METHODS

A PLASTIC SUPERCONDENSER (3D)

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Statement of the problem. Investigations on biogranules carried out in our Institute required improvements in the technic of microscopic observations. Our aim was to increase the resolving power of the microscope and to obtain undistorted plastic pictures of unfixed, unstained, living biological material.

According to Abbé's teaching the microscopic picture in stained preparations is due to absorption, and in unstained preparations to diffraction. In the latter case the rays of light, after passing through the fine structures of the object, undergo precisely the same changes as on passing through an optic grating. Light falling on the object is dispersed into the so-called diffraction beams. The center one of these diffraction beams (0) passes through the optic media of the object without changing its initial direction. The lateral beams are inclined to the optic axis at an angle α, whose value is greater the higher the serial number of the beam. (Fig. 1).

The magnitude of the angle α can be determined by considering the wavelength of the light (λ), the width of the tissue elements or the intervals of the grating (d) and, finally, the refractive index of the medium occupying the space between the object and the objective (n). For the first beam, this magnitude is determined by the expression:

$$\sin \alpha = \frac{\lambda}{n \cdot d}.$$

In accordance with the theoretical and experimental investigations of Abbé, in order to obtain an image similar within limits to the object it is essential that besides the median diffraction beam at least one other diffraction beam of light should fall on the microscope (Fig. 1). In a case where the objective is situated very close to the object (for high magnification), this can take place at an angle $\alpha = 70\text{-}72°$

$$(\sin \alpha\ 72° = 0.95).$$

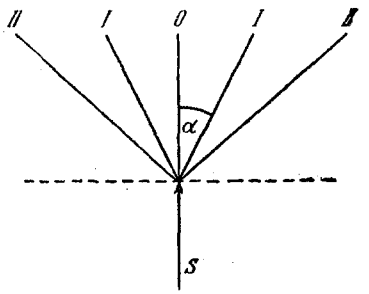

Fig. 1. Diagram showing the arrangement of the diffraction beams during passage of the light through the fine structures of an object (according to Abbé). For description see text.

The smallest distinguishable width of the spaces of the grating or the finest unit of structure of tissue (d) in microscopic examination corresponds to the limit of the resolving power of the microscope, i. e. is equal to the smallest distance between two points on the object from which it is possible to obtain separate points on the image

$$d = \frac{\lambda}{n \sin \alpha} = \frac{\lambda}{A}$$

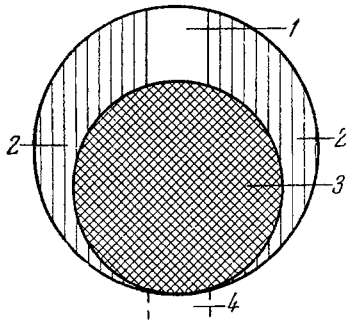

Fig. 2. Arrangement of the separate fields of illumination in the plane of the object in the method of combined illumination by Zselionka and Kiss. For description see text.

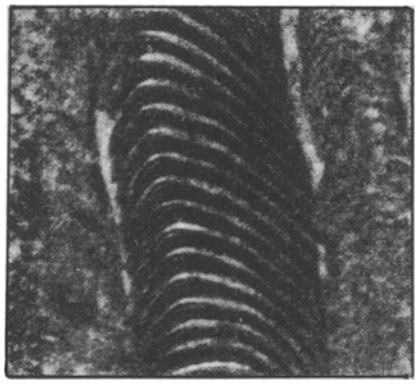

Fig. 3. An area of transversely striated muscle from a human cadaver exhumed 492 days after death (preparation of Prof. Ekresh).

Microphotograph produced by the use of the supercondenser (3D).

where A=n sin α; A is the so-called numerical aperture of the system.

If d is smaller than this value, then beam I does not fall on the microscope and no image is formed. The field of vision becomes uniformly illuminated. Thus the resolving power of the microscope (d) is greater the smaller λ and the greater A.

The wavelength of the greenish yellow light which acts most strongly on the eyes is (λ)=0.55 μ.

When a dry lens system is in use, n=1, since the light enters the objective from air. Consequently in this case A=1·sin 72°=1·0.95.

