

# Fundamental Knowledge

## Part 2 Optical Terminology

In part one (I&M Volume 9 issue 4) we looked at the physical principals of light and its interaction with optical surfaces. We considered such topics as wavelength, frequency and energy as well absorption, scatter, refraction, reflection, interference, polarisation and diffraction. Here, in part 2, we take a closer look at how these underlying principles manifest in the capabilities and limitations of microscope optical systems.

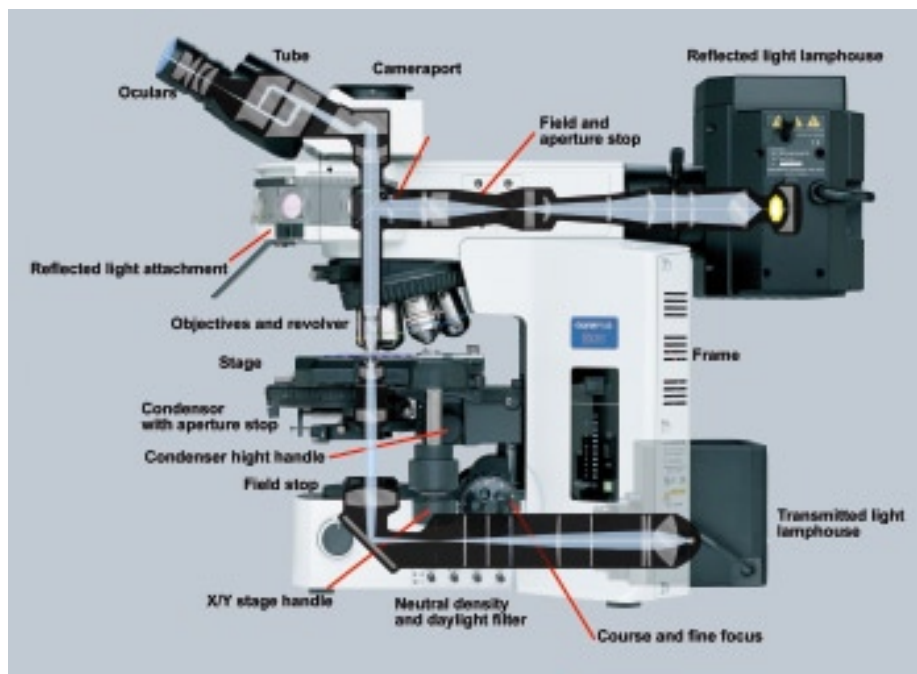


Figure 1

There are a number of figures quoted in relation to the optical components of a microscope, such as the objective, eyepiece, condenser and field lenses. Understanding what these figures mean will help you to make sense of microscopy and maximise the use of your microscope. Firstly though, it is essential to understand the basic structure of modern light microscopes. An object is placed

on a stage and illuminated either from above or below. Light from the object is captured by the objective lens which projects a magnified image through the tube lens to the eyepiece diaphragm where it is focused at the so called intermediate image plane. The eyepiece(s) then further magnify this image and project it onto the user's retina (see figure 1 for a schematic overview of typical upright microscope from the Olympus BX2 range).

### Field Number (F.N.) and Practical Field of View

The diameter of the field of view in an optical microscope is expressed by the field number (F.N.) This is the diameter of the viewable field in millimetres measured at the intermediate image plane. In most cases, this is determined by the diameter of the opening of the eyepiece field diaphragm. The field size in the specimen plane (the diameter of the part of the sample in view) is known as the field of view and is defined as the field number divided by the magnification of the objective as follows:

$$\text{F.O.V.} = \frac{\text{Eyepiece F.N.}}{\text{Objective lens}}$$

### Working Distance (W.D.) and Parfocal Distance

To maximise the amount of the light captured by objective lenses at the front lens element (also see Numerical Aperture), they are designed with small free working distances. This is the distance from the front lens element to the specimen surface when it is in sharp focus.

For applications such as chemical and metallurgical microscopy, where the objective front lens must be protected against environmental hazards (e.g. heat, caustic vapours, and volatile chemicals) by a thick coverslip, longer free working distances are often essential. Specialised objectives have been designed with 2–3 times the WD for such purposes, despite the subsequent difficulties in achieving the required large numerical apertures and suitable corrections for optical aberrations.

