

# ILLUMIN8

The newsletter for microscope users

## Welcome

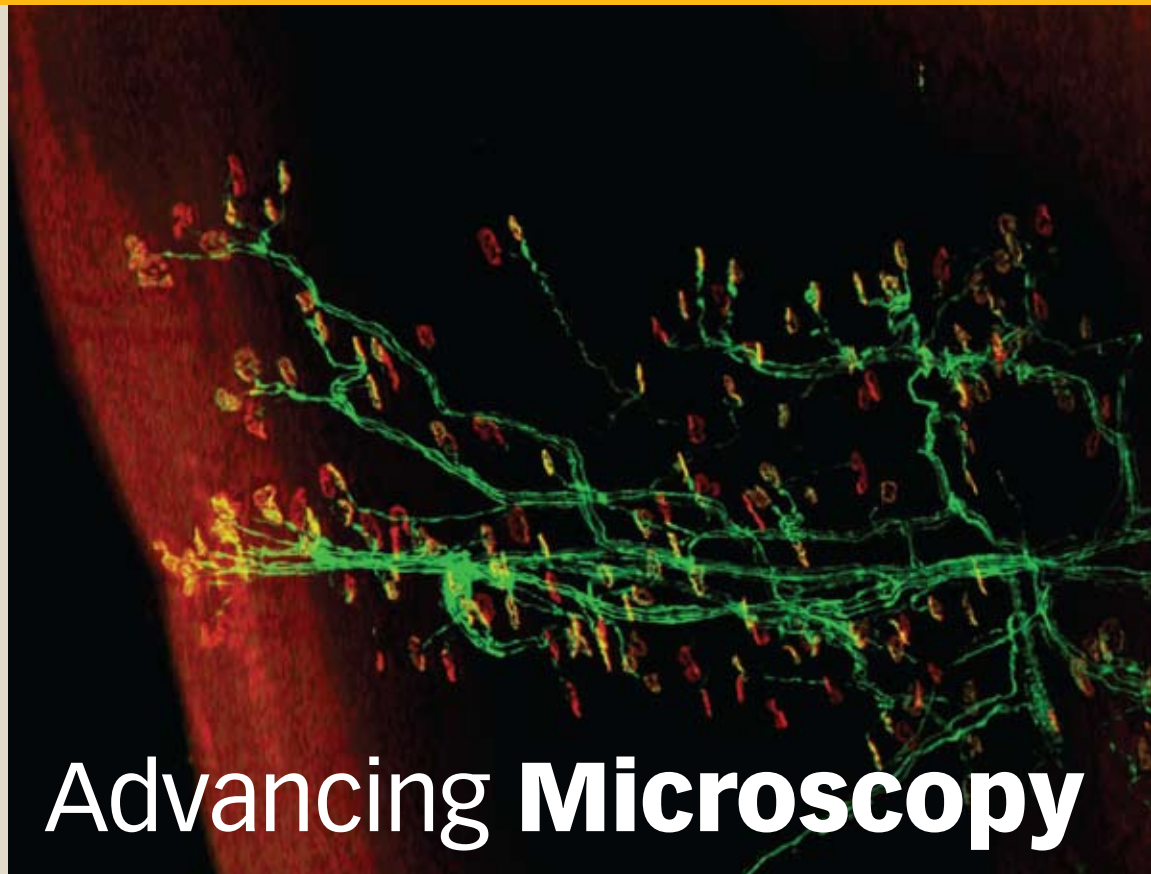
This issue of Illumin8 focuses on the use of microscopes in optical systems and specifically on some new innovations from Olympus. For future issues we would like to know what you want to read about. Moreover if you are doing any interesting research, or have a great microscopy tip, then send an email to

**microscopy@olympus.uk.com** or fill in the reply paid card. You can also use these to request your own copy of 'Illumin8' as well as the handy leaflet and poster series available. We hope you enjoy this issue and don't miss our competition to win a tough new Olympus  $\mu$ [mju:] 770 SW digital camera.



