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Letters to the Editor

Numerical apertures of light microscope objectives

We write with reference to the short technical note by Lehmann & Wachtel (1993). It seems rather surprising to us that the authors considered it necessary 'to develop the method described in this paper' since similar methods have been in use for almost 100 years; no references were quoted by Lehmann and Wachtel, but interested workers can find many references to techniques in the literature (see below). Very recently, there has been an article on this subject by Haselmann (1991) in the *Proceedings of the Royal Microscopical Society*, followed by an extensive correspondence (Martin, 1992; Sanderson, 1992; Speight, 1992) on the same subject.

Although the method described by Lehmann and Wachtel is workable, we have doubts as to its practical value to the non-specialist, since details of the photographic methods of measurement were not given. We consider that Lehmann and Wachtel's attempts to simplify their technique and its description have led to uncertainties as to what they actually measured, and its accuracy. Although they claim to have measured the radius 'of the rear lens', we suspect that, in fact, their values may refer to the fully illuminated back focal plane of the objective. This normally lies some distance below the rear element of the lens. Moreover, the position of the back focal plane differs from that of the rear optical element of the objective by a variable amount which depends on the type and maker of the objective. In consequence, the exit angle calculated by the authors may be based on an inappropriate distance.

It should also be noted that the method described by Lehmann and Wachtel is not new, being essentially the same as that of Nelson (1896/7). This was redescribed by Cheshire (1902, 1904, 1914), who, together with Ainslie (1914), used similar principles to develop direct-reading apertometers, either in rule or card form. A more recent account of Nelson's method can be found in Dade (1955). Such simple methods allow measurement of numerical apertures (accurate to the second decimal place) of 'dry' objectives. Similar methods using simple apparatus involving the addition of a block of glass of known refractive index (for example, the Beck apertometer marketed before World War II or the apertometer published in 1991 by Haselmann) may be used with immersion objectives too. Such devices allow any microscopist to measure the numerical aperture of objectives quickly, simply and accurately. Great accuracy in the determination of numerical apertures, however, seems of little practical value, especially as the values obtained depend to some extent (particularly with objectives of high numerical aperture) on the wavelength of the light with which the measurement is made. Abbe (1880) himself (who developed perhaps the most accurate apertometer ever made, marketed by Zeiss) commented:

> 'No microscopist in the world will be able to make out any difference in the performance of objectives, as long as the numerical apertures do not differ by *several* per cent, other circumstances being equal.
> For these reasons I consider all attempts at very accurate measurements of this kind to be useless.'

In their note Lehmann and Wachtel do not detail the various possible sources of errors in apertometry. Those interested in a detailed discussion of the possible errors in measurement of numerical aperture will find them fully covered in a paper by Hartridge (1918).

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Because of Dr Lehmann's untimely death, I am not able to reply to Bradbury *et al.*'s commentary in as much detail as I would desire. Nevertheless, I should point out the following.

(1) If our method was known almost 100 years ago, then at least one of our reviewers should have warned us about that.

(2) The commentary that precise knowledge of the NA of objectives is of little practical value does not address itself to the content of our paper. It is not aimed at determining the NA with great precision, but for the convenience of those who wish to make their own measurements and do not possess an apertometer.

(3) Concerning experimental detail, we performed our measurements with a knowledge of their limitations, but kept the description of this part as brief as possible. We did mention that other techniques of measurement are possible (e.g. callipers for the diameter of the rear lens), but we did not want greatly to lengthen such a short note. I had suggested a short-working-distance telescope with a calibrated eyepiece reticle, focused on the lens. However, I am sure that Dr Lehmann was aware of the photographic magnification necessary to minimize error by his technique. The object of our paper is described in the first sentence of the Summary. It does not primarily consist of a description of particular measurement techniques. The error limits, listed in Table I of our paper, are based on realistic evaluations of our measurements.

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Erratum

Milne, R.H., Hembree, G.G., Druker, J.S., Harland, C.J. & Venables, J.A. (1993) Surface studies in UHV SEM and STEM. *J. Microsc.* **170**, 193–199.

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Short technical note

Numerical apertures of light microscope objectives

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Key words. Objective, numerical aperture, magnification.

Summary

The numerical aperture of light-microscope objectives is measured via the exit angle of the rear lens towards the image space, and the magnification of the objective. The method is reliable because of its simplicity and is independent of special instrumentation such as apertometers. Results from eight commercially available objectives indicate fair agreement of nominal data with measured data for the magnifications, but not for all numerical apertures.

