

ILLUMIN

The newsletter for microscope users

Welcome

This issue of Illumin8 focuses on a range of different microscopy techniques. 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:] 1000 digital camera.



Molecules: The building blocks of engineers!

Plant cell immobilised on a semiconductor device

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The NIBSC is more than just the UK's biological standards establishment

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Olympus microscopes are certainly getting their 15 minutes of fame

For biologists, evolution and biological manipulation have provided all the materials for life and medicine. Physicists and engineers, on the other hand, have developed synthetic materials to build structures from integrated circuit boards to massive buildings. More and more though, the solution to difficult engineering problems, in particular at the ultimate molecular scale, lies in biology and vice versa, hence the two are coming closer together, especially for micro- and nano-scale manufacture.

Bio-electronics

Dr Christoph Walti and his group at Leeds University's School of Electronic and Electrical Engineering are running an ambitious research programme to investigate what can essentially be classed as 'bio-electronics'. Their aim is to develop hybrid bio-electronic devices, where electronics and biology integrate seamlessly with multiplex communication. Christoph commented, "Evolution

has given biological systems an excellent ability to detect molecules with very high specificity and react in defined ways." Many biological downstream control systems, such as the response to hormones or other chemicals, share similarities with electronics – feedback loops, cut-offs and even logic gates. He continued, "The aim of our research is to take the 'detection' and 'scaffold' parts of the biological systems e.g. the antibodies, receptors, proteins, nucleotides etc, and couple them with advanced electronic systems capable of making very rapid decisions, display answers or initiate further actions."

Applications

Technology based around this would be extremely important in the miniaturisation of diagnostic systems, for instance, where present technologies use various chemical reporting methods to identify a biological interaction. Other applications are limited

only by imagination and include such arenas as medical treatment, security, forensics and molecular computing.

Present considerations

These interactions though, are some way off reaching their full potential as the fundamental facets of such systems need to be fully studied. Christoph's group presently focuses on investigating many of the properties that will come into play with such devices. For example:

- How physical factors, like high frequency electrical fields, affect biomolecular structures. This may play a role in how such systems are designed but could also act as a switching method.
- Functionalisation of silicon devices so that DNA and proteins can bind to them with signals travelling between the synthetic and natural components.
- Developing biomolecular scaffolds out of branched DNA. These can act as 'beds' for accurately and securely holding other biological molecules such as proteins for multifunctional devices.
- Manipulating biomolecules, including DNA molecules and proteins, using electrical fields. This will provide a method for precisely placing and moving/manipulating biomolecules.

Essential tools

Investigating these areas takes more than just technical know-how – microscopes, for example, are essential tools for looking at what is actually happening. The group recently

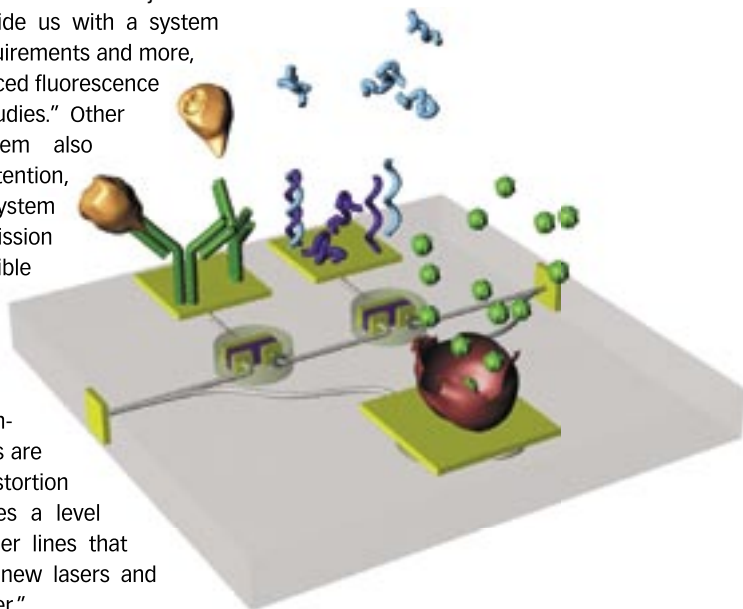
purchased an Olympus FV1000 FluoView laser scanning confocal microscope (LSCM), with SIM scanner and TIRFM modules. Christoph said, "Having spoken to a number of microscopy companies, Olympus were the only ones willing to engage with the challenges of our requirements. We are essentially physicists with a need for an excellent and flexible 'optical bench', to which we can add 'home-grown' attachments." He continued, "The secondary laser scanner and the advanced objective-based TIRFM unit provide us with a system that fulfils all of our requirements and more, allowing us to do advanced fluorescence bleaching and FRET studies." Other aspects of the system also grabbed the group's attention, "The lead-free optical system gives us better transmission across the entire visible light spectrum range and even into the near-UV and near-IR regions. Autofluorescence is also almost non-existent, and the images are completely flat and distortion free. The AOTF provides a level of control over the laser lines that makes the addition of new lasers and extra features a lot easier."

