

ILLUMIN8

The newsletter for microscope users

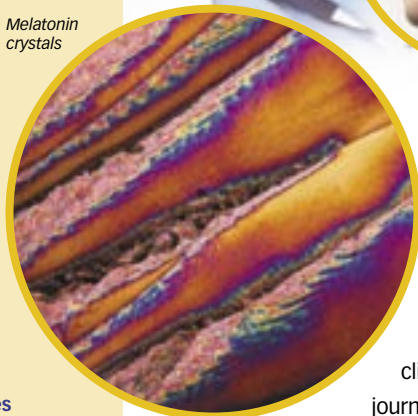
Welcome

to the second issue of 'Illumin8'. Taking over from our previous publication 'Objectives', Illumin8 is full of handy tips and interesting stories specifically for people who use microscopes on a day-to-day basis. We really want to hear about what you think should be covered in future issues. So if there's something interesting that you're doing, or you've got a great tip that you want to pass on, simply email microscopy@olympus.uk.com or fill in the reply paid card. And if you don't get your own copy of Illumin8, ask for it by using this email address or the reply card. We hope you enjoy this issue.

Melatonin crystals

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Dr David Barlow



The face that launched a thousand clips

You will most probably have never heard of Dr David Barlow, but many of you will have seen at least one of his TV film clips. If you've enjoyed a journey down a blood vessel or watched in amazement as you were taken through the valves of a pumping heart, then you have definitely seen his work. Dr Barlow is one of the most accomplished and respected biological film makers and has pioneered some innovative techniques to convey the complexities of biology in an understandable way. He has won two awards from the Royal Television Society, the Lennart Nilsson Award for scientific photography and has been nominated for a BAFTA.

He developed his unique filming skills during his PhD at the University of Southampton, where he was studying the locomotion of insects. This research work involved filming with microscopes and high speed cameras. It took him initially into natural history film making, shooting sequences for series such as the BBC's 'Natural World', 'Horizon' and 'Wildlife on One'. With Channel 4's 'Living Body' series, David branched out into medical subjects and started mixing straight scientific photography with accurate simulation to cover "unfilmable" sequences. The chairman of the Lennart Nilsson award committee, Michael Peres, noted that "David is an inventor on a lot of levels. He's created things that don't exist... nobody has made pictures like he has". Joseph Breaan of the Canadian National Post said "Until he devised ways to animate X-rays, film hairs growing and build scale models



David with his Olympus Vanox microscope

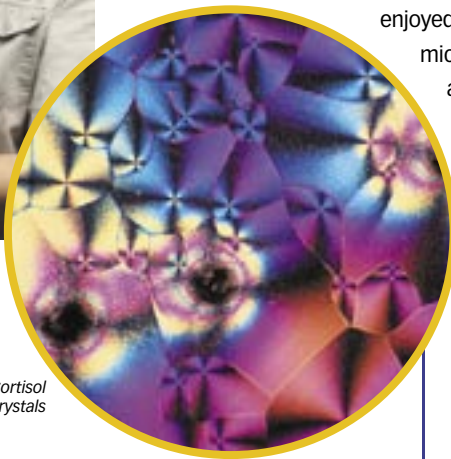
of hearts, few photographers have been able to capture the body's internal machinations". His creative scientific approach continues to this day and has been one of the reasons for his continued success and worldwide recognition.

David puts his success down to his scientific background. "I believe that my ability to film actual bodily processes or make realistic simulations has stemmed from my biology background – I am a scientist first and foremost, and a cinematographer second. I have also always

enjoyed filming using microscopes, and although 'special effects' filming is taking up more of my time, I still look forward to setting up my

Olympus Vanox microscope and filming real events. These are also the hardest to capture properly, since I have no real control over yeast fusion or when a blastocyst divides for example".

The BBC large format (IMAX) version of 'The Human Body' again pushed the envelope of scientific filming, with one powerful scene showing a sperm fertilizing an egg in real time, filmed at high resolution. Peter Georgi director and producer commented "we think it is the greatest purely optical magnification (70,000-80,000 times on the IMAX screen) in motion picture history". Most recently Dr Barlow has completed special effects sequences for the C4 film 'Krakatoa' and unique microscope footage of blastocyst development for the C4 programme 'In the Womb'. He is currently working with his microscope on a follow-up to 'In the Womb', which will once again push the frontiers of biological filming.



Cortisol crystals

Don't be in the dark



To help you make the most of your microscope we have produced a range of 'Illumination' leaflets on key points such as Cleaning & Maintenance, Phase Contrast and Nomarski DIC. Also available is a poster to help you select the right objective for your needs in upright, brightfield microscopy. To request leaflets or posters, please fill in the reply paid card or send an email to

microscopy@olympus.uk.com

We would also like to know what topics you want to see both within this newsletter and as separate support material such as leaflets and posters – so please send us your feedback in the same way.

