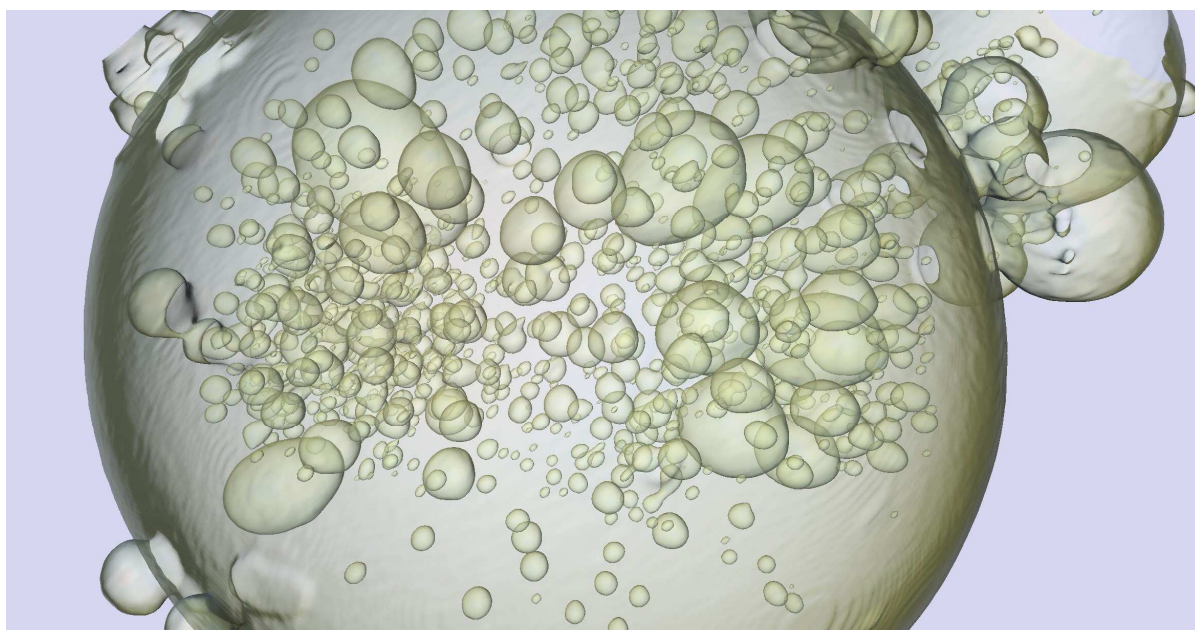


Aus Tomo II

Second Australian Tomography Workshop
Monash University, Melbourne
13-14 November, 2008



Program and Abstracts Book



Computational and
simulation sciences



Australian Research Council
**MOLECULAR + MATERIALS
STRUCTURE NETWORK**



Australian Synchrotron

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AUS TOMO II WORKSHOP PROGRAMME

Day 1: Thursday November 13th – Morning Sessions

8:30 Registration:

Foyer, Lecture Theatre S1, Monash University, Clayton Campus

8:50 Welcome

9:00 Mojette Discrete Tomography - how to cope with real devices and system noise by using redundancy and geometry

Professor Jean-Pierre Guédon

Nantes 'Polytech, France

9:30 Neutron tomography

John Banhart, Nikolay Kardjilov, Ingo Manke, Andre Hilger, Martin Dawson, Timur Kandemir

Helmholtz-Centre for Materials and Energy, Berlin

10:00 Microstructural characterization of the Al-Li-Mg-Cu alloy using 3-dimensional atom-probe tomography

Xiangyuan Xiong¹, Stavroula Moutsos², Russell King¹, Stan Lynch³ and Barry Muddle²

¹ Monash Centre for Electron Microscopy, Monash University

² ARC Centre of Excellence of Design in Light Metals, Monash University

³ Defence Science and Technology Organization

10:30 – 11:00 Morning Tea/Coffee

11:00 X-TRACT: software for simulation and reconstruction of X-ray phase-contrast CT

T.E. Gureyev¹, Ya.I. Nesterets¹, S.C. Mayo¹, A.W. Stevenson¹, D.M. Paganin², G.R. Myers² and S.W. Wilkins¹

¹ CSIRO Materials Science and Engineering

² School of Physics, Monash University

11:30 X μ CT LaTrobe Facility

B.D. Arhatari, A.G. Peele

Physics Department, La Trobe University

12:00 Imaging embryonic development in 3D Using optical projection tomography

Rob Bryson-Richardson^{1,2}, Silke Berger¹, Peter Currie^{1,3}

¹ Victor Chang Cardiac Research Institute

² School of Medical Sciences, Faculty of Medicine, University of New South Wales

³ St Vincent's Clinical School, Faculty of Medicine, University of New South Wales

12:30 – 1:30 Lunch

Day 1 – Afternoon Sessions

1:30 Katsevich's exact reconstruction algorithm and its application to various X-ray source scanning trajectories

Andrew Kingston and Trond Varslot

Research School of Physical Sciences and Engineering, Australian National University

2:00 Investigating 2D and 3D structure of aluminium alloys at the nanoscale and below

Matthew Weyland

Monash Centre for Electron Microscopy, Monash University

2:30 Formation of a Tomography society (subgroup within AMS)

Allan Jones (University of Sydney) and Tim Senden (Australian National University)

3:00 – 3:30 Afternoon Tea/Coffee

3:30 Drishti – A volume exploration and presentation tool

Ajay Limaye

Supercomputer Facility, Australian National University

4:00 Advanced high-resolution X-ray Computed Tomography using a laboratory system

