Systematic Survey on Optical Microscope Technology in Japan

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Chikara Nagano

■ Abstract

Microscopes are tools for observing infinitesimal objects that are invisible to the naked eye. Invented in the Dutch Republic in the late 16th century, handmade microscopes were used to discover cells and microorganisms in the 17th century, thus unveiling the microscopic world. Manufacturers of microscopes began to appear mostly in England in the 18th century, but microscope performance did not significantly improve until the second half of the 19th century. At the end of the 19th century, the German and Austrian lens manufacturers suddenly became the leading optics companies in the world, building on the foundation laid by Ernst Abbe, who established a theory of image formation and a method of designing lenses, and also drastically improved the performance of objective lenses. This improvement in the performance of microscopes led to further developments in medicine, especially bacteriology, and in addition to major achievements such as the discovery of the pathogens that have continuously plague mankind and ways of dealing with them, microscopy has made substantial contributions to the development of all other fields of science and industrial technologies.

In Japan, there was an increase in the importation of microscopes from the middle of the Meiji era, which were mainly used in the medical and sericulture industries, and this led to the appearance of Japanese who endeavored to make Japan's first microscopes during the period spanning the end of the Meiji and the beginning of the Taisho eras. Although reproducing foreign microscopes posed challenges, especially with regard to the production of high power objective lenses, which is the ultimate sign of high quality, this was overcome with the zeal and spirit of the craftsmen. Subsequent improvements and technological developments improved the quality of Japanese microscopes, paving the road to birth of this industry in Japan. The post-war period saw the emergence of a number of microscope manufacturers. Building on the base of pre-war technological standards, and due to the establishment of the Japan Microscope Manufacturers' Association and joint efforts by industry/government/academia in terms of technological research and standardization, slowly but surely, further improvements were made in terms of the level of functionality and performance. This eventually led to the development of research microscopes, compound microscopes and high-class microscopes for photography, and with improvements in quality control and customer service, Japanese microscopes have come to be recognized throughout the world, with annual exports to Europe and the US increasing year-on-year due to their superiority in terms of cost-performance. This was made possible not only by the passion and tenacity of the researchers and engineers who worked for the manufacturers, and the skill of the manufacturers, but also by thanks to the maturing of the domestic industrial environment in terms of lens design techniques, optical glass quality and electronics technologies, etc., and the support and expectations of personnel engaged in cutting edge research as users. Since the late 1970s, the top two leading microscope manufacturers in Japan have progressively developed new optical systems, and due to significant improvements in performance and usability with the introduction of electronics technologies and the increase in the number of units/accessories, both companies have now come to rank alongside the two German manufacturers as the top manufacturers in the world.

Thus the history of optical microscopes has been a tireless struggle to make visible the invisible. Beginning with the performance increase obtained in objective lenses by the use of geometric optics, a variety of methods of observation were established through the application of physical optics; interference, diffraction, polarization, and fluorescence, etc., which contributed greatly to the progress of science and technology, as well as industrial development. Many specialized types of microscopes exist; inverted microscopes, stereoscopic microscopes, metallurgical (or industrial) microscopes, and so on, and these continue to evolve. There exists a limit, imposed by the wavelength of light, on the resolving power of optical microscopes that rely on visible light, and as the performance of objective lenses had also all but reached its limit, many researchers held the view that further development of optical microscopes could not be expected, especially in light of the development of electron microscopes. However, new forms of optical microscope, such as the laser scanning microscope, emerged in the latter half of the 1980s, and optical microscopes made a comeback as the mainstay of cutting-edge research, such as bio-imaging, in light of their advantages, which include the ability to

dynamically observe living biological specimens. Even more advanced multi-photon excitation laser scanning microscopes and super-resolution microscopes have appeared, and further progress is expected.

In this paper I will report on the history of such optical microscopes and the spread of the technology in Japan, paying particular attention to optical technology for objective lenses. Following the introduction that is Chapter 1, I will then report on the fundamental principles of optics and microscopes in Chapter 2, the history of microscopes from their invention up until the 19th century in Chapter 3, the birth and growth of Japanese microscopes with particular regard to the spread of upright biological microscopes in Chapter 4, the history and development of the various microscopy techniques in Chapter 5, the history and development of the various types of microscope in Chapter 6, the development of optical technology for microscopes with particular regard to the development of objective lenses in Chapter 7, the latest optical microscopes in Chapter 8, and present a conclusion and a discussion in Chapter 9. Mechanical and electronic design and production, which are other important elements of optical microscope technology, have been omitted from this report as they are outside the Author's area of expertise.

The theory and application of the optical microscope has been the base for much Nobel prize-winning research. The awarding of the 2014 Nobel Prize in Chemistry was connected with the development of super- resolution microscopy, and even in Japan the optical microscope has played a part (amidst other important research) in securing the Nobel Prize in Physiology or Medicine. Thus the optical microscope has been a base for combining optical technology, electronics technology, and information technology, etc., to create technology and equipment for visualizing vital phenomena that were previously thought to be impossible to visualize. Researchers have long desired to have the ability to observe the invisible, and the struggle to push the boundaries of optical microscopy will undoubtedly continue. I look forward to seeing Japanese microscope R&D and manufacturing technology continue to lead the world in incorporating new theories, technologies and materials to meet the needs of the biosciences and other fields of research.

■ Profile

Chikara Nagano

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1974: Graduated from Osaka University School of Engineering, Department of Applied Physics
1976: Completed Master's Degree program in Applied Engineering at Osaka University Graduate School of Engineering
Started working for Olympus Optical Co., Ltd. (now Olympus Corporation), working on research and development of microscope optical systems in the optical research and development department
1996: Assigned to Olympus Japan Co., Ltd.
1999: Assigned to Olympus Research & Development Head Office
2005: Appointed as Chief Secretary of Japan Microscope Manufacturers' Association
2016: Senior Researcher, Center of the History of Japanese Industrial Technology, National Museum of Nature and Science
1994-2016: Expert on ISO/TC172/SC5 (microscopes and endoscopes)

■ Contents

1 Introduction

In March 2015, the National Museum of Nature and Science held a planned exhibition, “A Century of Japanese Microscopes – How Made-in-Japan Microscopes Took on the World” in conjunction with the Japan Microscope Manufacturers’ Association (Figure 1.1) [1]. This event was held to commemorate 100 years since the formation of M & Katera, which became the cornerstone of domestic microscope development in Japan. The event also showcased how, in the short space of a century, Japan came to occupy a world-leading position in optical microscopy, a field with a 420-year history.

While preparing for this exhibition, the author came to the strong sense that creating a record of the history of optical microscopes in Japan, with its unique track record of technological developments, would provide an extremely valuable source of information for future researchers from Japan and around the world. As it happened, this coincided with a request from the National Museum of Nature and Science to work on a systematization survey report on optical microscope technology. The author accepted this perfect opportunity.

An increasing number of Japanese researchers have been recipients of the Nobel Prize in Physiology or Medicine in recent years. While this is truly wonderful, it could never have happened without the optical microscope. Bearing this in mind, it is indeed very significant to systematize this technology at this point in time.

This paper is a survey report on optical microscopes that use light for imaging. Other types of microscopes outside the scope of this report include transmission electron microscopes that use electrons (see Vol. 11 2008, A Systematic Survey of the Technical Development of Transmission Electron Microscopes), scanning electron microscopes, scanning probe microscopes that operate on the interaction between the specimen and the probe (such as atomic force microscopes, scanning tunneling microscopes and scanning near-field optical microscopes), X-ray microscopes and ultrasonic microscopes. Optical microscopes also encompass a broad range of technologies, including optics, precision instruments, electronics, software and ultra-precision machining and assembly. While this technology systematization report should likewise encompass all of these technologies, due to the constraints of time and author expertise, it focuses on the development of optical technology and objective lenses in particular. In order to familiarize the reader with the development history of microscopes, a chapter is provided on the fundamental principles of optics and microscopes, with as many photographs and figures as possible. The author trusts readers will appreciate this.

References

1) National Museum of Nature and Science: Planned Exhibition “A Century of Japanese Microscopes” pamphlet, 2015

Fig. 1.1 A Century of Japanese Microscopes Exhibit Poster [1)]

2 Fundamental Principles of Optics and Microscopes [1) 2) 3)]

Understanding this report first requires an explanation of the fundamental principles of optics and microscopes. Please refer to Appendix 1 for a list of the microscope-related international standards (ISO), Japan Industrial Standards (JIS) and Japan Microscope Manufacturers' Association Microscope Industrial Standards (MIS) mentioned in this chapter and throughout the rest of this report.

2.1 Fundamental Principles of Optics

Optical technology comprises geometrical optics, which utilizes the straight-line, reflective and refractive properties of light, and physical optics, which treats light as a wave (light is a type of electromagnetic wave, the same as radio waves and X-rays). The fundamentals of these are explained below.

2.1.1 Light Wavelength and Color

Optical microscopes generally work within the range of visible light plus infrared light, with a longer wavelength, and ultraviolet light, with a shorter wavelength. Figure 2.1 shows the approximate color divisions of visible light. ISO 20473 defines visible light as being within the wavelength range of 380-780nm. ISO 7944 (JISB 7090) defines the reference wavelength of various optical instruments as the mercury e-line (546.07nm: green), although the helium d-line (587.56nm: yellow) is also recognized for some ophthalmic instruments. This standard also defines the recommended spectral lines for each color (Fraunhofer lines). Light travels in a straight line in a homogenous medium. When it reflects off the surface of another medium, the angle of reflection is equal to the angle of incidence. If the surface refracts the light, the ratio between the sine of the angle of incidence and the sine of the angle of reflection is constant (Figure 2.2). Supposing that medium 1 shown in the diagram were air (or strictly, a vacuum), the value is referred to as the refractive index of medium 2. The refractive index changes with the wavelength of light. This is referred to as dispersion. Dispersion varies according to the properties of the optical glass. Optical glass is often defined by its Abbe number, the indicator of the refractive index and dispersion of the material (Figure 2.3).

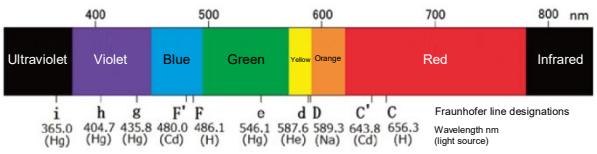


Fig. 2.1 Approximate Classification of Visible Light and Fraunhofer Lines

Figure 2.4 maps out the optical glass produced by Ohara, Japan's leading optical glass manufacturer (vertical axis shows the refractive index, horizontal axis shows the Abbe number). Glass with a higher Abbe number (lower dispersion) is known as crown glass, while glass with a lower Abbe number (higher dispersion) is known as flint glass.

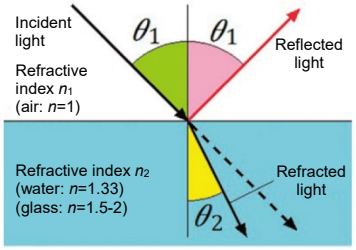


Fig. 2.2 Laws of Reflection and Refraction

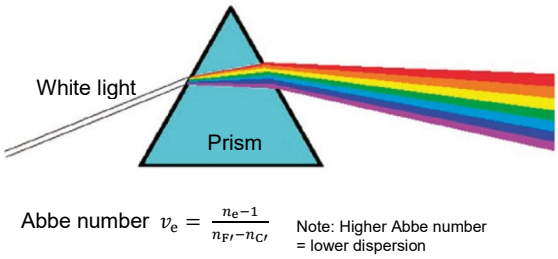


Fig. 2.3 Light Dispersion and Abbe Number

The ratio of dispersion of a third wavelength, e.g. violet (g-line), in relation to the dispersion of blue (F-line) and red (C-line) is referred to as the relative partial dispersion. This is represented by the formula $\theta_{g,F} = (n_g - n_F)/(n_F - n_C)$. Figure 2.5 shows the partial dispersion ratios of optical glass produced by Ohara. Regular optical glass follows the diagonal line in the figure quite closely, while anomalous dispersion glass deviates from the diagonal line. Where this glass is used, it is possible to design for an apochromatic lens (apochromat) instead of a normal achromatic lens (achromat). Special low dispersion glass with an Abbe number over 80 is known as extra-low dispersion (ED) glass. This glass works well for convex lenses.

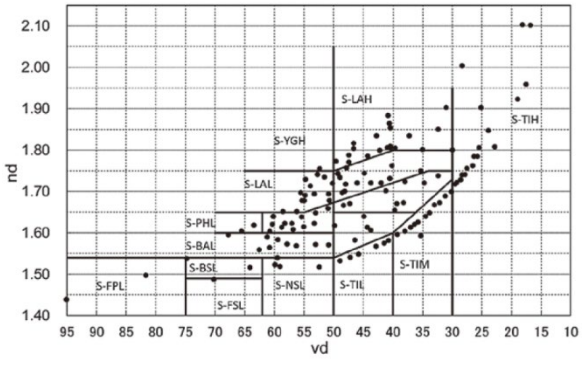


Fig. 2.4 Optical Glass Summary Chart [4)]

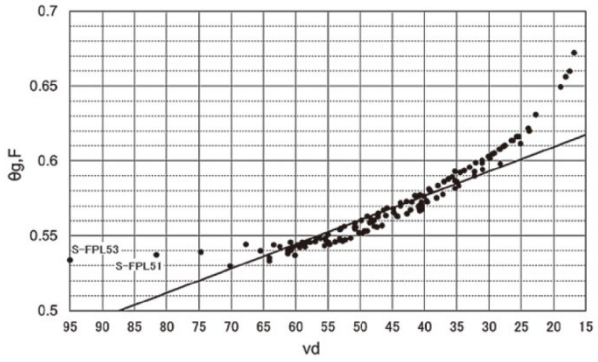


Fig. 2.5 Optical Glass Relative Partial Dispersion Map

(created from cited reference 4)

2.1.2 Lens Aberration

Lens aberration refers to a geometric optical deviation from the ideal image formed in an optical system. This is typically either chromatic aberration, caused by dispersion, or one of the five constituent aberrations published in 1855 by German mathematician P. L. Seidel (1821-1896).

1) Spherical aberration occurs when incident light entering an optical system from a point on the optical axis intersects the optical axis at a different position due to the distance from the point of incidence. For simple convex lenses, the light farther from the optical axis intersects closer to the image point. For simple concave lenses, the light intersects farther from the image point. Accordingly, correcting spherical aberration requires a combination of concave and convex lenses (Figure 2.6 a, b, c). In the figure, the vertical axis shows the numerical aperture (NA) and the horizontal axis shows the point the light intersects the optical axis.
2) Comatic aberration occurs when forming an image of an object point at a distance from the optical axis. The size of the image distorts and blurs into a comet-like shape due to the position of the incident light entering the lens (Figure 2.7).
3) Astigmatism occurs when an off-axis image point produced by light rays from the off-axis image point in the optical system does not focus. The meridional image point (the image formed on the plane containing the principal ray and the optical axis) and the sagittal image point (the image formed on the plane perpendicular to the meridional plane and containing the principal ray) do not correspond (Figure 2.8). The image forms straight lines at the two image points and a blurry elliptical shape at other points.
4) Curvature of field occurs when the image surface of a planar object curves (Figure 2.9). With this aberration, the outer edges do not focus even though the center focuses properly. Generally, in convex lens systems, the further the image surface is from the optical axis, the closer it curves towards the lens.
5) Distortion occurs when magnification varies with the size of the image. Distortion with increased magnification is called positive distortion (also called pincushion distortion), while distortion with decreased magnification is called negative distortion (also called barrel distortion) (Figure 2.10).

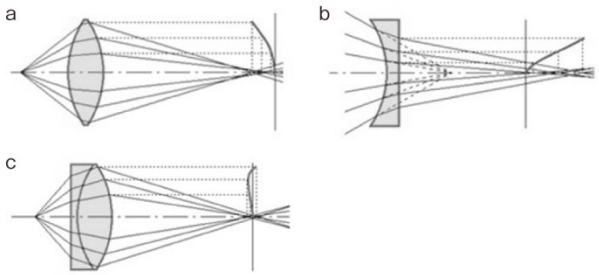


Fig. 2.6 Explanatory Diagram of Spherical Aberration

a Convex lens spherical distortion
b Concave lens spherical distortion
c Correction of spherical distortion using a combination of convex and concave lenses

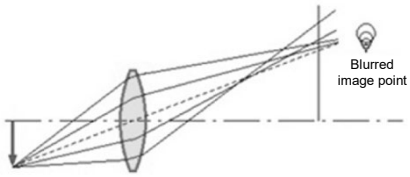


Fig. 2.7 Explanatory Diagram of Convex Lens Comatic Aberration

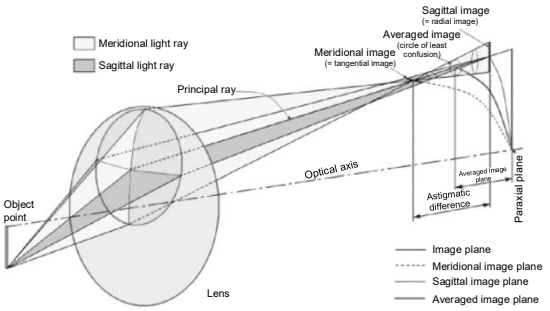


Fig. 2.8 Explanatory Diagram of Astigmatism

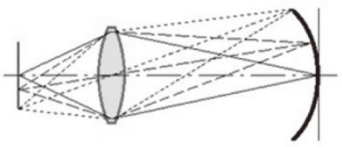


Fig. 2.9 Explanatory Diagram of Curvature of Field

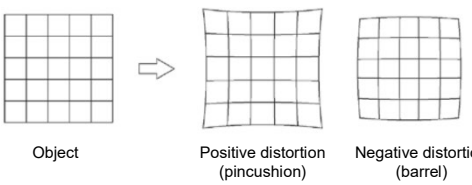


Fig. 2.10 Explanatory Diagram of Distortion

6) Chromatic aberration occurs when the image position and magnification of an image is formed in an optical system vary depending on the light wavelength. The former is referred to as axial or longitudinal chromatic aberration, while the latter is referred to as transverse or lateral chromatic aberration (Figure 2.11). Where aberration occurs due to optical glass dispersion, chromatic aberration can be rectified with a combination of optical glass with different properties (using low dispersion crown glass for convex lenses and using high dispersion flint glass for concave lenses) (Figure 2.12). If achromatism occurs for two colors, e.g. blue (F-line) and red (C-line), achromatism for a third color, e.g. violet (g-line), will remain unrectified. This residual chromatic aberration is called the secondary spectrum. It is rectified using anomalous dispersion glass, mentioned above.

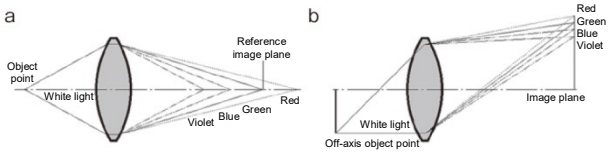


Fig. 2.11 Explanatory Diagram of Chromatic Aberration

a. Axial or longitudinal chromatic aberration
b. Transverse or lateral chromatic aberration

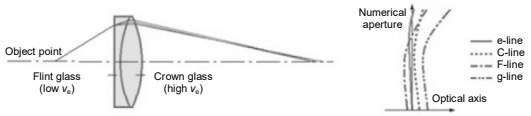


Fig. 2.12 Correction of Chromatic Aberration

The right shows spherical aberration after chromatic correction

2.1.3 Interference, Diffraction and Polarized Light

Since light has the properties of a wave, it is subject to phenomena such as interference, diffraction and polarized light.

(1) Light interference occurs when two or more light waves overlap at the same point and either strengthen or weaken each other. This phenomenon was discovered in 1803 by British scientist T. Young (1773-1829). His experiments confirmed that shining light onto a screen through two parallel slits would produce interference fringes. This provided a significant basis for the light wave theory that was under dispute at the time. Figure 2.13 shows applied examples of light interference. a) shows that when glass is coated with a thin layer of anti-reflection coating, interference causes a reflected light strength of 0 when the peaks and valleys of the light reflected off the surface coincide with those of the light reflected off the glass (the uncoated glass has a reflected light strength of 4-7%). b) shows what is called Newton's rings, concentric interference fringes that appear around the point of contact between two spherical surfaces with different radii of curvature (one surface in the figure is a planar surface). The overlapping reflected light from the two surfaces in contact causes multiple interference fringes. In the lens manufacturing process, the processing quality is often evaluated by overlaying the curved surface of a manufactured lens onto a prototype with guaranteed standard curvature and surface accuracy and assessing the number and shape of the Newton's rings.

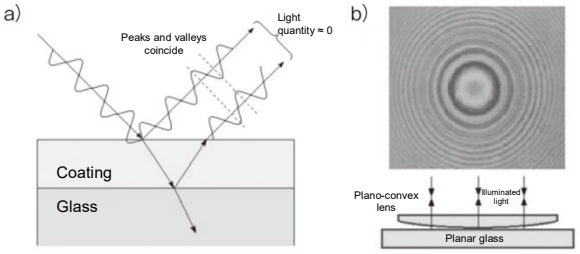


Fig. 2.13 Example Applications of Interference

a) Anti-reflection coating
b) Newton's rings

(2) Light diffraction occurs when light hits an object and, instead of travelling in a straight line, spreads out and even travels into the shadow area of the object. This phenomenon was discovered in 1665 by Italian physicist F. Grimaldi (1618-1663). Later scientists also carried out various studies on diffraction, including French physicist A. Fresnel (1788-1827), German physicist J. von Fraunhofer (1787-1826) and British astronomer G. Airy (1801-1892). Figure 2.14 a) illustrates this phenomenon. Because of diffraction, when light is concentrated through a circular aperture such as a lens, even without aberration the light will not form into a point, but spread out as shown in b). The bright circle at the center of the diffraction pattern is called the Airy disc. The diameter of this disc is given as $1.22\lambda/\mathrm{NA}$ (where λ is the light wavelength and NA is the numerical aperture of the lens. See 2.5).

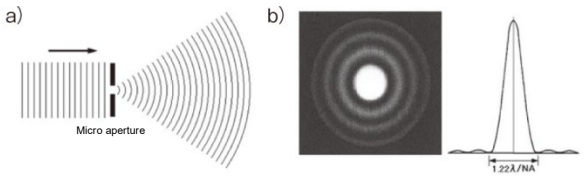


Fig. 2.14 Diffraction Phenomenon

a) Spread of light due to diffraction
b) Diffraction pattern and intensity distribution from circular aperture

(3) Polarized light is light with a regular wavelength (electric vector) oscillation direction. Light without polarized light characteristics is called natural light (Figure 2.15). This phenomenon was discovered in 1808 by French physicist E. Malus (1775-1812), while using calcite, a birefringent substance, to observe light

reflected from a window. Birefringence is a phenomenon in which incident light on a crystal or other substance disperses into ordinary rays that follow the law of refraction and extraordinary rays that do not (Figure 2.16). In 1828, Scottish geologist W. Nicol (1770-1851) pasted together two calcite prisms to form the Nicol prism (Figure 2.17). This and other polarized light apparatus he devised enabled him to turn natural light, which has a random oscillation direction, into linearly polarized light with a regular oscillation direction. The light could be cut by arranging a polarized light apparatus orthogonally to the linearly polarized light from another polarized light apparatus (this is referred to as crossed nicols). The original device is referred to as the polarizer, while the other device is referred to as the analyzer. If a crystal or other substance with optical properties that differ according to direction (anisotropic material) is placed between the two devices, the light in that area can be observed through the analyzer. The Nicol prism used in the device was large, expensive and difficult to use. In 1929, American inventor and physicist E. Land (1909-1991) invented a thin polarizing plate, which he named “polaroid”. Various improvements followed, resulting in an inexpensive and highly efficient polarizing element, used in sunglasses, photographic filters and polarized light microscopes. When there is an anisotropic material between the crossed nicols, retardation occurs between the two rays due to the thickness of the material and the difference in refractive index between ordinary and extraordinary rays. The two rays interfere with each other after passing through the analyzer. The intensity of the interference varies with wavelength and causes changes in color, referred to as interference color. Figure 2.18 shows an interference color chart, showing the relationship between the amount of retardation and the interference color. At a retardation of around 530nm, green is cut out altogether and slight changes in retardation can cause yellow and red to change rapidly to blue. This is called sensitive color.

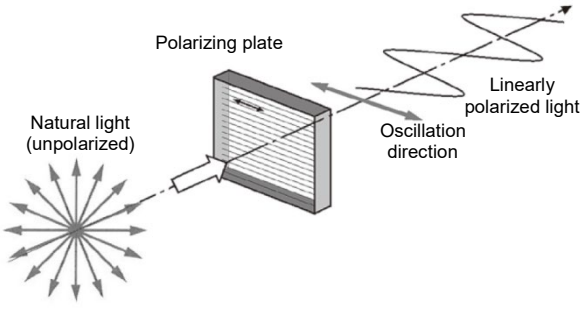


Fig. 2.15 Polarized Light

Fig. 2.16 Birefringence through Calcite [5)]

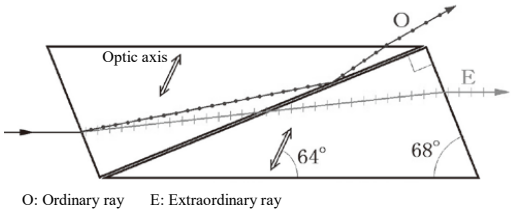


Fig. 2.17 Nicol Prism

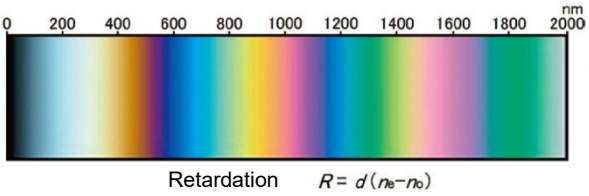


Fig. 2.18 Interference Color Chart

2.2 Images by Lens

There are two main ways to use a lens to produce an image. One method creates a real image on film or image sensor, such as in camera photography. The other method creates a virtual image, such as with a magnifier (or lupe). Figure 2.19 shows a real image being created using a convex lens.

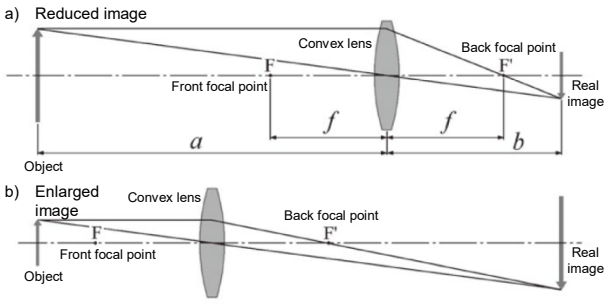


Fig. 2.19 Convex Lens and Real Image [1)]

a) Reduced image b) Enlarged image

a) shows that when the object is far away from the front focal point F, a small inverted image is produced. b) shows that when the object is slightly closer to front focal point F, a larger inverted image is produced. The following formula gives the relationship between the distance from the lens to the object a, the distance to the image b, the lens focal length f and the image magnification M.

$$1/a + 1/b = 1/f \qquad (2.1)$$

$$M = b/a \qquad (2.2)$$

By contrast, Figure 2.20 shows a virtual image being created using a convex lens. While moving the object slightly closer to the front focal point of the lens does not produce an image, placing the eye further back behind the lens reveals an enlarged positive virtual image. In this case, the size of the virtual image varies according to the positional relationship between the object and the front focal point of the lens. The magnifier display magnification M_L refers to the scale of magnification of the virtual image compared to the object when the lens is positioned 250mm from the eye (the reference viewing distance). If the focal length of the lens is f, then M_L is given as follows.

$$M_L = 250/f \qquad (2.3)$$

For some simple microscopes such as Leeuwenhoek microscopes (Figure 3.3), f is reduced by 1mm. While this allows higher magnification, observation becomes more difficult as the distance between the eye and the lens is too short.

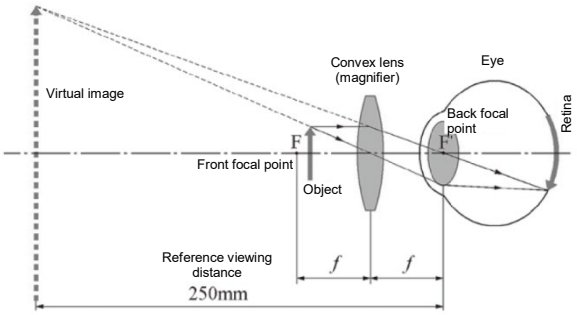


Fig. 2.20 Convex Lens and Virtual Image [1)]

2.3 Enlarging Images by Microscope

Ordinary optical microscopes (compound microscopes) have a system of two convex lenses. One lens is the objective lens, close to the specimen, and the other is the eyepiece or ocular lens, close to the eye. When an object AB is placed just outside the forward focal point Fo of the objective lens, as shown in Figure 2.21, an inverted real image A′B′ (the primary image) is formed, enlarged by the objective lens. When this image is brought just within forward focal point Fe of the eyepiece (by adjusting the focus), a virtual image A″B″ can be seen in the reference viewing distance, enlarged from A′B′.

The total magnification M_T of the microscope is given as:

M_T = objective lens magnification M_o × eyepiece magnification M_e (2.4)

The size of the observed object surface (diameter: mm) is given as:

Microscope field of view = eyepiece field number FN / objective lens magnification M_o (2.5)

Here the field number FN of the eyepiece is a value that determines the field stop diameter (mm). It is usually indicated on the eyepiece.

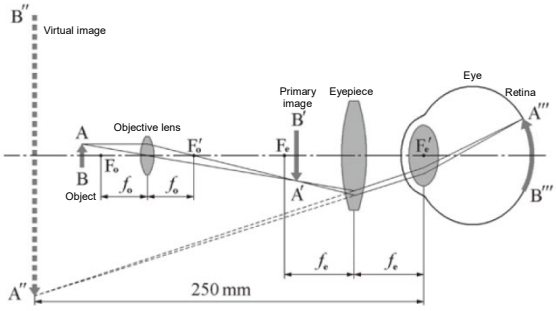


Fig. 2.21 Fundamental Optical System of a Microscope [1)]

2.4 Mechanical Tube Length and Parfocalizing Distance

Many microscopes have several objective lenses of different magnifications mounted in a revolving nosepiece for easy changing of magnification. In such cases, the distance from the locating flange of the objective lens to the locating flange of the eyepiece and to the specimen plate have fixed values to maintain focus when the objective lens is changed. The former is called mechanical tube length, while the latter is called parfocalizing distance. Mechanical tube length is categorized as either finite (e.g. 160mm) or infinite (Figure 2.22). In infinite mechanical tube length systems, the light from the objective lens travels in parallel (that is, an infinite image distance) and the primary image is formed by a tube lens. Even with various optical elements (such as filters, analyzers or mirrors) inserted into or removed from this parallel light section, the image does not offset or deteriorate (Figure 2.23). While metallurgical microscopes have a long history of use, there has been a growing emphasis on system scalability by combining various methods of observation using biological microscopes as well. This has meant that infinite mechanical tube length systems are becoming the norm for high-end microscopes. The magnification $M_{o\infty}$ of the objective lens in an infinite mechanical tube length system is given as:

$$M_{o\infty} = f_t / f_o \qquad (2.6)$$

where f_o is the objective lens focal length and f_t is the tube lens focal length. The tube lens focal length varies between manufacturers (e.g. 180mm for Olympus and 200mm for Nikon; see Table 7.1). Most major Japanese manufacturers use a parfocalizing distance of 45mm or 60mm (ISO 9345-2, JIS B 7132-2) and 95mm for industrial lenses. The distance from the apex of the objective lens to the specimen plate (or above it if a cover glass is used) is referred to as the working distance (*WD*). Generally, the higher the magnification, the smaller the working distance, although some commercially available objective lenses are easier to work with by allowing a longer working distance at high magnification.

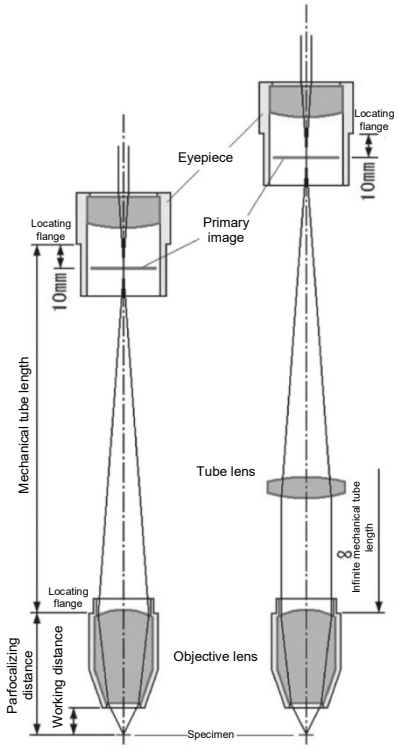


Fig. 2.22 Finite (left) and Infinite (right) Mechanical Tube Length [1)]

Table 2.1 shows the types of screw thread for objectives and related nosepieces. The Royal Microscopical Society (RMS) thread has long been used as the standard objective thread. As a result, objective lenses produced by many manufacturers can be mounted into a microscope revolving nosepiece if they match this standard. However, it is important to be aware that the correct magnification or performance may not be achieved due to differences in parfocalizing distance, tube lens or eyepiece. New standards, such as M25, M27 and M32, have also begun to grow in popularity with recent advances in performance and versatility.

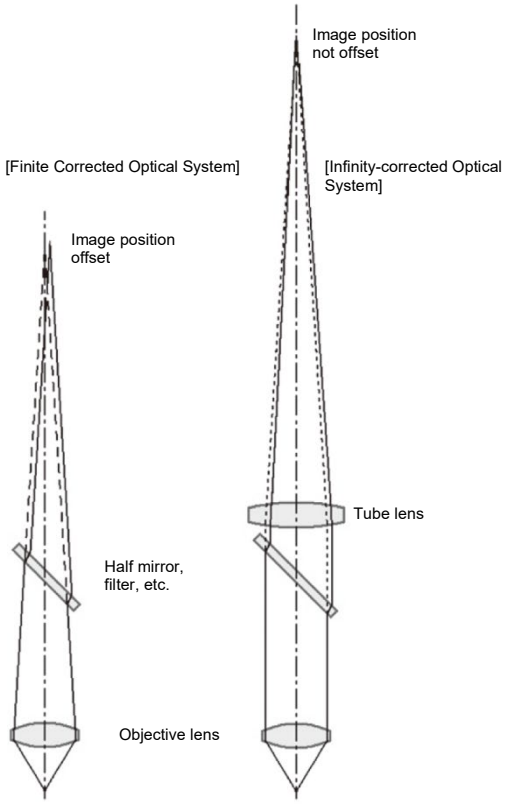


Fig. 2.23 Advantages of Infinite Mechanical Tube Length [1)]

2.5 Resolving Power and Numerical Aperture

Microscopes are devices for enlarging and observing objects. As such, the magnification value is obviously significant, as it indicates the performance of the device. However, it is even more important to be able to distinguish the details of the object. This capability is referred to as resolving power and is represented by the minimum distance distinguishable between two points that are infinitesimally close to each other. The minimum distance (resolution limit) δ is given as:

$$\delta = k\lambda/NA \qquad (2.7)$$

Table 2.1 Types of Screw Thread for Objectives and Related Nosepieces

Name of Thread		Nominal diameter	Pitch	Comments
Whitworth screw	RMS	20.32 mm	0.706 mm	General use
	W26	26 mm	0.706 mm	Industrial use, reflected-light, dark-field microscopy
Metric screw	M25	25 mm	0.75 mm	General use
	M27	27 mm	0.75 mm	General use, reflected-light, dark-field microscopy
	M32	32 mm	0.75 mm	Reflected-light, dark-field microscopy

(from ISO 8038 and JIS B 7141)

Here k is a coefficient that varies according to conditions, although a value of 0.61 or 0.5 is commonly used (Abbe derived this formula with a value of k = 0.5). λ is the light wavelength, usually 0.55μm for normal visible light (the wavelength of green light, to which the eye is most sensitive). *NA* is the numerical aperture, the most significant value in determining objective lens performance. It is given as:

$$NA = n \sin\theta \qquad (2.8)$$

Here n is the refractive index of the medium of the space on the object side and θ is the outermost angle of light entering the objective lens from a point on the axis, called the aperture angle (Figure 2.24). This formula shows that the higher the numerical aperture of the objective lens, the higher the resolving power (low δ value). For a dry objective lens, since the medium is air with a value of $n = 1$, *NA* does not go above a value of 1. In fact, it has a maximum value of 0.95 (θ = 72°). For an objective lens immersed in a liquid medium between the objective lens and the specimen (cover glass), the most commonly used homogenous immersion oil (oil with a refractive index approximately the same as the objective lens and the cover glass) has a value of $n = 1.52$, making the maximum value of *NA* around 1.45. For water immersion, the maximum value of *NA* is around 1.25, since water has a value of $n = 1.33$. Where observation is carried out under visible light using an oil-immersed objective lens with a numerical aperture of 1.45, formula 2.7 gives the resolving power as $\delta = 0.19$μm (assuming k = 0.5). This is the minimum resolving power of an ordinary optical microscope. However, as mentioned above, this is the two-point resolution value. In terms of the existence and movement of microscopic objects, it is possible to detect objects with far smaller values than this (see 5.2 and 5.7). There are objective lenses coming into commercial production, used for examining high-integration LSI circuits, that use far-ultraviolet light (with a wavelength around half that of visible light) to significantly increase the resolving power (see 7.4.5 (7)). Since resolving power has no direct relationship to magnification, microstructures beyond the resolving power cannot be distinguished by increasing the magnification alone. The appropriate magnification is determined by the numerical aperture of the objective lens. For observation, the total magnification falls between 500 *NA* and 1000 *NA*. Enlarging beyond this upper limit is known as empty magnification.

