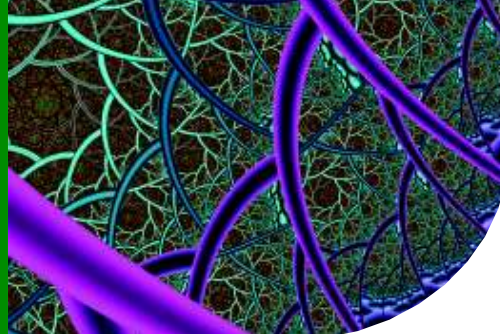


Microscopy Focus



Bringing Advanced Imaging In-Lab - Complex Microscopy Simplified

As pivotal techniques that provide significant advances to the world of science, fluorescence and confocal microscopy facilitate a wide range of complex imaging tasks. A variety of advanced microscopy systems have been successfully developed for the imaging of live as well as fixed cells and tissue samples. With uses in both medical and biological sciences, these microscopy systems are comprised of a series of components, which must all be optimised in order to produce a clear, high-resolution image. As such, the advanced capabilities of these microscopes are often inaccessible to less experienced users. The development of compact, easy-to-use systems, such as the all-in-one FSX100 fluorescence and FluoView FV10i cLSM microscopes from Olympus, enable simple step-by-step operation with clear on-screen instructions. With one-click operation, these self-contained microscopes have the ability to automatically locate the best-possible settings, removing the need for the user to adjust any complicated optical set-ups. Highly advanced imaging is therefore made accessible in-lab, to researchers of all experience levels.

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Fluorescence and confocal techniques both offer significant advances to the field of life-science microscopy.

FLUORESCENCE MICROSCOPY

Fluorescence illumination and observation has rapidly expanded, producing images based on the labelling of molecules with fluorescence dyes, which absorb light at one wavelength and emit light of a longer wavelength. The precise location of intracellular components can therefore be monitored and their associated interactions identified and subsequently imaged. This makes fluorescence microscopy pivotal for a number of applications within medical and biological sciences. A fluorescence microscope uses a single light source, such as a mercury arc burner, which is passed through wavelength specific filters.

CONFOCAL MICROSCOPY

Confocal microscopy is able to produce extremely high-quality images using samples prepared for conventional fluorescence. Confocal laser scanning microscopy (cLSM) systems operate by applying pinpoint illumination to a very small sample section. This provides precise excitation of the fluorophores, whose subsequent emitted light is focussed through a confocal pinhole to eliminate light from out of focus regions. The laser scans the field of view in a raster pattern to provide an extremely clear, high-resolution image.

ADVANCED IMAGING CAPABILITIES

In order to accurately obtain such fluorescence and cLSM images, the user must ensure that the microscope system is set-up to provide optimal optical capabilities. This includes the correct positioning of the stage for a focused image, as well as the use of advanced optics to provide the highest possible numerical aperture (NA) and light transmission. Furthermore, the occurrence of spherical and chromatic aberrations must be minimised (ideally eliminated) in order to produce a clear, accurate image of the sample. The illumination and image capture systems must be adjusted to produce the ideal exposure properties and obtain the best possible image clarity. This process in its entirety is not only lengthy, but also difficult for inexperienced users to perform successfully.



Figure 1: Olympus FV10i - Confocal microscopy for users of all experience levels.

As a result of these complexities, users often require extensive training and know-how in order to accurately manipulate the various components to obtain an image of the best possible quality. As such, use is often limited to a small number of advanced microscopists who have the relevant experience and knowledge. This can put a strain on these specialised users, as well as causing frustration to researchers who are awaiting results. Furthermore, as both systems utilise fluorescence, they will operate best if used in a darkened room, which can pose a number of additional issues. For example, transporting precious



Figure 2: The Olympus FSX100 all-in-one fluorescence microscope for one-click imaging.

samples to and from the microscope is inconvenient and can result in damage or disruption to the cells during the move itself.

INCREASING USABILITY

Making these complex techniques more easily accessible to the everyday researcher will not only alleviate the workload of experienced microscopists, but also enable users to maintain complete control over every aspect of their research. This increased usability may also encourage researchers to broaden the spectrum of study undertaken. Furthermore, if the complete process was able to take place conveniently in-lab, procedures could be effectively streamlined for an exceptionally smooth workflow. Simple and convenient access to complex microscopy techniques can be made available to a much wider audience, without any compromise on the quality of the image obtained.

MICROSCOPY IN A BOX

The novel idea of unifying all of the components required to perform either confocal or fluorescence microscopy into a single, compact 'box' removes some of the complexities, and subsequently increases the usability, of such microscopy systems. In order to meet this need, Olympus has recently introduced its all-in-one microscope series: the FSX100 fluorescence system and the FluoView FV10i cLSM system. Bringing this technology into the laboratory not only increases accessibility for the everyday researcher, but enables effortless production of high-quality images.

EASE-OF-USE WITHOUT COMPROMISE

All necessary components for high-end imaging are easily contained within the main body of the units. For example, the cLSM instrument includes a scanning unit, built-in vibration insulators, with an incubator and automatic water supply added for the water-immersion model. Furthermore, the accompanying software is user-orientated to facilitate easy operation. Once the sample has been placed on the stage and the lid closed, the microscope automates the rest of the process with user input at vital stages. Time-consuming tasks, such as focusing and setting the correct exposure levels are automated, enabling the production of an optimised image without the need for any additional user input. In the fluorescence system, the user can select the desired illumination wavelength on-screen and the correct filter is automatically used. The cLSM model automatically optimises detection bandwidths for a broad range of fluorophores. Both systems will automatically set themselves to image at optimal conditions, however these settings can be further adjusted, as required.

