University of Portsmouth and ZEISS Correlative Microscopy Workshop

2-3 May 2018

University of Portsmouth

Aim:

Imaging technologies have been instrumental to revealing structural and functional information across the biological, material and geosciences research fields.

To capture the essence of your sample, multiple analytical methods are often needed.

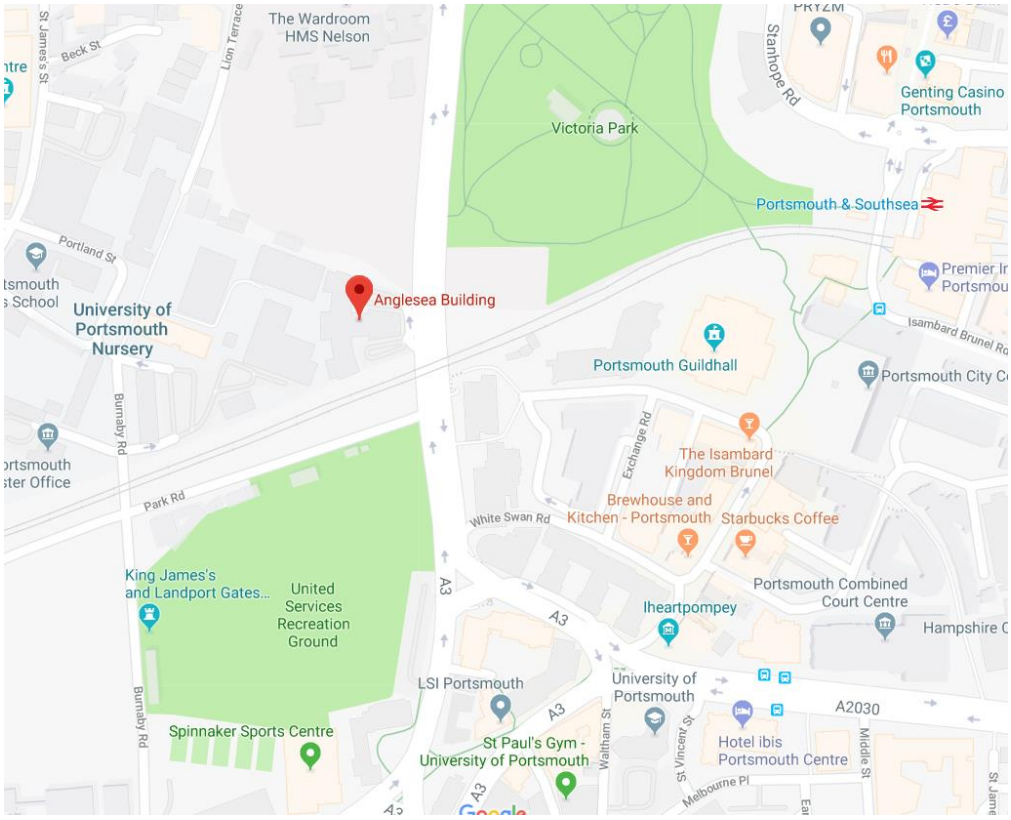
The University of Portsmouth offers a suite of cutting edge imaging tools - from light microscopes to confocal laser scanning microscopy (LSM), X-ray microscopy (XRM) and scanning electron microscopy (SEM). The University of Portsmouth is therefore optimally positioned to enable researchers to analyse a wide range of materials from the nano- to micro- scale.

In the past, correlating different techniques tended to be time consuming and labour intensive. However, ZEISS offers correlative microscopy (CorrMic) integrated solutions and seamless workflows. The process of correlating different microscopy techniques is now made streamlined and efficient.

This workshop aims to demonstrate the use of ZEISS CorrMic tools to streamline to correlative process through seminars and demonstrations.

