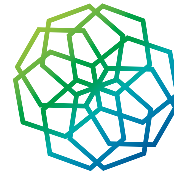
PHARMACOLOGY MATTERS



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Up close & personal:
Pharmacologists' perspectives on the role of imaging technology

Image of stained glass courtesy of Felicity N.E. Gavins - see page 27 for further details

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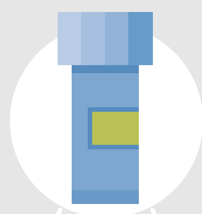
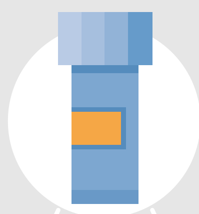
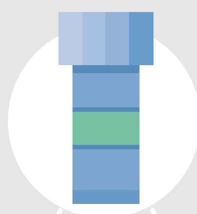
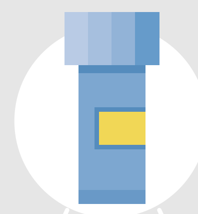
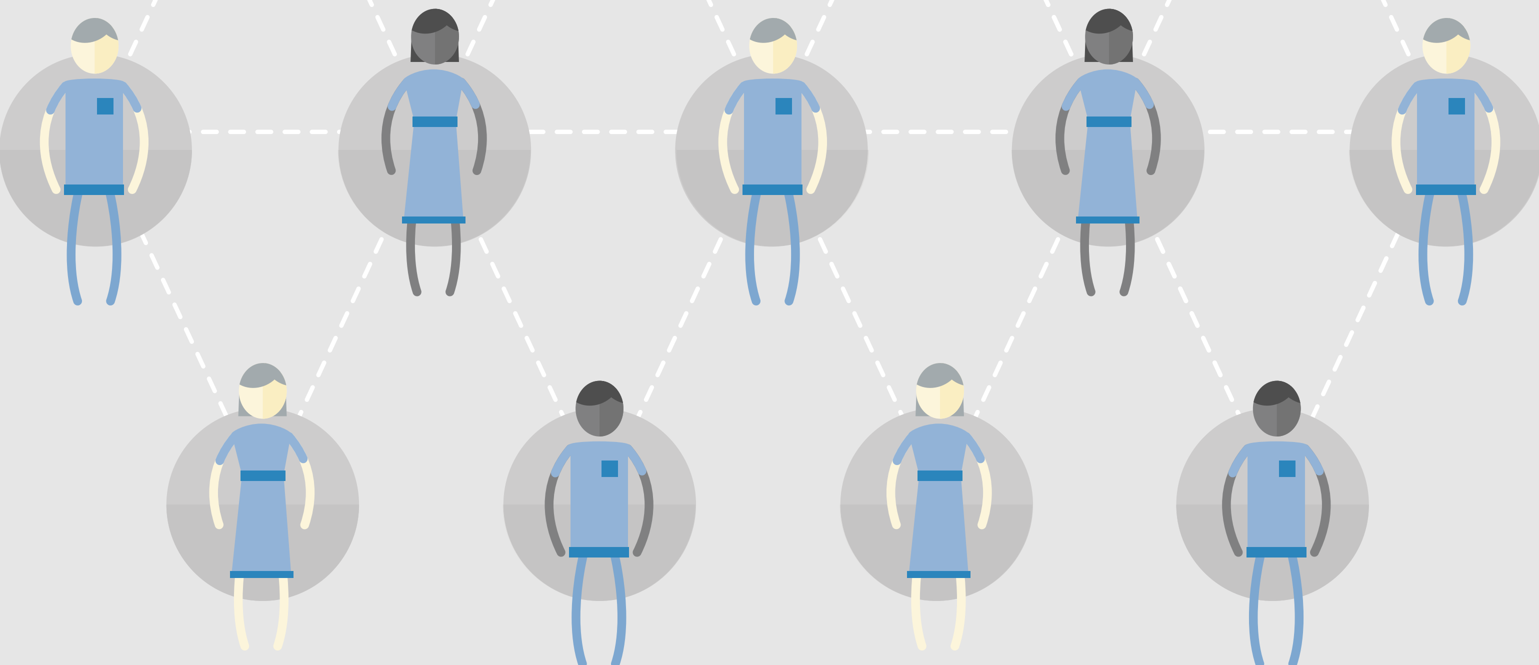

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Editorial

Felicity N.E. Gavins
Editor-in-Chief, Pharmacology Matters

What a year it has been, and how fantastic to finish off 2015 with another super issue of Pharmacology Matters! Our theme for this edition is "Pharmacologists' perspectives on the role of imaging technology", and we have a delightful range of articles, providing an interesting read over a festive mince pie!

Jono kicks off by providing an insightful overview of recent developments at the Society and what's to come in the year ahead, including the 'Focus on Pharmacology' initiative.

We have a thought-provoking selection of articles on a variety of different microscopy modalities – super resolution, intravital microscopy, electron microscopy, FRET and the mesoscope – from Tim Self and Robert Markus, Kim Chisholm, Kevin Mackenzie, Steve Tucker, and Gail McConnell.

A large number of members (including myself) use mice in their in vivo research. Alessandro Pristerà has kindly given us a helpful overview of the Cre-LoxP recombination system. This system and genetic engineering have been exploited to achieve conditional deletion of genes in specific cells, thus enabling researchers to answer their specific research questions. Following on from this, Sarah Bailey discusses the Concordat on Openness on Animal Research and what it means for the Society and its members.

How much do you know or think you know (!) about forensic pharmacology? Well, worry not, because Paul Skett and Steph Sharp fill us in about this interesting area of pharmacology (page 24), and in particular the role that a forensic pharmacologist plays as an expert witness in court.

Of course we have our regular features: Barbara McDermott and Talja Demster give us our meeting update (past and future), and Vedia Can provides us with an update from our younger members.

In our final article of the last issue of 2015, I give you all an insight into what I do in my spare time, along with the history of my hobby. What is my hobby? You will just have to turn to page 27 to find out!

So, it remains for me to offer a huge thank you to all the Editorial Board and everyone at the Schild Plot (especially Katharine – you have been a star!) for helping make Pharmacology Matters a great success, and also to you, the reader, for continuing to support your magazine.

Finally, I wish you all a very Merry Christmas, and an even better 2016, filled with peace and goodwill to all.

Best wishes,
Felicity

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Your BPS

Jono Brüün
BPS Chief Executive

As I write, Pharmacology 2015 is just around the corner and the team at the Schild Plot is focused on delivery another lively and successful annual meeting. If you are reading this issue online as you travel to the meeting, I look forward to seeing you there and celebrating another year together.

A full review of the Society's extensive activities during 2015 will be produced in the New Year, but a summary will be presented as part of our Annual General Meeting (AGM). Slides and materials from these AGM presentations will be available to members through the enhanced 'My Society' section of our new look website at www.bps.ac.uk, but I would encourage all of our members to attend the meeting itself to discover a little more about how the Society is managed on your behalf.

Looking ahead, our objectives for 2016 became significantly clearer during discussions at the Council meeting in June, when our Trustees and Vice Presidents came together to discuss how the principle of "being more ambitious" could be realised by the Society's different Committees and Groups in the future. The result was a truly exciting, forward-thinking programme of work, for the period taking us up to the end of 2017. We will be aiming higher than we ever have done in the coming years, but also carefully measuring the impact and value of how we commit resources during 2016 and 2017.

Two projects that demonstrate this expanded, forward-thinking approach are:

1. Focus on Pharmacology: As mentioned in the last issue of Pharmacology Matters, this two-year project aims to increase awareness of the importance of pharmacology in the modern world, and help decision-makers in universities, industry and government understand the scope and value of what pharmacology has to offer. It will begin with an extensive fact-finding exercise to establish how pharmacology is being taught, how its value is perceived by students, employers and policy-makers, and the career pathways followed by graduates – and you will see early signs of this activity before, during and after Pharmacology 2015.
2. Prescribing Safety & Skills: The Society continues to support prescribing skills through the development of resources in the UK, namely the Prescribing Safety Assessment (PSA), which is being adopted by all UK Medical Schools, and its associated e-learning resources: Prescribe and Prescribing Simulator. We also plan to develop versions of these resources for non-medical prescribers, and for use in other countries. We believe that this timely and successful initiative will stimulate efforts to improve teaching and research in basic and clinical pharmacology.

In preparation for expanding these and other initiatives, I have agreed with Council to restructure the Society office and expand the number of staff to 18 full time equivalents, five of whom will be on contracts up to the end of 2017. I believe this expansion will help us deliver the kinds of ambitious projects highlighted above, while improving the level of service offered to members and the public alike. New roles at our head office will help us:

- support high quality scientific publishing – Kathryn Wilson has joined as Head of Publishing
- build our engagement with policy makers and the public, and drive the Society's leadership of education, training and prescribing assessment programmes – Fatima Syed joined us as Clinical Programmes Manager, working alongside Cynthia Sam as our new Education, Training & Policy (ETP) Manager, and Chinara Rustamova returned from maternity leave to her new role of ETP Officer
- provide improved services for our existing members and reach out to groups of potential new members – Teesha Bhuruth has joined us as Membership & Community Officer

I'm delighted with the significant achievements made already by staff, working alongside our Trustees and members as part of our five-year strategy, and our expanded team will build on this progress and be able to explore an even greater number of opportunities. I would like to thank existing staff, as well as former colleagues who left the organisation over recent months, for their dedication and enthusiasm.

Finally, as many of you know, Professor Humphrey Rang's term as President will come to an end on 31 December. I should like to put on record my thanks to Humphrey for providing clear, supportive and consultative leadership over the past four years. Humphrey has been a pleasure to work with, and I wish him all the very best for the future.

At the same time, I should like to welcome Professor David Webb as the Society's new President. David and I have established a good working relationship during his term as President-Elect, and I am greatly looking forward to continuing that work with him over the next two exciting years at the Society.

On behalf of everyone at the Schild Plot in London, I wish you a Merry Christmas!

Super resolution microscopy – the bright side of molecular biology

Tim Self & **Robert Markus**,
University of Nottingham

With the significant breakthrough in resolution capabilities brought about by the 2014 Nobel Prize in Chemistry winners William E. Moerner, Stefan Hell and Eric Betzig, this is an exciting time to be working in microscopy. Not only is it one of the most fundamental techniques used by biologists, microscopy is also facilitating crucial advances in healthcare and drug discovery, allowing scientists to observe the micro world in ever increasing detail.

Many of the advances in life sciences have resulted from the use and application of microscopy and in particular light microscopy. The possibility to see and hence follow sub resolution processes within cells has always been limited by a physical limit to the resolution of light (Rayleigh Criterion) and has been a matter of frustration for researchers until now. In recent years this limit to resolution has been broken and scientists now have access to a new series of super resolution microscopes to enable them to start to investigate cellular processes at greater resolution with multiple labels even in living cells. The super resolution techniques so far are based on fluorescence microscopy and rely on the photochemical properties of the fluorescent labels used to specifically highlight the molecules of interest (Figure 1).

The resolution limit

Due to the diffraction of light the signal from one single fluorescent molecule is detected as a blurred spot (PSF – point spread function) at the focal plane of the camera attached to a microscope. The diameter of the spot is dependent on the wavelength (λ) and the numerical aperture (NA) of the microscope objective used and according to Abbe this is: 0.6λ/NA. Using green light this would be around 230nm. This means if we are looking at an E. coli bacterium (with an approx. size of 500nm x 2000nm) already ten sparsely distributed fluorescent molecules would cover the whole area (see Figure 2). The molecules closer than 230nm to each other will appear as one single spot. Thus it is practically impossible to resolve ultra-fine structures within a span of a micron using classical light microscopy, because the light emitted by the molecules close to each other is overlapping, which in turn will result in a blurred image.

To overcome the problem different imaging methods have been developed and implemented in microscopy, resulting in super resolution techniques.

Figure 1. *Structured illumination microscopy (SIM) image of bovine pulmonary epithelial cell. Subcellular structures are visualised using pseudo colouring. Labelling: DNA-DAPI (red), actin cytoskeleton-phalloidin (blue), and mitochondria-MitoTracker (yellow). Scale bar 10 μm.*

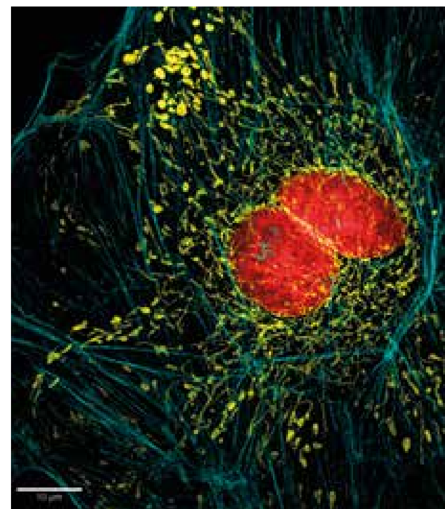
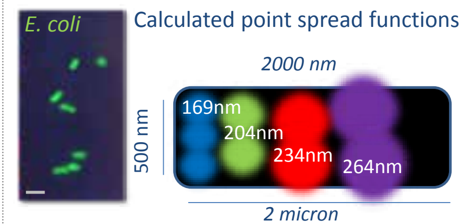


Figure 2. *Fluorescently labelled E coli. bacteria and modelling of signals emitted from single fluorescent molecules. Estimated diameter of point spread functions are shown at different wavelengths using an 100x/NA 1,46 microscope objective. Scale bar 2 μm.*



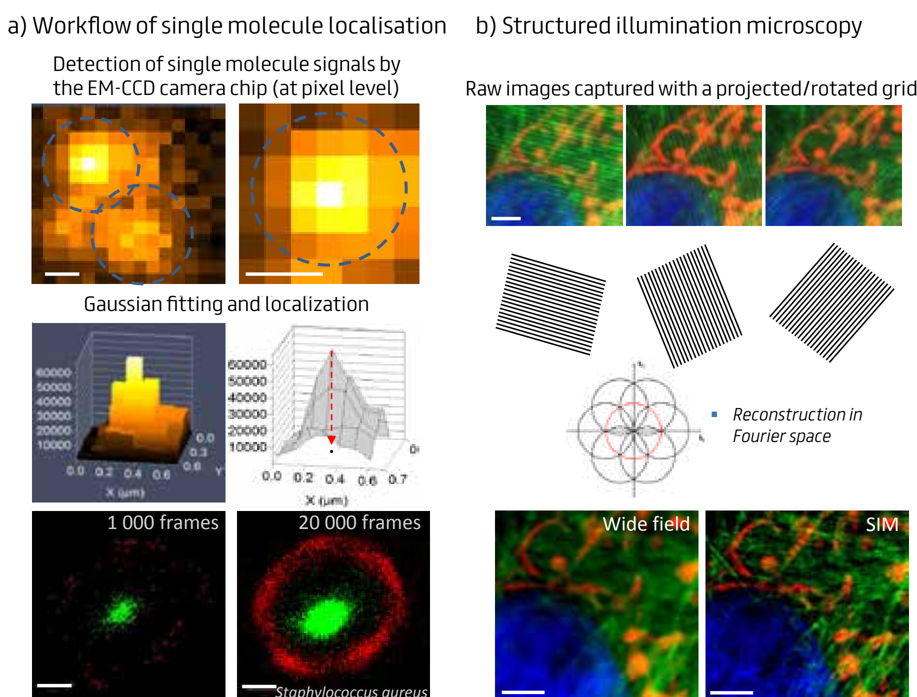
Principles of Single Molecule Localisation

With the advances in digital camera technology and enhanced computing power, now we can image even single molecules and calculate their position in the sample using fluorescence light microscopy. The principle is to randomly light up only a fraction of the fluorescent molecules (Figure 3a), image them and switch them off; by repeating the cycle a raw image set is generated from which, the new super resolution image is calculated, and visualised similar to pointillism. Hence the resulting images will contain high resolution information surpassing the resolution limit of an optical light microscope[1].

