OLYMPUS

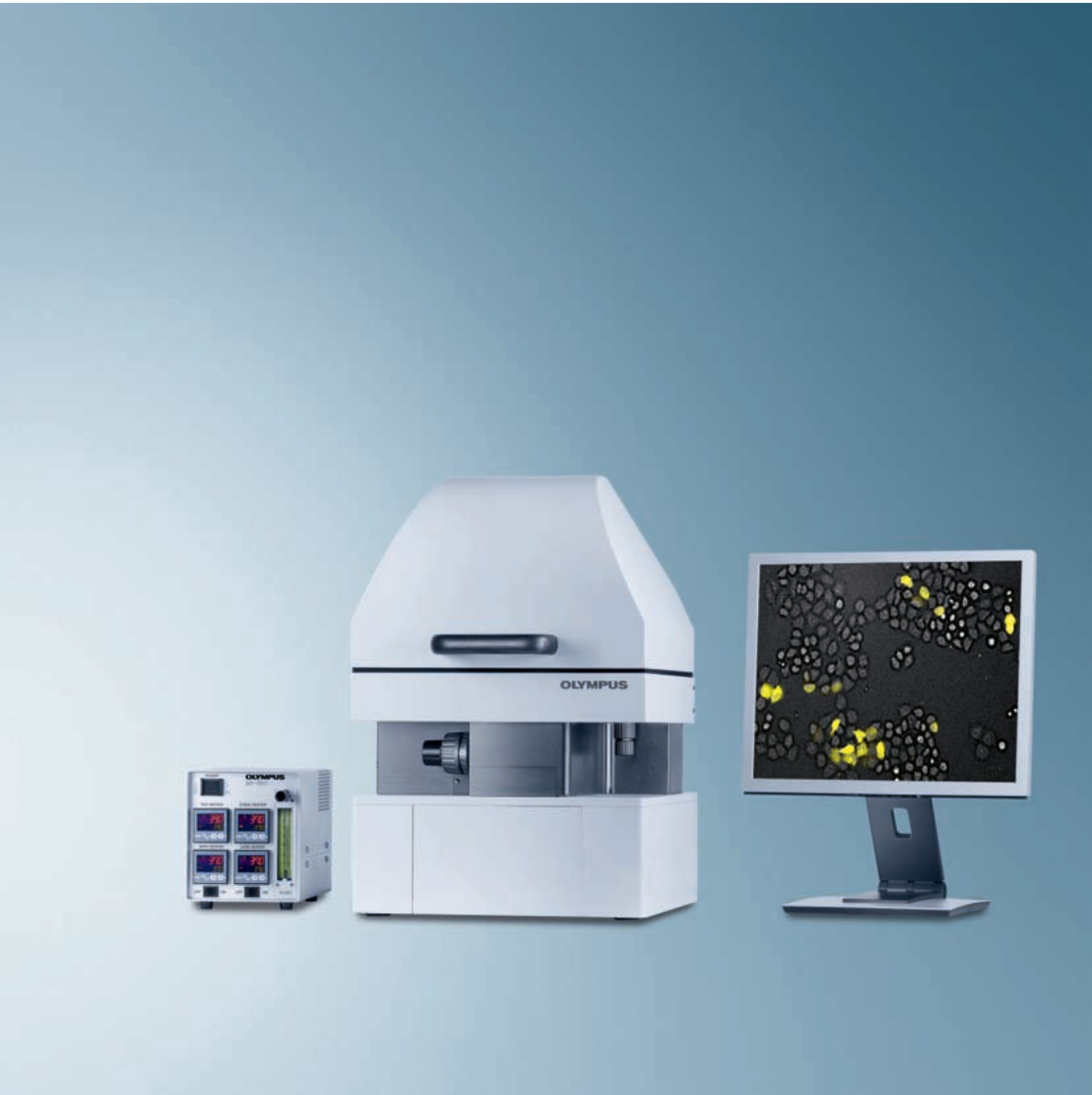
Your Vision, Our Future

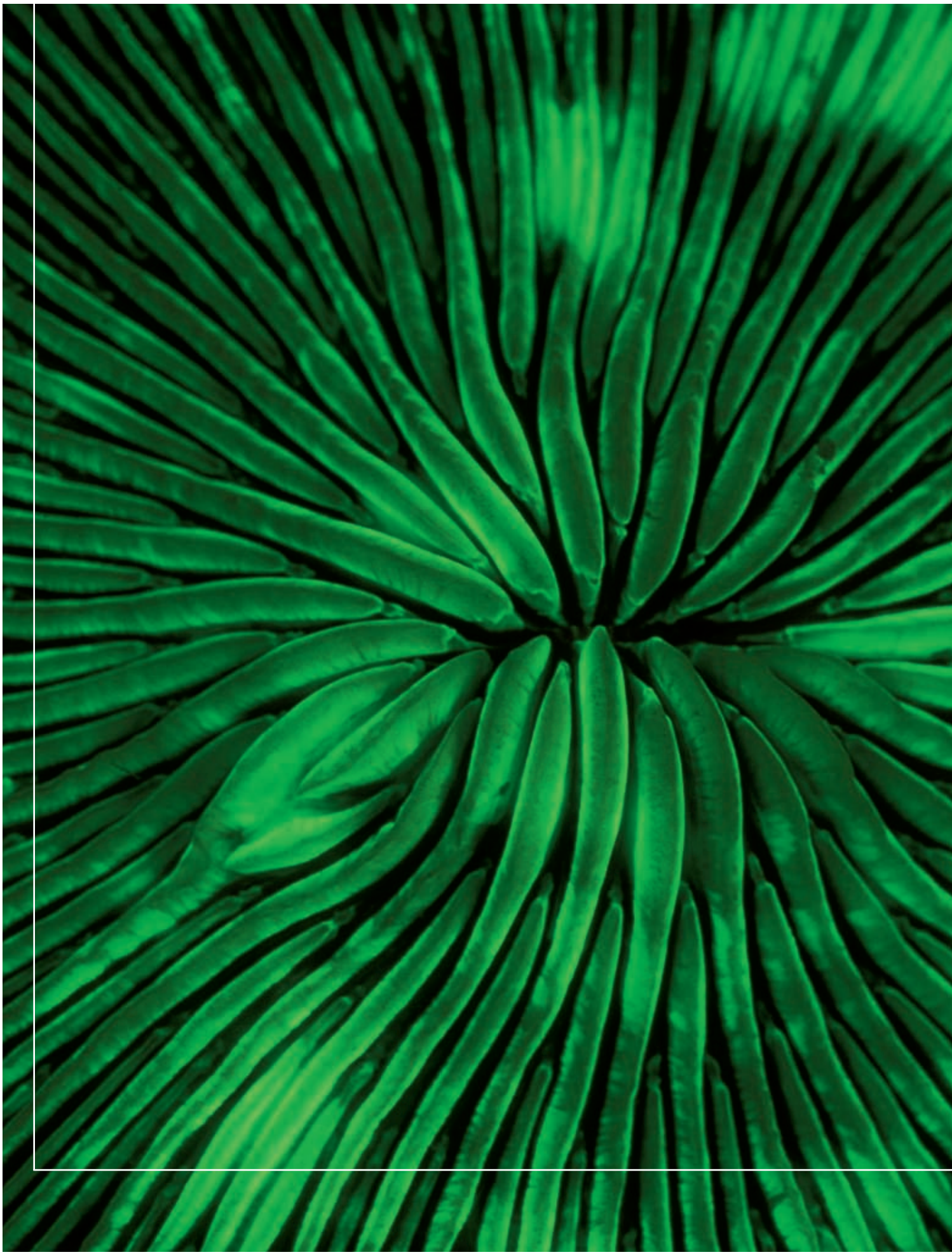
Bioluminescence Microscopy

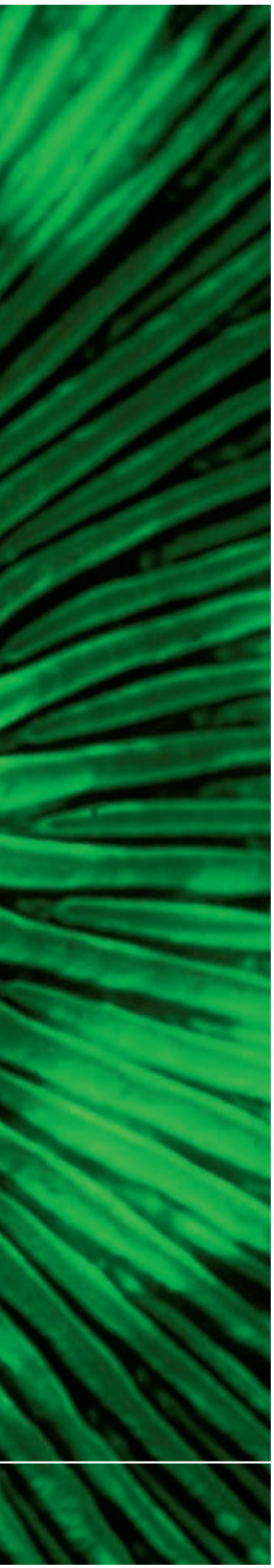
LV200 LUMINOVIEW

Luminescence Microscopes

A quantum leap in live cell imaging



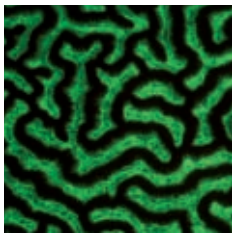


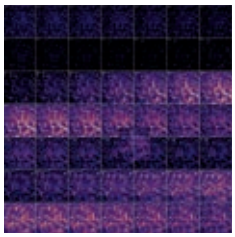


THE LIGHT WITHIN

Advanced bioluminescence microscopy

Illumination is an essential requirement for all forms of optical microscopy and a large number of recent scientific developments have been aided by the advancements made in lighting systems and techniques, many related to fluorescence. Luminescence, a close relation to fluorescence, promises much but has yet to prove its full potential due to a number of technical challenges. Luminescence provides the ideal signal-to-noise ratios and does not cause any toxicity to the sample, making it an excellent choice for following long-term changes in live systems. Olympus has now successfully harnessed the power of luminescence imaging for the first time in a commercial system – the LUMINOVIEW LV200.





