

ILLUMIN

The newsletter for microscope users

Welcome

This issue of Illumin8 is specifically focusing on imaging and analysis. 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

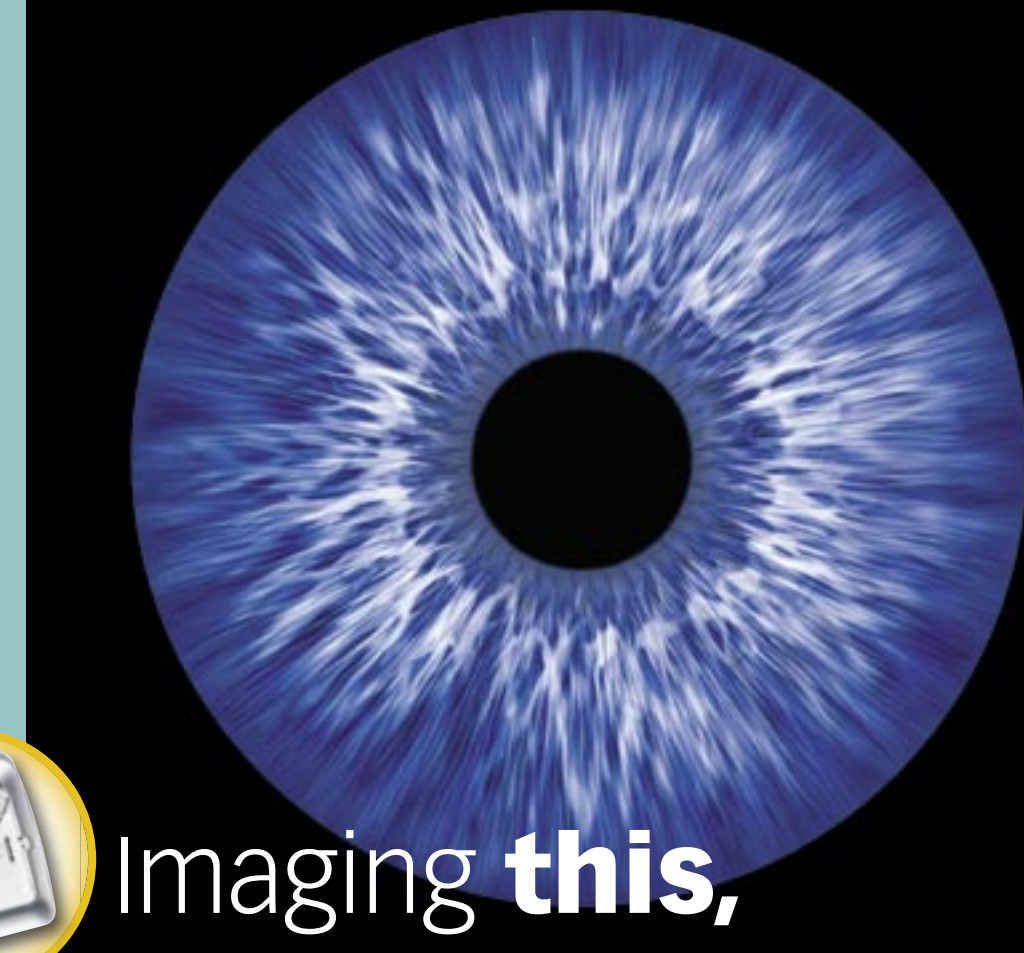
microscopy@olympus.uk.com

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 an Olympus μ [mju:] 720SW digital camera



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In this article we look at imaging and analysis with examples from bone and brain research
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A short overview of the new life science and materials science imaging software programmes available from Olympus



Imaging **this,**

Analysing **that**

Taking images using a microscope has changed immensely over the past few decades. Not only have film cameras been largely replaced by digital cameras, but software has been introduced that can enhance images and extract amazing amounts of information. These have revolutionised microscopy, opening up a new aspect - imaging. One may argue that the whole purpose of microscopy is to produce images and therefore the latter is fully covered by the former. In this article though, we will look specifically at imaging and analysis as opposed to microscopy.



