Upgrading Standard Bright-Field Microscopes for Dark-Field and Phase Contrast

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Introduction

Bright-field illumination is widely used as standard technique for examination of transparent specimens characterized by an appropriate optical density so that the transmitted light is partially absorbed when passing the specimens (for example, stained histological sections and blood smears). On the other hand, thin and colorless low-density specimens, such as unstained living cells that do not reduce the amplitude of the transmitted light, cannot be well perceived in bright field because of their ultra-low natural contrast. These so-called phase specimens can be examined when an apparatus for phase contrast is used. Phase contrast can be carried out with special objectives fitted with a phase ring situated within or near their back focal plane. Moreover, the condenser must be fitted with annular light masks (light annuli) that are projected onto the plane of the objective's phase ring so that both components, light annuli and phase rings, are optically congruent and conjugate.

Alternatively, low-density specimens can be examined in dark-field illumination. This technique can be carried out with the same standard objectives as used for bright-field examinations so long as the numerical aperture of the objective remains lower than the condenser's aperture. The condenser has to be modified for dark field so that the illuminating light runs in an oblique direction from the condenser's periphery to the specimen's periphery. The central part of the condenser's cross-sectional area is fitted with a black opaque insert leading to a black background. Thus, the specimen is illuminated by concentric oblique light and appears bright on a dark background.

Universal condensers are commonly used for examinations in phase contrast and dark-field microscopy. Such condensers typically consist of a set of several light annuli for phase contrast and a larger-sized light annulus for dark-field illumination all mounted on a revolving turret pivoted near the condenser's aperture diaphragm. The price of a well-designed phase contrast microscope is rather high when compared with more simply designed standard bright-field microscopes. Thus, students at schools and universities often are trained only with low-cost bright-field microscopes, especially where funds are low. Consequently, these students might not be experienced in phase contrast and dark-field examinations when leaving their institutions. In this article, low-cost solutions are suggested for upgrading bright-field microscopes, leading to useful phase contrast and dark-field imagery at low and medium magnifications.

Materials and Methods

Test microscope. A standard microscope manufactured by Celestron (model "Professional 44110") was used for our practical evaluations and tested as an example of a low- or moderate-cost bright-field microscope. This microscope is designed for routine bright-field examinations based on a set of four plane objectives 4/0.10, 10/0.25, 40/0.65, and oil 100/1.25 constructed for a 160 mm tube length. It is equipped with a binocular tube and two pairs of eyepieces 10×/18 and 15×/13. The condenser is designed as a standard bright-field condenser (a so-called Abbe condenser) consisting of base lenses and a removable head lens group (numerical aperture 1.25). Furthermore, the microscope is fitted with aperture and field diaphragms. A 20-watt halogen lamp is integrated in the microscope stand together with a transformer necessary for continuous regulation of the light source. For photomicrographs, one of the Celestron eyepieces was replaced by a Leitz Periplan 10× ocular (for eyeglass wearers) mounted with an Olympus Camedia C-7070 digital camera. In the captions of Figures 10–18, information is given about the sizes of the specimens shown. The total magnification can be calculated for all photomicrographs by multiplying the magnification of the objective by 10 (the 10× ocular).

Lenses for phase contrast. Several phase contrast lenses from E. Leitz Wetzlar, constructed for 160 or 170 mm tube lengths, were mounted on the Celestron microscope to achieve phase contrast illumination. The magnifications of these lenses were 10×, 16×, 20×, 32×, and 40×. Simply constructed achromatic lenses (Phaco 10×, Phaco 40×, Phaco L 20×, and Phaco L 32×) could be used for phase contrast as well as higher-corrected plane, semi-apochromatic, and apochromatic lenses. For visual controls of the alignment in phase contrast, we used a phase telescope from Leitz Wetzlar.

Lenses for dark field. For dark-field examinations, the original 4- and 10-fold magnifying Celestron lenses could be used. Moreover, the well-known plane achromatic lens PL 2.5/0.08 from E. Leitz Wetzlar was mounted for dark-field observations at very low magnification and a 20-fold magnifying special dark field objective from E. Leitz Wetzlar (L 20/0.32) fitted with an iris diaphragm instead of a phase ring was used for higher-magnification dark-field images.

The 10× and 15× magnifying eyepieces from Celestron work well with all the Leitz lenses used. The Leitz optics mentioned above can be supplied at relatively low prices from traders who specialize in secondhand microscopes and accessories, or they can be bought at web-based auctions.

Condenser and light masks. The original bright-field condenser from Celestron must be fitted with appropriate light masks (light annuli) to produce dark-field and phase contrast. Well-suited light masks are manufactured by Victor Gagiu (Los Angeles, CA) who sells these items on Ebay by the name "robytec2010." According to our practical tests, only two different light masks are needed to achieve phase contrast and dark-field illumination when the Celestron microscope is used with its original bright-field condenser in the manner described. Figures 1 and 2 show different sets of phase inserts offered by Gagiu and used for our tests. In both figures, the respective light masks necessary for dark-field or phase contrast are marked by arrows. The insert marked in Figure 1 can be successfully

