

**Nomarski differential
interference-contrast
microscopy**

by Walter Lang

I. Fundamentals and experimental designs

Since 1965, CARL ZEISS of Oberkochen has been supplying interference-contrast equipment for transmitted light and since 1967 for reflected light, both based on suggestions by Professor G. Nomarski. This equipment was developed in close cooperation with Nomarski and has meanwhile proven superior results in the manifold uses of optical interference-contrast techniques.

The increasingly spreading acceptance and use of Nomarski differential interference-contrast microscopy call for a comprehensive description of this technique. This is part one of a paper on the subject and it deals with the physical principles of the method and the instrumentation developed for it. A second paper will discuss the formation and interpretation of the differential interference-contrast image. Both together will serve as a basis for a comparison of Nomarski differential interference-contrast with phase-contrast, which will follow in a third paper. Finally, a fourth paper will deal with the applications of Nomarski differential interference-contrast. For easier comprehension we have avoided going into mathematical explanations. However, numerous references are given for readers who wish to pursue the study of the subject further.

First, the principle of interference-contrast microscopy will be explained. Then the most notable differences between the *Jamin-Lebedeff* and the *Nomarski* interference methods will be discussed. This will offer an opportunity to give a summary of the most important terms used in crystal optics and required to understand how the two methods operate. The design of the *Nomarski* interference-contrast microscope for transmitted light is described for two different techniques: one for double-beam interference microscopy, the other for the compensation of interference fringes. Both are also applicable to describe the equipment for reflected light.

1. Basic principles of interference-contrast microscopy

If in an interference microscope the distance between fringes (and thus also the width of

the fringes) is made so wide that one fringe covers the entire field of view, we speak of an infinite spreading of that particular interference fringe, or of interference contrast. Thereby the area of interest in a specimen is rendered visible by interference-contrast (6, 7, 8, 18, 23, 30). This can be explained with the aid of the micrographs shown in Fig. 1, which were taken by reflected light under the Interference Microscope made by CARL ZEISS, Oberkochen. This instrument, as a rule, is set in such a way that interference fringes are visible in the field of view. Under reflected light, the distance between fringes corresponds to half the wavelength of the monochromatic light used. Path differences, expressed in fractions or multiples of half a wavelength of the light used and produced by irregularities in the surface structure of the specimen, result in a displacement of the fringes. The amount of fringe shift is directly proportional to the path difference.

In the micrograph to the left in Fig. 1 the fringe spacing is very narrow; consequently, the fringe shift is also small, but the fringes are well defined and have relatively sharp contours. The micrograph in the center illustrates the transitional stage between finite and infinite fringe spacing: only five interference fringes can be seen in the field of view (as compared to 14 in the previous micrograph). Here, too, the path difference or fringe shift is expressed as fractions of half a wavelength of the light used. However, it is also obvious that the edges of the fringes are more or less "blurred". In the micrograph to the right, finally, the distance between two neighboring fringes is greater than the field of view. Here we speak of an infinitely wide distance between fringes or - in accordance with the definition given above - of interference contrast. A comparison with the other two micrographs clearly shows that in the case of interference contrast path differences are transformed into differences of brightness. This enhances the clarity of the microscopic image which appears to be almost three-dimensional, due to a certain shadow effect. On the other hand, it becomes also obvious that path differences can no longer be easily determined since no measurable fringe shift exists in interference contrast.

