MICROSCOPY

PlasDIC – a useful modification of the differential interference contrast according to Smith/Nomarski in transmitted light arrangement*)

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PlasDIC is a new polarization-optical transmitted light differential interference contrast method where, unlike conventional DIC according to Smith or Nomarski, linearly polarized light is only generated after the objective. Consequently, the differential interference contrast is invariant in relation to the unwanted optical anisotropies that may stem from the condenser, slide, object and objective. Cells grown in plastic petri dishes, for example, can be studied with DIC.

1 Introduction: The traditional DIC method according to Smith/Nomarski

In invisible structures microscopy, we are, of course, dealing with *phase objects* that, unlike *amplitude objects*, affect not the amplitude but the phase of the light with which they interact. For this reason, such structures, when viewed with a spherically and chromatically corrected microscope, are also depicted true to the object, i.e. invisibly or with a contrast approaching zero. So, in order to create high-contrast images of these invisible structures without actually interfering with them (e.g., using staining), phase-optic aids have to be used. Much has been written over the past century about the possibilities of high-contrast depiction of phase objects in light microscopes [1,4], and the differential interference contrast method according to Smith/Nomarski [2,3] certainly ranks among the best of them today.
The Smith/Nomarski interference contrast method is based on the principle of self-reference: With the help of a polarization-optical shearing interferometer (Wollaston prism), the light is split into two coherent component waves independently of the object. These waves are influenced equally by the object and finally, laterally displaced by a small ("differentially small") amount s (= shear), interfere with one another. To achieve the desired gray contrast, not just the color contrast, the oscillation directions of the polars, i.e. those of the polarizer and the analyzer, must be perpendicular to each other. The Wollaston prism is arranged diagonal to the crossed polars, so that the two component waves have equal amplitudes and, consequently, the image can appear with maximum interference contrast [5].
The typical relief contrast for this method is produced not by the geometry alone, but by the path difference profile of the object (for example, at its limits), which is proportional to the product of the thickness and the refractive index difference compared with its immediate environment.

*) Dedicated to Dr. Hans Tandler for his 65th birthday on 30.05.2004

Figure 1 illustrates the basic principle of the DIC arrangement according to Smith/Nomarski. The linearly polarized light leaving the polarizer 1 reaches Wollaston prism 2, where it is split equally into the ordinary beam and the extraordinary beam. The extraordinary beam oscillates in the principal section (plane that contains the crystallographic axis and the wave normal of the incident light beam) and the ordinary beam oscillates perpendicular to the principal section.
The beams, separated in this way according to their polarization direction and propagation speed, travel in the same direction in the first sub-prism because the crystallographic axis there lies parallel to the drawing surface. However, in the second sub-prism, the crystallographic axis lies perpendicular to that of the first sub-prism, and so the functions of the ordinary and extraordinary beams are reversed and the angular split of the two beams can be effected as desired. The backward projections of the split beams are sheared in the condenser focal plane 3, pass through object points OP_1 and OP_2 in the object plane 5 as parallel beams and are reunited again at Wollaston prism interface 7, which coincides with the objective focal plane 8.
The OP_1OP_2 distance corresponds to the object-plane-related shear s, which lies in the order of the objective's resolution limit. Wollaston prisms at the condenser side (2) and at the imaging side (7) must be harmonized such that the condition

$$f'_{\kappa} \cdot \varepsilon_{\kappa} = f'_{o} \cdot \varepsilon_{o} \qquad (\varepsilon = \text{sine } \varepsilon << 1) \quad \text{(equation 1)}$$

where f'_{κ} condenser focal distance, ε_{κ} splitting angle of the Wollaston prism at the condenser side, f'_{o} objective focal distance and ε_{o} splitting angle of the Wollaston prism at the imaging side, is satisfied.

Due to the inclined wave fronts in the condenser and objective focal planes, a pattern of interference fringes is created in each with the following fringe distances:

$$d_{\kappa} = \lambda/\varepsilon_{\kappa} \text{ and } d_{o} = \lambda/\varepsilon_{o}, \text{ respectively} \qquad \text{(equation 2)},$$

where λ is the wavelength of the light.

The fringe pattern of Wollaston prism 2 is depicted in the fringe pattern of Wollaston prism 7 such that the minus nth interference order of pattern 2 is depicted in the plus nth interference order of pattern 7. In this way, the path difference in the objective focal plane (objective exit pupil) is compensated for fully. Hence, the sole function of the Wollaston prism at the illumination side, namely to be able to work with full illumination aperture, becomes obvious.

