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# On the Modified Form of Phase-Contrast Microscope

By

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Recently one of the authors (T. H.) succeeded in converting the ordinary microscope into phase-contrast one with a simple method. The underlying principle and the technical procedure of conversion will be briefly explained.

## § 1. The Principle and the Method of Conversion.

In his theory of microscope Abbe assumed plane waves incident upon the object and regarded the image formation at the image plane as the superposition of the elementary waves emerging from each point of the Fraunhofer pattern which is formed at the focal plane of the objective lens. Mathematically speaking, the image can be expressed approximately by the twice repeated Fourier-transform of the function representing the object.

The physical idea involved in this theory, however, holds good also in the case of any convergent or divergent incident rays, since, if we conceive the Fraunhofer pattern in its generalized sense at the plane conjugate to a point source with respect to the intervening lenses, the elementary waves emerging from this pattern will be superposed at the image plane to form exactly the same image as in the case of parallel incident rays.

In the usual phase microscope originating from the theory of Zernike, which in turn is based upon the original form of Abbe's theory, the annular diaphragm is placed at the front focal plane of the condenser and the phase plate at the rear focal plane of the objective, in other words, the diaphragm and the phase-plate are so arranged that the beam passing through the object, which corresponds to each point of the diaphragm, should be parallel. Thus in this case one is obliged to make a new design of the objective and, what is worse, the insertion of the phase-plate is likely to have ill-effects on the resolution of the image.

If, on the other hand, we stand on the basis of the generalized conception of the microscope image formation, we need not necessarily mount the phase plate at a restricted position, but, generally speaking, we may put it at any position between the object and the image plane; then we have only to place the diaphragm at the plane which is conjugate to the phase plate with respect to the whole or a part of the objective lenses or of the lens system including both the objective and the condenser or even of only the condenser lenses. It can thus readily be seen that the conversion of the ordinary into phase-microscope, without inserting a phase plate and therefore without reducing the resolving power, will be achieved, if a suitably selected surface of one of the lenses composing the objective is coated with a "phase-film", as we may call it, and the annular diaphragm of proper size is placed in such a position as to be conjugate to the phase-film. Practically, the diaphragm may be attached to the substage condenser either in front of or at the rear surface of the lens; the correct position of the diaphragm can easily be found by observing the parallax, while adjusting the height of the substage.

The phase-film, with which one of lens surfaces of the objective is to be coated, may be made of transparent plastic substance such as celluloid, which is, if necessary, colored with dyes. A simple device enables one to make a film of uniform thickness. Even when one happens to get slight non-uniformity in thickness (of the order of a fraction of wave-length), it causes only negligible distortion in the shape of the image, as can be proved theoretically (see Appendix).

The usual simple method of analysis for the evaluation of image-contrast in phase microscopy[1] can be applied also in our case without any essential alteration of the argument. The only point to be observed is that, when we consider the wave starting from each point of the object as composed of the diffracted (deviated) and the background (undeviated) wave, the latter is to be understood as the wave which would start from the same point if the object were replaced by the same medium as the surrounding.

## § 2. Characteristic Features.

The distinctive features of the present method are summarized as follows:

(1) Any existing microscope in research institutes and schools (dry or immersed) can be converted into phase microscope without any change in its original form.

(2) The conversion brings about no reduction of resolving power.

(3) The condenser lenses may not necessarily be perfect, or even they may be lacking.

(4) The same (annular) diaphragm can be used for a set of objectives of different magnifying power.

(5) As the troublesome adjustment of the position of the diaphragm is not necessary, the manipulation is very simple.

(6) The converted microscope can be used also as the ordinary (bright field) microscope. Moreover, in the case of research microscope, the phase-contrast condition can be attained continuously from the bright field condition (simply by adjusting the height of the condenser).

(7) Even when the thickness of the object or its supporter amounts to about 1 cm., the phase-contrast condition can be secured.

(8) For illumination the ordinary electric light (made diffuse by a frosted glass) may be used ; no special light sourse is necessary.

(9) The cost of conversion is very low.