Richard Trett¹, T. Beetz, T. Case, S. Chen, Y. Feng, M. Feser, M. Freed, J. Gelb, B. Hornberger, G. Hsu, C. Huang, D. Hunt, A. Lyon, R. Pastrick, J. Rudati, S. Sassolini, A. Tkachuk, Y. Xu, W. Yun, X. Zeng

1. Australian X-Ray Tubes Pty Limited Unit 3/59 Myoora Road Terrey Hills NSW 2084
Xradia, Inc., 5052 Commercial Circle, Concord, CA 94520, USA

4:30 Imaging the inner ear with X-ray Micro-Tomography

Allan S. Jones¹, Hilal Uzun-Coruhlu¹, Christopher Wong¹ and Ian S. Curthoys²

¹ Electron Microscope Unit, University of Sydney

² School of Psychology, University of Sydney

5.00 Simulation of sandstone elastic properties using synchrotron images (TBC)

Sherry Mayo on behalf of Marina Pervukhina¹, Ben Clennell¹, Maxim Lebedev² and Boris Gurevich^{1,2}

¹ CSIRO Petroleum Resources

² Curtin University, Department of Exploration Geophysics

5:30 End of Workshop Programme Day 1

6:30 BBQ at Australian Synchrotron

Day 2: Friday November 14th – Morning Sessions

8:30 Quantitative phase-contrast tomography of few-material objects

Glenn Myers

School of Physics, Monash University

9:00 Whole cell imaging, an answer for the Maurer's cleft puzzle.

Eric Hanssen

Department of Biochemistry, La Trobe University

9:30 Performance measurements with modern medical x-ray CT machines

Stewart Midgley

Monash Centre for Synchrotron Science, Monash University

10:00 Synchrotron tomography of mouse tibia utilising the Elettra synchrotron light source

Andrew Stevenson¹, Stephen Wilkins¹, Jillian Cornish², Giuliana Tromba³, Lucia Mancini³, Luigi Rigon³, and Damian E Myers^{1,4}

¹ CSIRO Materials Science & Engineering

² Department of Medicine, University of Auckland, New Zealand

³ Sincrotrone Trieste, Trieste, Italy

⁴ Department of Medicine (RMH/WH), University of Melbourne

10:30 – 11:00 Morning Tea/Coffee

11:00 Exact, Non-iterative ghost removal techniques for Mojette inversion

Shekhar S. Chandra and Imants Svalbe

School of Physics, Monash University

11:30 In-vivo microCT imaging of rats for monitoring the efficacy of antiresorptive therapies on bone

E. Perilli¹, V. Le¹, B. Ma¹, P. Salmon², K. Reynolds³, and N. L. Fazzalari¹

¹ Bone and Joint Research Laboratory, Surgical Pathology, Institute of Medical and Veterinary Science and Hanson Institute, Adelaide
Discipline of Pathology, University of Adelaide

² Skyscan NV, Kontich, Belgium

³ School of Computer Science, Engineering & Mathematics, Flinders University

12:00 Imaging inclusion bodies in cell models of Huntington's and other related conformational diseases using full-field x-ray microscopy.

Philip Heraud^{1,2}, David Cram¹, Peter Guttmann³, Steve Bottomley⁴.

¹ Monash Immunology and Stem Cell Laboratories, Monash University

² Centre for Biospectroscopy, Monash University

³ Bessy II Synchrotron, Berlin, Germany

⁴ Department of Biochemistry, School of Biomedical Sciences, Monash University

12:30 – 1:30 Lunch

Day 2 - Afternoon Sessions

Tomography at the Australian Synchrotron, Welcome (Anton Maksimenko)

1:30 Progress towards fast fluorescence tomography

Martin de Jonge¹, Chris Ryan, Robin Kirkham, Jonathan McKinlay, Peter Siddons, David Paterson, Daryl Howard, Christian Holzner, Ian McNulty, Stefan Vogt, Chris Jacobsen

¹ Australian Synchrotron

2:00 Tomography at the imaging and medical therapy beamline

Anton Maksimenko and Daniel Häusermann,
Australian Synchrotron

2:30 How to achieve sub-micron resolution for tomography with x-rays?

Marian Cholewa¹, Rob Lewis¹, Chris Hall¹, Steve Wilkins², Sherry Mayo², Daniel Häusermann³

¹ Monash Centre for Synchrotron Science, Monash University

² CSIRO Materials Science and Engineering, Clayton

³ Australian Synchrotron

3:00 Australian Synchrotron Tomography User's Panel Meeting

chair: Marian Cholewa

Workshop Delegate Tours (concurrent with panel):

3:00 Australian Synchrotron

3:00 Monash Centre for Electron Microscopy

4:00 Australian Synchrotron

5:00 pm Close

POSTERS

Gold nano-particles as x-ray CT contrast markers for cells

C. J. Hall, E. Schultke, B. Juurlink, R. Menk, F. Arfelli, L. Rigon, A. Astolfo, School of Physics, Monash University

Hierarchical tomography of human bone

Thomas C David L¹, Hislop-Jambrich J¹, Hall CJ², Peele A³, Hannah K³, and Clement JG¹

¹ The Melbourne Dental School, University of Melbourne

² Monash University Centre for Synchrotron Science

³ Department of Physics, LaTrobe University

Visualisation of the Bast's valve and crista of the guinea pig using X-Ray Micro tomography

