Spotlight

Luminescence, UV & Microplate Readers

Although the concept of bio-luminescence microscopy (BLM) is not a novel one, the recent advances introduced with new specialised imaging systems have made it a more realistic procedure. BLM represents a quantum leap in microscopy since, as its name suggests, it uses luminescence rather than fluorescence. This may not seem like a big deal initially, but luminescence has a range of benefits over fluorescence. For example luminescence does not bleach the sample and is not toxic either, which makes imaging much more suitable to live cells and organisms as well as for long term imaging.

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A QUANTUM LEAP IN LIVE CELL IMAGING:
Advanced Bio-Luminescence Microscopy

BIO-LUMINESCENCE VERSUS FLUORESCENCE

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 - chemiluminsecence).

Fluorescent emissions tend be short lived and bright, requiring specific frequencies of light (shorter wavelength (higher energy)) for excitation. As a result, 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.

Luminescent 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.

IMAGING USING BIO-LUMINESCENCE

Bio-luminescence imaging has great advantages over fluorescence 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 of this measurements are absolute and directly quantitative.

RELATIVE FORTUNES

Due to the technology available, the range of the dyes and the more controllable nature of fluorescence, it has established a universal foothold in microscopy based imaging and has been used to move our knowledge of both molecular and cellular level events on at a great pace. Luminescence on the other hand has been largely overlooked. This is in part because, although the optical components are simpler, the highly sensitive imaging devices – special photon counting systems or liquid nitrogen cooled cameras are expensive and complicated. Furthermore, even with these specialised detectors, the lack of spatial resolution meant that it couldn't provide the cellular detail possible with fluorescence imaging. Therefore, most systems in use have been built specifically by the researchers using components that best suited their experimental requirements.

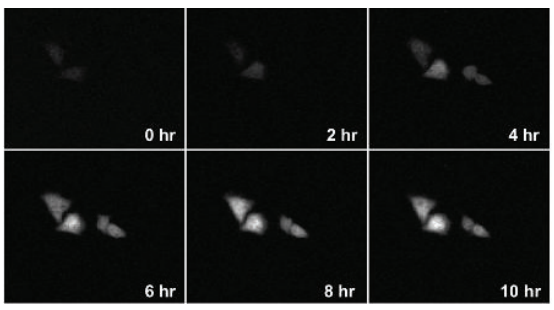


Figure 1. An exposure time series using HeLa cells transfected with Tet on-Luc vector

FROM DIFFUSE TO DETAILED

Recently though, companies like Olympus have developed bio-luminescent imaging systems that provide amazingly detailed images using standard CCDs rather than specialised photon-detectors. To do this, the optical designs of the systems are highly specialised to maximise light collection - essential for the low levels of light emitted. For example, 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 additional mirrors, filters or lenses to absorb light. 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 instruments, such as the Olympus LV200, to produce signal outputs many times higher than traditional systems and therefore use conventional CCD or EM-CCD cameras. These unique optical properties also ensure that high magnification objectives can be used, with suitable camera integration times, to provide exquisite single cell resolution not previously possible with luminescence imaging. These improvements, along with the number of luminescent probes now available, are promising to take microscope imaging to a different level.

ADDED EXTRAS

With optical components optimised for the detection of luminescent light, systems can be further designed to match the requirements of research. Therefore, it is possible to integrate excitation and emission filter wheels to enable dual-colour luminescence as well as transmitted light fluorescence imaging. With standard brightfield illumination and phase contrast inserts, targets 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 enables localisation and co-localisation capabilities that were not previously possible.

With the systems now capable of long term imaging, it is important that samples can be left on the stage for the entire time of the imaging experiment. Therefore an environmental chamber can be used to enable independent temperature control for the stage, incubation chamber, top cover and objective. Furthermore, a water reservoir can be used to maintain the correct humidity level and CO_2 flow control enables pH stability. 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.

DON'T LET THERE BE LIGHT

It is still important with this new breed of luminescence imaging system, to ensure that there is no ingress of external light, or any reflective surfaces within the box. The boxes are therefore incredibly 'light tight' so can be used in a standard laboratory.

EXAMPLE APPLICATIONS

Bio-luminescence microscope imaging has important applications across a diverse range of topics, providing a new insight and opening novel routes of discovery.

Gene Expression Analysis

With a system such as the LV200, it is possible to complete long term, quantitative analysis of gene expression in living cells. First of all, gene expression studies need a quantitative sensor, which does not accumulate in the cell. Firefly Luciferase, for example, has a very low half live in living cells and therefore allows the detection of both up and down regulation with excellent dynamics.

