

High-resolution episcopic microscopy

Dr Tim Mohun, senior scientist at the Medical Research Council National Institute for Medical Research, explains how to create three-dimensional images using high-resolution episcopic microscopy.

TM: So the conventional procedure for studying the structure of tissues or embryos is to use histology, and this is a process which has been used for well over a century and involves embedding the tissue in a block of material – either wax or plastic – and then cutting very thin sections or slices of the tissue. Those slices can then be looked at down a microscope, they can be stained to look at individual structures and that then gives you an image of that particular slice of the tissue. The problem then is how to build that series of images into the three-dimensional structure.

When you cut slices from your tissue and stain then an examine them down the microscope, those slices become slightly distorted, and they distort in ways that's a little bit unpredictable but it means that it's very difficult to recombine them back accurately. Also when you're recombining these slices together, or images of slices, it's very difficult to know how exactly you should align them with respect to each other. The net result has been that if you want to build a 3D model from this sort of data, that retains the level of accuracy and resolution that you get on your individual images, it's essentially proved to be impossible. And so that's really the problem we faced when we were looking at a series of slices through, at the time it was the developing frog heart, trying to understand the structure of the heart. In collaboration with a friend of mine, Wolfgang Weninger, who works in Vienna, in the anatomy department, we came up with a solution to these problems.

The method that we have ended up using takes the simple route of eliminating the problem of aligning individual images. Instead what we do is we look at the surface of the block of the tissue that we're cutting and capture an image of that. We then remove a slice, capture a second image and that second image is of course in perfect alignment with the first image because it's simply a view of the surface of the block. And you can imagine that if you do that successively, slicing all the way through your tissue, you will end up with a series of images that are in perfect alignment.

So here is the block moving past the blade, a section is being cut. We have a small fan here which blows the section back to be collected in this netting, and that's to enable us to take a photograph of the surface without a section obscuring the block surface. And an image is captured on screen here on the computer. It's going to take quite a few hours of automated actions by the machine to capture the data set.

You can see the samples are red in colour because of the dye that we put inside the plastic. We put the dye in the plastic because that dye is very fluorescent. When we view the block surface using fluorescent light, that dye fluoresces and appears very bright. Wherever there's tissue, that fluorescence from the dye is blocked and so the net effect is on the surface of the block we get a positive image of the tissue. So if you want to model the data, all you have to do is reduce the size of your images and then put them into – there are a number of different software packages available now, which enable you to model data sets on desktop computers. We've now reached the stage in developing the technology that we think that this could be a very useful imaging

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procedure, producing data that could be very useful for a wide range of studies and that's very exciting.

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