

# **ToScA 2017**

6 – 8 September 2017, University of Portsmouth, UK

# Programme









Thank you to the Natural History Museum for supplying the images used throughout this booklet.

### Welcome



#### Symposium Chair: Dr Farah Ahmed (Natural History Museum, UK)

A warm welcome to the fifth annual ToScA UK and Europe! It is my pleasure to announce that ToScA 2017 is being hosted by the University of Portsmouth. The scientific programme covers a broad range of applications and an increase in the range of pre-conference workshops. Since the foundation of ToScA the conference has had attendance from over 450 delegates from over 15 countries. I am pleased to report that the very first ToScA North America took place at the University of Texas, hosted by the Jackson School of Geosciences in June 2017. The successful conference was attended by over 70 delegates from 32 institutions. By sharing ideas, networking across Industry and demonstrating innovative developments, the conference provides an ideal arena for the international tomography community and associated Industry. ToScA UK and Europe will address key areas of science, including, Correlative Tomography, Additive Manufacturing, 4D Tomography, Computational Modelling, Earth and Space, Life Sciences, Materials Science, Healthcare and Cultural Heritage.

We are grateful to the University of Portsmouth for their support and I look forward to the presentations, lightning talks and posters. The evening dinner takes form in a banquet with the unique experience of dining on HMS Warrior, providing a perfect environment for networking. ToScA promises a full and diverse scientific programme, on behalf of the organising committee I hope you will find the conference interesting and stimulating.

We look forward to welcoming you all.

### Symposium Co-Chairs: Professor Asa Barber & Dr Gianluca Tozzi (University of Portsmouth)

We would like to welcome you all to the University of Portsmouth for the 5th annual conference on Tomography for Scientific Advancement (ToScA). The University of Portsmouth is a modern institution and provides an important focus for the city of Portsmouth and the region. Portsmouth is internationally recognized for its naval history that has grown into an area of global economic significance. The immediate locality around the university highlights this development, where HMS Warrior and the Mary Rose exhibition present a grandeur of the past, through to the new Queen Elizabeth aircraft carrier, the biggest naval vessel ever constructed in the UK, that reflects a promising future. Large multi-nationals sit next to supply chains that have cultivated new industries including high performance racing, with many formula one constructors and the America's cup represented, and healthcare rising to the second biggest sector of the region. This diversity of activity aligns well with the remit of ToScA, bringing together interests from cultural heritage and manufacturing to the life sciences. Translating best practice and excellence in the use of tomography both to the next generation of users as well as the broader community is of additional importance, and we are happy to see a significant growth in the pre-conference workshops in order to facilitate such ambitions. We are therefore enjoying the opportunity to develop further understanding of the discipline as well as support networking within the community.





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# Journal of Microscopy



# Recent Articles using Tomography



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**Time-lapse lab-based x-ray nano-CT study of corrosion damage** Microscopic dual-energy CT (microDECT): a flexible tool for

multichannel ex vivo 3D imaging of biological specimens Torsion estimation of particle paths through porous media observed by in-situ time-resolved microtomography

Application of sensitive, high-resolution imaging at a commercial lab-based X-ray micro-CT system using propagation-based phase retrieval

Micro-CT versus synchrotron radiation phase contrast imaging of human cochlea

The effect of X-ray micro computed tomography image resolution on flow properties of porous rocks

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### Workshop Programme Wednesday 6 September 2017

Registration for those who have booked to attend workshops will take place at 09:00 on Wednesday 6 September at the School of Engineering, The morning workshop sessions will start at 09:30 and the afternoon sessions will start at 13:30. A light lunch will be provided.

Confirmed workshops include:

## LaVision - Digital Volume Correlation (DVC) for volume strain mapping, defect detection, and crack tracing

Whilst people are familiar with X-ray CT and the ability to visualise features and structures in 3D, what's often not realised is that you can take sequences of volume images and quantify the deformation of the material. The ability to calculate full volume displacement and strain maps from volume images utilises a technique called Digital Volume Correlation (DVC). DVC essentially correlates pattern or features within the greyscale of the image in order to track the material deformation. The features may occur naturally within the material (such as voids, cellular material, graphite nodules) or may be artificially introduced (such as tin powder mixed with aluminium). DVC gives the user the ability to identify material properties, strain hotspots, and crack initiation points, and provides a powerful tool for FEA validation.

In this half day course LaVision will introduce the concept of DVC and basis of the computation. We will give examples of applications and work through example datasets within the group. This short course is suitable for newcomers to DVC, or those who already have some experience.

#### Amira-Avizo Software - Introductory Workshop (morning session)

This workshop is an introductory course focusing on the advanced 3D visualization and analysis capabilities of Amira and Avizo software for exploring and understanding scientific and industrial CT data. Participants will be offered an overview to data visualization, image processing and segmentation, measurements and statistics, and other advanced set of functionalities.

Attendees will have the opportunity to use the software through a hands-on session, accessible to first-time users of Amira and Avizo.

# Amira-Avizo Software - Meshing and Digital Volume Correlation Workshop (afternoon session)

Participants will be offered the chance to try some of the latest features of Amira and Avizo software. Amira and Avizo provide advanced workflows to collaborate with FEA solvers. During this workshop, participants will use the software to perform data visualization, image processing, and prepare a 3D mesh suitable for FEA simulation. Attendees will be also able to try the latest Digital Volume Correlation (DVC) extension to compute the precise internal displacement and strain maps from 3D images of materials acquired during a deformation process (in situ experiments).

#### **VGSTUDIO MAX Introductory Workshop (morning session)**

This workshop will introduce you to CT data analysis and visualization using VGSTUDIO MAX.Volume Graphics will present typical workflows which are of special interest for the scientific community for the fast and precise analysis of voxel data: you will accomplish the first steps of quantitative analysis options, segmentation, and visualization techniques. Use VGSTUDIO MAX to easily get the information contained in your data sets, whether acquired by laboratory X-ray CT, a synchrotron, with neutrons, or with another source. Use this special opportunity to speak personally with Volume Graphics experts!

#### **VGSTUDIO MAX Segmentation & Analysis Workshop (afternoon session)**

This workshop will cover image segmentation and selected analyses available in VGSTUDIO MAX. Participants should be familiar with the basic operations of the software or have attended the VGSTUDIO MAX Introductory Workshop the same day. Volume Graphics will present typical segmentation tasks and solutions and give you tips and tricks for challenging multimaterial datasets. The second part of the workshop will be dedicated to selected quantitative analysis options. Take the chance to speak personally with Volume Graphics experts!

#### BlueScientific Bruker Skyscan workshop

BlueScientific will be bringing a Bruker 1275 microCT which with advances in scanning and reconstruction software can scan objects and produce a 3D rendering in as little as 5 mins.

We shall be running morning and afternoon sessions, limited to 15 people each, which are designed to be complementary to the other workshops running that day.

#### Specimen Prep in Earth Sciences - Amin Garbout (NHM, London)

X-Ray Micro-computed tomography (micro-CT), is being used by a rapidly increasing number of researchers in earth sciences. Due to its non-destructive nature, little or no preparation of samples is needed for micro CT scanning. However, a few guidelines should be observed to enable optimal data acquisition.

Thus, an overview will be provided of the different parameters to consider when scanning specimens of various size, density or composition (filtering, exposure, voltage...). This hands-on workshop will showcase a range of sample mounting techniques with a variety of earth science specimens. The workshop aims to address how good sample mounting practise contribute to increased data quality, addressing factors such as low noise and reduced artefacts.

#### Microtomography for life sciences research - Brian Metscher (University of Vienna)

Applications of microCT in biological and biomedical research have expanded to include imaging of a wide variety of softtissue samples and other low-Z materials. Each different type of sample requires a different set of considerations for optimal imaging.

This workshop will introduce some methods for enhancing x-ray contrast in non-mineralised tissues and techniques for mounting biological samples for microCT imaging, especially embryos and other soft tissues, insects and other invertebrate specimens, and any samples of particular interest to the participants.

We will begin with some principles of x-ray imaging, discuss various types of samples and applications, and then work with your own interesting specimens.

#### 3D printing from CT data - University of Portsmouth

Additive layer manufacturing, commonly referred to as three-dimensional (3D) printing, shows significant potential in producing the complexity required for mimicking complex structures. At Portsmouth we are developing advanced 3D printing strategies employing multi-material technology to optimise morphological and mechanical performance of 3D structures captures using x-ray micro-CT. The aim of this workshop is to provide hands-on experience in the 3D modelling, meshing and printing of structures. An emphasis will be placed on biological structures, particularly bone, but the techniques developed in the workshop will be useful for a broad range of samples.

### **Invited Speakers**



#### Dr David Bacon, University of Portsmouth

David Bacon is a Reader in Cosmology at the Institute of Cosmology and Gravitation, University of Portsmouth. After his PhD research in Cambridge, he has held research fellowships in Edinburgh and Portsmouth. His interests include mapping the Universe, cosmology at radio wavelengths, gravitational lensing and theories of gravity.



#### Prof Marco Viceconti, University of Sheffield

Marco Viceconti holds the chair of Biomechanics at the Department of Mechanical Engineering of the University of Sheffield, UK, and serves as Executive Director of the Insigneo Institute for in silico Medicine, a joint initiative of the University of Sheffield and the Sheffield Teaching Hospital NHS Foundation Trust. He is also professor associate in the department of Oncology and Metabolism. Prof Viceconti is an expert of musculoskeletal biomechanics in general, and in particular in the use of subject-specific modelling to support the medical decision. He is one of the key figures in the Virtual Physiological Human (VPH) community: he is the President of the VPH Institute, an international no-profit organisation that coordinates this research community, and has recently concluded the coordination of the Avicenna action, which road-mapped the emerging topic of "in silico clinical trials", where subject-specific modelling is used in the development and assessment of biomedical products.



#### Prof Francois Hild, LTM-Cachan

François Hild is a Research Professor at the Laboratory of Mechanics and Technology in Cachan (France). He received his PhDs in Mechanical Engineering from the University of Paris 6 in 1992 and from the University of California in 1995, and his habilitation from the University of Paris 6 in 1998. His research interests include advanced experimental techniques, digital image and volume correlation, identification and validation procedures for material models.



#### Dr Brian Metscher, University of Vienna

Following bachelor's work in applied physics at Caltech and a first career as a research engineer at NASA/JPL, Dr. Metscher completed his Ph.D. in the then-new interdiscipline of evo-devo at the University of California, Irvine. He did postdoctoral research on the development and evolution of appendages and teeth at The Natural History Museum (London) and Penn State University, and then served five years as an Assistant Professor in southern Indiana. During the summers he carried out research at Yale University and came to the University of Vienna in 2006, to set up the imaging lab in the Theoretical Biology Department where he is now Senior Scientist. Dr. Metscher helped to establish X-ray microtomography as an essential method for imaging ex vivo biological samples, especially embryos and invertebrates. His lab is developing new and refined sample preparation and imaging methods, with applications including molecular imaging and imaging of specific cells types.



#### Dr Paul Zaslansky, Universitätsmedizin Berlin

Dr. Paul Zaslansky is a research scientist in the Department for Restorative and Preventive Dentistry of the dental school of the Charité Universitätsmedizin Berlin and a senior researcher in the Julius Wolff institute for Biomechanics and Musculoskeletal Regeneration of the Charité Universitätsmedizin Berlin of the Berlin-Brandenburg Center for Regenerative Therapies. He graduated as D.M.D from the Hebrew University - Hadassah School of Dental Medicine in Jerusalem in 1991 and initially worked as a dental surgeon focusing on composite restorative dentistry for more than a decade, before turning to an academic career. He completed his PhD in the Department of Structural Biology of the Weizmann Institute of Science in 2005 followed by post-doctoral training in the Biomaterials department of the Max-Planck Institute of Colloids and Interfaces in Potsdam, Germany.

Zaslansky's research centers on understanding structure-function relations in mammalian teeth and bones, with a strong emphasis on non-contact and non-destructive imaging and in-situ mechanical testing. For this he has combined classical microscopy with phase-contrast imaging by laser-speckle interferometry (ESPI) and high-resolution X-ray phase-contrast enhanced tomography. His work combines structural investigations using materials-science approaches with surface deformation real-time visualization and sub-micron volume imaging of the collagen-based composites in teeth and bone.



#### George Oates, Museum in a Box Ltd

George Oates is an award-winning designer who's worked on and around the web since 1996. She was the first designer of Flickr, and invented the innovative Flickr Commons programme, to help public institutions share their photography collections with the Flickr community.

She specialises in friendly, simple interaction design, and, after moving to London from San Francisco in 2014, has started two companies: Good, Form & Spectacle, a small but mighty digital agency focussed on experimental software development for the cultural heritage sector, and Museum in a Box, a tactile, fun, interactive edtech product company whose main goal is to increase access to cultural heritage collections.

## Programme

### Thursday 7 September - Quantitative Tomography

08:45 - 09:15	Registration, Tea & Coffee
09:15 - 09:30	Welcome and Introduction Farah Ahmed, Natural History Museum. Gianluca Tozzi & Asa Barber, University of Portsmouth

Session 1: Correlative Tomography (Session Chair: Philipp Schneider, University of Southampton)	
09:30 - 10:00	Keynote Talk: Recent Advancements in Correlative Microscopy. Jeff Gelb, Carl Zeiss Microscopy
10:00 - 10:15	Serial block-face scanning electron microscopy of human bone tissue – towards a correlative imaging workflow. <b>Patricia Goggin, University of Southampton</b>
10:15 - 10:30	Optimised lab-based in-line phase contrast micro-computed tomography for soft tissues. <b>Berit Zeller-Plumhoff, Helmholtz-Zentrum Geesthacht</b>
10:30 - 10:45	Novel reconstruction software for multispectral computed tomography. <b>Daniil Kazantsev,</b> <b>University of Manchester</b>
10:45 - 11:15	Tea & Coffee, Exhibition, Posters & Image Competition

# Session 2: 4D Tomography & Related Techniques (Session Chair: Enrico Dall'Ara, University of Sheffield)

11:15 - 11:45	Keynote Talk: Mechanics-based 4D tomography. Francois Hild, LTM-Cachan
11:45 - 12:00	A collection of X-ray tomography datasets for benchmarking sparsity-based reconstruction. Jakob Jorgensen, University of Manchester
12:00 - 12:15	Micromechanics and digital volume correlation of in vivo bone-biomaterial systems. <i>Marta</i> <b>Peña Fernández, University of Portsmouth</b>
12:15 - 12:30	Time-lapsed synchrotron micro-CT imaging of the proximal human femur under progressive load. <b>Egon Perilli, Flinders University</b>
12:30 - 13:45	Lunch, Exhibition, Posters & Image Competition

### Session 3: Tomography & Additive Manufacturing (Session Chair: Frank Witte, Charite' Berlin)

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13:45 - 14:15	<b>Keynote Talk:</b> Micro-CT Inspects the Structural Integrity of Additive Layer Manufactured Parts. <b>Andrew Ramsey, Nikon Metrology</b>
14:15 - 14:30	Comparative performance assessment of beam hardening correction algorithms applied on simulated data sets. Wenchao Cao, Nikon Metrology
14:30 - 14:45	X-ray tomography evaluations of failure in additive manufactured biomimetic structures. <i>Marco Curto, University of Portsmouth</i>
14:45 - 15:00	Structural stiffness estimation of replica cancellous bone models via finite element analysis of 3D ultrasound computed tomography data (UCT-FEA). <i>Christian Langton, Queensland University of Technology</i>
15:00 - 15:30	Tea & Coffee, Exhibition, Posters & Image Competition

# Session 4: Tomography-based Computational Modelling (Session Chair: Martino Pani, University of Portsmouth)

15:30 - 16:00	<b>Keynote Talk:</b> CT-based bone strength prediction: from human to mice. <i>Marco Viceconti,</i> <i>University of Sheffield</i>
16:00 - 16:15	Validation of micro-Finite-Element models with combination of microCT imaging and digital volume correlation. <b>Enrico Dall'Ara, University of Sheffield</b>
16:15 - 16:30	A parallel CT reconstruction algorithm using partial row and column blocks of the system matrix. <b>Yushan Gao, University of Southampton</b>
16:30 - 16:45	3D strain fields across the whole thickness of thoracic aortas using digital volume correlation combined with OCT. <i>Victor-Andres Acosta-Santamaria, SalnBioSE, INSERM</i>
16:45 - 17:30	Lightning Talks
19:00	Symposium Banquet at HMS Warrior

### Friday 8 September - Tomographic Applications

08:45 - 09:00	Tea & Coffee, Posters
Session 5: Materials Science (Session Chair: Egon Perilli, Flinders University)	
09:00 - 09:30	<b>Keynote Talk:</b> How Digital Volume Correlation helps better characterization of materials? <b>Jan</b> <b>Giesebrecht, Thermo Fisher Scientific</b>
09:30 - 09:45	<b>Keynote Talk:</b> YXLON'S new generation of smart Laboratory CT devices enabling HIGh precision and maximum user friendliness. <b>André Beerlink, YXLON</b>
09:45 - 10:00	Laminography with Robotic Manipulators for Composite Aerospace NDT. <b>Thomas Blumensath,</b> <b>University of Southampton</b>
10:00 - 10:15	Fabrication, characterization and high-resolution x-ray tomographic investigation of resorbable electrospun nanofibrous scaffolds for tendon regeneration. <i>Alberto Sensini, Università di Bologna</i>
10:15 - 10:45	Tea & Coffee, Exhibition, Posters & Image Competition