Venues:

- School of Pharmacy and Biomedical Sciences, St Michaels Building, White Swan Road
- School of Earth and Environmental Sciences, Burnaby Building, Burnaby Road
- School of Engineering, Anglesea Building, Anglesea Road



Agenda:

Date	Time	Session/Speaker/title	Venue
2 May	**9 - 10:30**	**Opening, Current state of the art**	**Burnaby Lecture Theatre** BB3.30
	9 - 9:10	Opening speech and explanation of the workshop programme, Dr Katherine Lau, ZEISS	
	9:10 - 9:30	Dr Gianluca Tozzi – 'XRM imaging of biological tissues and biomaterials'	
	9:30 - 9:50	Dr James Darling – 'SEM imaging, chemistry and structure of natural materials'	
	9:50 - 10:10	Dr Mohen Seifi – 'Using high resolution laser scanning microscopy (LSM) to inform the function of diverse neural pathways'	
		Coffee break	
	10:30 - 11:50	**Correlative Microscopy used in material sciences research**	
	10:30 - 11:15	Prof Phil Withers – 'Multifacetted correlative tomography of materials across time and lengthscale'	
	11:15 - 11:45	Stefanie Freitag – 'Additive Manufactured Parts: New Insights through Correlative Studies with X-ray, Light- and Electron Microscopy'	
	13:30 - 16:30	**Poster Session with refreshments**	**Burnaby Building BB2.26**
	13:15 - 16:45	**Demos – use of LSM/XRM/SEM for different areas of research** Demonstrators will be at different stations to introduce to researchers who are not familiar with the techniques how the microscopy technique can be used to benefit their research. Participants must register in advance and provide description of their research areas. Each slot is half an hour, with breaks every hour.	**LSM 880, St Michael's Building** **EVO MA at Burnaby Building** **Xradia Versa at Anglesea Building Room 0.22**
	13:15 - 13:45	Demo	
	14:00 - 14:30	Demo	
	14:45 - 15:15	Demo	
	15:30 - 16:00	Demo	
	16:15 - 16:45	Demo	

3 May	**9:00 - 10:00**	**Correlative Microscopy used in raw materials research**		**Burnaby Lecture Theatre BB3.30**
	9:00 - 9:30	Dr Matt Andrew – 'Multiscale analysis of unconventional shale reservoirs for the Oil & Gas industry'		
	9:30 - 10:00	Dr Matt Andrew – 'Multimodal mineral classification using machine learning'		
		Coffee break		
	10:15 - 11:50	**Correlative Microscopy used in life sciences research**		**Burnaby Lecture Theatre BB3.30**
	10:15 - 10:45	Dr Maria Hariolaki, Beamline B24, Diamond Synchrotron Centre – 'Correlative microscopy developments at Diamond Light Source beamline B24'		
	10:45 - 11:15	Ann-Katrin Unger – 'Array tomography - Possibilities for 3D imaging'		
	11:15 - 11:45	Ann-Katrin Unger – 'Recent advances in 3D correlative microscopy'		
		Coffee break		
	12:00 - 12:30	**Round table discussion** Invite all speakers to come on stage to discuss best use of CorrMic solutions for different research areas. Audience can ask questions.		
	14:15 - 15:15	**Poster session with refreshments**		**Burnaby Building BB2.26**
	13:15 - 16:45	**Correlative microscopy demos (half hour slots)** **LM -> SEM and XRM -> SEM (Atlas 5) at Burnaby Building** **LM to LM (live cells to fixed cells) at St Michael's Building** **Please refer to the demos time table.**		**Burnaby Building (SEM)** **Michael Swan Building 5th floor (LSM 880 Airyscan)**
	13:15 - 13:45	Demo at Burnaby Building	Demo at Burnaby Building	
	13:45 - 14:15	Demo at Burnaby Building	Demo at St Michael's Building	
	14:30 - 15:00	Demo at Burnaby Building	Demo at St Michael's Building	
	15:00 - 15:30	Demo at Burnaby Building	Demo at St Michael's Building	
	15:45 - 16:15	Demo at Burnaby Building	Demo at St Michael's Building	
	16:15 - 16:45	Demo at Burnaby Building	Demo at St Michael's Building	

Abstracts:

2nd May 2018

09:10 – 09:30 **XRM imaging of biological tissues and biomaterials**

Dr Gianluca Tozzi, Senior Lecturer in Mechanical Engineering, Director of Zeiss Global Centre, School of Engineering, University of Portsmouth

The Zeiss Global Centre (ZGC) at the University of Portsmouth is using high-resolution X-ray microscopy (XRM) to study a wide range of biological materials (i.e. bone). Advanced investigation is conducted via in situ testing protocols combined with digital volume correlation (DVC), for which the ZGC has gained worldwide recognition. The seminar is intended to provide insight on structure and mechanical competence of such materials, including novel workflows for subject-specific 3D printing and biomimetic engineering.