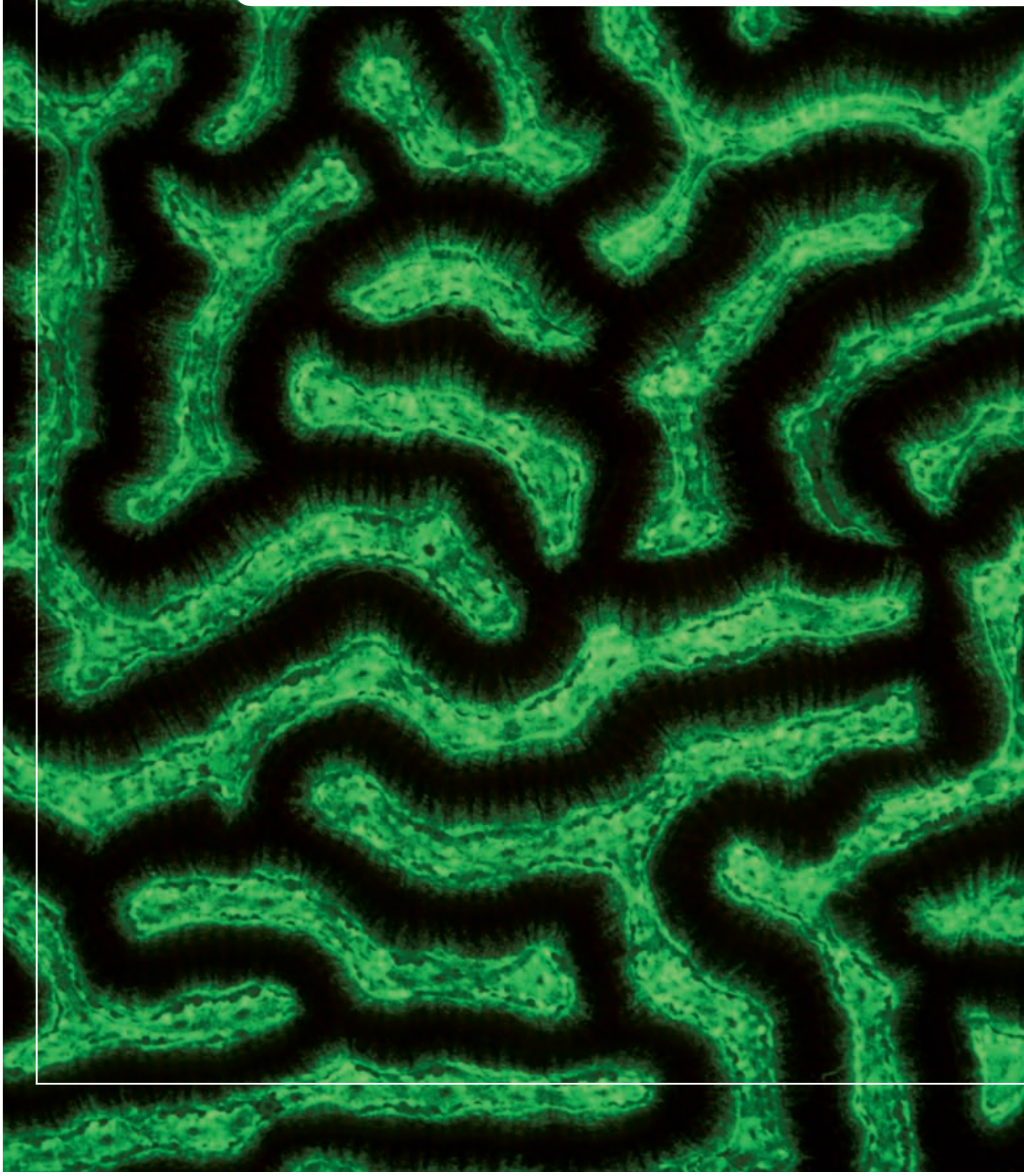
Your future is bright

As your partner for advanced scientific research, Olympus is dedicated to making state-of-the-art microscopes and accessories that are the best in their class, whatever the requirements. Our capabilities in R&D and quality manufacturing, and our attentive and informed customer support, are totally focused on success for your current and future experiments – making your future bright.

CELL-FRIENDLY IMAGING

The right light for living samples

Luminescence comes from within the sample and is therefore non-toxic to the cells. There is also no background noise or autofluorescence to worry about. Luminescence is therefore the right light for live cell imaging.



