Walter Lang
Laboratory for Microscopy
Carl Zeiss, Oberkochen

Nomarski Differential Interference-Contrast Microscopy

Summary of Part III: Comparison with phase-contrast method

Like a phase-contrast attachment, the Nomarski differential interference-contrast (DIC) attachment can be easily adapted to any ZEISS microscope of the STANDARD KK, RA, WL, UNIVERSAL, PHOTOMICROSCOPE or ULTRAPHOT series. Since phase-contrast observation will in some cases be a valuable supplement to DIC observation, the condenser for the Nomarski method also contains annular diaphragms for the phase-contrast technique. This guarantees quick and easy changeover from one of these differentiation methods to the other. However, this applies only to the equipment for substage illumination. With reflected light, there is no need for a combination of the two techniques, since the Nomarski method is here clearly superior to phase contrast.

The second part of the paper deals with a few characteristic features of phase-contrast and Nomarski DIC microscopy. If a rotating specimen stage is used, the azimuth effect of the Nomarski method, which may be noted quite clearly in the case of oriented linear phase structures, cannot be eliminated, but may be avoided. On the other hand, the formation of halos in phase contrast is a considerable drawback. It is known that halation will be all the more pronounced, and thus troublesome, the larger and steeper the change of optical path difference in adjacent specimen details. But it is precisely here that the Nomarski method gives excellent results.

While for reasons of sensitivity and unambiguity the phase-contrast method should primarily be used for microscopic specimens introducing only negligible optical path differences, there is no such limitation in the Nomarski DIC technique. However, in order to obtain optimum contrast, very thin transparent specimens should preferably be used in the Nomarski method as well. As in phase contrast, very thick transparent specimens will impair the reproduction of the contrast-producing elements, namely auxiliary prism on principal prism on the one hand and annular condenser diaphragm on objective phase plate on the other.

It is sometimes considered a disadvantage that in the DIC image the phase structures of directly adjacent object points will only become visible if they exhibit a gradient of optical thickness in the splitting direction. It should be remembered that there are cases in which phase objects become visible only on account of the halo effect, when the halation is not necessarily identical with the geometrical course of the phase structure.

A clear advantage of the DIC method over phase contrast is its different depth of focus. It is known that even with high illuminating and viewing apertures very good contrast can be achieved in the Nomarski method; thanks to the high apertures that are possible, the depth of focus is so shallow that in the DIC image so-called "optical sections" are hardly impaired by objects or object details which are in the light path but outside the focal plane.

The fact that polarized light is required for examining birefringent objects imposes a certain restriction on the practical uses of the Nomarski method.

The paper concludes with a summary explaining essential differences between transmitted and reflected-light microscopy in connection with equipment designed for the combined use of the two techniques.

III. Comparison with phase contrast

Part I of the general description explained the fundamentals and the experimental setup for Nomarski differential interference-contrast (DIC) microscopy (11). Part II dealt with the formation of the DIC image (12). The present part III is devoted to a comparison between the characteristics of DIC equipment and those of phase-contrast (PC) equipment. This comparison is limited to transmitted-light instrumentation. A comparison with reflected-light equipment will be published elsewhere. A final section, part IV, will discuss the uses of Nomarski DIC microscopy.

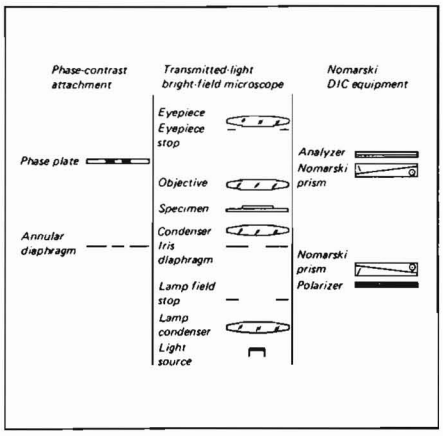


Fig. 1: Diagram illustrating the conversion of a ZEISS transmitted-light bright-field microscope for Zernike phase contrast and Nomarski differential interference contrast.

1. Experimental setup

The great majority of biological specimens are so-called phase objects. Pure phase objects (as compared to amplitude objects) do not affect the amplitude of the waves transmitted by the object. Apart from the diffraction of the light by object details, phase objects modify the path difference between the waves passing through the object field and those traversing the surrounding field. However, the human eye acting as a detector during visual observation of the microscopic bright-field image is unable to recognize these path differences. To make them visible, the light path has to be suitably modified.

The light path of ZEISS transmitted-light bright-field microscopes can be modified by the user, due to the availability of suitable accessories. (The same applies to ZEISS reflected-light microscopes.) To convert a bright-field microscope for phase-contrast observation (Fig. 1), it is necessary to exchange the condenser iris for an annular diaphragm and to mount a phase plate, optically conjugated to the condenser annulus, in the exit pupil of the objective. Since the pupil of microscope objectives, above all of high-aperture and high-power types, is in the interior of the optical system, special phase-contrast objectives are made for phase work, which, following a suggestion by K. Michel, have the phase plate in a cement layer between lens elements. (The history of the phase-contrast technique is discussed in [9, 10]).

To convert a bright-field microscope for interference microscopy (Fig. 1), it is only necessary to add a polarizer and a Nomarski prism below the front focal plane of the condenser and a second Nomarski prism as well as a second polarizer (as analyzer) above the objective (see 17).

2. Characteristics of ZEISS PC and DIC equipment

A comprehensive discussion of the differences between phase-contrast and interference-contrast accessories is beyond the scope of this series of papers. The following explanations will therefore be limited to the description of a few characteristic properties of these two optical staining methods.