09:30 - 09:30 **SEM imaging, chemistry and structure of natural materials**

Dr James Darling, Senior Lecturer, School of Earth and Environmental Sciences, University of Portsmouth

SEM: imaging, chemistry and structure of natural materials

Scanning electron microscopy (SEM) provides a versatile set of techniques to study natural and engineering materials. Different interactions between the primary electron beam and the sample can be exploited for imaging, chemical analysis and structural characterization at the micro- to nano-scale. These include secondary and backscattered electrons, cathodoluminescence, secondary X-Rays and diffracted electrons. Many modern SEMs are equipped with a range of detectors that readily allow for the integration of these datasets; providing tremendous opportunities for rapid characterization of complex materials. Here we will present example data from our LaB_6 Zeiss EVO MA10 SEM to show how this approach has helped to resolve how deformation influences chemical mobility in natural materials (e.g. zirconia, apatite) under extreme conditions. These examples include samples from hypervelocity impact events and major tectonic faults.

09:50 - 10:10 **Using high resolution laser scanning microscopy (LSM) to inform the function of diverse neural pathways in body's stress response**

Dr Mohsen Sefi, School of Pharmacy and Biomedical Sciences, University of Portsmouth

The nervous system is composed of two main divisions: the central division involving the brain and spinal cord and the peripheral division which controls the function of all major peripheral organs. The nervous system plays a pivotal role in mediating the body's physiological response to various stressors. The stress response is an adaptive mechanism that serves to maintain the stability of the internal milieu. However, experiencing stressors over a prolonged period of time, can result in a long-term drain on the body and lead to permanent molecular and functional changes in central and peripheral neural pathways. Indeed, psychosocial stress not only predisposes individuals to mental illnesses such as anxiety and depression but it is also a key contributor to the underlying pathology of various peripheral disorders such as inflammatory bowel disease.

The nervous system mediates the stress response by producing and releasing a whole host of molecules such as neurotransmitters and hormones. These molecules will in turn bind to their specific receptors which are expressed on various cell types throughout the body. Hence, understanding the stress induced alterations in location and expression levels of these molecules within the nervous system is a prerequisite to understanding and combating stress induced mental and peripheral disorders. Therefore, in our combinatorial research, we use immunohistochemistry and high resolution laser scanning microscopy in order to investigate such molecular changes and correlate them to the functional aspects of the stress response which we measure using various functional techniques.

10:30 - 11:15 **Multifacetted correlative tomography of materials across time and lengthscale**

Prof Philip Withers, Henry Royce Institute for Advanced Materials, University of Manchester, Manchester

Natural, and increasingly manufactured materials, rely on complex hierarchical structures to provide a suite of interesting properties and functionality. Often they exploit anisotropy and heterogeneity through interfaces, chemistry and crystallography from the nanoscale to the component scale. To date much has been learnt about materials behaviour from 2D images. However two dimensions don't always tell the whole story. Tomographic methods enable us to build up 3D pictures of structure. In many cases we need to be able to follow the response of these features to external loads and environments. Because it is non-destructive, X-ray computed tomography (CT) enables to acquire sequences of 3D images over time, to carry out time-lapse studies.

There are now a plethora of techniques able to characterise materials. Rather than apply them separately on different specimens, correlative tomography enables us to combine and integrate these methods to examine in detail a region of interest and to build up a multifaceted picture of how these hierarchical structures perform enabling us to identify the key length scales. We exploit multiscale correlative strategies to enable multiple chemical, structural and property datasets can be brought together at key regions of interest in three dimensions.

Through a series of examples I will look at how combining multiscale, multimodal and time lapse information can help us to investigate a very wide range of phenomena from the pupation of butterflies and the self-healing of ceramics, to corrosion and cracking in metals, to the nucleation, growth and accumulation of damage in 3D woven composites.

11:15 - 11:45 **Additive Manufactured Parts: New Insights through Correlative Studies with X-ray, Light- and Electron Microscopy**

Stefanie Freitag, Carl Zeiss Microscopy GmbH, Munich, Germany

The layer-by-layer build-up process, termed additive manufacturing, is mostly used for the production of spare parts and prototypes. The medical, automotive, aerospace and tooling industry are already using this process on a daily basis. The high geometric and constructive freedom, as well as the ability to generate curved cooling channels in parts that heat up during operation, are of big interests.