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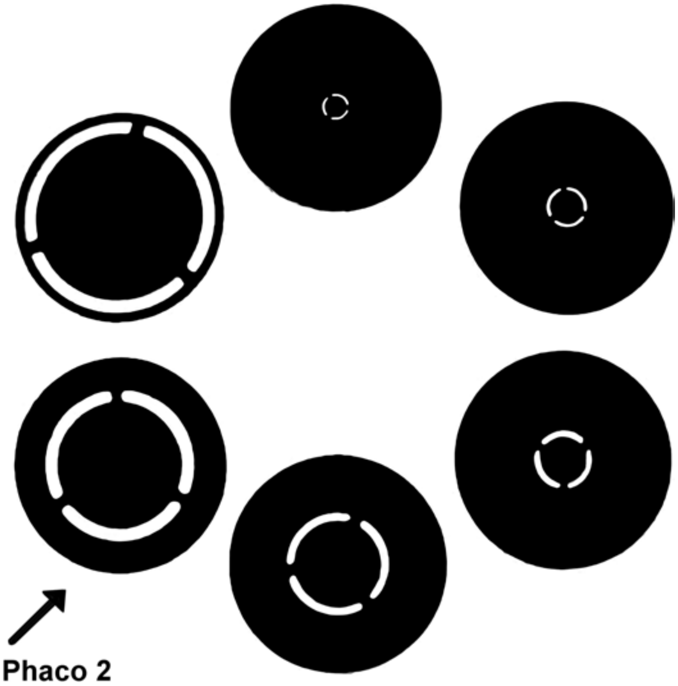

Figure 1: Phase Inserts for Leica condensers No. 1–6 from V. Gagiu, exterior diameter = 24 mm. Arrow: Insert No. 5, used as light annulus "Phaco 2" matching with 40× objectives for phase contrast. (Image contributed by V. Gagiu.)

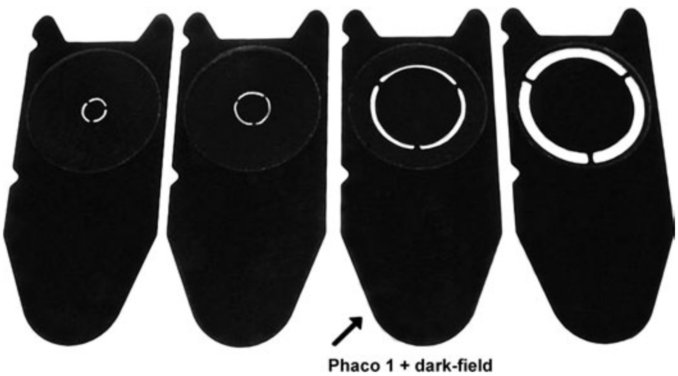

Figure 2: Holders with inserts for Leitz microscope condensers No. 1–4 manufactured by V. Gagiu (Ebay No. 2708415679698). Arrow: Holder No. 3, used for dark field or phase contrast as light annulus "Phaco 1" matching with 10–32× phase contrast lenses. (Image contributed by V. Gagiu.)

used for phase contrast illumination with 40× magnifying Leitz Phaco objectives; it works in the same manner as the Leitz light annulus "Phaco 2." The light mask of the holder marked in Figure 2 has to be removed from its slide, and then it can be used for phase contrast illumination with 10–32× Leitz Phaco objectives, in analogy with the Leitz light annulus "Phaco 1" when the condenser is shifted down. Moreover, the same light annulus can also be used for dark-field illumination when the condenser is elevated into its highest position. The differently sized inserts of the holders presented in Figure 2 can be removed from their slides so that they can be used also in the same way as the set of six 24 mm phase inserts shown in Figure 1, which are shipped without slides.

Technical data for all inserts in the set shown in Figure 1 are compiled in Table 1. Insert No. 5 is that which is marked with an arrow in Figure 1 and used for phase contrast with 40× phase contrast lenses from Leitz. Technical data for the four different holders for Leitz/Leica objectives shown in Figure 2 are presented in Table 2. Holder No. 3 (marked in Figure 2) can be used for dark-field or phase contrast with 10–32× magnifying phase contrast objectives from Leitz. The construction material for all light masks compiled in this article is Delrin. Light masks in other separate sets may be useful for upgrading other microscopes.

The insert of holder No. 4 (Table 1, section 2) was used for dark-field illumination and for phase contrast achieved with objectives from E. Leitz Wetzlar ranging from 10× up to 32× magnification. The 24 mm phase insert No. 5 (Table 1, section 1) was combined with 40-fold magnifying phase contrast lenses from E. Leitz Wetzlar. Thus, all applications in dark-field and phase contrast could be achieved with only two inserts (Figure 3).

Phase Contrast Microscopy

When Leitz objectives for phase contrast are mounted with the Celestron microscope, the head lens group of the Celestron bright-field condenser has to be removed or turned out of the illuminating light pathway. For phase contrast examinations with 10–32× magnifying objectives, the light mask from "Holder No. 3" (Table 2) can be put onto the black metal margin of the superior base lens mount. When examinations have to be made with 40× magnifying Leitz objectives, the 14 mm Insert No. 5 (Table 1) has to be used in the way described instead of the larger-sized light mask from "Holder No. 3."

The correct alignment of light annuli and corresponding phase rings can be controlled with a phase telescope. The position of the light annulus can be manually changed, and the size of its projection can be modified by turning the condenser up and down. The greater the distance between condenser annulus and phase ring, the smaller is the projection image of the light annulus. This simple procedure can be used as test to ascertain if the setup is correct.

