Issue 95 / Summer 2014

PN Physiology News

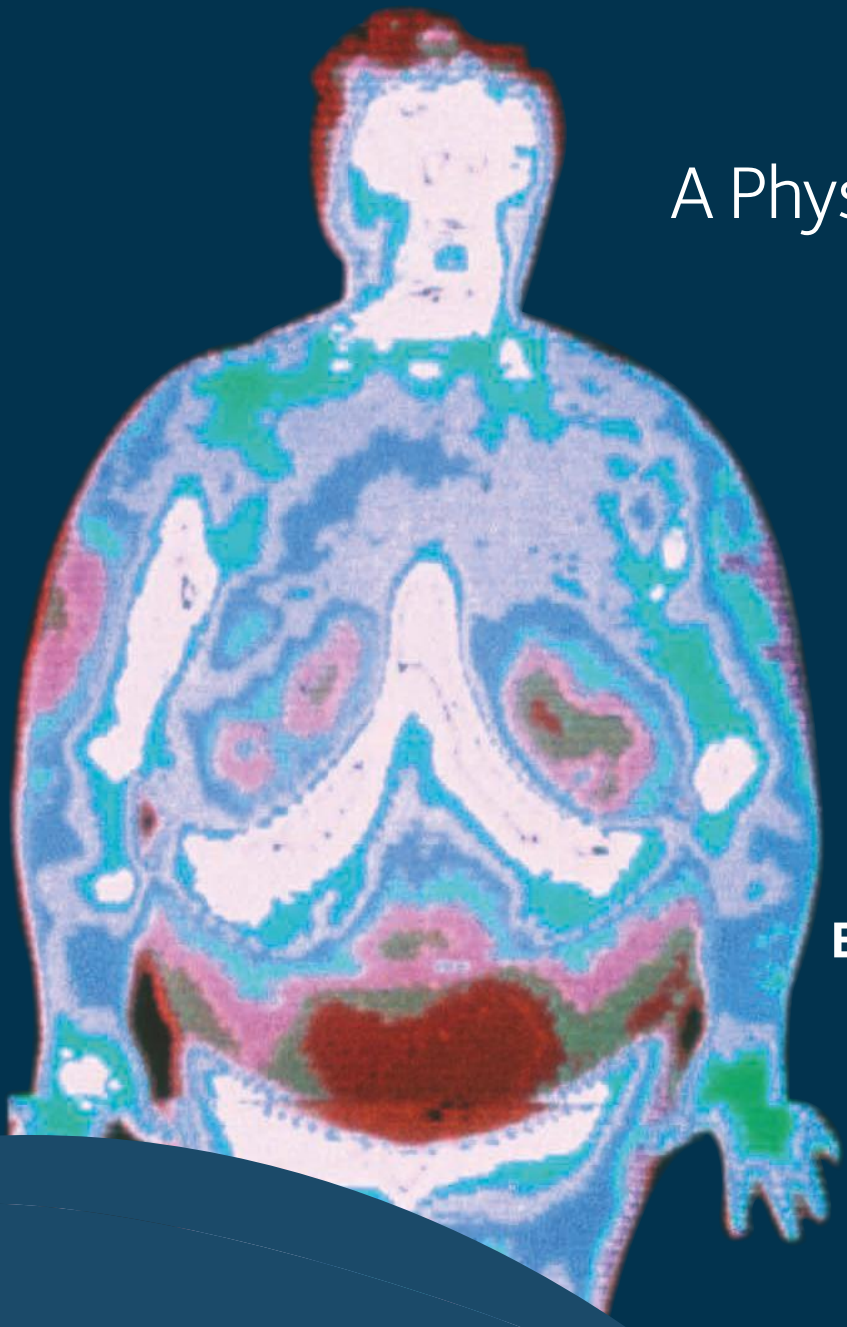
Being an artist and a scientist

Helium Ion Microscopy of the kidney

High content screening: It's all about numbers

Imaging special issue:
Seeing is believing

The Physiological Society



Obesity:
A Physiological Perspective

10–12 September 2014
Newcastle United Football Club
Newcastle upon Tyne, UK

Abstract submission
13 June to 11 July 2014

Early registration deadline
11 August 2014

Plenary lecturers

Steve Bloom (Imperial College London, UK)

Mark Walker (Newcastle University, UK)

John Blundell (University of Leeds, UK)

Giles Yeo (University of Cambridge, UK)

Roy Taylor (Newcastle University, UK)

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www.physoc.org/topicobesity

The Physiological Society
2014: Understanding Obesity

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Physiology 2015

6 - 8 July 2015

Motorpoint Arena Cardiff

Mary Ann Street, Cardiff, United Kingdom, CF10 2EQ

Registration Opens

1 January 2015

Abstract Submission

1 March - 31 March 2015

www.physiology2015.org

The Physiological Society

Physiology News

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Editorial

'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'
Robert Hooke, in *Micrographia*, 1665

Keith Siew

Guest Editor

It is my pleasure to welcome you, to this special imaging themed issue of *Physiology News*. Our aim is to introduce to both the expert and novice some of the exciting novel trends and emergent technologies in the field of Imaging, in particular what this plethora of new possibilities holds in store for physiological research.

Since the 1600s pioneers like Marcello Malpighi, the father of microscopic anatomy, utilised early microscopes to draw back the veil on the world hidden from the naked eye. Though it was not until the late 1800s that the optics breakthroughs of Ernst Abbe and developments in histological stains by Wissowzky, Golgi, Cajal and their ilk, would usher in a fundamentally new understanding of physiology, thereby cementing imaging as a cornerstone of our discipline.

In the midst of the 20th century, an explosion of new technologies led to a second revolution in biological imaging. For the first time cellular ultrastructure was revealed to us in exquisite detail with the electron microscope, the localisation and expression levels of specific proteins could be visualised by immunolabeling with conjugated antibodies, and diagnostic medicine was to be transformed by the birth of x-ray, ultrasound and magnetic resonance based imaging. As the century came to a close, the ability to track and image protein clusters in real-time and three dimensions took centre stage thanks to the discovery of fluorescent proteins and development of the confocal microscope.

Now over a decade into the post-genomic era, the time is ripe for the next great imaging revolution. Mechanical automation and computer assisted image analysis have seen microscopy become a truly quantitative technique (see high content screening microscopy on p28). Creative thinking has allowed us to overcome Abbe's resolution limit of light microscopy, gifting us with the ability to quantify and localise single molecules (see super-resolution microscopy on p24). The transition from electron to ion based microscopes has revealed yet greater detail in the ultra-anatomy of tissues (see Helium ion imaging on p32), while on the macro end of the spectrum it is now possible to render whole organs transparent to light (see CLARITY meeting report on p20) and with relatively simple DIY microscopes live specimens can be imaged *in toto* over the course of days if not weeks (see light sheet microscopy on p40).

In this age of big data there is an ever growing risk of losing valuable information to resource and storage space constraints, and even less attention is given to preserving the skill sets and legacy left to us by histologists past, a species that is fast dying out (see the preservation efforts at Glasgow on p10). Advances in technology can often outpace our rulebooks, for example the differences between image manipulation and acceptable practice can be many shades of grey (see imaging ethics on p9).

However we also have much cause for celebration, the myriad technologies have set a trend toward core imaging facilities at universities and presents many new career opportunities for future physiologists (see the member profile of a bio-imager/physiologist on p44). The internet has given rise to many open access and community developed projects which have become incredibly valuable resources for labs around the world or those wishing to acquire a new skill (see imaging tools and freebies on p12).

Human health has also benefited greatly from advances in imaging. In 1926 Werner Forssmann tested the first cardiac catheterisation on himself, an achievement that got him both fired from his job and a Nobel prize, and decades later angiography is still the gold standard though now surrounded by an arsenal of new imaging modalities (see adjunctive coronary imaging on p36).

Finally I leave you with the question: Are we effectively communicating our science to the public? Although arguably easier to reach the masses with the many forms of media available today, doing so in an engaging and accessible manner remains a challenge. Seeing is believing, and perhaps images hold the key to a second renaissance in which art and science are no longer mutually exclusive. I have had the pleasure to work with those like Mara Haseltine (**calamara.com**), who believe that harmonious use of science and art can communicate the importance of understanding the world around us and appreciating the inherent beauty in it (See the BHF Image awards on p14 and the fusion of artist and scientist on p16). To quote French mathematician, Henri Poincare, "The scientist does not study nature because it is useful to do so. He studies it because he takes pleasure in it; and he takes pleasure in it because it is beautiful".

We hope this issue will spark your imagination, perhaps bring insight into techniques you haven't encountered and give food for thought on future experiments... And, of course, the images make pretty viewing!

PhySoc Outreach Grant: Reading Science Slam

Gary Stephens & Alister McNeish

University of Reading, UK

A Physiological Society Outreach Grant helped to set up a gladiatorial battle between five young postgraduate scientists (Slammers) and one Faculty Outreach Facilitator (!) from the University of Reading on 22 March 2014. The inaugural Reading Science Slam played out in front of a 125-strong, sell-out public audience at Reading's South Street Arts Centre. The event was held together by the stern hand of local Physiological Society representative, Gary Stephens; there were no rules! Well, there were some rules:

- **MUST** be fun
- **MUST** be theatrical **AND** creative **AND** scientific
- **MUST NOT** use PowerPoint (it's not a lecture!)
- **MUST NOT** exceed 6 mins – the bell must be obeyed!

Ben Littlefield (a Faculty Outreach Facilitator) showed the Slammers how it was done, detailing the life of the unfortunate chemist, Carl Wilhelm Scheele, with added pyrotechnics, and teaching us to always use our senses in science. Ioannis Zoulias from the Department of Systems Engineering gave an impassioned talk entitled 'Senses and the Brain – How Do We Recognise Our Body?'. Who could top this energetic enthusiasm (and failure to remember the names of his volunteers)? Step forward Charlotte Hill from the Reading School of Pharmacy, a true (electro)physiologist, delivering 'How Cannabis Can Treat the Epileptic Brain' with only a couple of sticks as props. Charlotte had the audience under her control, instructing them how to behave like pyramidal neurones and their dominant inhibitory neighbours, the interneurons; luckily no drinks were spilled... Natalie Masento from Psychology presented 'The Hydration Exploration', asking how avoiding dehydration can increase our cognitive function. Natalie, a mild-mannered, forgetful PhD student by day, was transformed into 'Water Woman' once she got some water inside her, replete with increased cognitive powers and able to spot someone tweeting in the audience from a mile off!

Charlotte Hill (Reading Science Slam Winner)

The final two Slammers provided a change from life sciences. Meterology's Melody Sandells explained how size matters – for snowflakes, helping predict global snow cover and ice melt – with the use of ice, hammers and a blender. Mel battled the toll of the bell to finish her presentation. The final slammer was Emily Sonnex from Chemistry, who gave an expert demonstration of 'Chemistry in the Fight Against Crime', by revealing how to detect a fraudulent bank note. This required willing participants from the audience to do impressions of vibrating particles and their absorption spectra – the only question is – will Alister McNeish ever get his £20 note back?

Dr Stephens then explained the empirical, scientific voting system and, after a quick count, the scores were in. And the winner was Charlotte Hill, a Physiological Society Member no less, a well-deserved victory explaining a complex problem in a simple and enjoyable way. The public left happy; none of this could have happened without the support of The Physiological Society – we expect this event to run for many years to come! See the videos at **www.youtube.com/channel/UCPGv40B6QqHcwmvrReHJxlQ**

The inaugural Reading Science Slam. (L-R) Yentil Lim (Technical help), Natalie Masento (Slammer, 2nd prize), Gary Richens (Reading Rep), Melody Sandells (Slammer, Runner-Up), Dr Ben Littlefield (Slammer, Faculty Outreach Facilitator), Ioannis Zoulias (Front, Slammer, Runner-Up), Emily Sonnex (Slammer, Runner-Up), Dr Hilary Geoghegan (Organizer, Bell Ringer), Charlotte Hill (Front, Slammer, 1st Prize), Ann Westgarth (Main organiser, Community Relations Manager), Dr Gary Stephens (M.C), Gwynon Williams (Technical help)

Physiology 2014 workshop: Get into outreach!

13.15–14.15, Monday 30 June, QEII Conference Centre, London

Want to know more about outreach grants? Join us for our workshop 'Get into outreach' at Physiology 2014. Reading Science Slam organisers Gary Stephens and Alister McNeish, will join University of Leeds Society Representative Charlotte Haigh in telling us about what they got up to with their grants.

Annual General Meeting 2014

The Annual General Meeting (AGM) of The Physiological Society will be held on Tuesday 1 July 2014, 12.30–14.30 in the Caxton Lounge, The QEII Conference Centre, London, during The Society's annual meeting, Physiology 2014.

Ordinary and Honorary Members have the right to attend and vote at the AGM. Affiliates have the right to attend, but may not vote. Please note that you do not have to register for Physiology 2014 to attend the AGM. Questions can be submitted in advance online at **www.physoc.org/agm-2014-questions**

The Annual Review, Annual Report and Accounts, and agenda for the 2014 AGM, as well as minutes of last year's meeting, can be downloaded at **www.physoc.org/agm2014**. The Annual Report and Accounts, which received an unqualified audit opinion, should be consulted for a complete understanding of the financial affairs of The Society.

Vote now: 2014 Council elections

The Society has three vacancies on the Council of Trustees from July 2014.

Trustees are legally responsible for the overall governance, management and policy of The Society, ensuring that the charitable objects for which it has been set up are met. The Trustees are also the Directors of The Society.

Following a call for nominations earlier in the year, four members are standing for election:

- **Philip Irving Aaronson**
  Proposers: Jeremy PT Ward, Giovanni Mann, Chris Peers, Sergey Sminov and Rachel Tribe
- **Deborah Baines**
  Proposers: Douglas Bovell, Stuart Wilson, Matt Bailey, Emma Bake and Bonnie Blazer-Yost
- **David Eisner**
  Proposers: Jonathan Ashmore, Anne King, Godfrey Smith, Richard Vaughan-Jones and David Wyllie
- **Roger Christopher Thomas**
  Proposers: Christof Schwiening, Christopher Fry, William Colledge, Judy Harris and Jonathan Coles

All candidates have been proposed by five Ordinary or Honorary Members. The candidates' proposers, supporting statements and instructions to vote can be found at **www.physoc.org/council-trustees-election-2014**

All Ordinary and Honorary Members are encouraged to vote for the individuals they wish to fill these vacant roles. You may vote for up to three candidates. Voting closes at **noon on Thursday 26 June.**

Physiology Feed

Bringing you snippets of the latest intriguing research

Predicting facial features from DNA

Researchers have identified 24 single-nucleotide polymorphisms in 20 genes that are significantly associated with facial features and used this data to develop a program that can predict 3D facial structure on the basis of these markers.

DOI: 10.1038/nrg3727

Clostridium receptor found

This paper describes the discovery of the receptor responsible for smuggling the toxin of the gas gangrene and food poisoning bacterium *Clostridium perfringens* into the cell. The discovery should open the way for the development of an intervention to prevent the TpeL toxin from entering and killing the cell.

DOI: 10.1073/pnas.1323790111

Learning and memory protein identified

This study shows that the protein RGS7 works in concert with R7BP to regulate the signalling cascade that opens inhibitory channels known as GIRKs in the cell membrane. Knowing the identity of the critical modulator of GIRK channels may open therapeutic possibilities for neuropsychiatric conditions including drug addiction and Down's syndrome.

DOI: 10.7554/eLife.02053.001

Live from inside the cell

This paper describes a novel imaging technique that provides insights into the role of redox signalling and reactive oxygen species in living neurons, in real time. With this technique researchers have been able, for the first time, to study redox signal induction in response to neural damage, and to image spontaneous contractions of mitochondria that are accompanied by a rapid shift in the redox state of the organelle.

DOI: 10.1038/nm.3520

Printing 3D tumours

This paper describes a procedure for 3D printing research-grade models of cervical cancer tumours. This living model will give cancer researchers a better look at how tumours behave and a more accurate measure of how they respond to treatment.

DOI: 10.1088/1758-5082/6/3/035001

continues overleaf

Physiology Feed

Bringing you snippets of the latest intriguing research

Epigenetic insights into development in the womb

Genes inherited from the parents account for just 25 per cent of the epigenetic variation between babies, with the remaining 75 per cent best explained by the interaction of genetic differences and the prenatal environment, according to this study – the first to use DNA itself to estimate how big a role the prenatal environment plays in a child's development and how much is simply genetically determined.

DOI: 10.1101/gr.171439.113

Amino acid 'shield' regulates pacemakers of brain and heart

T-type channels in the pond snail can shift from using calcium ions to using sodium ions to generate their electrical signals thanks to an outer shield of amino acids called a turret situated above the channel's entrance. Understanding how these shields work will help to develop a new class of drugs for treating epilepsy, cardiovascular disease and cancer.

DOI: 10.1074/jbc.M114.551473

How our cells fight off rogue DNA

This study looks at an enzyme called APOBEC3A, which causes mutations within rogue DNA fragments, LINE-1 retrotransposons, preventing their invading other areas of DNA. The findings not only help explain a mystery of the body – they also may help in the development of cancer drugs.

DOI: 10.7554/eLife.02008

Old arteries appear young again with novel antioxidant

According to a new study in *The Journal of Physiology*, carried out at the University of Colorado, an antioxidant that targets mitochondria may be able to reverse some of the negative effects of ageing on arteries by affecting the endothelium, hence reducing the risk of heart disease.

DOI: 10.1113/jphysiol.2013.268680

If you spot some interesting research that you'd like to share with your fellow Members, please send it to us at **magazine@physoc.org**

Policy Corner

Come and meet us at Physiology 2014

The Policy Committee will have a stand at Physiology 2014, which is taking place at the QEII conference centre, London, from 30 June to 2 July. Whether you have an interest in our Engaging with Parliamentarians scheme (**www.physoc.org/engaging-parliamentarians**), want to raise a specific policy issue, or would just like to find out more about our activities, please come along for a chat.

Society signs Concordat on Openness on Animal Research

The Society is delighted to announce that it has joined fellow learned societies, funding organisations, universities and industry bodies in signing the Concordat on Openness on Animal Research. Over the past 12 months The Society, alongside a number of other stakeholders, has been heavily involved in the development of the Concordat on Openness on Animal Research. Following a public consultation, the Concordat was finalised in early 2014. It calls for signatories to be more open about the use of animals in scientific research and highlights practical steps that stakeholders can take to achieve this.

Society supports the Wellcome Trust in lobbying the EU parliament

In advance of a debate in the EU Parliament on the use of EU funding for research that involves the destruction of embryos, The Society joined 30 other organisations in signing a statement in support of stem cell and reproductive health research. The debate was triggered by an EU wide petition, which received over 1.7 million signatures, calling on a ban on EU funding for research that 'presuppose the destruction of human embryos'. The statement, co-ordinated by the Wellcome Trust, was circulated to MEPs and relevant Directorate-Generals in the European Commission ahead of the debate. Following the debate the EU Commission was due to provide its response to the petition by 28 May.

Parliamentary scientific poster competition

SET for Britain bioscience gold medal winner Graham Robertson, University of Strathclyde

In March, the annual SET for Britain parliamentary scientific poster competition took place, with The Society continuing its sponsorship of the event. The competition provides a unique platform to inform the significant number of MPs who attend the event about the cutting edge science taking place in the UK. The Society would like to thank Members Sarah Hall, Rachel Tribe and Sue Wray for helping in the difficult job of judging the 60 bioscience posters on display.

Status and valuation of HE teaching workshop

The Society, along with the Academy of Medical Sciences, the Society of Biology and the Heads of UK Biosciences hosted a workshop on the status and valuation of teaching in Higher Education. The workshop brought together key stakeholders from over 50 HEIs and affiliated organisations to share best practice and processes. Delegates heard perspectives from across the sector and examples of best practice before breaking out to discuss different processes for evaluating teaching effectively. The consensus view of the delegates was that a cultural shift was needed to encourage a greater amount of pride in teaching achievements and that new systems were required to recognise and highlight teaching excellence. The Society is continuing to work with partner organisations to decide how best to take this policy issue forward.

If you are interested in these or any other policy related issues please contact us at **policy@physoc.org**

Publishing images: Is technology progressing faster than our ethics?

Simon Rallison

Director of Publications,
The Physiological Society

A 1937 photograph of Stalin shows him, half-smiling in greatcoat and military cap, on the banks of the Moscow-Volga Canal. To his left stands the slight figure of Nikolai Yezhov, head of the NKVD. In later copies of the photo, though, Stalin's companion has vanished. After Yezhov was secretly executed in February 1940 (in an execution cell of his own design), all traces of him were removed from the official record by censors. Nikolai Yezhov was airbrushed out of Soviet history.

While the deliberate alteration of photographs is almost as old as photography itself, the potential for less detectable manipulation was hugely increased in the late 1980s and early 1990s by the arrival of digital cameras followed by software programs like Adobe's Photoshop and Illustrator. By the early 2000s, the editors of scientific journals knew that they could have a problem on their hands.

Good science requires reliable data. Fraudulently changing or omitting data or results so that the outcome of research is not accurately represented in the record is falsification or fabrication, serious forms of research misconduct and breaches of publishing ethics. At the less serious end of the spectrum are manipulations of image data that violate recognized guidelines without affecting the interpretation of the data.

The potential problems that digital image manipulation raised for journals, particularly those in cell and molecular biology, were first drawn to the attention of the wider community by Mike Rossner, the then Editor-in-Chief of *Journal of Cell Biology*. Rossner and Yamada's manifesto, set out in a 2004 editorial 'What's in a picture? The temptation of image manipulation' (*J Cell Biol* 166, 11–15), is recommended reading for authors, including those of The Society's own journals (it's cited in the Author Information).

The general rule in publishing images is that the final image must remain consistent with the original data, accurately representing the information as captured, and must conform to community standards. While manipulation of an image is acceptable in some circumstances, for instance the adjusting of contrast or brightness, any adjustment must be applied equally to the entire image and must be described in the paper.

Unacceptable manipulations generally include adding to, altering, moving or removing a specific feature of an image. The instructions for The Society's journals exclude using software to edit images such as micrographs or photographs of gel arrays, as these are primary data. However, it is acceptable to size and crop the images.

Although there are also technologies for detecting image manipulation, in many cases there are tell-tale signs that can be picked up by eye, for instance by spotting irregularities in the background signal or in the outer margins of images. Or the background signal is not uniform, with noticeable variations in pixilation, colour or texture. Or sometimes it is possible to see duplication of background features like dust particles.