Cutting corners?

With the advent of advanced image manipulation software, it is still important to prepare microscope samples of the highest quality and not

rely on the software to 'clean-up' the images later. The reasons for this are two-fold. Firstly, the better the initial data, the more extensive the analysis can be. Secondly, traceability requirements of GLP mean that any derived data must be inextricably linked to and sensibly produced from high quality raw data.

So, your fluorescently-labelled samples are finally on the stage after months of careful preparation, including many delicate steps. You have selected the correct objective for what you want to do and the DAPI filter cube is in place. Before you, are perfect blue nuclei, some of which are part way through cell division, confirming that your culture was healthy at the time it was fixed. You switch to the GFP filter cube and see exactly what you were looking for. You momentarily chastise yourself for not believing in your own abilities and then instantly

want to show everyone else – you need to record your image.

From the basic...

The image is sent to a digital camera and the software on the computer allows you to change the exposure time, contrast and brightness. A simple click of the mouse and the image is saved. This very simple example of where imaging software fits into microscopy, is just the tip of the iceberg; there is so much more that can be achieved with products such as the new Olympus *cell[^]F* software family.

...To the advanced

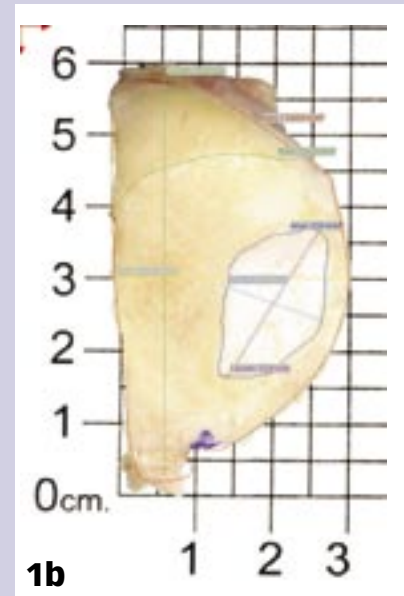
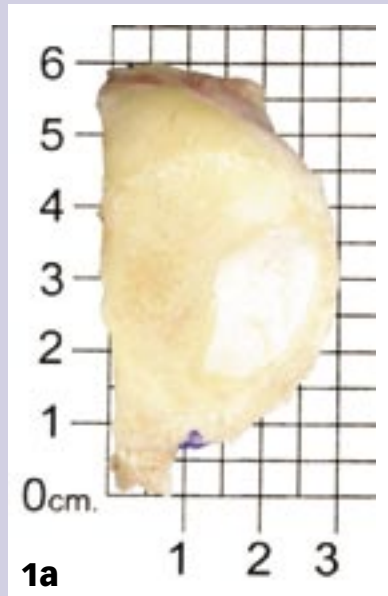
With the new advanced *cell[^]F* software, complex imaging processes can be carried out on carefully prepared samples. For this, the software needs to integrate more completely with the imaging system and control all the parameters such as the various shutters and filters, light sources and objectives, as well as the focus and stage position of the sample. This enables much finer control over multi-dimensional imaging, such as recording images taken at various depths through a sample (Z-stacks) at a number of positions using a range of fluorescence and brightfield methods.

Once an image (or series) has been taken, many different analyses can be performed to provide a wealth of extra information, from inclusion analysis to dimensional calculations.

