Title: "The Open Microscopy Environment: Open Image Informatics for the Life and Biomedical Sciences"
OME Consortium

Abstract: "Despite significant advances in biological imaging and analysis, major informatics challenges remain unsolved: file formats are proprietary, storage and analysis facilities are lacking, as are standards for sharing image data and results. The need to share large image sets using secure, cross-platform APIs, and for scalable applications for processing and visualisation, are shared across the life and biomedical sciences. The Open Microscopy Environment (OME) is an open-source software framework developed to address these challenges. OME tools include - an open data model for multi-dimensional imaging (OME Data Model); an open file format (OME-TIFF) and library (Bio-Formats) enabling free access to images (5D+) written in more than 145 formats from many imaging domains; and a data management server (OMERO)."
Let me know if you need anything else to register - there don't seem to be any guidelines on abstract length but I tried pasting this into the form and it didn't object so I'm assuming the length is fine. It's an edited version of the short SPIE abstract btw, with reference to FITS and astronomy removed.

Synaptobrevin-II trafficking is impaired in neurons expressing a synaptophysin mutation associated with a severe neurodevelopmental disorder

Callista Harper, Grazia M.S. Mancini & Michael A. Cousin

Abstract

Following exocytosis, synaptic vesicles (SVs) need to be reformed with the correct complement of proteins in the correct stoichiometry to ensure continued neurotransmission. Synaptophysin is a highly abundant, integral SV protein necessary for the efficient retrieval of the SV SNARE protein, synaptobrevin II (sybII). However the molecular mechanism underpinning synaptophysin-dependent sybII retrieval is still unclear. Human synaptophysin mutations identified in patients with X-linked intellectual disability (XLID) cannot support efficient sybII retrieval when expressed in neuronal culture. Thus dysfunctional sybII retrieval may underlie specific forms of XLID, and specific human synaptophysin mutations may inform studies regarding its mechanism. We recently identified a male patient with severe ID, hypotonia, epilepsy and callosal agenesis who has a point mutation in the juxtamembrane region of synaptophysin. The transmembrane domain is proposed to interact with sybII, suggesting this mutant may not support sybII retrieval. This mutation had no effect on the retrieval of synaptophysin tagged with a genetically-encoded pH-sensitive reporter (pHluorin) in synaptophysin knockout hippocampal cultures. This suggested this mutant has no global effect on SV endocytosis, a hypothesis confirmed when retrieval of the SV glutamate transporter was examined. However neurons expressing this mutant showed impaired sybII retrieval, similar to that observed in synaptophysin knockout neurons. Interestingly this impairment did not result in an increased surface stranding of sybII at the plasma membrane that is usually observed in synaptophysin knockout cultures. This novel human synaptophysin mutation has revealed a kinetic defect

in sybII retrieval, without affecting SV endocytosis. Furthermore it suggests that this mutation significantly impairs sybII binding and may be the first full-length synaptophysin mutant with altered sybII affinity.

Real-time and non-invasive measurements of cell mechanical behaviour with optical coherence phase microscopy

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BACKGROUND: There is an unmet need in tissue engineering for non-invasive, label-free monitoring of cell mechanical behaviour in their physiological environment[1]. We demonstrate that optical coherence phase microscopy (OCPM) can map relative cell mechanical properties in monolayers and 3D systems under cyclic stress.

METHODS: An OCPM system was developed around a commercial spectrometer. The custom scanning head can operate in an ad-hoc mode allowing the collection of varying phase over time, and in depth into the sample, with increased phase stability[2].

Cyclic stress was applied non-destructively and non-invasively to breast cancer cells (MCF-7) and mouse fibroblasts (3T3) within a microfluidic chip by a microfluidic pump.

A 4D data cube was captured with a frequency of 1.2kHz to sample varying phase over time which was converted to displacement with a custom designed set of algorithms.