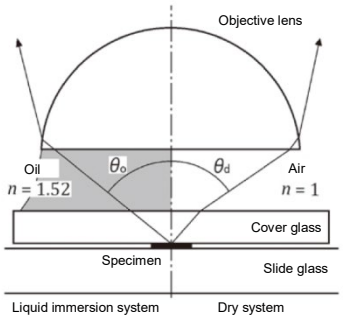


Fig. 2.24 Explanatory Diagram of Numerical Aperture [1)]

When a specimen is viewed through a microscope, there is a fixed range of focus in the direction of the thickness of the specimen. This is called the depth of focus in object space (*DOF*), typified by the following formula (the Berek formula) [6) 7)].

$$DOF = n \cdot \left(\frac{\lambda}{2 \times NA^2} + \frac{250000 \times \omega}{M \times NA} \right) \quad (\mu m) \qquad (2.9)$$

n: refractive index of the medium between the specimen and the objective lens
M: total magnification
ω: resolving power of the eye (2 minutes = 0.00058)

The first part of this formula is the depth determined from the resolving power, while the second part is the depth determined from the resolving power of the eye of the observer, which varies between individuals. The microscope image brightness I is determined by the brightness of the light source as well as the numerical aperture and total magnification.

$$I = I_O \times (NA / M_T)^2 \qquad (2.10)$$

I: image brightness I_O: specimen surface brightness
M_T: total magnification

2.6 Microscope Optical System Structure

The microscope optical system is fundamentally made up of the imaging system (objective lens, tube lens, etc.), the observing/recording system (eyepiece, projection lens, etc.) and the illumination system (collector lens, condenser lens, etc.). Figure 2.25 shows a ray diagram of a typical optical microscope (upright biological microscope).

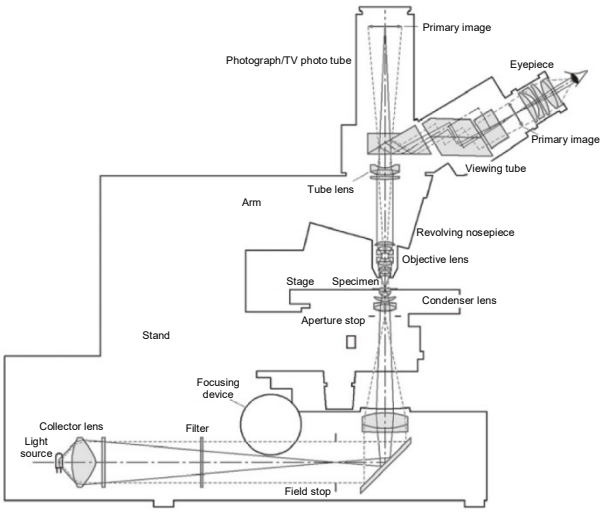


Fig. 2.25 Upright Biological Microscope Ray Diagram [1)]
(Olympus BX50: Fig. 4.47) [1)]

2.6.1 Objective Lens

As mentioned previously, the objective lens plays the most important role in determining microscope performance. Major microscope manufacturers have hundreds of types of objective lenses in their catalogs, categorized by performance, use and magnification. Performance is usually categorized by correction of aberration (difference from the

ideal image created by the lens).

1) Achromat: correction of chromatic aberration on two wavelengths (e.g. red and blue)
2) Apochromat: correction of chromatic aberration on three wavelengths (e.g. red, blue and green)
3) Semi-apochromat (fluorite): correction of mid-level between achromat and apochromat
4) Plan: correction of curvature of field and astigmatism to allow focusing on the center and peripheral edge of field at the same time

Accordingly, the most basic objective lens is an achromat and the highest quality objective lens is a plan apochromat. The respective lens structures and their differences, etc. are discussed in 7.4 with many examples.

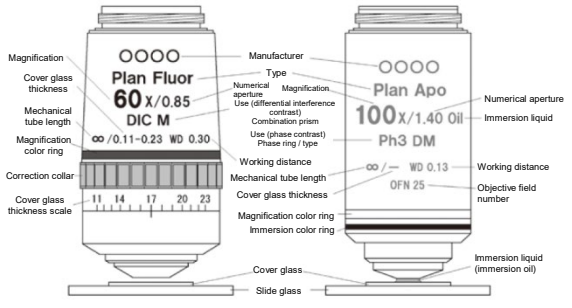
Categorization by use includes biological and industrial (metallurgical). Biological microscopes are designed to include the thickness of the cover glass (usually 0.17mm, or around 1mm for cultures). Industrial microscopes are not designed to include the cover glass (thickness 0mm). Nevertheless, there are some biological objective lenses that are designed to have no cover (such as for blood smear preparations) and some industrial objective lenses that are designed to be used with a cover glass (such as for examining LCD panels). Where mechanical tube length is finite, industrial objective lenses are less compatible than biological lenses, as the mechanical tube length is lengthened by the amount the reflected light illumination system is placed in the optical imaging path. This issue can be resolved by combining the two in an infinity-corrected optical system.

There are some specialized objective lenses categorized by method of observation (see Chapters 5 and 6). These include phase contrast objective lenses used in phase plates, objective lenses for differential interference contrast / polarized light with optical strain removed and epifluorescent objective lenses made of glass with high transmittance in the near-ultraviolet region and little autofluorescence. As these observation methods have begun to be used more frequently in combination, there has been a demand for an objective lens series that is compatible with all methods of observation. It is no easy task to develop a so-called universal objective lens that will meet all of these design constraints and demonstrate superior performance in all of these observation methods. Nevertheless, the major manufacturers are beginning to achieve this through advances in design and manufacturing technology, development of new types of optical glass and improvements in coating technology.

There are other types of objective lenses on the market with the following functions.

1) Objective lens with correction collar: When using a dry objective lens at very high magnification and high aperture to observe a specimen with a cover glass in place, lens performance can deteriorate if the thickness of the cover glass differs from the design value. This lens has a structure in place to correct any movement of the internal lens in the optic axis direction by means of a correction collar.
2) Objective lens with aperture stop: When using a high numerical aperture immersed lens for dark field observation, the dark field illumination can deteriorate the contrast in the objective lens. This lens incorporates an aperture stop mechanism within the objective lens.
3) Reflected-light, dark-field objective lens: An objective lens for dark field microscopy with industrial microscopes. The lens has an optical path around its periphery for reflected light, as well as a structure on the specimen side to illuminate the specimen against a dark field using mirrors or lenses (see 6.4.1 and Figure 6.38).
4) Long working distance objective lens: Objective lenses usually have a shorter working distance the higher the magnification and numerical aperture. This lens is designed to significantly increase the working distance, for use in industrial microscopes and culture microscopes.
5) Infrared objective lens: With high transmittance and imaging performance in the near-infrared region with wavelengths of 780-2000nm, this lens displays images using an infrared camera. This is useful for inspecting semiconductors and repairing lasers.
6) Ultraviolet objective lens: With high transmittance and imaging performance in the near-ultraviolet region with wavelengths of 240-380nm, this lens displays images using an ultraviolet camera.

Objective lens magnification is configured based on ISO and JIS designated values (ISO 8039, JIS B 7254). While magnification ranges from very low magnification (1×, 2.5×, etc.) to ultra-high magnification (150×, 250×, etc.), combinations of 4× (5×), 10×, 20×, 40× (50×), 60× and 100× are normal. There are also lenses with magnifications of 16×, 32×, 63×, etc. These are based on a series of R10 preferred numbers (10th root of 10: ISO 3). What is labelled on an objective lens is prescribed by ISO 8578 and JIS B 7252 and includes manufacturer name, type, magnification, numerical aperture, use, mechanical tube length, cover glass thickness and objective field number. Lenses are also colored to indicate magnification and immersion liquid. These are summarized in Figure 2.26 and Tables 2.2 and 2.3.



Main display codes: POL (P, PO) for polarization, EPI (M) for reflected illumination, UV for fluorescence/ultraviolet, IR for infrared, NCG for no cover glass, L (LWD) for long working distance
Display colors: Red for polarization, green for phase contrast

Fig. 2.26 Example Objective Lens Label [1)]

Table 2.2 Ring Color Classification for Objective Lens Magnification

Magnification value	1/1.25	1.6/2	2.5/3.2	4/5	6.3/8	10/12.5	16/20	25/32	40/50	60/63/80	≥100
Ring Color	black	gray	brown	red	orange	yellow	light green	dark green	light blue	dark blue	white

Table 2.3 Ring Color Classification for Objective Lens Immersion Medium

Medium	air	oil*	water	glycerol	others
Ring Color	none	black	white	orange	red

* Immersion oil designated in ISO 8036 and JIS K 2400

2.6.2 Tube Lens

The infinity-corrected objective lens discussed in 2.4 forms images in combination with a tube lens. Since the light pencil running between the objective lens and the tube lens is parallel (Figure 2.22), altering the distance between the lenses will not change the position and magnification of the primary image, although the edge of the image will start to darken beyond a certain distance. To prevent deterioration in performance at the peripheral edge of the image, the tube lens must adequately correct image edge aberration on its own (see Figure 2.25 and Figure 6.37).

2.6.3 Eyepiece

The eyepiece produces a further enlarged virtual image to enable the real image created by the objective (and tube lens) to be viewed by the eye. Eyepiece magnification is generally within the 8-15× range, although some are 5×, 20× and 30×. The field number (FN), which indicates the visible field of view, also varies from eyepiece to eyepiece. For standard 10× magnification, a field number of 18 or higher is called wide-field, while 23 or higher is called ultra (or super)-wide-field. The outer diameter of the part of the eyepiece inserted into the tube sleeve is a standard specified size of 23.2mm or 30mm (ISO 10937 and JIS B 7143). What is known as high eyepoint allows the position of the eye (eyepoint) to be farther from the end face of the eyepiece during observation, such as if the observer is wearing glasses. Some eyepieces also have a diopter adjustment mechanism to compensate for the difference in vision between each of the observer's eyes. There are also some eyepieces on the market that have a built-in (or detachable) graticule (Figure 2.27) at the field stop, etched with micrometers or a similar scale. Figure 2.28 shows an exterior view of an eyepiece, with each symbol defined by standards (ISO 8578, JIS B 7252). The types of eyepiece lenses are discussed in 7.5.

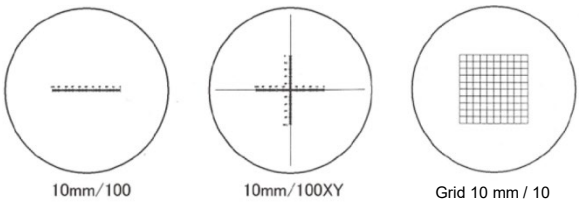


Fig. 2.27 Examples of Various Graticules (Micrometers) [1)]

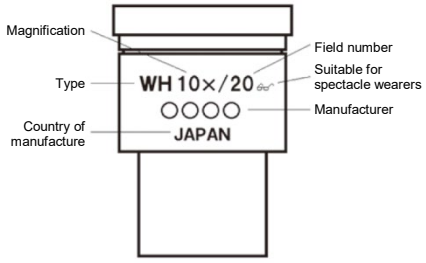


Fig. 2.28 Example Eyepiece Markings [1)]

2.6.4 Photographic Projection Lens

This lens projects the real image from the objective lens to a recording device such as photographic film or CCD. It is mounted on the straight portion of a trinocular tube system. For 35mm film photography, projection magnification is usually around 2.5-5×. With digital cameras now becoming widespread, imaging devices are much smaller in size, meaning projection magnification of 1× or less is now often used. Figure 2.29 shows the relationship between field of view and photographic film or imaging device capture range.

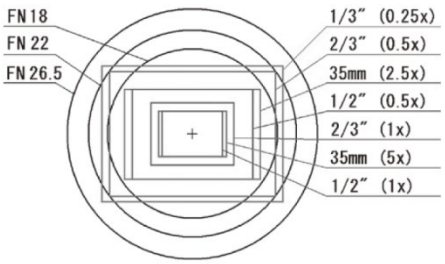


Fig. 2.29 Field of View and Capture Range [1)]

Projection lens magnification in ( )

2.6.5 Tube System

Viewing tubes can be monocular, binocular, trinocular etc., depending on the number of eyepieces and projection lenses fitted. Monocular tubes can be straight along the optical axis or inclined, using prisms or mirrors for a better viewing angle (Figure 2.30a). Binocular tubes split the light rays into two optical axes for the left and right eyepiece using either the Jentsch system (Figure 2.30b) or the Siedentopf system (Figure 2.30c). The former has a mechanism to

automatically compensate for the forward/backward shift of the intermediate image position caused by adjusting the interpupillary distance. Trinocular tubes (Figure 2.25 tube area) comprise an inclined binocular tube with an additional straight tube containing a photographic device or similar. Distributing the light path between this tube and the viewing tube is usually achieved by moving prisms. Other types of tubes available on the market include binocular systems with variable tilt angle (Figure 2.30d) and discussion tubes that allow observation by multiple people at the same time (Figure 2.30e: five-person intermediate tube).

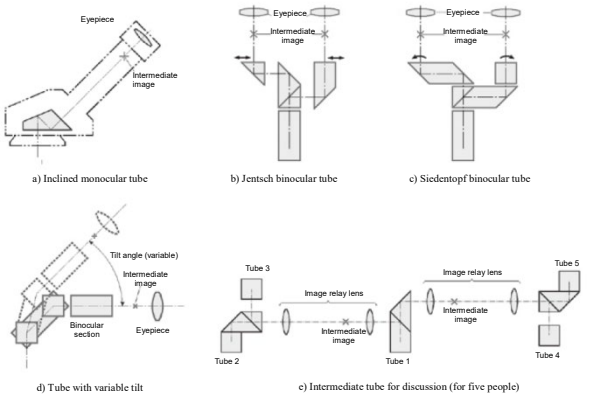


Fig. 2.30 Example Structures of Various Tubes [1)]

2.6.6 Illumination System

Since microscopes are used for enlarging and observing minute portions of a specimen, illumination is essential for maintaining brightness levels. The simplest illumination device is a reflecting mirror (Figures 4.5-14 show microscopes of this type) that makes use of a natural light source, such as a north-facing window or frosted glass window (avoiding direct sunlight), or an artificial light source, such as an electric lamp. The reflecting mirror often has a flat surface on one side and a concave surface on the other side, with the flat side used for low-magnification viewing and the concave side used for high-magnification viewing. A condenser lens is also used to concentrate the light onto the specimen surface.

Proper observation requires a dedicated artificial light source. The illumination system needs to meet certain conditions, such as adequate brightness of white light, uniform illumination of the entire observation range and meeting the maximum aperture of the objective lens. Ordinary light sources (such as tungsten lamps and halogen lamps) have a filament structure that produces conspicuously uneven lighting on the specimen surface when used as a direct light source. This makes them particularly unsuitable for low-magnification viewing and photography. A. Köhler (Figure 3.8) devised Köhler illumination, an illumination system to resolve this issue and satisfy the conditions given above. As shown in Figure 2.31, this system creates an image of the light source at the front focal point of the condenser lens, thus placing the light source image at an infinite distance from the specimen, thereby preventing uneven illumination. With Köhler illumination, the aperture stop can be placed at the light source image position, or the field stop can be placed at a point where it conjugates with the specimen surface (in relationship with the object and the image). Adjusting these produces the optimum level of contrast. Consequently, most high-end microscopes use Köhler illumination.

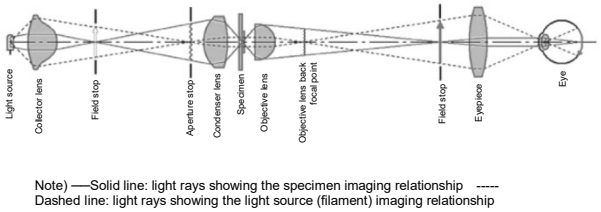


Fig. 2.31 Structure of Köhler Illumination System [1)]

Halogen lamps with high luminance and high color temperature (light source chromaticity) are often used as a light source. However, color balance filters (color temperature converters) should be used to produce completely white light. Since color temperature varies with lamp power voltage, any changes in brightness after adjusting the color temperature are carried out using a neutral density (ND) filter to maintain the color temperature.

2.6.7 Condenser Lens

The condenser lens is used to concentrate the illuminating light onto the specimen surface effectively. There are various types depending on the purpose (Figure 2.32). Regular condensers most commonly comprise a two-lens structure known as an Abbe condenser. However, to maximize high-end objective lens performance, it is recommended to use an achromatic aplanatic condenser (AAC) that corrects for spherical aberration and chromatic aberration regardless of the illumination system. According to optical theory, it is difficult to satisfy both the low-magnification microscope field of view and high-magnification numerical aperture of illumination with one condenser lens. This has resulted in widespread use of swing-out condensers, in which the top lens can be shifted in and out of the optical path when switching between low magnification and high magnification. There are also specialized condensers for the various methods of observation mentioned in the following chapters (see Chapter 5: dark-field microscopy, phase contrast microscopy, polarized light microscopy, differential interference contrast microscopy, modulation contrast microscopy, etc.), as well as universal condensers that combine all of these into one.

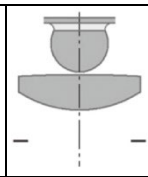
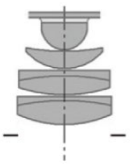
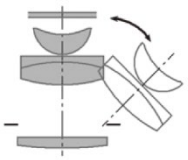
Lens configuration			
Name	Abbe condenser	Achromatic aplanatic condenser	Swing-out condenser
Numerical aperture	1.25	1.4	0.9 (top lens in)
Objective lens magnification	4x ~ 100x	10x ~ 100x	2x ~ 4x (top lens out) 10x ~ 100x (top lens in)

Fig. 2.32 Various Condenser Lens Examples [1)]

2.7 Microscope Types and Structures

Microscopes can be categorized by structure as upright microscopes, by which the specimen is observed from above, or inverted microscopes, by which the specimen is observed from underneath. They can also be categorized as biological microscopes, by which light transmitted through a biological specimen or other test piece, or industrial microscopes (metallurgical microscopes), by which light is reflected off an opaque specimen such as metal. Other types of microscopes include stereomicroscopes, which allow three-dimensional observation of a specimen. The respective characteristics are discussed below.

2.7.1 Upright Microscope

Throughout the long history of the microscope, many different types have been developed with various advances in form as science has progressed in medicine, biology, industry and other fields. The most widely used microscope today is the upright microscope. As shown in Figure 2.25, this microscope has the objective above the specimen and can either be a transmitted-light (trans-illumination) microscope or a reflected-light (epi-illumination) microscope, depending on the type of illumination. Upright transmitted-light microscopes are most commonly used in the fields of medicine and biology, although some research uses upright microscopes that are capable of both trans and epi-illumination. By contrast, upright reflected-light microscopes that incorporate epi-illumination systems between the base and the tube are used for inspecting industrial components such as semiconductors and materials.

2.7.2 Inverted Microscope

Unlike the upright microscope, the inverted microscope is designed so that the specimen is observed from underneath (see 6.1 and 6.4). The objective lens is underneath the stage. To allow observation in the same manner as for upright microscopes, the optical axis bends diagonally upwards to the eyepiece. Since this lengthens the optical path, an optical system is sometimes used to relay the primary image from the objective lens to the eyepiece. Inverted microscopes are also categorized by illumination method as transmitted light or reflected-light. Transmitted-light inverted microscopes are often used in the fields of medicine and biology, mainly for examining culture cells. Simple versions of these are known as culture microscopes. As these need to allow observation of the culture vessel (Petri dish) from underneath, they incorporate a trans-illumination system mounted above the stage and a condenser with a long working distance. The culture microscope's objective lens also has a long working distance, as it is designed to accommodate the dish being much thicker than an ordinary cover glass. The stage is stationary, while the objective lens moves up and down to focus. This is essential for performing tasks such as micromanipulator cell manipulation. Inverted biological microscopes are useful for observing living specimens and have become very widely used in recent cutting-edge bio research. In industrial fields, inverted microscopes are mainly used for studying and examining metal materials. Simply putting a polished metal surface face down on the stage can level the observation surface, while the stationary stage can take even large and heavy specimens.

2.7.3 Stereomicroscope

Observing a specimen three-dimensionally requires parallax between the left and right eyes. A stereomicroscope is a low-magnification, wide-field, long working distance microscope that uses two optical paths to create a three-dimensional image to observe a specimen from different directions (see 6.2). The observed image is turned upright by an erecting prism inside the tube. These microscopes are mainly used for assembly and inspection in the precision and electronics industries and for dissection and cell manipulation in the fields of medicine and biology. The surgical microscopes used in brain surgery and ophthalmology are also stereomicroscopes.

There are two types of stereomicroscopes: convergent microscopes (Figure 2.33a, also called Greenough microscopes), which have two optical systems, left and right, and two optical axes at a fixed convergence angle (around 12°), and parallel microscopes (Figure 2.33b, also called common main objective (CMO) microscopes), which have two optical systems, left and right, and two parallel optical axes from one objective lens. The former is widely used due to its compact structure and ease of operation. The latter has the advantage of being easily lengthened and has been widely adopted as a high-end microscope. Magnification is usually changed by zoom, although it can also be done by changing objective lenses and conversion lenses. While these have a

lower resolving power than ordinary microscopes due to a smaller numerical aperture at the same magnification, they have a high depth of focus and tend to be used in types of work relevant to this. Stereomicroscopes often have simple illumination systems using fluorescent lamps or light guides, although high-end stereomicroscopes may have trans-illumination devices, dark field illumination devices or coaxial epi-illumination devices.

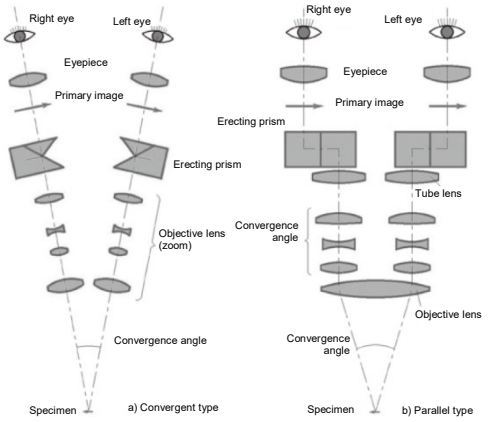


Fig. 2.33 Stereomicroscope Optical System [1)]

2.8 Microscope Components

Microscopes are made up of the objective lens and eyepiece for enlarging and viewing specimens, the light source for illuminating the specimen, the condenser lens and other parts of the optical system, as well as the mechanical system that supports these. Figure 2.34 lists the different parts of an upright biological microscope.

The various mechanical components of a microscope are discussed below.

(1) Stand

The stand is the main body of the microscope and is made up of the base underneath, which supports the whole stand, and the arm, which supports the optical system. The shape, size and weight of the base maintain the stability of the entire microscope. Many smaller microscopes have a U-shaped base, although newer microscopes with built-in illumination systems often have a rectangular, T-shaped or Y-shaped base. The arm is very solid, as it supports the stage and has the tube and camera device, etc. at the top. The focusing is also built into the arm.

(2) Stage

This component holds the specimen and allows smooth and accurate movement. The stage is either stationary, attached to the base, or moveable, moving up and down with focusing. Types of stage include the plain stage (Figure 2.35a), in which the specimen is held by two clips and moved by hand, and the mechanical stage (Figure 2.35b), in which the specimen is moved in four directions using knobs. There is also a 360° rotating stage (Figure 2.35c), used for polarized light microscopy and similar, and a large stage (Figure 2.35d), for examining large wafers or liquid crystal panels in industrial applications.

(3) Focusing

This component is for adjusting the focus on a specimen. It has a coarse focus knob for observation at low magnification and a fine focus knob for high magnification. In high-end microscopes, these are coaxial (Figure 2.36).

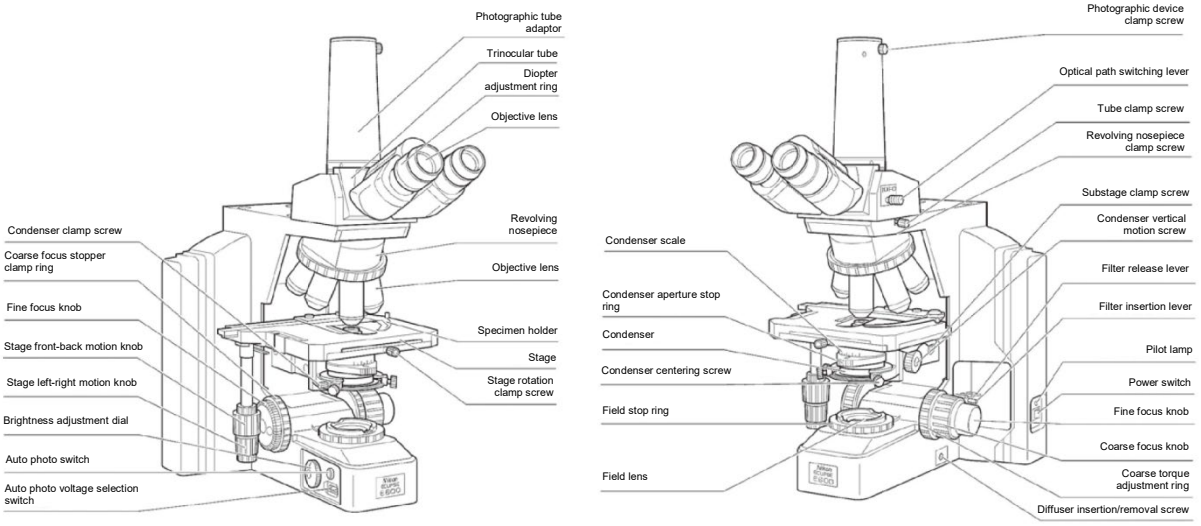


Fig. 2.34 List of Various Microscope Parts (Nikon E600: Fig. 4.50) [8)]

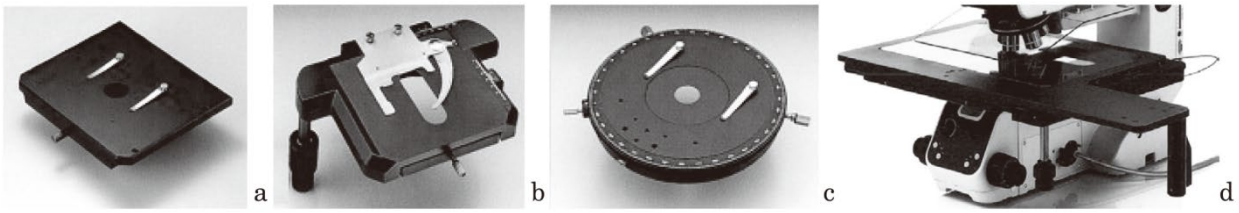


Fig. 2.35 Examples of Various Stages [9)]

a) Plain stage b) Mechanical stage c) Rotating stage d) Large stage

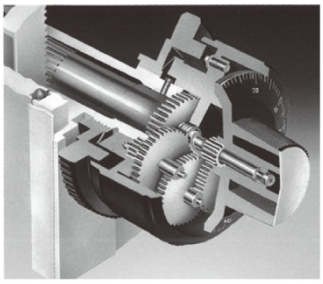


Fig. 2.36 Coaxial Focusing System [9)]

(4) Tube

This component is built into the upper section of the arm and has the eyepiece at the top and the revolving nosepiece and objective lens at the bottom. In older microscopes, the tube moves up and down on a rack and pion system together with the objective lens and eyepiece while focusing. In many newer microscopes, the tube is stationary and clamped to the arm (it can be rotated). Details are omitted here, as the types of tube are discussed in 2.6.5.

(5) Revolving Nosepiece

This component rotates objective lenses of different types and magnification. It can hold 2-7 objective lenses (Figure 2.37). It is machined very precisely to allow the objective lenses to be switched quickly and smoothly, with as little impact as possible on the focus or position of the specimen in the field of view.

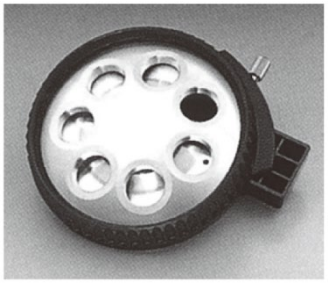


Fig. 2.37 Revolving Nosepiece (7 positions) [9)]

2.9 Microscope Accessories

There are all kinds of accessories for high-end microscopes. These form part of the microscope system to achieve various purposes, such as observing, recording and measuring. The following is a brief introduction to some typical accessories.

2.9.1 Photographic Devices

The importance of photography for recording microscope images goes without saying. Microscope cameras once used silver halide film and required various filming techniques and expertise to operate. As electronics technology developed, manufacturers improved photomicrographic devices and automated the following tasks. Figure 2.38 shows examples of automatic photographic devices.

1) Loading, winding and rewinding film
2) Setting film properties (= DX code reading)
3) Adjusting color temperature (= setting power voltage)
4) Switching illumination systems (adjusting field stop and aperture stop)
5) Focusing (autofocus)
6) Photometry (moving spot photometry, two-dimensional photometry)
7) Exposure (reciprocity law failure correction, auto bracketing)

It is also possible to mount multiple cameras, with functions including zoom magnification with easy framing and overlaying of scale data. These improvements have made photomicrography relatively easy, with the photographer only needing to choose the position of the specimen and the magnification of the photograph. However, with digital image recording now widespread, even these devices are falling out of use.

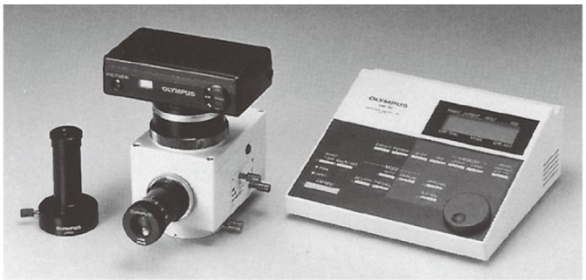


Fig. 2.38 Automatic Exposure Device [9)]

2.9.2 Cameras

Recording the dynamic behavior of specimens was once done by filming with 16mm cine devices. This was difficult and prone to failure and met its end with the appearance of video. Video devices grew rapidly in popularity for recording microscope images, as they were not only easy to use, but also offered technological advances in image quality and sensitivity. Cameras, particularly 3-CCD color video cameras, Hi-Vision HDTV cameras and high pixel digital cameras, have become an essential unit in the system, due to the ease of analyzing, processing and saving microscope images on computer.

2.9.3 Measuring Systems

When measuring specimen tissues and cells, it is easy to take larger measurements with the XY stage scale, but more detailed measuring requires an eyepiece with a graticule (Figure 2.27). For even greater accuracy, there are also micrometer eyepieces (Figure 2.39). Since there can be measurement errors such as magnification errors or lens distortion depending on the objective lens used, they need to be precisely calibrated with an objective micrometer. Measuring microscopes have many accessories for measuring, although details are omitted here.

Fig. 2.39 Micrometer Eyepiece [9)]

2.9.4 Microscope photometers

Microphotometry measures the fluorescence and transmittance of cells and is used for photometric analysis of DNA/RNA/protein quantities or Ca^{2+} and other ion concentrations. Microscope photometers were once large, specialized devices, but now come in the form of modules that can be mounted onto ordinary microscopes. They are made up of a high sensitivity photomultiplier and controller, a photometric unit with multiple pinholes to limit the Microphotometric section, and a photometric tube (or photometric finder) (Figure 2.40). There are also high-speed excitation switching units and dichroic mirror units for measuring Ca^{2+} concentrations. Other devices can be combined with monochromators for spectral photometry, or with auto scanning units for two-dimensional distribution photometry. A computer carries out various statistical processing, image processing and time-dependent analysis of the measurement data and displays it on a monitor.

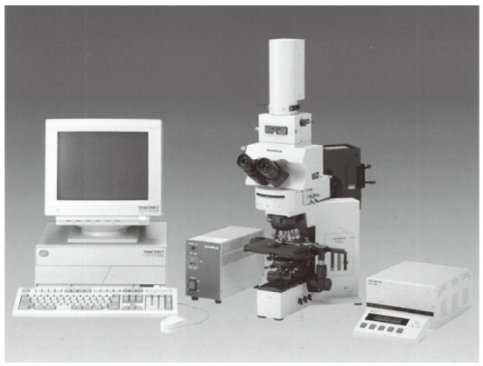


Fig. 2.40 Microscope photometer [9)]

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3 Invention and Development of Microscopes [1) 2) 3) 4) 5)]

3.1 Optics and Lenses

Various phenomena of light have caught the interest of philosophers since ancient times. The law of reflection, which forms the basis of geometric optics, was noted by Greek mathematicians Euclid (330?-275? BCE) and Archimedes (287?-212 BCE) in the third century BCE. The law of refraction was noted by Ptolemy of Alexandria in the second century CE, although the correct refraction relationship formula was not discovered until the 15th century, by Dutch astronomer W. Snellius (Snell). The later decline of Greece and growth of Christianity spread science to Arabia. Ibn al-Haytham (Latin name Alhazen, 965-1040) made a detailed record of the reflection and refraction of light rays in his "Book of Optics", published in the early 11th century. He also made reference to magnification by convex lenses and other phenomena. The work was translated into Latin and had a significant impact on later developments in optics. In the 13th century, British friar Roger Bacon (1214-1294) wrote about magnification by lenses and suggested the utility of this in reading glasses. The first pair of reading glasses was made in Venice, Italy in the 1280s. Glasses grew steadily in use by monks studying the Bible. The earliest record of glasses and magnifying glasses is a fresco (1352) in a church in northern Italy, which depicts a cardinal wearing glasses reading a book and a monk using a magnifier to read. The first glasses using concave lenses for nearsightedness appeared in the early 15th century. Groups of spectacle-makers began to emerge all across Europe.

3.2 Invention of the Microscope and Telescope

A commonly held view is that the first compound microscope using a combination of two convex lenses was invented around 1590 by Zacharias Janssen (c.1580-c.1638), a Dutch spectacle-maker from Middleburg, with his father, Hans Janssen, although this is subject to much debate. Janssen's microscope (Figure 3.1) is purported to have comprised a biconvex objective lens and a plano-convex eyepiece fitted inside a brass tube, with a total magnification of around 9×. In 1608, Janssen's neighbor, spectacle-maker Hans Lipperhey (1570-1619), became the inventor of the telescope with his patent of a telescope comprising a convex objective lens and a concave eyepiece. This device reached Italy, where Galileo Galilei (1564-1642) used a telescope of his own making to observe various astronomical phenomena the following year, opening the door to modern science, as is well known. In 1611, German astronomer Johannes Kepler (1571-1630) published his work "Dioptrice" theorizing on telescopes in which the objective lens and eyepiece are both convex lenses.

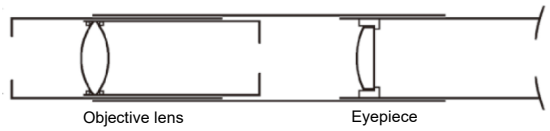

Fig. 3.1 Janssen Microscope Configuration

(created from cited reference 4)

Robert Hooke (1635-1703) of the Royal Society of London made a microscope of his own, comprising an objective lens and an eyepiece (both convex lenses). He published his biological observations in "Micrographia" [6)] in 1665. His detailed engravings of a flea, a fly, a louse and other creatures were very popular, and the work became a best seller. Hooke named the cavities in cork "cells" (Figure 3.2). Antonie van Leeuwenhoek (1632-1723), a draper from Delft in the Dutch Republic, made a large number of his own single-lens microscopes (Figure 3.3). His discoveries in the 1670s and 80s included red blood cells, microbes in water, yeast, bacteria and sperm (Figure 3.4) [7)]. He reported these findings in detail to the Royal Society of London and later became known as the "Father of Microbiology". Leeuwenhoek made improvements to his microscope by grinding his own tiny convex lenses. He is believed to have achieved an astonishingly high magnification of 275× with a single lens and a resolving power of 1.4μm, although he was apparently the only one who could work his microscopes properly. A number of microscope makers emerged in the later 17th century through to the 18th century, including Giuseppe Campani in Italy, John Marshall, Edmund Culpeper, John Cuff, Benjamin Martin and George Adams Sr. & Jr. in the United Kingdom, and Camille Sebastien Nachet in France. However, it being 200 years before the invention of achromatic objective lenses and the establishment of microscope resolution theory, these compound microscopes were no match for Leeuwenhoek's single-lens microscope.