2. Operating principle of ZEISS double-beam interference microscopes

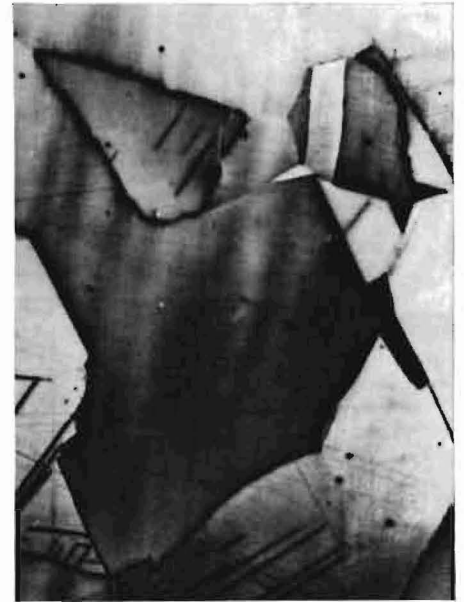
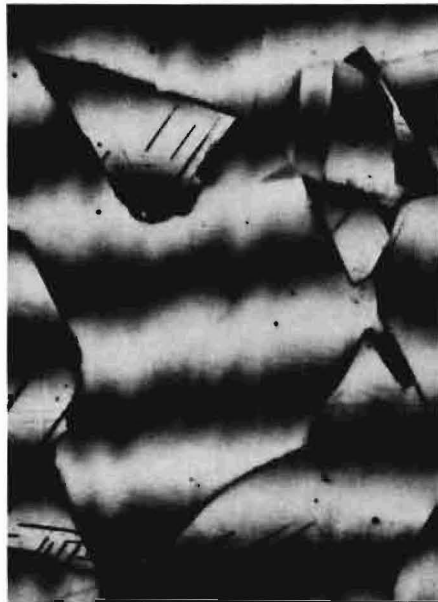
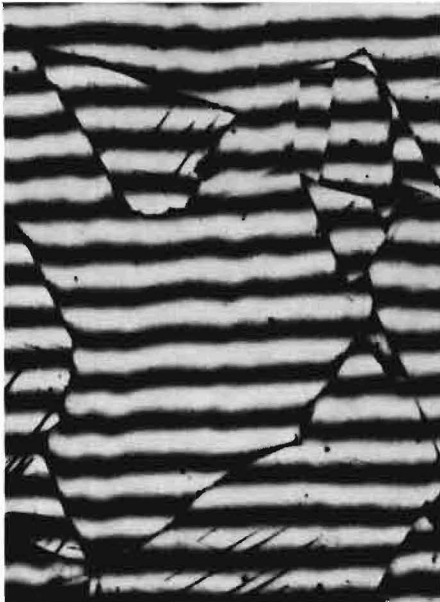
CARL ZEISS of Oberkochen, West Germany, produces three different double-beam interference microscopes: the *Michelson*, *Jamin-Lebedeff* and *Nomarski* types. The *Michelson*, which was used to take the micrographs shown in Fig. 1, is characteristic for the wide separation of the specimen and reference beams¹, which can reach an order of magnitude of up to a few centimeters. In the *Jamin-Lebedeff* interference microscope, the lateral separation of the reference beam in relation to the specimen beam is considerably smaller, namely a few millimeters (see below). Finally, in the *Nomarski* interference microscope, the lateral separation of the two beams is only a few microns, i. e., it is slightly smaller than the resolving power of the microscope. In this case we speak of differential splitting of beams.

In double-beam interference microscopes the magnitude of beam splitting is of great importance for the interference image and offers another classification of double-beam microscopes: in the case of the *Michelson*-type microscope we can always assume that the reference beam is not affected by the specimen. In interference microscopes of the *Jamin-Lebedeff* type, the reference beam is only then not influenced by the specimen if the specimen under examination is smaller than the distance between the two beams. However, if we leave to deal with a differential splitting of beams, as is the case in the *Nomarski* interference microscope, the terms "specimen beam" and "reference beam" have no meaning since both beams pass through the microscopic specimen. In other words, with differential beam splitting both beams are influenced by the specimen - a fact that must definitely be taken into account when interpreting the interference image.

In addition to the aforementioned differences existing in the double-beam interference

¹ By specimen or measuring beam we understand the bundle of coherent light which passes through the specimen. The second coherent bundle, called reference or comparison beam, does not pass through the specimen but bypasses it.

Fig. 1: Interference micrographs of polished chrome-nickel steel showing increasing spacing of interference fringes (see 27) produced by tilting a plane-parallel plate in the light path (36).



