Published in final edited form as:

J Neurosci Methods. 2008 June 30; 171(2): 207–213. doi:10.1016/j.jneumeth.2008.03.006.

3D micro-CT imaging of the Postmortem Brain

Alex de Crespigny^{1,#}, Hani Bou-Reslan^{2,#}, Merry C. Nishimura², Heidi Phillips², Richard A. D. Carano², and Helen E. D'Arceuil¹

¹ Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Charlestown, MA

² Tumor Biology and Angiogenesis, Genentech Inc., South San Francisco, CA

Abstract

Magnetic resonance microscopy (μ MRI) is becoming an important tool for non-destructive analysis of fixed brain tissue. However, unlike MRI, X-ray computed tomography (CT) scans show little native soft tissue contrast. In this paper, we explored the use of contrast enhanced (brains immersion staining in iodinated CT contrast media) micro-CT (μ CT) for high resolution 3D imaging of fixed normal and pathological brains, compared to μ MRI and standard histopathology. An optimum iodine concentration of 0.27M resulted in excellent contrast between gray and white matter in normal brain and a wide range of anatomical structures were identified. In glioma bearing mouse brains, there was clear delineation of tumor margin which closely matched that seen on histopathology sections. μ CT tumor volume was strongly correlated with histopathology volume. Our data suggests that μ CT image contrast in the immersion-stained brains is related to axonal density and myelin content. Compared to traditional histopathology, our μ CT approach is relatively rapid and less labor intensive. In addition, compared to μ MRI, μ CT is robust and requires much lower equipment and maintenance costs. For simple measurements, such as tumor volume and non-destructive post-mortem brain screening, μ CT may prove to be a valuable alternative to standard histopathology or μ MRI.

Keywords

brain; micro-CT; magnetic resonance microscopy; post mortem; pathology; tumor

Introduction

Conventional histopathology permits very high resolution, within the plane in which the tissue is sectioned, for a wide range of specific stains. However this method is destructive and two dimensional in nature; true three dimensional (3D) information is difficult to obtain with this approach. In addition, the thin sectioning and mounting process often results in significant tissue shrinkage and geometric distortions, and the staining process is time consuming and labor intensive. A considerable body of work exists on the use of high resolution MRI for nondestructive microscopic analysis of tissue specimens (Augustinack et al., 2005; Boyko et al., 1994; Delnomdedieu et al., 1996; Huesgen et al., 1993; Johnson and Hedlund, 2002; Johnson et al., 1993; Johnson et al., 2002; Lester et al., 1999). Although the in-plane resolution

Address for correspondence: Helen E. D'Arceuil, MGH-NMR Center, Rm 2301, Bldg 149, 13th St., Charlestown, MA 02129, USA, Telephone: 617 726 8005, Fax: 617 726 7422, E-mail: helen@nmr.mgh.harvard.edu.

[#]Contributed equally.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

of MRI is far inferior to that obtainable with light microscopy, its excellent soft tissue contrast, nondestructive nature and ability to provide true 3D image data makes “MRI Histology” (Johnson et al., 1993) a valuable tool for the study of fixed tissue specimens. However, high field small-bore MRI (μ MRI) scanners remain relatively expensive and difficult to site, and scans often require a significant amount of operator interaction during the ‘setup phase’. Conversely, X-ray micro computed tomography (μ CT) systems are around 5 fold cheaper than μ MRI systems, are straightforward to site in the laboratory and much cheaper to maintain. With this in mind, we have investigated the use of 3D μ CT for the noninvasive characterization of fixed brain tissue specimens, “ μ CT Histology”, as a means to differentiate between gray and white matter, along with tumor identification and quantification.