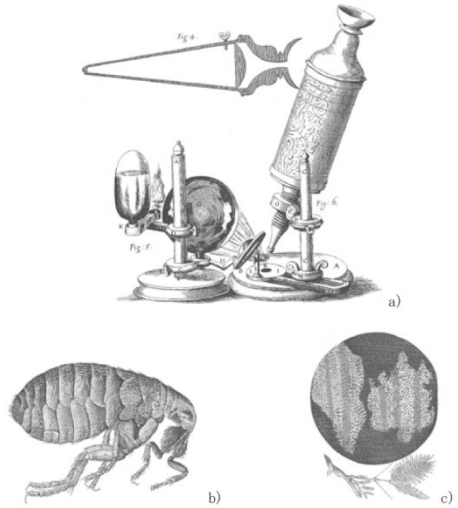

Fig. 3.2 Hooke's Microscope: from Micrographia [6)]

a) Microscope illustration b) Drawing of a flea
c) Drawing of cork

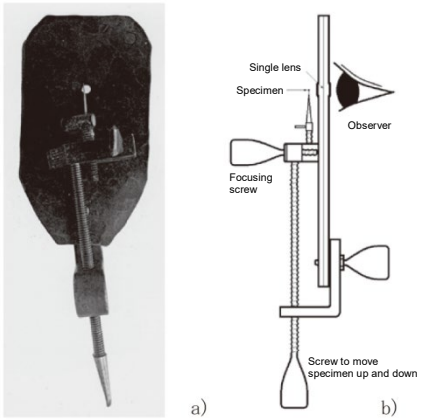

Fig. 3.3 Leeuwenhoek's Single-Lens Microscope

a) Exterior view
(replica held by the Japan Microscope Manufacturers' Association)
b) Usage diagram

(created with reference to cited reference 3)

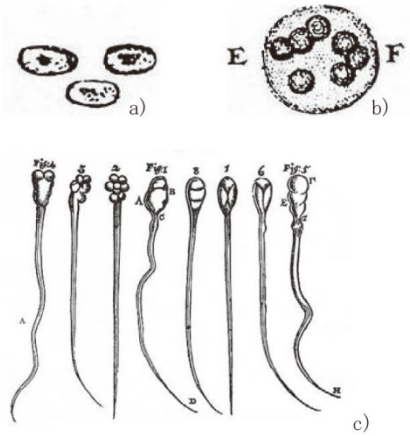

Fig. 3.4 Leeuwenhoek's Observational Drawings [2)]

a) Fish red blood cells b) Volvox
c) Human and canine sperm

3.3 Advances in Objective Lenses

The microscope is an instrument used to enlarge infinitesimal objects and observe their composition in detail. The performance of the microscope is determined by the performance of the objective lens. The first improvement to the objective lens was removing various aberrations, especially chromatic aberration. Sir Isaac Newton (1643-1727), who discovered the light spectrum, is well known for using a reflector to make a reflecting telescope (1668), with the idea that it would never be possible to eliminate chromatic aberration with an objective telescope lens that refracted light. Swiss mathematician and physicist Leonard Euler (1707-1783) proposed in 1747 that it would be possible to eliminate lens chromatic aberration by combing different materials. In 1758, British optician John Dollond (1706-1761) achieved an achromatic lens by combining a convex lens of low dispersion (refractive index varies with wavelength) crown glass with a concave lens of high dispersion flint glass. Later, achromatic lenses were also used for microscope objective lenses, but these were more difficult to design and produce than for telescope objective lenses. In 1830, British optician Joseph J. Lister (1786-1869) successfully produced a practical achromatic microscope objective lens (Figure 7.1). He also confirmed that it was necessary to increase the numerical aperture of the objective lens in order to increase resolving power. In 1837, Italian astronomer Giovanni B. Amici (1786-1863) designed an objective lens with even higher magnification by adding a hemispherical lens on the object side (Figure 7.2). Amici further increased the numerical aperture by filling the space between the object and the end lens with liquid (immersion technique) and devised an objective lens with even greater resolving power. These improvements to objective lenses finally gave the compound microscope greater resolving power than the simple microscope. The simple microscope is also harder to use, and only survived in the form of a high-magnification lupe (20-30×) used for a dissection microscope. From the start of the 19th century, the United Kingdom led the world in microscope production, with developments made by Andrew Ross, James Smith & Richard Beck, Hugh Powell & Peter Lealand, and William Watson etc. In 1839, Lister and others founded the Microscopical Society of London (renamed the Royal Microscopical Society (RMS) in 1866 after gaining its royal charter).

3.4 Rise of German Microscopes [8) 9) 10) 11)]

From the mid-19th century, businesses began to be established outside of the United Kingdom as well, some of which later became global microscope manufacturers. In the Unites States, Charles Spencer (1813-1881) founded the Spencer Lens Company in 1847 in Buffalo, New York. (This became the American Optical Company in 1835 and then part of the Leica Group in 1990). In 1853, John J. Bausch (1830-1926) and Henry Lomb (1828-1908) founded Bausch & Lomb in Rochester, New York (which became part of the Leica Group in 1990). Meanwhile, in Germany, Carl Zeiss (1816-1888, Figure 3.5) started an optical workshop in Jena in 1846, while Carl Kellner (1826-1855) did the same in Wetzlar in 1849.

Carl Zeiss AG (referred to below as Zeiss) worked constantly to improve its manufacturing of microscopes right from the founding of the company. The young physicist Ernst Abbe (1840-1905, Figure 3.6) was invited to work on theoretical research and practical development of objective lenses to make production even more scientific. As part of his work, Abbe published the sine condition that forms the basis of objective lens design in 1870 and a theory of microscope imaging in 1872 (see 2.5), as well as developing various measurement methods and devices. He achieved successive ground-breaking results that made dramatic improvements to microscopes, such as the Abbe condenser (Figure 2.32) in 1873, the homogenous oil immersion method (see 2.5) in 1877 and the apochromat objective (see 2.6.1) using calcium fluoride (CaF_2) in 1886. Abbe also founded a glass works in Jena in 1884 with Otto Schott (1851-1935, Figure 3.7), who had developed new type of glasses in 1879. In 1900, Abbe brought August Köhler (1866-1948, Figure 3.8), who had published a new illumination method (Figure 2.31) in 1893, into the company. In 1897, he developed the first stereomicroscope (see 2.7.3), based on an idea proposed by American biologist Horatio Greenough. Abbe continued running the company as the Carl Zeiss Foundation after Zeiss' death. He was an outstanding industrialist as well who established many modern working conditions. Köhler and Abbe's other successors were involved in later inventions and developments in microscopy, while Zeiss continues to be a world leading company in the optical industry.

The optical company founded by Kellner was taken over by Ernst Leitz (1843-1920, Figure 3.9) in 1869, under the name Ernst Leitz GmbH (the microscope division is now Leica Microsystems, referred to below as Leitz and from 1990 onwards as Leica).

Fig. 3.5 Zeiss [12)]

Fig. 3.6 Abbe [12)]

Fig. 3.7 Schott [12)]

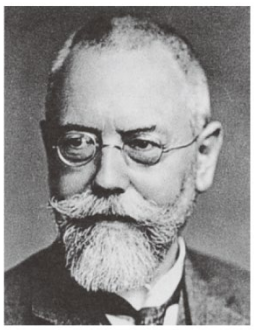

Fig. 3.8 Köhler [12)]

The company experienced significant growth in microscope production and developed as a global microscope manufacturer on par with Zeiss. Its objective lenses, designed by mathematician Carl Metz (1861-1941), were highly regarded. Leitz's son-in-law Carl Reichert (1851-1922, Figure 3.10) founded a new microscope company (part of the Leica Group since 1990) in Vienna, Austria, in 1876, which grew into a leading European microscope manufacturer after Zeiss and Leitz. The Leica 35mm camera, invented by Leitz engineer Oskar Barnack (1879-1936) and developed into a commercial product in 1925, dominated the camera market due to the lenses by optical designer Max Berek (1886-1949, Figure 3.11). In competition, Zeiss Ikon launched the Contax 35mm camera in 1932, with noted rivalry ensuing between the two companies. Since Zeiss was in located in the east of Germany, a month after Germany surrendered to the Allies in 1945, US armed forces transported 126 Zeiss supervisors and engineers to the western side to recreate a West German Zeiss company in the southern German town of Oberkochen. This meant that after the war, Zeiss resumed its production of optical instruments as a divided entity across East and West Germany, although in 1991 the company reunited in Oberkochen. Meanwhile, Leitz, based in West Germany, was quick to get up and running again after the war, having suffered minimal damage.

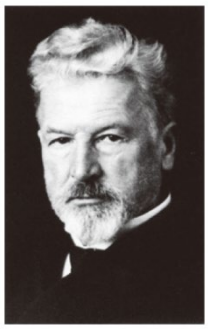

Fig. 3.9 Leitz [13)]

Fig. 3.10 Reichert [13)]

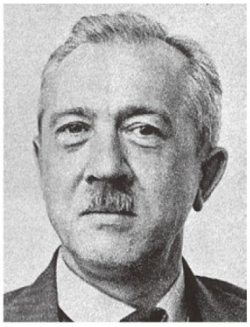

Fig. 3.11 Berek [13)]

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4 Birth and Growth of Japanese Microscopes

4.1 Japanese Microscopes from the Edo Period to the Meiji Period [1) 2) 3)]

Microscopes are estimated to have been brought to Japan around 1750. This was quite a late date compared to the telescope, which the British presented to Tokugawa Ieyasu in 1613, not long after its invention. The first documented mention of the microscope is in "Oranda Banashi [Dutch Tales]" by Gotō Rishun in 1765. In 1781, Nakai Riken of Osaka wrote "Kenbikyō-ki [Microscope Records]", stating that Hattori Eishaku and Aburaya Kichizaemon had made microscopes. Japan's oldest existing microscope, a wooden microscope (Figure 4.1) made by Kobayashi Kiemon that same year, is on display at the Shimadzu Foundation Memorial Hall (Kyoto). In 1787, Morishima Chūryō includes sketches of a "mikorasukobiyun" or Culpeper microscope (Figure 4.2), a mosquito, a flea and a mosquito larva in his work "Kōmō Zatsuwa [Various Accounts of the Dutch]". His brother Katsuragawa Hoshū used a microscope in medicine, and wrote "Kenbikyō Yōhō [How to Use a Microscope]" in 1802. Iinuma Yokusai published "Sōmoku Zusetu [Diagrams of Plants]" in 1832. In 1833, Udagawa Yōan, who had been given a microscope by Philipp F. von Siebold on a visit to Japan, published "Shokugaku Keigen [Origins of Plants]". Doi Toshitsura, feudal lord of Koga Domain, studied snow crystals under a microscope and published "Sekka Zusetsu [Diagrams of Snow crystals]" (Figure 4.3), which led to a later popular craze in snow crystal patterns. Thus, we see that microscopes in the Edo Period were only used for interest or for study by a few individuals of culture or scholars of Western knowledge.

Fig. 4.1 Japan's Oldest Existing Domestically Produced Microscope (held at Shimadzu Foundation Memorial Hall) [4)]

Fig. 4.2 From "Kōmō Zatsuwa [Various Accounts of the Dutch]"

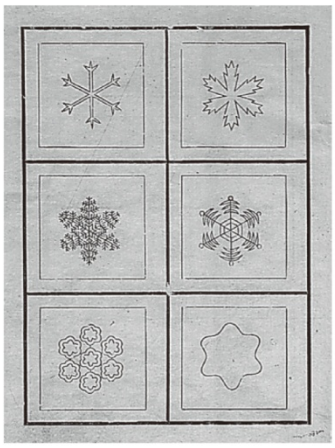

Fig. 4.3 From "Sekka Zusetsu [Diagrams of Snow crystals]"

In the Meiji Period, Western civilization started flooding into Japan. In the field of medicine, cutting-edge medical science from Germany was adopted without delay. Successive discoveries in the area of disease-causing pathogens using high-powered microscopes led to rapid developments in bacteriology and immunology in particular. As a result, Japanese universities and government institutions began to focus on experiments, research and education using microscopes, with many students studying abroad in Europe and the United States. This led to global-level achievements by Japanese scholars, such as Kitasato Shibasaburō (clean culture of tetanus bacillus in 1889, developed serotherapy in 1890, discovered plague bacillus in 1894), Shiga Kiyoshi (discovered dysentery bacillus in 1897), Takamine Jōkichi (invented the Takadiastase enzyme in 1894, discovered adrenalin in 1900) and Noguchi Hideyo (research on syphilis spirochete and yellow fever). Microscopes were also used in sericulture, which was growing into a major Japanese industry at that time, for quality control of silkworm eggs.

This was a major market for imported microscopes at the time. Microscope imports began to boom around 1887 (Figure 4.4). There was greater demand for the more affordable Leitz products than for the high-quality Zeiss products. Reichert products and American microscopes were also imported. Some attempts were also made at producing microscopes in Japan at this time.

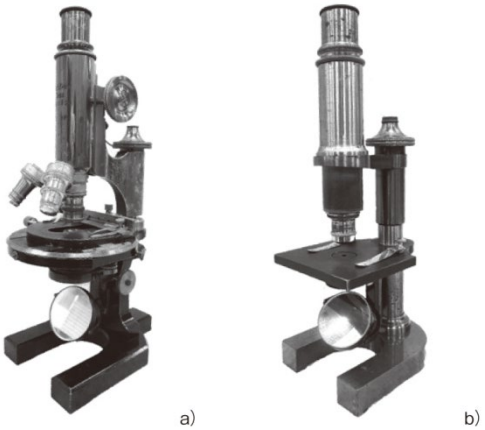

Fig. 4.4 Examples of Imported Microscopes from the Late Meiji Period [5]

a) Zeiss Ia b) Leitz IV (both held by Hamano Microscope Co., Ltd.)

4.2 The Birth of the Japanese Microscope [1) 6) 7)]

Although several Japanese microscopes were exhibited at the 4th National Industrial Exhibition held in 1895 in the late Meiji Period, these were far from practical, with magnifications of only around 20×. The first commercial advertisement for a Japanese microscope appeared in the medical journal "Chūgai Iji Shinpō Vol. 697" in 1909, for a microscope by Iwashiya Iwamoto Tokichi, listed as "the Iwamoto purely Japanese microscope". The quality of the microscope is unknown, although it had a maximum magnification of 600× and was about half the price of an imported microscope. As there are no existing versions of this product and no later records of it, it is thought to have probably been a limited edition [7) 8)].

The earliest Japanese microscope to be industrially mass produced was the "Tanaka microscope" (Figure 4.5, 600× dry), manufactured in 1907 by Tanaka Mokujirō (Tanaka Partnership) [8)]. It won gold at the Tokyo Industrial Exhibition held the same year. A catalog[9) 10)] was published the following year, and production grew to around 100 microscopes per month. It was also shown at the Japan-British Exhibition held in London in 1910 and won bronze. Katō Kakitsu (in charge of the lenses) and Shintō Shinkichi (in charge of the mechanisms) started out making microscope in 1910, modelled on the Leitz IV. After much trial and error, the two completed their first prototype in 1912. They were joined by Terada Shintarō, who worked hard to leverage their skills to present the microscope as a domestic product at an Exhibition. The three men worked through repeated difficulties and repeated improvements before finally presenting the microscope under Terada's name at the Tokyo Taisho Exhibition at Ueno Park in 1914, where they won bronze (the Tanaka microscope won silver at this Exhibition) [11)]. Matsumoto Fukumatsu of Iwashiya Matsumoto Kikaiten, the long-standing medical instrument store that had imported German microscopes, had long dreamed of a Japanese microscope that could hold its own against the products from overseas. Matsumoto met with registered owner Terada and manufacturers Katō and Shintō for some discussions about further improvements to the quality of the microscope and how to work towards manufacture and sales. Taking this on board, the three worked hard to improve their product and finally produced a microscope of satisfactory quality. They named this microscope (Figure 4.6) the "M & Katera", using the first letters of Matsumoto, Katō (& Shintō) and Terada. In 1915, the following year, Matsumoto established M & Katera Optical Works as the manufacturing division and started developing, selling and advertising microscopes. Coincidentally, the outbreak of the First World War in 1914 made it difficult to import microscopes into Japan, which meant a favorable start to production and sales. Tanaka Shoji Co., Ltd. (the new name of Tanaka Partnership) and M & Katera Optical Works both suffered heavy damage to their offices and factory in the Great Kanto Earthquake of 1923. While Tanaka withdrew from microscope manufacturing, Matsumoto was determined to turn disaster into opportunity and weathered the storm by engaging in technological developments to produce high quality microscopes.

Fig. 4.5 Tanaka Microscope [15)]

Fig. 4.6 M & Katera Microscope [16)]

Yamashita Takeshi, an associate of Terada, joined in the mission to produce a Japanese microscope that would equal those from overseas. In 1919, he established Takachiho Works (renamed Olympus Optical Co., Ltd. in 1949, then Olympus in 2003, referred to below as Olympus). Terada was a company director and served as technology supervisor for microscope manufacturing. Suzuki Taiichi, a former apprentice of Katō, was in charge of lenses and assembly. The following year, they completed 20 of their first microscope, the "Asahi" (Figure 4.7). The "Olympus"

trademark was registered that same year. After that, the company worked hard to develop high-performance technology and began to make great progress from the late 1920s [12) 13)].

Fig. 4.7 The Asahi [17)]

Katō worked with Shintō to establish Kalnew Optical Industries (now Shimadzu Device Corporation) in 1924. The company started manufacturing microscopes (Figure 4.8) and supplying lenses for the M & Katera. Suzuki of Olympus established the Toyo Optical Company in 1927 and announced its first microscope, the "Eliza" (company closed in 2002).

In 1934, M & Katera Optical Works changed the name of its microscope to the Tiyoda microscope, after a microscope developed by Shintō and the team. The company re-launched under the new name of Tiyoda Optical in 1942 (referred to below as Tiyoda). Thus, the three men who developed the M & Katera and its technologies laid the foundation for later developments in the Japanese microscope industry.

In 1917, Iwasaki Koyata, president of Mitsubishi Partnership, funded a merger between the optical instruments division of Tokyo Keiki and the reflector division of Iwaki Glass, aimed at domestic production of optical instruments. Nippon Kogaku K.K. (Japan Optical Industries Company) was established (renamed Nikon in 1988, referred to below as Nippon Kogaku and as Nikon after the name change) and very soon merged with the Fujii Lens Factory. In 1921, the company engaged eight optics and precision engineers from Germany and saw rapid improvement in its technology. One of these German engineers, Heinrich Acht, produced mathematical designs for various lenses, including microscope lenses and photography lenses. Acht's design data and German design methods served as a basic design resource for later photographic lenses. Nippon Kogaku released its first microscope, the "JOICO" (Figure 4.9), in 1925, based on Acht's design.

Fig. 4.8 Kalnew Microscope [5)]

Fig. 4.9 The JOICO Microscope [18)]

From the late 1920s onwards, with Japan growing increasingly militant, the main orders received by microscope manufacturers were military related. Portable microscopes were developed that could easily fold down into a wooden box. These were sold in great numbers. Companies made use of their optics technologies and began producing optical military equipment, such as binoculars, periscopes and sights. In 1938, Olympus developed the Super Photo, a universal microscope equipped with photographic equipment (the "Universal", Figure 4.10). Capable of both biological and industrial applications, the microscope was equipped for reflected and transmitted light bright-field/dark-field observation and had built in devices for photography, projection and drawing. This was the highest quality microscope produced in Japan before the war. Although only a few were sold, this model indicates the level of technology at the time and served as the forerunner to the company's postwar high-end universal measuring microscopes, the Photomax and the Vanox. Toward the end of the war, factories had to decentralize their operations due to frequent air raids. Olympus relocated its microscope division to a plant in Ina, Nagano in 1944. However, the main offices and factory were heavily damaged in an air raid on Tokyo in May 1945. Such was the situation when the war ended in August.

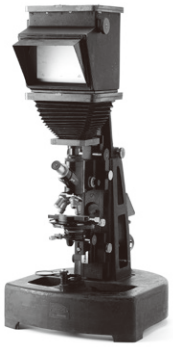

Fig. 4.10 The "Universal" Super Photo Universal Microscope Equipped with Photographic Equipment [17)]

4.3 Revival of the Microscope Industry

The end of the war in 1945 was followed by a time of

uncertainty. Optical companies faced drastic staff cuts (Nippon Kogaku went from 25,000 employees to 1,724 employees), factory closures and administrative cuts. On top of that, all industries had to transition from war industries to peace-time industries. However, the Japanese optical industry recovered relatively quickly, having made great technological progress before and during the war. In January 1946, members of 73 companies founded the Japan Optical and Precision Instruments Manufacturers' Association (president: Hatano Yoshio of Nippon Kogaku), with the aim of working together to develop the optical instruments industry in the six divisions of filming equipment, cameras, telescopes, microscopes, surveying equipment and precision measuring equipment. In June 1954, the Association changed its name to the Japan Optical Industry Association, while the divisions also changed their names, e.g. the Japan Microscope Manufacturers' Association, which continue to the present day (the association has seven divisions following the establishment of the Japan Photo and Imaging Accessory Association and the Japan Medical-Optical Equipment Industry Association and after the Japan Motion Picture Equipment Industry Association left the association). Microscope manufacturer Tiyoda had escaped any war damage to its factory and resumed production in December 1945 with Shintō as company president. In addition to its prewar high-end microscope, the ACM (Figure 4.11), and its portable microscopes, the company also developed a research microscope, the LCM (Figure 4.12), in 1947, based on the Zeiss L. Olympus resumed production at its factory in Ina, but had to refit new machinery, equipment and tools. It produced its first G microscope (Figure 4.13) in July 1946, while production of its prewar high-end microscope, the UCE, resumed at the end of 1948. Nippon Kogaku had the nation's best optical technology capabilities and went into full-scale microscope development and production right after the war. Production began on the popular O model (Figure 4.14) in 1948 and on the high-end oil-immersion K model (Figure 4.15) in 1949. Many other microscope manufacturers were established after the war (Appendix 2). With increasing demand for progress in all industries, the microscope industry began to revive and grow as postwar medical, welfare, research and education institutions were established and expanded.

Fig. 4.11 Tiyoda ACM [7)]

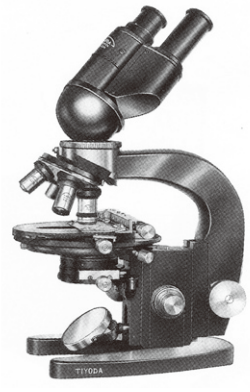

Fig. 4.12 Tiyoda LCM-Bi [7)]

Fig. 4.13 Olympus GK [17)]

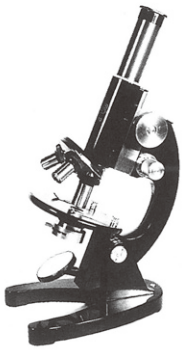

Fig. 4.14 Nikon O [14)]

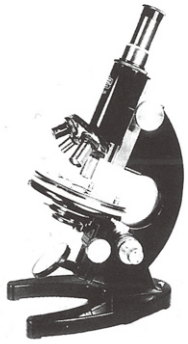

Fig. 4.15 Nikon K [14)]

4.4 Creation of Microscope-Related JIS

After the war, much attention was drawn to the importance and potential of Japanese microscopes as export products beyond domestic demand. Standards relating to their performance and accuracy were urgently needed. In January 1947, the microscope division of the Japan Optical and Precision Instruments Manufacturers' Association drafted some industrial standards for microscopes. These were put up for deliberation by the microscope committee of the Japanese Industrial Standards Committee precision instrument division (chairperson: Hiroshi Kubota, professor at the University of Tokyo) and enacted in October 1948 as JES 7132 Microscopes (JIS B 7132 from December 1949, now JMMA MIS 1001). The result of examining microscopes around the world, the standard defined specifications such as size tolerance and eccentricity of various components, magnification calculation error, image resolving power and peripheral performance. An issue at the time was that it was difficult to find something suitable for a test specimen to measure microscope resolving power, as it had to be mass produced identically, have the appropriate microstructure and not change over time. This was solved by Tiyoda with the production of a plastic replica diffraction grating, plated on an angle in a vacuum to form a light and dark grid, then coated with an emulsion of microparticles [7)]. Test plates No.1

(300 grooves/mm) and No. 2 (600 grooves/mm) were defined in JES 7140 (now MIS 8602). Figure 4.16 shows a later No. 1 test plate produced by Nippon Kogaku and a partial photograph of the magnified image. The defect parts of the grating pattern are useful to assess performance (resolution, aberration, etc.). By 1951, 11 JIS had been created, covering areas such as small biological microscopes, stereomicroscopes, objective thread and graticules for eyepieces, further completing the JIS coverage of microscopes. Appendix 1 shows a summary of the ISO, JIS and MIS standards relating to optical microscopes at the time of writing this report.

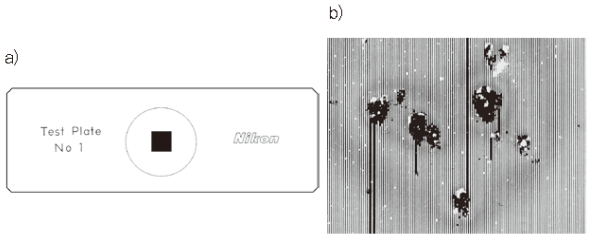

Fig. 4.16 Test Specimens [19]

a) Test plate No. 1 b) Partial photograph of magnified grating pattern

4.5 Development of Research Microscopes

Resuming operations after the war, the West German branch of Carl Zeiss GmbH released the Zeiss "Standard" microscope (Figure 4.17) in 1950. Each unit was interchangeable and further improvements in function and performance were added. This was literally the world standard for high-grade microscopes. In 1955, the company released the Photomicroscope (Figure 4.18), a high-end automatic photography microscope with a built-in camera and automatic exposure device. It was very well received as a high-status microscope by the top researchers around the world.

Fig. 4.17 Zeiss Standard [20]

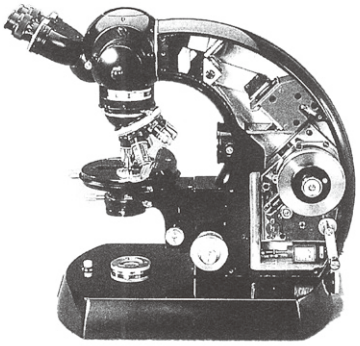

Fig. 4.18 Zeiss Photomicroscope (cut-open model to show the interior structure) [20]

Prompted by this, Japanese companies began working on improving their own microscopes further. Nippon Kogaku launched the S model (Figure 4.19) in 1956, with a coarse/fine movement function that moved the stage up and down. Later, the company standardized more than 30 types of units and incorporated them into an industry-leading multi-product / multi-function system. The company also developed Japan's first Köhler illumination system and incorporated it into the S-Ke model (Figure 4.20), launched in 1961. In 1957, Olympus developed a new research microscope, the DF model (Figure 4.21), which had a moveable stage. In 1958, the company launched a clinical research microscope, the E model (Figure 4.22), which was widely adopted for its high cost performance. By 1963, the E model had expanded into the FH model (Figure 4.23) with the addition of a high precision coaxial coarse/fine movement device and a built-in light source. Meanwhile, Tiyoda released a large-scale photography microscope, the Polyphoto (Figure 4.24) in 1958. This was equipped with two 35mm cameras, a revolving nosepiece fitted with six objective lenses and a built-in Köhler illumination system. These ground-breaking specifications for a microscope were very well received. In 1959, the company launched the R model (Figure 4.25) research microscope together with a specialized photographic device.

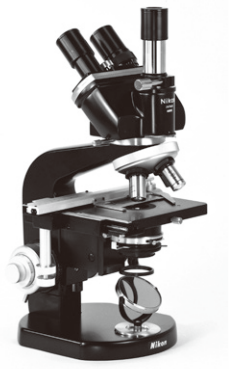

Fig. 4.19 Nikon S [18]

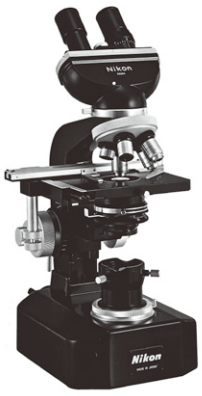

Fig. 4.20 Nikon S-Ke [18]

Fig. 4.21 Olympus DF [17]

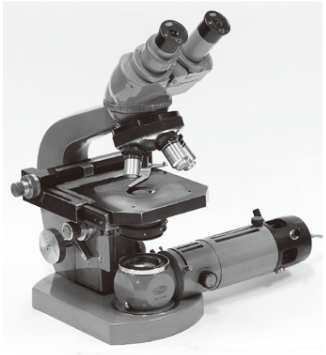

Fig. 4.22 Olympus E [17]

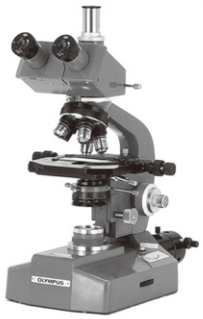

Fig. 4.23 Olympus FH [17]

Fig. 4.24 Tiyoda Polyphoto [7]

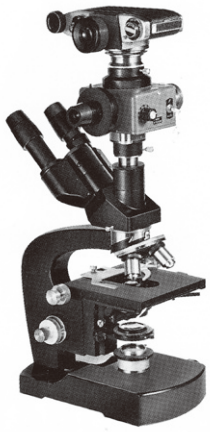

Fig. 4.25 Tiyoda R + Photographic Device [7]

Amidst these Japanese developments in the microscope industry, the National Museum of Nature and Science and the Japan Microscope Manufacturers' Association held the "History of Japanese Microscopes" exhibition from September 8 to October 13, 1963. The exhibition showcased many microscopes from overseas, antique microscopes and Japanese microscopes. The successful event was even visited by the Emperor Shōwa and the Crown Prince. A commemorative book, "Wagakuni no Kenbikyō no Ayumi [History of Japanese Microscopes]" [1] was also published.

Later, as various different methods of observation emerged, large flagship model research microscopes were developed, featuring combinations of various units and multiple functions. Zeiss brought out the Ultraphot, a much larger version of the Photomicroscope, while Leitz released the Ortholux and Reichert the Zetopan. These were world-leading high-end universal microscopes.

Olympus set out to develop a high-end universal microscope to rival these. In 1966, the company launched the Photomax (Figure 4.26). With standard units integrated into the main body of the microscope, this could be used in three ways, as a biological microscope, a metallurgical microscope or a polarized light microscope. It also had over 20 accessories fitted, including automatic exposure photographic equipment, making it truly universal. The objective lenses were also from the newly completed Plan series. In 1967, Nippon Kogaku launched its own high-end universal microscope, the Apophot (Figure 4.27). Capable of trans-illumination, epi-illumination and simultaneous illumination, it used a Köhler zoom system to match the numerical aperture of the objective lenses for trans-illumination. With detachable photographic and film devices included as well as all kinds of observation methods possible, this functional and unique design was produced by an industrial designer.

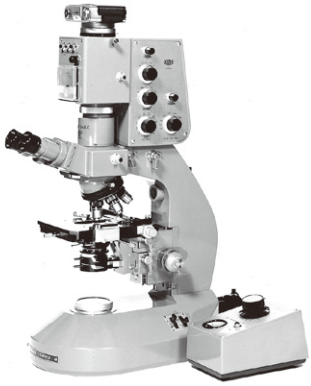

Fig. 4.26 Olympus Photomax [17]

Fig. 4.27 Nikon Apophot [18]

Olympus launched the Vanox AH (Figure 4.28) in 1971, with a stronger frame and greater systematicity. It was also the first Japanese microscope to incorporate an epifluorescence device and epi-illumination phase contrast device. In 1974, the company further developed the systematicity of the E model and released it as the BH series (Figure 4.29). Both the AH and BH had PlanApo series (1975) and LB series objective lenses. In 1976, the company launched the CH series for clinical examination and student training. Japanese high-end research microscopes were improving in function and performance, steadily catching up to their European counterparts.

Tiyoda had spearheaded the Japanese microscope industry, both before and after the war, ever since the commercialization of the M & Katera. The company developed the MT-A research microscope in 1974 and released a further improved model, the MT-B (Figure 4.30), the following year. Significant improvements were also made in operability, such as a super wide field of view (field number 26.5) at magnification levels from 1× to very high, with parfocal viewing also possible. Although it was well received for its unique technological specifications, Tiyoda had ongoing operational difficulties and had to close the factory in 1976. Sakura Seiki took over its operations, but the microscope business did not survive. Sakura Finetek Japan is now in charge of this microscope legacy.

Fig. 4.28 Olympus Vanox AHB [17)]

Fig. 4.29 Olympus BHA [17)]

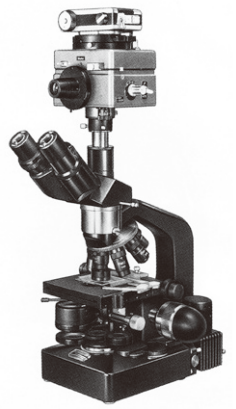

Fig. 4.30 Tiyoda MT-B [7)]

4.6 New Optical Systems and Advances in Biological Microscopes

In the late 1970s, major progress was made with Japanese microscopes. Nippon Kogaku adopted a completely new CF optical system (see 7.4.3) and launched the Microphot V series in 1976 as the successor to its Apophot high-end research microscope. The V series consisted of the Biophot (Figure 4.31) for biological and medical applications, the Metaphot for metallurgical and industrial applications, and the Fluophot for fluorescence microscopy. In July 1978, the company launched the Optiphot (X model, Figure 4.32) and the Labophot (Y model, Figure 4.33) as successors to the standard high-grade L and S models that had been on the market since 1956. With the superior performance of the CF optical system and a novel functional design, these microscopes were well received and significantly boosted the global status of Nikon microscopes.

Fig. 4.31 Nikon Biophot V [18)]

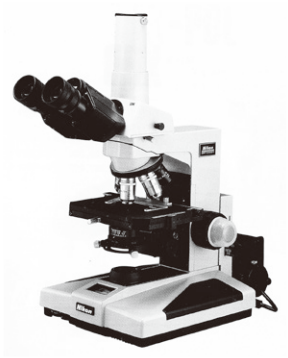

Fig. 4.32 Nikon Optiphot X [18)]

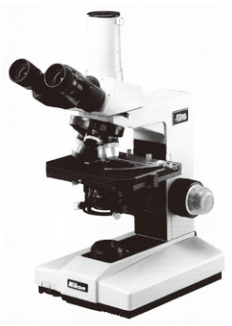

Fig. 4.33 Nikon Labophot Y [18)]

In 1975, Olympus also started working on a completely revised design of its microscope optical system (the LB series, see 7.4.3) and alterations to the microscope bodies in the AH, BH and CH series. However, when Nippon Kogaku announced its CF optical system and V series, Olympus hurriedly made some minor changes to the AH (Figure 4.28) and BH (Figure 4.29) to be able to incorporate the new LB optical system and released the AH-LB and BH-LB in 1978,

thereby preventing any major loss in market share. The development schedule prioritized the later BH model over the flagship AH model, as it had a greater operational impact. Thus, the BH2 series was launched in 1980. There four types of BH2 microscope: the BHS (Figure 4.34) and BHSU, which had a high luminance 12V 100W halogen light, and the BHT and BHTU (Figure 4.35), which were slightly smaller and had a 6V 20W halogen light. The BHSU and BHTU featured a revolving nosepiece that oriented towards the arm. The BH2 became a worldwide best seller, being well received for its high-performance LB optical system and its operability and systematicity. Student microscopes included the Alphaphot YS (Figure 4.36) with a CF optical system, launched by Nippon Kogaku in 1983, and the CH2 (Figure 4.37), launched by Olympus in 1986 with an LB optical system. Major developments were also made in metallurgical (industrial) microscopes following the changes in biological microscopes. These are discussed in 6.4.

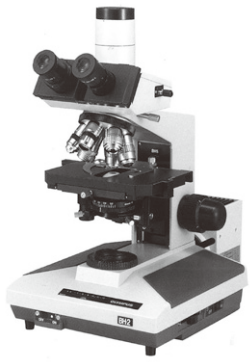

Fig. 4.34 Olympus BHS [17)]

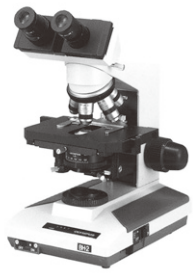

Fig. 4.35 Olympus BHTU [17)]

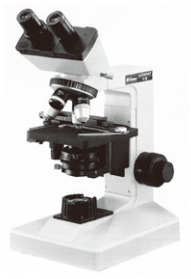

Fig. 4.36 Nikon Alphaphot YS [18)]

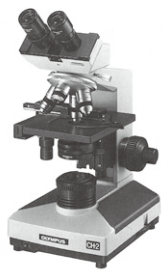

Fig. 4.37 Olympus CH2 [17)]

After developing the BH2, Olympus started working on a completely revised design for the high-end AH (Vanox). The basic concept was to produce a universal photography microscope with high-powered optical performance and as many operations as possible automated or electrified.

1) When changing objective lenses (by pushing a button), the illumination lens changes to match the magnification. At the same time, the aperture stop narrows to around 80% of the numerical aperture of the objective lens and the field stop circumscribes the field of vision. To ensure constant color temperature, changes in brightness are done by ND filter selection without changing the lamp voltage. The above are set automatically and instantly.
2) Selecting the light path and photographic lens for the camera (up to three) and video camera is done electrically by pushing the respective buttons.
3) When photographing under low magnification, the difficulty of focusing due to the film and the eye having different depths of focus is resolved with the world's first microscope autofocus mechanism. This is based on the principle of the image phase detection method (Figure 4.38), in which a chopper situated at the exit pupil area of the photographic lens is rotated at high speed and the movement of the image is used to determine the amount by which the focus is out [21)].
4) A finder optical system is also incorporated to directly determine the photographic range. This is devised with a specially shaped prism to reflect twice and return an erected image, as the image rotates by 30° when the circulating light path returns to the viewing tube prism.
5) Various kinds of accessories are included to ensure universality, such as an epifluorescence unit and a phase contrast unit.