Depending on the application area, various functional tests are performed after production. However, research institutes and quality assurance laboratories found that pure mechanical tests could lead to incorrect part failure interpretations. To bring additive manufacturing to

the next stage, from prototype production to serial production, materials researchers are aiming to analyse various properties within the material. These properties include particle residues, porosity, impurities and roughness.

The current study shows the use of a new analysis approach – combining X-ray microscopy, targeted preparation methods, as well as focused ion beam (FIB)-scanning electron microscopy (SEM), in a correlative manner. The experiments were performed on a 3D printed gear wheel (AlSi10Mg), using X-ray microscopy to non-destructively image the whole part. This allowed an understanding of the overall part quality, thus evaluation of criticality and quantity of defects. The location of one specific suspicious area (with higher X-ray absorption) was approximated in the X-ray image. This enabled a highly precise (5 µm accuracy) and targeted preparation process for further analysis, to grind and polish towards the region of interest. Subsequent correlative FIB-SEM studies revealed an inclusion with a high atomic number. The microstructure around the impurity was noticeably affected and a molten alloy could be assumed. EDX mapping verified this inclusion to be metallic and identified it as H13 steel. With this information it was possible to draw the conclusion that the impurity originated from the predecessor process.

This study shows that X-ray microscopes can be used to visualize the structure and distribution of part porosity as well as impurities. This study outlines the suitability and benefits of using various microscopic methods for the evaluation of additively manufactured metal parts. It also showed that the correlation of contextual, chemical and microstructure analysis allows for in depth interpretation. The results facilitate the understanding of the root cause of defects in parts, and enables a targeted optimization of their production and preparation process is possible.

3rd May 2018

9:00 - 9:30 **Multiscale analysis of unconventional shale reservoirs for the Oil & Gas industry**

Dr Matt Andrew, Carl Zeiss X-ray Microscopy, Pleasanton, USA

The advent of shale production has transformed the energy industry, however to date a generic pore scale recovery mechanisms (governed by pore network structure) has been lacking. Two qualitatively different pore systems were compared and contrasted using scale independent techniques, finding organic hosted porosity (nanometer scale and common in unconventional reservoirs) to be much better connected than intergranular porosity (micrometer scale and common in conventional reservoirs). This contrast is explained by variations in pore shape, caused in turn by variations in the geological processes responsible for pore network genesis.

These processes were examined by creating a suite synthetic pore geometries with strong statistical agreement between imaged and synthetic pore networks. Synthetic pore networks were then used to examine how connectivity changes with porosity (as may arise by varying degrees of shale maturity, or by reservoir diagenesis), showing that network connectivity, needed for effective capillary imbibition, is much easier to achieve in organic-type pore networks than in the intergranular-type pore networks. We then review how such techniques might be applied to solve problems within the petroleum industry, including the development of new pore-scale core analysis techniques and the application of wellsite imaging to aid production operations.

