OLYMPUS

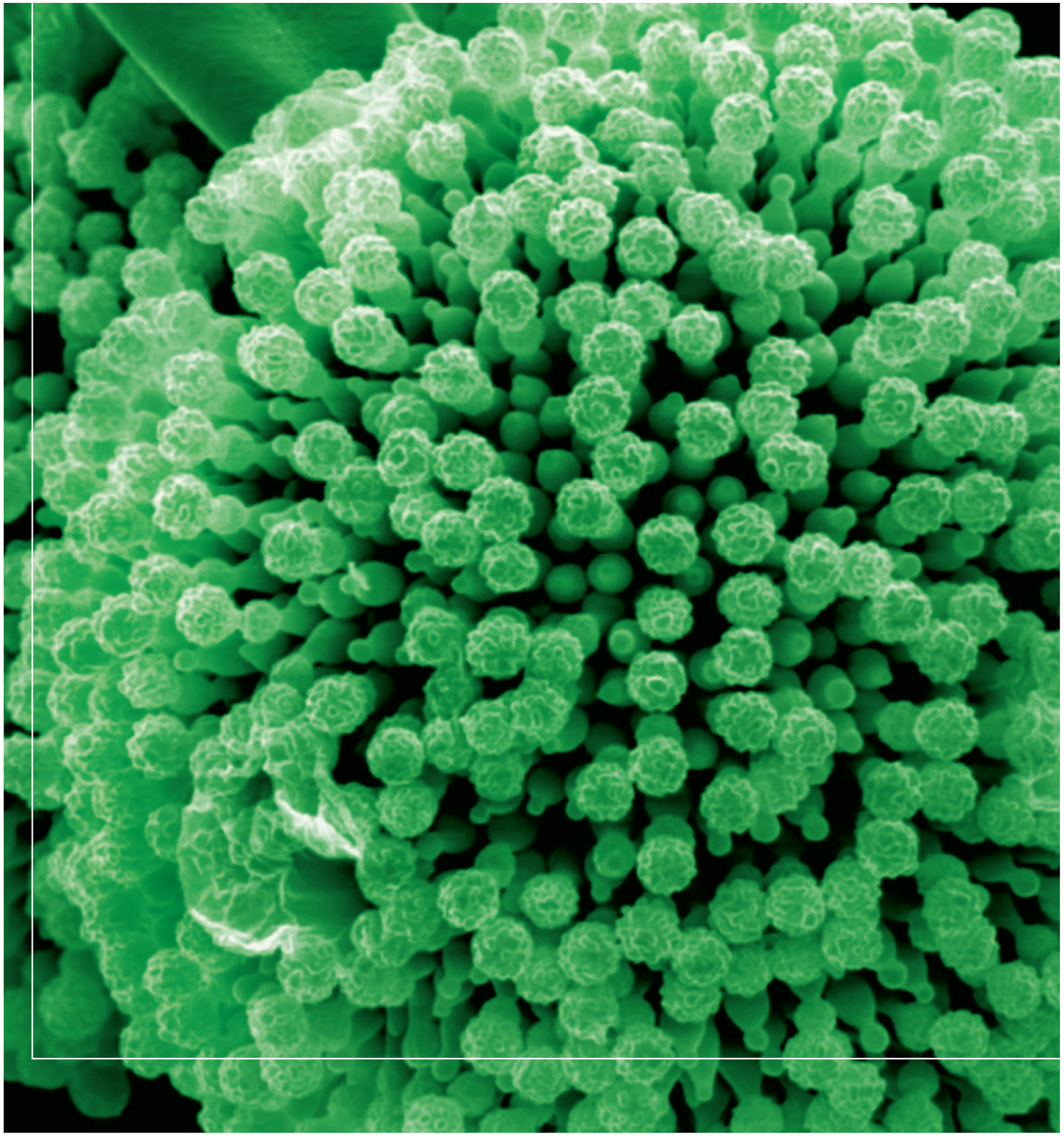
Your Vision, Our Future

Advanced Live Cell Imaging Systems

xcellence, cell^{tirf}, cell^{frap}

Widefield, Confocal and TIRF Microscopy

Expanding the possibilities of fluorescence microscopy



EXCELLENT SAMPLES NEED AN XCELLENCE SOLUTION

The Olympus xcellence platform for advanced, flexible fluorescence microscopy

Olympus always strives to provide systems that don't just do the job; they do it faster, better and with greater flexibility. As a result, the xcellence system covers an amazing array of advanced fluorescence techniques, presenting users with an easy-to use interface and peerless options. Whatever the fluorescence challenges, the xcellence platform is the perfect expandable system solution.

Meeting your microscopy needs

Olympus is dedicated to making state-of-the-art microscopes, accessories and imaging system solutions to support your research at all levels. We have therefore worked closely with customers to produce the ultimate in flexible and accessible microscopes. Our goal is your success, now and in the future.

SOLID FOUNDATION

Appreciating accuracy

The Olympus xcellence platform enables fluorescence imaging at an unprecedented level of speed and accuracy. This is made possible by the class-leading components and optics built as standard into every system.

POWER IS NOTHING WITHOUT CONTROL

Accurate, insightful experiments require high positional and temporal resolution. Therefore, the Olympus xcellence platform incorporates precise motorised controllers and a dedicated real-time computer processor, providing optimised image capture.

Application: multidimensional fluorescence live cell imaging

A B C Cells are dynamic, 3D entities that change and develop over time. Therefore, to truly appreciate cell behaviour and accurately analyse the function of cellular components, it is necessary to perform studies that work in five dimensions – X, Y, Z, time and position.

Challenges: speed, accuracy and flexibility

In order to collect reliable 3D time-lapse data, a microscope system must be fast and accurate, capturing many consecutive images at a range of Z positions without losing focus and with minimal sample bleaching. In addition, as some experiments involve ultra fast, sub-second imaging, whereas others require consecutive images to be captured over days or even weeks, the system must be flexible enough to provide both. This includes the option to easily modulate imaging parameters on the fly, in order to adjust to changing or unexpected experimental conditions.

Solution: the xcellence rapid imaging system

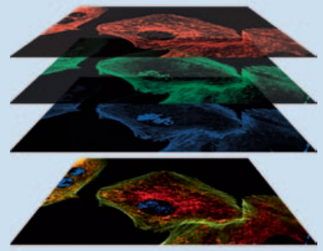
The powerful xcellence pro and rt systems combine high-end microscope optics with advanced components and motorisation via the comprehensive xcellence software. The software also brings together extensive imaging and analysis tools along with logical archiving. As a result, the xcellence platforms are the ideal starting point for a broad range of live-cell fluorescence imaging processes.

The real-time control concept
For complex multi-device experiments xcellence rt uses a real-time controller board offering µ second timing precision for conducting multiple processes in parallel, guaranteeing hardware interplay and efficiency. For procedures that do not require such high speed or parallel operation, xcellence pro implements a system controller board, which provides <5 ms precision with processes controlled sequentially.

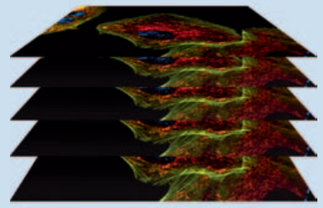
System Comparison

	xcellence pro	xcellence rt
Controller	System coordinator	Real-time controller
Operation	Sequentially	All components in parallel
Digital I/O Panel	Standard	Standard
Temporal resolution	10 ms (camera 1 ms)	0.1 ms
Timing precision	About 15 ms	<0.01 ms
Multitask imaging	3–4 frames/s	19 frames/s
	@ 50 ms exposure	@ 50 ms exposure
Imaging	up to 500 frames/s	up to 500 frames/s
Illumination system	MT10	MT20

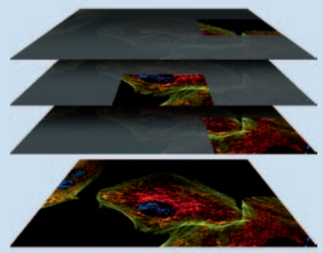
A Multichannel
Flexible acquisition of multiple fluorescence and transmission channels



B Multidimension
Real-time time-lapse imaging of multiple channels and Z-layers



C Multi-position
5D imaging of multiple positions and panoramic images

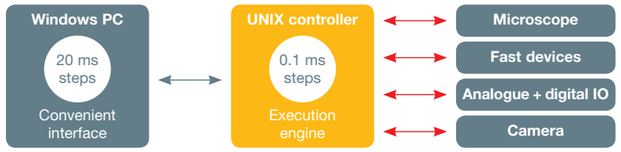


A **xcellence real-time controller**

PC integrated real-time controller and digital I/O board

Both these control systems ensure that experiment flow and timing precision are maintained while changing hardware settings. For example, filter wheels, attenuators and shutters are optimally synchronised with the camera, as well as with optional piezo Z-drives and other devices. By using independent and intelligent control boards, xcellence does not rely on the imaging computer to control the hardware. This is essential for high-speed imaging as the independent CPU boards can perform calculations with greater rapidity than standard PC CPUs, increasing timing precision, data transfer consistency and therefore experiment accuracy and reproducibility.

Real-time controller: state-of-the-art accuracy and system speed



ODB – Olympus digital bus

All advanced Olympus imaging hardware is connected via the proprietary Olympus digital bus (ODB), an intelligent connection capable of automatic device detection. Each component is linked by a single pathway of cables, terminating at the real-time controller which provides power and control to the system.

Intelligent integration provides flexibility and freedom

A Controlling a number of peripheral components efficiently and with great speed requires a dedicated interface system. The xcellence platforms are fitted with a real-time controller with seven-port TTL panel featuring three dedicated trigger-out ports and four fully digital I/O ports. Multiple sockets are also provided for the integration of external devices such as motorised microscope controllers (UCB), motorised stages or an emission filter wheel.

LET THERE BE LIGHT

The adoption of fluorescent proteins as markers for cellular factors has revolutionised live cell imaging. The Olympus xcellence system is optimised to take advantage of these techniques, taking your live cell studies to the next level.

Application: imaging sensitive living samples

B Visualising protein localisation and movement in living cells is essential for studying important processes such as secretion, vesicle transport and cell division. The use of fluorescent molecules to tag proteins of interest is particularly powerful, as this approach does not require any harmful cell preparation or fixation steps.

