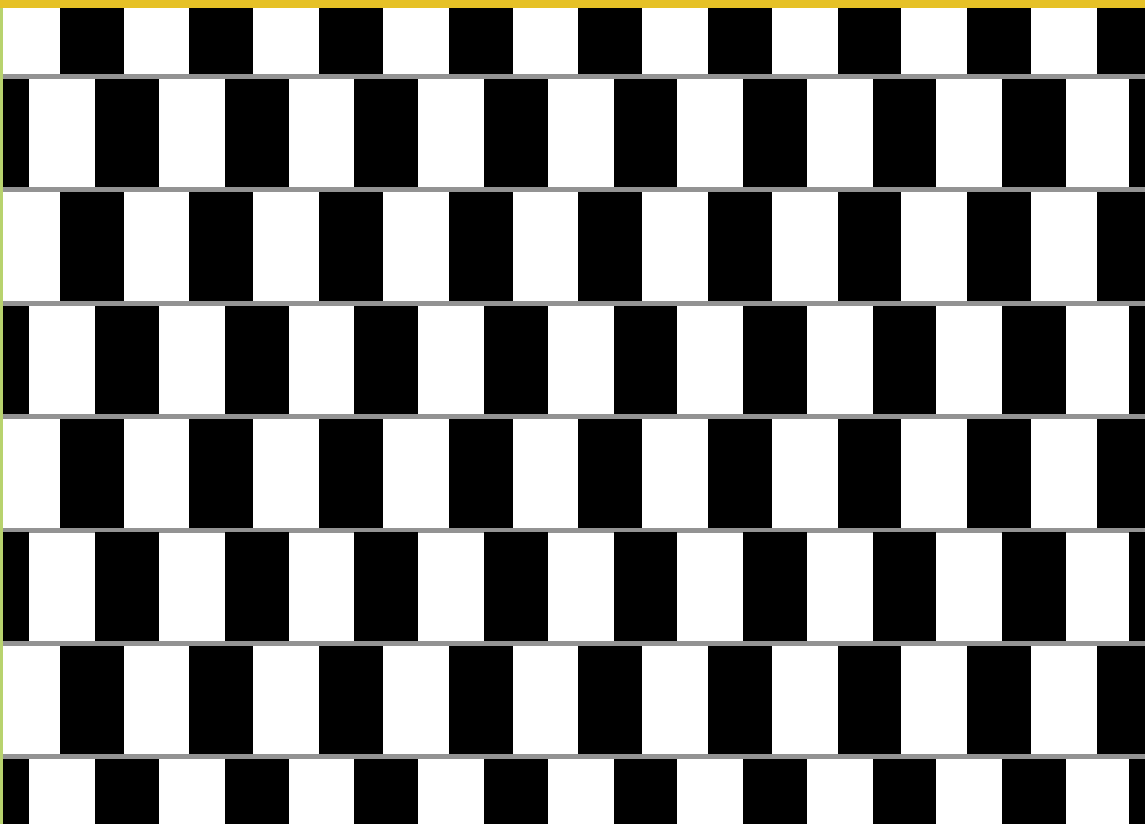
OLYMPUS
Your Vision, Our Future

Issue 3 • November 2005

ILLUMIN8

The newsletter for microscope users



An example of 2D illusion - the 'Cafe Wall'. Courtesy of Professor Richard Gregory

Welcome

to the third issue of 'Illumin8'. This is a newsletter written especially for microscope users and we want to know what you would like to read about. Moreover, if you are doing some 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' and supplementary material such as our 'Illumination' booklets and poster series. We hope you enjoy this issue and don't miss our competition to win one of five 512MB Olympus xD Memory cards.

