Principles and Applications of Differential Interference Contrast Light Microscopy

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BIOGRAPHY

After graduating from Leicester University with a degree in Biological Sciences Alan Lasslett went on to work in pharmaceutical research. Subsequently he has undertaken several commercial roles, and has held the position of marketing manager with Olympus UK for a number of years. He is actively involved in running microscopy courses in research institutes and hospital laboratories.

ABSTRACT

Differential interference contrast (DIC) light microscopy is a technique which produces impressive 3D-like images of unstained specimens. The shadowing effects of the technique are remarkable, yet often misunderstood. The additional components required to enable DIC observation on a research-level microscope are precision-made and expensive, so DIC is often overlooked in favour of the more common phase contrast technique. This article provides an overview of DIC microscopy, including guidelines for setting up the system correctly.

KEYWORDS

light microscopy, polarized light, differential interference contrast, phase contrast, Nomarski, Wollaston prism

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INTRODUCTION

Most life scientists recognise phase-contrast images of unstained specimens and many will be adept at adjusting the microscope to achieve good images. Differential interference contrast (DIC), on the other hand, is much less frequently encountered, yet is in many ways a superior technique. Possibly the main reason why DIC is not often used is familiarity. Day-to-day microscopy remains, on the whole, a subjective matter so knowing what a specimen should look like is an advantage. Switching to a new technique may lead to difficulties in interpretation, or worse, the acceptance of poor images. The aim of this article is to introduce DIC to those life scientists who are not well acquainted with the technique and to give tips on how to achieve the best images.

ORIGINS AND ADVANTAGES OF DIFFERENTIAL INTERFERENCE CONTRAST

Differential interference contrast optics have been with us since the 1950s. Georges Nomarski, a French physicist, modified the Wollaston prism (used to detect optical gradients in specimens) in order to create differences in intensity. The DIC microscope as such was devised by Francis Smith in 1955.

The major advantage of DIC over phase contrast is that the full aperture of the microscope is used. In phase contrast the condenser's annular stop restricts the aperture, and therefore the resolution of the system.

Compared with phase-contrast images, differential interference contrast:

- Produces high resolution images
- Shows good contrast
- Can be used with thick specimens
- Lacks the distracting halo of phase contrast
- Can be further processed (video enhanced).

HOW DOES DIC WORK?

DIC microscopy is essentially a development of polarising microscopy which employs sophisticated optical principles to produce 3D-like images of unstained cells and tissues. However, it is essential to realise that these images are not all they seem; the apparent peaks and troughs seen in the image are not actual representations of the morphology of the cell or tissue. Instead, they are the product of the optical gradient through the specimen and the wavefront path distance. So while DIC images are impressive, care must be taken in their interpretation.

This is briefly how Nomarski DIC images are produced (see Figure 3 for a schematic):

1. Light passes through a standard polarizer before entering the condenser, producing plane-polarized light.

2. This light enters a Wollaston prism situated in the front focal plane of the condenser. The actual components are described below. The prism interacts with the polarized light to produce two separate wavefronts polarised perpendicularly to each other. These are termed the ordinary (O) and extraordinary (E) rays. Furthermore, these two wavefronts are separated by a very small difference (less than the resolution of the system). This separation is termed shear and is an important characteristic of the system (Figure 3).

3. The two wavefronts pass through the specimen, and are retarded to varying extents in doing so.

4. The light now enters a second Wollaston prism set-up which recombines the wavefronts. If there has been a phase shift between the two rays as they pass through areas of different refractive index then elliptically polarised light is the result.

5. Finally the light enters a second polarizing filter, termed an analyzer. The initial polarizer and this analyzer form crossed polars. The analyzer will permit the passage of some of the elliptically polarized light to form the final image. All the remaining light will be blocked by the analyzer.

An additional component, a first-order red plate, may also be part of this arrangement. It permits adjustment of contrast and colour.

The above description merely touches on the physics of the technique; for a fuller description refer to the excellent Florida State University Molecular Expressions website.

Figure 1:
A typical research-level upright microscope for brightfield, darkfield, phase contrast, DIC and fluorescence applications.

THE DIC MICROSCOPE

A typical research level upright microscope for brightfield, darkfield, phase contrast, DIC and fluorescence applications is shown in Figure1.

The configuration for DIC varies between models and manufacturers, and in particular between upright and inverted microscopes. While most utilise the conventional Nomarski DIC system others use the related de Sénarmont set-up. The following description refers to the Olympus BX upright microscope configuration; other makes and models will have comparable components.