Super resolution microscopy techniques especially single molecule localisation techniques (dSTORM – direct Stochastical Optical Reconstruction Microscopy and FPALM – Fluorescent Photo Activation Localisation Microscopy) break the resolution barrier taking advantage of behaviour of the fluorescent molecules and using high computing power[1,2,3].

Therefore a combination of optical, chemical and mathematical knowledge leads to the generation of images which contain super resolution information. Other techniques like Stimulated Emission Depletion (STED)[4] and Structured Illumination Microscopy (SIM)[5] rely on different concepts to narrow down the size of the detection area. STED uses confocal technology by superposing a depletion laser ring around the excited area, therefore the size of the scanning spot can be adjusted to be much smaller than in confocal microscopy alone. In SIM, an optical interference is generated, modulated and rotated; the raw images are then computer processed and the resultant image is therefore a calculated / reconstructed super resolution image, with two fold increase in resolution compared to confocal microscopy (Figure 1 and Figure 3b).

Figure 3. *Workflow of the super resolution techniques. (a) In single molecule localisation (e.g. dSTORM) a high power laser is used to illuminate the sample which will drive the fluorescent molecules into an intermediate dark state. When molecules stochastically return to their ground state the photons are emitted and are captured with the high sensitivity EM-CCD camera. The mathematical centre of these events is calculated, stored and plotted. Running the analysis on 20 000 frames of images will yield a super resolution image. Scale bars 200 nm. (b) In structured illumination microscopy (SIM) the sample is illuminated in stripes. These rotate and modulate creating Moire patterns and in total at least 15 images are captured in each focal position. The image set is processed and assembled from the high resolution information contained within Fourier space. The images show subcellular structure of a bovine epithelial cell, DNA-Blue, cytoskeleton-green, mitochondria-red. Scale bars 2 μm.*



At the moment the sensitivity of SIM is similar or better than the confocal, however a high contrast fluorescent sample is a must in order to deliver high resolution data. Further developments in the sensitivity of the digital cameras (C-MOS) used in SIM will increase the sensitivity over confocal microscopy. A different optical approach has been adopted in the development of Instant SIM, where the high resolution image is generated utilising spinning disk micro lens/pinhole optics; bypassing the time consuming computing, therefore live high resolution microscopy becomes available, with the limitations inherent from a spinning disk microscope[6].

Super resolution techniques used in Nottingham

Dr Kim Hardie, Mahmoud Ashawesh & Dr Christopher Penfold

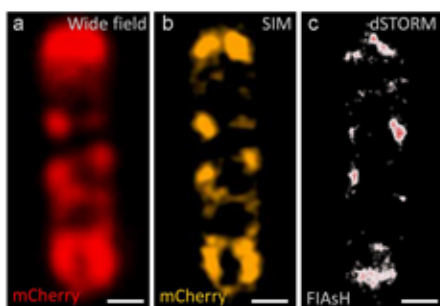
Bacteria imaged at single molecule level

Microbiology research and biotechnological innovation has benefited greatly in recent years from molecular cloning technologies and protein biochemistry. However because bacteria are inherently small, classical microscopy could not provide much more information than the morphological changes and approximate localisation of molecules of interest. The use of super resolution techniques alongside in situ fluorescent labelling of individual molecules has helped to visualize and precisely localise structures within the bacterial cell with minimal or no disturbance of those structures. PhD student Mahmoud Ashawesh (under the supervision of Associate Prof. Kim Hardie and Dr Christopher Penfold) has fluorescently labelled proteins located in the outer membrane of Gram negative bacteria with the fluorescein Arsenical Hairpin Binder (FIAsH) which interacts with proteins via the tetracysteine tag technology[7]. Insertion of the motif Cys-Cys-Pro-Gly-Cys-Cys into the protein by genetic engineering provides a recognition sequence for attachment of the FIAsH substrate which emits a green fluorescence on binding of the tetracysteine motif.

This mode of labelling proteins reduces any potential disruption to protein structure and function compared to tagging with a large fluorescent protein such as GFP or mCherry (27-30kDa) (Figure 4a-b). Consequently, the tetracysteine fusion proteins are more likely to be correctly folded, localised and functional. This avoids the pitfalls of aggregation of overproduced fusion proteins which tend to detrimentally affect the overall structure of bacterial compartments and hence cell viability.

Using SIM we generated a more precise 3D model of the localisation of the tagged protein within the bacterium, and observed that it was distributed as a spiral structure that stretches along the cytoskeleton from pole to pole of a bacterial cell (Ashawesh & Hardie unpublished). dSTORM was used in parallel with the same tag, creating a high precision XY localisation map of the distribution of these molecules within the bacterium (Figure 4c).

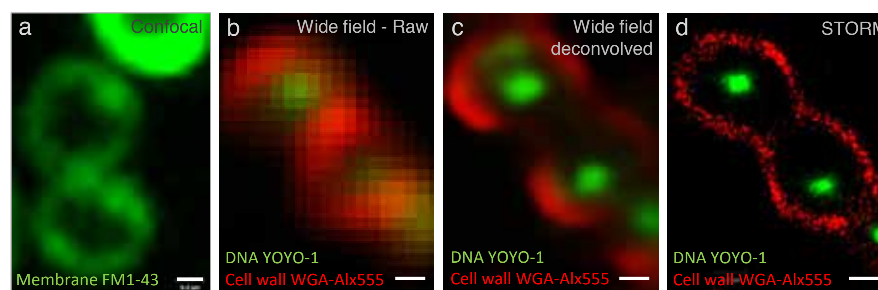
Figure 4. *Localisation of proteins in Gram negative bacteria. (a) Wide field, (b) SIM image of Gram negative bacteria expressing mCherry and (c) STORM image of FIAsH labelled proteins. Red pixels are the centroids of the localised molecules the white clusters are the molecules displayed with their localisation error rendered to a Gaussian function.*



Dr Phil Hill

Our model organism Staphylococcus aureus is an important human pathogen that relies upon surface-expressed proteins to help initiate infection. Super resolution microscopy provides a tool to investigate the location and density of surface protein loading onto the bacteria and allows us to evaluate agents that disrupt this process. Now we are using super resolution microscopy techniques (dSTORM) to investigate the localisation of expressed and covalently bound proteins on the surface of the bacteria with 5-10 fold higher at precision than before (Figure 5). Additionally we implemented multi-colour dSTORM to visualise the main compartments, the DNA and the cell wall. In relation to these we will define the localisation of the expressed / engineered proteins, which will serve as scaffold for fluorescent molecules or other chemical labelling, allowing us to trace bacteria *in vivo*.

Figure 5. *Staphylococcus aureus bacteria imaged with various techniques. (a) Laser confocal image of bacteria labelled for the cell membrane with FM 1-43 dye. (b) Raw wide field image of the bacteria shown in c and d; (c) single optical slice from a deconvolved image stack (focus stack), (d) two colour dSTORM image of Staphylococcus aureus bacteria, labelled for DNA in green (YOYO-1) and cell wall in red (WGA-Alexa Fluor 555). Scale bars: 200 nm.*



Acknowledgements

We thank the BBSRC for founding the establishment of the Multidisciplinary Super Resolution Microscopy Facility at Nottingham University (Grant no.BB/L013827/1), and for the Libyan Government for supporting Mahmoud Ashawesh PhD program. We thank Dr Phil Hill, Dr Kim Hardie and Mahmoud Ashawesh for samples and examples from their projects, and Seema Rajani for her help in the figure preparation and image processing.

About the authors

Tim Self is a Chief Experimental Officer and Head of the core School of Life Sciences Imaging facilities (SLIM) at the University of Nottingham. He has over 30 years' experience in imaging microscopy and has publications in diverse research areas such as, pharmacology, hereditary deafness, neural degeneration, microbiology and stem cells.

Robert Markus is a Senior Imaging Technician working for the SLIM team at the University of Nottingham, where he runs the super resolution microscopy platform. Prior to this, he has worked in Hungary and Sweden on several research projects investigating innate immunity, and chromatin organisation of cancer and stem cells using microscopy and image analysis. Now, his interests lie in multicolour single molecule localisation techniques.

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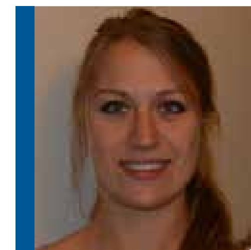
In vivo microscopy: The intravital assessment of functional networks through calcium imaging in the CNS



Kim Chisholm, King's College London

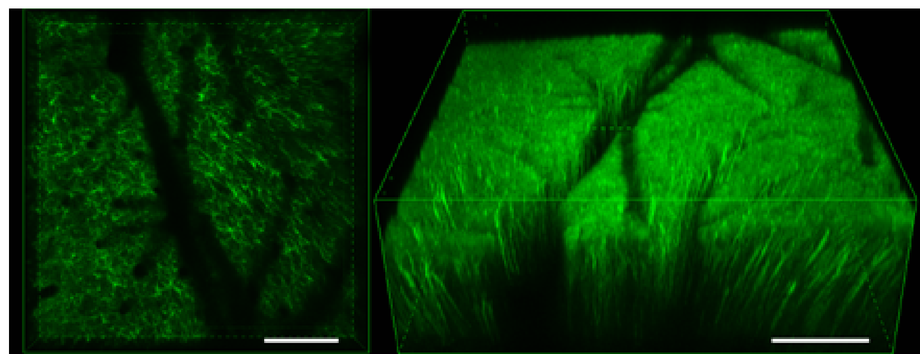
Intravital fluorescence microscopy is the ability to visualise structures in the whole animal, using the fluorescent or phosphorescent properties of molecules inherent to tissues or artificially added. This technique allows the visualisation of individual structures, cells and subcellular organelles within a heterogeneous and physiological environment. In addition the ability to dynamically alter conditions while imaging, for example by electrical stimulation or application of treatments, offers very powerful experimental designs. Combined with the high spatial and temporal resolution of modern microscopes, *in vivo* microscopy therefore represents an important tool to answer vital questions that other techniques struggle to address.

What do we need for *in vivo* microscopy?

The first step is the identification of a suitable model organism. Most intravital imaging studies are undertaken on animals smaller than rats, including mice and drosophila. Even with small rodents (the focus of this review) a specialised stage is often needed to allow sufficient distance between the stage and microscope objective to fit the animal.

In addition, one needs to consider the surgical exposure/stabilisation of the organ of choice. The cerebral cortex (example in Figure 1) has been a much quoted system for intravital microscopy: cranial windows or thinned-skull preparations provide relatively easy techniques for repeated assessment over long periods even in awake and behaving animals[1]. Other organs (e.g. the spinal cord) are more difficult to visualise due to greater movement artefacts, necessitating sophisticated stabilisation techniques, both through physical stabilisation and post-acquisition image processing. Other organs may require externalisation and superfusion if they are located deeper within the organism.

Figure 1. *In vivo GCaMP expressed in the mouse cortex and imaged using two-photon microscopy, 1 month post injection of a viral vector containing a GCaMP transgene. Left: top-down view onto cortex. Right: three dimensional representation of z-stack. Scale bar = 200μm.*



Many *in vivo* microscopy studies use two-photon microscopes which employ near-infrared femtosecond pulsed lasers. As the beams coincide, in a diffraction-limited focal volume, they provide enough energy to excite the fluorophore. As any emitted light therefore originates from a known focal source there is no need for the addition of a pinhole (as in confocal microscopy), consequently requiring less laser intensity. Additionally, longer wavelengths have better tissue penetration, with structures being visualised to depths of 600-1000μm[2]. Confocal microscopy in turn, is more suited to red-shifted signals (which can be difficult to detect with two-photon microscopy), and is often cheaper and more widely available.

A final consideration is the choice of fluorophore. Huge advances in the development of fluorescent labels and a greater understanding of the sources of autofluorescence have provided a vast bank of fluorescent probes, ranging from vital dyes directly applied to tissues, or injected systemically to a huge array of genetically encodable dyes.

An example of the application of in vivo fluorescence microscopy – Calcium imaging in the CNS

As the communication between cells and other non-cellular compartments is not disrupted, intravital microscopy is a particularly valuable technique when investigating the spatial and temporal relationship between components of a complex network and their environment. Such questions are especially important to the central nervous system where network interaction is essential to its main function: signal conduction. More traditional techniques, including electrophysiology, suffer from strong limitations such as the selectivity of certain cellular classes and neglect of silent cells.