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microscopes, there is another distinguishing characteristic, that of splitting and recombining the beam. In the interference microscope of the *Michelson* type a beam-splitting prism is used. (For further details, see 11, 13, 16, 17, 28, 35, 36, 37, 40 and 41). In the *Jamin-Lebedeff* and *Nomarski* double-beam interference microscopes, on the other hand, the beams are split and recombined by birefringent crystals. Birefringent or doubly refracting crystals (3, 4, 23, 34) are crystals which split an incident light wave into two components which are plane-polarized and whose vibration planes run perpendicular to each other. Fig. 2 may serve as an explanation. It also illustrates the principle of wave splitting in the *Jamin-Lebedeff* microscope. Let us assume that a light wave, of which the diagram shows only the axis, hits a plane-parallel calcite plate perpendicularly. Furthermore, we assume that the incident wave is plane-polarized and its vibration plane is inclined by 45° to the plane of the diagram. The optic axis² of the calcite plate – marked by the double arrow – runs parallel to the plane of the diagram. As the beam enters the crystal, the wave is split into two parts, the axes of which are entered in the diagram as

a principal section³. The so-called ordinary wave is transmitted by the crystal without any deflection. The so-called extraordinary wave is deflected to one side, in spite of its vertical (90°) incidence on the plane-parallel plate. It emerges from the crystal parallel to the axis of the ordinary wave. If the direction in which the crystal is cut is known, the separation between the two waves is a function of the thickness of the crystal plate. In the case of the ZEISS *Jamin-Lebedeff* transmitted-light interference equipment it amounts to minimum 0.05 mm (Achromat Pol Int., 100 x, 1.0 N.A., oil) and maximum 0.5 mm (Achromat Pol Int., 10 x, 0.22 N.A.). As is evident from Fig. 2, a birefringent plate is characterized by another feature in addition to lateral beam splitting. The plane-polarized light wave which hits the crystal and whose vibration direction is offset by 45° to the plane of the diagram, is split into two plane-polarized components. The vibration direction of the ordinary wave is perpendicular to the plane of the diagram, while the vibration direction of the extraordinary wave coincides with the plane of the diagram. This is a fact which must be taken into account in the design of the *Jamin-Lebedeff* interference

microscope, because, after recombination, the two coherent bundles can only interfere and produce an interference image, if the vibration directions of the two plane-polarized waves lie in one and the same plane⁴.

Similar considerations also apply to the *Nomarski* differential interference microscope. They are of decisive importance not only for the formation, but also for the

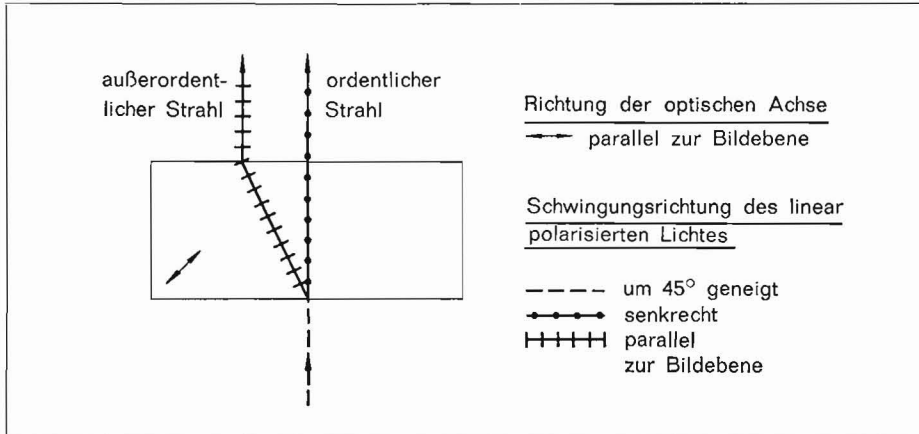
² The optic axis of a birefringent crystal indicates the direction in which the ordinary and extraordinary waves coincide. This direction, in which the crystal behaves like an isotropic, i. e., not birefringent medium, is also called the isotropic axis. In the case of calcite and quartz it is identical with the optic axis of the crystal. Crystals which have only a single optic axis, e. g., calcite or quartz, are called uniaxial crystals.

³ Any plane through the crystallographic axis is a principal section of a crystal.

(Following general parlance, the term "beam" taken from geometrical optics is here and in the following frequently used instead of the more appropriate terms "bundle of rays" or "light wave".)