RESULTS: Cyclic stress was successfully applied directly to cells, and corresponding displacement was recorded in real-time at the nanometre scale for each pixel of the cell. Change in amplitude and/or frequency of the stimuli was translated to corresponding cell response. Differences were observed in relative strain rates between the cell lines under investigation.

DISCUSSION & CONCLUSIONS: We have described a new method to monitor cell response to cyclic hydrostatic pressure in real-time and non-destructively. This can be directly related to the biomechanical properties of cells.

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[1] A. Conway and D.V. Schaffer (2012) *Stem Cell Research and Therapy* **3**:50

[2] C. Holmes, M. Tabrizian, and P. O. Bagnaninchi (2015) *J Tissue Eng Regen Med* **9**(5): 641- 45.

Visualising osteoclast ruffled border formation using electron tomography

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Osteoclasts are large multinucleated cells responsible for bone resorption and a vital component of bone remodelling. Following activation, osteoclasts attach onto the bone surface and forms distinct plasma membrane domains. The ruffled border (RB) domain forms at the bone/cell interface and is indispensable for bone resorption. Here, osteolytic enzymes are released onto the bone surface and degraded bone material is endocytosed. The RB is a highly convoluted and complex membrane consisting of many densely packed cytoplasmic projections, but it is not known how it forms or where the membrane comes from. We aimed to investigate RB formation using TEM (transmission electron microscopy) and electron tomography. Rabbit osteoclasts were cultured on dentine discs and treated with calcitonin, a potent inhibitor of osteoclast resorption that causes the RB to disappear. Following treatment, the calcitonin was washed out, replaced with fresh medium and the cells were allowed to recover. The samples were processed for TEM at different timepoints following calcitonin washout to capture the stages of RB reformation. Using TEM we observed that immediately following calcitonin treatment, the RB was abolished and the osteoclast cytoplasm became highly vacuolised. 80 minutes post-washout, vacuolisation decreased and an intracellular membrane complex formed near the bone surface. By 120 minutes, channels appeared to connect the complex with the bone-facing plasma membrane. After 120 minutes, more channels began to appear eventually forming the distinct cytoplasmic projections of a mature RB. Electron tomography was used to visualise the 3D anatomy of these structures and their relationship with neighbouring organelles.

NEUBIAS - A New Network of Bioimage Analysts to Advance Life Science Imaging

Dr. Graeme Ball, Image Analyst, Dundee Imaging Facility

NEUBIAS is a new EU COST action to establish a network and provide a stronger identity for BioImage Analysts, to play a well-defined role in bridging the gap between Image Analysis technology and Life Sciences. In addition building a BioImage Analyst network and organizing meetings, this COST action will deliver open publications, image analysis webtools and training; and there will be funding for Short Term Scientific Missions involving exchange visits between researchers involved in this COST action.

Nanoscopic localization of the components of the origin recognition complex during *Trypanosome brucei* cell cycle

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Abstract

Origin recognition complex (ORC) architecture has only been explored in the opisthokont, a supergroup of eukaryotes that includes yeast and mammals, with little work in protists. Kinetoplastida is a protozoa order containing several important human parasites, such as *Trypanosoma brucei*. Genome sequencing of *T. brucei* identified only a single ORC-related protein – TbORC1/CDC6. A number of interacting factors have been identified, though highly diverged in sequence from canonical ORC subunits; and none has been shown to have a role in replication. To compare the localization pattern of TbORC1/CDC6 with these factors (TbORC1b, TbORC4, TbMCM3), each protein was tagged at its endogenous locus. The ratio and morphology of the nucleus and kinetoplast delineates the cell cycle stage: 1N1K for G1 phase; 1N1eK (elongated - replicating - but not yet divided) for S phase; 1N2K cells for S-G2 phase; and 2N2K cells for postmitosis. Using Super-resolution structured illumination microscopy (SR-SIM) we characterized the distribution pattern and compared them with replicating DNA, which was detected with EdU labeling. Images confirmed the cell cycle dependence of TbORC1B nuclear localization and revealed a comparable pattern to TbORC1/CDC6 and TbORC4 in replicating cells, with signal again seen throughout the nucleus in a similarly large number of puncta and with some overlap with EdU. TbMCM3 signal was more abundant and more homogenous in the nucleus that any of the others factors, and displayed little obvious variation in the different cell cycle stages, indicating that each of TbORC1/CDC6, TbORC4 and TbORC1B display distinct sub-nuclear localization to that of TbMCM3.