The resolving power of the microscope when working with a dry lens system is thus:

$$d = \frac{0{,}55}{0{,}95} = 0{,}58\ \mu.$$

For an oil immersion objective this value is:

$$d = \frac{0{,}55}{1{,}51 \cdot 0{,}95} = \frac{0{,}55}{1{,}4} = 0{,}4\ \mu.$$

With lateral illumination:

$$d = \frac{\lambda}{2A},$$

i. e.

$$d = \frac{0{,}55}{2 \cdot 0{,}95} = \frac{0{,}55}{1{,}9} = 0{,}3\ \mu \text{ (for dry objectives)}.$$

$$d = \frac{0{,}55}{2{,}8} = 0{,}2\ \mu \text{ (for oil immersion)}.$$

These values are the limits, determined on theoretical grounds, of the resolving power of the microscope. Further increase in this power is possible only by means of reduction of the wavelength (λ) (Abbé).

If d is equal to 0.4 μ, then in a microscope image of an area of the object 10 μ in length, 25 distinct points can be made out; if d=0.2 μ, then in this distance of 10 μ 50 separate points can be distinguished optically.

By the use of ultraviolet light (λ=0.275 μ) the resolving power of the microscope is increased to

$$d = \frac{0{,}275}{2 \cdot 1{,}4} = 0{,}1\ \mu \qquad \text{(Köhler)}$$

In this case quartz objectives are used and the source of light is an arc lamp with cadmium electrodes.

From these considerations it is understandable that improvements in the microscopic image can be obtained not only by changes in the objective and ocular of the microscope but also by improving the illumination of the object. We chose this latter route in the construction of our supercondenser 3D, which is based on a novel combined illumination.

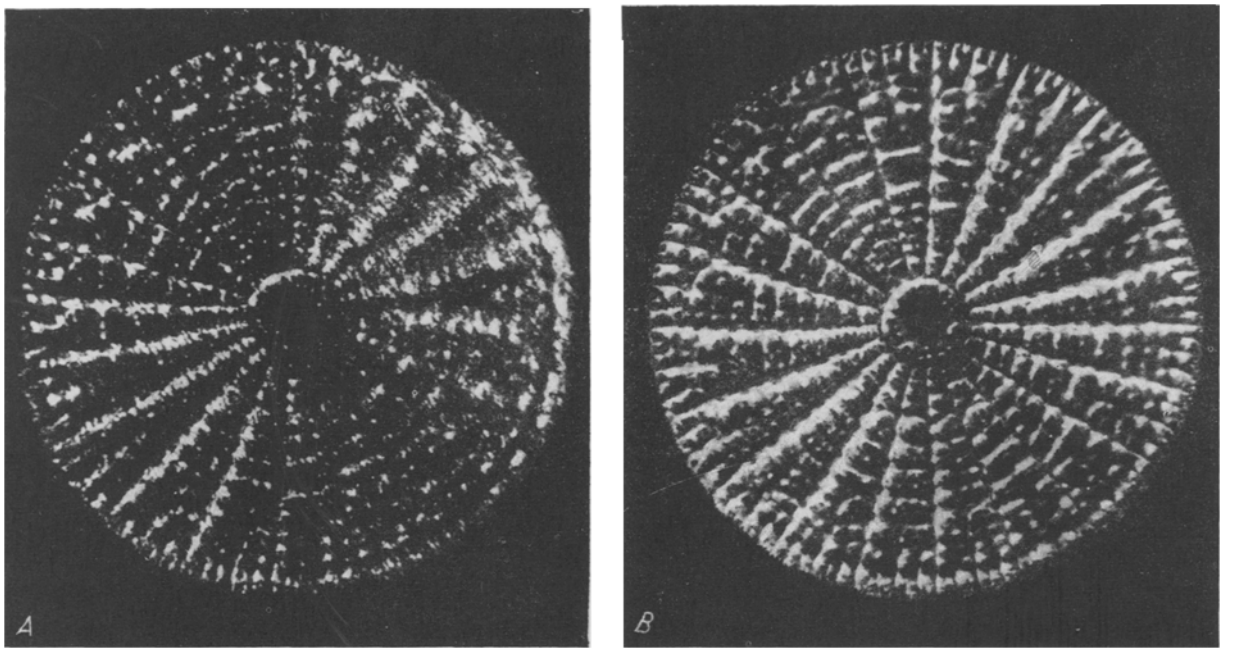

Fig. 4. A — microphotograph produced by means of a cardioid condenser; B — microphotograph produced by means of the supercondenser (3D).

