OLYMPUS
Your Vision, Our Future

Issue 10

ILLUMIN8

The newsletter for microscope users

Welcome

This issue of Illumin8 focuses on novel life science and environmental research. 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 pair of 8x25 WP I waterproof binoculars

IN THIS ISSUE

And You Think You're **Stressed!**

Mougeotia sp. showing cell membrane stained with FITC (green) after lectin staining and chloroplast autofluorescence at 660nm (red)

Algae report on environmental damage

Algae represent one of the most important photosynthetic groups and contribute significantly to global oxygen production as well as being a potentially important natural environmental detoxification system for aqueous environments (for example heavy metal removal). As a result, abiotic stressors which affect these organisms may have potentially profound effects on the environment.

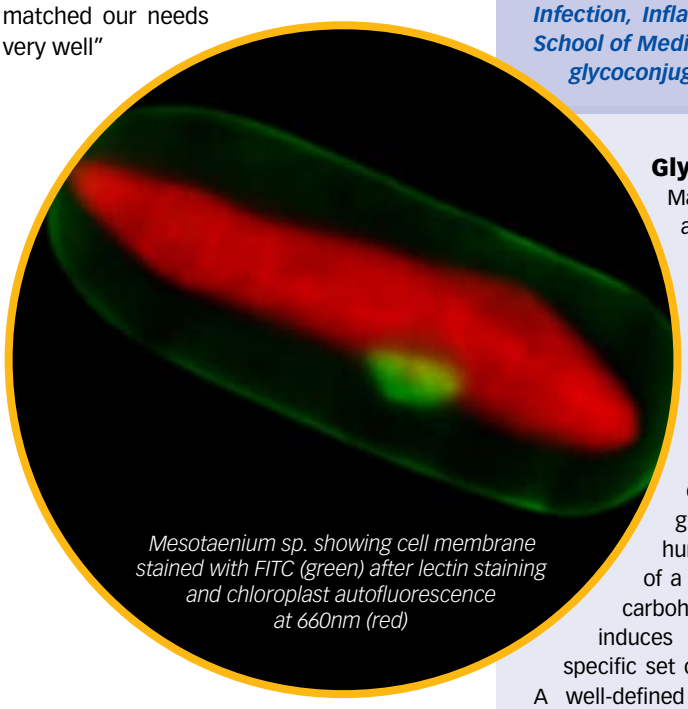
The Paradigm

The algal species, *Mougeotia* and *Mesotaenium*, are abundant in canals and drainage ditches and are the basis of the food web in these environments. Therefore anything that affects their biology or chemical composition will, in turn, affect the rest of the ecological web in these locations including invertebrates, amphibians and fish. Dr Mark Brickley, Director of The Somerset Cell Biology Research Group (SCBRG) commented, "People are becoming more aware of environmental problems, such as chemical accumulation and the increasing levels of damaging UV due to the weakening ozone layer. Both these factors have a direct impact on *Mougeotia* and *Mesotaenium* because they rely on the sun for energy and thrive in places that collect chemical, especially herbicide, run-off." Mark added, "Furthermore, the growing use of genetically engineered crops with tolerance to potent herbicides, increases their use and subsequent run-off, making this a contemporary concern."

The investigation

The SCBRG is studying the effects of high light levels as well as Bromoxynil and DCMU herbicides on *Mougeotia* and *Mesotaenium* in the laboratory. Mark commented, "The aim is to produce a model of the interaction between these abiotic stressors." The group uses an array of techniques to investigate these changes, including HPLC and proteomics. To assess the changes at a subcellular level, the group are using an Olympus FluoView FV300 confocal laser scanning microscope (cLSM). This versatile three channel microscope was selected for its flexibility and cost effectiveness. The FV300 offers two confocal fluorescence detection channels and a third transmitted light detection channel, making it an excellent entry-level confocal system for all applications. Mark said, "We like the simplicity that the FV300 brings to confocal microscopy, and its

versatility means that we can incorporate existing components thus reducing the investment we needed to make." He continued, "We are looking at the 3D morphological changes to algal chloroplasts and mitochondria under different abiotic stresses. To do this we needed a microscope capable of live cell 3D reconstruction at 0.5 µm resolution. Since we only need to use one or two fluorescent markers at a time, the capabilities of the FV300 (two confocal channels and a transmitted light channel) matched our needs very well"

