OLYMPUS

Your Vision, Our Future

Specialised Microscopy Illumination

Optigrid M

Structured Illumination System

Making Fine Fluorescence a Reality

TAKING LIFE SCIENCE FURTHER

Better fluorescence microscopy

The advent of fluorescence microscopy gave scientists a completely different view on biological interactions, both at the cellular and subcellular levels. Indeed, many important biochemical and molecular discoveries have been made as direct result of the information gleaned through fluorescence microscopy. Confocal instruments have taken fluorescence microscopy one step further by providing a solution to the out-of-focus blur commonly associated with standard fluorescence illumination, which in turn has greatly increased clarity and resolution. They do, though, represent a more significant investment for the users and are also more complex to use. A new structured illumination system available from Olympus, the Optigrid M, enables confocal-like images without the initial investment or complexity.

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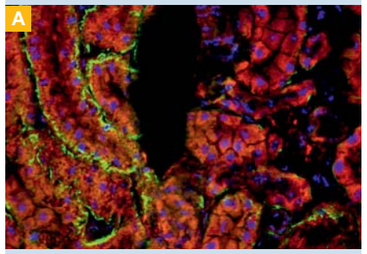

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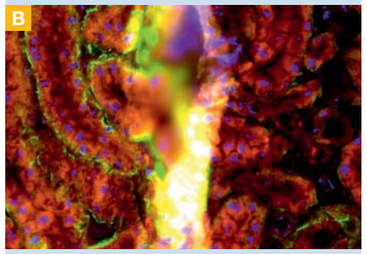


Rat kidney section.

Image A was acquired with Optigrid M, image B with conventional fluorescence equipment.

Hoechst Alexa 44, laminin CD31, Cy3.

SEEING THROUGH THE BLUR

Standard arc-burner-based microscopy illumination excites fluorophores throughout the entire sample and not just within the field and plane of view. The fluorescence light emitted by excited fluorophores gets distorted by the optical system. This distribution of light from a very small point like a fluorophore is known as the point spread function (PSF). As a result, fluorophores above and below the focal plane contribute unwanted light, which blurs the image and reduces the resolution and clarity. A number of methods have been developed to de-blur these images, such as deconvolution and confocal microscopy.

Deconvolution

Deconvolution is a mathematical process that removes blur, simply by calculating all the convolved light back into the position from which it was emitted, based on the PSF of the optical system. This is most accurately achieved by deconvolving a series of optical sections (z-stack) of images and combining the resultant in-focus parts of the images into one sharp, three-dimensional image.

Confocal microscopy

Confocal microscopy uses two physical techniques to reduce out-of-focus information, generally producing better resolution than deconvolution. Firstly, a focused and refraction-limited beam of light (usually a laser beam) illuminates just one point of the sample. Fluorophores which are not in the focus of this beam are not excited and cannot contribute with out-of-focus information. The image is built up by moving the laser spot over an area and detecting the signals one after the other. Secondly, a minute pinhole in an optically conjugate plane blocks out-of-focus light and therefore the image is formed only from in-focus information.

The new Optigrid M

C D Optigrid M uses a combination of physical and mathematical processing to produce near-confocal-quality images using a stabilised arc burner illumination source. This is achieved by using a one-dimensional optical grid mounted on a piezo-electronically driven actuator to project a line pattern onto the specimen. The grid is moved perpendicularly to the grid lines, in steps 1/3 of its period length so that three grid movements cover one optical section. This process returns a strong signal where focus is sharp and a weak signal where focus is soft.

These optically clear image portions are recombined using a specially developed substractive algorithm, which ensures that only in-focus pixels are used and that the overlapping sections of each image portion line up. This results, in most cases, in images possessing the same clarity and resolution as those produced using a confocal laser scanning microscope (CLSM).

A z-stack taken through a sample can be combined to create a haze-free, ultra sharp composite image. Furthermore, image stacks can also be used to produce 3-D reconstructions using cell* or additional post-processing software.

C **Optigrid M**

On a BX61 microscope

D **Optigrid M paddle**

Creating ulta sharp images

APPLICATIONS

E F G The Optigrid M is perfect for a broad range of samples but excels with thick and multicellular samples such as nematodes, brain slices, embryos and fish. Due to its reliance on an arc-burner-based illumination source, Optigrid M is perfect for all procedures normally completed without the Optigrid M installed. The additional resolution and clarity it provides also make it highly suited to other tasks.