The base lens group of the Celestron bright-field condenser is shown in Figure 4a. The position of an appropriate light mask (14 mm insert No. 5) is demonstrated in Figure 4b. Figure 5 shows the light annulus from Figure 4b controlled by a phase

Table 1: Set of 6 inserts from V. Gagiu, fitted with differently sized light annuli and designed for 24 mm exterior insertion diameter (see Figure 1).

Item / Set	Diameter Center Stop (mm)	Width of Light Ring (mm)	Exterior/Insertion Diameter (mm)
24 mm Phase Inserts, No holders (6 pieces)			
Insert No. 1	2.6	0.28	24
Insert No. 2	3.75	0.45	24
Insert No. 3	5.0	0.80	24
Insert No. 4	9.5	1.00	24
Insert No. 5	14.0	1.50	24
Insert No. 6	19.0	2.00	24

Table 2: Set of 4 holders for Leitz / Leica from V. Gagiu, fitted with differently sized light annuli and designed for 27 mm exterior insertion diameter (see Figure 2).

Item / Set	Diameter Center Stop (mm)	Width of Light Ring (mm)	Exterior/Insertion Diameter (mm)
Holders for Leitz, Leica (4 pieces)			
Holder No. 1	4.7	0.3	27
Holder No. 2	6.5	0.4	27
Holder No. 3	17.0	0.85	27
Holder No. 4	21.0	2.0	27

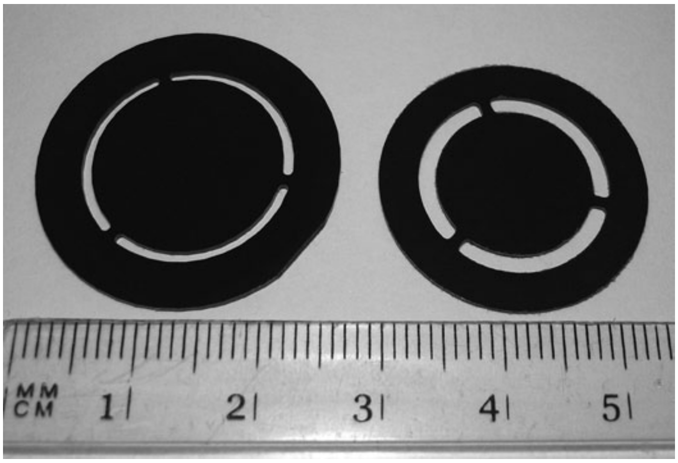

Figure 3: Two inserts used for our evaluations. (left) Insert from Holder No. 3 shown in Figure 2. (right) 24 mm phase Insert No. 5 shown in Figure 1.

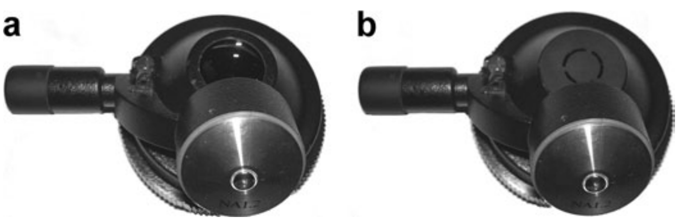

Figure 4: Abbe condenser from Celestron, head lens group switched out. (a) Mounting of the superior base lens in normal condition. (b) Covered with a phase insert (24 mm Insert No. 5) from Figure 1.

telescope when properly aligned with the phase ring of a Leitz 40× phase contrast objective.

The contrast of the resulting image can be optimized with the help of the field diaphragm. Moreover, contrast and focal depth can be enhanced further when the aperture diaphragm is regulated in tiny steps so that the transparent area of the light annulus is slightly reduced (aperture reduction phase contrast, ARPC).

In the arrangement described, the phase inserts also can be easily modified for oblique phase contrast illumination (so-called “relief phase contrast”). This variant of phase contrast has been published and extensively discussed in a prior contribution [1]. To achieve this, the light annulus on the condenser has to be partially covered by a larger-sized non-transparent plate so that the specimen is illuminated by a small light segment coming from a well-defined direction instead of a circular light cone. Figure 6 gives examples for such modifications taken with a phase telescope. In the arrangements shown here, light annulus and larger-sized opaque plates are eccentrically superimposed on each other so that small sickle-shaped light gaps result. The smaller and shorter the remaining transparent light segment, the higher the enhancement of contrast and relief effects will be.

When phase contrast lenses are used that are manufactured elsewhere and they contain phase rings that do not match with any standard inserts available, the size of an appropriate light annulus can be measured as follows (Figure 7): The condenser’s head lens group is switched out of the light path, and the scale of a small transparent ruler is put on the mount of the superior condenser base lens instead of a light mask. A stained specimen is focused in bright field with the respective phase contrast lens. Then the objective's phase ring is focused with the phase telescope. Last, the ruler scale is focused as sharp as possible by turning the condenser up and down. In this arrangement, the size of a congruent light annulus (diameter and breadth) can be approximately estimated using the ruler's scale so that a matching light mask can be individually manufactured. Remaining potential small differences between the sizes of the phase ring and the projection image of the light annulus can be equalized by moderate vertical shifts of the condenser as described.

