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Meeting Report – Imaging in Cell Biology: Where Next?

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The Company of Biologists Workshop entitled 'Imaging in Cell Biology: Where Next?' was held in October 2012 at Cumberland Lodge in Windsor, UK. The meeting was a forum for leaders in different areas of single-molecule analysis, high- and super-resolution imaging and data processing both at cellular level and within intact tissues, with an emphasis on cross-fertilisation between fields. Here, we review the proceedings at this meeting and highlight the issues raised during a discussion on the future of imaging in cell biology.

Held in the historic surroundings of Cumberland Lodge, this workshop provided a unique opportunity for a small group of 30 scientists to communicate their work and discuss the future direction of imaging technology in cell biology. Leaders in the development of cutting-edge imaging methodologies constituted the bulk of the attendees. An emphasis was placed on highlighting the current challenges associated with each of these different methodologies and the need for a unified approach to imaging within the life sciences. Also present were several early-career scientists, who were provided with an excellent opportunity to mingle with leaders in their field.

Proceedings commenced on Sunday evening with dinner, introductions from each of the attendees and a plenary lecture from John Sedat, whose team at University of California, San Francisco (UCSF, CA) developed the technology behind the super-resolution OMX microscope. He demonstrated, using a yeast model, that the exposure levels commonly used experimentally can lead to an arrest in the cell cycle. By contrast, a decrease of four orders of magnitude in excitation intensity allowed imaging of cells without impacting on their ability to divide during and after the acquisition process. However, such low exposure results in a low signal-to-noise ratio. He introduced a de-noising procedure based on identifying regions of similar intensity in three (space), four (time) or five (wavelength) dimensions that allows the cell to be successfully distinguished. By exploiting the OMX, a microscope platform that enables sub-second multicolour super-resolution imaging through structured illumination, coupled to low-dose illumination and this novel de-noising procedure, he demonstrated that it is possible to image sub-cellular processes at a high frame rate while avoiding photo-toxic effects.

The following two days featured a total of 15 talks, divided into four manageable sessions, allowing plenty of time and energy for discussion over (a rather elaborate) dinner and drinks each evening. Jennifer Lippincott-Schwartz (National Institutes of Health, NIH, MD) began on Monday morning, discussing a variety of imaging techniques applicable to the study of the spatiotemporal dynamics of molecules in cells. These techniques include photo-activated localization microscopy (PALM), single-particle tracking PALM (sptPALM) and blinking/bleaching-assisted localisation microscopy (BALM). By exploiting the blinking and bleaching characteristics of conventional fluorescent probes, BALM permits point-localisation super-resolution imaging without the need for photo-activatable or photo-switchable probes. It is possible to obtain localisation precision on the order of tens of nanometres with such a technique.

Following a brief pause for a group photograph, the second stand-out talk of Monday morning was delivered by Ernst Stelzer (Goethe University, Frankfurt, Germany), who presented high-resolution images of large, dispersed biological systems acquired through light-sheet-based fluorescence microscopy (LSFM). Some advantages of this technology – of which there are a number of implementations, such as selective plane illumination microscopy (SPIM) – include a high signal-to-noise ratio, low level of bleaching (three orders of magnitude lower light intensity relative to confocal microscopy) and imaging speed (~1 stack/second). However, one must consider that this approach generates massive volumes of data: ~72 terabytes/day. The talk concluded with an impressive demonstration of 3D-live imaging of an *Arabidopsis thaliana* growing root.

Later on Monday afternoon, we heard Graham Knott (École Polytechnique Fédérale de Lausanne, EPFL, Switzerland) speak about analysing the ultra-structure of adult brain neuronal networks using scanning electron microscopy and focussed ion milling. Scanning electron microscopy scans the top of a block of a fixed sample embedded in resin. To allow for a sequential imaging of several sections of the same block, the face is milled every 5 nm with a focussed Ga^+ beam and an image acquired after each milling step. Using specialised software packages, the images are aligned and stacked to create a 3D volume that can be semi-automatically segmented using ilastik (www.ilastik.org), revealing the neuronal organisation. This volume has a resolution close to 5 nm, allowing the visualisation of complex membrane formations as well as single microtubules, all included in a large volume containing whole neurons.

Although these imaging techniques are primarily used in cultured cells, Michiyuki Matsuda discussed the ability to express fluorescence resonance energy transfer (FRET) biosensors *in vivo*. FRET usually requires the expression of two proteins tagged with fluorescent proteins, but Matsuda's laboratory developed probes for intramolecular FRET that fuse two proteins. These FRET biosensors enable the activation of a number of proteins, such as small GTPases, to be monitored in living tissue.