One way to address the complex network of neuronal and non-neuronal cells is through intravital calcium imaging. Calcium is taken into cells through voltage- and neurotransmitter-gated ion channels. Thanks to these short, action potential-linked calcium transients, as well as the development of various fluorescent calcium indicators and their use *in vivo*, our understanding of the response properties of cells and neuronal networks has advanced considerably. Indeed, intravital calcium imaging, with its variable protocols, is a useful method to exemplify some of the features discussed above, which are common to all *in vivo* microscopy preparations.

Calcium sensitive dyes: One of the techniques to assess calcium signals is through the use of calcium sensitive dyes. In a pioneering study Stosiek et al. bulk loaded a number of acetoxymethyl (AM) esters conjugated dyes into the mouse cerebral cortex[3]. AM esters offer membrane permeability and, after cleavage of the AM groups through endogenous esterases, provide intracellular stability. Using this multicellular bolus loading technique, Dombeck et al. were able to assess neuronal function in awake and moving mice[4]. Here, the use of sulforhodamine 101 to label cortical astrocytes, provided a stable image for residual motion corrections which was present even after mechanical stabilisation.

However, bulk loading of calcium dyes involves non-discriminate labelling of the entire tissue. This makes it difficult to distinguish finer structures, such as dendrites, necessitating the use of additional dyes or transgenic labels to discriminate cellular populations. Not only does this complicate the set-up, but it is also limiting due to the number of labels available and their spectral interference. Additionally, dyes tend to leak out over time, providing strict temporal limits on repeated measurements of calcium signals.

Genetically encoded calcium indicators: These limitations can be overcome by the use of genetically encoded calcium indicators (GECIs). These calcium indicators remain stably expressed over months and allow selective assessment of almost any subgroup of cells.

As one of the most widely used examples of intravital calcium sensors, GCaMP proteins have seen numerous iterations. In the presence of calcium their fluorescence intensity increases and they currently equal or even surpass some of the commonly used synthetic dyes in sensitivity and speed, allowing the detection of dendritic calcium signals and single action potentials *in vivo*. Virally-driven GCaMP can be stably expressed in any CNS tissue, for example in the murine cortex following vector injection (Figure 1). In another example Dombeck et al. were even able to image calcium transients in hippocampal neurons in awake mice, through surgical aspiration of the cortex and its replacement with a stabilising metal cannula[1]. Despite such flexibility associated with viral constructs this technique can result in spatially and temporally heterogeneous gradients of GCaMP expression as protein levels are higher near the injection site and can increase over time. This heterogeneity can lead to inconsistencies between animals and even affect cellular health.

In contrast, transgenic animals have the potential for more consistent and stable expression of GCaMP in cellular or subcellular structures. For example Chen et al. were able demonstrate non-toxic levels of GCaMP expression in their *Thy1* driven mouse lines[5]. Zariwala et al. in turn showed stable expression of GCaMP in numerous CNS tissues, including the retina and the cerebellum by crossing floxed GCaMP reporter mice with Cre lines, suggesting that the only limitation for tissue specific expression is the quickly expanding availability of Cre driver lines[6].

It is also possible to use Förster resonance energy transfer GECIs to visualise changes in cellular calcium. Using this ratiometric technique for example, Nishida et al. were able to attenuate the remaining movement artefact associated with *in vivo* spinal cord microscopy even after stabilisation[7].

However, it should be remembered that calcium indicators are also calcium buffers and therefore influence cellular dynamics. Toxicity can be monitored by nuclear calcium signals which indicate altered physiology and impaired indicator function[8]. Additionally, the use of modern indicators, in utero electroporation and transgenic expression seem to lead to less toxicity[5,9,10].

Concluding remarks

Despite some of the complications associated with intravital microscopy, the benefits of this approach are numerous and include the high spatial and temporal resolution of modern microscopes, the intact and physiological nature of the preparation, the ability to visualise multiple network components simultaneously, including active and silent cells, and the possibility to image the same regions over extended periods of time. Therefore, intravital microscopy has the potential to answer questions that more traditional methods struggle to address.

About the author

Kim is a postdoctoral researcher in the Wolfson Centre for Age-Related Diseases in King's College London. She obtained her PhD in the Institute of Neurology at University College London, studying the effect of inflammation and hypoxia on cortical mitochondria *in vivo*. Currently she is working on visualising the functional connectivity of spinal cord projection neurons and assessing their role in the development and progression of chronic pain. Throughout her research Kim has investigated multiple CNS tissues by *in vivo* optical imaging, spanning the retina, the cerebral cortex and the spinal cord of mice using multiple intravital methods including confocal, two-photon and fluorescence lifetime imaging microscopy.

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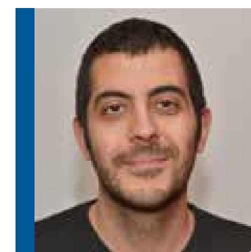
Cre-LoxP strategy, oldie but goldie



Alessandro Pristerà, Francis Crick Institute

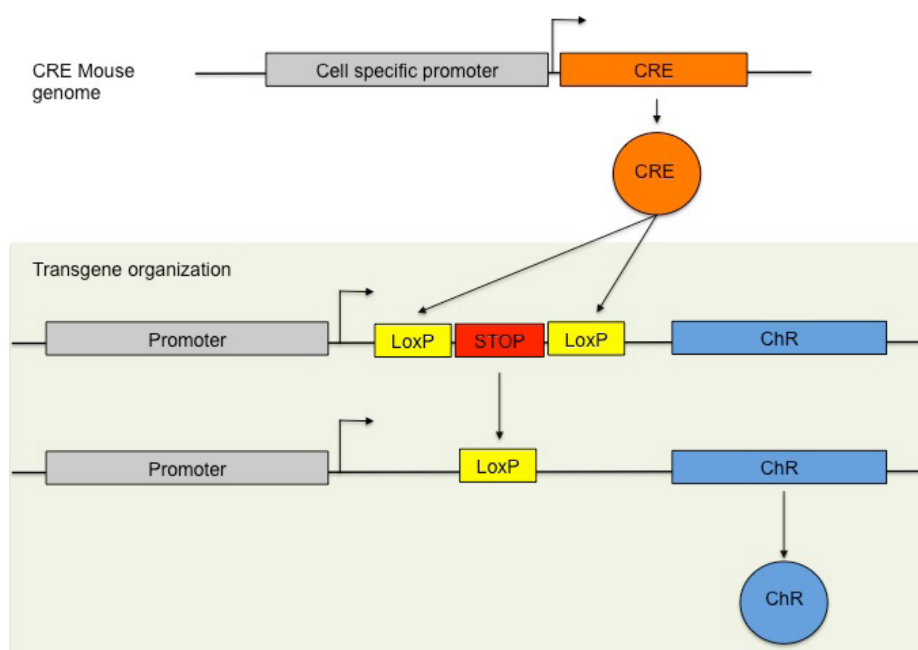
In the last twenty-five years genetically modified mice have proved to be an invaluable tool to study gene function, model human disease, test potential therapeutic targets and aid drug development. A tremendous amount of data has been generated by deleting or over-expressing genes of interest in defined loci in the mouse genome. This strategy has permitted researchers to link any gene of interest to its function, both in physiological conditions and in the development of diseases. To achieve spatial resolution (e.g. deletion only in certain tissues or cells) and temporal resolution (deletion occurring at a certain stage determined by the experimenter), scientists have harnessed a natural occurring phenomenon, the Cre-LoxP recombination system[1].

Cre is a viral recombinase derived from P1 bacteriophage that catalyses the site specific recombination event between two LoxP sites, its DNA recognition sequences. LoxP sites are short (34 base pairs) DNA sequences containing two palindromic fragments. The orientation of the two LoxP sites determines the fate of the DNA sequence that they flank, upon recombination. If the LoxP sites are oriented in the same direction, the DNA sequence in-between is excised, circularized and deleted after Cre-mediated recombination. Conversely, if the two LoxP sites are oriented opposite to each other, the DNA sequence in between is inverted upon Cre-mediated recombination.

Cre-LoxP recombination and genetic engineering of mice has been exploited to achieve conditional deletion of genes in specific cells. To do so, mice strains that express Cre under the regulation of the promoter of a cell-specific marker (e.g. Slc17a6 for excitatory glutamatergic neurons, Slc32a1 for inhibitory GABAergic neurons, Slc6a3 for dopaminergic neurons) are bred with mice strains that have LoxP sites flanking a region of DNA critical for protein function (Fig. 1).

In the resulting offspring, Cre-LoxP recombination and DNA excision, leading to gene deletion, will only occur in cells that express the Cre recombinase. The obvious advantage of this strategy is the gain in tissue-specificity, in the case of a widespread expression of the gene of interest in other regions of the body. Cre-LoxP permits investigation of the role of a gene in a more precise fashion (e.g. the role of the gene of interest in a specific tissue) and can circumvent the lethality issue that may occur in knock-out mice, when a vital gene of interest is deleted in every cell of the body.

Figure 1. *The schematic shows the genomic organization of Cre and LoxP sites in the genome and the breeding strategy to generate conditional knock-out mice. Source: Wikimedia commons (https://commons.wikimedia.org/wiki/File:CreLoxP_experiment.png)*



Scientists are also able to control the temporal specificity of Cre-LoxP recombination[2]. This has been made possible by combining genetic engineering of a modified version of the Cre recombinase and pharmacological treatment. Cre recombinase fused to a mutant estrogen-ligand binding domain (CreERt2) is normally sequestered into the cytoplasm of the cells where it is expressed, hence it is not in the proximity of genomic DNA and cannot mediate recombination. In this scenario, the DNA that is to be deleted, flanked by the LoxP sites, continues to be expressed under the endogenous cell programme. To induce CreERt2 mediated recombination, tamoxifen, a specific ligand of the mutated estrogen-ligand binding domain, is delivered to the mice through injection or via the diet. Upon binding of tamoxifen to CreERt2, the latter translocates into the nucleus where it can mediate recombination and gene deletion. With this strategy it is therefore possible to control the time of deletion, which can be particularly important in circumstances where the gene of interest is involved in developmental processes. By allowing normal development and inducing gene deletion in adult mice, scientists can discriminate the role of certain genes between developmental stages and cell homeostasis during adulthood.

In our lab, we have employed the CreERt2-LoxP strategy to study the role of FOXA transcription factors in the maintenance of ventral midbrain dopaminergic neuronal properties in adult mice. FOXA genes are necessary for the development of dopaminergic neurons in the brain, and their deletion during development results in a lack of generation of dopaminergic neurons, while FOXA deletion during adulthood (when developmental processes are complete and dopaminergic neurons are formed) results in functional impairment of dopaminergic neurons, without leading to neuronal loss[3].

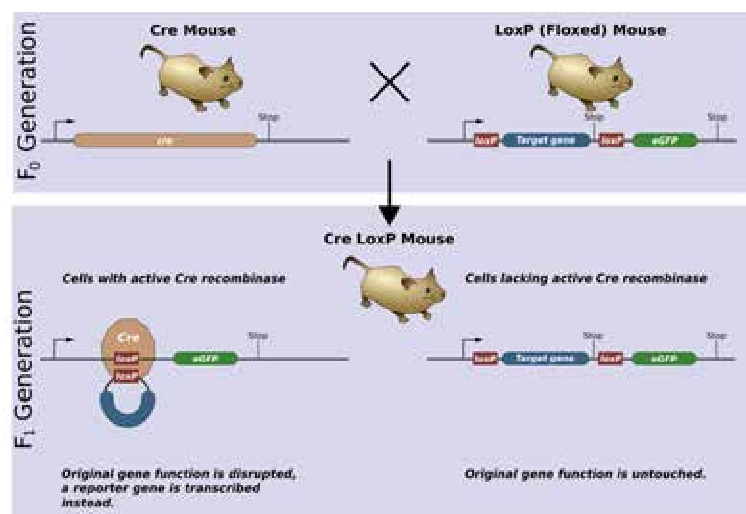
More recently, genetically modified mice have been exploited not only to define gene function, but also to gain specific access to defined populations of cells for manipulation. Particularly in the field of neuroscience, transgenic mice have proved invaluable in determining neuronal function and, importantly, to prove causal relationships between neuron activity and behaviour.

Together with tools designed to quantify and manipulate the activity of defined sub-sets of neurons, the Cre-LoxP strategy has given scientists a robust tool to study neuronal function in the brain, their circuitry and resultant role in specific behaviours. The strategy bridges molecular biology, genetics and physics and relies on Cre-dependent, genetically encoded tools, to bi-directionally control neurons, by forcing them to fire or to fall silent. A major breakthrough in the last decade in the field of neuroscience was the development of optogenetics, which is the name given to the plethora of techniques used to control neurons with light stimuli. Optogenetics relies on expressing ion channels named channelrhodopsins, derived from green algae, in a defined population of neurons. When these proteins are exposed to specific wavelengths they allow ion flux into the neuron and action potentials are triggered. Therefore optogenetic techniques allow specific manipulation of neuronal activity using light[4]. A seminal study showed, for example, that channelrhodopsin-evoked burst firing in midbrain dopaminergic neurons is sufficient to drive behavioural conditioning[5].

A fruitful tactic being exploited by many neuroscientists relies on specific viral delivery of a transgene encoding channelrhodopsins, whose expression is impeded by a DNA sequence, a STOP cassette, in front of the transgene itself. STOP cassettes are usually flanked by LoxP sites, so that upon delivery of the transgene into Cre-expressing mice lines, only the desired neuronal sub-types expressing Cre will allow deletion of the STOP cassette and drive expression of the channelrhodopsin (Fig. 2). This method allows specific targeting and manipulation of neuronal sub-sets. By forcing the selected neurons to fire, or to suppress their activity, and assessing the behavioural response, it is possible to establish causal relationships. Thanks to this strategy new causative links have been made between neuronal populations, higher cognitive functions and behaviours like feeding, sleeping, reward and motivation mechanisms, fear, depression, anxiety and drug addiction. An elegant example is provided by the identification of a sparse neuronal population of the hippocampus, whose channelrhodopsin-mediated activation is sufficient to recall a fearful memory[6]. Another study showed that activation or inhibition of midbrain dopaminergic neurons immediately modulates depression states caused by chronic stress[7].

The classic Cre-LoxP strategy has not only made the conditional knock-out possible, with both spatial and temporal resolution, but has also paved the way for even more sophisticated manipulation of cell functions, thanks to the development of tools to interfere with cellular processes.