Second, in a long term analysis of living cells, phototoxic effects should be kept to a minimum to avoid side effects from phototoxicity. With BLM, cells are simply observed and never disturbed by high light intensities. For example, in chronobiology bio-luminescence is used since it takes a long time to observe the oscillation of clock genes in photosensitive cells.

Ca^{2+} Imaging

Imaging and following Ca^{2+} patterns and fluxes is a key topic in physiology, neuroscience and signalling. Different fluorescence techniques have been established such as ratiometric measurements using Fura-2. As with gene expression studies, phototoxic effects can not be excluded.

Using bioluminescent markers like aequorin, obelin or BRED probes, the Ca^{2+} concentration in sensitive cells can be measured without the influence of phototoxic side effects.

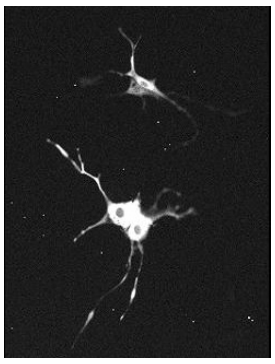


Figure 2. NIH/3T3 cell transfected Per2-Luc vector

ATP Imaging

Similarly, ATP and ATP-coupled measurements can be achieved with a high spatial resolution in single cells and extracellular matrix using luminescence instead of fluorescence, which is a key development for many metabolic and G-protein coupled receptor (GPCR) researchers.

Imaging in Auto-Fluorescence Environments

A different problem exists with some plants and embryos – there is a large amount of autofluorescence and therefore if imaging using fluorescent dyes, the excitation required can lead to a large amount of diffuse background noise.

With luminescent dyes this is no longer a significant problem since there is no excitatory illumination and therefore the only light being put into the sample is from the luminescent dyes, which is not strong enough to elicit any significant autofluorescence. BLM has been used for evaluating photosensitive receptor expression in *Arabidopsis thaliana* seedlings and gene expression in *Xenopus spp.* embryos.

Intracellular Protein Imaging at the Tissue Level

The intracellular localisation of proteins can be observed at high-resolution using luminescence imaging, even at the level of a whole tissue.

Application Example

The nuclear localisation signal (NLS) and endoplasmic reticulum (ER) localisation signal (calreticulin) sequences are fused with the luciferase gene in the mammalian expression vector. These are pGL3/NLS and pGL3/calreticulin, respectively. HeLa cells are transfected with these vectors in a φ35 mm glass-bottomed dish.

After overnight incubation, luminescence images are recorded using the LV200 with the following conditions: [Objective lens magnification: 40x, exposure time: 5 min., binning: 1x1, CCD camera: DP30 (Olympus), Luciferin: 1 mM]

As a result, localisation of the luciferase tagged signal sequence is visualised in the nucleus and ER as a luminescence image.

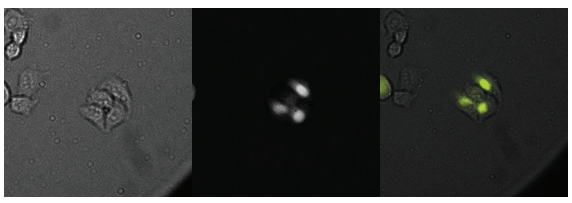
Imaging Bacteria and Parasites

Luminescence is also gaining ground in the imaging of active bacteria and micro-organisms, which is very important for microbiology and food technology laboratories, as well as those studying parasites. For example, malaria parasites can be imaged inside in the midgut wall of Anopheles mosquito or in red blood cells.

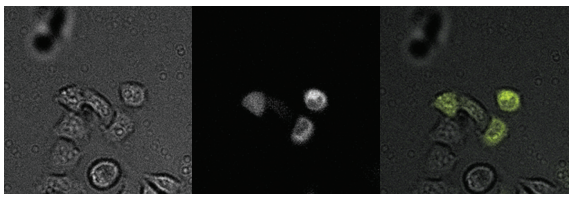
A SYSTEM FOR SUCCESS

The first commercially available system for bio-luminescent microscopy, the Olympus LV200, can provide unprecedented sensitivity and resolution whatever the magnification. The LV200 is most suited to imaging small organisms, slice cultures and live cells from the gross level down to the single cell scale.