Three-dimensional μ CT has proven to be a powerful technique for imaging and analysis of bone structure and density in small animals. Morphometric parameters such as bone volume and surface density, and trabecular thickness and separation were significantly correlated to the corresponding measures made by conventional histomorphometry (Muller et al., 1998). In addition, μ CT trabecular bone analysis has become a valuable tool in osteoporosis studies (Kapadia et al., 1998) and has also been used to quantify callus formation during bone repair (Street et al., 2002) and bone destruction (Barck et al., 2004) in a mouse model of rheumatoid arthritis. Although initial μ CT imaging was performed *ex vivo*, recent advances allow for similar imaging protocols to be performed *in vivo* (Barck et al., 2004). While μ CT has proven to be a very effective tool for imaging of bone, soft tissue imaging usually requires the use of X-ray absorbing contrast agents because there is very little difference in density and X-ray absorption over different tissue types, i.e., no native CT contrast, especially in the brain (e.g. cerebral gray or white matter, tumors etc.). μ CT angiography has been performed in small animals with the aid of intravascular contrast agents to provide high-resolution ex-vivo images of vascular structures (Garcia-Sanz et al., 1998; Kwon et al., 1999; Kwon et al., 1998). In fixed tissue samples, injected X-ray contrast agents have been used to enhance joint spaces in knee specimens (Schnier et al., 1997) and in rodent lungs (Langheinrich et al., 2004).

MR microscopy studies of whole mouse bodies which were perfused and soaked in gadopentetate dimeglumine (GdDTPA) MR contrast agent showed good soft tissue contrast in the axial, coronal and longitudinal sections through the head, chest and abdomen (Johnson et al., 2002). Previous high resolution MR diffusion tensor studies of perfusion fixed rodent, macaque and rabbit brains which were immersion stained with GdDTPA showed excellent contrast between gray and white matter (D’Arceuil and de Crespigny, 2007; D’Arceuil et al., 2007a; D’Arceuil et al., 2007b). The purpose of the present work was to determine whether a similar approach involving immersion of brain tissue in a CT contrast agent prior to μ CT imaging can yield images with useful anatomical contrast, comparable to that obtained by MR microscopy. In addition, we sought to evaluate this method for measuring the volumes of intracranial gliomas in a murine model.

Methods

Experimental animals: brain immersion protocol

Rabbits—The μ CT tissue immersion and imaging protocol were evaluated in 5 normal fixed adult male New Zealand White rabbit brains (perfusion fixed with 4% paraformaldehyde). All brains were soaked in their respective solutions at 4°C for 5 days. One rabbit brain was soaked in GdDTPA MR contrast agent (Magnevist®, Berlex Labs., Richmond, CA, USA) diluted 1:67 with phosphate buffered saline solution (PBS) to a concentration of 7.5mM GdDTPA as previously described (D’Arceuil et al., 2007b). Another brain was soaked in pure PBS, while the remaining three rabbit brains were soaked in Hypaque®-76, a hydrophilic iodinated contrast agent (Diatrizoate Meglumine and Diatrizoate Sodium, GE Healthcare Inc. Milwaukee, WI,

USA) diluted 1:20, 1:10 or 1:5 with PBS, resulting in I^{127} concentrations of 0.14M, 0.27M and 0.49M respectively.

Mice—Male CD1 nude mice (n=5) received intracranial implants of a human glioma cell line. Under 2% Isoflurane anesthesia mice were placed into the Kopf stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA) and U87 cells (2.5×10^5 cells/5 μ l) were injected intracerebrally into the right striatum. After 3 weeks, animals were anesthetized with sodium pentobarbital (40–70mg/kg IP) and transcardially perfused fixed with 10% buffered formalin. The brains were removed and placed into 10% buffered formalin overnight. Based on the results from the rabbit brain experiments (above), the mouse brains were then soaked in Hypaque diluted 1:10 with PBS for a period of 5 days prior to μ CT imaging.

All animal procedures were approved by the institutional review boards of the contributing institutions.

μ MRI Scanning—The fixed, GdDTPA-soaked rabbit brain was immobilized in a molded plastic holder and placed in a sealable custom built plastic chamber. The chamber was filled with Fomblin[®] LC8 liquid ((Solvay Solexis, Thorofare, NJ), an MRI susceptibility matching fluid. MR Imaging was done on a 4.7T Bruker Biospec Avance scanner (General Electric/ Bruker Instruments, Fremont, CA) equipped with 40Gauss/cm gradients. Signal reception used a custom built solenoid coil (2cm $\times$ 3cm) which allowed a high filling factor. High resolution anatomical scans were acquired with a 3D FLASH sequence (Frahm, 1987) with the following imaging parameters: TE 11ms, TR 40ms, bandwidth 30 kHz, flip angle 55°, 10 averages, matrix 512 $\times$ 512 $\times$ 256, scan time 14.5 hours, 75 μ m isotropic resolution.