Further perspectives on the field of super-resolution fluorescence microscopy were provided on Tuesday morning by Markus Sauer (Universität Würzburg, Germany), whose group developed the direct stochastic optical reconstruction microscopy (dSTORM) technique. Their freely available rapidSTORM software (www.super-resolution.de) can process 2D and 3D data sets derived from dSTORM or other point-localization microscopy data. He pointed out that the size of an antibody label has become an issue in super-resolution approaches: an electron microscope measures the thickness of a microtubule as 25 nm; however, it is measured as 35–40 nm by

dSTORM. Moreover, he introduced his recent work on 3D whole-cell super-resolution imaging, SPIM-dSTORM, live-cell dual-colour dSTORM and 3D nanomaps of synaptic proteins and 'super-super-resolution': correlative electron and dSTORM microscopy. Markus Sauer also revealed his conceptual 70-nm-resolution microscope that can be built for less than €10,000, and kindly offered to share the know-how with the scientific community on his website.

Ilan Davis (University of Oxford) then spoke about how his laboratory is employing super-resolution microscopy to investigate the composition of P bodies, the RNA 'factories' in *Drosophila melanogaster*. Super-fast microscopy is being used, in conjunction with bespoke software (www.ParticleStats.com), to understand how RNAs are transported across long distances to specific locations in *Drosophila* oocytes. His laboratory intends to combine super-resolution and super-fast microscopy in order to understand biological problems at the molecular level *in vivo*. For this, they intend to build a new microscope based on John Sedat's design, in which structural illumination is combined with single-plane illumination microscopy to create a microscope that can provide super-resolution both in space and time.

Although all these novel approaches to light microscopy are providing tremendous insight into biological processes, reliable quantitative analysis is essential to ensure robust data and reproducibility of results. A key component, therefore, in the implementation of new imaging modalities is open-source bioimage informatics. The developmental biologist Pavel Tomancak (Max Planck Institute for Molecular Cell Biology and Genetics, MPI-CBG, Dresden, Germany) shared his perspectives on this very subject. He drew attention to the frequent lack of acknowledgement bestowed upon scientific software developers, as scientists often regard writing computer programs as a non-scientific activity. He called for the necessary changes to be made in funding and long-term career-path development for bioimage informaticians. Discussing his laboratory's current contributions to the SPIM field, he unveiled the 'SPIM in a suitcase' concept, with the promise that the results of the OpenSPIM project will be available to the scientific community.

Following on from Pavel Tomancak's talk, the final sessions of the workshop commenced with Daniel Gerlich (ETH Zürich, Switzerland) discussing his laboratory's development of the CellCognition platform (http://cellcognition.org), a computational framework for annotating cellular dynamics. His talk focussed on attempts to develop unsupervised learning methods, on the basis of constrained combinatorial clustering, Gaussian mixture modelling and hidden Markov models, for the analysis and labelling of cellular phenotypes. Classification by unsupervised learning was demonstrated to yield labels that closely matched user annotated data (~87% accurate).

The final talk of the workshop was given by Kurt Anderson (Beatson Institute, Glasgow, UK), who kindly stepped in following a last-minute cancellation. He described the difficulties associated with studying tumour-cell migration *in vivo*, in that the migration of cells from tumours occurs randomly over large scales of space and time. He also advised caution in interpreting the results of *in vitro* studies. Using fluorescence recovery after photobleaching (FRAP) dynamics at cellular junctions as an example, he emphasised the significant discrepancies that can emerge between experimental results *in vitro* and *in vivo*. With this in mind, Kurt Anderson proposed an experimental 'pipeline', whereby systems are initially studied *in vitro*, the experimental design is then progressively increased in complexity to mimic a gradual transition to *in vivo*, culminating in a pure *in vivo* study.

The workshop concluded with an open discussion on the future of imaging in cell biology. Among the issues raised was the lack of standards surrounding the submission of imaging data to journals and the frequent omission of details pertaining to image-analysis methods used. Furthermore, questions remain as to when it is acceptable for a reviewer to request additional imaging data – for example, when should it be considered (un)necessary for super-resolution analysis to be included in a study? Does it help to address any fundamental questions? Concern was also expressed regarding the lack of an imaging community, particularly in the UK, where the Euro-Bioimaging initiative (www.eurobioimaging.eu), for example, has failed to gain ground. The practicality of storing and sharing raw imaging data was also discussed. As there remains much to be done in addressing these questions, it was suggested that perhaps The Company of Biologists (and *Journal of Cell Science* in particular) lead the way in setting imaging standards in scientific publication.

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