Session 6: Healthcare (Session Chair: Christian Langton, Queensland University of Technology)	
10:45 - 11:15	<b>Keynote Talk:</b> Human tooth micro anatomy and root canal treatment failure: insights by X-ray phase contrast enhanced microCT. <i>Paul Zaslansky, Universitätsmedizin Berlin</i>
11:15 - 11:30	A new era of virtual histology: contrast-enhanced microCT to simultaneously visualize and quantify in 3D soft and mineralized biological tissues. <b>Greet Kerckhofs, KU Leuven</b>
11:30 - 11:45	Microstructure and Mechanics of Intervertebral Discs from Phase Contrast Synchrotron X-ray Tomography. <b>Catherine Disney, University of Manchester</b>
11:45 - 12:00	Linking regional proximal tibia 3D bone microarchitecture and in vivo joint loads in end- stage knee osteoarthritis. <b>Egon Perilli, Flinders University</b>
12:00 - 13:00	Lunch, Exhibition, Posters & Image Competition

Session 7: Life Sciences (Session Chair: Greet Kerckhofs, KU Leuven)	
13:00 - 13:30	<b>Keynote Talk:</b> From whole animals and micromorphology to cell types and molecular probes: MicroCT as a new standard tool in bioscience research. <b>Brian Metscher, University of Vienna</b>
13:30 - 13:45	A novel approach for studying 3D embryo development of crustaceans (freshwater shrimp Neocaridina heteropoda) using the X-ray Microtomography. <i>Lidia Sonakowska, University of Silesia</i>
13:45 - 14:00	The developmental biology of extremely strong limpet tooth material. <b>Robin Rumney,</b> <b>University of Portsmouth</b>
14:00 - 14:15	Exploring the potential of neutron imaging and complementary techniques for life sciences applications at the neutron spallation source, ISIS, UK. <i>Genoveva Burca, STFC-Rutherford Appleton Laboratory</i>
14:15 - 14:45	Tea & Coffee, Exhibition, Posters & Image Competition

Session 8: Earth & Space (Session Chair: Asa Barber, University of Portsmouth)	
14:45 - 15:15	Keynote Talk: Tomographic mapping of the Universe. David Bacon, University of Portsmouth
15:15 - 15:30	Optimisation of gold nanoparticles as a novel contrast medium for plant root and soil X-ray CT imaging. <i>Callum Scotson, University of Southampton</i>
15:30 - 15:45	The usage of modern data science in segmentation and classification: Machine Learning and Microscopy. <i>Matthew Andrew, Carl Zeiss Microscopy</i>
15:45 - 16:00	Revealing the complexity of tephra horizon structures in soft sediment sequences. Elizabeth Evans, University of Swansea

Session 9: Cultural Heritage & Public Engagement (Session Chair: Farah Ahmed Natural History Museum)	
16:00 - 16:30	Keynote Talk: 3D Museums: tactile learning, greater access. George Oates, Museum in a Box Ltd
16:30 - 16:45	A Whale of a Project: How to Scan the World's Largest Animal. <i>Kate Burton, Natural History Museum</i>
16:45 - 17:00	Ageing fossil birds using high-resolution synchrotron-based computed tomography for virtual histology. <i>Katherine Williams, University of Southampton</i>
17:00 - 17:15	MorphoSource: A Virtual Museum and Digital Repository for 3D Specimen Data. <b>Julie</b> <b>Winchester, Duke University</b>
17:15 -	Final Remarks

### **Oral Abstracts**

### Session 1: Correlative Tomography

#### **Recent Advancements in Correlative Microscopy**

Jeff Gelb<sup>1</sup> and Tobias Volkenandt<sup>2</sup>

<sup>1</sup>Carl Zeiss Microscopy, Pleasanton, CA, USA <sup>2</sup>Carl Zeiss Microscopy, Oberkochen, Germany

In a typical material characterization study, the question of scale is of paramount importance. A material system may contain tiny features, requiring a high-resolution imaging approach, but may need to simultaneously characterize the volumetric dispersion of those features, necessitating a large field of view and 3D imaging capabilities. Thus, a typical material system may require characterization across a range of length scales, spanning from the millimeters to the nanometers. Furthermore, complementary data may be desired, such as targeted EDS or EBSD mapping, but this data must be kept within the volumetric context of the overall material system. Unfortunately, no single tool exists capable of delivering this type of multi-scale information in a reasonable length of time.

To address the needs for multi-scale characterization, many researchers are turning toward correlative microscopy as a holistic approach to bridge between available imaging resolutions and accessible imaging volumes. While the correlative microscopy approach has a rich history in the literature, it is only in recent years that this has been made accessible to a wide variety of scientists and engineers.

Here, we present the latest advancements in correlative microscopy, including the development of an integrated characterization framework for blending instruments and modalities. We will present several key use cases where a multi-scale, multi-modal approach has been employed, including applications in metal alloys, composite systems, and even packaged devices, such as Li-ion batteries. This correlative microscopy approach represents a paradigm shift in material characterization strategies, shifting focus away from the capabilities of individual instruments and, instead, centering on the needs of each study, incorporating the necessary characterization tools along the way.

# Serial block-face scanning electron microscopy of human bone tissue – towards a correlative imaging workflow

#### Patricia Goggin<sup>1</sup>, Richard O.C. Oreffo<sup>2</sup> and Philipp Schneider<sup>1</sup>

<sup>1</sup>Bioengineering Science Research Group, Faculty of Engineering and the Environment, University of Southampton, UK; <sup>2</sup> Bone and Joint Research Group, Centre for Human Development, Stem Cells and Regeneration, Faculty of Medicine, University of Southampton, UK

Keywords: Bone, osteocytes, correlative microscopy, serial block-face scanning electron microscopy, 3D

**MOTIVATION:** Osteocytes form an interconnected network within mineralised bone, pivotal for bone health. To better understand bone mechanobiology high-resolution 3D imaging of osteocytes and their surrounding matrix across multiple scales is necessary [1]. Therefore, a correlative microscopy approach is needed. X-ray based computed tomography (CT) is an established technique that can cover thousands of osteocyte lacunae [2], yet osteocytes and their interconnections are not accessible due to lacking soft tissue contrast and limited spatial resolution. Serial block-face scanning electron microscopy (SBF SEM) should be able to fill this gap. In SBF SEM resin-embedded tissue is alternately imaged using SEM and sliced by an automated ultramicrotome producing a stack of 2D images [3]. We have developed a protocol for SBF SEM of human bone tissue as a step towards developing a correlative imaging workflow when combined with X-ray CT.

**METHODS:** Blocks of tissue (< 2 mm3) from a human femoral head obtained during hip replacement surgery were fixed for EM with 3% glutaraldehyde, decalcified, processed using heavy metals to add conductivity and contrast, then dehydrated

and embedded in epoxy resin [4]. A block was trimmed, mounted on a pin and imaged using a Gatan 3ViewXP2 system in a Quanta 250 FEGSEM. An osteocyte cell body, processes, nucleus and mitochondria were manually segmented by tracing the outlines in sequential slices using the FIJI plug-in TrakEM2 [5, 6]. The segmented data was used to render volumes and generate surfaces in Amira.



**RESULTS:** Figure 1:An osteocyte reconstructed from SBF SEM data. The cell body is shown in transparent yellow, processes in green, the nucleus in blue and mitochondria in orange. Scale bar =  $5 \mu m$ .

**DISCUSSION:** SBF SEM provides stacks of high-resolution images which allow 3D reconstruction of cell ultrastructure (Figure 1). The mineralised matrix and soft cellular elements of bone can be imaged simultaneously.

**OUTLOOK:** A correlative microscopy workflow combing X-ray-based CT and SBF SEM needs to be established, providing 3D data for computational modelling and quantitative morphological analysis of osteocytes in health and disease.

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- [5] A Cardona, S Saalfeld, J Schindelin, et al. (2012). PloS One 7: e38011.
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# Optimised lab-based in-line phase contrast micro-computed tomography for soft tissues

#### B. Zeller-Plumhoff<sup>1</sup>, J.L. Mead<sup>2</sup>, M. Wallis<sup>2</sup>, D. Tan<sup>2</sup>, T. Roose<sup>2</sup>, G.F. Clough<sup>3</sup>, R.P. Boardman<sup>4</sup> and P. Schneider<sup>2,4</sup>

<sup>1</sup>Helmholtz-Zentrum Geesthacht, Centre for Coastal and Materials Research, Geesthacht, Germany
 <sup>2</sup>Bioengineering Research Group, Faculty of Engineering and the Environment, University of Southampton, Southampton, UK
 <sup>3</sup>Institute for Health Sciences, University of Southampton, Southampton, UK
 <sup>4</sup>µ-VIS X-ray Imaging Centre, University of Southampton, Southampton, UK

Phase contrast-based X-ray computed tomography (CT) imaging can be used to enhance the understanding of 3D anatomic structure and related function of soft tissues. Unfortunately, phase contrast X-ray imaging is often limited to synchrotron radiation sources offering coherent X-rays, to which access is limited and highly competitive. Therefore, it is important to enable high-quality phase contrast CT using lab-based X-ray sources. Here, we present an optimised approach for in-line phase contrast imaging of soft tissues, going beyond those presented by Bidola and colleagues [1]. This includes an analysis on the influence of the exposure time, the X-ray propagation distance and the specific choice of parameters during Paganin phase retrieval [2]. The optimal experimental CT settings have been determined using a mouse soleus muscle [3]. The quality of the reconstructed CT data after phase retrieval was also directly compared to reconstructed CT data based on X-ray absorption only to quantify the benefit of phase retrieval for soft tissue imaging.

CT imaging was performed using a ZEISS/Xradia Versa 510 system at an X-ray energy of 40 keV and a voxel size of 0.9  $\mu$ m. For gold-standard comparison, CT imaging of the same sample has been performed at the TOMCAT beamline of the Swiss Light Source using a monochromatic beam at 15 keV. Paganin phase retrieval was performed using Octopus 8.4 (Inside Matters, Belgium) with the  $\delta/\mu$  ratio varying between 1.48E-8 and 9.12E-12 (see Fig I for signal-to-noise (SNR) and contrastto-noise ratio (CNR) characterising image quality).





Fig 2: Comparison of lab-based (a) and synchrotron-based (b) phase contrast CT data of mouse soleus muscle (\* marks nerves).

Fig 1: Signal-to-noise and contrast-to-noise ratio of reconstructed lab-based CT data dependent on  $\delta/\mu$  ratio chosen for Paganin phase retrieval [2]. The dashed red line shows the optimum value.

We found that larger propagation distances in combination with larger exposure times result in higher image quality. Image quality measures such as SNR and CNR were well below those obtained using synchrotron radiation [3]. However, important soft tissue features such as nerves, could be distinguished from surrounding tissue (see Fig 2), thus making lab-based X-ray sources a viable alternative to synchrotron sources for soft tissue imaging.

#### References

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#### Novel reconstruction software for multispectral computed tomography

#### Daniil Kazantsev<sup>1,2</sup>, Jakob S. Jørgensen<sup>1,4</sup>, Martin J. Turner<sup>3</sup>, William R.B. Lionheart<sup>4</sup>, and Philip J. Withers<sup>1</sup>

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Keywords: vectorial image reconstruction, multi-energetic imaging, correlative regularization

Conventional X-ray Computed Tomographic (CT) imaging is mostly stuck in a black and white (single channel) era, just as it was with the first image Röntgen captured in 1895! However, technological breakthroughs in energy-sensitive detectors and time-of-flight technologies enable a new era of tomographic imaging in 'colours' (multiple channels) [1], see Fig.1. Hundreds or thousands of energy channels can be acquired simultaneously, leading to chemical tomography extracting materials linked to their unique k-edge signatures. This delivers a significant advantage over single-channel (grey-scale) imaging but also poses great challenges for multispectral image reconstruction.



Fig I. Conventional X-ray projection of a USB dongle (left) and multispectral X-ray projection, where each colour represents a different element (right) [1].



Fig. 2. Multispectral data and reconstruction of materials with state-of-the-art iterative methods.

Current software only allows us to reconstruct each channel independently which is a significant limitation since the energy channels are mutually correlated, just like the red-green-blue (RGB) channels of a colour image. Therefore, noise and other inaccuracies in spectral measurements should be treated holistically across the spectral domain, leading to massive improvements in imaging quality (higher signal-to-noise ratio and resolution, see dTV channel-correlated recovery in Fig. 2)

The new CCPi [2] Flagship project: A Reconstruction Toolkit for Multichannel CT (RT-MCT) aims to deliver the set of novel methods and software tools, which are specifically designed to extract maximum information across the spectral dimension within an open source framework [3]. Three major imaging facilities are key collaborators and committed initial users of the RT-MCT: I) Manchester X-ray Imaging Facility; 2) Neutron Imaging and Diffraction Facility (IMAT) at the ISIS; and 3) Diamond Light Source.

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### Session 2: 4D Tomography & Related Techniques

#### **Mechanics-based 4D tomography**

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X-ray microtomography coupled with Digital Volume Correlation (DVC) offers the possibility of performing and characterizing exhaustively in-situ mechanical tests. Three dimensional displacement fields are measured from the registration of two tomographic images. Once a series of acquired scans is obtained, 4D analyses can be performed. Integrated digital volume correlation (I-DVC), which incorporates a finite element modeling of the test, thereby performing a mechanical integration in 4D registration of a series of 3D images, is a possible route to bridge the gap between experiments and simulations. In the present case a non-intrusive procedure is developed in which the 4D sensitivity fields are obtained with finite element codes, allowing for a large versatility in meshing and incorporation of complex constitutive laws. A tensile test on cast iron is analyzed and the plasticity parameters are calibrated thanks to the present framework.

One limitation of the previous analyses is the time needed to acquire the entire set of radiographs for a 3D reconstruction. When the sample cannot be regarded as motionless during the entire scan, classical image reconstruction software cannot

be used any longer. This renders very difficult to capture even slow motions occurring during a scan. A novel approach is presented where DVC and reconstruction are associated together with a mechanical model in order to reformulate the temporal evolution of a sample with a small but suited set of parameters. Using such an approach, the problem is reduced to the registration of a few (down to two) radiographs provided the reference state has been scanned once. This procedure drastically reduces the required number of radiographs, and hence it opens the way to a much improved time resolution without any hardware change. The methodology is illustrated on a cast iron sample subjected to an in situ tensile test.

# A collection of X-ray tomography datasets for benchmarking sparsity-based reconstruction

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Keywords: Open-access CT data, image reconstruction, sparsity regularisation, total variation, Compressed Sensing

In recent years tremendous progress has been achieved on reconstruction algorithms for X-ray Computed Tomography (CT). An important class of algorithms utilise sparsity regularisation (SR), such as total variation (TV) minimisation, to enable high-quality reconstructions from substantially fewer projections than required by conventional methods such as filtered backprojection. However the full potential as well as limitations of these methods have not yet been systematically established. For example, it remains poorly understood how few projections suffice and how image characteristics such as sparsity (number of zero or close-to-zero pixel or gradient values) affect performance.

We present a collection of 48 X-ray CT datasets called SparseBeads designed specifically for benchmarking SR reconstruction algorithms. Beadpacks comprising glass beads of 5 different sizes (representing different gradient sparsity levels) as well as mixtures were scanned in a micro-CT scanner in the Henry Moseley X-ray Imaging Facility at the University of Manchester. The resulting raw data as well as manufacturer reconstructions are made available from zenodo.org using DOI: 10.5281/ zenodo.290117.The datasets form a highly structured collection with variable image sparsity levels, numbers of projections and noise levels to allow the systematic assessment of parameters affecting performance of SR reconstruction algorithms.

As an example of studies enabled by the SparseBeads dataset, the present work establishes for the first time for real CT data a direct relation between gradient sparsity and the sufficient number of projections for accurate TV-regularized reconstruction. Image quality for TV-regularized reconstruction was assessed as function of numbers of projections and gradient sparsity. The critical number of projections for satisfactory TV-regularized reconstruction was found to increase almost linearly with the gradient sparsity. This may be of utility in planning of dose- or time-critical experiments, such as 4D tomography, as a quantitative guideline of how few projections to acquire based on expected sample sparsity level.

#### Micromechanics and digital volume correlation of in vivo bone-biomaterial systems

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Novel osteoregenerative biomaterials for tissue engineering are currently being developed, with research aims ranging from optimal bone integration in scaffolds to complete bone formation. However, very little is known about the ability of such biomaterials to produce bone comparable with native to provide support in load-bearing regions. The combination of high-resolution synchrotron micro-computed tomography (SR-microCT), in situ mechanical testing and digital volume correlation (DVC) enables a detailed investigation of the three-dimensional deformation of bone-biomaterial systems under loading. This study aims to characterize the mechanical performance of regenerated trabecular bone in bone-biomaterial systems after implantation of osteoregenerative commercial grafts.

Bone defects were surgically created in ovine femoral condyles and four biomaterials were implanted. Cylindrical samples (D:4mm, L:8mm) of bone-biomaterial were cored. SR-microCT was performed at the II3-2 beamline (Diamond Light Source, UK) with an effective voxel size of 2.6µm. Tomographic datasets were obtained with at an exposure time of 64 ms/projection (1800 projections/tomography). In situ mechanical testing (CT5000, Deben Ltd, UK) was carried out with four compression steps under displacement control (0.1, 0.25, 0.5 and 1 mm). Tomographic datasets were acquired at each loading step. DVC software (DaVis 8.3, LaVision, Germany) was used to obtain full-field strain distribution throughout each bone-biomaterial composite.

The largest microfailure events were localized close to or within newly formed bone tissue. When damage appeared in the native tissue, DVC successfully correlated it to large levels of compressive strains, above the typical values of trabecular bone yielding (Figure 1). Further evaluation needs to be conducted to better understand the specific effect of each biomaterial on the quality of bone produced in vivo. This study will allow the first exploration of some important aspects of ex vivo integrated bone-biomaterial constructs, including the identification of microdamage initiation at the bone-biomaterial interface and its contribution to the overall mechanics of the newly formed tissue.