μ CT Scanning

All brains were imaged with a μ CT40 (SCANCO Medical, Basserdorf, Switzerland) X-ray micro-CT system. The brains were removed from their respective solutions, blotted dry and placed in a sample holder for imaging. The sample holder was sealed with a plastic film to prevent sample dehydration. A sagittal scout image, comparable with a conventional planar X-ray, was obtained to define the start and end point for the acquisition of a series of coronal slices through the brains. The location and number of axial images were chosen to provide complete coverage of the brain. The brains were imaged with air as the background media. Images were generated by operating the X-ray tube at an X-ray voltage of 45 kVp, a current of 177 μ A. Images were generated by the acquisition of 1000 projections with an integration time of 300 milliseconds and 7 signal averages. The total acquisition time was 5 hours per brain. Images were obtained at an isotropic resolution of 30 μ m for the rabbit brain scans and 16 μ m for the mouse brain scans.

H&E staining

After μ CT scanning, all mouse brains were processed for standard histological Hematoxylin and Eosin (H&E) staining. The brains were put in 10% formalin (≥ 1 day) and subsequently placed into 30% sucrose for 2–3 days. Fifty micron coronal sections through the forebrain were cut on a freezing/sliding microtome and every 6th section was mounted and stained.

Data processing

μ MRI—The contrast-to-noise ratio (CNR) was measured between regions-of-interest (ROIs) placed in cortical gray matter (on 5 slices in fronto-parietal cortex, bilaterally) and in the body of the corpus callosum on the μ MRI scan of the normal rabbit brain. The noise level was defined as the standard deviation of voxels within a large artifact free ROI placed in the background outside of the brain.

μCT—ROIs were measured on the rabbit brain μCT images in locations matching those used for the μMRI measurements. The noise level was defined as the standard deviation of voxels in a homogenous region of cortical gray matter. CNR calculations were done on the image signal intensity values (i.e. 'CT number) for convenience. For all other measurements, CT numbers were converted into standard X-ray attenuation units (Hounsfield Units, HU).

The 3D μMRI and μCT data volumes were compared visually to identify known anatomical structures such as the corpus callosum and internal capsule using Amira software (Mercury Computer Systems Inc., San Diego, CA, USA).

Tumor volume estimation

The mouse tumor volume estimates were obtained from μCT data down sampled from 16 to 40 μm in order to provide a better signal to noise ratio. Tumor borders were manually traced on all brains using Analyze software (AnalyzeDirect Inc., Lenexa, KS, USA) to act as reference borders for the Analyze Region-of-Interest interpolation function. The complete tumor border was generated by interpolation across all relevant slices contained within the reference borders, and the tumor volume calculated. On the H&E stained sections, tumor area was analyzed on successive tumor-containing slides using the Ariol (Applied Imaging Corporation, San Jose, CA, USA) microscopy software. Tumor volume was obtained by multiplying these areas by the sum of the section thickness plus the intersection distance, and summing. A linear regression analysis was performed between the μCT and histological tumor volume measurements (JMP, SAS Institute Inc., Cary, NC, USA).

Results

Figure 1 shows 2D coronal sections through each 3D μCT data volume (each section at approximately the same anatomical level) of the normal rabbit brains at the different contrast agent concentrations, along with a plot of the gray/white matter CNR. For comparison, an anatomically matched μMRI image from the GdDTPA soaked brain is also shown. As expected, there is no native μCT tissue contrast in the brain soaked in pure PBS. Soaking the brains in Hypaque results in a clear difference in signal between the gray matter, the white matter and the ventricular spaces in the brain. The 1:10 Hypaque/PBS solution (0.27M I¹²⁷) yielded images with the best CNR, 4.2.

A qualitative comparison between matching axial sections extracted from the 3D μCT and μMRI scans of fixed rabbit brains is provided in Figure 2. The mean CNR between gray and white matter in the μMRI image was 14.9 which is 3.5 times greater than for the μCT. Nevertheless, visual inspection of the images shows a high degree of similarity in the overall contrast for the two modalities.