Conclusions

Microscopes are used in both biology and engineering but often with different specifications. In the research conducted by Dr Walti and colleagues, the all round advanced

features available with the Olympus FluoView FV1000 LSCM and the interest of the Olympus personnel, proved an excellent combination for their fascinating bio-engineering research.

To find out more about the research, visit Dr Walti's webpage at www.bioelectronics.leeds.ac.uk. To find out more about the FluoView FV1000, please fill in the reply paid card or email microscopy@olympus.uk.com



Schematic of a hybrid bio-electronic device. Information arising from biomolecular recognition events is processed by the underlying electronics. Logic decisions are made, triggering further biomolecular events such as release of a chemical species

One to Watch



Following a cameo role in 'Jimmy's Farm', an Olympus microscope has shot to further fame in a new series, 'Grime Scene Investigation.' (GSI), running on the digital channel - BBC3 - over October and November. The series looks at our everyday lives and how grime is an integral and sometimes disgusting part. The power of the show lies in the visual impact provided via an Olympus CX41 upright microscope and a JEOL scanning electron microscope. Simon Kerfoot, Producer and Director of the series said, "Without the invaluable contribution of companies like Olympus and JEOL, it would have been impossible for the Grime Scene Investigation team to reveal the secrets of the hidden world that surrounds us."

The programme airs every Tuesday at 20:00 for 8 weeks (from 3rd October), visit www.bbc.co.uk/bbcthree/tv/grime_scene/index.shtml for more information.

To find out more about the CX41 and other Olympus products, please fill in the reply paid card or email microscopy@olympus.uk.com

More than just **Standard**

The National Biological Standards Board (NBSB) is a non-departmental public body (NDPB) of the UK government, established in 1975 as a Statutory Body by Act of Parliament. The Board is responsible for safeguarding and advancing public health by assuring the quality and safety of biologicals, through its management of the National Institute for Biological Standards and Control (NIBSC).

NIBSC provides independent testing of biological medicines for the UK market, in particular with vaccines for the UK children's vaccination programme, and operates as an Official Medicines Control Laboratory (OMCL) of the European Union for release of medicines onto the EU market. The NIBSC also produces standards for use in the research of many key public health issues such as vCJD, AIDS and influenza, as well as running an extensive regenerative medicine programme.

