Differential Interference Contrast Light Microscopy

Ronald J Oldfield, *Macquarie University, Sydney, Australia*

Differential interference contrast (DIC) is a technique in light microscopy that maintains high resolving power because it introduces contrast optically into images of transparent specimens. The image is characterized by a three-dimensional appearance (a pseudo-relief) in which contrast and colour may be varied.

Secondary article

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The Problem of Visualizing Phase Objects

By interacting with light in various ways, a microscope specimen becomes visible – either as a colour distinction from the background or as changing light/dark intensity effects. To explain these various observations, Ernst Abbe (1840–1905) introduced the concepts of direct and diffracted waves for microscope image formation, best studied with the abrupt intensity boundaries (i.e. amplitude changes) of gratings. Diffraction causes some unevenness in intensity in the rays emerging from the specimen, explained in terms of interference effects. There is a redistribution of energy ($1 + 1 = 0$ in parts of the system, $1 + 1 = 4$ in other parts of the system). If focused, the direct and diffracted waves interfere to present light and dark areas in the image.

Living biological material in the microscope, however, has little variation in light absorption; but small changes in phase are introduced through variation in specimen thickness or refractive index. If light waves are refocused in the microscope image to appear exactly as they were when they left a transparent specimen, they will be uniformly bright and will not readily reveal aspects of the object's structure. The image of the specimen is not completely invisible, however, and by methods such as restricting the aperture of the system or by defocusing the objective, an attempt can be made to introduce semblance of structure into the image, however poorly. Acceptable imaging may demand some additional effects such as phase changes or differential absorption between the direct and diffracted waves. This is the technique employed in phase contrast microscopy. Differential interference contrast (DIC) microscopy is another technique that is used to introduce contrast, and sometimes colour, into specimens that lack contrast.

Design of a DIC Microscope

Several 'interference microscope' systems have been designed using the principle of existing optical instrumentation such as the Mach–Zehnder interferometer. By shearing an incident beam, passing one ray through the specimen and passing the other (reference) ray through the mountant, they were quantitative devices measuring optical path differences in the specimen. Such microscopes are little used today. DIC is a shearing technique in which both sheared beams pass through the specimen, but shearing is of the same lateral dimensions as the minimum resolved distance (resolving power) of the objective. When the two beams are recombined, there are two images of the specimen but, because the beam separation is so small, two distinct images are not perceived.

In the system developed by Georges Nomarski, beam separation is by a cemented pair of double-refracting prisms, a modification of a design by William Wollaston. A polarizer provides plane-polarized light at 45° to the lower Wollaston prism, called in this work the beam splitter, from which emerge two sets of rays, ordinary and extraordinary, polarized at right angles to each other (**Figure 1**). A separate prism is provided for each objective, the prisms being held in a turret beneath the condenser. In some instruments a 'Universal' condenser has provision for a selection of DIC prisms and/or phase-contrast annuli, as well as a 'zero' bright-field position. The condenser aperture diaphragm is fully functional whether or not the beam splitter is engaged. For the phase-contrast function of such a condenser, the aperture diaphragm is not functional.

A beam combiner, placed between the objective and eyepiece, is another (modified) Wollaston prism, similar to the beam splitter. Only one beam-combining complex is needed, which suffices for all objectives that may be used. The beam-combining prisms can be displaced laterally by a lever or screw movement, which introduces contrast or colour change in the image. Above the beam combiner, and frequently part of its construction there is an analyser set at 135° to the beam-combining prisms.

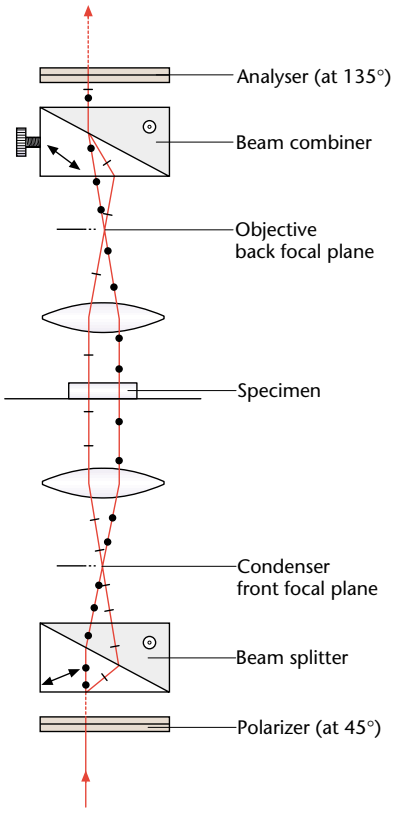

Figure 1 A diagrammatic representation of the transmitted-light Nomarski DIC system.