Bone

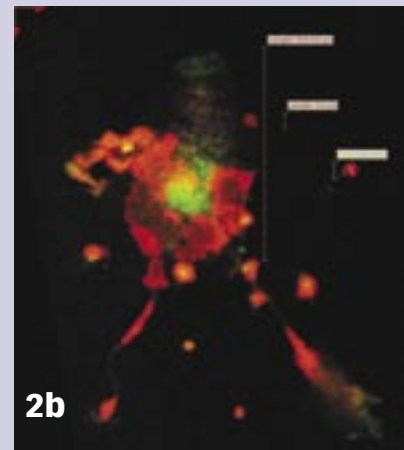
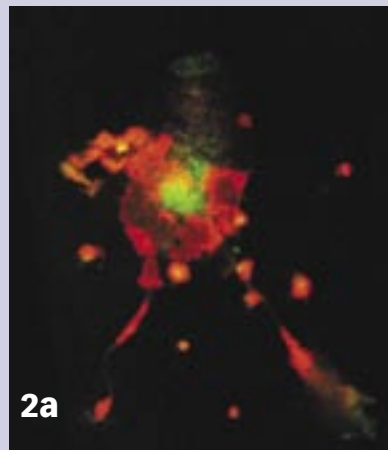
Dr Philippa Hulley (PhD), Dr Iannis Adamopoulos (PhD) and Mr Stephen McDonnell (MRCS) at the University of Oxford's Botnar Research Centre, Nuffield Department of Orthopaedic Surgery, carry out research into bone and joint diseases. They use the *cell[^]F* software to analyse camera images of specimens following surgical procedures and microscope images of signalling processes in both skeletal cells and resection specimens from damaged joints. Philippa commented "Being able to use the software for both micrographs and photographs significantly improves our analysis capacity and provides crucial data for our research." Stephen added "Following surgery, we need to analyse the dimensions of resection specimens. The *cell[^]F* software provides a very accurate way of doing this quickly and easily, even though it is designed for micrograph rather than photo analysis."

Philippa's work is based on exploring the survival and differentiation control pathways in osteoblasts and chondrocytes and establishing the molecular mechanisms by which commonly used bone-sparing and bone-building treatments interact with these pathways. This facilitates rational improvements in drug design and is of value in both stem cell technologies and tissue engineering. Philippa said, "Microscope imaging plays a large role in my work and with the advanced analysis programmes now available, my results are



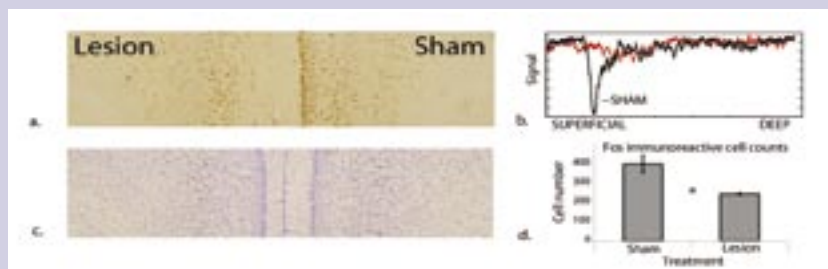
1

a Photo of bone taken on a digital camera and imported into *cell[^]F*.
b Scaled measurements made. This method was used over a series of specimens to enable the creation of a detailed log and to allow mapping of this pattern of disease.
Image Courtesy of Mr Stephen McDonnell (MRCS) Botnar Research Centre, Nuffield Department of Orthopaedic Surgery, University of Oxford.



2

a Image of a double stain (Live/Dead-molecular probes) showing terminally differentiated osteoclast (green) from peripheral blood monocytes (red).
b as 'a' but with measurements using the *cell[^]F* software.
Images courtesy of Dr Iannis Adamopoulos, Institute of Musculoskeletal Sciences, Botnar Research Centre, University of Oxford.



3

a Fos reduction in the superficial layers of the retrosplenial cortex
b Horizontal mean profile of signal strength
c Nissl stain (note normal appearance of tissue on lesion side)
d Two-sample t-test (one-tailed) revealed a significant difference in Fos immunoreactive cells between Sham and Lesion ($t=9.387$, $p<.05$). Profile analysis reveals that the reduction in protein expression is confined to a specific lamina.
Images Courtesy of Guillaume Poirier, School of Psychology, University of Cardiff.



much easier to demonstrate and I can spend more time on things other than counting cells." She continued, "Bone and cartilage are not passive tissues; there is a whole network of cells either resorbing or synthesising the many components. It is essential to understand all the various signalling pathways involved. This means that we need to follow the appearance, movement and disappearance of many molecules both within cells and in the extra-cellular matrix (ECM). The *cell^F* software enables us to do this and analyse the results easily."