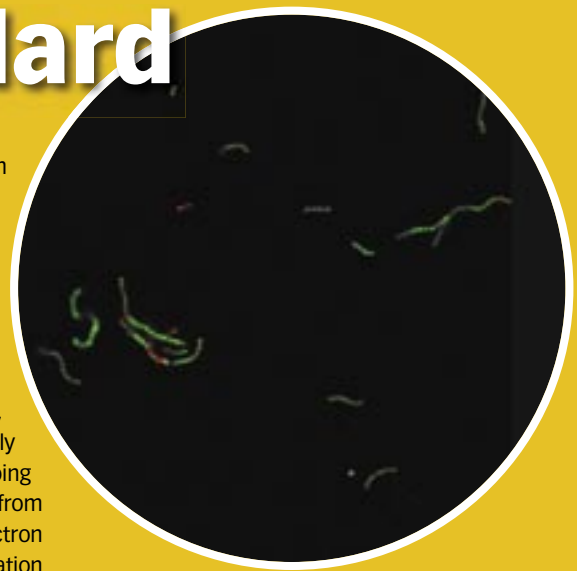
Imaging input

Imaging has a role in many ongoing projects at the NIBSC and is managed as both a central resource and through dedicated instrumentation located in each of the laboratories of the many specialist groups at the NIBSC. As a central resource, the Imaging Group headed by Dr Roland Fleck, is equipped with extensive facilities for all standard light-, confocal-, freeze drying- and electron-microscopy techniques. Roland commented, "We are involved in many projects at the NIBSC since so much unique information about all types of cells and viruses can be gleaned from the various microscopical techniques we use. Light microscopy and confocal techniques

produce a wealth of data about cell growth (be it enhanced, normal or inhibited), transcription and translation events as well as morphological changes." He continued, "By combining light and electron microscopy we can provide even more powerful results since the effects of pathogens, such as viruses, can be seen on cells under light microscopy, but the pathogens themselves are only visible in great detail using EM." The ongoing value of imaging to the institute is evident from the recent investment in new JEOL electron microscopes and Bal-tec specimen preparation equipment.

International kudos

Roland and his colleagues travel to many meetings presenting their findings and also provide courses on relevant techniques. Roland said, "Although we are essentially an extension of the UK Government, our influence is worldwide and we have an excellent reputation to maintain. We do this by presenting our research as often as possible and by giving instruction on a whole array of standard techniques."



Optimisation of Pneumococcal assays. Live cells fluoresce green due to SYTO9 stain and dead cells fluoresce red due to propidium iodide stain

For a more detailed explanation of the activities of the NIBSC, please visit www.nibsc.ac.uk. To find out more about Olympus Microscopy systems, please fill in the reply paid card and return. Alternatively, email microscopy@olympus.uk.com

Cooperation with Olympus

Michael Czempiel, Managing Director, Microscopy, Olympus Life and Material Science Europa GmbH commented, "We understand the value and importance of the research carried out at the NIBSC and as a leading microscopy company wanted to ensure that they had the very best equipment available to them so they can maintain and increase their essential work. We will therefore provide them with an advanced cell[^]R live cell imaging package." There will be more about this collaboration in future issues.

'Crypto' course



Far from being the spooky underworld of cryptography, this Crypto course held at the University of Strathclyde from 12th-14th September, dealt with the mandatory screening of UK domestic water for the pathogenic *Cryptosporidium* spp. micro-organisms. The 11th annual 'Cryptosporidium Size Matters' course was organised by Professor Huw Smith, Scottish Parasite Diagnostic Laboratory and Dr Tony Grimason, University of Strathclyde, with support from Olympus. Attending the course were new and experienced cryptosporidium screeners from various UK water authorities.

Professor Huw Smith said, "This is one of the most difficult analyses that I have performed. By the time that analysts look at oocysts they have been flattened and distorted by air drying onto microscope slides. It's like trying to identify a three dimensional object that has been flattened by a steam roller! We use fluorogenic reporters to identify the location

and approximate size of an oocyst and whether it possesses sporozoite nuclei, but DIC is the only microscopy contrast tool available for *Cryptosporidium* spp. screening that can accurately determine the morphology and morphometry of oocysts. Many *Cryptosporidium* analysts in the water industry find using DIC difficult, and our 'Cryptosporidium size matters' workshop is meant to address this specific issue and generate confidence in analysts. The specialist knowledge provided by our Olympus trainers always convinces delegates that they can optimise their microscopes for maximum performance. So with the help of Olympus, we have addressed this issue successfully, over that last 11 years.