# Time-lapsed synchrotron micro-CT imaging of the proximal human femur under progressive load

#### Egon Perilli and Saulo Martelli

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Keywords: Time-lapsed imaging, synchrotron micro-CT, mechanical testing, human, bone

#### Introduction

Time-lapsed micro-computed-tomography (micro-CT) with concomitant mechanical testing is increasingly used to study the bone deformation and fracture mechanism. However, previous femur studies were limited, imaging only small cores under load [1]. We developed a protocol for time-lapsed synchrotron micro-CT imaging of entire human femoral epiphyses under incremental load.

#### Methods

Twelve human femurs from elderly female donors (56-91 years) were obtained. Fracture loads were estimated using clinical CT images and finite-element modelling [2]. A compression stage was custom-built, including an aluminium compression chamber, a 6-degree-of-freedom load cell and a screw-jack mechanism. Samples were mounted inside the compression stage, replicating a single-leg stance configuration. Micro-CT scans (29.81 µm/voxel, isotropic) were performed at the Australian Synchrotron (Clayton,VIC,Australia). One-fifth of the predicted fracture load was incrementally applied to the femur from the initial unloaded condition, with one complete micro-CT scan performed at each load step. For each step, the total volume scanned was 160 mm in diameter and 130 mm in height, scanning time 25 min. Four femurs were loaded to fracture, while 8 femurs were loaded non-destructively. The 6-component-force over time was recorded during the experiment.

#### Results

Fractures were experimentally obtained in 5-6 load increments as predicted, with loads within the predicted range (1998-8636 N). The 2D and 3D micro-CT images showed first deformation, then fracturing of the trabeculae and cortex (figure 1). Sub-capital femoral neck fractures, consistent with observed patterns of clinical fractures, were obtained and were visible in the micro-CT images.

#### Discussion

Time-elapsed synchrotron micro-CT imaging of entire human femoral epiphyses with concomitant step-wise mechanical testing was successfully performed, at 29.81 µm/voxel. Clinically relevant fracture patterns were experimentally replicated and visible in the micro-CT images, together with the bone microarchitecture. Morphometric and micro-finite-element analyses are being undertaken, to investigate the contribution of the different microstructural compartments to withstand load.

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### Session 3: Tomography & Additive Manufacturing

### Micro-CT Inspects the Structural Integrity of Additive Layer Manufactured Parts

#### Andrew Ramsey

#### Nikon Metrology

Additive Layer Manufacturing (ALM), or "3D printing" is being now used to manufacture high value low volume components in industries such as aerospace, medical implants and automotive.

ALM parts are being increasingly used today to lower the weight of components without compromising on strength, for example in aerospace applications where decreased weight leads to increased efficiency.

For safety-critical components it is essential that failure-free parts are created in order to preserve the structural integrity.

It is important to know whether there are voids and inclusions present, how large they are (both individually and in total), and where they are – and also whether the dimensions of the part conform to those of the design.

By giving a full 3D density map of the samples micro-CT gives all this information in an easy-to-read visual format.

As ALM continues to rewrite the manufacturing rulebook, X-ray computed tomography can be a powerful partner for the non-destructively assurance of geometrical tolerances and the assessment of internal defects.

# Comparative performance assessment of beam hardening correction algorithms applied on simulated data sets

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Keywords: beam hardening, performance assessment, dimensional metrology, computed tomography

Beam hardening artefacts caused by the polychromatic nature of the normal X-ray spectra are known to deteriorate the reconstructed image quality in industrial computed tomography (CT). A variety of beam hardening correction (BHC) algorithms exist, yet their relative performance in the correction of multi-material reconstructions has not been benchmarked. This work addresses the need for such a comparison by comparing three BHC algorithms of different types: a segmentation based multi-material linearisation algorithm, a dual energy algorithm, and an iterative reconstruction algorithm [1-3]. Each BHC algorithm is applied to simulated data sets of a dual-material phantom consisting of multiple cylinders. The comparison is performed on data sets simulated both under ideal conditions and with the addition of noise. The relative performance of each algorithm is assessed with respect to its effect upon the final image quality (contrast to noise ratio, spatial resolution), artefact reduction (streaks, cupping effects), and dimensional metrology. The metrics have been carefully designed in order to achieve a quantifiable assessment. The results suggest that the segmentation based linearisation algorithm for multimaterial correction can effectively reduce beam hardening artefacts, but it tends to degrade the contrast resolution of the reconstructed images. The dual energy method yields the best results for all investigated indicators, yet data acquisition and processing are sophisticated. The iterative algorithm is able to eliminate beam hardening streaks in the cross section. However, it seems to introduce aliasing patterns around the object edge, and its performance proves very dependent on computer capabilities. The dual energy method and the segmentation based multi-material linearisation algorithm give more accurate results in dimensional metrology than the iterative reconstruction algorithm. Overall, the dual energy method works best among the three. As expected the addition of noise is found to degrade contrast to noise ratio and spatial resolution. Noise also increases the uncertainty of the dimensional measurements. Nonetheless the presence of noise does not adversely affect the relative performance of any of the BHC algorithms.

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# X-ray tomography evaluations of failure in additive manufactured biomimetic structures

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#### Keywords: x-rat tomography, additive manufacturing, biomimetics, composites, mechanics

Biology is adept at producing structures optimized for mechanical function [1]. Understanding the design concepts found within biological structures and reproducing through manufacturing routes therefore presents significant opportunities in developing novel engineered structures. Additive manufacturing has recently become prevalent in producing complex engineered structures that exhibit high fidelity with their biological source, and has been applied to produce biomimetic bone, shell and teeth [2-4]. However, evaluating both the resultant 3D structural organization and corresponding mechanical performance is lacking. X-ray computed tomography (XCT) provides considerable opportunities to validate resultant structural organization as well as understanding the deformation and failure of the structure due to mechanical loading.

In this work we exploit the latest state-of-the-art jetting technology to produce a biomimetic structure based on the nacreous region of sea shells. Nacre is widely accepted as being tough due to a bricks and mortar structure consisting of hexagonal hard mineral platelets organized into layers and separated by considerably smaller volume fractions of soft material [5]. The toughening mechanism within nacre is contentious but controlled by failure between the hard platelets

during mechanical loading. Parametric design is exploited to generate a biomimetic nacreous structure of hexagonal platelets occupying approximately 90% by volume. A commercial high resolution multi-jetting material printer (ProJet 5500X, 3D Systems, USA) is used to produce biomimetic structures through depositing hard plastic for the platelet geometries and soft rubber materials at the interfaces between the platelets. The hard plastic and soft rubber are produced using a UV curable step and are polymers of similar attenuation under an x-ray probe. Figure 1 shows XCT evaluations of the resultant biomimetic nacre structure with an emphasis on maximizing contrast between the similar polymeric materials.





Regular hexagonal platelets in a repeating organization are clearly identified in this figure as well as the lower volume fraction rubber material between the hard platelets. Subsequent XCT imaging of the biomimetic nacre is performed in situ under mechanical deformation to failure, which provides direct evidence of the dominant failure mode. Our results demonstrate considerable interfacial failure within the biomimetic nacre confirmed by XCT that is comparable to the failure found in biologically formed nacreous regions of shell. This failure is highlighted as being effective for enhancing the toughness of additive manufactured structures. The incorporation of XCT into workflows to validate additively manufactured parts is powerful especially when composite structure incorporating multiple polymeric materials are used such as for biomimetic design.

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### Structural stiffness estimation of replica cancellous bone models via finite element analysis of 3D ultrasound computed tomography data (UCT-FEA)

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Keywords: bone, structural stiffness, ultrasound computed tomography, finite element analysis, mechanical testing

Several non-invasive methods are used to assess a subject's bone status including X-ray absorptiometry (bone mineral density) and quantitative ultrasound (speed of sound and broadband ultrasound attenuation). Noting that to date there is no

non-invasive method of accurately measuring the structural stiffness of bone, here we report the feasibility of its estimation using finite element analysis of 3D ultrasound computed tomography data.

X-ray microCT scan data of four human cancellous bone samples (femoral head, iliac crest, calcaneus, lumbar spine) were replicated using 3D-printing (VisiJet M3 Crystal material, ProJet 3510 SD printer) into eight 15x magnified orthogonal cylindrical models. Each was scanned by our prototype UCT system, consisting of two 64-element 5MHz transducer arrays connected to an Olympus Omniscan unit; with translation and rotation of each model facilitated by a Motoman HP6 robotic arm.Variable-displacement FEA (ANSYS®) was performed to predict the structural stiffness of each model; incorporating the UCT image data along with the corresponding Young's modulus (1463 MPa) and Poisson's ratio (0.35) data for the 3D-print material. For comparison to the gold-standard of destructive mechanical testing, the structural stiffness of each model was experimentally measured under compressive physical loading.

We obtained a coefficient of determination (R2%) of 84% for UCT-FEA to estimate mechanical test derived structural stiffness. This result suggests that UCT-FEA may have the potential to provide a non-invasive and non-destructive estimate of the structural stiffness of bone, with significant potential to improve prediction of osteoporotic fracture risk and the clinical management of post-amputation prosthetics.

### Session 4: Tomography-based Computational Modelling

#### CT-based bone strength prediction: from human to mice

#### Marco Viceconti, Xinshan Li, Muhammed Qasim, Zainab Altai, and Enrico dall'Ara

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Computed Tomography (CT) is a powerful imaging technology that measure x-ray attenuation of a 3D space. Its use as medical imaging modality is well established, and special systems are available for small animals and for specimens, which operate at microscopic resolutions. In addition of providing the 3D morphology of the sample being scanned, when mineralised tissues are involved CT provides also a quantification of the mineral content of the tissues, and from it their elastic properties. In this keynote, we will review three applications where CT images of bone tissue are converted into finite element models, and used to explore non-destructively the mechanical properties of the skeleton. First, we will review the extensive work done on the development and validation of the CT2S technology, which uses calibrated clinical CT to provide accurate estimate of the strength of femurs, tibias, and vertebral bodies of osteoporotic patients. Then we will describe an adaptation of this technology to develop a normative table for skeletal strength in infants. Last, we will show how these methods were adapted to test new bone drugs in mice. CT-based bone strength prediction is now a well-established methodology which can be safely adopted in pre-clinical and clinical research.

# Validation of micro-Finite-Element models with combination of microCT imaging and digital volume correlation

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Understanding the relationship between local mechanical properties of bone and its microarchitecture is fundamental to study bone remodeling and for optimizing implants and injectable biomaterials. Due to the complexity of bone, the only way we have to study its local deformations is with microCT based computational finite element models (microFE) [1]. Before their applications models should be validated against accurate experiments. In particular, the digital volume correlation (DVC) techniques combined with ex vivo stepwise loading of bone samples provide an experimental framework to test how well the microFE models predict the local displacements. In this study three independent datasets of bone specimens (three trabecular bone cylinders, four porcine vertebral bodies and two murine tibiae) were microCT scanned (voxel size between 10 and 40 micrometers) and stepwise compressed in situ. For each dataset the accuracy and precision of a global DVC approach was measured [2, 3] and DVC was applied to map the displacement field under the induced deformation. From the undeformed microCT images microFE models were generated with the same boundary conditions of the experiments. The microCT images were segmented and each bone voxels was converted into an 8-nodes hexahedral element. Bone was considered

isotropic and homogeneous. The predicted displacements were compared in the location of the DVC nodes, which fell within the middle portion of the specimens and within the bone tissue. The microFE showed good to excellent predictions of local displacements in all three directions, for all specimen types and for each specimen, with linear regressions close to the 1:1 relationship (slopes between 0.75 and 1.38; intercepts between -34 and 21 µm), R2 close to 1 (between 0.84 and 0.99) and RMSE below 38 µm.As example comparison between predicted and measured displacements along the compressive direction for the four vertebral bodies are reported in Figure 1.

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Figure I: Example of linear regressions between predicted and measured axial displacements for the four vertebral bodies (left) and example of distributions of maximum and minimum principal strains from the models and the DVC data (in the sagittal plane).

# A parallel CT reconstruction algorithm using partial row and column blocks of the system matrix

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Keywords: Algebraic CT reconstruction; parallel computing; gradient descent; coordinate descent

Despite the relatively low computational efficiency, iterative methods have shown advantages in CT image reconstruction, especially when data is noisy [1], when limited data is available or when unusual scan trajectories are used. To address the challenge of big data sets in CT, parallel computing schemes are widely applied. Both row-action and column-action methods fit parallel computing architectures by dividing the linear system matrix into several row or column blocks and then assigning these blocks to several computing nodes [2]. Row-action schemes, such as SIRT [3], require each computing node to keep a full copy of the reconstructed volume. Communication cost between nodes thus can be unacceptably high when the reconstructed volume. However, they require each node to have access to all the projection data at once. This can be computationally prohibitive in large-scale situations when the matrix is repeatedly computed in parallel by each computing node. To avoid significant inter-node communication, parallel column action methods also pose strict requirements on scan trajectories, which should be circular or helical and on the reconstruction volume slicing direction, which should be perpendicular to the rotation axis. These restrictions can be difficult to achieve when the reconstructed volume is large.

We here present a parallel algorithm called coordinate-reduced steepest gradient descent (CSGD) by combining steepest gradient descent method in a row-action form and a partial update strategy adapted from column-action strategies. Each node only has access to a small row and column subset of the whole system matrix (i.e. each node only needs access to a subset of observations and a portion of the reconstruction volume). CSGD does not pose any restrictions on either scanning strategy

or volume, data slicing and the computational burden on each node is significantly reduced. We also apply non-homogeneous selection criteria [5] when selecting sub-matrices from the entire system matrix, ensuring that denser sub-matrices are more frequently chosen. This is shown to further increase the convergence rate. One of the simulations is shown in Fig. I to demonstrate the effectiveness of the CSGD method.



Fig. 1: CSGD with or without importance sampling strategy and a diagram showing difference in pixel values along a line through a 2D reconstruction after 500 epochs.

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## **3D** strain fields across the whole thickness of thoracic aortas using digital volume correlation combined with **OCT**.

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Optical Coherence Tomography (OCT) is a suitable technique to reconstruct the microstructure of biological tissues (cartilage, cornea, vessel walls, skin, oesophageal or gastric tissues). Through imaging techniques, elastography consists in reconstructing strain and stiffness distributions of soft tissues. To reconstruct deformation or strain fields from 3D images of soft tissues, Digital Volume Correlation (DVC) can be applied. The DVC algorithms require though sufficient contrast to deduce deformations with appropriate accuracy. The penetration depth, imaging contrast and high scattering (normally seen in biological tissues), are current OCT limitations. The use of osmotic agents (propylene glycol), can improve the optical scattering properties and increase the imaging contrast and depth capability (Tissue Clearing technique)... Jiawei et al. (2013) combined OCT and DVC under a tensile test to measure in depth deformations, using silicone gels. The goal of the present work is to introduce and show the proof of concept of a novel immersed tensile tests combining DVC and OCT with tissue clearing, permitting to image tissues of 2 mm thick. Results show for the first time the strain distribution across the whole wall of a porcine thoracic aorta with a sub-millimiter spatial resolution.

### **Session 5: Materials Science**

#### How Digital Volume Correlation helps better characterization of materials?

#### Jan Giesebrecht

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Digital Volume Correlation (DVC) was introduced in 1999 by the group of researchers Bay, Smith, Fyhrie, and Saad. Since then the method has been widely accepted and has expanded to different imaging techniques. To date it has been used with MRI imaging, Computer Tomography (CT), and microCT but also recently, confocal microscopy, for testing of live tissue. DVC technique is preferred because of the non-invasiveness of the method.

DVC technique computes 3D full-field continuous displacement and strain maps from volume images acquired during a deformation process of an object. DVC can for instance be used to visualize and quantify deformation-induced microstructural changes during dynamic processes, such as localization phenomena induced by heterogeneities or thermal expansion mismatch between materials. Furthermore, the output displacement field can be used to enrich a numerical simulation by using measured boundary conditions, or to optimize this simulation by comparing numerical and measured data.

Avizo Software introduces two DVC algorithms: a traditional one using a subset-based approach and a more advanced FE-based continuous approach. The 2 approaches can be used independently or combined, in order to tackle for instance phenomena where large deformation is expected. These algorithms can be used in conjunction with all visualization and quantification modules, to track for instance pore network changes in between the different steps of the experiment. Powerful combination with Avizo Software meshing tools allows for setting up simulation with displacement and strain coming from real experiment.

This presentation will focus on 3 use cases demonstrating how DVC can help understand load transfer in bone-cement interface, measure crack opening displacement in a polymer or how particles influence the mechanical behavior of nodular cast iron.

### YXLON's New Generation of Smart Laboratory CT Devices Enabling High Precision and Maximum User Friendliness

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Keywords: micro-focus X-ray computed tomography (CT), laboratory CT system, industrial NDT, metrology

The YXLON FF35 CT system, see Fig. 1(a), is designed to achieve extremely precise X-ray inspection results for a wide range of applications while at the same time it offers user friendliness at highest level by a new intuitive touch interface control concept and further smart functionalities. Available in a single or dual tube configuration, it is perfect for very small to medium size parts inspection in the automotive, electronics, aerospace and material science industries and research [1].

#### Introduction

Computed tomography goes light years beyond regular 2-D X-ray technology to deliver accurate three-dimensional images of scanned objects, including their voids and areas of differing density. It has become one of the most important and powerful non-destructive testing (NDT) methods - an achievement resulting from the continuous improvement of CT scanning and reconstruction methods, the enabling of increased precision and resolution, the manufacturing of cheaper and more compact devices and finally innovative concepts allowing for highest user friendliness to simplify the execution of successful CT experiments with high quality results.