A more detailed evaluation of the visibility of specific anatomical structures is provided in Figure 3, which shows coronal sections from a 3D μCT data set compared to standard histological myelin (Loyez) stained sections taken from a rabbit brain atlas (Shek et al., 1986). Of 23 structures annotated on the myelin stained sections from the atlas, 22 structures are also apparent on the μCT images. The only structure not visible on μCT was the lateral olfactory tract, which was detached from the surface of the brain during removal from the skull. This suggests that the CT contrast derived from the Hypaque-soaked fixed brains is strongly related to tissue myelin content.

In the tumor bearing, Hypaque-soaked mouse brains, visual inspection of the μCT images shows marked contrast between the tumor and the surrounding gray and white matter, Figure 4.. The tumor location and extent on the μCT images are in excellent agreement with the corresponding H&E sections. A linear regression analysis of tumor volumes measured by

histology and μ CT showed a significant correlation ($R^2 = 0.79$, $p=0.0003$, with the Y intercept fixed at 0.0). The slope of the regression line was 1.27 which was not significantly different from 1.0.

Discussion

To our knowledge, this report is the first to demonstrate the use of an X-ray contrast agent for direct immersion staining of fixed brain tissue, providing high resolution μ CT images with good gray/white matter definition. This study also demonstrates the utility of 3D gross histopathology using μ CT rather than μ MRI.

The process of chemical fixation using formaldehyde or any other fixative, causes coagulation of the tissue's proteins (as a result of protein crosslinking) and constituents (Srinivasan et al., 2002). The process of protein cross linking significantly reduces brain tissue T_1 and T_2 MR relaxation times, (Pfefferbaum et al., 2004; Tovi and Ericsson, 1992) thereby reducing image contrast between gray and white matter compared to that seen in scans of live brain (*in vivo*, both proton density and T_2 are lower in white matter than in gray matter (Just and Thelen, 1988)). In fixed brain tissue, the native MRI contrast results from differences in the proton density (Augustinack et al., 2005) and T_2^* (D'Arceuil et al., 2007b) of the MR-visible mobile water pool. Soaking the tissue in exogenous Gd-based contrast agents enhances gray-white matter contrast, because the T_2 relaxation effect (relaxivity) of GdDTPA is greater in fixed white matter than in fixed gray matter (D'Arceuil et al., 2007b).

Unlike MRI, there is little soft tissue contrast in CT images of normal *live* brain (in quantitative terms, gray matter 20–35 HU, white matter 30–40 HU). In our method, *ex vivo* μ CT contrast is simply due to differential uptake of the contrast agent into the tissue as a result of passive diffusion. Our data indicates that, after soaking the tissue in Hypaque, the resultant contrast enhancement is greatest in gray matter, intermediate in peripheral white matter and least in the densely myelinated white matter tracts (such as the corpus callosum). Assuming that, after sufficient time, all of the mobile (i.e. unbound) water within the sample has reached an equilibrium contrast agent concentration equal to that of the bulk water in the sample container, variations in tissue X-ray attenuation will then be proportional to the concentration of hydrophilic Iodine anion (radiopaque component of the hypaque/PBS solution) in the mobile water in each voxel. Since water content varies with cell type and is lower in axons, particularly highly myelinated axons (Fenrich, 1992), the contrast in our contrast-enhanced μ CT images of normal brain reflects local axonal, and particularly myelin density.

In effect, the μ CT contrast we see in Hypaque soaked fixed brain is inverted and amplified compared to that seen *in vivo* in non-contrast CT: the denser tissue (white matter) that attenuates slightly more than gray matter *in vivo*, is less attenuating after soaking *ex vivo* precisely because of its lower water content. This picture helps in our understanding of the appearance of brain pathology using our proposed method. In general we would expect any pathology than is visible in non-contrast CT *in vivo* to be seen with greater contrast in fixed tissue using our approach. Tumor tissue that is edematous has, by definition, a higher mobile water content than normal tissue, and this leads to a greater contrast agent concentration after soaking in Hypaque, as we in fact observe (see Fig. 4). In addition, the H&E images exhibited heterogeneous regions within the tumors, which were regions of necrosis. These regions showed no cystic transformation. Heterogeneity within the tumor area on the μ CT images was also apparent but was too subtle to quantify.