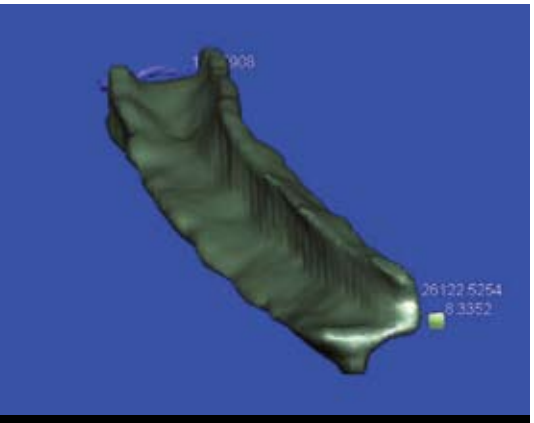


Mesotaenium sp. showing cell membrane stained with FITC (green) after lectin staining and chloroplast autofluorescence at 660nm (red)

Conclusion

Algae sit at the bottom of a complex and necessary food web, therefore the effect of abiotic stresses such as light and herbicides on their proliferation can have a serious knock-on effect for the entire food web. Studying the effects of these stresses requires the use of good quality, entry level confocal laser scanning microscope, a requirement met by the Olympus FluoView FV300. This provides the group with the right level capabilities without being excessive to requirements.

To find out more about the SCBRG or any of the Olympus confocal systems, please fill-in the reply paid card or email **microscopy@olympus.uk.com**



3D reconstruction of intact Mougeotia sp. chloroplast

Not All **Carbohydrates** Are Bad!

A closer look epithelial repair.

The upper airways provide a comprehensive protective barrier to environmental contaminants and contain elements of both the acquired and innate immune systems. The surface epithelial cells also facilitate the clearance of material landing on the airway. In conditions such as asthma, damage to the airway can result in a loss of function or structural integrity and an associated local mucosal activation. Following damage in normal airways, epithelial repair involves the rapid and autonomous migration of the surrounding cells to cover the damaged area. Dr Peter Lackie and his team in the Infection, Inflammation and Repair Division within the University of Southampton's School of Medicine are studying the essential role of cell surface carbohydrates, called glycoconjugates, on these repair processes.

Glycoconjugates

Many aspects of epithelial repair and the effects of disease are not yet fully understood. It is clear though, that many of the important interactions between neighbouring cells and with itinerant cells of the immune system are based on or modified by cell surface carbohydrate moieties called glycoconjugates. For example, human Galectin I (a member of a family of structurally related carbohydrate-binding proteins) induces apoptosis by binding to a specific set of cell surface glycoproteins. A well-defined group of glycoconjugates containing fucose ($C_6H_{12}O_5$) are widely expressed in mammals with several key biological processes attributed to them.

The conformation of the fucose linkage provides a further level of discrimination – the roles of glycoconjugates (complex molecules containing carbohydrates) can only be fully understood if their structures are known. During leukocyte binding to endothelium at sites of inflammation for instance, fucose containing glycoconjugates are specific ligands for E-selectin (a cell adhesion molecule) which is present on vascular endothelial cells. Selectins tether the leukocytes to the endothelium and are essential for the cell crawling and extravasation required for their movement to a site of inflammation. The important role of fucose-containing glycoconjugates, has been further shown by the higher prevalence of asthma and wheezing in the absence of key antigens.

Investigations

The lectin from the mushroom *Aleuria aurantia* (AAL) has a high affinity and is very selective for α1,6 linked fucose found in disaccharides, oligosaccharides and glycoproteins on the surface of non-secretory columnar and basal epithelial cells, but importantly does not bind to the basement membrane. (Lectins are naturally occurring glycoconjugate binding molecules which provide highly selective tools to identify or block specific glycoconjugate motifs.)

Dr Lackie and his co-workers are using this knowledge to conduct a series of experiments looking at wound repair following the addition of AAL. They used a simple protocol where a 'pulse' of FITC labelled AAL is 'chased' with unlabelled fucose to block lectin binding. This proved that AAL seriously inhibits repair while adding fucose enabled the repair processes to happen as normal (providing the fucose is added in the first few hours). They also investigated the fate of the AAL showing that, although it is internalised relatively quickly by the cells, its impact on the repair mechanism is enough to seriously disrupt successful repair.