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## Advancing Microscopy

*Microscope manufacturers are constantly striving to push the bounds of microscopy and imaging – we would like to take this opportunity to tell you about some of our recent innovations at Olympus.*

### So why now?

The basic optical layouts used on present microscopes - upright, inverted and stereo (Greenough and Galileo) - have been around, essentially unchanged, for many years. Despite this, microscopy has evolved immensely over this period and the last decade has seen a rapid increase in its development and application. Companies, such as Olympus have been at the forefront of this recent renaissance with both the development of improved materials and applications, and the advancement of micro-imaging concepts, often in collaboration with the academic sector e.g. multi-photon imaging. So we think that now is a good time to give you an overview of what we have been doing.

### So what has changed?

Over the last few years the general focus of microscopy has been on improving resolution. This has been applied throughout the magnification range from whole organism 'macro-views' to

wavelength-limited 'micro-views'. This has led to innovative advances finally reaching the physical limitations of standard optical systems, as in the examples below:

#### *A touch of glass*

Glass tends to absorb light to increasing amounts as the wavelength gets shorter i.e. ultra violet wavelengths are almost totally absorbed (hence why you don't get sunburnt inside your car with the windows shut). This is obviously a problem when using shorter wavelength lasers and means that they are often used at high power to generate any results at all. Olympus has developed a new glass for its UIS2 objective range which has significantly improved transmission across the entire spectrum. This glass also greatly reduces the autofluorescence which often mars fluorescence work. Together these new features reduce the laser power and background subtraction needed for successful results.

This becomes even more important when you consider that resolution in standard optical systems is, in part, dependant on the wavelength of the light being used. As a rule of thumb, the maximum resolving power of a perfectly set-up system (without oil) is proportional to half the wavelength of the illuminating light e.g. 400 nm light will allow a

Continued from front page

resolution of approximately 200 nm. Therefore to see more detail requires shorter wavelengths of light and preferably near-UV.

#### Numerical aperture

Related to the wavelength is one of the most important concepts in microscopy and one that many people know absolutely nothing about – Numerical Aperture (NA). The NA of an objective lens is a measure of its ability to gather light and resolve fine specimen detail at a fixed object distance – the higher the NA the better the resolution and brighter the image. Olympus has maximised the NAs of its objectives, be they achromatic, fluorite or apochromatic.

NAs are also a very important factor in objective-based Total Internal Reflection Fluorescence (TIRF) microscopy, where they need be at least 1.45. Olympus has developed a range of TIRF objectives which contains two 'firsts': A 150x objective, the highest available magnification in a TIRF objective; a 60x objective with a peerless NA of 1.65.

#### And what else?

##### Yours ergonomically

Amongst a number of key improvements implemented to Olympus microscopes, such as more extensive illumination choices and complete software solutions, ergonomics has been paid a lot of attention. Many people sit in unnatural positions at their microscopes, which is due to many factors, some of which are out of their control. Olympus microscopes are now designed with a great deal of flexibility and modularity because one size does not always fit all. These features include (depending on the microscope): different eyepiece tubes; nosepiece focus control instead of stage focusing; low-down and easy to use control dials, as well as ComfortView eyepieces for more relaxed eye positions.

#### As a result

Microscopes, such as the new SZX16 stereo, provide stunning images that were not previously possible and thus are able to advance our understanding of life processes. For example, at a recent conference Prof Richard Ribchester from the University of Edinburgh viewed one of his samples under fluorescence on the SZX16 and was amazed at the clarity and detail at all stages of magnification. The micrograph on page 1 is a whole-mount preparation of a toe muscle showing neuromuscular junctions in a transgenic mouse expressing Yellow Fluorescent Protein in motor neurones. Intramuscular axons and motor nerve terminals emit green fluorescence. Acetylcholine receptors at the sites of neuromuscular contact were counter stained with TRITC-[alpha]-bungarotoxin (red fluorescence). Where motor nerve terminals overlay the motor endplates the combined fluorescence appears yellow. Each neuromuscular junction is about 40 micrometres in length. Prof Ribchester commented, "This microscope yielded impressive fluorescence images over the entire range of objective lens power. It is ergonomically designed and has potential applications in my work for high-throughput genotyping based on fluorescence; for monitoring nerve development, degeneration, disease progression and regeneration in animal models *in vivo* as well as in organ, tissue or cell culture; and for electrophysiological recording from identified, fluorescent-labelled neuromuscular synapses in isolated nerve-muscle preparations *in vitro*."