Erratum:

In Illumin8 Issue 1 (July 2005), the main figure on page 1 was incorrectly labelled. It should read 'Abnormal endocervical cells from an LBC preparation - courtesy of Andrew Evered, Welsh Cytology Training School.' We apologise for any confusion this may have caused.

The 'Illumination' leaflets cover a wide range of important 'How to' topics

Must visit events in September

The Biomedical Science Congress - the most important exhibition for hospital scientists



From 26-28th September, the ICC in Birmingham is once again the venue for the Biomedical Science Congress (www.ibms.org). This year the IBMS will again be running their successful 'parallel sessions' formula to

ensure there is plenty of interesting content on offer at any given time. All eight scientific disciplines are fully represented, with their own series of lectures, workshops and interactive sessions. The scientific content is complemented by education and management sessions covering a range of current issues.

The 44th Annual Scientific Meeting of the British Society for Clinical Cytology (BSCC) is being held at the Quayside in Gateshead from 11th-14th September 2005 (www.bsccevents.co.uk). As well as the customary trade show and society dinner, an extensive list of workshops is being run alongside the topical, stimulating and educational scientific programme. This will include acclaimed speakers from the UK and abroad on a variety of topics of interest in the field of gynaecological and non-gynaecological cytology.

Olympus is exhibiting at both events, so please visit us at our stands.

Matching your light source, microscope resolution and digital camera can be the difference between catching an event and missing it. All three are available in many different types and specifications, but combining them to create the perfect system is not always a case of buying the most sophisticated instruments.

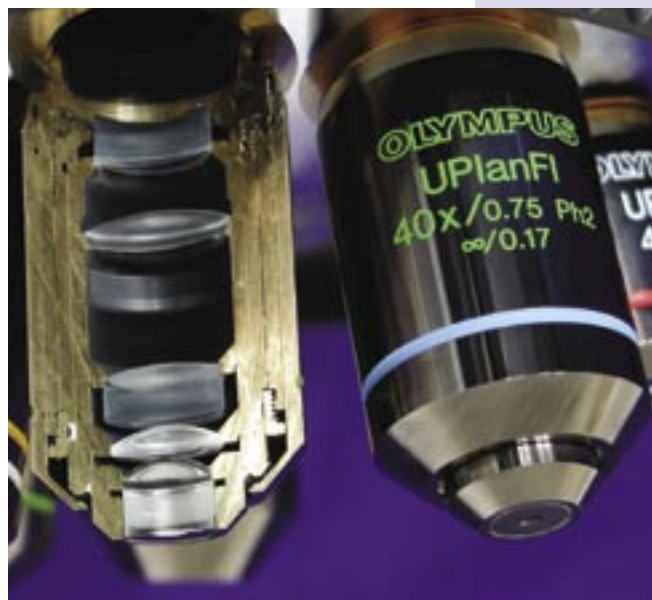


Figure 1

See the light

Most microscopes come supplied with voltage-controlled tungsten-halogen light sources. These provide a good level of illumination for the majority of routine brightfield applications. At a high voltage a tungsten-halogen bulb may give a slightly blue tinted light, but at a low voltage it will be yellow or red tinted. By using neutral density filters light intensity can be varied without changing voltage, so maintaining a constant colour temperature.

Another common light source for some microscopy techniques is the mercury burner, which has illumination peaks at 313, 334, 365, 406, 435, 546 and 578nm. This feature means that it is the most used light source for fluorescence applications. Whenever the peak emissions of the burner match the excitation needs of the fluorochromes, a good signal can be achieved (depending on the specimen).

Xenon burners are the first choice light sources when very bright light is needed, moreover they show an even intensity across the visible spectrum.

Reaching your objectives

The single most important part of any microscope set-up is the objective lens. Using the correct one is essential for getting a decent optical image for screening and observation, but also for recording that image using analogue or digital cameras (and other detection means such as photo-multiplier tubes). Objectives are very complex arrangements of lenses, available in different magnifications, in addition to a range of chromatic corrections. The result is a set of values on each objective that may at first appear slightly confusing. See Figure 1, an example of an Olympus objective.

The power to resolve

Resolution is defined as the ability to distinguish two closely-spaced points.

In the transmitted light microscope, the resolution is determined essentially by three parameters: the wavelength of the illuminating light, the numerical aperture (NA) of the objective and the NA of the condenser. Note that resolution is not directly dependent on the magnification. Furthermore the end magnification should not be higher than 1000x the NA of the objective, because then the image will be only enlarged but no further resolution will be visible (empty magnification).

The numbers game

So with the light source providing the correct wavelength and evenness of illumination (either with or without filters), the resolution of the system comes down to the NA of the objective and condenser. Of these, the most important consideration is the NA of the objective, which can be basically described as a measure of its ability to gather light and thus resolve fine specimen detail.

