

Depth of Field and depth of Focus Explained

What is the difference between 'depth of field' and 'depth of focus'? - I have seen both terms used in textbooks

This is a good question, which prompts an answer that encompasses more than simply terminology. In the past there has been some confusion about these terms, and they have been used rather indiscriminately. Recently, usage has become more consistent, and when we published the *RMS Dictionary of light Microscopy* in 1989, we felt able to make an unambiguous distinction in our somewhat ponderous definitions:

- Depth of field is the axial depth of the space on both sides of the **object plane** within which the object can be moved without detectable loss of sharpness in the image, and within which features of the object appear acceptably sharp in the image while the position of the image plane is maintained.
- Depth of focus is the axial depth of the space on both sides of the **image plane** within which the image appears acceptably sharp while the positions of the object plane and of the objective are maintained.

The essential distinction between the terms is clear: depth of **field** refers to **object space** and depth of **focus** to **image space**. A possibly useful mnemonic is that the *field* of view is that part of the *object* that is being examined, and the *focus* is the point at which parallel rays converge *after passing through a lens*.

These definitions contain two rather imprecise terms: detectable loss of sharpness, and acceptably sharp; clearly it all depends on what can be detected and what is considered acceptable. Several equations for calculating depths of field appear in the literature, but a mathematical discussion is not appropriate here. In fact, the values of depth of field and focus depend on a complex of factors, consideration of which involves both geometrical and wave optics, as well as the accommodation of the eye in the case of visual rather than recorded images. Two important practical points are:

- a. High-magnification objectives (because of their large apertures) have extremely limited depth of field, yet have relatively large depth of *focus*, and
- b. Low-magnification objectives (usually of small aperture) have considerable depth of field, but extremely shallow depth of *focus*.

The practical implications of both of these points can easily be demonstrated.

Concerning *depth of field*, it is hardly necessary to remind readers that the fine focus adjustment must be set extremely delicately and precisely for a high-magnification (and large aperture) objective. For a low-magnification (small aperture) objective, a few turns of the knob in either direction may seem to make no noticeable difference to the image.

The effects on *depth of focus* are less well known, and are well worth exploring. Set up a microscope carefully using the 40x objective, and focus precisely. While observing through one eyepiece, withdraw this eyepiece from the tube by about 10mm and note the effect on the sharpness of the image. Repeat the

procedure with a low power objective, as low as 4 if available. It will be seen that in the case of the low-power lens, the image becomes considerably more defocused for the same movement of the eyepiece: in other words the depth of focus is much shallower.

The restricted depth of focus of low-magnification objectives provides the best criterion for correct setting of microscope eyepiece tubes. A set of objectives, or a zoom- or step-change stereomicroscope, will maintain focus throughout the magnification range *only* when operated at the correct tubelength. Users frequently blame the microscope when it fails to maintain focus throughout its range, not understanding that a simple user adjustment is required. It is a fundamental and crucial procedure, but one that appears to be rarely taught and almost never learned. Suitable procedures are as follows:

For the **stereomicroscope**:

1. Observing a fine-detailed specimen, raise magnification to maximum using the zoom or step changer.
2. Focus precisely using the microscope's main focus control (which usually moves the whole optical system up or down).
3. Lower magnification to minimum but **do not** readjust microscope focus control.
4. Focus image for each eye by using the individual adjustment for each eyepiece.
5. Repeat steps 1. to 4., and the microscope should then maintain focus throughout its magnification range.

For the **conventional microscope** the procedure is similar:

1. Set microscope up carefully using a fine-detailed specimen, and change to the highest dry objective.
2. Set interocular distance on binocular head (if fitted) for comfortable use.
3. Focus precisely using the fine focus adjustment.
4. Change to lowest magnification objective but **do not** readjust microscope focus controls.
5. Focus image for each eye by using the individual adjustment for each eyepiece.
6. Repeat steps 1. to 5., and the microscope should then maintain focus throughout its magnification range.

Where a microscope of either type is fitted with one or more eyepieces with built-in focusing adjustments for a photographic frame or a graticule, these should ideally be used in eyepiece tubes of adjustable length, and should be set before step 4. is carried out. For correct adjustment, microscopes having eyepiece tubes of fixed length should always be fitted with a pair of eyepieces with focusing adjustments. If the microscope is fitted with a camera tube, a similar, adjustment should be made for its 'eyepiece'.

Considerations of depth of field and depth of focus are particularly important in photomicrography where three dimensional objects are imaged on to two-dimensional film. It is an unfortunate fact of optics that high resolution and large depth of field are incompatible, being dependent in opposite ways on numerical aperture. As is pointed out in Roy Freere's article in this issue (Freere, 1996), it is important to avoid restricting depth of field by using a system of unnecessarily high resolving power, which will provide detail in the image finer than the viewing system (e.g. eye or television camera) or recording medium (e.g. film) can handle.

I have an expensive photomicrographic camera system on my microscope, but my low-magnification micrographs are often out of focus; I have no problem with higher-powered ones. Why is this?

I have decided to answer this frequently asked question here, since in part it shares an explanation with the previous one: it is principally due to the shallow depth of focus of low-magnification objectives.

When a normal healthy eye is observing a microscope image it will *accommodate* - i.e. adjust the strength of its lens as it does when it focuses on objects at different distances. This enables the eye to see clearly features which are imaged slightly above or below the normal primary image plane. Combined with small movements of the fine-focus control, this permits three-dimensional or unflat objects to be studied without discomfort. Since a photograph will record the image from only *one* plane, it can be expected to contain slightly less detail than was seen by the eye.

However, it is another result of accommodation that is relevant to the question asked. Quite simply, the problem is that the microscope user is adjusting the microscope so that the image falls in the wrong plane, so that it will be out of focus on the film, but this is not apparent, because the observer's eye accommodates to-render the image clearly. The same happens whichever objective is in use but, as explained and demonstrated in the reply above, the out-of-focus effect is much more noticeable with the low-magnification objective because of its shallow depth of focus. It should be noted here that one of the very few advantages of increasing years is that one becomes better able to focus low-power micrographs as presbyopia removes the power of accommodation.

So that is the explanation; what can we do about it? There are several possibilities. First and best, if your camera system offers a focusing magnifier designed to fit between the viewing lens and the eye, buy one and use it carefully. Even a relatively modest magnification, up to about 4x, will help considerably. First focus carefully through the magnifier on to the cross-lines which are usually in the centre of the frame, doing this with no specimen on the microscope to avoid being distracted by an image lying, possibly in the wrong plane. Then replace the specimen and adjust the microscope focus so that the image of the specimen falls in precisely the same plane as the graticule. If you are in any doubt about this, move your head very slightly from side to side; if their planes are not coincident the image will be seen to move against the graticule, due to parallax.

If you do not have a focusing magnifier with your camera system, appropriate magnification can be provided by a monocular, a small telescope, or one side of a pair of binoculars, focused on infinity and used between the viewing lens and the eye. It may also be possible to make the similar use of the telescope often supplied with the microscope for setting up phase contrast and observing the back focal plane of the objective. Some of these telescopes have sufficient adjustment to enable them become short enough to focus on infinity; they provide another inversion of the image, but microscopists should be familiar with that! The problems of using a conventional SLR camera for photomicrography will be dealt with in a future issue.

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Reference

Freere, Roy (1996) Stereomicroscopes, macroscopes and low magnification imaging: a review of the advantages and limitations of these and similar devices. *Proc. Royal Microsc. Soc.* Vol. 31/pt.1 pp 9-15.