Anoptral phase contrast applied to diatoms: a study by means of test diatoms on the advantages originally described by its discoverer Alvar Wilska, over the standard (positive) phase contrast

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Introduction

Anoptral phase contrast was invented in 1953 by Alvar Wilska (1911-1987), a great Finnish inventor, scientist and professor. In positive (Zernike) phase contrast the specimens generally appear with a lighter gray background and often have a bright halo along their edges; at the opposite, in negative phase contrast the same specimens appear lighter with a dark background and usually have a dark halo along their edges. According to Zeiss, "*for some applications, such as examining sperm cells,* ***negative phase contrast may produce more specimen detail than the traditional positive phase contrast***" (1). **Anoptral phase contrast is a variant of negative phase contrast** (5) and produces a typical sepia / golden brown background, much lighter than the standard, classic negative phase contrast.

A. Wilska used the following words to describe Anoptral phase contrast: "*As a result, the background of the microscope image appears in an* ***agreeable sepia tone****, often giving the illusion of sky-illuminated objects lying on the sand floor of a shallow sea. Many features which are generally invisible with the normal use of the microscope are* ***rendered visible ; for example, influenza virus on red cell ghosts, bacterial flagella, sperm tail pencil, and delicate membranous margins in living thrombocytes***" (6), moreover in Anoptral phase contrast *"the image becomes "negative" in the ordinary sense; physiologically, however, it is* ***much more natural than the "positive" one*** *because the* ***haloes unavoidable in the latter are converted into shadow borders giving the image an illusion of depth****. In addition to this,* ***the gold-brown tint of the Anoptral image is very agreeable to the eye****"* (7).

Although at Diatom Lab I work with top of the range modern microscopes and accessories to guarantee customers the best quality and accuracy, in the laboratory there is also a rich collection of vintage and antique microscopes and accessories for the following purposes:

a) to understand how and at what level of quality diatoms and other microscopic objects were observed throughout history of microscopy;

b) to understand how and at what level of quality test diatoms were resolved throughout the history of microscopy;

c) to understand the technical evolution and the optical aberrations of the microscope objectives and accessories throughout the history of microscopy.

In order to correctly compare the Anoptral phase contrast with the positive phase contrast, I have used two vintage Soviet Lomo sets, the the КФ-4 set and the (unfortunately very rare nowadays) МФА-2 set (each one includes a special condenser, four microscope objectives, a centering telescope and a green filter as you can see in pictures 7 and 8). The first set I have mentioned is for positive phase contrast, while the second is for a negative phase contrast without a doubt definable as Anoptral phase contrast, because it gives the typical golden brown background: in fact many authors have called the constrast technique from Lomo МФА-2 as Anoptral, such as the Russian Yuri B. Okolodkov in his publication "Cryopelagic flora of the Chukchi, East Siberian and Laptev

seas" (Proc. NIPR Symp. Polar Biol., 5, 28-43, 1992). In the Lomo instruction manual for МФА-2 set this contrast technique is generically and curiously described with the words "*phase-dark-field method"* (but no real dark-field illumination is technically involved as it is an Anoptral phase contrast)*:* "Фазовотемнопольный метод имеет повышенную чувствительность, большую разрешающую способность) и применяется в основном для исследования объектов с малыми изменениями оптической длины пути. При этом на золотисто-коричневом фоне поля выявляются светлые детали объекта; чем выше показатель преломления, тем светлее изображение", translatable as follows: "*The phase-dark-field method has* ***increased sensitivity, high resolution*** *and is mainly used to study objects with small changes in the optical path length. At the same time, light details of the object are revealed against the* ***golden-brown background*** *of the field; the higher the refractive index, the brighter the image*." (2).