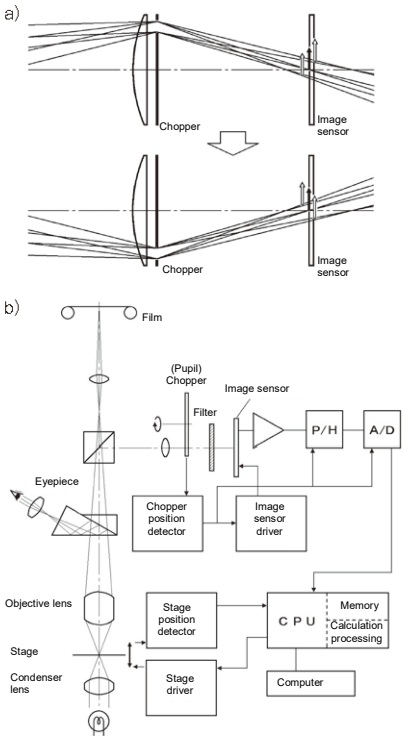

Fig. 4.38 Microscope Autofocus a) Principle and b) Configuration

(created from cited reference 21)

With features such as the illumination system, imaging system (objective lens and eyepiece), photographic system and finder system all included, this was an unprecedented complex optical system for a microscope (Figure 4.39). The AH2 (New Vanox) series was developed to incorporate these features, with three models launched in 1983: the automatic AHBS (Figure 4.40), the electric/manual AHBT and the industrial AHMT. The innovation was welcomed by users, especially those who took frequent micrographs, and took German manufacturers by surprise.

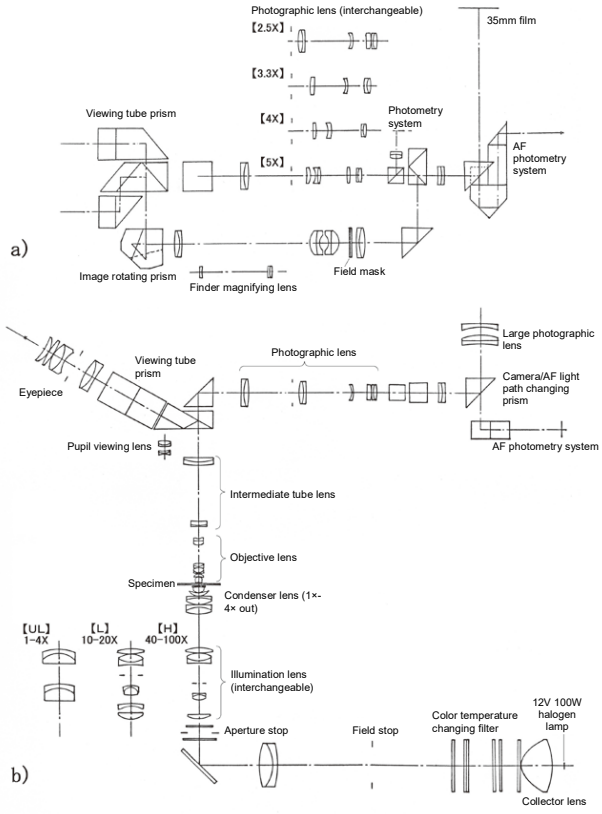

Fig. 4.39 Olympus AHBS Optical Diagram [17]

a) Plan view b) Side view

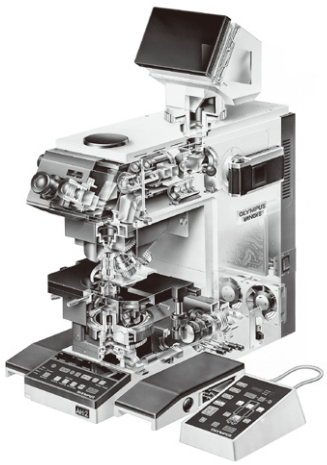

Fig. 4.40 Olympus AHBS and Optical System [17]

In 1985, Nippon Kogaku launched the Microphot FX, a high-end universal photography microscope featuring an automatic exposure function and the NCF series optical system (see 7.4), with improved resolution and contrast than the CF system. In 1988, the company released the Microphot FXA (Figure 4.41), with features including autofocus (image contrast method), an electronic revolving nosepiece and a communication function. The Alphaphot YS2 (Figure 4.42) was also released the same year, followed by the new Optiphot 2 X2 (Figure 4.43) and the Labophot 2 Y2 in 1990.

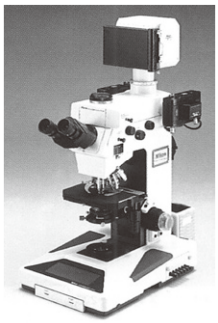

Fig. 4.41 Nikon Microphot FXA [18]

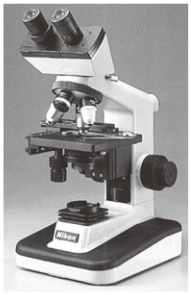

Fig. 4.42 Nikon Alphaphot YS2 [18]

Fig. 4.43 Nikon Optiphot 2 X2 [18]

Thus we see that as a result of Nippon Kogaku and Olympus spurring each other on to remodel their microscopes and develop new optical systems in the late 1970s and further improving the functions and performance of Japanese microscopes, they came on par with their German counterparts, which had dominated the world since the late 19th century.

4.7 Infinity-Corrected Optical Systems and Remodeling of Biological Microscopes

Following the Photomicroscope and Ultraphoto, Zeiss went for a long time without producing any further world-class research microscopes. In 1973, the company released the Axiomat (Figure 4.44), an innovative large-scale microscope. Unlike conventional objective lenses with a mechanical tube length of 160mm, this used a special

infinity-corrected optical system (see 2.4) that allows an infinite mechanical tube length. The main body of the microscope was a solid block that offered a variety of observation methods through different combinations. While the technology was highly regarded, the model was not a great success, as it was very expensive and a little difficult to operate. In 1986, Zeiss launched the highly anticipated Axio series. This was made up of three models: the Axiophot high-end photography microscope (Figure 4.45), the Axioplan research microscope and the Axiotron industrial microscope. These featured a new infinity-corrected optical system called the infinity color-corrected system (ICS). The Axio series had a huge impact on the industry, once again proving Zeiss' technological prowess and heightening the company's reputation. In 1992, Leica brought out a high-end microscope of its own, the DM series (Figure 4.46), featuring its own Delta (Δ) infinity-corrected optical system.

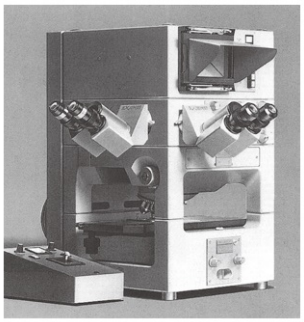

Fig. 4.44 Zeiss Axiomat [20)]

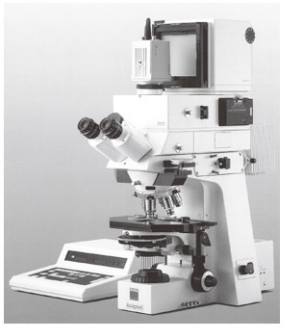

Fig. 4.45 Zeiss Axiophot [20)]

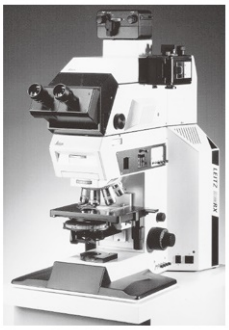

Fig. 4.46 Leica DAS Mikroskop DMRX [22)]

Olympus considered and debated whether it should compete against Zeiss' new optical system by enhancing its LB system and improving functions or by designing a completely new infinity-corrected optical system. In 1988, the company opted for the latter. The concept and characteristics of this new Universal Infinity System (UIS) are discussed in 7.4.4. As well as the UIS, the company also redesigned its microscope bodies, with the successors to the AH2, BH2, CH2 and IMT2 (inverted microscope, see 6.1) named the AX, BX, CX and IX respectively. The new design began with the launch of the BX and UIS in 1993, followed by the AX and IX in 1994 and the CX in 1997. The body shape of each series had an ergonomic Y shape design. There were three models in the BX series: the standard BX40, the high-grade BX50 (Figure 4.47) and the BX60 epifluorescence microscope. Many new units and accessories were also developed, such as a variable tilt angle tube to allow adjustable viewing positions (Figure 2.30d) and various camera adaptors. The AX (Provis) series also included the AX70 research microscope and the fully automated AX80 (Figure 4.48). These superseded the previous BH2 and AH2 models with even greater popularity.

Fig. 4.47 Olympus BX50 [17)]

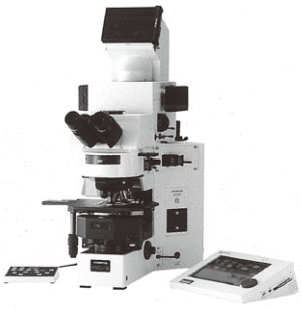

Fig. 4.48 Olympus AX80 [17)]

Nikon changed from its conventional NCF optical system to its new CFI_{60} infinity-corrected optical system in 1996. The objective lens parfocalizing distance (see 2.4) also increased from 45mm to 60mm (see 7.4.4 for details). The company incorporated this system into the new Eclipse biological microscope series, made up of the premium grade E800 (Figure 4.49), the high-grade E600 (Figure 4.50) and the E400, all launched at the same time. In 1997, these were followed by the premium fully automated E1000. The main Japanese high-end biological microscopes had all transitioned to infinity-corrected optical systems by around the year 2000.

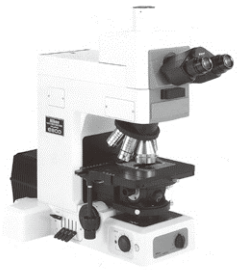

Fig. 4.49 Nikon Eclipse E800 [18)]

Fig. 4.50 Nikon Eclipse E600 [18)]

In 2000, Olympus released the Power BX Plus series, a complete redesign of the BX series. The series had 12 models in total, including industrial microscopes such as the BX51, 52, 61, 62, 41 and 45. Key features included improved functionality in fluorescence imaging and differential interference contrast imaging, computerized control for digital imaging and an ergonomic design that was easier to use. In 2010, the company launched the BX3 series, a further complete redesign of this series. This comprised three models: the BX43, 53 and 63. The BX43 is the efficient viewing model, with a newly developed LED light source offering improved color reproducibility. The BX53 has improved illumination and an ecology mode operated by motion sensor. The BX63 (Figure 5.39) features all kinds of electronic units and touch panel operation.

Fig. 4.51 Nikon Eclipse 90i with DIH [18)]

Fig. 4.52 Nikon Eclipse 55i [18)]

Meanwhile, Nikon launched its Eclipse 80i and 90i upright research microscopes in 1994, as well as the Eclipse 50i and 55i clinical upright microscopes. The 80i featured improved functionality in fluorescence imaging and differential interference contrast imaging, improved illumination and a new digital imaging head (DIH) that integrated the optical systems for the eyepiece, fluorescence device, photography port and zoom. The 90i (Figure 4.51), an electronic version of the 80i, was the premium model in the series, incorporating new software in an easy-to-use digital imaging system. The 50i and 55i (Figure 4.52) offered improved operability as clinical microscopes. The 50i used halogen illumination, while the 55i used LED illumination. In 2011, the company completely redesigned its series, launching the Eclipse Ni research microscope series and the Eclipse Ci clinical microscope series. The Ni series came with a full complement of electronic accessories to further improve operability, as well as better optical performance through the addition of the new CFI Plan Apo λ series objective lenses (see 7.4.5 (4)). The electronic Ni-E (Figure 4.53) and the manual Ni-U were the two models in this series. The Ci series comprised three clinical microscope models: the LED-lit Ci-E (electronic), the manual Ci-L and the halogen-lit Ci-S (manual, Figure 4.54).

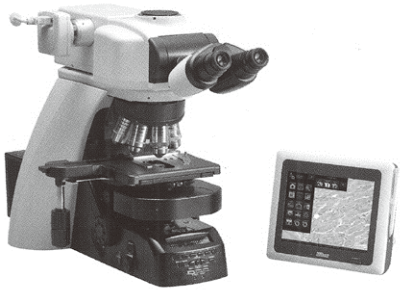

Fig. 4.53 Nikon Eclipse Ni-E [18)]

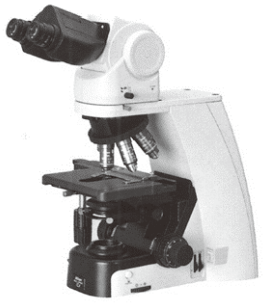

Fig. 4.54 Nikon Eclipse Ci-S [18)]

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16) Supplied by Sakura Finetek Co., Ltd.
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5 Development of Various Microscopy Techniques [1) 2) 3)]

The 20th century has seen the development of various methods of viewing objects by microscope. This has gone beyond simply viewing objects under ordinary natural light to applying various characteristics of light, such as refraction, scattering, diffraction, interference, polarized light and fluorescence, to bring out objects and information that would otherwise be invisible to the naked eye. It is no overstatement to say that no other optical instrument makes use of as broad a range of the properties of light as the optical microscope. This chapter discusses the principles of different microscopy techniques and the history of their development in Japan and overseas.

5.1 Bright Field Microscopy

Bright field microscopy is the most common method used with optical microscopes. The illuminated specimen is enlarged by the objective lens and further enlarged by the eyepiece, and then either displayed or recorded by camera or video. Many specimens are viewed directly (Figure 5.1a) with bright field microscopy. Anything that is too transparent to see easily can be viewed with contrast by narrowing the aperture stop of the condenser. However, viewing more infinitesimal structures requires fixing the specimen in place and slicing off sections with a device called a microtome. Since this causes most specimens to become transparent, a process called staining is used to color the specimen with a special dye. The techniques for this process were established in the late 19th century. Even today, bright field microscopy for observing stained specimens is a common use for optical microscopes, widely used in medical and biological research and examinations (Figure 5.1b,c). However, since the staining method requires the specimen tissue to be fixed in place for coloring, the drawback is that it either destroys the organism or severely impairs its functions, meaning it cannot be used to observe specimens in a living state.

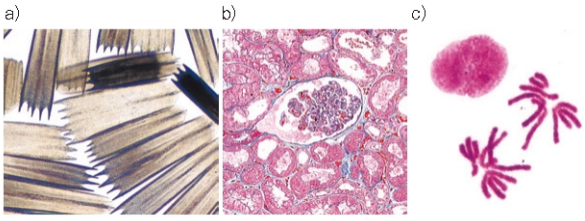

Fig. 5.1 Example Photos of Bright Field Microscopy [1)]

a) Butterfly scales (not stained) b) Kidney (H&E stained) c) Chromosomes (Giemsa stained)

5.2 Dark Field Microscopy

If the light illuminating the specimen is prevented from entering the objective lens, the only light entering the objective lens is that which is reflected, scattered or diffracted from the specimen against a pitch-black field. The advantage is that this requires no fixing in place, staining or other pre-processing of specimens, meaning that specimens can be observed live. This makes it possible to view the presence and movement of colloidal particles (several nanometers in diameter) and bacteria flagella (around 20nm in diameter), far smaller than the microscope resolution limit (around 200nm).

Dark field microscopy is achieved by simply including a dark field condenser, which is designed with a greater numerical aperture (NA) than the objective lens, so that the light only shines on the specimen. Types of dark field condensers include the simple type (Figure 5.2a), which has a ring stop on the light source side of an ordinary large aperture condenser, and special dry (Figure 5.2b) and oil (Figure 5.2c) types. Special condensers concentrate the light on the specimen and have a cardioid or similar spherical surface (Figure 5.2b-c, second reflection). Dark field microscopy using an oil immersion objective with a high NA of around 100× requires an iris diaphragm built into the objective lens itself.

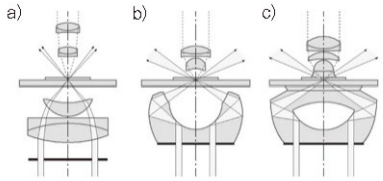

Fig. 5.2 Different Types of Condensers [1)]

a) Simple b) Special (dry) c) Special (oil)

The first dark field condenser is thought to have been a paraboloid reflector made by British engineer F. H. Wenham in 1850. Austrian-born chemist R. Zsigmondy (1865-1929) worked with Zeiss optical researcher H. Siedentopf to invent and develop the ultramicroscope in 1903 for studying colloids. This dark field microscope prototype illuminated the specimen from the side, so that light scattered off the particles of the specimen instead of directly entering the objective lens. Zsigmondy used the ultramicroscope to research colloidal particles far smaller than the resolution limit of the optical microscope and was awarded the Nobel Prize in Chemistry in 1925. Dark field microscopy is still

used to study things in the nanometer order, such as bacteria flagella or gold colloid indications in biological and chemical examinations. Figure 5.3 shows an example photograph using dark field microscopy.

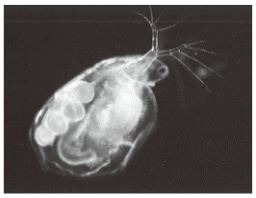

Fig. 5.3 Example Photograph using Dark Field Microscopy (Water Flea) [1)]

5.3 Phase Contrast Microscopy

Light has the physical properties of a wave. When light is sensed by the human eye, photographic film or electronic image sensor, the amplitude is perceived as brightness contrast and the wavelength is perceived as color contrast. However, phase objects that absorb very little of the light passing through them, such as biological tissue sections and culture cells, can be difficult to perceive, as they are colorless and transparent (Figure 5.4). Phase contrast microscopy makes these infinitesimal, colorless and transparent specimens visible by using light diffraction and interference to change the light and dark contrast.

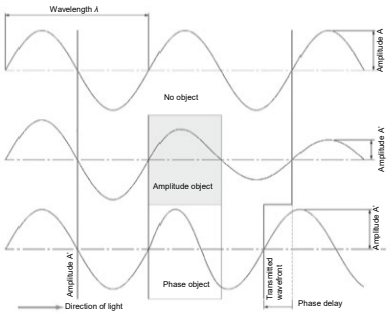

Fig. 5.4 Changes in Light Passing Through an Object [1)]

Phase contrast microscopes are structured as shown in Figure 5.5, with a ring stop at the front focal point of the condenser and a phase plate with a ring-shaped phase film at the conjugate back focal point of the objective lens. Figure 5.6 provides a simple explanation of the principle. The phase-shifted wave P that passes through the phase object can be considered as the sum of the direct wave S that is unaffected by the object and the diffraction wave D. If the difference in phase is small enough at this point, the phase of the diffraction wave D is only delayed by one quarter of a wavelength behind the direct wave S. While direct wave S passes through the phase film, diffraction wave D is diffracted by the phase object and only part of it passes through the phase film. As a result, if the phase of the light passing through the phase film is set to move forward by only quarter of a wavelength, the phases of the direct wave and the diffraction wave differ by half a wavelength and the amplitude I' of the compound wave is less than that of the direct wave (background brightness). That is, a dark contrast image is formed of the phase object, which has a higher refractive index than its surroundings. The contrast can be further increased by having an absorption film on the phase plate and reducing the intensity of the surround wave (Figure 5.6b). This is called positive (or dark) contrast. Similarly, delaying the phase of the light passing through the phase plate by only one quarter of a wavelength causes the phase of the direct wave and diffraction wave to coincide, thereby increasing the compound wave amplitude I'' to greater than that of the direct wave, making the phase object brighter than its surroundings (Figure 5.6c). This is called negative (or bright) contrast. Figure 5.7 shows photographs comparing the two. Phase contrast microscopy is widely used for studying live specimens such as culture cells.

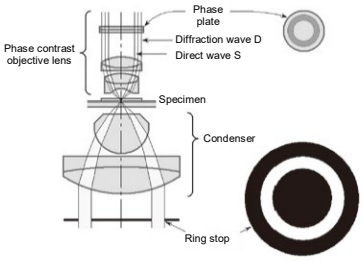

Fig. 5.5 Structure of a Phase Contrast Microscope[1)]

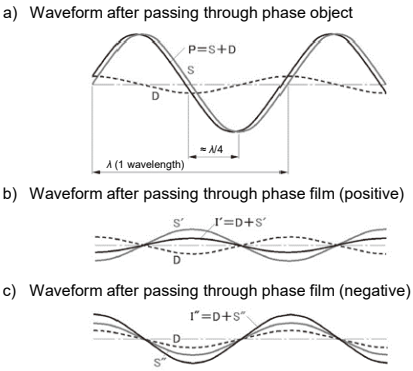

Fig. 5.6 Phase Contrast Principle [1)]

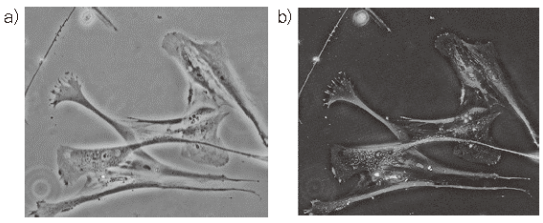

Fig. 5.7 Phase Contrast (fibroblast) [1)]
a) Positive (Dark) b) Negative (Bright)

Dutch physicist F. Zernike (1888-1966) studied Abbe's theory of microscope imaging and published the principle of phase contrast microscopy in 1932. Zeiss completed a phase contrast microscope prototype in 1936 and published a paper on it in 1941, during the Second World War. In 1943, the company also successfully filmed cell division, which gained attention worldwide. As soon as the war was over, the United States began building phase contrast microscopes. In 1953, Zernike was awarded the Nobel Prize in Physics for his work on the ground-breaking method of viewing living things, such as culture cells and chromosomes (so called because they were previously invisible without being stained), in clear contrast.

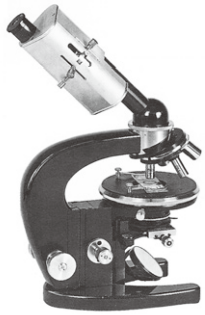

Fig. 5.8 Zeiss Phase Contrast Microscope Prototype [5)]

In Japan, Prof. Kubota Hiroshi of the University of Tokyo Second Faculty of Engineering took note of Zeiss' paper and made recommendations to Tiyoda. Joint research began in 1948 [6)]. Miyata Shōichi of Olympus also took note of Zeiss' paper and began research and development of his own. Prof. Dan Katsuma, then professor at the University of Tokyo Misaki Marine Biological Station in Aburatsubo Bay, Kanagawa (later president of Tokyo Metropolitan University), owned Japan's only phase contrast microscope, an early model made by American company Bausch & Lomb brought over by Dan's American wife, Jean. They were visited so frequently by developers from Tiyoda and Olympus observing and comparing prototypes that it became known as the "Misaki pilgrimage". In April 1949, Tiyoda presented Japan's first phase contrast microscope to the Japan Society of Applied Physics[7)] and caused a sensation (Figure 5.9). Olympus put out a 30th anniversary publication in October that year, although its adoption of a vacuum deposition method that made it possible to mass produce phase film (Figure 5.10) gained more attention [8)]. In December the same year, the Phase Contrast Microscope Symposium (later Research Society) was founded. Through its publication [9) 10)], the group made significant contributions to users and manufacturers, such as spreading information on the utility of phase contrast microscopes and how to use them. Nippon Kogaku and other Japanese microscope manufacturers also began developing and producing phase contrast microscopes. These microscopes spread rapidly throughout Japan, contributing to developments in biology.

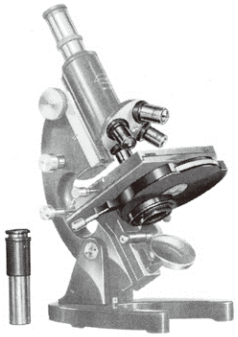

Fig. 5.9 Tiyoda Phase Contrast Device PI [6)]

Fig. 5.10 Olympus Phase Contrast Device PA [8)]

5.4 Polarized Light Microscopy [11) 12) 13)]

The polarized light phenomenon, whereby light polarizes in the direction of oscillation (see 2.1.3 (3)), was discovered in 1808 by French physicist E. Malus (1775-1812) observing light reflected off a palace window through a birefringent calcite crystal. In 1828, Scottish geologist W. Nicol (1770-1851) invented the Nicol prism (Figure 2.17) by cementing together two calcite prisms, enabling him to turn natural light, which has a random oscillation direction, into linearly polarized light with a regular oscillation direction. He also devised other polarizing equipment and studied other rocks and minerals. In 1834, British scientist H. F. Talbot (1800-1877, also known for his invention of the calotype photographic process) invented the polarized light microscope using Nicol prisms in the illumination optical path and the viewing optical path. In 1851, British geologist H. C. Sorby (1826-1908), a known pioneer in specimen staining, began using a polarized light microscope to study the crystalline structure of rocks and minerals. This work was continued by German geologists F. Zirkel (1838-1912) and H. Rosenbusch (1836-1914), laying the foundations for the golden age of petrography. However, the Nicol prisms used in polarized light microscopes were expensive and had a narrow viewing angle. These issues were resolved in 1929 with the invention of a thin polarizing plate by American physicist E. H. Land (1909-1991), who named his invention "polaroid". Various improvements followed, resulting in an inexpensive and highly efficient polarizing element, used in

sunglasses and photographic filters and eventually becoming standard in polarized light microscopes.

The basic structure of a polarized light microscope comprises a bright field microscope with two polarizing plates, the polarizer on the condenser side and the analyzer on the objective lens side. The polarizer and analyzer are placed so that their direction of oscillation is at right angles (called crossed nicols), allowing clear viewing of an optically anisotropic (birefringent) material against a darkened background. There are two main types of polarized light microscopy. The basic method involves lowering the numerical aperture of the illumination (placing the top lens of the polarization condenser out to the side) and viewing the birefringence of the specimen with a low-magnification objective lens of around 4-10×. This is called the orthoscope (Figure 5.11a) and uses test plates (wave plate, sensitive tint plate) and a compensator (compensation plate). The other method involves increasing the numerical aperture of the illumination (placing the top lens of the polarization condenser into the optical path) and viewing the interference fringes (representing the optical characteristics of the crystal specimen) produced near the back focal point of the high NA objective lens. This is called the conoscope (Figure 5.11b) and uses a Bertrand lens in the viewing optical path to make it easier to view the conoscopic image. Professional polarized light microscopes also have other components in addition to the polarizer and analyzer, including a rotary stage to precisely determine the orientation of the sample, test plates for changing the polarization state, compensators (compensating plates) to measure the birefringence of the specimen and an eyepiece with cross lines.

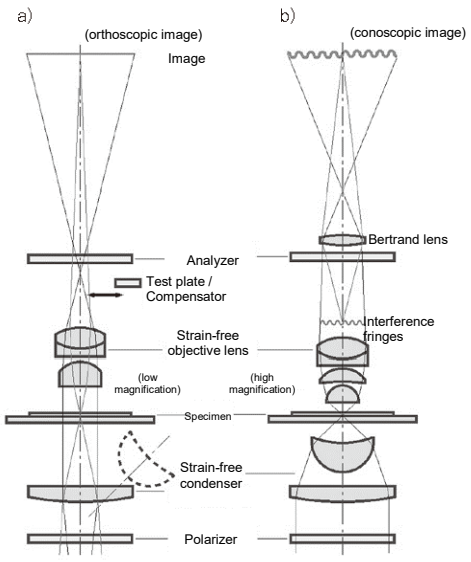

Fig. 5.11 Polarized Light Microscope Structures [1)]

a) Orthoscope b) Conoscope

The objective and condenser lenses between the polarizer and analyzer also need to be specially designed for polarization to prevent any optical strain that can cause birefringence. Test plates can include quarter wave plates, which change the polarization from linear to circular (or vice versa), half wave plates, which change the direction of linear polarization or the rotation of circular polarization, and sensitive tint plates (or one wave plates), which change slight differences in birefringence into clear colors such as yellow, red or blue. These plates are made by sandwiching sections of mica or crystal between two glass plates, although more are now being made from polymer materials. The compensator is a device used to measure the birefringence (retardation, see 2.1.3 (3)) of the specimen. These vary by measurement range and precision, and include the Berek (Figure 3.11) type, the Sénarmont type and the Bräce-Köhler type. Figure 5.12 a) shows an orthoscopic image (biotite gneiss, using a sensitive tint plate), b) shows a conoscopic image of a uniaxial crystal (calcite) and c) shows a conoscopic image of a biaxial crystal (topaz).

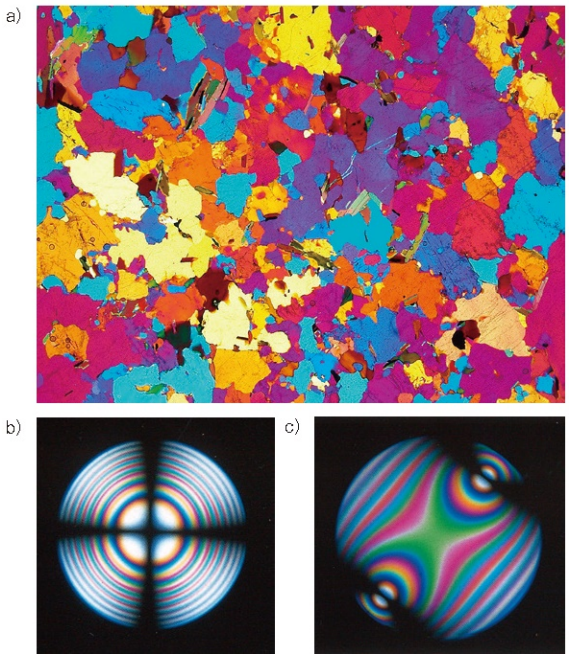

Fig. 5.12 Polarized Light Photomicrographs [1)]

a) Orthoscopic image: biotite gneiss
b) Conoscopic image: calcite (uniaxial crystal)
c) Conoscopic image: topaz (biaxial crystal)

As previously mentioned, polarized light microscopes have mainly been used for studying rocks and minerals. As such, they are also called ore microscopes. In Japan, Takachiho and Shimadzu jointly produced a mineral microscope (Figure 5.13) in 1925. In 1949, the Polarization Microscope Committee was established (with Prof. Emeritus Tsuboi Seitarō of the University of Tokyo as president) with

the aim of producing proper polarized light microscopes in Japan. In 1951, Nippon Kogaku released its POB polarized light microscope, funded by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture. This was followed by the proper POH polarized light microscope (Figure 5.14) in 1952 [14]. Olympus launched the POM (Figure 5.15) in 1960, followed by the POS in 1963. Since then, each company has produced its own polarized light microscope system as part of its lineup of units.

Fig. 5.13 Olympus/Shimadzu Ore Microscope [4]

Fig. 5.14 Nippon Kogaku POH Polarized Light Microscope [17]

Fig. 5.15 Olympus POM Polarized Light Microscope [4]

Objective and condenser lenses for polarized light microscopes are designed to prevent optical strain. However, rotation of polarization is inevitable due to the spherical surface of the lens. The amount of rotation is proportionate to the numerical aperture of the objective lens. The highest amount of light leakage occurs at an angle of ±45° to the oscillation direction of the polarizer. Even after the light passes through the analyzer, the pupil of the objective lens is not completely dark, which causes the formation of a dark cross, known as an isogyre, and deterioration in polarization performance. This issue was resolved with a rectifier produced by Inoue Shinya et al. in 1957 [15) 16)]. As shown in Figure 5.16, the rectifier comprises a combination of a half wave plate paired with a lens with no refractive power. The rectifier compensates for the rotation of the polarized light from the lens and is situated with the condenser on the object side (Figure 5.17). This allows a highly polarized optical system. Inoue also developed video microscopy using image processing to amplify images with poor contrast. By making faint birefringence visible for cell division, he was able to identify the presence of spindles and the cell division mechanism. The rectifier was commercialized by Nippon Kogaku in 1975 with the Apophot (Figure 5.18).

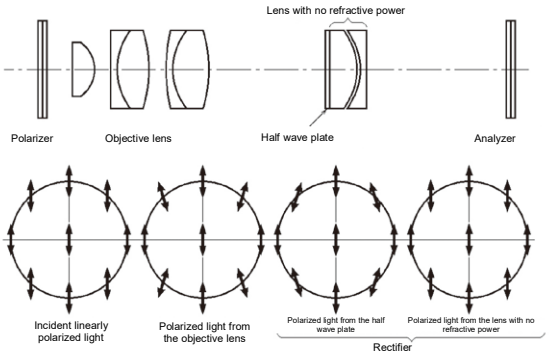

Fig. 5.16 Explanatory Diagram of a Rectifier (objective lens section only)

(created with reference to cited reference 15)

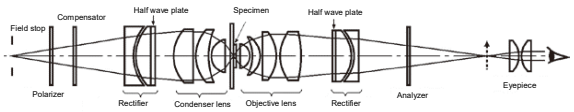

Fig. 5.17 Rectifier Optical System

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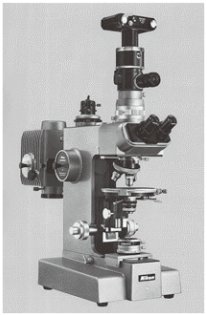

Fig. 5.18 Nippon Kogaku Rectifier Microscope [17]

Recent methods have been proposed for enabling microscopes to directly compensate for deterioration in polarization using a polarizing liquid crystal element combined with image processing technology. It is now becoming possible to sharpen the viewing image and take various quantitative measurements.

5.5 Differential Interference Contrast Microscopy (DIC)

Differential interference contrast (DIC) microscopy makes it possible to see phase information of a colorless and transparent object using interference color contrasts on polarization interference. Figure 5.19 shows the structure and principle of how this works.

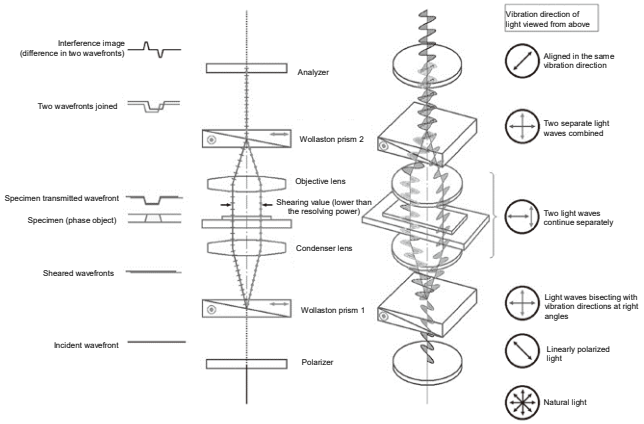

Figure 5.19 Structure and Principle of Differential Interference Contrast Microscope

(created with reference to cited reference 19)

The linearly polarized light passing through the polarizer below the condenser (vibration direction shown to the right of the image) first travels into the first Wollaston prism (Figure 5.20a, a combination of two crystal prisms joined together that are cut at a narrow wedge angle to have a specific direction) and splits into two linearly polarized light waves with vibration directions at right angles to each other. The two light waves pass through the phase object, undergo wavefront deformation and then pass through the objective lens before being combined again by the second Wollaston prism. The analyzer aligns the vibration direction, causing interference corresponding to the difference in the two wavefronts. At this point, if the separation (shearing value) between the two light waves passing through the phase object is less than the resolving power of the objective lens, the image does not double up and has an interference contrast corresponding to the phase difference (the differential coefficient of the transmitted wavefront). As a result, differential interference contrast images have a relief-like three-dimensional feel. This method of differential interference contrast microscopy was invented by British microscope designer F. Smith and French physicist M. Françon in 1947. The first and second Wollaston prisms must be located at the front focal point of the condenser lens and the back focal point of the objective lens, respectively. However, this is difficult in practice, as the back focal point of many objective lenses falls inside the lens itself. In 1952, Polish and naturalized French physicist G. Nomarski (1919-1997) resolved this with the invention of the Nomarski prism (Figure 5.20b), which has one of the Wollaston prism optic axis on an angle [18]. The Nomarski system is the main type of differential interference microscope currently used. Nomarski DIC microscopes have respective prisms for the condenser lens and the objective lens, although this can be done in two ways. One way is to have a prism on the condenser side for each objective lens and one shared prism on the objective lens side. The other way is to have the prisms on the condenser side categorized by the numerical aperture of the objective lens and have separate prisms on the objective lens side. Adjusting the background contrast can be done either by shifting in the lateral direction of the prism on the objective lens side or by the Sénarmont method, which includes a polarizer that rotates with a quarter wave plate below the condenser. These microscopes are most commonly used with a gray contrast background, as this makes the specimen the most visible, although they can be further adjusted using sensitive color to give bright yellow, red or blue contrast. The Nomarski prism is made of crystal material and needs to be polished with minute-level accuracy for the optic axis and second-level accuracy for the wedge angle. This makes it one of the most difficult optical components to manufacture. Figure 5.21a shows a DIC photomicrograph of a diatom. Figure 5.21b shows a comparison photomicrograph taken using a phase contrast microscope.

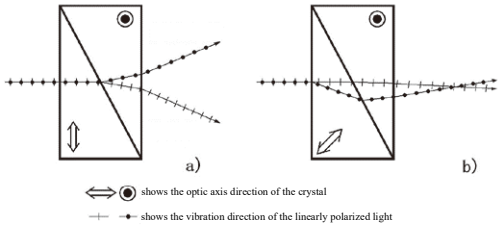

Fig. 5.20 a) Wollaston Prism and b) Nomarski Prism

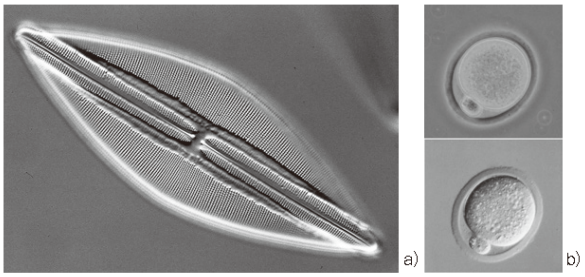

Fig. 5.21 DIC Photomicrographs [1]

a) Diatom b) Rat embryo (top: phase contrast; bottom: DIC)

The Nomarski DIC device for microscopes was commercialized by Zeiss in 1965 [20]. In Japan, Yamamoto Tadaaki of Nippon Kogaku, together with Françon, published their own theory on differential interference contrast in 1962 [21) 22)]. In 1966, Nippon Kogaku released the T model biological DIC microscope (transmitted light type, Figure 5.22a) and the R model metallurgical DIC microscope (reflected light type, Figure 5.22b). Meanwhile, Union Optical obtained a license for the Nomarski system and developed it for industrial microscopes in 1971 (Figure 6.54). Olympus also obtained a sublicense and launched its own Nomarski interference contrast (NIC) device in 1973 for industrial microscopes (Figure 6.55) and in 1974 for biological microscopes. These were used with the Vanox (AH) and the BH. Figure 5.23 shows a NIC device for the BH. From the left, the items are an intermediate tube (Nomarski prism and analyzer on the objective lens side), a centering telescope, a condenser and three Nomarski objective lenses.