Key examples of procedures where the Optigrid M provides excellent images:

- Basic deblurring of fluorescence specimens
- Concentration on the structures within a focal layer
- Removal of non-focused fluorescent intensities
- Image enhancement
- Optical sections through thick specimens
- 3-D reconstruction of cell groups

These peerless capabilities make the Optigrid M ideal for research into key topics such as neurobiology, developmental biology and cell biology.

System solution

C D The Optigrid M consists of a control unit, a paddle slider and a field stop insert. The slider is inserted into the field diaphragm slot of a standard illuminator, which enables it to be easily removed for standard microscope functions. As a result, the Optigrid M system is less complex than a confocal set-up and doesn't require as much bench space either. It does, though, need a stabilised arc burner illumination source, as fluctuations in the illumination intensity over the three image portions can lead to banding patterns in the final image. As a result, the Optigrid M is available with the X-Cite or MT10/MT20 illumination systems, for use on the inverted IX2, upright BX2 and MVX10 Macroview Olympus microscopes. The microscopes themselves also need to be isolated from vibrations and therefore special anti-vibration plates are available. The Optigrid M is essentially a module that can be added to the advanced arc-burner-based fluorescence microscope, and can therefore be used across the full range of wavelengths normally available to the user, from UV to IR. However, the better the chromatic correction of an objective lens is, the better the spectral range for application will be. We recommend objectives from the U-PLANSAPO range for best results.

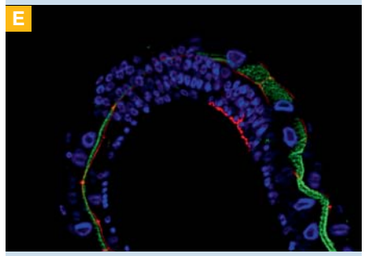
Chromatic compensation

Different wavelengths require the grid position to be adjusted slightly to compensate for chromatic effects. The Optigrid M only needs to be set once for each wavelength using the calibration slide. Once set, the grid position changes automatically depending on the fluorescence wavelength selected.

Component integration

The Optigrid M can be easily integrated into an overall imaging system, since the control unit interfaces seamlessly with the Olympus cell* family. This provides users with excellent imaging throughput and programmable experiment repeatability for parallel multiple-specimen imaging.

E



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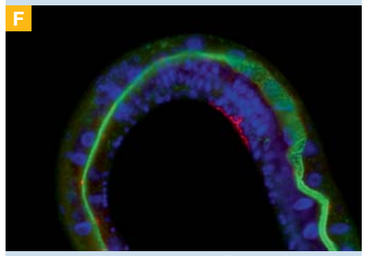


Immunofluorescence, *C. Elegans* hermaphrodite in the late L2 stage.

Image A was acquired with Optigrid M, image B with standard fluorescence equipment.

Green: Antibodies recognising the PDZ-protein TAG-60. Expression detected on the lumenal side of intestinal cells. The function of TAG-60 during *C. elegans* development is under investigation.

Red: Antibodies recognising the well-characterised protein AJM-1, which is expressed in the junctions of epithelial cells.

Blue: DNA stained by DAPI.

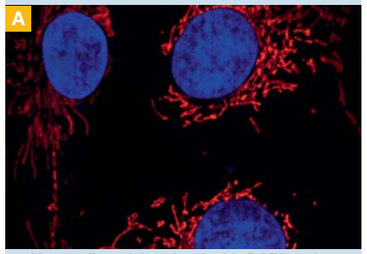
Images courtesy of Prof. Hajnal et al., Institute of Zoology of the University of Zurich, Switzerland

G

Mouse embryo (*mus musculus*)

Image courtesy of Instituto de Neurosciencias de Alicante, Spain

A



Vero cell nuclei stained with DAPI and mitochondria stained with MitoTracker® red

CONTROLLED

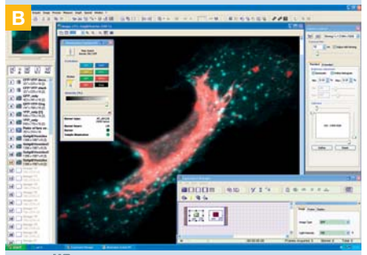
Power is nothing without control! The Olympus cell* software programs provide an abundance of control and analysis options.