Multispectral transgenic mice and zebrafish to visualise vascular regeneration

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In 1997, the observation of a circulating endothelial progenitor cell (EPC) capable of postnatal neovasculogenesis challenged long-standing beliefs regarding the body's innate capacity for vascular regeneration. However, little is known about EPC including their origin, *in situ* niche residence, and mechanisms through which they maintain structural and functional integrity of the vessel wall. The development of 'Brainbow' technology provides a means of understanding clonal dynamics of healthy and regenerating tissues at the single cell level. We used endothelial-specific multispectral transgenic mice and zebrafish to investigate the hypothesis that EPC reside within a vessel wall niche and drive vascular cell turnover in the heart through clonal expansion to form differentiated progeny.

The *Pdgfb-iCreER^{T2}-R26R-Brainbow2.1* mouse has stochastic expression of green, red, cyan or yellow fluorescent proteins specifically in endothelial cells. Upon Cre-recombination, endothelial cells express a unique spectral barcode, inherited by their progeny following division. This model permits 3-dimensional visualisation of the clonal architecture of the vessel wall during homeostasis, and quantification of EPC proliferative kinetics and their contribution to cardiovascular regeneration e.g. following myocardial infarction. We report the generation of *Kdrl-CreER^{T2}-ubi-Brainbow* zebrafish, a unique model system for intravital visualisation of vascular clonal growth in homeostasis and in response to injury. Our preliminary analyses using these models indicate that endothelial cells in the heart are not terminally differentiated and can undergo expansion to form clonal progeny. Further evaluation of the mechanisms through which vessel wall EPC maintain vascular integrity will facilitate significant advances in regenerative medicine for the treatment of ischaemic disease.

Word limit: 250/250 words

The role of LC3 and autophagy in bone resorption by osteoclasts

Anh Tran, Fraser Coxon, Emma McDermott, Ian Ganley, Paul Odgren, Jennifer Martinez, Douglas Green, Miep Helfrich

The autophagy protein LC3 is necessary for bone resorption by osteoclasts, although it has been suggested that this may be through a novel, autophagy-independent process, by promoting lysosomal fusion at the ruffled border (RB). This process would be analogous to LC3-associated phagocytosis (LAP), in which LC3 is acquired by phagosomes through an autophagy-independent process, and controls phagosome maturation by promoting fusion with lysosomes. We have investigated this possibility by using novel mouse models for monitoring LC3 localisation and a model in which autophagy is selectively ablated. In vitro, LC3 localises to the RB in 30% of actively resorbing osteoclasts. Most of these osteoclasts are at an early stage of RB formation; LC3 did not localise to the RB in osteoclasts associated with extensive resorption pits. We further investigated this by using an autophagy-deficient mouse model in which FIP200 is deleted in the myeloid lineage; FIP200 is essential for autophagy, but is not required for LAP. FIP200 null osteoclasts were able to target LC3 to the RB and resorb dentine despite impaired autophagy, indicating that a process similar to LAP, rather than autophagy, controls RB formation. The Rab7 effector Plekhm1 may also be involved in this process; mutations in Plekhm1 cause osteopetrosis, due to the failure of osteoclasts to form RBs and resorb bone. Furthermore, Plekhm1 binds to LC3 and plays a role in autophagosome-lysosome fusion by bridging the membranes of these two vesicle types. Surprisingly, we found little difference in autophagy in osteoclasts derived from patients with Plekhm1 mutations or in mice lacking Plekhm1, compared to family or littermate controls, respectively. By contrast, Plekhm1-deficient osteoclasts exhibited defective RBs and profoundly impaired resorptive activity. These data suggest that Plekhm1 may play a redundant role in autophagy in osteoclasts, but is essential for lysosomal fusion at the RB through interactions with LC3.

