

Light microscopy

Spike Walker, microscopist, explains the process of light microscopy.

SW: I was given my first microscope when I was a 12, a tiny thing, and then a larger one a few years later, and it was then that I decided to take photographs, largely because, while people whose interest was football could easily strike up conversations even with complete strangers on buses about whatever Tottenham had done over the weekend or whatever, my enthusiasm, which was largely at that time for things that lived in dirty water, was not something that was intelligible to other people, and I thought if I take photographs I can at least show people, even if it isn't strangers on buses, and that's what happened.

This is an example of a fairly simple microscope, this would be an advanced student's microscope, probably from the 1930s, 1940s, and you can fairly easily see its components. These are the objective lenses, these are the things that put the detail into the image, and they also do some magnification and usually on this turret or nose piece you've got three, four, five of these objectives of different magnification and with different resolving powers. And those objectives are on the end of a tube, which traditionally was six inches long, and in the top there's an eyepiece and the eyepiece merely magnifies the image produced by these objectives.

Here we have the stage; now underneath the stage we've got the sub-stage, and the sub-stage has a condenser in it and that has the job of concentrating the light onto the specimen. Underneath there is an Irish diaphragm, and people sometimes use that for making the image darker or lighter, but its real job is to control the behaviour of the light as it goes through the specimen and into the objective. Underneath that, because this was before microscopes had built-in light systems, it has a mirror and so you'd have an external lamp.

Well here, certainly as far as size is concerned, we're at the other end of the scale. This is a microscope designed in the 1950s for serious research and the reason really why it's so large is that originally it had a plate camera in here and the light went over mirrors in here. One of the big advances of this microscope type over that is that you can not only look at transparent things with light going through, but you can have light coming through here and bounce it off the top of the specimen, so we can look at materials, at silicon chips, at metal specimens and so on. We've got a camera which is anchored on this very substantial bracket here to reduce vibration, but contrary to what you might think, you need very long exposures, not very short ones. By having a very long exposure you lose the first half-second while the camera's waving about and then most of the exposure is where everything is still and then it shuts at the end. So usually four or six seconds exposures on this.

Specimen preparation is of great importance to the final result. It's true to say that most biological material is too thick to get an image. For the best results, then sections one micrometre or 1.5 micrometres, what I call semi-thins, reveal far more detail. If you can't cut sections then of course there are other ways of preparing a thin object. For instance, in the case of blood, you wouldn't have it in a test-tube, you would smear it out into a very, very thin layer on a slide and then you can stain it.

These are just some of the stains that it's possible to use. I may use a computer actually to fire the shutter and the image appears on my monitor screen, once I've collected the images, and I may need to take several, of a specimen at different levels because the depth of field of a microscope is very small and if you're looking at something thick and then you may have to take photographs at different depths through this specimen, and then use software to stack all the images so you get a much clearer image of what the thing really looks like. But most of the colours that you see in images done with Rheinberg illumination and cross polars and so on, these are just the consequence of the laws of physics rattling around in the microscope.

Rheinberg illumination's named after its inventor, and he invented it in the late 19th century. It's very, very effective because it gives brightly coloured images full of contrast and it's very simple to do as long as you have a suitable condenser. And all that you need to do to modify it is to put a Rheinberg disc, made in this case of gels, you can do it with toffee paper stuck together with sellotape and that fits somehow or other into the filter tray under the condenser or I've made this special magnetic one and in this case because it has a dark red patched up and a pale green annulus it will make a colourless object appear green against a dark red background. So if you're prepared to play around, this will produce the most colourful images you'll ever see. In fact the images are as colourful as the bits of toffee paper that you can find.

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