Christopher Wong¹, Allan S. Jones¹, Ian S. Curthoys²

¹ Electron Microscope Unit, The University of Sydney

² University of Sydney, School of Psychology, The University of Sydney

Observations and simulations of imaging techniques with an X-ray laboratory source

K.M. Hannah, B.D. Arhatari, A.G. Peele,
Department of Physics, La Trobe University

Precise measurement of radial dimensional variation in macroscopic cylindrical objects from their x-ray CT images

R. B. Horney¹ and I. D. Svalbe²

¹ Department of Medical Imaging and Radiation Sciences, Monash University

² School of Physics, Monash University

"Mojette Discrete Tomography - how to cope with real devices and system noise by using redundancy and geometry"

*Professor Jean-Pierre Guédon,
Nantes 'Polytech, France*

From the beginning of this century, discrete tomography has made a stream of inventive advances. While, by the end of the previous century, data analysis had made good progress, such as the use of wavelet transforms to characterize and discriminate signals, the increasing demand was to design new technologies that continue to function even when all the information is not available. The Radon transform, discovered one century ago, was, by nature, a redundant transform. Its ability to use a set of different projected views (that only have their mean value in common) has been restricted because the ill-posed constraints prohibited exact reconstruction. Discrete projective geometry profoundly changed this status; new, discrete exact Radon transforms such as the Mojette Transform and the Discrete Radon Transform are sufficiently flexible and robust for many complex situations and they have a low computational overhead, involving only additions and subtractions.

In this talk, we will present:

- * The definition and properties of the French Mojette transform and its Australian counterpart, the DRT
- * The main reason to use these transforms in many different engineering situations; to cope with redundancy
- * Applications of discrete projections in medical imaging, watermarking, distributed information storage and multiple description data transmission

Neutron Tomography

*John Banhart, Nikolay Kardjilov, Ingo Manke, Andre Hilger, Martin Dawson, Timur Kandemir
Helmholtz-Centre for Materials and Energy Berlin
Contact email: banhart@helmholtz-berlin.de*

Neutron tomography can be a useful imaging tool when the specific interaction of neutrons with matter can be exploited. This is often the case when thick metallic parts have to be penetrated or an imaging contrast between light elements such as hydrogen and metals is required. Such standard imaging allows us to achieve spatial resolutions down to 50 μm . Some applications obtained at the Cold Neutron Radiography and Tomography beamline of the Hahn-Meitner Reactor in Berlin will be presented. Recent new developments include the use of polarised neutrons to obtain a 3D picture of magnetic fields inside or outside bulk materials. Furthermore, energy-selective tomography around the Bragg edge of a material such as iron can reveal subtle microstructural changes during processing in 3D and also provide information on textures in simple geometrical situations.

Microstructural characterization of the Al-Li-Mg-Cu alloy using 3-dimensional atom probe tomography

Xiangyuan Xiong ¹, *Stavroula Moutsos* ², *Russell King* ¹, *Stan Lynch* ³,
and *Barry Muddle* ²

¹ *Monash Centre for Electron Microscopy, Monash University,
Clayton, Victoria 3800;*

² *ARC Centre of Excellence of Design in Light Metals, Monash
University, Clayton, Victoria 3800.*

³ *Defence Science and Technology Organization, Melbourne,
Australia.*

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The mechanical properties and microstructure of Al-Li-Cu-Mg alloys have been studied extensively due to their low density and high stiffness, compared with the conventional aluminium alloys used in aircraft structures. However, the low ductility and toughness of these alloys have restricted their applications. The microstructure and local composition distributions are important for optimizing these mechanical properties. Previous studies have shown that a homogeneous distribution of coherent spherical δ' (Al_3Li) precipitates was formed together with other semi-coherent phases S' (rod or lath-like) and T_1 (plate-like) after being aged at 200°C. Because these precipitates are very small, ~20 nm in diameter for the δ' precipitates and even smaller for the S' and T_1 phases in the thickness direction, the accurate compositions of the precipitates in these alloys have not yet been determined. The 3-dimensional atom probe (3DAP) is a unique technique for determining the local composition distribution on a nano-meter scale. In this presentation, we will introduce briefly the 3D atom probes at the Monash Centre for Electron Microscopy, and present detailed studies of the compositions of the various phases in the Al-Li-Mg-Cu alloy analysed using the new generation 3DAP recently installed at the centre.

X-TRACT: software for simulation and reconstruction of X-ray phase-contrast CT

T.E.Gureyev, Ya.I.Nesterets, S.C.Mayo, A.W.Stevenson, D.M.Paganin, G.R.Myers and S.W.Wilkins

*CSIRO Materials Science and Engineering; School of Physics, Monash University
Tim.Gureyev@csiro.au*

We will present an overview of X-TRACT software that has been developed in CSIRO MSE over the last 8 years. The software can be accessed over the Internet and can utilize a compute cluster or GPUs for high-performance computations. It has a rich graphical user interface and tools for 2D image display and manipulation. X-TRACT can simulate a variety of phase-contrast imaging modes (in-line, ABI/DEI, Zernike, DIC, etc) and can perform phase retrieval using a number of different methods based on the Transport of Intensity equation, first Born approximation and Gerchberg-Saxton-Fienup algorithms. The program can also simulate polychromatic tomographic projections in parallel and cone-beam modes, where the sample is represented as a stack of 2D slices with a known spatial distribution of real and imaginary parts of the refractive index, or a distribution of known material components. X-TRACT contains algorithms for parallel-beam and cone-beam (FDK) CT reconstruction. Additional tools for quantitative CT imaging include batch pre-processing of input files (that can account for image drift, background illumination, bad pixels in the detector, phase contrast, etc.), a separate tool for sinogram creation, a generic image calculator with over 50 different image operations and a scripting capability, and many more.