2.1 Azimuth effect

The specific components required for phase contrast are rotation-symmetric. As a result, the PC image of a phase object is independent of the angular, i. e. azimuth orientation of the object in relation to the PC system. By contrast, the Nomarski DIC system is not rotationally symmetric but has a pronounced preferential direction (1, 8, 23). This direction is given by the design of the Nomarski prism and its fixed angular orientation relative to the polarizer and analyzer. Owing to the asymmetry of the Nomarski prism in relation to the optical axis of the microscope, the DIC effect is produced in the direction of the prism edges, but not perpendicular to them, because the differential retardation of waves is effective only in the direction of the prism edges (1, 11, 12, 19, 20, 21). The effect of this phenomenon is illustrated in Fig. 2.

However, this disadvantage of DIC equipment is rarely found disturbing. It is particularly pronounced in linear phase objects extending in the direction of shear.

If a rotary specimen stage is used, the linear object can always be oriented so that the detail of interest is imaged with optimum differential interference contrast. Non-linear objects hardly show this azimuth effect (see Figs. 4 to 7).

2.2 Halo effect

Haloes in the image of object edges are typical of phase contrast. In positive phase contrast[1], the edge of an object of higher refractive index than its surroundings has a bright fringe on the outside and a dark one on the inside (halo effect). The opposite is the case when an object of lower refractive index than its surroundings is viewed in positive phase contrast. Brief mention should here be made of the causes of the halo phenomenon[2]. Objects of a pronounced phase nature can be recognized in a bright-field microscope only with difficulty – if at all – since they hardly attenuate the light incident on them. However, a small portion of the incident radiation is deflected out of its original direction; it is diffracted by the phase object. By comparison with the non-diffracted light, the diffracted rays are shifted in phase by 90°. In Zernike phase contrast (28, 29)

[1] All ZEISS phase-contrast accessories are designed for positive phase contrast. As a result, objects whose optical thickness is greater than that of the surrounding field appear dark against a bright background.

[2] For references, see, for example, 2, 4, 5, 6, 7, 13, 14, 15, 16, 22, 26.

a) the direct light is also shifted in phase by 90°,

b) the intensity of the direct light is reduced until it is comparable to that of the diffracted light,

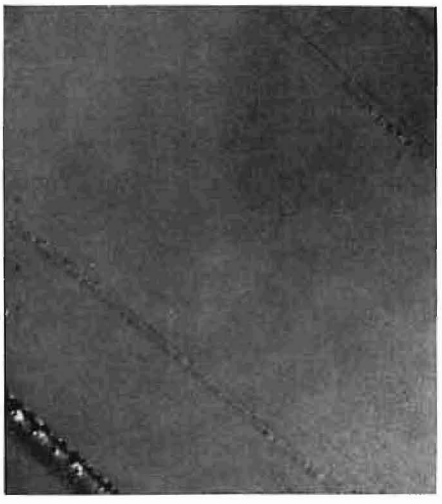
c) the diffracted light and the direct light of reduced intensity and shifted phase are superimposed on each other for interference.

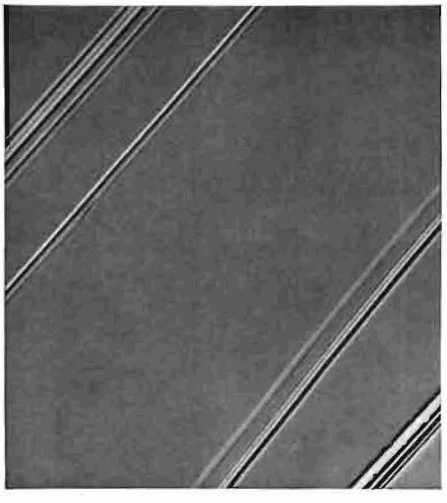
The ZEISS phase-contrast equipment satisfies all these conditions with the aid of an absorbing annular phase plate in the front focal plane of the objective.

The phase plate accelerates the light by 90° (positive phase contrast). In order to reduce the effect of the phase plate on the direct light as much as possible, a hollow cone of ligth produced by the annular condenser diaphragm is used for illumination. In spite of this precaution, a certain part of the diffracted light will also pass through the phase plate because whenever radiation is transmitted by the specimen, every point of the phase object becomes a wave center from which the diffracted light is deflected in certain directions. The smaller the object detail, the larger is the angle of diffraction. If the phase object is of appreciable extension and differentiated structure (which is practically always the case with biological objects), the diffracted light will also pass through the phase plate (shaded beam in Fig. 3 [see 24]). An additional path difference of 90° (undesirable but unavoidable) is imparted to this light. It interferes constructively with the direct light in the intermediate image plane, i. e. its intensity is increased (bright fringe). On the one hand, the intensity and extent of the halo effect are equipment factors determined by the amount to which the undiffracted light is absorbed and shifted in phase by the phase plate. On the other hand, the halo effect varies with the size of the object (23), a phenomenon that will be discussed in greater detail in the next paragraph. In addition, however, the halo effect is also a function of the difference in refractive index between the object and its surrounding field (8, 23), as is evident from Fig. 7.

On the whole, the halo effect is thus partly due to equipment conditions. While it can be reduced to a certain extent by suitable design of the phase-contrast accessories, it cannot be eliminated altogether.

A one-sided lightening of object edges similar to the halo effect is sometimes observed in differential interference contrast also. However, this phenomenon is due to entirely different causes which were explained in Part II in connection with the description of DIC image formation (12).