Condenser

Although a separate polarizer is often described, in fact for practical reasons condensers designed for DIC usually have a built-in polarizer. This can be slid out of the light path for brightfield illumination. The polarizer can fully be rotated, but is marked to permit correct east-west orientation and a locking screw is provided.

The main body of the condenser is the rotating, phase contrast type, but supplied without inserts. The insert used for DIC is a Wollaston prism, a kind of beamsplitter (Figure 2a). Each prism consists of two precision made wedges of quartz, cemented together so that their axes of birefringence are at right angles to each other. The prism itself is mounted in a circular cell.

Appropriate Wollaston prisms need to be inserted, taking care to orientate correctly using the protruding pin as a guide. These prisms are specific for the objectives to be used, so if DIC observation at 10×, 40× and 100× is required then three matching prisms need to be installed.

One advantage of this condenser type is that generally there are additional mounting positions so phase-contrast annuli can be installed. This will permit observation of a specimen first by DIC then by phase contrast – a very instructive exercise.

Objectives

Theoretically any objectives can be used, but in practice higher grade objectives (Fluorite and apochromatic types) are generally specified to benefit from the high-resolution potential. In many cases phase-contrast fluorite objectives are chosen, permitting brightfield, DIC, phase contrast and fluorescence observation with a single set of objectives.

Note that since DIC is a polarized-light technique then ideally strain-free objectives should be used (these are to be found on the manufacturer's list of objectives for quantitative polarized-light microscopy). However, most users find standard objectives acceptable.

Specimen slides and vessels

One important restriction of DIC is that plastic vessels cannot be used due to the strain they exhibit under crossed polars. For upright microscopes this is not an issue, but users of inverted microscopes may consider using plastic vessels which have a glass insert of coverslip thickness – these are available commercially.

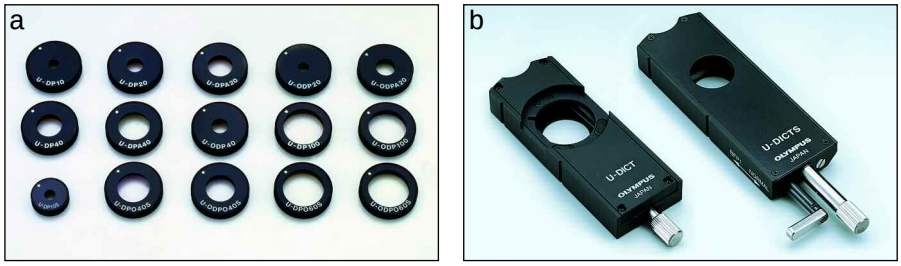

Figure 2:
(a) DIC prisms designed to fit the condensers of Olympus BX and IX microscopes.

(b) DIC sliders which are inserted into the nosepiece of Olympus BX microscopes. The lefthand slider has a recess to accept an analyzer.

DIC slider

The second Wollaston prism arrangement is a slider (Figure 2b) fitted above the objectives but below the Telan lens (in an infinity-corrected microscope). In this case only one prism is required, and it is provided with a means of sliding it across the light path. The DIC slider is orientated northwest – south east, i.e. diagonally in the light path.

Analyzer

The second polarizer in the system, termed the analyzer, is installed above the DIC slider. In most cases it is fitted directly into the slider, and its north – south orientation is guaranteed by a lug and corresponding slot.

The optical components are now in the configuration shown in Figure 3.

SETTING UP DIC

Although differences exist between models and manufacturers, the following procedure (for Olympus BX microscopes) will have parallels in most other models.

Köhler illumination

It is a prerequisite for DIC microscopy that correct Köhler illumination is first set up. To do this move all DIC elements out of the light path (the polarizer can be left) then focus on a stained specimen using a 10× objective.

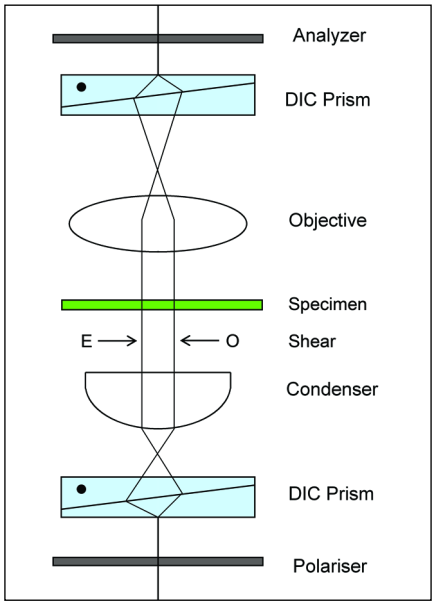

Figure 3:
Schematic showing the major wavefront-splitting optical components and pathways in the differential interference contrast microscope.

