

ILLUMIN8

The newsletter for microscope users

Welcome

This issue of Illumin8 focuses on the use of microscopes in the bioscience research setting. For future issues we would like to know what you want to read about. Moreover if you are doing any interesting research, or have a great microscopy tip, then send an email to microscopy@olympus.uk.com or fill in the reply paid card. You can also use these to request your own copy of 'Illumin8' as well as the handy leaflet and poster series available. We hope you enjoy this issue and don't miss our competition to win...



...an Olympus µ1030 SW digital camera

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Introducing an exciting new award - a boost to any career

**We hope to see you at
MICROSCIENCE 2008
Stand H5**

23-26 June 2008, ExCel London

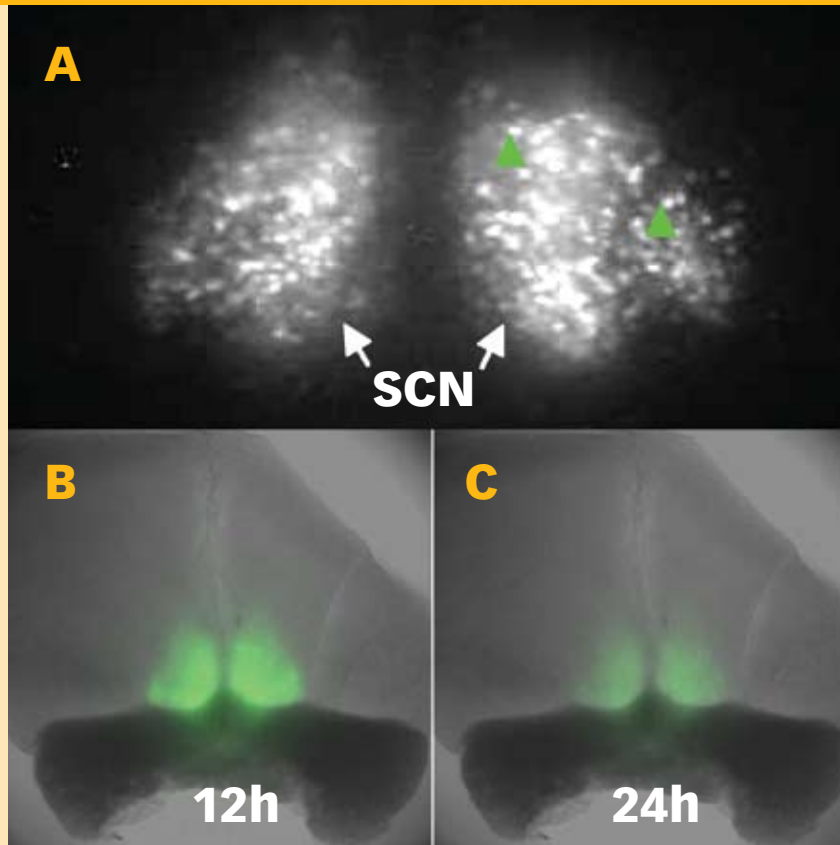


Figure 1
Bio-luminescence microscopy imaging of the suprachiasmatic nucleus using the unique Olympus LV200 (please see text for full explanation)

It's about **time**

If you have ever wondered why we have physiological and behavioural traits related to day- or night-time? Is there an underlying biochemical clock modulating this or is it just connected to your degree of tiredness? Prof Hugh Piggins, Faculty of Life Sciences, University of Manchester and his team, have these and many other related questions in mind.

Clocking on

It has been clearly established that a structure in the hypothalamus of the mammalian brain, called the suprachiasmatic nucleus (SCN), contains a 'daily clock' which generates near 24 hour (i.e., circadian) rhythmic variations in both physiology and behaviour. This clock is synchronized to changes in environmental illumination (i.e., day/night variations) by light information, which is conveyed directly to the SCN by a specialized input from the eye. The SCN neurons send clock information to the rest of the brain and body via nerve pathways and secretion of particular chemicals.

