DESIGN OF OPTICAL MICROSCOPES—SOME GUIDING FACTORS

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The essential parameters of different types of microscopes, eg. magnification, resolving power and numerical aperture, have been highlighted and relations among these parameters have been established. Effort has been made to correlate focal length and numerical aperture of objectives, which may prove useful to the microscope designers in the country.

In designing microscopes the magnification factor plays an important role. At first sight it seems that the higher the magnification, the better is the performance of the microscope, but it is a misleading concept. By merely increasing the magnification, two points in the object space may be enlarged more and more but a limit will be reached when their images become ill-defined and finally merge into a single point. In order that the eyes can see the images separately, the objective must have reasonable resolving power. Obviously, the finer the details are resolved, the more they must be magnified in order that the eyes can see them clearly and comfortably. In other words, higher magnification must be accompanied with higher resolving power.

RESOLVING POWER AND NUMERICAL APERTURE

The minimum linear separation R, between the two points in a specimen, that can be resolved by a lens, is given by $R = \frac{0{\cdot}5\lambda}{N.A.}$ where λ is the wavelength of the radiation illuminating the specimen and $N.A.$ is the Numerical Aperture of the lens. In the case of optical microscopes, it is only $N.A.$ of the objective which controls the Resolving Power ($R.P.$). From the above expression it is clear that $R.P.$ and $N.A.$ are directly proportional to each other.

Numerical aperture is considered as a measure of light gathering power of the objective and is expressed as $n \sin u$, where n is the refractive index of the medium between the object and objective and u is half of the apical angle.

In the case of dry objectives the value of n (i.e refractive index of air) is one. As light rays pass from cover glass to air, most of the rays which exceed the critical angle of 41·5° are totally internally reflected at the glass-air surface. It has been experimentally found that the maximum value[1] of u is only 72°. This leads to the maximum value of $N.A.$ in the case of dry objectives to be 0·95.

This value can, however, be increased by using a medium of greater refractive index between the cover glass and objective. In the case of water immersion objectives the total internal reflection takes place at 61·5°. The maximum value[1] of u in this case has been found to be 64° thereby giving maximum value of $N.A.$ to be 1·20.

The $N.A.$ is further increased by using oil in place of water. This eliminates the possibility of total internal reflection and subsequently increases the value of u. If we take, for example, cedar wood oil for which the maximum value[1] of u has been found to be 67°, the maximum $N.A.$ is calculated to be 1·40.

LIMITS OF RESOLUTION

In the expression for resolving power, if we take mercury green radiation as a representative of λ value and the maximum $N.A.$ as 1·40, the maximum $R.P.$ in the case of an optical microscope can be calculated as 2000 A°. However, this limit of $R.P.$ can be increased by using radiations of shorter wavelengths. In the case of ultra-violet microscopes, using quartz lenses, the maximum[2] $R.P.$ is 1000A°. In the case of electron microscopes the maximum value is further enhanced[3] to 4A° and in some cases even upto 2A° due to electrons behaving like a wave having wavelength even smaller than that of X-rays, when they are accelerated by applying high voltages after their ejection from the source.

LIMITS OF MAGNIFICATION

Overall magnification

An objective can just resolve two points in the object space if the linear separation between them is not less than $\frac{0{\cdot}5\lambda}{N.A.}$. Taking M to be the overall magnification of the microscope, the separation between those points in the image formed at the least distance of distinct vision D, will be given by $M \times \frac{0{\cdot}5\lambda}{N.A.}$. In order that the eye can resolve this separation, the angle formed at the eye should be equal to or more than the resolving power of the eye.