According to our experience, the "pure minus", "pure A", "A plus" and "pure plus" types of phase-film are most useful and these are the very types that we can prepare with the easiest procedure.

In Photo. 1–9 are shown some examples of microphotographs taken with various types of phase objective.

## APPENDIX

### On the Influence of Slightly Non-uniform Phase Retardation in the Phase Plate on the Phase-Microscopic Image

by J. HORI

The phase- and amplitude distribution of the one-dimensional phase-microscopic image is given by

$$F(x) = \int_{-\infty}^{\infty} e^{2\pi i \xi(\nu)} \Psi(\nu) e^{-2\pi i \nu x} d\nu , \qquad (1)$$

where $\xi(\nu)$ is phase modulation characteristic of the phase plate, $\Psi(\nu)$ the Fourier-transform of the amplitude transmission characteristic of the object $\psi(x)$. We assume as the object a "phase-slit" having phase retardation $\alpha$ and width $X$, namely that

$$\left.\begin{aligned} \psi(x) &= (e^{2\pi i\alpha}-1)\,\Delta'(x)+1\,, \\ \Delta'(x) &= 1\,, \qquad |x|<X/2\,, \\ &= 1/2\,, \qquad |x|=X/2\,, \\ &= 0\,, \qquad |x|>X/2\,, \end{aligned}\right\} \tag{2}$$

and,

$$\Psi(\nu) = (e^{2\pi i\alpha}-1)\,\frac{\sin\pi\nu X}{\pi\nu} + \delta(\nu)\,, \tag{3}$$

$\delta(\nu)$ being Dirac's $\delta$–function. Then, by inserting (3) in (1), we get

$$F(x) = \frac{e^{2\pi i\alpha}-1}{\pi}\int_0^\infty \frac{e^{2\pi i\xi(\nu)}}{\nu}\left\{\sin 2\pi\nu\left(x+\frac{X}{2}\right)\right.$$
$$\left.-\sin 2\pi\nu\left(x-\frac{X}{2}\right)\right\}d\nu + e^{2\pi i\theta_0}\,, \tag{4}$$

where $\theta_0$ is the phase retardation at the centre of the phase plate.

Now let us consider the phase plate whose characteristic is shown by Fig. 1. The phase retardation within the interval $-N/2 < \nu < N/2$ is constant $2\pi\theta_0$, but outside this region it varies linearly

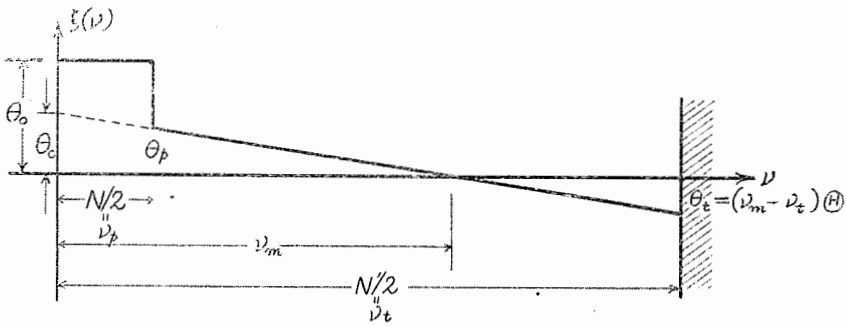


Fig. 1.

with $\nu$ up to the limb of the aperture. As the measure for the "non-uniformity" serves the gradient $\Theta$, which in practical cases is very small. The amplitude distribution of the image is then given by