Gels and blots are subject to a recognized set of rules. For instance if gels, blots or fields are rearranged, then dividing lines should indicate this. Pasting-in or rearranging individual bands is simply a non-starter.

While there are no figures for the prevalence of undisclosed image manipulation in physiology, it is estimated that in cell biology 25% of papers accepted for publication have one or more figures that have been 'inappropriately modified'. *Journal of Cell Biology*, *Nature* and some other journals check all figures for manipulation on acceptance for publication.

Despite the efforts of the journals, not all authors are aware of the prohibitions on image manipulation. They may naively be hoping to make their point more clearly or even just trying to make their photographs more pleasing to look at. Because of this, journals try not to be heavy-handed in dealing with suspected cases of image manipulation. Authors are generally given the opportunity to correct an unsatisfactory figure, either by replacing it with another example from their dataset or where fitting by explaining in the text the manipulation it has undergone. Transparency is always at a premium in issues of publishing ethics. The author should be prepared to provide the journal with the original, unedited data and images, plus details of what software was used in acquiring and processing the image and what was done with it. These measures almost always resolve the issue.

Science has been fortunate in being allowed to police itself to a very large degree. Maintaining high standards of integrity is a small price to pay for this. Institutions, societies, journals and editors can play their part but it is up to individual scientists to keep themselves informed of ethical issues and to question their own actions.

To quote Rossner and Yamada:

'Just because the tools exist to clean up sloppy work digitally, that is no excuse to do sloppy work... If you would have redone an experiment to generate a presentation-quality image in the days before the digital age, you should probably redo it now.'

Changing teaching practices of functional micro-anatomy in physiology

Hugh Elder, David McEwan Jenkinson & David Russell

University of Glasgow, UK

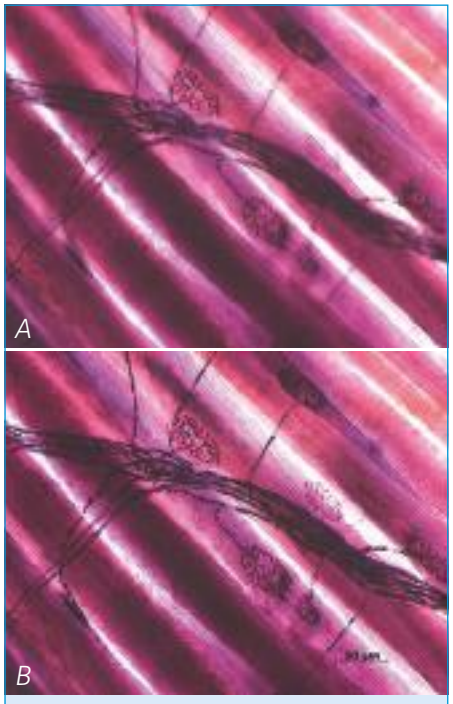


Figure 1. A, a single frame from serial images of motor end plates in a teased, whole mount preparation of skeletal muscle fibres stained with the Gairns gold chloride heavy metal staining technique. This shows the limitation of focal depth provided in a single focal plane of the original specimen. *B*, the more detailed information provided by a Z dimension stack through the more than 50 µm thick specimen. ©University of Glasgow.

In the last two decades in particular, a range of factors including financial constraints, technical and instrumental advances, and research strategies have combined to radically change the way that the teaching of histology and functional micro-anatomy is now conducted in universities. However, it is essential that invaluable teaching material is not lost as a consequence.

Many will still remember the time when every student had a class box of slides prepared to illustrate the structure of the major organs of the body and an individual microscope with which to examine them. Instruction was given by staff with an expansive knowledge of functional micro-anatomy and cytology, the correct use of the microscope (e.g. setting up Köhler illumination), the significance of the stain combinations used on the tissue sections, and ultimately the architecture and function of the organs at cellular level.

The histologist conducting the class usually first demonstrated the specific tissue and cellular characteristics by transparency projection or more recently by PowerPoint computer projection. Two of the authors well remember in Glasgow Physiology that, even for a decade after the Second World War, projection was accomplished by means of a horizontally mounted microscope; the intense light required was from a carbon arc between two sharpened carbon electrode rods, struck by a skilled technician.

Throughout the years, the conventional sectioning and staining techniques for optical microscopy have been greatly augmented by many technical advances such as confocal microscopy, fluorescence and phase contrast microscopy, enzyme and immuno-fluorescence techniques, GFP gene product tagging, and correlative strategies relating the optical level knowledge to electron microscopy.

In addition, computer software like PowerPoint and sophisticated scanning programs such as SlidePath or PathXL to record, display and disseminate images have expanded the options for histological instruction.

Influence of resource constraints

The resource demands to purchase and maintain microscopes of adequate quality and quantity, and to retain the trained histological technicians and service labs required to provide boxed slides for each student have presented an irresistible attraction to cash-strapped institutions, which have dispensed with them, especially when alternative and effective learning strategies are available. The advent of well illustrated texts together with provision of metadata and annotated computer displays has contributed to a steep decline in the number of trained conventional histologists in universities; only pockets remain in specialist institutes.

In Glasgow there are no longer any dedicated histologists, as opposed to pathologists, within the university, although we have a rare slide collection stretching back over the last hundred years. These are not simply 'H & E' stained sections but specimens that exhibit particularly clear specific features donated by successive generations of histologists as demonstration examples in their chosen field. The collection includes early material donated by HSD Garven, who learned heavy metal staining techniques for the nervous system during a period with Camillo Golgi in Italy and who with FW Gairns subsequently continued peripheral nerve work in Glasgow including the development of the gold chloride technique (Figs 1 and 2). Early enzymatic demonstration of the location of the hydrolytic enzyme responsible for the degradation of ACh in the endplate membranes (Fig. 3) supplements the anatomical information.

The collection also contains examples of the historical development of histology from early spread preparations and thick hand cut sections that predate reliable microtomy (Fig. 4). The collection has become scattered as departments have combined into schools and colleges and it is gradually deteriorating in storage. Its extent and content are no longer known and some of the accompanying metadata have been lost.

Digital archiving

However, although teaching methods have changed, an understanding of cellular structure and function is still an essential component in the training of clinical students and research biologists and the need for the best histological images remains.

Hence, somewhat belatedly, in recent years we have embarked upon a core objective of digital archiving to 'save' the collection. Initially the best of the slides are being selected for scanning, using the SlidePath system, and we are adding available metadata. Sadly some 23% of the slides examined so far have deteriorated to the extent that they are no longer usable. A concomitant objective of making the images of the collection as widely accessible as possible to both staff and students for educational purposes is served by the addition of further annotation as time and resources permit. When particular cytological detail of the best quality is found it is being augmented with images taken at the limit of conventional optical resolution (~0.2 µm point to point resolution) with best quality oil immersion objectives (Fig. 5).

With the project now well advanced we hope shortly to encourage staff with personal collections to augment the collection and add to the basic labelling, thereby creating a really useful teaching resource as well as preserving the collection in a readily accessible, immediately useable digital archive. This annotation stage is undoubtedly the most time and effort consuming phase of the project and we are currently seeking further financial support to complete it.

Further phases are anticipated as we expand the project to the slide collections in the medical, veterinary, dental, biomedical and pathological disciplines to provide ultimately a centralised university teaching and research resource.

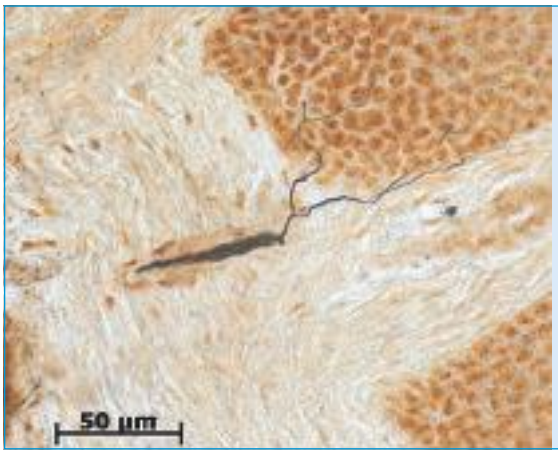


Figure 2. Stacked image from a thick specimen (~15 µm) of human fingertip skin stained by the Bielschowski–Gros heavy metal silver technique for peripheral nerves. It demonstrates that the free nerve endings progress upwards through the basal layer and into the stratum spinosum further than previously described; they have now been followed with stack imaging to near the stratum granulosum.
©University of Glasgow.

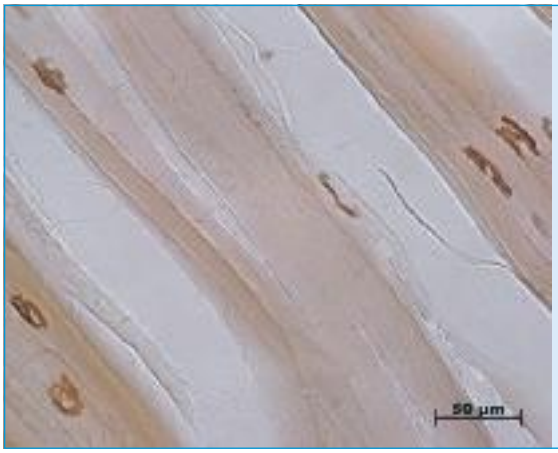


Figure 3. Brown reaction product showing the location of non-specific cholinesterase at motor endplate 'footprints'. This is an early enzymatic application demonstrating the location of the hydrolytic enzyme responsible for the degradation of ACh in the endplate membranes.
©University of Glasgow.

Figure 4. The microcirculation of villi in a whole mount preparation of the intestinal wall from a specimen perfused post mortem with gelatine–carmine. Images taken at a sequence of focal planes through the thick specimen have been stacked to show the arboresent capillary supply to each villus. Deep with respect to the villi, vessels at right angles serve, respectively, the circular and longitudinal smooth muscles.
©University of Glasgow.

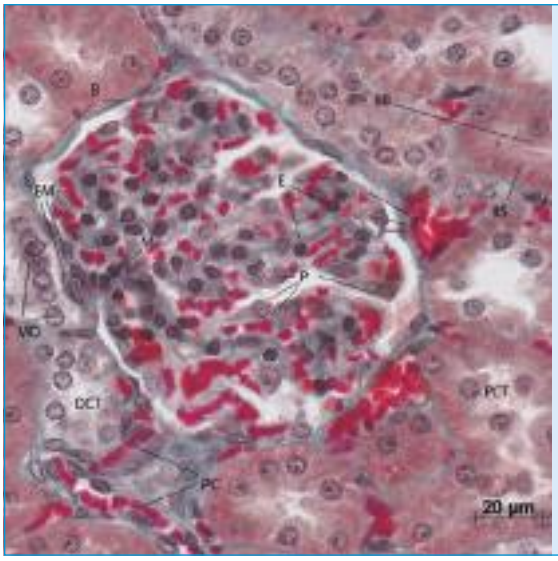
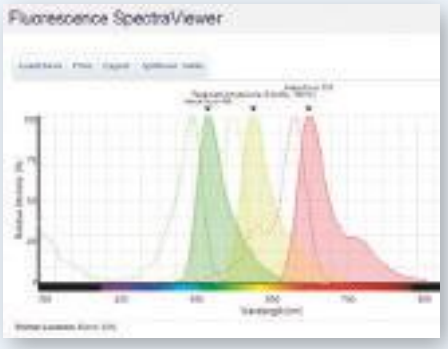


Figure 5. Section of rabbit kidney cortex stained by the Masson technique and imaged with an oil immersion lens. It shows some of the wealth of cytological features that link with functional information: B, Bowman's capsule squamous epithelium; BB, brush border; BS, mitochondria causing basal striations; DCT, distal convoluted tubule; E, glomerular endothelial cell; EM, extra-glomerular mesangial cells; F, 'feet' of a podocyte straddling 4 capillaries; M, mesangial cells; MD, macula densa; P, podocytes; PC, red blood cells in peritubular capillaries; PCT, proximal convoluted tubule.
©University of Glasgow.

Imaging tools and freebies

Keith Siew
University of Cambridge, UK

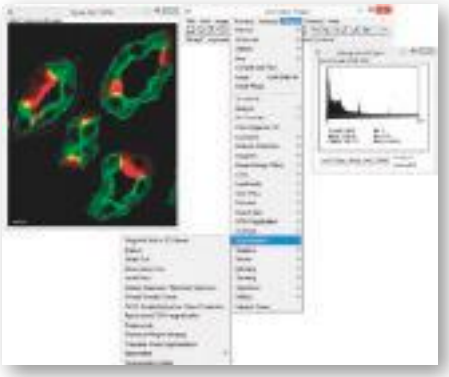
Unbeknownst to many there is a veritable treasure trove of free online resources and tools for those who engage in biological imaging. The list below is hardly exhaustive, but I've endeavoured to share with you the ones that I've found most useful in my own work, and that will hopefully save you both time and money!



Fluorescence Spectraviewer & Stain Your Own Cell

www.lifetechnologies.com/uk/en/home/support/research-tools.html

Two extremely useful tools to help with experimental design are offered free online with Invitrogen (Life Technologies). The Fluorescence SpectraViewer allows you to check compatibility of your fluorescent dyes with one another to prevent potential overlap and bleed through. You can also determine what dyes may be suitable for your imaging set-up with the excitation/emission settings. The cell stainer is great for getting an idea of what subcellular structures you may want to counterstain and informs you what reagents are suitable for live or fixed cell purposes.



ImageJ

Image Processing and Analysis in Java

http://imagej.nih.gov/ij

ImageJ is a powerful, free, scientific, Java-based image processing and analysis software package. It is completely customisable with numerous plugins available that have been developed and are maintained by an active research community. Many scientists will use FIJI (Fiji Is Just ImageJ) which is an open source package based on ImageJ that comes preloaded with many bundled plugins and is slightly more user friendly (**http://fiji.sc/Fiji**). Both of these versions will enable you to write your own code and record macro functions to facilitate data processing and analysis. There is also a dedicated ImageJ Wiki (**http://imagejdocu.tudor.lu**) with video tutorials, how to manuals, details of all the available plugins and even a wish list of processing and analysis procedures that are in development. This is a must for every lab delving into imaging; there is little offered by expensive commercial products like Adobe Photoshop or Bitplane IMARIS that ImageJ can't do!

The Embryonic Mouse Atlas

www.emouseatlas.org

The e-Mouse Atlas (EMA) project was established to provide both a detailed anatomical model and characterisation of spatiotemporal protein expression in the developing mouse. The EMA has similar search functions, but unlike the Human Protein Atlas, the expression data are typically derived from *in situ* hybridisation data in publications, and it has limited data on the adult mouse.

IHCWORLD
Life Science Products & Services

www.ihcworld.com/

ImmunoHistoChemistry (IHC) World is an amazing repository of knowledge and a vital resource for anyone dabbling in histology/cytology. The website hosts a database of universal protocols and techniques for histological staining, buffers, immunohistochemistry, immunocytochemistry and immunofluorescences work, as well as a litany of specialised protocols categorised by the leading laboratories from which they originated. IHC world also boast a very active forum where you can troubleshoot your problems with the research community and benefit from the experience of the experts in the field.

THE HUMAN PROTEIN ATLAS

www.proteinatlas.org

The Swedish Human Protein Atlas project was setup to allow for a systematic exploration of the human proteome using antibody-based proteomics. The atlas database will show you the location and expression levels of gene products from the subcellular to the organ level in healthy male and female tissues, various cancers and several cell lines. The atlas also characterises all the antibodies it uses, both commercial and those they generate in-house. This is an invaluable resource and always worth a quick search to check before embarking on your own studies.

MOOC LIST

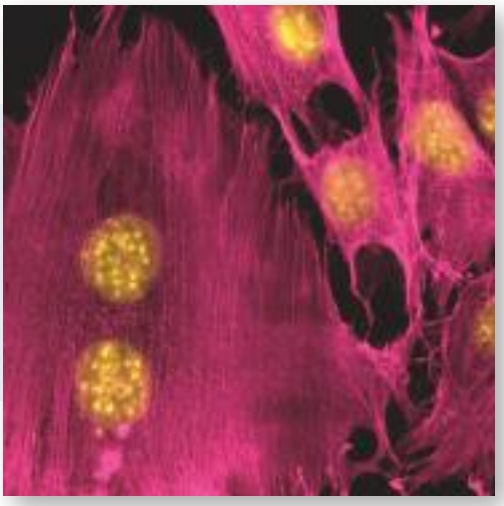
www.mooc-list.com

For those of you who have never heard of a Massive Online Open Course (MOOC) before, well they do exactly what they say on the tin. The number of participants at any one time can be in the thousands, they are usually free of charge (unless you want to obtain certification in some cases) and consist of a video lecture series and often some interactive elements run by a group of tutors. MOOC List provides links to many excellent courses on histology, microscopic techniques and image analysis offered by universities such as MIT and Stanford. Quite useful for those who want to learn a new skill or brush up on their knowledge on a variety of subjects (not just imaging)!

The British Heart Foundation's Reflections of Research competition

The British Heart Foundation (BHF)'s Reflections of Research competition seeks out the most extraordinary and surprising views of the heart and blood vessels amongst the research the charity funds. The annual competition is open to researchers supported by the BHF, and accepts images from all areas of cardiovascular research.

Simon Gillespie, Chief Executive at the BHF, said of last year's competition: 'This isn't just visually arresting art; these pictures are reflections of our life-saving research, which makes them even more beautiful.'



Pink in art

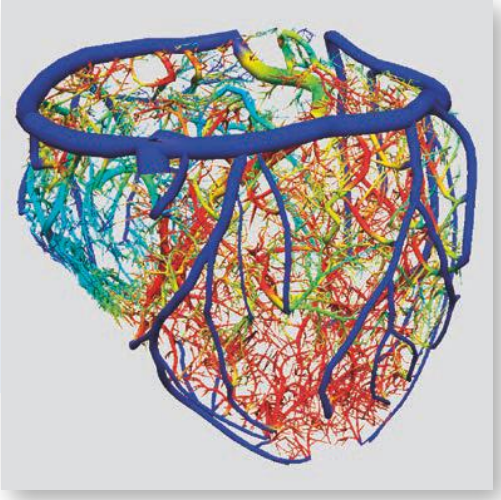
Fluorescence staining of the nuclei (yellow) and actin filaments of endothelial cells (in pink) which provide a defensive wall against toxins to keep blood vessels healthy.

Kenneth Cheung, Queen Mary, University of London, UK

Heart strings

Diffusion Tensor Imaging from an MRI scan of a heart. The tracks show movement of water molecules in the heart muscle, which reveals how the muscle cells are aligned. Resultantly, the streamlines shown in the image represent the orientation of 'muscle fibres' in the heart's left ventricle.

Patrick Hales, University of Oxford, UK



Feeding the heart

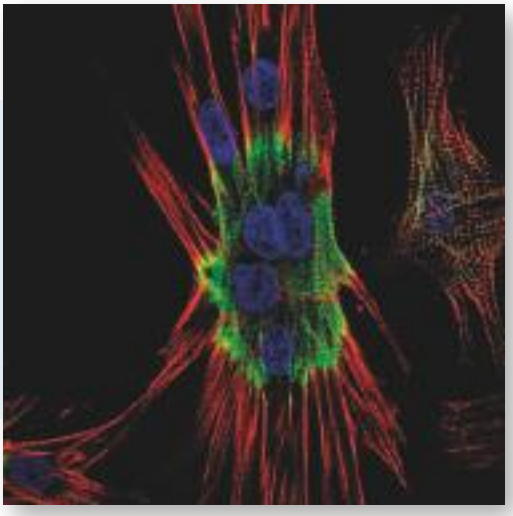
This virtual model shows the blood flow through vessels serving the heart.

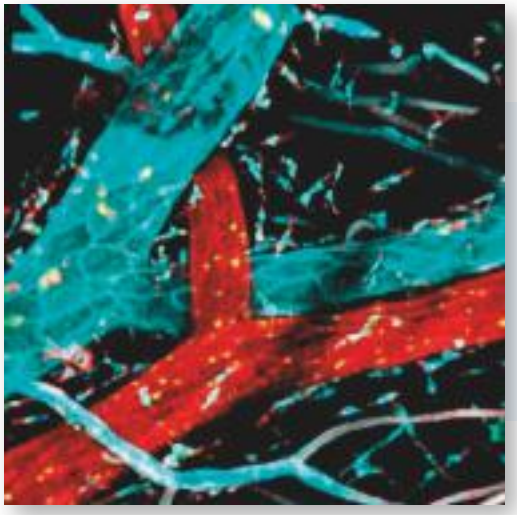
Nic Smith, Kings College London and University of Oxford, UK

The ‘B’ of the Bang

Cultured cardiomyocytes stained for subcellular structures; nuclei (blue) are surrounded by the cytoskeletal (red) and beating elements of cells (green).

Elizabeth Ehler, King’s College London, UK





Opposites attract

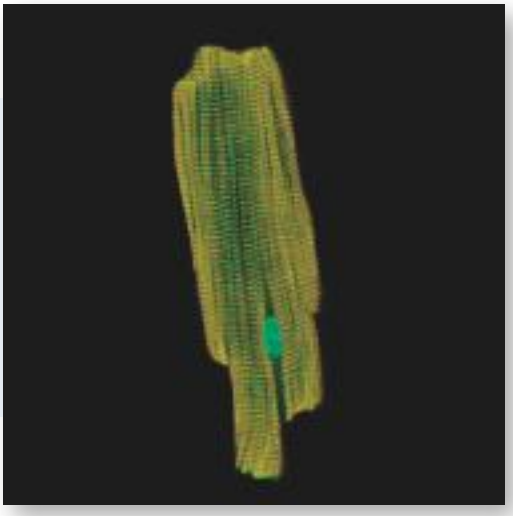
Blood vessels stained for arterial (red) and venous (blue) markers.

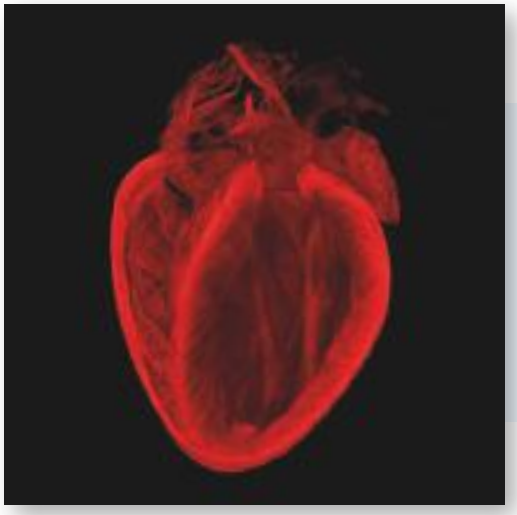
Krishma Halai, Queen Mary, University of London, UK

Pulling together

Confocal image of a mouse cardiomyocyte stained for the nucleus (cyan) and two different proteins, alpha-actinin (red) and titin (green) involved in the contractile function of the cells.