While the water soluble Hypaque contrast agent enhances the mobile water pool of the postmortem brain as discussed above, lipophilic contrast agents may have different enhancement effects in the brain as well as other postmortem tissues. For example, Osmium

tetroxide (a lipid stain commonly used in transmission electron microscopy) has been employed to enhance anatomical structures (whole body) in fixed normal and genetically modified mouse embryos. Using methodology similar that described in our study, the embryos were immersion stained in an Osmium tetroxide solution then subsequently scanned using high resolution volumetric CT to quantitatively assess developmental patterning defects (Johnson et al., 2006).

There are significant limitations to our proposed technique and clearly it is not intended to replace conventional histopathology. Conventional histopathology on 5µm tissue sections provides a wealth of detailed information on cellular morphology that is not accessible at the resolution of our data (16µm). In many cases however, gross measurements on thicker tissue sections are adequate for determining, for example, lesion load in each organ. Here, our method may prove a time-saving and nondestructive alternative. We have shown that we can detect glioma in murine brain 3 weeks after implantation. Future studies will explore the visibility of other tumor types as well as other pathologies in different organs.

Conclusions

This technique permits soft tissue characterization in biological specimens with contrast and spatial resolution comparable to that of MR microscopy scans. An advantage of this approach lies in the fact that µCT scanners are much cheaper than high field (microscopy capable) MRI scanners and are much cheaper to site and maintain. It therefore has the potential for more widespread application than the µMRI (e.g. a laboratory may site 3 or 4 µCT systems in the space required for 1 high field MRI scanner and still at less than the total cost of one MRI scanner).

The technique is suitable for defining tumor boundaries by providing contrast between tumor and normal (gray and white matter) brain tissue. This technique provides a tumor volume estimate that is strongly correlated with histology, making it well suited accessing tumor burden. The technique is nondestructive which will allow a detailed histological analysis to still be performed (as we have shown) if additional information is needed beyond tumor burden. In cases where a simple measure such as total tumor volume is required, our µCT approach may prove useful, since it is quick (compared to performing histopathology) and sample analysis can be highly automated. µCT may prove valuable in the field of drug discovery or drug screening for 3D morphological characterization of whole organs or whole animals (e.g. mice) after drug injections. It may also prove valuable for rapid characterization of morphological changes resulting from genetic manipulation of animals (e.g. KO mice) and thus find application in a wide range of studies involving genetics with histopathological correlation.

Acknowledgments

This work was supported in part by NIH Grant EB00790 and the Athinoula A. Martinos Center for Biomedical Imaging (NIH/NCRR: P41RR14075, 1S10RR016811 and the MIND Institute). We authors would like to thank Mark Does for helpful discussions, Ben Reichardt and Rajiv Gupta for initial volume CT feasibility data and Jeffrey Eastham-Anderson (Genentech, Inc.) for his assistance in use of the Ariol software for H&E analysis.

References

- Augustinack JC, van der Kouwe AJ, Blackwell ML, Salat DH, Wiggins CJ, Frosch MP, Wiggins GC, Potthast A, Wald LL, Fischl BR. Detection of entorhinal layer II using 7Tesla magnetic resonance imaging. *Ann Neurol* 2005;57:489–94. [PubMed: 15786476]
- Barck KH, Lee WP, Diehl LJ, Ross J, Gribling P, Zhang Y, Nguyen K, van Bruggen N, Hurst S, Carano RA. Quantification of cortical bone loss and repair for therapeutic evaluation in collagen-induced

arthritis, by micro-computed tomography and automated image analysis. *Arthritis Rheum* 2004;50:3377–86. [PubMed: 15476252]

Boyko OB, Alston SR, Fuller GN, Hulette CM, Johnson GA, Burger PC. Utility of postmortem magnetic resonance imaging in clinical neuropathology. *Arch Pathol Lab Med* 1994;118:219–25. [PubMed: 8135623]

D'Arceuil H, de Crespigny A. The effects of brain tissue decomposition on diffusion tensor imaging and tractography. *Neuroimage* 2007;36:64–8. [PubMed: 17433879]

D'Arceuil H, Liu C, Levitt P, Thompson B, Kosofsky B, de Crespigny A. Three-Dimensional High-Resolution Diffusion Tensor Imaging and Tractography of the Developing Rabbit Brain. *Dev Neurosci*. 2007a

D'Arceuil HE, Westmoreland S, de Crespigny AJ. An approach to high resolution diffusion tensor imaging in fixed primate brain. *Neuroimage* 2007b;35:553–65.