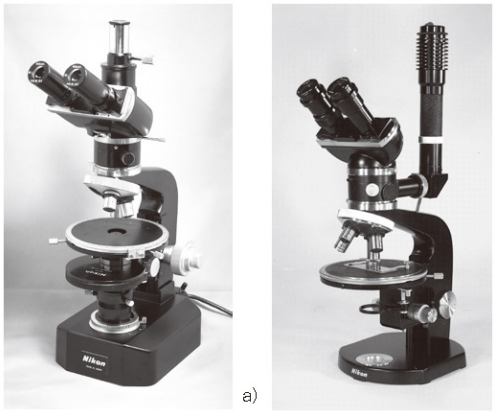

Fig. 5.22 Nippon Kogaku Yamamoto-Françon DIC Microscopes [17)]

a) Transmitted light type S-Ke-T b) Reflected light type S-R

Fig. 5.23 Olympus Differential Interference Contrast Device BH-NIC [4)]

Since biological DIC microscopes make it possible to view infinitesimal living phase objects in clear contrast, they are often used for similar purposes as phase contrast microscopes. However, differential interference contrast microscopy requires polarized light, so the condenser, specimen and objective lens must produce as little optical strain as possible, meaning specimens in plastic containers cannot be used. Nevertheless, unlike phase contrast microscopy, it allows viewing of thick specimens without difficulty and without a halo appearing. The two systems of microscopy have complementary image characteristics and are used according to purpose as required. In recent years, microscopes are being designed in response to user needs, such as having prisms with different shearing values, depending on specimen thickness.

5.6 Modulation Contrast Microscopy (MC)

Another method for viewing phase objects is modulation contrast (MC) microscopy (also called relief contrast microscopy) [23)], devised by American R. Hoffmann in 1975. As shown in Figure 5.24, this is structured with a rectangular slit off the optical axis placed at the front focal point of the condenser lens and a modulator placed at the conjugate back focal point of the objective lens. The modulator has three areas, bright (B), gray (G) and dark (D), as shown in the figure. The image from the slit is adjusted so that it is projected into the G area. Polarizing plate P1 is attached to the slit and P2 is below it. Rotating P2 alters the width of the slit and the amount of light, making it possible to adjust the contrast of the image. If the specimen has a low refractive index gradient, light refracts from the slit into the B or D areas of the modulator. Adding a light-dark contrast to that part produces an image with a three-dimensional feel, like DIC microscopy (Figure 5.25). Modulation contrast microscopy has an advantage over phase contrast microscopy in that there are no image halos, and an advantage over DIC microscopy in that it is possible to use plastic containers. Although the resolving power is lower than that of DIC microscopes, it is comparatively lower priced and is steadily growing in popularity, particularly for studying culture cells.

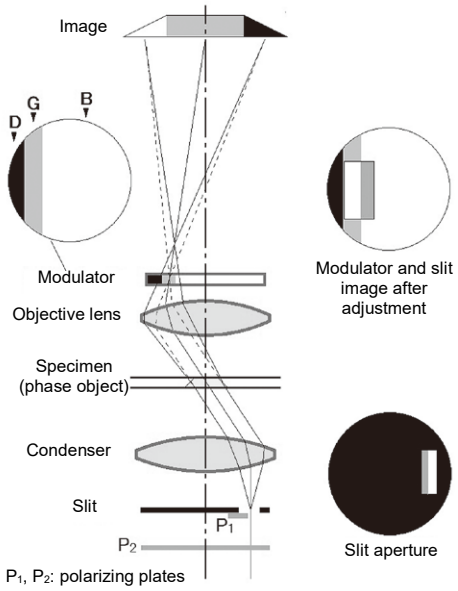

Fig. 5.24 Modulation Contrast Principle [1)]

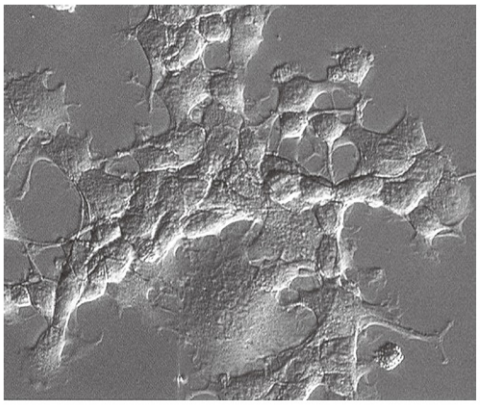

Fig. 5.25 Modulation Contrast Image (nerve cell) [1)]

5.7 Fluorescence Microscopy

5.7.1 Transmitted Light Fluorescence Microscopes

Fluorescence is a type of photoluminescence, whereby light (ultraviolet or visible) energy is absorbed (excitation) and released. Photoluminescence with a short (near non-existent) light lifespan after the excitation stops is called fluorescence (photoluminescence with a long light lifespan is called phosphorescence). Fluorescence has a longer wavelength than the excitation light (Stokes law, 1852) and is also far weaker. A. Köhler of Zeiss built an ultraviolet microscope in 1904 that used objective lenses made from a combination of fluorite and quartz to improve the resolving power. Prompted by this, H. Lehmann et al. worked on developing a fluorescence microscope, which Zeiss announced in 1913. Reichert was quicker off the mark, having commercialized the fluorescence microscope in 1911 [24)]. At first, fluorescence microscopes were mainly used to view autofluorescence (also called primary fluorescence) emitted by microbes and plant tissues. In 1933, Austrian researcher M. Haitinger discovered fluorochromes and developed a fluorescent staining technique of staining certain parts of tissues or cells with fluorochromes and viewing the secondary fluorescence. Many types of fluorescent dyes were later discovered, expanding the practical applications for fluorescence microscopes. In 1941, American immunologist A. H. Coons discovered a method of staining antibodies specifically bound to antigens with fluorochrome and then identifying those antigens using a fluorescence microscope. The establishment of a fluorescent antibody technique using fluorescein isothiocyanate (FITC) in 1950 [25)] led to major breakthroughs in immunology. Thus, through the development of various fluorochromes, the fluorescence microscope has become an essential part of the latest medical and biological research.

Japanese fluorescence microscopes began with the release of an ultra-high voltage mercury lamp illumination system by Nippon Kogaku in 1953. This was followed by the Tiyoda H-200 in 1954 and the Olympus HLS in 1963. The first specialized fluorescence microscopes were the Tiyoda FM-200A (Figure 5.26) and the Nippon Kogaku S-FS (Figure 5.27), both launched in 1965, followed by the Olympus FLM (Figure 5.28) in 1970, in response to growing demand, such as fluorescent antibody techniques.

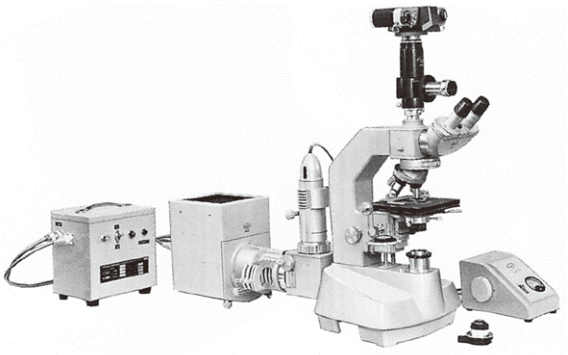

Fig. 5.26 Tiyoda Fluorescence Microscope FM-200A [6)]

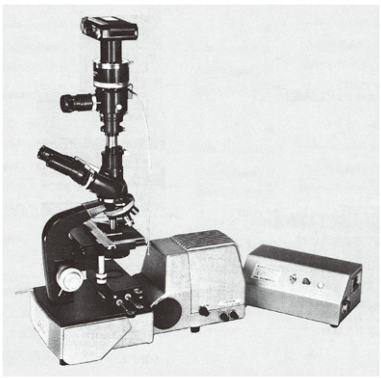

Fig. 5.27 Nikon Transmitted Light Fluorescence Microscope S-FS [17)]

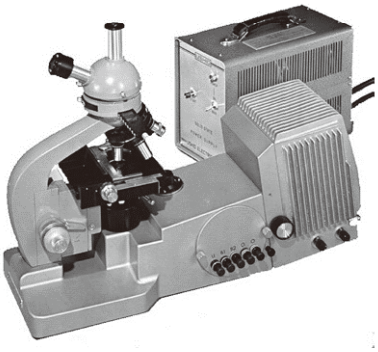

Fig. 5.28 Olympus Transmitted Light Fluorescence Microscope FLM [4)]

Figure 5.29 shows the optical path of a transmitted light fluorescence microscope. Figure 5.30 shows the characteristics of the specimen excitation light absorption spectrum and the accompanying fluorescence spectrum (FITC example, intensity normalized respectively). Since fluorescence is far weaker than excitation light, an ultra-high voltage mercury lamp is often used for illumination, as it has a strong bright line from near ultraviolet to visible light. The excitation light irradiating the specimen is cut from the viewing optical path using a dark field condenser so that only the fluorescence is observed. To completely isolate the overlap between the excitation and fluorescence spectra, an excitation filter is placed on the illumination side to select the excitation wavelength, while a barrier filter is placed on the viewing side to cut out any excitation light entering the optical path and only transmit the fluorescence wavelength (Figure 5.30, thin line). The excitation filter in the figure is an interference filter, which had just begun to be manufactured at the time. Viewing at high magnification required oil immersion between the objective lens and dark field condenser and the specimen. As the immersion liquid could not have any autofluorescence, glycerol (refractive index 1.450) was used in place of regular oil (refractive index 1.518). To cut out the excitation light in dark field microscopy, the glycerol objective lens had a built-in iris diaphragm to lower the numerical aperture (NA) when viewing.

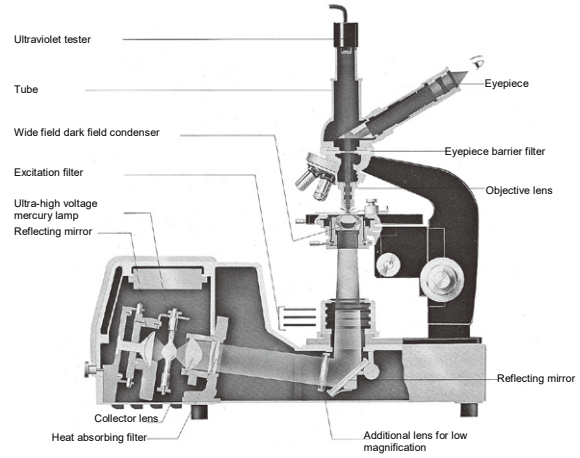

Fig. 5.29 Optical Path of a Transmitted Light Fluorescence Microscope (Nikon S-FS)

(partially revised from cited reference 26)

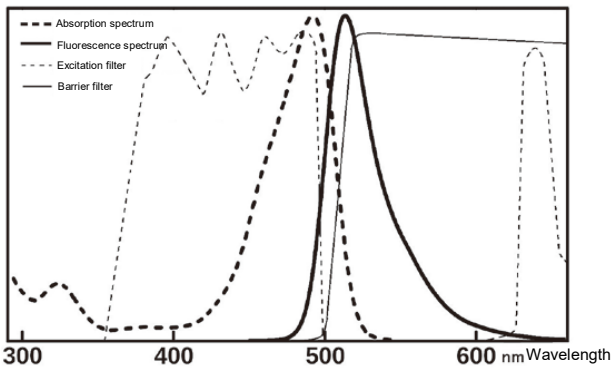

Fig. 5.30 FITC Absorption/Excitation Spectra and Excitation/Barrier filter Spectrum Characteristics

5.7.2 Epifluorescence Microscopes

Dutch scientist J. S. Ploem (1927-) published a method of epifluorescence illumination incorporating a dichroic mirror to reflect short wavelength light and transmit long wavelength light in 1967 [27]. Shortly afterwards, Leitz released an epifluorescence device with the brand name Ploemopak, which had four excitation units that could be switched easily by rotating. This epifluorescence illumination system had many advantages over transmitted light systems.

1) Since the objective lens also served as the condenser, the NA and illumination scope of the excitation illumination was identical to the NA and viewing scope of the objective lens. This made for a bright, high resolution fluorescence image with limited photobleaching (phenomenon of gradually decreasing fluorescence intensity under excitation light).
2) Transmitted light systems used highly viscous immersion liquids such as glycerol on the dark field condenser side as well, making them more difficult use. Epifluorescence systems alleviated this difficulty somewhat by only having this on the objective lens side.

However, because the excitation light shines directly on the objective lens in epifluorescence systems, the objective lens must have as little autofluorescence as possible, otherwise the background of the fluorescence image becomes brighter and the contrast decreases. Accordingly, the objective lens must be made from nonfluorescent glass.

To increase the brightness of the fluorescence, it is preferable for the objective lens to have a high NA and low chromatic aberration. Once companies overcame these hurdles, the epifluorescence microscopes they developed became the mainstream models that have continued to the present day.

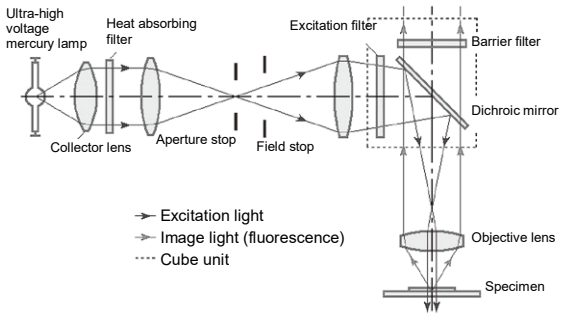

Fig. 5.31 Epifluorescence Microscope Structure [1]

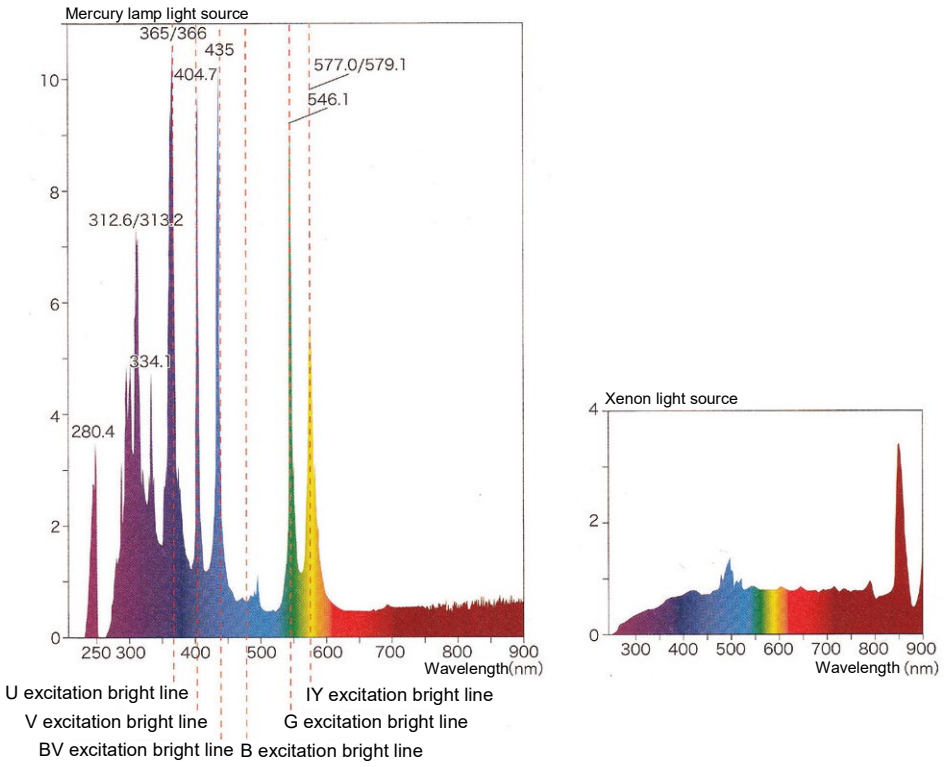

Fig. 5.32 Ultra-High Voltage Mercury Lamp and Xenon Lamp Spectral Properties [4)]

The following outlines the structure of epifluorescence microscopes, based on Figure 5.31. The epifluorescence illumination device is placed between the base and the tube. Light from the light source passes through the excitation filter that selects the wavelength, is reflected by the dichroic mirror through the objective lens to illuminate (excite) the specimen. Fluorescence from the excited specimen passes through the objective lens and transmits the dichroic mirror. Although most of the excitation light is cut out at this point, only the fluorescence passes through the barrier filter to form the fluorescence image to be viewed or recorded. The excitation light wavelength is selected according to the specimen and the fluorochrome. Although ultra-high voltage mercury lamps have been the main light source, improvements in optical systems and image sensor sensitivity have seen an increase in the use of xenon lamps, which have a broader wavelength range.

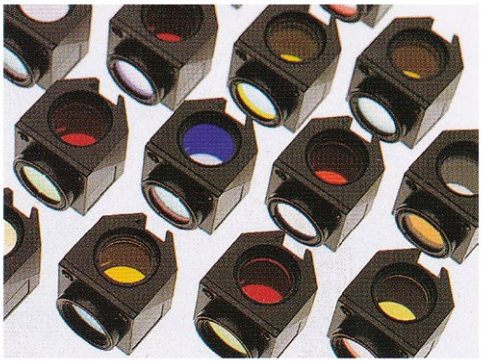

Fig. 5.33 Fluorescence Cubes [4)]

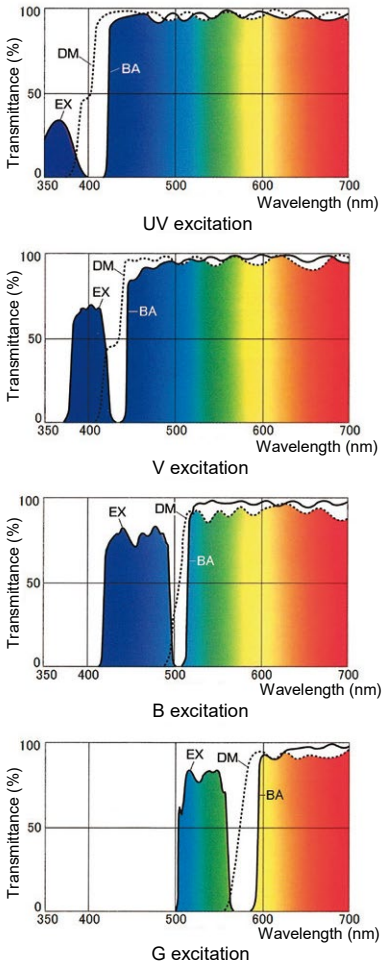

Fig. 5.34 Spectral Properties of Different Excitation Types [1)]

Figure 5.32 shows the spectral properties of both lamps. The main types of excitation include U (365nm), V (405nm), BV (436nm), B (490nm) and G (546nm) (main excitation wavelength shown in brackets). The excitation filters, shown by the dotted lines in the figure, combine with the dichroic mirror and the barrier filter to form a cube unit (Figure 5.33). These combinations can be interchanged easily, according to the different types of excitation. Figure 5.34 shows the properties of the different excitation filters (EX) for U, V, B and G, the dichroic mirror (DM) and the barrier filter (BA). Figure 5.35 shows fluorescence image photographs using U, B and G excitation. Recent developments in interference filter technology have made it possible to produce systems that reflect/transmit several different wavelengths. This makes it possible to view fluorescence from double or triple excitation using different fluorochromes at the same time (Figure 5.36).

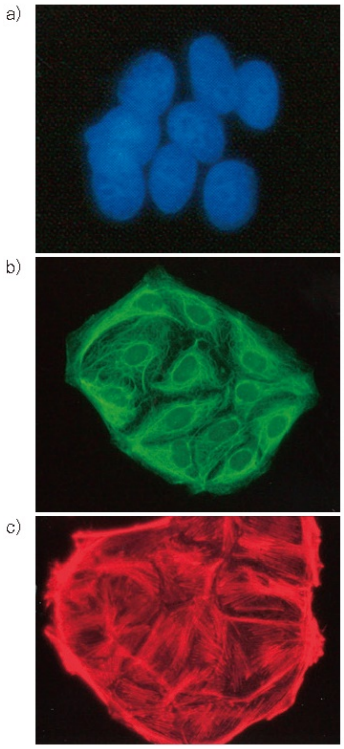

Fig. 5.35 Epifluorescence Photomicrographs Using Different Excitation Types [1)]
a) U excitation b) B excitation c) G excitation

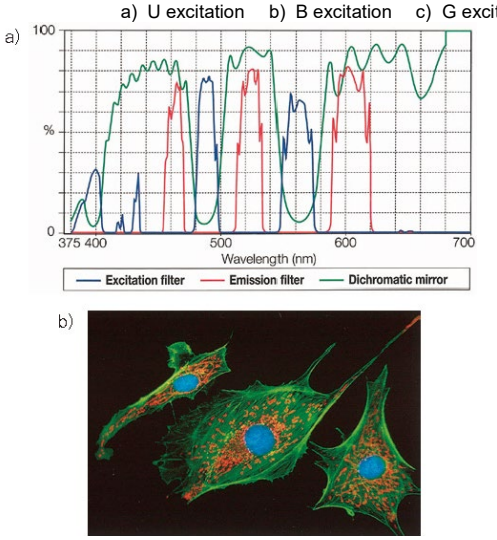

Fig. 5.36 a) Spectral properties of a 3-band filter for U, B and G excitation (blue), dichroic mirror (green) and barrier filter (red) [4)]
b) Fluorescence photomicrograph of triple excitation filter for U (DAPI stain, cell nucleus), B (FITC stain, microtubule) and G (TRITC stain, actin filament) [1)]

The first Japanese epifluorescence microscope was released by Olympus in 1973 as an attachment unit for the Vanox (AH) (Figure 5.37). Relatively low autofluorescence objective lenses were used. Olympus also developed a specialized 100× objective lens using a specially developed silicon oil (less viscous and easier to use than glycerol) for the immersion liquid. In 1976, Nippon Kogaku launched the Fluophot epifluorescence microscope (Figure 5.38) with a special CF UV-F epifluorescence objective lens (Figure 7.22d). As epifluorescence microscopes gained importance in fields of research, biological microscopes with built in epifluorescence devices began appearing in product lineups. Figure 5.39 shows the Olympus BX63, released in 2010.

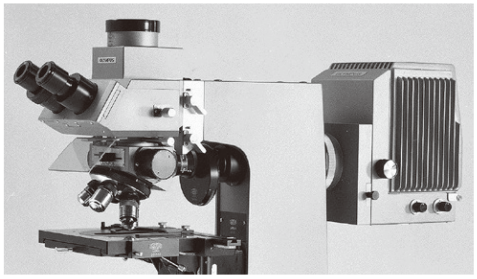

Fig. 5.37 Olympus Epifluorescence Device AH-RFL [4)]

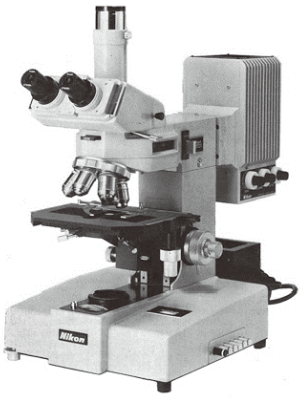

Fig. 5.38 Nikon Fluophot [17)]

Fig. 5.39 Olympus BX63 [4)]

5.7.3 Example Applications of Fluorescence Imaging [28) 29)]

As mentioned above, as successive new fluorochromes and fluorescent proteins were developed for fluorescence microscopes, new phenomena came into view that had never been seen before. Some typical examples are given below.

1) Shimomura Osamu (1928-) discovered green fluorescent protein (GFP) in the crystal jelly Aequorea Victoria in 1962 [30)] and identified its luminescence mechanism. GFP demonstrated outstanding properties in detecting specific single molecules when used in live cells and tissue. In 1992, M. Chalfie (USA) introduced a GFP coded gene to E. coli and nematodes, successfully expressed GFP in live cells. After that, GFP spread rapidly as an effective technology for observing biological activity at molecular level. Shimomura and Chalfie were awarded the Nobel Prize in Chemistry in 2008, together with R. Y. Tsien (USA), who identified the structure and mechanism of GFP. Successive other fluorescent proteins have been discovered since, such as BFP (blue), CFP (cyan), YFP (yellow) and RFP (red).
2) Calcium ion (Ca^{2+}) controls various cell functions and its dynamic state is essential to understanding biological functions. In the 1960s, a method was reported of measuring changes in Ca^{2+} by directly inserting a fluorescent probe into muscle fiber. In the 1980s, many more fluorescent probes were developed, and the method became essential for visualizing the dynamic state of Ca^{2+} in cells. When Fura-2, a typical fluorescent probe, binds with Ca^{2+}, its fluorescence intensity (510nm) under a 380nm excitation light decreases, but increases under a 340nm excitation light. Measuring the fluorescence intensity ratios makes it possible to determine the Ca^{2+} concentration. Another fluorescent probe, Indo-1, has a fluorescent spectrum under a 340nm excitation light that changes depending on the Ca^{2+} concentration. Comparing the fluorescence intensity at 480nm and 420nm determines the Ca^{2+} concentration. There are many other fluorescent probes for measuring Ca^{2+} with one wavelength for excitation and one wavelength for photometry.
3) Fluorescence in situ hybridization (FISH) is a method for determining where a particular DNA segment is in a chromosome. The chromosome (DNA) is fixed to a microscope slide and reacted with a probe marked with a fluorescent reagent to form a hybrid, while viewing under fluorescence microscopy to determine the location and number of copies. Since the method was published in 1986, it has been widely used in biomedical fields for DNA mapping and detecting genetic abnormalities, as it is a very simple method yet is highly accurate in analysis.

Total internal reflection fluorescence microscopy (TIRFM) is a new method of fluorescence microscopy. When the incident light is shone from a medium with a high refractive index to a medium with a low refractive index at an angle of incidence greater than a critical angle, total internal reflection occurs. At this time, a very small amount of light leaks to the medium on the low refractive index side at the interface; this is called evanescent light (or near field light). TIRFM uses evanescent light as excitation light to excite only the fluorescent molecules in a limited area of around 150nm on the cover glass (or specimen surface). This allows viewing with little fluorescence from the background and high S/N ratio. Figure 5.40 shows this structure. The critical angles of total internal reflection for water (refractive index 1.33) and cells (refractive index 1.37-1.38) on a cover glass (refractive index 1.52) are 61° and 64.3° respectively, which equate to objective lens NAs of 1.33 and 1.37. Since even high-end plan apochromats at 60× and 100× have a NA of 1.40, there is very little area for total reflection. As such, a NA 1.49 objective lens has been developed especially for TIRFM. An objective lens has also been developed that combines with a special high refractive index oil and cover glass to give a NA of 1.7 (see 7.4.5 (4)). A TIRFM using prism illumination method was announced in 1989, followed by the development of the special high NA objective lenses described above, the main TIRFM system using the objective illumination method that can be combined with other devices and operated easily from above.

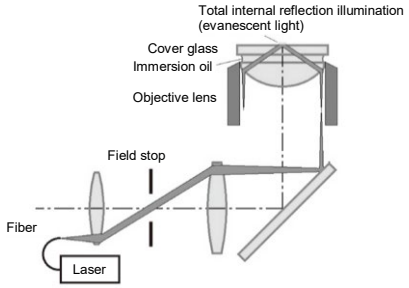

Fig. 5.40 Structure of a Total Internal Reflection Fluorescence Microscope (inverted)

(created with reference to cited reference 31)

Fluorescence microscopy has thus become an essential tool for research, development and inspection in biomedicine and other cutting-edge fields, due to various developments in fluorescence probes and rapid progress in new viewing methods, ultra-sensitive image sensors and image processing software. This rapid progress in fluorescence microscopy was aided by laser scanning microscopes, multi photon excitation microscopes and super resolution microscopes, discussed in 8.1-8.3.

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6 Development of Various Types of Microscopes

Chapter 4 discussed the birth and development of optical microscopes in Japan with a focus on biological upright microscopes. As mentioned in 2.7, there are various types of microscopes with different forms and uses. This chapter discusses inverted microscopes, stereomicroscopes, educational microscopes and metallurgical microscopes (including industrial microscopes) and their development in Japan.

6.1 Inverted Microscopes

As mentioned in 2.7.2, inverted microscopes are a type of microscope in which the specimen is viewed from underneath, with the objective lens located under the stage. For transmitted light systems, the light source, condenser and other parts of the illumination system are positioned above the specimen. The first inverted microscope prototype was made by French optical instrument maker C. Chevalier (1804-1859) in 1834. His compatriot, French chemist H. Le Chatélier (1850-1936, known for his chemical equilibrium principle) used an inverted microscope to observe metal specimens face down, after which inverted metallurgical microscopes also became known as Chatélier microscopes. Later, inverted microscopes spread in popularity, mainly for observing metal specimens. Eventually, with the emergence of phase contrast microscopes and as people started studying culture cells, inverted biological microscopes became more popular, as it was easier to view cells at the bottom of the culture fluid on a fixed stage. This section discusses the development of inverted biological microscopes that are now the main type used in bio research and inspection. Inverted metallurgical microscopes are discussed in 6.4.2.

The first inverted biological microscope in Japan was the PMB (Figure 6.1), produced by Olympus in 1958, based on an inverted universal metallurgical microscope and incorporating a trans-illumination system. In 1964, Nippon Kogaku developed the MD (Figure 6.2), a dedicated inverted biological microscope, in collaboration with the University of Tokyo Institute of Medical Science [2]. This had a sideport, which allowed greater systematicity, while the fixed stage and moving objective lens focusing mechanism eliminated any timewise focal loss, which made it possible to perform time lapse filming. This was a trailblazing inverted biological research microscope, fully equipped with all the specifications needed for cell research at the time. In 1967, the MD was systemized into the multipurpose Model M, which could be used for biological or metallurgical microscopy, or both. Tiyoda launched its T-2 inverted microscope (Figure 6.3) in 1965. As the study of culture cells became more widespread, a succession of simpler inverted microscopes called culture microscopes emerged, starting in 1966. The Olympus CK (Figure 6.4) and Nippon Kogaku MSD (Figure 6.5) were this type. In 1976, Olympus launched the IMT inverted biological microscope (Figure 6.6) and a special long working distance objective lens, the LWDC Plan40×.

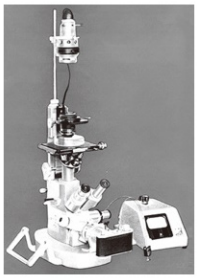

Fig. 6.1 Olympus PMB [1]

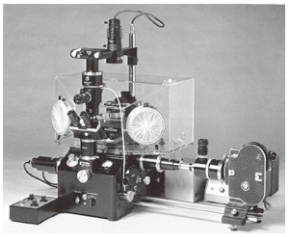

Fig. 6.2 Nikon MD [3]

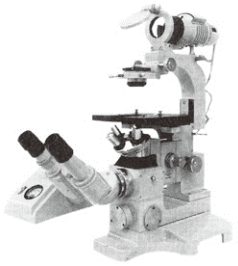

Fig. 6.3 Tiyoda T-2 [4]

Fig. 6.4 Olympus CK [1]

Fig. 6.5 Nippon Kogaku MSD [3]

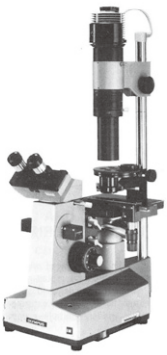

Fig. 6.6 Olympus IMT [1]

As discussed in 4.6, Japanese upright microscopes underwent a complete redesign of the objective lens and other parts of the optical system from the late 1970s, resulting in new microscope performance, function and design. New inverted microscopes were also launched as a result. In 1976, Nippon Kogaku introduced the CF optical system to the market. The company launched the Diaphot TMD inverted biological microscope in 1980 (Figure 6.7), followed by the TMS culture microscope in 1984. The TMD was highly regarded for its superior optical performance, its consistency of form and the diversity of its system, which included epifluorescence and differential interference contrast. The model took an increasing share of the bioresearch domain, both in Japan and overseas. In 1978, Olympus released the new LB optical system, followed by the IMT2 (Figure 6.8) and CK2 (Figure 6.9) microscopes in 1984. In 1993, Nikon launched the TMD300 (Figure 6.10), an improved version of the Diaphot. In 1994, Olympus switched to a new infinity corrected optical system and released the IX50 and IX70 (Figure 6.11) inverted biological microscopes with the UIS optical system. Nikon responded with the launch of the Eclipse TE300 in 1997 (Figure 6.12), featuring the CFI_{60} optical system. Nikon later released the Eclipse Ti-E in 2007 (Figure 6.13), while Olympus launched the IX2 series in 2002 and the IX3 series (Figure 6.14) in 2012, each model progressing in response to the latest needs in the field.

Fig. 6.7 Nikon TMD [3)]

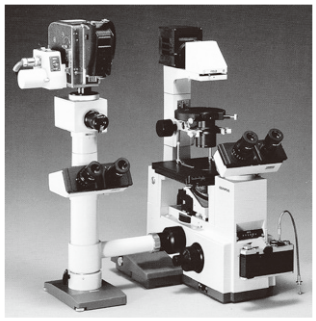

Fig. 6.8 Olympus IMT2 [1)]

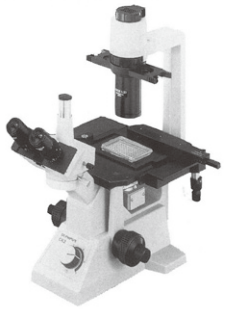

Fig. 6.9 Olympus CK2 [1)]

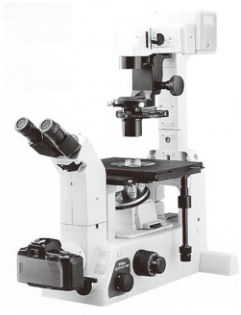

Fig. 6.10 Nikon TMD300 [3)]

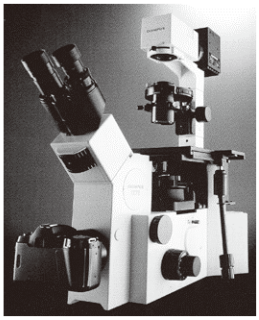

Fig. 6.11 Olympus IX70 [1)]

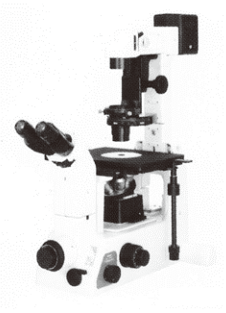

Fig. 6.12 Nikon TE300 [3)]

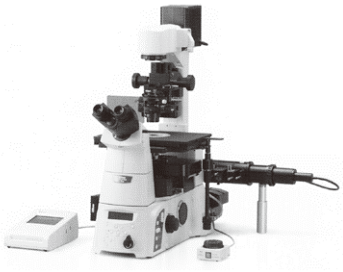

Fig. 6.13 Nikon Ti-E [3)]

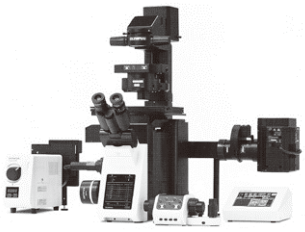

Fig. 6.14 Olympus IX83 [1)]

One important application for inverted microscopes is infertility treatment. Unlike artificial insemination, in which active sperm are selected and placed artificially into the uterus to aid impregnation, in vitro fertilization (IVF) involves extracting eggs that have grown to near ovulation (ovum collection), fertilizing them with sperm and then placing them back in the uterus once they have undergone cell division (embryo transfer). Following the birth of the first child conceived by in vitro fertilization in the United Kingdom in 1978, the process spread rapidly around the world. In Japan, around 40,000 babies are born each year through IVF. Intra-cytoplasmic sperm injection (ICSI)

involves injecting a sperm directly into an extracted egg under microscope. The process was first performed in 1992 (using a Nikon TMD). Figure 6.15 shows an example inverted microscope fitted with a micromanipulator. Figure 6.16 shows a sperm being injected into a human embryo. In 1996, Dolly, the cloned sheep, was born in the United Kingdom in 1996 (announced in 1997). The process involved taking the nucleus of a somatic cell from a sheep, transferring it into the enucleated unfertilized germ cell of another sheep and, following cell fusion, placing the embryo into the uterus of a surrogate mother ewe (a Nikon TMD was used). Although cloning technology has since been used on larger mammals, such as cattle and horses, Japan announced in 2000 that human cloning technology would be prohibited by law.

Fig. 6.15 Olympus IX71 + Manipulation System [1)]

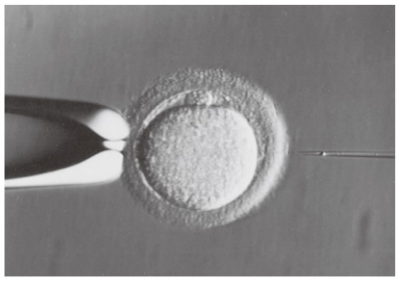

Fig. 6.16 Injecting a Sperm into a Human Embryo [5)]

6.2 Stereomicroscopes

Stereomicroscopes are microscopes with two separate optical axes from the specimen to the left and right eye. The parallax caused by the angle between the optical axes creates a stereoscopic view. Since it is used for tasks such as inspection and dissection, the viewing image must be an erect image with the same orientation as the specimen. American biologist H. S. Greenough devised a method for stereoscopic viewing of an erect image and took it to Zeiss. His idea was to have an objective lens on each optical axis as well as a relay lens to erect the respective images. However, Zeiss had already made binoculars using the Porro prism (an erecting prism invented by Italian optical instrument inventor I. Porro (1801-1875)) in 1893. The company replaced the relay lens with this system to produce a compact prototype of the modern stereomicroscope (1897) and marketed it as the Greenough microscope (Figure 6.17).