Thomas Cahill, University of Oxford, UK





The Broken Heart

3D structure of an adult mouse heart. The imaging technique used here is being developed to allow us to better measure the extent of injury after heart attack, and to assess repair.

Gillian Gray, Megan Swim, and Harris Morrison, University of Edinburgh, UK

The winning images can be viewed online at **bhf.org.uk/reflections**. You can support the BHF’s life-saving science by donating at **bhf.org.uk**

Being an artist and a scientist

Stefanie Reichelt
University of Cambridge, UK

Stefanie Reichelt and her Light Microscopy team (Alexander Schreiner, Imran Patel, Patrice Mascalchi) at CRUK Cambridge Institute. ©Stefanie Reichelt.

I am a Cambridge-based researcher and photographer. I have always been an observer and a dreamer, and I have combined the qualities of both to become a scientist and a photographer.

For me art and science have never been separate but complimentary ways of seeing. As a biology undergraduate, I learned the craft of scientific illustration, producing accurate but beautiful drawings and paintings of beetles and butterflies. Photography was a means to document processes in Petri dishes as well as in life. Electron microscopy and light microscopy both use large format film, and dark room work was essential. I experimented with a pinhole camera and making my own photographic paper processes.

For 5 years I worked with one of the UK-based inventors of the confocal microscope, Brad Amos, at the MRC-LMB in Cambridge. As a biologist I had not been trained to take microscopes apart and put them back together, but soon became caught up in optical design and testing work. I was one of the first to use a 405 nm (violet) laser in a confocal microscope and also worked on a high-resolution spectral confocal development, as well as a compact point-scanning confocal microscope, which was commercialized by a company (BioRAD).

Since 2005 I have been the Head of the Light Microscopy Laboratory at the CRUK Cambridge Research Institute. My research includes the development of new imaging techniques, which will enable the visualisation of molecules in cells for cancer diagnostics. See **www.cruk.cam.ac.uk/core-facilities/light-microscopy-core**

My group at the CRUK Cambridge Institute uses confocal point-scanning, spinning disc systems and non-linear imaging techniques to study live cell divisions and cell development, tumour development and biopsy material. As head of the Microscopy Laboratory, I am in charge of a team of imaging specialists who work in collaboration with researchers, obtaining results that are crucial to developing new treatments for cancer patients.

Last year we developed with a group of physicists a CARS Raman multiphoton imaging system, which could help in eliminating the bane of staining in translational imaging experiments

For over 300 years optical microscopy has been one of the fundamental tools to study biology. Staining with dyes or fluorescent labels has remained one of the main ways of visualisation and diagnostic imaging of cells ever since the first use of dyes in the mid-nineteenth century. However, many such stains affect the normal function of cells making unfeasible their use with live cells.

The multi-photon microscopy set-up at the CRUK Cambridge Institute is helping researchers to image cell and tissue structures in live cells without the requirement of stains. Through the overlaying of lasers and exploiting how tissues respond to certain wavelengths,

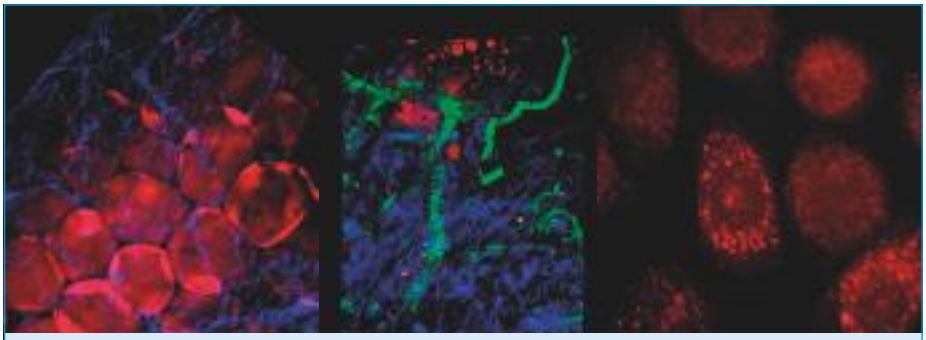


Figure 1. CARS of lipid (red), SHG of collagen (blue) and of two photon excitation fluoresence of actin GFP (green) in cells and tissues. © Stefanie Reichelt and Imran Patel. See Steuwe *et al.* (2014) and Patel *et al.* (2013).

we can acquire structures such as collagen, muscle and lipids stain free (Fig. 1). These processes are acquired through techniques called second harmonic generation (SHG) and coherent anti-stoke raman scattering (CARS).

In a similar concept to the function of a microwave oven, where absorption of microwaves causes vibration of water molecules and heating of food, CARS works by the absorption of light and detection of vibrational movements of molecules. This opens up a whole field of potential stain free imaging, currently unavailable by conventional techniques. Imaging drug localisation is capable of furthering understanding of pharmacodynamic properties in treating diseases.

My photographic work explores perception in photography and our conscious and unconscious reactions to the images we see. It raises such questions as: is seeing believing? is what we see what we perceive? and what is the relationship between our subjective and objective realities? Leaving photographic interpretations to observers' imaginations, as with fairy tales, I seek to depict photographic stories with clues, hints and surprises, so that the perception of what lies within the photographs elicits desires, fears and unease in the observer. I have exhibited widely and my projects are features in photography and science magazines.

As a scientist I take microscopic images of the human body, of small cells that move and divide, and contain even smaller molecules. These objective images, both informative and beautiful, enhance the knowledge of our physical self and help researchers develop cures for cancer. My images were projected onto Senate House during the 800-year Cambridge Anniversary Celebration and are part of the science photo library collection (**vimeo.com/8768073**).

I make hand-made photo books from my projects and also experiment with alternative display options using LCD screens or viewing boxes with sound (**www.stefaniereichelt-photographyandprints.com/projects.php**) (Fig. 2 & 3). A recent project 'Traces of Genius' can be found here: **tracesofgenius.wordpress.com**. The aim was to record the 'traces' left behind by the scientists of the famous MRC Laboratory of Molecular Biology and create a visual 'memento mori' for the old LMB building. The project has resulted in a photographic archive of the human traces and marks in the vacated old LMB building. These were exhibited in the new LMB building following the scientists' move from the old to the new space. A collaborative literary art book will be published soon combining photographs from the project with short stories inspired by them.

When joining CRUK, I founded ArtCell Gallery, which provides exhibition space on the Addenbrooke's Hospital site for local artists and the science community. I am the curator and have organized and designed the ArtCell exhibitions and events (**www.stefaniereichelt-photographyandprints.com/artcell.html**).

In March 2014, as part of the 2014 Cambridge University Science Festival, I organized and hosted a day of talks, lectures, demonstrations and exhibitions on 'the Art of Scientific Imaging' with the Royal Photographic Society. As part of the festival the 'International Images for Science exhibition 2013' will be on display until May. We also had the amazing Camper Obscura to demonstrate the basic principles of imaging: **camperobscura.co.uk**

Figure 2. The Snow Queen – a photographic portrait through ice. ©Stefanie Reichelt.

Figure 3. Waterfleas and Artificial Cells. © Stefanie Reichelt.

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Steuwe C, Patel II, Ul-Hasan M, Schreiner A, Boren J, Brindle KM, Reichelt S & Mahajan S (2014). CARS based label-free assay for assessment of drugs by monitoring lipid droplets in tumour cells. *J Biophotonics* (in press, doi: 10.1002/jbio.201300110).

2014 *Forthcoming events*

4–5 Sept

Special symposium to mark the retirement of Robert W Banks - Something old, something new, something borrowed, something... Else!
Collingwood College,
Durham, UK
www.physoc.org/robertbanks

10–12 Sept

Obesity: A Physiological Perspective
Newcastle Upon Tyne,
United Kingdom
www.physoc.org/topicobesity

19 Sept

H^3 symposium:
Public engagement as a 'Pathway to Impact'
Hodgkin Huxley House,
London, UK
www.physoc.org/publicengagementh3

2015

10–12 Apr

Ageing and Degeneration: A Physiological Perspective
Royal College of Physicians,
Edinburgh, UK
www.physoc.org/ageingtopic

Meeting Preview

Ageing and Degeneration: A Physiological Perspective

Part of The Society's 'Understanding Ageing' year in 2015

10–12 April 2015
Royal College of Physicians,
Edinburgh, UK
www.physoc.org/ageingtopic

Following the upcoming launch of The Society's Topic Meeting 'Obesity: A Physiological Perspective' in September 2014, The Society are excited to announce the second Topic Meeting taking place in April 2015 in Edinburgh on 'Ageing and Degeneration'.

Topic Meetings are a new style of meeting aimed at attracting physiologists from diverse backgrounds in an attempt to promote cross-fertilisation and integration.

The programme will be announced in summer 2014 and will look at the hot topic of Ageing and Degeneration from a physiological viewpoint. Abstract submission will open in January 2015 and poster communications are welcome.

Alongside the scientific programme The Society will be holding education and outreach activities and a special issue of *The Journal of Physiology* will also be published.

During the meeting there will be social activities, a great chance for delegates to network and have an enjoyable evening out in the beautiful city of Edinburgh.

Make sure you mark the date in your diary and watch the website for regular updates.

The Great Hall at the Royal College of Physicians, Edinburgh

Meeting Notes

Inaugural H³ symposium 'Cellular approaches for cardiac repair: A physiological perspective'

4 April 2014,
Hodgkin Huxley House, London

Cesare M Terracciano

Imperial College London, UK

I have recently had the opportunity, together with Susan Currie (University of Strathclyde) and Sandra Jones (University of Hull), to organise the inaugural H³ symposium. The symposium took place in the brand new headquarters of The Physiological Society, named Hodgkin Huxley House (hence the H³) in London, and was a full day of oral and poster presentations on a subject that is topical, fascinating and controversial.

Stem and progenitor cells in cardiac physiology and medicine are very likely to change our understanding of cardiac development, function and disease, and to provide new, more relevant physiological and pathological models which may become a real alternative to animal experimentation. In the last decade, fuelled by enormous public interest and significant funding from government agencies and charity organisations, the field of cardiac regenerative medicine has expanded massively and every scientific cardiology meeting now contains sizeable sessions on this topic, predominantly focusing on stem cell origin, differentiation, signalling, disease modelling and clinical applications. However, specific meetings on the physiological mechanisms involved in regenerative approaches are rare and this H³ symposium gave us the platform to bring together major experts from across the globe to specifically discuss the physiological perspective.

The programme was divided into three parts: the sources of cellular regenerative elements, the use of stem and progenitor cells for *in vitro* studies, and the clinical applications. I was particularly proud of the phenomenal line up of speakers who took part to the symposium. From cardiac physiologists to bioengineers, from developmental biologists to clinical cardiologists, a multidisciplinary faculty dissected the current evidence, and presented new and exciting results and proposed future directions. At the end of the meeting, several concepts were more defined in my mind: the high heterogeneity in the origin and function of the populations of cells studied, the need to define parameters that are relevant for physiology, the importance of more complex, multicellular systems with tissue engineering as a priority, and the necessity of redefining the clinical goals in cell therapy trials. There is clearly a lot of future work in this field and this symposium was a very intense learning opportunity for the novices and an important check point for the experts.

The new venue at the Hodgkin Huxley House provided a cosy environment for a meeting that was extremely popular and sold out many days in advance. The Society is intending to live cast these events in the future following the overwhelming demand. The nice environment, the convenience of the central London location and the helpful staff of The Physiological Society contributed to the success of the meeting and will ensure a bright future for the H³ symposia series.

Call for 2015 Topic Meeting symposia proposals

We are calling for proposals for symposia for the 2015 Topic Meeting on 'Imaging' that The Society is running; please visit **www.physoc.org/suggest-symposium** for further information and to submit your proposal (deadline 8 September 2014).

Meeting Notes

CLARITY users meeting

7 April 2014
University College London, UK

Bertrand Vernay

University College London, UK

Imaging large samples at high resolution is a common problem in life science. To this effect multiphoton microscopy has been successfully used up to 1 mm of imaging depth but is then limited to genetically encoded fluorescent proteins as antibodies cannot penetrate deep enough into the samples. The recently published CLARITY protocol (Chung *et al.* 2013) from the Deisseroth research team in Stanford now enable biologists to image several mm into the sample at high resolution. Samples are rendered optically transparent and macromolecule permeable by removing the lipids impeding both light and antibody penetration into the samples, but otherwise maintaining the tissue organisation with a nanoporous hydrogel.

The first London CLARITY Users' meeting was held at the UCL Institute of Child Health in April. The meeting attracted attendees from London, Cambridge, Oxford and further afield in Europe (Netherlands, Germany, Austria and Spain). It was a unique opportunity for researchers to exchange their experiences of the CLARITY protocol in a friendly and informal atmosphere. A year after the initial publication, the enthusiasm for this promising new method was reinforced by several speakers presenting preliminary results obtained with the CLARITY protocol.

More information is available at:
http://clarityresourcecenter.org

References

Chung K, Wallace J, Kim SY, Kalyanasundaram S, Andalman AS, Davidson TJ, Mirzabekov JJ, Zalocusky KA, Mattis J, Denisin AJ, Pak S, Bernstein H, Ramakrishnan C, Grosenick L, Gradinaru V & Deisseroth K (2013). Structural and molecular interrogation of intact biological systems. *Nature* **497**, 332–337.

Meeting Notes

The 33rd Annual Conference of the Physiological Society of Nigeria

12–14 February 2014,
University of Ibadan, Nigeria

Bamidele Owoyele

University of Ilorin, Nigeria

The members of the Physiological Society of Nigeria (PSN), which was founded in 1979, met at Ibadan, the largest city in West Africa. The Head of Department, Adesoji Fasanmade, was quite delighted to welcome members to the conference, which took place at the newly commissioned ultra modern international conference centre of the University. The meeting started on 12 February with the arrival of participants and a guided tour of Ibadan land. However, the opening ceremony was held on 13 February and the president of the Society, Eme Osim, gave a speech highlighting the developments in the Society during the past four years of his leadership. He appreciated the efforts of the Local Organizing Committee as well as the Department of Physiology. He also pointed out that the theme (see thematic lecture below) was very topical, emphasizing the link physiology has with the laboratory, hospital and society. He congratulated seven members of the society who were elevated to the rank of Professor in the last 12 months. The key note address was subsequently delivered by Olatunde Farombi titled 'The role of selected phytochemicals in chemoprevention and their clinical outcomes'. This lecture focused on the chemopreventive effects of phytochemicals such as kolaviron, epivernodalol and curcumin and their potential in animal models as well as the various efforts that have been made to translate the basic research to clinical applications. The lecture was followed by a thematic lecture delivered by Olusoga Sofola titled 'Physiology: The laboratory, the Hospital and Society'.

For the first time, prizes were introduced for the Society's conferences. The prizes were for younger physiologists and included best overall poster presenter, best first time poster presenter, best overall oral presenter, best first time oral presenter, best presentation in the field of renal physiology, and most outstanding research output. There was a plenary session followed by oral and poster sessions.

On 14 February, Frank Mojiminiyi delivered a plenary lecture on the use of a dietary approach for the treatment of hypertension.

Executives of The Society at the Annual General Meeting, from left to right Kolawole Olorunshola, Samuel Olaleye, Frank Mojiminiyi, Arthur Nwafor, Eme Osim, A. Adelaiye and Ifedayo Ajayi.

Cultural dance at the Dinner

The session was followed by four parallel oral sessions as well as a poster session. Later in the evening, the Annual General Meeting was held and one of the most important issues discussed was the need to mobilize members for the forthcoming IUPS/PSN Regional Teaching & Research Workshop which has been slated for October 2014 in Lagos, the commercial capital of Nigeria. The workshop is expected to draw participants from across the world. The preparations for this meeting led to the postponement of elections of new executives for the Society.

The last event of the conference was the Society dinner where there were lots of local dishes and drinks for members. The members were entertained by a traditional music band after which the various prizes were awarded to the winners. The secretary of the Local Organizing Committee (Samual Olaleye) informed members that 336 participants attended the meeting with 95 attendees applying to become members. A total of 130 oral and poster presentations were made. The meeting was truly refreshing and I had my debut as a rapporteur for one of the plenary sessions.

Acknowledgement

I would like to express my appreciation to the University of Ilorin for providing funding support for my attendance of the meeting. Special thanks to Frank Mojiminiyi for going through the draft of the report and to Samuel Olaleye for his encouragement.

Meeting Notes

The Young Life Scientist Ireland (YLSI) Symposium 2014

1 March 2014,
Trinity College Biomedical Science Institute, Ireland

Sinead Merrigan

National University of Ireland, Ireland

The ethos behind the symposium was to create an environment where young researchers could come together to share their work and connect with fellow scientists.

This year's committee (pictured) was formed in May 2013 and consists of students from seven different Irish colleges. Our efforts were well rewarded when we welcomed more than 150 young life scientists from around Ireland for the symposium. Over the course of the day parallel oral sessions covering various themes, workshops, keynote talks and poster sessions were held.

Inspiring words spoken by our keynote speakers left a tangible buzz in the lecture theatre. Kingston Mills talked in depth and with incredible passion about his work on immunotherapeutics and recent advances in this area. Moreover, Sir Stephen O' Rahilly gave an entertaining yet incredibly inspiring talk on his research in the areas of genetics and obesity (pictured top right).

In the aftermath of the conference, the committee is proud of their success in creating an environment where young researchers could share their knowledge and create bridges for collaboration. Not only was the turnout overwhelming, the standard of oral presentations and posters was a testament to our young researcher's capabilities, leaving no doubt that research in Ireland is thriving.

The committee would like to take this opportunity to thank all sponsors and supporters. We would especially like to thank our delegates who certainly were the most important element of the symposium.

Back: Aidan Kaar (UCC), Andrew O'Leary (UCC), Daniel Hurley (UCD), Tadhg McGivern (RCSI, Co-Chair). Front: Aideen Allen (TCD), Ines Freitas (UCD), Lydia Dyck (TCD, Chair), Prof Sir Stephen O'Rahilly (University of Cambridge), Paul Carlyle (DIT), Sinead Merrigan (NUIM). Not in picture: Michelle Breathnach (LIT)

Meeting Notes

The Biophysical Society's 58th Annual Meeting

15–19 February 2014,
San Francisco, USA

Sally Howells & Nick Boross-Toby

The Physiological Society

After the howling gales and floods that battered the UK in January and February 2014, it was great to go to San Francisco to confirm that the sun still shone somewhere in the world. The Moscone Center in San Francisco was this year's host for the Biophysical Society's annual meeting, and after setting up the stand in preparation for the exhibition, we took a tour of Alcatraz. Although sunny during the day, it wasn't long before the fog rolled in over the bay and the notorious island was shrouded in an eerie mist. We were, of course, on our best behaviour to ensure we weren't incarcerated or unable to man the stand.

Our stand was extremely well positioned opposite a busy and lively poster area, and we had a steady stream of scientists wanting to learn more about *The Journal* and The Society. We were pleased to be able to tell them that *The Journal* has no restrictions on page count or the number of figures allowed, and that we impose no submission or page charges – a great competitive advantage.

The Journal of Physiology really is the 'home of biophysics' so in addition to featuring some of our best biophysics content published in the last 12 months by way of a virtual issue, we were also proud to display the original copies of some Nobel Prize winning papers published in *The Journal* in the last century. Having these historic copies on show really helped to pull in the crowds, and some of the younger delegates were amazed to see the hardback format of *The Journal*, and some of the more senior scientists remembered reading these seminal papers during their studies. At this conference we launched our Oral History series that features interview transcripts of notable physiologists talking about their life and work. These can be found here: **http://bit.ly/PhysHist**

Several of our loyal reviewers and authors stopped by the stand to say hello, as did some of *The Journal*'s Reviewing and Senior Editors. David Paterson, Editor-in-Chief, was impressed with the quality of science on offer at this meeting, and we are expecting to receive more top biophysics submissions as a result of attending the conference, which attracted over 7,000 delegates.

Director, Events and Marketing, Nick Boross-Toby, and Managing Editor of *The Journal of Physiology*, Sally Howells

Due to the success of this meeting, we are hoping to attend the 59th Annual Meeting next year in Baltimore, where the first umbrella factory in the USA was opened in 1828 – which doesn't bode well for the forecast!

Meeting Preview

Physiology 2014: An insider's view

Ahead of The Society's annual conference in London, Physiology 2014 (P14), *PN* gets the inside scoop from three of our prize lecturers. We find out about the hot topics they will be presenting and what else they are looking forward to in the diverse and exciting programme.

Robert Winston

Imperial College London, UK

Annual Public Lecture: Shall we be human in the next century?

1 July | 18.15–19.15

What is the purpose of meetings like P14?
Science is no longer a series of silos of biology, physics, chemistry, mathematics and engineering: they are all blurred. That is of course why meetings are valuable – you share, and see the interfaces between what have been separate subjects in the past. You see how they work and interact together and that's important.

Also, I think to get more science engagement is an important thing for our society – it has to be something which is of increasing necessity if people are to control the science which is done for them by other people that think they know better!

What can we look forward to hearing from you in your public lecture?
The issue really is that humans, ever since the beginning of time – or before historical time – have manipulated their own evolution. They did so by producing technology, like the hand axe, which of course has impacted the modern human brain. So really what I'm going to be arguing is that we are possibly now in the position to manipulate our evolution again using molecular biology. The question I will be proposing is whether this is a good thing or a bad thing. And what we might find are some of the issues involved in genetics of this kind, and where the pitfalls are.

Why do you think it's important to highlight this topic to the general public?
The topic that's important is how we handle our technology and it is significant to everybody. What is crucial is that, in an age which is increasingly driven by science, we ensure that people are as literate as possible about the science that has been done in their name. So that in a democracy they can have some control over it.

A-level students will present research projects at Physiology 2014. What advice would you give to students that may consider a career in research?
Remember that science is full of impossible role models. We are taught again and again of the great scientists: the Darwins and the Einsteins and the Newtons. Actually, science isn't really like that. The great names really are unimportant and real science is not done by those people. Real science can be done by these students – the next generation – because science essentially is not a difficult pursuit. People like myself are not geniuses, we are very ordinary and how we achieve the science that we do is by collaboration with a group of people. We work together, rather like a termite mound: each termite has a pinhead of a brain, but actually working collectively they can make a 10-foot edifice which is full of tunnels and air conditioning! So I think it's important for young people to understand that actually if they're really interested in science, they can do it and they can do it to a very high level.