IN THIS ISSUE

Seeing is believing

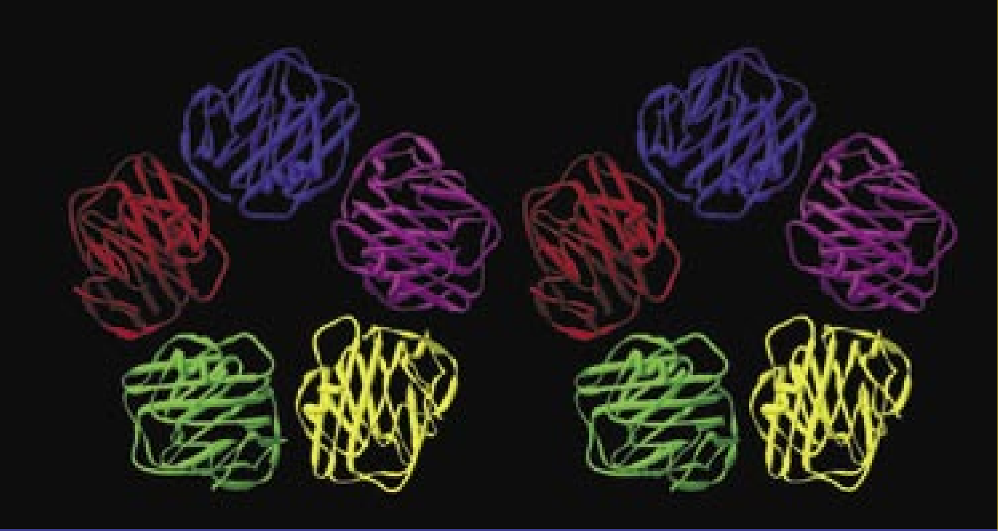
For the majority of people reading this article, their eyes work in perfect unison. This harmony generates our perception of depth, which not only allows us to judge relative sizes, but also to estimate distances and thus accurately reach for objects or drive a car for example. More commonly, we say that humans see in three dimensions (3D): height (X), width (Y) and depth (Z). Most microscopes though, generate flat, 2D images which can sometimes provide a significant amount of extra data if software is used to reconstruct a 3D image.

Acquired, not innate

The ability to 'see' depth is generated by each of our eyes viewing an object from a slightly different angle – so the brain receives two offset views of the same object and has to merge them seamlessly. The neuronal circuitry producing the stereo view is extremely complex and unique to each individual. For this reason, stereo vision is not innate – it must be learnt and this is usually achieved in the first year of life. For example, you may notice babies reaching for their toes and eventually being able to grab them – they receive sensory feedback when they have actually touched them, so their eyes and muscles become accustomed to the X, Y and Z movement planes.

It's all an illusion

The intricacies of human vision have been extensively studied and many experiments have shown how the brain interprets both 2D and 3D images. For example, some fascinating 2D illusions confuse our brains into seeing static images as if they are moving, or parallel lines as if they are converging. It is also possible to produce stereo illusions – as typified by 'magic eye' images. These involve using a process known as parallel viewing, where the hidden 3D image only appears when



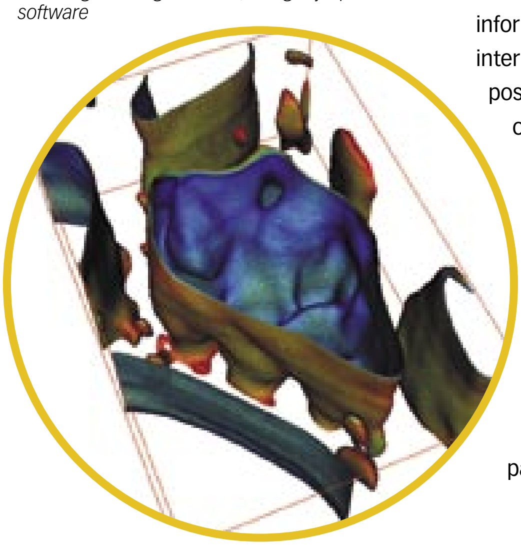
'Cross-view' stereo image of the C-reactive Protein (CRP) structure: cross your eyes and focus on the central, 3D image that appears. Courtesy of Dr Neil Ravenhill

focusing beyond the plane of the 2D image. A more natural method of seeing images in stereo is to use cross-viewing, where two images taken at offset angles are merged into a third 3D image by crossing your eyes (as shown by the molecule image above). The most common way of generating the illusion of depth though, is by creating anaglyphs (as used in 3D movies) where two offset views are displayed on top of one another, one in blue/green and one in red. By blocking each eye from seeing one of the coloured images with blue/green or red filters, the brain can generate a stereo view without having to focus in front or beyond the image.

Designed for stereo

With humans designed primarily for seeing in stereo, 2D images can sometimes be harder for us to interpret since we have no depth information. The methods mentioned above work very well for printed or displayed images, but not for viewing down a microscope and therefore most light microscopy is carried out in 2D. Stereo microscopes (such as the Olympus SZ2 and SZX series) can be used for larger objects where meaningful thin slices cannot be produced. This is due to them maintaining two separate light paths and hence two offset images. Above a certain magnification though, it is not easy to maintain two separate paths and therefore stereo visualisation is not possible. Moreover at high magnifications the depth of focus is too shallow to permit depth perception.