Brain

The most neurologically dynamic organ in the body, the brain, has always intrigued researchers. Its delicate intricacies form both our physical and psychological identity. Prof John Aggleton's team at Cardiff University's School of Psychology are carrying out research to show the links between biochemical and cellular changes with learning and memory. One of the Cardiff researchers, Guillaume Poirier, said, "The brain shows a highly ordered structure with physiologically and biochemically distinct regions. Communications both within and between these enable the formation and processing of thoughts into actions and memories." He continued, "The biochemistry involved in this is both intricate and heavily influenced by many factors. We are looking at the expression levels of a number of key genes using immuno-histochemistry and immuno-fluorescence techniques."

Essentially, Prof Aggleton's team are not looking at these molecules on the biochemical level per se, but on a brain-architecture scale. To do this, they need to analyse the proteins in terms of their distribution within the different layers or laminae of the cortex. Guillaume commented "The Olympus *cell^P* software provides extended programming facilities, which enable us to both expand the complexity of the analysis we carry-out while simplifying the steps involved. The intensity profiles produced allow us to instantly get a feel for the expression levels of the target proteins in each cell lamina. What we have been able to achieve is rapid characterisation of the cells expressing the proteins, using parameters such as intensity, shape and distribution."

Conclusion

It has always been important to use the correct microscope to produce the best images. It is now equally essential to pick the right software for imaging and analysis processes. The Olympus *cell^** family of imaging and analysis software, provides the perfect range of software solutions from basic acquisition through to advanced, real-time imaging.

To find out more about the software solutions, such as the *cell^** family, available from Olympus, please fill in the reply paid card or email microscopy@olympus.uk.com

Babraham1-2-3

Olympus has been developing closer links with the UK's research community. In this issue we look at how we have worked with the BBSRC's Babraham Institute near Cambridge.

On your marks

The Babraham Institute took part in the Cambridge Science Festival in March 2006. Scientists were on hand in the Biology Zone to talk about the science behind heart failure, neurodegenerative disorders, infertility and cancer. An interactive computer presentation enabled children to pit their wits against sheep demonstrating how we, and they, recognise faces.

And biology was brought to life using the Olympus SZ61 stereomicroscope, enabling children to zoom in on the activities of fruit flies and marvel at a range of other interesting biological specimens.

Get Set

For the fifth year running, Olympus has provided training courses for post-graduate and post-doctoral scientists in and around Cambridge. Both the Babraham and Gurdon Institutes saw a team of experts from Olympus demystify microscopy. Practical, hands-on demonstrations showed students essential processes such as centring a mercury burner, setting up Köhler illumination, as well as cleaning and maintenance.

Go!

Olympus is launching a new microscopy journal which details the microscopy achievements of key researchers within Britain's leading research centres. The first issue of 'Research Insights' concentrates on the work of the scientists, such as Dr Martin Bootman, at the Babraham Institute.



Children explore the fascinating world of microscopy at the Cambridge Science Festival. Picture courtesy of Dr Claire Cockcroft.



Babraham postgraduates learn the intricacies of modern microscopy. Picture courtesy of Alan Lasslett.

Heading for the hills

Dr Andy Irving, a lecturer at the University of Dundee's Neurosciences Institute, attended this year's European Winter Conference on Brain Research. "With the help of sponsorship from Olympus, I was able to attend the EWCBR conference in Villars, Switzerland, in March this year. The conference focused on neuroscience research and I hosted a session on the CNS actions of cannabinoids. This highlighted some of the recent, exciting advances in the field and illustrated how new developments in microscopy and imaging are driving research in this area. The EWCBR conference is organised on a yearly basis and offers researchers the chance to present and discuss science within the idyllic setting of the snow-covered Swiss Alps."



From L to R: Dr Andy Irving (Dundee), Dr Lene Martini (San Francisco) and Dr Ken Mackie (Seattle), on the piste at EWCBR in Villars.