$$
\begin{aligned}
F(x) = {} & \frac{e^{2\pi i\alpha}-1}{\pi} e^{-\pi i\theta_0} \int_0^{\nu_p} \frac{1}{\nu} \left\{ \sin 2\pi\nu\left(x+\frac{X}{2}\right) - \sin 2\pi\nu\left(x-\frac{X}{2}\right) \right\} d\nu + e^{2\pi i\theta_0} \\
& + \frac{e^{2\pi i\alpha}-1}{\pi} e^{2\pi i\theta_c} \int_{\nu_p}^{\nu_t} \frac{e^{-2\pi i\Theta\nu}}{\nu} \left\{ \sin 2\pi\nu\left(x+\frac{X}{2}\right) - \sin 2\pi\nu\left(x-\frac{X}{2}\right) \right\} d\nu \\
= {} & e^{2\pi i\theta_0} + \frac{e^{2\pi i\theta_0}(e^{2\pi i\alpha}-1)}{\pi} \left[ \mathrm{Si}\left\{\pi\left(x+\frac{X}{2}\right)N\right\} - \mathrm{Si}\left\{\pi\left(x-\frac{X}{2}\right)N\right\} \right] \\
& + \frac{e^{2\pi i\theta_c}(e^{2\pi i\alpha}-1)}{2\pi} \times \\
& \times \begin{bmatrix}
-\mathrm{Si}\left\{\pi N\left(x+\frac{X}{2}+\Theta\right)\right\} - \mathrm{Si}\left\{\pi N\left(x+\frac{X}{2}-\Theta\right)\right\} \\
+\mathrm{Si}\left\{\pi N\left(x-\frac{X}{2}+\Theta\right)\right\} + \mathrm{Si}\left\{\pi N\left(x-\frac{X}{2}-\Theta\right)\right\} \\
+\mathrm{Si}\left\{\pi N'\left(x+\frac{X}{2}+\Theta\right)\right\} + \mathrm{Si}\left\{\pi N'\left(x+\frac{X}{2}-\Theta\right)\right\} \\
-\mathrm{Si}\left\{\pi N'\left(x-\frac{X}{2}+\Theta\right)\right\} - \mathrm{Si}\left\{\pi N'\left(x-\frac{X}{2}-\Theta\right)\right\} \\
+i\mathrm{Ci}\left\{\pi N\left(x+\frac{X}{2}-\Theta\right)\right\} - i\mathrm{Ci}\left\{\pi N\left(x+\frac{X}{2}+\Theta\right)\right\} \\
-i\mathrm{Ci}\left\{\pi N\left(x-\frac{X}{2}-\Theta\right)\right\} + i\mathrm{Ci}\left\{\pi N\left(x-\frac{X}{2}+\Theta\right)\right\} \\
-i\mathrm{Ci}\left\{\pi N'\left(x+\frac{X}{2}-\Theta\right)\right\} + i\mathrm{Ci}\left\{\pi N'\left(x+\frac{X}{2}+\Theta\right)\right\} \\
+i\mathrm{Ci}\left\{\pi N'\left(x-\frac{X}{2}-\Theta\right)\right\} - i\mathrm{Ci}\left\{\pi N'\left(x-\frac{X}{2}+\Theta\right)\right\}
\end{bmatrix} . \qquad (5)
\end{aligned}
$$

It can readily be seen from this expression that the modification of the image due to this type of non-uniformity is almost confined to the region $\pm\frac{X}{2}-\Theta < x < \pm\frac{X}{2}+\Theta$. When $\Theta$ becomes zero, (5) reduces to the formula (16) of the former paper.[2] Numerical evaluation of the expression (5) for the case $2\pi\theta_0 = -\pi/3$, $2\pi\alpha = \pi/3$, $2\nu_p = N = 1$, $2\nu_t = N' = 40$, $X = 1$, $\Theta = 1/20$, $2\pi\theta_c = \pi/20$ gives the result shown

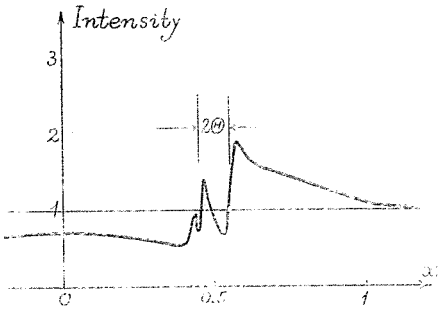


Fig. 2.

in Fig.2. When this is compared with Fig. 12 (§. 4) of the former paper,[2] the difference strikes the eye at once. There appears namely an excess modification with complicated structure in the interval mentioned above. But since in actual cases $\theta$ is extremely small as compared with the value of $X$, it may safely be concluded that the distortion of the image does not manifest itself in any appreciable amount.