X μ CT LaTrobe Facility

*B.D. Arhatari, A.G. Peele
Physics Department, La Trobe University
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X-ray micro computed tomography as an imaging tool plays an important role in many diverse applications such as bio-medical imaging, metal industries, polymer science, material science and fibre innovations. This is a unique three dimensional imaging technique for measuring and characterising internal structures as a non-destructive evaluation for materials with high resolution results. Our group was recently funded by the Victorian State Government to acquire a laboratory-based X-ray micro Computed Tomography system (X μ CT) with micron scale resolution. Coupled with a high quality detector, our system can achieve resolution smaller than the source size.

In most tomography work that has been done, the reconstruction is obtained from intensity measurements based on absorption through the object. Later we also put efforts to develop tomographic reconstructions based on phase information. This allows the visualization of phase based on refractive index features in a material. In this talk we will show both absorption and phase based tomography results using our machine.

Imaging Embryonic Development in 3D Using Optical Projection Tomography

Rob Bryson-Richardson^{1,2}, Silke Berger¹, Peter Currie^{1,3}

¹*Victor Chang Cardiac Research Institute*

²*School of Medical Sciences, Faculty of Medicine, University of New South Wales*

³*St Vincent's Clinical School, Faculty of Medicine, University of New South Wales*
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A holistic understanding of the anatomy of an organism is critical to dissecting the development and function of different organs and tissues in space and time. Information about the spatio-temporal patterning of an anatomical structure, gene, or protein expression pattern can be invaluable in elucidating function. The most complete mechanism for generating this understanding is to represent in 3D the development of a tissue or structure.

In order to image the developing embryo in 3D we have utilized the technique of Optical Projection Tomography (OPT). OPT is a non-destructive 3D imaging technique that can be applied to samples ranging from 1mm to 10mm and can be used to analyse a wide range of commonly used labeling techniques. Using OPT we have created a complete anatomical reference for the zebrafish, and the first 3D reference for any species covering development from embryo to adult. I will describe the imaging process and our work investigating zebrafish anatomy and heart regeneration, as well as lungfish and wallaby development using OPT.

Katsevich's exact reconstruction algorithm and its application to various X-ray source scanning trajectories.

Andrew Kingston and Trond Varslot

Research School of Physical Sciences and Engineering (RSPHysSE), Dept. of Applied Math. The Australian National University (ANU)

andrew.kingston@anu.edu.au

ANU has a custom-built micro-CT using a circular orbit scanning trajectory, and employ the Felkamp-Davis-Kress (FDK) filtered back-projection (FBP) algorithm for image reconstruction; a common set-up. To improve image resolution, we are about to upgrade the source from diameter $D=4$ to $D=1$ micron. However, X-ray flux decreases with D . To maintain the overall flux of the current system, we can either increase the required scanning time, or decrease the source-detector distance. The latter solution implies a larger cone-beam angle which degrades the already approximate FDK-FBP reconstruction. In anticipation of the new source we have been investigating alternative scanning trajectories and reconstruction algorithms.

The sufficiency condition for scanning trajectories to facilitate exact reconstruction was discovered by Tuy in 1983 [1]. Trajectories satisfying Tuy's condition include helical, saddle, and 2 connected circular orbits. A major breakthrough came in 2002, when Katsevich developed an exact FBP algorithm for helical trajectories [2]. Prior to this, despite extensive use of helical scanning in the medical community for over a decade, all practical algorithms were approximate. Since then Katsevich's principles have been applied to other trajectories. We will present an overview of these developments.

References:

[1] H. K. Tuy, "An inverse formula for cone-beam reconstruction," SIAM (Soc. Ind. Appl. Math.) J. Appl. Math. **43**, 546 – 552 (1983).

[2] A. Katsevich, "Theoretically exact filtered backprojection-type inversion algorithm for Spiral CT," SIAM (Soc. Ind. Appl. Math.) J. Appl. Math. **62**, 2012 – 2026 (2002).

Investigating 2D and 3D structure of Aluminium Alloys at the nanoscale and below

Matthew Weyland

Monash Centre for Electron Microscopy, Bldg 81, Monash University, Vic 3800

The importance of Aluminium alloys as engineering materials cannot be overstated, their high specific strength, formability and environmental resistance make them invaluable for a range of critical applications. While many of their macroscopic properties are well understood there are still many uncertainties of how many of these properties can be explained by their microstructure. High performance alloys gain these properties from the growth, via heat/mechanical treatment, of nanoscale precipitates. There are many unknowns about these precipitates; their atomic structure, their nucleation mechanism and even their exact nanoscale morphology and volume fractions.

Next generation electron microscopy is uniquely suited to revealing these unknowns. In particular two developments will lead to an enhanced understating of these materials; aberration corrected electron microscopy and electron tomography. The former will allow the characterisation of a specimens structure and chemistry at sub-Å resolution and the latter will reveal their morphology and allow quantitative characterisation of volume fractions, spacings and arrangements. Both of these will be demonstrated, combining results from HAADF-STEM tomography and initial high resolution studies using the first aberration corrected TEM in Australia at the Monash Centre for Electron Microscopy.

