Remarks on the Effect of the Aperture of the Condenser on Resolution by the Microscope

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SUMMARY

Variation of the aperture of the condenser makes somewhat less difference to microscopical resolution than is usually supposed.

IN theoretical discussions of resolution by the microscope, it is conventionally assumed that two points can just be resolved as separate by a particular optical system, when the geometrical image of one of the points lies on the edge of the diffraction-disk representing the other point in the image-plane. The distance apart of the two points when this is so is generally known by the Greek letter ρ. In most books on microscopy ρ is stated to be related to λ (the wave-length of the light used) and to the numerical aperture of the objective thus:

$$\rho = \frac{0{\cdot}61\,\lambda}{n \sin.\ \alpha}$$

where α is half the angle of the rays accepted by the objective and n the refractive index of the medium in which the angle is measured.

When this equation is used, it is generally understood that the numerical aperture of the condenser is the same as that of the objective. It is often stated that when the two apertures differ, their mean should be used as the denominator of the fraction in the equation. No practical microscopist, however, would accept this. If it were true, one could get *better* resolution with an objective of N.A. 0·1 and a condenser of N.A. 1·2, than with an objective of N.A. 0·7 and a condenser of N.A. 0·5. In fact, of course, the latter arrangement would give quite good resolution and the former very poor.

Hopkins and Barham have recently (1950) provided a theoretical treatment of the influence of condenser-aperture on resolution. They denote by the letter s the N.A. of the condenser divided by the N.A. of the objective. When $s = 1$, the equation given above is correct (on the assumption, always made in discussions of this subject, that perfect spherical correction is achieved in the objective). When s is not 1, however, the figure 0·61 in the equation is no longer applicable. It is changed to a variable which Hopkins and Barham represent by the letter K. They give a graph which shows how K varies with different values of s. The graph is a curved line, crossing the abscissa-value $s = 1$ at the K value 0·61.

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The treatment by Hopkins and Barham is quite general. Many microscopists would probably like to know the conclusions to be drawn from their work in terms of practical microscopy, and it is the main purpose of the present paper to provide this information. When an object is too small to be resolved by a particular objective, another of higher aperture is usually substituted until the oil-immersion lens of N.A. 1·3 or 1·4 is reached. These two numerical apertures are therefore of particular importance in matters connected with resolution, and they alone will be considered here. For simplicity, the wave-length of light used may be standardized at 550 mμ (yellowish green). The value of K for different values of s may be obtained from Hopkins's and Barham's graph (their fig. 2). Table 1 in the present paper shows the results of calculations made on this basis.

TABLE 1. *This shows how resolution is affected by the aperture of the condenser.*

	ρ (*expressed in* mμ)	
N.A. of condenser	*with objective of N.A. 1·4*	*with objective of N.A. 1·3*
0·0	327	352
0·1	325	349
0·2	321	344
0·3	315	337
0·4	308	329
0·5	301	320
0·6	293	311
0·7	285	302
0·8	277	294
0·9	270	286
1·0	263	278
1·1	256	270
1·2	250	264
1·3	244	258
1·4	240	253

If a wave-length other than 550 mμ is used, the value of ρ must be multiplied by $\frac{x}{550}$, where x is the wave-length of the light used, expressed in mμ.

The figures in the table show that the influence of the aperture of the condenser on resolution is somewhat less than many microscopists have supposed. For instance, if an objective of N.A. 1·4 is used with a condenser-aperture of 1·4, and the iris diaphragm is then closed to give N.A. 0·7, ρ only changes from 240 to 285 mμ; or, to express the same facts in another way, the resolution falls from 417 to 351 lines per 100 μ, a drop of only 16 per cent. If one had adopted the old practice of using the mean of N.A. 1·4 and N.A. 0·7 as the denominator of the fraction in the equation $\rho = \frac{0{\cdot}61\,\lambda}{n \sin.\,\alpha}$, the value of ρ would have increased to 320 mμ and the resolution would have fallen to 313 lines per 100 μ, 25 per cent. (instead of 16) below the full-aperture figure.

It must be remarked that ordinary microscopical images are affected by a factor separate from those concerned in the theoretical basis of the table. If any object is of markedly different refractive index from the surrounding mounting-medium, it will tend (as is well known) to appear with a Becke line round it, and this will necessarily interfere with the perfection of the image. The effect will be increased if a narrow cone of light is used for illumination, whether that cone is central (that is, central light of low numerical aperture) or not (oblique light of high numerical aperture). Whenever one opens the iris diaphragm, this undesirable Becke effect will be reduced.

There are theoretical reasons, however, for supposing that it is usually undesirable to use an illuminating cone of quite so high a numerical aperture as the objective, even if the extreme marginal zone of the latter be assumed to be perfectly corrected for spherical aberration (see Oettlé, 1950). This accords with the experience of most practical microscopists. Oettlé's explanation is that when direct light enters the marginal zone of an objective, only half of the diffracted rays are concerned in image-formation, the remainder being too oblique to enter the lens-system.

REFERENCES

HOPKINS, H. H., and BARHAM, P. M., 1950. Proc. Phys. Soc. B, **63**, 737.
OETTLÉ, A. G., 1950. Quart. J. micr. Sci., **91**, 348.