analysed and so may not always produce the same phylogeny as that based on hybridization. The fact that the phylogenies shown by Diamond are alike, and so probably correctly represent the pattern of primate evolution, must mean that mutation accumulation rates in different chromosomal regions of primates have been similar.

J. FILIPSKI

Institute Jacques Monod,
2 Place Jussieu — Tour 43,
75251 Paris,
France

1. Diamond, J.M. *Nature* **332**, 685–686 (1988).
2. Beintema, J.J. & Campagne, R.N. *Molec. biol. Evol.* **4**, 10–18 (1987).
3. Li, W.H. & Tanimura, M. *Nature* **336**, 93–96 (1987).
4. Goldman, M.A. *et al. Science* **226**, 686–692 (1984).
5. Filipski, J. *FEBS Lett.* **217**, 184–186 (1987).

Scanning optical microscopy of low-contrast samples

SIR—A recent letter[1] by Rothenhäusler and Knoll considered the microscopic imaging of low-contrast samples and presented images of molecular films formed by the technique of surface-plasmon microscopy. It was stated that monolayer films do not provide sufficient contrast to be visible with either phase-contrast or Nomarski microscopy. We have examined the imaging of monomolecular films using various techniques of scanning optical microscopy[2] and our results lead us to dispute this claim. In particular the differential phase-contrast method[3], using a detector split into two halves, is especially suitable for such applications. Here we show that the technique provides high sensitivity to specimens of low contrast.

The specimen used was a monolayer film of ω-trisenoic acid ($CH_2{=}CH_2(CH_2)_{20}COOH$) deposited onto a glass substrate. The thickness of the film, 3.15 ± 0.05 nm (ref. 4), is a function of the length of the molecule and the angle at which the molecules are aligned. The optical system used is shown in Fig. 1. Illumination is provided by a HeNe laser (λ=632.8 nm) and the objective lens is of numerical aperture 0.5. The system has both a transmitted-light split detector for differential phase contrast and a reflected-light detector which can be used either for bright-field imaging or, with a pinhole placed in front of it, for confocal imaging[2].

The output of either detector can be connected to an LSI–11 computer to be sampled and quantized over a 256 × 256 grid by an eight-bit analogue-to-digital converter and stored in a frame memory. Images are generated by scanning the specimen mechanically in an xy raster through the focused spot. Imaging time is ~3 s per frame.

A differential phase contrast image of the edge of the film is shown in Fig. 2: the glass substrate is on the left and the monolayer on the right. The dark vertical line between these two areas is the edge of the film. To the right of this line can be seen areas where the film has not adhered to the substrate during dipping. These areas are clearly depressions: the sign of the gradient at their edges is bright on the left, indicating a downward slope, and dark on the right, indicating an upward slope. Not only does this method image the 3–nm phase steps easily, but it also shows up smaller detail on the surface of the slide and of the film.

To demonstrate the sensitivity of this technique to phase variations a confocal reflection image was observed for comparison. Despite a 25-fold enhancement in electronic contrast the confocal image showed the film edge only very weakly and also contained much less information about the surface structure of the film. A reflected light bright-field image exhibited even less detail than the confocal image despite a 30-fold contrast enhancement.

In Fig. 2 the dark line representing the edge of the film has a width of about 2 μm. If the edge is assumed to be a linear ramp rising 3 nm vertically in 2 μm horizontally, the normalized intensity signal is estimated[2] as 0.002, agreeing well with the observed value. The theoretical contrast in the edge in a bright-field image is also 0.002, and in a confocal image it has twice this value[2]. These also agreed well with observed values, demonstrating the improved sensitivity of confocal microscopy to small phase changes.

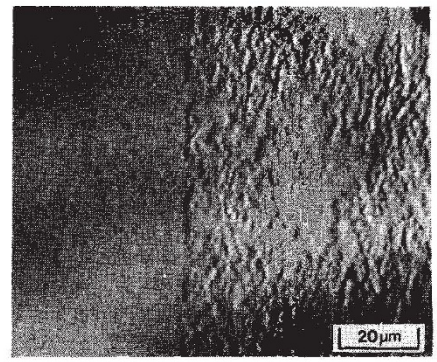

Fig. 2 A differential phase contrast image of a single monolayer.

The differential phase contrast method has been demonstrated to detect phase gradients of about 1 in 1,000, which is equivalent to a height change, over a resolution element, of less than 1 nm. One reason for this high sensitivity is that mechanical scanning avoids the appearance of mottle in the image[5]. The sensitivity can be improved further by using an annular split detector[6], giving an increase in sensitivity of about a factor of 10, or by using two photomultipliers or photodiodes instead of the solar cell detectors actually used. If small photodiodes are used it is then necessary to focus each half-beam individually onto its corresponding photodiode.

C.J.R. SHEPPARD
D.K. HAMILTON
H.J. MATTHEWS

Department of Engineering Science,
Oxford University,
Parks Road,
Oxford OX1 3PJ, UK

1. Rothenhäusler, B. & Knoll, W. *Nature* **332**, 615–617 (1988).
2. Sheppard, C.J.R. in *Advances in Optical and Electron Microscopes* Vol. 10 (eds Barer, R. & Cosslett, V.) 1–98 (Academic, London 1987).
3. Hamilton, D.K. & Sheppard, C.J.R. *J. Microsc.* **133**, 27–39 (1984).
4. Bonnerot, A., Chollet, P.A., Frisby, H. & Mollet, M. *Chem. Phys.* **97**, 365 (1985).
5. Allen, R.D. & Allen, N.S. *J. Microsc.* **129**, 3–17 (1983).
6. Hamilton, D.K., Sheppard, C.J.R. & Wilson, T. *J. Microsc.* **153**, 275–286 (1984).

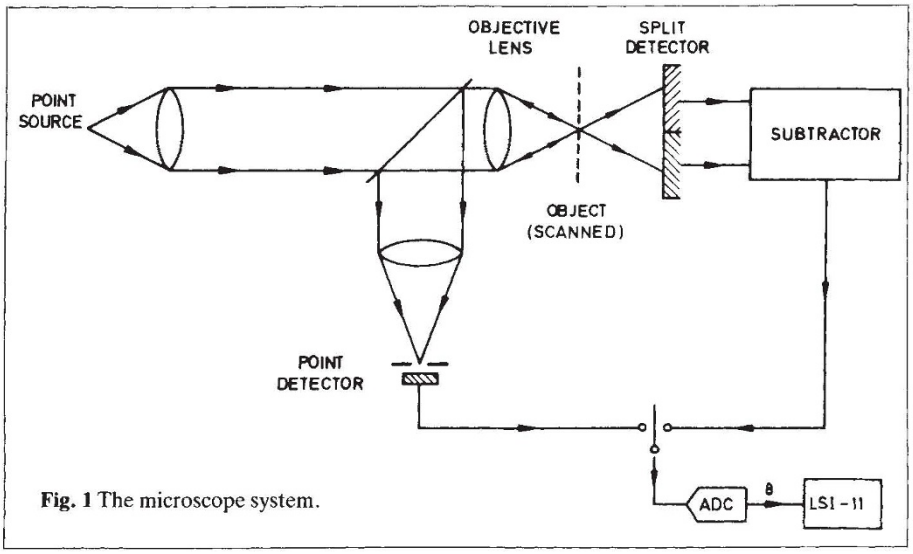

Fig. 1 The microscope system.

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of less than 500 words and five references. □