Box 7.5 Depth of Field and Depth of Focus

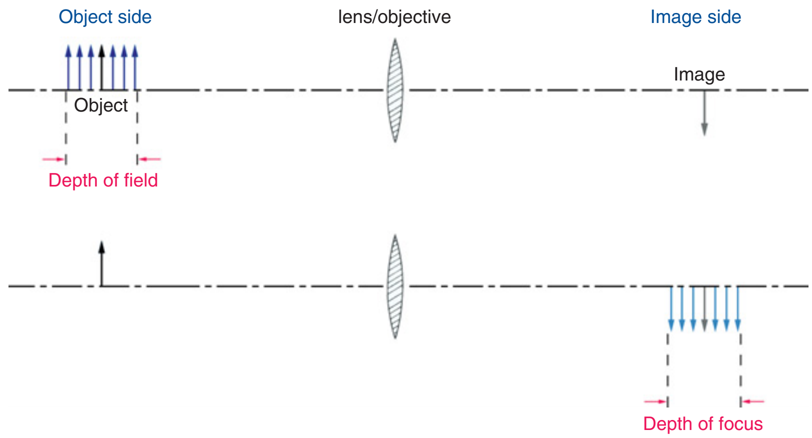
There is wide discrepancy in the literature in the formulae cited for calculating depth of field, partly because different assumptions are made regarding the limits to depth of field, the setting accuracy of the microscope, physiological and psychophysiological factors and the minimum angle of visual resolution.

The simplest formula for depth of field is:

$$\text{DoF} = \lambda \cdot n/2(\text{NA}_{obj})^2,$$

where λ is the wavelength of illumination, n is the refractive index of the immersion medium and NA_{obj} is the objective numerical aperture. The proof is given in Pluta (1988) pages 351–352.

The eye, however, can accommodate, and therefore *visual* depth of field is greater than that recorded photographically or by an electronic detector. From work done by Max Berek, chief designer for Leitz in 1927, an extra term must be added, which is $(n.250\,000.\omega/\text{Mag}_{TOTAL}.\text{NA}_{obj})$,



where 250 000 is the reference viewing distance in micrometres; ω = the tangent of the limit of visual acuity (usually taken to be 0.0003) and Mag_{TOTAL} is the overall magnification.

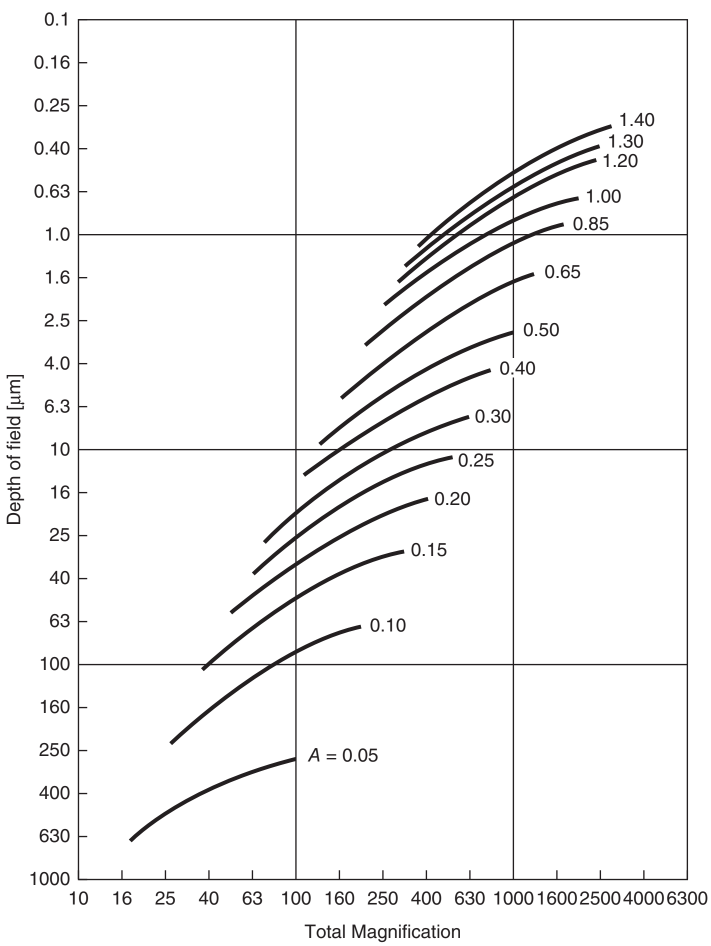
Therefore, for visual observation the total DoF = $\lambda.n/(2\text{NA}_{obj})^2 + n.75/(\text{Mag}_{TOTAL}.\text{NA}_{obj})$

The figure shows values for visual depth of field taken from Figure 3.44 in Pluta (1988) according to overall magnification for objectives of different numerical aperture (A) which agree broadly with the expression above.

These values for the depth of field apply to incoherently illuminated (i.e. emitting) point sources (i.e. $\text{NA}_{cond} \approx \text{NA}_{obj}$); the depth of field increases by up to a factor of 2 as the coherence of illumination increases (i.e. as $\text{NA}_{cond} \to 0$). The diffraction-limited 'wave-optical' depth of field term $(\lambda.n/(\text{NA}_{obj})^2)$ shrinks inversely with the square of the numerical aperture. The result is that axial resolution (see values in Table 7.5) and the thickness of optical sections in the image are affected by the numerical aperture much more than is the lateral resolution of the microscope.

The depth of focus, on the image side, is given by:

$$\left[\lambda n/\text{NA}^2_{obj}\right] \times \text{Mag}^2_{TOTAL}$$



Source: Pluta 1988.

References

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