For phase-contrast or bright-field observation, it is necessary to slide out of the light path the polarizer, any beam splitter, the beam combiner and the analyser.

Since the back focal plane of objectives for DIC needs to be located in a special plane with respect to the beam combiner, not all objectives are suitable for the technique.

Operations of a DIC Microscope: Theory and Practice

The wavefronts emerging two from the beam splitter pass through closely adjacent points in the specimen; the two waves are then recombined in the upper prisms and analyser. If there is no path difference between the two wavefronts, extinction (darkness) occurs. Where there is a path difference, the image will be bright, possibly coloured. DIC gets its name because it shows only optical gradients or path differentials in the specimen. DIC reveals changing refractive index or changing thickness at the site(s) of transmission.

Moving the upper prisms laterally gives the two wavefronts a path difference additional to that introduced by the specimen. When the two Wollaston prisms are first aligned and centred, when there is zero path difference between the two sets of rays, the field is maximally dark (**Figure 2c**). As the beam combiner is adjusted laterally in either direction from this zero position, an optical path difference is introduced so that the field colour changes through the well-recognized sequence of Newton colours as seen in polarization microscopy – through the greys, white, yellow, red–blue and higher-order colours. Best

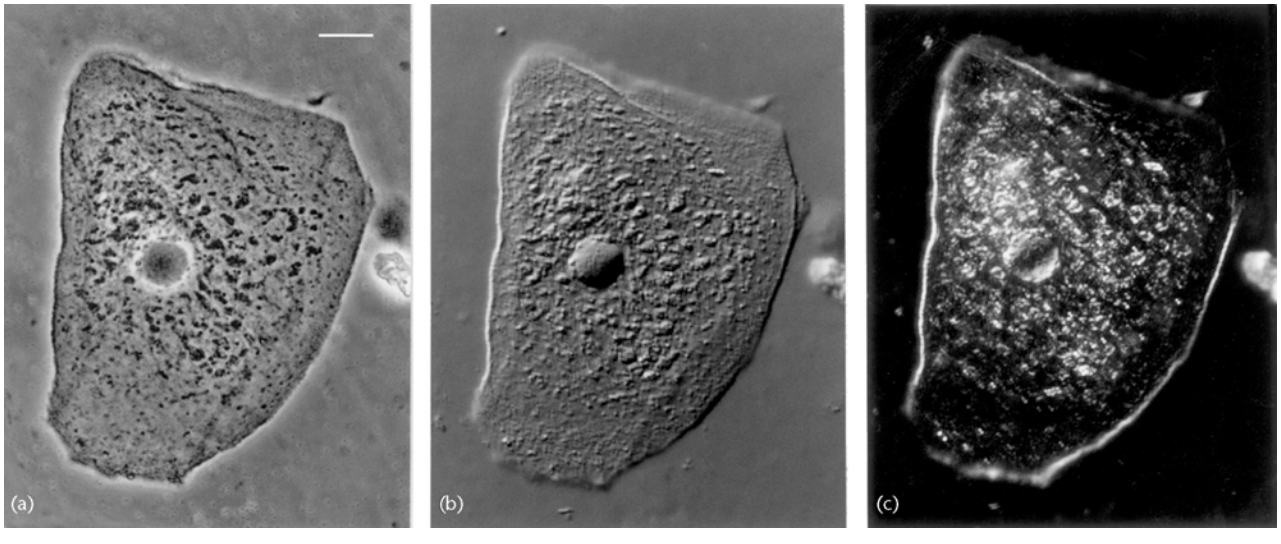

Figure 2 Epithelial cheek cells in phase contrast (a), in DIC pseudo-relief adjusted for most effective contrast (b), and in DIC adjusted for the zero or 'dark-field' position (c). The bar represents 10 μm.

contrast of thin specimens is likely to be in the early greys; colour effects have more limited scientific use but may be very beautiful indeed and make attractive presentations. Quantitative information is a most significant feature of polarization microscopy, but DIC images, like those of phase-contrast microscopy, are valued for their qualitative utility and appeal.