Figure 2: *The schematic shows the strategy to achieve Cre-mediated expression of Channelrhodopsin (ChR) only in the cells expressing Cre. (Adapted from Zeng & Madisen 2012)*



About the author

Alessandro Pristerà received his degree in biological sciences at the University of Rome "La Sapienza" in 2005. He joined the London Pain Consortium in 2006, when he was awarded a Wellcome trust PhD studentship. During his PhD he investigated the role of lipid rafts and ion channels in primary sensory neurons and completed his thesis in 2011 with the title: Association of $Na_v1.8$ with Lipid Rafts in DRG Sensory Neurons. Presently, Alessandro is based at the Francis Crick Institute as a career development fellow, where he is interested in dopaminergic neurons in the brain.

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Biological Electron Microscopy at the University of Aberdeen

Kevin Mackenzie, University of Aberdeen

The University of Aberdeen's Microscopy and Histology Facility provides researchers with access to a range of services and resources, one of which is Electron Microscopy.

For the uninitiated, Electron Microscopy allows researchers to view specially prepared samples under a high vacuum using a beam of electrons. This technique allows them to then capture black and white images of small regions of cells at a much higher magnification than a light microscope. Because samples are under vacuum however, it is not possible to look at wet or live cells.

The facility has a JEOL JEM-1400 plus Transmission Electron Microscope (TEM) with an AMT camera system (Figure1), and a Zeiss MA10 Scanning Electron Microscope (SEM) (Figure 2).

Figure 1: *JEOL JEM-1400 plus TEM*

Figure 2: *Zeiss MA10 SEM*

Currently, researchers are using these microscopes to look at a diverse range of samples, including bone, fungal cells, and macrophages. Some of the more unusual samples to pass through the lab include seaweed and sea water plankton.

Let's have a closer look at both TEM and SEM and compare methods of sample preparation and the advantages of each.

Transmission Electron Microscope (TEM)

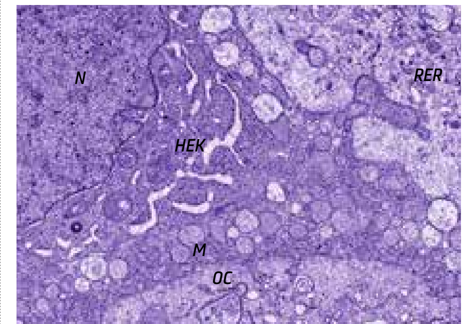
We chemically fix small tissue samples of 1mm3 in size to preserve them in as life-like a state as possible, then using ethanol, we dehydrate them before embedding them in a hard resin.

We cut ultrathin sections of 70-100 nm and place them on 3 mm round copper grids. The electron beam is able to pass through these thin sections and gives good contrast, especially if the sections are stained with heavy metal salts (Uranyl Acetate and Lead Citrate).

The resolution of TEM for biological samples can easily be 0.5 nm in biological samples – this is a 400 times improvement compared to Light Microscopy resolution of 0.2 microns (200 nm). We are able to examine the internal ultra-structural details of tissues and cells with a magnification range from x 10 – x 1,000,000

Our new AMT camera has an image resolution of 4872 x 3248 pixels (Figure 3) – a huge improvement on our previous system which gave us just 1024 x 1024 pixel images.

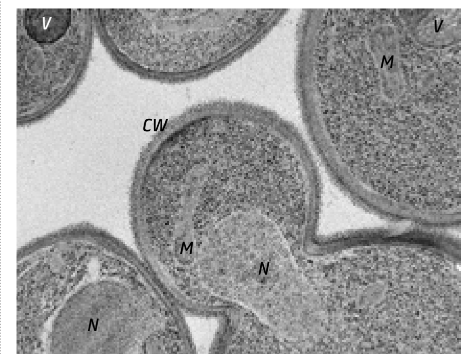
Figure 3: *Osteoclast and HEK cell pellet. N nucleus, RER rough endoplasmic reticulum, M membrane of Osteoclast, OC osteoclast cell, HEK human embryotic kidney cells – kindly provided by Dr Debbie Wilkinson*



Other TEM techniques available are:

- **High Pressure Freezing (HPF):** The sample is first frozen under high pressure with liquid nitrogen (avoiding preserving chemicals) then subjected to Freeze Substitution, where it is slowly warmed up to room temperature allowing the resin to replace the ice. This is particularly useful for preserving fine structures ie cell walls of yeast (Figure 4).

Figure 4: *Candida albicans cells growing in the yeast form. V vacuole, M mitochondria, CW cell wall, N nuclei - kindly provided by Dr Megan Lenardon*



- **Tomography:** This involves taking a succession of images while tilting the specimen through increasing angles +/-70 degrees. The resulting image stack can then be combined to form a three-dimensional image of the specimen.

Scanning Electron Microscopy (SEM)

Similar to TEM, SEM samples first need to be fixed and dehydrated. We then dry the samples using the critical point drying method, where we substitute the ethanol with liquid carbon dioxide in a pressure vessel, then sputter coat them, covering them with a very thin layer of metal such as gold or platinum to make them conducting as we scan the electrons across the specimen.

By preparing the samples in this way, we are then able to view details on the surface of the specimen at a magnification range of x 20 to x 50,000 (Figures 5 – 7).

On our microscope, the resolution of the SEM for biological samples is round 5 nm. Other microscopes can do better, but this is still a 40 times improvement compared to Light Microscope resolution of 0.2 microns (200 nm).

Figure 5: *Scanning electron micrograph of a moth fly (Psychodidae), also known as a drain fly – Kevin Mackenzie*

Figure 6: *Scales on the wing of a Madagascan moon moth, Argema mittrei – Kevin Mackenzie*

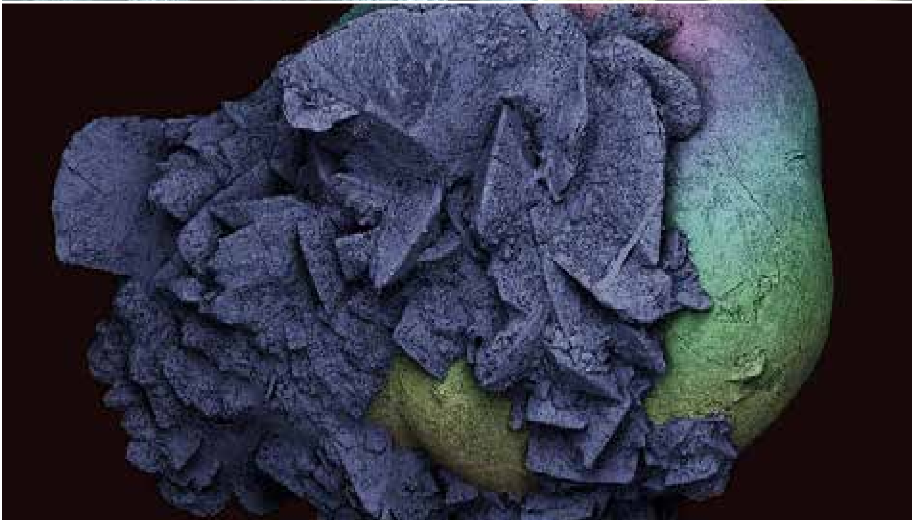


Figure 7: *Human Kidney stone – Kevin Mackenzie*

Find out more

- www.abdn.ac.uk/ims/microscopy-histology/
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- wellcomeimages.org

Recent developments in Electron Microscopy (EM)

Over the last few years there have been some exciting new developments in EM. These include:

- **Correlative Light and Electron Microscopy (CLEM):** This is essentially the ability to locate and look at the same cells under both fluorescence microscopy and Electron Microscopy. CLEM combines the advantages of both techniques, allowing scientists to spot cellular structures and processes of interest in whole cell images with LM and then zoom in for a closer look with EM.
- **Serial Block-Face EM (SBFEM):** This technique allows direct imaging of a resin block within the SEM chamber. The chamber contains a diamond knife that can cut a section of 30-50 nm thick. It is the cut surface of the block that is then imaged. This process is repeated, giving you the ability to serial section through the block and allowing an automated 3D data acquisition process.

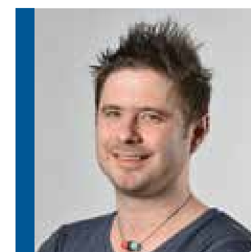
And the images produced?

All EM images are captured in black and white; however, because nowadays the resulting images are in digital format, it is relatively straightforward to add false colour using image editing software such as Adobe Photoshop.

About the author

Kevin Mackenzie has worked at the University of Aberdeen for 36 years, across various disciplines including Anatomy, Plant Science, and Zoology. He currently manages the Microscopy and Histology facility at the University's Institute of Medical Sciences, where he has worked for the past 11 years. His wonderful microscope images have been used on journal covers (EMBO) and also selected for Wellcome images awards.

Don’t worry, just FRET



Steve Tucker, University of Aberdeen

Fluorescence resonance energy transfer (FRET) is a modern molecular technique with its roots firmly embedded in physics, chemistry, molecular engineering and imaging technology. Indeed, this marriage of disciplinary inputs make it broadly relevant as an application in chemistry, physics and biology, where its measurement of structural proximity permit high resolution and quantitative study of inter- and intra-molecular interactions.

The theoretical basis of FRET is relatively straightforward, where 2 different fluorophores with overlapping fluorescence spectra are attached to the target molecule(s). When these are in close proximity, excitation of fluorophore 1 (the *donor* fluorophore) will result in emission of fluorescent energy at a wavelength corresponding to the excitation wavelength of fluorophore 2 (the *acceptor* wavelength). The result is fluorescence emitted through the *acceptor* fluorophore and not the *donor* fluorophore, as the latter has much of its emitted energy transmitted to the *acceptor* (illustrated in Figure 1). By measuring the ratio of *donor* to *acceptor* fluorescence emission following excitation of the *donor* fluorophore, the molecular intimacy of the fluorescent labels can be determined. Such information aids in elucidating interactions between specific chemical moieties, details of transduction pathways and mechanisms of protein activation/inactivation through folding/unfolding conformational shifts. Additionally, visualisation in real time and in live cells imposes this molecular level information on a relevant and physiologically applicable backdrop. While the use of FRET extends far and wide across a plethora of scientific disciplines, it is in context with live imaging of signal transduction events that this article will focus. However, that is not to say that the principles outlined do not apply across other FRET contexts and applications.

In establishing a robust and reliable FRET reporter system, there are three central considerations to address and that may require some preparatory work. By careful and considered installation of these the overall reproducibility and impact of FRET as an experimental research tool will be enhanced[1,2].

Figure 1: Fluorescence resonance energy transfer (FRET)*. The efficiency of FRET depends upon the donor and acceptor fluorophores being closely associated. Energy transfer is primarily nonradiative and occurs between paired dipoles.*

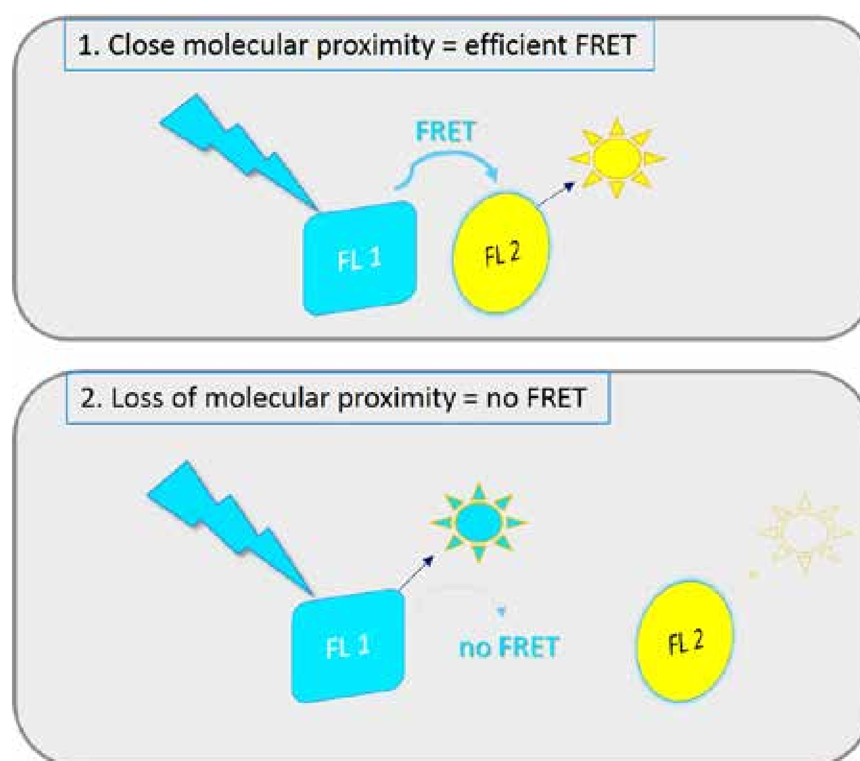
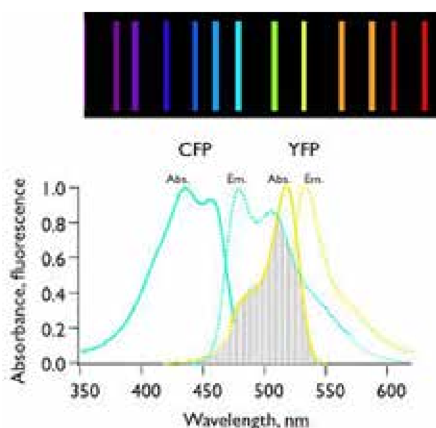


Figure 2: The overlapping fluorescence spectra of cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) make them an ideal FRET coupling*. The shaded region shows the overlap between the CFP emission peak and the YFP absorption (excitation) maxima.*