Challenge: bleaching and phototoxicity

In order to monitor living cells using fluorescent proteins, the amount of light shone onto the sample should be minimised. Firstly, the continual radiation of cells with fluorescent light is toxic, at best inhibiting the normal function of cells and at worst causing cell death. Similarly, the continual exposure of fluorescent proteins to light causes a phenomenon known as photobleaching, which makes it more difficult to accurately monitor experimental changes over the course of a prolonged study. Therefore, minimising these effects is essential for accurate and reliable live cell imaging.

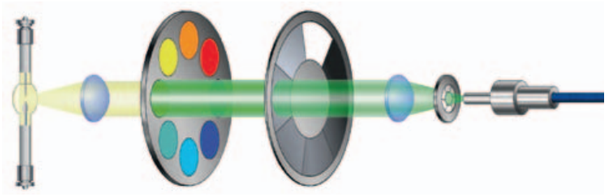
Solutions: 1 ms-fast shutter and microsecond fast real-time controller

C Fast switching between excitation wavelengths is crucial for many applications, e.g., dual excitation ratio measurements or fast multi-colour time-lapse experiments. The Olympus all-in-one illumination systems, MT20 (integrates with the xcellence rt) and MT10 (integrates with the xcellence pro), match these exacting requirements with filter-switching times of 58 and 85 ms, respectively between the eight excitation filter positions. This minimises the amount of time the sample must be exposed to light in order to carry out multi-wavelength experiments. Due to fast control via the xcellence system components, live cell experiments are optimised to provide insightful data without compromising cell viability.

Controlling illumination

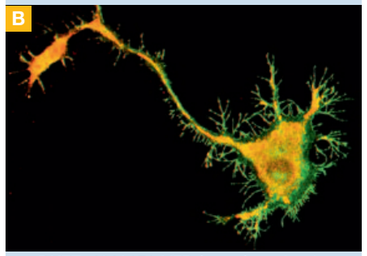
The integrated attenuators switch between intensity grades (7 for the MT10 and 14 for the MT20) at even faster speeds than the filter wheel. Furthermore, the built-in shutters have exceptional on/off intervals of only 1 ms for the MT 20 and < 5 ms for the MT10, eliminating photobleaching when not acquiring an image.

Schematic array of the MT10/MT20 modules



From left to right: arc burner, filter wheel, attenuator, shutter and fibre

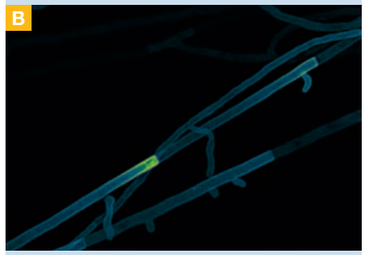
B



DKFZp434A171-YFP (green) and red staining of the cytoplasm in PC12 cells.

Image courtesy of Vibor Laketa, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

B



Hyphae of filamentous fungi with GFP tagged ApsA forming gradients towards septa.

Courtesy of D. Veith and R. Fischer, Institute of Technology (KIT), Karlsruhe, Germany.

C **MT20 illumination system**

Highly stabilised, fast-switching fluorescence light source

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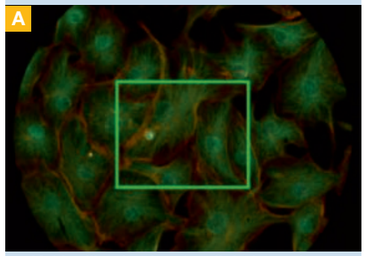
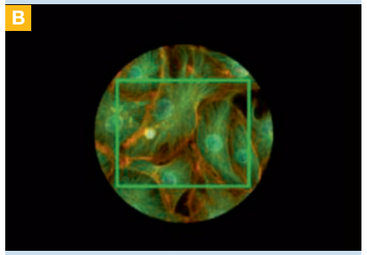


Kohler illumination: low light intensity and large distribution of light. The area for camera detection is marked in green.

B



xcellence critical illumination: all light is intensified to the area of camera detection.

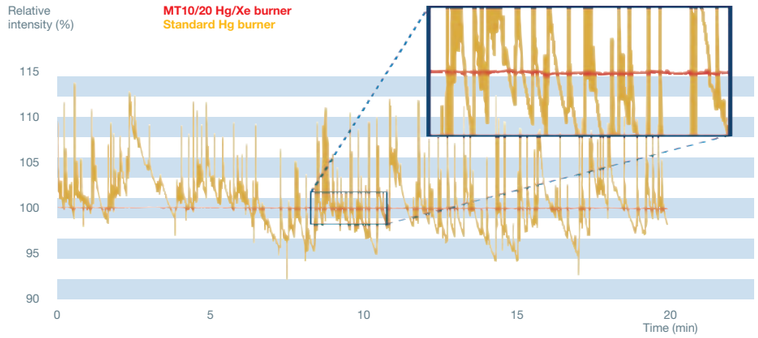
C **Fibre illuminator**

For critical illumination

Outstanding flexibility and stability

Two different arc burners are available for both illumination systems: The Xenon burner provides stabilised broadband illumination from near-UV to near-IR. The highly stable mercury/xenon burner provides the same broadband coverage with the addition of five very intense peaks that guarantee low exposure times for samples with corresponding fluorophores. The stability of both types is much superior to that of standard mercury burners and is further enhanced by specialised electronics. Minimising exposure time simultaneously minimises phototoxicity and photobleaching.

Highly stable light output
Comparison with standard Hg burners



Light output normalised to the average intensity (100%).

Fibre-coupled illumination maximises light efficiency without compromising quality

A B C The use of critical illumination instead of traditional Kohler illumination delivers all light to the area of interest and generates a much stronger signal intensity. Achieved by routing the fluorescent light to the microscope via a high-quality, single-quartz fibre coupling and achieving much shorter camera exposure times.

Comparison of the MT10 and MT20 illumination systems

	MT10	MT20
Arc burners	150 W, optionally xenon or mercury-xenon, output-stabilising electronics	150 W, optionally xenon or mercury-xenon, output-stabilising electronics
Illuminators	Critical epi-illumination, intensity optimisation via integrated photodiode	Critical epi-illumination, intensity optimisation via integrated photodiode
Filters	8 positions, 25 mm, quick tool-free exchange	8 positions, 25 mm, quick tool-free exchange
Light fiber	2 m (optionally 3 m = single quartz)	2 m (optionally 3 m = single quartz)
Operation	modules sequentially	all modules in parallel
Filter switch	min 85 ms (neighbouring positions)	min 58 ms (neighbouring positions)
Attenuation	7 levels, < 85 ms switch	14 levels, < 58 ms switch
Shutter	< 5 ms on/off time	1 ms on/off time

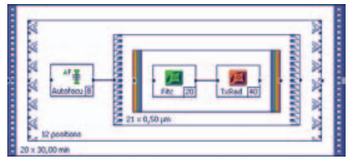
THE EXPERIMENT MANAGER

With full control of the microscope and all the components, the Olympus xcellence Experiment Manager provides a universal planning and execution tool, enabling even the most complex tasks to be completed with ease.

E F Experiments can be set up via the intuitive drag-and-drop assembly of command icons, such as “image acquisition”, “Z-stack” or “time-loop”. This simple procedure controls the whole series of required hardware actions, so the user does not have to consider these in detail. Simple tasks such as a time series of monochrome images can be easily defined, as well as complex data acquisitions requiring multi-device systems, including motorised microscopes and automated components.

The design concept is very intuitive and self-explanatory, which minimises training requirements — an ideal solution for multi-user environments and imaging facilities. Furthermore, once set, the complete experiment plan is visible at a glance and is stored with the data in the database. As a result, key imaging tasks can be repeated with ease simply by loading the experiment plan, adjusting any variable parameters (such as regions of interest) and then rerunning the programme. This also ensures a very high level of consistency between experiments.

Example experiment plan for dual-colour Z-stack acquisitions at different stage positions with autofocus, repeated over time.



All that is needed to define this complex experiment: three icons (for autofocus and dual/multi-channel image acquisitions) and four frames (for fluorescence overlay, Z-stack acquisition, stage positions and time-lapse repetition).

Keeping an eye on your experiments

The movement and flux of labelled cellular ions can provide real insight into biological processes. The speed and accuracy of the Olympus xcellence system means it is well suited to performing these sorts of studies.

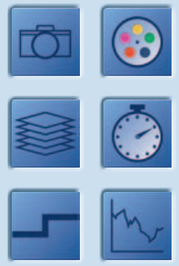
E

Live cell imaging experiment
Example image of a time-lapse experiment. Overlay of GFP-labelled microtubules and DIC image of the filamentous fungi Aspergillus nidulans.

Courtesy of R. Fischer, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany.

F **Experiment Manager icons**

Simple experiment creation to fulfill complex tasks, using icons for camera acquisition, changing fluorescence channel, Z-stack, time lapse, setting trigger and online kinetics.



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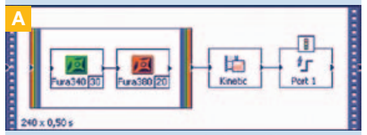


Time-lapse experiment plan with online kinetic and triggered event (for example a microinjection)

Applications: Ca++ imaging and other physiological measurements

The fluorescence behaviour of several dyes is dependent on the concentration of certain ions such as calcium (Fura-2) or on the pH value (BCECF). The detection, quantification and analysis of changes in fluorescence intensity are thus an indirect means of studying important physiological processes such as neuronal signal transmission and ion channel activity.

Challenges: accurate, rapid detection of small fluorescence changes

In order to accurately infer changes in ion concentration via fluorescence changes, the system must be able to rapidly capture images, while minimising the detrimental influence of photobleaching.

Solution: maximising the information provided by each photon

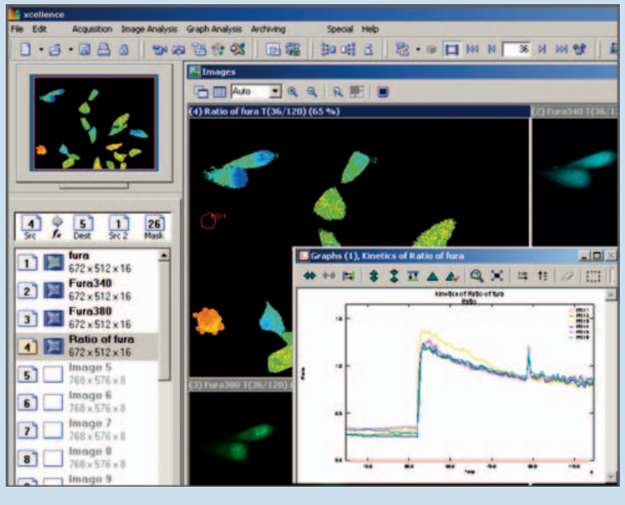
A B The Olympus Experiment Manager combines an easy experiment set-up with high functionality. An experiment plan for a dedicated ratio imaging experiment including online kinetics, ratio image display and trigger output can be set-up with ease and the real-time controller runs the experiment with the highest possible speed and accuracy.