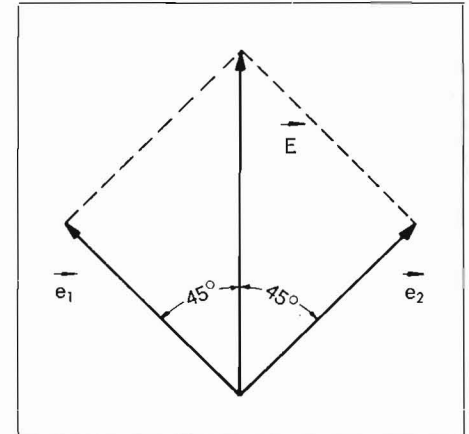
⁴ The term "plane of polarization" (plane perpendicular to the vibration direction of the electric vector) used in earlier literature can be dispensed with and is not used in this paper.

Fig. 2: Beam path in the principal section of a birefringent plate.
 (außerordentlicher Strahl = Extraordinary ray, ordentlicher Strahl = Ordinary ray, Richtung der optischen Achse = Direction of optic axis, parallel zur Bildebene = parallel to image plane, Schwingungsrichtung des linear polarisierten Lichtes = Vibration direction of plane-polarized light, um 45° geneigt = inclined by 45°, senkrecht = perpendicular, parallel zur Bildebene = parallel to image plane)



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Fig. 3: Splitting the vector \vec{E} into \vec{e}_1 and \vec{e}_2 , the last two at right angles to each other.



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interpretation of the interference image. Therefore, the splitting of a plane-polarized wave into two plane-polarized components of different vibration directions will be discussed in greater detail (see also 6, 21, 22, 34). The opposite process, namely the recombining of two plane-polarized components into a plane-polarized wave, will be dealt with in connection with the interpretation of the differential-interference image.

The vibration direction of a plane-polarized light wave is identical with the plane in which the vector of the electric field strength \vec{E} vibrates. In accordance with the rules governing the calculus of vectors, \vec{E} can be split up into two vectors, \vec{e}_1 and \vec{e}_2 , which are at right angles to each other and each of which forms an angle of 45° with \vec{E} (Fig. 3).

If we apply this formalism to a plane-polarized light wave, the electric vector of which changes sinusoidally with time, we obtain the diagrammatic representation in Fig. 4: it shows a sine wave (center curve) proceeding from the left foreground to the right background; the vibrations represented by the two inclined curves are equivalent to it.

The form of representation chosen in Fig. 5 is particularly advantageous for interpreting the differential interference-contrast image.

It summarizes the most essential results of Figs. 3 and 4. Let W in (a) be a plane-polarized wave normal to the plane of the diagram. Following the scheme of Fig. 3, this wave with the vectors \vec{E} and \vec{E}' (b) can be split into the two components w_1 and w_2 with the vectors \vec{e}_1 , \vec{e}_1' and \vec{e}_2 , \vec{e}_2' . In (c) the vibration plane of the wave W coincides with the plane of the diagram.

As was mentioned above, a strong lateral beam-splitting effect is achieved in the *Jamin-Lebedeff* interference microscope with the aid of a birefringent plane-parallel plate. (Questions of the beam recombination and compensation cannot be discussed here. For more information interested readers are referred to the pertinent literature: 9, 10, 14, 20, 23. For numerous references, see 25).

If a so-called *Wollaston* prism is used as a beam splitter instead of a birefringent plane-parallel plate, the beam splitting that results will not be lateral but angular (Fig. 6). A *Wollaston* prism consists of two prisms cemented together and made of birefringent, uniaxial material (preferably quartz or calcite). The optic axes of the two prisms are at right angles to each other. If a plane-polarized bundle of light (vibration plane inclined by 45° to the plane of the drawing) hits the *Wollaston* prism perpendicularly, as is shown in Fig. 6, it will be split into two plane-polarized waves in the lower prism, the vibration planes of which each make an