A LEADING LIGHT

Luminescence has been kept in the shadows over the years by the amazing success of fluorescence microscopy and the many related techniques. With the new Olympus LV200, luminescence now has the right platform to excel and prove the full extent of what it can offer to microscopy and imaging.

The same, only different!

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.

Fluorescence

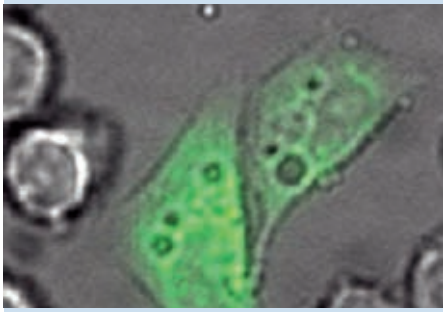
Fluorescent emissions tend to be short-lived and bright, requiring specific frequencies of light for excitation. As a result, this illumination is required at the time of imaging, which means that the optical system must be able to supply strong and fully controllable light at one wavelength and project the emitted light on a different wavelength to the user's eyes and/or camera. Despite these relatively complex optical requirements, fluorescence techniques have flourished and are enabling groundbreaking discoveries in many research areas.

Luminescence

Luminescence emissions tend to have varying lifetimes and are often quite faint, but due to the lack of background or fluorescence they can be measured with a high signal-to-noise (S/N) ratio. This makes luminescence ideal for applications where there is strong autofluorescence, such as whole animal imaging or in samples containing various compounds from chemical libraries. *In vivo* imaging systems and microplate luminescence readers have shown great success in these areas.

Fascinating microscopy without excitation

Bioluminescence imaging has great advantages over fluorescence imaging since it combines a high S/N ratio with no 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, bioluminescent measurements are ideal for sensitive living cells and are absolute and directly quantitative. These advantages are now no longer limited to macro imaging and bulk measurement, but can now also be applied to high-resolution microscopy.

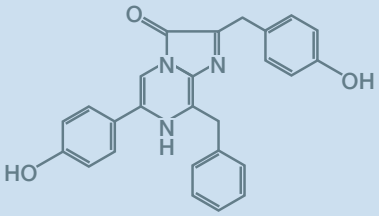


Luminescent signal from HeLa cells expressing firefly luciferase in peroxisomes

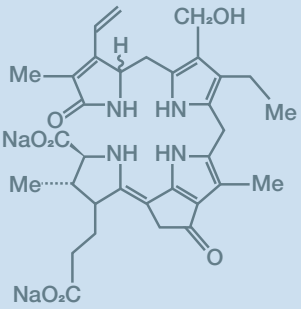
A **Substrates**

For bioluminescent proteins

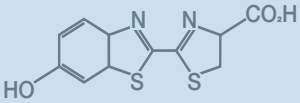
Coelenterazine



Dinoflagellate luciferin



Firefly luciferin



A **Olympus DP72**

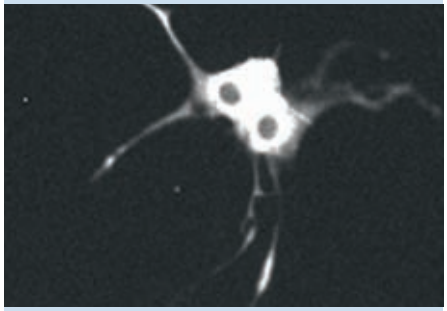
Highly sensitive microscope camera

THE OLYMPUS LV200 LUMINOVIEW

Olympus has developed the LV200 LUMINOVIEW bioluminescent imaging system, which provides amazingly detailed images using standard CCDs rather than specialised photon detectors. To do this, the optical design of the system is highly specialised to maximise light collection – essential given the low levels of light emitted.

Taking the right path

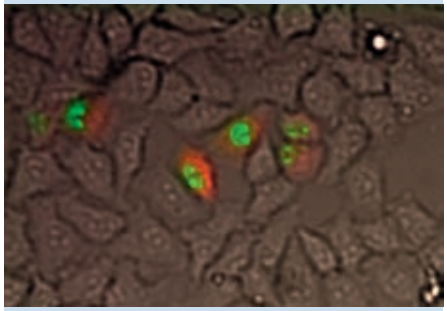
The light path from the object to the camera is straight and as short as it can be to ensure that as much light as possible reaches the CCD chip. There is also no need for any additional mirrors, filters or lenses which absorb light, reducing the signal further still. What is more, the tube lens has been designed with an extremely high numerical aperture (NA), which affords a vast increase in sensitivity when compared to conventional microscope optics. As a result, the LV200 produces signal outputs many times higher than traditional systems and can therefore be used with conventional CCD or EM-CCD cameras. The LV200 can use different magnifications from 0.8x to 20x, enabling it to clearly image samples from huge brain sections down to individual cells.



NIH/3T3 cell transfected with a fusion of CLOCK gene PER2 and luciferase

Seeing much more

These unique optical properties ensure exquisite single-cell resolution not previously possible with luminescence imaging. These improvements, along with the number of luminescent probes now available, promise to take microscope imaging to a different level. These properties also enable excellent spatial resolution, so that weak signals near areas with high signals can be differentiated with great ease, which is not possible on a luminometer.