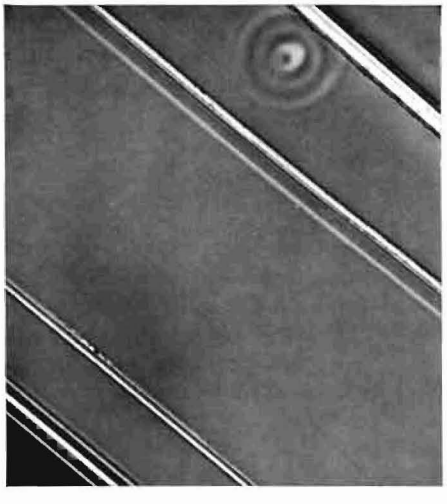


Fig.: 2 Optical staining of oriented linear phase objects (scratches in specimen slide).
Top: DIC image. The grooves extend in the direction of shear. Only details which exhibit very pronounced changes of optical thickness in a minimum of space stand out against the background.
Center: DIC image. Object turned through 90° and thus aligned for optimum contrast.
Bottom: PC image. Alignment of the object has no effect on contrast. Photomicroscope II, 40x N. A. 0.65 Planachromat and 40x N. A. 0.75 Ph-2 Neofluar, total magnification approx. 530x.

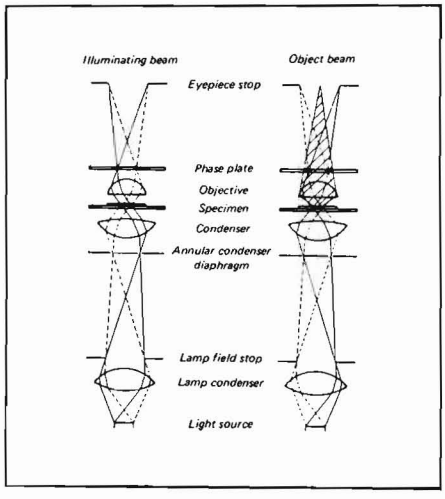
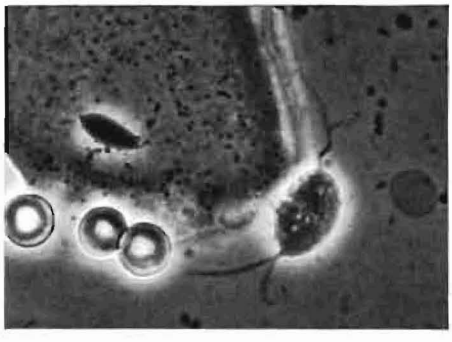


Fig. 3: Optical diagram of transmitted-light microscope with phase-contrast equipment.



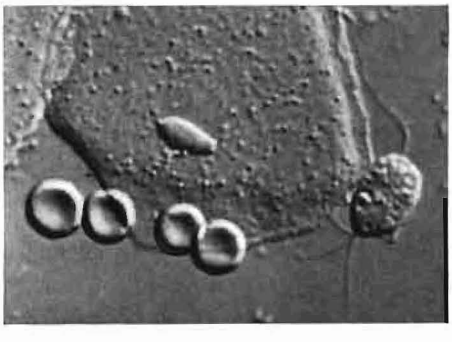


Fig. 4: The halo effect in PC microscopy with phase objects of "medium" size. The illustration[3] shows a gynecological smear in a saline solution: living trichomonad beside an epithelial cell and erythrocytes. Top: phase contrast, bottom: differential interference contrast. Photomicroscope. 40x N. A. 0.75 Neofluar and 40x N. A. 0.65 Planachromat. Total magnification approx. 530x.

[3] Figs. 4 and 7 courtesy of Prof. Dr. P. Stoll and Dr. H. Gundlach.