Locate the field iris diaphragm in the base of the microscope and partly close it. Use the controls on the condenser to focus and centre the image of the blades of the iris. Open the iris fully after this procedure.

Initial procedure

Firstly ensure that the aperture iris fitted into the condenser is fully open. Next, insert the polarizer into the light path but ensure that the condenser is in the brightfield position (i.e. no Wollaston prism in the light path).

Slide the upper Wollaston prism into position (this is the the DIC slider, usually in the nosepiece) along with the analyzer.

Remove an eyepiece and look down the eyepiece tube; you can see the back focal plane of the objective. If required a centring telescope can be inserted into the eyepiece tube to provide a magnified view.

Turn the knob on the DIC slider until a black band on a light background extends diagonally across the back focal plane. This is the initial starting point for correct set-up; if the diagonal band does not appear check for errors elsewhere, for example the polarizer may not be correctly orientated. Replace the eyepiece.

Finally rotate the condenser to bring the appropriate Wollaston prism into the light path; for example select the DIC20 position if a 20× objective is being used.

DIC IMAGES

Monochrome or colour?

A correctly set up a DIC microscope will produce monochrome images with good resolution and contrast. However more aesthetically pleasing images can be achieved by moving the DIC slider through the light path. Colours varying from magenta to blue and yellow are formed, reflecting the optical gradients in the specimen. Note, however, that while these images are very attractive (and frequently published) they do not tell us anything useful about the specimen. From a scientific point of view the best DIC images are monochrome.

Peaks and troughs

Another consideration when interpreting these images is understanding how the shadow effects are formed. They result from two characteristics: the difference in refractive index within the specimen or between specimen and medium, and the distance the wave path travels within these regions. Thus a 'hill' may indeed be a raised feature, or it may sim-

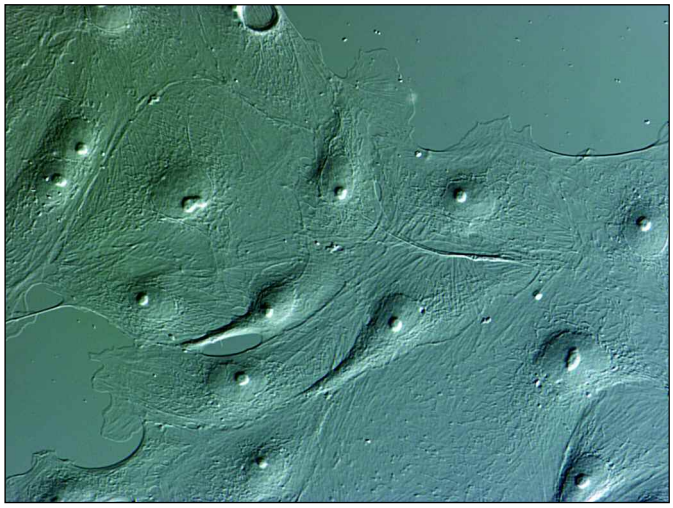

Figure 4:
A thin layer of cultured cells imaged using a DIC system designed to enhance contrast.

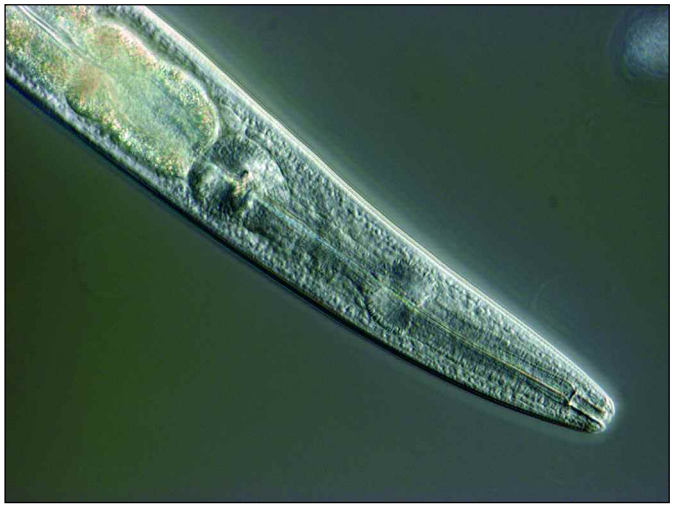

Figure 5:
Anterior region of a nematode worm – a thick specimen imaged using high-resolution DIC.

ply be an area of high refractive index. Similarly a 'crater' may be a hollow or may be a vacuole of very low refractive index.

