it can greatly extend the power of the other imaging methods over what they can achieve alone. Use of other aspects of X-ray/matter interaction as the basis for X-ray imaging has potential for higher sensitivity and specificity of image content.

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REFERENCES

Beckman F, Bonse U, Busch F, Gunnewig O. 1997. X-ray microtomography (μCT) using phase contrast for the investigation of organic matter. J Comput Assist Tomogr 21:539–553.

Calder WA III. 1984. Size function and life history. Cambridge: Harvard University Press.

Chatziioannou A, Tai YC, Doshi N, Cherry SR. 2001. Detector development for microPET II: A 1 microL resolution PET scanner for small animal imaging. Phys Med Biol 46:2899–2910.

Chesler DA, Riederer SJ, Pelc NJ. 1977. Noise due to photon counting statistics in computed X-ray tomography. J Comput Assist Tomogr 1:64–77.

Dilmanian FA. 1992. Computed tomography with monochromatic X-rays. Am J Physiol Imaging 7:175–193.

Dowseth DJ, Kenny PA, Johnston RE. 1998. The physics of diagnostic imaging. London: Chapman and Hall Medical.
Faridani A, Ritman EL. 2002. High-resolution computed tomography from efficient sampling. J Inverse Probl 16: 635–650.
Feldkamp LA, Davis LC, Kress JW. 1984. Practical cone-beam algorithm. J Opt Soc Am A 1:612–619.
Fenster A. 1978. Split xenon detector for tomochemistry in computed tomography. J Comput Assist Tomogr 2: 243–252.
Gonzalez CF, Osterholm JL, Triolo AJ, Bell RD, Menghetti RA. 1986. Fluorocarbon emulsion as a potential contrast medium in the subarachnoid space and brain tissue. Experiments in cats. Acta Radiol Suppl 369:554–557.
Grodzins L. 1983. Optimal energies for X-ray transmission tomography of small samples. Nucl Instr Methods 12: 541–545.
Harding G, Kosanetzky J, Neitzel U. 1987. X-ray diffraction computed tomography. Med Phys 14:515–525.
Kantor B, Jorgensen SM, Lund PE, Chmelik MS, Reyes DA, Ritman EL. 2002. Cryostatic micro-computed tomography imaging of arterial wall perfusion. Scanning 24: 186–190.
Kinney JH, Lane NE, Haupt DL. 1995. Three dimensional in vivo microscopy of trabecular bone. J Bone Miner Res 10:264–270.
Lerman LO, Rodriguez-Porcel M, Romero JC. 1999. The development of X-ray imaging to study renal function. Kidney Int 55:400–416.
Maran A, Khosla S, Riggs BL, Zhang M, Ritman EL, Turner RT. 2003. Measurement of gene expression following cryogenic μ-CT scanning of human iliac crest biopsies. J Musculoskeletal Neuronal Interact (in press).
Margaritondo G. 1988. Introduction to synchrotron radiation. New York: Oxford University Press.
Paroz Z, Moncada R, Sovak M. 1987. Contrast media: Biological effects and clinical applications. Boca Raton, Florida: CRC Press. Vols. 1–3.
Rice BW, Cable MD, Nelson MB. 2001. In vivo imaging of light emitting probes. J Biomed Opt 6:432–440.
Riederer SJ, Mistretta CA. 1977. Selective iodine imaging using K-edge energies in computerised X-ray tomography. Med Phys 4:474–481.
Ritman EL. 1994. Myocardial capillary permeability to iohexol: Evaluation with fast X-ray computed tomography. Invest Radiol 29:612–617.
Ritman EL, Peyrin F. 2002. Micro-imaging of small animals and biological specimens. Proc 2002 IEEE International Symposium on Biomed Imaging, Washington DC, July 7-10, 2002, pp. 361-364 [IEEE Cat. Nr. 02EX608C–CD-ROM].
Ritman EL, Dunsmuir JH, Faridani A, Finch DV, Smith KT, Thomas PJ. 1997. Local reconstruction applied to X-ray microtomography. In: Chavent G, Papanicolaov G, Sacks P, Symes W, editors. New York: Springer-Verlag. IMA Volumes in Mathematics and Its Applications, Inverse Problems in Wave Propagation 90:443–452.
Ritman EL, Bolander ME, Fitzpatrick LA, Turner RT. 1998. Micro-CT imaging of structure-to-function relationship of bone microstructure and associated vascular involvement. Technol Health Care 6:403–412.
Rutherford RA, Pullan BR, Isherwood I. 1976. Measurement of effective atomic number and electron density using an EMI scanner. Neuroradiol 11:15–21.
Service RF. 1999. Scanners get a fix in lab animals. Science 286:2261–2262.
Smith BR, Shattuck MD, Hedlund LW, Johnson DA. 1998. Time course imaging of rat embryos in utero with magnetic resonance microscopy. MRM 39;673–677 1998 Comput Med Imaging Graph 20:483–490.
Stampanoni M, Borchert G, Abela R, Rüegsegger P. 2002. Bragg magnifier: A detector for sub-micrometer X-ray computer tomography. J Appl Phys (in press).
Takeda T, Maeda T, Yusa T, Akatsuka T, Iot K, Kishi K, Wu J, Kazama M, Hyodo K, Itai Y. 1996. Fluorescent scanning X-ray tomographic image with monochromatic synchrotron X-ray. Med Imag Tech 14:183–194.
Turnbull DHT, Foster FS. 2002. In vivo ultrasound biomicroscopy in developmental biology. Trends Biochem Sci 20:S1–S5.
Wang LH, Chen WR, Nordquist RE. 1997. Optimal beam size for light delivery to absorption-enhanced tumors buried in biological tissues and effect of multiple beam delivery–A Monte Carlo study. Appl Opt 36: 8286–8291.
Weber DA, Ivanovic M. 1999. Ultra-high-resolution imaging of small animals: Implications for preclinical and research studies. J Nucl Med 6:332–344.
Wernick MN, Wirjadi O, Chapman D, Oltulu O, Zhong Z, Yang Y. 2002. Preliminary investigation of a multiple-image radiography method. Proc 2002 IEEE International Symposium on Biomed Imaging, Washington DC, July 7–10, 2002, pp. 129–132 [IEEE Cat. Nr. 02EX608C–CD-ROM].
Westmore MS, Fenster A, Cunningham IA. 1997. Tomographic imaging of the angular-dependant coherent scatter cross section. Med Phys 24:3–10.
Wu J, Sundearesan G, Lyer M, Gambhir SS. 2001. Noninvasive optimal imaging of firefly luciferase reporter gene expression in skeletal muscles of living mice. Mol Ther 4:297–306.