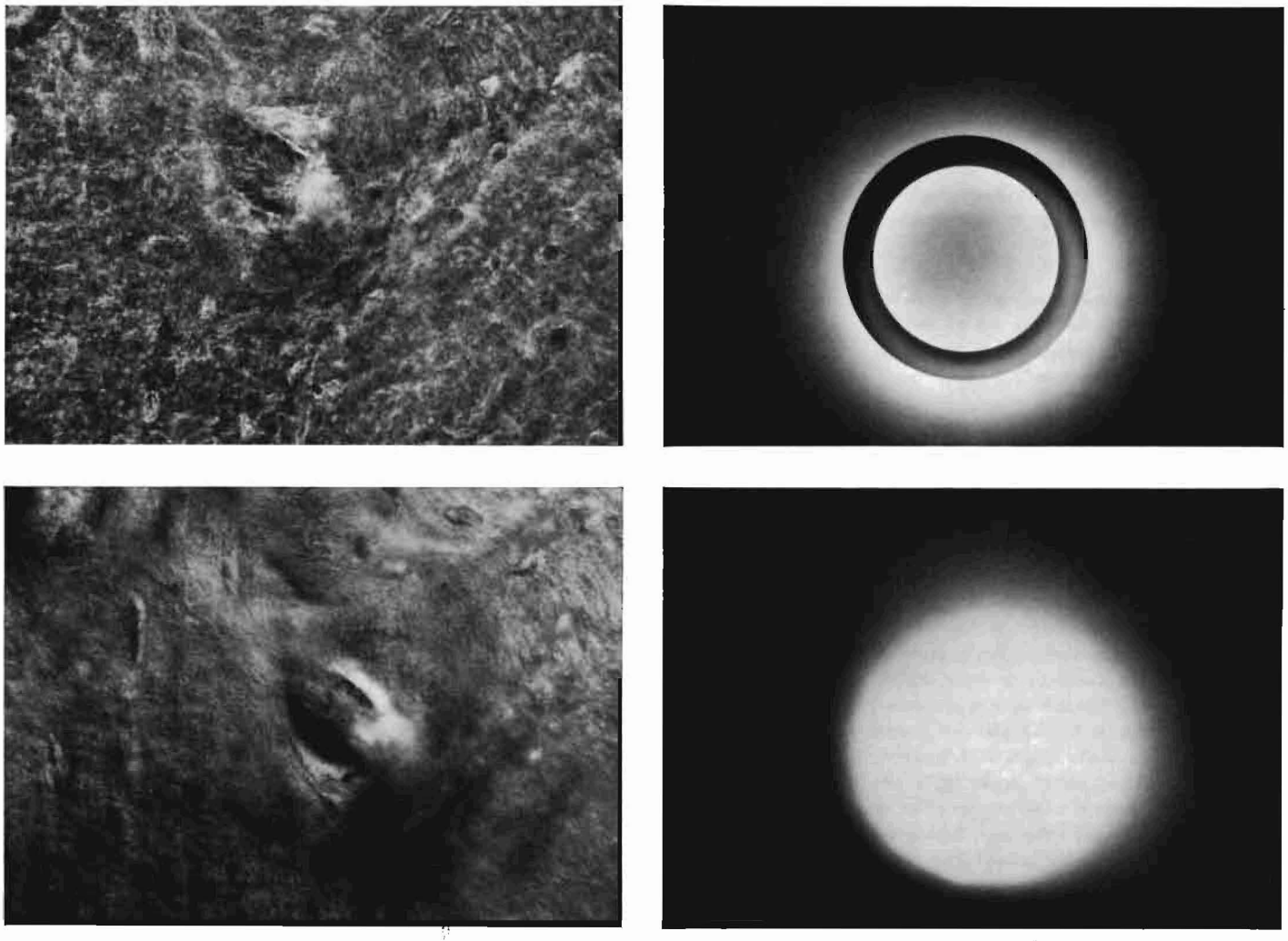


Fig. 5: This specimen (polished bone, tetracycline-labeled for fluorescence microscopy by reflected light) is unsuitable for observation by transmitted light because it is too thick and does not lie flat on the specimen slide. Exact reproduction of the contrast-generating PC or DIC elements is not possible under these conditions.
Top left: PC image. Right: pupil. Bottom left: DIC image. Right: pupil. Photomicroscope. 16x N. A. 0.35 Planachromat and 16x N. A. 0.40 Ph-2 Neofluar; Optovar 1.25x. Total magnification approx. 170x.

2.3 Object size and differences of refractive index

There is a direct connection between the halo effect in phase-contrast microscopy and the limited range of object sizes suitable for optimum reproduction in phase contrast (1, 7, 23). For the reasons mentioned under 2.2, the phase structure of phase objects of „medium" size is not reproduced with high fidelity because the phase plate of the Ph-objective has an undesirable effect on the light they diffract. Which object size should in practice be considered as "medium" depends on one hand on the size of the annular condenser diaphragm (with conjugate phase plate) and on the other on the magnification of the PC system used. A phase object, for example, which reveals the halo effect when observed with type Ph-2 phase accessories, should be considered as of "medium" size. If the same object is examined with a phase-contrast objective of higher power (Ph-3 with appropriate annular condenser diaphragm), it may then be considered as large. It is thus quite possible that one and the same object may show haloes under medium magnification but be free from haloes at high powers.

However, it should be noted that object size alone (for a given PC system) is not enough to explain the halo effect. Another factor to be taken into account is the difference in refractive index between the object and the mounting medium. The greater this difference, the more pronounced the halo effect (8, 14). It is therefore quite possible that not only objects of medium size but also small objects, for instance, exhibit a pronounced halo effect (see Fig. 7). By adapting the refractive index of the mounting medium to that of the object, these haloes can be drastically reduced.

Contrary to phase work, DIC microscopy is not characterized by such a pronounced dependence of image quality on the size of phase objects. DIC microscopy can be equally well applied to small, medium and large microscopic phase objects without any impairment of image quality (1, 7, 23). However, this applies only to interference microscopes using the principle of differential shearing, i. e. in which the lateral shift of wave fronts (12) is smaller or equivalent to the microscope's resolution (7). In the case of interference microscopes based on total splitting – e. g. the ZEISS Jamin-Lebedeff transmitted-light interference attachment – the admissible object size must be smaller than the separation between the measuring beam and the reference beam (11) to give satisfactory results.

Another advantage of Nomarski DIC microscopy comes as a welcome supplement to PC microscopy: pronounced differences of refractive index between the object and the mounting medium, which give rise to disturbing haloes in the phase-contrast image, are highly desirable for DIC work. They give images of excellent contrast and allow minute details to be recognized (e. g. Fig. 7, lower part of picture) which in phase contrast are hidden by bright fringes around the object.