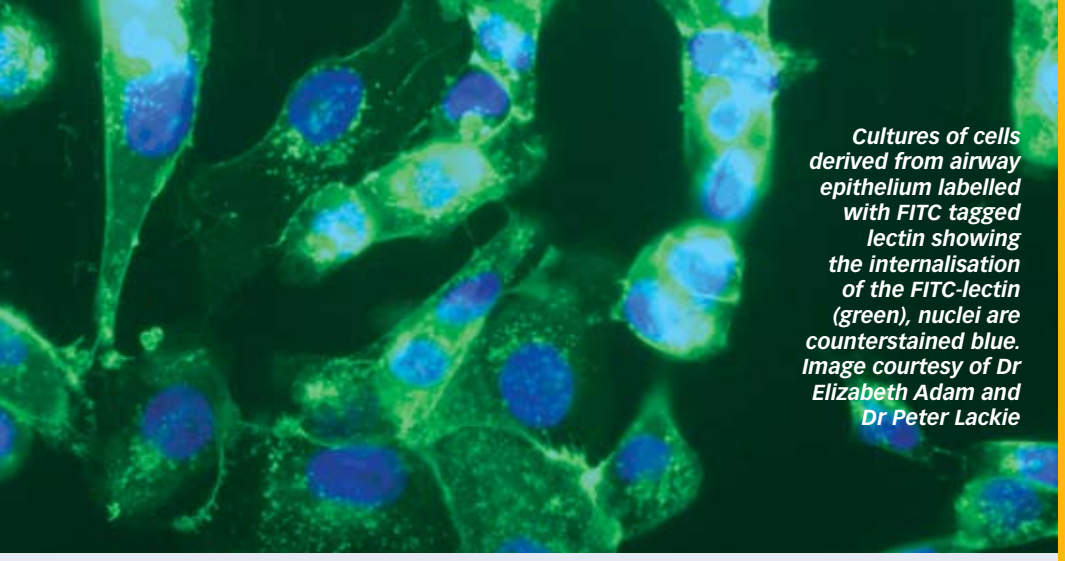
Investigative tools

Microscopes are amongst the most essential tools for any cellular investigation and especially these repair studies. Peter and his team use a number of different microscopes, including a standard Olympus inverted for examining cultures and an advanced motorised IX81 for fluorescence-based time-lapse investigation. This IX81 is fitted with an Olympus FView II high sensitivity, black and white digital camera to record the fluorescent images and is operated via the cell^P software programme. The system is also fitted with an automated stage and a Solent Scientific incubation system to provide environmental control, producing an excellent live cell imaging station for time-lapse experiments.

Time-lapse experiments using the IX81-based system enable the team to follow the molecular and cellular events involved in the repair process, as well as to assess the viability of the cells following repair. Peter commented, "Time-lapse imaging has allowed us to establish the time course of important processes such as the internalisation of the AAL and similar lectins, while confirming that, although the repair process may be inhibited by AAL binding, the inhibition does not affect cell survival rates."

An 'optical bench'

The modular design of the microscope ensures that many components can be added and will work together via a range of different software packages, including cell^P, providing control over aspect of microscopy and imaging required. For Peter's group this



Cultures of cells derived from airway epithelium labelled with FITC tagged lectin showing the internalisation of the FITC-lectin (green), nuclei are counterstained blue. Image courtesy of Dr Elizabeth Adam and Dr Peter Lackie

includes the microscope, nosepiece, stage and imaging. More importantly, the software is designed to allow the easy integration of user's own experimental protocols and to let them run automatically. Peter said, "The microscope set-up enables us to complete multiple time-courses in parallel, which is useful from both time and experimental points of view. We can now maximise our throughput to run between six and eight experiments simultaneously, providing us with a huge amount of data for each different parameter." He continued, "When comparing results from the same 'run', this set-up also allows us to compare identical samples with different experimental treatments greatly reducing variability and increasing the value of our data."

The Olympus IX81 inverted research microscope with Solent Scientific Incubation chamber

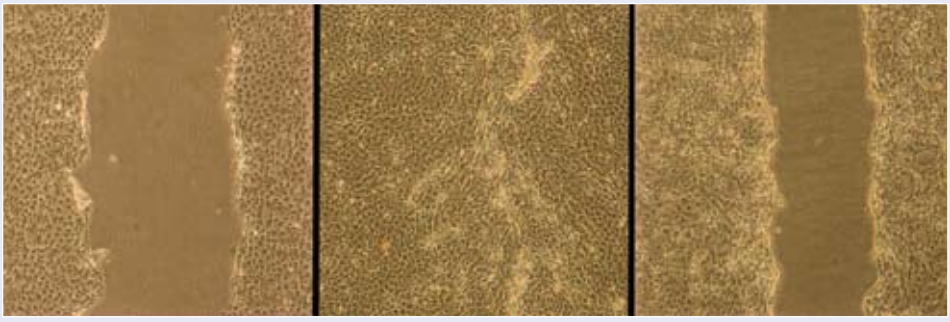
Conclusions

Dr Peter Lackie and his colleagues, Dr Elizabeth Adam and Prof Stephen Holgate, have used advanced microscopy techniques to help demonstrated the importance of α1,6 linked fucose in the repair of damage to epithelial cell layers. They showed that the addition of AAL to damaged monolayers of epithelial cells blocked, or significantly inhibited, the normal ability of the layer to repair. Any delay in repairing minor damage could significantly increase the already heightened risk of infection at sites of trauma. The AAL binding sites therefore appear to have an important role in the normal epithelial repair process. Downstream from their studies, these observations could prove very useful in the identification of novel drug targets for diseases such as asthma.