Today's range of NDT applications is applied to nearly every industry, i.e. automotive, electronics, aerospace and material science. Here, CT is used for research and development, failure analysis, process and quality control, small series inspection, combined DR-CT inspection, defect and material analysis, assembly checks and, becoming more and more important, metrology, i.e. dimensional measurements. To comply with such a variety of demands, YXLON has developed the powerful FF35 CT with an optional dual-tube-configuration (nano-focus transmission tube and high power micro-focus tube), which makes the FF35 CT extremely versatile. With a simple touch of a button, each tube can be adjusted independently. Intuitive system control is accomplished via two touchscreens displaying easy-to-understand graphics, see Fig. 1 (b). Intelligent functionality supports the user: As an example, "IntelliGuard" avoids collision with X-ray tube and detector by automatically determining the outer-shape of the inspection item during a 360° rotation. CT trajectories such as "HeliExtend" (Helical CT scan and reconstruction method) provide consistently good image quality from top to bottom, and capture elongated

specimen with a high magnification scan. The ability to select a custom centre of scan rotation using 'virtual axis rotation' ("FlexCenter") offers considerable comfort and saves time. The inspected item does not need to be repositioned if regions of interest fall outside the physical centre of rotation. A system health monitor provides information on various parameters, see Fig. 1 (c). Results are consolidated and displayed in a 'traffic light' representation. Additionally, one can access this health-state of the system and the progress of the CT (and reconstruction) from everywhere through remote monitoring software.

#### **Experimental Method**

Experiments are performed by choosing one out of two different x-ray sources mounted side-by-side in the high precision laboratory CT system FF35. The nano-focus transmission x-ray tube with maximum photon energy of 190 kV and a maximum output power of 15 W enables highest resolution by accessing sub-micron length scales. The more powerful directional x-ray source performs up to 225 kV and a maximum target power of 280 W. The FF35 CT is equipped with a 300 mm x 250 mm (w x h) flat panel detector with a pixel size of 139 µm and various scintillator materials. A granite base enables precise manipulation of the inspection part which can weight up to 30 kg. By using the horizontal scan field extension or the helical CT option for vertical scan field extension the maximum scan field can be 300 mm in diameter or 500 mm in height. The additional detector axis for movements along the beam direction (focus to detector distance (FDD)) allows for the optimal combination of magnification (up to factor 200 and more) and collected photons for highly resolved CT results with an optimal signal-to-noise ratio. Reconstruction of the CT volumes is carried out by a filtered back projection algorithm. Data quality can be improved by applying a numerous variety of correction filters for beam-hardening, ring-artefacts, noise, bad pixels, focal spot drift, and more.

#### Results

Results of representative applications, e.g. from biology, additive manufacturing, carbon fibre composites and metrology will be highlighted during the presentation to demonstrate the performance of today's laboratory CT devices, such as the YXLON FF35 CT.

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Figure 1: (a) The YXLON FF35 CT represents a new generation of high resolution and precision CT inspection devices and it features an innovative and highly intuitive touch screen control concept (b). (c) System parameter and status are shown in the health monitor while the radioscopic image is displayed below.

### Laminography with Robotic Manipulators for Composite Aerospace NDT

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Keywords: Computed Laminography, Planar, Carbon Fibre Reinforced Polymer, Non-destructive Testing, Robot, Impact Damage

There is a growing requirement within the aerospace industry to perform 3D scans of composite, carbon fibre reinforced polymer (CFRP) parts as part of a non-destructive testing (NDT) regime. Many such specimens have a laterally-extended shape and are therefore ill-suited to scanning with conventional computed tomography (CT). Where specimen dimensions permit a full rotation within a scanner, parts may be stacked or techniques such as variable exposure or variable energy CT scanning may be employed to mitigate some of the issues caused by the high variability in attenuation between different specimen orientations. For specimens where these approaches are not feasible, computed laminography (CL) offers the potential to achieve 3D imaging using trajectories other than conventional full-rotation CT and may therefore be more practical in some applications. CL has been in use at synchrotrons for some time but here we focus our attention on a cone-beam lab-based system.

We have designed and built a demonstrator system that allows the implementation of a number of non-standard trajectories to perform region-of-interest laminographic scans on planar specimens. The manipulator systems are designed as add-in modules for an existing, custom bay CT scanner at the University of Southampton, so that they may be installed and removed quickly and without interfering with usual CT scanning operations. We will discuss the design and implementation of our two sample manipulators, based around a hexapod and an industrial robot arm. We will then share some illustrative examples of reconstructed volumes, from scans of both machined specimens with known features and of impact-damaged CFRP specimens. Whilst the quality of the reconstructed image is below that of an optimised CT scan, the results demonstrate the technique's ability to provide better information for NDT than radiographs alone, in scenarios where full CT is impossible or inappropriate.



The image shows our hexapod-based manipulator system mounted within the Nikon/X-Tek custom bay CT scanner at the  $\mu$ -VIS X-Ray Imaging Centre, University of Southampton

# Fabrication, characterization and high-resolution x-ray tomographic investigation of resorbable electrospun nanofibrous scaffolds for tendon regeneration

## <u>Alberto Sensini</u><sup>1</sup>, Maria Letizia Focarete<sup>2</sup>, Chiara Gualandi<sup>2</sup>, Andrea Zucchelli<sup>1</sup>, Gianluca Tozzi<sup>3</sup>, Liam Boyle<sup>4</sup>, Gwendolen Reilly<sup>4</sup> and Luca Cristofolini<sup>1</sup>

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#### Keywords: Electrospinning, tendon tissue engineering, high-resolution x-ray tomography, cell viability

Tendon injuries represent an unsolved clinical need. Recent preliminary studies confirmed the suitability of resorbable electrospun scaffolds (bundles), for mimicking the multiscale structure of tendon fascicles [1-2]. In the present work resorbable electrospun crosslinked scaffolds (bundles) of aligned nanofibers, made of poly-L-lactic acid (PLLA) and collagen (Coll) blends in different percentages (PLLA/Coll 75/25 (w/w) and 50/50 (w/w)) were produced. Nanofibers (370-400 nm) and bundles (550-650  $\mu$ m) in the same range of diameters of human Achilles' tendon collagen fibrils and fascicles were obtained. The mechanical properties of the bundles after crosslinking and ageing in physiological environment were in line with those of human Achilles' tendon fascicles [3]. Human fibroblast proliferation on the bundles was also characterised with positive outcome. In order to investigate the full morphology of the scaffolds and the effects of the crosslinking process high-resolution x-ray tomography was used, with two different voxel sizes (1  $\mu$ m and 0.4  $\mu$ m). The tomographic analysis confirmed the expected hierarchical structure for the bundles, in terms of morphology and alignment of the nanofibers. The promising results for such resorbable electrospun nanofibrous bundles confirmed their suitability for tendon tissue reconstruction.





Example of a high-resolution x-ray tomography of a PLLA/Coll 75/25 crosslinked bundle (voxel size: 0.4  $\mu$ m):A) full volume of the bundle; B) internal volume of the bundle with fibers detail.

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### Session 6: Healthcare

# Human tooth micro anatomy and root canal treatment failure: insights by X-ray phase contrast enhanced microCT

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X-rays are essential for medical diagnosis in general and form an important component of many forms of dental treatment. The advent of digital radiography and an understanding that 2D X-rays are often not enough have lead to routine implementations of 3D imaging configurations by CT, for example dental CBCT. And while resolutions and detail visibility continue to increase, medical and dental CT solutions are still limited. High resolution and high flux ex-vivo CT solutions are providing complementary diagnostic information with important potential to improve treatment based on evidence based 3D measurements. Synchrotrons provides laser-like coherent X-rays that can be used to produce contrast between areas of similar material densities, disclosing inhomogeneities by highlighting X-ray interference effects. For example, interactions of X-rays with root filling materials reveal significant details using so-called phase contrast-enhanced images. This makes it possible to investigate the interfaces between the root canal filling and the tooth tissues with exquisite detail and at high resolution. For example, while absorption of X-rays due to materials used in root canal fillings often conceals low density gaps, phase contrast enhancement reveals morphology and extent of significant micron sized gaps. This presentation will showcase clinically important observations in healthy and treated human teeth based on a range of X-ray tomography approaches, and will present some of the advantages, disadvantages and possible clinical relevance. Ongoing potential benefits to future applied dental treatment will be discussed while highlighting how tomographic X-ray imaging in teeth helps reveal invisible treatment challenges, linking the patient-side setting to the lab.

# A new era of virtual histology: contrast-enhanced microCT to simultaneously visualize and quantify in 3D soft and mineralized biological tissues

#### Greet Kerckhofs<sup>1,2,3</sup>

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#### Keywords: contrast-enhanced microCT, virtual 3D histology, soft tissue visualization

**Introduction:** Biological tissues are 3D structures with complex spatial heterogeneity, for which traditional 2D imaging techniques such as standard histomorphometry are insufficient to comprehensively characterize them or assess their quality. In this overview, the potential and added value compared to standard histomorphometry of contrast-enhanced microfocus computed tomography (CE-CT) is presented. This recent development in the microCT imaging field allows virtual 3D quantitative histology of both soft and mineralized tissues.

**Methods and results:** For cartilaginous tissues, both an anionic (Hexabrix 320) and cationic (CA4+) contrast agent have been validated on murine knee joints (as blueprint)[1,2] and explants of cell-based bone tissue engineering (TE) constructs respectively. CE-CT using these contrast agents not only allowed to visualize the cartilage (both mineralized and non-mineralized) along with the bone in a single, 3D dataset, but it also enabled to quantify the 3D structure of the different cartilage types and the bone, as well as the glycosaminoglycan content of the non-mineralized cartilage. For bioreactor-driven TE construct development, we showed that CE-CT using Hexabrix staining can be used as a 'whole-construct' imaging technique allowing to quantify in vitro formed, neo-tissue (cells and extracellular matrix) in large 3D TE constructs in a perfusion bioreactor [3,4]. Staining with polyoxometalates (POMs – both commercially available as well as in-house developed) enabled to visualize and quantify in 3D the architecture of the blood vessel network and the adipocytes in the bone marrow compartment along with the bone in murine long bones (as blueprint) [5] and in explants of TE constructs (Fig. 1).

**Conclusion:** CE-CT is an important and innovative enabling technology to get a better understanding of the complex mechanisms behind tissue formation and regeneration, both in vitro and in vivo, and it sets the stage for a new era of virtual histology.



Fig. 1:A 3D representation of the blood vessel network (in red) in the bone marrow compartment of an explanted TE construct (using POM staining). In grey-scale, a cross-section through the explants is shown.

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#### Microstructure and Mechanics of Intervertebral Discs from Phase Contrast Synchrotron X-ray Tomography

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Keywords: soft tissue imaging, phase contrast synchrotron X-ray tomography, volume correlation

Intervertebral discs (IVD) are distributed throughout the spine, providing flexibility while supporting loads from body weight and physical activity. This mechanical function is directly related to their complex microstructure, and degeneration of these structures is associated with low back pain. In order to understand the pathological mechanisms and develop new treatments it is necessary to characterise the 3D microstructure of the IVD under different loading conditions. MicroCT is commonly used to resolve microstructure in mineralised samples, however IVDs have low X-ray absorption contrast. Here we describe the strategies which we have adopted to improve image contrast and to characterise the mechanical properties of sequentially loaded discs.

Staining of discs improved contrast but had uneven penetration and induced structural and mechanical changes. Therefore, we next employed in-line phase contrast microCT to image native (fresh) samples under compression and mapped the resulting strain. Rat spines were dissected to include the disc with adjacent vertebrae attached. Samples were set in holders and compressed in stages using displacement control steps (0.02mm, 2% strain). The Diamond-Manchester Branchline 113-2 was used for in-line phase contrast imaging at each step.

Phase contrast imaging clearly resolved the main anatomical components – calcified vertebral endplates, nucleus pulposus, annulus fibrosus – in the native discs (Fig. IA&B). Finer structural details including individual lamellae and collagen-fibre bundles (Fig. IC) were consistently resolved in the outer annulus throughout the in situ loading sequences, and supported the use of digital volume correlation for displacement and strain mapping. Results show differences in shear strains between adjacent lamellae and a relationship between strain patterns and collagen bundle orientations. This degree of strain heterogeneity within the annulus has not been previously documented.

Measuring volumetric strain using microCT provides detailed insight into micromechanics of native IVD tissue. This method has the potential to bridge the gap between measures of macro-mechanical properties and the local 3D micro-mechanical environment experienced by cells. In future these studies will impact on and may be used for the design and testing of potential IVD replacements.



Figure I.A) Schematic of the intervertebral disc. B) A single transverse tomography slice showing the main anatomical components. C) Magnified region showing concentric lamellae and bundle organisation.

### Linking regional proximal tibia 3D bone microarchitecture and in vivo joint loads in end-stage knee osteoarthritis

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Keywords: bone microarchitecture, in vivo joint loading, micro-CT, osteoarthritis, tibia

This ongoing study aims to examine, on end-stage knee osteoarthritis (OA) patients, relationships between knee joint loads measured in vivo using gait analysis prior to knee replacement surgery and the 3D bone microarchitecture of their excised tibial plateau quantified with micro-computed tomography (micro-CT).

Twenty-five knee-OA patients (age  $68\pm7$  years, mean $\pm$ SD) underwent pre-operative walking gait analysis: peak external (ERM) rotation moment, knee adduction moment (KAM) and mechanical axis deviation (MAD) were determined. After surgery, their entire tibial plateaus were retrieved and scanned with micro-CT ( $17 \mu$ m/pixel) [1]: subchondral bone 3D microarchitecture (bone volume fraction (BV/TV), trabecular thickness, trabecular number and structure model index (SMI)) was analysed in four subregions, in antero-medial, antero-lateral, postero-medial and postero-lateral condyles, and compared among them. Relationships between gait measurements and regional bone microarchitecture were examined.

The four anatomical subregions differed significantly in tibial subchondral bone microarchitecture aong them (p<0.05): antero-medial followed by postero-medial, exhibited highest BV/TV (up to +75% and +43%, respectively), trabecular number, trabecular thickness and lowest SMI, compared to other subregions. The BV/TV correlated negatively and the SMI positively with the peak ERM, in particular in the antero-medial (r=-0.74, p<0.01, r=0.67, p<0.01) and postero-medial (r=-0.75, p<0.01, r=0.53, p<0.01) condyles. Medial:lateral BV/TV ratio was significantly associated with ERM, KAM and MAD (r=-0.74, r=-0.60 and r=0.74, p<0.01).

This study is the first examining relationships between knee joint loading in vivo and knee bone microarchitecture, on the same patient. Our findings suggest that in knee-OA, during stance, peak ERM is significantly correlated with subchondral BV/ TV in the antero-medial and postero-medial tibial plateau, the anatomical locations where BV/TV was highest. This could be linked to microstructural bone adaptation to altered loading patterns that generate increased mechanical stresses in this condyle. Analysis is ongoing and if confirmed, gait analysis parameters could be suggested as non-invasive indicators of disease progression.

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### **Session 7: Life Sciences**

### From whole animals and micromorphology to cell types and molecular probes: MicroCT as a new standard tool in bioscience research

#### Brian Metscher

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Driven by the fundamental necessity of detailed and realistic visualisations for the study of anatomy in development, function, evolution, and diversity, technologies for making 3D images have progressed from the meticulously reconstructed wax-plate models of the late 19th century to high-resolution direct 3D microscopy, while increasingly sophisticated tools for rendering and analysing volume images have allowed us to move from depicting organs and tissues as simple coloured blobs to viewing anatomical structures with their full internal complexity.

X-ray microtomography (microCT) was the first direct non-destructive 3D imaging method and has now come of age as a general-purpose tool for anatomy, especially as a non-destructive 3D complement to other important imaging modalities, particularly EM, conventional histology, and block-face imaging methods. With soft-tissue contrast enhancement, microCT offers 3D in statu imaging of complete embryos and other intact specimens at histological scales. Digital 3D models of embryos can serve as virtual specimens in teaching and research, and we are currently generating high-resolution 3D embryo images for atlases of rat and chick developmental anatomy. Related projects include characterising a teratogenic rat model of oesophageal atresia; a study of the relationship between the vestigial lung and pulmonary vasculature in snakes; research on horse early renal development, combining microCT and TEM to maximize the information obtained from rare specimens; and demonstrating the role of gut endoderm in the development of cement organs and other extreme anterior structures in basal fishes. MicroCT is remarkably effective for imaging insects and other arthropods, and internal micro-morphological characters can be essential for distinguishing and describing species as well as for analysing function. We have recently published the first new millipede species description to be based partly on its cybertype – a set of virtual specimens made from microCT images of the physical type material for the species.

Our other current efforts include refinement of molecular probe imaging with microCT, developing X-ray-dense tissueselective stains, and imaging of melanocytes in development and in tumours. These methods will work effectively with dualenergy (spectrally-sensitive, "two-colour") microCT, which allows simultaneous high-resolution imaging of different tissues or contrast agents using lab-based systems.

# A novel approach for studying 3D embryo development of crustaceans (freshwater shrimp Neocaridina heteropoda) using the X-ray Microtomography

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#### Keywords: embryogenesis, crustaceans, X-ray Microtomography

Embryogenesis is an important stage during animal development, because during this stage the embryo's body undergoes significant transformation which can prepare the entire organism for proper functioning along adult stages. Despite the fact, that research studies on embryogenesis and postembryonic development are very interesting, they are also very difficult to conduct in practical terms due to relatively small specimen sizes and high sensitivity of the material. Therefore, a carefully chosen set of non-destructive microscopy techniques has to be implemented in such studies in order to maximize the imaging results without the need for complex sample preparation procedures. Up to now, mainly the light and fluorescence microscopes have been used in order to present the anatomy of embryos, while scanning electron microscopy (SEM) has been used for preparing images of embryos morphology. However, these methods are very time-consuming and invasive – the embryos must be cut and completely destroyed. One such non-destructive technique is Microtomography technique which has only recently begun to be used for studying this kind of material.

