Photomicrography

A COMPREHENSIVE TREATISE

VOLUME I

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John Wiley & Sons, Inc.

NEW YORK | LONDON | SYDNEY | TORONTO

objectives of newer design are purchased independently of a microscope, one should look for the manufacturer's recommendations for the oculars to be used with them. These are not always easy to get because of the common assumption of the companies that only their equipment should be used anyway.

Such special objective-ocular combinations include the following:

Objective	Ocular
Leitz Plano	Periplan and Periplan GF
Zeiss Neofluar	Compensating Plano, KPl
Zeiss Planachromat	Komplan
Zeiss Planapochromat	Komplan

Leitz particularly states the point that "use of the negative oculars for photography (Homals and Ampliplans) with plano objectives would be wrong."

Distance of Microscope Image. Now the microscope designer *could* design the ocular and the positions of its lenses so that the final image of the microscope would lie at any desired distance from the eyelens. In practice, except for some special types, **the optics of microscopes have been designed as if they were only to be used visually**, that is, **with parallel beams** emerging from the exit pupil (eyepoint) of the microscope **to form a divergent cone of light. Each beam** (having the diameter of the eyepoint at that location) **comes from one point in the object**, and the plane on which these individual beams of the bundle focus determines the location of the image. Therefore this statement concerning parallel beams is equivalent to saying that the **image is at infinity**. Note that this is entirely independent of the statement that the magnification of the microscope has its rated value at a projection distance (bellows length) of 10 in. or 250 mm; this is merely an accepted convention (see Chapter 1).

In order to focus to a real image at some shorter distance from the microscope, the eyelens of the ocular could be withdrawn a bit until the new projection distance is obtained. This would leave the objective, and also its primary image, undisturbed and as the lens designer intended. **If the microscope is refocused as a whole** (*fixed mechanical tube length*) to refocus the final image, **the objective will no longer be working at its prescribed condition**. The tolerance for this common practice is discussed in this chapter. **Any change in the distance of the ocular that represents a different tube length will not only degrade the image but also change the magnification.** The use of an incorrect tube length can be considered as introducing only spherical aberration in a well-centered system. **The first noticeable effect is a lowering of the contrast of the image;** its definition *might* even improve slightly. However, more spherical aberration will degrade definition and can make it very poor. The most sensitive test, and one that will identify pure spherical aberration, is the "star test," made with a pinhole in an opaque slide (qv). It will also indicate the way to change the tube length to reduce the aberration. **A tube length that is too short introduces undercorrection, a characteristic of a simple lens, whereas a tube length that is too long introduces overcorrection.**

There is an optimum tube length equivalent to the mechanical tube length of 160 or 170 mm, at which the optical characteristics of the object space are assumed to be correct. In practice there is some tolerance which can be expressed as a length on each side of the optimum position ($\pm\Delta L$) within which the observer's eye cannot detect the degradation from the optimum. This **tolerance to change of tube length is affected only by the numerical aperture*** of the objective, in good objectives. Values for this tolerance, as determined by the star test and for dry objectives with the correct cover slip, are given in Figure 2-2, line A. Line B shows the tolerances allowed by immersion objectives of various apertures, **with or without a cover slip.** When the acceptability of an image of a specimen rather than the star test is the criterion, the tolerance may become about eight times greater. In a photomicrograph to be made with a 4 mm, 0.95 NA objective this allowable variation from the strict optimum would be about ± 8 mm. Because the tolerance graph is linear on logarithmic paper, the optical demand for exactly the correct tube length diminishes rather rapidly with decrease in objective aperture, that is, for objectives used at lower magnifications.

When classifying particles according to size by means of a reticle in the eyepiece, it is common practice to alter the tube length until the units of the reticle are equal to exact integral numbers. This is usually wise, but the allowable tolerance, as given in Figure 2-2, must be kept in mind.