The SCN is composed different cell types, differentiated in part on the basis of the kinds of neurochemicals that they make as well as their location within the SCN. Further, some SCN cells contain the necessary molecular apparatus to function as single cell timekeepers ('clock cells'), whereas others lack this property. Key to progressing with research is to be able to identify clock cells and the chemicals via which they communicate to one another as well as the rest of the brain.

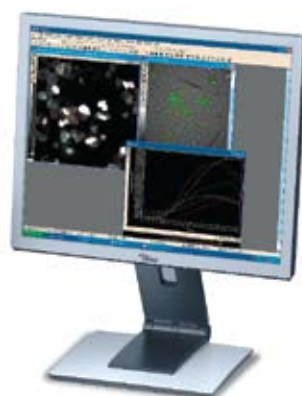
Investigating clocks

In Prof Piggins's group, Dr Alun Hughes and Dr Clare Guilding are looking at the long term expression of the protein Period-2 (PER2), one of the proteins coded by the period2 gene, a key 'clock' gene. They do this using tissue from a mouse created by Dr. Joe Takahashi's lab at NorthWestern University in the US in which Luciferase is made when PER2 is synthesized and therefore the luminescence generated by the luciferase can be used to 'report' on the gene expression. This so-called PER2::LUC

Continued overleaf



*The Olympus LV200
Bio-Luminescence Microscope*



SCN can be used to identify where clock cells are in the SCN and to determine what chemicals they use to communicate to one another. Capturing and analysing this bioluminescent signal presents its own challenges:

Luminescence vs. Fluorescence

There are two basic types of reporter groups that are used in live-cell imaging - fluorescent and luminescence reporter systems. Luminescent and fluorescent molecules both use the same process to emit light: electrons in an excited state emit a photon as they return to their ground state. This light is emitted within defined wavelength ranges depending on the molecular structure and therefore different compounds can be used as markers for different events, processes or molecules. The fundamental difference between luminescence and fluorescence is the way in which the excited state is generated in the first place. Fluorescence occurs when the excited state is caused by external stimulation by light, whereas luminescence is caused by a chemical reaction (either a natural, biological one - bio-luminescence - or a purely chemistry based one - chemiluminescence).

- Fluorescence emissions tend to be short lived and bright, requiring specific frequencies of light (shorter wavelength (higher energy)) for excitation. As a result, this illumination is required at the time of imaging, which means that the optical system must be able to supply fully controllable light at the excitation wavelength and project the emitted wavelength to the user's eyes and/or camera without any crossover between the two. This requires the use of dichroic mirror sets, which are designed specifically for the excitation/emission combination.
- Luminescence emissions tend to have varying lifetimes and are often quite faint, but due to their nature have a high signal-to-noise ratio (S/N). This makes them ideal for long exposures or long term imaging since there is little or no 'background' to worry about. Basic luminescent systems therefore need no illumination source or dichroic mirrors, but do need highly sensitive light detection equipment and a very dark chamber.

As a result bio-luminescence has great advantages over fluorescence for long term live cell imaging, since it combines a high signal-to-noise (S/N) ratio with no background light emission or bleaching/phototoxic effects. What is more, only viable cells emit luminescence signals since emission is only possible with a functioning metabolism. As a result, measurements are absolute and directly quantitative.

The model of a modern bio-luminescence microscope

The Olympus LV200 Luminoview has been optimised for collecting the faint light associated with bio-luminescence providing consistent cellular level clarity for the first time, without the need for users to build their own, expensive, systems. In essence, bio-luminescence microscopes need only have a very simple optical design but because a standard microscope is not ideal, the LV200 has been built from the ground up. The LV200 development team considered all the optical designs necessary to maximise light collection - essential for the low levels of light emitted. As a result, the path from the object to the camera is straight and as short as possible to ensure that as much light as possible reaches the CCD chip. Therefore there are no necessary additional mirrors, filters or lenses which all contribute to light absorption. What is more, the tube lens has been designed with an extremely high numerical aperture (N.A) which affords a vast increase in sensitivity when compared to conventional microscope optics. This enables the Olympus LV200 to produce signal outputs many times higher than traditional systems and therefore use conventional CCD or EM-CCD cameras, rather than expensive liquid nitrogen cooled devices. These unique optical properties also ensure that high magnification objective lenses can be used, with suitable camera integration times, to provide exquisite single cell resolution not previously possible with luminescence imaging.