In actual cases the non-uniformity may not be of such a simple type as assumed here, but since any sufficiently gradual variation may be approximated as the combination of several linear variations, it might be well expected that the above argument holds in general.

We wish to acknowledge our indebtedness to Mr. K. OGURA for his cooperation in the experiment associated with this work. We are also much indebted to Professor S. MAKINO and Mr. T. YOSHIDA who have taken much interest in the work and have supplied necessary specimens for the experiment.

## References.

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3) J. HORI: *Journ. Facul. Sci., Hokkaido Univ.*, Ser. II, 4 (1951), 29.

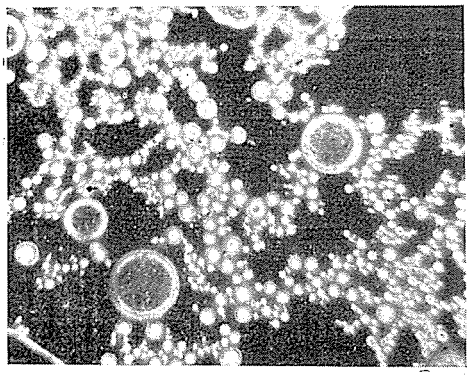


Photo. 1. (Pure A)

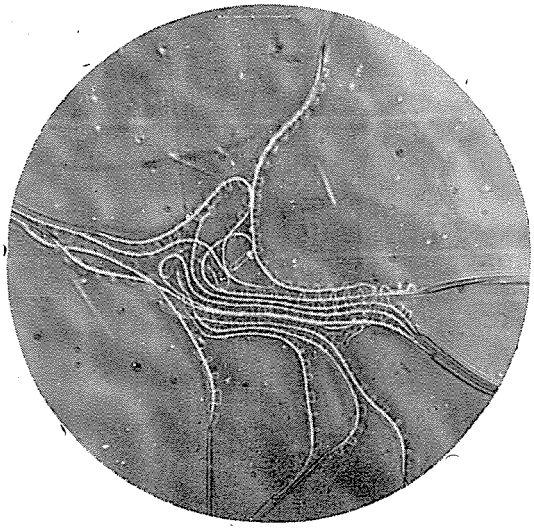


Photo. 4. (A plus)

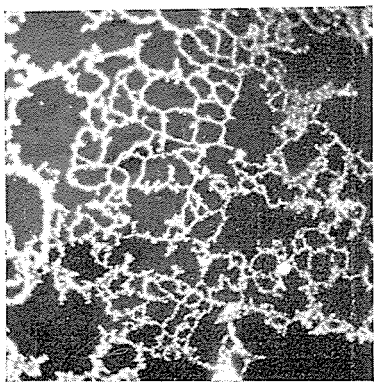
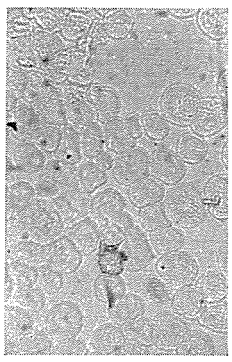
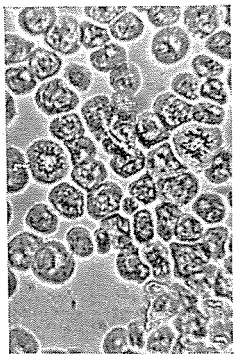


Photo. 2. (Pure A)





(Ordinary bright field) (Pure minus)

Photo. 5.