Richard Tsien

New York University, US

Annual Review Prize Lecture: Excitation–transcription coupling: Novel mechanisms and implications for brain disease

30 June | 18.00–19.00

What do you feel is the purpose of meetings like P14?
Meetings like this one – the programme looks superb – serve several important functions. Scientific interchange in our own fields and in areas we didn't realize we needed to know about. Socializing; making new, sometimes lifelong friends. I vividly remember Denis Noble telling me about his first physiology congress, in Leiden, around 1960.

What can we look forward to hearing about in your lecture?
I will speak about how neuronal firing is linked to the expression of genes. We have found a new mechanism for sending signals from the surface of excitable cells to their nucleus – an express package for calcium/calmodulin.

It's important to highlight this topic because excitation–transcription (E-T) coupling is universal to excitable cells but much less understood than excitation-secretion coupling (think Bernard Katz) and excitation–contraction coupling (think Alan Hodgkin and Andrew Huxley, working independently). All cells have genes to regulate, and dysfunction of E-T coupling may lead to diseases of the brain.

What are the latest developments in this field?
There is an upwelling of discovery about new players in the regulation of signalling from synapse or surface to nucleus, from multiple groups. We are also seeing calcium signalling, so important for non-excitable as well as excitable cells, in a new light.

Which other symposia and workshops stand out to you at P14?
Well, everyone will have their own picks, but I like:

Coupling cellular metabolism to neuronal signalling

Physiology meets clinic: From bench to bedside and from bed to benchside

The heart is lost without the brain: The autonomic perspective
Of course, the reverse is also true!

The axon initial segment, a plastic gatekeeper for neuronal activity

Get into outreach: Practical advice from Society grant success stories
This is something we're very keen on here in NYC under the leadership of two Heathers (http://comebebrainy.com/brainy-blogs).

Finding the hook in your research

What advice would you give to students presenting posters at P14?
Make sure your most important points are stated clearly and simply at the beginning and, in different words, at the end. Work hard on reaching a broad audience, both for its own sake as well as career advancement. Be open to branching out into areas you were never trained in.

Peter Sleight
University of Oxford, UK

Paton Prize Lecture: A historical perspective on reflex cardiovascular control in man

2 July | 11.15–12.15

What do you feel is the purpose of meetings like Physiology 2014?
For young people, it's an opportunity to put faces to names and make contacts with other people who are working in the same field. For older people, it's fun to meet old friends and chew the fat and discuss things. It's a sort of club really. You undoubtedly get new things out of it – you can't always take in everything that happens, but you can hear what people think.

What can we look forward to hearing about in your lecture?
It's about the history of the baroreceptor control of blood pressure. It's quite an interesting time because of new developments in treating hypertension. The problem with drug treatment for hypertension is that we have a lot of choices – they are very effective, but people don't stick with it – the flower beds outside hospital wards are full of pills! For some people in other countries, the price of drug treatments is a big burden. Treatment with cardiac pacemakers, supplied to the carotid sinus nerve, has now become a real practical proposition and a very good alternative to drugs.

What are the latest developments in this field?
The hot topic is renal denervation, where you pass a catheter up from the groin and burn the inside of the renal arteries carefully. That destroys the sympathetic nerves of the kidney and has been touted as a very simple and effective treatment – even better than a pacemaker. This has become very popular and they estimate something like 10,000 people have had that treatment. But the problem is that the American Food and Drug Administration were not going to license this treatment without a better trial. It was double blind – the patients all had a catheter but didn't know if their renal nerves had been denervated. This trial has reported recently (called 'SYMPLICITY 3') and it proved negative, which has raised a lot of questions and has put it on hold. They found that black participants in fact had increased blood pressure, which did not occur in white patients. So it's now a very interesting topic, and I'll be talking about that and what needs to happen next. It's not dead, but it's certainly stunned!

Which other symposia and workshops stand out to you at P14?

The heart is lost without the brain: The autonomic perspective
It's an interesting area; we're learning much more about how the brain works and how it affects cardiac-related things.

Co-morbidities associated with the sleep apnoea syndrome and their mechanisms
I spent seven terrible years of my life studying sleep! Intelligent people study sleep in animals like cats that sleep during the day and it doesn't disturb your sleep – but I spent seven years or more studying sleep in man, where I lost a lot of sleep myself!

Effective Presentation Skills
I just think this is very important for everyone in their career.

Statistics: the Emperor's clothes
If you're doing research, you've got to understand statistics. Even if you're looking at published papers, you've got to understand statistics. I'm not great on it – I must have been to three courses in my life but if I need any statistics on my own papers, I go and ask somebody else!

A-level students will present research projects at Physiology 2014. What advice would you give to students that may consider a career in research?
The main thing when you consider your career is to do something you're going to enjoy. Physiology is something that people will really enjoy. It's a tough field because, when you're young, getting grants isn't easy. But if you are successful and get grants, I can guarantee you're going to have an interesting life!

Nominations for 2015 prize lectures

Nominations open on 30 June and close on 31 August for:

- Annual Review Prize Lecture
- GL Brown Prize Lecture
- Hodgkin–Huxley–Katz Prize Lecture
- Michael de Burgh Daly Prize Lecture
- Otto Hutter Teaching Prize
- The Paton Prize Lecture
- The Annual Public Lecture

For more information please visit:
www.physoc.org/prize-lectures

Single molecule super-resolution microscopy: All for one and one for all

In light microscopy, the ability to resolve two objects from one another is ultimately limited by the physical properties of light. Recent breakthroughs in the field of super-resolution microscopy have overcome this limitation using clever workarounds to localise single molecules.

Matthieu Palayret

University of Cambridge, UK

'Super-resolution microscopy bridges the nanoscopic world of structural biology and electron microscopy with the live cell microscopic world of confocal fluorescence microscopy'

All for one: invading the diffraction-limited world

The diffraction limit of light

Microscopes, together with telescopes, have been aiming at increasing their magnification in order to see deeper into the infinitely small or the infinitely large. However, both optical tools are limited by the physical diffraction limit of light as described by Abbe (1873): due to the wave nature of the light, even the smallest point that emits light will be observed as a blurred blob, or point-spread function (PSF) (Fig. 1*C* and *D*), with a size approximately half the wavelength of emitted light. This limit is approximately 250 nm in modern optical microscopes (e.g. green light from a single GFP at 500nm). Similarly, the size of a light source (e.g. spot size of a focused excitation laser) is also limited by this same physical restriction. This means that no object below ~300 nm can be directly resolved optically. Electron microscopy solves this issue by using much smaller wavelengths (10^{-10} m instead of 10^{-7} m); however it requires fixation of the sample, which kills the sample by physically or chemically altering it.

Two arms in optical super-resolution

Today fluorescence microscopy is a key tool used in many labs and has been proven to be very efficient for observing phenomena in live cells. Two main strategies have thus been developed to overcome the Abbe limit in this field: reducing the illumination of the sample to subdiffraction regions (STED, SIM), or separating in time single molecules that can be localised with a subdiffraction precision (STORM, PALM, PAINT). This article will mainly focus on the latter pointillism techniques. Good reviews for STED and SIM methods can be found in Allen *et al.* (2014) and Müller *et al.* (2012).

Concept of single molecule localisation microscopy

The concept behind single molecule localisation microscopy (SMLM) is that the position of an object can be known with greater precision than its size. The size of a table is in the order of metres, but its position can be easily known with a precision of centimetres. Similarly, Newton localised the Earth with a greater precision than its actual size. This is similar with fluorophores. Although they are observed as a 250 nm wide PSF, one can fit the observed blob very accurately with a 2D-Gaussian and localise its centre with great precision (Fig. 1*C*–*G*). This precision mainly depends on the pixel size of the camera and the intensity of fluorescent signal (the number of photons emitted by the fluorophore and detected by the camera above the background noise) (Thompson *et al.* 2002).

However, this trick is only useful if separate PSFs can be distinguished from one another. If the density of labelled proteins brings the proteins closer than the diffraction limit (e.g. protein complexes), then the different single PSFs merge and determining the number of PSFs and the positions of their centres is almost impossible. This is made harder by the fact that the intensity of a fluorophore can vary considerably, depending on its position and its excitation. This means that one cannot simply infer that a PSF twice as bright as another has twice as many labelled proteins.

In order to circumvent this density issue, three labs (Hess *et al.* 2006; Betzig *et al.* 2006; Rust *et al.* 2006) independently published in 2006 different methods which

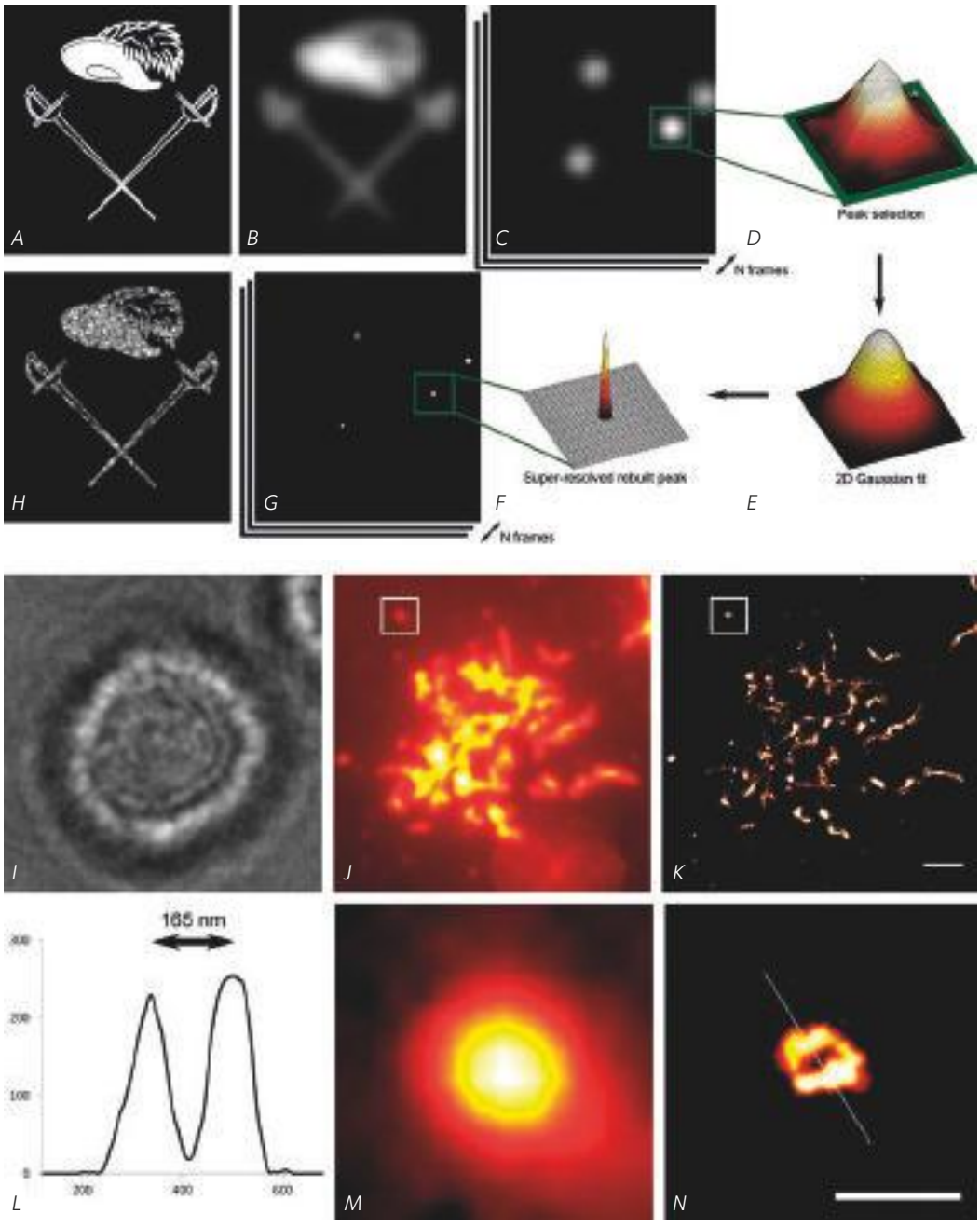

Figure 1. Concept of single molecule localisation microscopy. A musketeer coat of arms structure was simulated (*A*) from which 10,000 fluorophores were stochastically activated. A few fluorophores only are activated in each frame (*C*). Each PSF is then fitted (*D–E*) and re-plotted as a 2D-Gaussian whose width corresponds to the precision of the localisation (*F* and *G*). Many frames allow the rebuilding of a super-resolved picture (*H*) which is much more precise than the diffraction limited one (*B*). A live T cell (white light transmission given in *I*) labelled with α-CD45 ATTO655 Fabs was dropped onto a coverslip coated with non-specific antibodies. The super-resolution picture reveals 165 nm diameter ring-like structures (*K, L* and *N*) that could not be seen in the diffraction limited pictures (*J* and *M*). *M* and *N* are respectively close-ups of the white square from *J* and *K*. *L* is the intensity plot (arbitrary units) alongside the white line in *N*. Scale bars: 2 µm for *J* and *K*, 500 nm for *M* and *N*.

theoretically consist of randomly separating the fluorophores in time so that only a few appear in each frame (Fig. 1*A–H*). This separation of PSFs in time can be done in multiple ways, using dyes or fluorescent proteins with specific photo-physical properties: some can be stochastically switched 'on' and 'off' either optically or chemically, either reversibly or irreversibly (fPALM and PALM); some can blink in specific buffers, i.e. go in a long-lived dark 'off' state and only switch stochastically back into their fluorescent 'on' states for short periods of time, allowing only few to be 'on' at any given moment (STORM); some can only emit fluorescence when bound to their ligand (PAINT) (Sharonov & Hochstrasser, 2006). Many methods have already been described, and many more will be, but they all are based on this initial concept.

In a typical SMLM experiment thousands of consecutive images of the sample are acquired, with each frame only imaging a few labelled proteins at a time. The data containing the position and precision of localisation of each protein is then extracted from these images, plotted and merged into a single composite super-resolved image (Fig. 1*I–N*).

Going 3D

As most biological processes happen in three dimensions, observing their 2D projections is not always very useful. However the depth information of a fluorophore can be obtained from the shape of its PSF. Four main methods have been developed (Fig. 2*A–D*), listed here following their easiness of implementation and use (or, in the reverse order, on their axial precision): biplane microscopy, astigmatism, the double-helix PSF and interferometric PALM. For example, a cylindrical lens can be inserted into the optical path to introduce an astigmatism causing the round PSF of the fluorophore to become elliptical and the axial position can be obtained from this ellipticity.

They all share the same drawbacks, however; the axial position is gained to the detriment of both precision and resolution (Fig. 2*E–J*). Indeed, adding extra optical elements, dilating the PSF over more pixels or the increase in out-of-focus noise automatically decreases the signal-to-noise ratio, which is directly correlated to the quality of precision. Similarly, since a volume is then observed, more labelled proteins have to be separated, and an increased sampling is therefore necessary to prevent a decrease in resolution of the final super-resolved picture.

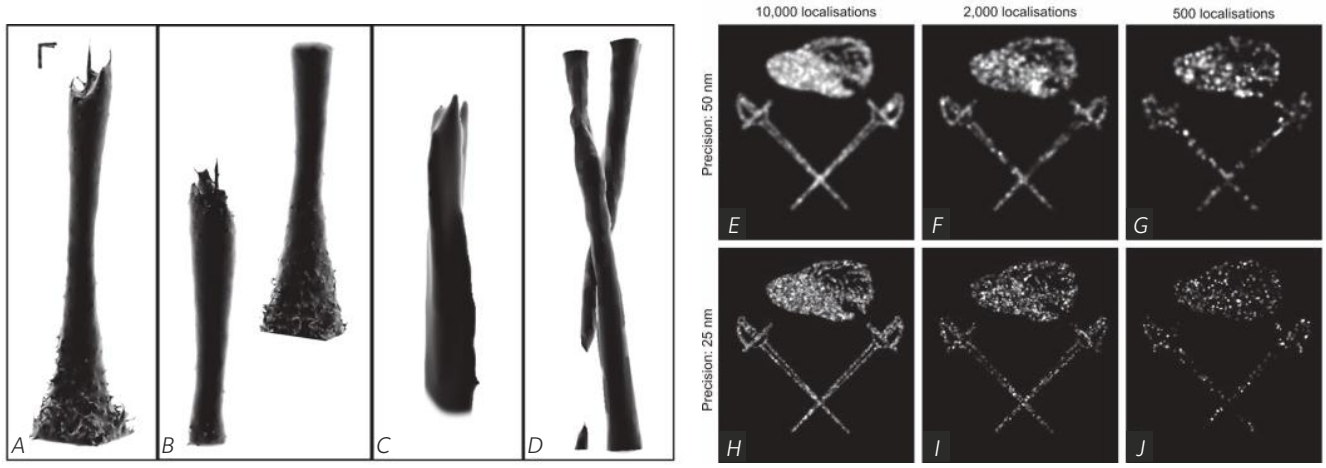

Figure 2. 3D super-resolution and resolution problem. Different 3D PSFs are used to get the extra information about the axial position of the fluorophore: usual PSF (*A*), biplane microscopy (*B*), astigmatism (*C*), and the double-helix PSF (*D*). The form of the 2D PSF observed in the image plane on the camera indicates the axial position from where the fluorophore was emitting. Simulations reveal the importance of both precision and resolution (or density of labels). For the same figure as in Fig. 1*A*, different numbers of fluorophores were activated (10,000 for *E* and *H*, 2,000 for *F* and *I*, 500 for *G* and *J*). This was simulated for different imaging protocols were the average precision of localisation would either be 25 nm (*E–G*) or 50 nm (*H–J*).

One for all: getting more than subdiffraction resolution

Advantages

Super-resolution microscopy bridges the nanoscopic world of structural biology and electron microscopy with the live cell microscopic world of confocal fluorescence microscopy. It allows observation of proteins at a typical precision of 20 nm in live cells, i.e. a 10-fold better precision than confocal microscopy, without any need to fix the sample. It has to be mentioned that precisions of 0.2 nm in a single dimension were described, through image averaging, and helped to rule out some models of the electron microscopy structure of the nucleopore complex (Szymborska *et al.* 2013).

By allowing imaging of live cells, SMLM specifically allows observation of moving proteins and study of the diffusion of single molecules *in vivo*. Single particle tracking is an easy and natural feature that can be added to any SMLM experiment.

Moreover, SMLM methods are relatively easy to implement and use. On the hardware side, a simple but stable total internal reflection fluorescence microscopy (TIRFM) platform together with a 405 nm laser is enough for 2D SMLM. Adding a single cylindrical lens would be enough to gain 3D information for most experiments. On the biological side, many usual fluorophores (such as most of the dyes from the Alexa, Cy or ATTO families) can blink in specific buffers, and primary antibodies (or nanobodies) are commonly used, as are GFP-like fluorescent molecules such as mEos, mDendra or PAmCherry. A last option that combines the brightness of organic dyes to the specificity of fluorescent proteins are the Halo and Snap enzyme systems that are genetically tagged to the protein of interest and accept with a very high affinity a ligand that can be chemically linked to theoretically any dye.

Quantification

One of the least known but major advantage of SMLM is the quantification it brings to the microscopy field. Some fluorophores such as caged dyes or mEos can be photo-activated once and imaged until they bleach. Those fluorophores thus allow counting of the precise number of labelled molecules in a cell, a cluster or a protein complex (Sengupta *et al.* 2011; Lee *et al.* 2012). The technique is still in its infancy but is giving interesting results about the stoichiometry of protein complexes *in vivo*.

Another way of determining the stoichiometry of a protein complex is to localise each of the proteins with higher precision than the size of the complex. To prevent under-counting due to non-perfect labelling efficiency, nanobodies (tiny single-domain antibodies from camelids or cartilaginous fish) with very high affinities have been used to observe the eightfold symmetry of the nucleopore complex (Szymborska *et al.* 2013).

Finally, at the core of SMLM is the ability to track and study low probability events. This is the American school bus problem (Fig. 3): when viewing Earth from space, looking specifically at the actions of American school buses on average over a day (as in bulk imaging), an observer would see them aligned in car parks, static and aggregated. This observation would not help the observer to understand the function of such devices on Earth. However, if the observer could follow only a few buses over one day, he would realise that they leave their static state and very regularly visit the same schools. These tracks would help us understand the role of the school bus. Therefore SMLM can elucidate the functions of rare events much better than bulk imaging (through either confocal or epi-fluorescence microscopy).

Drawbacks

However, SMLM is still in the early stages of development and has a few drawbacks. The first of which is the importance of the signal-to-noise ratio, which is critical in single molecule experiments and thus in SMLM. To increase the signal, very sensitive EMCCD cameras are used together with high-numerical aperture objectives (up to 1.49, in order to get as many photons as possible from each single fluorophore). Both are very expensive. However, a new statistical analysis has recently been developed to use sCMOS cameras, which are cheaper and offer a higher frame rate (Huang *et al.* 2013).

The signal can also be increased by using the brightest dyes and fluorescent proteins. However, this sometimes comes with drawbacks such as more photo-switching, less specificity, worse labelling efficiency or cell permeability issues.

Finally, decreasing the background noise is also of prime importance. This is done by using labelling protocols offering high specificity (fusion proteins with a fluorescent

Figure 3. Illustration of the American school bus problem (Google Maps®). School buses seen from the space on average during the day would be observed aligned in car parks (top panel). Without better resolution (bottom panel), the observer could not even see a single bus (arrow) and would only observe them when clustered. Without following single buses over a day, they would not be able to observe the 'rare' event of regularly driving to specific schools which would indicate their function.

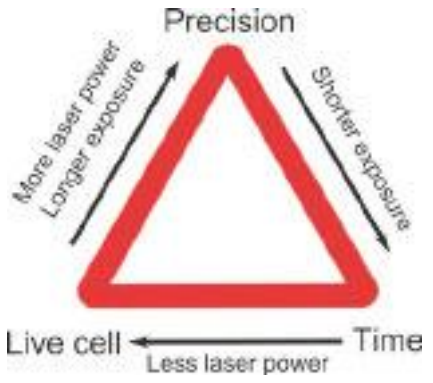

Figure 4. The triangle of compromise.

protein or a Snap/Halo enzyme tag, or very high affinity nanobodies) and by illuminating as thin as possible a region of the sample. Indeed, in epi-fluorescence, since the whole sample is illuminated, the objective collects all the out-of-focus light and the background usually obscures the signal. However, TIRFM is a technique which allows illumination of only 100 nm of the sample above the coverslip. It exploits evanescent waves produced at the site of total internal reflection of light (e.g. rays of light reflected on a lake) to excite fluorophores, the intensity of these waves decreases exponentially when moving away from the surface where the laser strikes. Similarly, a light-sheet system (illumination by a sheet of light about 1 µm thick from the side of the sample) only illuminates the focal plane, considerably reducing the out-of-focus background.