A broad range of Olympus and third-party CCD and intensifying EMCCD cameras are supported by the software. What is more, the xcellence platform has been developed to leverage the huge capabilities of each camera, enabling features such as pixel binning, photon counting and partial readout area. The software also supports advanced features such as overlapping readout and EM gain control as well as real gain control.

In combination, these features ensure that every last photon is used for the analysis of physiological processes, maximising the accuracy and validity of these studies.

B **Ca++ time-lapse experiment**

With online ratio image and online kinetics.



LIVE CELL IMAGING – FROM OBSERVING TO UNDERSTANDING

Images generated by microscopy are inherently 2D, but cells are 3D entities. Therefore, to understand cellular processes, it is necessary to generate high-quality 3D representations of cells. In addition, these data sets should be amenable to precise quantification via measurement and sophisticated image analysis. The Olympus xcellence system provides both, generating accurate, insightful information for in-depth study.

Application: imaging complex processes such as mitosis

C To fully understand complicated processes such as mitosis, it is necessary to generate high-quality images at different focal positions. These images can then be used to investigate the process with the naked eye, while providing the best possible source material for generating quantifiable data such as distances, angles and areas.

Challenge: accurately investigating a complex process in three dimensions

The understanding of complex processes is significantly facilitated by obtaining the highest-quality data possible. In the case of three-dimensional imaging, this means generating the clearest images possible for each Z-position. A flexible, programmable solution that provides information in an intuitive format is the best answer, as it provides the freedom to measure any variable of interest, while making it easy to view and analyse findings.

Solutions: powerful image analysis and handling

Image processing and 3D visualisation

A broad array of image processing functions are included in the xcellence software, such as background subtraction, shading correction, display adjustment, as well as the filtering and overlay of fluorescence and transmission images. The powerful deblurring algorithms (no neighbour, nearest neighbour and Wiener filter) enhance the spatial resolution of widefield microscopy by reducing out-of-focus blur, giving images with greatly improved clarity. The xcellence SliceViewer generates slices through image stacks and the VoxelViewer renders three-dimensional images and isosurfaces.

Measurements

Measurements are becoming increasingly important in life science imaging as they enable the researcher to clearly define the various features in terms of physical size, cellular location and proximity to other features. The xcellence software programme offers a unique measurement environment suitable for interactive measurement tasks and dimension calculations. Results can be presented as graphs or sheets and evaluated directly to determine mean values, extremes and standard deviation. The “Image Statistics” button takes a set of 10 automated measurements to assess either regions of interest or complete images at the click of a button.

Programming

The Imaging C programming language offers advanced users a complete, integrated development environment. This includes a programming library with access to a wide range of image acquisition and processing functions.

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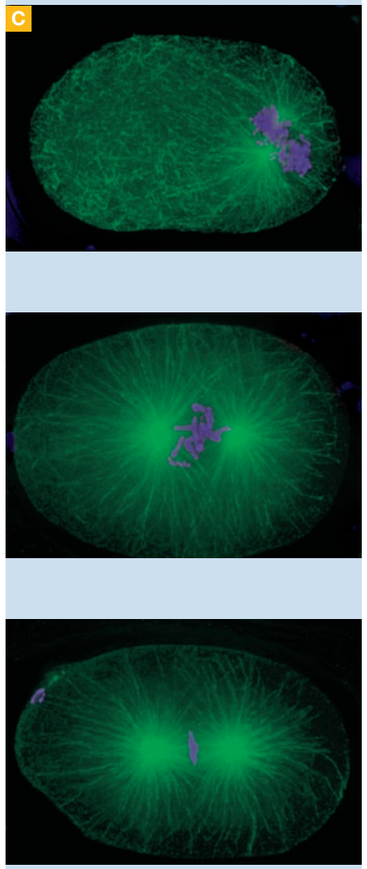

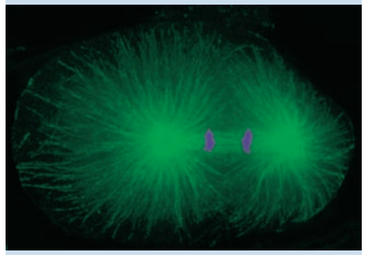

Cell division in the early C. elegans embryo; microtubules in red, DNA in blue.

Courtesy of K. Oegema, T. Hyman group, Max Planck Institute, Dresden, Germany.

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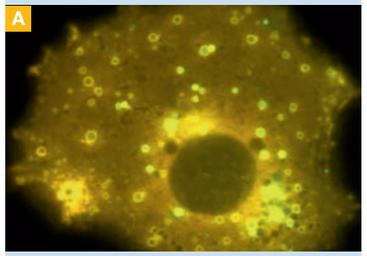
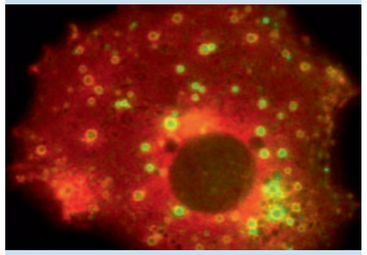




Spectral unmixing:
GFP-labelled vesicles and YFP-labelled membrane proteins; YFP displayed in red for clarity. Centre: original image; bottom: after spectral unmixing.

Courtesy of Y. Okada, Grad. School of Medicine, Univ. Tokyo, Japan.

Report generation
The report generator makes creating reports quick and easy via a simple drag-and-drop process. These reports can contain graphical elements such as images, sheets and diagrams and also offers uncomplicated text input. Placeholders are automatically filled with the contents of various fields – from the archive or from analytical results.

Archiving
The integrated database archives multi-dimensional image series along with analytical data, reports and associated documents in a well-structured system. The system has been designed to make access fast and navigation easy, while keeping network capacity requirements low.

Advanced analysis

Fluorescence analysis and kinetics
More complex analysis routines required for processes such as ion imaging are included within the xcellence software suite. These include intensity kinetics, colocalisation statistics as well as ratio and ΔF/F analyses. Results are provided as false-colour images, graphs of regions of interest and data sheets.

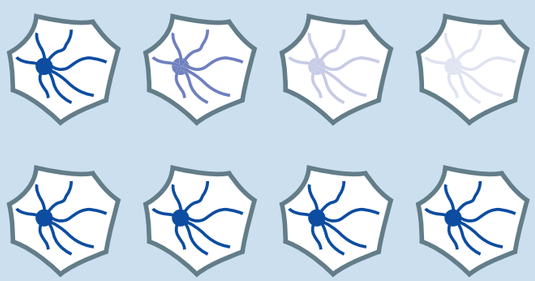
Spectral unmixing: enhanced colour resolution
A Pronounced spectral overlap of the excitation and emission characteristics of certain fluorescent dyes often prevents their combined use when investigating a sample. Linear spectral unmixing determines the contribution of each fluorochrome to the total signal and, by chromatic redistribution of the intensity, restores a clear signal for each colour channel, undisturbed by the emission from other fluorochromes. This procedure uses the inherent information within the data set, and the results are therefore not simply embellished images. The spectral redistribution maintains the overall intensity of the raw data and facilitates quantitative analyses, for example in colocalisation and FRET studies.

Bleaching correction
B The xcellence software can be used to apply bleaching correction algorithms across time series to ensure that during long term imaging, the effect of any unwanted bleaching is corrected. The adjustment can be made to the entire image or just specific regions of interest.

Kymogram
The advanced kymogram which features in the xcellence software enables the precise calculation of the speed of movement of molecules within a cell, even along convoluted pathways.

B **Bleaching correction**

Schematic drawing of a cell with intracellular structures loosing fluorescence signal over time (upper row, left to right). The lower row shows the result after bleaching correction.



FUNCTIONAL MODULES

The solid foundation provided by the Olympus xcellence system is powerful and flexible enough for answering most biological questions. However, the platform can also be adapted to meet your more specific research needs, as and when they appear. This is achieved through a range of specialised modules that provide additional functionality.

trackIT! Easy-to-use vesicle tracking module

C The movement of molecules within biological systems is often mediated by vesicles, which have an important role in controlling cellular processes. The tracking of vesicle movement and activity is a complex process that requires intelligent automated analysis tools and high-quality 3D image capture. The Olympus xcellence trackIT! solution module is capable of detecting moving objects automatically, tracking the paths taken by these bodies over a given period of time. Results are presented as movies illustrating the path taken by each object, accompanied by charts and histograms providing further details of parameters such as velocity, direction, path length and total distance travelled.

Application: Fluorescence Resonance Energy Transfer (FRET)

D The measurement of fluorescence energy transfer from one fluorochrome molecule to another right next to it can be used for the investigation of molecular interactions. This powerful technique allows you to indirectly observe cellular processes far beyond the resolution of traditional light microscopy.

Challenges: fast processes and low signal difference

The energy transfer from the donor to the acceptor can be reflected by a very low change in the emissions of the two fluorophores. FRET requires the simultaneous or very fast acquisition of images with different excitation and emission wavelengths under absolutely stable conditions. To calculate the distance of the two proteins, sophisticated algorithms are needed.

Solutions: FRET module, DualView and DualCam module

FRET module
The FRET measurement package is easy to use and very flexible, offering both predefined or custom algorithms to calculate the distance between the two proteins of interest down to several nanometers.

DualView™/DualCam
E The DualView™ micro-imager beam-splitting device allows the simultaneous acquisition of two chromatically separated fluorescence images at half the normal frame size. This makes it possible to capture emission wavelengths for multiple excitation wavelengths at the same time, providing the fastest possible FRET image capture. Spectral unmixing of the captured emission light restores a clear signal for each colour channel, undisturbed by the emission from other fluorochromes, thereby facilitating more accurate analysis. With the DualCam™ micro-imager beam-splitting device the simultaneous acquisition of two fluorophores can be imaged with the full frame size using two cameras.