The project as the basic one is connected with the anatomy and morphology development and differentiation in freshwater shrimp N. heteropoda (Crustacea, Malacostraca), one of the most preferring bred freshwater invertebrate all over the world and great example for comparative developmental study for other crustaceans.

Obtained information give us much more precise understanding of body plan development. Moreover, produced dataset allows for preparation of detailed 3D reconstructions which can be subjected to interactive manipulation and precise analysis.

Up to now, studies of the crustaceans embryo development using X-ray Microtomography have not been conducted.

### The developmental biology of extremely strong limpet tooth material

#### Robin Rumney, Alex Kao, Rachna Parwani, Alex Ford, Dareck Gorecki, and Asa Barber

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The tooth of the common limpet (Patella vulgata) has highest recorded elastic modulus and tensile strength of any biologically occurring material (120+/-30 GPa and 4.90+/-1.90 GPa respectively) (1). These unique properties arise from its composite nanostructure consisting of a chitin polysaccharide matrix strengthened with goethite (FeO(OH)) nanofibres and amorphous silica granules.

Limpet teeth grow in ~200 rows along a tongue-like organ known as the radula. At the base of the radula is a body of soft tissue known as the odontophore. The rest of the radula has previously been divided into stages with stage I (rows 0 to 15-20) being non-mineralised, stage II (next 12 rows) early mineralising, stage III (next 30 rows) late mineralising and stage IV (the remainder) containing mature teeth (2). However, depending upon the size of the limpet, the radula can vary in length from 35 to 80mm. It is vital to be able to consistently identify each stage of limpet radula prior to any cell culture or gene expression studies.

Light microscopy reveals a clear boundary between stages I and II of the radula, with maturing teeth visible in stage III and mature teeth in stage IV. Staining for iron using Prussian blue dye shows a distinct lack of iron in stage I, a gradient of iron in stage II and a saturation in stage III. The exceptional power of X-ray microscopy allows the visualisation of rows of developing teeth within stage II radula which would not otherwise be observed (Figure 1).

The observations made via light and X-ray microscopy not only validate previous studies on each stage of the developing limpet radula but uniquely reveal the presence of tooth precursors in stage II radula.



Figure 1: Reconstructed x-ray microscope image of limpet radula

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# Exploring the potential of neutron imaging and complementary techniques for life sciences applications at the neutron spallation source, ISIS, UK

#### Genoveva Burca

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Neutrons form a highly penetrating radiation passing through matter without damaging or structurally modifying it, a property that makes them the ideal tool for many kinds of complementary material investigations. Neutron imaging is one of the experimental techniques in which captured or scattered neutrons provide important information about the inner structure and indirectly about the composition of a sample considering the fact that different materials attenuate neutrons to a greater or lesser extent. The strong interaction of neutrons with hydrogen and their ability to distinguish between hydrogen and deuterium with no radiation damage make neutrons a good probe for imaging biological specimens.

This talk aims to present preliminary results acquired from the first neutron imaging measurements on different biological samples which I have done at the Rutherford Appleton Laboratory during the scientific commissioning of the IMAT beamline [1-3] (e.g. non-invasive imaging of heavy water distribution in organs, plants or soils) and explore potential applications of neutron imaging in other fields such as geology, palaeontology or forensic research.

The advantages of other complementary techniques, XRF (X-ray fluorescence) for elemental composition analysis or NAA (neutron activation analysis) for determining the concentrations of elements in various materials on the other hand are also discussed based on initial experimental results obtained.

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### Session 8: Earth & Space

#### Tomographic mapping of the Universe

#### David Bacon

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The study of the Universe is progressing at an astonishing rate. Perhaps surprisingly, some of the questions and techniques in Cosmology will sound very familiar to participants at this symposium. We want to make a 3-dimensional (or manydimensional) map of the contents of a region; that map can be inferred by measuring the scattering of light or particles; and then the map is used to answer fundamental questions about the content, origin and future of the region. I will introduce these steps in their cosmological context, seeking to set up a dialogue between your discipline and mine - what do you do which we don't, and vice versa? I will also present tomographic maps of some of the largest volumes ever charted.

## Optimisation of gold nanoparticles as a novel contrast medium for plant root and soil X-ray CT imaging

### <u>Callum Scotson</u><sup>1</sup>, Arjen van Veelen<sup>1</sup>, Simon Duncan<sup>1</sup>, Iain Dunlop<sup>2</sup>, Maria Munoz-Hernando<sup>2</sup>, Samuel Keyes<sup>1</sup> and Tiina Roose<sup>1</sup>

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Keywords: nanoparticle, contrast medium, porous media

Better understanding of plant root systems and their interactions with soil is vital to our future food security. The use of X-ray CT imaging to study soil and root systems is now widespread, but the poor contrast inherent to such systems currently limits the scope of these investigations. In biomedical imaging, contrast media have come to play a fundamental role in enabling fine structures and dynamic processes to be imaged. However, the use of contrast media in plant and soil

imaging has until now been little-explored. Gold nanoparticles are a highly promising candidate for these applications, since they exhibit low toxicity and can be functionalised to target specific tissues or sites of biophysicochemical interest. However, the characteristics of their transport and stability in the challenging environments of soils and plants are largely unknown. Since nanoparticle stability is compromised by salinity, acidity and strong surface charges, a major hurdle to their use in environmental applications is the development of suitable coatings to mitigate the effect of these influences. We have undertaken a suite of experiments to quantify the stability of coated and uncoated gold nanoparticles in a range of soils, soil solutions and soil analogues, using complementary 4D X-ray CT, UV-Vis, ICP-MS, SEM and Zeta particle size analyses. We show how coating strategies were developed using these screening tools, producing suspensions with suitable stability for use in real soil environments. We then show how 4D imaging of nanoparticle transport in soils and soil analogues allows the dynamics of flow and surface interactions in these media to be quantified. Using these data, we will discuss the optimisation of nanoparticle coatings and X-ray imaging protocols for soil and plant applications, and discuss the possibilities for using functionalised gold nanoparticles to probe nutrient and water dynamics in these systems using multi-scale X-ray CT.



a) Column of silica and alumina grains after being perfused with gold nanoparticle suspension in a flow experiment. Particle binding to grains is observed on the upper surface. b) A section across the dashed line in (a) shows the nanoparticle coatings, seen as bright fringes around individual grains.

# The usage of modern data science in segmentation and classification: Machine Learning and Microscopy

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Keywords: machine learning, multiscale simulation, mineralogy

One of the most challenging stages in any microscopy workflow is the ability to transform images into rich digital models of segmented data. These models enable quantification of features of interest and power data-driven analysis. Frequently, the greyscale output from detectors carry both a variety of modality-specific artifacts and noise that cause, as resulting images become more complex, the failure of threshold-based segmentation approaches [1]. The last 20 years has seen a
transformation in a wide range of fields, widely grouped together under the umbrella of "Machine Learning". While these technologies have transformed many areas of data science ranging from medical diagnosis to stock market analysis, frequently image analysis for microscopy (outside some specific areas of application) has lagged behind developments in other fields. The power of such algorithms, when applied to segmentation and classification problems in microscopy lie in their ability to create arbitrary classifiers which operate in much higher dimensional space than simply the image output from a specific microscope detector.

In this paper, we show results enabled by these techniques in two applications in geological analysis; lithological classification of heterogeneous rocks and 3D mineralogy. In the first of these, we use a multi-scale approach to deal with the heterogeneity inherent in subsurface rock [2]. First we characterize heterogeneity at low resolution using machine learning before finally zooming to image specific locations based on the macroscopic map (figure 1). This can be used to not only drive the downscaling locations for high resolution interior tomography, but also upscaling and creation of a computational composite model [3]. In the second application, we show how multimodal imaging can be used extend 2D mineralogical information into 3 dimensions on a multi-mineralogical sandstone sample. By spatially co-correlating an EDS based mineral map with a 3D XRM image, we can use machine learning to extrapolate the 2D mineralogical information into 3D (figure 2).

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Figure 1. Lithological classification of rock sample, using Machine Learning techniques. High porosity regions are shown in green, low porosity regions are shown in red and a fracture running through the sample shown in blue.



Figure 2. 3D Mineralogical distribution found by classifying 3D XRM datasets, with training guided by correlated 2D mineralogical information. The red shows quartz regions, blue feldspar, green clay minerals and yellow pyrite.

#### Revealing the complexity of tephra horizon structures in soft sediment sequences

#### Elizabeth Evans<sup>1</sup>, Siwan Davies<sup>1</sup>, Sabine Wulf<sup>2</sup>, Millie Watts<sup>3</sup>, Peter Talling<sup>4</sup> and Richard Johnston<sup>1</sup>

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Keywords: geology; volcanic ash; tephra; sedimentary structures; bioturbation;

Volcanic ash, or 'tephra', ejected from volcanic eruptions are deposited instantaneously (on geological time scales) creating a layer of equal age in the sedimentary record, an isochron, which can then be used as age markers. The use of tephra layers to define a chronological framework is termed 'tephrochronology'. A key principle of this technique relies on identifying deposits that relate to the primary air fall events that are undisturbed in the sediment record. Some deposits, however, are prone to disturbance and reworking and so assessing the depositional integrity of these ash layers is essential. We present visualisations of post-depositional movement and reworking of tephra deposits in sedimentary sequences using X-ray microtomography.

The samples investigated in this study were recovered as drill cores from a number of North Atlantic marine and European lake sedimentary environments. The structures uncovered include evidence of bioturbation and downward movement of the volcanic ash that were not identified using conventional one and two-dimensional approaches (e.g. thin-section analysis and

linear shard concentration profiles). This highlights the need for careful identification of isochron positions for chronological purposes. We also present a selection of samples taken across the same tephra horizon situated below one of the North Sea's Storegga landslides. Notable features observed between the cores include lateral variation in deposit thickness and the amount and type of bioturbation observed. This shows that there is variation in the integrity of a deposit and, therefore, a particular horizon in one core may be unsuitable for dating but should not be ruled out as useful in subsequent cores.

Our work shows that X-ray microtomography and three-dimensional visualisation has significant potential for the investigation of sedimentary and depositional processes associated with tephra deposits. We also expect this work to be of particular interest to the wider geological field especially sedimentologists, as well as biologists and palaeontologists working with burrow structures and trace fossils.



The region in opaque red shows a tephra horizon with numerous burrows

#### Session 9: Cultural Heritage & Public Engagement

#### 3D Museums: tactile learning, greater access

#### George Oates

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Museum in a Box is on a mission to help museums increase access to their collections. Even as we digitize more of our treasures, these resources can also end up "in the dark" like physical objects that can't be displayed because of a lack of space. Alongside the growth of 3D digitization, Museum in a Box is updating the old idea of a museum handling collection using replica 3D printed objects and Raspberry Pi to trigger audio responses as people touch the object in a simple, satisfying interaction.

From educational outreach to oral history collection, we have a growing list of case studies about how folks are able to fill the box with their own collection materials and stories and engage audiences in a fresh way.

#### A Whale of a Project: How to Scan the World's Largest Animal

#### Kate Burton

#### Natural History Museum

Keywords: surface scanning, laser scanning, specimen, structured light, lasers, micron, polygon, 3d modelling, data points, polygonal mesh, blue whale, skeleton, bone material

This talk will be a brief discussion into how I completed the task of 3D surface scanning the entirety of a disarticulated blue whale skeleton. This involves using a combination of laser and structured light scanners which are mounted and hand-held. First it involves capturing the images in real time. These machines are accurate to research-quality levels and are able to produce details down to the micron level. The next stage is when the data is cleaned up. At this point, extraneous data points like the props used to hold up the object are deleted. The last stage for 3D modelling is the transformation of raw data points into a polygonal mesh that can be used for a large variety of purposes. Once in mesh form, the object is again inspected for any missed or bad data, then is polished up and ready for distribution. I will discuss what worked, what did not work, and plans for the future.

## Ageing fossil birds using high-resolution synchrotron-based computed tomography for virtual histology

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#### Keywords: palaeobiology, birds, virtual histology, high-resolution

Accurately estimating developmental age and life history traits of fossils is crucial for identifying and classifying extinct species and understanding how key biological attributes developed. In birds, the evolution of life history traits such as growth pattern is far from clear, and only a handful of modern species have been studied developmentally. The exceptionally rapid growth of modern birds means ageing methods based on annual incremental growth lines, used in other vertebrates, are inapplicable to birds and robust alternative ageing methods remain to be established.

Analysis of avian intracortical bone microstructure, which varies with both age and tissue deposition rate [1], provides a promising approach. However, current thin-section-based histological methods are destructive and, to date, most microstructural studies in avian bone are qualitative, two-dimensional, involving a limited range of extant species. We aim to use minimally-destructive high-resolution three-dimensional imaging to test correlations between cortical bone microstructure, and developmental age and life history traits in living birds, to identify phenotypes which may help to estimate these in avian fossils.

We imaged cortical bone from the midshaft of the femur, tibiotarsus, and humerus in a full growth series of modern domestic ducks (Anas platyrhynchos) using high-resolution synchrotron-based computed tomography. 21 individuals were measured, between I day and 2 years old. Visualisation in 2D (Figure 1) and 3D (thresholded using a fixed greyscale value) (Figure 2) show cortical microstructure changes through development.

Next steps are to quantify variation in bone microstructure through development, including estimating bone porosity and cell number density using volume measures in ImageJ. These measures will be tested for correlation with developmental age to identify phenotypes that could be used to estimate age. Future work will test whether phenotypes identified also relate to body mass, developmental mode, and phylogeny, by comparing between modern species including pheasant, rock dove, and starling.



Figure 1: Cortical bone samples of duck (Anas platyrhynchos) femurs through development, imaged using synchrotron-based computed tomography at the TOMCAT beamline of the Swiss Light Source (1.3 µm voxel size at an energy of 21 keV, exposure of 180 ms per projection over 1501 projections). Ducks (left to right) are 1 day old, 2 weeks old, 6 months old, 2 years old. Bone porosity and heterogeneity appears to change through development, from exceptionally porous to densely mineralised. Scale bar 1mm.



Figure 2: Cortical bone sample of duck (Anas platyrhynchos) femurs (left 2 weeks old, right 2 years old) imaged using synchrotron-based computed tomography at the TOMCAT beamline of the Swiss Light Source (1.3  $\mu$ m voxel size at an energy of 21 keV, exposure of 180 ms per projection over 1501 projections). Segmented intracortical canals are shown in red and osteocyte lacunae in yellow. Scale bar 200  $\mu$ m

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#### MorphoSource: A Virtual Museum and Digital Repository for 3D Specimen Data

Julie M. Winchester<sup>1</sup>, Gregg F. Gunnell<sup>2</sup>, Seth Kaufman<sup>3</sup>, Timothy M. McGeary<sup>4</sup>, and Doug M. Boyer<sup>1</sup>

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Keywords: 3D data, data stewardship, data access, digital repository, biological database, morphology, surface scan, microCT, laser scan, MorphoSource

Over the past several decades, the use of 3D data has become increasingly common in biological and paleontological research, and techniques leveraging 3D data offer great promise for scientific advancement. Increasing availability of this data through sharing and archiving is a crucial step in breaking down barriers to progress, but achieving this goal poses challenges concerning implementation, stewardship, and incentivization. We describe MorphoSource, a web-based virtual museum and digital repository that has existed since 2013 and adheres to standards of data quality and format recently established by the community of scientists working with 3D data. At the time of writing, it holds over 25,000 3D datasets representing over 1,200 taxa. Users can upload and download high fidelity 3D renderings of specimens derived from a variety of scanning modalities. Specimens (ideally physically vouchered in a collection) comprise the basic unit of organization. Specimens are represented by media files and associated with metadata including project, scanning facility, institution code, and taxonomy, using best-practice standards where possible. Users who upload data maintain control over access to that data, and DOI identifiers can be generated that allow uploaders to be cited for usage of data when they share it. Shared data can be easily searched and downloaded, and downloaded data can be used by researchers, educators, or other interested parties. MorphoSource in its current form represents a proof-of-concept for how to address challenges relating to increasing data access. Subsequent updates will implement standardized digital repository architecture and enable distributed multiinstitutional models for storage management. MorphoSource is increasingly endorsed by major American specimen collections as an appropriate solution for hosting researcher-generated 3D data, and journal reviewers, editors, and grant officers have started to suggest or require that data be made available through this site. Its existence is beginning to change data transparency standards in comparative biology and paleontology.



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Engineering and Physical Sciences Research Council

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## **Poster Abstracts**

## Mapping the radiation dose received by bone during X-ray microCT and its importance for imaging ancient remains

Sarah Aldridge<sup>1,2</sup>, Richard Hugtenburg<sup>3</sup>, Nick Owen<sup>2</sup>, Sarah Forbes-Robertson<sup>4</sup>, Alex Hildred<sup>5</sup> and Richard Johnston<sup>1</sup>

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A common application for micro-CT is for virtual analysis and preservation of objects of scientific or cultural significance, in particular, human and animal remains. High resolution tomograms can yield important information for the studies of bone quality, disease, activity, and diet. Imaging can expose these samples to the X-ray beam for extended periods. But the interaction of high energy X-rays with organic materials is potentially destructive, and has been documented to cause damage in the form of colour change and molecular alteration. Many of these collections of remains are unique and of cultural and historical significance. It is therefore important to establish the level of dose through a material, so that areas receiving a proportionately higher dose can be monitored. This is particularly applicable to the field of ancient DNA; informing prospective sampling locations.

The modelling of dose was performed using a Monte Carlo simulation to model the transport of photons and electrons through the bone matrix. The results showed a broad variation in received dose for the different tissues.