Dark-Field Illumination

For examinations in dark field, the condenser has to be turned into its top position so that the distance from the specimen is as short as possible. When the condenser’s head lens group is switched out of the light path, the light mask from “Holder No. 3” (see Table 2) is placed onto the condenser’s superior base lens as described above. The adjustment of the light mask can be visually controlled by looking at the condenser; it is correct when a small circular

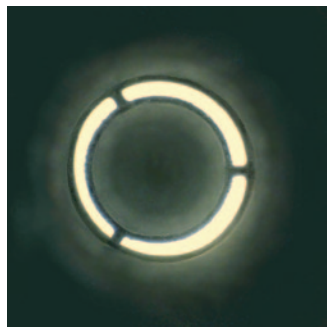

Figure 5: Correct alignment of the insert from Figure 4b used with a Leitz Phaco 40/0.65 objective. Image taken with a phase telescope.

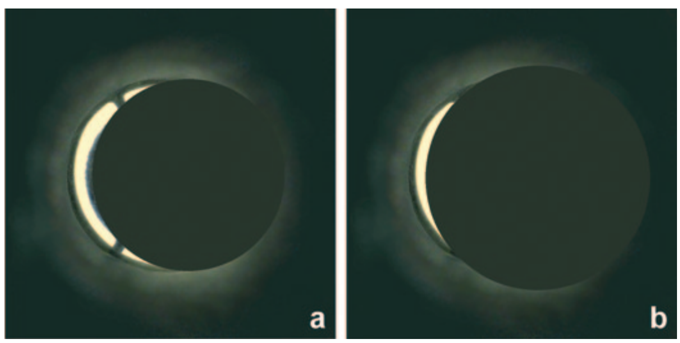

Figure 6: Achievement of relief phase contrast, image taken with a phase telescope, light annulus from Figure 4, properly aligned, eccentrically covered by circular opaque plate with long (a) and short (b) light sickle.

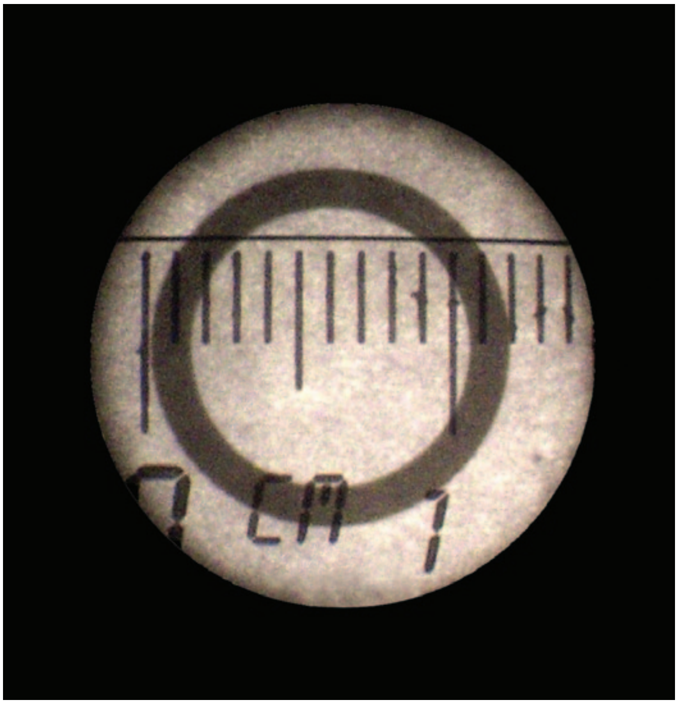

Figure 7: Measurement of the dimensions for an individual light annulus matching with a phase ring (see further explanations in the text).

peripheral light cone results. When the back focal plane of the objective is focused with a phase telescope, the light annulus has to be projected outside the objective's cross-sectional area for dark-field illumination. Thus, a phase telescope can also be used for testing the alignment to ascertain that this setup is correct. The optimum position of the light mask can also be controlled further and modulated in tiny steps by observation of the microscope live image when the mask is slightly shifted in a horizontal direction and/or the condenser is moderately shifted in a vertical direction. The field diaphragm should be regulated in the usual manner so that the background is maximally darkened and the contrast optimized. In some cases, image contrast and focal depth can be enhanced still more when the condenser's aperture diaphragm is turned into smaller positions in tiny steps. The character of the respective dark-field image can be modulated further when the condenser is slightly shifted in a vertical direction. The 4× and 10× magnifying Celestron objectives can be used, as well as lower magnifying lenses from other manufacturers (for example, Pl 2.5/0.08 from E. Leitz Wetzlar) or higher magnifying lenses fitted with an iris diaphragm for reducing the objective's aperture (for example, L 20/0.32 from E. Leitz Wetzlar).

Production of Self-Made Light Annuli

When the internal and external diameters of an appropriate condenser annulus necessary for phase contrast, and matching with a particular phase contrast lens, are measured by use of a small ruler in the way described, the respective light mask can also be made by the user himself from a piece of opaque black cardboard. A number of small equidistant holes acting as light perforations can be made by a needle or a fine drill as a substitute for a standard light annulus. Larger-sized light masks containing circular perforations can also be made for dark-field illumination. Phase contrast and dark-field images resulting from such modified light outlets are not different from those generated with standard light masks. When two concentric light outlets are made in appropriate sizes (see Figure 8c), the external light annulus can generate a dark-field image, whereas the internal light annulus can act as a light mask for phase contrast. By this means, two partial images, one dark-field and one phase contrast, can be superimposed. The breadth and area of the external light annulus, i.e. the intensity of the dark-field image, can be influenced with the help of the condenser aperture diaphragm. Thus, the resulting composite image can be equalized (well balanced) or dominated by phase contrast or dark field (variable phase-darkfield-contrast, VPDC [2]).