2 The limitations of the conventional DIC setup according to *Smith/Nomarski*

As already mentioned above, the relief-contrasted image corresponds to the product of the object thickness and the refractive index difference compared with its immediate environment, provided that the object is isotropic. This is only the case if the refractive index is constant for all transmission directions and the refraction of light follows Snell's law. A double refractive object, whose refractive index is direction-dependent, would produce a phase profile that, depending on its direction of oscillation and illumination aperture, contains an additional term, namely the product of object thickness and *refractive index difference between ordinary and extraordinary light*. This, however, leads to a considerable loss of contrast and the disappearance of the relief effect. In addition, there should be no further anisotropic (i.e. double refractive) elements between the polars, such as plastic slides or high-tension condensers and objectives, which, as they are also orientation- and aperture-dependent, have a "phase-shifting" and, consequently, contrast-reducing effect.
The only remaining logical step would be to shift the polarizer to a plane where the light has already passed through the object and objective. But can a DIC setup that has been modified in this way actually work?

3 The PlasDIC principle

The arrangement we are imagining is set up in accordance with **Figure 2**. The aperture stop in the condenser focal plane 1 is closed up to the point where a paraxial light beam passes through the object point OP in the object plane 3, the objective 4 and the polarizer 5. At the Wollaston prism interface 6 the beam is split, in the same way as in Figure 1, by angle ε, with the result that the backward projections of the

beams s_1, s_2 generate two object points that are laterally displaced by the shear s and coincide in the intermediate image plane (not depicted). Through the microscope the observer therefore sees the same duplication of the image that he would see on a conventional Smith/Nomarski microscope. This also means, however, that the object can be illuminated using natural light, and consequently *it is only necessary to generate linearly polarized light immediately in front of the Wollaston prism on the imaging side!* [6]
The image quality is, however, greatly restricted, because, as initially stated, point illumination, i.e. coherent illumination, was used. But what happens to the contrast of the interferogram? Can this actually be set for incoherent, or at least partially coherent, illumination with an “unarmed”, i.e. “prism-free”, condenser?

Whereas in Figure 2 only the axis point was included in the calculation, we now expand the illumination by a point far from the axis (distance b) in the condenser focal plane (**Figure 3**) so that the object point OP is illuminated at aperture angle α. The inclined beams through OP or OP_1 and OP_2 reach the objective focal plane with a path difference Δ that is dependent on the shear s (=OP_1OP_2) and the illumination aperture (sine α). This path difference would be expected to be the product of both variables:

$$\Delta = s \cdot \sin \alpha \qquad \text{(equation 3)}$$

Beam pairs with a path difference Δ of between zero and $s \cdot \sin \alpha$ therefore interfere at the same image point. In the least favorable scenario, namely $\Delta = \lambda/2$, the same number of beam pairs are present, and these intensify and extinguish each other with the result that the contrast (quotient of the difference and sum of the maximum and minimum intensity = Michelson contrast definition) disappears completely. The illumination aperture cannot therefore be rotationally symmetric, as it is only in one direction, namely the direction of shear, that it may not exceed a certain threshold value. It has therefore been established that the condenser iris diaphragm has to be replaced by a slit diaphragm. The width of the slit determines the path difference Δ, where the image contrast K is proportional to a sinc function (**Figure 4**):

$$K = \frac{I_{max} - I_{min}}{I_{max} + I_{min}} \sim \operatorname{sinc}(\Delta\, 2\pi/\lambda) \qquad \text{(equation 4)}$$

It can be seen from the function curve in Figure 4 that, in order to generate a sufficient contrast of 0.64, the width of the slit should correspond to no more than a quarter of the interference fringe distance d_O in the objective exit pupil. This requirement has been published in the literature [1] under the term “quarter-wave condition”. If, by way of example, the object-plane-related shear size is the same as the resolution limit of the objective (= 0.61 λ/nA), the illumination aperture sine α in the direction of shear

$$\sin \alpha = \frac{\lambda}{4\,s} = \frac{nA}{2.44} \qquad \text{(equation 5)}$$

may amount to around 40% of the objective’s numerical aperture (nA) without falling below the stated contrast value. For practical application, however, this necessary reduction in the illumination aperture represents a balanced compromise in terms of interference contrast, image quality and lateral resolution, especially as the illumination aperture remains perpendicular to the direction of shear without restriction.