The example used is a medical human ulna bone sample, which demonstrates the variation across a dry sample, and the differences in dose between the cortical and trabecular bone tissue. This is of significance for molecular purposes, since the endogenous DNA yield between the two materials varies. This has potential implications for ancient DNA extraction, and directly informs our current work in partnership with the Mary Rose Trust, imaging and analysing these unique human remains.

## New insights into Brassicaceae seed coat structure and function using synchrotron radiation X-ray tomographic microscopy (SRXTM)

Arshad W.<sup>1</sup>, Collinson M.<sup>2</sup>, Marone F.<sup>3</sup>, Leubner-Metzger G.<sup>1</sup> and Steinbrecher T.<sup>1</sup>

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 <sup>3</sup>Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland.

#### Keywords: Seeds, Aethionema arabicum, mucilage, Lepidium sativum, proanthocyanidins

Seeds are the beginning and end of most food supply chains, central to human and animal existence. As reproductive units of both angiosperms and gymnosperms, seeds are eminently adapted to a highly varied and changeable environment. Understanding seed germination traits is therefore fundamental for successful dispersal, germination, and seedling establishment. Unravelling the detailed structure of seeds will help link internal morphology to physiological and biophysical traits. In this study, we performed Synchrotron Radiation X-ray Tomographic Microscopy (SRXTM) at the Swiss Light Source to visualise the internal morphology, tissue anatomy, and the detailed cellular structure of different Brassicaceae seeds. We explored the dimorphic seeds of Aethionema arabicum, a new model system for seed and fruit dimorphism. This annual species, belonging to the most basal lineage of the Brassicaceae family, has the remarkable ability to produce two distinct fruits harbouring two different seed types ("M+" and "M–"), with unique anatomical, ecophysiological and biomechanical differences. Mature "M+" seeds produce mucilage from the outer cell walls of the seed coat epidermal cells, thought to have a role

relating to seed hydration, germination and/or dispersal. Mature "M–" seeds lack this distinctive morphological adaptation. We also determined the presence and localisation of structural modifications in the seed coat of a unique set of Lepidium sativum seeds. Paler seeds lacking proanthocyanidins (complex flavonoid compounds that confer a brownish colour to the seed coat) pose problems with abnormal germination, early dormancy release, and potential losses in crop yields. By employing SRXTM to investigate the linkage between proanthocyanidins, biomechanical properties, and unique morphological structures of the seed coat, we show the potential for high-resolution scanning technologies to be used in cross-species seed and seed coat morphology studies of extant taxa.

## Sample preparation and imaging strategy for microtomography of central nervous system tissues

## <u>Andrew J Bodey</u><sup>1\*</sup>, Merrick Strotton<sup>2\*</sup>, Kazamir Wanelik<sup>1</sup>, Shawn Mikula<sup>3</sup>, Christoph Rau<sup>1</sup> and Elizabeth J Bradbury<sup>2</sup>

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#### Keywords: X-ray tomography, central nervous system

Synchrotron X-ray microtomography can yield 3D, micron-level information across extended regions of intact soft tissues such as those of the central nervous system (CNS). Macro features of CNS samples (such as white-grey matter boundaries and large, hollow components of the vascular and ventricular systems) can be easily revealed, but visualisation of microstructures (including capillary-level vasculature, large cell bodies, myelinated axons and organelles) is less straightforward and only made possible by combining effective specimen preparation with an optimised imaging strategy. Specimens must be beam resistant, physically stable and enable imaging with high contrast, high signal:noise and minimal artefacts; it is also important that the physiological accuracy of specimens is minimally compromised by the meeting of these requirements.

Sample characteristics and experimental aims (including subsequent use of tissues for other purposes such as histology) will dictate the best methods by which to conduct experiments, and it is therefore desirable to have a range of preparation options by which to achieve these goals. The relative merits of a variety of contrast methodologies (both chemical and optical) are explored, as well as a range of sample mounting options which were trialled on murine spinal cord and dorsal root ganglia samples.

Whole tissue samples were imaged at Diamond Light Source (DLS) with an 8-30 keV X-ray beam which was filtered to balance sample damage with image acquisition times. Data were reconstructed with DLS's new modular reconstruction pipeline Savu, incorporating optical distortion correction and both Raven and wavelet ring artefact suppression. A simple method to derive the optimal number of projections from a single iteratively downsampled 'set-up scan' is also outlined; this will enable experimenters to optimally balance signal:noise with acquisition time and its associated beam damage.

By optimising sample preparation and imaging strategy, we have visualised capillary level vasculature alongside neuronal cell bodies at lower magnification across extended regions of the spinal cord. Higher magnifications have enabled visualisation of different diameter myelinated axons and subcellular organelles, including nuclei (Fig 1). The methods outlined should be applicable to soft tissue tomography more generally.



Fig 1. Microtomogram of a dorsal root ganglia sample, showing different diameter myelinated axons and cell bodies with nuclei and other organelles.

#### Automatic Diameter and Orientation Distribution Determination of Fibrous Materials in micro X-Ray CT Imaging Data

#### John Chiverton<sup>1</sup>, Gianluca Tozzi<sup>1</sup>, Alexander Kao<sup>1</sup>, and Marta Roldo<sup>2</sup>

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Fibrous nano-materials such as electrospun materials have many uses ranging from tissue engineering to biosensors. Highresolution imaging is an important component in determining the resulting properties of these materials. Some important parameters for fibre rich materials include the distribution of the orientations of the fibres and also the diameters of fibres and the spacing between fibres. The orientations and the relative dimensions of the fibres can be measured via specially designed imaging software. Difficulties in this measurement process can arise if fibres are distributed in close proximity to each other in relation to the dimensions of the imaging modality. This is particularly true if some automation is required in the measurement process if it is not adapted to situations where the fibres are in close proximity to each other. This work is therefore concerned with the development of automated measurement techniques to provide estimates of the diameters and spacing of fibres and also the orientation distribution. Software automatically detects special points in the fibrous materials where fibres can be considered to have some delineation from surrounding fibres. These sparse points are considered to be points at which estimates of the fibres' properties can be quantified. Imaging of Poly-CaproLactone (PCL) electrospun fibres is undertaken with a Zeiss Xradia Versa 520 Microtomography X-Ray Computer Tomography (XCT) system at the University of Portsmouth's Zeiss Global Centre. Image acquisition is performed with an imaging resolution of 0.4x0.4x0.4mm3.An exemplar fibre diameter distribution can be seen in Fig.1 (left) along with an exemplar fibre orientation distribution also in Fig.1 (right).



Figure 1 Frequency distribution of diameter for electrospun fibres and orientation distribution for the directionality of the detected fibres.

# Joint assessment of soil hydraulic properties by constraining geoelectrical tomography measurements with X-ray Computed Tomography pore architecture information

#### Cimpoiasu, M. O.<sup>1,2</sup>, Kuras, O.<sup>1</sup>, Mooney, S. J.<sup>2</sup> and Pridmore, T. P.<sup>3</sup>

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Keywords: Electrical Resistivity Tomography; X-ray Computed Tomography; Spatial Resolution; 3D characterisation; Hydraulic properties; Non-invasive soil core study; Time-lapse monitoring

Developing a better understanding of hydraulic properties of soils is of significant importance for such diverse fields as agriculture, soil and ecosystems management, vadose zone hydrology, contaminant transport, civil engineering and geotechnics

and the tourism and leisure industries. We propose a new method of investigating soil hydraulic properties by the joint appraisal of two advanced tomography technologies: Electrical Resistivity Tomography (ERT) and X-ray Computed Tomography (CT). Both methods are non-invasive and allow properties measurements without disturbing the structural integrity of the sample. The method implies contemporaneous moisture dynamics measurements in soil columns. ERT enables the continuous time-lapse measurement of the 4D resistivity profile. This allows monitoring and modelling the fluid preferential pathways inside the column. X-ray CT has the ability to determine the pore structure and matrix architecture of the samples. The information obtained from the X-ray scanner is used to constrain the ERT models and refine the mesh used to reconstruct the 4D resistivity profile. Synthetic modelling has been run integrating spatial information extracted from X-ray scans into ERT inversion models. Constrained models obtained as such have been compared with unconstrained scenarios. The results suggest that the constrained models provide a higher accuracy and resolution. This innovative methodology of mapping and monitoring fluids, which combines the advantages of two established techniques, holds promise for soil science application and related fields.

## Utilising 3D Xray to help identify gut mechanics and structure in marine wood boring organisms

#### **Elizabeth Clutton** and Simon Cragg

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#### Keywords; Limnoria, Lyrodus, wood borers

Marine wood boring organisms frequently attack coastal sea defence timber structures, expanding their range whilst colonising floating drift wood. Extensive research into understanding the various feeding mechanisms employed by marine wood borers during wood colonisation is ongoing. Whilst some species employ the aid of gut microbes to help in the breakdown of wood lignocellulose (Lyrodus pedicellatus), in other species no additional microbial activity has been observed (Limnoria quadripunctata). Utilising 3D Xray to image both species, provides better clarity of the gut structure and function, allowing better determination of the mechanics of the gut and to locate possible sources of enzyme activity within the gut. The main objective of using 3D Xray is to achieve clear digital dissections of the gut area from each species, to help characterise the functioning of the gut and therefore, characterise digestion of wood by marine wood boring organisms. The use of 3D Xray compliments a multiple method approach combining it with SEM. This provides us with a rounded picture as the 3D X-ray allows delicate structures to be analysed in three dimensions, whereas SEM is excellent for revealing surface detail of dissected or sectioned specimens. In addition, 3D Xray has been utilised to digitally quantify the anatomy of the brooding larvae structure in L. pedicellatus Mediterranean species, believed to be a cryptic species. Further image analysis of this species is ongoing, but initial images support the theory of a cryptic species by demonstrating larvae broods of varying developmental stages.



Whole body 3D Xray using Zeiss Xradia 520 Versa, species L. quadripunctata



Whole body 3D Xray using Zeiss Xradia 520 Versa, species L. pedicellatus Mediterranean species

#### Approaches to 3D Printing of Teeth from X-Ray Microtomography

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Keywords: X-ray Microtomography, Additive Manufacturing, Artificial Teeth, Dental Materials, Pre-Clinical Teaching

**Objectives:** Artificial teeth have several advantages in pre-clinical training. The aim of this study is to 3D print accurate artificial teeth using scans from x-ray microtomography (XMT), that mimic the properties of real teeth. In this first stage, the emphasis is on geometry only.

**Methods:** Extracted teeth were imaged by the in-house XMT system at Queen Mary (MuCAT2) at 90kV, to create detailed high contrast scans. The dataset is then viewed in Drishti, where both internal and external meshes can be exported to 3D modelling software (Meshmixer), for modification before finally exporting to a slicing program (Cura) for printing. After appropriate parameter setting, the printer deposits material in specific locations layer by layer, to create a 3D model.

**Results:** XMT produces a high-resolution scan of the tooth, which is easily converted into a workable 3D model using multiple software. Excess material was removed, to ensure a clean model was produced, then was imported into the slicing software, where layer height could be determined to replicate the high resolution that was seen in the XMT scans. The model was then printed in two different materials, changing settings dependent on the material. A multi-material print was created to show the different physical characteristics between enamel and dentine.

**Conclusion:** The study so far has demonstrated a way to use XMT to create high-resolution scans and print a replica. The 3D modelling process ensures any imperfections or excess material, scanned in the XMT can be removed leaving only the original state. The slicing software managed to recreate a high-resolution print but was limited by the resolution of the printer (50 µm). A printer with a higher resolution would be able to recreate the resolution of the original scan (20 µm). Future work will include force testing and material testing on extracted and artificial teeth.

#### Manufacturing of a Voronoi-Based Biomimetic Bone Scaffold by means of Direct Metal Laser Sintering

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#### Introduction

In the field of Bone Tissue Engineering (BTE), synthetic scaffolds are going to replace bone grafts [1][2]. Synthetic porous scaffolds can be directly manufactured with biocompatible materials by means of advanced Additive Manufacturing (AM) technologies [3][4][5][6]. Coupled with the emerging field of Design for Additive Manufacturing (DFAM) the digital fabrication offers numerous benefits for innovative design solutions, suitable for 3D modeling anatomic structures, including shape complexity, material complexity, hierarchical complexity, and functional complexity [7][8]. Together with the design, also materials and manufacturing processes play a fundamental role to obtain efficient biocompatible scaffolds with porous and interconnected architectures. For this reason, the capabilities of AM, according to the biomaterials used, have to be proven to assure the proper manufacturability of these biomimetic scaffolds in terms of porosity, pores size, pores interconnection and trabecular thickness. For that reason, the purpose of this work was the actual fabrication of a Voronoi-based scaffold [9] through AM technologies to directly assess its manufacturability and the deviation from the nominal digital surface.

#### Methods

A biomimetic cubic bone scaffold  $(10 \times 10 \times 10 \text{ mm})$  with controlled porosity (P% = 80%) and mean pores size (Dp = 0.800 mm) was designed through an interactive Generative Design (GD) process and then a sample of Ti6Al4V scaffold was manufactured by means of the Direct Metal Laser Sintering (DMLS) system EOSINT M270 (Figure 1). The surface morphology of the scaffold was analyzed by a Scanning Electron Microscopy (SEM), equipped with an Energy Dispersive X-ray Spectrometry (EDX), while the internal morphology was examined through a high-resolution micro-CT SkyScan 1172. Finally, the morphometric assessment of the scaffold was carried out using ImageJ and BoneJ [10], a tool for image bone analysis, by measuring the main indices for the characterization of trabecular bone structure, such as Trabecular thickness (Tb.Th.), Trabecular separation (Tb.Sp.), Degree of anisotropy (DA) and Connectivity density (Conn.D) [11][12].

#### Results

The sample of Ti6Al4V scaffold, even though with reduced porosity (P% = 73%) and mean pores size (Dp = 0.695 mm) respect to expected (Table I), was successfully fabricated by DMLS and presented a fully interconnected porous architecture with an intact trabecular skeleton, therefore proving the proper manufacturability of such kind of biomimetic structures. Moreover, the main indices for the characterization of trabecular bone structure were well congruent with the actual natural bone.



Figure 1: a) 3D mesh model of the designed scaffold; b) Ti6Al4V sample of the scaffold; c) Projection image captured by Skyscan 1172 micro-CT scanner

Indices	Unit	Reconstruct.	Designed	Variation
Porosity	%	73	80	-7%
Pores Size	mm	0.695	0.800	-13%
Surf.Area	mm <sup>2</sup>	3499	3911	-5%
Volume	mm <sup>3</sup>	272	201	+35%

Table 1: Reconstructed vs Designed scaffold comparison

#### Discussion

A viable and reproducible method to fabricate Ti6Al4V biomimetic bone scaffolds, with controlled porosity and mean pores size, was presented. These kind of scaffolds allowed reproducing the actual architecture of the trabecular bone and could be suitable for Bone Tissue Engineering (BTE), according to specific surgical requirements.

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#### Hydrogels silver nanowires for bone regeneration

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Keywords: hydrogels, silvernanowires, calcification, high-resolution x-ray tomography.

The reconstruction of large bone defects remains one of the most challenging topics in orthopaedics, where bone surgical treatments are often compromised by bacterial infections [1]. To such extent, silver nanowires (AgNWs) are considered promising agents against a wide range of bacteria species. However, the practical application of AgNWs is weakened by self-aggregation, precipitation or loss of antibacterial activity [2]. This is why an appropriate carrier for controlled drug release and for dispersing the nanowires is necessary. Hence, composite materials, made of an organic polymer (Chitosan-CS) and an osteoconductive ceramic (Hydroxyapatite-HA), can be combined for a dual purpose: controlled antibiotic release and stimulation of new bone formation. In this study, we have combined AgNWs (L:  $5.03 \pm 1.85 \ \mum$ ; D:  $99 \pm 20 \ nm$ ) into CS-[HACS]-based hydrogels in order to obtain a scaffold able to prevent infections and to allow good mechanical strength, Ca/P deposition and cell ingrowth. Mineralization induced by the biomaterials following incubation in SBF solution at 7, 14 and 21 days and its relation to nanowires aggregates was documented by means of high-resolution x-ray tomography (Versa 510, Zeiss, CA), with voxel sizes ranging 10 $\mu$ m-70nm.

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#### Laboratory X-ray computed laminography for planar object imaging

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#### Keywords: Laminography, imaging of planar objects, reconstruction

Most non-destructive testing using X-ray Computed Tomography (CT) in a laboratory setting uses a cone-beam, circular source path scan configuration. However, this conventional scan configuration produces sub-optimal results when scanning planar objects due to the object's large aspect ratio. One solution to this problem is to tilt the object so that the axis of rotation is at an acute angle to the vertical, and acquire a series of projection images. This is known as computed laminography (CL) and has shown great potential in a parallel-beam synchrotron setting and to less extent on dedicated laminography laboratory systems, both of which are not widely accessible.

We present a proof-of-concept CL system implemented on a general-purpose Nikon 320/225kV Bay micro-CT instrument at the Henry Moseley X-ray Imaging Facility reprogrammed using a VBA interface. A dedicated Simultaneous Iterative Reconstruction Technique (SIRT) method has been implemented using the GPU-accelerated toolbox ASTRA to allow reconstruction in this non-conventional configuration. The system has been tested using a planar test object built from Lego bricks. Computer simulations were also used to understand and mitigate image artefacts, and to investigate the effect of the laminography tilt angle on reconstruction image quality. The results indicate that the developed CL configuration can produce reconstructions with fewer artefacts and more isotropic resolution compared to alternative limited-angle scan configurations otherwise used for planar samples.

Laboratory-based CL is of particular interest for use in manufacturing, as it can be used to identify defects in products such as printed circuit boards and composite panels. It also provides a way of imaging paintings and planar fossils.