Delnomdedieu M, Hedlund LW, Johnson GA, Maronpot RR. Magnetic resonance microscopy--a new tool for the toxicologic pathologist. *Toxicol Pathol* 1996;24:36–44. [PubMed: 8839279]

Fenrich, FRE. Medical Physics. University of Alberta: Edmonton, Alberta; 1992. MSc: A simulation and experimental study of water proton relaxation in a white matter model.

Frahm J. Rapid FLASH NMR imaging. *Naturwissenschaften* 1987;74:415–22. [PubMed: 3683588]

Garcia-Sanz A, Rodriguez-Barbero A, Bentley MD, Ritman EL, Romero JC. Three-dimensional microcomputed tomography of renal vasculature in rats. *Hypertension* 1998;31:440–4. [PubMed: 9453342]

Huesgen CT, Burger PC, Crain BJ, Johnson GA. In vitro MR microscopy of the hippocampus in Alzheimer's disease. *Neurology* 1993;43:145–52. [PubMed: 8423879]

Johnson G, Hedlund L. MR Microscopy of whole specimens, individual organs or tissue specimens perfused simultaneously with fixative and MRI contrast agent. 2002

Johnson GA, Benveniste H, Black RD, Hedlund LW, Maronpot RR, Smith BR. Histology by magnetic resonance microscopy. *Magn Reson Q* 1993;9:1–30. [PubMed: 8512830]

Johnson GA, Cofer GP, Gewalt SL, Hedlund LW. Morphologic phenotyping with MR microscopy: the visible mouse. *Radiology* 2002;222:789–93. [PubMed: 11867802]

Johnson JT, Hansen MS, Wu I, Healy LJ, Johnson CR, Jones GM, Capecchi MR, Keller C. Virtual histology of transgenic mouse embryos for high-throughput phenotyping. *PLoS Genet* 2006;2:e61. [PubMed: 16683035]

Just M, Thelen M. Tissue characterization with T1, T2, and proton density values: results in 160 patients with brain tumors. *Radiology* 1988;169:779–85. [PubMed: 3187000]

Kapadia RD, Stroup GB, Badger AM, Koller B, Levin JM, Coatney RW, Dodds RA, Liang X, Lark MW, Gowen M. Applications of micro-CT and MR microscopy to study pre-clinical models of osteoporosis and osteoarthritis. *Technol Health Care* 1998;6:361–72. [PubMed: 10100939]

Kwon HM, Hong BK, Jang GJ, Kim DS, Choi EY, Kim IJ, McKenna CJ, Ritman EL, Schwartz RS. Percutaneous transmyocardial revascularization induces angiogenesis: a histologic and 3-dimensional micro computed tomography study. *J Korean Med Sci* 1999;14:502–10. [PubMed: 10576145]

Kwon HM, Sangiorgi G, Ritman EL, McKenna C, Holmes DR Jr, Schwartz RS, Lerman A. Enhanced coronary vasa vasorum neovascularization in experimental hypercholesterolemia. *J Clin Invest* 1998;101:1551–6. [PubMed: 9541483]

Langheinrich AC, Leithauser B, Greschus S, Von Gerlach S, Breithecker A, Matthias FR, Rau WS, Bohle RM. Acute rat lung injury: feasibility of assessment with micro-CT. *Radiology* 2004;233:165–71. [PubMed: 15317950]

Lester DS, Lyon RC, McGregor GN, Engelhardt RT, Schmued LC, Johnson GA, Johannessen JN. 3-Dimensional visualization of lesions in rat brain using magnetic resonance imaging microscopy. *Neuroreport* 1999;10:737–41. [PubMed: 10208540]

Muller R, Van Campenhout H, Van Damme B, Van Der Perre G, Dequeker J, Hildebrand T, Rueggsegger P. Morphometric analysis of human bone biopsies: a quantitative structural comparison of histological sections and micro-computed tomography. *Bone* 1998;23:59–66. [PubMed: 9662131]