3D reconstruction of a cell from a clematis stem with neighbouring cell walls, using Olympus software



Reconstructive power

With more advanced microscopy techniques, cells can be stained or biochemically tagged with probes and dyes. This means that a 3D picture of a sample can be reconstructed by taking a series of 2D (X,Y) images at known depths (Z) and rebuilding a 3D approximation using Olympus software. Imaging a sample in 3D allows a user to more accurately assess the proximity of items of interest, which in 2D may appear on top of one another. This is very important for investigations into protein co-localisation, where a 2D image may show a clear overlap but a 3D image would show otherwise.

Conclusions

We see in stereo to generate 3D images because our world has depth. Some 2D microscope images therefore lack certain key information which may be essential to the interpretation of some samples. It is therefore possible to use either stereo microscopes or software reconstruction of 3D images to create a more representative picture of the sample in view. To find out more about the stereo microscopes or software packages available from Olympus, or more specifically on reconstructing 3D images using Olympus software, email us on **microscopy@olympus.uk.com** or fill-in and return the attached reply paid card.

A microscope for all reasons

The new Olympus MVX10 'Macroview' Microscope

Choosing a microscope best suited to the work of a laboratory is not always an easy task. With so many accessories and add-ons, such as the plethora of different objectives and illumination sources, one must find a balance between specialised and general applications. Before reaching a decision on additional items though, the single most important choice to make is the type of microscope – Upright, Inverted or Stereo. This will then not only influence your choice of add-ons but also determine the flexibility of your imaging capabilities.

Classically, the lines dividing these three microscope types are very clear. For anything requiring high power magnification, the choice is reduced to either upright or inverted, since stereo microscopes are generally used for low power observation in 3D. The choice between upright and inverted though, is traditionally down to the type of object being observed. For fixed cells on slides, be they unstained, stained or labelled, it is more common to use an upright microscope (such as the Olympus BX and CX series), whereas for cells in liquid culture an inverted microscope (such as the Olympus IX and CKX series) is more suitable since the objective can focus on the cells through the bottom of the culture vessel.

With some microscopes though, the distinction is not so clear. A unique new microscope – the Olympus MVX10 – is capable of brightfield and fluorescence observation on whole organisms. This is ideal for work on common research species such as: *A. thaliana, Xenopus spp., M. musculus, D. rerio, C. elegans and D. melanogaster*, generating much better images and data.

Many heads
are better than one

Biomedical scientists using microscopes for cytology screening undergo extensive and ongoing training at regional schools. To provide a better learning experience and meet the requirements of the National Health Service Cervical Screening Programme (NHSCSP)[1-5], these establishments use multi-header microscopes suitable for at least 5 participants.

For Training...

Although highly desirable, a large multi-head microscope takes up a significant amount of space and can only be accommodated in regional training schools. The largest Olympus multi placement is an 18 head microscope at the Sheffield Cytology Training School. There is though, a growing impetus behind the placement of smaller multi-header set-ups in every establishment conducting clinical screening, especially those using liquid-based cytology (LBC). Andrew Evered, General Secretary of the National Association of Cytologists (NAC) and Manager of the Welsh Cytology Training School is a firm advocate and said "I am asked to visit many establishments to conduct ongoing training programmes for various numbers of staff. Due to the requirements of the NHSCSP, any training I provide must include a significant amount of live microscope work on multi-header systems." Andrew continued "I used to transport a system with me, but this has become impractical. I can therefore now only conduct certified on-site training at establishments with their own facilities."

...and much more

Dr Lesley Turnbull, Director of the Liverpool Cytology Training Centre, strongly believes that multi-header microscopes are not just a training tool. She commented "In addition to any training requirements, the presence of a multi-header system at a screening laboratory would make a big difference to all levels of staff. Many cytology slides require one-to-one and group discussions, and although most consultants have the equipment required for one-to-ones, very few establishments have adequate facilities for groups." Dr Turnbull continued "Local training sessions also take on a different perspective when carried out on a multi-header and thus become much more effective."

Head Count

As mentioned above, the NHSCSP recommends that for accreditation, a training establishment should be able to accommodate at least 5 people on its multi-head facilities[1-5]. With all cervical screening set to be carried out by using LBC procedures by 2008, the necessity for local multi-header set-ups will increase, since ongoing training will be even more essential until all staff become fully proficient. In Wales, where LBC has been fully integrated, the experience of the training staff such as Andrew Evered will be of great value to the rest of the UK. Andrew remarked, "I would encourage most screening units to accommodate a six place multi-head microscope on the grounds that it will be one of the most valuable purchases they will ever make." He continued "The multi-header set-up we have at the Welsh Cytology Training School is used everyday by the cytologists in Llandough Hospital and has made a real difference to their efficiency." Dr Turnbull is of the same opinion, saying "A small multi-header microscope set-up would definitely enhance the local provision of LBC, and when our 14 head training microscope is not in use for classes, it is being constantly used for live case discussions".