Figure 8 shows examples of a handmade light mask suitable for ARPC (Figures 8a and 8b) and well-balanced VPDC (Figure 8c). Figure 9 shows typical alignments of special light masks from the Figures 8b and 8c used for ARPC and VPDC (images taken by a phase telescope); the light masks shown in Figure 8c consist of three small perforations for phase contrast illumination and additional peripheral perforations for dark field that are projected outside the objective and therefore invisible when controlled with a phase telescope. The VPDC shown here can lead to enhanced visual information when compared with phase

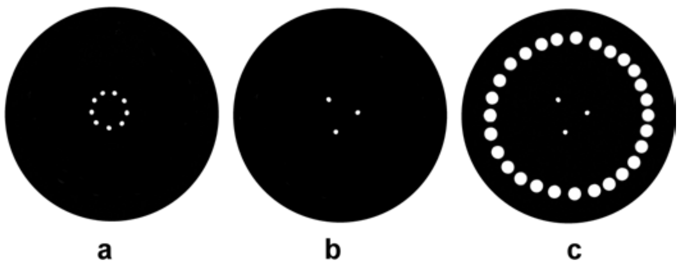

Figure 8: Handmade light masks for moderate (a) and high-magnification (b) ARPC and (c) VPDC.

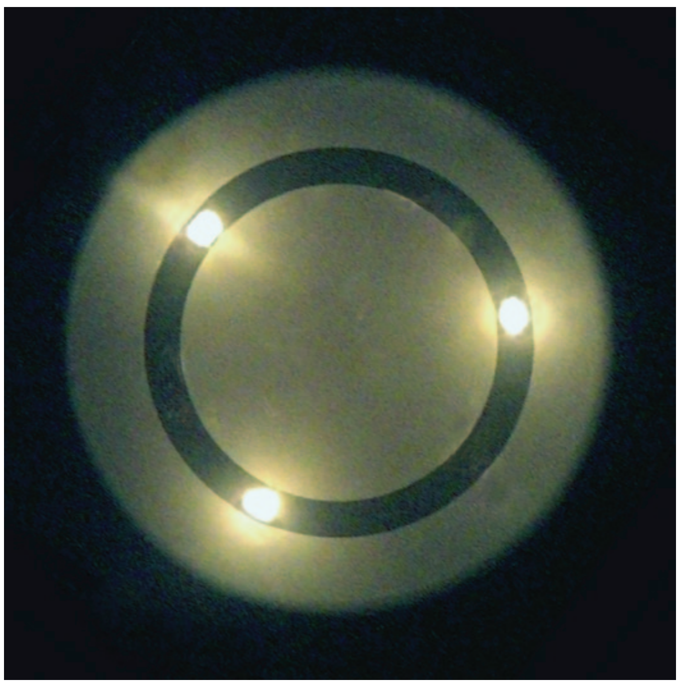

Figure 9: Alignment of light masks from Figures 8b and 8c, controlled with a phase telescope.

contrast and dark-field illumination carried out separately. More aspects of this interesting illumination technique have been published recently [2].

Practical Results

Several specimens often examined in phase contrast or dark-field illumination were used for practical tests carried out with the Celestron microscope and the additional equipment described. All optics from E. Leitz worked well with the original components from Celestron—the objectives as well as the Periplan eyepiece used for photomicrographs. Figures 10–12 give examples of typical dark-field observations; they demonstrate that good image quality is achievable for magnifications up to 300× (20× objective with the 15× Celestron ocular). Representative images taken in phase contrast are shown in Figures 13 and 14 taken with 10- and 40-fold magnifying achromatic objective lenses. For these examples, contrast, sharpness, and planarity are adequate for total magnifications up to 600×. In Figure 15, normal phase contrast is compared with relief phase contrast according to the arrangements shown in Figure 6. The three-dimensional architecture of the crystallization shown here is much more accentuated in relief phase contrast when compared with the standard mode.

Fundamental improvements in image quality can be achieved with ARPC. Figure 16 shows alum precipitations situated at the surface of a glass ramp (angle of inclination: 20°) in normal phase contrast and ARPC. The oblique situated surface of the transparent glass carrying the crystallizations causes the vertical sharpness to be low in normal phase contrast (depth of field: 0.17 mm). In ARPC, the depth of field can be dramatically enhanced (up to circa 0.77 mm).

An advantage of variable-phase dark field is the ability to obtain good contrast on specimen features of different density. Figure 17 shows a preparation of living algae taken in four contrast modes: bright-field, dark-field, normal phase contrast (use of a standard light annulus), and VPDC (based on a special light mask consisting of a couple of concentric light outlets). In the latter technique, the low-density clear algae are well contrasted, and the high-density algae colored in red are illuminated in best clarity showing their natural colors. Figure 18 gives an additional example for the advantages of VPDC. The section of a potato shown here consists of low-density phase structures and high-density pigmentations. Also in this example, all colorless and colored low- and high-density details are visualized in the best manner when VPDC is carried out.