Added extras

With the optical components optimised for the detection of luminescent light, the LV200 is housed inside a light-tight box which also serves as precisely controllable environmental chamber. The system provides:

- independent temperature controls for the stage, incubation chamber, top cover and objective
- a water reservoir can be used to maintain the correct humidity level
- CO₂ flow control enables pH stability
- Culture medium pumping for replenishing/ changing the growth medium.

Such environmental control enables samples to be continuously monitored over days or even weeks, without the need to move the sample between the microscope and an incubator.

The optical path has also been designed with the ability to place excitation and emission filter wheels in the light path to enable dual-colour luminescence as well as transmitted light fluorescence imaging. Furthermore, with standard brightfield illumination and phase contrast inserts, target areas of the sample can be found easily before switching to luminescence detection. It is therefore also possible to produce luminescent and fluorescent overlays on phase contrast brightfield images, which provides users with the capability to localise and co-localise proteins more fully.

Luminescence in action

Drs Hughes and Guilding have only been using the LV200 for a short time, but have already generated meaningful data. They use it to follow and analyse their experiments, recording images once every three minutes for up to seven days on end. They can then analyse the gross expression of PER2 over time within the entire culture, as well as view each individual cell to look for variations from and similarities with the gross expression. Figure 1 (on the front page), shows in panel A, a higher magnification image of the bilateral SCN, with individual cells clearly visible (green arrowheads). The lower panels, B and C, show PER2::LUC expression (green) superimposed on a light-transmission image of the SCN culture. Note that the levels of PER2::LUC bioluminescence show large changes across the 24h day (high at 12h, lower at 24h).

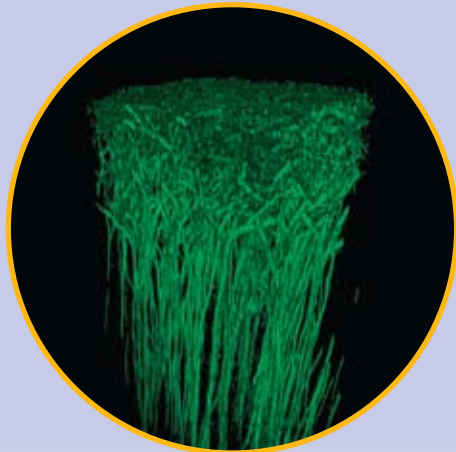
Conclusions

Luminescence has been overshadowed by fluorescence for many years, mostly due to the expensive image collection systems required. The advantages that luminescence offers over fluorescence for long-term live cell imaging through, make it very attractive to researchers. The LV200 system represents a sea-change in luminescence imaging capabilities since its optics have been optimised for luminescence. Prof Hugh Piggins and co-workers at the University of Manchester have been using the LV200 to look at long-term expression patterns of the Per2 clock protein, a process that requires acutely cultured brain slices to be incubated and imaged for extended periods of time.

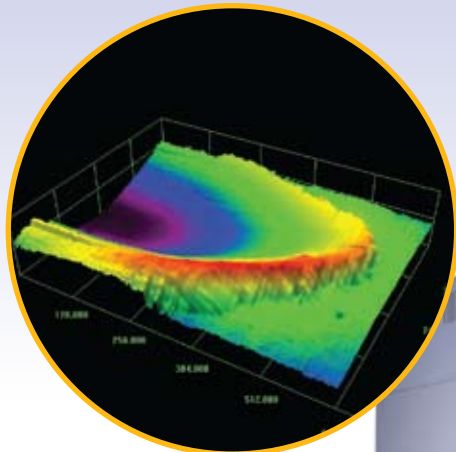
To find out more about Prof Piggins' research, please visit his group's webpage: <http://www.manchester.ac.uk/research/Hugh.d.piggins/research>
If you think the LV200 would benefit your research, or you would like to know more, please fill in the reply paid card or email microscopy@olympus.uk.com