Video-enhanced DIC

Since the 1970s, DIC images have been favoured for the representation and interpretation of living cellular material. However, as mentioned below, compared with phase-contrast microscopy, there is some lesser contrast with DIC, and heating of the specimen may limit the light intensities that can be tolerated. These disadvantages can be countered with electronic image-recording; the use of highly sensitive video cameras and the possibility of tweaking contrast with the turn of a dial or the touch of a keyboard have extended the importance and range of applications of the DIC microscope. With video systems, increasing the camera gain (sensitivity or amplification) automatically increases the contrast in the image. Video camera images, digitized and stored in a computer, permit image-processing, the adjustment of the 'black level', the sharpening of boundaries and the possibility of removing image artefacts such as random noise. Time-lapse images with video-enhanced DIC are among the most exciting microscope revelations of division and growth processes in living cells. Transient cellular processes may be slowed down in playback.

Interpretation and Comparison of Phase and DIC Images

DIC gives visual representation of gradients, i.e. differential coefficients of optical path length or lateral shearing. Hence the name *differential* interference contrast. Where such gradients or slopes occur in a specimen, DIC will show that change. Where the specimen has uniform, unchanging path length, it will be indistinguishable from other parts of the field (such as the mounting fluid or background) that also have no gradients.

The most obvious difference from other images in the microscope is the three-dimensional appearance of a DIC image (**Figures 2** and **3**). In transmitted light DIC, the appearance should be regarded as pseudo-relief, for in almost every instance it is not indicative of any actual topographic profile in the specimen.

The condenser aperture diaphragm retains its full function in DIC. For maximum resolution the 7/8th position as judged in the objective back focal plane is still appropriate, but the aperture stop quite properly can be used to adjust depth of field and contrast as required. It is the particular advantage of being able to achieve minimum depth of field, i.e. optical sectioning with full objective NA, that is a feature most appreciated with DIC, especially with thick specimens.

It is simplistic to suggest that DIC has replaced phase contrast as a microscope accessory. The two techniques complement each other, and both systems are likely to find application in a general laboratory. Phase-contrast attachments are relatively cheap and, once aligned, quite simple to operate. This has to be compared with the considerable

