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Video Microscopy

Chapter 11 of *Light Microscopy* (D.J. Rawlins)

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There has been a tremendous expansion in the use of video in microscopy in recent years. This is probably not due to the most obvious advantage of video, that of real-time imaging of moving events, but to its use in image analysis systems. The cheapness of PCs and the associated hardware and software to store and manipulate images, coupled with the availability of low-cost CCD cameras has made the analysis of microscope images commonplace. In the article the equipment used and the sorts of analysis which can be performed will be briefly outlined. The standard text on video is that of Inoué[1]; a more recent review is by Weiss *et al.*[2] while the major video equipment manufacturers will also provide literature and advice on the more up-to-date equipment.

Types of Video Cameras

Video cameras using pick-up tubes work by detecting electrical charge built up on a photoreactive plate when light falls on it. The light first passes through a positively charged, transparent window and then hits a deposited layer of a photoreactive substance. This changes its conductance, depending on the amount of light that falls on it, and causes charge to be drawn from the charged window and stored at the back of the layer. An electron beam is then scanned over the back of the layer and interacts with the stored charge, producing a potential difference which gives rise to the current that makes the picture. The difference between the different types of video camera is basically the substance used to make the photoreactive layer. These give different sensitivities for different applications, for example, infra-red, UV, low-level visible light, etc. The most commonly used cameras for transmitted light microscopy are Vidicon and Chalnicon, the latter being more sensitive. For low light levels, for example, fluorescence, SIt (silicon intensifier target) cameras are often used. These usually come with external control electronics to allow control of brightness and contrast (called gain and black level). The most sensitive video camera is the ISIT (which stands for intensified SIT).

The phrase video camera now also encompasses cameras based on a type of silicon chip called a charge-coupled device (CCD). This can be thought of as a block containing an array of very small wells. When a photon of light enters a well, an electron is stored there and these electrons build up all the time the camera shutter is open. When the shutter closes, the number of electrons in each well determines the brightness of the corresponding picture element (pixel) which make up the image when it is displayed. One of the main advantages of CCD cameras is that because there is no electron gun, they are lighter and more cost-effective. Using a slow scan (say 1 image per second), rather than at video rates, the sensitivity of the best CCD cameras is such that single photons can be detected.

Most cameras used for viedo microscopy are monochrome, although the home video market has meant that color video cameras (which are all CCD based) are now affordable and useful for applications where light levels are reasonably high.

Fitting a Video Camera to a Microscope

Most video camera systems come ready fitted with appropriate connectors and adaptors to fit onto your microscope if it already has a 35 mm camera setup. If not, the main problem is to project the image though the camera window in such a way that the image is focused on and fills the pick-up tube or CCD chip. This can be done either with or without another lens. As a first step, remove the cine-lens from the camera (if there is one) and support the camera 20-30 cm from the eyepiece of the microscope. Shield the gap between the two with black paper or card to keep out stray light and adjust the gap between the two until a focused image is produced. Most commercial systems incorporate a low power (x1 to x4) eyepiece or projector lens as for photomicrography. If using a photomicroscope, the phototube will need to have a male "C-mount" adaptor at the top to screw into the camera. This is a fairly uniform standard for video cameras but not camcorders. Another standard is the size of the target, which is normally 1 inch.

Tube cameras as opposed to CCD cameras can be ruined by focusing too much light onto the target. Therefore, with whatever projection system is used, make sure the lamp is turned right down before diverting the light to the camera.

The simplest video system is a camera, a black and white monitor (not a television unless it has a video input) and a length of coaxial cable with a suitable connector on each end.

Image Capture, Display, Processing and Analysis

Video images can be "captured", that is, converted to a stream of digital information and stored as files on a computer using a frame-store (also called a frame-grabber). These are often PC based and are simply an extra card for a PC with connections for the camera and monitor. There are now many manufacturers of frame-stores either as stand-alone units or as a part of image analysis packages. All frame-stores have the facility to display the images stored and often to adjust the brightness and contrast too. This is usually, but not always, on a separate monitor from the computer.

Video images can be displayed on a black and white monitor, or, depending on the frame-store, on a color monitor using either "composite" video or separate channels for red, green and blue (RGB). The size of screen is not as important as the number of dots (pixels) that make up the image.