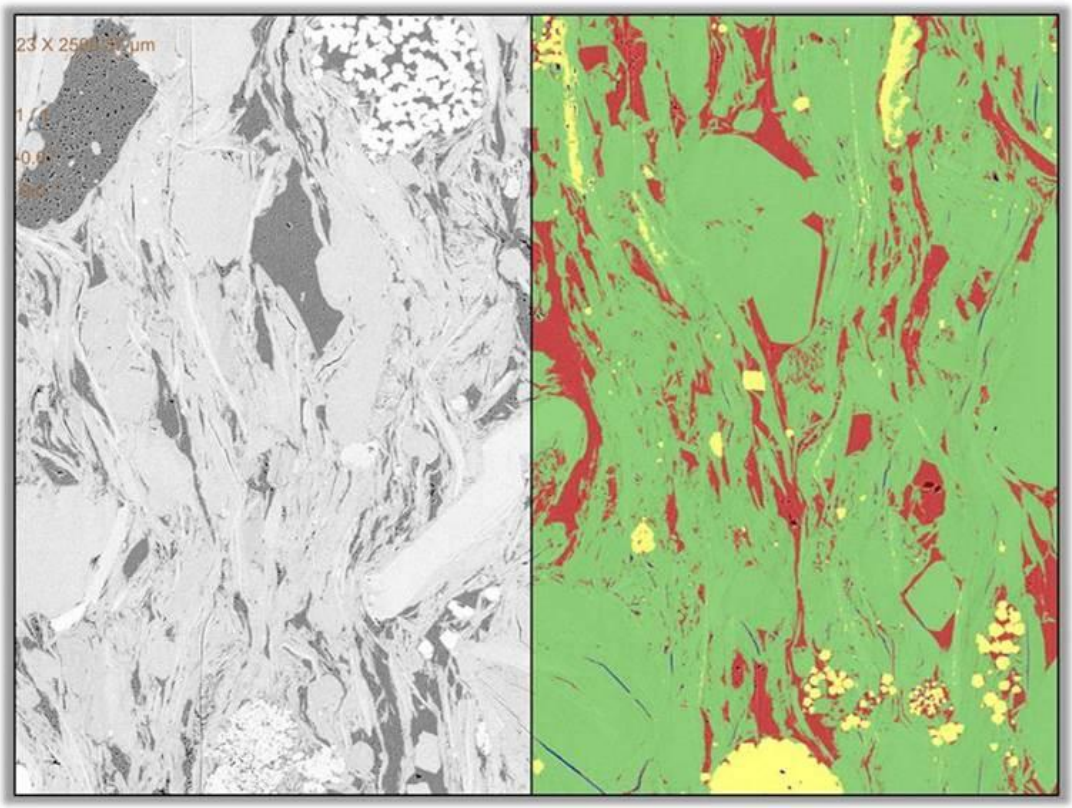


Figure 1 Large area electron microscopy map, classified using a machine learning algorithm, showing organic hosted pore structures (red), silicate mineral phases (green), pyrite (yellow) and microfractures (blue).

9:30 – 10:00 **Machine learning and microscopy: views, applications and techniques**

Dr Matt Andrew, Carl Zeiss X-ray Microscopy, Pleasanton, USA

Great technological progress has been made over the last 20 years in the development of pore-scale imaging and modelling to address challenges in the petroleum geosciences. One of the principal challenges has been that these techniques are challenging to scale and automate, usually because the continuous outputs of the imaging techniques in question have to be ultimately classified into discrete phases for subsequent analysis and interpretation. These image outputs carry a variety of artifacts and noise that cause traditional analytical techniques to fail as the images become more complex.

During visually examination, the brain of a trained petrographer, petrophysicist or mineralogist acts to integrate the rich, potentially multimodal datasets to extract the desired information. Such an approach is challenging to capture and express in a computational form, making microscopy challenging and expensive to scale across the many 1,000s of feet of core required to effectively describe reservoir behavior. Machine learning techniques give us, for the first time, a powerful set of tools to capture the complex set of processes involved in analyzing the rich datasets available to microscopic imaging in a way computational scalable to a much large range of samples.

In this study we will show, with the use of quantitative performance metrics, how such machine learning techniques perform when compared to more traditional image processing, segmentation and analysis techniques for a suite of different images, including both X-ray microscopy and nano-scale FIB-SEM imaging across a wide range of different noise levels. We will even show how such machine learning can be used to discriminate features which have little or no difference in their greyscale values, but instead are discriminated by textural features alone. We will also review a range of different applications of machine learning technologies to geological microstructural examination. First, we will show how it can be used to classify micro-CT volumes into different lithological regions, which are then used as a macroscopic map of geological heterogeneity, making resulting petrophysical pore-scale simulations more predictive of core scale behavior.

We will then show how we can use machine learning to reduce acquisition time (and so cost-per-sample) for such petrophysical analysis. Machine learning based classifiers are much more noise tolerant than their traditional counterparts, and single high fidelity datasets can be used to train classifiers operating across a wide range of core sample. Finally, we will show how, by integrating automated mineralogical analysis with optical petrography, we can automatically extract mineralogical information from traditional cross-polarized light microscopy techniques. This is particularly exciting, as optical petrographic techniques are cheap, provide rich data about a wide range of reservoir petrophysics and geology, and can easily be scaled across extended sections of core.