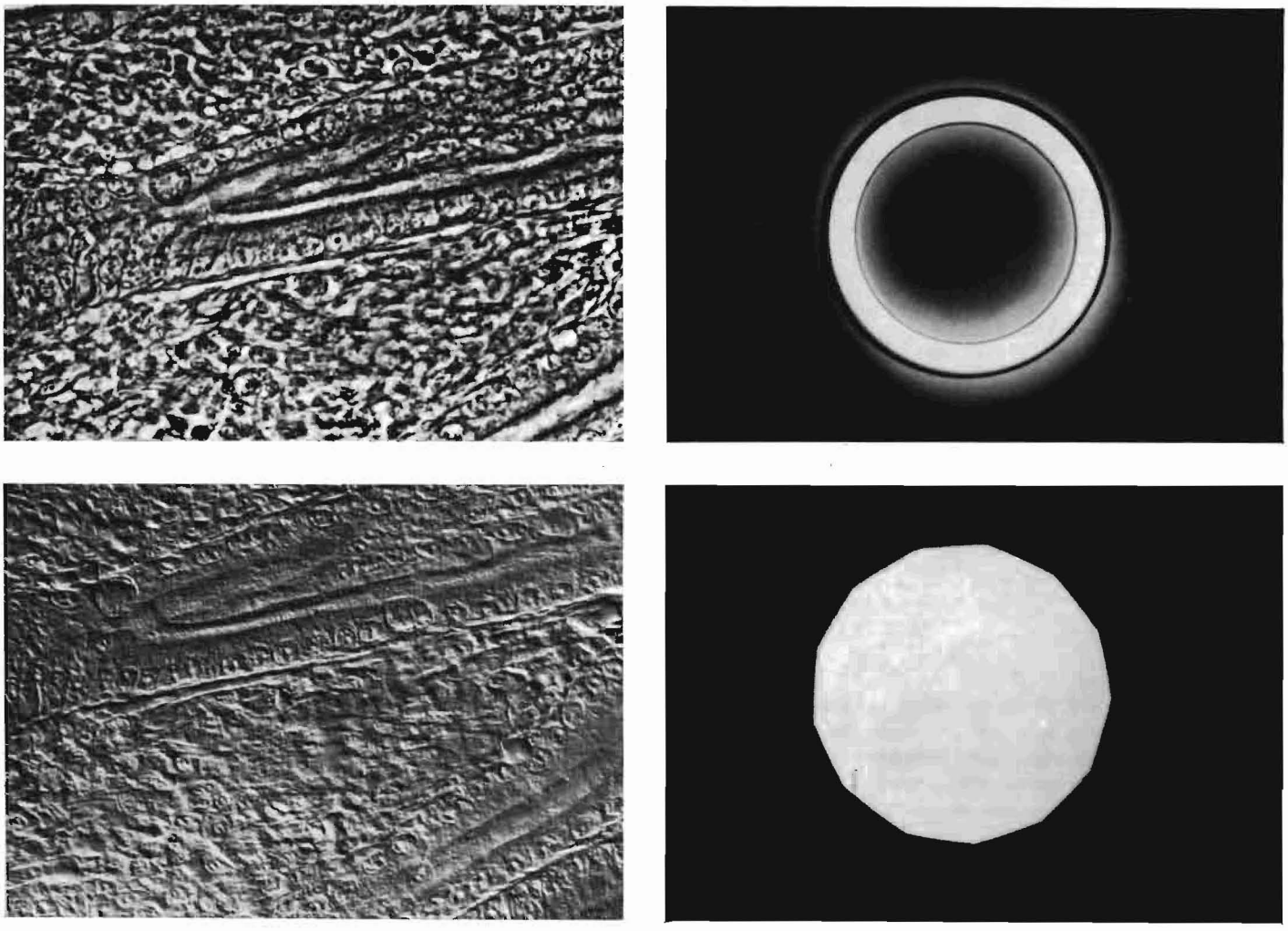


Fig. 6: Specimen suitable for examination by transmitted light (rat's tongue, unstained). Top left: PC image. Right: pupil. Bottom left: DIC image. Right: pupil. Photomicroscope, 16x N. A. 0.35 Planachromat and 16x N. A. 0.40 Ph-2 Neofluar; Optovar 1.25x. Total magnification approx. 170x.

2.4 Optical thickness of the object

The difference between the optical thickness (product of refractive index and geometrical path length) of the object field and the surrounding field determines the optical path difference Γ between object wave and field wave. The phase angle φ in degrees can be computed, as is known, from the relationship $\varphi = \Gamma\ 360°/\lambda$ where λ is the wavelength of the monochromatic light used. Let the expression $K = (E_{max} - E_{min})/E_{max}$ be the contrast, with E_{max} and E_{min} the maximum and minimum radiant intensity, respectively, of the microscopic image. Plotting contrast against phase angle, we obtain information on the optimum range in which a microscopic technique should be used. According to Michel (14, p. 110), a phase plate of 64% absorption introducing a phase shift of 90° will theoretically enhance contrast from 0 to 0.9 if the phase angle is increased from 0 to 20°. For very small phase angles contrast will even change linearly with φ. This range is most sensitive to changes of phase angle. Maximum contrast is obtained between 30° and 35°. Beyond these values, K drops to 0 at 180° as φ increases. For phase angles between 180° and 360° (negative phase contrast), the curve is inverted. The diagram also shows that even at path differences of up to half a wavelength ($\varphi = 180°$) ambiguous phase images may be produced due to the fact that very different phase angles have the same degree of contrast (25). Thus, for example, a contrast of 0.4 corresponds to phase angles of both 5° and 130°. In practice this means that under the aforementioned conditions points of different optical thickness in the phase object cannot be distinguished because they are of absolutely equal phase contrast.

In order to ensure unambiguous and accurate results, the phase-contrast method should therefore preferably be used for phase objects with small phase angles not exceeding 30°, which is equivalent to a path difference of not more than $\lambda/12$. According to Michel (14, p. 119), thickness differences of $^1/_{100}$ µm (= 10 nm = 100 Å) can still be distinguished with a contrast of 0.3 in a phase object with a refractive index of 1.5; if the geometrical thickness of the phase object is 5 µm, differences of refractive index of 0.001 in the object can be detected.

The above explanation shows that thick specimens are unsuitable for examination by the phase-contrast technique (14, p. 120). The same applies to specimens of wedge-shaped texture: in both cases, the exact reproduction of the annular condenser diaphragm on the phase plate in the microscope objective is made difficult if not impossible (Fig. 5). In these unfavorable conditions, phase contrast loses its experimental basis and becomes more and more of a bright-field method with all the disadvantages this holds for the reproduction of phase objects.

