Confocal and multiphoton microscopy

Stuart Townsend, of the bioimaging team at MRC Harwell, explains the use of confocal and multiphoton microscopy to create images.

ST: Whereas a conventional light microscope, when you look down it, will give you information, there's a lot of excess fluorescence that gets in the way, it gets into the picture and the way the confocal works is it removes this excess fluorescence and you get a really nice clear image.

Well it's essentially a standard fluorescence microscope but instead of using a white light source like a light bulb to illuminate your sample, you're using lasers of various colours to illuminate your sample and to get the information from the sample. The tissue you get will arrive, probably on a microscope slide, maybe in a dish, which you put on to the microscope. You would probably view it through the microscope first of all, just to orientate yourself and get the sample in the right place. You would choose the laser or the lasers that you're going to use to excite the sample. Normal biological material you can only go perhaps a hundred microns or so through on a conventional confocal; if you go into multiphoton, you can go further, and multiphoton microscopy uses lasers but it uses a red laser in the near infrared, it uses a longer-wavelength laser. This gives the ability to penetrate deeper into tissue, but also, because you're using a red laser, you don't cause as much damage to a specimen. An image will appear on the screen; you can then move it around, focus it, move up and down until you collect the image that you're happy with and then you save it. You're also able to optically slice through a sample – a bit like going up in a lift I suppose. You go through a sample stage by stage by stage, and you can go all the way through and get a series of optical slices, which you can reconstruct later on to produce three-dimensional images.

The lasers have all got colours and the fluorescent compounds that you're looking at, the markers you're looking at have got colours, but the image comes out black and white, so you just have to add a false colour and it's – you're just adding false colour to the image.

You can't say what makes a perfect confocal image because it depends what tissue you're looking at; you're looking to be able to see structures within the cell, you're looking for things that you recognise.

Right, this is an image of some muscle, mouse muscle tissue, but it's been stained with three different fluorochromes: the blue is detecting the nucleus and the green and the red are staining other parts of the muscle. It's been acquired using three lasers, a laser to excite each of the three colours, and once you've got your three separate images you can merge it to give all the three together – and just something you just couldn't see with any other conventional microscope and that's what makes confocal so good.

[End of transcript]

183 Euston Road London NW1 2BE UK
T +44 (0)20 7611 8348 F +44 (0)20 7611 8577 E images@wellcome.ac.uk
www.wellcomeimageawards.org images.wellcome.ac.uk

wellcome images

wellcome collection