Moreover, if we observe microscope objectives for Anoptral phase contrast such as the Lomo ФА lenses, we can note that – unlike those for positive phase contrast – the phase rings are very close to the edge of the lens (the distance proportionally decreases with increasing lens magnification), it means that most of the zero order or undiffracted component is present: this fact gives a technical explanation to the previous quotes on more specimen details given by Anoptral phase contrast! Now let's get into the highlight of the study…

Materials and methods

The experiments at Diatom Lab were performed using the Lomo МФА-2 and КФ-4 sets mounted on one of the several available Lomo Biolam microscopes used in the laboratory, in this case the Biolam 70 Д1 (D1) travel microscope, equipped with the Lomo ОИ-35 illuminator that allows Köhler illumination (see image number 6). All this Lomo equipment is like new (stock fund) to ensure the homogeneity of the tests. The МФА-2 set includes four microscope objectives characterized by the acronym ФА: the Achromatic objectives 20x/0.40 ФА, 40x/0.65 ФА, and the Achromatic 90x/1.25 ФА for oil immersion, plus the Apochromatic 70x/1.23 ФА for water immersion. The КФ-4 set includes four Achromatic microscope objectives characterized by the acronym Ф: 10x/0.30 Ф, 20x/0.40 Ф, 40x/0.65 Ф and 90x/1.25 Ф. For the comparison experiments it was necessary to have lenses with the same magnification and numerical aperture, and for my tests I preferred the 40x/0.65 ФА to be compared with the 40x/0.65 Ф, plus the 90x/1.25 ФА to be compared with the 90x/1.25 Ф, discarding the 20x lenses as they give much lower magnification. Köhler illumination has been used in all cases. The upper lenses of both condensers have not been oiled in case of oil immersion microscopy (just the 90x lenses have been oiled with original Lomo immersion oil).

I love to define diatoms as merciless test objects for microscopes, or more calmly the best test objects for microscopes, and for this study I opted for the following Diatom Lab preparations, all mounted in Diatom Cubed high refractive index mountant:

Stauroneis phoenicenteron (Nitzsch) Ehrenberg 1843 (belonging to a sample with striae in 10µm: 12-15 longitudinal. Details to resolve: areolae);

Craticula cuspidata (Kützing) DG Mann ex Rotonda et al. 1990 (belonging to a sample with striae in 10µm: 16-17 longitudinal. Details to resolve: areolae)

Other test diatom species have been used, but the two mentioned above are the ones that gave the best results for this demonstration.

Results

No focus stacking has been used for the imaging, but the best possible focal plane for the resolution of the areolae was chosen for each image, thanks to a careful use of the fine adjustment knob.

If we look at the images of *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg 1843, obtained with the dry 40x lenses, we understand that Anoptral phase contrast permits to resolve the areolas a little better, especially those near the raphe, which appear very clear and defined. But the most striking difference appears during oil immersion with 90x lenses: *Craticula cuspidata* (Kützing) DG Mann ex Rotonda et al. 1990 shows much more resolved areolae by Anoptral phase contrast, moreover the fact that the latter appear light against the golden brown background ensures much more contrast. **These recent tests show once again that the "old" Anoptral phase contrast was in some cases more efficient that the "old" phase contrast**. I am rightly referring to the "old" phase contrast as modern, good quality phase contrast lenses are generally better than those of the past and in several cases the phase rings of today have a different, innovative manufacture.

It is necessary to specify that the same two diatom species can be better resolved with modern top-of-the-range microscopes (such as the in-house Zeiss Axio Imager.A2 with infinite M27 objectives) even in bright field, without using special contrast techniques. Moreover better results would have been achieved if LED lighting had been used instead of the incandescent bulb that is inside the Lomo ОИ-35 illuminator, but the current experiment wanted to correctly re-propose the technique of the past with the means of the past, without any modern improvements whatsoever.

Finally, the leap into the past through vintage (and antique) microscopy has several salient aspects:

- first of all it always generates a strong emotion, especially if it is possible to observe from old but like-new instruments (because we understand how the observations of the past were really like and we can put on the clothes of the ancestors. It's a kind of priceless time machine journey);

- we realize that many old scientific discoveries have been made despite using instruments with much inferior performances;

- we understand how much genius, care and passion the past scientists and opticians had in building masterpieces despite having less means than today;

- we can directly verify how the optics have made great strides over time, in this way we can appreciate the present microscopy even more.