Where WD varies for each objective, the parfocal distance for the majority of objectives conforms to the convention set by the Royal Microscopical Society (RMS) of 45.0 mm. This is the distance between the objective lens mounting plane and the specimen. Olympus UIS2/UIS objectives all follow this convention. This means that with an image focused prop-

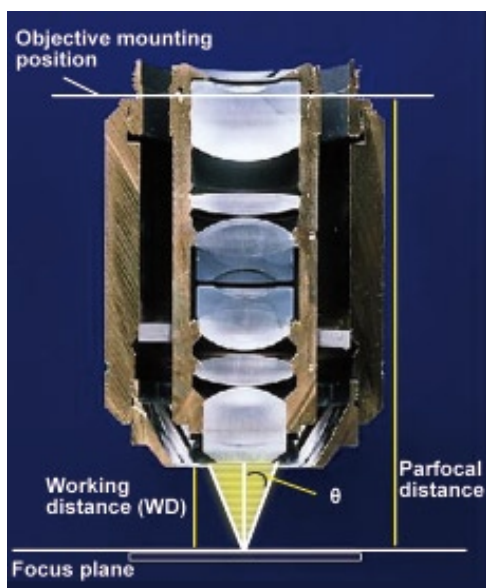


Figure 2

Figure 3:  
A: Imaging Device size

Camera Format	Diagonal	Horizontal	Vertical
1/3"	6.0 mm	4.8 mm	3.6 mm
1/2"	8.0 mm	6.4 mm	4.8 mm
2/3"	11.0 mm	8.8 mm	6.6 mm

B: Imaging device size and monitor magnifications

Camera Format	Monitor Size (Diagonal)				
	9"	12"	14"	21"	27"
1/3"	38.1x	50.8x	59.2x	84.6x	114.1x
1/2"	28.6x	38.1x	44.5x	63.5x	85.7x
2/3"	20.8x	27.7x	32.3x	46.2x	62.3x

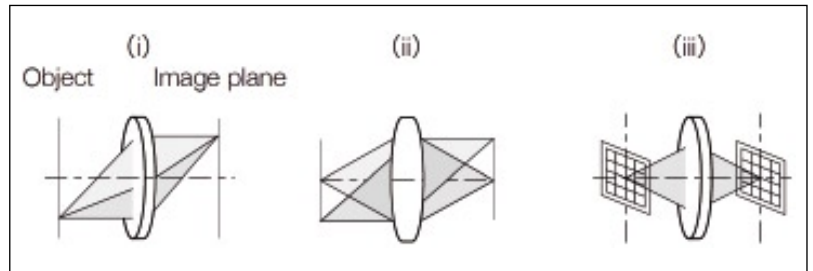


Figure 4

erly, changing between objectives should not require major refocusing. Figure 2 shows a cut-away of a modern multi-element objective lens and details the working distance and parfocal distance.

**Optical Lengths and Magnification of Objective**

Early microscopes utilised finite tube lens lengths, where objectives were designed to focus (as well as magnify) the image at the intermediate image plane. Modern microscopes tend to use infinity corrected optics, where the objectives project the image to infinity rather than focusing it. This is advantageous for many reasons e.g. intermediate components such as polarization prisms and beam-splitters can be placed in the light path without the need for additional corrective optics. The length of the tube lens is defined as the distance from the nosepiece opening (where the objective is mounted) to the top edge of the observation tubes where the eyepieces are inserted. Olympus microscopes utilise infinity corrected optical systems with a tube lens length of 180 mm. The magnification power of the objective lens is the product of the focal length of the tube lens divided by the focal length of the objective:

$$M_{(ob)} = \frac{\text{Objective lens magnification}}{f}$$

$M_{(ob)}$ : Objective lens magnification  
f: Objective lens's focal length

**Calculating the Total Magnification**

With a number of optical elements contributing to the overall image magnification, it is important to remember that your final image is not just magnified by the objective.