Introduction

The numerical aperture (NA) of a light-microscope objective determines the resolution limit of object details in the image at a given wavelength of illumination. It is customarily defined as $\mathrm{NA} = n \sin \alpha$, where n is the refractive index of the medium between the object and the front lens of the objective, and α is half the front lens acceptance angle.

Concern regarding the reliability of the NA values engraved on the sides of commercially available objectives led us to develop the method described in this paper. We propose to call this the 'rear-angle method' because it is based on a measurement of the exit angle of the objective towards the image. It is, essentially, a simple method to determine an NA, which would otherwise be difficult to measure without specially calibrated instruments, such as apertometers etc.

Figure 1 shows the parameters required for the calculation. The dimensions denote the optical path lengths, which are somewhat longer than the geometrical lengths because of the refractive indices of the immersion oil (if used) and of the objective lens glass.

Aberration-free imaging requires that the entire path length, $a + b$, and the magnification ratio, $M = b/a$, are independent of the path the light rays take through the objective. Figure 1 provides the equation for a dry objective: $a \sin \alpha = b \sin \beta$, from which it follows that

$$\mathrm{NA} = \sin \alpha = M \sin \beta.$$

The corresponding equation for an oil-immersion objective is

$$\mathrm{NA} = n \sin \alpha = M \sin \beta. \qquad (1)$$

This equation shows that, for the determination of the NA, only two quantities, M and β, need to be known.

The front angle, α, can be substantial, especially for high-power objectives, but the rear angle, β, is usually sufficiently small that, very approximately, $\sin \beta = \tan \beta = r/c$, where r is the radius of the rear lens and c is the distance from the rear lens to the image. This equation demands that the light bundle emerging from the rear lens equals its diameter. This is normally so, unless a physical obstruction (e.g. a narrow aperture) is present in the objective. The final result is

$$\mathrm{NA} = Mr/c. \qquad (2)$$

The magnification, M, was measured by comparing the magnified image of the stage micrometer scale with a millimetre scale on frosted glass held against the open end (i.e. without ocular) of the microscope tube. The radius, r, of the rear lens was measured by means of a photographic method: the image of the fully lighted rear lens was projected by another lens (at the end of the tube) against film in a camera. The objective to be tested was then replaced by a millimetre scale in the same position as the rear lens, and also photographed. Enlarged pictures of both, rear lens and scale, were then compared so that the diameter, $2r$, could be determined with an accuracy of about $\pm 0{\cdot}1$ mm. Other methods, such as calipers, are possible, of course.

The distance, c, from the rear lens to the image at the end of the tube is the sum of the standard tube length of

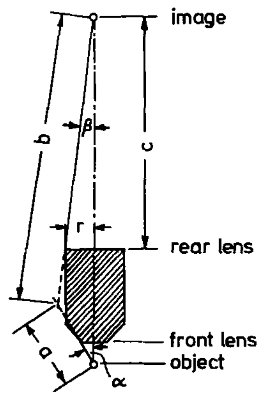

Fig. 1. Parameters used in the calculations.

160 mm plus the small distance from the rear lens to the rim of the objective. The latter is normally in the range of about 10–40 mm and is easily measured to about ±1 mm or better.

In practice, whenever a microscope is used with an ocular, the image is not observed at the end of the tube, but in the focal plane of the ocular. Therefore, the real objective magnification also depends on the ocular used, and is slightly lower than the nominal magnification. This difference has little or no effect on the determination of the NA using Eq. (2), because it affects both quantities, M and c, similarly, and therefore cancels out.

Table 1. Nominal and measured values of some commercial objectives.

Magnification		Numerical aperture	
Nominal	Measured	Nominal	Measured
5× dry	5·5×	0·10	0·11 ± 0·02
10× dry	9·7×	0·30	0·23 ± 0·02*
20× dry	22×	0·40	0·45 ± 0·02*
40× dry	40×	0·65	0·64 ± 0.02
60× dry	62×	0·85	0.83 ± 0.03
100× oil	102×	1·25	0·97 ± 0·04*
100× oil	100×	1·25	1·23 ± 0·05
120× oil	120×	1·30	1·30 ± 0·07

The range of uncertainties of the measured values of NA is estimated from the uncertainties of the measured values of r and c and from the approximation $\sin\beta \approx \tan\beta$. The nominal values of NA of the three objectives marked by an asterisk deviate from the measured values beyond the stated uncertainties.

Results

Results of magnifications and values of NA measured in this way are given in Table 1 for eight different objectives from four different manufacturers. The nominal magnifications agree fairly well with our measured magnifications, but the nominal NA values disagree with the measured NA values in three of the eight examples.