Figure 10: Gnat, total length about 4 mm, on a permanent slide. Dark field taken with Leitz Pl 2.5/0.08 objective.

Figure 11: Foraminiferida, total diameter of the arranged circle about 1 mm. Dark field taken with Celestron 4/0.10 objective (preparation: E. Raap, Germany).

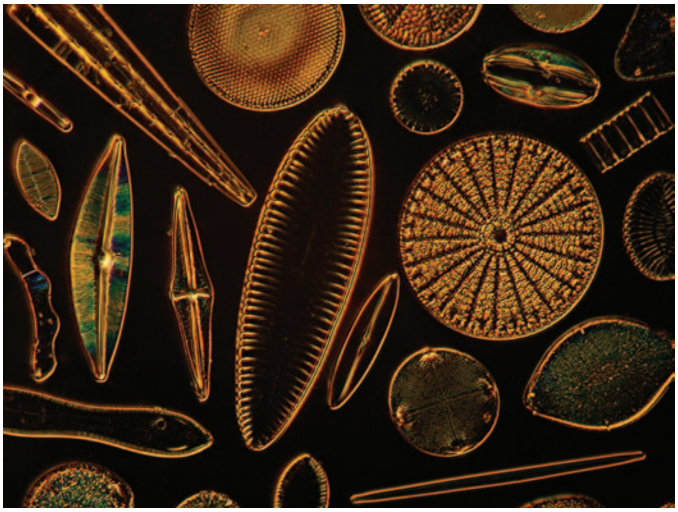

Figure 12: Diatoms, horizontal field width = 0.4 mm. Dark field taken with Leitz Iris L 20/0.32 objective (L = long working distance) (arranged slide prepared by K. Kemp, UK). Iris diaphragm used for reductions of marginal blooming and scattering.

Discussion

In low-budget conditions, easy technical solutions may be of interest for routine tasks in light microscopy based on different illumination techniques. Other authors presented a low-cost microscope in a previous article targeted to specific tasks in the developing world based on bright-field and

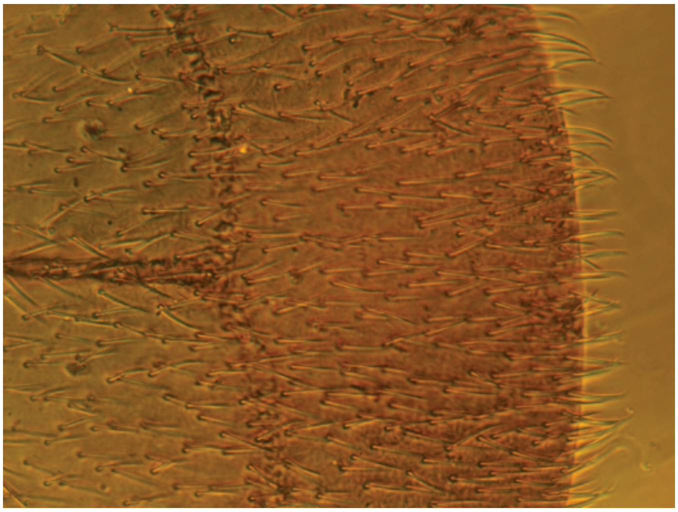

Figure 13: Wing of an insect, horizontal field width = 0.7 mm, permanent slide. Phase contrast image taken with a Leitz Phaco 10/0.25 objective.

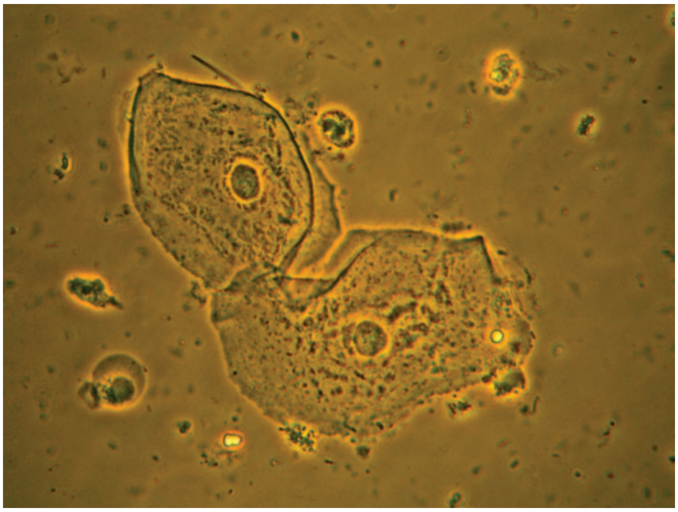

Figure 14: Epithelial cells, plasma cells, and bacteria, horizontal field width = 0.17 mm, cover slip preparation of native saliva. Phase contrast image taken with a Leitz Phaco 40/0.65 objective.