The next course takes place in Hamburg in February 2007, if you are interested in attending or have any special microscopy requirements please fill in the reply paid card or email microscopy@olympus.uk.com

Competition time: **Olympus μ [mju:] 1000 digital camera**

Be amongst the first to own an Olympus μ [mju:] 1000 digital camera

The new Olympus μ [mju:] 1000 digital camera pushes the very boundaries of technical achievement, without skimping on the style. It features a weatherproof metal body, beneath which is a ten million pixel CCD and a 3x zoom lens, which together deliver razor-sharp results. Suited to low light photography, its light sensitivity can be boosted to an amazing 6400 ISO. To be in with a chance of winning one, you will need to answer these three questions correctly on the reply paid card and return it to us by the 4th December 2006.

Question 1:

Which module for the Olympus FluoView FV1000 LSCM enables processes such as advanced bleaching studies and FRET?

Question 2:

Which two contrast techniques are used for screening for *Cryptosporidium spp.*?

Question 3:

Which microscopy technique was devised by Zernicke?



Congratulations to Pavel Drevinek at the Bioscience Department, Cardiff University, for winning the Olympus 10-30x25 Zoom PC I binoculars from the last issue of Illumin8.

Keystone techniques

Brightfield microscopy

This is "normal" microscopy, when no optical contrast technique is employed. As cells and tissues have insufficient contrast in themselves, staining techniques must be used. Common stains include *haematoxylin and eosin (H&E)*, used to stain histological sections, *Papanicolaou's stain*, for cervical smears (hence "Pap test") and a range of *Romanowsky stains* for haematology. To achieve optimal resolution and contrast most microscopes need to be set up for *Koehler illumination*.

Darkfield microscopy

The specimen is illuminated obliquely, with no direct light entering the objective. Features in the specimen plane which scatter light can clearly be seen against a dark background. Darkfield illumination is provided by either a simple *patch stop*, a darkfield element in a phase contrast condenser or purpose-built darkfield condenser. The latter is required for high-resolution objectives to prevent the oblique rays entering the wide aperture of the objective. Applications include detection of micro-organisms in unstained smear preparations and classical diatom studies.

Phase contrast

Devised by Zernicke, this technique exploits the fact that light slows slightly when passing through biological specimens. The specimen is illuminated by a hollow cone of light coming through a *phase annulus* in the condenser. Phase contrast objectives must be used, which have a corresponding *phase plate*. Light rays passing through the specimen are slightly retarded, and further retardation takes place in the phase plate. When these rays combine with rays which have not taken this path degrees of *constructive and destructive interference* occur which produce the characteristic light and dark features in the image.

Differential Interference Contrast

In this complex form of polarised light microscopy two slightly separate, plane polarised beams of light are used to create a 3D-like image with shades of grey. *Wollaston prisms* situated in the condenser and above the objective produce the effect, and additional elements add colour to the image. Care must be taken interpreting DIC images as the apparent hills and valleys in the specimen can be misleading. The height of a "hill" (e.g. the nucleus) is a product of both the actual thickness of the feature (i.e. ray path length) and its refractive index. Variations of the DIC system are named after their originators, *Nomarski* and *de Sénarmont*. Options can be selected to maximise either resolution or contrast. *For a full description of DIC, please use the reply paid card to request a reprint of an article which appeared in Microscopy & Analysis (M&A), September 2006.*

Fluorescence microscopy

Specimens labelled in some way with a *fluorochrome* such as fluorescein or green fluorescent protein (GFP) are illuminated with the relevant wavelength of light (blue in these examples) and emit the energy as a longer wavelength (green). The key feature of fluorescence microscopy is that it employs *reflected* rather than transmitted light, which means transmitted light techniques such as phase contrast and DIC can be combined with fluorescence. At the heart of the fluorescence microscope is the *dichroic mirror cube* which comprises three components: a *dichroic mirror*, an *excitation filter* and a *barrier filter*.

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(3) _____

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Focus lock Koehler Illumination

Phase Contrast Cleaning & Maintenance

Please send me the DIC Reprint from M&A Sept 06

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