# Microscope or Tool?

## Laser Microdissection

Collecting contamination-free biological material for further analysis has always been a problem with not wholly satisfactory solutions, usually involving various chemical and gravitation steps. Laser Microdissection uses a high power laser focused through a microscope objective to cut out the desired tissue section, cell or even cell fragment. Contamination free collection ensures that the sample used is as pure as possible.

Olympus offerings: CellCut Plus - research level system and SmartCut Plus – entry level system.



MultiCap Collecting unit on Olympus CellCut Plus



The Olympus cell^R

## High Speed Imaging

Capturing sub-cellular microsecond events in live cells is very important for advancing our knowledge of how cells operate and communicate and yet it is very difficult to visualise or even record such events. By combining high power computers, top-end motorised microscopes and illumination systems with high quality digital cameras it is possible to not only see these events but also record and measure them.

Olympus offerings: cell^R high-level research system & cell^M research system.

## Screening Station

Screening large numbers of samples for huge amounts of data may seem completely opposite to more sedate manual scanning of a single sample generally associated with microscopy, but it is a growing requirement. The integration of high powered software algorithms with advanced microscopy systems has made this a reality.

Olympus offering: scan^R screening station for life science.



The Olympus scan^R screening station for life science

*Microscopes have always been seen as instruments in their own right. Increasingly though, they are becoming integral parts of other systems and even being completely redesigned to create novel tools. Olympus has a number of new instruments which utilise the advanced optics of their microscopes:*

To find out more about any of these Olympus products, please fill in the reply paid card or email [microscopy@olympus.uk.com](mailto:microscopy@olympus.uk.com)

## Whole Animal Imaging

With so much now known about processes at a sub-cellular level, scientists have started to go back to studying the effect of known changes and diseases at the 'whole organism' level using a range of different paradigms.

Microscopes such as the Olympus SZX2 stereo range and the MVX10 Macroview are used on small animals such as the zebrafish (*Danio rerio*), whereas the new OV100 small animal imaging system is designed to allow researchers to observe fluorescent markers in live animals e.g. rodents, at the whole animal, organ, tissue, and cell level, with a single optical instrument.



*The Olympus SZX16*

## Multi-photon

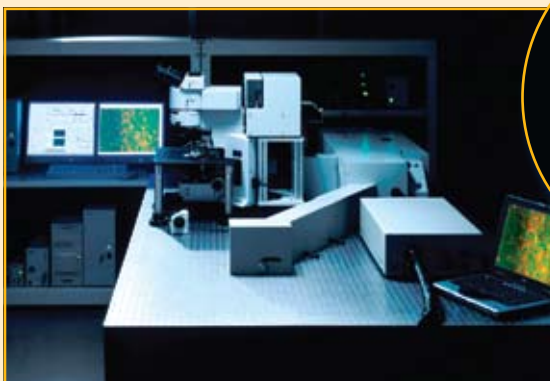
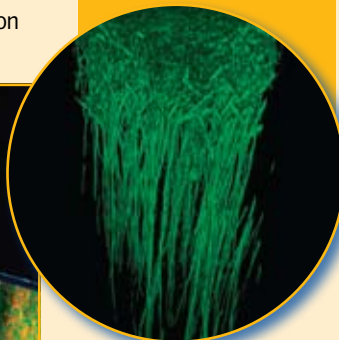
As well as the drive to get a 'holistic' view of life processes with whole animal imaging, there is another to see deeper into living tissues at very high magnifications – especially within the extreme complexity of the brain. Standard illumination procedures and even confocal systems have very shallow penetration depths due to a number of physical factors which send two or more low powered-photons (not strong enough to elicit fluorescence on their own) to meet at a specific position simultaneously, producing the energy required to generate fluorescence. This extends the penetration depth to a couple of hundred nanometres.