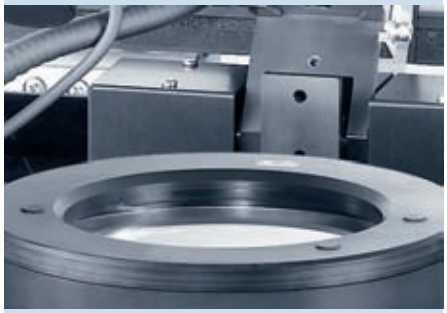
Overlay of luminescence signal (luciferase in red), fluorescent signal (GFP in green) and brightfield image

Expansive system

With optical components optimised for the detection of luminescent light, the LV200 is further designed to match the requirements of a broad range of research. It has integrated 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, 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 brightfield images, which enables localisation and colocalisation capabilities that were not previously possible.

Incubated

With the system 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. The LV200 is designed so that samples can be placed inside a highly accurate environmental chamber, which has independent temperature control for the stage, incubation chamber, top cover and objective. Furthermore, a water reservoir is 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.



Integrated environmental chamber

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. Therefore the entire system is incredibly "light tight", so it can be used in a standard laboratory.

A peerless system

In summary, the Olympus LV200 represents a quantum leap in microscope-based imaging. It effectively unleashes the full power of luminescence imaging, offering scientists the capability to not only measure molecular events, but also follow them precisely for the first time at the cellular level. The key advantages of bioluminescence microscopy on the LV200:

A Long-term live cell observation using bioluminescent reporter genes

Due to the lack of phototoxic effects, cultured cells or tissue slices can be imaged for days and even for weeks to measure fluctuations in gene expression.

B Imaging of photosensitive samples

Besides the general phototoxic effects, some samples can't be imaged effectively if illuminated with a strong light: for example, photoreceptors, early embryos and light-regulated systems.

C High S/N ratios and quantitative analysis, even in difficult samples

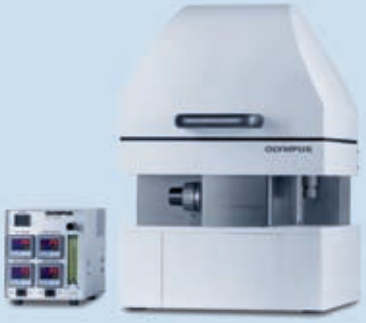
Quantifying weak fluorescence signals in an environment with strong or variable auto-fluorescence is always challenging and needs a lot of controls. Luminescence gives a clear and straight signal.

D Visual inspection of any bioluminescent assays on a cellular level

Due to the easy assay set-up and high S/N ratio, many bioluminescent assays have been developed for high-throughput screening. The ability to detect signals at a cellular resolution assists with assay development time.

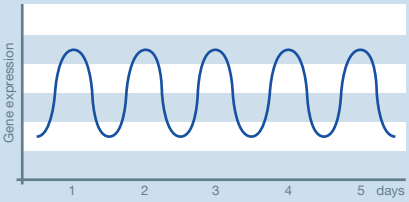
A LV200 LUMINOVIEW

System



A Long-term observation

of live cells, using bioluminescent reporter genes



B Imaging

of photosensitive samples

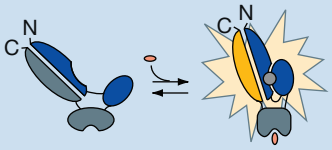
C High S/N ratio

and quantitative analysis, even in difficult samples

S/N

D Visual inspection

of any bioluminescent assays on a cellular level



APPLICATIONS FOR BIOLUMINESCENCE IMAGING

Limited only by your imagination

The LV200 presents researchers with a real opportunity to produce insightful and original research. Whether it's a technique unique to luminescence, or an investigation where it can replace fluorescence, the LV200 delivers breathtaking images.

LONG-TERM GENE EXPRESSION

Chronobiology (the study of biological timing or circadian rhythm) was arguably the first research area to use bioluminescence microscopy. Measuring the upregulation and downregulation of a gene over days or even weeks needs a reporter system with a short half-life, stable substrates and an analysis method lacking any toxic effect to the cells. The luciferin-luciferase bioluminescent system covers these requirements perfectly.

Importance of rhythm

Circadian timing is a complex process, and in mammals most vital processes are subject to circadian variations. Thus sleep-wake cycles, locomotor activity, heartbeat, blood pressure, renal plasma flow, body temperature, sensorial perception and the secretion of many hormones fluctuate during the day in an orderly fashion. The daily timing of these physiological parameters persists under constant conditions and must therefore be controlled by one or more circadian pacemakers.