Key considerations

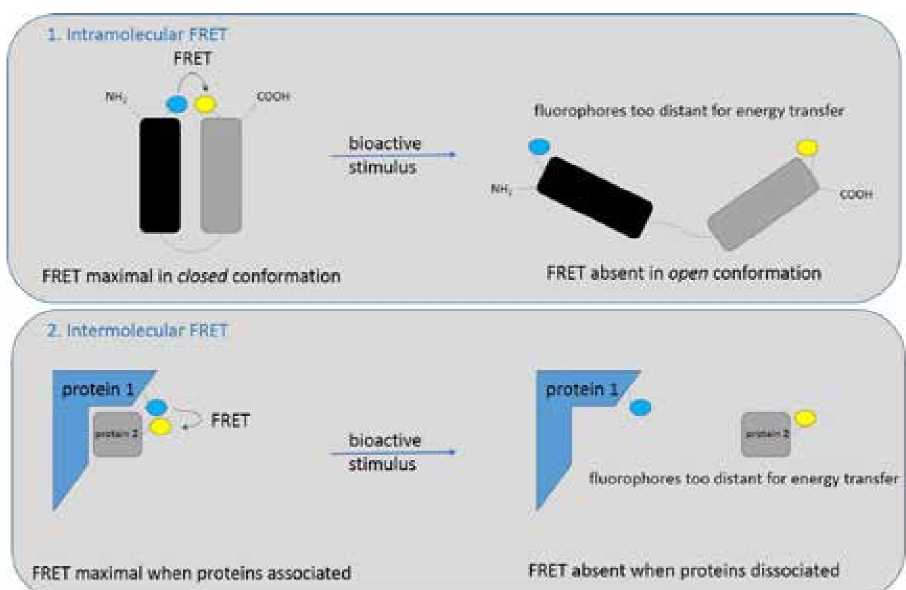
1. Molecule(s) of interest
This is primarily driven by the precise area of research interest and expertise, but fundamental to the use of FRET is the conformation and arrangement of these target molecule(s), which must have the fluorophores located within 10-100 Å of one another. Of note, the efficiency of this energy transfer is inversely proportional to the sixth power of this distance, and therefore is very sensitive and dynamic. Termed the Förster radius (after the German Physicist Theodor Förster), this distance must also change (although not by much) during conformational changes or molecular interactions to produce a measurable alteration in energy transfer. Indeed, fluorescence resonance energy transfer is a specific example of Förster resonance energy transfer that involves fluorescent molecules, although the transfer of energy is nonradiative and not a function of the fluorescence directly. Instead, the energy passes between coupled dipoles on the fluorophores and can also occur between paired non-fluorescent chromophores that possess dipole-dipole coupling[1]. Hence, Forster resonance energy transfer is a more inclusive, umbrella term covering all such applications, although the term FRET in this article specifically describes fluorescence resonance energy transfer in the context of my experience with the use of fluorescent chromophores.

In terms of choosing appropriate fluorophore (or chromophore) pairs, the excitation and emission spectra must be adjacent and overlapping such that emission from the higher energy (shorter wavelength) fluorophore excites the lower energy (longer wavelength) fluorophore effectively. The most common fluorescent pair used are derivatives of green fluorescent protein (GFP), namely cyan fluorescent protein (CFP), the higher energy *donor* fluorophore (excitation ~440 nm; emission ~480 nm) and yellow fluorescent protein (YFP), the lower energy acceptor fluorophore (excitation ~480 nm; emission ~540 nm). The relative spectral overlap is shown in Figure 2 below. Engineering of these constructs can be a laborious task, although once successfully created, these tools provide a flexible and effective means of answering research questions[3]. A further important consideration relating to the use of these constructs is how to express them efficiently in target cells. Established cell lines typically can be transfected effectively using commercially available lipofection systems, and resistance to such methods amongst primary cell lines can be overcome using electroporation-mediated transfection e.g. use of Nucleofector™ technology (Lonza)[2].

2. Intra- and inter-molecular FRET
Typically, two types of FRET operate at a molecular level, namely: intramolecular and intermolecular FRET. The former describes tethering of both donor and acceptor fluorophores to a single molecule to measure conformational changes, whereas the latter labels different molecules with separate fluorophores to measure interactions. Using the example of signalling proteins, intramolecular FRET investigations might apply fluorescent moieties to the amino and carboxyl termini of a single signalling protein such that high levels of FRET indicate the protein folded in a closed conformation and the termini close together, with the opposite response detected when the protein is open and its functional domains exposed.

Intermolecular FRET is used to confirm the close association of different proteins such that elevated FRET signal would indicate that *donor* fluorophore-labelled protein 1 was closely associated with *acceptor* fluorophore-labelled protein 2. The choice between these approaches is obviously driven by the experimental design and the molecular questions posed, but both are often used pharmacologically to piece together the sequence of molecular events following application of a ligand, drug or other bioactive molecule to a cellular system. Figure 3 below compares the processes involved in intra- and inter-molecular FRET applications[3,4].

Figure 3: Intra- and Intermolecular FRET and their use to investigate pharmacological signalling. *Intramolecular FRET with the fluorophores tagged to domains on a single protein compared to intermolecular FRET that involves fluorophore tethering to separate proteins. The resultant detection of the FRET signal permits conclusions to be drawn about the protein conformation or interactions between independent proteins.*

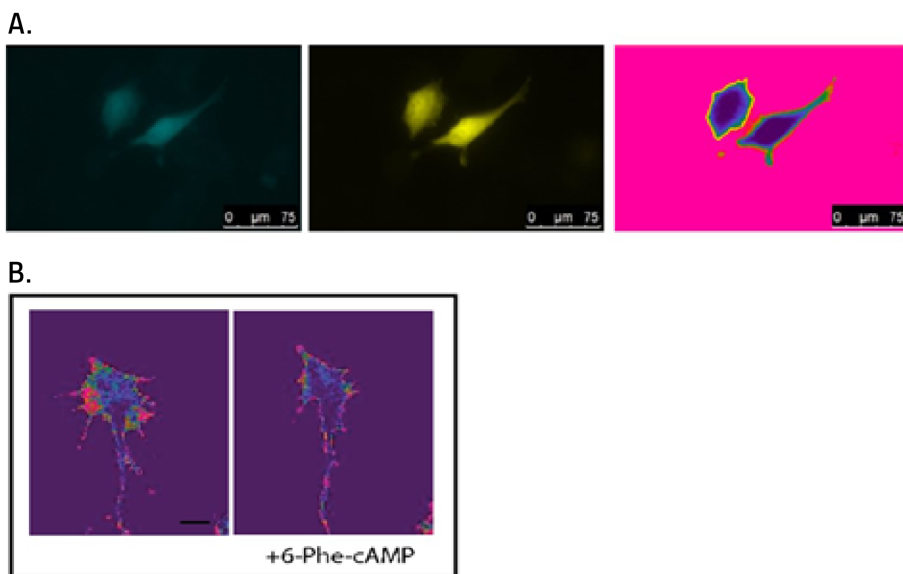


3. Quantification methods
A typical FRET imaging system comprises a fluorescence-enabled microscope, CCD-camera, appropriate filters, an incubated stage (for live time-lapse) and software/processing power to capture, store and compute appropriate images. A typical configuration for a standard CFP/YFP paired FRET system captures three channels consecutively; a *donor* (CFP) channel (excitation and emission matched to the CFP fluorophore), an *acceptor* (YFP) channel (excitation and emission matched to the YFP fluorophore) and a FRET channel (excites at the CFP excitation wavelength (440 nm) and captures emission at the YFP emission wavelength (540 nm) (Figure 4A). These images are then processed according to algorithms[5]. The result is a pseudocolour

quantified image representing the FRET efficiency corrected for channel/fluorophore cross-talk and background signal (Figure 4A). Due to the fact that this method measures the intensity of FRET as a function of *acceptor* emission across time and during stimulated molecular re-arrangement, it is often referred to as *sensitized emission* or FRET-SE. It can be used to monitor real time changes in molecular interactions following various experimental interventions (Figure 4B).

Other measurement approaches involve progressive bleaching of the *donor* fluorophore in the presence and absence of *acceptor*, where the excitability of the donor fluorophore is preserved by FRET, and therefore a delay in bleaching time indicates efficient transfer to the acceptor when present. This is termed *photobleaching FRET*. Fluorescence-lifetime imaging microscopy (FLIM)-based FRET is a method that measures the decay in *donor* fluorophore energy with time and activation. As FRET contributes to decay in the energy capabilities of the fluorophore, more efficient FRET is proportionate to a greater rate of fluorescent-lifetime decline[1].

Figure 4: Sample FRET sensitized emission (FRET-SE) images. *A) Collection of three sequential fluorescent channels (CFP, YFP and FRET from left to right). The FRET image shows pseudocolour representation of the FRET efficiency across the cells. A border applied to the upper cell permits isolation of measurement to a single region of interest. B) Example of an experimental use of FRET SE, where primary dorsal root ganglion neurons were transfected with an intermolecular FRET system reporting protein kinase A (PKA) activation. Inactive PKA shows maximal FRET as labelled regulatory and catalytic subunits are in close proximity. Application of PKA activating analogue (6-Phe-cAMP) activates PKA by dissociating the complex and reducing FRET efficiency. Scale bar indicates 30 µM.*



Overall, FRET represents a powerful and effective means of studying molecular events in real time and in live samples. As such, it permits visualisation and enhanced understanding of mechanisms associated with signal transduction, but also any other process involving changes in molecular proximity. In essence this make it applicable to an inordinate number of contexts across biological and chemical disciplines. While aspects of establishing an effective and reproducible FRET system can be laborious and time consuming, they are critical components of developing an experimental approach that is flexible, reliable and abiding. *FRET? No worries!*

About the author

Prior to becoming a senior teaching fellow and the pharmacology degree programme coordinator at the University of Aberdeen, Steve established the use of fluorescence resonance energy transfer (FRET) as a means of measuring molecular events in axon growth and guidance as part of an MRC-funded post with Derryck Shewan. His current research is developing the use of FRET alongside migration assays in cancer cells, so molecular events and cell motility can be temporally aligned and resolved to enhance our understanding of cancer cell movement. He is also a British Pharmacological Society Ambassador, and uses the exciting pseudocolour and fluorescent images as a way of engaging the public with pharmacology!

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Mesoscope: a novel instrument for imaging microscopic detail in a huge volume of tissue

Gail McConnell , Centre for Biophotonics, SIPBS, University of Strathclyde

In pharmacology, quantities are important. A problem with the conventional optical microscope (which has remained essentially unchanged for more than 100 years) is that there is a choice between seeing a tiny sample of the specimen in good detail, or a large amount with insufficient detail. For example, to identify cell nuclei, a histologist examining one slide of a thick section of tissue with a high-power objective lens and focussing up and down in 200 different areas would have to spend a full day studying the slide. At the end of this day, no more than one cubic millimetre of tissue would have been examined. This means that not only important or rare structures may have gone undetected, but unconscious bias may have occurred in area selection.

In the Centre for Biophotonics at the University of Strathclyde, we are developing a lens system which we term the Mesoscope, which allows the capture of 3D structure with subcellular detail in 59 cubic millimetres of tissue in a single dataset. Fig. 1 shows how, with this system, every cell can be seen at each focal level in a whole mount of a mouse embryo. The word 'Mesoscope' was chosen to indicate that the new microscope has the fine detail of a microscope but the large field of a macrophotography lens.

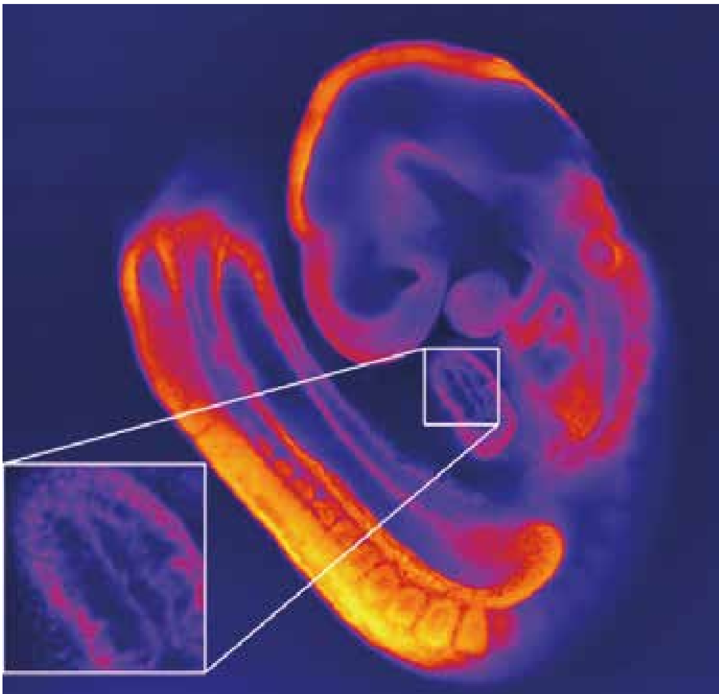

Figure 1: *Confocal mesoscopy of an 11-day mouse embryo stained for Golgi bodies shows in a single image a median optical section through the whole organism, which is 5mm in diameter, with sub-micron resolution. The full size image shows the microanatomy of the whole embryo, while in the enlarged portion at the bottom left, bright dots corresponding to the Golgi bodies of individual cells can be seen in the developing heart.*

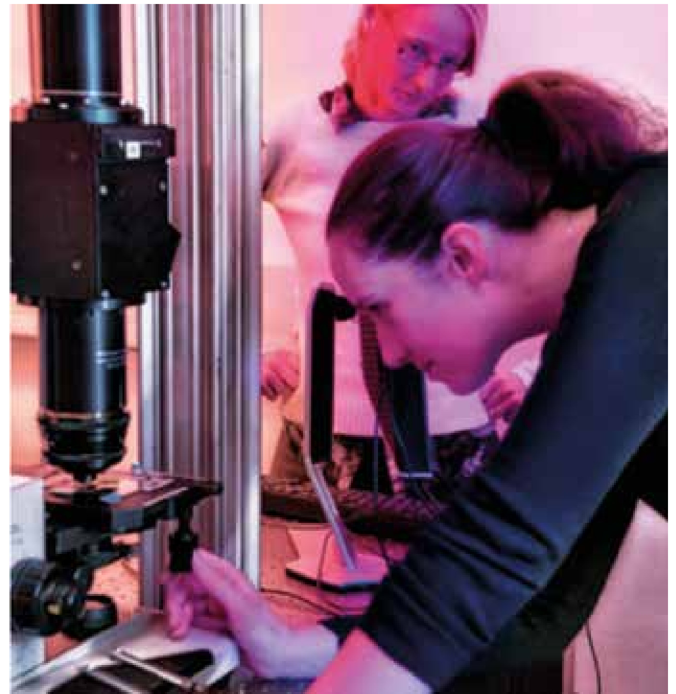

Figure 2: *The author adjusting the prototype Mesolens (with colleague Dr Johanna Trägårdh): note the large size of the Mesolens: it is the black tube to the left of the photograph. The square box in the middle of the tube houses spectral reflectors used for epifluorescence with a camera.*

The need for a Mesoscope was realised by Dr Brad Amos FRS, working in the MRC Lab of Molecular Biology in the late 80s. Amos had found that all attempts to record large volumes, such as 5mm-long embryos or small tumours or organs, in the confocal microscope (which he had co-developed) failed. This was because the available low-power microscope objective lenses (the only ones which could show a large enough area) had very poor discrimination of depth as well as slightly reduced lateral resolution [Amos et al, 2011]. Optically, the reason was that the numerical aperture of these simple lenses was too low. Amos worked with a lens designer, Mr Esmond Reid, to develop the so-called 'Mesolens', with a field diameter of 5mm, a working distance of 3mm and lateral and depth resolutions of 0.75 microns and 6 microns. It is the first 4x objective ever developed with a numerical aperture of 0.5 and colour correction good enough for biomedical work.