Olympus has developed a series of three multi-photon excitation (MPE) systems which provide the greatest penetration depths on the market. The highest specification system utilises the unique Olympus SIM scanner to provide two laser scanners. This enables experiments requiring photobleaching, photoactivation and uncaging.

*Single neuron imaged on Olympus FV1000MPE*



*Dense network of neurons imaged on Olympus FV1000MPE*



*Olympus FV1000MPE Multi-photon microscope*

## Virtual Slide

The internet, and especially broadband, have greatly increased the ability of professionals to collaborate around the globe, but data is still often shared in a limited format – micrographs, extracted and calculated data, etc.

The new dotSlide system from Olympus creates an electronic copy of the entire slide, be it one sample or many (such as a tumour microarray). These are then accessible anywhere around the world with the functionality of a normal microscope. So a collaborator on the other side of the globe can access the virtual slide and carry out their own observations and measurements as if they were using the real slide. The software also enables live discussions and records the notes of the meeting – a truly revolutionary tool.

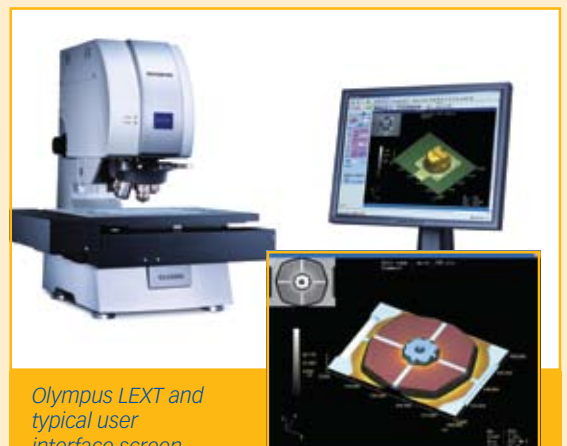


*Olympus dotSlide and typical user interface screen*

## Metrology

Surface analysis plays a key role in many R&D procedures and includes important measurements and analyses such as profile, roughness, faults and inclusions, often on one-off production test pieces. Despite this, the instruments available for metrology generally require some sort of damage to the sample.

The Olympus LEXT confocal metrology instrument provides up to 14,400x magnification (overlapping with electron microscopy) with resolutions of 0.12µm (XY) and 0.01µm (Z), without needing to damage the sample in any way.



*Olympus LEXT and typical user interface screen*

# Competition time: Your chance to win a **tough camera**

Accidents happen! You'll have a hard time damaging the new Olympus  $\mu$ [mju:] 770 SW digital camera though - it is one tough cookie! It will still work if dropped 1.5 metres, submerged up to 10 metres, frozen to  $-10^{\circ}\text{C}$  or crushed by 100kg, and yet it is still very stylish and takes excellent pictures. We are giving one away, so to be in with a chance of winning you will need to answer the three questions below correctly on the reply paid card and return it to us by the 2nd July 2007.



### Question 1:

Which microscope technique requires objectives with NAs of more than 1.45?

### Question 2:

Which microscopy technique enables a deeper and higher resolution view inside the brain?

### Question 3:

Which microscope did Peter Wilberforce use to discover five new species of fungus?

**Congratulations to Mrs J Todd of Weston Turville, Buckinghamshire, for winning a pair of Olympus 8x25 WP I waterproof binoculars from the last issue of Illumin8.**



Olympus Microscopy has partnered with the Nature Publishing Group to present the "Nature Reviews Fluorescence Imaging Collection". This compilation of leading articles from Nature's portfolio of Reviews journals has been carefully selected to support cell biologists using fluorescence microscopy techniques in their research and is the latest in Olympus's ongoing programme of activities in support of the scientific community.

For more information please fill in the reply paid card or email [microscopy@olympus.uk.com](mailto:microscopy@olympus.uk.com)

# In with **the new...** but not out with **the old**

As an example of the foundations upon which Olympus is building state-of-the-art instruments, we recently had a letter from Peter Wilberforce, a retired dentist, who purchased an Olympus BHS upright microscope 25 years ago. The instrument has been used virtually every day since and amazingly the original bulb is still going strong!