All the best things **come in 3s**

Such as: **Olympus Workshops at MICROSCIENCE 2008**



Dense network of neurons imaged on Olympus FV1000MPE

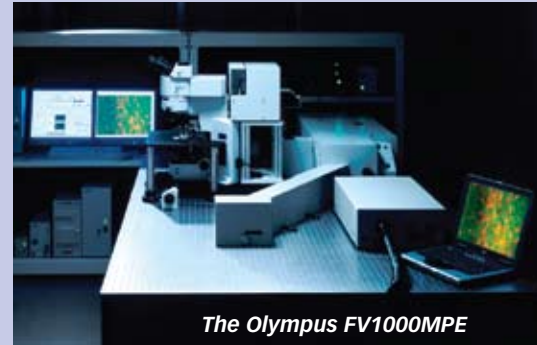


3D height map of 'pile up' around a scratch as visualised on an Olympus LEXT OLS3100.
(Crown copyright ©2008)

Microscopy has come a long way since the early days of Robert Hooke and Antony van Leeuwenhoek. For example, resolutions have reached their theoretical limit (and moved beyond in certain circumstances), almost everything has been motorised/automated and there are now myriad different methods, protocols and procedures to pick from. In fact, microscopy now offers researchers so many unique opportunities to push the boundaries of science that it could be considered a rock solid tool, applicable to many different subject areas.

The most recent improvements in microscopy will be showcased during the conference talks and on the exhibition stands at MICROSCIENCE 2008, taking place at ExCeL from 23-26th June. Olympus will be exhibiting at the show (stand H5) and is running three workshops, two of which will be presented by customers:

Dr Martin Tewinkel, Group manager – laser scanning microscopy, Olympus Life Science Europa, will present on the exciting topic of multiphoton microscopy. His talk "Deep *In Vivo* Imaging & Simultaneous IR Laser Uncaging or Bleaching" covers the advantages of multiphoton microscopy for looking deep into thick samples such as organotypic cultures. It also addresses the functionality offerings



The Olympus FV1000MPE

of the Olympus FV1000 with SIM scanner, for simultaneous uncaging/bleaching and imaging.

Dr Louise Brown, National Physical Laboratory, will be talking on the "Applications of Laser Scanning Materials Microscopy". She will cover her work within the Performance Materials group at the NPL where she researches the properties of the latest materials, with the aid of the Olympus LEXT – see the last issue of *Illumin8* for more details).

Professor Hugh Piggins, Manchester University will be talking about "Imaging Brain Clocks". During this he will highlight his groundbreaking research into circadian rhythms using the LV100 advanced bio-luminescent imaging system from Olympus. See first article in this issue.



The LEXT OLS3100



MICROSCIENCE 2008 will also see the launch of the

Olympus Early Career Scientist Microscopy Award

A high-value microscopy system for your research

The Olympus Early Career Scientist Microscopy Award has been set up to help life scientists seeking to establish their careers, by providing the long-term loan of a specific item of Olympus microscopy equipment for their research.

The equipment on offer ranges from a basic light microscope to a highly-specified FluoView 1000 confocal microscopy system. All equipment will be loaned free of charge for a period of up to 2 years, with full training and technical support.

Depending on the applications received, Olympus may make several awards of basic microscopy systems, or loan a single piece of high-end equipment.

What is the purpose of this award?

Olympus is seeking to provide support to scientists at a time in their careers when it is most needed. The loan of equipment is intended to allow a successful applicant to have unrestricted use of an imaging resource, to which they may otherwise have little or no access. It is anticipated that the equipment will be used to obtain data for publications and grant applications that will increase the standing and future capacity of the successful applicant's laboratory.

Who is eligible?