Drishti – A Volume Exploration and Presentation Tool

Ajay Limaye

Australian National University, Supercomputer Facility

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Drishti is graphics hardware-based direct volume rendering application for real-time exploration of large volumetric data.

Drishti provides multi-resolution zooming and allows the users to view arbitrarily large data sets by allowing visualization of smaller sub-volumes. This open-source software (<http://anusf.anu.edu.au/Vizlab/drishti>) is continually being developed with inputs from its users.

Some of the salient features of Drishti include:

- Handling of large scalar and vector volumes and volumetric time series.
- Handling photographic volumes.
- Advanced transfer function interface.
- Advanced animation interface.
- Semi-automatic segmentation facility.
- Mesh generation and operations on meshes.
- Advanced rendering and lighting capability.
- On-line help.

Imaging the Inner Ear with X-ray Micro-Tomography

Allan S. Jones¹, Hilal Uzun-Coruhlu¹, Christopher Wong¹ and Ian S. Curthoys²

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The inner ear is one of the most important sensory structures of the body. It not only provides auditory sensation but it is also the primary sensory control for our balance mechanism - and yet our understanding of its structure and function in both normal and pathological states is still incomplete in many ways. This is largely due to the difficulty in examining its structure by dissection and histology without losing spatial context or inducing distortion.

In this presentation we will show how X-ray micro-tomography is overcoming this difficulty and providing new insights. We will show how the boney structures can be accurately imaged. We will show how the delicate membranous structures of the labyrinth and cochlear can be imaged in 3D following density enhancement by application of Osmium tetroxide. We will also show how the spatial relationships between specific sensory structures can be seen in situ and detail how this new information is helping to clarify anatomical understanding of this complex organ.

Advanced High-Resolution X-ray Computed Tomography using a Laboratory System

T. Beetz, T. Case, S. Chen, Y. Feng, M. Feser, M. Freed, J. Gelb, B. Hornberger, G. Hsu, C. Huang, D. Hunt, A. Lyon, R. Pastrick, J. Rudati, S. Sassolini, A. Tkachuk, Y. Xu, W. Yun, X. Zeng

Xradia, Inc., 5052 Commercial Circle, Concord, CA 94520, USA

High-resolution x-ray computed tomography (XCT) enables non-invasive 3D imaging of complex structures. Today's applications in research and industrial processing place increasingly higher demands on XCT imaging resolution and sensitivity for low-contrast samples. To address these demands, Xradia's nanoXCT™ system makes use of high-efficiency, high-resolution x-ray optics (capillary condenser and zone plate optics) to deliver sub-50 nm resolution on the laboratory system. The resolution can be further increased by using the microscope in combination with a synchrotron source where sub-30 nm has been demonstrated. The nanoXCT system uses multi-keV X rays for high sample penetration. Zernike phase-contrast imaging provides high image contrast even for low-contrast materials. A graphical user interface combined with automated acquisition and reconstruction tools. We will discuss the technical innovations of the nanoXCT and show a range of applications such as fuel cell research and die level imaging in the semiconductor industry among many others.

Simulation of sandstone elastic properties using synchrotron images (TBC)

Marina Pervukhina¹, Ben Clennell¹, Maxim Lebedev² and Boris Gurevich^{1,2}

¹ CSIRO Petroleum Resources; ² Curtin University, Department of Exploration Geophysics

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Predicting of elastic properties of rocks relies on the availability of accurate microstructural models. Synchrotron images of two sandstones are segmented to separate grain from pore space. The porosity obtained as a result of the segmentation process is compared with the measured porosity for the segmentation quality control. Parallelized 3D finite difference code is used to simulate elastic wave propagation through the digitized two phase media where the total solid phase is supposed to have elastic properties of intact quartz and the pore space is either dry or saturated with water. Attenuation and dispersion of acoustic velocities are obtained at a range of frequencies. The obtained results are compared with the results of the laboratory experiments at ultrasonic frequencies.

Quantitative phase-contrast tomography of few-material objects

Glenn Myers
Monash university
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I will discuss several new techniques for quantitative phase-contrast tomography using, for example, unfiltered radiation from a polychromatic X-ray microfocus source. The proposed algorithms allow one to reconstruct the three-dimensional distribution of complex refractive index in a sample composed of one or more materials. If the number of constituent materials is sufficiently low, significantly fewer projections are required for an unambiguous quantitative reconstruction.

Whole cell imaging, an answer for the Maurer's cleft puzzle.

Eric Hanssen,
Department of biochemistry , La Trobe University, Melbourne, Australia

During intra-erythrocytic development, the human malaria parasite, *Plasmodium falciparum*, reorganizes its host's cell by exporting proteins beyond its own plasma membrane. It is faced with a complete lack of endogenous protein trafficking machinery within the host red blood cell, and therefore has to install its own apparatus. The Maurer's clefts (MC) are a crucial component of this protein sorting and trafficking machinery. MC are parasite-derived membranous whose architecture is not well characterized, current model range from single isolated membranous structures linked by vesicular trafficking through to a highly interconnected network that in some models link the parasitophorous membrane to the erythrocyte surface.

