ADVANCED BIO-LUMINESCENCE MICROSCOPY

Microscopy Focus

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 synchronised to changes in environmental illumination (i.e. day/night variations) by light information, which is conveyed directly to the SCN by a specialised 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 of 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

Prof Hugh Piggins, Dr Alun Hughes and Dr Clare Guilding at the University of Manchester's Faculty of Life Science, 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 synthesised and therefore the luminescence generated by the luciferase can be used to 'report' on the gene expression. This so-called PER2::LUC 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 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 (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. 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 digital CCD camera chip. Therefore there are no necessary additional mirrors, filters or lenses which can all contribute to light absorption. What is more, the tube lens has 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 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 a precisely controllable environmental chamber.

The system provides:

- Independent temperature controls for the stage, incubation chamber, top cover and objective;
- A water reservoir to maintain the correct humidity level;
- CO_2 flow control for pH stability; and
- 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 optical path has also been designed with the flexibility 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 luminescence and fluorescence overlays on phase contrast brightfield images, which provides users with the capability to localise and co-localise proteins more fully.

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LUMINESCENCE IN ACTION

Drs Hughes and Guilding have been using the LV200 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* 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).

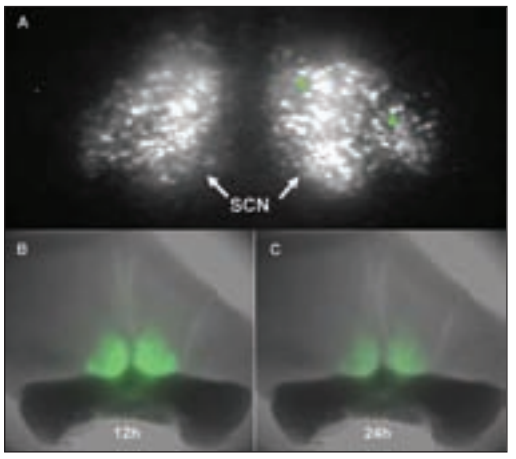

Figure 1. Panel A, a higher magnification image of the bilateral SCN, with individual cells clearly visible (green arrowheads). The lower panels, B and C, show PER2::LUC expression (green) superimposed on a light-transmission image of the SCN culture at 12h and 24h. Image courtesy of Prof Hugh Piggins.

Figure 2. The Olympus LV200 Luminoview.

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

Analysing Ointment Particles in Fraction of Time

The Morphologi high sensitivity particle characterisation system from **Malvern Instruments** has slashed the time required to analyse particles in a pharmaceutical ointment from two hours to just 15 minutes. The commercially available ointment contains needle-shaped particles of active pharmaceutical ingredient in a paraffin-based formulation. Microscopy-based quality control specifications for the product require that in a 3mg sample there are no particles longer than 250 µm, and not more than 100 particles over 100 µm. With traditional manual microscopy, analysis can take well over 2 hours per sample. Using the Morphologi this was reduced to less than 15 minutes. Morphologi provides microscope quality images and delivers statistically significant data through the rapid analysis of hundreds of thousands of particles, with little or no user intervention. It gives information on both particle size and shape. As a result it identifies, measures, counts and classifies these needle-shaped crystals in a manner that avoids human subjectivity. In contrast to manual microscopy, where only a small number of representative fields can be photographed, Morphologi also maintains a permanent record of every measured particle.

The automation of QC microscope methods such as this eliminates analyst-to-analyst and even site-to-site variability. It can improve lab throughput and reduce measurement times without compromising the integrity of the analytical procedure. In addition, the ability to classify on the basis of both size and shape is valuable in allowing formulators to more closely tailor QC specifications to product performance.

Circle no. 159

Imaging System has Worldwide Appeal

UVP's BioDoc-It™ Imaging System was recently spotted on hit TV show Heroes. Not only is this show released in the United States, but also in many other countries including England. In addition to Heroes, the BioDoc-It system has been used in other TV shows. The system's popularity is due to its compact size and the visual appeal with the ability to show DNA gels on the LCD screen.

Outside of the TV world, laboratory researchers worldwide regularly use the BioDoc-It Imaging System for visualising and documenting DNA stained with fluorescent Ethidium Bromide. To visualise the DNA stained gel, the gel is placed on an ultraviolet transilluminator where distinct bands in the DNA become visible with the fluorescent stain. The system's high-resolution camera takes a picture of the fluorescent gel and displays the image on the screen. Researchers can then save the image for documentation, presentation or analysis.

Circle no. 160

Modular LED Light Source Now Available

CoolLED Ltd is pleased to announce a licensing agreement with Carl Zeiss MicroImaging GmbH for the sale of the precisExcite LED fluorescence excitation system in the USA.

The granting of the license by Carl Zeiss now allows all microscope users in the USA access to the LED excitation technology developed by CoolLED. Excitation by LEDs offers better control, integration under imaging software, and the benefits of long lifetime LEDs. With LEDs, there is no need to replace, align and dispose of mercury-based bulbs.

precisExcite is a flexible and modular system using interchangeable LAMs (LED Array Modules) to provide excitation across the visible spectrum. precisExcite is integrated with many of the imaging software packages from leading microscope and imaging companies.

CoolLED is a UK specialist manufacturer based in Andover, Hants. It provides products and solutions using opto-mechanical assemblies based on LED technology.

Jim Beacher, Business Manager for precisExcite, stated that they are pleased to have reached this agreement with Zeiss as LEDs are the future for fluorescence excitation. There is considerable interest from US cell biologists and they can now supply precisExcite to meet their needs.

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