Image processing means altering the stored image, or in real time on a live image. At its simplest, this means altering the brightness and contrast. In addition, there are programs for improving noisy images, sharpening the edges of objects within an image, cutting and pasting parts of an image, merging one image with another, rotation and mirror imaging, negative contrast, producing diffraction patterns, 3D reconstruction of optical sections, making pseudo-colored images and many more. More details on image processing can be found in references 1-4.

Image analysis is subtly different from image processing but the two techniques are closely related and the terms often interchanged. Analysis of an image could include plotting the intensities of all the pixels within a defined area or along a line (*e.g. Figure 1*), measuring parameters of an area of the image traced with a mouse (e.g. perimeter, area, average pixel intensity with standard deviation), counting the number of a particular object, perhaps defined by "thresholding" at a particular intensity (an image analysis function giving a particular color to all pixels within a specified intensity range) and particle analysis.

Magnification, Pixel Size and Resolution.

Typically, an image will be displayed on a 512 x 512 array of pixels. The resolution of the light microscope itself is about 0.2 μm in the x and y planes so that is the minimum distance that can be represented on two adjacent pixels without producing empty magnification. This means that the minimum width that a whole screen can represent is 512x0.2 = 102.4 μm. Therefore, there is no advantage in the camera and projection system producing an image much smaller than this (i.e. a much greater magnification) as no more resolution will be achieved. This corresponds to a magnification of about x40 for the objective and projector lens combined. However, the optical system of the microscope (effectively the objective NA) has to be capable of the best resolution resolving two points that far apart, and as this requires a high NA lens (e.g. x63/1.4), in practice it is best to use the highest NA lens possible and ignore any empty magnification produced. This strategy will also ensure the brightest image possible. Of course, a larger image may also be more pleasing to look at.

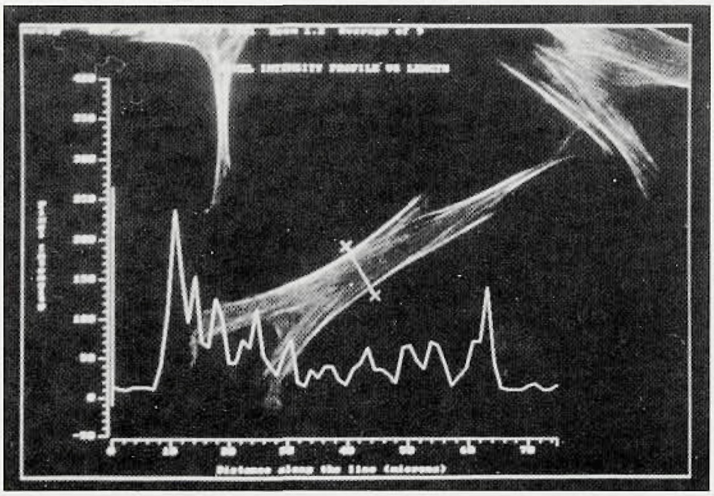

Figure 1: MRC5 cell using confocal imaging of the rhodamine fluorescence Plot of the pixel intensity (y-axis) along a line defined by the operator (x-axis). This gives an indication of the spacing of the stress fiber bundles.

Recording Dynamic Processes

It is straightforward to connect a video camera to a domestic VHS video recorder for recording moving events. However, the quality of the image produced will be better with one of the professional formats (e.g. Super VHS or U-matic). It is also worth considering a machine that will produce and record text and possibly simple graphics such as arrows on the screen.

For long term dynamic events, a VHS time-lapse video recorder is used. This records a single video frame at intervals up to 8 sec or more and also moves the tape much more slowly than normal. In this way, 1000 h of action can be reduced to 3 h of tape played back at the normal speed. Most time-lapse recorders have a text-recording facility.

Aesthetic considerations are particularly important if video-recorded material is to be published because as yet, the only practical way to do it is to take photographs. The resolution of VHS is much less even than a standard monitor and so it is important to frame the shot and adjust the brightness and contrast carefully.

The major alternative to video-recorders are laserdisks which can store many hundreds of 512 x 512 images on a single disk and retrieve them within a second or less without loss of resolution. This rapidly developing area of technology is likely to produce real-time playback soon. ■

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