Since the minimum angle that the eye can resolve is of the order of 1 minute of arc[4], the minimum overall magnification M_{min} will be given by :

$$M_{min} = \frac{1 \text{ minute of arc (in radians)} \times D}{\frac{0{\cdot}5\lambda}{N.A.}}$$

$$= \frac{\frac{1}{60} \times \frac{\pi}{180} \times 250}{0{\cdot}5 \times 5461 \times 10^{-7}} \times N.A.$$

$$\simeq 250 \times N.A. \quad (\because \lambda = 5461 \times 10^{-7} \text{ mm for mercury green radiation and } D = 250 \text{ mm.})$$

The minimum angle of 1 minute, as considered above, is somewhat rigid and cannot be adopted by most of the observers. Relaxing this value to 4 minutes[1], the maximum overall magnification comes out to be:

$$M_{max} \simeq 4 \times 250\ N.A. \simeq 1000 \times N.A.$$

For comfortable vision, the angle of 2 minutes of arc[4] is quite suitable and hence the reasonable value of magnification will be $2 \times 250\ N.A. \simeq 500 \times N.A.$

Primary magnification

Considering eyepieces having magnifications between $5X$ and $15X$, we can now calculate the limits of primary magnification assuming the reasonable value of overall magnification to be $500 \times N.A.$ The above two limits of eyepiece magnification are quite reasonable. Eye pieces having magnifications less than $5X$ are long and restrict the field of view, while those having magnifications more than $15X$ have uncomfortable short eye-clearances. Thus the lower and upper limits of primary magnification shall be $(500 \times N.A.)/15$ and $(500 \times N.A.)/5$ respectively. The primary magnifications should, therefore, lie between $33 \times N.A.$ and $100 \times N.A.$

OBJECTIVE FOCAL LENGTH AND N.A.

An objective of short focal length has high primary magnification. A high power objective requires high resolving power which is achieved by increasing $N.A.$ It is thus clear that the numerical aperture increases with decrease in focal length. As there is no strict relation between the two, it is very difficult to say what should be the exact value of $N.A.$ for any particular focal length. Different leading microscope manufacturers have used slightly different values of $N.A.$ for the same focal length. This is clear from the great differences between the minimum and the maximum values of $N.A.$ for the same focal lengths as shown in Table 1. The data tabulated have been used by different leading manufacturers, such as Bausch & Lomb, Spencer, Zeiss, Leitz, Beck, Swift, Watson, Reichert & Fuess[5].

Using the data of Table 1 two graphs have been plotted as shown in Fig. 1 and 2.

From Fig. 1 and 2, one can easily see how the points (values) are widely scattered. This confirms that there is no strict rule which has been followed by manufacturers in determining the values of $N.A.$ However, the nature of the two curves reveals the fact that the focal length and $N.A.$ are inversely proportional to each other. To test that the two curves are true representatives, let us find the minimum and maximum values of $N.A.$ for some most common focal lengths and see whether the corresponding primary magnifications are between $33 \times N.A.$ and $100 \times N.A.$ (Table 2). These two limits of primary magnification have already been calculated.

From Table 2 it is clear that the values of primary magnifications shown in column 2 are well within the two extreme limits as given in columns 4 and 6; except at S. Nos. 2 and

3 in column 6 where the lower limits are slightly higher but cannot be considered appreciable. This illustrates that the minimum and maximum values of *N.A.* obtained from the two Figures and shown in columns 3 and 5 respectively are quite reasonable and acceptable. Hence the curves drawn in Fig. 1 and 2 can be considered true representatives and may prove useful to the microscope manufacturers in choosing the value of numerical aperture consistent with the objective focal length.

TABLE 1

MINIMUM AND MAXIMUM VALUES OF *N.A.* FOR DIFFERENT OBJECTIVE FOCAL LENGTHS[5]