Peter commented, "Admittedly I am a very careful and considerate user and always switch on at the lowest

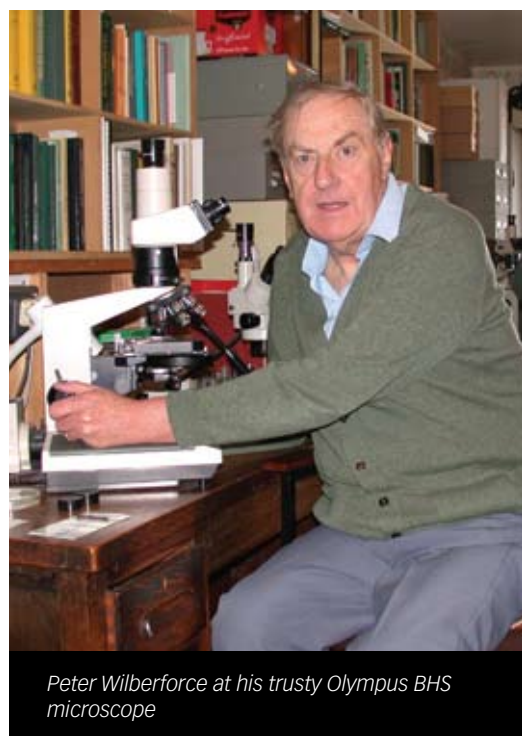
setting and then gradually increase to the working level of about 8 volts. I always wind it down slowly to the lowest level before switching off. Even so, it is a tribute to the excellence of Olympus that it has lasted so long."

Peter though, is not just a casual user; he has discovered and described 5 species of fungus with the help of his BHS (*Triblidium crarense*, *Ceratosphaeria fragilis*, *Karstenia inconspicua*, *Taphrophila argyllensis* and *Lachnum luteovinosum*) and is still fascinated by the views he sees through his eyepieces. The micrograph he captured above is rather unusual as it shows the spores from a minute ascomycete called *Podospora decipiens*. The fungus itself is black, pear shaped and about  $38\mu\text{m}$  long. It is a common species found in the dung of cows, deer and rabbits. The unusual feature is the ability to see the secondary appendages, which show up white under Nomarski DIC but are normally very-short lived and quickly detach. Peter said, "It is pure luck to find spores completely intact."

As well as the Silver anniversary for his microscope (and bulb), Peter and his wife Sheila have just celebrated their Golden wedding anniversary, so many congratulations from Olympus! Obviously we no longer produce the BHS microscope, instead the BX2 range is available and to find out more, please fill in the reply paid card or email [microscopy@olympus.uk.com](mailto:microscopy@olympus.uk.com)



*Podospora decipiens* showing secondary appendages taking using Nomarski DIC on Olympus BHS (Image courtesy of Peter Wilberforce)



Peter Wilberforce at his trusty Olympus BHS microscope

# OLYMPUS

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### To request further information:

Olympus UK Ltd  
Microscopy Division  
Dean Way  
Great Western Industrial Park  
Southall  
Middlesex  
UB2 4SB

Phone: 020 7250 0179  
Fax: 020 7250 4677

Email: [microscopy@olympus.uk.com](mailto:microscopy@olympus.uk.com)  
Web: [www.olympus.co.uk](http://www.olympus.co.uk)

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Dept. \_\_\_\_\_  
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My answers to the competition are:

- (1) \_\_\_\_\_  
(2) \_\_\_\_\_  
(3) \_\_\_\_\_

Please send me future issues of Illumin8  by post  by email

Please send me the leaflets on:  Darkfield

Focus lock  Koehler Illumination

Phase Contrast  Cleaning & Maintenance

Please send me

Nature Reviews Fluorescence Imaging Collection

Please send me more information on:

\_\_\_\_\_  
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I would like to receive regular Olympus eNewsletters

June 07

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June 07

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Microscopy Division  
Dean Way  
Great Western Industrial Park  
Southall  
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UB2 4BR

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