The triangle of compromise

These illumination strategies are also important for both preventing photo-bleaching of fluorophores deeper in the sample and limiting the light damage to the imaged live cell. This underlines the compromise that one has to make for each experiment between the precision required, the speed of the phenomenon to be observed and how robust the live cells are to light damage (Fig. 4). Indeed, to keep cells happily alive, the laser power and the exposure of the cells to the illumination laser need to be decreased. But to get better precision, a higher signal-to-noise ratio is required, meaning higher laser powers and higher exposure (lower frame rate). Finally, to observe a quick phenomenon, the frame rate has to be increased (lower exposure time), but to consequently keep a similar precision, the laser power needs to be increased. Thus, these three parameters have to be carefully considered for each experiment, and a compromise needs to be found.

The future

The field of SMLM is moving towards resolving these drawbacks. 3D microscopy is mostly adopted and standardisation introduced for its most sophisticated forms. Different fourth dimensions are investigated to provide even more information about the single molecules: lifetime, spectrum, dipole orientation, etc. On the hardware side, light-sheets are developed to look deeper into cells and replace TIRFM, quicker and cheaper sCMOS camera are studied to correct for their unequal noise, and adaptive optics are more frequently used to compensate for light scattering and look deeper into samples without losing precision.

On the analysis side, numerous quick and precise software packages have been developed in the last few years (Small & Stahlheber, 2014). There is a focus on the detection of higher densities of fluorophores in order to increase the sampling rate and observe faster phenomena. Also, the integration of the super-resolution data with electron microscopy and structural data is just beginning. Finally, the field is constantly developing still better controlled switchable or activatable fluorophores to increase the accuracy of quantification.

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High content screening microscopy: It's all about numbers

High content screening microscopy is a rapidly evolving technology that can be applied to extract rich quantitative information about cell form and function. This article introduces the basic concepts of this powerful imaging modality, and presents a perspective on its current use and possible future direction.

Jeremy Simpson

University College Dublin, Ireland

Biological imaging has long been a powerful tool for scientists to gain a greater understanding of the world around them. Although sensitive imaging devices such as cameras and photomultipliers are a relatively recent invention in the timeline of biological research, the early pioneers designing and constructing the first microscopes already realised the importance of being able to accurately transpose their microscopy observations into a format that could be appreciated and studied by all.

'Surely the time is right to visualise and understand these networks in a truly systematic and quantitative way?'

The first microscopists such as Robert Hooke and Anton van Leeuwenhoek, working in the late seventeenth century, were not only great technical innovators, but also immensely skilled at being able to precisely draw their observations to scale, with an incredible degree of accuracy and detail. The further refinement of microscopes and microscopy resolution through the nineteenth and early twentieth centuries allowed biologists to gain yet further insight into the organisation of cells and tissues, producing micrographs with ever more detail. Two of the great scientists in the latter part of this era were Camillo Golgi and Ramon y Cajal, whose work developing and using stains, for example silver nitrate, allowed the visualisation of cellular distribution in complex tissues such as the brain, in addition to revealing the presence of various subcellular structures, most notably the Golgi complex. Arguably the most impressive aspect of these experiments is the attention to detail in the drawings that were produced, allowing these scientists to already attempt to reconcile cell biology with physiology. However, it has been the introduction of fluorescence which has truly enhanced our ability to understand cell structure and function at the subcellular level. Fluorescence microscopy – and all its variants – is now a mainstream technology found in cell biology, developmental biology, and physiology laboratories around the world. 'Seeing is believing' is a phrase widely used in this context, reflecting the power and importance of the image to our understanding of biological systems and structures.

Despite being widely embraced by the scientific community, remarkably fluorescence microscopy has largely remained a qualitative method. This is surprising, particularly from a cell biology perspective, as the complex arrangement of molecules and organelles within cells clearly warrants a quantitative description (Murphy, 2010). The reason for this general lack of quantification is unclear – perhaps researchers believe that simply presenting a fluorescence image of a cell conveys enough information, or that quantification from an image is unnecessarily time consuming or potentially not meaningful. Fluorescence cellular imaging is also a relatively slow process, requiring sample preparation, manual identification of suitable and representative cells, careful focusing and then image acquisition and processing. As such, at first glance, it does not particularly well lend itself as a technique to rapid or systematic analysis of large numbers of samples in a quantitative manner. The

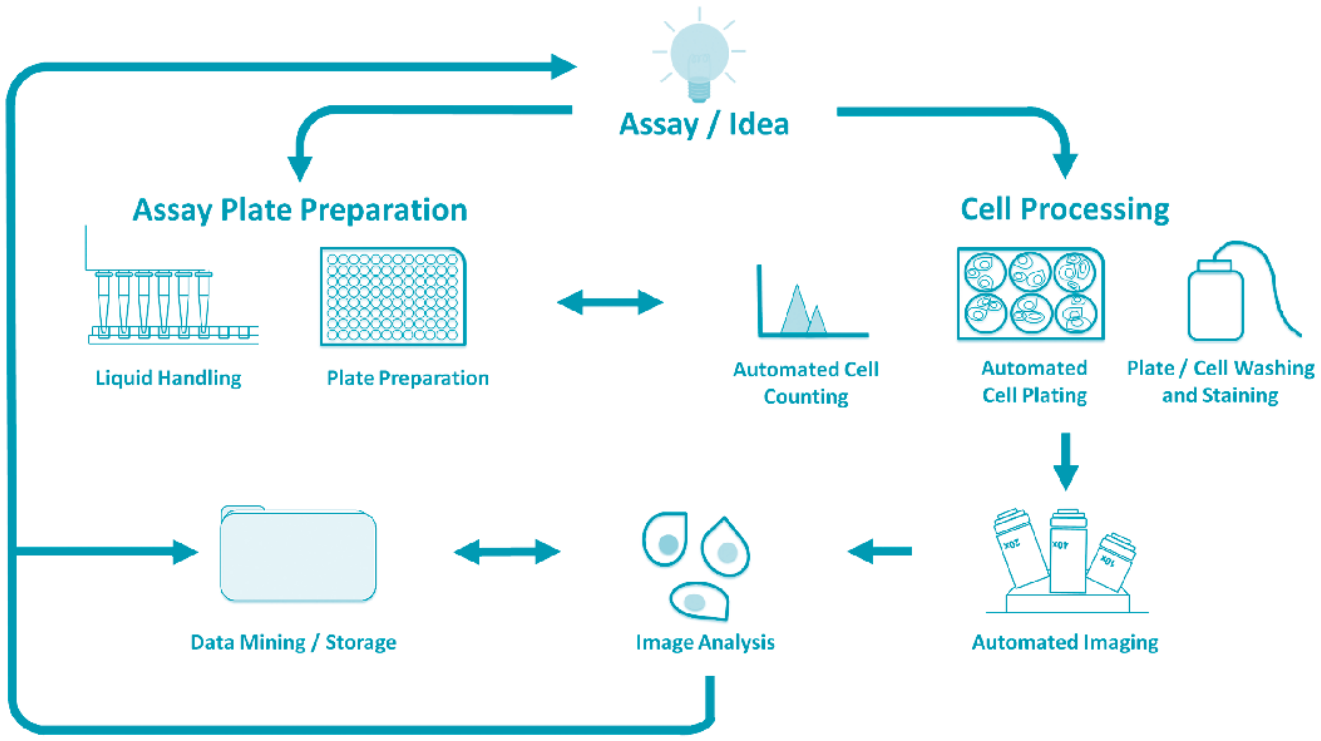

Figure 1. Schematic diagram showing a typical HCS workflow. From the initial assay or idea, multi-well plates containing assay reagents are prepared, typically using liquid handling robotics. Automation is also used for cell counting, plating and any cell processing steps. On completion of the assay, an automated microscope acquires images, which are then analysed by HCS software. The quantitative data generated are used to inform subsequent experiments.

sequencing of various genomes, most notably the human genome, potentially serves as a driver to change this perspective. Now we have knowledge of the entire complement of genes and proteins within cells, so surely the time is right to visualise and understand these networks in a truly systematic and quantitative way?

The twenty-first century has seen a remarkable evolution in fluorescence microscopy, as a response to the potentially huge amount of data that knowledge of genomes can provide. Specifically it was realised that manual microscopy – including both image acquisition and analysis – was simply not feasible if cells, their components and pathways were to be studied at a systems-wide level. The response, from a variety of individual laboratories and microscope hardware manufacturers, has been the development of totally automated microscopes (Liebel *et al.* 2003; Lee & Howell, 2006), equipped with high levels of robotics allowing plate loading, stage movement, filter changing, autofocusing and image acquisition, all without manual intervention. The development of such hardware has also aligned well with the increasing miniaturisation of experiments, with wider use of 96-, 384- and 1536-well plates for biological experiments (Mayr & Bojanic, 2009). The consequence of this evolution, of course, has been that now we are confronted with potentially several thousand or even millions of images – certainly too great a number to be manually assessed, and therefore automated image analysis methods (discussed below) have also had to be developed. Together, this integration of automated microscope hardware, coupled with automated single-cell image analysis routines encompasses the technology that we now term high content screening (HCS) microscopy. This is ever more becoming the method of choice for quantitative systems biology (Pepperkok & Ellenberg, 2006).

One of the real strengths of HCS microscopy is that it can be applied in a wide variety of biological applications, ranging from fundamental cellular pathway analysis through to drug discovery and testing. The starting point for any HCS regime is the biological assay (Fig. 1). Typically this is an experiment that has been proven to work with samples on an individual basis, or in a manual low-throughput scale. Generally the assay is simple, providing a clear and robust readout that can be easily quantified. The majority of HCS assays are fluorescence based, and utilise either cell lines stably expressing a fluorescent marker, or feature the addition of a fluorescent component (dye or antibody) that acts as a reporter for the assay (Wolff *et al.* 1996). HCS experiments are usually carried out in multi-well plates, and so robotics, and in particular liquid handling, is often employed at this stage. Plate preparation can include the dilution and dispensing of molecular reagents (such as DNA constructs for over-expression experiments or RNA interference (RNAi) reagents for gene depletion studies) through to chemical compound libraries as would be used in drug/target identification studies. Robotics at this stage can be particularly important, as generally this ensures high levels of reproducibility both across individual plates and between plates. Cells can then be added to the assay plates, and again various levels of automation (for example cell counting and cell plating) can be employed. Once the plated cells have been incubated with the reagents and the assay is complete, the plates are transferred to an automated HCS microscope. The number of images required for a screen is very much dependent on the assay itself and the type of quantification required, but a typical primary RNAi screen for example may involve the acquisition of as many as 20 sub-positions (fields of view) within each well, meaning that from a 96-well plate and in a three-colour assay,

'Converting image information into unbiased quantitative data is essential if we are to fully understand cell physiology at a complete systems level'

a total of 5760 images are collected from a single plate. The next stage in the pipeline is the analysis of the images (discussed below), and the storage and subsequent mining or interrogation of the results. In turn these results are usually used to inform subsequent experimental design and candidate selection.

At the heart of the HCS technology is the image analysis component. Indeed the 'content' in HCS refers to the fact that the image requires (quantitative) content to be extracted from it, and to be true HCS this needs to be at the single cell level. It is in this key respect that HCS differs from high-throughput screening (HTS), a similar technology in terms of general approach and throughput, but which has no requirement for extracting fluorescence data from individual cells. The typical first step in HCS image analysis is cell segmentation, often based on the signal in one of the fluorescence channels, and which allows the outline (plasma membrane) of each cell to be recognised (Fig. 2). Most commercial HCS software packages have accurate segmentation algorithms within them, although there are also several good examples of open-source solutions (Fenistein *et al.* 2008). Following this step it is then usual to identify internal structures within the cells, for example the nucleus and any other membranes of interest that have been fluorescently labelled (Fig. 2). Once relevant structures have been identified they can be quantified. The range of metrics available is now vast, but they broadly fall into three categories – object intensity, object morphology and object texture measurements. Intensity measurements are probably the most straightforward to calculate, with mean fluorescence intensity in a particular region being a relatively well-established HCS parameter. Such measurements have found particular favour with researchers investigating translocation events, for example between the cytoplasm and nucleus. These two compartments are relatively easy to segment accurately, and such assays monitoring changes in fluorescence intensity between them (in response to a perturbation) have been established. A good example of this is an assay to measure quantitatively the translocation of the tumour suppressor protein FOXO (Zanella & Carnero, 2009). Other intensity-based measurements include object and radial intensity distribution metrics, and also quantitative co-localisation of two markers. Morphological measurements include length, width, area, aspect ratio and circularity, and can be applied at either whole cell or subcellular levels. These metrics are particularly powerful to describe quantitatively changes in cell shape and even changes in the organisation of subcellular structures such as focal adhesions (Prager-Khoutorsky *et al.* 2011). The third category of quantification is object texture feature analysis, which utilises a complex series of algorithms (for example Gabor and Haralick) to highlight patterns and topographical elements within fluorescently labelled objects (Fig. 2). This quantification type is relatively widely used for the analysis of medical images, but is only now gaining traction for the analysis of biological images. It is incredibly powerful however, as it can potentially discriminate subtle cellular phenotypes from very complex images, including from cells of neuronal origin that traditionally have proved difficult to quantify (Wu *et al.* 2010). Altogether, this is now an exciting time for image analysis of HCS data, and further integration and application of these various methods is eagerly anticipated (Singh *et al.* 2014).

So what impact has HCS had in the biological sciences and what is its likely future? It is probably too early in the life of HCS to truly appreciate the richness and relevance of data that this imaging modality is giving us; however, HCS has proved to be particularly powerful when aligned with gene depletion studies (Conrad & Gerlich, 2010). HCS in combination with genome-wide RNAi experiments has been used to dissect fundamental cellular events such as endocytosis (Collinet *et al.* 2010), protein secretion (Simpson *et al.* 2012), and cell division (Neumann *et al.* 2010), providing a truly systems-level perspective on how these processes are regulated. HCS also has clear applications in understanding disease and infection (Brodin & Christophe, 2011), and recently has been applied to developmental biology, specifically the study of embryonic morphogenesis (Truong & Supatto, 2011). In the future it is a technology that is likely to be applied to more sophisticated cellular models, including three-dimensional cell assemblies such as spheroids, and also to cells growing in environments that can be tightly controlled, for example in microfluidic devices (Cheong *et al.* 2010). The image analysis aspects of HCS will also be further refined, which is important if we are to extract more of the rich information that is contained within an image. Those early microscopy pioneers embarked on a great journey of discovery, already then appreciating the importance of producing accurate biological images. This journey now continues – the image remains at the heart of everything, but the scale is now different, meaning that converting image information into unbiased quantitative data is essential if we are to fully understand cell physiology at a complete systems level.

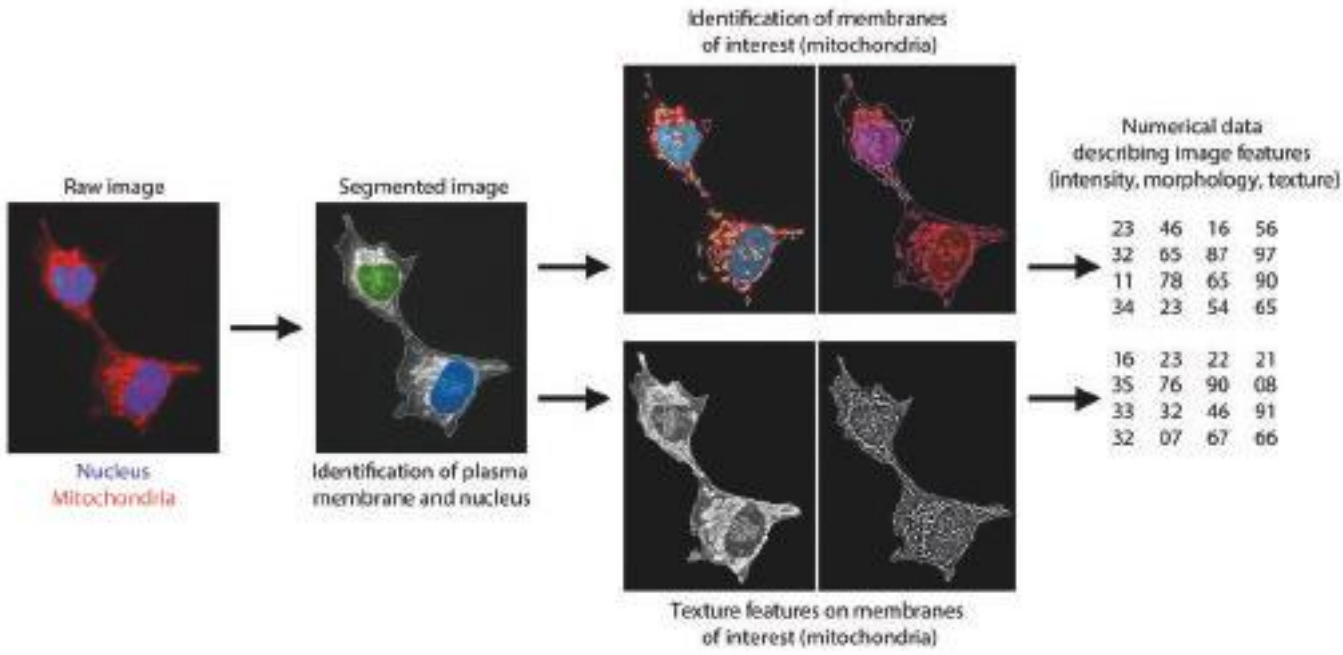

Figure 2. Example HCS image analysis routine. Raw image data (nuclei stained blue and mitochondria stained red) are analysed by software to initially segment the cell boundaries and nuclei. This allows identification of the cytoplasm containing the membranes (mitochondria) of interest. A spot detection algorithm then identifies the mitochondrial membranes and makes measurements describing their morphology; in parallel a texture feature algorithm identifies topographical features of interest. Both analysis routines provide a numerical output describing the fluorescence patterns in each cell.

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HIMaging the kidney: High resolution helium ion microscopy

Various imaging techniques have been widely used to investigate kidney structure and function. **H**elium **I**on scanning **M**icroscopy (HIMaging) constitutes a major technological breakthrough that can potentially push the imaging resolution limit beyond that of any other currently available technique.

Teodor G. Păunescu, Sylvie Breton & Dennis Brown

Massachusetts General Hospital and Harvard Medical School, USA

Maintaining body homeostasis for pH, fluid osmolality and volume, and electrolyte balance is accomplished by the kidneys through a number of active and passive membrane transport mechanisms that occur in specialized cell types located in distinct tubule segments (Brenner & Rector, 2012). Accordingly, the intricacy of kidney structure underlines this functional complexity. For example, most of the filtered salt, amino acids and glucose are reabsorbed in the proximal convoluted tubule (PT) and thick ascending limb of Henle, whereas water is reabsorbed in the PT, thin descending limb, and principal cells of the cortical and medullary collecting duct (CD). On the other hand, acid–base homeostasis is achieved by the kidneys, in concert with the lungs, via proton secretion and bicarbonate reabsorption in the PT and by intercalated cells of the CD.

'The kidney samples do not need to be covered with a conducting metal coat, thus allowing for a clearer visualization of the actual cell surface architecture'

Given the strong structure–function relationship of renal epithelial cells, exploring kidney morphology, structure and ultrastructure, including cell membrane topography, has been for decades a major way of investigating their functional characteristics. For higher resolution, conventional bright-field and fluorescence microscopy gives way to scanning or transmission electron microscopy, or to atomic force microscopy.

In recent years, scanning helium ion microscopy (HIM) has emerged as a technology that can potentially offer higher resolution imaging compared to electron microscopy (Ward *et al.* 2007). This is achieved by scanning the sample with a narrow beam of high energy helium ions instead of electrons, thus allowing the beam to focus to subnanometer dimensions. HIM has been used significantly in materials science, but only recently has it also been applied to study biological samples, such as butterfly scales (Boden *et al.* 2012), fly cuticle (Boseman *et al.* 2013), mammalian articular cartilage (Vanden Berg-Foels *et al.* 2012), cancer cells (Bazou *et al.* 2011) and renal epithelial cells (Rice *et al.* 2013).

Tissue preparation for HIM

Preparation of kidney samples from adult male Sprague–Dawley rats and adult male and female C57/BL6 mice for HIM imaging has been described in detail previously (Rice *et al.* 2013). Briefly, it involves tissue fixation by transcardial perfusion followed by immersion in aldehyde fixatives, such as glutaraldehyde (GA), modified paraformaldehyde (PFA)–lysine–periodate (PLP) (Rice *et al.* 2013), or modified Karnovsky's fixative containing 2.5% GA and 2% PFA. Small pieces of fixed tissues are then dehydrated in graded alcohol (methanol or ethanol) and subjected to critical point drying. An important feature of HIM, compared to conventional scanning electron microscopy (SEM), is that the kidney samples do not need to be covered with a conducting metal coat,

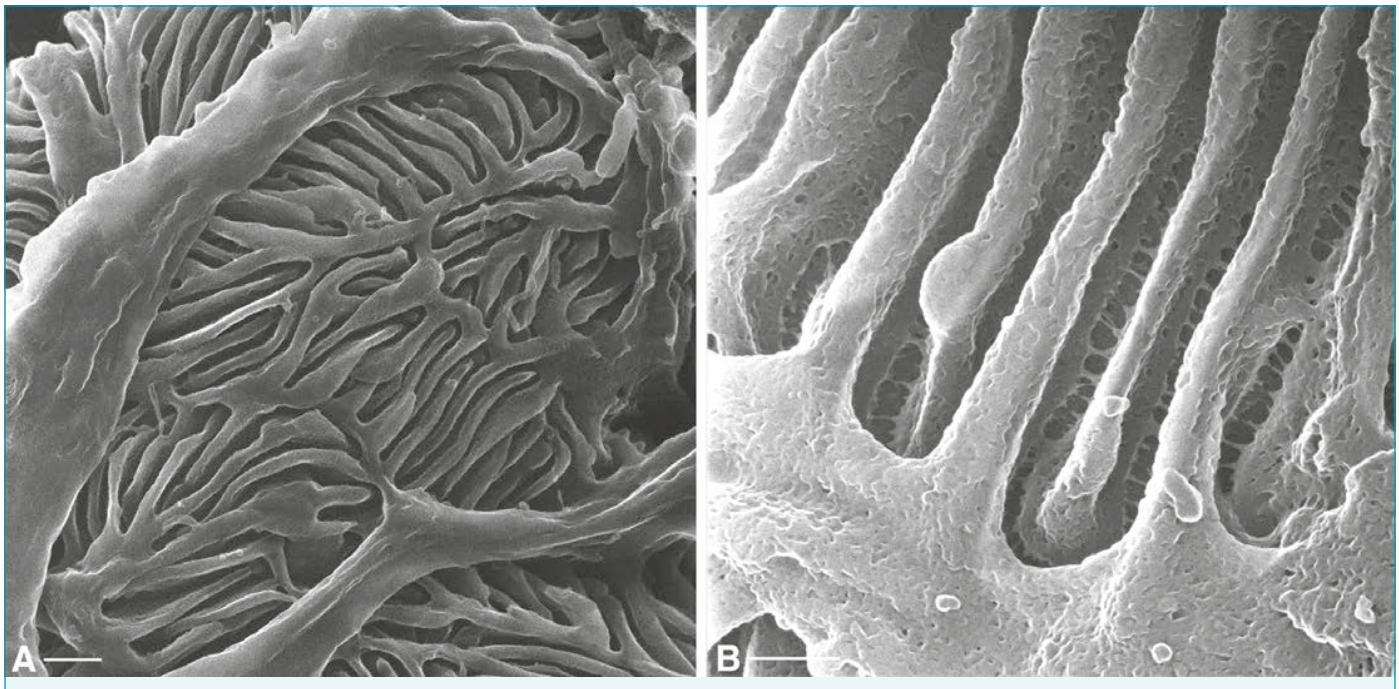

Figure 1. HIM imaging of a rat kidney glomerulus. *A*, podocyte interdigitations and foot processes (scale bar, 500 nm). *B*, higher magnification image of a kidney reveals the filtration slit diaphragm of the podocyte which appears as numerous lateral 'bridges' crossing between adjacent podocyte foot processes (scale bar, 200 nm).