Figure 2 shows the author, with Strathclyde colleague Dr Johanna Trägårdh, working on the 'Mesoscope' which has been built to permit the use of the Mesolens for either camera imaging or confocal laser scanning detection. The Figure shows how the requirement of large field and large working distance drive the lens design towards a much larger lens than is normal in microscopy: the prototype Mesolens was 50cm long and the current design is longer and has a folded light path.

The 'Mesolab' in the University of Strathclyde has been set up to allow Amos and Reid to manufacture improved Mesolens systems, which are intended to be set up in Strathclyde in autumn 2015. Funding has been provided by the MRC and the University of Strathclyde, while a Leverhulme Emeritus Fellowship allows Amos to travel from Cambridge to the University of Strathclyde frequently to collaborate. Researchers throughout the UK will be using these instruments in the Mesolab, with initial emphasis on micro-anatomical study of mouse and chick embryos, for which the lens system was designed.

The Mesolens is likely to have an impact throughout science, and has already shown promise in areas as diverse as the visualization of individual tumour cells carrying luciferase genes, the measurement of 'comet' assays in acrylamide gels with vastly greater speed than standard methods, and the direct visualization of fluorescent protein expressing parasites in intact mouse ears. Of particular interest is the new ability to record rare events, such as mitoses in tissues where the mitotic index is low. In pharmacology, applications may be expected in all areas where better statistical microscopical data is required, such as in the cellular effects of drugs, and where the interactions of cells in an intact and complex tissue need to be studied, rather than isolated cells in culture.

As yet, none of the major microscope companies is offering this type of equipment, so Amos and Reid have set up a small company to manufacture the instruments, which require lens manufacture to unusually stringent tolerances. Manufacture is divided between the company and the University of Strathclyde, where the laser expertise of the group and software, developed by Dr John Dempster, are indispensable. At present, the University of Strathclyde is the only place in the world being equipped with Mesoscopes, but we hope and expect to see them spread widely in the same way as the confocal microscope. This will require improvements in the handling of data: the Mesoscope generates image information of the order of 200 Mb per focal level per channel, which might be repeated at 200 levels of focus per millimetre of depth!

About the author

Gail McConnell is Chair of Biophotonics at the Strathclyde Institute of Pharmacy and Biomedical Sciences at the University of Strathclyde. Following a first degree in Laser Physics and Optoelectronics (1998) and PhD in Physics from the University of Strathclyde (2002), she obtained a Personal Research Fellowship from the Royal Society of Edinburgh (2003) and a Research Councils UK Academic Fellowship (2005), securing a readership in 2008. Since 2004, Gail has received over £9M of research funding from a range of sources including EPSRC, MRC, BBSRC, EU Framework Programme and industry. The work in Gail's group involves the design, development and application of linear and nonlinear optical instrumentation for biomedical imaging, from the nanoscale to the whole organism. She is a Fellow of the Institute of Physics, and a Fellow of the Royal Microscopical Society.

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Amos WB, McConnell G, Wilson T. Confocal Microscopy, chapter in *Handbook of Comprehensive Biophysics*. Elsevier; 2011.

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Help promote research in developing countries. Whether you are a dynamic young pharmacologist or an old codger like me you can play your part in training early career scientists in developing countries by joining the AuthorAID programme and becoming a mentor.

AuthorAID **(http://www.authoraid.info/en/about/)** is an NGO based in Oxford funded by the UK and Swedish governments. It runs an online programme that puts young scientists in developing countries in contact with experienced researchers willing to act as mentors and give constructive advice. AuthorAID also provides online courses on research writing and proposal writing for researchers in developing countries that complements the monitoring programme.

IUPHAR wishes to promote this mentoring scheme to early career pharmacologists across Africa through the *PharfA* initiative as well as to pharmacologists in other developing countries. But first we need to recruit experienced pharmacologists to act as mentors.

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Professor Graeme Henderson
Vice President
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- Presentations and posters
- Preparing grant proposals
- Scientific communication in general

How does the mentoring process work?

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- Register for AuthorAID at **www.authoraid.info** and indicate that you are interested in becoming a mentor
- Fill in your AuthorAID profile with as much detail as possible so that potential mentees can find you
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- Start a conversation and establish rapport with your potential mentee using AuthorAID's messaging system
- If you both agree to mentoring, follow a link to set up a formal online learning agreement and use the task-based mentoring system

What sort of potential mentor is AuthorAID looking for?

- An experienced researcher, with a track record of publications
- Someone who is diplomatic and can encourage and support early-career researchers in developing countries
- Someone who is committed and enthusiastic and who is not looking for academic gain.

Need more help?

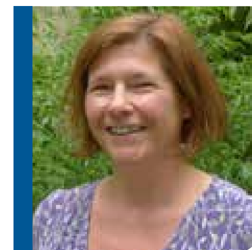
Visit **www.authoraid.info/help** and see the AuthorAID guidelines for more information. For a general example of how AuthorAID might work, follow Rahim on his mentoring journey at: **www.authoraid.info/en/mentoring/authoraid-mentoring-journey**

If you have specific questions, please feel free to contact us at: **authoraid@inasp.info**

www.authoraid.info

What does the Concordat on Openness on Animal Research mean for the British Pharmacological Society and its members?

Sarah Bailey, Department of Pharmacy & Pharmacology, University of Bath
& Education & Training Committee/ Animal Welfare & *In Vivo* Pharmacology Sub-Committee



Drug discovery research requires the use of animals; both at the level of isolated cells and tissues, as well as whole organisms. In the UK the use of animals in scientific procedures is highly regulated and the legislation places the welfare of the animals at the heart of every research project. Despite this, there is widespread public feeling that unregulated experiments are conducted, that scientists may be causing unnecessary harm to animals and that unnecessary duplication of animal experiments occurs. The "public attitudes to the use of animals in scientific research" survey[1], conducted by IPSOS MORI from 1999-2014, shows that two thirds of the public support the use of animals in medical research. But this support is waning (2014:64% vs 2010:76%). How can the scientific community ensure continuing support for the vital use of animals in drug discovery research?

Understanding Animal Research is an organisation that aims to achieve broad understanding of the humane use of animals in biomedical research in the UK. It is a not for profit organisation funded by academic institutions, medical charities, pharmaceutical industry and learned societies including the British Pharmacological Society. Concern about the waning support for animal research and the lack of trust in scientists led to Understanding Animal Research working with its members and partners to develop the Concordat on Openness on Animal Research[2]. The Concordat was launched in Spring 2014. As well as being instrumental in the discussions leading up to the Concordat, the British Pharmacological Society was among the first signatories. The Concordat has four key commitments to promote being more open about the use of animals in research:

Commitment 1: We will be clear about when, how and why we use animals in research

Commitment 2: We will enhance our communications with the media and the public about our research using animals

Commitment 3: We will be proactive in providing opportunities for the public to find out about research using animals

Commitment 4: We will report on progress annually and share our experiences

Perhaps the cleverest of these commitments is the last one, the requirement to report annually on progress. When the British Pharmacological Society had to complete the annual report in May this year, we had to consider what we had been doing as a Society to meet our commitments during the Concordat's first year. The full report can be viewed on the UAR website[3]. Learned societies and professional bodies are in an unusual position in this regard – they do not carry out research but act in the interests of their members. Of course the British Pharmacological Society is also a direct funder of animal usage in research and education through its grant schemes – so what can the British Pharmacological Society do to meet its commitments under the Concordat? How can it support its members to be more open?

I use animals in my research into mechanisms and treatments of depression. I believe that it is important to be open with the public about how we use animals to ensure continued support and funding for this valuable work. The first time I was open about animal research I was fortunate enough to be able to work with the Science Media Centre in London who supported me during my first ever press briefing. I was anxious that journalists were going to take my research and challenge me about using animals at all, or take the research and extrapolate the findings into humans when we were a long way from that. My experience was very positive and I found my research reported accurately and fairly in all the major newspapers, on radio and even on TV. Now, I don't do research that is that newsworthy every day but I had realised that talking about animals in research was not something that attracted negative attention. So I did some training with Understanding Animal Research and became one of their school ambassadors. The training really helped me rehearse some of the arguments I might face which was very useful as GCSE students can be very direct with their questions! I have continued to work with Understanding Animal Research over several years now and we have arranged visits of our local MP, patient groups and school children to our animal facilities at the University of Bath. I have recently joined the Animal Welfare and *In Vivo* Pharmacology (AWIVP) sub-committee at the British Pharmacological Society and am looking forward to working with the group as they consider how to develop a strategy for the Society to meet its commitments under the Concordat.

The British Pharmacological Society has started with some "quick wins" that are relatively easily achieved – there is a statement on the Society's webpage and links to publicity for the general public e.g. UAR literature, NC3Rs literature[4]. There are plans to use ***Pharmacology Matters*** to communicate with the membership and discuss issues around the Concordat and openness, amongst other *in vivo* pharmacology issues.

Also you will be able to catch up on developments around the Concordat at Pharmacology 2015 where Bella Williams from Understanding Animal Research will update us on progress[5].

The British Pharmacological Society has also tried to be proactive in engaging with the media. Working with the Science Media Centre, in connection with a special issue[6] of the *British Journal of Pharmacology*, Graeme Henderson, Emma Robinson and myself all attended a background briefing on "What animals tell us about psychiatry". It is perhaps testament to how far we have come in being open about animal research that only a handful of journalists turned up and they only wanted to hear the very latest developments in research. No journalists were interested in arguing the rights and wrongs of using animals at all. In terms of moving forwards, rather than print media, perhaps there is more that BPS can be doing using social media. The *British Journal of Pharmacology* also published a series of editorials[7] discussing how it is responding to the 'Transparency Agenda' set out in the Concordat, including changes to its reporting requirements.

There is also scope for learned societies to work together to look at ways of providing a forum for sharing best practice in animal research, to provide a national focus to promote the benefits of animal research and possibly even having a national pro-animal research day. One thing is clear – the Concordat is a starting point – the expectation from funders, learned societies, animal welfare and animal rights activists is that the scientific community will need to go much further with regard to openness in the future.

This is exactly what the Society has committed to. Over the coming months, the AWIVP Sub-Committee will examine how we can continue and develop its work to deliver the Concordat commitments. One mechanism is to ensure that we highlight and support the openness work of our members. Many of you reading this will yourselves be involved in animal research, and we would like to hear from you about your openness activities and the support you would value – so we can work towards upholding the Concordat together.

If you would like to discuss your activities and ideas, please email the team using **education@bps.ac.uk**.

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About the author

Sarah's research focuses on understanding molecular mechanisms underlying depression and anxiety. At the University of Bath, she is chair of the Animal Users Forum and a Public Engagement Advocate, which involves supporting innovative ways to increase engagement with research. She is also a Schools Ambassador for Understanding Animal Research and has worked with the Science Media Centre to promote the importance of being open about animal research in the development of medicines. In addition to her involvement in the British Pharmacological Society, she is also a member and elected to the Council (2013–2017) of the British Association for Psychopharmacology.

Meetings update

Barbara McDermott, Vice President – Meetings
Talja Dempster, Head of Meetings & Events

It has been a busy few months for the Meetings Team at the British Pharmacological Society with a nice mix of our own meetings, meetings or symposia run jointly with other societies, or meetings to which we have contributed funding. A short summary of those meetings and events are below:

Past meetings

21st Scientific Symposium of the Austrian Pharmacological Society (APHAR)

The 21st APHAR Meeting of was jointly organised with the British Pharmacological Society and the pharmacological societies of Serbia, Slovenia and Croatia. The meeting took place in September in Graz with around 100 attendees from the five host societies attending.

We ran our first Diploma Workshop outside of the UK as a precursor to the APHAR meeting and this was attended by students from Austria, the UK and Egypt.

You will also hear about the Young Pharmacologists Advisory Group's "speed dating" event that was held as part of this meeting in Austria as part of the Group's update (on page 26).

British Hypertension Society (BHS) Annual Meeting

The Society ran a joint symposium at the BHS Annual Meeting in Stratford upon Avon in late September. The symposium was well attended and the topic 'Medicines optimisation in blood pressure and diabetes' was well addressed by speakers from Scotland, Birmingham, Oxford and Copenhagen.

Stratified medicine and prevention of adverse drug reactions

This joint meeting between the Society and the British Toxicology Society took place in early October at the Royal College of Physicians in Edinburgh.

The meeting attracted 60 attendees from all over the UK. Feedback received has been very positive, with 'great meeting' and 'excellent conference' being the most heard parting words as the attendees headed back into the everyday world.

Poster session at our 'Stratified medicine and prevention of adverse drug reactions' meeting

Networking at our 'Stratified medicine and prevention of adverse drug reactions' meeting

Future meetings

Pharmacology 2015

There have been a record breaking number of abstract submissions for Pharmacology 2015, with 418 submissions in total, an impressive 11.5% increase on 2014. The programme scheduling is now well underway and we will upload the detailed version to the sparkly new website (**www.bps.ac.uk**) just as soon as it is done. If you have yet to register, you can sign up on the website too – please make sure you do sooner rather than later as this year is anticipated to be bigger and better than ever before, and we are really looking forward to seeing you there!