Expression of Dbp transcription factor

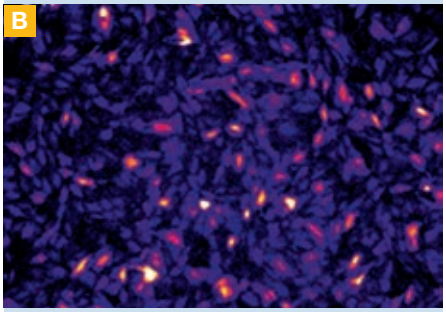
A B Mouse NIH3T3 fibroblasts, stably expressing the full-length circadian Dbp gene fused to luciferase, were visualised for four days in the Olympus LV200. Image A (left) shows the low-resolution display of the whole time series. Image B shows a display of individual cells. The luminescence signal can be measured for any cells over time. Microscope settings: Objective 20x, Hamamatsu ImageEM, no pixel binning, EM gain 150, 15 min acquisition time (courtesy of M. Stratmann, U. Schibler, Department of Molecular Biology, University of Geneva, Switzerland).

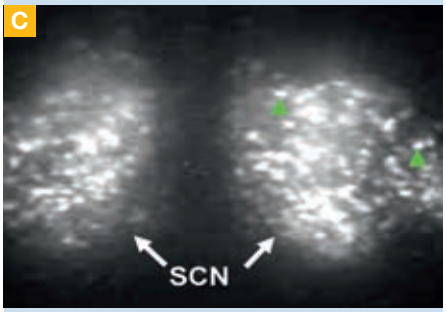
Promoter studies in tissue slices

C D E Hugh Piggins, Alun Hughes and Clare Guilding at the University of Manchester's Faculty of Life Sciences are looking at the long-term expression of the protein Period-2 (PER2) in mice, a protein encoded by the PER2 gene, a key CLOCK gene. They have been using the Olympus LV200 to look at long-term expression patterns of the PER2 protein, a process that requires acutely cultured brain slices to be incubated and imaged for extended periods of time. Recording images once every 3 minutes for up to 7 days, they can then analyse the gross expression of PER2 over these times within the entire culture, as well as view each individual cell (C) to look for variations from and similarities to the gross expression (D, E).

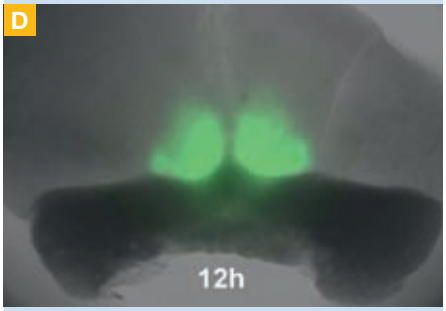
New insights through increased resolution

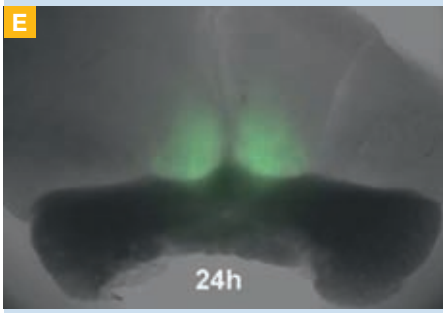
As a result of these studies, it is clear that the Olympus LV200 LUMINOVIEW enables the measurement of light intensity over prolonged periods of time and, more importantly, detailed information on the morphogenesis and gene expression patterns of individual cells to be obtained.





High-magnification image of the bilateral SCN, with individual cells highlighted by green arrowheads





PER2::LUC luciferase fusion protein 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 24-hour day (high at 12 hours, lower at 24 hours).

Images courtesy of H. Piggins, Faculty of Life Sciences, University of Manchester, England

Imaging embryos

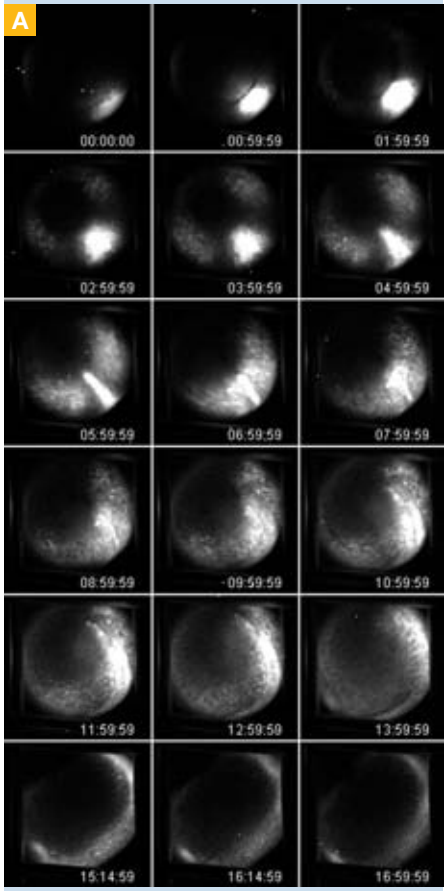
One of the most important live cell paradigms being studied is vertebrate and invertebrate embryos. Mammalian embryos are generally very susceptible to changes in their environments and can be acutely affected in various different ways. They therefore need to be carefully incubated and handled as little as possible during an experiment. Furthermore, they are very sensitive to strong light. This makes it very difficult to image them, since development experiments require long-term observation with frequent imaging. Bioluminescence microscopy offers the perfect tool to follow gene expression during the early embryo development without affecting the embryo's viability.