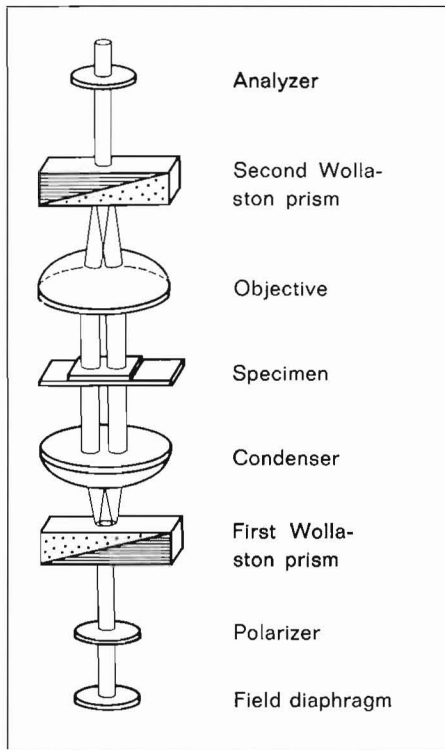
angle of 45° with the incident wave (see Figs. 3 and 5). At the cemented surface of the *Wollaston* prism the two component waves are deflected in two directions at a certain, relatively small angle. The ordinary wave⁵ is deflected towards the base of the upper prism, while the extraordinary wave⁵ is deviated towards the edge of the upper prism. The two component beams encounter different indices of refraction so that the wavefronts travel with different speed.

3. Double-beam interference-contrast microscope with two Wollaston prisms

On the basis of the explanatory remarks in the previous section, the design of a double-beam interference-contrast microscope can be easily explained with the aid of Fig. 7 (see also 5, 8, 30, 31, 32). The unpolarized light emerging from the field diaphragm is plane-polarized by the polarizer and strikes the first *Wollaston* prism. As was explained

⁵ The ordinary wave is the component vibration perpendicular to the plane of the crystal's principal section. Conversely, an extraordinary wave vibrates parallel to the plane of the principal section. Since the *Wollaston* prism is composed of two prism elements, the optic axes of which form an angle of 90°, it has two different, mutually perpendicular principal planes. Since, moreover, the vibration direction of the two components is fully preserved on the way from the lower to the upper prism, the ordinary wave of the lower prism becomes the extraordinary wave in the upper prism, while the extraordinary wave of the lower becomes the ordinary wave in the upper prism.

Fig. 7: Diagrammatic representation of a double-beam interference-contrast microscope as suggested by Smith, with two Wollaston prisms.

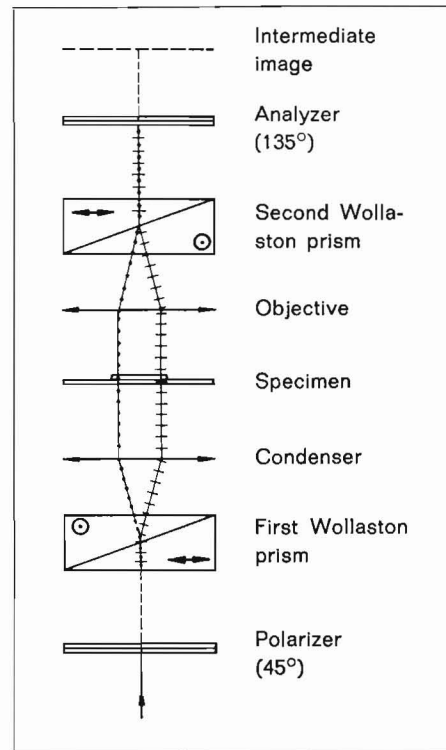


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the microscope objective and the second *Wollaston* prism. It becomes obvious that the condenser with the first *Wollaston* prism and the objective with the second *Wollaston* prism are functionally correlated. The analyzer is oriented so as to form an angle of 45° with the vibration plane of each of the entering waves. In accordance with Figs. 4 and 5 this ensures that both beam components act with the same intensity. What happens when the analyzer forms an angle other than 45° with the two beam components will be discussed in detail in connection with the interpretation of the differential interference-contrast image.