4 Examples of applications

The PlasDIC method was launched onto the market by Carl Zeiss in the fall of 2003 for inverted microscopes.
The only difference between PlasDIC sliders and conventional DIC sliders are the polarizers that are cemented to the Wollaston prisms (**Figure 5, left**). For each condenser there is only one slit diaphragm (**Figure 5, right**), the dimensions of which have been set such that the λ/4 condition at least is met for all of the three PlasDIC sliders used and the objectives A-Plan 10x/0.25, LD A-Plan 20x/0.30 and 40x/0.50.
The first test object was a polished quartz section with a thickness of around 40μm. In this case the polarization-optical path difference R (product of thickness and double refraction, i.e. R ≈ 400 nm) is already

so great that fine thickness inhomogeneities, which can be attributed to the polishing process, can no longer be detected in conventional DIC (**Figure 6, left**). With PlasDIC, on the other hand, it is possible to display the desired phase profile, which is proportional to the product of the section thickness and the refractive index difference between the environment (cement) and the average refractive index of quartz (**Figure 6, right**). The most promising applications of the PlasDIC method at the moment are in the fields of biology and medicine. Here analyses are often performed on living cells, which, due to costs, are cultivated in plastic petri dishes. We know that plastic behaves in an optically anisotropic way, ruling out conventional DIC for the analysis of these cells (**Figures 7a and b**). PlasDIC, on the other hand, has now made high-quality DIC imaging of individual cells (**Figures 7c and 7d**), cell clusters and thick individual cells in plastic cell-culture vessels possible for the first time.

The PlasDIC method is particularly suitable for reproductive medicine, e.g., in-vitro fertilization.

5 Summary

The generation of polarized light only after the light has passed through the condenser, object, objective and suitable aperture stop geometries means that for the first time the conditions have been met for realizing high-quality polarization-optical differential interference contrast, even when the condenser, slide, object and objective are optically anisotropic in nature.

Thanks to:

We would like to thank Mr. Gerhard Börner, Jena, Dr. Martin Völcker and Dr. Michael Zölffel, Göttingen, for their critical checking of the manuscript.

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[6] Danz, R. et al.: *Anordnung und Verfahren zum polarisations-optischen Interferenzkontrast,* European patent application EP 1 359 453, 2003

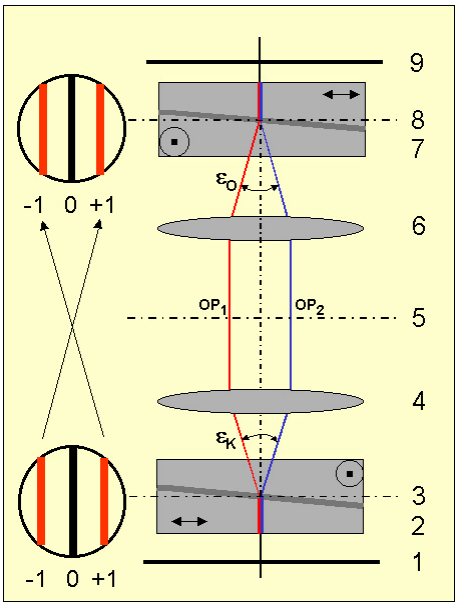

***Figure 1**: Principle of DIC with pupil compensation.*
1 Polarizer, 2 Wollaston prism at the illumination side, 3 condenser focal plane, 4 Condenser, OP_1 and OP_2 object-plane-related image and twin image of object point OP in the object plane 5, 6 Objective, 7 Wollaston prism at the imaging side, 8 Objective focal plane, 9 Analyzer, ε_K, ε_O splitting angles of Wollaston prisms 2 and 7. Due to the angular split into ordinary and extraordinary beams in the second sub-prism, a pattern of interference fringes is created in both the condenser and objective focal planes. The two fringe patterns are depicted interlaced such that the path difference in the objective focal plane (exit pupil) is compensated for fully (Smith principle).

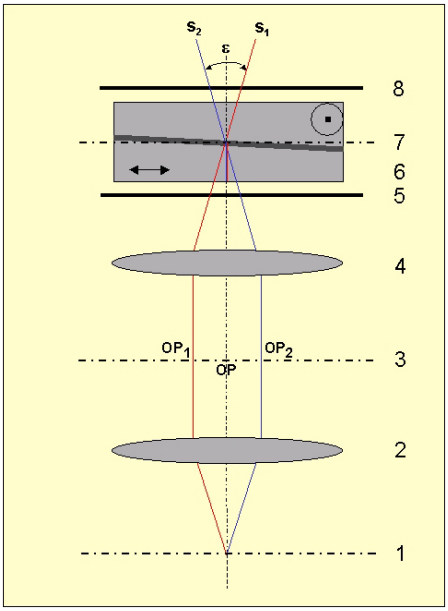

***Figure 2**: The PlasDIC principle.*
1 Condenser focal plane,
2 Condenser,
3 Object plane, OP_1 and OP_2 object-plane-related image and twin image of object point OP,
4 Objective,