Takachiho produced Japan's first stereomicroscope, the XA, in 1933 (Figure 6.18) [3)]. It had three objective lenses and a maximum total magnification of 48×, as well as a hand rest for easier operation. Nippon Kogaku launched its first stereomicroscope, the SM, in 1954 (Figure 6.19). Equipped with a three-level magnification system of the Galileo telescope type (combination of convex and concave lenses) and designed to prevent loss of focus while changing magnification, the microscope also had an epi-illumination device. Olympus released its X model (Figure 6.20) with similar specifications in 1959.

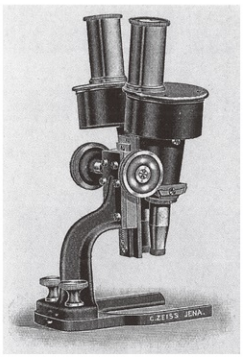

Fig. 6.17 Zeiss Stereomicroscope (1897) [6)]

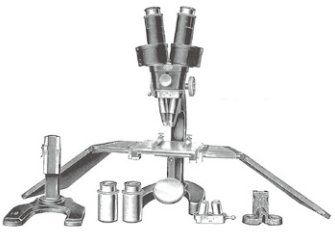

Fig. 6.18 Olympus XA [1) 7)]

Fig. 6.19 Nippon Kogaku SM [3)]

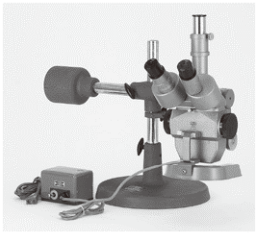

Fig. 6.20 Olympus X [1)]

In 1957, American Optical launched a stereomicroscope under the brand name Cycloptic. It had two parallel optical axes from the objective lens and a variable magnification optical system (five levels ranging from 0.7× to 2.5×, by rotating two Galileo telescopes). In contrast to the Greenough (convergent) system, this arrangement is called the common main objective (CMO) system or parallel optical axis system. This stereomicroscope was rapidly adopted, particularly for use in the fledgling semiconductor manufacturing process. Two years later in 1959, rival American company Bausch & Lomb brought out its own stereomicroscope under the brand name StereoZoom, with a Greenough system and a consecutive zoom mechanism. The objective lens had a magnification range of 0.7-3× (zoom ratio of 4.3), while the erect image was produced by an arrangement of four reflecting mirrors in the tube. As this system was able to keep up with current needs, such as providing more interchangeable lenses, an illumination system, an arm, a stand etc., it was well received in the semiconductor industry and other industrial fields, as well as in biology and medicine. Production and sales continued for more than 40 years until 1990, when Bausch & Lomb merged with the Leica Group, along with other companies, including American Optical.

Japanese stereomicroscopes adapted early to zoom capabilities. While zoom lenses had been used in TV cameras and small-scale video cameras that did not need high resolution, in 1959, Nippon Kogaku brought out the Nikkor Zoom 8.5-2.5cm for use in the Nikon F single-lens-reflex (SLR) camera. This demonstrated the high standard of optical design technology at the time. This was developed for use as a stereomicroscope zoom lens and went on the market in 1961 in the SMZ (Figure 6.21). The magnification was 0.8-4× (zoom ratio of 5). Meanwhile, Olympus had made its own progress on stereomicroscope zoom lenses, having pioneered camera lenses, and unveiled the SZ (Figure 6.22), Japan's first stereomicroscope with zoom capability, in 1960. The microscope was released onto the market in 1961, the same year as the SMZ. In 1966, the SZ was the first Japanese microscope to be awarded the Good Design Award (inaugurated in 1957 by the Ministry of International Trade and Industry, symbolized by the "G Mark" system). Both of these models were easier to use than the existing stepped magnification system and many were adopted into assembly processes in electronics industries such as precision technology and semiconductors. The microscopes were also widely adopted in biomedical research and other fields and have since proven to be long sellers. The SMZ is still being manufactured and sold today, more than 50 years after it was launched. In 2011, it was awarded the Good Design Long Life Design Award. The convergent (Greenough) system has continued to the present day in popular model stereomicroscopes, manufactured and exported by Olympus and Nikon, as well as mid-sized microscope manufacturers such as Meiji Techno (formerly Meiji Labax), Carton Optical, Kyowa Optical, Seiwa Optical, Minato Optical, Optart and Nissho Optical. Since around the turn of the century, an increasing number of microscopes have been fitted with white LED illumination for energy conservation and longevity.

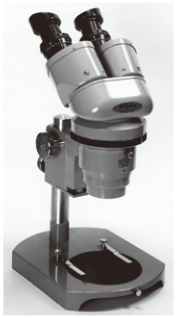

Fig. 6.21 Nikon SMZ [3)]

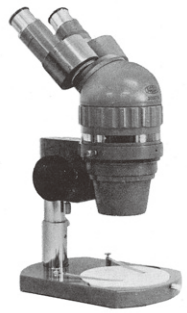

Fig. 6.22 Olympus SZ [1)]

With growing demand for stereomicroscopes to have higher magnification and resolution for applications, such as integrating semiconductors and handling tissue cells, the limitations of the Greenough system started becoming more apparent. Drawbacks included difficulty in correcting optical aberration due to the optical axis biasing towards the specimen and difficulty in adding various units into the middle of the optical system. These were proving to be major limitations to its systematization. To resolve these issues, a single-objective, parallel optical path system was reconsidered. The first dedicated single-objective stereomicroscope in Japan was the SMZ-10 (Figure 6.23), released by Nippon Kogaku in 1977. The objective lenses had a magnification of 0.66-4× (zoom ratio of 6). By changing the objective lens and the eyepiece, the total magnification was 3.5-160×. Various illumination systems, including epi-illumination using the same axis, and accessories such as a stand were added to the line up to meet the needs of stereomicroscope users in the research market. Although the global market for high-end stereomicroscopes was dominated by Swiss company Wild (later Wild Leitz, now Leica Group), the SMZ-10 was the forerunner of high-end Japanese stereomicroscopes. By way of competition, Olympus brought out the SZH high-end stereomicroscope in 1985 (Figure 6.24). The objective lens had a high zoom ratio of 8.5, with magnification of 0.7-6× (total magnification of 3.5-360×). The single objective lens maintained high optical performance by taking measures to prevent image distortion from the large decentralized optical system and incorporating technology such as semi apochromatic lenses to prevent chromatic aberration.

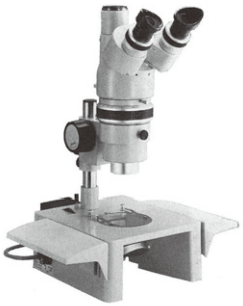

Fig. 6.23 Nikon SMZ-10 [3)]

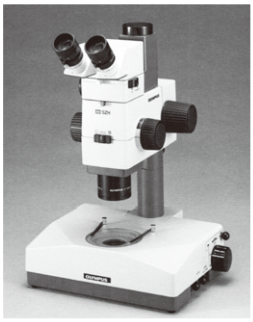

Fig. 6.24 Olympus SZH [1)]

It was a high-end system that included many accessories as well. In 1990, Nikon released the SMZ-U stereomicroscope system (Figure 6.25) with a zoom ratio of 10 (0.75-7.5× objective lens, 3.75-450× total magnification). After that, Olympus brought out the SZH10 with a zoom ratio of 10 (0.7-7×), followed by other products with even higher zoom ratios, such as the SZX12 (0.7-9×, zoom ratio of 12.86, Figure 6.26) and the SZX16 (0.7-11.5×, zoom ratio of 16.5, Figure 6.27). Following the SMZ800 in 1999, Nikon also brought out the SMZ1000 and SMZ1500 (0.75-11.25×, zoom ratio of 15, Figure 6.28), and then the SMZ25 in 2013 (0.63-15.75×, zoom ratio of 25, Figure 6.29, combined with the epifluorescence set). Throughout this time, both companies were also producing additional accessories such as epifluorescence units, revolving nosepieces for changing objective lenses, variable tilt tubes and electronic focusing devices, as well as steadily building up their optical performance by changing over to apochromatic lenses and higher resolution (currently, the highest NA in the world for a stereomicroscope is 0.312).

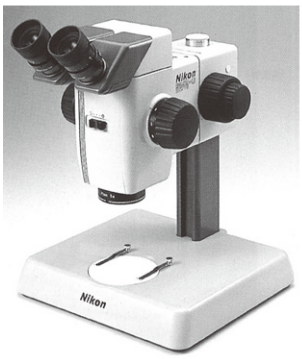

Fig. 6.25 Nikon SMZ-U [3)]

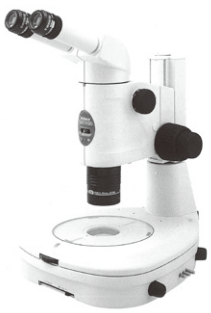

Fig. 6.26 Olympus SZH12 [1)]

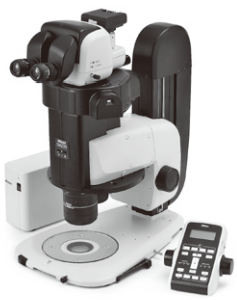

Fig. 6.27 Olympus SZH16 [1)]

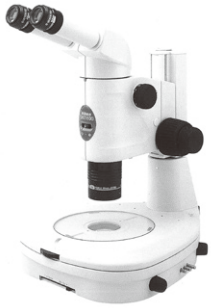

Fig. 6.28 Nikon SMZ1500 [3)]

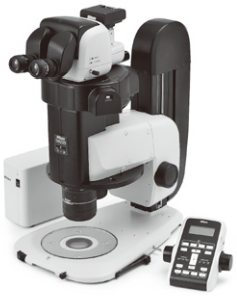

Fig. 6.29 Nikon SMZ25 [3)]

As a result of Olympus and Nikon working together to develop products with higher specifications and better performance, Japanese products now lead the world also in the area of stereomicroscopes.

6.3 Educational Microscopes

There is no clear definition of educational microscopes. They are usually microscopes that are used for science education in elementary and secondary schools. These microscopes are equipped with the minimum required functions and performance, are robust, durable and moderately priced. In elementary school, children start using microscopes in their science classes from 5th grade. The next step up, used by university science and medical students, are called student microscopes and are outside the scope of this chapter. Japan's first specifically educational microscope was the Sakura (Figure 6.30), produced by Takachiho Optical in 1922 at the request of a scientific instruments dealer in Kyoto for a simple microscope to use in elementary and secondary schools. Modelled on a German-made student microscope, it only had one objective lens. It was later developed into the Hatsukaze on an ST model.

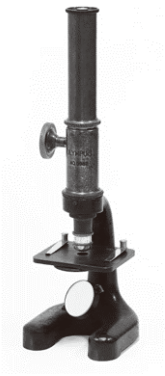

Fig. 6.30 Olympus Sakura [1)]

After the war, with the view that science education would play an important part in laying the foundation for a cultural nation, the Japanese government established the Science Education Promotion Act in 1953 and executed it the following year. Japan Industrial Standards also began to be formulated for microscopes, with JIS B 7132 Biological microscopes enacted in 1949, followed by 7133 Biological microscopes for dry objectives, 7134 Small size biological microscopes, 7139 Stereo microscopes etc. in 1951 (see Appendix 1). As this took place, microscope manufacturers focused their efforts on developing educational microscopes based on these JIS standards. Olympus released the MK (MIC) low-cost, simple microscope in 1959 (Figure 6.31), aimed also at ordinary household use. With objective lenses able to be changed by turning a handle, it had four magnification levels and a total magnification of 40-300×. Designed for ease of use, the tube was tilted at 30° and focusing was performed by moving the stage up and down. In 1960, this microscope was designated by both the JIS and the Science Education Promotion Act. The same year, the Microscope Observation Contest started by Olympus in collaboration with children's newspaper Mainichi Shogakusei Shimbun changed its name to the Natural Science Observation Contest (Shizecon). To this day, the contest continues to contribute to promoting science education for schoolchildren. Kalnew Optical released the SGL-600 educational microscope in 1960 (Figure 6.32), the year after it became an affiliated company of Shimadzu. Developed with guidance from Tokyo University of Education (now the University of Tsukuba), the microscope was fully designed for children, including its overall size, the viewing angle and the up and down movement of the stage, making it the exemplary educational microscope of the day. The same year, Nippon Kogaku also launched an educational microscope, the E model (Figure 6.33, E stands for Elementary). Based on the S research microscope and designed for science classes, this microscope was packed away into a unique rocket shell shape storage box to make it easy for children to handle. As other Japanese microscope manufacturers also began bringing out their own educational microscopes, the major companies stopped investing in new products for this market. This allowed major educational equipment companies such as Uchida Yoko and Shimadzu Rika to dominate this area. Recent changes have been seen with ICT entering the science classroom, such as microscopes with digital displays (Figure 6.34 shows a microscope with a digital display, released in 2014) and improved educational software.

Fig. 6.31 Olympus MK (MIC) [1)]

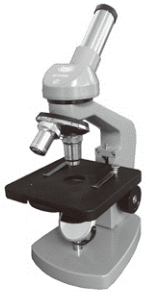

Fig. 6.32 Shimadzu Kalnew SGL-600 [8)]

Japan has always aimed to be a nation of science and technology. Microscopes that focus on science education are an important educational tool and a first step in getting children interested in science. Microscopes make it possible for children to enjoy their learning as they observe the micro world with their own eyes. With more microscopes in this market now being imported from China and other emerging nations, the hope is that the mid-sized Japanese manufacturers will still hold their ground in this country, the microscope superpower.

Fig. 6.33 Nippon Kogaku E [3)] Fig. 6.34 Uchida D-EL4N [9)]

6.4 Metallurgical (Industrial) Microscopes

6.4.1 Reflected Light (Incident Light, Epi-Illumination) Microscopy [10)]

Thus far, we have discussed microscopy of biological specimens by transmitted light illumination or fluorescence. For viewing specimens made of metal or ceramic material that do not transmit light, reflected light illumination is used. Figure 6.35 shows an illumination optical system for standard reflected light microscopy. Light from the light source is reflected off the half mirror and illuminates the specimen through the objective lens. The light reflected from the specimen passes through the objective lens and is transmitted by the half mirror to form an image. Dark field and differential interference contrast are also often used in reflected light microscopy.

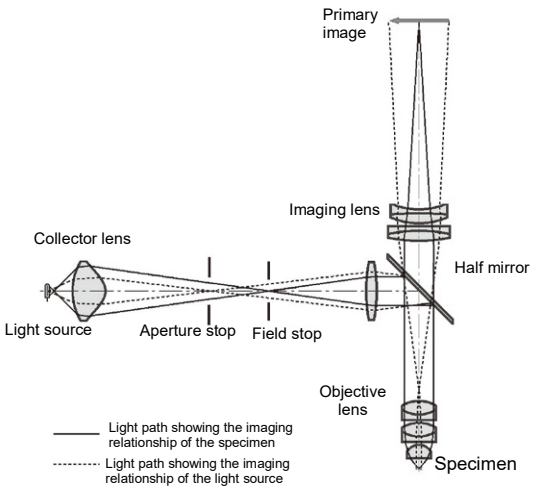

Fig. 6.35 Reflected Bright Field Optical System [10)]

Figure 6.36 shows a reflected dark field optical system. The incident light forms a ring through the aperture and is reflected by a mirror with a hole. The light travelling along the illumination optical path through around the dark field objective lens illuminates the specimen by means of a ring-shaped lens or mirror. The image is formed by only the light scattered or diffracted by the specimen passing through the objective lens. Any small flaws or level differences stand out as visible by sparkling, making this very effective for inspecting the surface of the specimen. Figure 6.37 shows a reflected differential interference contrast optical system. Light from the light source is linearly polarized by the polarizer on the illumination optical path and then reflected by the half mirror to the Nomarski prism, which divides it into two linearly polarized light rays vibrating at right angles to each other that illuminate the specimen at a separation (shearing value) lower than the resolving power. The two linearly polarized light rays reflected by the specimen are combined once more by the Nomarski prism. After passing through the analyzer, a contrast image is obtained that corresponds to the differential coefficient of the level difference (phase contrast) of the specimen with interference. The contrast, including sensitive color, can also be adjusted by moving the prism horizontally. Since reflected differential interference contrast makes it possible to detect minute irregularities that could not be detected under reflected bright field microscopy, it is widely used in inspection processes.

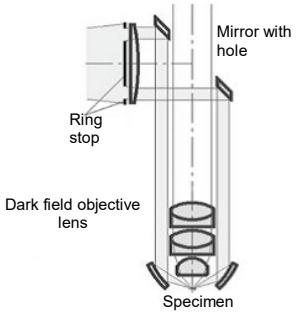

Fig. 6.36 Reflected Dark Field Microscopy [10)]

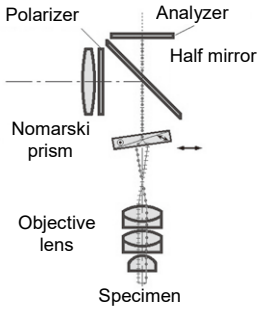

Fig. 6.37 Reflected Differential Interference Contrast Microscopy [10)]

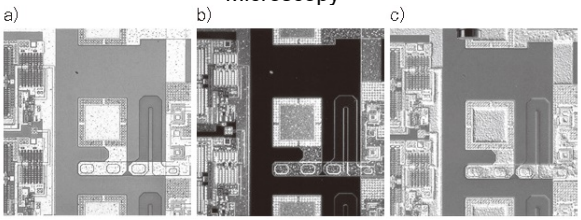

Fig. 6.38 Photographs of an Integrated Circuit under Reflected Light Microscopy [10)]

a) Bright Field b) Dark Field c) Differential Interference Contrast

Figure 6.38 shows the same view of an integrated circuit under reflected bright field, reflected dark field and reflected differential interference contrast microscopy.

6.4.2 History and Development of Metallurgical (Industrial) Microscopes

French chemist and mathematician F. de Réaumur (1683-1757) made observations of the surface structure of fractures in iron under microscope and published “L’Art de Convertir le Fer Forgé en Acier [The Art of Converting Forged Iron into Steel]” in 1722. German physician J. N. Lieberkühn (1711-1756) fitted a concave mirror (called a Lieberkühn mirror) to a microscope to perform animal dissections under reflected light (Figure 6.39). British geologist H. C. Sorby (see 5.4) used a microscope fitted with a special reflected illumination system to examine microstructures. He published his findings on the crystalline structure of minerals in 1858 and on the structure of iron and steel in 1863. After that, the United Kingdom began to produce many metallurgical microscopes. Specimens view under metallurgical microscopes are often larger and heavier than those view under biological microscopes. The surface of the specimen also needs to be perpendicular to the viewing optical axis. To resolve these issues, the inverted metallurgical microscope (Chatélier microscope) was devised, with a fixed stage and the specimen observed from underneath (see 6.1). This has a coaxial system in which the objective lens itself serves as the condenser lens to allow the incident light to illuminate the specimen perpendicularly. There are two ways to achieve this: using a thin plate, as shown in Figure 6.40 a), or using a prism, as shown in Figure 6.40 b).

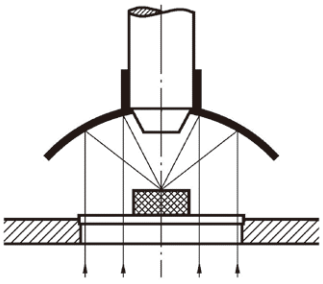

Fig. 6.39 Lieberkühn Mirror Reflected Illumination

(created with reference to cited reference 11)

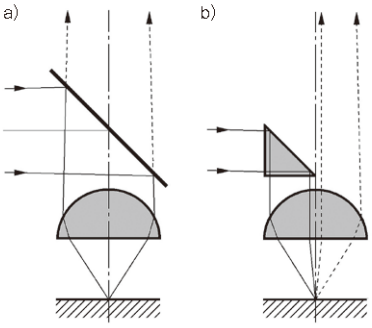

Fig. 6.40 Reflected Illumination

a) Thin Plate Method b) Prism Method

(created with reference to cited reference 11)

At first, semi-transmissive mirrors were difficult to manufacture, so the thin plate method only resulted in dark images, as much of the illumination light was lost. While the prism afforded brighter illumination through total reflection, the resolving power was lower, as half of the objective lens aperture was hidden, resulting in uneven image brightness and lateral shift during focusing. Ultimately, once it became possible to produce uncolored semi-transmissive mirrors, the thin plate method took precedence. There are two ways in reflected dark field illumination to have the light passing around the objective to illuminate the specimen: using a ring-shaped concave mirror, as shown in Figure 6.41 a), or using a ring-shaped lens, as shown in Figure 6.41 b). This prototype, Ultrapak, made by Leitz, was originally made for biological use, but was also used in metallurgical microscopes as well.

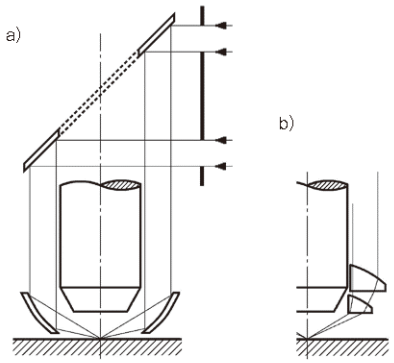

Fig. 6.41 Reflected Dark Field Illumination

a) Concave Mirror Method b) Lens Method

(created with reference to cited reference 11)

Japanese development of metallurgical microscopes began with Takachiho Works in 1928, with a subsidy from the Ministry of Commerce and Industry. Developers studied the metallurgical microscopes made by Reichert, the most reputable in the world at the time, and completed the Olympus MC in 1930 (Figure 6.42) [12]. The company also developed the PMA photomicrograph apparatus (Figure 6.43) for inverted metallurgical microscopes. This was a horizontal device, like many photomicrographic devices at the time. In 1938, the company developed the Super Photo “Universal” (Figure 4.10), equipped for reflected light and transmitted light bright-field/dark-field observation and with built in devices for photography.

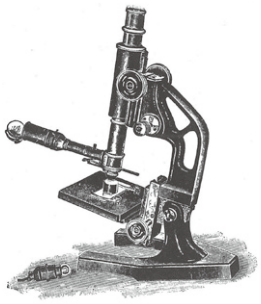

Fig. 6.42 Olympus Metallurgical Microscope MC [13]

After the war, the first company to establish itself in metallurgical microscopes was Union Optical (referred to below as Union, the company went insolvent in 2010), founded in 1948. The company launched the UM inverted metallurgical microscope (Figure 6.44 shows the NUM, an improved version of the UM) in 1952. This was followed by a succession of small to medium metallurgical microscopes, including the MeC, and the company emerged as a prominent national metallurgical microscope manufacturer [14]. Meanwhile, Olympus brought out the PMF universal inverted metallurgical microscope in 1954 (Figure 6.45). This microscope shifted away from the horizontal design with a new vertical style. The photomicrographic equipment was also compact and easier to use, having switched from the larger dry plates to 35mm film. Capable of reflected light phase contrast and polarized light microscopy, this truly was the pinnacle of Japanese metallurgical microscopes at the time. Olympus released the MF in 1959 (Figure 6.46), which would become the benchmark standard for upright metallurgical microscopes. In 1964, the company further developed the PMF to produce the PMG (Figure 6.47), the high-end universal inverted microscope with an inbuilt photometer for photomicrography. In 1967, the company also launched the Neopak MN bright/dark field metallurgical microscope (Figure 6.48).

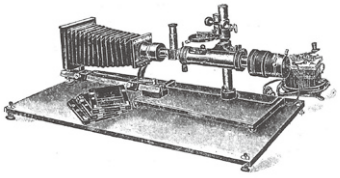

Fig. 6.43 Olympus Inverted Metallurgical Microscope Photomicrograph Apparatus PMA [13]

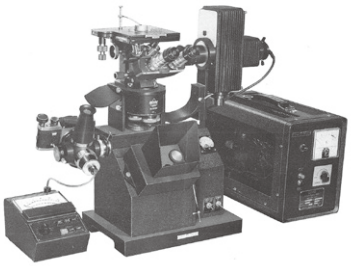

Fig. 6.44 Union Universal Inverted Microscope NUM [15]

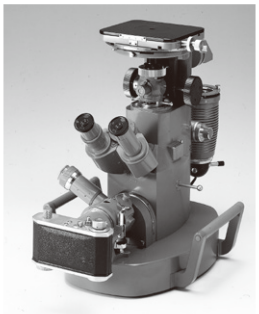

Fig. 6.45 Olympus Universal Inverted Microscope PMF [1]

Union announced the UPM universal upright microscope around 1967 (Figure 6.49). This and other high-end models for reflected/transmitted bright field illumination, as well as phase contrast, interference, polarized light and dark field illumination, led the company to dominate the Japanese metallurgical microscope market alongside Olympus. Nippon Kogaku added a reflected light illumination system to its S biological microscope system for research and stepped into the arena with the S-M upright metallurgical microscope in 1961 (Figure 6.50). In 1964, the company brought out the ME inverted metallurgical microscope.

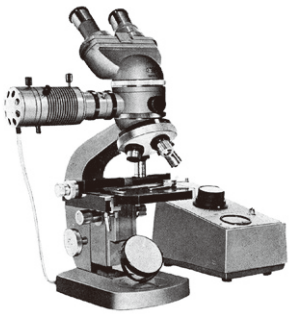

Fig. 6.46 Olympus Metallurgical Microscope MF [1)]

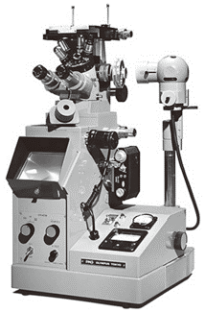

Fig. 6.47 Olympus Universal Inverted Microscope PMG [1)]

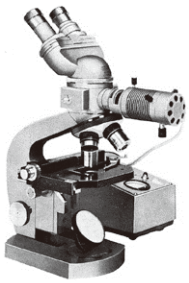

Fig. 6.48 Olympus Bright/Dark Field Metallurgical Microscope Neopak MN [1)]

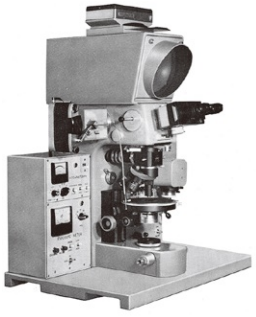

Fig. 6.49 Union Universal Upright Microscope UPM [14)]

Metallurgical microscopes were also developed as optical instruments for inspecting and measuring precision parts in various industrial fields. Relatively simple versions are toolmakers' microscopes and factory microscopes. Nippon Kogaku pioneered this field in Japan, developing various measuring instruments for its own use as inspection tools from 1920. With the Second Sino-Japanese War making it difficult to import measuring instruments from the West, the company answered the call of the nation and in 1938 began supplying others with many kinds of optical instruments [16)]. In 1939, the No. 1 universal projector (also called a measuring projector or profile projector) (Figure 6.51) was completed. After the war, as the manufacturing industry advanced, various manufacturers started putting out these kinds of measuring microscopes in full force. Typical models include the SM factory microscope (Figure 6.52), released by Union in 1955, and the MTM large moiré fringes toolmakers' microscope (Figure 6.53). These are optical instruments that use microscope technology. The developments in measuring methods and advances in technology and company products are very interesting. However, no further detail is given here, as the topic of this report is optical microscopes.

As mentioned in 5.5, Nippon Kogaku released the Yamamoto-Françon type S-R reflected light differential interference contrast microscope in 1966 (Figure 5.22b).

Fig. 6.50 Nikon Metallurgical Microscope S-M [3)]

Fig. 6.51 Nippon Kogaku Universal Microscope No. 1 [16)]

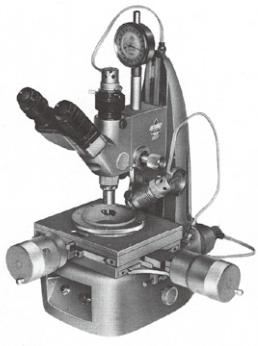

Fig. 6.52 Union Factory Microscope SM [15)]

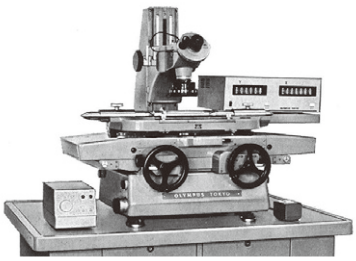

Fig. 6.53 Olympus Large Toolmakers' Microscope MTM [1)]

Union developed a Nomarski type reflected light differential interference contrast system and launched it in the form of the ME-IC in 1971 (Figure 6.54) [17)]. Olympus sublicensed the Nomarski patent from Union and added the M-NIC incident differential interference contrast unit (Figure 6.55) to its lineup in 1973 for use in the MF and universal AHM microscopes. Nippon Kogaku went for a long time without putting out any new industrial microscopes except for an IC inspection microscope in 1974. In 1976, the company launched the Metaphot metallurgical/industrial microscope (Figure 6.57) at the same time as the new CF optical system. This was followed by the Optiphot XP industrial microscope in 1978 and the Optiphot-55 IC inspection microscope the following year (Figure 6.58).

Fig. 6.54 Union ME-IC [15)]

Fig. 6.55 Olympus M-NIC [1)]

Later, as the market for metallurgical microscopes expanded, metallurgical microscopes other than inverted microscopes began to be known as industrial microscopes, as it was becoming common to use them for applications other than examining metal specimens. The Olympus BHM industrial microscope (Figure 6.56), launched in 1975 based on the high-end BH microscope system, became the benchmark standard.

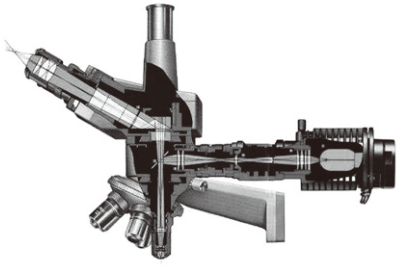

Fig. 6.56 Olympus BHM (optical path diagram) [1)]

The company strengthened its assault on the industrial microscope market by responding to market needs, such as better optical performance, dark field and differential interference contrast (using the Nomarski system) capabilities and adding a large stage for handling wafers (the 55 in the name of the microscope stands for the stage size of 5″×5″). Olympus responded by developing the new infinity correction (IC) series of lenses for industrial microscopes (see 7.4.3). The lenses were launched in 1981 with the BH2-UMA universal reflected light microscope (Figure 6.59), capable of switching easily between bright field, dark field, polarized light and differential interference contrast. The following year, Nikon launched the Optistation wafer inspection microscope device (Figure 6.60), mainly used for visual inspection during the lithography and etching processes in semiconductor manufacturing. This vastly increased the industrial microscope's share of the semiconductor manufacturing industry. Both companies continued to bring out new innovations, such as more types of objective lenses, larger stages to match larger sizes of liquid crystals and wafers (Nikon brought out the Optiphot-88 in 1987), and automating various operations.

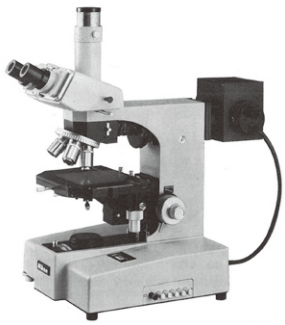

Fig. 6.57 Nikon Metaphot [3)]

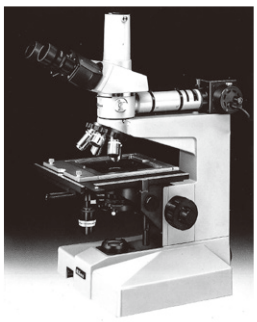

Fig. 6.58 Nikon Optiphot-55 [3)]

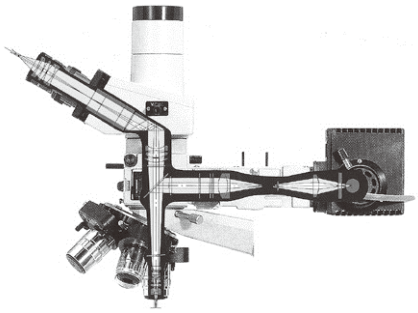

Fig. 6.59 Olympus BH2M (optical path diagram) [1)]

Fig. 6.60 Nikon Optistation [15)]

In 1993, Olympus brought out the new UIS infinity corrected mechanical tube length optical system for biological microscopes. The following year, the company launched the UIS optical system for industrial microscopes, together with the BX60M industrial microscope (Figure 6.61). The year after that, Olympus launched the MX50 semiconductor inspection microscope (Figure 6.62), which had a large stage capable of holding 6″ and 8″ wafers, and the MX40 ordinary class industrial inspection microscope. The MX50 had improved operability, such as a variable tilt tube (0-35°). The MX80 (Figure 6.63) could be extended to inspect a 300mm wafer, photo mask or flat panel display (FPD).

Fig. 6.61 Olympus BX60M [1)]

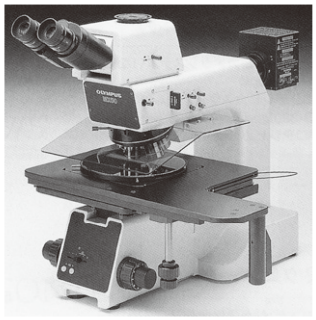

Fig. 6.62 Olympus MX50 [1)]

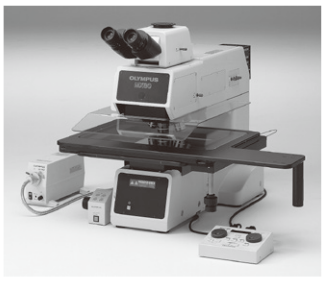

Fig. 6.63 Olympus MX80 [1)]

It also had more automated and electronic functions, such as auto focus. Nikon responded by incorporating the CF & IC series developed in 1994 into the Optiphot 100S metallurgical and LSI inspection microscope (Figure 6.64). In 2000, the company launched the Eclipse L200 LSI inspection microscope, with the newly developed CFI_{60} optical system built in and capable of handling 200mm wafers. The company also put out the L200A the same year (Figure 6.65), with more automated functions and greater system scalability. In 2001, Nikon released the Eclipse L150 and L150A industrial microscopes capable of handling 150mm wafers. These were followed by the Eclipse L300D FPD inspection microscope in 2004 (Figure 6.66), capable of handling a 400mm × 300mm sample size. In 2005, the company followed with the Eclipse LV150 industrial microscope (Figure 6.67), successor to the Optiphot 100S, and the 100 series.

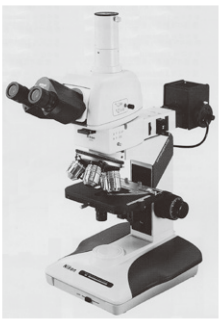

Fig. 6.64 Nikon Optiphot 100S [3)]

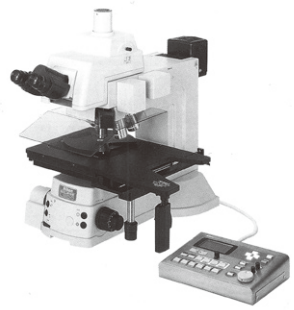

Fig. 6.65 Nikon Eclipse L200A [3)]

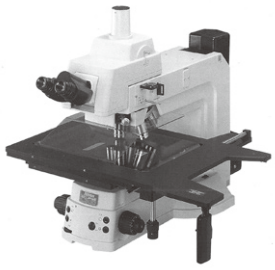

Fig. 6.66 Nikon Eclipse L300D [3)]

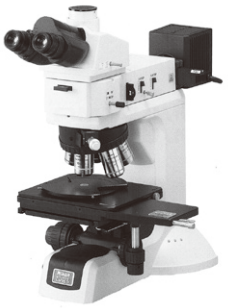

Fig. 6.67 Nikon Eclipse LV150A [3)]

Despite the emergence of a new optical system in the late 1970s, new models of inverted metallurgical microscopes were slower to be developed than upright/inverted biological microscopes or upright metallurgical microscopes. Nippon Kogaku launched its first inverted metallurgical microscope, the Epiphot TME, in 1981 (Figure 6.68). Until then, Olympus and Union had dominated the Japanese inverted metallurgical microscope arena. However, the high performance of the CF optical system and the robust design were well regarded by users. Olympus brought out the IC series infinity corrected optical system in 1981 and launched the PMG3 high-end inverted metallurgical microscope in 1987 (Figure 6.69). Inverting the previous reverse image and incorporating a zoom mechanism, this was a drastically improved design from the PMG2 high-end universal microscope released in 1972. The IC optical system also offered improved performance and was well received by many users. The following year, the company also launched the popular PME3 (Figure 6.70).

Fig. 6.68 Nikon Epiphot TME [3)]

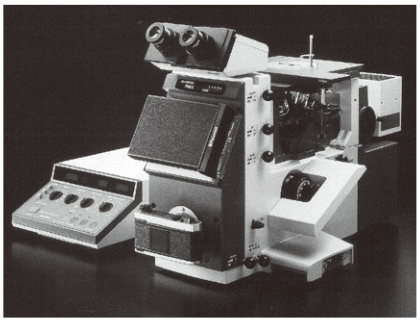

Fig. 6.69 Olympus PMG3 [1)]

Union incorporated its new SPLM series infinity corrected optical system into the Versamet-3 high-end inverted metallurgical microscope, launched in 1990 (Figure 6.71).