10:15 - 10:45 **Correlative microscopy developments at Diamond Light Source beamline B24**

Dr Maria Hariolaki, Beamline Scientist, Beamline B24, Diamond Synchrotron Centre, Oxfordshire

B24 is the full field X-ray tomography beamline at Diamond currently delivering X-ray absorption contrast imaging of biological material (cells and tissues) to a resolution of 40nm. The resulting 3D data allows the unambiguous delineation of cellular ultrastructure and is employed in the interpretation of the effects of biological chemical and mechanical cues depending on the subject matter. The method provides cellular context without necessarily

any further insight as to the molecules that specifically effect subcellular architecture.

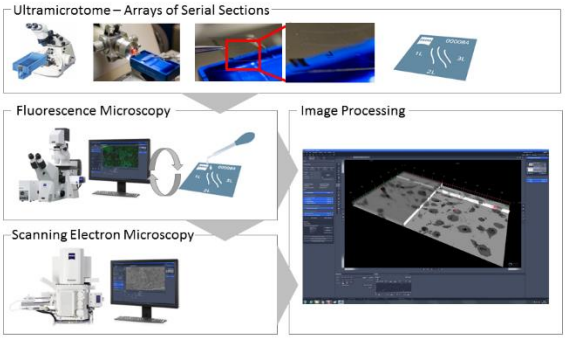
That is where fluorescence microscopy can provide invaluable insight as to the molecular localisation of key parameters relevant to the system under study. To that effect, at B24, a bespoke cryo fluorescence super resolution module has been developed offering both cryo-Structured Illumination microscopy (cryoSIM) and dSTORM. The particular attraction of the system is that samples that are due to be used for X-ray imaging can be processed there first to generate 3D fluorescence information at high resolution on identified areas of interest before taken to the transmission X-ray microscope allowing for the accumulation of directly correlated localisation data (the same sample is imaged through a variety of methods and the results are directly correlated avoiding sample to sample variations and therefore allowing the unambiguous interpretation of data across modalities).

10:45 – 11:15 **Array tomography - Possibilities for 3D imaging**

Ann-Katrin Unger, Carl Zeiss Microscopy, Oberkochen, Germany

In array tomography ordered, ribbon-like assemblies of ultrathin serial sections are deposited on a solid substrate and imaged afterwards. The resulting images are then aligned and reconstructed into a three-dimensional representation of the object. Depending on the preparation and labelling regime, different imaging modalities can be applied. When using light microscopy, the labelling with fluorescent markers would be the obvious choice, whereas the imaging in a scanning electron microscope would require impregnation with heavy metals. Depending on preparative constraints, the combinationof diverse imaging modalities or truly correlative imaging is possible. To allow easy fast forward sample preparation for Array tomography Zeiss offers a complete software and hardware solution allowing array tomography in a fast and easy way. An introduction into the world of array tomography approaches for 3D EM and correlative imaging will be presented.

Beginning from fast and easy sample preparation using the serial section Tape collector (ATUMTOME) or the advanced micromanipulator (ASH) for correlative Array tomomgraphy different imaging modalities can be involved. A joined software and hardware approach allows gentle, flexible and fast image acquisition and the generation of the 3D volumes of the region of interest.



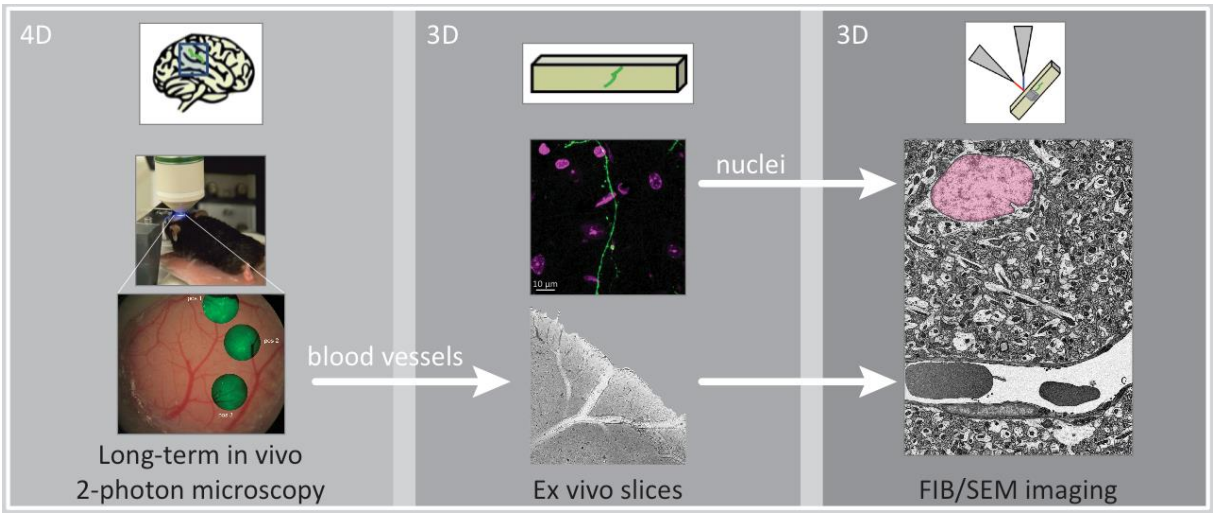
11:15 - 11:45 **Recent advances in 3D correlative microscopy for life sciences**