Nucleus



Endoplasmic reticulum



Bright Field *Luminescence* *Merged Image*

Figure 3. Localisation of luciferase: Merged image from luminescence and bright field images

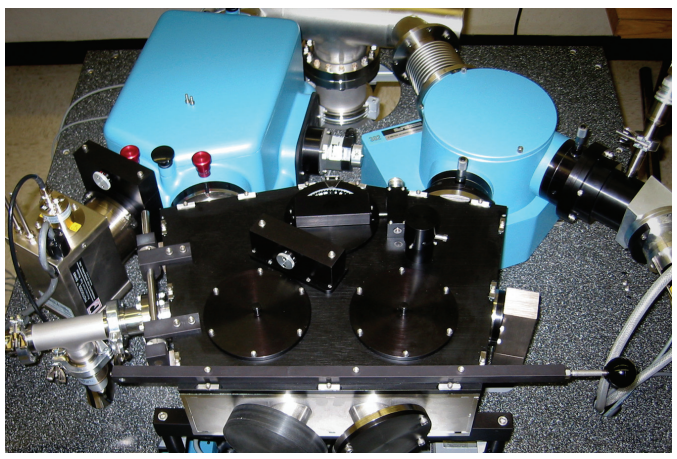
Ultra-Fast Fluorescence Lifetime Analysis

The R&D100 award winning MF2 multi-frequency fluorometer from **Horiba Jobin Yvon** revolutionises fluorescence lifetime analysis with orders of magnitude increases in acquisition speeds. Lifetime analysis provides scientists with valuable information on a molecule's interaction with the surrounding environment, such as molecular binding and interactions, rotational diffusion, quenching, and energy transfer (FRET).

With the MF2 these phenomena can be investigated more rapidly than ever – lifetime measurements can now be completed on the millisecond timescale, providing the researcher with dramatic increases in sample throughput and hence results. Such speed opens up new opportunities for analysis, for example, real time analysis of millisecond/second reaction kinetics based on reagent fluorescence lifetimes is no longer a dream. The MF2 makes it a simple reality.

Circle no. 290

Vacuum Ultraviolet Optical Crystal Characterisation System



McPherson, Inc received a contract from the Optical Crystals group in the Solid State Physics Division of the Institute of Physics and Academy of Science (Czech Republic.) Under the contract, McPherson fabricated and delivered a versatile vacuum ultraviolet spectrometer system for transmission, reflection, and emission measurements of diverse samples.

The McPherson system works with vacuum from 120 to 930nm. The Czech group, led by researcher Dr. Martin Nikl, uses the McPherson spectrometer to measure and characterise the optical and luminescence properties of optical crystals, films and single crystals.

The McPherson instrument enables research efforts by allowing tunable, monochromatic vacuum ultraviolet wavelength sample excitation and subsequent emission measurements from the vacuum ultraviolet to the near infrared. The detection system is cooled and capable of 5nanosecond time resolution in the time-resolved signal collection mode.

To increase the potential scope of future work, the sample chamber is prepared, ready for future augmentation. Ports are provided to allow introduction of cryogenically cooled samples as well as Excimer laser and x-ray excitation sources.

Circle no. 291

Spotlight

Luminescence, UV & Microplate Readers

Simple UV-Vis and Fluorescence Measurement

Thermo Fisher Scientific Inc. recently announced the introduction of the Thermo Scientific NanoDrop line of micro-volume spectroscopy systems. These unique instruments utilise a patented technology that enables easy measurement of UV-Vis and fluorescence without the use of cuvettes. The Thermo Scientific NanoDrop family of products, recently acquired by Thermo Fisher, extends the company's molecular spectroscopy portfolio for increasing applications involving small sample volumes.

The Thermo Scientific NanoDrop instruments eliminate cuvettes and associated dilutions, resulting in more reliable measurements. An undiluted one-microliter sample is pipetted directly onto the measurement surface. After a quick spectral reading, the sample is simply wiped away in preparation for the next sample. The instruments are used to measure the quantity and purity of nucleic acids and proteins, as well as associated fluorescent labels. Such measurements are routinely needed for quality control and sample preparation at multiple process points in many applications, including microarray probe preparations, PCR template normalisation, small molecule crystallisation, sequencing, antibodies, microgenomics, proteomics, genotyping and FRET (Fluorescence Resonance Energy Transfer). These applications are used in such diverse fields as cancer research, microbiology, drug discovery, forensics, histocompatibility and diagnostics.

The Thermo Scientific NanoDrop line of products includes the Thermo Scientific NanoDrop 1000 Spectrophotometer, Thermo Scientific NanoDrop 8000 Spectrophotometer and the Thermo Scientific NanoDrop 3300 Fluorospectrometer.