If possible, thin objects should be used also for DIC microscopy. In the case of very thick objects which, moreover, do not lie flat on

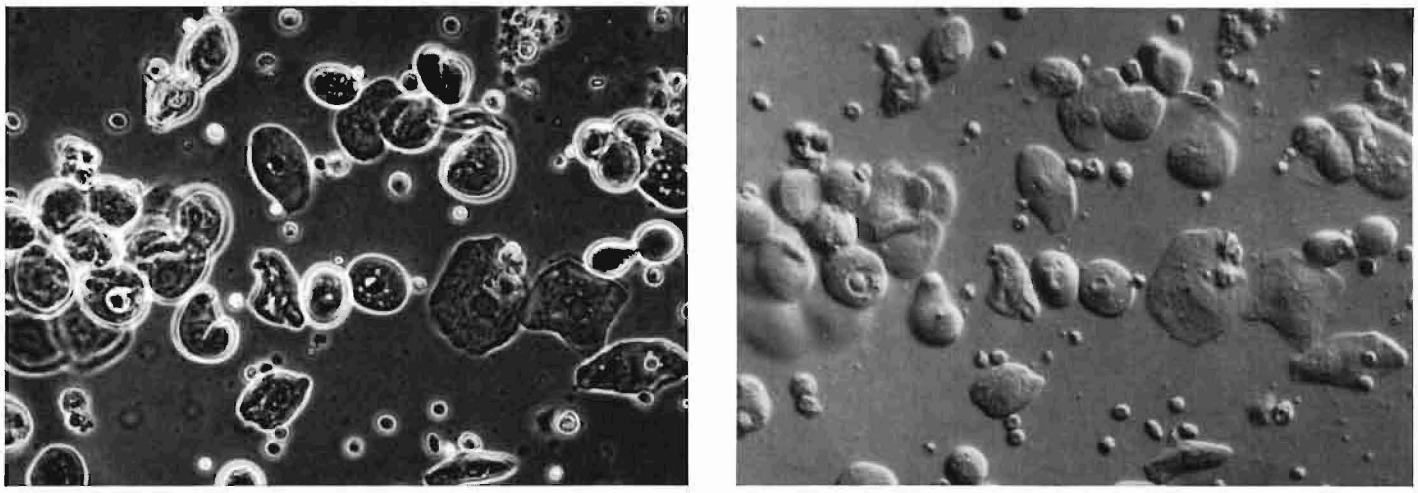


Fig. 7: Reproduction of detail in stratified phase objects. Gynecological smear in a saline solution; immature cells of lower epithelium (basal and parabasal cells). Left: PC image. Right: DIC image. Photomicroscope. 16x N. A. 0.40 Ph-2 Neofluar and 16x N. A. 0.35 Planachromat; Optovar 1.25x. Total magnification approx. 170x.

the specimen slide (Fig. 5), the interference plane of the auxiliary prism in the condenser can no longer be accurately focused on the conjugate interference plane of the principal prism above the objective. For comparison, a thin, flat phase specimen is shown in Fig. 6. In this case, the pupil image of the PC microscope also shows a sharply defined annular condenser diaphragm and objective phase plate; in the DIC microscope, a sharply defined image of the aperture (iris) diaphagm of the condenser is likewise visible in the pupil plane.

A comparison of the photomicrographs published in this journal (27) may serve as an example of the different image quality secured by phase-contrast and differential interference-contrast microscopy. This comparison also shows that the DIC method can be used over a far greater range of path differences in the object than would be practical with the PC method. If in this connection we look at Michel-Lévy's chromaticity diagram, the clear marking of the phase object by interference colors becomes evident over a wide range of path differences. Small path differences of about 50 nm (i. e. approx. $^1/_{10}$ wavelength of green light) fall in the area of first-order gray. The gray tone changes only very slowly with increasing path difference (e. g. up to 100 nm). Inexperienced observers will recognize these changes only with difficulty. In the area of first-order red, however, even slight changes of path difference by about 10 to 20 nm (equivalent to 2 to 4 % of the wavelength of green light) give rise to variations in color which are marked enough to be detected even by inexperienced observers. Since the Nomarski DIC equipment allows one of the Nomarski prisms to be shifted so that the image background can, within certain limits, be made perpendicular to the microscope axis (see 11, 12), phase objects can always be reproduced with optimum contrast.

If Michel-Lévy's chromaticity diagram alone were used to assess the DIC method's suitability for distinguishing optical thickness, the impression might be created that DIC microscopy is suitable only for relatively great path differences (such as 40 nm and larger). However, this is not so. Experience has shown that even very small path differences can be made visible. Fig. 2 may again serve as an example. The extraordinary capabilities of DIC microscopy are probably due to the fact that under favorable conditions[4] phase objects can be reproduced with contrast 1. Owing to this wide range of contrast, the observer is able to detect minor brightness differences and thus differences in optical thickness.

2.5 Gradient of optical thickness

An essential difference between DIC and PC microscopy is due to the lateral variation of optical thickness in a phase object; in this case we also speak of the effect of the gradient of optical thickness on the appearance of the DIC image (1, 8). For better understanding it should be recalled that DIC microscopy may be considered as two-beam interference microscopy with differential shearing (7, 11). If both waves traverse identical optical paths, they will produce identical intensity in the DIC image; in the special case in which the Nomarski prisms are in center position (zero path difference) with polarizer and analyzer crossed, the intensity in the DIC image will be zero. In other words, a variation of intensity (in the aforementioned case, lightening of the DIC image) is possible only if the two waves cover different optical paths. However, since the two waves are separated by only a minute distance – a distance roughly equivalent to the resolution of the microscope – a variation of intensity can occur only if there is a marked change in the optical thickness of the object even over this short distance. Or we may say that the partial differential quotient of the optical path in the phase object as referred to lateral shearing in the DIC system must differ from zero in order to reveal the phase structure of the object in the DIC image. (It is known that no such requirement exists for the phase-contrast technique [see 8].) Naturally, this requirement is easier to satisfy at the edges of objects than in extensive phase objects. It is therefore quite possible that only the boundaries of a phase object will appear in the DIC image. This was explained with a few examples in the discussion of DIC image formation (12, Fig. 4, case A, and Fig. 5, detail I). But it has been found that even the phase-contrast method is not completely free of this complication in regard to image interpretation. For in the DIC image of an extensive phase object of uniform optical thickness the intensity distribution in the interior of the PC object approaches that of the surrounding field, with increasing object size. In an extreme case, the object will therefore only stand out against the background due to the halo effect (26), and it should be noted that the boundary between the bright and the dark fringe is not necessarily identical with the actual limits of the phase structure (see 25).