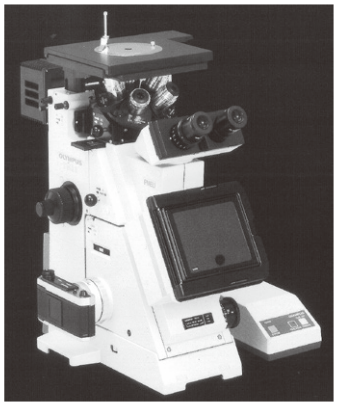

Fig. 6.70 Olympus PME3 [1)]

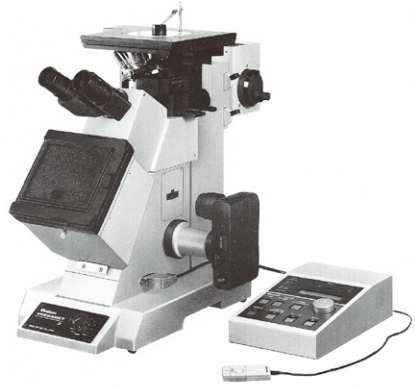

Fig. 6.71 Union Versamet-3 [14)]

Nikon launched the TME300 universal research microscope (Figure 6.72) and the TME200 as successors to the TME from 1995, using the CF & IC optical system. In 2006, the company released the Eclipse MA100 popular inverted metallurgical microscope using the new CFI_{60} optical system. This was followed by the MA200 in 2008 (Figure 6.73), with a new box design. These microscope models have continued to the present day.

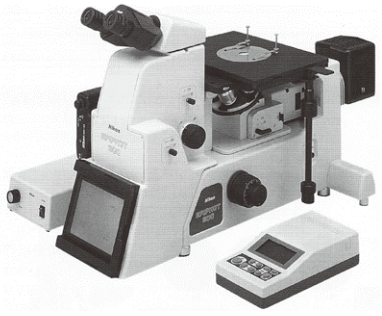

Fig. 6.72 Nikon Epiphot TME300 [3)]

Olympus launched the GX71 high-end system inverted metallurgical microscope (Figure 6.74) and the GX51 metallurgical microscope system, using the industrial UIS optical system introduced in 1994. The company also launched the GX41 small-scale inverted metallurgical microscope in 1994 (Figure 6.75). All new products since 2000 have changed from the 35mm camera and large size in favor of digital systems. This has the advantage of improved performance and operability.

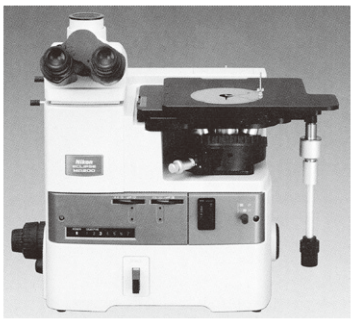

Fig. 6.73 Nikon Eclipse MA200 [3)]

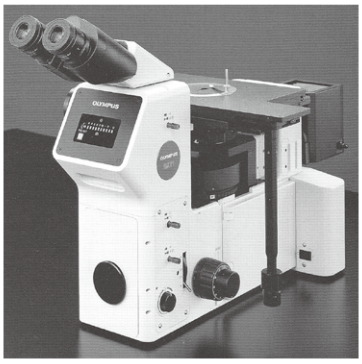

Fig. 6.74 Olympus GX71 [1)]

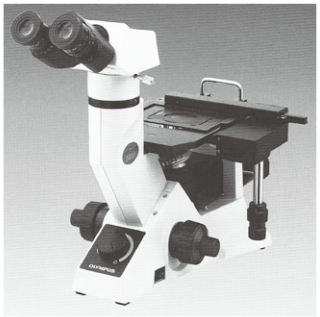

Fig. 6.75 Olympus GX41 [1)]

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7 Development of Optical Technology for Microscopes

Technological components of microscopes include optical systems, mechanical systems, electrical/electronic systems and software. Of these, it is the optical system that most determines microscope performance. This chapter discusses the history and development of the optical system, particularly the central component at the heart of the microscope: the objective lens. This is also the author's field of expertise.

7.1 History of Objective Lenses [1)]

7.1.1 Advances in Basic Performance

As discussed in 3.2, following the invention of the compound microscope around 1600, the objective lenses used in microscopes were single lenses (Figures 3.1, 3.2) until the invention of achromatic lenses. These single lenses were not capable of high magnification. Even after the successful manufacture of achromatic lenses by Dollond in 1758, as mentioned in 3.3, no high magnification objective lenses were immediately forthcoming that were capable of satisfactorily eliminating the spherical aberration caused by increasing the numerical aperture. This was resolved by Lister in 1830. Figure 7.1 shows how a virtual image O′ of an object O is created by plano-convex achromatic lens A, after which a real image of O′ is created by plano-convex achromatic lens B. This made it possible to manufacture objective lenses with relatively high numerical aperture and low spherical and chromatic aberration.

In 1837, Amici added a hemispherical lens on the object side of Lister's objective lens to successfully produce a high-NA, high-magnification objective lens that corrected spherical and chromatic aberration (Figure 7.2). On the understanding that increasing the numerical aperture would increase the resolving power, he also came up with the idea of immersion lenses, in which the space between the specimen and the apex of the objective lens is filled with liquid to increase the numerical aperture. He successfully completed the first water-immersion objective lens in 1853.

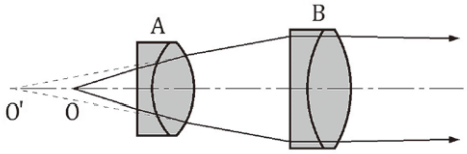


Fig. 7.1 Lister Objective Lens
(created with reference to cited reference 1)

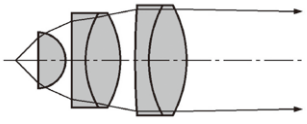


Fig. 7.2 Amici Objective Lens
(created with reference to cited reference 1)

Although these gradual improvements were being made to microscope objective lenses, it was Abbe (Figure 3.6) who transformed Zeiss into a top microscope manufacturer. From the 1870s, he established microscope image formation theory, came up with designs by objective lens ray tracing and set up testing systems in the manufacturing process. Lens design by ray tracing was practiced in part at the start of the 18th century and is still used today. In 1819, J. Fraunhofer (1787-1826) designed a large-diameter (φ244mm) refracting telescope objective lens using glass data (refractive index and dispersion properties). In 1870, Abbe adopted this method of design for microscope objective lenses and found a way of correcting comatic aberration by meeting the sine condition (the ratio of *NA* between the object side and image side is constant in whole *NA* and equal to the magnification value). Using this method, he completed designs for a series of objective lenses, correcting spherical aberration and meeting the sine condition (aplanat), as well as correcting chromatic aberration (achromat). He also developed and introduced many measuring tools taking after his name, such as the Abbe refractometer, the Abbe apertometer and the Abbe sine condition test chart, to check material quality and that processing and assembly were being carried out according to the design values. In 1872, Zeiss abandoned its conventional manufacturing method based on the experience and intuition of craftspeople and switched completely to using Abbe's designs, securing its position as the world's leading manufacturer. That year, using a vast number of calculations and experiments, Abbe derived a theory of image formation that the numerical aperture had to be larger for the objective lens to capture high-order diffraction rays from the object, which was necessary for accurately reproducing microstructures as images. He showed that microscope resolving power δ could be represented as $\delta = \lambda/2NA$ (where λ is the wavelength). Abbe also developed the Abbe condenser (Figure 2.32) the same year, having found during this research that the illumination system also needed to perform better. In 1877, Abbe developed homogenous immersion (HI), using cedar oil, which has a similar refractive index to the cover glass and lens apex. This design made it possible to eliminate aberration, even with a high NA, since the second spherical surface of the objective lens worked as a refractive surface, not the first surface. Recognizing the importance of optical materials, Abbe met with Schott (Figure 3.7) and in 1882 the two jointly established an optical glass factory in Jena to research and develop new types of glass. In 1886, Abbe used a new type of barium crown glass and natural

fluorite (CaF_2) to produce an apochromatic objective lens that was achromatic over the entire range of visible light. Unlike optical glass with regular dispersion properties, fluorite has anomalous dispersion properties (see 2.1.1) and can achieve effective achromaticity for a third wavelength when used as a convex lens. However, natural fluorite is a difficult material to work with, as it is difficult to get superior transparency and homogeneity, it scratches easily (4 on the Mohs hardness scale) and, being a crystal, does not lend itself well to high precision polishing. Figure 7.3 shows the structure of an apochromatic lens 60× (dry system = not oil immersion, × represents magnification). The shaded lenses in the figure are made of fluorite.

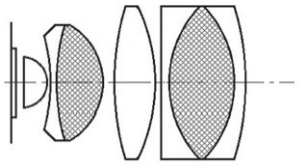


Fig. 7.3 Zeiss Apochromat 60× /0.95

(created with reference to cited reference 2)

If the Lister and Amici achromatic lenses were the first step in microscope objective lens development and the apochromatic lens mentioned above was the second step, then the third step would be the plan lens. Microscope objective lenses do not have as wide a view angle as photographic lenses. High magnification lenses in particular have such a short focal length that the curvature of field cannot readily be corrected. Focusing on the center of the field of view makes the peripheral edges blurry. With the increase in photomicrography in the 20th century, there has been a growing need to have the entire field of view in focus. Although a method was used to correct field curvature using a negative projection lens for photography (Zeiss called this the Homal), there was strong demand to be able to correct field curvature and peripheral aberration using the objective lens alone. Other methods to correct curvature of field include using a convex lens with a high refractive index and a concave lens with a low refractive index or using strong negative meniscus lenses (lenses with surfaces on both sides curving in the same direction). Since microscope objective lenses are often made from low refractive index, low dispersion glass to correct chromatic aberration, the first method was difficult to design and deploy. The second method required a high level of competence in complex lens design to produce a well-balanced design to also compensate for peripheral aberrations. The first Plan objective lens was designed by P. H. Bögehold (1876-1965) of Zeiss and patented in 1938 (Figure 7.4)[3]. The first lens on the object side and the last lens on the image side are strong meniscus lenses. Later, Zeiss and Leitz added Plan objective lens series to their lineups. The development of the highest quality Plan Apochromat objective lenses demonstrated the unwavering predominance of German microscopes.

Fig. 7.4 Zeiss Plan Achromat 40× /0.65

(created with reference to cited reference 3)

7.1.2 Advances in Objective Lens Related Standards and Technology (see 2.4)

(1) Mechanical Tube Length

The mechanical tube length is the distance from the objective lens revolving nosepiece mount to the eyepiece tube mount. The distance of 160mm, recommended by the Royal Microscopical Society (RMS) in 1882, was adopted by Zeiss and many other manufacturers, although not all. For example, Leitz used a distance of 170mm. The related German industrial standard Deutsches Institut für Normung (DIN) 58887 was published in 1975, stipulating 160mm and infinite distance. At that point, Leitz changed from 170mm to 160mm. Infinity-corrected mechanical tube length optical systems emerged in the 1920s and 30s and had been sold by Zeiss and Leitz for metallurgical microscopes. However, this system saw little growth in popularity over the next 60 years. It was not until Zeiss announced the ICS optical system in 1986 that major manufacturers, including Japanese companies, began adopting infinity-corrected optical systems.

(2) Parfocalizing Distance

The parfocalizing distance is the distance between the specimen and the objective lens revolving nosepiece mount. This is designed with a constant value to ensure that the focus is maintained when the revolving nosepiece is turned to change the objective lens. Many companies previously used a value of around 37mm, although most German manufacturers used 45mm. After DIN 58887 was published, stipulating 45mm, Japanese companies also transitioned to new optical systems with a 45mm parfocalizing distance. However, Nikon adopted a 60mm parfocalizing distance for its CFI_{60} series in 1996 and has continued to use this distance ever since.

(3) Screw Thread Shape (see Table 2.1)

The shape of the screw thread for mounting the objective lens onto the revolving nosepiece affects the mechanical compatibility of objective lenses from different companies. The dimensions specified by the RMS have long been used as the standard dimensions and are still used by many manufacturers today. This specification is known as the Whitworth screw, with a thread angle of 55°, outer diameter of 20.32mm (0.8″) and pitch of 0.706mm ($^1/_{36}$″). However, with objective lenses growing in diameter in recent years, M25 and M27 threads are now also being used. Reflected light dark field objective lenses have a larger thread diameter to allow illumination light around them. These use W26, M27 or M32 diameter values. All of these, including factors such as dimensional tolerance, are stipulated in ISO 8038 (JIS B 7141). Table 7.1 shows the focal length, parfocalizing distance, screw thread shape and year of adoption for the infinity-corrected mechanical tube length optical systems of major microscope manufacturers.

7.2 Advances in Lens Design

After Abbe started developing microscope lenses at Zeiss using ray tracing, other companies in the West began adopting this method and producing microscopes with superior optical performance. In Japan, the objective lenses used in the Tanaka microscopes and M & Katera were manufactured by engineers taking apart, analyzing, measuring and reproducing these Western lenses by trial and error to ensure their performance by any means possible. It was a natural progression for these business owners to prioritize optical design research. M & Katera Optical Works founded the M & Katera Optical Research Laboratory in 1931 and began working on lens design using manual calculation [4) 5)]. In 1935, Takachiho founded Mizuho Optical Research Laboratory and began working on mathematical designs for photographic lenses. The following year, the company named a successful prototype lens the “Zuikō”, a Japanese abbreviation of the name of the laboratory [6)].Prior to that, Nippon Kogaku had employed eight engineers from Germany in 1921, after Germany was defeated in the First World War. These engineers worked on research and introducing technology for optical designs, design drawings, glass processing and machine processing [7) 8)]. Among the engineers were optical design expert M. Lange and microscope designer H. Acht. As well as working on various camera lenses (the prototypes for the later “Nikkor” lens), these two, especially Acht, worked on designing and developing the objective lenses for Nippon Kogaku’s first microscope, the JOICO (Figure 4.9). Acht’s design data and German design methods served as a basic design resource for later photographic lenses. Later, as Japan grew increasingly militant, optical companies began devoting their resources to designing and manufacturing optical military equipment, such as binoculars, sights, range finders and periscopes. Great progress was made in Japanese optical technology.

After the war, optical instruments transitioned into a peacetime industry. With growing demand for binoculars and camera lenses, optical companies devoted their efforts to developing new products. Designing new lenses was done by coming up with different lens types and glass combinations. This meant tracing several hundred light rays using trigonometric functions, which required vast amounts of mathematical calculations. These were all completed manually, logarithm tables on hand (Figure 7.5). One high-end lens could take three years to design. Designers also acquired knowledge and techniques from lens design literature, such as “Applied Optics and Optical Design” (Conrady, 1929, Part 1) [9)], “Grundlagen der praktischen Optik” (Berek, 1930) [10)] and “Renzu no Sekkei to Sokutei [Lens Design and Measurement]” (Ashida Seima, 1940) [11)]. In 1951, Suzuki Tatsurō (1919-1993) et al. published an academic paper on a lens design method [12) 13)].

Table 7.1 Optical System Related Dimensions of Major Microscope Manufacturers

Manufacturer (optical system)		Tube lens focal length (mm)*	Parfocalizing distance (mm)	Screw thread shape ( ) means reflected light dark field	Year transitioned to infinity corrected optical system	
					Biological	Metallurgical / industrial
Leica	E. Leitz	250	45	RMS	—	1931, 1972
	(DELTA)	200	45	M25 (M32)	1992	
	(HC)	200	45	M25 (M32)	1997	
Nikon	(CF)	(MTL = 160, 210)	45	RMS	1976	
	(CF & IC)	200	45	RMS (M27)	—	1994
	(CFI_{60})	200	60	M25 (M32)	1996	1999
Olympus	(LB)	(MTL = 160)	45	RMS	1978	—
	(IC)	180	45	RMS (W26)	—	1981
	(UIS)	180	45	RMS (W26)	1993	1994
Zeiss					—	1924
	For Axiomat	250	45	M27	1973	
	(ICS)	164.5	45	RMS, M27 (M27)	1986	

(* MTL in parentheses stands for mechanical tube length in finite corrected optical system)

Fig. 7.5 Female Employees Working on Lens Design Calculations [7)]

This method used the differential calculation coefficients of each lens component to effectively estimate the rates of variability for different lens aberrations. The introduction of Monroe electronic calculators (Figure 7.6) from 1953 increased the calculation efficiency several times over that of calculation by hand. Fuji Photo Film (now FUJIFILM) devised a way to automate lens design and spent seven years developing the FUJIC, Japan's first working electronic digital computer, completed in March 1956 (Figure 7.7). This machine increased the calculation efficiency around 2000 times over that of calculation by hand. Relay automatic calculators were introduced to the production system in 1957. Olympus bought a FACOM 138, the first automatic calculator commercialized in Japan (Fuji Tsushinki, now Fujitsu), while Nippon Kogaku bought a similar German machine the same year. Nippon Kogaku introduced an electronic computer in 1962 and Olympus did the same in 1963, dramatically increasing calculation speeds. Research on how to use electronic computers to correct lens aberration had begun around 1955.

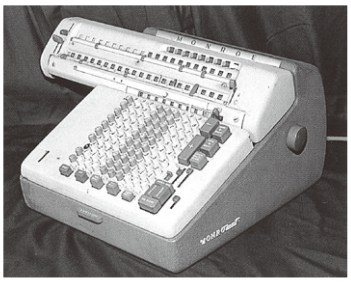


Fig. 7.6 Monroe Electronic Calculator [7)]

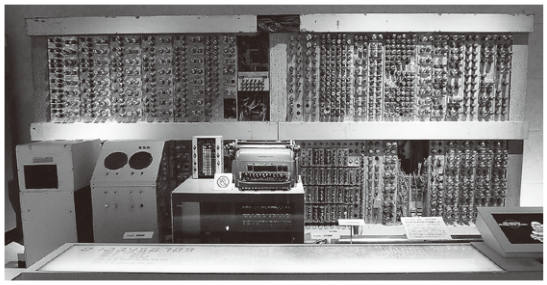


Fig. 7.7 The FUJIC, Japan's First Automatic Calculator

(held by the National Museum of Nature and Science, photographed by the author)

Major optical manufacturers started building their own lens design computer programs, incorporating aspherical surfaces, zoom lenses and other design considerations to achieve top-level, world-class optical design technology. Later advances in supercomputers, microcomputers and personal computers and the accompanying increase in processing power and decrease in cost gave designers greater control to produce high-end lens designs. Commercial lens design software also grew in popularity, making it now possible even for individuals to design high-end lenses.

7.3 Advances in Optical Glass [14)]

The first research on glass used in lenses, or optical glass production methods, was carried out by P. L. Guinand (1748-1824) of Switzerland. After the discovery of achromatic lenses and as lead-oxide-rich flint glass (used widely by craftspeople) began to be used in lenses, there were issues with cords or striae and other refractive index irregularities and issues with visible light transmittance. Around 1790, Guinand came up with a method of melting the materials in a crucible at high temperature and stirring them mechanically. This method is still used today. Fraunhofer took this method and further improved it to successfully produce large amounts homogenous optical glass in 1812, which he used to complete a 175mm diameter achromatic objective lens for a telescope. He also made detailed refractive index readings of the dark lines he discovered in the solar spectrum (Fraunhofer lines) and based his lens design and production on this data. French company Parra-Mantois and British company Chance Brothers continued with Guinand's production method and the two countries became major suppliers of optical glass in the late 19th century. There were further advances in lens design theory and high hopes for new types of optical glass with refractive index and dispersion properties not found in the existing optical glass to correct various aberrations in a balanced manner. German optical glass researcher Schott met with Abbe of Zeiss, who had established the design theory for microscope objective lenses and observed the properties of optical glass, and in 1884 the two jointly developed new types of glass, such as barium crown glass, and also established an optical glass factory in Jena (see 3.4). This glass had a higher refractive index than existing glass, which made it very useful for designing apochromatic objective lenses and high-performance photographic lenses. A company catalog published in 1886 lists 44 types of optical glass and their characteristics and prices, including 19 new types of glass. By 1888, 24 more types had been added. The following year, dense crown glass was successfully developed. The 1913 catalog lists 97 types of glass, including 17 new types, while the 1923 catalog lists 114 types, including 22 new types of glass. Figure 7.8 shows an optical glass map from the 1923 catalog, believed to be the earliest

by Schott. The existing types of glass form an arc from the upper right of the figure to the lower left, with many new types of high refractive index glass widely distributed. Due to Schott's development of new glass and superior product quality control, Schott glass gained a high reputation as "Jena glass", surpassing optical glass from Britain and France. Later, G. W. Morey of the United States developed high refractive index lanthanum glasses, containing rare elements such as lanthanum (La), thorium (Th) and tantalum (Ta). These glasses dramatically improved the potential of optical design.

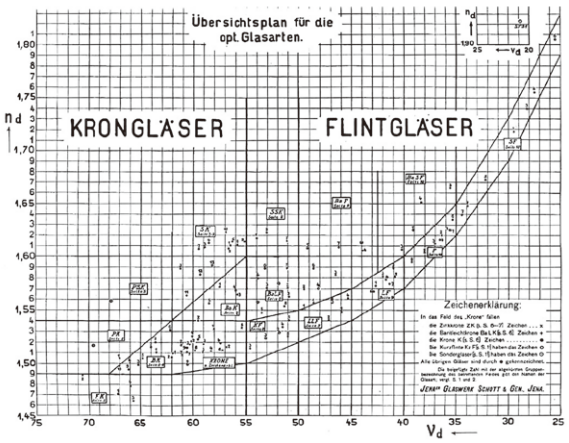


Fig. 7.8 Schott 1923 Optical Glass Map [15)]

In Japan, many ordinary glass industries came into being in the early 20th century, but manufacturers of colorless, homogenous optical glass were nonexistent. This glass had to be imported. The first optical glass was successfully produced by the naval arsenal in Tokyo (Tsukiji) in 1918. After that, Nippon Kogaku took the lead in developing and producing glass, achieving success in high volume melting in 1927 and producing 15 types of glass by around 1935. That year, principal researcher Ohara Jinpachi (1888-1968) founded the Ohara Optical Glass Manufacturing Company (now Ohara Inc., referred to below as Ohara) and became Japan's first specialized optical glass manufacturer. The company continues to supply optical glass to many optical instrument manufacturers today. Fuji Photo Film (now FUJIFILM) started producing optical glass in 1938, while the Chiyoda Optical Seiko (now Konica Minolta) did the same in 1944. Toyo Optical Glass Manufacturing (now HOYA) was founded in 1941 to produce optical glass, as was the Sumita Optical Glass Manufacturing Company (now Sumita Optical Glass Inc.) in 1953.

When optical glass production resumed in Japan after the war, there was a growing demand for optical glass, mainly for camera lenses. There was also strong demand for high refractive index glass. Optical glass manufacturers began sharing technology and collaborating on research with universities and government research stations. In 1948, collaborative research by five members of the Japan Optical Glass Manufacturers' Association resulted in the development of a new type of glass. To achieve a high refractive index required glass containing elements such as lanthanum (La), tantalum (Ta), titanium (Ti) or zirconium (Zr). As this glass was difficult to melt in conventional clay crucibles, platinum crucibles began to be used in glass production. Prominent manufacturer Ohara successfully developed LaSF glass (now called S-LAH) in 1958, with a refractive index of 1.8. The number of glass types increased to 138 by 1962, 207 by 1967 (Figure 7.9) and 229 by 1986. Meanwhile, Schott successfully developed a low refractive index, low dispersion glass (ED glass) called FK01 (now S-FPL51) in 1975. followed by FK03 (now S-FPL53) in 1989, which had almost identical optical properties to fluorite. This made it possible to transition away from fluorite, which was expensive and difficult to work with, and contributed greatly to the design and development of apochromats, semi-apochromats and other high-quality microscope objective lenses.

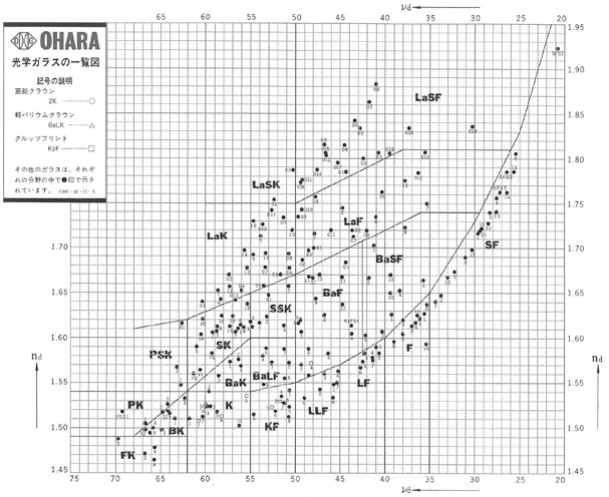


Fig. 7.9 Glass Chart from 1967 (n_d – ν_d) Ohara Optical Glass 16)

The Ohara Optical Glass Manufacturing Company changed its name to Ohara Inc. in 1985. In 1990, the company named its own glass instead of using names designated by Schott. It also led the world in developing lead-free and arsenic-free glass as an environmental measure, announcing this initiative in 1991. After that, out of consideration for eco-friendly resource conservation, the types of glass dropped from around 250 to 112. By 1997, all

recommended optical glass was eco-friendly. Short flint (KzF, KzFS), the anomalous dispersion glass used in microscope objective lenses, also ceased production. However, research on an eco-friendly alternative resulted in the successful development of S-NBH and S-NBM glass in 1998. Initiatives to develop an optical glass with low autofluorescence to use in fluorescence microscopes culminated in the announcement of a new type of S-NBH glass in 2007. Further progress was made on high refractive index glass and now there are several types of glass on the market with a refractive index of over 2.0. Figure 2.4 shows the latest optical glass map (Ohara).

Ohara, HOYA, Sumita Optical Glass, Hikari Glass (Nikon Group since 2004) and other outstanding optical glass manufacturers have been proactive in understanding global trends and the needs of optical instrument manufacturers. These Japanese companies have worked on developing new types of optical glass and making improvements to product quality and cost. This has been a key contributing factor in Japan continuing to remain at the top of the global optical industry.

7.4 Japanese Development of Objective Lenses

7.4.1 To 1945 [5) 6)]

Industrial production of microscopes in Japan, as mentioned in 4.2, began with copying products imported from Leitz and other overseas companies. However, this was a constant struggle, as there were significant differences in performance and there was no access to design data including optical materials, especially for producing objective lenses. According to Suzuki Taiichi, an apprentice to Katō Kakitsu in charge of lens machining and assembly at M & Katera, early objective lens manufacturing was as follows, “We would cut Leitz objective lenses through the center and measure their thickness, curvature and distances, etc. and then grind out several types of glass we had in stock and replace them for the Leitz lenses one by one to decide which glass was best. We repeated this endlessly and managed to produce what looked like objective lenses using only the glass we had. But ordinary grinding methods were no good for the top high magnification objective lens, which was hemispherical or thicker with a narrow radius of curvature, so we would stick the lens on the head of a nail on the end of a pole and manage to grind it that way. Our glass was made up of different materials from Leitz lenses, so some of our lenses would not focus, even when touching the specimen cover glass. Lens manufacturing in those early days was done in a special small room in the factory with a contact window. The technology was almost like a secret art.” Improvements were made through these vast amounts of trial and error. Objective lens machining and assembly technology steadily improved. M & Katera Optical Works released its first oil-immersed 1/12 objective lens in 1918 (focal length in inches, around 90×). Along with this, improvements were also being made to the accuracy of the fine movement mechanism for focusing and the cross-motion stage mechanism for moving the specimen in the XY direction. In 1924, a high-end microscope was developed with a total magnification of 1000×. Takachiho had produced a 100× objective lens in 1920 (Figure 7.10) and launched the GK high-end oil-immersion microscope (“Showa”) in 1927.

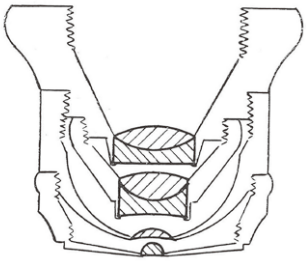


Fig. 7.10 Achromat 100×oil-Immersion Lens Cross Section [17)]

However, the highest quality objective lens in the world was the Apochromat, completed by Abbe in 1879. Due to the distinctive characteristics of the glass, it was thought to be impossible to manufacture in Japan. In 1928, Takachiho Works started researching the Apochromat, funded by an industrial research grant from the Ministry of Commerce and Industry. One of the materials in the convex lens was colorless and transparent natural fluorite. The company did not know where to obtain this and searched everywhere for a source. It was found to be naturally present at Obira mine (Ōita prefecture). As fluorite is a crystal with low hardness, processing it for high-precision lenses required a lot of skill and expertise. Alum was also used as another material for a concave lens. There were anecdotes of this substance deliquescing and disappearing during research and analysis. The grinding method and frame structure for the alum lens also needed new technological development.

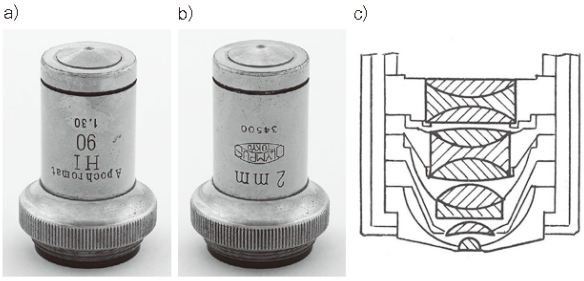


Fig. 7.11 Apochromat 90×oil-Immersion Objective Lens [18) 19)]

a) Front side b) Reverse side c) Cross section [17)]

Researchers persevered through these setbacks to produce a 90× Apochromat oil-immersion objective lens prototype in 1931 (NA 1.30, Figure 7.11), followed by 10× (NA 0.30) and 20× (NA 0.65) Apochromats and a 40× Apochromat dry objective lens (NA 0.95) with a correction collar in 1934 (Figure 7.12). These were launched in combination with the "Fuji OCE" high-end microscope [6) 17)].Completing these Apochromats was a ground-breaking result, indicating the rising level of optical technology in Japan at the time.

Fig. 7.12 Apochromat Objective Lenses [19)]

a) 10× b) 20× c) 40× with correction collar d) Ditto, Reverse side

7.4.2 Development from 1945 to 1975

After the war, the Japanese microscope industry resumed production of its prewar models, but as demand grew, there was also demand for higher performance. While manufacturers of course worked on mechanical precision, a major challenge was improving the optical components, especially the objective lens. In 1950, the first issue of the Journal of the Mechanical Testing Laboratory was published, "Kokusan Kenbikyō no Genjō [Current State of Domestically Produced Microscopes]", including promising results of a review of objective lenses indicating that Japanese lenses were not inferior to Western lenses. However, the objective lenses reviewed were only achromats, not apochromats or semi-achromats (fluorite). Flat-surfaced plan achromats had also not yet been developed by any company. Thus, it was conceded that overall microscope optical performance in Japan was still largely trailing behind the West.

Olympus and Nippon Kogaku both added plan objective lenses to their lineup between 1963 and 1967. In contrast, a paper published in 1964 described a total of 66 types of biological and industrial plan objectives produced by Leitz, ranging from 1× to 160×, showing a stark contrast in technological development capabilities [20)]. Figures 7.13 and 7.14 show the structures of Olympus and Nippon Kogaku Achromat objectives (Ach) at the time, while Figures 7.15 and 7.16 show the two companies' Plan Achromat objectives (Plan). The horizontal lines in the upper section of each figure indicate the objective lens mounting position (revolving nosepiece mounting position), the lenses with diagonal lines and cross hatching indicate ED glass and fluorite, respectively (the same applies below). Olympus completed its Plan objective lens series for industrial microscopes in 1966 (mechanical tube length 200mm, parfocalizing distance 42.5mm), while Nippon Kogaku did the same around 1970 (mechanical tube length 210mm, parfocalizing distance 33.6mm). Compared to Achromat objective lenses, these have more lenses, including meniscus lenses, and use more fluorite and ED glass with higher magnification.

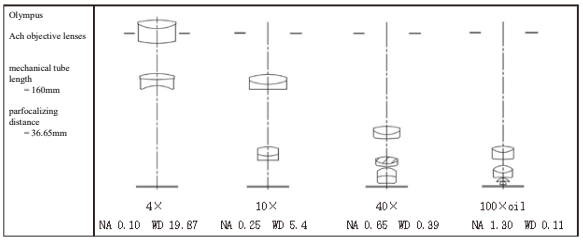


Fig. 7.13 Structure of Achromat Objective Lenses (Olympus) [21)]

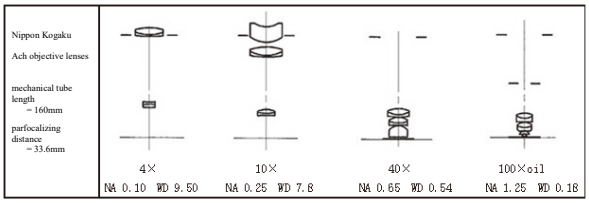


Fig. 7.14 Structure of Achromat Objective Lenses (Nippon Kogaku) [22)]

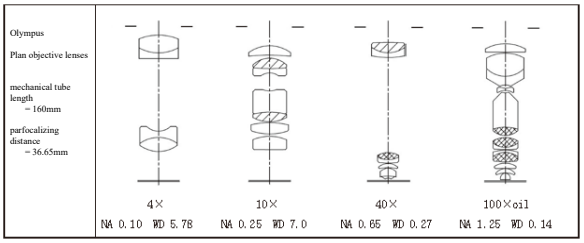


Fig. 7.15 Structure of Plan Achromat Objective Lenses (Olympus) [21)]

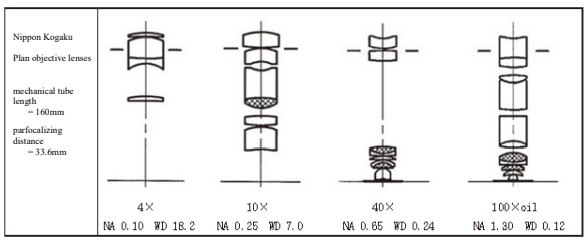


Fig. 7.16 Structure of Plan Achromat Objective Lenses (Nippon Kogaku) [22)]

The highest quality plan apochromat (PlanApo) objective lens had been manufactured in series in Germany since the 1950s. In Japan, Nippon Kogaku launched the first PlanApo 100×oil-immersion objective lens (Figure 7.17) and plan

fluorite PlanFl 40× in 1964. Olympus had completed an apochromat objective lens series before the war, but after the war only continued with semi-apochromat objective lenses using fluorite, the Fl 100× (NA 1.30, oil-immersion) and Fl 40× (NA 0.77), released in 1953-1955. The company completed a plan apochromat series comprising 4×, 10× and 20× lenses in 1972, followed by a 40× with a correction collar and an oil-immersion 100× in 1974 (Figs. 7.18 and 7.19). The most difficult of these was the 100×, made up of a tiny hemispheric front lens of crown glass with a refractive index almost identical to that of the immersion oil and a meniscus lens made of flint glass with a very high refractive index (Figure 7.20). The rearmost concave lens was made up of three layers, consisting of alum with anomalous dispersion properties (n=1.456) sandwiched between regular glass lenses (the dotted area shown in the figure). Engineers had to study in Germany to learn the grinding technology to achieve this. Japanese objective lenses seemed to catch up the German lenses technically, but were still no match in terms of actual overall performance.

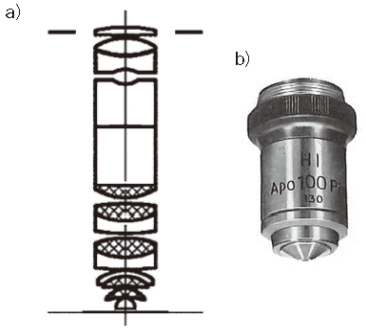


Fig. 7.17 Nippon Kogaku Plan Apochromat 100×oil [22)]

a) structural view b) external view

(Mechanical tube length: 160mm; parfocalizing distance: 33.6mm; NA: 1.30; WD: 0.12)

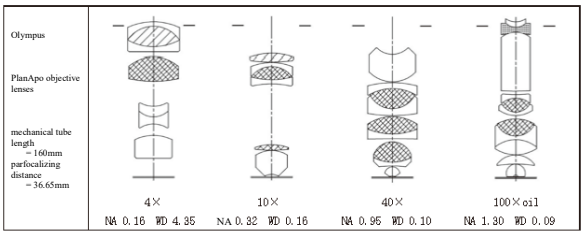


Fig. 7.18 Structure of Plan Apochromat Objective Lenses (Olympus) [21)]

Fig. 7.19 Plan Apochromats
(from left) 4×, 10×, 20×, 100×oil [21)]

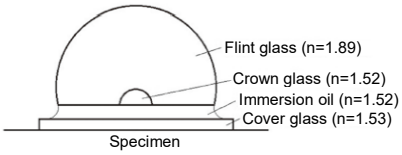


Fig. 7.20 Structure of Plan Apochromat 100×oil Front Lens

7.4.3 Development from 1976 to 1992

Japanese microscope manufacturers added new models to their lineups, from ordinary educational and student microscopes to high-end microscopes for cutting-edge research. These were well received and their market share grew, although they still were not as highly regarded as the world-leading German microscopes, especially in fields such as biomedical research. To overcome this situation, Japanese manufacturers had to engage in research and development to rebuild their entire microscope systems, and above all, to achieve top-ranking optical performance.