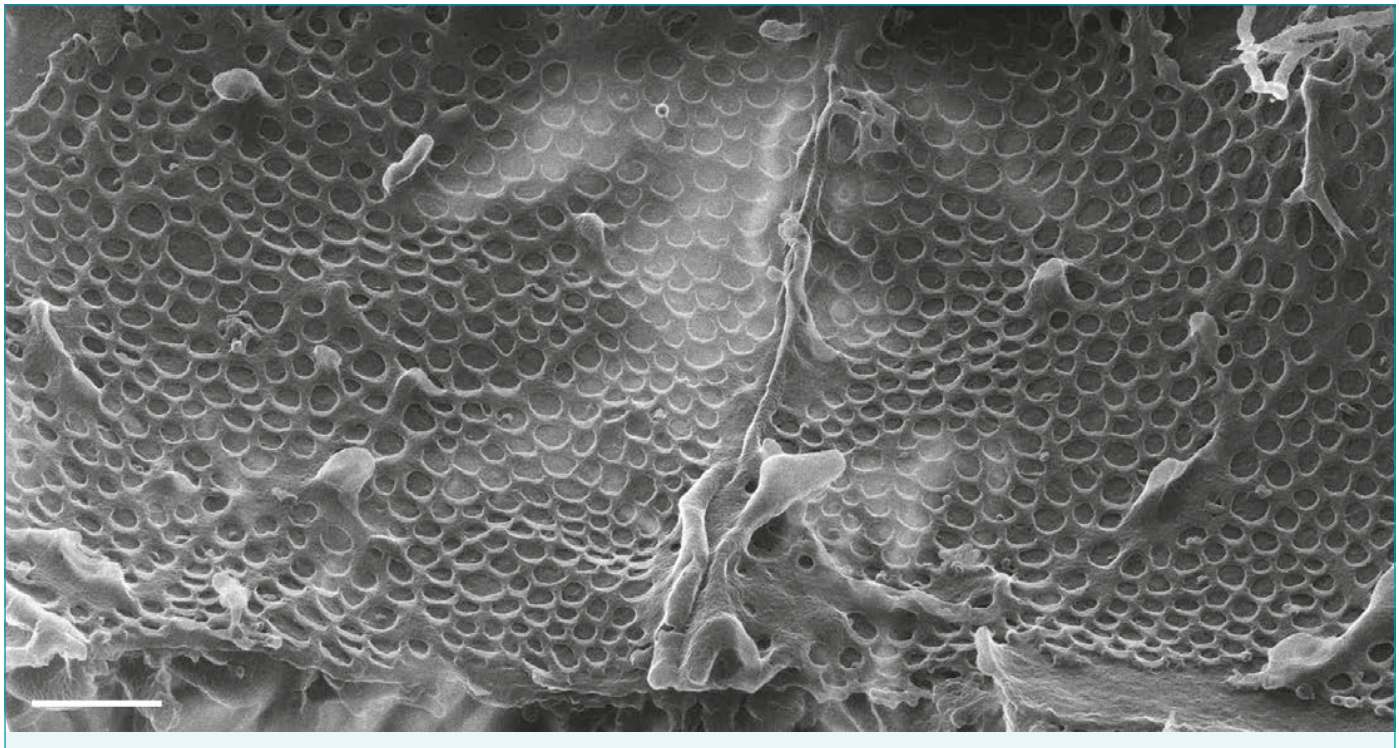

Figure 2. Luminal view of a glomerular capillary from the rat kidney shown in Fig. 1*B* revealing adjacent endothelial cells covered with numerous round fenestrations. The tight junction separating the two cells appears as an elevated ridge (scale bar, 500 nm).

thus allowing for a clearer visualization of the actual cell surface architecture. HIM was performed as previously described (Rice *et al.* 2013), yielding high resolution images of the renal glomerulus, PT and CD, whose quality and sharpness greatly exceed those of images obtained by standard SEM (see for example Madsen *et al.* 1988).

HIMaging of the glomerulus

The renal glomerulus is the site of plasma ultrafiltration across capillaries. The blood vessels in the glomerulus are surrounded by podocytes, specialized epithelial cells characterized by the presence of highly interdigitated, branching foot processes. The spaces between these processes represent the location of the filtration slit diaphragm (Brenner & Rector, 2012). Figure 1*A* shows the glomerular podocyte foot processes imaged by HIM in the cortex of a rat kidney. Increasing the magnification allows visualization of the podocyte filtration slit, which appears as a ladder-like structure

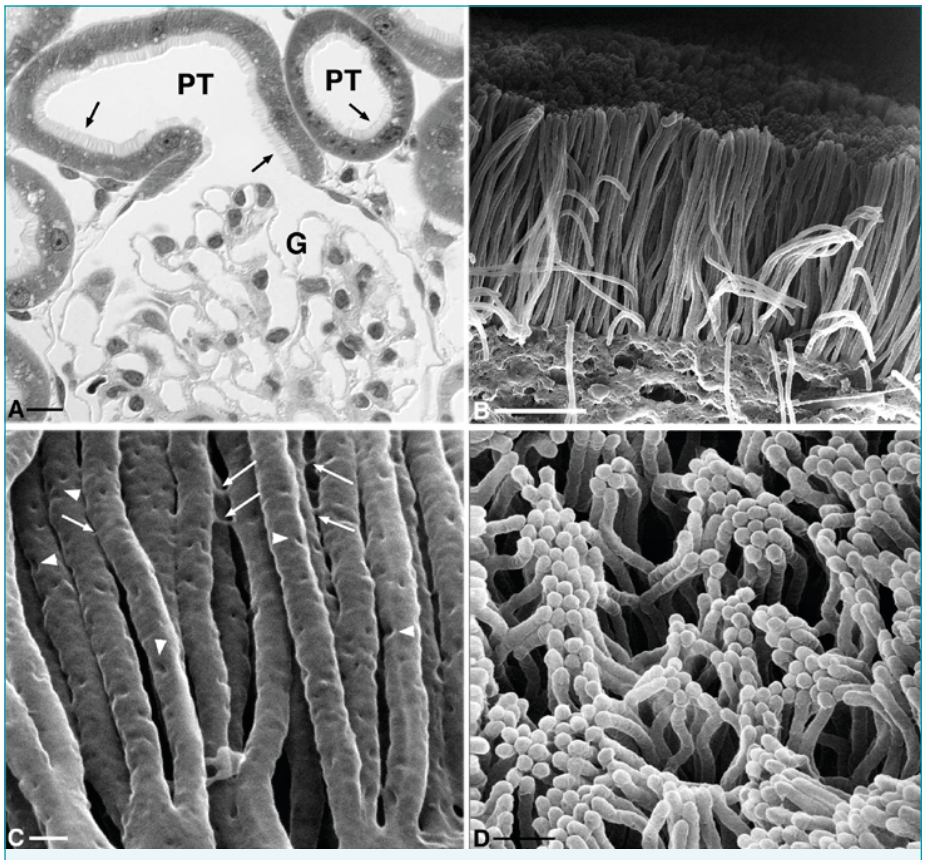

Figure 3. The brush border microvilli of the rat proximal convoluted tubule. *A*, phase contrast micrograph showing a glomerulus (G) and adjoining proximal tubules (PT) with the brush border indicated by arrows (scale bar, 10 µm). *B–D*, HIM imaging of PT brush border microvilli . *B*, low magnification image, showing that the apical surface of PT cells is covered by a dense array of microvilli (scale bar, 1 µm); *C*, high magnification revealing micropits (arrowheads) on the microvillar surface as well as thin filaments joining adjacent microvilli (arrows) (scale bar, 50 nm); and *D*, luminal view showing the clustering of the apices of the microvilli (scale bar, 200 nm).

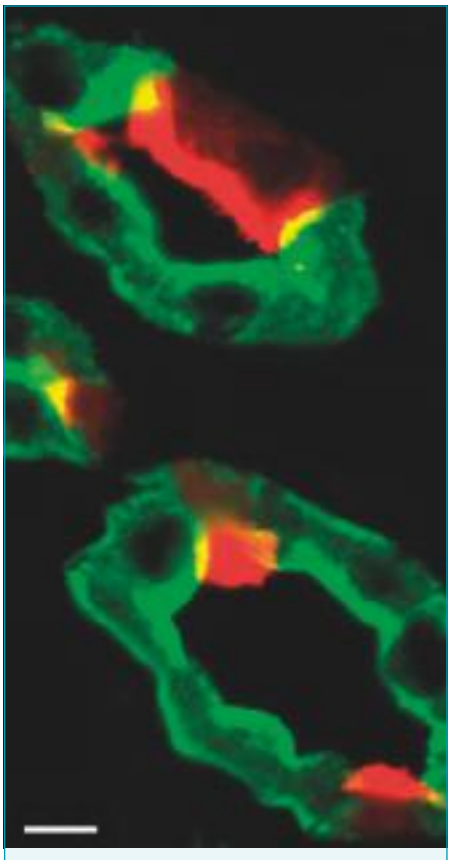

Figure 4. Dual immunofluorescence staining for collecting duct cell type markers in rat kidney inner medulla. Principal cells are identified by staining with an antibody against the aquaporin-2 water channel (green), whereas the less numerous intercalated cells express the proton pump, or vacuolar H^+-ATPase (V-ATPase, red) (scale bar, 10 µm).

stretching between adjacent foot processes (Fig. 1*B*). The blood-facing surface of endothelial cells of the renal glomerular capillary from a rat kidney (Fig. 2) shows the tight junction separating two adjacent cells and the round fenestrations that typify these glomerular endothelial cells. These fenestrations are 60–90 nm in diameter (Rice *et al.* 2013) and they render the endothelium freely permeable to water and small solutes.

HIMaging of renal tubules

The proximal convoluted tubule (PT), the first segment of the renal tubule after the glomerulus, is highly active in membrane transport processes that undertake the reabsorption of the ultrafiltrate that is delivered to the PT from the glomerulus. The PT is involved in the reabsorption of water, ions and organic solutes, including glucose and amino acids (Brenner & Rector, 2012). PT cells are characterized by the presence of a highly developed brush border – a layer of densely packed microvilli that cover the apical surface of the cell, considerably increasing its surface area (Fig. 3*A*). The brush border is seen in great detail in HIM images: Fig. 3*B* shows the apical pole of a PT cell from a rat kidney. Increasing the magnification reveals the detailed morphology of PT brush border microvilli, including microscopic depressions on their lateral surface, which we termed micropits, and also thin filaments that appear to connect adjacent microvilli (Fig. 3*C*). When imaged from the luminal side, the tips of the microvilli are seen aggregating together in clusters of variable numbers (Fig. 3*D*).

The collecting duct (CD) is the most distal portion of the renal tubule, and is active in the transport of water, sodium, chloride, potassium, protons and bicarbonate. The CD comprises two distinct cell types that fulfil two major physiological functions: principal cells (PCs) mediate water reabsorption, thus regulating body water balance, and intercalated cells (ICs) are responsible for proton secretion and bicarbonate reabsorption, and hence body pH homeostasis, as reviewed extensively elsewhere (Wagner *et al.* 2004; Fenton & Knepper, 2007; Brown et al. 2012). PCs and ICs express different sets of transport proteins (Fig. 4), including the proton pump V-ATPase (red) located in the apical pole of ICs, and aquaporin 2 (green) located in the apical and basolateral membrane of PCs. ICs and PCs also show considerable differences in their respective morphologies, which are highlighted very clearly by HIM. A PC is characterized by the presence of a single central cilium, on average around 3 µm long (Rice *et al.* 2013) and numerous short microvilli, whereas an IC has no cilium, but instead an elaborate network of apical microplicae and infoldings (Fig. 5*A*). The size and number of these microplicae correlate with the level of activation of transmembrane transport in IC, which in turn correlates with the rate of exocytosis of specialized vesicles that contain the V-ATPase (Wagner *et al.* 2004; Brown et al. 2009). In this respect, the IC shown at higher magnification in Fig. 5*B* is more representative of a stimulated animal – e.g. in response to elevated cAMP levels (Paunescu *et al.* 2010) – although different ICs usually reveal various degrees of activation even under 'baseline' conditions.

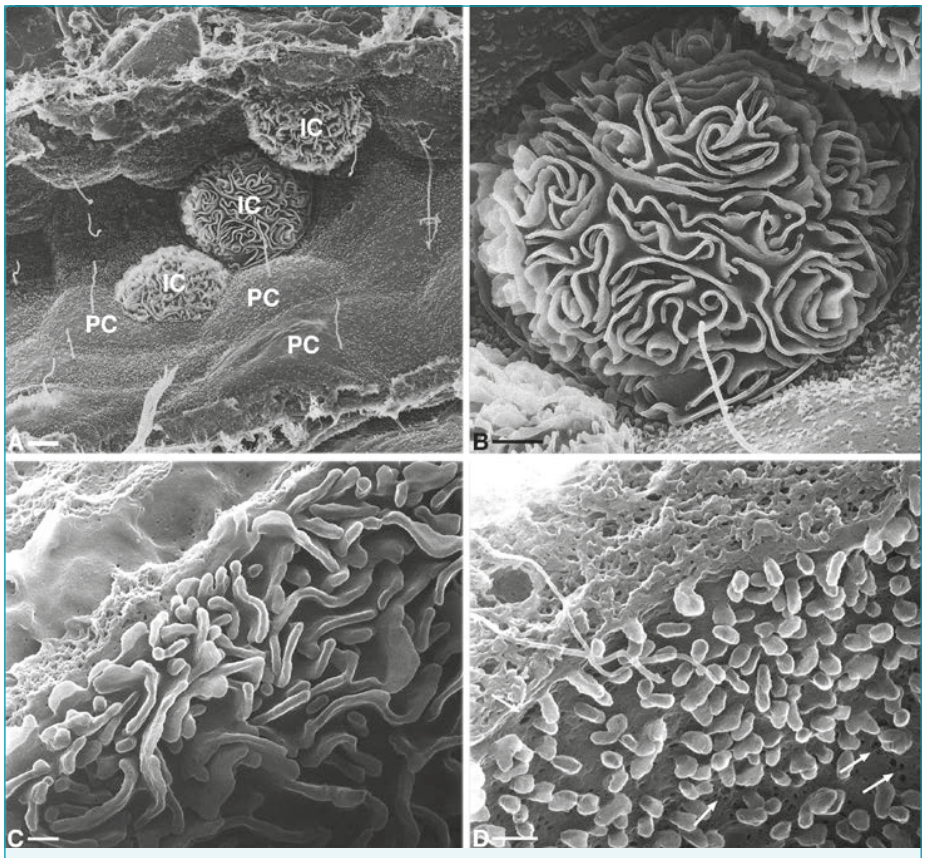

Figure 5. HIM imaging of the different cell types of the collecting duct of rat kidney. *A*, lower magnification image showing principal cells (PCs) whose apical surface features a long central cilium and short microvilli, whereas intercalated cells (ICs) are characterized by a complex network of apical microplicae (scale bar, 2 µm). *B*, intermediate magnification image of the central IC shown in *A* (scale bar, 1 µm). *C* and *D*, high resolution images of rat collecting duct cell apical membranes showing intercalated cell dense ruffles and microplicae (*C*), and principal cell short microvilli and membrane depressions (arrows) which may constitute the sites of exocytic or endocytic events (*D*) (scale bars, 200 nm).

Given the number and size of the microplicae, the flat region of the IC apical plasma membrane is barely visible (Fig. 5*C*). High resolution HIM imaging of a PC (Fig. 5*D*) better illustrates their stubby apical microvilli and exposes subapical cytosolic structures. The depressions seen on the surface of the PC membrane, frequently found at the base of the microvilli, are thought to constitute the sites of exocytic or endocytic events.

Conclusion

We applied the relatively new technique of helium ion scanning microscopy successfully to study the rodent kidney. Our results show that this novel technology advances the field appreciably by improving the resolution with which uncoated biological specimens can be imaged. We refer to the resulting micrographs as 'HIMages'. Given the remarkable quality, detail definition, and sharpness of such HIMages, we anticipate that expanding the use of this technology for investigating biological samples has the potential to further our understanding of their detailed structure and function.

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Adjunctive intracoronary imaging

Coronary heart disease is the UK's biggest killer. The Imaging modalities discussed in this article not only play an important role in pathophysiological research, but also in the effective diagnosis and treatment of patients.

Kalpa De Silva

Ashford & St Peter's Hospitals NHS Foundation Trust, UK

Philippa Howlett, Fiona Hatch & Michael Mahmoudi

University of Surrey, UK

'The development of adjunctive intravascular imaging techniques has enabled clinicians and scientists to gain a better anatomical understanding of any given coronary lesion'

Coronary angiography is an x-ray based technique whereby a catheter is passed through either the radial or the femoral artery into the coronary circulation and visualization is achieved through the injection of iodine-based contrast into the lumen under x-ray guidance. It is currently the gold standard method for imaging the epicardial coronary arteries. It remains the investigation of choice in patients suspected of having obstructive narrowings in the coronary arteries.

Such narrowings are referred to as 'lesions' and are characterized by the build-up of cholesterol, vascular smooth muscle cells and inflammatory cells. The efficacy of coronary angiography is limited by its two-dimensional representation of a highly dynamic three-dimensional structure. The development of adjunctive intravascular imaging techniques has enabled clinicians and scientists to gain a better anatomical understanding of any given coronary lesion, and to optimize the management of patients presenting with stable angina, unstable angina, or myocardial infarction who undergo coronary stenting as part of their treatment. The three main adjunctive intracoronary imaging modalities include greyscale intravascular ultrasound (IVUS), frequency domain optical coherence tomography (FD-OCT), and near-infrared spectroscopy (NIRS).

Intravascular ultrasound

Intravascular ultrasound (IVUS) is a catheter-based device that utilizes ultrasound frequencies of 20–40 MHz to create an image within a coronary vessel lumen (Fig. 1). The technology has been in clinical use for approximately two decades and is now the mainstream adjunctive technology in the cardiac catheterization laboratory. The technique enables precise measurements of the vessel and plaque anatomy. The addition of advanced spectral analysis, so called virtual histology IVUS (VH-IVUS), to this methodology has enabled quantification of the contents of coronary lesions into the necrotic core consisting predominantly of cholesterol-rich particles, calcium, fibrous tissue and fibro-fatty tissue (Fig. 2).

IVUS luminal area measurements may be utilized in the assessment of lesions of intermediate severity. As IVUS provides morphological assessment of coronary lesions, its role in haemodynamic assessment is limited by its inability to include lesion length and to assess lesion geometry and the amount of myocardium supplied by the coronary artery in question. This has been borne out in recent studies demonstrating that IVUS accurately identifies non-functionally significant lesions for which coronary stenting can be safely deferred, but it cannot accurately predict haemodynamically significant lesions and therefore should not solely be used to justify treatment of a coronary lesion (Kang *et al.* 2011).

IVUS is being increasingly recognized as an important tool to optimize the treatment of patients undergoing coronary stenting. This is particularly the case where complex coronary artery disease is present and subsequent stenting procedures will involve complex lesions such as bifurcation lesions and lesions

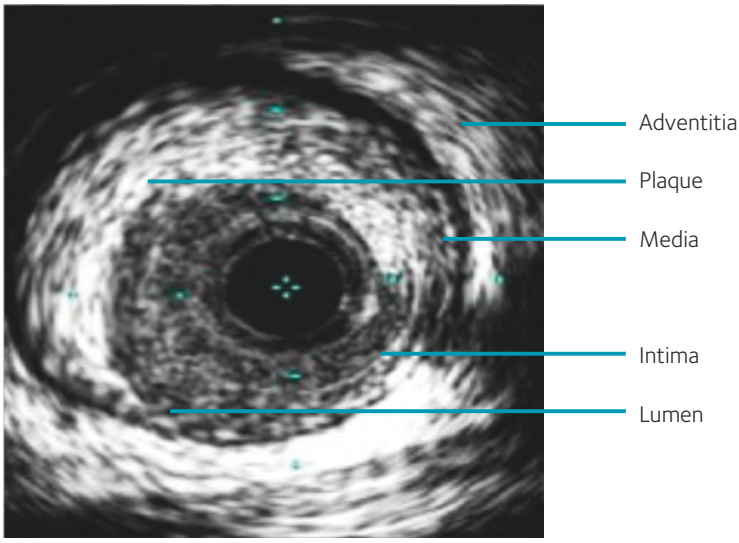

Figure 1. Greyscale intravascular ultrasound of a coronary artery demonstrating the lumen, intima, media, adventitia and plaque.