In the equipment for transmitted light, two *Wollaston* prisms are needed for beam splitting and recombining, whereas only one *Wollaston* prism is required for a reflected-light setup, since the light passes through the prism twice in opposite directions (30, 31, 32). The splitting of the beam when it passes through the prism the first time is

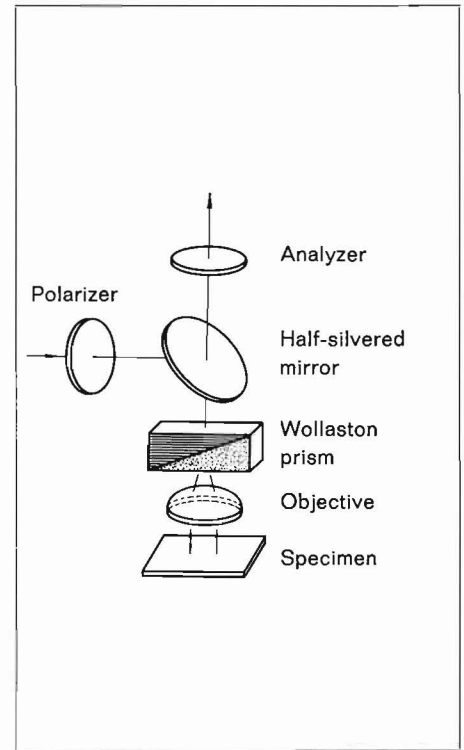
Fig. 8: Principle of a double-beam interference-contrast microscope with two Wollaston prisms. The symbols used for the optic axes of the crystals and the vibration directions of the plane-polarized waves are the same as in Fig. 6.



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cancelled out when it passes the prism the second time. In other words, when the beam travels the first time through the prism the reflected-light objective and the *Wollaston* prism act in the same manner as the condenser and the first *Wollaston* prism of the transmitted-light setup illustrated in Figs. 7 and 8, while when it travels through the prism the second time, these components correspond to the objective and the second *Wollaston* prism in those figures. This is indicated by the diagram in Fig. 9: the beam that is plane-polarized by the polarizer is deflected to the *Wollaston* prism by a half-silvered mirror. The two beam components emerge from the reflected-light objective parallel to each other and with a slight lateral separation. The beams reflected by the specimen are then recombined with the aid of the objective and the *Wollaston* prism, pass through the half-silvered mirror and, by means of an analyzer, are brought to vibrate in one plane. The interference-contrast image of the specimen is formed in

Fig. 9: Diagrammatic representation of a double-beam interference-contrast setup for reflected light using one Wollaston prism. For greater clarity, the lateral separation of the two beams striking the specimen has been exaggerated.



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the conventional manner in the Intermediate image plane (not shown) and viewed through an eyepiece.

As regards the diagram in Fig. 9, it may be mentioned that the beam passing through the polarizer is inclined by 45° to the plane of the diagram. The polarizer should therefore be imagined as lying below the plane of the drawing. For this reason – and because of the perspective view – the vibration directions of the polarized components have not been indicated. In principle, however, conditions are similar to those shown in Figs. 7 and 8 for transmitted light.

4. The Nomarski double-beam interference-contrast microscope

The preceding remarks were primarily devoted to the design and operation of the birefringent components used to split and recombine the beams, while nothing was said about the effect of the specimen on the

beams. This will be discussed together with the formation and interpretation of the interference-contrast image in the second paper to be published on this subject in this journal. We shall now examine what requirements have to be fulfilled by the image-forming optical system.

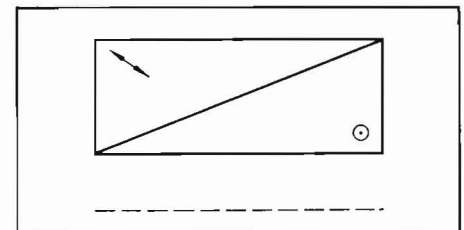
When discussing the double-beam interference-contrast microscope with two *Wollaston* prisms for transmitted light, we assumed that the lamp-side focal plane of the condenser and the eyepiece-side focal plane of the objective are located in the central planes of the *Wollaston* prisms (8, 15, 30, 31, 32). In addition, we assumed the interference planes of the *Wollaston* prisms, which coincide with their central planes (parallel to the upper and lower surfaces of the prism) to be exactly superimposed. The first of these requirements presents fundamental difficulties: with high-power objectives, the image-side focal plane lies in the objective, below the thread seat: a *Wollaston* prism can therefore not be located in that plane. This is equally applicable to transmitted-light and reflected-light objectives. But neither do transmitted-light condensers of high numerical aperture and correspondingly small focal length allow a *Wollaston* prism to be located in their front focal plane (i. e. on the side of the light source). As a result, differential interference-contrast microscopy with the aid of *Wollaston* prisms is limited to very low magnifications (30).