References

(1) http://zeiss-campus.magnet.fsu.edu/articles/basics/contrast.html

(2) Lomo instruction manual for МФА-2 set (edition from 1983)

(3) Lomo instruction manual for КФ-4 set (edition from 1983)

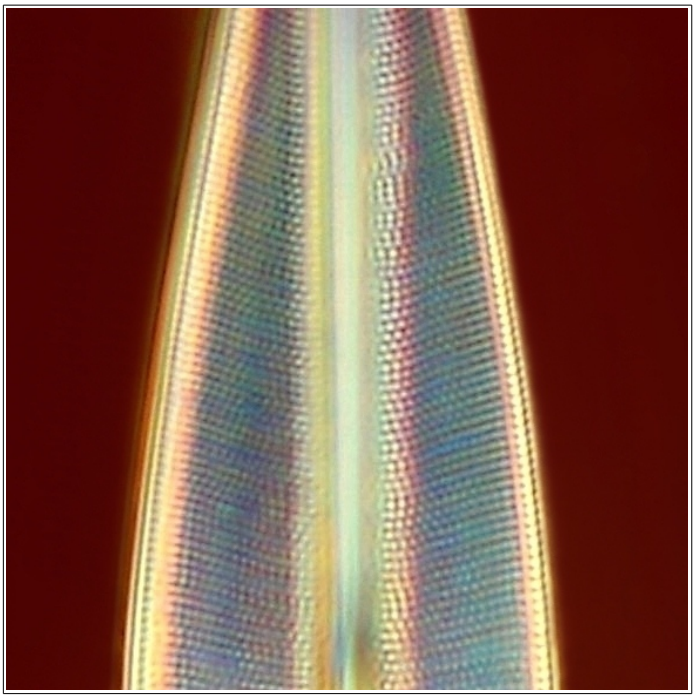
(4) Lomo instruction manual for ОИ-35 illuminator (edition from 1979)

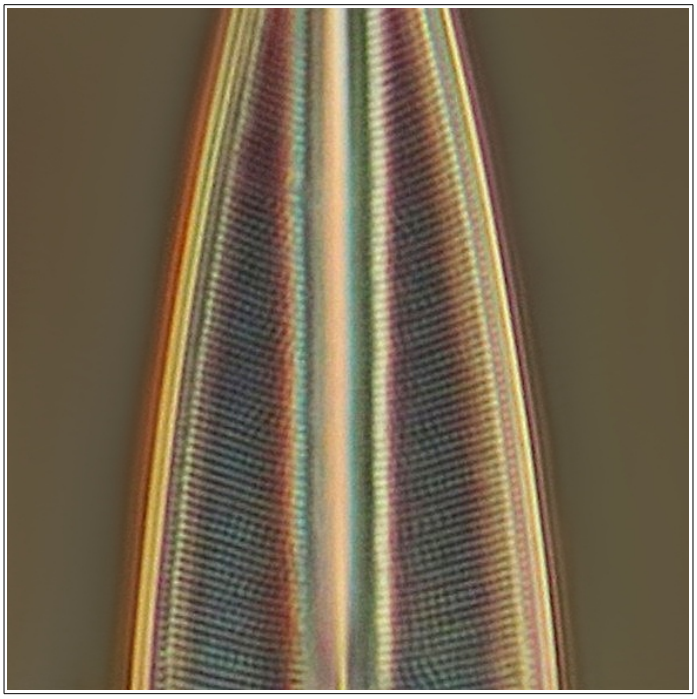
(5) Pelc, Z. Hostounský, T. Otaki and K. Katoh (2020) Conventional, Apodized, and Relief Phase Contrast Microscopy Chapter 10 Neurohistology and Imaging, Techniques, Neuromethods, vol 153. Springer.

(6) Wilska, A. *A New Method of Light Microscopy.* Nature171, 353 (1953)

(7) Wilska, A. *Observations with the anoptral microscope.* Mikroskopie. 9(1-4):1-80 (1954)