Ann-Katrin Unger, Carl Zeiss Microscopy, Oberkochen, Germany

“By the help of microscopes, there is nothing so small, as to escape our inquiry; hence there is a new visible world discovered to the understanding”. The words of Robert Hooke reflect in a perfect sense the developments science has made to understand the subcellular world in its most specific details.

Developments in microscopy in the centuries after Robert Hooke have brought light into different new fascinating worlds of Life. The rapid progression of new innovative techniques in light microscopy has opened new horizons but also to much bigger extent new inquiries. New routes have opened to enter the 3D subcellular space with the use of scanning electron microscopes. Serial blockface and focused ion beam techniques have opened a new dimension in imaging large 3D volumes. Latest scientific approaches tend to connect the world of light and electron microscopy. Correlative microscopy opens a completely new horizon in microscopy which leads once again into a new world of scientific understanding. An overview about new approaches connecting the two worlds will be presented.

Starting from living samples we want to show new workflows to enter the 3D correlative ultrastructural space and relocate the dynamics in a ultrastructural correlative context. Since 170 years Carl Zeiss opens new perspectives in resolution to further enlight our understanding of Life.



Demos:

Please go to your demo(s) per the timetable below. Please note that the LSM 880, EVO and Xradia Versa are in different buildings. Please refer to the maps for information.

2nd May 2018

	St Michael's Building 5th floor		Burnaby Building B2.26		Anglesea Building 0.22		St Michael's Building SM 0.10	
	Nick Sergent		Joe Dunlop, Steve Furzeland, Jacob Hoster		Alex Kao, Matt Andrew, John Flynn		Katherine Lau, Paul Wetton	
Time	**LSM Airyscan (topic)**	**Attendees**	**SEM (topic)**	**Attendees**	**XRM (topic)**	**Attendees**	**LSM (topic)**	**Attendees**
13:15 – 13:45			**Geosciences**	Prof Craig Storey	**Life sciences**	Dr David Gibbs	**Material sciences**	Katerina Karali
				Ines Pereira		Prof Gordon Blunn		Nikolay Zhelev
				Dr Philip Benson		Julia Wells		Dr Ria Mitchell
				Ricardo Thomas		Dr Lin Wang		Cherie Morrison
				Sean Feist		Dr Helen Fillmore		Dr Hari Arora
				Emily Butcher		Antonio de Grazia		Dr Esmeil Namvar
				Glenn Chapman		Monique Marylin		Peter Davies
								Rachel Boardman
14:00 – 14:30	**Life sciences**	Giulia Consolandi	**Life sciences**	Dr Robin Rumney	**Material sciences**	Peter Davies		
		Dr Sepinoud Firouzmand		Gillian Whitaker		Vili Grigorova		
		Prof Gordon Blunn		Dr Anne Gesell		Nicola Thomas		
		Julia Wells		Lowrie Vayro		Dr Hari Arora		
		Dr James Smith		Francesca Noyce		James Russell		
				Dr Helen Fillmore		Rachel Boardman		
				Dr Lin Wang				
14:45 – 15:15	**Life sciences**	Dr Helen Fillmore	**Life sciences**	Dr James Smith	**Material sciences**	Dr Antigoni Barouni		
		Francesca Noyce		Dr Sepinoud Firouzmand		Cherrie Morrison		
		Dr Lin Wang		Julia Wells		Dr Ria Mitchell		
		Dr Gianluca Tozzi		Prof Gordon Blunn		Nikolay Zhelev		
				Giulia Consolandi		Dr Aikaterini Lalatsa		
				Antonio de Grazia		Dr Marta Roldo		
				Dr Katherine Brown				
15:30 – 16:00	**Life sciences**	Antonio de Grazie	**Material sci**	Peter Davies	**Life sciences**	Lowrie Vayro		
		Jill Rice		Nikolay Zhelev		Dr Anne Gesell		
		Monique Marylin		Nicola Thomas		Amber Collings		
		Dr Aikaterini Lalatsa		Dr Ria Mitchell		Dr Katherine Brown		
		Dr Marta Roldo		Katerina Karali		Dr James Smith		
				James Russell				
				Hari Arora				
16:15 – 16:45			**Material sci**	Vili Grigorova	**Geosciences**	Natalia Walasek		
				Gemma Lawson		Dr Tony Butcher		
				Hugo Moreira		Prof Craig Storey		
				Jack Muchemi		Emily Butcher		
				Dr Catherine Mottram		Ines Pereira		
				Cherie Morrison		Dr Philip Benson		
				Stephanie Griffiths		Dr James Darling		
						Ricardo Tomas		