## Overcoming challenges in experiment and analysis for high resolution x-ray tomography of pharmaceutical beads

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Within the pharmaceutical industry the manufacture of multi-particulates is an acceptable dosage form. Multi-particulates consist of beads which have typical diameters in the 100's of micrometres, and are often produced by the application of different layers on to an inert core. The components in each of the layers applied will have a significant impact on how the drug will be released from the bead. Porosity of these layers is one of many important factors in the release rate and consequently the speed of drug delivery. While the bead microstructure may be visualised at high resolution with conventional SEM, this technique is limited to surface observations only, requiring physical slicing of the sample for any internal measurements. For a non-destructive and fully volumetric measurement we look to x-ray micro-tomography, allowing for porosity analysis in 3D across the entire bead volume. Even with the highest resolution micro-tomography achievable at a synchrotron x-ray source, with pore sizes of only a few micrometres or less across a sample nearly 1 mm in diameter, there are considerable challenges involved. This is especially true when the key porosity measure of interest is within the outermost layer which is only 7-10 micrometres thick and of low image contrast.

In this study a number of optimisations were performed as part of the experimental process, performed at Diamond Light Source's imaging beamline II3-2. These include choice of energy, scan times, use of in-line phase contrast, and lens distortion measurements. For the tomographic analysis, various image filtering and processing steps were required in combination with multiple segmentation tools before performing a label analysis within Avizo. With a view towards future higher throughput studies of different manufacturing batches, we consider ways to reduce the level of manual intervention in the analysis pipeline. An interpretation of the porosity layer statistics is also presented, together with the limitations of the method.

## Abrasion induced volume loss in teeth measured by X-Ray microcomputed tomography

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#### Keywords: X-ray microtomography, teeth, abrasion

Tooth brushing is a common part of oral health care with the purpose of mechanically removing dental plaque to prevent the onset of dental disease and decay [1]. The abrasion between the tooth surface and the brush serves as a contributing factor to tooth wear [2]. The aim of this work is to use computed tomography techniques to characterise the volumetric changes in enamel after normal brushing with a powered toothbrush. X-ray microcomputed tomography (XRT) provides micrometer length scale resolution of an entire volume of material enabling the evaluation of complex biological structures in three dimensions (3D). Evaluation of structures in 3D is particularly important when considering mechanically induced damage or deformation, as the location of the damage can be highlighted and analysed fully. Tooth volume loss is typically quantified using two dimensional measurement techniques such as profilometry, or point based techniques such as indentation [3], [4].

These techniques result in localised information rather than providing information about the total volume of the enamel. XRT captures three dimensional information about the whole volume of the enamel allowing for quantification of the change volume over subsequent abrasive tests. XRT is required to reach the required resolutions to observe the changes in the volume of the teeth. The XRT data is captured prior to and directly following brushing with a powered toothbrush at multiple time steps. Reconstructed images and subsequent segmentation are shown in Figure 1. The results of this work show that XRT can be accurately used to quantify and visualise the volume loss of teeth during brushing. The techniques used throughout this work can be extended to investigate the effect of the environment on the abrasive effects the brushing.



Figure 1.Top) Reconstructed XRT images of the (left) tooth before brushing and (right) after brushing with a powered toothbrush. Bottom) Segmented images used to evaluate the volume change in the tooth as a result of the brushing.

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#### Nanoindentation and digital volume correlation of trabecular bone

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Nanoindentation has been widely used to identify the mechanical properties such as hardness and elastic modulus of bone and other biological tissues. In addition, Digital Volume Correlation (DVC) is gaining great popularity due to its uniqueness as a 3D full-field displacement and strain measuring technique. The purpose of this study is to combine the results of these two experimental methodologies to further characterise the properties of bone tissue from porcine vertebral bodies. Bone cylinders (D: 3 mm; L: 8 mm) where cored from different locations within the vertebral body along the axial direction. The samples were dried, embedded in epoxy resin and then polished with sandpapers and Alumina paste.

Indentations were performed in the middle of the trabeculae, which were visually selected, using a Berkovich tip in the NanoTest indenter (Micro Materials Ltd, UK). The maximum depth was set to 1 µm, with a 1 mN/s loading rate. High–resolution X-ray computed tomography (microCT) was conducted pre- and post- indentation Versa 520 system (Zeiss, CA) and achieving a pixel size of 0.3 µm.

Twenty indents were performed along the length of one trabecula, in the transverse direction. This analysis resulted in hardness of  $0.7\pm0.1$  GPa and elastic modulus of  $21.1\pm3.2$  GPa, with an elastic recovery of ~0.1 in line with published literature.

Current work is focusing on combining nanoindentation measurements with full-field accumulated strain from DVC, applied to microCT pre- and post-indentation for 3D mapping of bone tissue.

## Integrated reconstruction, quantification and visualisation of solidification microstructures in 4D

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Keywords: iterative image reconstruction, model-based, segmentation, visualisation, CCPi

Many synchrotron X-ray microtomography ( $\mu$ CT) experiments are performed dynamically while tracking the evolution of structure or microstructure as a function of time, defining the field of 4D (3D plus time)  $\mu$ CT imaging. One important 4D  $\mu$ CT imaging application is the quantification of solidification processes, which is a core focus of materials science as it defines the initial length and chemical segregation scales [1]. We focus on the formation of metallic dendritic structures and their evolution during melting since this can significantly affect the properties of the material. The dendrite usually has a complex 3D morphology that evolves over time, depending on various conditions including solute elements, cooling rates, etc. Due to motion and the lack of temporal and spatial resolution involved at the early stages of solidification, filtered back projection reconstruction can create blurred and noisy surfaces. This makes the detailed quantitative analysis of the 4D dynamic changes in the dendrite evolution very difficult. Additionally, the surfaces of dendrites are relatively smooth and therefore it is important to ensure a smooth and physically faithful recovery of the shapes.

In this work we present a new model-based iterative reconstruction (MBIR) algorithm which ensures piecewise-smooth recovery of dendrites (see Fig. 1) by incorporating a cost function for simultaneous removal of ring artefacts and retain smoothness through special regularisation term. When the conventional reconstruction methods, such as filtered back-projection (FBP) fail to recover segmentable images, MBIR provides smooth recovery with much improved signal-to-noise ratio and with the absence of ring artifacts [2].



Fig. 1. left to right: FBP (conventional reconstruction), MBIR recovery, segmentation, and profile across the area to show the smoothness of the recovery. The bar scale corresponds to 50 µm.

We demonstrate that the obtained reconstructed images can be easily segmented, quantified, and visualised (see Fig. 2).



Fig. 2. Visualisation direct from the MBIR reconstructed 4D datasets using the Drishti toolkit [3] along with CCPi guides [3].

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#### Neutron tomography on IMAT: Project Status and Future Plans

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A new neutron imaging beamline, called IMAT, has recently been taken into operation on TS-2 at ISIS at the Rutherford Appleton Laboratory at Harwell [1]. The instrument provides white-beam radiography and tomography, energy-selective and energy-dispersive imaging. Intended applications are: non-destructive testing of industrial components; in-situ processing and analysis in engineering materials science; studies of water and solvent transport in biomaterials and soft matter; studies in other fields as varied as geology, archaeology, and physics. The high penetration power of neutrons allows for non-destructive testing of bulky materials, and for locating hydrogen, water and fluids in the bulk of components.



Figure 1: Design of the IMAT sample area, showing a camera box on a robotic arm and 90-degree diffraction detectors.

The installation of various components on IMAT was completed, such as the large sample positioning system and a robot arm to carry and exchange imaging cameras. Current activities on IMAT are concerned with the development of new imaging cameras, data analysis tools, and preparations for the operation of loading rigs. IMAT has completed the first round of commissioning where key instrument parameters have been determined. After a period of further commissioning and operation with 'friendly-users', there will be a first call for IMAT proposals in October 2017 for using the imaging options only. In the near future the imaging setup will be complemented with diffraction detectors for spatially-resolved phase, strain and texture analyses on regions of interest (Figure 1).

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## An X-ray CT derived 3D-printed ultrasound phase-interference compensator (UPIC) for reduced wave degradation through bone

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#### Keywords: Ultrasound Wave Degradation, Bone, Phase Interference, Spatial-Temporal, Complex Media, Computed Tomography

Transcranial ultrasound wave degradation created by variations in both thickness and tissue composition significantly impedes diagnostic and therapeutic interventions of the brain. The current 'active' solution is to vary the transmission delay of ultrasound pulses, inherently necessitating electronic control of each individual transducer element. Applying the sonic-ray concept of ultrasound wave propagation [1], it has recently been demonstrated that wave degradation may be significantly reduced if both the transit-time and propagation path-length for all sonic-rays are made constant [2]. The 'passive' ultrasound phase-interference compensator (UPIC) consists of a 3D-printed device that is positioned between the ultrasound transducer and test sample.

Both 'active' and 'passive' approaches require detailed knowledge of the variations in thickness and composition of bone tissue, which may be obtained from an X-ray CT scan of the region of interest. The spatial resolution of the 'active' approach is determined by the dimensions of the transducer elements (typically a few mm), whereas the 'passive' UPIC approach is limited by the spatial resolutions of the X-ray CT scan and the 3D-printer (typically sub-mm) [3].

The aim of this study was to utilise externally-sourced µCT-derived binary data sets (bone/void) of four complex porous human cancellous bone samples (femoral head, calcaneus, iliac crest and vertebral body) to investigate the potential for UPIC to reduce ultrasound wave degradation. A 'spatial profile' along the direction of ultrasound propagation was first calculated for each bone sample. Since both replica bone model and UPIC were created from the same 3D-print material, the UPIC design was the inverted spatial profile.

Normalised broadband ultrasound attenuation was used as a quantitative measure of wave degradation, performed in transmission mode at a frequency of 1 MHz, yielding a reduction ranging from 57% to 74% when the UPIC was incorporated. We propose that the passive UPIC offers a broad utility, where it may be applied to any ultrasound transducer, of any complexity (single element or array), frequency and dimension.

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## Correlative microfocus computed tomography and fluorescence microscopy of fixed human lung tissue

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Keywords: Correlative imaging, computed tomography, fluorescence, human lung tissue

Microfocus computed tomography ( $\mu$ CT) can be used to visualise the 3D microstructure of the lungs relevant to the understanding of respiratory diseases including COPD, pulmonary fibrosis and lung cancer. Correlation with fluorescence microscopy imaging allows cellular and molecular localisation.

Using a prototype µCT system optimised for unstained soft tissue imaging (provided by Nikon Metrology UK) we imaged formalin fixed paraffin embedded unstained human lung tissue. Wax tissue blocks were scanned using low energy X-rays generated using a molybdenum (Mo) target. Approximately 4000 radiographs of the specimen were captured during a 360° rotation. Reconstructed volumes at 6-10µm voxel (3D pixel) size were sufficient to visualise lung microstructure.

For histology, tissue was re-embedded in wax, maintaining the same tissue orientation as in the  $\mu$ CT scan. Fluorescence microscopy images were digitised using a dotSlide 2.1 virtual microscopy system (Olympus, UK) and correlated to the  $\mu$ CT using point to point landmark recognition on the slice tool in Amira software (V6.1.1, FEI). Tissue autofluorescence proved useful to identify overall tissue structure including collagen and blood vessels. Immunofluorescence was used to identify specific features in the tissue. A cytokeratin 8/18 primary antibody was used to stain the airway epithelium. A secondary antibody that fluorescence provides high staining specificity, good signal to noise ratio and images can be produced in the same greyscale format as the  $\mu$ CT.

The 2D immunofluorescence images were successfully correlated to the  $\mu$ CT dataset. Information from the autofluorescence and immunostaining was used to segment key features in the 3D structure using Amira, including airways and blood vessels.

In summary, correlation of 2D immunofluorescence and 3D  $\mu$ CT data permits localisation and segmentation of key lung features such as airway epithelial thickness in fixed human lung tissue.

#### Investigating the Micro Structure of Plant Leaves in 3D, with X-ray µCT

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#### Keywords: Plant Leaves, X-Ray µCT, Low Density, High Resolution, Photosynthesis

It is estimated that a doubling in agricultural productivity will be required over the coming decades to meet the increasing demand for food by a rapidly growing global population. Photosynthesis is the prime driver of food production in crops, however crop responses to climate change need to be evaluated now in order to sustainably maintain global food security. The cellular architecture and associated extracellular air space of plant leaves is thought to play a key role in gas exchange and carbon assimilation, thus influencing their photosynthetic capacity and efficiency. However, successfully imaging the cellular architecture of plant leaves is challenging due to their low density, fine structure and fragile nature.

Classical optical techniques, such as histology, light sheet and confocal microscopy can provide valuable information about the structure of plant materials, however these approaches are destructive, time consuming and are limited by optical depth, typically producing 2D cross sectional data.

Here, we employ X-ray micro Computed Tomography ( $\mu$ CT) to visualise and quantify differences in plant leaf extracellular airspace in 3D at high resolution (2.5  $\mu$ m). Image analysis techniques have been developed to provide descriptors of plant leaf airspace, including bulk leaf porosity, porosity distribution, pore thickness, pore surface area and channel network connectivity. All of these provide insights into gas flow which in turn influences the photosynthetic efficiency of the plant.

These data, in conjunction with physiological studies, will help provide plant breeders with the necessary information to produce more efficient crops and moreover, those that can cope and even thrive in response to climate change.

Our current studies include elucidating the influence of leaf structure on the photosynthetic efficiency of both wildtype and transgenic rice and Arabidopsis lines; the influence of different CO2 levels on the intercellular airspace of wildtype rice leaves, and the influence of mesophyll structure manipulation on the photosynthetic capacity of Arabidopsis leaves.

## Correlative imaging to elucidate biological form and function: bio-inspiration from barnacle shells

#### Mitchell, R. L<sup>1</sup>, Pleydell-Pearce, C<sup>1</sup>, Coleman, M<sup>1</sup>, North, L<sup>1</sup>, Gelb, J<sup>2</sup>, Davies, P<sup>1</sup>, and Johnston, R<sup>1</sup>

<sup>1</sup>Advanced Imaging of Materials (AIM) Facility, College of Engineering, Swansea University, Swansea, SA1 8EN, UK. <sup>2</sup>Carl Zeiss X-ray Microscopy, Pleasanton, CA, USA

Biomaterials in nature can be subject to extremes of moisture, loading, temperature flux, and attack from predators. Yet, they form in ambient conditions, using only the resources available to the organism. Nature has developed an incredibly diverse range of strategies to survive despite these conditions, including complex microstructural forms and subsequent structure-property relationships. Correlative imaging provides an opportunity to discover the intimate interactions and mechanisms involved with the structure of complex biological systems at varying length scales. Correlative imaging allows us to identify sites of interest across various 2D and 3D imaging and analytical platforms by combining light, electron and X-ray microscopy to generate diverse information taken directly from nature.

Here, we have used the correlative potential of numerous coupled systems: X-ray microscopy, scanning electron microscopy, optical light microscopy, and focussed ion beam microscopy to ascertain the chemistry, microstructure, and crystallographic orientation of plate joints in the external 'shell' of the barnacle Semibalanus balanoides at different length scales. The joints are the meeting point of two interlocking plates, which are often 'shared' between neighbouring organisms providing strength and the ability to expand in size. X-ray microscopy ( $\mu$ CT) reveals complex interactions between joints that can only be identified via non-destructive methods, and light and SEM microscopy indicates specific chemical and crystallographic orientations at the tip of joints, which has implications for natural strengthening mechanisms. FIB is used to section areas of interest and generate chemical profiles, and to investigate anisotropy. Understanding the organism's behaviour is essential to consider the functions of these complex forms. The work demonstrates that correlative methods such as this that span different platforms and techniques enables the extension of the fundamental basics of material science to broaden the application of bioinspiration in human-made engineering practices.

#### Visualising liver pathology of Schistosomiasis in mice using micro-CT

## James D B O'Sullivan, Andrew S MacDonald, Alexander T Phythian-Adams, Julia Behnsen, Sheena M Cruickschank, Kathryn J Else and Philip J Withers

#### University of Manchester

#### Key words: schistosome, vasculature, inflammation, pathology, micro-CT

Schistosomiasis is a debilitating and potentially fatal disease caused by flatworms of the genus Schistosoma. Adult flatworms live in the mesenteric venules surrounding the large intestine and sustain themselves on the rich supply of host blood. The eggs laid by the adult flatworms may be swept by the blood flow towards the liver, where they produce damaging pathology. Eggs become lodged in the liver parenchyma inducing an inflammatory response localised around each egg (granuloma). The granulomas are known to cause remodelling of liver vasculature and an increased systemic blood pressure, both of which are risk factors for fatal internal bleeding (varices). Despite the potentially fatal nature of schistosome infection, little is known about the relationship between the granuloma response of the host and the downstream clinical risk factors. The 3D imaging capabilities of X-ray micro-computed tomography (micro-CT) provide an excellent opportunity to put vascular remodelling and blockage caused by the flatworm eggs into the context of the inflammatory host response. In mice infected with Schistosoma mansoni, we visualise the characteristic liver pathology of schistosomiasis. Mouse livers were dissected and stained with a 5% solution of Lugol's iodine. Inspection of slices allows distinction and quantification of both the eggs and surrounding inflammation against healthy tissue. This therefore allowed a non-stereological approach to assessing liver pathology not previously attempted in this tissue. Furthermore, contrast between blood vessels and parenchyma allows a first view of the changes in gross vascular morphology in the context of the inflammatory pathology of the surrounding parenchyma, including blockage caused by egg deposition. Such developments represent the first steps in relating the wellknown immune pathology of this disease to the clinical risk factors.