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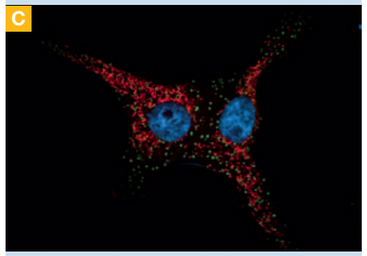
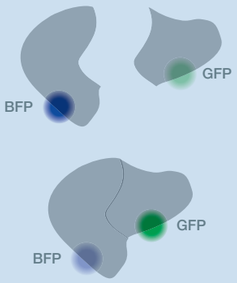


Example of vesicle detection. Generating reliable tracking data of vesicles.

D **FRET Detection**

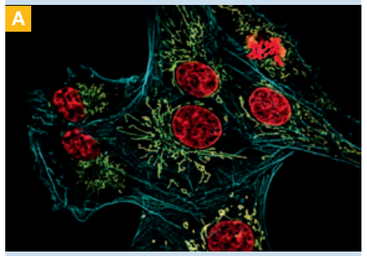
Of in vivo protein-protein interactions



E **Simultaneous Multi-colour Imaging**

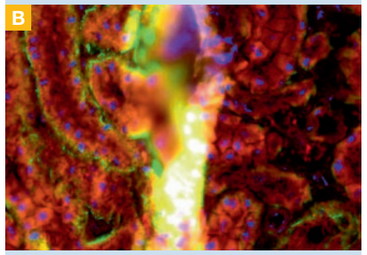
Olympus IX81microscope with beam-splitting device and Andor iXon camera for simultaneous dual-colour imaging.

A



Deconvolved three-colour fluorescence image of VERO cells.

B



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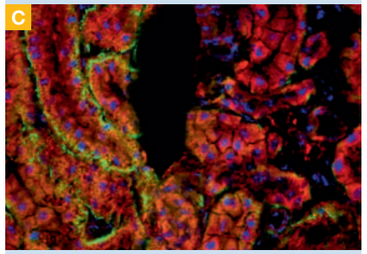


Section of rat kidney

Above with conventional fluorescence equipment, below with Optigrid M

Hoechst Alexa 44, laminin CD31, Cy3.

Multi-dimensional image analysis

Quantifiable data is a powerful tool for making meaningful inferences about the biology of your samples. This often involves counting features of interest including cells and nuclei, as well as differentiating between multiple cell types. The perfect solution is one that is powerful enough to carry out sophisticated analysis but flexible enough to meet new requirements as they arise. The Olympus xcellence Multidimensional Object Detection tool analyses 4D image data, taking into account Z-stacks and time-lapse series. Objects can be detected and classified based on diverse parameters such as area, size, shape, position, density and intensity. The tool has multiple applications including automatic cell classification, cell counting, mitotic and apoptotic analysis, drug discovery and many more.

Application: 3D imaging of thick specimen

The xcellence system is adept at providing high-quality 3D time-lapse images, and several additional modules are available to further improve the data collected by these experiments. These xcellence modules are capable of providing near-confocal-quality images at a fraction of the cost and without the need for laser hardware.

Challenges: imaging speed, out-of-focus blur

In order to provide high-resolution time-lapse data, imaging speed must be increased, while minimising photobleaching and phototoxicity. Out of focus blur is also a significant factor affecting the quality of 4D studies, a problem which can be solved by reducing the point spread function of the imaging system and by taking additional steps to remove out-of-focus light from the image captured.

Solutions: deconvolution, Optigrid, DSU

A The new XRide 3D deconvolution algorithm included in the xcellence software provides faster and more accurate deconvolution whilst ensuring outstanding usability. Faster than all other algorithms currently available (e.g. no neighbour, nearest neighbour, Wiener) and providing near-confocal results, the Olympus xcellence software provides a new level of deconvolution precision.

Optigrid

B C The Optigrid M is an ideal solution for thick and multicellular samples such as nematodes, brain slices, embryos and fish. It is designed to work with a stabilised arc burner-based illumination source, and uses a combination of physical and mathematical processing to produce near-confocal-quality images. 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 subtractive algorithm, which ensures that only in-focus pixels are used and that the overlapping sections of each image portion line up.

DSU (spinning disk system)

D E The DSU (disk-scanning unit) offers cost-efficient, semi-confocal image observation with a cooled CCD or EMCCD camera and one of the illumination systems: MT10 or MT20. The DSU disk contains a pattern of slits that creates a virtual pinhole as the disk spins at 3000–5000 rpm. The system removes out-of-focus blur, thereby enhancing resolution to yield clear, continuous, optically cross-sectioned images. The Olympus DSU is especially suitable for automated Z-sectioning followed by post-image processing steps such as 3D reconstruction.

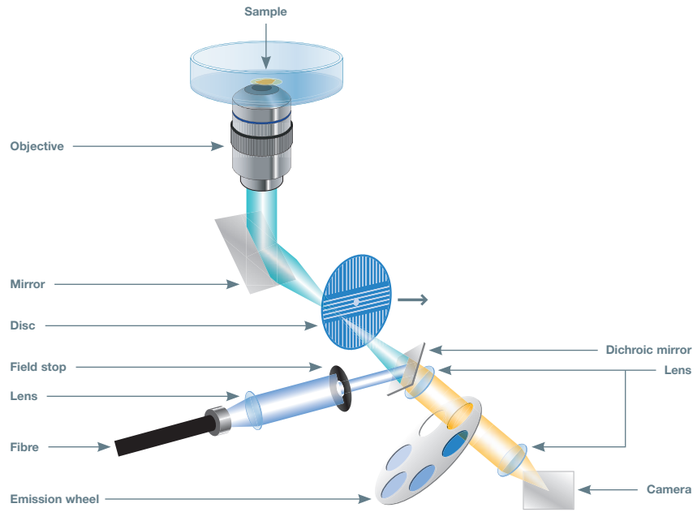
Real-time ZDC2 zero drift module

Controlling the focus 100 times per second and correcting it in real time with up to 70 nm precision makes the ZDC2 the perfect device for all types of live cell imaging applications. It stabilises the focus automatically in the complete slide, and even in temperature shift experiments.

The Olympus ZDC2 zero drift module uses a 785 nm laser diode to locate the uppermost surface of the coverslip, multi-well tissue culture plate, glass bottom culture dish or other glass substrate. The system then automatically moves to a user-defined focal plane relative to the coverslip and maintains specimen focus for extended periods of time. The process repeats to capture images with the utmost precision throughout the experiment, which can last for minutes, hours, days or even weeks.

D

Light path of spinning disk system



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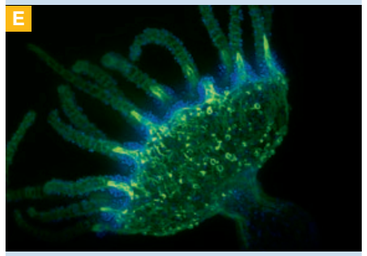

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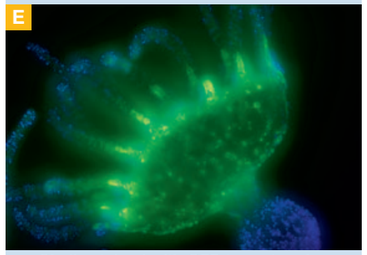


Obelia stained with GFP (green) and DAPI (blue) taken on the Olympus DSU with and without the DSU engaged.

BEYOND FLUORESCENCE

Pushing the boundaries

Modern imaging techniques have taken fluorescence microscopy to a whole new level creating unique ways of imaging with greater resolution, clarity and precision: moving the microscope away from being a tool for observation, and making it an instrument for discovery.

cell tirf

As our understanding of the inner workings of the cell has grown, so has our need for imaging techniques that provide greater resolution, precision and clarity. One such technique, total internal reflection fluorescence microscopy (TIRFM), has firmly established itself over the last few years as an ideal method for investigating molecular interactions at or near the cell surface. Many important biological events occur here, including adhesion, cell binding, cell signalling and transmembrane transport. The system provides a high signal-to-noise ratio and is less phototoxic than other illumination approaches, making it perfect for imaging living cells in fine detail down to the level of single molecules.

What is TIRF?

A Total internal reflection fluorescence (TIRF) is a method of microscopy designed to reduce the amount of background signal produced when using fluorescence. The system works by shining one or more lasers onto a sample at a specific angle in order to excite fluorescent molecules at the surface. At this critical angle, which is dependent on the wavelength of light being used, the beam reflects off the surface of the sample, producing an evanescent wave. This wave selectively illuminates and excites fluorophores in a restricted region of the specimen right next to surface of the coverslip. The effect only penetrates approximately 50 to 200 nm into the sample, ensuring that only fluorescent molecules in that area are excited enough to produce emission spectra. This provides a technique with an exceptional signal-to-noise ratio and makes it possible to investigate the surface of cellular membranes with great accuracy and clarity. The technique is facilitated by the use of high numerical aperture objective lenses, such as those available as part of the Olympus TIRF objective range.

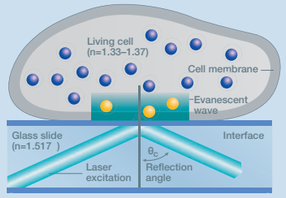
The pioneers in TIRF Microscopy ushering in the next generation of TIRF technologies

In 1997, Olympus designed the first turnkey objective-based TIRF illuminator for commercial applications. As pioneers of TIRF, we have remained committed to the field and are continuously developing cutting-edge advancements to push the limits of science.

B The Olympus cell tirf illuminator represents the very latest in TIRF technology and is complemented by the largest portfolio of specially designed TIRF optics. The cell tirf illuminator is unique, offering independent and simultaneous control of the critical angle for four separate evanescent waves, allowing optimisation of the angle for different wavelengths. Users can preset calculated penetration depths for all lasers with a single mouse click, such that the angle of each laser will be individually adjusted to simultaneously capture TIRF across each channel. Fine-tuning and angle adjustment are made as simple as the scroll of a mouse wheel.

A TIRF illumination

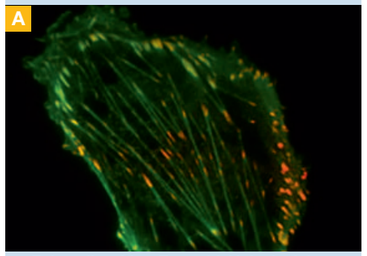
Light is reflected from the cover-slip/sample surface and only fluorophores close to the glass emit light.