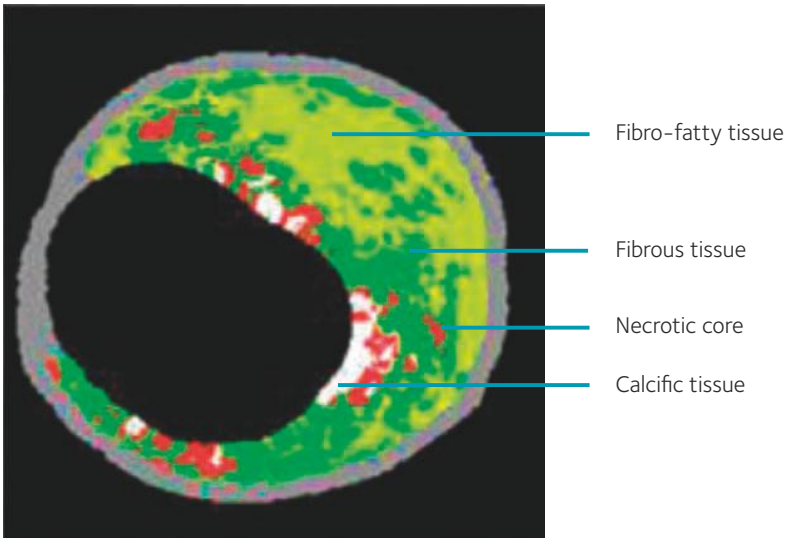

Figure 2. Virtual histology intravascular ultrasound provides additional data regarding the composition of the plaque. The colour coding describes the components of the plaque with the necrotic core appearing as red, calcium appearing as white, fibrous tissue appearing as green and fibro-fatty tissue appearing as lime.

located within the left main coronary artery. In such populations, IVUS has been shown to reduce the rates of stent thrombosis in both bare-metal and drug-coated stent platforms, which contain anti-proliferative agents on their surface in order to reduce the rate of stent narrowing. It does so by the identification of inadequate stent expansion and/or stent apposition against the vessel wall, as well as by reducing mortality (Uren *et al.* 2002; Cheneau *et al.* 2003; Steigen *et al.* 2006; Doi *et al.* 2009; Park *et al.* 2009). Furthermore, VH-IVUS has been used to identify coronary lesions at risk of rupturing or eroding and leading to unstable angina or myocardial infarction (Kovanen *et al.* 1995).

Frequency domain optical coherence tomography (FD-OCT)

The use of VH-IVUS is being progressively surpassed by FD-OCT. This technology utilizes a near-infrared light source to image 54 mm of any blood vessel in just 2.7 s with image resolution (10–15 μm) that is 10-fold greater than IVUS (Yabushita *et al.* 2002) (Fig. 3). In clinical studies of patients presenting with unstable angina or myocardial infarction, FD-OCT has been demonstrated to be superior to IVUS in detecting fibrous cap disruption and erosion as well as identifying intracoronary thrombus (Kubo *et al.* 2007). Although FD-OCT cannot penetrate into the

Glossary

Coronary stents:

Alloy scaffolds that stretch coronary narrowings & keep them patent.

Complex CAD:

Extensive coronary narrowing caused by the deposition of cholesterol in the wall of these arteries.

Fibrous cap:

Connective tissue matrix enclosing the atherosclerotic plaques.

Bioabsorable scaffolds:

Biodegradable stents that are currently being studied as an alternative to alloy scaffolds.

Showering the distal vasculature:

The downstream spread of thrombo-embolic debris that can lead to capillary occlusion.

'IVUS, FD-OCT and NIRS have provided a paradigm shift in the invasive management of patients presenting with obstructive coronary artery disease'

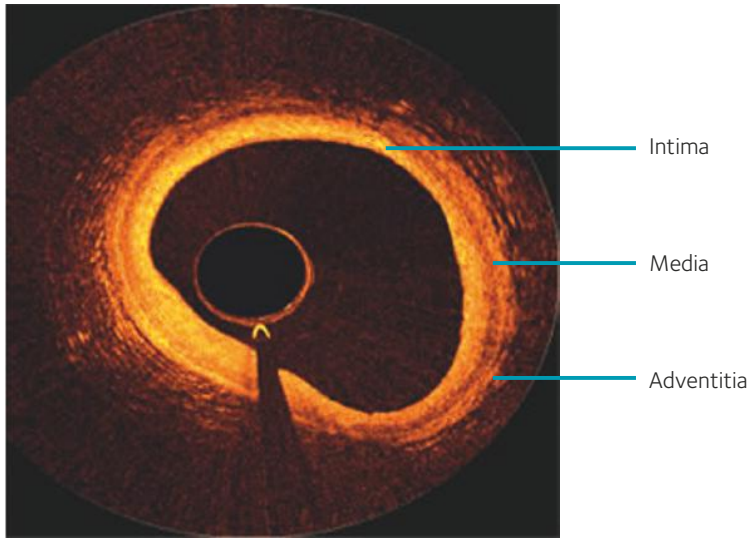

Figure 3. Optical coherence tomography allowing precise endoluminal definition.

vessel wall as far as IVUS, it provides superior endoluminal definition and thus accurately delineates all aspects of plaque morphology including fibrous tissue, lipid accumulation and calcific deposition. In view of the improved spatial resolution, FD-OCT can be selectively used in specific scenarios.

Prior to stent implantation, FD-OCT can provide quantitative measurements of lesion length and reference vessel lumen diameter, which may guide stent selection. Moreover, when undertaken after stent deployment, FD-OCT may be used to optimize results by evaluating for under-expansion, strut mal-apposition, and edge dissection, which are important potential substrates for stent thrombosis. FD-OCT is also playing an increasing role in the assessment and deployment of evolving stent platforms such as the bioabsorbable scaffold technology.

Near-infrared spectroscopy

NIRS provides a colour map of the arterial wall permitting visualization and quantification of cholesterol core-containing coronary plaques (LCP), which is not seen using conventional coronary angiography alone (Fig. 4). In one study, of those lesions containing LCP, 16% were found to extend beyond conventional angiographic margins (Dixon *et al.* 2012). LCPs are prone to rupture and can complicate stenting procedures due to showering and obstructing the distal vasculature with lipid containing particles and lead to procedure-related myocardial infarction. In this setting, identification of LCPs using NIRS has been shown to predict adverse events. Patients with large LCPs have a 12-fold increased relative risk of peri-procedural myocardial infarction. This contrasts with a 3.5-fold relative risk using plaque characteristics assessed by standard angiography (Goldstein *et al.* 2011).

The clinical niche of NIRS remains to be determined. The CANARY trial (NCT01268319), a phase-2, prospective, randomized trial investigating a NIRS-guided embolic protection device to prevent peri-procedural myocardial infarction in patients with high-risk LCPs, will shed further light in the clinical applicability of NIRS.

Conclusion

IVUS, FD-OCT and NIRS have provided a paradigm shift in the invasive management of patients presenting with obstructive coronary artery disease. The clinical studies have been unanimous in demonstrating that the use of such technologies is associated with improved patient outcomes. Advances in technology are likely to ultimately provide multiple imaging modalities that can be combined on a single delivery system to allow a more comprehensive intravascular assessment and further optimize procedural outcomes. A number of studies are currently underway to determine whether characterization of any given coronary lesion using such techniques may be combined with novel, blood based biomarkers that could be used as a potential tool in identifying patients at risk of a myocardial infarction. Such patients include those with conventional risk factors for coronary artery disease such as hypertension, diabetes mellitus, hyperlipidaemia, smoking and a positive family history for coronary artery disease.

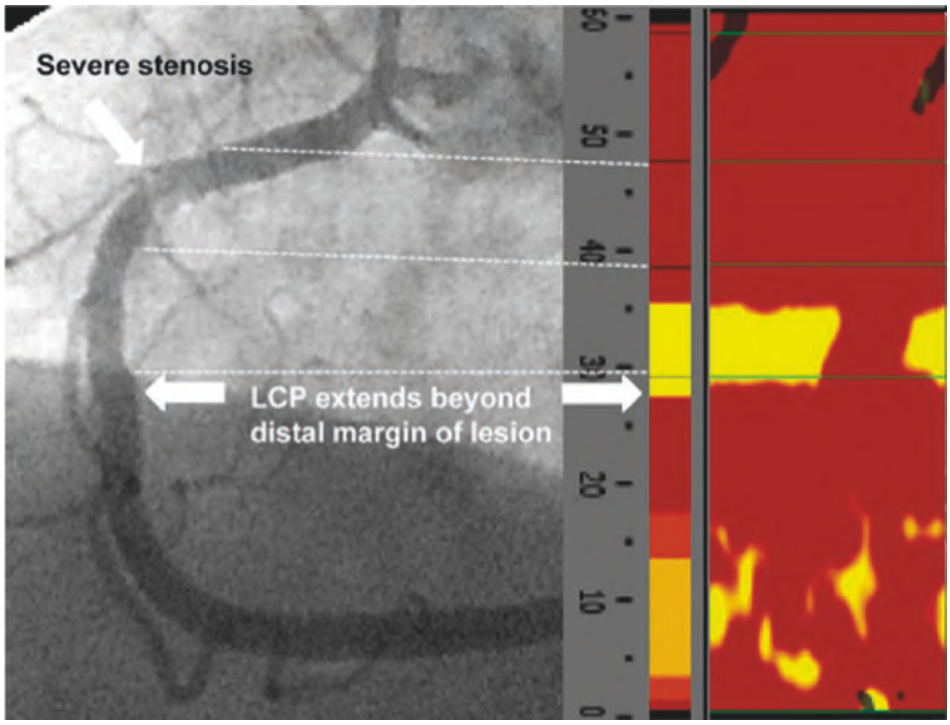

Figure 4. Left, an angiographic appearance of the right coronary artery with a severe lesion (stenosis) in the proximal segment of this vessel. Right, a near-infrared spectroscopy of the right coronary artery. Lipid core-containing coronary plaques can be seen on the colour map in yellow extending well beyond the lesion, an appearance that cannot be appreciated using conventional angiography. Image kindly provided by Dixon *et al.*

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Developing light sheet microscopy in an advanced imaging facility

Light Sheet Microscopy offers unprecedented temporal resolution for in toto imaging of live specimens. While the advantages of this relatively simple microscopic set up are self-evident, users should be mindful of the sheer quantity of data generated and careful planning is necessary.

Kevin O'Holleran
University of Cambridge, UK

At the Cambridge Advanced Imaging Centre (CAIC) we are building a complete portfolio of advanced fluorescence imaging, from two-photon to super-resolution and light sheet microscopes (LSMs). The mission of the centre is to make available the most advanced imaging techniques to a wide variety of life sciences by designing and developing the instruments in-house by an interdisciplinary team of physicists, engineers, computing scientists and biologists. Of all the techniques we are implementing, light sheet microscopy is our primary focus due to the incredible range of bioimaging applications it enables.

'The relative simplicity of a LSM to other systems has made it popular as a DIY microscope'

There has been a significant amount of excitement surrounding light sheet microscopy over the last few years. Although not a new technique, having appeared in various guises since the early 1900s, it has recently gone from strength to strength, enabling a broad range of biological imaging with unprecedented temporal resolution of live specimens, both *in vivo* and *in toto*. The primary advantage to light sheet microscopy is its combination of speed and low photodamaging effects, the latter being particularly essential for imaging of whole live organisms.

In recent years there has been an abundance of incredible imaging made possible by LSMs. Through use of multiple light sheets an entire larval zebrafish brain was imaged, via a genetically encoded calcium indicator, in 3D at a speed of 0.8 Hz continuously for an hour whilst capturing 80% of all neurons at a single-cell resolution (Ahrens *et al.* 2013). This fast and long term imaging combined with multiple sheets also makes cell lineage tracking possible in full *Drosophila* embryo development, by imaging in 3D once every 20 s over a number of hours (Krzic *et al.* 2012). Speed, in the case of imaging *Drosophila*, is essential given how often and quickly mitotic divisions occur, taking up to only a minute to complete. An example of a *Drosophila* embryo imaged with our LSM (see Fig. 2C). LSMs equipped with high NA excitation and observation lenses have also enabled fast 3D imaging of mitosis in single cells, with each volume measurement taking only one second (Planchon *et al.* 2011).

In order to operate over a wide variety of specimens and applications, LSMs come in many different configurations, but the principle with which they all work is the same. At this point I will discuss briefly the general principles of how light sheet microscopes work and some common issues with their application but I will keep the technical details brief and concentrate on the practicalities, benefits and impact of using LSMs in an imaging facility. For a fuller review of different types of LSM configurations and their use in developmental biology see Huisken & Stainier (2009).

The mechanism through which LSMs achieve fast, low bleaching, imaging is through the illumination of a fluorescent sample from a direction orthogonal to the detection objective lens. See Fig. 1 for an illustration of

Figure 1. Light sheet microscopy works by illuminating a sample from a direction orthogonal to the observation lens and detection path. The illustration shows how this compares with a typical confocal microscope configuration which uses the same objective lens for both excitation of the sample and collection of emitted fluorescence. For clarity, the confocal configuration (left) only shows three points being excited (with blue light) and one point emitting light (green); in reality the entire plane requires scanning, so a 512 × 512 image requires 262,144 points to be separately excited, with the emitted fluorescence being measured by a photomultiplier tube. A typical light sheet configuration (right) only requires one excitation using a sheet of light (shown in blue) generated by a separate lens orthogonal to the collection objective. The emitted light (green) is collected and imaged onto a camera chip where the entire 2D section is imaged in one exposure.

such a geometry compared with a confocal system. This illumination forms a sheet of light which excites only a thin slice of the sample. The excited fluorescent markers emit light and the detection objective images the fluorescence onto a large multi-megapixel camera chip. The result is an optically sectioned image, captured with a single exposure of laser light formed into a sheet and imaged with a camera exposure typically of tens of milliseconds.

Compare this with a laser scanning confocal microscope: a 1000 × 1000 image requires a million measurements, each measurement requiring the sharp focus of laser light for several microseconds, with sectioning achieved by the rejection of out-of-focus light. This comparison is a little over-simplified but it demonstrates clearly the principle advantages over laser scanning confocal systems.

Like all techniques, LSMs have their disadvantages. A significant one is non-uniformity of image properties caused by non-uniformity of the light sheet used. A common non-uniformity is the varying thickness, and intensity, of the light sheet across the direction of propagation. Light sheets are thinner, and brighter, at the focal plane and gradually get thicker either side of the focus. Exciting a sample with such a light sheet results in an optically sectioned image with varying section thickness, making the edges of the image appear more blurred than the centre. Other non-uniformities are caused by variations in the structure of the sample being excited. As a light sheet propagates in a sample, it can be absorbed and scattered, leading to artefacts such as shadows being cast across the image as streaks and further thickening of the light sheet. These problems can be overcome to some extent by using different types of beams, such as Bessel beams (Fahrbach *et al.* 2010), which have a longer depth of field than normal Gaussian beams; by using multiple light sheets incident from different directions (Huisken & Stainier, 2007); by using two-photon excitation (Truong *et al.* 2011) for deeper penetration and less scattering; or even by using a combination of the above. All these approaches can indeed both improve image quality deeper into the sample (as seen from the excitation lens) and provide more isotropic image resolution, but at a cost of higher exposure to laser light, albeit still orders of magnitude less than confocal laser scanning systems. However, these improvements come at a cost of increased instrument complexity and expense.

Putting the more advanced variants of LSMs aside, the relative simplicity of a LSM to other systems has made it popular as a DIY microscope, as evidenced by the openSPIM project (Pitrone *et al.* 2013) which details exactly how to build your own selective plane illumination microscope (SPIM), a type of light sheet microscope that utilises a cylindrical lens to form a fixed light sheet with samples mounted vertically in a rotatable holder (Huisken *et al.* 2004). The openSPIM project lists what parts to use and how to put them together, and even provides control software through the Micromanager platform. A typical openSPIM experiment requires samples to be suspended in an agarose cylinder which can then be immersed in water (or other similar refractive index medium) and moved and rotated relative to horizontally mounted lenses and a vertical fixed light sheet. Although suitable for *Drosophila* and zebrafish embryos it is not suitable for samples that cannot be suspended in agarose such as chick or mouse embryos. Deviating from the openSPIM design does, however, require a reasonable knowledge of optics and the hardware and software platform being used for controlling the system.

At CAIC we are building different types of LSMs, designed by ourselves and our collaborators. The first system we have built is aimed at imaging a wide range of model organisms and as such was built to allow flexible sample mounting. The objective lenses are mounted at 45 degrees from the vertical and samples are simply mounted on a bar in a Petri dish (the configuration can be seen in Fig. 2A). This only allows illumination from one direction and as such we will soon be including a two-photon imaging mode to allow deeper penetration of the light sheet into the sample and removal of some shadowing effects.

Although the speed and low bleaching offered by LSMs enable a wide range of exciting biological imaging investigations it does leave the user with one big headache: enormous amounts of data. For example, the LSM we have built at CAIC can generate just over 800 Mb per second at its fastest (full frame at 100 fps or smaller frames even faster) and although different applications require different numbers of time points, colour channels and duration, we find that typical experiments result in several terabytes of raw image data. For example, imaging the retina of a 1.5 day post-fertilisation zebrafish embryo (Fig. 2B) for 48 h imaging 532 µm × 532 µm × 100 µm at a resolution of 0.26 µm × 0.26 µm × 0.5 µm every 2 min amounts to approximately 2 terabytes per channel. It won't be uncommon for multi-channel LSM data sets to reach 5 Tb of data and many hundreds of thousands of images, particularly for developmental biology applications.

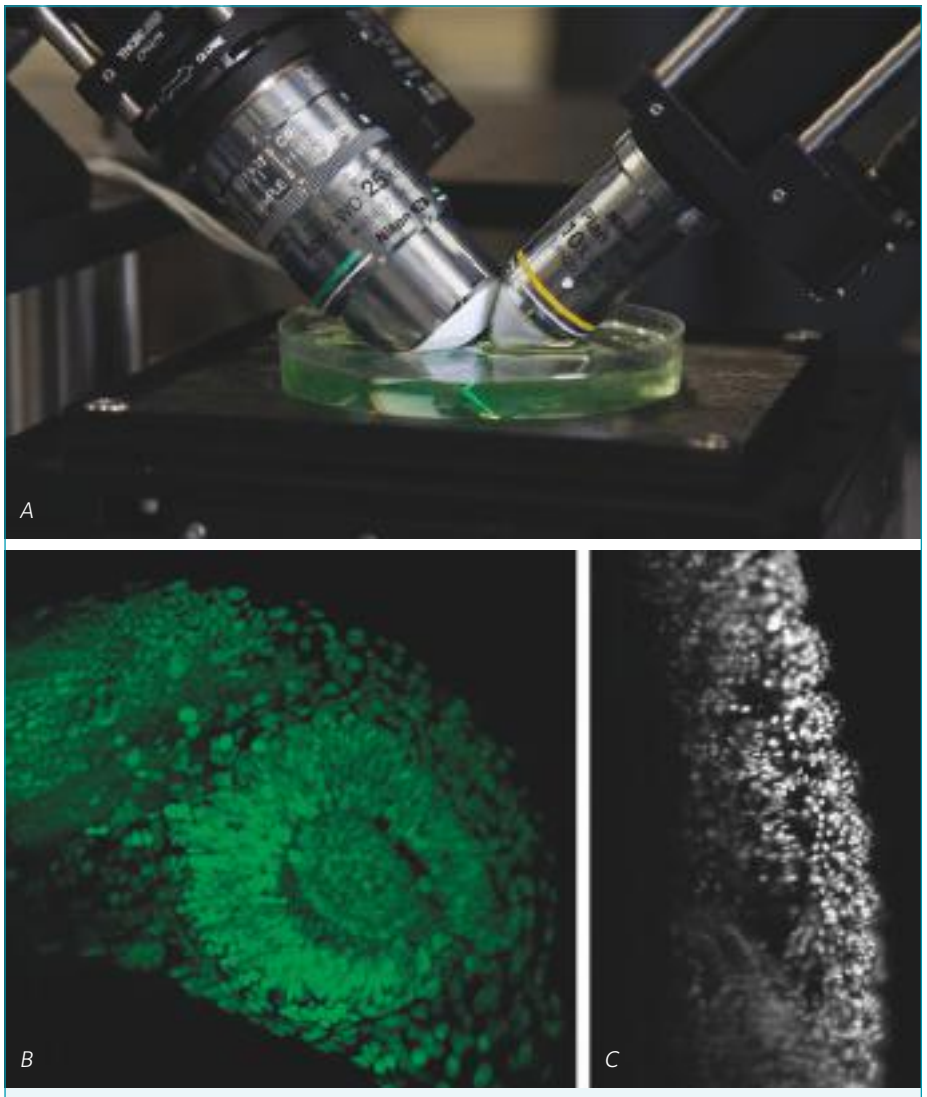

Figure 2. A, the configuration of the light sheet microscope at CAIC. The objectives are oriented at 45 degrees from the horizontal and allow samples to be mounted in a Petri dish in a water based medium. The ×10 objective on the right forms the light sheet. In this particular configuration a beam is scanned quickly in a plane to create a sheet of excitation light. The beam forming the sheet can be seen exciting fluorescein in solution. *B*, a rendered viewpoint of a 3D data set captured by imaging a 1.5 day post-fertilisation zebrafish embryo with H2B-GFP fusion protein. The volume shown is approximately 330 µm × 330 µm × 100 µm and was measured in several seconds. *C*, a single plane of a *Drosophila* embryo with H2B-GFP fusion protein imaged with a scanned light sheet.

Having enormous amounts of data may be a good problem to have, but the sheer scale of data is likely to require users to redesign their data analysis workflow. This side of light sheet microscopy underlines the importance of interdisciplinary collaborations, involving scientists skilled in dealing with big data and volumetric image processing.

The leap in data sizes also has longer term ramifications, with data archiving for 10 years being a condition for many funding bodies. Given the costs of long term data archival it is important to discard, crop and compress as much as possible before committing data to an archive. Although these points are just as true for long time-lapse confocal scanning, and any other type of microscopy image data, as they are for light sheet data, the difference is the step in magnitude that must be dealt with if LSMs are used to their full temporal and volumetric imaging potential.

I have no doubt that light sheet microscopes will soon become a commonly used tool in the life sciences. They come with tremendous benefits, but also with significant challenges in unlocking the valuable information contained within the vast amounts of data that they can produce. Our place, as an advanced imaging centre, is to develop and facilitate the use of such techniques, from sample preparation to acquisition, analysis and storage. We must meet the challenges presented by these techniques to ensure that the life sciences community can benefit from them and that the techniques themselves evolve to better suit the biological questions being asked.