Circle no. 292

HTRF® - Certification for the Multimode Microplate Reader

Berthold Technologies is ready to announce the HTRF®-certification for the multimode microplate reader Mithras LB 940. The HTRF® assay platform from Cisbio offers the researchers a highly sensitive, robust technology for the detection of molecular interactions of proteins in vitro and widely used by the pharmaceutical and biotech industry for the high throughput screening stage of drug development. With its HTRF® technology, Cisbio offers a comprehensive technological platform for the screening and investigation of biological interactions such as G-Protein Coupled Receptor (GPCR), kinase and imflammation biomarkers among others. Berthold Technologies has based its development on DOPS – Dedicated Optical Path System - to ensure the best available performance for each technology.

Mithras LB 940 offers all comprehensive technologies used in modern research including: absorbance; luminescence; BRET; AlphaScreen®; fluorescence; FRET; fluorescence Polarisation (FP); time-resolved fluorescence; and HTRF®. Mithras can be supplemented with up to 4 reagent injectors – based on the proven and highly precise and accurate JET injection technology – and a temperature control unit for the microplates. All these features offer extensive opportunities to the users to perform a huge number of applications including kinase activities, second messengers (e.g. cAMP, IP-One), GPCRs, phagocytosis, calcium flux, cell viability, apoptosis, protein and DNA determinations, reporter gene assays or protein-protein interactions. The Time-Resolved Fluorescence module uses a high-power flash lamp. Optical emission filters can be used in Mithras´s high-efficiency luminescence optics (<6 attomoles ATP) to get unsurpassed results in BRET (e.g. functional assays for GPCR research) and multicolour luminescence (luciferase reporter gene assays). Alphascreen™ can also be measured on the Mithras multimode reader. The software is supporting a multitude of functions including standard curves and kinetic data reduction and meets the 21 CFR part 11 requirements. For higher throughput Mithras LB 940 may be equipped with the Stacker unit LB 931 or the BUTLER LB 930 plate handler or it can be integrated into a laboratory automation system.

Circle no. 293

Flexible SPE Microplate Enhances Methods Development

The Development Microlute™ from Porvair Sciences provides R&D laboratories with a uniquely flexible tool for developing the Solid Phase Extraction (SPE) methods to best suit an analytical process.

The Development Microlute offers the user a choice of 8 or 12 different phases and sorbent volumes (10 -100mg) in one plate. This allows the user to rapidly and simply identify the optimal sorbent and loading for their specific application. By supplying a complete methods development product that does not need to be constructed by the user, Porvair delivers considerable savings in valuable laboratory time.

The novel design of the Development Microlute offers all the advantages of automated and high throughput SPE sample preparation in a convenient microplate format capable of rapidly processing 96 samples in one go repeatedly and precisely.

Constructed from a single piece of moulded high quality polypropylene, a Development Microlute plate will not bend or distort because individual SPE cartridges do not have to be repeatedly plugged in and out.

Using a proprietary sorbent slurry loading technique, Porvair has eliminated the channeling effects often limiting the performance of dry powder loaded SPE columns. Each well on a Microlute Development plate has an individual drain spout ensuring 100% sample transfer and zero crossover contamination.

Working closely with the leading automated liquid handling companies has ensured that Porvair Microlute Development plates deliver high productivity, trouble-free operation with all robotic sample handling and preparation systems.

Circle no. 294

The Optimum Tool for Assay Development

Introducing the new FDSS7000 modular and versatile imaging based plate reader from Hamamatsu, the optimum tool for assay development and high throughput screening. The versatility of the new FDSS7000 can be seen in features such as the new 1536 dispensing head, and the ability to run both fluorescent and flash luminescent assays in the same instrument. It can also run protocols for assay development and High Throughput Screening.

The FDSS7000 incorporates a set of xenon light sources and filter sets. This is a key benefit for assay development as it allows flexibility on selection of excitation wavelengths. therefore, this allows the user to easily develop novel assays and perform HTS with a large variety of dyes such as Fluo-3/4, Fura-2, FRET (VSP).

For optimum performance, the FDSS7000 includes two separate Hamamatsu detectors: a cooled CCD camera for fluorescence and a photon-counting camera for luminescence. The detectors are optimised to give the best performance for fluorescent applications such as intra-cellular calcium measurements and luminescence applications such as Aequorin. A new sensor, which provides 20 x increased detection sensitivity at 700nm, is featured making it ideal for weak luminescence applications.

The system has two dispensing heads that can operate independently, to allow the user to perform contamination free agonist/antagonist assays. The FDSS7000 can achieve throughput as fast as 52 seconds, making it ideal for integration into a robotic platform for large-scale HTS applications. The new FDSS7000 is the ideal solution for all screening needs.

Circle no. 295

The Spotlight could be on you!

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