Candid cameras

There are key differences between a high street digital camera and the ones used on microscopes to view and record images. It is therefore advisable to use a camera tailored to microscopy for recording images faithfully. Such cameras are now available in a whole range of sizes, capacities and technologies including cooled and uncooled electronics and with the ability to record up to 12 million pixels. It maybe surprising to learn therefore, that as the resolution of the objective lens increases, the necessary resolution of the digital camera actually decreases. This is a very important fact to bear in mind, since it is often necessary to take pictures quickly at high magnifications to catch short lived events – the higher the resolution of the captured image, the longer it will take to be processed by the camera and then stored by the computer. If the digital image size is decreased, there is no loss of resolving ability, but there will be a significant increase in the speed of capture – perhaps the difference between capturing or missing that important image. Table 1 shows the suggested resolutions for digital cameras with ½ inch chips when using different microscope magnifications. This equates to a camera capable of recording 4 million pixels for the lower magnifications and under 1 million pixels for the higher magnifications.

Table 1

Objective	N.A	Necessary resolution
PlanApo 2x	0.08	2289 x 1717
UPlanApo 4x	0.16	2289 x 1717
UPlanApo 10x	0.4	2289 x 1717
UPlanApo 20x	0.7	2003 x 1502
UPlanApo 40x	0.9	1288 x 966
UPlanApo 100x	1.4	801 x 601

Conclusions

Picking the correct combination of light source, objective and digital camera can make a huge difference to a recorded image, but need not make a big dent in your budget. For more advice on creating your own fully-optimised Olympus imaging system, fill in and return the reply paid card or email us at

microscopy@olympus.uk.com

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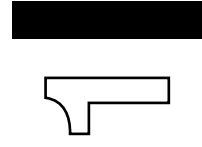
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Win an Olympus VN-240 PC voice recorder

Each issue we give you the chance to win an Olympus branded prize. This issue we're giving away an Olympus VN-240 PC voice recorder, so you can record all your 'Eureka' thoughts as you have them. For your chance to win, all you have to do is answer the following questions correctly on the reply paid card and return it to us by the 3rd October 2005.

We are pleased to announce that last month's draw prize was won by Glenda Monson of Bedford Hospital.



Olympus VN-240 PC
voice recorder

Question 1:

How many illumination peaks does the mercury burner have?

Question 2:

Using a PlanApo 100x objective, what is the necessary resolution for digital camera image capture?

Question 3:

Which model of Olympus microscope does Dr David Barlow use?

SHORT TIPS

Breaking glass

Have you ever experienced the alarming feeling as the objective goes "crunch" through your microscope slide? Most of us have. What many of us don't realise is that microscope makers fit a device to prevent this - the Focus Lock.

The majority of modern microscopes have a lever or similar mechanism which, when operated, restricts the movement of the coarse focus control. By setting the lock after focusing with a low power objective you can

safely move a high power objective without worrying about breaking the slide. The fine focus is not affected by this process.

A full explanation of the focus lock and its use will be available shortly in the "Illumination" series.



Optical Personalities

No one can be exactly sure when man first discovered the optical properties of solids and liquids. However, with the writings of classical scholars we have an excellent chronology for some of the stages on the road to the development of the modern microscope. Here are some of the key moments:

Aristophanes (450?-388? BC) – in one of his satires, a character reflects the sun's rays to secretly melt an I.O.U. on a wax tablet

Democritus (460-370 BC) - attempted to explain perception and colour

Aristotle (384-322 BC) – rejected the Euclidian theory that vision was solely due to rays emanating from the eyes and "touching" the object

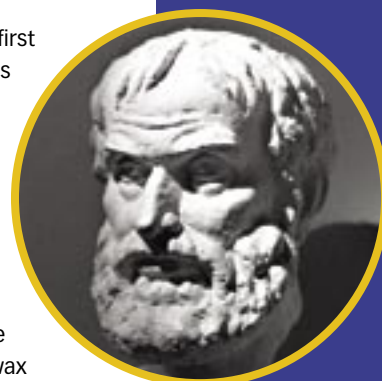
Archimedes (290?-212? BC) – wrote about catoptrics (reflections from surfaces) and refraction

Lucius Annaeus Seneca (4 BC-65 AD) - wrote about the magnifying effects of liquids in transparent vessels

Nero (37-68 AD) - used an emerald while watching combat in the arena - the first recorded incidence of the use of a monacle

Ptolemy (85-165 AD) - investigated refraction and obtained the small angle approximation to Snell's law, concluding that the ratio of the angles of incident and refracted light were constant

Alhazan (965-1040 AD) - researched reflections from spherical and parabolic mirrors and disproved Ptolemy's law of refraction, disagreeing also with his theory of vision



Aristotle

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Our thanks to Professor Taylor at the University of Maryland for helping to compile this list