To find out more about the 'Research Insights' journal, or other Olympus literature, please fill in the reply paid card and return. Alternatively, email microscopy@olympus.uk.com

The Olympus *cell** and *analysis* software families

We hope to see you
at **MICROSCIENCE 2006**
27th-29th June, Excel London

Life Science	Materials Science
<i>cell</i> ^A Acquisition	<i>analysis</i> Start Acquisition
<i>cell</i> ^B Basic Imaging	<i>analysis</i> Work Basic Imaging
<i>cell</i> ^D Documentation	<i>analysis</i> Docu Documentation
<i>cell</i> ^F Fluorescence	<i>analysis</i> Auto Particle Analysis
<i>cell</i> ^P Professional	<i>analysis</i> Pro Professional
<i>cell</i> ^M Modular Fast	
<i>cell</i> ^R Real-time	

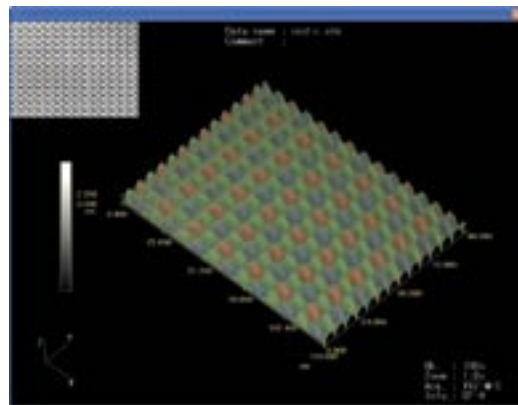
FUNCTIONALITY

Both software families can be supplemented with add-on modules to enhance functionality for specific tasks.

To discover more about the Olympus *cell** and *analysis* software families, please fill in the reply paid card or email microscopy@olympus.uk.com

Did you know?

...That the humble Pixel is 52 years old this year! A portmanteau word for Picture Element, the Pixel was first proposed and demonstrated by researchers at Princeton's Institute for Advanced Study in 1954. Since that time the pixel has become more than just a method of displaying the results of a computation and in some ways has shaped our present world. Initially they were very visible single points of light, but now they are virtually invisible to the naked eye and we can control many millions at once to generate images with extremely high fidelity. Our view of the modern world is now mediated via these minute elements, and the word has



Micrograph of a CCD digital camera pixel array taken using the Olympus LEXT metrology system

become part of everyday language – for example digital cameras are classified by their mega-pixel value (see competition) and enlarged images can be 'pixelated'.

The modern technology behind pixels though, isn't as simple as it may at first seem. Not only have we generated high resolution screens,

but ones capable of showing more shades (referred to as bit depth) than our eyes can perceive. Also, most computer pixels are now formed from triads of subpixels representing the three primary colours: red, green and blue (RGB) and therefore it could be argued that the pixel has actually disappeared all together!

Win the perfect holiday camera

Where's the camera? Oops, it's fallen into the sea. Not to worry, the waterproof Olympus μ [mju:] 720SW can be submerged up to 3 metres without affecting the camera – it can even take pictures underwater! More impressively, the impact resistant sleek body enables you to capture 7.1 mega pixel images and comes in a choice of colours. To be in with a chance of winning the camera you will need to answer three questions correctly on the attached reply paid card, to be received by us before the 3 July 2006.

- Question 1:** What Olympus imaging software package enables real-time imaging?
- Question 2:** At which UK research institutes did Olympus recently provide training?
- Question 3:** What does the word Pixel stand for?

Congratulations to Mrs Alison Knight at the Royal Devon & Exeter Hospital, for winning the WS-300M voice recorder from the last issue of Illumin8



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Institute/Company _____
Address _____

Telephone _____
Email _____

My answers to the competition are:

- (1) _____
(2) _____
(3) _____

Please send me future issues of Illumin8 by post by email

Please send me 'Research Insights'

Please send me the leaflets on: Darkfield

Focus lock Koehler Illumination

Phase Contrast Cleaning & Maintenance

Please send me more information on:

Suggestions for future issues of Illumin8:

I would like to receive regular Olympus eNewsletters

June 06

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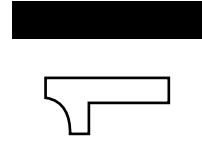
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