From a viewpoint of high-fidelity reproduction of phase structures, the aforementioned characteristic of differential interference-con-

[4] By favorable conditions we here understand, for example, a single phase object with relatively few structural details on a homogeneous, i. e. textureless background.

trast microscopy would seem to be a shortcoming. However, it is precisely this apparent drawback which is very helpful in the examination of microscopic objects of greatly varying phase structure, because fine phase detail, which in the PC image passes unnoticed or is seen only with difficulty, occasionally stands out with extraordinary clarity in the DIC image (see Fig. 7, bottom). This is due to the above mentioned fact that in the DIC image the intensity distribution is determined by the difference in path length between the (plane) reference wave and the (deformed) differential wave (12). This explains why even with heavily structured object fields of greatly varying optical thickness the background will appear fairly plane ("flat"). Local optical path differences stand out with apparent relief from this "plane".

2.6 Depth of field

An essential advantage of Nomarski DIC microscopy over PC microscopy is due to the shallow depth of field involved in this method. We know that in the PC system the illuminating (and viewing) aperture is determined by the dimensions of the PC attachment; it cannot be varied. In Nomarski DIC microscopy, on the other hand, the diameter of the aperture diaphragm in the condenser can easily be adapted to the requirements of the specimen (1), as in bright-field work. A relatively large aperture (about $^2/_3$ of the objective aperture) can generally be used without any loss of contrast. Owing to this high illuminating aperture, the DIC method offers only shallow depth of field, which is particularly welcome for thick objects. Details outside the focal plane are thus less disturbing in the microscopic image than in phase contrast (see Fig. 2). As a result, DIC images of excellent quality can be obtained even under unfavorable conditions when PC images – due to their great depth of field – make the identification of phase structures impossible because of overlapping details above and below the objects of interest and, in addition, due to the halo effect. Here again, the bottom portion of Fig. 7 may serve as an example (see also 23).

2.7 Dichroic objects

One source of errors in the DIC method is the necessity of using polarized light. We can distinguish between an ordinary and an extraordinary ray, as was described in the preceding parts of this paper (11, 12). In so-called dichroic objects, the ordinary and extraordinary rays are absorbed to different degrees. In other words, they interfere with different intensity so that the DIC image is not only a function of the difference of optical path length for the two rays, which would normally be of interest, but also of the different absorption in the two beams. This effect is comparable to a setup in which the planes of transmission of polarizer and analyzer are not perfectly perpendicular to each other (see 12). Phase contrast, on the other hand, does not require the use of polarized light. Consequently, the PC method is free from possible disturbance due to dichroic substances. It may generally be said that in practice it will only rarely be necessary to examine dichroic (i. e. absorbing) objects with microscopes designed for phase work.

3. Summary

In addition to the outstanding features of the ZEISS DIC accessories explained in this series of papers on Nomarski DIC microscopy there are quite a number of aspects which cannot be discussed here. Apart from these theoretical considerations, practical experience also advises against the classification of the Nomarski DIC method at this stage, because it has been found that Nomarski DIC microscopy is being used increasingly in fields in which conventional methods of light microscopy have failed or give only unsatisfactory results.

However, we already know beyond any doubt that Nomarski DIC microscopy has gained a firm footing in reflected-light microscopy because it is clearly superior to incident phase-contrast microscopy in a great number of cases. In transmitted-light microscopy, on the other hand, the two methods would appear, as before, to complement each other. This once more justifies the ZEISS concept of combining annular diaphragms for PC microscopy with auxiliary Nomarski prisms for DIC microscopy in the type V Z achromatic-aplanatic substage condenser.

It is also noteworthy that the PC and DIC accessories by ZEISS differ in one essential point: The ZEISS phase-contrast systems are equipped with phase plates for constant phase shift and constant absorption. The ZEISS Nomarski DIC systems, on the other hand, allow both the phase of the light and its amplitude to be varied (the former by adjusting one of the Nomarski prisms, the latter by moving the analyzer out of its crossed position in relation to the polarizer). If in spite of this the phase-contrast technique has lost hardly any of its importance, this is probably due to two reasons:

a) Phase-contrast techniques are primarily used for the examination of biological and medical objects, and
b) biological and medical phase objects generally vary so greatly in local optical thickness that it would be neither reasonable nor possible to obtain optimum contrast at every point in the entire phase object by means of a variable phase-contrast system (see 14, p. 117). A compromise solution will thus be inevitable in these cases.

However, the situation is apparently quite different in reflected-light microscopy. Here the microscopic objects to be examined are "plane" from the start, and their relief varies only within relatively narrow limits. (With transparent objects, this relief is equivalent to geometrical thickness.) A second variable is then the locally different phase retardation upon reflection of the incident light from the surface of the opaque object. (In the case of transparent objects, the refractive index has to be taken into account instead.) Contrary to transparent objects, the interesting detail in opaque objects is frequently a small phase object on a homogeneous phase background. In this case, an extremely useful feature of the Nomarski DIC system is the fact that by suitable selection of path difference with the aid of one of the Nomarski prisms the object can be made to stand out optimally from the surroundings by means of interference (see 8). With biological objects, however, the range within which path-difference staining can be used is considerably smaller; it is limited to fractions of a wavelength (usually below $\lambda/4$). This is why in the case of (biological) transparent specimens the need of a microscopic method allowing variable staining is by far less pressing than with (non-biological) opaque objects.