The orientation of features in the specimen with respect to the DIC elements is also an important aspect of the image. Rotating the stage (possible to a limited extent with most standard stages) will often produce significantly different images.

Contrast versus resolution

The shear distance between the two wavefronts emerging from the condenser Wollaston prism is a fixed feature of the system and determines the balance between contrast and resolution. Most systems are optimised for specimens of medium thickness, such as tissue sections. However if the specimen is very thin, such as cultured cells spread on a slide, it is useful to employ a DIC slider designed to enhance contrast and permit fine structures to be observed (Figure 4). Likewise, thick specimens such as small animals and plants are best observed using a slider designed to reduce contrast and enhance resolution with the specimen (Figure 5).

APPLICATIONS OF DIC

Like phase contrast, DIC is a very useful tool for visualising unstained specimens. This is clearly an advantage when observing living specimens, such as small organisms, tissues or cells. In addition to simply observing such specimens DIC can be used effectively in several other specialised applications listed below.

Fluorescence microscopy

Locating the specimen or even the focal plane using fluorescence illumination can be a challenge. DIC, like phase contrast, can be used at low illumination levels for this task, but more importantly for indication where in the specimen a labelled component resides.

Infrared DIC

One interesting application of DIC is the imaging of cells inside tissues, such as brain slices used in electrophysiology. Here, infrared (IR) light is used as it penetrates deeper into the tissue slice than visible light. Appropriate optics with high IR transmission must be utilised, and the image is captured using an infrared camera. To the untrained eye these IR-DIC images look blurred but they are highly valued by neurophysiologists.

A further advantage of this technique is that the microscope can be equipped with a sensitive camera to capture the fluorescence image (often of very short duration) while the IR camera gives structural information.

DIC in reflected-light microscopy

Although this article is written primarily with life scientists in mind there are, of course, applications of DIC in materials science using reflected light. Here, the principles are the same but the configuration is easier. A non-rotating polarizer is fitted into the illuminator. A reflected-light DIC slider fits into the nosepiece (as for life sciences) but in this case it has the function of both Wollaston prisms in the life-science configuration. Finally a rotating analyzer is installed just below the Telan lens in the observation tube.

DIC is used in metallurgy, materials and semiconductors, producing good images of surface features such as scratches (Figure 6).

CONCLUSIONS

Differential interference contrast is a technique which deserves wider applications in life science. Most modern research level microscopes, both upright and inverted, can be updated to carry out DIC observation, in many cases with just the addition of DIC prisms and sliders. The drawbacks of complexity and cost of DIC are offset by the impressive high resolution, high contrast images, and there is ample scope for combining DIC with other applications such as fluorescence.

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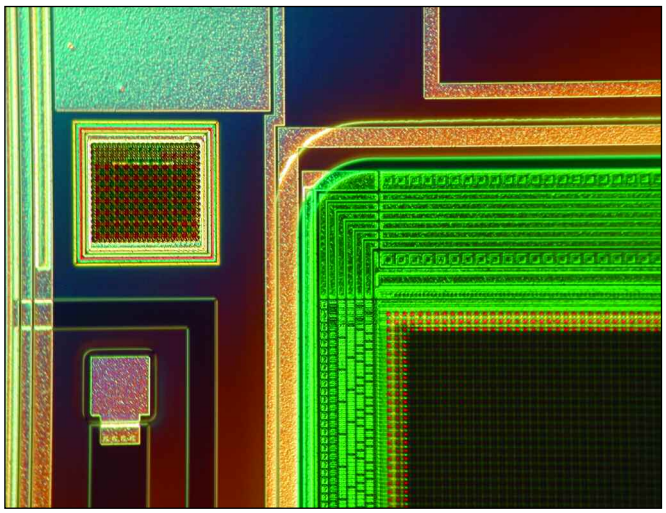

Figure 6:
Closed-circuit television chip imaged using DIC to show surface features which would otherwise be difficult to distinguish.