Immediate signal

Luciferase is active immediately after translation and has a short half-life, which enables very good resolution of the dynamics of gene expression. The LV200 has been used to look at luciferase expression in early stages (2–4 cell) of mouse embryos (data not shown).

Don't worry about autofluorescence

Another key point here is that embryos such as those of *Xenopus spp.* have a high level of autofluorescence due to the large concentration of yolk proteins. Nevertheless, luminescence imaging can generate a clear and quantitative signal in such difficult samples.



LEF1 gene activity in Xenopus embryos

Image courtesy of Ms. Chiyo Takagi, Naoto Ueno Ph.D, Division of Morphogenesis, National Institute for Basic Biology, Japan

A LEF1 expression pattern in Xenopus embryo
Luciferase expression, driven by LEF1 promoter, was followed in transgenic embryos using the Olympus LV200. Lymphocyte enhancer factor 1 (LEF1) is one of the transcriptional factors related to Wnt/ß-catenin signalling. Images were captured every 15 minutes for 17 hours with a 15-minute exposure time using a 20x objective (NA 0.75) and a Hamamatsu ORCA-AG camera.

SIGNALLING

Research on intercellular communication belongs to one of the most important and most complicated fields in biology and pharmacology. Therefore, many assays have been developed to measure signaling events such as receptor activation or intracellular Ca^{2+} concentrations.

Ca^{2+} imaging with bioluminescence

Intracellular Ca^{2+} concentration acts as a second messenger that regulates numerous physiological phenomena including development, differentiation and apoptosis. A number of fluorescence-based imaging methods are commonly used, including ratiometric measurement with FURA-2 or fluorescent indicator proteins such as the chameleon labels. Ca^{2+} signal fluctuations can display durations between milliseconds and minutes and can occur at unpredictable intervals (minutes to hours) during otherwise long periods of quiescence. Therefore fluorescent-based methods, with their need of strong excitation light and their sometimes toxic effects, are not suitable for continuous and non-invasive imaging.

Observation without limits

In contrast to fluorescent indicators, Ca^{2+}-dependent photo proteins like aequorin, aequorin-GFP, obelin or Photina® do not need any excitation and can be imaged after loading with their substrate – coelenterazine – for an extended time period. In combination with the optimised Bioluminescence Microscope LV200 and high-sensitivity EMCCD cameras, bioluminescent Ca^{2+} signals can now be measured at high speed, with frame capture times of 20 to 500 ms, depending on cell type, the photo protein used and the image resolution required.

Example Ca^{2+} imaging with Photina®

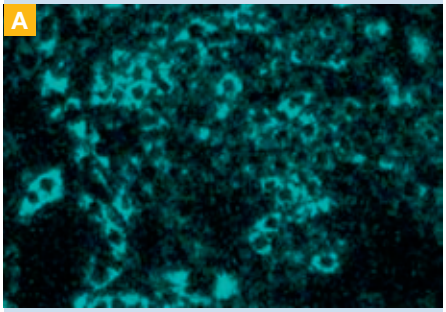
A Chinese hamster ovary (CHO) cells expressing Photina® protein (Axxam, Milan, Italy, www.axxam.com). The cells were stimulated with 10 µM ATP, which leads to phospholipase C activation through the endogenous P2Y receptor and G-protein activation. Phospholipase C cleaves PIP2 into diacylglycerol (DAG) and inositol-3-phosphate (IP_3). IP_3 releases intracellular Ca^{2+} which is stored in the endoplasmic reticulum (ER). The Ca^{2+} reacts with the Photina® and the substrate, coelenterazine, to generate a bioluminescent signal.

B The bioluminescent signal of five individual cells was plotted against time. The experiment was performed by Dr Marc Spehr at the Inst. of Zellphysiologie, Ruhr-University Bochum.

Advancing bioluminescence for physiological studies

C D E Due to the high S/N ratio and the easy set-up, bioluminescence assays are used widely for high-throughput screening (HTS). The ability to capture luminescence images on a cellular level can give important additional information for assay development and validation. An example of cAMP measurement, a key signalling molecule for many G-protein-coupled receptors, is shown. The GloSensor™ cAMP Assay from Promega Corp. (www.promega.com/glosensor) presents a novel approach to measuring cAMP levels in live cells. It is based on the GloSensor™ technology, a genetically modified form of firefly luciferase into which a cAMP-binding protein moiety has been inserted. Binding of cAMP results in light output directly proportional to the intracellular concentration at cAMP.