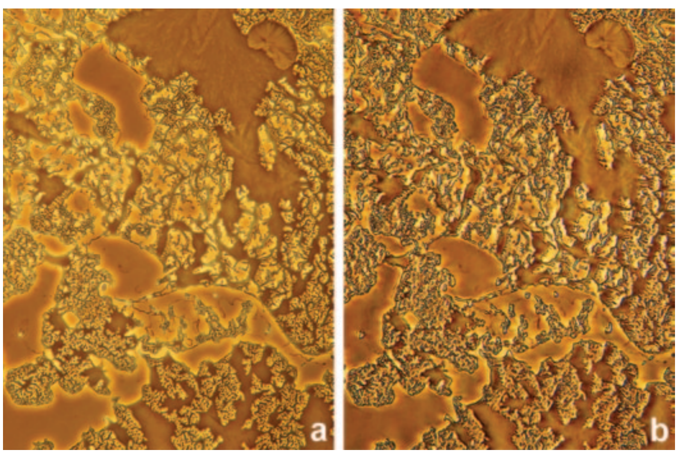

Figure 15: Thin-layer crystallization in a cover slip preparation, vertical field width = 0.17 mm. Image taken with a Leitz Phaco 40/0.65 objective, (a) normal phase contrast, (b) relief phase contrast.

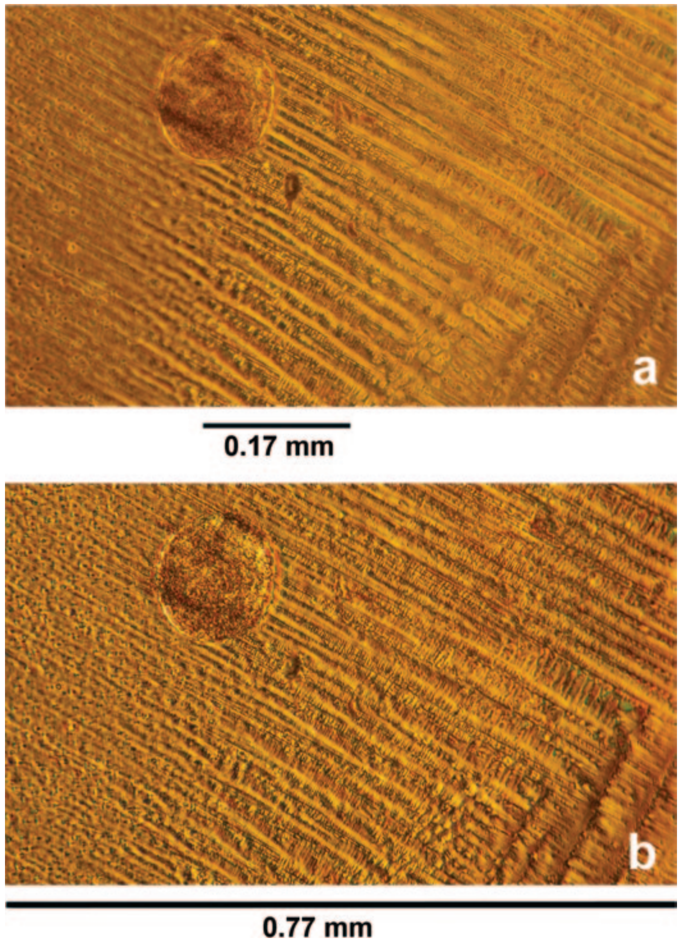

Figure 16: Colorless crystallizations (alum) at the surface of a transparent glass ramp, angle of inclination: 10°, objective 10×/0,25, (a) normal phase contrast, narrow field width of sharpness: depth of field 0.17 mm, (b) ARPC, wide field width of sharpness: depth of field 0.77 mm.

fluorescence illumination [3]. In this paper, complementary techniques are reported for achieving phase contrast and dark-field illumination in standard bright-field microscopes up to medium magnifications. It should be taken into account that phase contrast and dark field are relevant methods in routine diagnostics and thus important for several tasks. At present, low- or moderate-cost bright-field microscopes are manufactured by several companies. Microscopes fitted with a simple bright-field condenser, achromatic lenses, and monocular tubes are offered for about $300–400; more expensive bright-field microscopes designed with plane lenses, binocular tubes, and higher aperture Abbe condensers are available for prices of about $800–900. The Celestron microscope used for our tests is representative of the latter type. Most of these instruments were designed only for bright field, thus additional optical equipment such as dark-field or universal condensers and phase contrast lenses are not offered by their respective manufacturers. On the other hand, such stand-alone microscopes are widely available in educational institutions, developing countries, and laboratories of general practitioners or hobbyists. For these users, the methods described here may be an attractive low-cost alternative way for implementing phase contrast and dark field. Microscopes produced by other manufacturers might be upgraded in a comparable way. In each microscope intended for upgrade the