B cell tirf

Motorised multiline TIRFM illuminator for precise control of the evanescent field

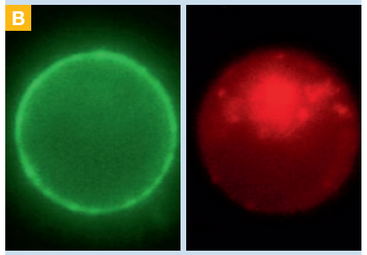
A



Folu cells with mEmerald AlphaActinin and mCherry LifeAct.

Image courtesy of Michael W. Davidson, NHMFL, The Florida State University

B



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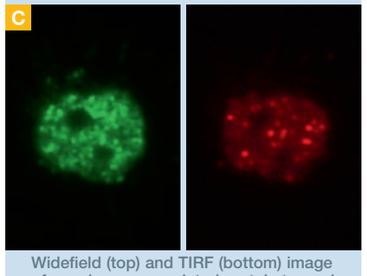


Widefield (top) and TIRF (bottom) image of membrane-associated protein tagged with Venus (green) and neuropeptide Y tagged with mCherry (red).

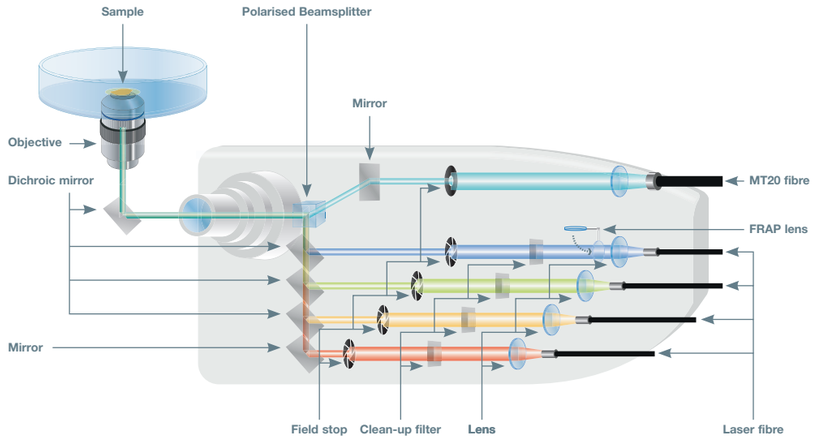
Courtesy of T. Cijsouw, M. Verhage and R. F. Toonen, Center for Neurogenomics and Cognitive Research (CNCR) and VU Medical Center (VUmc), Amsterdam, The Netherlands

THE ONLY TRUE SIMULTANEOUS MULTICOLOUR TIRF SYSTEM

The development of fluorophores operating in discrete areas of the light spectrum has opened up a new world of colocalisation studies, providing significant biological insight. The Olympus xcellence TIRF system can be used to perform multicolour TIRFM, combining the power of multi-fluorophore approaches with the resolution and clarity of TIRFM.

Application: imaging cell surface processes using multi-colour TIRFM

Visualising these processes is often difficult due to interference from intracellular fluorescence. TIRFM circumvents this and, due to the high signal-to-noise ratio obtained, even single molecules can be visualised, thereby providing high axial resolution. Using technological innovations pioneered by Olympus, it is now possible to perform TIRFM using multiple fluorophores, providing the opportunity to carry out colocalisation studies at cell surface membranes.



Challenge: TIRFM with multiple fluorophores requires lasers shone at different angles

A B C In order to reflect off the cover slip and induce an evanescent wave, each laser beam must be projected at a specific angle. The angle required to obtain an evanescent wave with a defined penetration depth varies depending on the wavelength of light being used. Therefore, in order to perform true multicolour TIRFM with the same penetration depth for different wavelengths, independent lasers must be irradiated onto the sample at different angles. In addition, TIRF microscopy needs a perfect collimated laser beam otherwise the TIRF image contains optical artefacts, reducing the signal-to-noise and image quality. This can be avoided with separate laser coupling to the illuminator to correct the laser fibre position according to a given wavelength.

Solution: the Olympus cell^{tirf} module

Four independent laser light paths

The Olympus cell^{tirf} system enables ultra sensitive simultaneous multi-colour total internal reflection fluorescence microscopy by offering four laser channels with independent beam paths and individual motorised angle controls. The xcellence cell^{tirf} system can acquire up to four fluorophores simultaneously with one exposure. Intelligent automation makes it possible to define a precise penetration depth for each laser, ensuring that comparable data is harvested from each channel in order to facilitate accurate and reliable colocalisation studies.

Olympus laser systems

D A choice of 14 compact, silent and long-life diode and DPSS lasers are available, covering wavelengths from 405 to 640 nm. The lasers, available with up to 150 mW of power, self-configure within the xcellence software using the intelligent ODB (Olympus digital bus) connection. Laser shutterring, including communication with the real-time controller is up to 200 µs fast (depending on the laser).

Intelligent and intuitive TIRF control

E The intuitive and intelligent system software ensures that only one button is required to switch between critical angle, defined penetration depth and laser widefield illumination. In addition, shuttering and attenuation can be performed in real time from the xcellence Experiment Manager. Lastly, fine adjustments are made via the mouse or keyboard, removing the need for screwdriver adjustments. Complete integration within the Experiment Manager software also enables specialised TIRFM commands for sophisticated applications.

Olympus TIRF objectives

F This superior multi-laser independent beam path technology, combined with the broadest selection of TIRFM objectives available, ensures that cell^{tirf} delivers a new level of sensitivity and precision in multicolour TIRFM. Olympus offers four purpose-built TIRFM objectives of exceptional optical quality. Newly available objectives offering increased numerical apertures of 1.49 at 100x and 60x magnification, ensure accurate angle control and precise knowledge of the depth of TIRFM penetration, which is highly advantageous for cell membrane studies.

Our APO 100x TIRF objective provides the highest Z-resolution and best signal-to-noise ratio available, with a numerical aperture of 1.65. The UAPON 150x TIRF objective gives highest magnification, 150x (1.45 NA), to the user. This objective is the only TIRF objective capable of getting the best out of the most sensitive cameras on the market (16 µm pixel size), making it perfect for single-molecule studies, from tracking through to super-resolution. Exceptional mechanics, along with high-quality objectives, means that users can precisely and automatically adjust laser penetration with 1 nm accuracy, all at the click of a button. This is possible as cell^{tirf} is fully integrated into the xcellence real-time imaging system with automatic device configuration via ODB, enabling fast switching and the precise control of laser lines.

Olympus TIRF objective comparison

Objective	Magnification	NA	PD	Correction ring
APON TIRF 60xO	60	1.49	64nm	x
UAPON TIRF 100xO	100	1.49	64nm	x
APO TIRF 100xO	100	1.65	42nm	o
UAPON TIRF 150xO	150	1.45	78nm	x

PD = Penetration Depth for 488 nm, and sample refraction index 1.36

D Laser system

Wide variety of different laser types with highest beam quality. Suitable for TIRF microscopy.

E

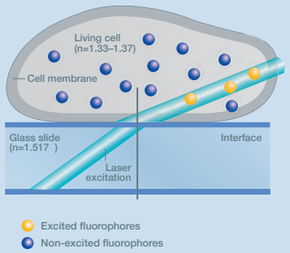
Intuitive TIRF control. All TIRF-relevant parameters can be set and read out with ease.

F Olympus TIRF objectives

An extensive range of purpose-built objectives for TIRF microscopy.

A **Light sheet illumination**

The light is introduced into the coverslip at a near-critical angle and forms a light sheet inside the sample.



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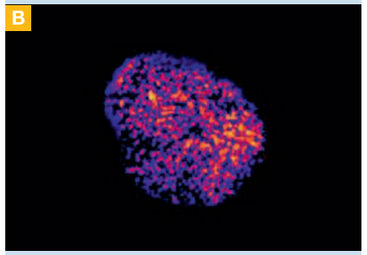
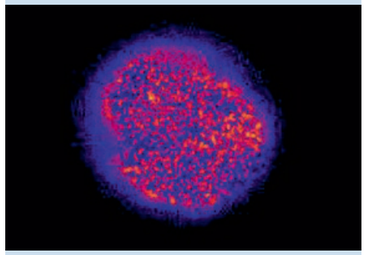
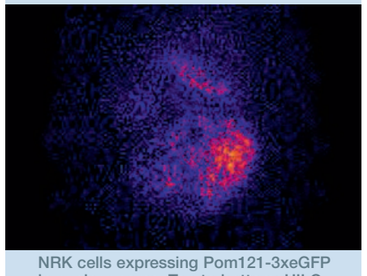






NRK cells expressing Pom121-3xeGFP in nuclear pores. Top to bottom: HILO, widefield and TIRF illumination.

Courtesy of A. Szymborska and J. Ellenberg, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

Point FRAP

Olympus cell^{tirf} has integrated point FRAP optics for the first laser line. This enables the user to carry out FRAP and photoswitching experiments without any additional costs. Turning a knob on the TIRF illuminator switches the light of one laser into a refraction-limited spot. In the experiment this laser can be used for photo manipulation and all the others for TIRF and widefield microscopy. The real-time controller offers a 100 µs switching time and outstanding data quality for these types of experiments.

Imagine what you could achieve if you could obtain TIRF levels of clarity, deep into your sample ...

Application: the study of nuclear pore complex formation

The nucleus contains the genetic information of an organism and in many ways can be considered the control centre of the cell. Factors synthesised in the nucleus such as ribosomal RNA and mRNAs must be transported via nuclear pores into the cytoplasm where protein synthesis takes place. The study of this transport is therefore essential to provide a full understanding of gene expression.

Challenge: a high signal-to-noise ratio with deep penetration

As the nucleus of a cell lies within the cytoplasm and is surrounded by the cellular membrane, it can be difficult to image clearly due to the out-of-focus blur caused by fluorescent molecules in the rest of the sample. Therefore, in order to study the nuclear cell membrane it would be ideal to have a version of TIRF that is not restricted to the short penetration depths of traditional TIRF.

Solution: Olympus light sheet illumination

A **B** Due to the angle setting that's precise to the nanometre, and freely X,Y adjustable field-stops for each laser beam, the xcellence cell^{tirf} can be used to generate light sheet illumination like highly inclined and laminated optical sheet (HILO) illumination. HILO uses a different refraction strategy to that of TIRFM, where the laser beam is converted into a thin sheet that passes through the specimen at an oblique angle. As a result, targets deep within the sample can be visualised.