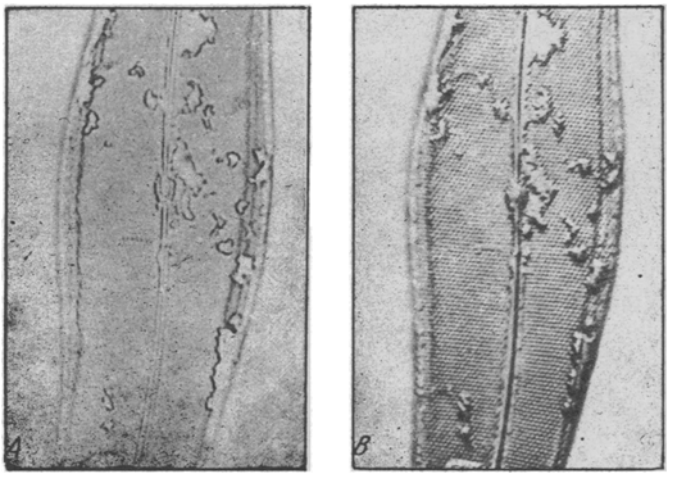

Fig. 5. A — microphotograph produced with an Abbé condenser; B — microphotograph produced with the supercondenser (3D).

Principle of the condenser 3D. The supercondenser 3D is suitable for any form of illumination by transmitted light with the exception of phase-contrast illumination. It may be used, for example, to obtain a central light field, for dark field, and in addition, for the so-called combined illumination.

Our combined illumination does away with the defects of the unilateral or circular oblique illumination in use at the present time. It is well known that with unilateral oblique illumination, adjacent points in the microscopic picture which coincide with the direction of the illuminating light, merge into a line, but a line running mainly in this direction becomes completely or almost completely imperceptible. In consequence of

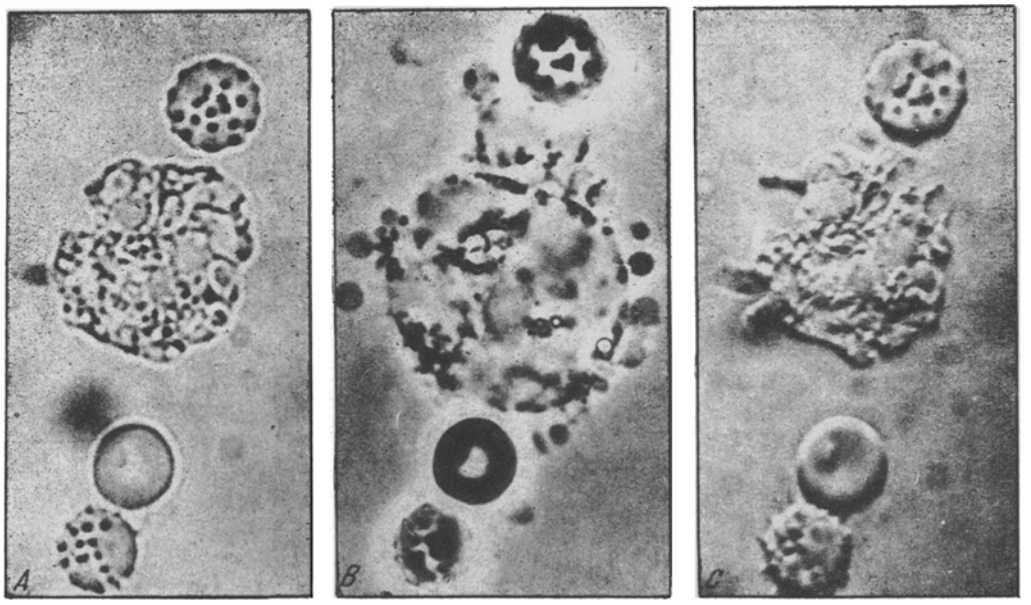

Fig. 6. Fresh, unstained human blood in a wet chamber. A — microphotograph produced by means of an Abbé condenser; B — phase contrast microscopy; C — microphotograph using the supercondenser (3D).

this,serious optical aberrations result, giving a false idea of the true structure of the object. With circular oblique illumination the optical aberrations are reduced to a minimum, but in certain cases, primarily with light from a wide aperture, the illumination of the object becomes excessive, and this leads to veiling of the image and to marked diminution of its contrast. The principle of our combined illumination is as follows: unilateral oblique illumination of considerable power is combined with light from the opposite side, leading in the same direction and creating the effect of a dark field. To this is added illumination of the field by means of crescentic beams of light of smaller intensity around it, which are partly diffuse and undergo a phase change towards a shorter wavelength of light (Fig. 2).

In Fig. 2 is shown the arrangement of the separate fields of illumination in the plane of the object with our combined method of illumination. In the 1st field is shown the action of an intense unilateral oblique illumination, whereas rays of light act in a 4th field which is arranged in opposition to the first field, to give the effect of dark-ground illumination. In a 2nd field, surrounding the 1st and 4th fields, rays of light of smaller intensity are in action, and these to some degree are filtered and become partially diffused. In the completely dark field (the 3rd field in Fig. 2) the central light (i. e. the light from a small aperture) are completely removed.

