Reprinted from Applied Optics, Vol. *26*, Page 2043, June 1, 1987

Microscope objectives, cover slips, and spherical aberration

Christopher W. Oates and Matt Young

U.S. National Bureau of Standards, Electromagnetic Technology Division, Boulder, Colorado 80303.
Received 16 March 1987.

In our laboratories, we have lots of garden variety microscope objectives. These range in numerical aperture from 0.15 to 0.9 or higher. We use them for spatially filtering laser beams, imaging the ends of optical fibers, and other functions for which they were never intended.

Most common objectives are designed for use with a cover slip that is 170 μm thick and has a refractive index of 1.522.[1] When they are used without a cover slip, their image quality is apt to suffer as a result of spherical aberration. (A metallurgical objective, which is designed for use without a cover slip, displays spherical aberration if it is used with a cover slip.)

The spherical aberration of the cover slip increases with increasing numerical aperture (N.A.). For a low enough numerical aperture, spherical aberration will not be a factor, and a low N.A. objective may be used with or without a cover slip. We wanted to know the highest N.A. for which the spherical aberration of the cover slip is not a factor.

We used the geometry of Fig. 1 to calculate the transverse spherical aberration introduced by the cover slip. A cone of rays converges to a virtual object point O. Its paraxial image is point O', which for our case lies 170 μm beyond the air–glass interface.

A marginal ray strikes the interface with angle U, where $n \sin U$ = N.A. This ray intersects the axis at a point O''. The transverse aberration we seek is the line segment δ, which is given by the relation

$$\delta = (n \tan U - n' \tan U')\,(l'/n'), \qquad (1)$$

where $n' \sin U' = n \sin U$. For our purposes, $n = 1$, $n' = 1.522$, $l' = 170\ \mu$m, and U is determined by the numerical aperture of the focusing optics.

A microscope objective has, built in, a spherical aberration equal and opposite to that of the cover slip; removing the cover slip, therefore, results in an aberration equal in magnitude to that of Eq. (1). (Using a metallurgical objective with a cover slip causes the same result.)

We calculated δ for various numerical apertures. As long as δ remains less than the theoretical resolution limit $RL = 0.61\lambda/\text{N.A.}$, the system remains diffraction-limited when we remove the cover slip. Table I shows values of the ratio δ/RL for typical wavelengths and numerical apertures. These values may be scaled proportionally for other thicknesses of glass, such as the 1-mm windows that often cover detectors. We are tacitly (and possibly incorrectly) assuming that an objective designed for visible light remains diffraction-limited in monochromatic light with IR wavelengths of interest in optical communications.

Table I. Ratio of Transverse Aberration to Resolution Limit

Typical power N.A.	10× 0.25	20× 0.45	40× 0.65	60× 0.85
Wavelength (μm)				
0.45	0.48	6.0	36	200
0.55	0.40	4.9	30	170
0.63	0.35	4.3	26	150
0.85	0.26	3.2	19	110
1.30	0.17	2.1	13	71

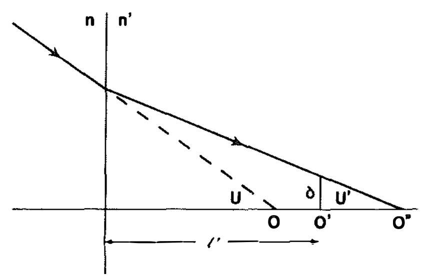

Fig. 1. Marginal ray striking a planar interface. l' is the paraxial image distance, and $n \sin U$ is the numerical aperture. The transverse aberration is δ.

The value $\delta/RL = 1.6$ corresponds to a wavefront aberration of $\lambda/4$; therefore, only the 10× 0.25-N.A. objectives may be used without a cover slip. Objectives with N.A. = 0.45 are at best marginal, and the others are far from diffraction-limited.

When the numbers in Table I are considerably greater than 1, they are approximately equal to the aberrated resolution limit in units of $0.61\lambda/\text{N.A.}$ In reality, the plane of best focus may not be the same as the paraxial focal plane, and the resolution will be slightly better than Table I suggests.

The point, however, is that, when resolution is important, microscope objectives of 20× and higher powers should be used only as intended. Generally, this means with a cover slip, unless the objective is labeled metallurgical.

Reference

1. J. R. Benford and H. E. Rosenberger, "Microscope Objectives and Eyepieces," in *Handbook of Optics*, W. G. Driscoll and W. Vaughan, Eds. (McGraw-Hill, New York, 1978), p. 6-3.