This competition is intended for UK life scientists who are at the beginning of their careers. Specifically, the competition is aimed at researchers who hold either personal fellowships or are within three years of their first tenured academic appointment, and have not yet secured substantial grant funding. There is no limit on age.

How to apply

Application forms and full terms and conditions are available to download at

www.olympus.co.uk/oecsm

Alternatively, an application form can be requested by emailing

microscopy@olympus.uk.com



OLYMPUS EARLY CAREER SCIENTIST

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June 08

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Olympus and Nature Team Up Again for the **Nature Neurotechniques Collection**

Olympus and the Nature Publishing Group are teaming up again to bring researchers free access to the Nature Neurotechniques collection. Advances in genetics and molecular and cellular biology, together with the development of increasingly sophisticated imaging techniques such as those from Olympus, have allowed neuroscientists to view and manipulate the nervous system in unprecedented ways. The Neurotechniques Collection brings together both original research and relevant, timely reviews that have been published in Nature Reviews Neuroscience and Nature Methods in the last two years. There is also a compilation of Research Highlights written by the editors describing some of the most important advances in this primary research area.



As well as being freely available in a printed supplement, the Neurotechniques Collection will also be accessible as a Web Focus at www.nature.com/focus/neurotechniques and on the Nature Reviews Neuroscience homepage - www.nature.com/nrn/index.html.

To request a copy of the Nature Neurotechniques Collection direct from Olympus, please fill in the reply paid card or email microscopy@olympus.uk.com

Product **UPDATE**

The essence of **fluorescence!**

The right lighting is not only essential for your home and office, but also for your microscopy. Olympus offers a broad range of specialised fluorescence illumination systems that have functionalities above and beyond the more routine mercury burners. Here we look at a new system that produces confocal-like images without the need for lasers or spinning disks.

Introducing a little structure

The new Optigrig M structured illumination system from Olympus yields ultra-rich, multi-channel fluorescence and 3D/4D imaging, whilst still using a standard stabilised illumination source. Compatible with Olympus BX2, IX2 and MVX10 microscopes, Optigrig M controls are fully integrated into Olympus cell* imaging software to provide all users with excellent confocal-like images. The Optigrig M uses a one-dimensional optical grid mounted on a piezo-electronically driven actuator to project a line pattern onto the specimen. The grid is then moved perpendicularly to the grid lines, in 1/3 steps of its period length so that three



The Optigrig M structured illumination system

grid movements result in one optical section. The structured illumination process returns a strong signal wherever focus is sharp and a weak signal where focus is soft. Furthermore, a patented algorithm is used to combine these strong signals from the grid images so that each optical section contains data that is exactly within the focal plane. A series of optical sections taken through a sample (z-stack) can then be combined to create a haze-free ultra-sharp composite image. Image stacks can also be used to produce 3D reconstructions using post-processing software.

To find out more about the OptiGrid M or any other Olympus product, please fill in the reply paid card or email microscopy@olympus.uk.com



Win the Tough Olympus μ 1030 SW **Digital Camera**

Adventure is yours for the taking with the Olympus μ 1030 SW. Waterproof, shockproof, freezeproof and crushproof, this camera goes wherever you do. Yet despite its tough attributes, image quality isn't compromised. Precision Olympus optics deliver true-to-life results. Integrated cutting-edge technologies provide intelligent solutions to ensure the flawless reproduction of subjects. We are giving one away, so to be in with a chance of winning you will need to answer the following three questions correctly on the reply paid card and return it to us by the 1st July 2008.

Question 1:

Which Olympus system is Prof Hugh Piggins' team using?

Question 2:

Who will be speaking about Multiphoton microscopy at MICROSCIENCE?

Question 3:

What is the focus of the Olympus and Nature collaboration?

Congratulations to Mr Sami Joviano, from the Dept of Biomedical Sciences at the University of Wolverhampton, for winning the Olympus μ [mju:] 790 SW digital camera from the last issue of Illumin8.



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Your Vision, Our Future

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