Through eyepieces (binocular observation)

$$M_{(bino)} = M_{(ob)} \times M_{(oc)}$$

$M_{(bino)}$ : Total magnification for binocular observation

$M_{(ob)}$ : Objective lens magnification

$M_{(oc)}$ : Eyepiece magnification

Video monitor observation

$$M_{(video\ monitor)} = M_{(ob)} \times M_{(video\ camera\ adapter)} \times \text{Monitor Magnification}^*$$

$M_{(video\ monitor)}$ : Total magnification on the video monitor

$M_{(ob)}$ : Objective lens magnification

$M_{(video\ camera\ adapter)}$ : Projected magnification for video camera adapter including photo eyepiece

\* Refer to Figure 3b for „Monitor magnification“  
Using video monitors to view samples also changes the practical field of view calculation as follows:

$$\text{Practical field of view for video} = \frac{\text{Imaging device size}^*}{M_{(ob)} \times M_{(video\ camera\ adapter)}}$$

$M_{(ob)}$ : Objective lens magnification

$M_{(video\ camera\ adapter)}$ : Projected magnification for video camera adapter including photo eyepiece (refer to Figure 1 for projected magnifications)

\* Refer to Figure 3a for imaging device size

**Example**

What is total magnification for a video monitor when a 50x objective lens, a 0.5xC video camera adapter and a 2/3" video camera are used?

Total magnification on the video monitor:

$$M_{(ob)} = 50 \times$$

$$M_{(video\ camera\ adapter)} = 0.5 \times$$

monitor magnification = 46.2 × (from Figure 3)

$$M_{(video\ monitor)} = 50 \times 0.5 \times 46.2 = 1155 \times$$

*Practical field of view for video observation (horizontal side):*

$$M_{(ob)} = 50 \times$$

$$M_{(video\ camera\ adapter)} = 0.5 \times$$

horizontal side of 2/3" imaging device = 8.8 mm (from Figure 2)

$$\text{Practical field of view for video} = \frac{8.8}{(50 \times 0.5)} = 352 \mu\text{m}$$

**Numerical Aperture, Brightness and Resolution**

The numerical aperture of a microscope objective is a measure of its ability to gather light (brightness) and resolve fine specimen detail (resolution) at a fixed object distance. The N.A. is determined by the following formula:

$$N.A. = n \times \sin \theta$$

n = Refraction rate of the medium between the specimen and objective lenses e.g. for: Air n=1; water n=1.33; glycerin n=1.47; oil n=1.515

θ: Is an angle that represents one-half of the angular aperture (maximum cone of light that can enter the lens).

**Brightness**

The visual field brightness (B) of the microscope is determined by the following formula in relation to the objective lens magnification ( $M_{(ob)}$ ). Therefore brightness will increase as the N.A. increases and/or as the objective magnification decreases.

$$B \propto \frac{N.A.^2}{M^2}$$

**Resolving Power**

The resolving power of an objective lens is measured by its ability to differentiate