3rd May 2018

			Michael Swan Building 5th floor		Burnaby Building BB2.26
Time			1 880 / LM-LM live cells to fixed c		EVO MA / LM-SEM material sci
13:15-13:45	Demonstrator: Nick Sergent	Prof Darek Gorecki		Demonstrator: Stefanie Freitag, Ann-Katrin Unger	Peter Davies
		Dr Anne Gesell			Dr Gianluca Tozzi
		Dr Robin Rumney			Dr Eesmaeil Namvar
		Dr Lin Wang			Prof Phil Withers
		Dr James Smith			Dr Hari Arora
					Dr Aikaterini Lalatsa
					Nikolay Zhelev
13:45-14:15	LSM 880 / LM-LM live cells to fixed cells				EVO MA / LM-SEM material sci
	Demonstrator: Nick Sergent	Julia Wells		Demonstrator: Stefanie Freitag, Ann-Katrin Unger	Nicola Thomas
		Dr Helen Fillmore			Dr Ria Mitchell
		Francesca Noyce			Dr Antigoni Barouni
		Lowrie Vayro			Vili Grigorova
		Dr Mohsen Sefi			James Russell
					Rachel Boardman
					Sam Kersley
14:30-15:00	LSM 880 / geo sample demo				] MA / LM-SEM and XRM-SEM life
	Demonstrator: Nick Sergent, Matt Andrew	Dr Sabine Wulf		Demonstrator: Ann-Katrin Unger, Steve Furzeland	Dr Zhengyi Yang
					Julia Wells
					Dr Anne Gesell
					Dr Maria Hariolaki
					Dr Lin Wang
					Amber Collings
					Dr Katherine Brown
					Dr Helen Fillmore
15:00-15:30					] MA / LM-SEM and XRM-SEM life
				Demonstrator: Ann-Katrin Unger, Steve Furzeland	Dr Gianluca Tozzi
					Francesca Noyce
					Dr Alex Kao
					Dr Robin Rumney
					Prof Darek Gorecki
					Dr James Smith
					Antonio de Grazia
					Lowrie Vayro
15:45-16:15					] MA / LM-SEM and XRM-SEM geo
	Demonstrator: Nick Sergent	Antonio de Grazia		Demonstrator: Matt Andrew	Dr James Darling
		Jill Rice			Joseph Dunlop
		Monique Marylin			James Coyne
		Dr Aikaterini Lalatsa			Ricardo Tomas
		Dr Marta Roldo			Dr Tony Butcher
					Ines Pereira
16:15-16:45					] MA / LM-SEM and XRM-SEM geo
				Demonstrator: Matt Andrew	Dr Philip Benson
					Dr Sabine Wulf
					Sean Feist

Feedback:

We hope you find the workshop useful.

We appreciate your comments. Please feel free to send your feedback to Dr Katherine Lau, katherine.lau@zeiss.com.