Software modules

cell^{F} and cell^{P}

A The Olympus cell^{F} and cell^{P} programs are designed to work with Optigrid M and the X-Cite imaging unit for the documentation, visualisation, processing and analysis of multichannel fluorescence images. This enables analysis and presentation of many immunofluorescence techniques, like z-sectioning and image acquisition at different focal positions to visualise specimens in 3-D. The cell^{P} version is the ideal system for automated image capture of multichannel analyses of fluorescent probes and dyes. It enables the user to carry out a range of complex and highly sophisticated experiments. These include automated capture of multi-fluorescence images, time-lapse photography, 3-D image composites, as well as automatic scanning of multiwell plates with a motorised XY-stage.

B



cell^{R} software

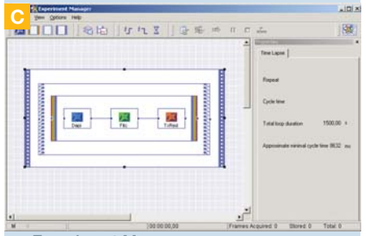
cell^{R} and cell^{M}

B Advanced live cell imaging applications, such as cell growth and metabolic transport, multicolour-time-lapse imaging, z-sectioning, multidimensional imaging, ion imaging, FRET, TIRF, etc., need illumination systems with bright light for efficient illumination, fast switching to different wavelengths, high-speed shutters for maximum specimen protection and high precision for analysis. These two software programs are designed specifically to work with the Olympus MT10 and MT20 light sources respectively, providing highly stabilised and easily controlled light from either a 150 W xenon or 150 W mercury/xenon burner.

Experiment Manager

C With both the cell^{M} and cell^{R} software programs, the powerful Experiment Manager provides precise planning and execution tools via an intuitive drag-and-drop system that enables easy assembly of command icons such as image acquisition, z-stack and time loop.

C



Experiment Manager

D

THE ILLUMINATION SOURCES

The Optigrid M concept is best exploited with a highly stable fluorescence light source. This ensures that each of the grid images is captured with exactly the same light intensity. Olympus offers a range of stabilised mercury and mercury/xenon arc-burner-based fluorescence light sources.

X-Cite

E The X-Cite uses an alignment-free metal halide burner, the unique “electronic control gear” and IntelliLamp™ facilities. Thus it provides the same fluorescence spectrum as and similar intensities to a standard mercury burner, while ensuring an additional level of consistency and safety. Furthermore, the units are thermally uncoupled from the microscope, with a liquid light guide delivering the light through collimator optics.

MT10

F The MT10 is designed for absolutely stable illumination of fast events and incorporates an 8-position filter wheel which can be moved between neighbouring filters in just 85 ms. Intensity can be controlled even more quickly via the 7-position attenuator (4–100%) and the high-speed shutter can be opened or closed in under 5 ms. The cell^{M} system also incorporates a system coordinator (PC control board), which provides 10 ms temporal resolution with a timing precision of 15 ms. The combination of the MT10, system coordinator and cell^{M} software makes even the most complex data acquisitions straightforward and repeatable.

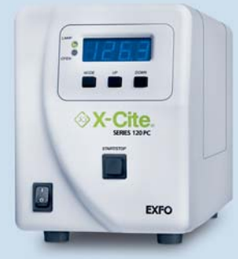
MT20

G The MT20 (and cell^{R}) is designed especially for high-speed live cell imaging and uses a faster filter wheel than the MT10, moving between each of the 8 neighbouring filters in just 58 ms. The expanded attenuator set includes 14 positions, offering intensities between 1% and 100%. The shutter is also much quicker with on/off times of less than 1 ms. The more powerful real-time controller PC board ensures, via parallel command execution, temporal resolutions of 1 ms and precision in the microsecond range.

The Optigrid M is an excellent and cost-efficient system for expanding the imaging capabilities of new or existing microscopes, without taking up valuable laboratory space. Offering confocal-like image quality, the Optigrid M integrates quickly and easily with Olympus BX2, IX2 and MVX10 microscopes.