Equivalent focal length (mm)	*N.A.*		Equivalent focal length (mm)	*N.A.*	
	Min.	Max.		Min.	Max.
75·0	0·09	0·11	6·0	0·65	0·95
60·0	0·07	0·09	5·0	0·75	0·85
50·0	0·08	0·17	4·4	0·65	0·85
45·0	0·10	0·17	4·3	0·75	1·00
40·0	0·08	0·16	4·0	0·65	0·95
35·0	0·17	0·19	3·5	0·85	0·95
32·0	0·10	0·20	3·2	0·75	0·85
25·0	0·12	0·30	3·0	0·85	1·40
24·0	0·08	0·30	2·9	0·85	1·40
16·0	0·17	0·45	2·7	1·00	1·24
15·6	0·28	0·30	2·6	0·90	1·30
14·0	0·35	0·40	2·2	0·90	1·25
12·0	0·34	0·65	2·0	0·90	1·40
8·3	0·40	0·65	1·8	0·80	1·30
8·0	0·50	0·65	1·5	1·30	1·32

TABLE 2

MINIMUM AND MAXIMUM VALUES OF *N.A.* EVALUATED FROM FIG. 1 AND 2 FOR COMMONLY USED OBJECTIVE FOCAL LENGTHS

1	2	3	4		5	6	
Equivalent focal length of commonly used objectives (mm)	Primary magnification at 160 mm tube-length	Minimum *N.A.* obtained from Fig. 1	Limits of primary magnification for minimum *N.A.*		Maximum *N.A.* obtained from Fig. 2	Limits of primary magnification for maximum *N.A.*	
			Lower $33\times N.A.$	Upper $100\times N.A.$		Lower $33\times N.A.$	Upper $100\times N.A.$
64	2·5X	0·055	1·8X	5·5X	0·06	2·0X	6X
32	5X	0·10	3·3X	10X	0·18	5·9X	18X
16	10X	0·25	8·2X	25X	0·37	12·2X	37X
8	20X	0·44	14·5X	44X	0·60	19·8X	60X
4	40X	0·68	22·4X	68X	0·90	29·7X	90X
2	80X	1·00	33·0X	100X	1·40	46·2X	140X

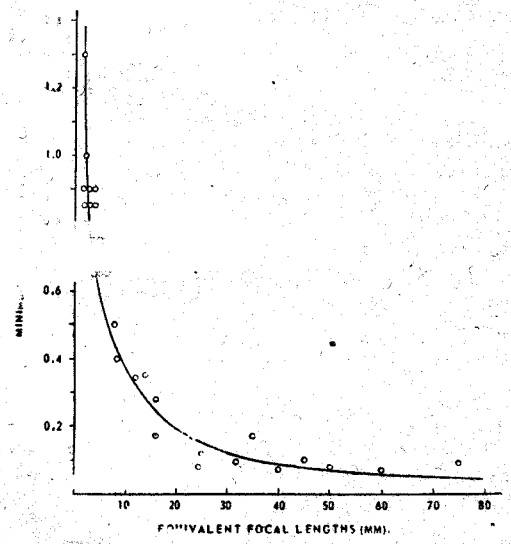

Fig. 1—Graph showing minimum values of *N.A.* plotted against equivalent focal lengths of objectives.

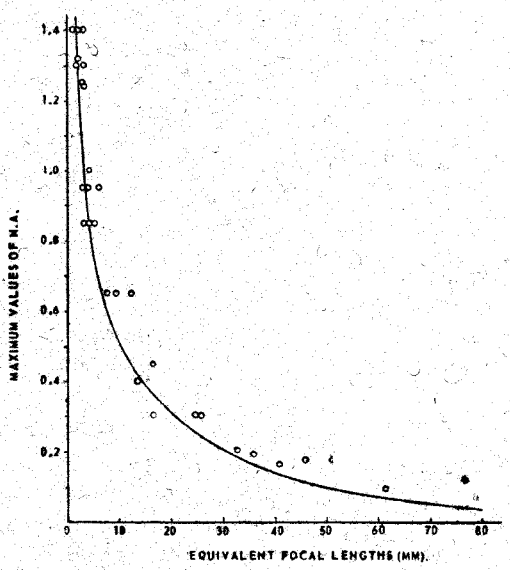

Fig. 2—Graph showing maximum values of *N.A.* plotted against equivalent focal lengths of objectives.

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