References

[1] *Allen, R. D., G. B. David* and *G. Nomarski:* The ZEISS-Nomarski Differential Interference Equipment for Transmitted-Light Microscopy, Z. wiss. Mikr. (at press)

[2] *Barer, R.:* Phase Contrast, Interference Contrast, and Polarizing Microscopy, in Analytical Cytology (Pub. R. C. Mellors) McGraw-Hill Book Comp., New York 1955

[3] *Barer, R.:* Phase Contrast and Interference Microscopy in Cytology, in Physical Techniques in Biological Research (Pub. G. von Oster und A. W. Pollister), Academic Press, New York 1956, S. 29

[4] *Beyer, H.:* Theorie und Praxis des Phasenkontrastverfahrens, Akad. Verlagsgesellschaft Frankfurt am Main 1965

[5] *Françon, M.:* Le contraste de phase en optique et en microscopie, Paris 1951

[6] *Françon, M.:* Le microscope à contraste de phase et le microscope interferentiel, Paris 1954

[7] *Françon, M.:* Einführung in die neueren Methoden der Lichtmikroskopie, übersetzt von L. Albert, Verlag G. Braun, Karlsruhe 1967

[8] *Gabler, F.* und *F. Herzog:* Eine neue Interferenzkontrasteinrichtung für Arbeiten im Durchlicht, Druckschrift SD Interf. Kontr. DL D 8/66 bzw. SD Interf. Kontr. DL E 2/67

[9] *Haselmann, H.:* 20 Jahre Phasenkontrastmikroskopie, Historischer Rückblick und aktuelle Sonderfragen, Z. wiss. Mikrosk. **63** (1957) 140

[10] *Haselmann, H.:* Phasenkontrast-Mikroskopie, Betrachtungen zur Entwicklung einer wissenschaftlichen Apparatur, ZEISS-Werkzeitschrift 5 Vol. 26 (1957) S. 109

[11] *Lang, W.:* Differential-Interferenzkontrast-Mikroskopie nach Nomarski, Teil I: Grundlagen und experimentelle Ausführung, ZEISS Information Vol. 70 (1968) 114

[12] *Lang, W.:* Differential-Interferenzkontrast-Mikroskopie nach Nomarski, Teil II: Entstehung des Interferenzbildes. ZEISS Information Vol. 71 (1969) 12

[13] *Michel, K.:* Die Grundzüge der Theorie des Mikroskops in elementarer Darstellung, 2. Aufl., Bd. I der Reihe Physik und Technik, Herausgeber F. Gößler, Wissenschaftliche Verlagsgesellschaft mbH., Stuttgart 1964

[14] *Michel, K.:* Die Mikrophotographie, 3. Aufl., Bd. X der Reihe „Die wissenschaftliche und angewandte Photographie", Pub. J. Stüper, Springer-Verlag Wien-New York 1967

[15] *Menzel, E.:* Phasenkontrast-Verfahren, Z. f. angew. Phys. **3** (1951) 308

[16] *Menzel, E.:* Phasenkontrast-Verfahren, in Hdb. d. Mikroskopie in der Technik (Pub. H. Freund), Vol. I, Part 1, Frankfurt a. Main 1957

[17] *Neupert, H.:* Interferenzkontrast-Einrichtung nach Nomarski (Lizenz CNRS), ZEISS Information Vol. 65 (1967) 96

[18] No author: Supplement to catalog S 40–554 of CARL ZEISS

[19] *Nomarski, G.:* Interféromètre à polarisation, Brevet français No. 1 059 123, 1952

[20] *Nomarski, G.:* Microinterféromètre differentiel à ondes polarisées, J. Phys. Radium, Paris **16** (1955) 9

[21] *Nomarski, G.* et Mme. *A. R. Weill:* Application à la métallographie des méthodes interférentielles à deux ondes polarisées, Rev. Métallurg. **52** (1955) 121

[22] *Osterberg, H.* and *O. W. Richards:* Phase microscopy, New York, London 1951

[23] *Padawer, J.:* The Nomarski interference-contrast microscope. An experimental basis for image interpretation. J. Roy. Micr. Soc. **88** (1968) 305

[24] *Richter, R.:* Eine einfache Erklärung des Phasenkontrastmikroskops, Optik **2** (1947) 342

[25] *Rienitz, J.:* Phasenkontrastverfahren, in „ABC der Optik" (Pub.: K. Mütze, L. Foitzik, W. Krug und G. Schreiber), Verlag Werner Dausien, Hanau/Main 1961

[26] *Wolter, H.:* Schlieren-, Phasenkontrast- und Lichtschnittverfahren in Hdb. d. Physik (Pub. S. Flügge) Vol. 24, Berlin, Göttingen, Heidelberg 1956

[27] *Wunderer, A.* und *S. Witte:* Zur Anwendung der Interferenzkontrastmikroskopie in der Zytologie, ZEISS Information Vol. 70 (1968) 121

[28] *Zernike, F.:* Das Phasenkontrastverfahren bei der mikroskopischen Beobachtung, Z. Phys. **36** (1934) 848

[29] *Zernike, F.:* Das Phasenkontrastverfahren bei der mikroskopischen Beobachtung, Z. techn. Physik **16** (1935) 454