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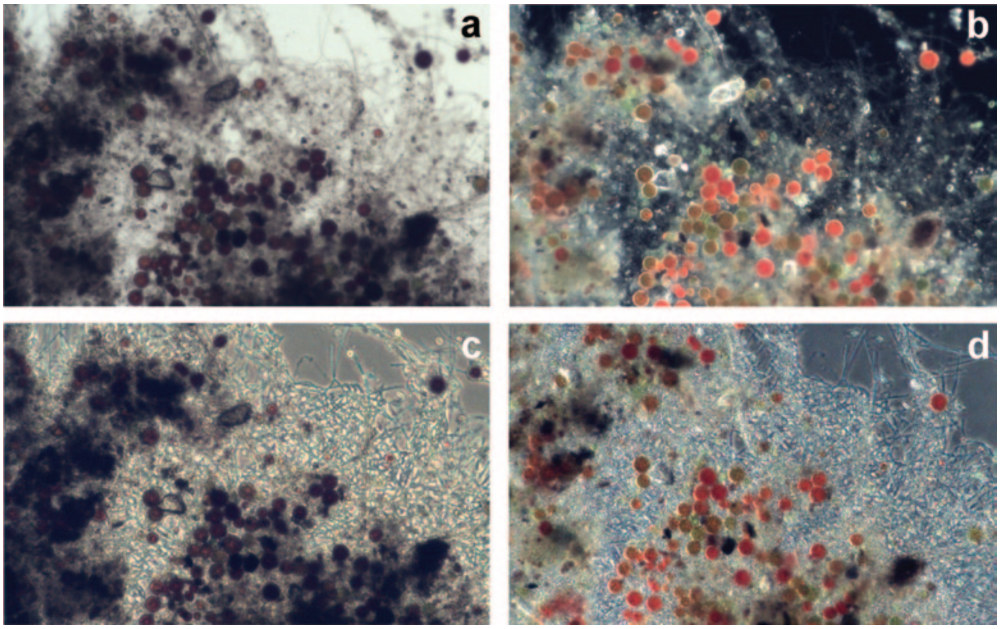

Figure 17: Native algae, prepared with a cover slip, diagonal width: 0.4 mm, objective Leitz Phaco L 20/0.32, (a) bright field, (b) dark field, (c) phase contrast, (d) VPDC. Photographs taken by Timm Piper.

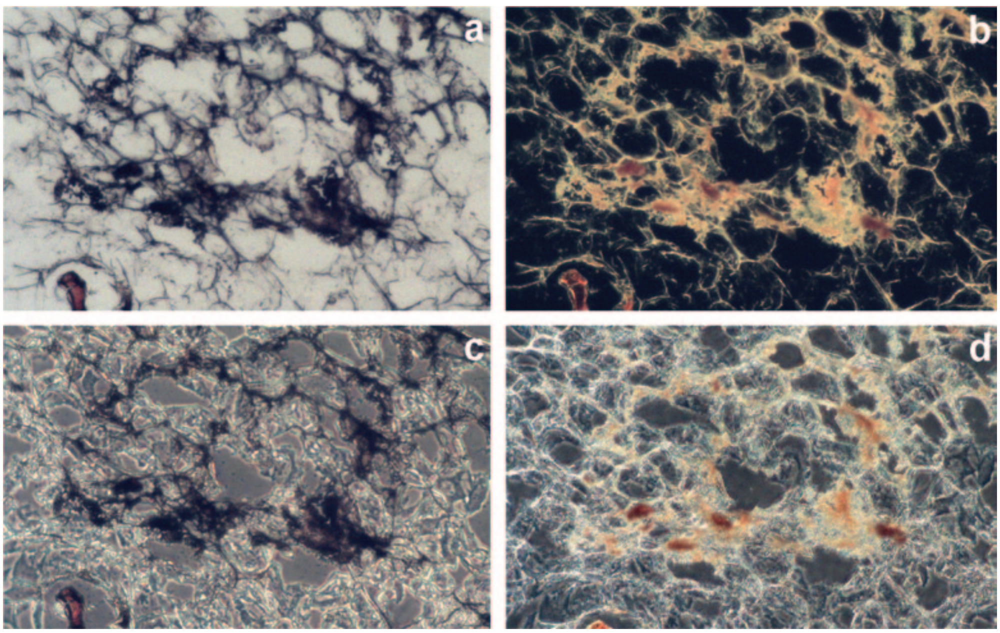

Figure 18: Section of a potato, diagonal field width of 0.8 mm, objective Leitz Phaco 10/0.25, (a) bright field, (b) dark field, (c) phase contrast, (d) VPDC. Photographs taken by Timm Piper.

user must evaluate which position of the light mask will be the best one.

The light masks (phase inserts) used for our practical tests were manufactured with high precision at low cost. Moreover, individual inserts can be produced on demand. An additional advantage is that light annuli also can be hand-made by the user. The Leitz objectives used for our tests can be purchased also for low prices from several sources; they work well with the instrument from Celestron. Lenses from other manufacturers, such as finite objectives from Zeiss, Olympus, or Hund, for instance, should be compatible in a similar way. We expect bright-field microscopes from other manufacturers could be upgraded for phase contrast and dark field in a similar same way. This article intended to show a way for upgrading a single microscope or a few microscopes. When a classroom has to be fitted with microscopes, it might be difficult to obtain an appropriate number of "old" objectives designed for a finite tube length. In such a situation, it might be necessary to buy original phase contrast microscopes.

Conclusion

Standard microscopes designed for routine bright-field illumination can be upgraded at low cost for medium-magnification phase contrast and dark field when they are fitted with compatible phase contrast lenses and light masks. Most such microscopes are designed for finite lenses based on a 160 mm tube length. Therefore, "old" phase contrast lenses from well-established manufacturers may be compatible with these routine microscopes. Appropriately sized light masks can be obtained from a specialized manufacturer mentioned in this article or made by the user. Although the costs for the equipment described are significantly lower than those of a specialized phase contrast microscope, the quality of phase contrast and dark-field images that were made using these modifications seems to be suitable for several tasks in routine microscopy. Moreover, special contrast effects can be achieved based on user-designed light masks.

Acknowledgments

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