Flexibility and value

Olympus' optical expertise allows cell^{tirf} to be used in combination with other applications to provide exceptional value for money. For example, the xcellence system provides real-time live cell fluorescence imaging and can also accommodate the cell^{frap} photo control and spinning disk confocal modules. Finally, the system is fully compatible with most microscope tables, Piezo drives and AFM stages.

cell^{frap}

C **The inside of a cell is a complex, dynamic place, requiring the careful control of molecule and organelle transport. Therefore, the precise measurement of protein movement and binding can tell us a great deal about the biological processes taking place there, for example via the calculation of binding constants, coefficients of diffusion and mobile fraction percentages. Such analyses require high levels of accuracy and reproducibility. For this reason, the Olympus xcellence cell^{frap} module is fast, precise and flexible, allowing complex cellular dynamics to be investigated via photobleaching.**

Applications: FRAP, FLIP, FLAP

FRAP

D Fluorescence recovery after photobleaching (FRAP) provides an ideal method for calculating the coefficient of diffusion of a particular molecule within a certain target area of the cell. This is achieved by photobleaching the fluorophore attached to a specific molecule within the target area and then assessing the dynamics of the return of fluorescence (recovery) to that area.

FLIP

Fluorescence loss in photobleaching (FLIP) is useful for studying molecular movement along cell membranes (lateral membrane fluidity) and membrane continuity, especially for membranous organelles. A defined area of the membrane/ cell is bleached and the loss of fluorescence from the remainder is measured as the unbleached molecules are exchanged with those from the bleached region.

FLAP

Fluorescence localisation after photobleaching (FLAP) is a clever adaptation of FRAP where the dynamics of a particular molecular species are of interest. The target molecule is coexpressed with two different fluorophores such that distribution and localisation overlap. One of the fluorophores is then bleached at a defined location and the movement of the molecule can be followed by looking at the relative and absolute distribution of both fluorophores.

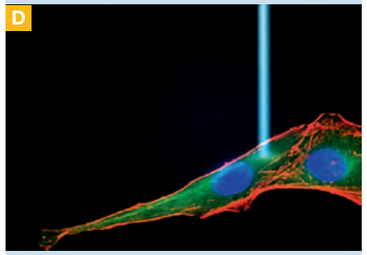
Challenges: FRAP requires fast switching speeds, whereas FLIP needs continuous rapid switching

The molecules within a cell are capable of moving at considerable speeds. In order to accurately interpret FRAP data, the microscope system needs to be able to bleach an area of interest quickly, before rapidly switching back to acquiring images to accurately assess the recovery of fluorescence. Similarly, FLIP is effectively a series of multiple FLAP experiments. Therefore, FLIP requires the ability to provide multiple bleaching and acquisition steps at fast speeds with continual switching between the two. To provide the most accurate analysis possible, it is important to capture the first few microseconds after bleaching, a time period often missed by other microscope systems as they cannot bleach and acquire subsequent images with enough speed.

C **FRAP and FLIP**

Schematic drawing of photobleaching experiments

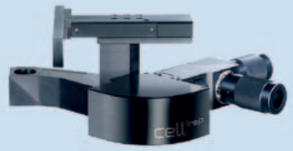
D



FRAP (Fluorescence recovery after photobleaching) experiment of fluorescent HELA cells

A cell^frap

Galvanometre-based fast-scanning photomanipulation unit

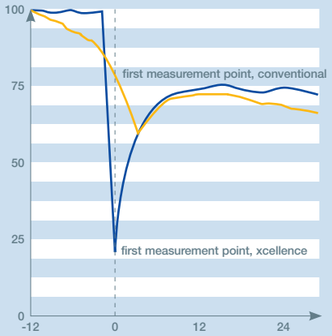


B Experiment Manager FRAP experiment

Easy drag-and-drop experiment set-up

C FRAP recovery curve

Conventional (yellow): significant bleaching from imaging and late start of post-bleach acquisition cell^frap (blue): reduced bleaching from imaging and fast imaging after bleaching



Solution: the Olympus cell^frap module

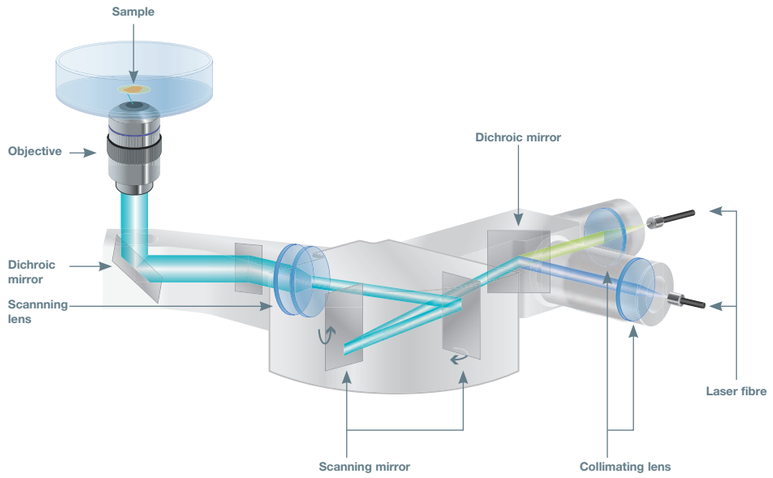
A **C** The Olympus cell^frap module is capable of incredibly fast switching between bleaching and image capture, making it perfect for all your FRAP, FLIP and FLAP needs.

Fast switching time

The Olympus real-time controller allows switching between imaging and FRAP in just 200 µs. By scanning just the region of interest, high-speed bleaching is achieved, and multiple areas can be bleached very quickly. This allows accurate FRAP studies to be conducted, with the flexibility to bleach multiple regions of interest – all within one experiment. Fast shutter and light source control of the xcellence system also reduces the bleaching generally obtained from image acquisition, as a result the FRAP data are more precise.

Flexible experiment set-up with Experiment Manger

B The best approach to conducting an insightful FRAP experiment is to begin a time-lapse series collecting images at a slow rate. After bleaching, the time-lapse series should be captured at a fast rate in order to accurately monitor the recovery of fluorescence. After recovery, a third slow-capture time lapse should be performed, completing the data sequence. In order to facilitate the planning and execution of complicated experimental protocols such as this, the xcellence Experiment Manager includes a series of easy-to-use tools for setting up FRAP/FLIP studies. Using a simple drag-and-drop approach, powerful experiments can be conducted with just a few clicks of the mouse.



Application: the tracking of fast-moving individual particles using photoswitching

C Many cellular particles can be fast-moving and unpredictable, for example vesicles or intracellular pathogens such as viruses. For this reason fluorescent molecules exhibiting photoactivation and photoconversion properties are used as markers to track the dynamic behaviour. For example, fluorophores are available that can be activated using one wavelength of light and visualised with another e.g. PA-GFP and KFP1. This is particularly useful for investigating the movement of a protein since existing tagged molecules can be activated and their fate followed, without the constant addition of fluorescence from newly synthesised tagged molecules. Similar to photoactivation, photoconvertible fluorescent molecules such as kaede change their emission wavelength profile when illuminated by an activation wavelength. Again, these molecules enable the clear visualisation of the diffusion of molecules.

Challenge: dealing with large numbers of difficult-to-distinguish particles

While single particles can be bleached or activated with a suitable rapid response system, the systematic targeting of multiple features can be time-consuming and technically challenging. Ideally, a FRAP system should be able to achieve both using an intuitive control system.

Solutions: point and shoot, pattern bleaching

Point and shoot

The xcellence cell^frap system enables the user to photomanipulate a target by clicking on it on a live image using the mouse. Multiple points can be bleached or activated and the resultant images can be captured during, or immediately after, the bleaching procedure.

Pattern bleaching

The cell^frap system is capable of bleaching a large number of points in a defined pattern at high speed. Combined with diffraction-limited optics, this method provides a powerful alternative to fluorescent speckle microscopy (FSM) and is therefore very useful in the study of macromolecular assemblies such as cytoskeletal elements.

Application: subcellular laser cutting

D As well as bleaching, the laser mounted in the cell^frap unit can be used to cut cell membranes and subcellular structures. This is achieved using pulses of short wavelengths, with high-energy laser light directed onto the element being manipulated. Such a methodology allows you to directly influence cellular processes by cutting out structures such as growth cones, filopodia, actin fibres, microtubules, endoplasmic reticulum or mitochondria, opening the door to a new world of powerful functional studies.

Challenges: simultaneous imaging and photomanipulation

In order to accurately influence cellular processes using the cutting laser, it is preferable to simultaneously image the structures you are manipulating. This makes it easier to ensure that you only affect those membranes and organelles that you intend to target. Maximal accuracy is achieved if the cutting laser is active at the same time as the imaging system.

Solution: the optically advanced cell^frap system

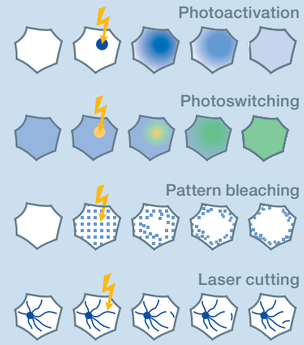
The Olympus xcellence cell^frap system uses an independent light path, enabling simultaneous fluorescence imaging and photomanipulation. The incorporation of two laser fibre inputs for multiple lasers provides excellent versatility and multichannel bleaching and cutting capabilities. The system makes it possible to visualise those areas you are targeting at the same time as you interact with them, ensuring the highest possible levels of accuracy.

Multifunctional but easy to use

The xcellence software puts all cell^frap features within easy reach via the intuitive graphical interface. Planning and executing complex FRAP, FLIP, photoconversion, point-and-shoot, laser cutting and pattern bleaching experiments couldn't be easier.

C Advanced photo-manipulation experiments

Schematic drawing of different photomanipulation experiments.



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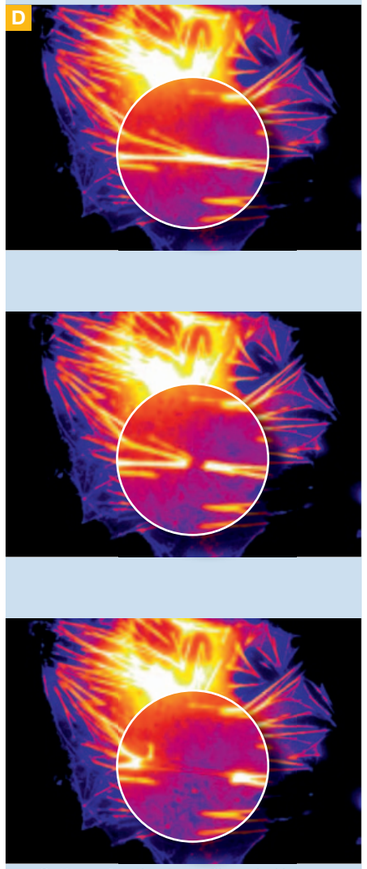


Cutting of actin stress fibres in HeLa cells transfected with actin-GFP.