Combining a fully-automated system with user-friendly software and advanced optics ensures that while the usability of the systems is simplified, the end-result remains of a high standard. Using superior, high resolution apochromatic objectives ensures that any aberrations are fully-compensated for. This, in combination with versatile imaging systems, enables fluorescence, phase contrast and brightfield images to be obtained with ease. Furthermore, these compact systems can capture snapshot images, z-stacks, time-lapses and perform stitching of several high-resolution images. Fluorescent channels can also be directly overlaid in live mode for highly-resolved, real-time observation. In addition, these systems are able to produce highly advanced multi-area time-lapse images. This enables real-time observations and advanced processing for noise reduction and sharpness.



Figure 3: All-in-one - Olympus presents a completely new concept in microscopy.

FLEXIBILITY

The ability to accept standard slides as well as a range of culture dishes further increases the versatility and usability of such systems. As such, dry, as well as water- and oil-immersion slides are all fully compatible. Both water and oil interfaces can be used to increase the achievable resolution by enabling the use of objectives with NAs above 1.0. The cLSM water-immersion model, even completely automates water dispensing, removing the need for any further user input.

IN-LAB IMAGING

Self-contained, independent and mobile microscope systems are a highly advantageous addition to any laboratory due to their space-saving design, making them easily portable between different laboratories. Such systems can therefore be kept on a mobile trolley to be easily transported to various locations, if required. The sealed, light proof design of a self-contained microscopy system provides an ideal imaging compartment for maximising the sensitivity of such systems. This eliminates the need to transport samples to a fixed microscope system in a darkened

room for analysis and enables detailed examination of samples to occur in daylight at the point of discovery. Furthermore, the additional incorporation of an incubator with the water immersion cLSM makes it possible to perform time-lapse imaging of live cells without lengthy equipment set-ups. Constant sample temperatures can be maintained, along with stable humidity and specified CO₂ levels.

CONCLUSION

Both fluorescence and confocal imaging techniques are widely acknowledged as being pivotal to a broad range of applications. Performing such complex microscopy has previously been tasked only to experienced and knowledgeable microscopists. As a result, such personnel may be overloaded with requests from researchers that do not have the in-depth knowledge of fluorescence or confocal microscopy to image samples themselves. These complex instruments provide extremely high-quality images, but require extensive training to operate efficiently. As a result, technology has been developed to simplify the interaction with such complex microscopy to make it accessible to a wider audience. Self-contained, fully automated systems have been combined with user-friendly software to guide the researcher through the complete process, without compromising on image quality.

Virtual Microscopy Made Real

Olympus has introduced its cutting-edge dotSlide 2.1 virtual microscopy system, offering advanced functionality with outstanding image quality. The Olympus dotSlide 2.1 system is based on the upright BX51 microscope, which ensures an outstanding optical performance. Furthermore, the peltier cooled dotSlide camera ensures high resolutions with fast frame rates and high sensitivity. In combination with a PC-based workstation, slides can be scanned and uploaded with great ease. This easy-to-use system provides superior functionality, usability and performance through the incorporation of an intuitive Scan Wizard, which guides the user step-by-step through the virtual slide acquisition process.

The standard dotSlide 2.1 MD (manual) system is suitable for fluorescent samples and allows slides and any meta-data to be manually loaded. The dotSlide 2.1 SL (slide loader) system is fitted with a slide loader, which holds up to 50 slides and is integrated with a barcode reader for full traceability and hands-off scanning, for the quick and easy archiving of slides. The dotSlide TMA is now a complete system, incorporating a tissue microarray (TMA) module with a new client server database Net Image Server (NIS) SQL. This combination enables the user to acquire small tissue cores as single images, and upload them together with the relevant metadata and TMA slide overview to the NIS SQL database. This allows users to perform effortless analysis of the TMA and meta-data, and select individual cores from the overview TMA slide image for analysis.

Multiple large specimens can be scanned in up to 15 horizontal or Z-planes. The dotSlide autofocus function offers outstanding autofocus capabilities. Focus points can also be manually set to achieve superior results for difficult samples. In addition the extended focal imaging feature (EFI) automatically produces extremely sharp and focused images.



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Bibliography Demonstrates Applications of UV Area Imaging



Paraytec Ltd has announced a major expansion of their online applications bibliography and technical downloads section.

Paraytec have assembled a selection of application notes, technical articles, posters and presentations that demonstrate the growing array of applications that benefit from their ActiPix™ technology.

Paraytec's multi-award winning product, the ActiPix D100, is the world's first quantitative UV area imaging system. As well as providing superior performance over existing micro and nano flow detectors the patented ActiPix opens up intriguing new possibilities never before possible using conventional detectors.

Applications for the company's patented ActiPix technology can be broadly classified into two types, area imaging or capillary based. Area imaging applications include real time study of diffusion processes, in-line quantification and sizing of biopharmaceuticals, dissolution/solubility testing and membrane transport studies. Capillary based applications include use of the ActiPix D100 as a sensitive and selective detector for multiplexed capillary electrophoresis (CE), nano-liquid chromatography (nano-LC) and capillary LC experiments.

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