5 Polarizer,
6 Wollaston prism,
7 Objective focal plane,
8 Analyzer,
ε splitting angle of the Wollaston prism.

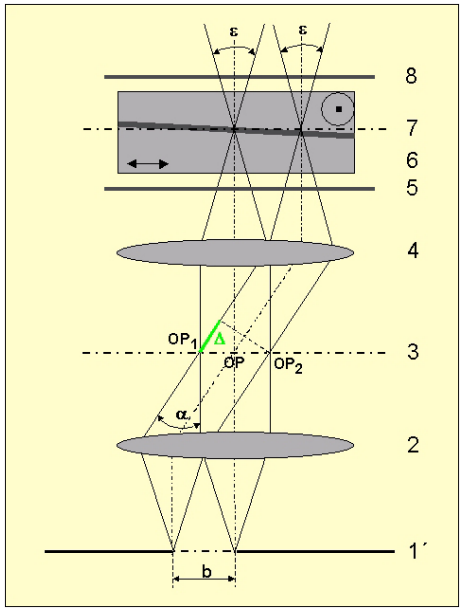

Figure 3*: Explanation of the λ/4 condition. 1´ slit-shaped aperture stop in the condenser focal plane, b width of slit, α aperture angle, Δ path difference between inclined and paraxial beam pair, otherwise as in Figure 2. For contrast reasons, the path difference Δ = OP_1OP_2 sine α should become no greater than λ/4 (see text).*

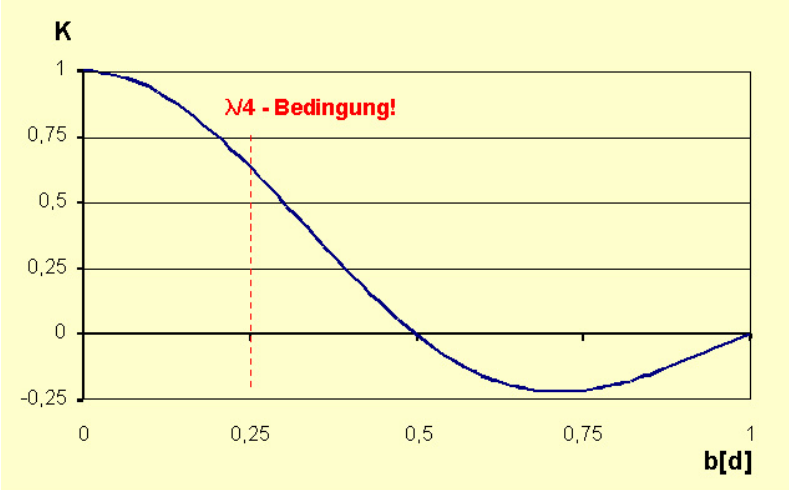

Figure 4: *Image contrast K as a function of width of slit b. Contrast K = f (width of slit) ~ sinc (Δ· 2π/λ). Width of slit b in units of interference fringe distance d in the back objective focal plane.*