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Acknowledgements

The Cambridge Advanced Imaging Centre is a new research facility within the School of Biological Sciences at the University of Cambridge and its creation was made possible by the support of The Wolfson Foundation. CAIC's light sheet microscope design is based on a system designed and built by Jan-Felix Evers, now at the University of Heidelberg. Ongoing light sheet microscopy development in the centre is supported by the Wellcome Trust.

Lab profile: Arthur Butt's laboratory, University of Portsmouth

Society Member James Hallett, a post-doc at the University of Portsmouth, gives an insight into the multinational laboratory and how it trains the next generation of budding gliophysiologists.

James Hallett

University of Portsmouth, UK

Portsmouth is found in the south of England and is the UK's only 'island city'. Famous for being a major military hub, it is gaining a lot of attention as a major university town, and is the perfect place to house a laboratory that specialises in glia.

We are a multinational (currently UK, France, Italy, Greece, Iran, Algeria, Singapore), multidisciplinary team interested in all aspects of the physiology of glial cells. The glia community have a difficult job to convince neurophysiologists that glia are not just 'support cells', but play essential roles in neurophysiology and pathology of various CNS diseases.

Between me and four PhD students, we are working on various projects including ion channel physiology in astrocytes and oligodendrocytes (Maria Papanikolaou, me), glial glutamate metabotropic receptors and the role of glia in Alzheimer's disease (Ilaria Vanzulli), and properties of drugs in glial proliferation and differentiation (Andrea Rivera, Francesca Pieropan). To answer these questions, we use a variety of techniques including optic nerve explant and organotypic slice cultures, global and conditional knock-out mice, immunohistochemistry, qPCR, Western blot, microarray, and electrophysiology including patch clamping and action potential recordings.

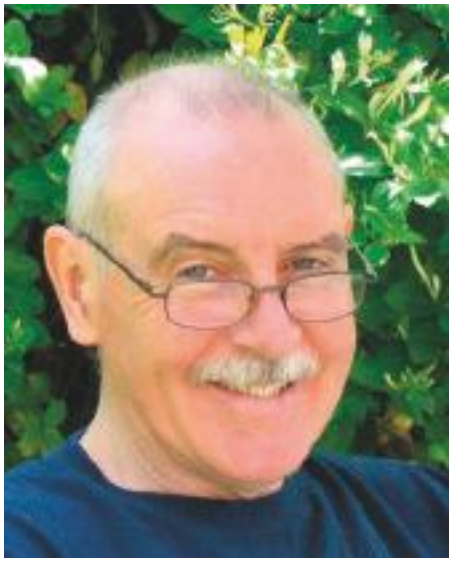
The university is a relatively small but significant hub of neuroscience and ion channel research; across the corridor from us is one of the UK's leading brain tumour research groups, and there are also other groups whose interests span from yeast ion channels to depression. As the unit is small, everybody knows everybody, which creates a collaborative environment with many shared resources between groups. We also get a high number of students and interns from the UK and abroad; this academic year, we have had three undergraduates and one taught Masters student from UoP, as well as two Erasmus students from France (University of Rouen and College de France) who both got here on the ferry, post-Masters interns from the UK, Spain and Italy, a high school student from Singapore and a Masters student from King's College London. As can be imagined, a lab with so many nationalities, cultures and backgrounds comes with its own sets of advantages and disadvantages. Some students from hotter countries sometimes see photos of Portsmouth in the sun and are slightly disappointed when they arrive in a flurry of wind and rain!

We also have a number of collaborations, with some examples in King's, UCL, Warwick, Southampton and Bilbao, as well as closer to home in Portsmouth itself. The high number of techniques employed and the high number of students coming in and out of the lab ensure that we have to continually strive to create a proficient training environment. We gladly share our technical expertise with our students to create a new generation of budding gliophysiologists. However, when we don't feel like sharing, you can find us at the beach and/or pub.

James and his lab colleagues (from left-right): Maria Papanikolaou, Francesca Pieropan, Andrea Rivera, Ilaria Vanzulli, James Hallett.

Member profile: Paul Thomas

The Imaging Facility Manager at the University of East Anglia, UK, tells of his journey from biochemist to 'born-again' physiologist to bioimager, and the gratification of hosting trainee imaging courses (funded by The Society).



Paul Thomas

Imaging Facility Manager,
University of East Anglia, UK

'...thank you for the wonderful imaging course... all of us as participants will always have it as a reference point as we begin our "voyage of discovery" in the world of biomedical imaging.' These words were written by a trainee at the end of one of my annual Society-funded workshops on live-cell imaging. In addition to being a rather nice compliment, for me the words also encapsulate one of the two most gratifying things about doing academic science: helping a young scientist at the start of his or her research career. During my own research career, I've been fortunate to find mentors who were both brilliant teachers and extremely generous with their time and knowledge. And it was while working with the first of these individuals that I chanced upon the second area of science that I find especially rewarding: that intoxicating (and generally elusive) moment when you see your own experimental predictions proved correct.

It happened during my first job after graduation. I was sitting rather nervously in front of an electron microscope with my boss, David Allan, and our collaborator Tony Limbrick. I had prepared 'Triton shells' from chicken erythrocytes treated with the ionophore A23187 in the presence of various inhibitors, and we were looking to see the effects on the cytoskeleton. First, we checked the controls, which to my relief showed a large black nucleus encircled by the marginal band of microtubules, with a meshwork of intermediate filaments sandwiched in between. Next came the sample treated with ionophore alone and, as expected, all we saw were bare nuclei – the increase in intracellular calcium had caused the total disappearance of the cells' cytoskeletons. Finally, as Tony loaded one of the samples with the inhibitors, I piped up with, 'Now in this one we should see the nucleus and intermediate filaments, but no marginal band.' And for the first time in my career, I got it right! The images we obtained that day took centre stage in my first publication as first author. Coincidentally, it was also the first publication in which I had a direct role in the microscopy.

Nevertheless, the path to my present position as a dedicated bio-imager was by no means straightforward. Starting out with a joint honours degree in Physiology and Biochemistry, I had always considered myself a physiologist rather than a biochemist. However, after graduation, I discovered that jobs in biochemistry were more plentiful, so when David (a newly appointed lecturer and lipid biochemist) offered me the opportunity to work in his lab at University College Hospital Medical School, I snapped it up. As I've already alluded to, David's interests were in how intracellular calcium affected erythrocyte shape, and from then on calcium would form the focus of my research career.

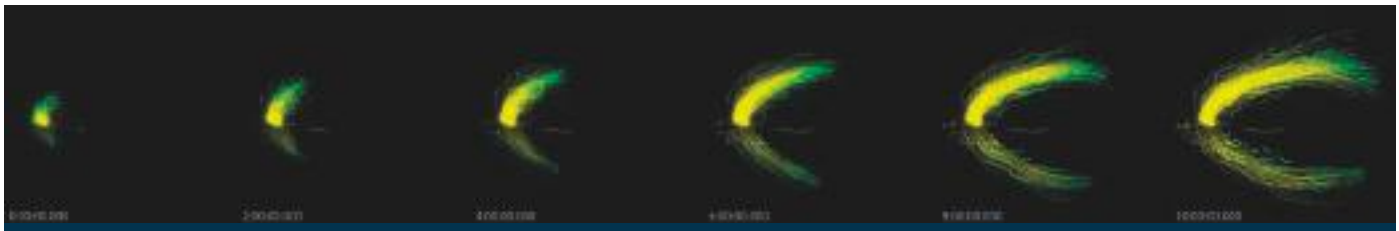
From London I ventured across the Atlantic to California where I used my experience in inositol-lipid metabolism to investigate calcium signalling in spermatozoa. My professor, Stanley Meizel, was one the most generous mentors anyone could wish for, and he convinced me to stay and study for my PhD while providing me with the vital support I needed. Ever since, I've tried to emulate his thoughtful approach in working with young scientists.

If Stan was the most inspiring influence in my career, it was Wolfhard Almers who was the most influential in developing my quantitative skills as a research scientist. On finishing my PhD I decided I could either go the 'molecular' route, or the 'single-cell' route. I chose the latter after having been profoundly impressed by a seminar in which Wolf described his work on measuring the properties of the fusion pore of single secretory vesicles. It was during my time with Wolf that I was 'born-again' as a physiologist and my work with him increasingly relied on microscopy. Nevertheless, it would be several more years and two more jobs before I would become an out-and-out 'imager'.

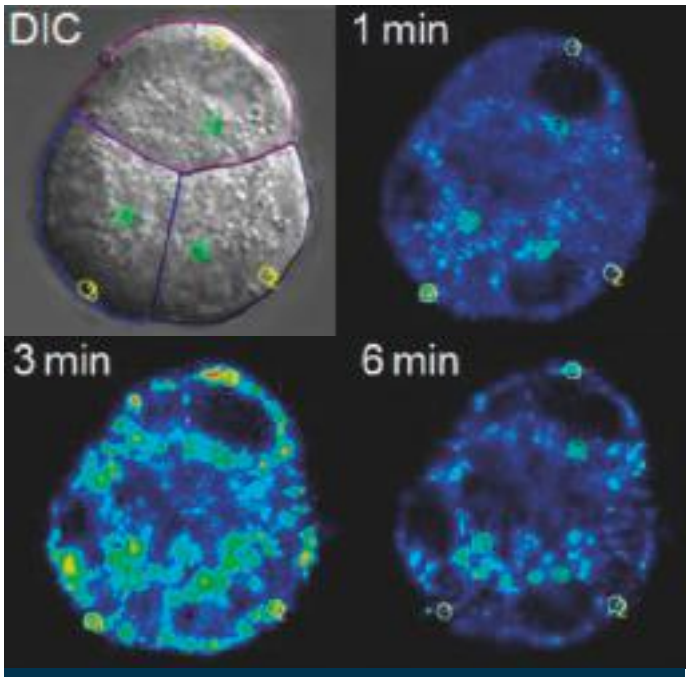
Returning to England via another stint in California, I obtained a Fellowship in Pharmacology at Cambridge University. While there I was awarded a grant in 'Bioimaging' – an initiative run by the BBSRC. In this work, my post-docs and I used time-differential analysis of brightfield images, in conjunction with fura-2 measurements, to investigate the regulation of exocytosis in individual pancreatic acinar cells. The results were subsequently published in *The Journal of Physiology*.

And so it was the acquisition of these imaging skills that led inexorably to my present appointment as the Manager of the Imaging Facility at UEA. In this role my greatest satisfaction comes from the training of young scientists, especially those that I'm privileged to teach in the course of running The Society's workshop every summer. Obviously it's always a pleasure to receive positive feedback from workshop participants, but it's even more rewarding to know that I might possibly have made a critical contribution to someone's research career as they embark on their own 'voyage of discovery'.

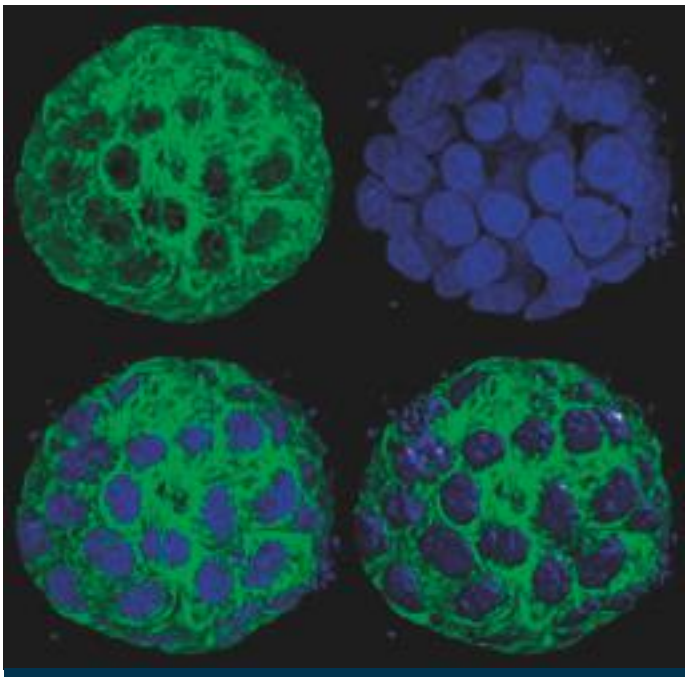
More information on Paul's workshops is available at **www.physoc.org/live-cell-imaging-17-19-june-2014**



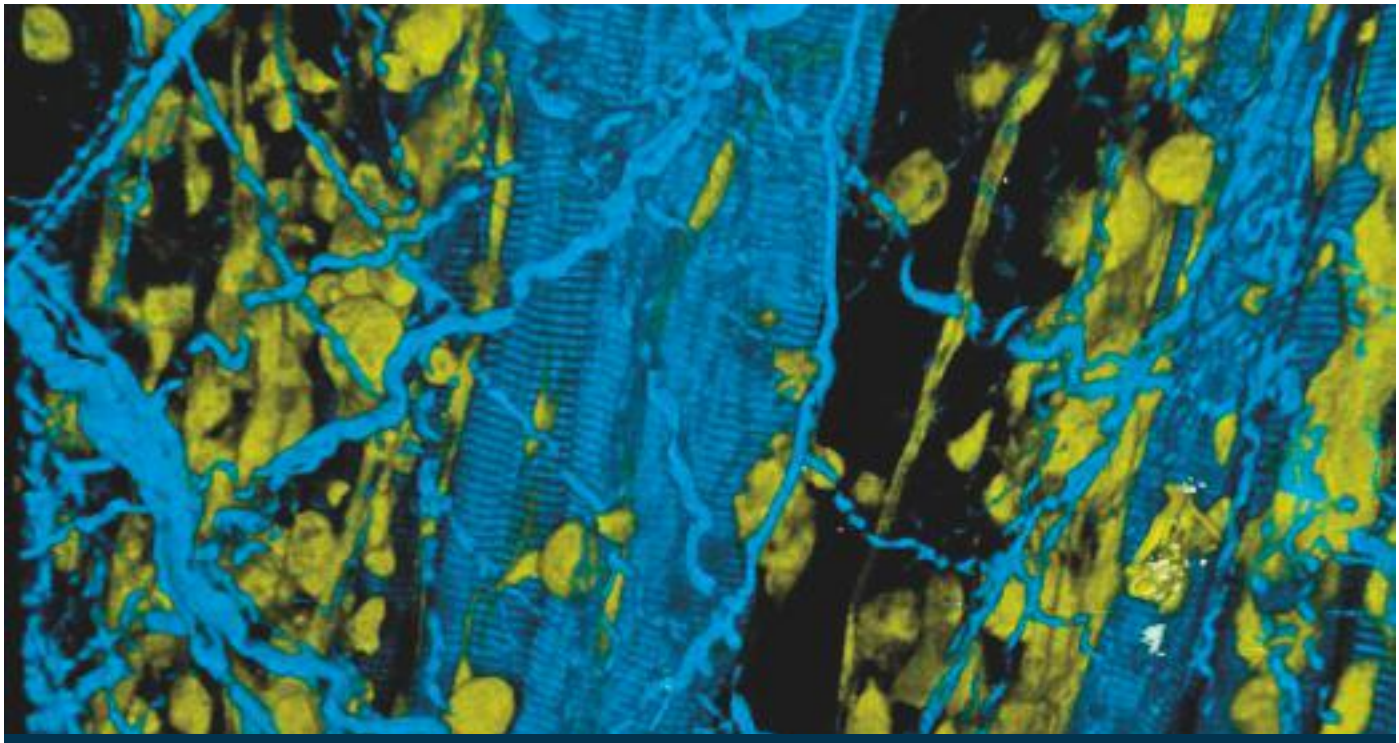
Migration of cells (GFP-labelled) during early development of a chicken embryo. Junfang Song, Paul Thomas & Andrea Münsterberg (UEA).



Mitochondrial calcium dynamics in pancreatic acinar cells stimulated with acetylcholine revealed with dihydro-Rhod-FF. Mega Okotete, Nafisa Shah (undergraduate students) & Paul Thomas (UEA).



MDCK cells grown in 3D culture (Matrigel), stained for DNA (blue) and α-tubulin (green). Paul Thomas, Deborah Goldspink & Mette Mogensen (UEA).



Multi-photon imaging of mouse muscle (Tibialis anterior) during induced tissue regeneration: stem cell population expressing YFP, and collagen fibrils and contractile apparatus (cyan) revealed by second-harmonically-generated (SHG) signal. Paul Thomas, Isabelle Piec, Ernst Pöschl & Uli Mayer (UEA).

Peter Mott

1930 – 2014

Many long-standing Members of The Society will be saddened to learn that Peter Mott died on 14 March. He was 84 and had been ill for some time.

Peter worked at Cambridge University Press and was responsible for overseeing the publication of many journals including *The Journal of Physiology* and the Meetings Abstracts. Later, when The Society took over the ailing *Quarterly Journal of Experimental Physiology* (now *Experimental Physiology*) Peter contributed to discussions on the change to monthly publication with the resulting need for a new title and a new cover (an eye-catching red rather than the old sludge-green). I remember that when *The Journal* had to change to Persil-white paper from its traditional cream (suddenly no longer available) he managed to ensure that this happened between issues rather than within a run.

Those of us who were Editorial Board members at the relevant time will probably remember how Peter's dry sense of humour helped defuse many a contentious argument. The Boards also benefited from his knowledge of food and wine, exercised to full effect when he organised the Annual Lunch that CUP gave to the Boards. Peter was always happy to show people round the Printing House after lunch and to explain its many facets. The machine that picked up single sheets of thin Bible paper, and the one that magically folded printed quarto pages, stick in my mind.

Things have changed since Peter's day but those of us lucky enough to have known him, and his work for The Society, will look back with warm gratitude.

Ann Silver

Peter Mott was one of the guests at a celebratory *Experimental Physiology* Centenary Dinner (at Peterhouse College, Cambridge) in July 2008

Left to right: Julian Paton, Cecil Kidd, Peter Mott, Emma Ward, David Paterson, Ann Silver, Carol Huxley, Ole Peterson, John Coote, Ian McGrath, Mike Spyer

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Physiol Rep is creating virtual issues to coincide with major international meetings; these are collections of papers already published that are selected by the editors to promote the journals. For EB2014 in April, the theme was Gender and Age and at Physiology 2014 it will be New Directions in Muscle Research. All virtual issues are available at **bit.ly/1iL5N6F**

Meet the Editors

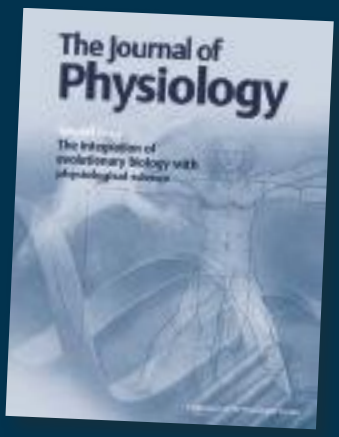
Those attending EB2014 were able to meet the *Physiol Rep* editors over ice cream. At Physiology 2014 there will be a breakfast with the editors on Tuesday 1 July. This is a chance for authors and potential authors to meet members of the editorial team. There will also be a sponsored lunchtime workshop on open data on Wednesday 2 July. For details see **bit.ly/1dQfsDB**

New website

Visit physreports.physiology.org to see our enhanced interface and user experience.

The Journal of Physiology

Special Issue



The Journal of Physiology has just published (1 June issue) a Special Issue called 'The integration of evolutionary biology with physiological science'. This issue, led by Consulting Editor Denis Noble, contains papers that provide an insight into a new holistic approach to evolution, which encompasses multiple processes that depend on multi-scale interactions. Incorporating the knowledge that genes are not isolated from the organism and the environment, and that acquired characteristics are heritable, it reveals the Selfish Gene to be, in fact, the Servant gene. The issue highlights the role of physiology in the changing theory of evolution and calls for a replacement framework based on a systems view of evolution.

Sponsored symposium

The Journal of Physiology is sponsoring the following symposium at Physiology 2014:

Coupling cellular metabolism to neuronal signalling

Tuesday 1 July 2014, 09.00–11.00, Queen Elizabeth II Conference Centre, London, UK

Organisers: Derek Bowie (McGill University, Canada) and David Attwell (University College London, UK). Speakers: David Attwell, Roger Thompson, Margaret Rice, Irene Llorente-Foch, Derek Bowie

Review papers from this symposium will be submitted to *The Journal* with publication planned for later in 2014 or early 2015

Early Investigator Prize

We are pleased to announce the winner of *The Journal of Physiology*'s second Early Investigator Prize: Lasse Gliemann for his paper 'Resveratrol blunts the positive effects of exercise training on cardiovascular health in aged men'. October 15, 2013 *The Journal of Physiology*, 591, 5047–5059.

Two runners up are: Daniel Wilkinson, for the paper 'Effects of leucine and its metabolite β-hydroxy-β-methylbutyrate on human skeletal muscle protein metabolism'. June 1, 2013 *The Journal of Physiology*, 591, 2911–2923. Also Aiste Adomaviciene, for the paper 'Putative pore-loops of TMEM16/anoctamin channels affect channel density in cell membranes'. July 15, 2013 *The Journal of Physiology*, 591, 3487–3505.

Entry requirement details can be found on *The Journal*'s website.

Nuffield Foundation: Provide a 4–6 week summer placement for sixth form students

Think back to when you were 17. What did you know about scientific research as a career? How did you get where you are today? It's likely there were key individuals and experiences that motivated and inspired you, and it's those experiences that we want to give to young people through our Nuffield Research Placements.

For more information visit **www.nuffieldfoundation.org/project-providers**

Engaging with Parliamentarians

The Society is offering an exciting new programme for Members at all career levels wanting to engage with policymakers and make a difference for physiology. Those in the early stages of their careers are especially welcome.

The programme will...

- Provide you with training on how best to engage with MPs and policymakers
- Provide you with a better understanding of the policymaking process
- Create opportunities so that you can engage with policymakers to increase their awareness of physiology and of the issues it faces

Key dates

30 June 2014 (11.30–14.15) – Introduction to the programme at Physiology 2014, including a reception at the Houses of Parliament

1 September 2014 (11.00–17.30) – Training event, Hodgkin Huxley House, London

9 February 2015 (13.00–17.30) – Programme evaluation and feedback session, Hodgkin Huxley House, London

Expenses

The Society will cover reasonable expenses associated with your participation in the programme, and registration is free to Society Members.

For further information, or to join the programme, please visit **www.physoc.org/engaging-parliamentarians** or contact Ed Hayes at **policy@physoc.org**

The Physiological Society
Hodgkin Huxley House
30 Farringdon Lane
London EC1R 3AW
United Kingdom

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and Wales No. 323575
020 7269 5710
www.physoc.org

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