References

1. Qualifications and training for non-medical laboratory staff in the UK cervical screening programmes. NHS Cervical Screening Programme (2000). NHSCSP Publication No 12.
2. Cervical Cytopathology Training Log, 2nd edition. NHS Cervical Screening Programme (1999).
3. Accreditation Handbook (Version7). Clinical Pathology Accreditation (UK) Ltd (1999).
4. Achievable Standards, Benchmarks for Reporting and Criteria for Evaluating Cervical Cytopathology. NHS Cervical Screening Programme (1995). NHSCSP Publication No 1 (currently under revision).
5. Guidelines for Introductory Courses in Cervical Cytopathology. Joint BSCC/IMLS Working Party (1990).

Group discussion of an interesting case by the staff at Llandough hospital. Courtesy of Andrew Evered

Win one of five Olympus 512MB xD **Memory Cards**

With each issue we offer the chance to win a different product from the Olympus range. This time, by correctly answering the three questions and returning the completed reply card by 30th November, you can win one of five Olympus 512MB xD memory cards, essential for many digital cameras such as those in the Olympus range.

We are pleased to announce that the winner of the VN-240 voice recorder from the last issue of 'Illumin8' is: Louise Hall of the University Hospital of North Tees

Question 1:
How many 'heads' has the largest multi-header microscope set-up in the UK?

Question 2:
What do the initials LBC stand for?

Question 3:
The Olympus inverted microscope range consists of which two series?

SHORT TIPS

Seeing **Straight**

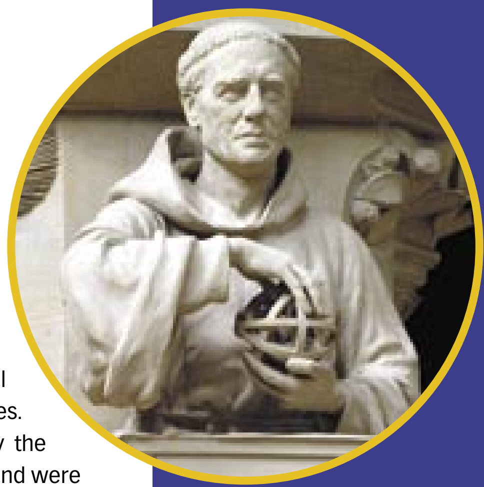
If peering down a microscope gives you a headache, here are some tips on setting up the binocular head and eyepieces to suit you.

1. Interpupillary distance. To set the distance correctly, first move the eyepieces too far apart, look down and then move them together slowly until a single, comfortable image is seen.

2. Eyepiece focusing. First find the eyepiece that doesn't have individual focusing and focus the microscope whilst looking down this side. Next look through the second eyepiece with the other eye and make adjustments using the focus ring on that eyepiece.

Finally, if you wear spectacles we recommend that you keep them on while looking down the microscope, especially if you are astigmatic.

Farsighted individuals


Statue of Roger Bacon at the Oxford University Museum of Natural History

In the last issue of Illumin8 we reviewed the 'Classical personalities' involved in the early development of optical theory. This time we look at those who started to apply this to correct visual defects – the first consistent use of lenses. Although eyeglasses were first used by the Chinese, they contained coloured glass and were only for adornment or supposed magical powers. Corrective eyeglasses appeared first in Florence (traditionally assigned to the work of Alessandro di Spina) in about 1280 and their use spread rapidly. The following individuals played a large part in the development of glasses:

Roger Bacon (1220 - 1292) made the first recorded reference to the magnifying properties of lenses in 1262, having carried out some experiments with lenses and mirrors and described the principles of reflection and refraction.

Johannes Kepler (1571 - 1630) provided a correct explanation of vision and the functions of the pupil, cornea and retina. Also, after more than three centuries, he gave the first correct explanation of how eyeglasses work.

Benjamin Franklin (1706 - 1790) invented bifocal glasses.

Sir George Biddell Airy (1801 - 1892) was the first to use a cylindrical lens to correct (his own) astigmatism. Airy made other contributions to optics and the diffraction pattern of a circular aperture, which he derived, is named after him (Airy discs).

Adolf Eugen Fick (1829 - 1901) was one of the first people to actually experiment with contact lenses on animals and then, finally, fit contact lenses to human eyes.

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