Figure 5: *PlasDIC slider and slit diaphragm*

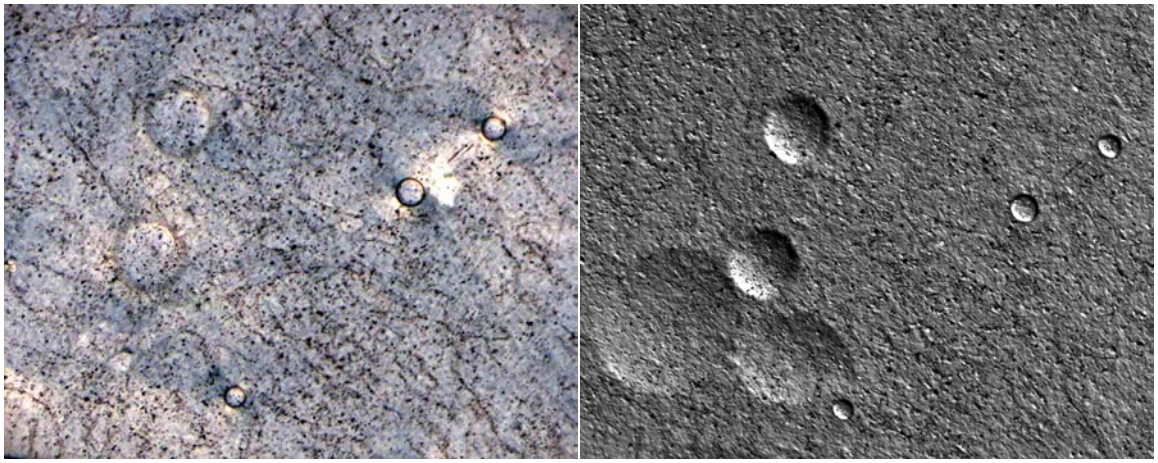

Figure 6: *polished quartz section*
left *Conventional DIC with compensation prism, Plan-Neofluar 10x/0.30 ∞/0.17: the path difference profile is essentially determined by the product of: double refraction (≈ 0.01) and thickness of polished section (≈ 40 µm).*
right *PlasDIC, A-Plan 10x/0.25 ∞/-: the object path difference profile is no longer disrupted by the double refraction of the object; only now can the isodiametric gradient details be portrayed in relief.*

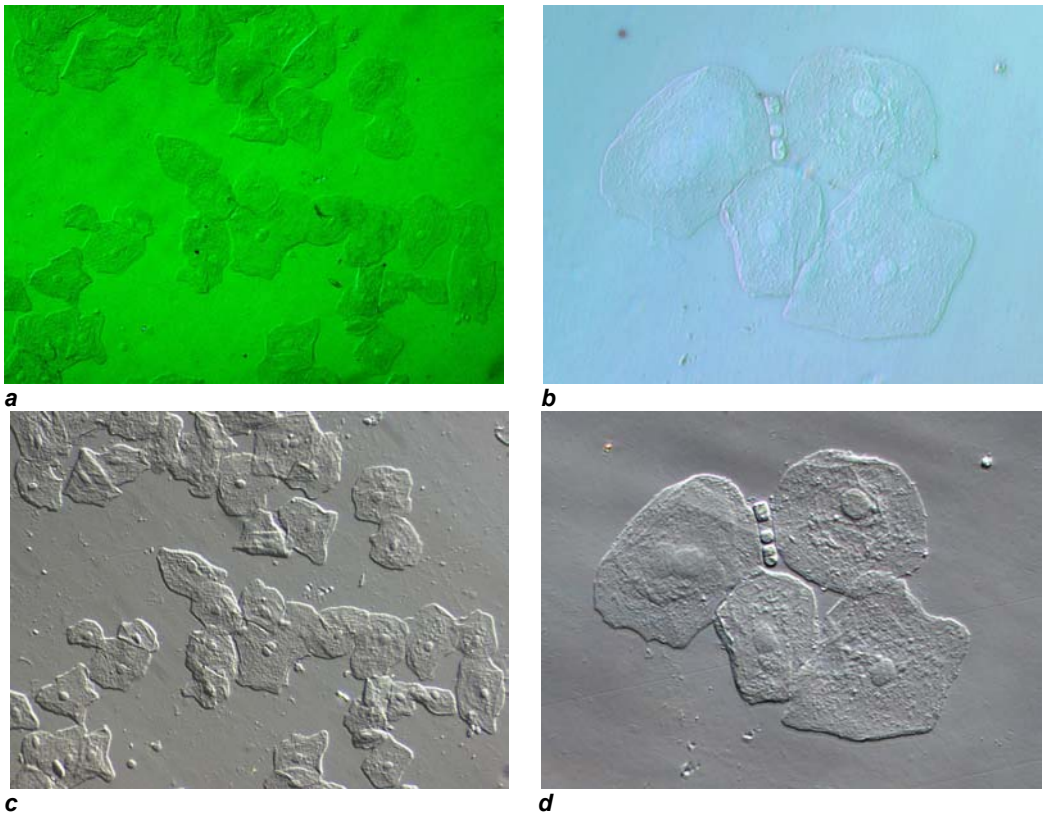

Figure 7: *Human epithel cells from oral mucous membrane, prepared in plastic petri dishes.*
a *Conventional DIC with compensation prism, AxioSkop 2 plus, Plan-Neofluar 10x/0.30 ∞/0.17, achromatic-aplanatic condenser 0.9, bottom of petri dish facing condenser. The anisotropic plastic petri dish has a strong phase-shifting effect, with the result that the relief effect of the DIC is hardly visible.*
b *As a, but Plan-Neofluar 40x/0.75 ∞/0.17. The larger illumination aperture causes an additional reduction in the relief contrast!*
c *PlasDIC, Axiovert 40, A-Plan 10x/0.25 ∞/-, bottom of petri dish facing objective. The anisotropy of the plastic petri dish has no influence on the DIC image!*
d *As c, but LD A-Plan 40x/0.50 ∞/1.0.*

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