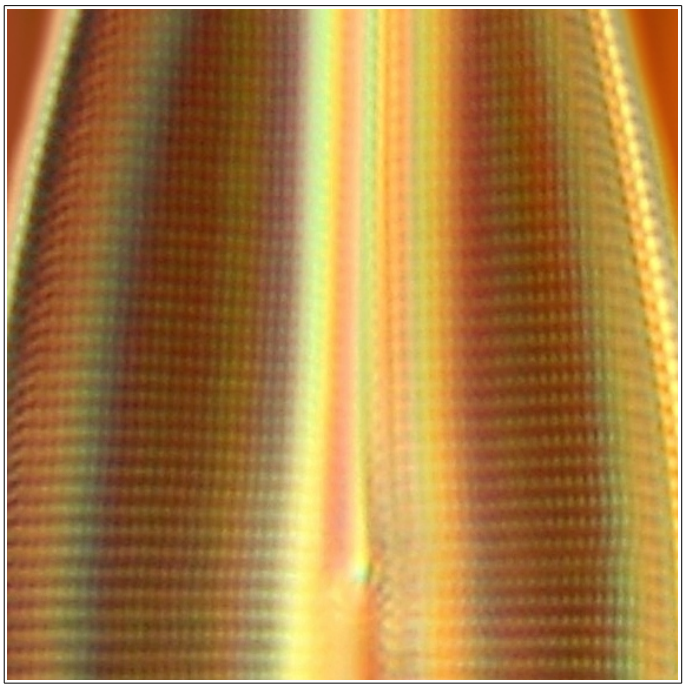
Images

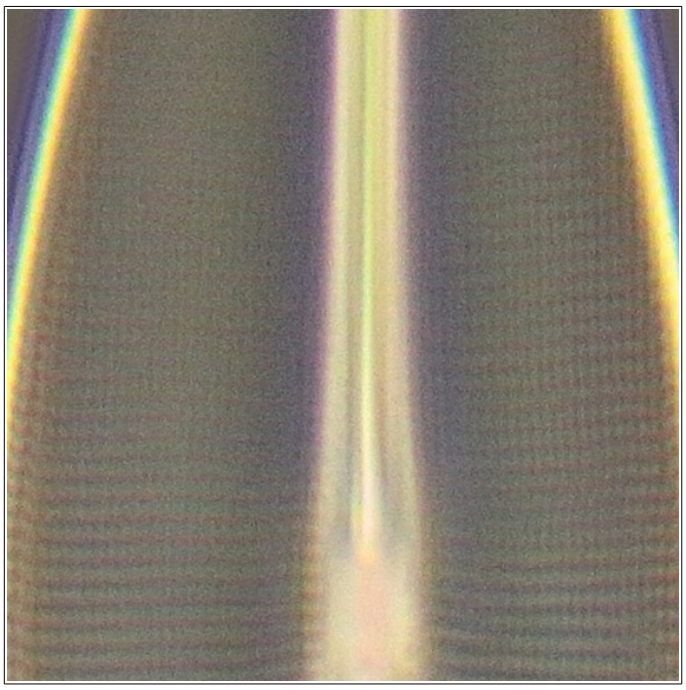




1. Diatom *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg 1843 mounted in Diatom Cubed mountant, from the Diatom Test Slide version 2.0 by Diatom Lab. Lomo Anoptral phase contrast, Lomo 40x/0.65 ΦA Achromatic lens.

2. Diatom *Stauroneis phoenicenteron* (Nitzsch) Ehrenberg 1843 mounted in Diatom Cubed mountant, from the Diatom Test Slide version 2.0 by Diatom Lab. Lomo phase contrast, Lomo 40x/0.65 Φ Achromatic lens.

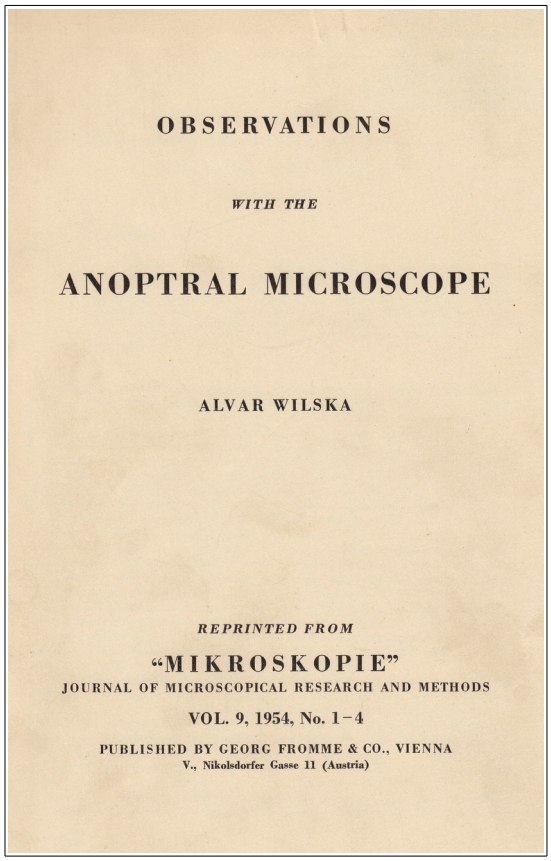




3. Diatom *Craticula cuspidata* (Kützing) DG Mann ex Rotonda et al. (1990), mounted in Diatom Cubed mountant. Test slide by Diatom Lab. Lomo Anoptral phase contrast, Lomo 90x/1.25 ΦA Achromatic lens.