We have conducted electron tomographic reconstruction in order to characterize new isolated structures in this trafficking route. But in order to describe the MC "network" we had to investigate means of imaging whole cells. While we have previously shown, using confocal microscopy, that the interconnection between MC is limited, structural illumination, serial section tomography, X-ray tomography and dual beam microscopy in conjunction with mutagenesis have led to new and unexpected insight in the MC architecture

Performance measurements with modern medical x-ray CT machines

Stewart Midgley

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A model is presented describing the dose efficiency of an X-ray transmission computed tomography (CT) system and then used to investigate the performance of a modern medical scanner. Scanner design and CT data sampling principles are reviewed, and the image performance parameters are defined in terms of the reconstructed spatial resolution and the noise-to-signal (NSR) ratio. There is an associated cost for the procedure, which is determined by the number of photons involved, and quantified as the radiation dose. This model is tested against performance measurements with a modern medical CT system over a range of operating conditions and patient doses. The system is found to have additional noise contributions arising from noise in the pre-amp, and from afterglow in the ceramic scintillation detector. Strategies are presented for minimising the patient dose whilst maintaining the reconstructed image quality.

Synchrotron tomography of mouse tibia utilising the Elettra synchrotron light source

Andrew Stevenson¹, Stephen Wilkins¹, Jillian Cornish², Giuliana Tromba³, Lucia Mancini³, Luigi Rigon³, and Damian E Myers^{1,4}

¹CSIRO Materials Science & Engineering, Clayton, Victoria; ²Department of Medicine, University of Auckland, New Zealand; ³Sincrotrone Trieste, Basovizza SS, 34012 Trieste, Italy; ⁴Department of Medicine (RMH/WH), University of Melbourne, Parkville, Victoria. damianem@unimelb.edu.au

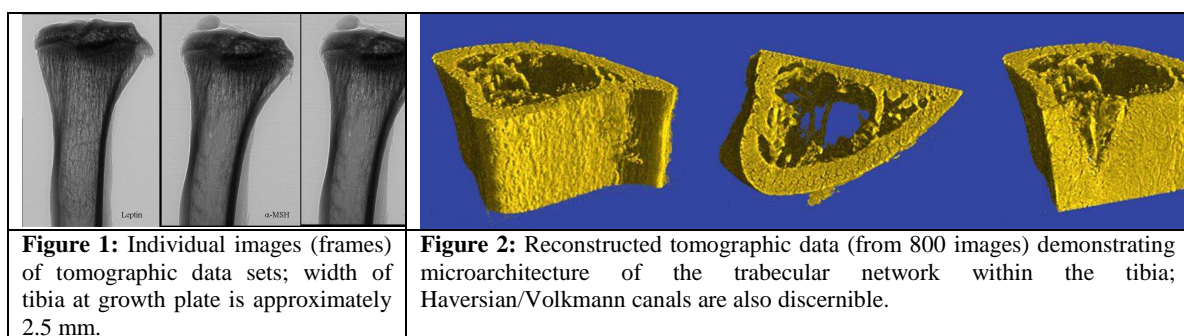
Introduction: Synchrotron X-ray microtomography has been used to study bone samples at micron resolution to produce 3D images [1]. Curved monochromator crystals have been used to facilitate phase-contrast effect as well as conventional absorption contrast X-ray imaging to improve spatial resolution and enable tomographic reconstruction of soft tissue to assess bone biomaterials [2].

Purpose: The current synchrotron study was performed to establish experimental conditions for imaging of mouse tibiae. The overall aim was to determine the parameters for imaging and analysis of the microarchitecture of the trabecular bone in a study of osteoporosis [3].

Methods: After treatment with cytokines of interest, the animals were transcardially perfused with phosphate-buffered 4% paraformaldehyde (PFA) and the bones were then excised and fixed in 4% PFA before storing in 70% v/v ethanol. The X-ray imaging was performed on the SYRMEP beam line at the Elettra Synchrotron in Italy.

Results: Figure 1 depicts typical images obtained for each of the three sample groups. Figure 2 shows rendered versions of the reconstructed tomographic data from one of the samples, presented in three different orientations. Microstructural components are clearly discernible and the Haversian/Volkmann channels can be delineated.

Conclusion: Synchrotron-based X-ray imaging provides high-resolution images of bone microstructures and depiction of the trabecular network as well as features of soft tissue. This data provides quantitative assessment of progressive bone changes and, in temporal studies, may enable assessment of therapeutic efficacy of drugs used to treat metabolic bone disorders.



References:

1. Weiss, P., et al., *Synchrotron X-ray microtomography (on a micron scale) provides three-dimensional imaging representation of bone ingrowth in calcium phosphate biomaterials*. *Biomaterials*, 2003. **24**(25): p. 4591-601.
2. Colonna, S., et al., *Curved optics for x-ray phase contrast imaging by synchrotron radiation*. *Phys Med Biol*, 2001. **46**(4): p. 967-74.
3. Cornish, J., et al., *alpha -melanocyte-stimulating hormone is a novel regulator of bone*. *Am J Physiol Endocrinol Metab*, 2003. **284**(6): p. E1181-90.

Exact, Non-iterative Ghost removal techniques for Mojette Inversion

Shekhar S. Chandra and Imants Svalbe

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The sufficiency of real-space projection data that is deemed adequate to reconstruct an accurate image of an object is open to debate. In contrast, the process of reconstructing discrete images from discrete projections quantify the precise criterion for sufficient projection information to ensure an exact reconstruction.

Using incomplete or insufficient projection data results in ambiguous reconstructed images, because more than one image solution satisfies the given projections. The variation in allowed solutions can be modelled as the addition of “ghost functions” that can be added to or span the solution space. These ghosts then quantify the detailed form of errors in the image reconstruction and their presence can be visualised in projection space as missing projection data.