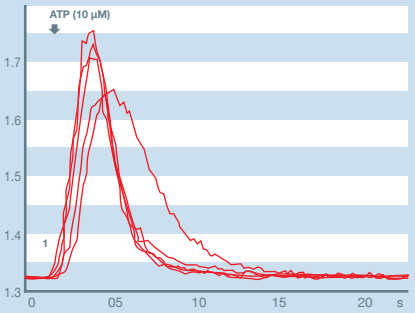
A



Ca^{2+} assay using CHO cells expressing Photina®

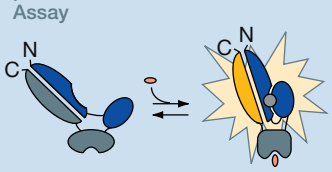
B Ca^{2+} activation

measured in five individual cells

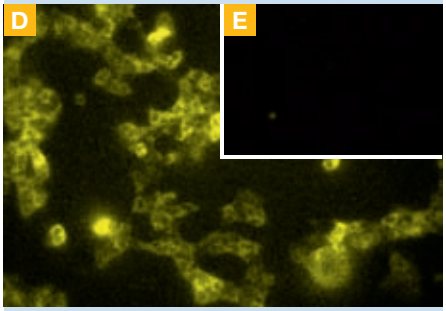


C Visual inspection

Model of the light-generating process of the GloSensor™ cAMP Assay



D



E

HEK293 cells transiently expressing the GloSensor™ cAMP protein were imaged with LV200

D: treated with 10 µM isoproterenol; inset, untreated
E: inserted box: untreated

Image courtesy of Promega Corporation. GloSensor™ is a trademark of Promega Corporation.

LV200 LUMINOVIEW specifications

Observation methods	Luminescence imaging, transmitted brightfield, basic transmitted fluorescence			
LV200 main body	Light-tight dark box			
	Manual objective lens focusing			
	Coaxial XY stage			
	Motorised exciting filter wheel with 3 positions for standard 25 mm optical filters			
	Motorised emission filter wheel with 6 positions for standard 25 mm optical filters			
	Condenser for transmission brightfield coupled to light guide			
	C-mount for camera			
	Tube lens optimised for luminescence imaging, 0.2x magnification			
Accessories and options				
Illumination unit	External halogen lamp housing coupled with light guide			
Objectives	Integration of all standard-size Olympus objectives possible			
	Recommended			
	objective	**NA**	**WD (mm)**	**Correction (mm)**
	UPLSAPO 10x	0.4	3.1	0.17
	UPLSAPO 20x	0.75	0.6	0.17
	UPLSAPO 40x	0.9	0.18	0.11– 0.23
	UPLSAPO 60xO	1.35	0.15	0.17
	UPLSAPO 100xOI	1.4	0.13	0.17
	LUCPFLN 20x	0.45	6.6–7.8	0–2
	LUCPFLN 40x	0.6	2.7–4	0–2
	PLAPON 60xO	1.42	0.15	0.17
Controller	IX-UCB controller for filter wheels and illumination			
Hand switch	Hand switch for filter wheels and illumination			
Environmental control	Double-layered chamber type incubator for 35 mm dish including controller, stage heater, top cover heater,			
	main body heater, objective heater, flow meter for 5% CO_2, 95% air			
System table	Approx. 700 (H) x 600 (W) x 600 (D) mm (needed if large-size camera is used)			
Motorised z-focus drive	Adapter for motorised z-focus, controller			
Computation	Imaging computer with minimum: Intel Core 2 Duo, 2 GHz, 1.5 GB RAM, 80 GB and 250 GB hard disk, dual-head video			
	board, DVD RW, USB, serial, parallel, LAN, video, audio onboard, keyboard (GB), optical mouse, MS Windows XP			
Device controller	Controller board for timing of experiments, mounted inside imaging computer. Controls CCD trigger,			
	filter wheels and peripherals			
Monitor	Computer flat-panel monitor 20" TFT			
Software	Imaging software cell* for Windows. Graphical interface for experiment planning and execution			
	(“Experiment Manager”). Structured database for multidimensional data handling.			
Camera options	Depending on application and required sensitivity, cooled CCD cameras, EM-CCD cameras or deep-cooled slow-scan			
	CCD cameras can be used. (Please ask about camera/software compatibility.)			
Power consumption	Main unit 850 VA			
	Controller 840 VA			
Installation space	Approx. 1500 (W) x 750 (D) mm (depending on configuration)			
Total mass weight	Approx. 70 kg (depending on configuration)			

The manufacturer reserves the right to make technical changes without prior notice.
To learn more about the new GloSensor cAMP Assay, please see www.promega.com/glosensor. GloSensor is a trademark of Promega Corporation.

www.olympus-europa.com

Art. code: E0430969 • Printed in Germany 03/2009

OLYMPUS

OLYMPUS LIFE SCIENCE EUROPA GMBH
Postfach 10 49 08, 20034 Hamburg, Germany
Wendenstrasse 14–18, 20097 Hamburg, Germany
Phone: +49 40 23773-0, Fax: +49 40 23773-4647
E-mail: microscopy@olympus-europa.com