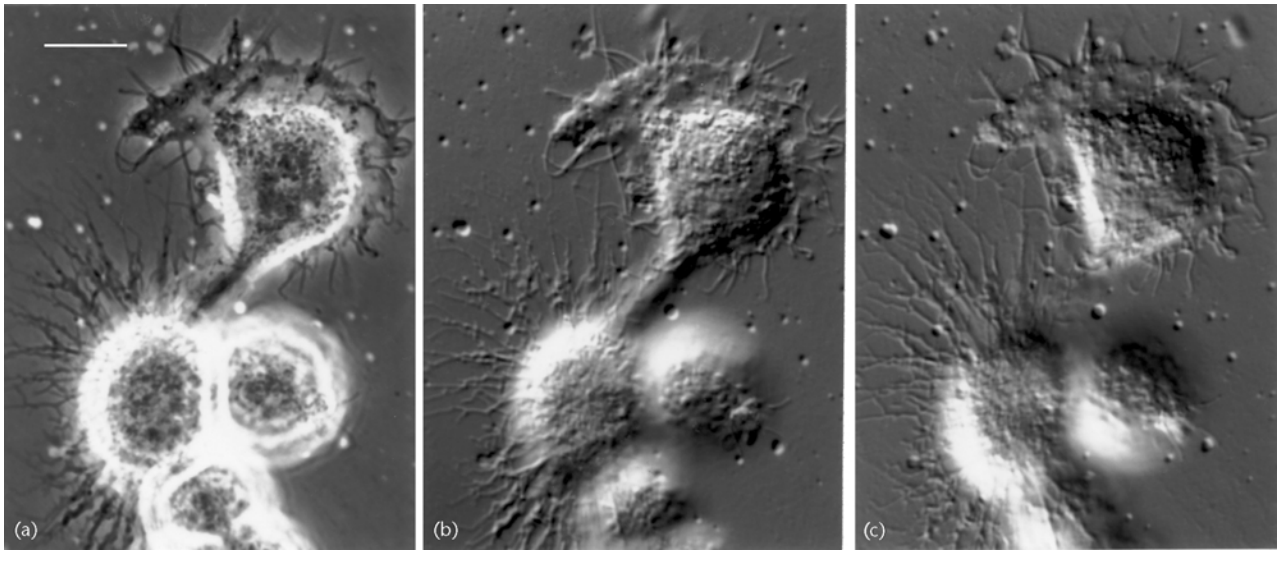

Figure 3 Cultured fibroblasts from *Monodelphus domestica*: phase contrast (a), compared with DIC. (b, c). In the two very different DIC images, the same focus has been maintained but the shearing direction has been rotated through 90° (equivalent to rotating the specimen through 90°). The bar represents 10 μm.

expense of the DIC condenser, the intermediate (beam combiner) attachment, and possibly additional Plan objectives; a pricing factor of 10× may be involved comparing the price of a full DIC attachment with that of a phase set. For the optimal use of DIC, its complexity requires much more operational skill and manual dexterity, even after correct assembly of the equipment. For any observation, attention must be paid to selective focusing, adjusting the aperture diaphragm to compromise resolution, contrast and depth of field, optimizing the displacement of the beam combiner and checking specimen orientation for contrast effects.

In phase-contrast microscopy, refractive index boundaries of the specimen that are above or below the plane of focus seriously degrade the ability to see clearly the in-focus image. DIC avoids the problem to a considerable extent, and successive planes of the specimen at high resolution can be examined and recorded.

Phase contrast seldom performs satisfactorily with a stained specimen. DIC is suited to both stained and unstained material; the phase halo is absent.

The DIC effect is directional, markedly so with some specimens. Image gradients need to be aligned at right angles to the direction of shear. A linear structure may well disappear if it is oriented along the direction of shear, but will show maximum contrast if rotated 90°, normal to the shear direction (**Figure 3**). A rotating stage is recommended for DIC work, but the placement of XY stage controls on a rotating stage is ergonomically inconvenient for extended sessions at the microscope.

Because of the polarization phenomena that make Nomarski DIC possible, specimens that are themselves anisotropic (doubly refracting) may prove unsuitable for DIC examination. For this reason, plastic dishes that are routinely used for cell culturing are not acceptable for DIC imaging, a very serious disadvantage of DIC. Some other (nonpolarization) contrast techniques, e.g. Hoffman modulation contrast and Varel (of Carl Zeiss) can avoid this problem.

Phase contrast may be more useful than DIC for specimens with very little phase retardation, and is said to work best with retardations no greater than $\lambda/10$. DIC is more appropriate for specimens with retardation from $\lambda/10$ to λ, i.e. either thicker specimens or specimens with a greater range of phase retardation.

Applications of DIC in Molecular and Cellular Biology

Phase-contrast microscopy was slow to be adopted as a routine microscopical technique, possibly because an understanding of image formation is appropriate to the final year of a physics major. Simple teaching models have been developed for phase contrast, but there are no convincing simplifications of the even more complicated theoretical basis for DIC. DIC remains a specialist tool, but its application can be mastered by anyone interested in applied technical aspects of microscopy.

Further Reading

Inoué S and Spring K R (1997) *Video Microscopy. The Fundamentals*, 2nd edn. New York: Plenum Press.

Oldfield R (1994) *Light Microscopy. An Illustrated Guide*. London: Wolfe Publications.

Pluta M (1989) *Specialised Methods. Advanced Light Microscopy*, vol. 2. Amsterdam: Elsevier.