The Finite Radon Transform (FRT) is a discrete reconstruction method that ensures exact reconstruction of image data within a square prime-sized array (i.e., it admits no ghosts). We would like to use the strong exact inversion properties of the FRT and the Mojette Transform (MT), an asymmetric generalisation of the FRT, to reconstruct better images from real projection data. Mojette projections approximate experimental parallel-beam x-ray intensity absorption profiles quite well. The cyclic projections of the FRT space can be adapted directly from any set of asymmetric, but sufficient set of Mojette projections. This adaptation results in a sufficient, but incomplete FRT projection set, which, when reconstructed, leads to the formation of discrete ghosts. We can eliminate these ghosts by properly symmetrising the Mojette projection set.

We have constructed two new direct and exact techniques to perform this projection symmetrisation. They both exploit the known sufficiency of the Mojette data to remove discrete ghosts through the use of redundant or "calibrated" image areas. Once the Mojette data is fully symmetrized, we can exploit the exact inversion property of the FRT to reconstruct the data.

The first "de-ghosting" method uses a simple shift and subtract algorithm that is applied to complete rows or columns of calibrated image data. The data is used to untangle and solve for the full content of each missing projection in the FRT projection set. The second method involves the use of Latin squares to find one or more complete sets of redundant data pixels that enable untangling of the ghosts that affect one image pixel. Each solution that is found is translation invariant and can be applied to the remaining image pixels.

In-vivo micro-CT imaging of rats for monitoring the efficacy of antiresorptive therapies on bone

E. Perilli¹, V. Le¹, B. Ma¹, P. Salmon², K. Reynolds³, and N. L. Fazzalari¹

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Osteoporosis is a significant health problem in the increasingly elder population. The ovariectomized rat is an animal model used for osteoporosis studies, to investigating the effects of bone loss and the efficacy of treatments against this loss. In the majority of these studies, the bone histomorphometry is assessed by histology or by in-vitro micro-CT using a cross-sectional study design, in which animals are euthanased at given time points for analysis.

In-vivo micro-CT studies, formerly carried out at synchrotron facilities, have shown that it is possible to track bony changes on the individual animal over time, enabling a longitudinal study design. Recent developments in X-ray micro-CT made compact in-vivo systems available, making longitudinal studies possible also to laboratories.

This study was done using a compact in-vivo micro-CT system to monitor, for three months, the in-vivo changes of the cancellous bone structures of three groups of rats: ovariectomized rats, ovariectomized rats subjected to antiresorptive treatment (zoledronic acid), and control rats.

Ovariectomy showed a striking bone loss within the first weeks, whereas the antiresorptive treatment restored the otherwise deteriorating bone structure. The longitudinal study design (repeated measures ANOVA), when compared to the cross-sectional study design (ANOVA without repeated measurements), provided earlier detection of the bone changes.

This confirms that in-vivo micro-CT is an effective tool for the early detection of bony changes and the effects of antiresorptive treatments on ovariectomized rats, with the advantage given by the longitudinal study design.

Imaging inclusion bodies in cell models of Huntington's and other related conformational diseases using full-field x-ray microscopy.

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Protein conformational diseases such as Huntington's Disease and spinocerebellar ataxias (SCA) are characterised by mutations of wild type genes leading to the expression of proteins that have expanded poly-glutamine domains. The expression of poly-Q mutant proteins results in the formation of intra-cytoplasmic and intra-nuclear inclusion bodies in cells. These are believed to result from misfolding of the poly-Q proteins in addition to the formation of beta-amyloid protein within the bodies. It is assumed that the accumulation of inclusion bodies in neurons results in neurodegeneration and clinical symptoms, however this link is far from being clearly understood. A major limitation in this area of research is the ability to image inclusion body formation, particularly at the early stages. Confocal fluorescence microscopy, which is the major tool presently employed, has a spatial resolution close to the size of fully formed inclusions. We have been investigating the use of full x-ray microscopy/tomography at beamline U41-TXM at Bessy II in Berlin to image inclusion bodies in various prokaryote and human cell models. Preliminary studies where SCA3 is expressed in *E. coli* bacteria will be presented.

Progress towards fast fluorescence tomography

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High-resolution scanning fluorescence microscopy provides invaluable information for investigations in the biological, environmental, forensic, and geological sciences. While 3D information is greatly desired, practice is restricted to 2D by a number of experimental factors including detector overheads, dynamic range, and sensitivity. A collaboration between the AS, CSIRO, and BNL is developing the Maia detector to overcome these limitations.

This presentation will focus on several outstanding challenges that remain to be solved before scanning fluorescence tomography can be realised.

The traditional failing of 3D scanning tomography is that the measurement scales as the inverse cube of the resolution. The Maia detector will reduce the minimum per-pixel dwell by over 3 orders of magnitude, but will only increase sensitivity by one order, leaving us with significantly reduced signal levels. We will use the dose fractionation theorem to obtain statistically significant reconstructions; however, we still need to align these projections. Alignment in software poses a significant challenge when each projection may comprise several hundred counts distributed amongst 100^2 pixels. We will use a differential phase contrast imaging technique to provide high-contrast images with which to align the fluorescence projections. Latest results and future at the Australian Synchrotron Microspectroscopy beamline will be outlined.

How to achieve sub-micron resolution for tomography with x-rays?