Title: Developing FRET based reporters to study spatio-temporal regulation of the PI 3-Kinase regulated Rac1 activator, SWAP-70

Abstract

Class I PI 3-Kinase (PI3K) and PTEN influence cell behavior through a large and diverse set of proteins which are able to bind selectively to the PI3K lipid product, PIP3. These PIP3-binding proteins include a set of Guanine Nucleotide Exchange Factors (GEFs) which are able to activate the small GTPase Rac1. We show that Rac1 but not AKT regulates glioma cell invasion and epithelial cell architecture downstream of PTEN. To understand further the underlying mechanism, we have developed FRET based biosensors to reveal conformational changes in the Rac1 specific guanine nucleotide exchange factor (GEF), SWAP-70. This SWAP-70 reporter translocates onto cell membranes in response to PI3K activation and also shows a higher FRET signal at the leading edge of migrating U87MG cell which is unrelated to the concentration of the reporter and absent from controls. We are investigating the hypothesis that these FRET changes indicate localized higher SWAP-70 GEF activity towards Rac1.

Three-dimensional reconstruction of multi-planar standing wave red blood cell images

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Multi-planar standing wave microscopy with the simple addition of a mirror below the specimen allows the generation of multiple super-resolved planes of illumination within a specimen. These multiple planes of light are spaced at regular intervals of $\lambda/2n$ and provide an axial resolution of $\lambda/4n$ [1]. When these planes of illumination intersect a fluorescently labelled specimen the resulting image can reveal a contour map of the surface geometry of the specimen by extracting the three dimensional information encoded in the image [2]. The work of Amor et al. demonstrated that by imaging a fluorescently stained red blood cell membrane using the standing wave method resulted in a standing wave pattern at points where the antinodal planes intersect the membrane of the red blood cell specimen. Using the resultant red blood cell standing wave images we have been able to generate a 3D reconstruction of the bi-concave section of a red blood cell using MATLAB®.

[1] B. Bailey, D. L. Farkas, D. L. Taylor, and F. Lanni, "Enhancement of axial resolution in fluorescence microscopy by standing-wave excitation.," *Nature*, vol. 366, no. 6450, pp. 44–8, 1993.

[2] R. Amor, S. Mahajan, W. B. Amos, and G. McConnell, "Standing-wave-excited multiplanar fluorescence in a laser scanning microscope reveals 3D information on red blood cells.," *Sci. Rep.*, vol. 4, p. 7359, 2014.

Multimodal, multiphoton platform for label-free nonlinear optical imaging

The identification of different molecular species on the microscopic scale is still a considerable challenge in many areas of biology. Typical optical analysis of biological systems is invasive and often relies on synthetic or genetic fluorescent labels, to provide contrast which can be far from ideal for imaging cells in their *in vivo* state. To address the need for minimally-invasive, high-speed, label-free chemical imaging, we have developed a multimodal nonlinear optical platform that combines coherent anti-Stokes Raman scattering (CARS), stimulated Raman spectroscopy (SRS), two photon excitation fluorescence (TPEF), second harmonic generation (SHG) and sum-frequency generation (SFG). This hybrid microscope offers an extremely wide range of complementary information on biological systems with deeper imaging and reduced photo-chemical damage compared to conventional fluorescence microscopes. In addition, we can also combine this with atomic force microscopy (AFM) to measure the surface topography and to map the local mechanical and chemical properties of live cells, tissue and biomolecules with approximately 1 nm resolution.

These microscopies are all available for use by students and researchers at the BioImaging Small Research Facility in the Institute for BioEngineering at the University of Edinburgh. If you wish to learn more about these techniques, please come see this poster or contact our unit at bioimaging@ed.ac.uk.