A very neat and technically feasible solution to these problems was the introduction of *Nomarski's* modified *Wollaston* prism which in the following will be briefly called "*Nomarski* prism". This prism (Fig. 10) consists of two cemented components of a uniaxial, birefringent crystal, such as calcite or quartz. The optic axis of one of these prisms (the lower one in Fig. 10) is parallel to the wedge side, as in the *Wollaston* prism. The optic axis of the other prism, (the upper one in Fig. 10), however, is inclined at a certain angle to the upper bounding face. As a result, the interference plane is outside the compound prism (dashed line in Fig. 10). By suitable orientation of the *Nomarski* prism its interference plane can be made to lie in the eyepiece-

side focal plane of the objective, although the *Nomarski* prism itself is located at a relatively long distance from the objective. This is of considerable importance for the design of a differential interference-contrast microscope: since the parfocal distance of the objectives, i. e. the distance between the object plane and the objective seating face, is given and constant, the *Nomarski* prism can likewise be mounted at a given distance from the objective, e. g. in an interference-contrast slide. Moreover, if the position of the eyepiece-side focal plane of the objectives used only slightly deviates axially from a certain mean position, a single *Nomarski* prism will suffice to recombine the beams with all available transmitted-light objectives. With the ZEISS interference-contrast equipment for transmitted light, one and the same *Nomarski* prism, i. e. the same interference-contrast slide⁶, is used for objectives of 16 x, 40 x and 100 x. For this reason – and for other reasons which cannot be explained in detail here – this is called the principal prism, to distinguish it from the so-called secondary prisms accommodated in the substage condenser. In the ZEISS transmitted-light equipment, there are three secondary prisms which are contained in the achromatic-aplanatic condenser (type VZ) for interference contrast, phase contrast and bright field and designated as I, II and III. Like the different sizes of annular condenser diaphragms for phase contrast, the secondary prisms in the condenser are adapted to the numerical aperture (and the initial magnification) of the objectives to be used.

The terms "principal prism" and "secondary or compensating prism" are used in connection with an entirely different way of describing the differential interference-contrast microscope (8, 26, 30, 32). In the present paper, the design of the *Nomarski* differential interference-contrast microscope has for didactic reasons been described as that of a double-beam instrument. The aforementioned second approach is useful sup-

Fig. 10: *Nomarski* prism with direction of optic axes and position of interference plane indicated.



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plement and should therefore be briefly discussed: first, imagine all optical components such as the condenser (with secondary prism), specimen, objective and principal prism between the polarizer and the analyzer removed from the light path. The field of view will then be completely dark. If the principal prism, that is a birefringent component, is now inserted diagonally between polarizer and analyzer, the field will be partly illuminated; interference fringes will be visible parallel to the wedge sides of the component prisms; the fringes are black or colored, depending on whether monochromatic or polychromatic light is used for illumination (only the fringe or zero path difference will always be black). If the secondary prism is then moved into the light path together with the image-forming optics, the interference fringes of the secondary prism can be made to cancel out the fringes of the principal prism in the objective pupil, provided that the two prisms are suitably dimensioned and oriented: the field is once more completely dark. This corresponds to interference contrast explained above in connection with the interference microscope, which exists in the case of infinite fringe spacing.

While in the transmitted-light setup the principal and secondary prisms are separate, the principal prism of the reflected-light setup also acts as secondary prism. It is contained in the interference-contrast equipment into which the proper reflected-light objectives are screwed. The prisms are adapted to the numerical aperture of the objectives. – For further technical details and practical hints consult the literature listed overleaf (1, 2, 8, 24, 26, 29).

⁶ The fact that the interference-contrast slide for the big STANDARD UNIVERSAL, PHOTOMICROSCOPE and ULTRAPHOT II is different from that of the STANDARD microscopes of the WL, RA and KL series is due to the different distance of the interference-contrast slides from the objectives (and other tube optics).

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