The value of these microscopical approaches has been recognised by the School of Medicine at the University of Southampton, who provided the funding to allow this equipment to be used as a core facility for research throughout the Medical School. The facility is housed in the Biomedical Imaging Unit at Southampton General Hospital.

Dr Lackie's homepage:
http://www.som.soton.ac.uk/research/iir/air/members/pml1/LackiePM.htm

To find out more about the Olympus IX81, please fill in the reply paid card or email **microscopy@olympus.uk.com**



Cultures of cell derived from airway epithelium, grown until confluent and then damaged (a) after 18 hours the area of damage has repaired (b) except in the presence of lectin when repair is inhibited (c). Image courtesy of Dr Elizabeth Adam and Dr Peter Lackie.

Come and see us

Olympus will be attending a number of conferences, meetings and exhibitions over the next few months:

APRIL

1st-4th: British Neuroscience Association, Harrogate

17th-20th: European Light Microscopy Initiative (ELMI) Meeting on Advanced Light Microscopy, University of York.

18th: University of Leicester Trade Exhibition, Leicester
19th: Memorial Symposium, Glasgow
24th: European Histopathology Forum, Northampton

MAY

1st: West Midlands Cervical QAS, Birmingham
8th: Association of Clinical Pathologists, Chester
9th: Functional Cellular Imaging, Open University, Milton Keynes

For more information about any Olympus products, or to organise a meeting with an Olympus representative at one of these events, please fill in the reply paid card or email **microscopy@olympus.uk.com**

Olympus Partners with Nature

Researchers given free access to major fluorescence imaging papers

Olympus Microscopy has partnered with the Nature Publishing Group to present the "Nature Reviews Fluorescence Imaging Collection". This compilation of leading articles from the Nature's portfolio of Reviews journals has been carefully selected to support cell biologists using fluorescence microscopy techniques in their research. Featured research includes:

- Cleavage pattern and emerging asymmetry of the mouse embryo, Magdalena Zernicka-Goetz
- The multiple uses of fluorescent proteins to visualize cancer in vivo, Robert M. Hoffman
- In vivo imaging of the diseased nervous system, Thomas Misgeld and Martin Kerschensteiner
- High-throughput fluorescence microscopy for system biology, Rainer Pepperkok and Jan Ellenberg

This partnership is the latest in Olympus's ongoing programme of activities in support of the scientific community.

For more information about Olympus Partner with Nature, please fill in the reply paid card or email **microscopy@olympus.uk.com**

Competition time: A chance to win a pair of **Olympus 8x25 WP I** waterproof binoculars

To be in with a chance of winning a pair of professional Olympus 8x25 WP I binoculars, you will need to answer the three questions below correctly on the reply paid card and return it to us by the 1st May 2007.

Question 1:
Which confocal system is being used to investigate abiotic stress in algae?

Question 2:
Dr Lackie is using which microscope for fluorescence-based time-lapse investigations?

Question 3:
Where did the recent Teenage Cancer Conference take place?

Congratulations to James Shipp from the Dept of Technical Service, Scapa UK limited, for winning the Olympus μ [mju:] 1000 digital

Finding Their **Sense of Tumour**

Teenage Cancer Conference

The Teenage Cancer Trust (TCT) is a charity devoted to improving the lives of teenagers and young adults with cancer, a severely medically and socially underserved group. TCT achieves its goals through a number of initiatives, all dependent on private fundraising efforts that range from high profile concerts and sponsored treks to local community events and personal charitable giving. The TCT sponsors a Teenage Cancer Conference so that participants from all over the country can attend free of charge.

Sue Morgan, Lead Macmillan Clinical Nurse Specialist, is one of the professionals who organise the conference entitled 'Find Your Sense of Tumour' – a name chosen by teenagers with a dark humour! This year's conference was held from the 2nd-5th March at the CenterParcs Resort near Nottingham. Sue commented, "Every 18 months we organise this conference for around 500 'teenagers' (aged 14 -25 years) – around 250 of those will have cancer and they can bring a friend or a sibling with them. The conference provides access to the most up to date information and talks from professionals who are world experts in treating cancer." Sue continued, "The aim is clear: to inform these young people, give them a platform to tell us their views and to provide them a forum where they can meet other young people who are in the same position as themselves - and to have fun!"

This year, Olympus provided several microscopes including one equipped with a digital camera and projector to allow the children to see the cancer cells causing their illness. Sue said, "Before they look at their cancer they imagine it to be black, mouldy with evil eyes and teeth! After they have seen it they see that it is pink and, at times, beautiful and it makes them feel psychologically better about having the disease. One young man had always imagined that his leukaemia was 'aliens' that controlled him – his life has turned around now that he knows they are like 'beautiful planets!' – Not an alien in sight!

Further information about the TCT and the conference can be found on the website **www.teenagecancertrust.org**

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(2) ______________________

(3) ______________________

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