RESULTS ACHIEVED WITH OUR CONDENSER

1). Since the combined illumination produced by our method has an azimuth of 360°, optical distortions are reduced to a minimum.

2). The deep shadowing occurring with unilateral oblique illumination is weakened by the oblique light of the 4th field and also by the light of the 2nd field. In consequence of this the optically sharp borders in the individual structures of the object do not suffer any great softening of their contours.

3). The partially diffused light of the 2nd field, lower in intensity and modified towards a shorter wavelength, brings about uniformity of illumination and, in addition, increases the resolving power; on the other hand, thanks to its low intensity, it reduces the degree of illumination of the object, which increases the contrast of the image.

Thanks to the combined illumination the microscopic image of the object takes on a solid appearance; the individual details literally stand out above the surface, which creates the effect of plasticity, which is even more expressed by the use of the binocular attachment. Since our condenser provides mainly oblique rather

than central illumination, the resolving power of the system is doubled. Thanks to this we were able to approach the theoretical maximum of resolving power of the microscope more closely than by using the unilateral oblique illumination which has been used up to the present time.

The plasticity of the image is particularly suitable for investigations of the detailed structure of a surface and also in the solution of certain technological problems (Fig. 3).

The greatest external diameter of the mounting of the condenser 3D is 39.5 mm, in consequence of which it can be used in any ordinary microscope. The lenses of the condenser form an aplanatic system, whose aperture is 1.25. The supercondenser 3D has three easily replaceable frontal lenses. With one of these lenses a so-called light field similar to that with the traditional Abbé condenser may be obtained; the second frontal lens gives a plastic image; the third lens permits the effect of dark-ground illumination to be obtained.

It will be clear from this account that our condenser makes the use of the cardioid condenser unnecessary. Working with the 3D condenser is simpler than with the cardioid condenser, and the accuracy of the image obtained is in certain respects superior to that obtained by the use of the cardioid condenser (Figs. 4, 5, 6).

ADVANTAGES OF THE SUPERCONDENSER 3D

We will briefly summarize the advantages of our condenser.

1). A plastic image may be obtained, free from distortion and with a uniformly illuminated field of vision. The plasticity of the image obtained gives considerable advantages when investigating the surface of a specimen, which has so far been visible only in two dimensions even at high magnifications.

2). Increase in the resolving power of the microscope practically to the maximum attainable and, consequently, the attainment of more considerable degrees of magnification.

3). Increase in the resolving power and the use of an original method of illumination enables the production of an image by the use of achromatic objectives of a quality which is obtainable with other condenser systems only by the use of the much more expensive apochromatic objectives.

4). The construction of our condenser enables a perfect dark field to be obtained by means of simple manipulation without the use of any accessories. This is possible both for work with dry or oil immersion systems, and furthermore thanks to interference between the rays of light, individual preparations give a many-colored light.

IMPORTANCE OF THE SUPERCONDENSER 3D

Our condenser opens up new possibilities not only in the realm of biological and medical microscopic research but also in industry and technology, in the investigation of synthetic fibers, in geology and so on. With the aid of this condenser we have already been able to obtain significant morphological results, mainly in the field of hematological investigation.

Our condenser will show, for example, particles which in the Abbé field appear only in the form of points (for example the granules in the red blood corpuscles and the leucocytes, and also certain details of the structure of diatoms).

Our condenser completely excludes the haloes characteristic of phase contract images obtained by the method of Zernik. On the basis of our microphotographs we believe that all the results of investigations carried out by the use of the phase contrast principle require critical review.

Our microphotographs obtained by the use of the 3D condenser and immersion objectives demonstrate the possibility of obtaining a degree of magnification which is 2 or 3 times greater than that attainable with previous condenser systems. It is understandable that this considerably increases the instructive and demonstrational value of the microscope as well as its documentational importance.

SUMMARY

A new construction of a microscope condenser – supercondenser (3D) based on combined illumination has been offered. The condenser consists of three frontal lenses forming an aplanatic system with a numerical aperture of 1.25.

Supercondenser 3D possesses the following advantages:

1). It improves the resolving power of the microscope.

2). It eliminates optical distortions.

3). It forms a plastic image of a three-dimensional object.

4). It is simple in use and may be utilized with ordinary microscopes-both for dry or immersion objectives.