Image provided courtesy of Dr Stefan Terjung from the European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.

Image acquisition

Live image acquisition	Captures live images in various formats	●	●	-
5D multidimensional image acquisition	Enables automatic acquisition of 5D images in X, Y, Z, as multichannel with transmission	●	●	-
	overlay functionality and with time lapse			
Position lists and stage navigator	Captures images at multiple stage positions or over stage areas	●	●	-
Automatic MIA	Creates panoramic images over areas (requires motorised stage)	●	●	-
Experiment Manager	Enables universal experiment planning tool	●	●	-
Interactive experiment	Enables interactive change of acquisition settings	●	●	-
Online kinetics	Performs online measurements of intensities on ROIs	●	●	-
Online ratio imaging	Performs online ratio images with predefined settings	●	●	-
FRET acquisition	Enables the acquisition of two colours simultaneously	●	●	-
Overlapping exposure	Enables simultaneous acquisition and read-out of cameras	●	●	-
RealGain™ EMCCD	Enables linear EM gains	●	●	-
Photon imaging mode	Enables signal enhancement for low light	●	●	-
Illumination timer	Enables timer to switch burner off in overnight experiments	●	●	-
Dual-camera acquisition	Enables simultaneous acquisition with two cameras	●	●	-
Motorised EFI	Automatically creates an EFI image via predefined numbers of slices, step size and	●	●	-
	top/bottom range (requires motorised Z)			

Image processing and data management

Image history and properties	Displays image history and properties	●	●	-
Image navigator	Enables tool window for image navigation and zooming	●	●	-
Adjusting display	Adjusts display settings automatically	●	●	-
Combining images	Enables the combining of multiple images in one multilayer image	●	●	-
Static annotations	Draws text, arrows, lines, rectangles and ellipses on the image	●	●	-
Image geometry	Enables the mirroring, rotating, resizing and cropping of images, and channel shifting.	●	●	-
	Enables the adjustment of image stacks			
Extended image processing filters and tools	Enables contrast adjustment, smoothing (lowpass) and sharpening of images, and	●	●	-
	noise correction			
Mode	Enables the conversion of bit-depth and colour space	●	●	-
Multidimensional image processing	Enables the combination, extraction and separation of frames, channels and RGB	●	●	-
Ratio measurement	Performs detection of ratio changes in two channels	●	●	-
ΔF/F	Normalises signal amplitudes in fluorescence images	●	●	-
Kymogram	Enables 2-dimensional display of dynamic behaviour	●	●	-
Bleaching correction	Performs subtraction of photobleaching effects in images and image series	●	●	-
Imaging C	Enables functional programming	●	●	-
Fluorescence unmixing	Enables spectral unmixing to correct overlapping fluorescence signals by isolating signals	●	●	-
	from the emission of other fluochromes			
Image arithmetic	Performs arithmetic and logical operations with images	●	●	-
Advanced image processing filters and tools	Projection calculations (min, max, mean on time, wavelength	●	●	-
	or Z) and further smoothing functions			
3D slice view	Performs basic 3D visualisation	●	●	-
3D voxel view	Performs basic 3D volume visualisation	●	●	-
Intensity calibration	Performs intensity calibration of channels	●	●	-
Deblur	Enables the deblurring of images by no neighbour, nearest neighbour and Wiener filter	●	●	-
Image database	Provides image and data management solution	●	●	-
Report composer	Interactively creates reports, which can be modified	●	●	-
Data export and statistics	Exports measurement data to MS Excel and enables the statistical analysis	●	●	-
	of measurements			

Image measurement and analysis

Object analysis	Performs threshold-based object detection and classification	○	○	●
Colocalisation	Enables colocalisation analysis of objects	●	●	-
Measurement	Performs extended measurement functions	●	●	-
Image statistics	Performs the ten most important measurements in one click	●	●	-
Deconvolution	Performs deconvolution with no neighbour and Wiener filter	●	●	-
Blind deconvolution	Performs deconvolution of images by using the constrained iterative method	○	○	●
FRET analysis	Performs measurement of fluorescent energy transfer	○	○	●
trackIT!	Enables the manual and automated tracking of particles over time	○	○	●

Control

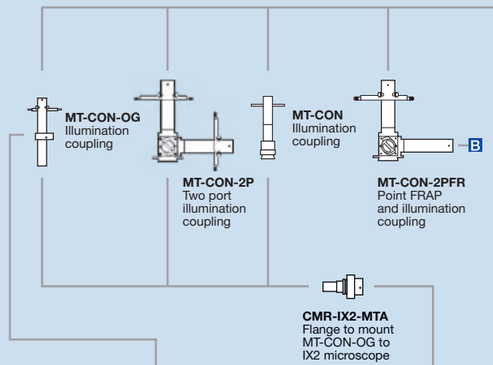
System coordinator	Controls the xcellence system coordinator	●	-	-
Real-time controller	Controls the xcellence real-time controller	-	●	-
Light sources	Controls the MT10 illumination system	●	-	-
	Controls the MT20 illumination system	-	●	-
	Enables Olympus laser control (shutter and intensity) for cell^TIRF, cell^FRAP	●	●	-
	Controls the TILL photonics monochromator Poly5000	○	○	●
Olympus cameras	Controls Olympus cameras DP72, XM10, XM10IR, XM10T, XM10TIR and old RoHS-	●	●	-
	compatible model F-View II			
Olympus microscope control	Controls motorised Olympus microscope systems BX2, IX2	●	●	-
Shutter	Olympus UFSHA, UNIBLITZ shutter control via TTL signal	●	●	-
Non-Olympus cameras	Controls non-Olympus cameras. Hamamatsu cameras Orca AG, Orca ER, Orca R2,	●	●	-
	Orca Flash 2.8, ImagEM, C9100-02, C9300-211, C9300-201, Orca II BT-512G;			
	Andor cameras: iXon 897, iXon 888, iXon 885, Luca R, Luca S, iKon M,			
	Photometrics Evolve 512x512			
3rd-party light sources	Controls CoolLED 3 and 4-channel light sources via TTL signal, Lumencor SpectraX	●	●	-
Olympus Spinning Disk control	Controls Olympus devices DSU	●	●	-
3rd-party stage controls	Controls X/Y-stage controllers Olympus STC for Märzhäuser stages, Prior ProScan,	●	●	-
	OptoScan and ITK Corvus			-
Optigrid M	Controls motorised structured illumination device	○	○	●
DualView™	Controls the TILL photonics micro-imager splitting device to divide two fluorescence	●	●	-
	channels on one camera			
DualCam™	Controls the TILL photonics micro-imager splitting device to divide two fluorescence	●	●	-
	channels on one camera (with two Hamamatsu ImageEM cameras)			
Piezo stepper devices	Controls Physic Instruments PIFOC Piezo steppers (built-in frame, nosepiece or stage)	●	●	-
Fast communication standard	TTL out and TTL in control	●	●	-
ZDC and ZDC2	Controls Z-drift compensation devices ZDC and ZDC2	●	●	-
cell^TIRF	Controls motorised TIRF illuminator	●	●	-
cell^FRAP	Controls galvanometric scanning device	○	○	●