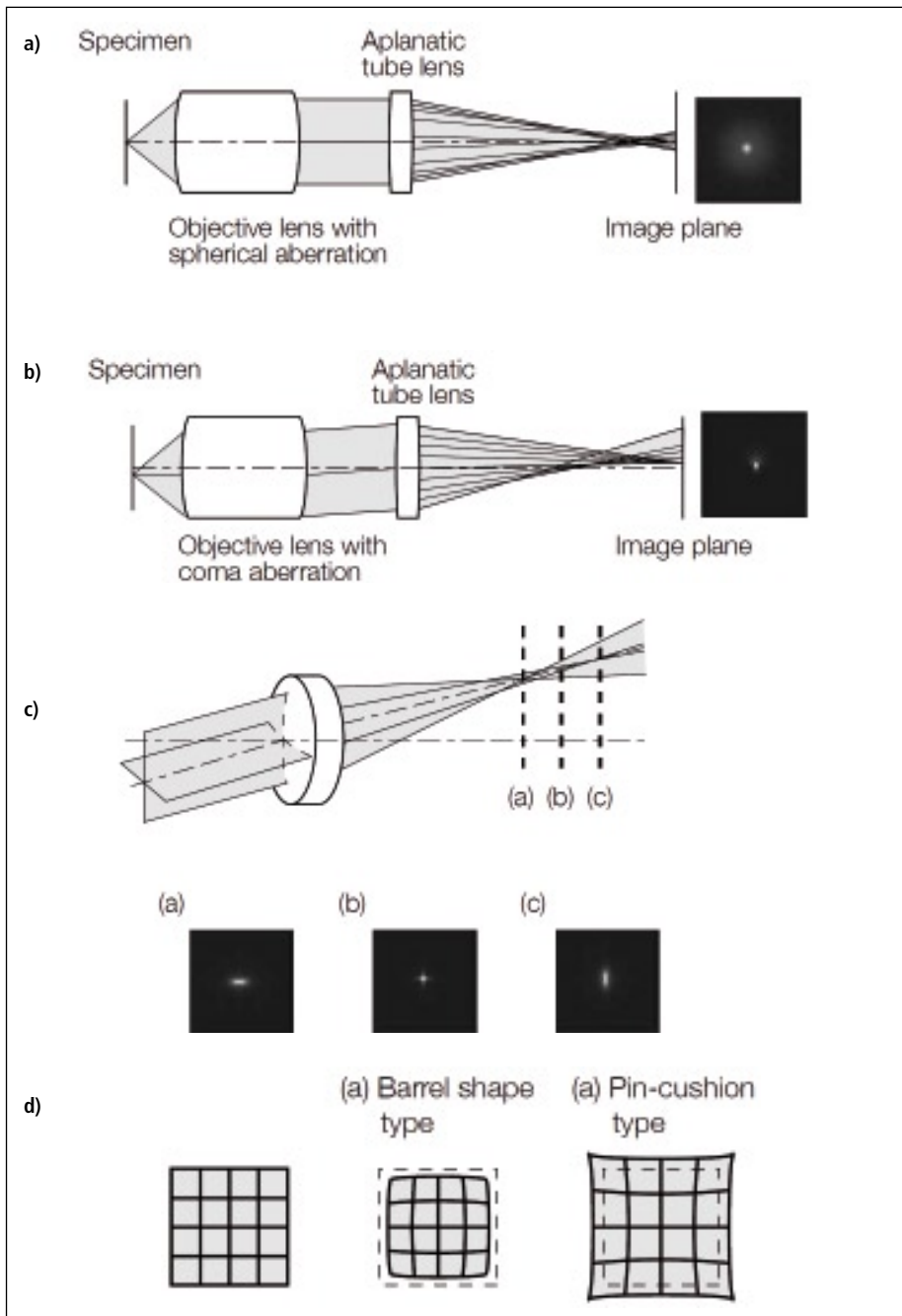


Figure 5

two lines or points in an object. As resolving power increases, the smaller the distance between the two lines or points to be distinguished can be. The more light that is collected by the objective lens, the better the resolution and therefore as N.A. increases, so does the resolving power.

**Resolving power formula**

The following formula is generally used for determining resolution.

$$\epsilon = 0.61 \times \frac{\lambda}{N.A.} \text{ Rayleigh formula}$$

$\lambda$ : Wavelength or radiation in use ( $\lambda=0.55 \mu\text{m}$  is used for visible light)

N.A.: Objective lens N.A.

Example

100 × MPlan Fluorite objective with an

N.A. = 0.90.

$\lambda = 0.55 \mu\text{m}$

**Depth of Field of Microscope**

$$e = 0.61 \times \frac{0.55}{0.90} = \frac{0.3355}{0.90} = 0.37 \mu\text{m}$$

The depth of field (also named focal depth) of a microscope is the depth of the specimen layer which is in sharp focus at the same time. This figure is not straight forward to calculate since each person's eyes are different in their focus adjust-

ment capabilities. As a result each person's perception of the focal depth varies. At present, the Berek formula is generally used, as it provides focal depth values that more closely match actual measured values than other similar calculations.

**Depth of field formula**

Visual observation (Berek formula)

$$\pm \text{D.O.F.} = \frac{\omega \times 250000}{N.A. \times M} + \frac{\lambda}{2(N.A.)^2} (\mu\text{m})$$

D.O.F.: Depth Of Field

$\omega$ : Resolving power of eyes 0.0014 (when optical angle is 0.5 degrees)

M: Total magnification (objective lens magnification x eyepiece magnification)

$$\Rightarrow \pm \text{D.O.F.} = \frac{350}{N.A. \times M} + \frac{0.275}{(N.A.)^2} (\lambda = 0.55 \mu\text{m})$$

This indicates that the depth of field becomes smaller as the numerical aperture becomes larger.