4. Diatom *Craticula cuspidata* (Kützing) DG Mann ex Rotonda et al. (1990), mounted in Diatom Cubed mountant. Test slide by Diatom Lab. Lomo phase contrast, Lomo 90x/1.25 Φ Achromatic lens.

5. A rare reprint of “Wilska A. *Observations with the anoptral microscope*. Mikroskopie. 1954;9(1-4):1-80” kept at Diatom Lab.



OBSERVATIONS

WITH THE

ANOPTRAL MICROSCOPE

ALVAR WILSKA

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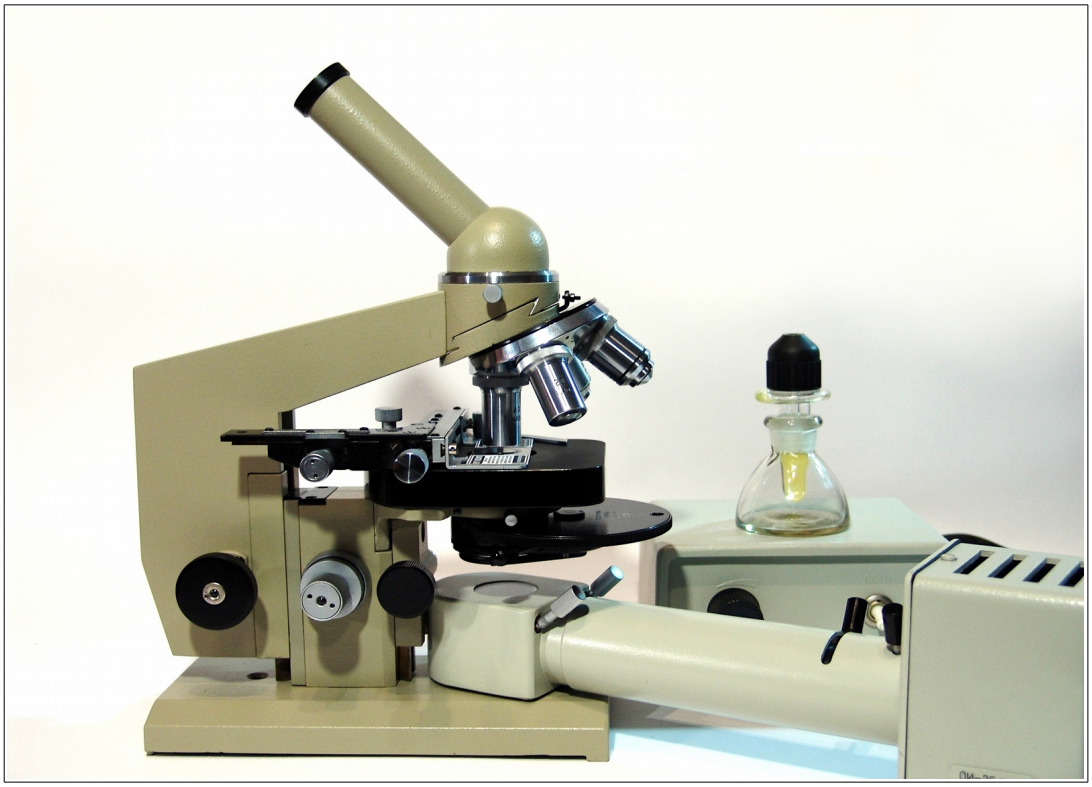

6.The Lomo Biolam 70 Д1 (D1) travel microscope used for the experiments. In this picture it is equipped with the Lomo МФА-2 condenser, the Lomo ФА objectives and the Lomo ОИ-35 illuminator, that allows Köhler illumination. All this Lomo equipment is like new (stock fund) to ensure the homogeneity of the tests. On the right you can see a small bottle containing the original Lomo immersion oil.

7. The Lomo МФА-2 set used for the experiments.

8. The Lomo КФ-4 set used the experiments.

9. Cover of the Lomo instruction manual for МФА-2 set (edition from 1983).

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