The optimum tube length of a commercial objective may not be exactly the standard 160 or 170 mm with a standard cover slip (or without one, if so corrected). This is largely because the separation of some lens components affects the value of the optimum tube length so powerfully that the elements would have to be adjusted within microns of the specified separation. Therefore, **when optimum tube length is very important,** as it may well be at high magnifications and high objective apertures, the optimum tube length **should be determined for the particular**

*See Chapter 1 for a definition of NA. It is discussed in more detail later in this chapter. In this and the next few pages the NA value referred to is that engraved on all objectives.

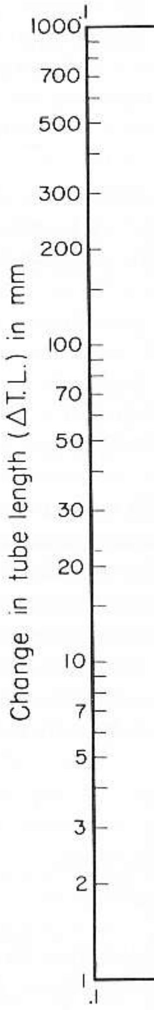

Figure 2

objective and, if
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defined structur
will do. The ad
contrast with no

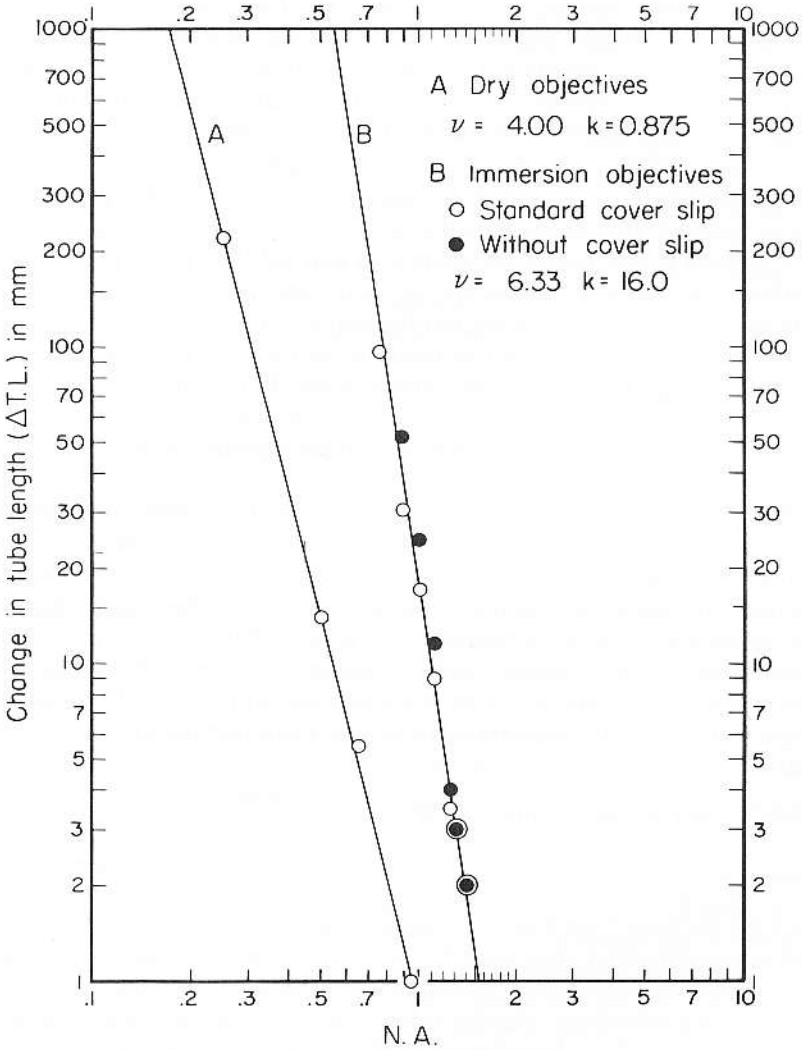

Figure 2-2 Tolerance to tube length change versus objective N.A.

objective and, if possible, for the particular specimen and its cover glass. Although this is most sensitively done with the star test, any fine, well-defined structure will do; very fine particles, including dirt in the field, will do. The adjustment, in this case, is for maximum sharpness and contrast with no loss in resolution.