**For example**

With a 100 × MPlan Fluorite (N.A.=0.90) objective lens, and a 10× eyepiece lens :

$$\Rightarrow \pm \text{D.O.F.} = \frac{350}{0.90 \times 1000} + \frac{0.275}{0.81} (\lambda = 0.55 \mu\text{m})$$

**Aberrations**

A quick and easy way of defining an aberration is: a difference between an 'ideal' image and the actual image that is processed through an optical system.

Requirements for Ideal Image Formation

Figure 4 shows the three requirements that must be satisfied to form an image with no aberration - an ideal image.

- (i) All the light rays coming from a single point and passing through an image formation optical system, converge on a single point.
- (ii) Image points, which correspond to object points on the same plane perpendicular to the optical axis, are present on the image plane.
- (iii) The planar shape of an object and the planar shape of an image that are on the same plane perpendicular to the optical axis have a similarity relation.

In an actual optical system, however, it is very difficult to strictly meet the requirements for ideal image formation and this

causes “aberrations” that interfere with image forming performance.

#### Classification of aberrations

A range of aberrations interfere with image forming performance and are summarised in the table below. They fall into three groups – Seidel’s aberrations, Chromatic aberrations and Wavefront aberrations (not covered here).

#### Seidel’s aberrations

In a seminal research paper from 1857, the eminent German mathematician, Philipp Ludwig von Seidel, defined five basic types of aberration for monochromatic light based on three different properties:

- Expansion of a point image
- Curvature of image plane
- Deformation.

The five Seidel aberrations are:

#### Spherical aberration

When light rays coming out of an axial object point enter a lens, the light rays with a larger numerical aperture (N.A.) are subjected to stronger refraction power and cross the optical axis in positions with larger differences from the ideal image formation position (see figure 5a). As a result spherical aberrations are proportional to the N.A.<sup>3</sup>.

It is said that objective lenses with larger N.A. have better resolution but worsen spherical aberration. Olympus’s advanced design and manufacturing techniques have minimised such aberrations and provide excellent optical performance even with large numerical aperture.

#### Coma aberration

Even though spherical aberration can be reduced to an absolute minimum, there are cases where light rays coming out of an off-axis object point are not condensed to a single point on the image plane but generate asymmetric blur just like a comet leaving traces (see figure 5b). This is called coma aberration and can also be compensated for.

#### Astigmatism

Even if a lens is compensated for spherical and coma aberrations, there are cases where an image of an off-axis object point is not focused to a single point but separated to a concentric line image and a radial line image. This is called astigmatism and will make a point image blur vertically and horizontally, before and after the focus position (see figure 5c).

#### Field curvature

Even when light from every point in the object is brought to a sharp focus, the points at which they are brought into focus might lie on a curved surface instead of a flat plane. When field curvature is present, the image is more displaced as it becomes closer to the periphery of the visual field. Therefore, when the centre of an image is brought into focus, blur occurs in the peripheral areas of the image. To bring the entire image, including the periphery, into clear focus, it is necessary to adequately compensate for this type of aberration.

#### Distortion

When there is no similar relation between a planar shape on an object and a shape on the image plane, this is called “distortion.” When distortion is present, a square image appears in a shape of a barrel or pin-cushion as shown in Figure 5d.

The microscope optical system contains some distortion. When distortion is present, it can bring erroneous results of shape measurements. When a microscope is used for precision measurements, pay close attention to this aberration, for example, by providing it with an aberration compensation function.

#### Chromatic aberrations

Glasses used for optical systems have different refractive indices depending on the wavelength. This causes differences in focal length between wavelengths and generates displacement of image forming position. This phenomenon is called “chromatic aberration,” which is sometimes subdivided into axial displacement on the optical axis, called “axial chromatic aberration” (or lateral chromatic aberration) and displacement on the image plane, called “chromatic aberration of magnitude.”

Many special glass materials are used, e.g., for apochromats and fluorites to eliminate chromatic aberration in a wide range from violet light to red light).

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