E **X-Cite 120PC**

With alignment-free burner



F **MT10**

Fast wavelength changer

G **MT20**

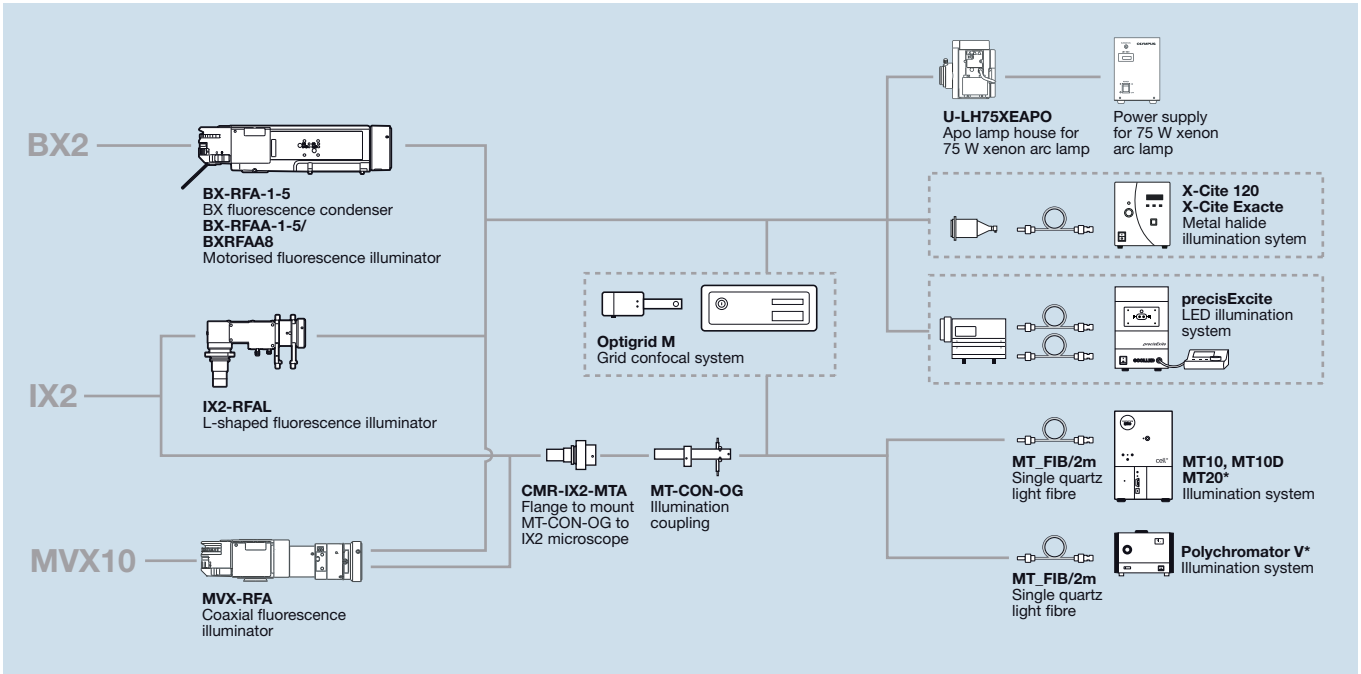
High-speed wavelength changer

Specifications

Confocal method	Structured illumination microscopy
	Optical system combined with mathematical algorithm
Observation method	Fluorescence microscopy
BX51/61 and BXWI	cell^F or cell^P imaging software and OGM BX
MVX	cell^F, cell^P, cell^M and cell^R imaging software and OGM IX
IX71/81	cell^F, cell^P, cell^M and cell^R imaging software and OGM IX
Objectives*	UPlanSApo 10x
	UPlanSApo 20x
	UPlanSApo 40x
	UPlanSApo 60xO
	UPlanSApo 60xW
Illumination	75 W Xenon arc lamp
	X-Cite
	preciseExcite LED illumination
	MT10 and MT20 illumination system
Recommended accessories	Antivibration plate
Recommended camera	DP72, XC10, XM10, Hamamatsu Orca R2, XM10, XM10TIR
Environmental conditions	Temperature and humidity: 10°C–35°C, 30%–80%

* For compatibility of other objectives, please consult your Olympus supplier.

System chart



* To use Optigrid M with cell^M or cell^R imaging systems, special controllers are needed. Please consult your Olympus supplier for details.

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