xcellence pro

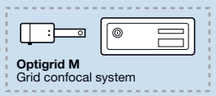
xcellence RT

xcellence advanced modules

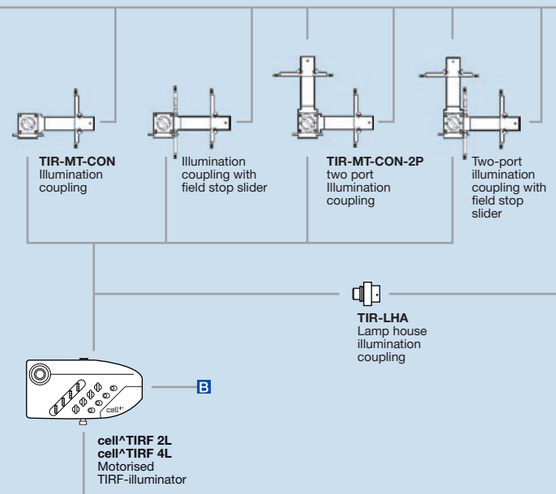
xcellence illuminations



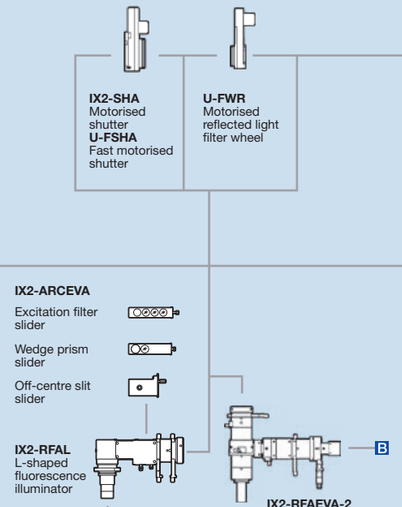
Structured illumination



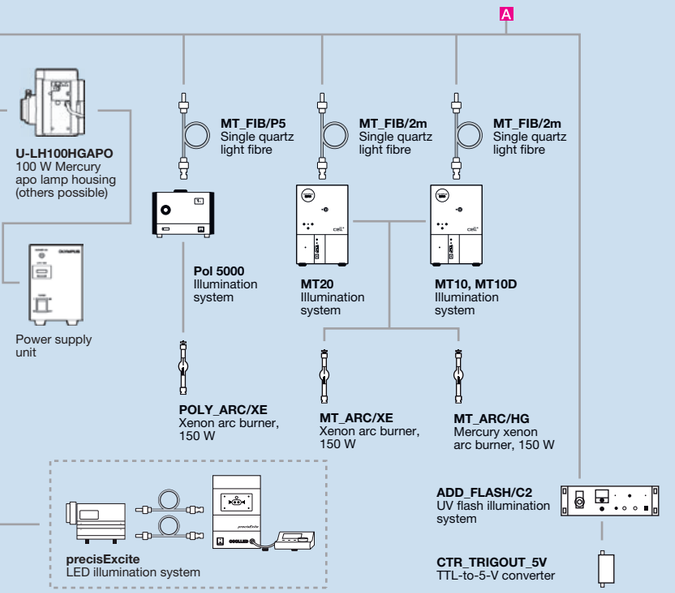
cell^tirf systems



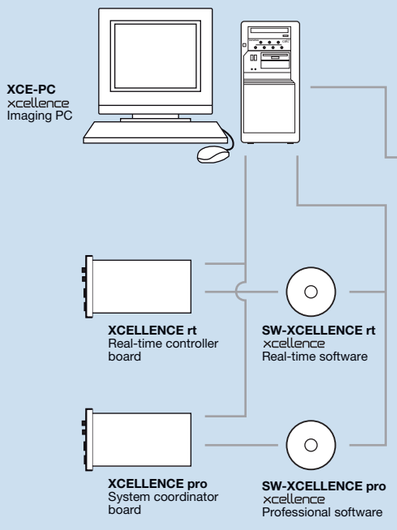
Stand-alone TIRFM illumination modules



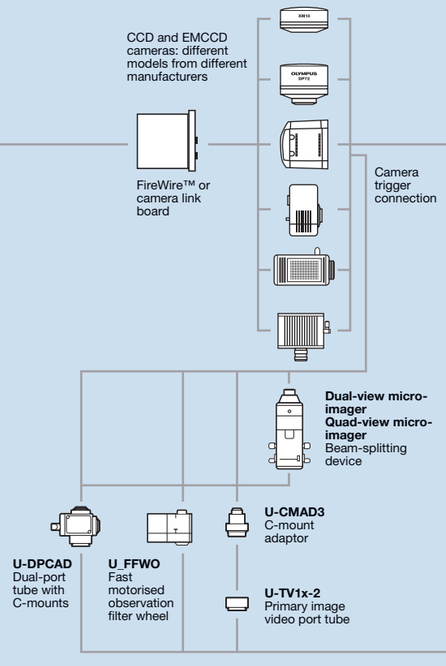
Widefield illumination systems



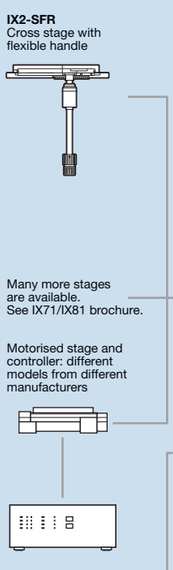
System control



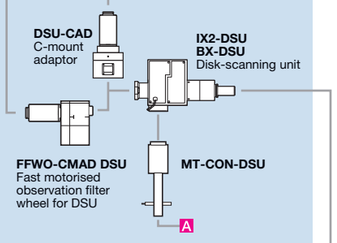
Cameras and observation equipment

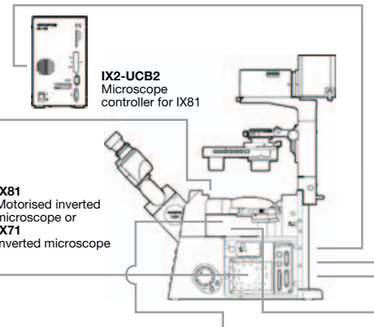


Stages

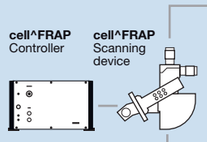


DSU

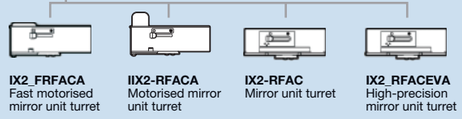




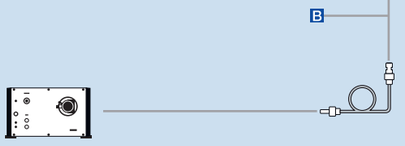
cell^frap



Mirror turrets



Laser systems



CMR-LAS-405-60-D
405 nm, 60 mW diode laser

CMR-LAS-405-100-D
405 nm, 100 mW diode laser

CMR-LAS-445-50-D
445 nm, 50 mW diode laser

CMR-LAS-473-25-D
473 nm, 25 mW diode laser

CMR-LAS-473-50
473 nm, 50 mW solid state laser

CMR-LAS-488-60-D
488 nm, 60 mW diode laser

CMR-LAS-488-150
488 nm, 150 mW solid state laser

CMR-LAS-491-50
491 nm, 50 mW solid state laser

CMR-LAS-491-100
491 nm, 100 mW solid state laser

CMR-LAS-514-50
514 nm, 50 mW solid state laser

CMR-LAS-532-50
532 nm, 50 mW solid state laser

CMR-LAS-561-50
561 nm, 50 mW solid state laser

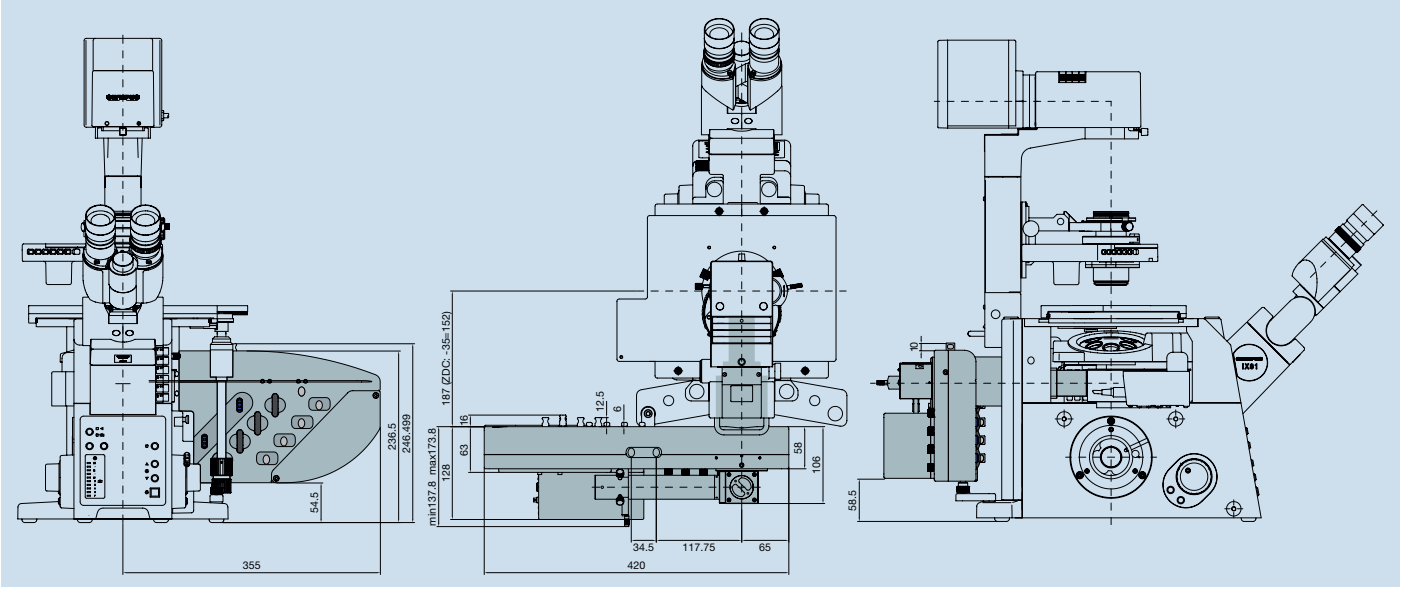
CMR-LAS-561-100
561 nm, 100 mW solid state laser

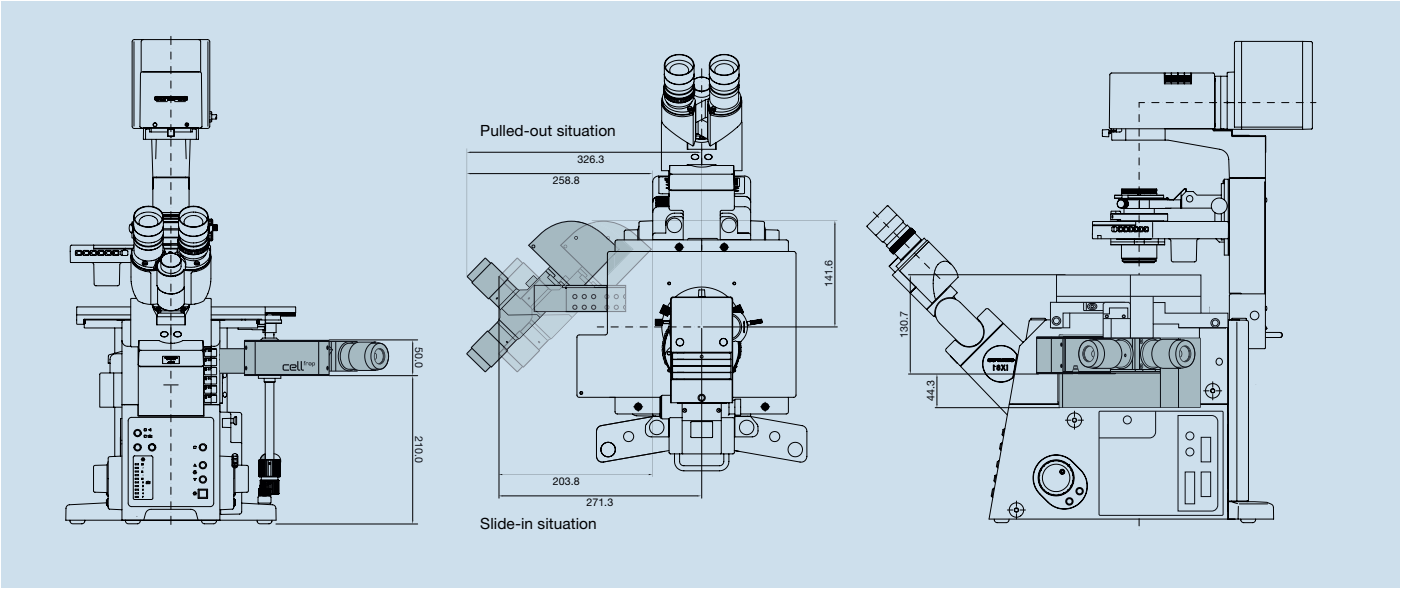
CMR-LAS-561-150
561 nm, 150 mW solid state laser

CMR-LAS-594-50
594 nm, 50 mW solid state laser

CMR-LAS-640-100-D
640 nm, 100 mW diode laser

Dimensions





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