The programme is packed with exciting symposia that are being run in conjunction with a number of sister societies, including the British Association for Psychopharmacology, British Society for Cardiovascular Research, ASCEPT and the Medicines and Healthcare Products Regulatory Agency. There are also three dedicated poster sessions and a number of networking events – plenty to keep you occupied over the three days!

Drug Metabolism and Pharmacokinetics (DMPK) Meeting

The 3rd DMPK (Drug Metabolism and Pharmacokinetics) meeting will take place on 8–9 February at Burlington House in Piccadilly, London. The meeting is being jointly organised by the British Pharmacological Society, the Royal Society of Chemistry BMCS (Biological & Medicinal Chemistry Sector), the Drug Metabolism Discussion Group and the Drug Metabolism Group and will provide a forum for the exchange of ideas between principal members of the DMPK research community. Keynote talks from industry leaders will provide global perspectives on how DMPK can bring value to the process of informing drug discovery. The emphasis will be on building dialogue between participants, who will range from experienced project/laboratory leaders to early-career researchers. Visit **www.bps.ac.uk** for more information, abstract submission and registration details.

6th Focused Meeting on Cell Signalling

The Society's 6th Focus Meeting on Cell Signalling will take place on 18–19 April 2016 at the University of Leicester. In addition to talks from an international speaker line up, the event will feature the 2016 Paton Memorial Lecture presented by Professor Michel Bouvier. More information on this event will be available on the website shortly.

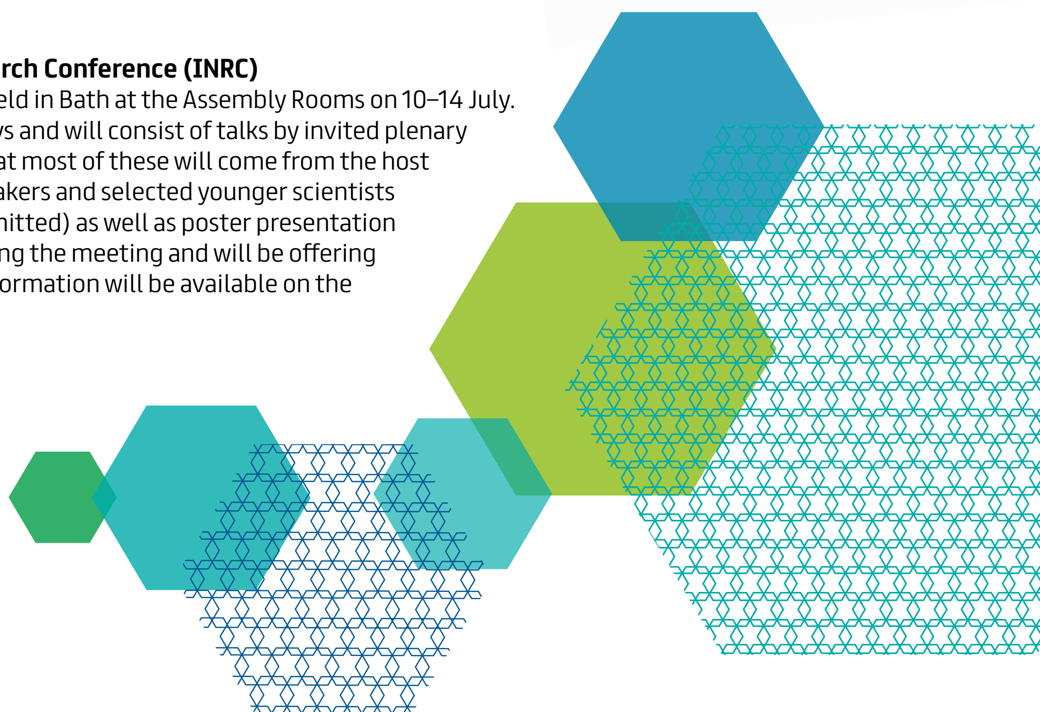
International Narcotics Research Conference (INRC)

The 2016 INRC Meeting will be held in Bath at the Assembly Rooms on 10–14 July. The meeting will run for four days and will consist of talks by invited plenary speakers (the tradition being that most of these will come from the host nation), invited symposium speakers and selected younger scientists (chosen from the abstracts submitted) as well as poster presentation sessions. The Society is supporting the meeting and will be offering bursaries for members. More information will be available on the website shortly.

Hello from Talja

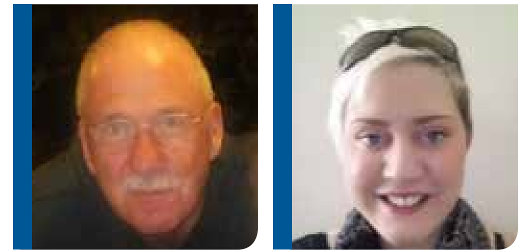
Hello! This is just a very brief message to introduce myself as the new Head of Meetings and Events. I joined the Society in August, prior to which I worked at the Society for Experimental Biology for six years managing their programme of events. Before that I spent two years working for 4Children, a national children's charity delivering a series of conferences, workshops and seminars for those working in the childcare sector. I have been made to feel very welcome by the team here in the office and by the members I've had the pleasure of meeting so far and I'm really looking forward to meeting many more of you at Pharmacology 2015 in December.

Talja



The Role of the Forensic Pharmacologist as an Expert Witness in the UK

Paul Skett & **Steph Sharp**,
Glasgow Expert Witness Service



Forensic Pharmacology is the application of pharmacological knowledge to legal matters, including criminal or civil cases such as drink driving or medical negligence/inquests where drugs are involved. So anyone with higher degree (PhD) training in pharmacology can, in theory, become a Forensic Pharmacologist. However, it requires training in legal report writing, courtroom technique and the legal role of the expert witness. The Court employs Forensic Pharmacologists to assist in matters within their area of expertise, to explain pharmacological principles and guide the Court through the intricacies of scientific knowledge related to the case in hand.

It is frequently the case in courtroom trials that additional information or clarification regarding specialist evidence may be required by the decision maker or jury in order to reach a verdict. The expert witness is a qualified specialist whose qualifications and experience in a given field deems their opinion on relevant aspects of courtroom evidence credible by the court . This article will summarise the role of the pharmacologist in legal cases involving drugs or alcohol.

Drugs and alcohol are involved in a great number of legal cases, both criminal and civil and they frequently require the assistance of a forensic pharmacologist. Such proceedings usually begin with instructions from the acting solicitor, requesting a report to be written on the matter in question. The submitted report may raise further questions for the court, and the expert may be required to attend the trial in person to explain any relevant queries raised by the defence, prosecution, jury or decision maker. Common areas of questioning range from basic information regarding the function and effects of prescribed or illicit drugs and alcohol, to more complex issues of polypharmacy, drug interactions, issues of medical negligence and illicit drugs in typical and atypical users. In some instances, these queries may appear to be common sense, however, it must be remembered that only a credible pharmacologist is qualified to give a statement in a court of law. In more complicated cases, it is pertinent that the expert can explain their opinion in a manner which is easily understood to all parties in the court.

In order to maintain credibility as an expert pharmacologist, responses to any question posed by the court must be based on evidence that has been appropriately evaluated and referenced from the published literature. The use of out of date information, or weak or underpowered studies may result in ill-informed opinions and inaccurate answers being communicated to the decision maker, which may be detriment to the court and the credibility of the witness.

Case Studies

Case 1

Alcohol is a common element in many cases that a forensic pharmacologist may encounter. Drink driving is, unfortunately, prevalent in the UK. One common defence in cases in which drivers have been apprehended under the influence of alcohol is 'post incident drinking', which is also referred to as a 'hip-flask defence'. In such cases, the position of the client is that they had consumed alcohol after a road traffic accident as opposed to having been driving while intoxicated. It is then up to the forensic pharmacologist to determine whether or not this is plausible.

One recent example of such a case involved a middle aged female, who had consumed a small amount of alcohol on the evening prior to driving to a shop. The shopkeeper contacted the police after the subject left the shop and entered her vehicle to return home, on the basis the subject appeared intoxicated in some way. The subject then returned home and drank vodka, prior to the arrival of the police. In court, the subject had argued that she was grieving, and hence appeared upset while in the shop, and admitted to consuming alcohol the evening prior to, and immediately following driving. This consumption was brought to our attention, and we were able to calculate that the breath alcohol concentration in the subject would have been well below the legal driving limit at the time of driving. The volume of alcohol consumed post driving stated by her was in keeping with the Intoximeter Breathalyzer reading obtained from her by the police. This confirmation given by the expert, based upon alcohol metabolism calculations was integral in the 'not guilty' verdict that was handed down by the decision maker.

Case 2

While alcohol is often a rather straightforward drug to explain, other drugs are typically far more complex in terms of function, metabolism and elimination. Drugs, whether prescribed or acquired illegally, are often taken in combinations, and often with alcohol. These cases require consideration of all drugs in question, separately and in combination.

One such case regards a murder trial. The aggressor had allegedly used diazepam, alongside various other drugs and alcohol, prior to committing a violent murder. The question posed was regarding the likelihood of diazepam to

elicit severe aggression in an individual user, to the point of provoking murder. While it is a rare occurrence, despite being a sedative, diazepam may have paradoxical effects on some users which manifest as severe aggression. Upon entering the court and liaising with the instructing solicitor, we were informed that the client had in fact consumed 30 milligrammes of diazepam, as opposed to the 90 milligrammes originally stated. The focus then turned into whether or not a lower dose of diazepam would have caused a severe enough aggressive reaction. The answer was 'incredibly unlikely, approaching No', and the experts were dismissed from the court.

We present here two unique cases in which the expert forensic pharmacologist is recruited in legal cases. In summary, the role of the expert pharmacologist is to address queries by the court, providing that those questions fall within his area of expertise. The expert must not be tempted nor persuaded to comment on matters out of their field of expertise. The expert must also refrain from stating a "guilty" or "not guilty" opinion when asked by either the prosecution or defence solicitors, and as such the report and evidence given ought not to differ.

About the author

Dr Sharp holds a MSci degree in Pharmacology from the University of Glasgow, and a PhD in Cancer Research from the University of Dundee. She currently works with the Glasgow Expert Witness Service as a co-director, and researcher and specialises in drugs of abuse.

Dr Skett is a pharmacologist and Honorary Senior Lecturer in Pharmacology in the College of Medicine, Veterinary Medicine and Life Sciences at the University of Glasgow, and specialises in the clearance of drugs from the body and the adverse effects of drugs. He has a BSc (Honours) degree in Biochemistry from the University of Liverpool, a PhD in Medical Chemistry from the Karolinska Institute, Stockholm. He founded the Glasgow Expert Witness Service in 1991.

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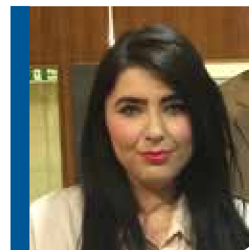
Young Pharmacologists update



Vedia Can, University of Westminster & member of the Young Pharmacologists Advisory Group

Pharmacology @ Pathways Careers Event

As part of the week-long careers festival held at the University of Westminster, the newly formed Westminster Pharmacology Society hosted the Pathways Careers Event supported by the British Pharmacological Society on the 8 October 2015. The event was hosted in the Faculty of Science and Technology, and students across all disciplines were invited. The keynote talk delivered by Professor Martin Michel, a member of the British Pharmacological Society's Industry Sub-Committee, who inspired both our students and academics about their future job opportunities. The evening proved to be a huge success across our faculty, and it encouraged the pharmacology students to network with their peers and seek further guidance on employability matters.

"Studying Pharmacology opens doors to a multitude of jobs both in the industry and in academia – you need to decide on what you enjoy doing"

Martin Michel

Pathways Careers Event

Speed dating

As part of the 21st Scientific Symposium of the Austrian Pharmacological Society, the British Pharmacological Society's Young Pharmacologists Advisory Group held the "Speed dating" event for careers in Pharmacology. Students and early career scientists were warmly invited to attend this special occasion on the 17th September 2015, to mark the closure of the second day of the popular symposium held in University of Graz, Austria. The event

Joanne Carter, on behalf of the British Pharmacological Society's Young Pharmacologists Advisory Group, led a "speed dating"' event: this was an opportunity for young people attending the meeting to come together and talk about their research and careers to date. The event was attended by 16 young and enthusiastic researchers, and the room buzzed with conversation until well after the allocated time had elapsed!

Speed dating

Thank-yous and welcomes

The Young Pharmacologists Advisory Group is grateful for the enthusiasm and contribution of Oliver Keown and chair Tim Warner, whose terms ended in late 2015. I'm confident that they will both continue to support our activities in the future. With Tim demitting, the group will be appointing a new chair at the start of 2016, following a call for expressions of interest in November 2015. Watch this space!

The Group was pleased to welcome Teesha Bhuruth as the Society's new Membership & Community Officer, who will be supporting our activities and acting as the main point of contact in the office for younger members.

Next year, the Group will involve the Society's new Young Member Trustee: this new post was created by Council as an exciting opportunity for a younger member to ensure that decision-making at the Society is guided by representation from all of our members. I'm pleased that Aidan Seeley, PhD student from Queen's University Belfast was appointed and his three-year term will begin on 1 January 2016, when he'll start attending Council and the meetings of the Young Pharmacologists Advisory Group.

About the author

Vedia C Can is a second year PhD student at the University of Westminster, specialising in immunopharmacology. This involves profiling novel compounds in the hope of inhibiting the inflammatory pathways found in Osteoarthritis. Prior to pursuing a PhD in this field she completed a Bachelor's degree in Biomedical Sciences and a Master's degree in Medical Molecular Biology.

Scientists and their hobbies: Glass is the Window of my Hobby

Felicity N. E. Gavins, Editor-in-Chief & Assistant Professor and Director of Small Animal imaging at Louisiana State University Health Sciences Center, Shreveport, USA.

"And what do you do for a living?" the lady asked as she turned towards me, having just finished discussing her ailments with the doctor next to us. (It always fascinates me how some people feel the need to share their "ailments" with a total stranger!)

"I am a scientist, well, a pharmacologist to be more precise," I said with pride.

"Oh, how interesting, although I imagine your job takes up all your time," the lady replied in a rather bored tone, as she proceeded to turn back to the doctor to continue her conversation about her back pain.