## Micro-computed tomography optimised for soft tissues: first steps towards early diagnosis of colorectal cancer

## <u>C. Rossides</u><sup>1,2</sup>, O.L. Katsamenis<sup>3</sup>, O.J. Larkin<sup>4</sup>, B. Smit<sup>4</sup>, I.G. Haig<sup>4</sup>, I. Sinclair<sup>3,5</sup>, S.L.F. Pender<sup>2</sup> and P. Schneider<sup>1,3</sup>

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Keywords: Colorectal cancer (CRC), Inflammatory Bowel Disease (IBD), soft tissue, micro-computed tomography

Introduction - One in five patients who suffer from Inflammatory Bowel Disease (IBD) for more than 20 years will eventually be diagnosed with inflammation-associated colorectal cancer (CRC) [1, 2]. Even though CRC is treatable if diagnosed at an early stage, it is the third most lethal cancer worldwide and the second in Europe [3]. Moreover, although IBD patients are usually screened by regular surveillance colonoscopies (annually or bi-annually) and random multiple biopsies, advanced stages of CRC are still reported [1]. To better understand the early stage of CRC, we propose the use of high-resolution computed tomography ( $\mu$ CT) to identify the early onset of structural changes in the gut mucosa. The goal of this study is to investigate if soft tissue-optimised  $\mu$ CT of standard formalin-fixed and paraffin-embedded (unstained) tissue [4], as used in clinical histopathology, can reveal microstructures of the colon in a mouse model for inflammation-associated CRC.

Methods - An azoxymethane/dextran sodium sulphate mouse model [5, 6] is used to mimic the conditions of IBD/CRC in patients. Murine colonic tissue samples have been collected at different stages of CRC and fixed in 10% formal saline. One group of samples was first stained using 25% Lugol's iodine [7, 8] ("stained" group), a diffusible contrast agent that increases X-ray absorption and thus CT image contrast, and embedded in paraffin wax blocks according to standard procedures. The second group of samples was embedded unstained in paraffin wax ("unstained" group). All samples have been imaged using a prototype µCT scanner, provided by Nikon Metrology UK, optimised for soft tissue X-ray contrast imaging [9].

Results and Discussion - Preliminary results show that it is possible to obtain high-fidelity 3D images of the colon (Figure 1: stained, Figures 2 & 3: unstained), including the underlying mucosal layer structure (Figures 2 & 3: unstained).

Conclusion and Outlook - 3D high-resolution imaging of the colon using soft-tissue optimised µCT can reveal microstructural details in a mouse model for CRC. Clinical translation will be facilitated as the proposed imaging approach is fully compatible with standard clinical workflows. In this spirit, we will start investigating abnormalities on the colonic microstructure as precursors of CRC and potential phenotypes for early diagnosis of the disease.





Top, Left. Figure 1: 3D rendering of murine colon tissue sample stained in 25% Lugol's iodine. High contrast and spatial resolution reveal the surface texture and the tissue structure. Voxel size of  $\mu$ CT scan = 5.5  $\mu$ m.

Top, Right. Figure 2: 3D rending of unstained murine colon tissue sample. Structure and surface texture of the mouse colon can clearly be identified. Voxel size of  $\mu$ CT scan = 3.25  $\mu$ m.

Bottom. Figure 3: Rendering of unstained murine colon tissue sample. Microstructure is visible in the mucosal layer. Voxel size of  $\mu$ CT scan = 4  $\mu$ m.

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#### Fracture mechanics of arthropod cuticle

#### Dan Sykes<sup>1</sup>, Russell J. Garwood<sup>2</sup>, Shelley Rawson<sup>1</sup> and Philip J. Withers<sup>1</sup>

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Cuticle is a natural, lightweight composite that forms the exoskeleton of arthropods: it is present in ~66% of all known species on Earth. Nanofibres of chitin (a long-chain polysaccharide) occur within a structural protein matrix (fibrous proteins that increase stiffness or elasticity). Cuticle has many diverse functions including: providing protection, giving structural support, controlling water content, mastication, penetrating prey cuticle with fangs, adhering to surfaces for climbing, forming wings for flight and sensory perception. As a result cuticle displays a remarkable range of properties, for example, elastic modulus varies over seven orders of magnitude, yet the basic macrostructure and composition of cuticle is consistent across all arthropods. As such, it is a valuable material for biomimetic design. The chitin-protein fibres are arranged in helicoid layers, themselves incorporated into three ultrastructural layers: epicuticle, exocuticle and endocuticle, each of which possesses different properties. In situ imaging of crack propagation during mechanical testing highlights how this structure affects fracture toughness. In this study, we used time-lapse (4D) nano-computed tomography combined with tensile testing to visualise 3D crack deflection along the chitin-protein fibres. By visualising the crack in 3D we can also observe the benefits of the helicoid structure (commonly found in biological structures) in reducing the risk of complete failure by fracture in biomaterials. Applying this technique to other types of arthropod cuticle will allow us to see how changes to structure and composition at the micro- and nano-scale affect material properties such as fracture toughness.

#### Collaborative Computational Project in Tomographic Imaging's Core Imaging Library (CIL) Release

#### Srikanth Nagella<sup>1</sup>, Ron Fowler<sup>1</sup>, Edoardo Pasca<sup>1</sup>, <u>Martin J. Turner<sup>1,2</sup></u> and Erica Yang<sup>1</sup>

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Keywords: iterative image reconstruction, segmentation, beam-hardening, visualisation, quantification, CCP

This poster will present the Core Imaging Library (CIL), a collection of algorithms (released in June 2017 as a binary Anaconda python distribution [1,2]) that provides modules for several steps involved in the Computed Tomography process. Supported by the Collaborative Computational Project in Tomographic Imaging (CCPi [3] which is funded by the EPSRC) the algorithms are contributed by the community where the code is then re-engineered for easy distribution and maintainability. A list of current routines in the initial version:

#### I. Pre-Processing Module:

Beam Hardening Correction (CarouselFit) software takes X-ray image data for a number of known samples, and fits them to a model of the beam when a broad spectrum source is used to image an unknown sample [4].

#### 2. Iterative Reconstruction Algorithm Module:

There are three main iterative reconstructions available in this package; Conjugate Gradient Least Squares (CGLS), Maximum Likelihood Estimation Method (MLEM) and Simultaneous Iterative Reconstructive Technique (SIRT); with three variants of CGLS available; CGLS with Convolution, CGLS with Tikhonov regularization and CGLS with Total Variation Regularisation (see Fig I left). These reconstruction algorithms work on parallel beam datasets although following versions will support cone beam datasets.

#### 3. Quantification Module:

Two algorithms are included; Accessible Volume that takes in a binary 3D image (usually scaffold images) and calculates the accessible volume for a range of sphere sizes [5] and Label Quantification that takes in a labelled image and calculates multiple characteristics for each label [6].

#### 4. Post-Processing Module:

Included is Simpleflex Segmentation algorithm described by Carr et al. [7] (see Fig I right) that is based on the calculation of the complete contour tree allowing for multiple isosurface structures to be interactively and simultaneously displayed.



Figure 1. left to right: example of different reconstruction techniques from the collection of CIL Iterative Reconstruction algorithms and a contour tree visualisation of the segmentation using Simpleflex.

We wish to acknowledge extensive contribution from Daniil Kazantsev's Iterative Tomographic Reconstruction CCPForge https://ccpforge.cse.rl.ac.uk/gf/project/ccpi\_itr/ and use of datasets from; Diamond Light Source https://github.com/ DiamondLightSource/Savu/blob/master/test\_data/data/24737\_fd.nxs and VTKData https://github.com/naucoin/VTKData/tree/ master/Data/headsq

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## Low – Z materials and in-situ microscopic structural investigation with a three dimensional X-ray microscope: Nano3DX

## <u>Paul Vanden Branden</u><sup>1</sup>, Peter Oberta<sup>2</sup>, Kazuhiko Omote<sup>3</sup>, Yoshihiro Takeda<sup>3</sup>, Raita Hirose<sup>3</sup> and Joseph D. Ferrara<sup>4</sup>

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<sup>3</sup>X-ray Research Laboratory, Rigaku Corporation
<sup>4</sup>Rigaku Americas Corporation

It is well known that X-rays can penetrate opaque objects and show the internal structure without destroying the object. Thus, X-rays are widely used for medical imaging, security and industrial inspection. X-ray computed tomography (CT) is a powerful technique for visualizing internal structure of various specimens including the human body, in 3D. Recently a high spatial resolution X-ray microscope has been developed as a result of improvements in microfocus X-ray sources and high resolution X-ray detectors, making it possible to determine the internal 3D structure. Using a quasi-parallel beam technique, with a rotating anode high power X-ray source and a sub-micron resolution detector. Using low energy X-rays (Cr, Cu and Mo) high-contrast CT images for low – Z materials is obtained. Adding techniques like phase contrast we even increased the imaging potential of the X-ray microscope. There is also demand for precise investigation of structural changes induced by changes in environmental conditions, like temperature variations or application of compression and tensile stress. In order to investigate structural changes under real environmental conditions, it was necessary to build in-situ attachments., which can be combined with the CT measurement.

#### Markov Random Fields for XCT image segmentation

#### J. M. Warnett<sup>1</sup>, A. Jaiswal<sup>2</sup>, A. Bhalerao<sup>1</sup> and M. A. Williams<sup>1</sup>

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Traditional approaches to image segmentation in X-ray CT such as ISO50 and Otsu use a global threshold to distinguish between material and background. Global approaches work well for clean data, but where there are imaging artefacts such as scatter they often incorrectly identify boundaries and pores. Particularly when imaging metallic objects there are additional inhomogenities in the reconstructions to include non-uniform illumination and streak artefacts that further complicate the segmentation process. To overcome this, information on the local context must be incorporated in the procedure to produce a more faithful segmentation. Markov Random Fields (MRF) is such a method that considers not only intensity but the current label assignment of neighbours to iteratively update the segmentation. Here the example of a steel column with porosity and a titanium cube with known defects are used to demonstrate the capabilities of such methods. In the case of the steel column there is significant streaking and variation of grey value within the material and pores, caused by the heavily scattering nature of the material. Global methods fails definitively identify boundaries between pore and material that renders analysis impossible without manual intervention, and smaller voids are lost completely. MRF is capable of realising the true boundaries that enables subsequent analysis. A titanium cuboid with prescribed defects is used as a second example. The sharp corners of the object result in streaks within the images, and the scattering has caused the pores to be of notably higher grey value than the background. The latter is in particular problematic for global methods, missing the pores almost in their entirety. By using three classes instead of two in the MRF segmentation method, the pores are successfully retrieved.

## Evaluating Megalosaurus bucklandii: X-Ray Computed Tomography (XCT) as a tool for Heritage Conservation

#### P. F. Wilson<sup>1</sup>, M.A. Williams<sup>1</sup>, J. M. Warnett<sup>1</sup>, A. Attridge<sup>1</sup>, H. Ketchum<sup>2</sup>, J. Hay<sup>2</sup> and M. P. Smith<sup>2</sup>

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#### Keywords: Conservation, Megalosaurus, Dinosaur, Heritage, X-Ray CT

Arguably the most important task for any cultural institution is the practice of conservation, the method by which specimens at risk of severe degradation or destruction are treated to ensure that they survive to be enjoyed by future generations. However, surface inspection is often insufficient to properly inform conservators of the internal structure of the artefact and where records of conservation are typically incomplete or have been destroyed over time, conservators find themselves challenged to identify what approaches have been previously undertaken. This can be problematic when planning the best approach to stabilise a museum artefact through remedial conservation and may also leave museums vulnerable to fraudulent specimens. However, X-Ray Computed Tomography (XCT) grants a way to overcome these issues by allowing conservators to non-destructively investigate the subsurface details of an artefact to provide essential information on the condition of a specimen. Here, the potential of this approach is demonstrated using the first XCT scans of the iconic dentary of Megalosaurus bucklandii Mantell, 1827; the first scientifically described dinosaur. This antiquated specimen is of particular interest due to its long residence time at the Oxford University Museum of Natural History (OUMNH) and, due to its antiquity, the extremely incomplete records of its repair history, providing a perfect case study for the use of XCT as a conservational tool. XCT analysis reveals that the degree of repair is less extensive than previously thought and also highlights two different material types, MI and M2, thought to be representative of at least two phases of repair. Energy Dispersive X-Ray Spectroscopy (EDX) also elucidates the composition of these two different materials. Finally the potential of this approach is further explored, highlighting its importance for conservation practice, identifying forgeries and hoaxes in addition to potential applications in public engagement.

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## **Imaging Competition**

Thank you to those who entered the ToScA Imaging Competition. The images will be on display throughout the symposium and the winners will be announced at the Symposium Banquet on HMS Warrior.



#### The Orchid

The Orchidaceae flower was scanned at the Natural History Museum for Kew Gardens. The image comprised of three individual scans of the same plant. The final image consists of two separate renders merged together using Photoshop. Both Drishti and Avizo software were used to create the final image.

Brett Clark, Natural History Museum, London

Acknowledgements: Kew Gardens, Imaging and Analysis Centre (The Natural History Museum), Farah Ahmed and Amin Garbout.



#### Jaw Bone Replacement (The Tree of Love)

The picture represents a micro CT scanning of a synthetic trabecular bone replacement to be applied to the field of Bone Tissue Engineering (B.T.E.). The process, based on Voronoi diagrams, has been implemented in Rhinoceros 5 (McNeal, Seattle, WA, USA) with the graphical algorithm plug-in editor Grasshopper (v. 0.9.0076). In this way, 3D geometrical heterogeneous structures can be generated resulting such as a really biomimetic shape, as it appears in actual natural trabecular bone. The generated stl file was UV cured with a photopolymer 3D printer called Form 2 (Formlabs) 25 microns layer resolution. After being 3D printed the scaffold was scanned with a microCT Xradia Versa 520 (ZEISS) setting a Voxel size of 24 microns. The picture has been taken rough with a very simple grayscale threshold segmentation.

Marco Curto, University of Portsmouth (UK), School of Engineering, Technology

Acknowledgments: Zeiss Global Centre, Formlabs, Rhinoceros McNeel & Associates



#### I spine with my little eye; in-line phase contrast microCT of fresh rat spine

Low back pain affects over 80% of the population at some point in their lives. A large number of these cases are related to irreversible structural degeneration of the intervertebral disc in the spine. The tomogram shows central cartilage-like gel surrounded by layers of collagen bundles and sandwiched between calcified endplates and vertebra. Importantly, phase contrast microCT allows imaging of soft tissue structures in a segment of fresh spine which preserves native structure and mechanics. Resolving these structures facilitates 3D structural and mechanical studies at resolutions in the micron range. A full understanding of structural disc mechanics during progressive degeneration and assessing efficacy of the first tissue replacements is required for successful low back pain treatments.

Catherine Disney, University of Manchester



#### What was for lunch, Archimedes?

An owl pellet is one of the few things I've always wanted to CT scan so I couldn't believe my luck when I found one on the Crymlyn Burrows nature reserve right next to the University. Owl pellets like this sample are dissected regularly by biologists to determine an owl's diet but I was also interested in how the pellet is packed together before dissection. Is the bone orientation significant? Does that aid regurgitation or is it caused by regurgitation? I don't know, but it would be exciting to find out and perhaps discover new insights into the biology of owls.

Elizabeth Emma Evans, Swansea University

**Acknowledgements:** Scanned at Advanced Imaging of Materials (AIM) Facility, Swansea University; Image produced in Drishti 2.6.3.



#### An osteocyte imaged using serial block-face scanning electron microscopy (SBF SEM)

Osteocytes are stellate cells found inside bone which coordinate bone homeostasis and are thought to have a role in the pathogenesis of osteoporosis. The many processes shown in green connect with other osteocytes, surface bone cells and the vasculature. Serial block-face scanning electron microscopy involves automated sequential imaging and slicing of a stained resin block. Segmentation of the cell from the 2D images and volume rendering produces high resolution 3D images. Scale bar =  $5 \mu m$ 

Patricia Goggin, University of Southampton

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#### Large scale pore structure measurements of ursine heattreated starch tuber products

Crisp pore structure analysis and visualisation was performed using Avizo software on tomographic data collected by Leigh Connor and Sally Irvine on beamline I12-JEEP at Diamond Light Source.

Dr. Sally Irvine, Diamond Light Source



#### Looking at the fibers

High-resolution x-ray tomography image of an electrospun scaffold, called bundle (diameter = 550  $\mu$ m), made of Poly-L-lactic acid aligned nanofibers (diameter = 0.47 ± 0.14  $\mu$ m). The image consists of two halves of the same scaffold, obtained using two different transfer function values: the left side shows the totality of nanofibers that compose the bundle, while the right side only shows the nanofibers inside the scaffold.

Many thanks to the Alma Mater Studiorum - Università di Bologna for the production of the scaffold, and the Zeiss Global Centre of the University of Portsmouth for the use of the high resolution x-ray tomograph.

Alberto Sensini, Universita di Bologna and Gianluca Tozzi, University of Portsmouth



#### Inside a duckling bone

Synchrotron-based CT image of the mid shaft of a duckling (Anas platyrhynchos) tibiotarsus, 1.3 micrometer voxel size. Transparent white indicates bone volume, red intracortical soft tissue, yellow osteocyte lacunae. Image taken at TOMCAT beam line of the Swiss Light Source.

Katherine Williams, University of Southampton



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The Royal Microscopical Society and the ToScA group of the Natural History Museum now offer joint membership. Many attendees to this ToScA 2017 Symposium have already signed up.

ToScA, Tomography for Scientific Advancement, is a rapidly expanding group focussing on the practice of tomography and its many applications in both life and physical sciences. Their annual symposiums, hosted by the RMS in the UK and now the United States, always boast a top line-up of speakers and engaging submitted talks as well as extra opportunities such as training workshops.

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