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The potential of x-ray microscopy was well understood half a century ago: new developments in x-ray optics would make high-resolution imaging possible. However, it was the advent of synchrotron radiation that spurred the development of hard x-ray microprobe systems, and soft x-ray microscopes using zone plate optics. Especially in the past decade, soft x-ray (<1 keV) microscope systems have advanced to provide images of biological specimens at 30 nm resolution in 2D and sub-100 nm resolution in 3D. More recently, hard x-ray (>1 keV) microscopes have also approached 50 nm resolution. These and other applications of x-ray microscopy have led to the development of multiple microscope systems at nearly all synchrotron radiation centres worldwide, and commercially available systems using laboratory x-ray sources.

There are two types of high-resolution x-ray microscopes: full-field transmission (TXM) and scanning transmission microscopes (STXM). A TXM images the entire field of view in parallel using an objective such as a zone plate lens while a scanning STXM forms an image in series in a point-by-point fashion.

In the light of these worldwide advances in x-ray microscopy techniques and applications, Australian Synchrotron is now poised to offer such a facility to its users. The creation of the X-ray microscope/microprobe will enable science and industry in Australia to take full advantage of this new method. Authors will explore in this talk different options for obtaining sub-micron resolution for high resolution and high speed tomography.

Gold nano-particles as x-ray CT contrast markers for cells

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In biomedical research any technique which allows the identification of cancer cells or pluripotent cells *in vivo* is being employed in an escalating effort to understand and combat disease. Most of the methods are limited to the analysis of tissues or cells *ex vivo* and are not capable of producing *in-vivo* images. We believe the use of x-ray tomography for pre-clinical longitudinal studies of disease progress, and of cell therapy efficacy would be embraced if an appropriate cell marker was available. One such potential marker is gold nano-particles (GNPs). Initial work has shown that test cells can be made visible in X-ray microCT if they have a number of GNPs incorporated within them. Studies to date have shown that this number is entirely compatible with the normal function of the cell. Some 26,000 GNPs of an average of 50nm in diameter have been taken up by these cells and shown not to affect their proliferation. Both C6 glioma and murine olfactory ensheathing cells have been successfully imaged in the head, liver and spine of rats using both synchrotron and micro-focus x-ray sources in CT. Some results are shown and the prospects for future development are discussed.

Hierarchical Tomography of Human Bone

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In order to understand age-related bone loss in humans it is necessary to integrate data from studies conducted at a hierarchy of scales. These scales are chosen to match those which divide bone structure into biologically meaningful compartments thus:

1. The whole-organ level, with sample dimensions in centimetres and voxels around 1mm.
2. The level that allows visualisation of the vascular structure of the cortex with samples of 1-2cm and voxels size of 10 μ m or so.
3. Tomography that resolves the lacunae enclosing osteocytes (bone-forming cells that have become entombed during bone formation) where the samples have dimensions of a few millimetres and voxels need to be around 1 μ m.
4. Diffraction studies of crystal and protein structure at micron and nanometre scales.

Results will be presented from studies at the first three of these scales:

1. Quantitative, helical-scan, multi-slice clinical CT (Toshiba Aquilion) of the proximal human femur.
2. Synchrotron micro-CT of centimetre scale blocks of cortex using 12 μ m voxels (Spring8).
3. Synchrotron micro-CT of 2x2x2mm blocks from the same bones with isotropic voxels of 1.4 μ m (Advanced Photon Source).

Data will be shown for clinically relevant bone mineral density measurements, anthropological dimensions, vascular structure topology and cell spacing.

Visualisation of the Inner Ear Microanatomy using X-Ray Micro tomography

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In the area of ear research, histological slicing and staining of thin sections have, been the dominant method in visualising and understanding the relationship between anatomically significant structures. The decalcification, slicing and staining processes can often create artefacts which alter the delicate membranes of the inner ear. This method requires intense spatial thinking and interpretation to visualise spatial arrangement of the sensory structures.

In the present study osmium tetroxide staining techniques, along with high resolution X-ray micro tomography and 3D- reconstruction software to isolate several structures within the membranous labyrinth and show their spatial interrelationships. The results include minimal artefact, easy identifiable 3D structure, which can be analysed at different orientations to better understand anatomical relationship and function.

Observations and simulations of imaging techniques with an X-ray laboratory source

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The Micro X-ray Computed Tomography (MXCT) laboratory source at La Trobe University has been used to image a various array of samples. Each sample has specific properties that are ideally examined with either absorption or phase contrast imaging. Recently Arhatari et al.(in preparation) showed that phase retrieval of a sample imaged with a polychromatic source can be achieved through an extended single plane TIE (Transport of Intensity Equation) based method under certain conditions. As the MXCT itself is further examined, the technical details specific to this device can be used in simulation to further improve the imaging process. This study looks at images produced by the MXCT under differing conditions and compares both the standard and the extended TIE based phase retrieval methods through simulations and images taken using each of these processes.

Precise measurement of radial dimensional variation in macroscopic cylindrical objects from their x-ray CT images

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A technique for the precise measurement of radial dimensional variation in macroscopic objects of nominally circular section from their CT images is described. The method measures, here, the deflection of a 140 mm diameter pipe, laterally compressed over a 300 micron range (three quarters of one pixel) in ten steps to a precision of approximately 10 microns. The method is based on the so-called patch-circle *PLUS* procedure which creates a one dimensional profile to locate the radial edge of the pipe precisely. The resulting deflections were compared with micrometer measurements. The method displays a full radius or diameter profile of the object and can measure edge distortion curvatures of either sign. It has shown itself to be bias-free (with suitable calibration), robust and reproducible. The method has potential applications in precision quality control of the radial variation in industrial production of objects such as pipes, shafts and circular or cylindrical machine components.

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