Pfefferbaum A, Sullivan EV, Adalsteinsson E, Garrick T, Harper C. Postmortem MR imaging of formalin-fixed human brain. *Neuroimage* 2004;21:1585–95. [PubMed: 15050582]

- Schnier M, Eckstein F, Priebisch J, Haubner M, Sittek H, Becker C, Putz R, Englmeier KH, Reiser M. Three-dimensional thickness and volume measurements of the knee joint cartilage using MRI: validation in an anatomical specimen by CT arthrography. *Rofo* 1997;167:521–6. [PubMed: 9440900]
- Shek, J.; Wen, G.; Wisniewski, H. *Atlas of the Rabbit Brain and Spinal Cord*. Karger: Basel, New York; 1986.
- Srinivasan M, Sedmak D, Jewell S. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. *Am J Pathol* 2002;161:1961–71. [PubMed: 12466110]
- Street J, Bao M, deGuzman L, Bunting S, Peale FV Jr, Ferrara N, Steinmetz H, Hoeffel J, Cleland JL, Daugherty A, van Bruggen N, Redmond HP, Carano RA, Filvaroff EH. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. *Proc Natl Acad Sci U S A* 2002;99:9656–61. [PubMed: 12118119]
- Tovi M, Ericsson A. Measurements of T1 and T2 over time in formalin-fixed human whole-brain specimens. *Acta Radiol* 1992;33:400–4. [PubMed: 1389643]

Figure 1.

(A) 2D coronal sections through 3D μ CT images of the normal rabbit brains. Brains have been soaked in various concentrations of Hypaque or GdDTPA in PBS. Left to right: 100% PBS, 1:20 Hypaque/PBS, 1:10 Hypaque/PBS, 1:5 Hypaque/PBS, 1:67 GdDTPA/PBS. There is no native μ CT contrast in the brain soaked in PBS while there is greatest gray/white contrast in the brain soaked in 1:10 Hypaque/PBS. (B) signal (Hounsfield Units) against I^{127} concentration for ROIs in cortical gray matter and white matter (corpus callosum), as well as the difference signal. (C) image noise (gray matter) and gray/white matter contrast-to-noise ratio against I^{127} concentration.

Figure 2.

Axial sections through normal rabbit brains showing the tissue contrast throughout the brain (A) μ CT and (B) μ MRI. In spite of the lower resolution obtained with μ MRI (75 μ m) compared to μ CT (30 μ m), the μ MR image shows excellent gray/white contrast. Overall, image contrast in the μ CT and μ MR scans is similar.

Figure 3.

Comparison of myelin stained (Loyez) histological sections* (A) and contrast enhanced (Hypaque-76 1:10 dilution) μ CT scans through the caudal (top) and rostral (bottom) aspects of a formalin fixed rabbit brain (B). Annotations show some of the brain structures identifiable on both images. Key to structures: (a) Tractus geniculocalcarine, (b) Tractus habenulointercruentalis, (c) Radiatio optica, (d) Pedunculus thalami dorsalis, (e) Tractus mamillothalamicus, (f) Tractus opticus, (g) Tapetum, (h) Columna descendens fornicis, (i) Ventriculus tertius, (j) Stria medullaris thalami, (k) Alveus hippocampi, (l) Fimbria hippocampi, (m) Lamina medullaris ventralis thalami, (n) Capsula interna, (o) Zona incerta, (p) Capsula externa, (q) Pes pedunculi cerebri, crus cerebri, (r) Tractus optici accessorii, (s) Corpus callosum, (t) Centrum semiovale, (u) Corona radiata, (v) Tractus olfactorius lateralis, (w) Commissura anterior. (*reproduced with permission from Shek et al. "Atlas of the Rabbit Brain and Spinal Cord", Karger, 1986)

Figure 4. Coronal Hematoxylin and Eosin stained sections and visually matched μ CT images through the brain of a mouse with a large glioma in the left hemisphere. The boundary of the tumor is clearly delineated by both imaging methods.

Figure 5.

Linear regression of tumor volumes measured by histology and μ CT. With the intercept fixed at zero, there was a significant correlation between the two measurement techniques ($p=0.0003$, $R^2 = 0.79$). The slope was 1.27 which was not significantly different from 1.0.