This would appear to be an age-old scenario, and I do wonder why. Generally scientists, by and large do not really have the reputation as being fun loving people. I feel that this is slowly changing, but we are still rather hampered in the "fun" department by some of our greatest scientists. Sir Isaac Newton for example, whilst a brilliant scientist, was not known to possess a fun-loving personality, but more a stern and skeptical persona. Many images of scientists (e.g. Michael Faraday and the Swedish chemist, Jöns Jacob Berzelius) working long days in the laboratory still prevail, and whilst this is still the case, it is also extremely important to take time out to pursue other interests. Not only is it important, but also scientists have now proven that by having a hobby (or even more than one!), we can actually *increase* our potential and enjoy ourselves in the process. This is due to the fact that by learning new skills the brain creates new neural pathways that make it work faster and better.

Some of our most successful scientists did have hobbies, Albert Einstein taught himself to play the violin. Karl Ernst Ludwig Marx Planck (or Max Planck), the founder of quantum mechanics, was an excellent pianist (also he was an extremely competent organist, cellist, singer and composer of songs and even operas), so much so, that he nearly chose music as his career over physics. Einstein and Planck frequently played music together, and I am sure discussed the implications of their many brilliant findings.

In my case, I have always had a great love for interior design and making things. At school and university I made various pieces of furniture, one being a solid oak coffee table (with legs carefully turned on a wood lathe), which currently stands with pride in my parents' drawing room. I think for the simple fact that I am a creative person and I like to see an end product, something tangible and visual.

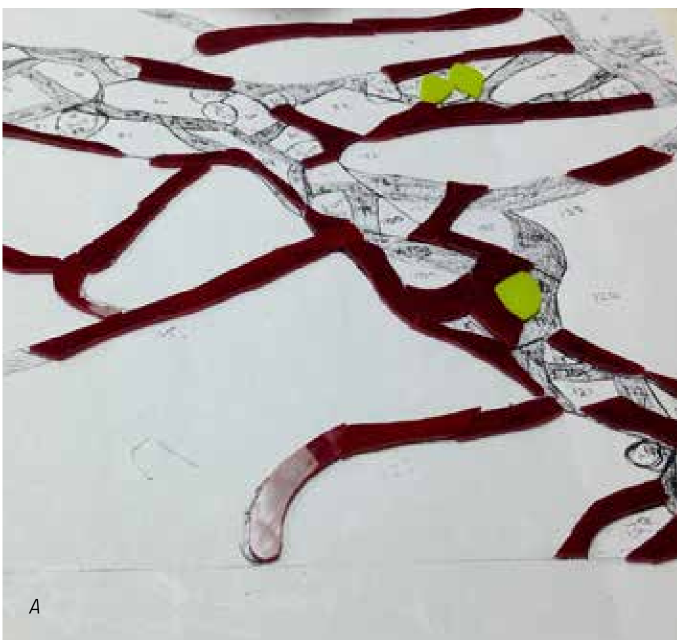


Figure 1: *A glass window of how the murine cerebral microcirculation looks following a stroke. A) a picture of how the glass window was made. Glass pieces are cut out and placed on a pattern. B) The final version. The bright green cells represent neutrophils that are labeled with green fluorescent protein*

As neuroscientist and Nobel laureate Ramon y Cajal once stated, "Scientists are endowed with an abundance of restless imagination." In my case this may indeed be true, so I use art as a tool with which to moderate day-to-day stresses, thus enabling relaxation.

As an academic, one spends a great deal of time reading and writing papers and applying for research funding. These, whilst exciting and "a part of the job", often result in having to wait months for an end result, which is not always positive! As such, I like to have some form of 'art' on the go, as a balance to my job.

Over the last couple of years, I have become very passionate about making stained glass windows. This idea was, in actual fact, introduced to me by a colleague who is equally creative. I liked the sound of it, and the thought of making art in a new medium very much interested me. So, I started attending classes, and now it has become my latest hobby.

The origin of my latest hobby is less than clear. Shards of glass dating back to 7000 B.C. have been unearthed in Egypt and Iraq, and it is known that the Egyptians developed reliable systems for glass making sometime between 3000 and 1500 B.C. However, the earliest stained glass window to have survived intact dates back to the 11th century A.D. and currently resides in Strasbourg (Germany). This window, which depicts the face of Christ, was actually originally installed in the Wissembourg Abbey (Alsace, France).

Across the ages, a great deal of stained glass was reserved for religious use between the 11th and 16th centuries, as it was believed that the light, which beamed through these windows, was spiritual light from heaven. However, I am sad to say that in the 17th and 18th centuries, puritans and fundamentalists who objected to how the Bible was being portrayed destroyed many pieces of glass. This demolition lead to coloured glass being virtually unobtainable in the 17th century. Moving through the early 19th century, a revival in glass started to happen, in particular led by two American glassmakers, John La Farge and Louis Comfort Tiffany, who developed a glass called opalescent. This glass was different to the European glass (or *antique glass*), as it had a translucent 'milky' quality. In the early 20th century, the art nouveau movement spread throughout Europe and North America, with lamps and windows being produced by many companies, including the more famous L.C. Tiffany Company (in particular their 'Tiffany lamp'). Not a huge amount of glass was produced during the 1930s-1950s, but today, there is a great demand for this art form, and I just love it!

So what is glass? It is basically made from silica sand, limestone and soda ash. These ingredients are mixed together and then heated to 3,000°F (1,650°C) to form a liquid which can be formed into glass sheets. I am constantly in ore of the beautiful colours and types of glass that I can buy for my different projects. To colour glass, various metal oxides (including selenium or gold for red, yellow and pink; cobalt for blue; and sulphur for amber) are added to the raw materials whilst the glass is being made. The hot colours (i.e. red, yellow, orange and pink) are the most expensive to buy as gold and selenium are expensive ingredients.

Figure 2: *Latest brain image I am working on*

***Figure 3:** Ginko leaf*

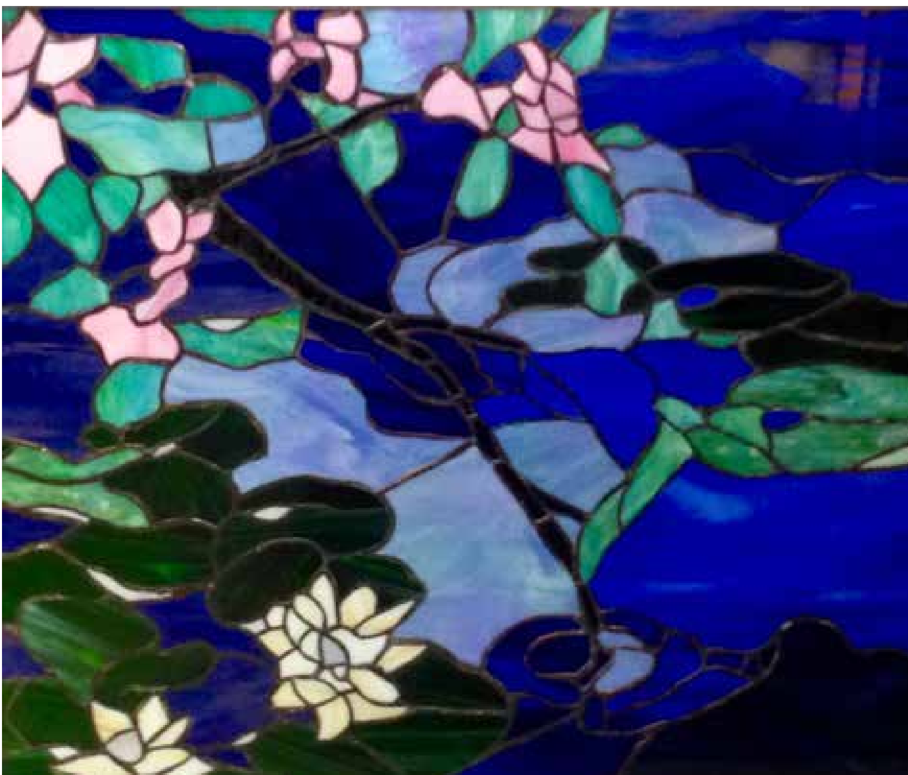


***Figure 4:** An adapted version of Monet's water lilies in glass*

So now that you know a little bit more about glass making and its history, why am I so fascinated by it all? I am a pharmacologist by trade and my research area focuses on inflammation and its resolution. I have devoted my time to understanding cellular trafficking in the microcirculation, particularly related to ischaemia reperfusion injuries e.g. stroke and myocardial infarction. With respect to stain glass window, my original vision was to be able to use the glass windows/ displays as a tool for outreach. In my experience, I have found that when you can translate your work into another medium in order to discuss ideas/ concepts it adds a new dimension, which I have found to have more receptive effects from the public. So, I started to make images in glass of e.g. things I saw down the microscope (e.g. the cover of this issue, and Figure 1A in this article), or the anatomical structure of an organ of interest (e.g. when talking about the brain, it is very nice to be able to point to areas in a brain made of glass, in order to describe what these areas do. (Figure 2 shows the latest brain image I am working on).

I make different types of stained glass: leaded panels, and copper foil panels. Leading (or glazing as it is sometimes called) involves assembling the glass pieces using lead *came* (channels) that is forced into the shape of a U or an H. Copper foil on the other hand, which was developed in the late 1800s (and used by the Tiffany Company to produce their intricate lamps) and as the name suggests, involves wrapping the edges of glass pieces in a thin copper sheet. The pieces are then placed on a pattern and soldered together. Both methods have their pros and cons, but generally, copper foil allows for smaller prices of glass to be used, which enables more intricate patterns to be used and translated into glass.

Both styles require two identical patterns to be made: one to be kept in tact and one in which all the pieces are cut out. Once cut, the pieces can be glued on to the glass and the glass cut in the correct shapes. After this, the glass edges first need to be ground: traditionally this was done by hand, but I'm pleased to say there are machines nowadays.

Although like with any craft, it is good to know both the traditional and the more modern methods. The glass is then cleaned, and the edges covered in copper foil (for soldering), or placed in the lead came and gently (and you soon realize how gentle this needs to be!) hammered into place, and then soldered together. Having the complete pattern enables the artist to place the glass pieces on top of it, so that everything fits into place beautifully (or at least that's the idea!) and it can be soldered together perfectly (Figure 1B).

There are certainly lots of things to think about when making a stained glass piece, such as planning the colour scheme, balancing the colours in a piece, the actual texture of the glass to be used, and whether the piece will be made using lead or copper foil. The pattern is another thought provoking area, and one that I thoroughly enjoy. Once one has decided on lead or copper foil, the pattern can then be constructed accordingly (as above, smaller pieces of glass can be used with copper foil). It is important to make sure that the joins are put in the correct places to not only add stability, but also to ensure that they flow with the piece. If it is a large piece of stained glass, then stability becomes more of an issue, and again one that needs to be thought about very carefully.

Once I started to show/use my pieces of art, I began to branch out and make other images and pictures. Some I design myself (Figure 3), and others are taken from paintings that I have seen in galleries (Figure 4) or pictures of wildlife (Figure 5). I am also very interested in the works of other artists that link art and science together (sometimes unconsciously or unknowingly), and I often recreate their paintings in glass. For example, the artist, Piet Mondrian used straight lines as the major feature of his compositions, as he believed that straight lines are constituents of all forms (Figure 6). What is particularly interesting regarding Mondrian's thoughts and concepts is the fact that years later physiologists discovered orientation-selective cells. These cells respond selectively to straight lines, and are widely thought to be the physiological "building blocks" of form perception (i.e. the recognition of objects in a particular form within a certain environment).

So, now you have an insight into what I like to do in my spare time. Whilst I find that my 'spare' time rather fluctuates depending upon my job, I really do enjoy both, and that is a very nice feeling indeed!

Figure 5: *An owl*

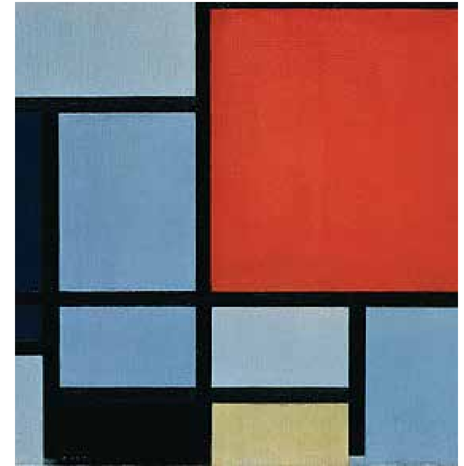


Figure 6: *Piet Mondrian. A) Original work Piet Mondrian entitled "Composition 1921 I". B) My version of Piet Mondrian's 'Composition 1921' in glass*

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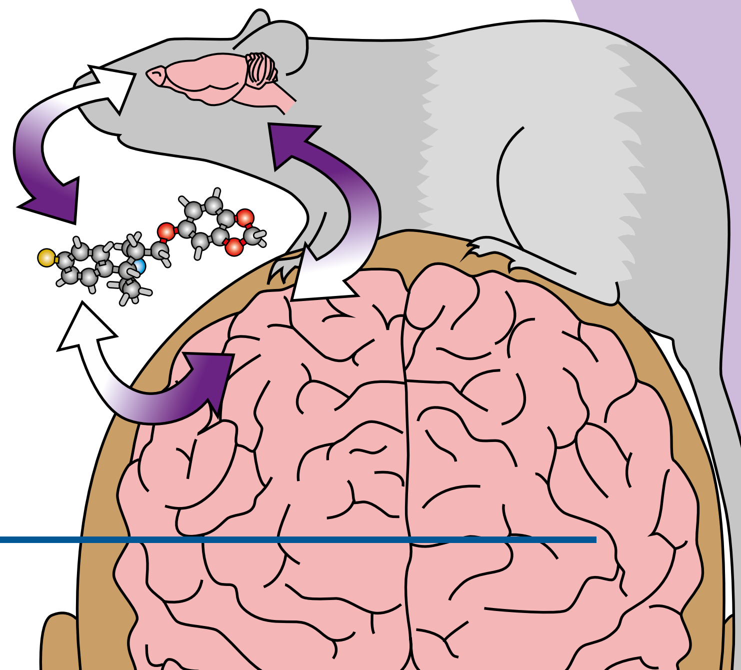

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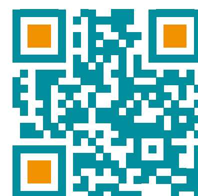

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