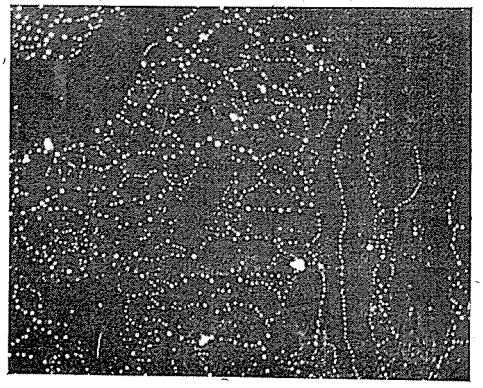


Photo. 3. (Pure A)

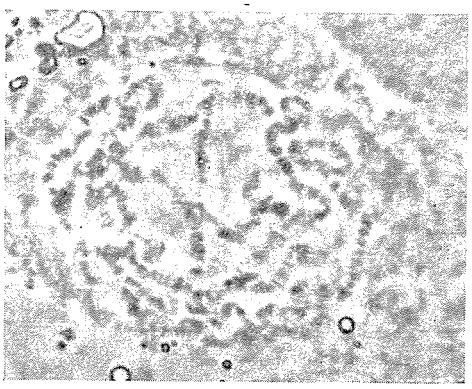


Photo. 6. (Pure minus)

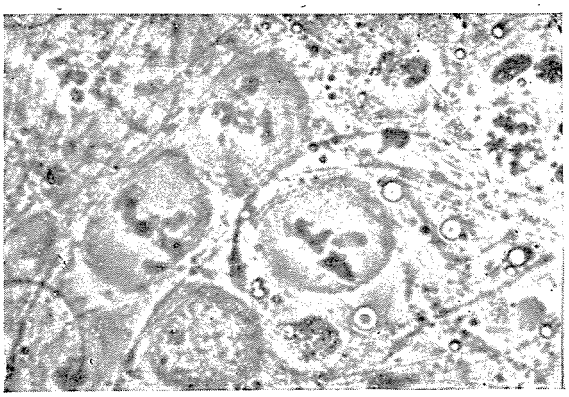


Photo. 7. (Pure minus)

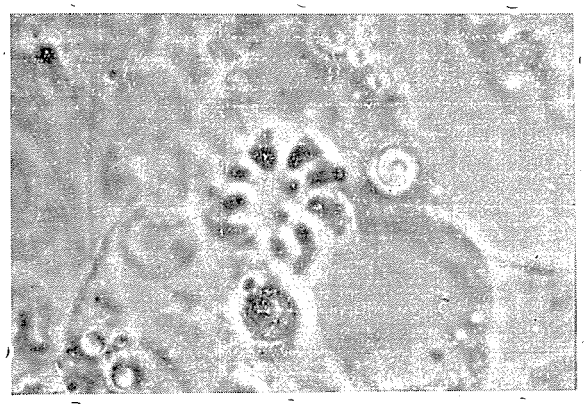


Photo. 8. (Pure minus)

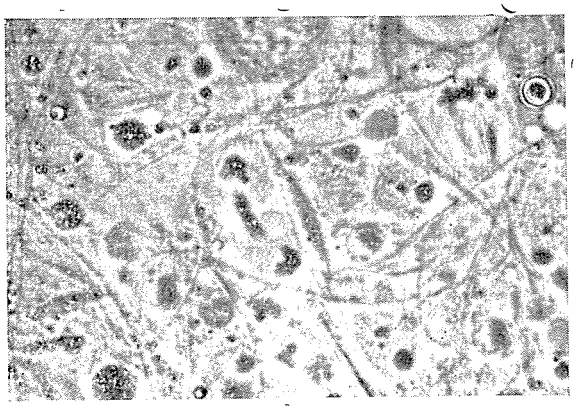


Photo. 9. (Pure minus)

Photo. 1. Chain-like structure of extremely thin film of polystyrene floating on water surface.

Photo. 2. Similar state of raw rubber.

Photo. 3. Similar state of vacuum pump oil.

Photo. 4. Spermatozoa of Hynobius retardatus.

Photo. 5. Yoshida sarcoma.

Photo. 6. Salivary gland chromosome of Drosophila virilis.

Photo. 7. First maturation division of Oxya yezoensis Shiraki (side view).

Photo. 8. Second maturation division of the same (polar view).

Photo. 9. Anaphase of second maturation division of the same (side view).

(Photo. 6~9. were taken by Dr. S. MAKINO and Mr. T. YOSHIDA of Zoological Institute).