Nippon Kogaku led out by changing the parfocalizing distance of its objective lenses from 33.6mm to the 45mm specified by the German industrial standard (DIN) (no change to the 160mm mechanical tube length). The company also made plans to redesign the optical system and embarked on a project with members from business, design, technology and manufacturing. The change in parfocalizing distance was useful in adding a greater degree of freedom in optical design, but it disadvantaged users in terms of compatibility with other microscope systems. The decision was made based on this issue. It was common practice in optical design to use the compensation method established by Abbe in the late 19th century to correct lateral chromatic aberration between the objective lens and the eyepiece. By contrast, Nippon Kogaku designed a new chromatic aberration free system (CF system), in which chromatic aberration in the objective lens and eyepiece are corrected separately. After testing this new design against the conventional system, the decision was made to transition to the new system and development began [23) 24)]. The CF system has the following five distinguishing characteristics.

1) Lateral chromatic aberration is corrected properly from the center of the field all the way to the peripheral edge.
2) There are no changes in color at field stops of eyepiece, allowing 100% effective field viewing (in the conventional system, there are red tinges inside of the stops).
3) When a graticule is inserted into the eyepiece, there are no color changes around the graduated scale or numbers.
4) Longitudinal chromatic aberration is improved for the objective lens and eyepiece.

5) Both the objective lens and eyepiece are their own complete optical systems and can be applied to any field.

The Abbe compensation method was adopted because it was difficult to correct lateral chromatic aberration, particularly for high magnification objective lenses. This was resolved by dividing the objective lenses into front and rear groups and over-correcting the front group, then inversely correcting the rear group. Figure 7.21 shows the structures of CF system Achromat (Ach) objective lenses, Plan Achromat objective lenses (Plan) and Plan Apochromat objective lenses (PlanApo). The distinguishing characteristics are clearly shown when compared to the conventional structure of the Ach 40× and 100×oil (e.g. Figure 7.14). For high-quality Plan and PlanApo objective lenses, aberrations such as strong chromatic aberration and curvature of field could be corrected even at high numerical aperture by using fluorite or the newly developed ED glass. For epifluorescence, the CF UV-F series was also added to the lineup, lenses with low autofluorescence and high transmittance in the near-ultraviolet range. Figure 7.22 shows the external view of different biological CF series objective lenses. The CF MPlan (structure shown in Figure 7.23) and CF MPlanApo metallurgical objective lens series were also developed (mechanical tube length 210mm, parfocalizing distance 45mm), as well as the CF BD Plan and CF BD PlanApo series for reflected light bright/dark field microscopy. The CF system was launched in 1976 in three microscopes in the newly developed high-end Microphot V series: the Biophot biological microscope (Figure 4.31), the Fluophot fluorescence microscope (Figure 5.38) and the Metaphot metallurgical/industrial microscope (Figure 6.57).

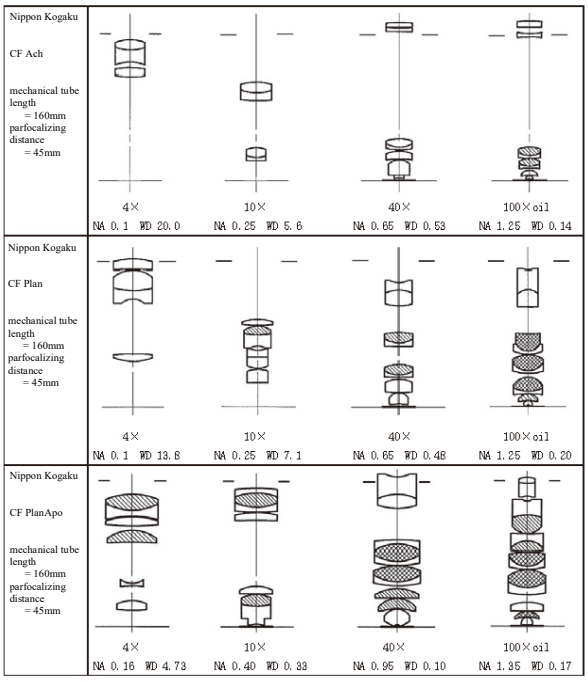


Fig. 7.21 Structure of Nippon Kogaku CF Objective Lenses

(extract from Ach, Plan, PlanApo Series) [22)]

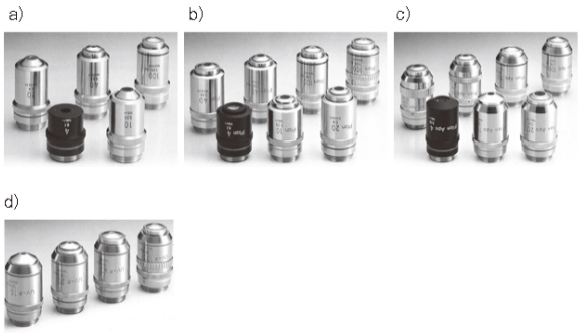


Fig. 7.22 Nippon Kogaku Biological CF Objective Lenses [22)]

a) Ach series: front row 4× 10×; back row 20×, 40×, 100×oil
b) Plan series: front row 4×, 10×, 20×; back row 40×, 40×NCG (no cover glass), 100×oil, 100×oil with iris
c) PlanApo series: front row 4×, 10×, 20×; back row 40×, 60×, 100×oil, 100×oil NCG (no cover glass)
d) UV-F series:10×, 20×, 40×gly, 100×gly (gly refers to glycerol immersion)

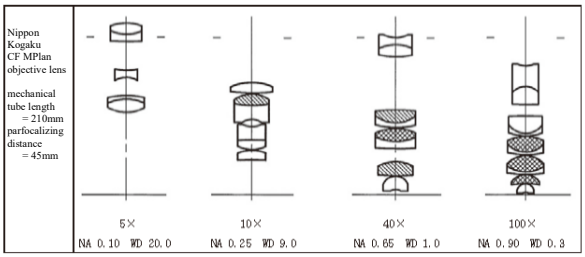


Fig. 7.23 Structure of Nippon Kogaku CF MPlan Objectives

(extract from series) [22)]

Olympus started planning development of a new optical system in 1974, changing the parfocalizing distance from the existing 36.65mm (42.5mm for metallurgical) to the 45mm specified by the DIN. The distinguishing characteristics of this long barrel (LB) biological microscope objective lens system are given below [25)].

1) The main Plan objective lens series are the high-grade SPlan and the regular DPlan series.
2) The high-end SPlanApo series increases the NA and completely corrects chromatic aberration.
3) The standard field number increased from 18 to 20, intended to enlarge objective lens working distance and improve operability.
4) SPlanFl 1× and 2× very low magnification objective lenses added to the lineup with the same parfocalizing distance as other magnifications.
5) Improved image contrast range using new ED glass and new multicoating technology.
6) Ach and regular Plan objective lenses designed to reduce costs while maintaining or improving existing performance.
7) Basic optical design concept is that lateral chromatic aberration remains the same as the compensation method with 160mm mechanical tube length.

However, not long after this design was established, Nippon Kogaku announced the CF optical system and a new microscope series. Olympus hurriedly pushed forward with the SPlan series and released it on the market in 1978 (the microscopes were the AH-LB and the BH-LB). In developing the LB, the company had formed an internal advisory committee for matters relating to design, processing and evaluation. The committee was involved in the development of various technical elements, including development and theoretical optimization of a new phase film for phase contrast microscopy and development of non-fluorescent oil (silicone oil) for epifluorescence microscopy. Figure 7.24 shows the structure of the DAch, SPlan and SPlanApo in the LB objective lens series. Figure 7.25 shows photographs of these lenses.

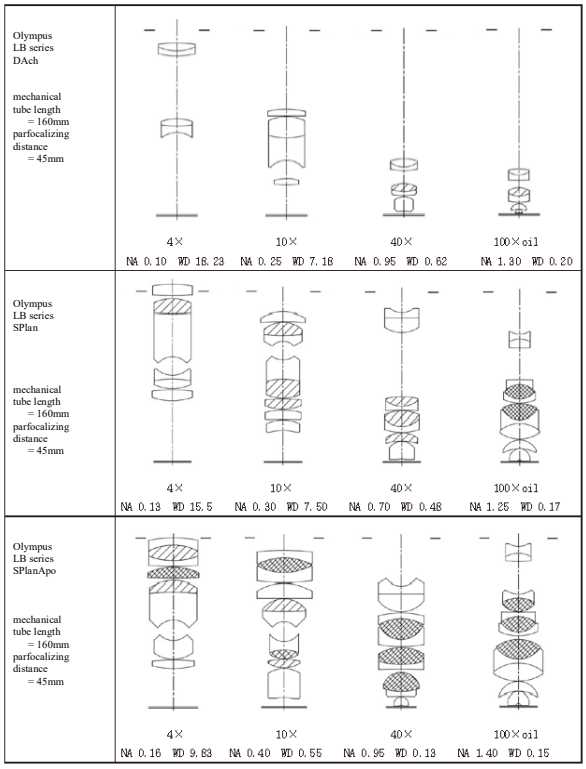


Fig. 7.24 Structure of Olympus LB Objective Lenses

(extract from DAch, SPlan, SPlanApo series) [21]

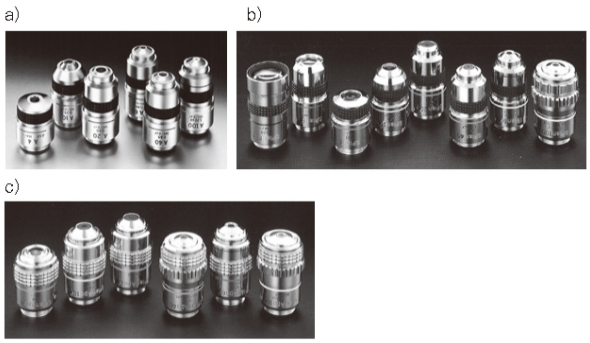


Fig. 7.25 Olympus LB Objective Lenses (Biological) [21]

a) DAch 4×10×, 20×, 60×, 40×, 100×oil
b) SPlanFl 1×, 2×, SPlan 4×, 10×, 20×, 40×, 100×oil, 100× dry
c) SPlanApo 4×, 10×, 20×, 40×, 60×oil, 100×oil (with iris)

After bringing the LB optical system to a certain point of completion, Olympus moved on to developing metallurgical/industrial objective lenses. The design concept was not simply lengthening the parfocalizing distance from 42.5mm to 45mm, but changing the mechanical tube length from the conventional 200mm to an infinity corrected optical system (see 2.4). The objective lens series was named the infinity corrected (IC) optics [26]. Since the tube and eyepiece were the same as for the LB optical system, the compensation method was used for correcting lateral chromatic aberration. The focal length of the tube lens was set at 180mm after various testing. Like the biological LB system, the plan objective lenses were divided into the high-grade MSPlan series (Figure 7.26a) and the regular MDPlan series. These were joined in the lineup by the bright and dark field NeoSPlan series (Figure 7.26b) and NeoDPlan series. These were all launched in 1981, together with the MSPlan-NIC and NeoSPlan-NIC series with reduced optical strain for reflected polarized light / differential interference contrast microscopy and the ultra-long working distance ULWDMSPlan (Figure 7.26c) and ULWDNeoSPlan (Figure7.26d). Including later additions, the number of objective lenses has grown to over 50 types.

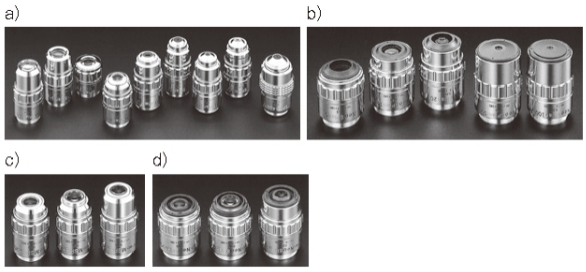


Fig. 7.26 Olympus IC Objective Lenses [21]

a) MSPlan 1.5×, 2.5×, 5×, 10×, 20×, 50×, 100×dry, 100×oil, MSPlanApo 50×
b) NeoSPlan 5×, 10×, 20×, 50×, 100×
c) ULWDMSPlan 20×, 50×, 80×
d) ULWDNeoSPlan 20×, 50×, 80×

Precision measuring instrument manufacturer Mitutoyo Manufacturing (name changed to Mitutoyo Corporation in 1987, referred to as Mitutoyo below) joined the microscope industry in 1984 with the launch of the FS optical microscope series, mainly aimed at the industrial market. The most prominent feature of this series was the infinity corrected objective lens series (tube lens focal length 200m), with a parfocalizing distance 95mm, more than double the standard parfocalizing distance (not stipulated in ISO 9345-2 or JIS B 7132-2) [27) 28)]. The standard MPlanApo and the bright/dark field BD PlanApo series were designed with an ultra-long working distance to make good use of the particularly long parfocalizing distance. The series also featured Apochromat lenses using many ED glasses.

Table 7.2 Mitutoyo MPlanApo Objective Lens Specifications

	1×	2×	5×	7.5×	10×	20×	50×	80×	100×	200×
MPlanApo	0.025 11	0.055 34	0.14 34	0.21 35	0.28 33.5	0.42 20	0.55 13		0.7 6	
MPlanApo-SL						0.28 30.5	0.42 20.5	0.5 15	0.55 13	0.62 13
MPlanApo-HR			0.21 25.5		0.42 15		0.75 5.2		0.9 1.3	

(top line: NA, bottom line: WD)

Table 7.2 shows the specifications of the MPlanApo, the long-working-distance MPlanApo-SL and the high-NA MPlanApo-HR series. Figure 7.27 shows photographs of the MPlanApo series. Maintaining its presence in the industrial market, Mitutoyo later expanded its lineup with the MPlan-NIR and LCD inspection LCDPlan-NIR series with chromatic aberration correction for visible and near-infrared light and increased near-infrared transmittance, the MPlan-NUV and LCD inspection LCDPlan-NUV series with chromatic aberration correction for visible and near-ultraviolet light and increased near-ultraviolet transmittance, the MPlan-UV series with fluorite and synthetic quartz lenses to ensure UV transmittance and performance, and the GPlanApo series, designed for viewing through a thick 3.5mm cover glass.

Fig. 7.27 Mitutoyo MPlanApo (back row) and MPlanApo-HR (front row) Series [29)]

Nippon Kogaku launched the Microphot FX high-end universal photography microscope in 1985 together with new NCF series objective lenses, which had better contrast and resolving power than the existing CF series. The NCF Plan and NCF PlanApo series (Figure 7.28) had higher numerical apertures than the CF Plan and CF PlanApo, rivalling the SPlan and SPlanApo by Olympus.

7.4.4 Developments Since 1993

Changes in parfocalizing distance and the transition of metallurgical/industrial objective lenses to infinite mechanical tube length continued in Japan. In 1986, as this came to near completion, Zeiss announced a succession of new microscopes featuring a completely new infinity color-corrected system (ICS) [30)], including the Axiophot, Axioplan, Axiotron and Axiovert (see 4.7). The ICS system was designed for infinity corrected mechanical tube length as well as lateral chromatic aberration correction in both biological and metallurgical/industrial microscopes.

All the objective lenses and eyepieces were completely new designs, evidence of the company's credibility as a leader in high-end microscopes. The tube lens was combined with the objective lens and corrected all aberrations. The idea of an infinity corrected optical system for biological microscopes was not new, but the full-scale adoption of it by Zeiss gained the approval of biomedical researchers around the world. Leica announced the Delta optics system [31)] using a similar idea in 1992 and developed it into the harmonic compound system (HCS) in 1998.

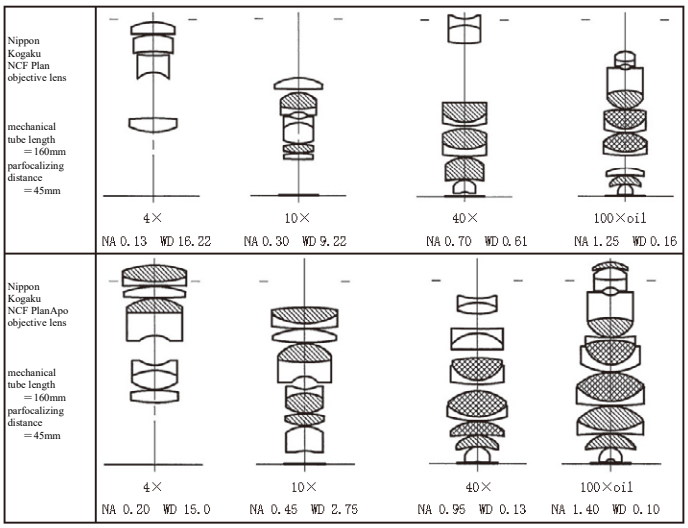


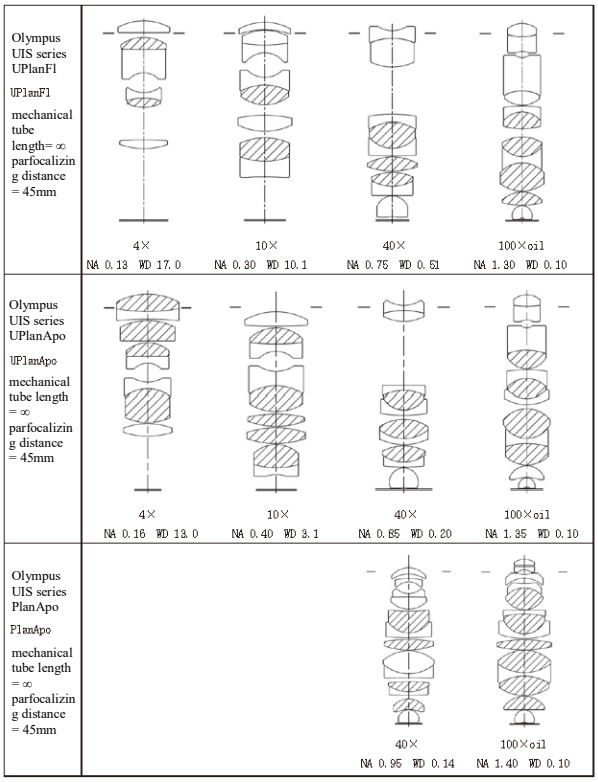
Fig. 7.28 Structure of Nippon Kogaku NCF Objective Lens

(extract from NCF Plan, NCF PlanApo Series) [22)]

In Japan, Olympus started investigating how to adopt this optical system. In 1988, after studying the technological and business aspects of it, the company started a development project, having decided to use the new concept to completely redesign its objective lenses and eyepieces for biological and metallurgical/industrial microscopes. The optical system was named the universal infinity system (UIS), designed for system scalability in order to incorporate various needs and perform at a world-class level [32) 33)]. The distinguishing characteristics of the system are given below, with respect to how it differs from the prior ICS series.

1) It offers world-class resolving power and contrast for all types of microscopy.
2) It has high system scalability through infinity correction, with biological and metallurgical/industrial microscopes using the same design concept.
3) It is a highly scalable, fully flexible system that completely corrects all aberration in the objective lens, tube lens and eyepiece respectively.
4) It has a lineup of universal objective lenses in one series that can be used for various different types of microscopy.
5) It is designed for significantly improved operability, with the standard field number increased to 22 (previously 20) and the objective lens working distance (WD) also increased.

Following a variety of design tests and prototype evaluations, the tube lens focal length was set at 180mm, the same as the IC optical system. There were two series of universal objective lens, the Plan Apochromat and the Plan Fluorite. This was difficult to design, as these needed to maintain the original high level of bright field performance as well as achieve improved near-ultraviolet transmittance and polarized light characteristics. The degree of design freedom was limited by the selection of glass, which had to have high near-ultraviolet transmittance and low autofluorescence, especially for fluorescence microscopy. It was around this time that special optical glass with near-identical properties to fluorite emerged, such as FK03 (made by Ohara, now called S-FPL53) and GFK70 (made by Sumita Optical Glass, also called Gadron) (see 7.3). These types of glass were used in lens design as a substitute for the difficult-to-process fluorite, which led to improved productivity. The development of broadband anti-reflection coating technology, including for the near-ultraviolet range, also served as a useful resource. The introduction of high-speed, high-accuracy and highly efficient automated lens processing lines to factories also played a significant role in improved productivity. In 1993, the UIS system was launched with the newly developed BX microscope series (see 4.7). The following year, UIS systems for inverted biological microscopes and metallurgical/industrial microscopes was released with the AX, IX and BXM series of microscopes. These were highly regarded by users in Japan and overseas.



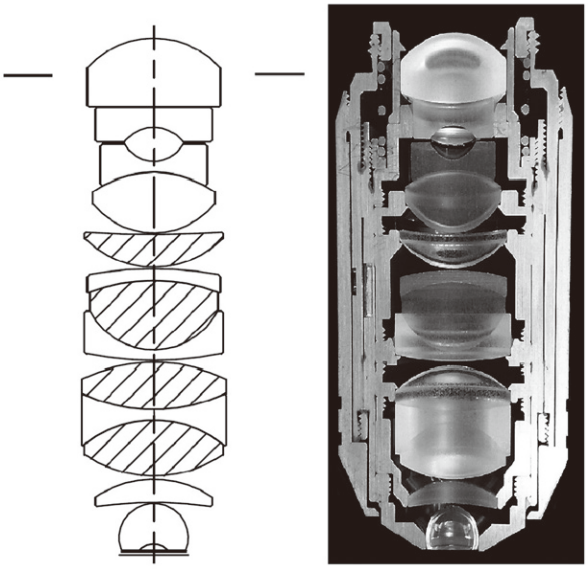


Fig. 7.29 Structure of Olympus UIS Objective Lenses [21)]

a) extract from the UPlanFl, UPlanApo and PlanApo series

b) Structure and cross-sectional photograph of the PlanApo 60×oil

Figure 7.29 shows the structure of UIS objective lenses and a cross-sectional photograph of the PlanApo 60×oil. Figures 7.30 and 7.31 show photographs of the biological and metallurgical/industrial series.

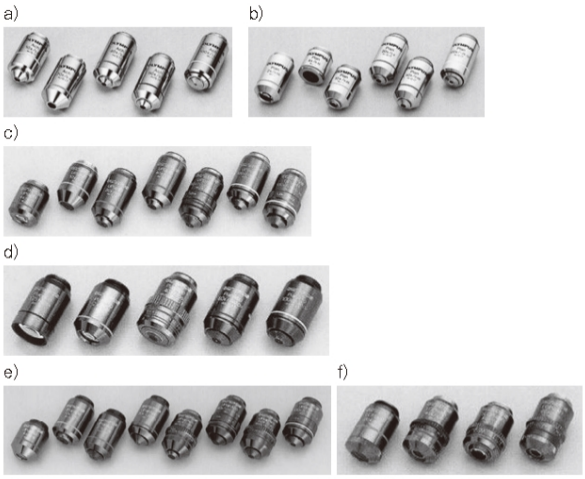


Fig. 7.30 Olympus UIS Objective Lenses (Biological) [21)]

a) Ach 10×, 20×, 40×, 60×, 100×oil
b) Plan 10×, 20×, 40×, 60×, 100×oil
c) UPlanFl 4×, 10×, 20×, 40×, 60×oil iris, 100× oil, 100×oil iris
d) PlanApo 1.25×, 2×, 40×, 60×oil, 100×oil
e) UPlanApo 4×, 10×, 20×, 20×oil, 40×, 40×oil, 60×, 100×oil
f) LCPlanFl 20×, 40×, S-40×, 60×c

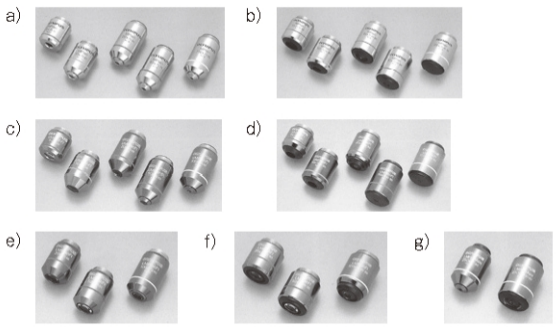


Fig. 7.31 Olympus UIS Objective Lenses (Metallurgical/Industrial) [21)]

a) MPlan 5×, 10×, 20×, 50×, 100×
b) MPlan-BD 5×, 10×, 20×, 50×, 100×
c) UMPlanFl 5×, 10×, 20×, 50×, 100×
d) UMPlanFl-BD 5×, 10×, 20×, 50×, 100×
e) LMPlanFl 20×, 50×, 100×
f) LMPlanFl-BD 20×, 50×, 100×
g) LMPlanApo(-BD) 250×

Meanwhile, Nikon kept up with the transition to mechanical tube length infinity correction by developing the new CF & IC optical system for metallurgical/industrial objective lenses in 1994, combining CF objective lenses with infinity correction. The tube lens had a focal length of 200mm. With a higher NA and better resolving power and contrast that the previous CF MPlan series, the new technology comprised the CF IC EPI Plan, the long-working-distance CF IC EPI Plan ELWD and the super-long-working-distance CF IC EPI Plan SLWD, as well as the bright/dark field CF IC BD Plan and CF IC BD Plan ELWD series (Figure 7.32).

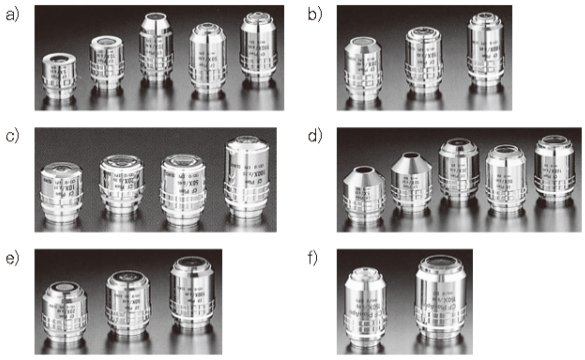


Fig. 7.32 Nikon CF & IC Objective Lenses [22)]

a) CF IC EPI Plan 5×, 10×, 20×, 50×, 100×
b) CF IC EPI Plan ELWD 20×, 50×, 100×
c) CF IC EPI Plan SLWD 10×, 20×, 50×, 100×
d) CF IC BD Plan 5×, 10×, 20×, 50×, 100×
e) CF IC BD Plan ELWD 20×, 50×, 100×
f) CF IC EPI PlanApo/CF IC BD PlanApo 150×

Nikon also developed the new CFI_{60} optical system, with fully redesigned biological and metallurgical/industrial objective lenses. The company announced the system in 1996 with the new Eclipse microscope series. Designed with improved specifications and performance, the major features of the system included the biological objective lenses also having infinity corrected mechanical tube length, the parfocalizing distance lengthening from the previous 45mm to 60mm and the revolving nosepiece mounting thread changing from RMS to M25. Table 7.3 compares the specifications of NCF series (Plan and PlanApo) and CFI_{60} series (Plan Fluor and PlanApo) biological objective lenses. The working distance (WD) clearly increases at low and medium magnification.

Table 7.3 Comparison of Nikon Biological Objective Lens CFI_{60} and NCF Series Specifications [22)]

	Plan (Fluor) series						PlanApo series							
	4×	10×	20×	40×	60×	100×oil	2×	4×	10×	20×	40×	60×	60×oil	100×oil
NCF series	0.13	0.30	0.50	0.70		1.25	0.05	0.20	0.45	0.75	0.95		1.40	1.40
	16.22	9.22	1.78	0.61		0.16	5.80	15.0	2.75	0.64	0.13		0.17	0.1
CFI60 series	0.13	0.30	0.50	0.75	0.85	1.30	0.10	0.20	0.45	0.75	0.95	0.95	1.40	1.40
	17.1	16.0	2.10	0.66	0.30	0.16	8.50	20.0	4.00	1.00	0.14	0.15	0.13	0.13

Figure 7.33 shows photographs of the main lineup of the CFI_{60} series.

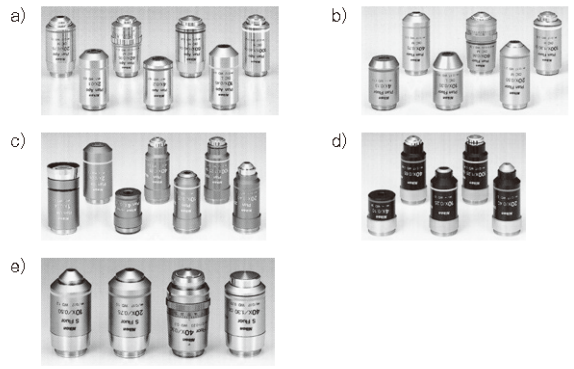


Fig. 7.33 Nikon CFI_{60} Objective Lens (Biological) [22)]

a) CFI Plan Apo 20×, 2×, 40×, 4×, 60×oil, 10×, 100×oil
b) CFI Plan Fluor 4×, 40×, 10×, 60×, 20×, 100×oil
c) CFI Plan 1×, 2×, 4×, 40×, 10×, 100×oil, 20×
d) CFI Ach 4×, 40×, 10×, 100×oil, 20×
e) CFI S Fluor 10×, 20×, 40×, 40×oil

Olympus revised the UIS optical system in 2004 and developed the new UIS2 optical system, launched with the Power BX Plus upright biological microscope and the Power IX Plus inverted biological microscope. The main features are given below.

1) Improved fluorescence S/N ratio through new lens coating technology and careful selection of glass.
2) High-end objective lens UPLSAPO series offers advanced correction of chromatic aberration from 430nm visible light to 1000nm near-infrared, as well as improved transmittance from near-ultraviolet to near-infrared, suitable for cutting-edge applications such as multicolor fluorescence microscopy.
3) Uniform illumination system and more accurate color representation of the specimen for digital imaging.
4) Environmentally friendly, with all glass material used in the optical system made from eco glass, free from lead, arsenic and other toxic substances.

Figure 7.34 shows a photograph of the UIS2 series.

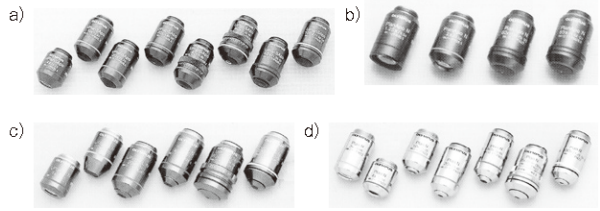


Fig. 7.34 Olympus UIS2 Objective Lens [21)]

a) UPlan SApo 4×, 10×, 20×, 20×oil, 40×, 60×w, 60×oil, 100×oil
b) PlanApo N 1.25×, 2×, 60×oil, 60×oil SC
c) UPlanFL N 4×, 10×, 20×, 40×, 60×oil, 100×oil
d) Plan N 2×, 4×, 10×, 20×, 40×, 50×oil, 100×oil

In 2012, Nikon launched the industrial CFI_{60}-2 objective lens series using its own phase Fresnel lens technology, with even lower chromatic aberration and longer working distance [34)]. The phase Fresnel lens is a type of diffractive optical element (DOE) with negative dispersion properties (for convex lenses, the shorter the wavelength, the longer the focal length), unlike regular refracting lenses. This combination makes it possible to offset color shift, thereby achieving powerful correction of chromatic aberration. The CFI_{60}-2 objective lens series comprises the long-working-distance TU Plan EPI/BD ELWD, the super-long-working-distance T Plan EPI SLWD, the high-end long-working-distance TU Plan Apo EPI/BD, the standard design TU Plan Fluor EPI/BD, the very low magnification T Plan EPI and the polarized light TU Plan Fluor EPI P, all with phase Fresnel lenses. Figure 7.35 shows photographs of the CFI_{60}-2 series.

Fig. 7.35 Nikon CFI_{60}-2 Objective Lens

a) T Plan 1×, 2×
b) T Plan SLWD 10×, 20×, 50×, 100×
c) TU Plan Fluor 5×, 10×, 20×, 50×, 100×
d) TU Plan Apo 50×, 100×, 150×

7.4.5 Objective Lenses with Special Characteristics

Thus far, this chapter has discussed standard objective lenses of various companies. However, as design technology has progressed, other objective lenses with various specifications and characteristics have been developed to meet a diverse range of needs. Below is a discussion of some of these lenses with special characteristics.

(1) Very Low Magnification Objective Lenses

While biological microscopes usually have magnifications of 4× or higher, some very low magnification objectives lenses have been developed with magnifications of 2× or lower to allow viewing and recording of specimens at a wider field. These lenses have a longer focal length, making it harder to design them with the same parfocalizing distance as other magnification levels. For example, the Olympus Plan 1.3× had a parfocalizing distance of 60.6mm instead of the usual 36.65mm, which made it less user-friendly. In 1975, Tiyoda became the first company in Japan to achieve success with very low magnification objective lens parfocalizing distance, announcing the Plan 1× objective lens (NA 0.035, WD 1.6mm) with a field number of 26.5 together with its

new MT-B microscope. In 1976, Nippon Kogaku included the CF Plan 1× to its lineup of CF objective lenses. Olympus launched the SPlanFl 1× with its LB series and the PlanApo 1.25× (NA 0.04, WD 5.1mm) with its UIS series. Figure 7.36 shows the structure of the SPlanFl 1×. As the figure shows, a lens with a focal length of 138mm has been made to fit within a parfocalizing distance of 45mm and the exit pupil (back focal point) must fit on the same mounting surface as lenses of other magnifications. This is a special type of lens made up of a convex lens group, a concave lens group and another convex lens group. Nikon developed the Macro 0.5× objective lens for its CFI series (NA 0.025, WD 7.0mm, Figure 7.37; even smaller than the other figure). This system had an additional lens (the lens at the top of the figure) separate from the objective lens, while the objective lens itself had a larger parfocalizing distance of 60mm, which made the design possible, although the specifications of a 400mm combined focal length and a φ50mm microscope field of view are not seen anywhere else in the world.

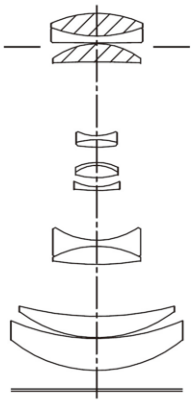


Fig. 7.36 Olympus SPlanFl 1× [21)]

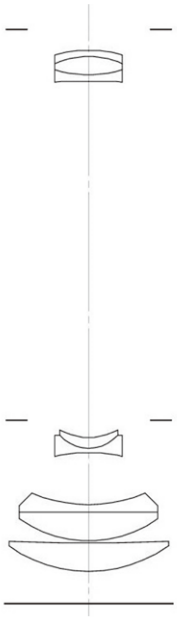


Fig. 7.37 Nikon CFI Macro Plan 0.5× [22)]

(2) Ultra-High Magnification Objective Lenses

As microscope resolving power is mainly determined by the objective lens NA, indiscriminately increasing the magnification does not necessarily make it possible to view the specimen in high detail. Generally, the effective range of the total magnification M is between 500NA and 1000NA. Magnification beyond this is called empty magnification (see 2.5). With the highest magnification objective lenses achieving 100×, or at best 160×, the need arose for an easier way to view higher levels of magnification as monitor viewing became more commonplace, primarily in the industrial inspection market. This need was met by the CF MPlanApo 200× (NA 0.95, WD 0.2mm) and the UIS series LMPlanApo 250× (NA 0.90, WD 0.80mm, Figure 7.38).

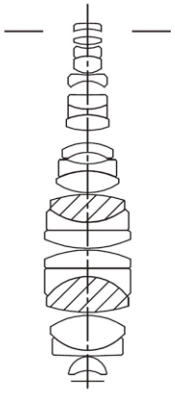


Fig. 7.38 Olympus LMPlanApo250× [21)]

(3) Ultra-Long Working Distance Objective Lenses

Microscope objective lens working distance (WD) generally gets shorter as magnification increases, although the longer the working distance, the more convenient and reliable it is to use in terms of specimen visibility and concerns over the objective lens colliding with the specimen. Accordingly, long WD objective lenses have been added to product lineups, especially for metallurgical/industrial microscopes and culture microscopes. These lenses usually have L or LWD in the name. Figure 7.39 shows the structure of an ultra-long WD objective lens. While the NA of 0.45 is low for 50×, the 15mm WD is around 4.2 times longer than the focal length. The lens is made with many ED glasses, as the front lens inevitably increases the beam height, making it susceptible to chromatic aberration. The back lenses include a strong concave power lens. This example shows a parfocalizing distance of 45mm, although long WD objective lens design is easier for objective lenses with parfocalizing distances of 60mm or 95mm, mentioned previously.

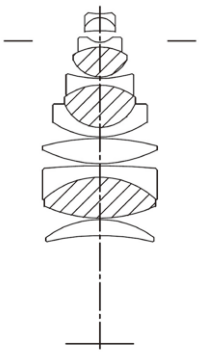


Fig. 7.39 Olympus SLMPlan 50× [21)]

(4) High Numerical Aperture (NA) Objective Lenses

Since the immersion oil used for increasing NA has a refractive index n_e of 1.518, for a long time the maximum limits for increasing objective lens aperture NA in optical design were thought to be 1.30-1.40. However, recent developments include the Olympus PlanApo N60×oil (NA 1.42) in the UIS2 series (2004) and the Nikon 100×oil H (NA 1.45) in the CFI PlanApoλ series (2011). With the emergence of total internal reflection fluorescence microscopy (TIRF, see 5.7.3) increasing the demand for objective lenses to have a high NA, manufacturers began to sacrifice image flatness for high NA in their high magnification objective lenses. Nikon brought out the CFI Apo TIRF 60×oil and 100×oil (both NA 1.49), while Olympus brought out the Apo N 60×oil TIRF, 100×oil TIRF (both NNA 1.49) and the 150×oil TIRF (NA 1.45), as well as the Apo 100×oil HR (NA 1.65, Figure 7.40) and Apo N 100×Hoil TIRF (NA 1.70), which used special oil (n_d = 1.780, ν_d = 19.1) and special cover glasses.

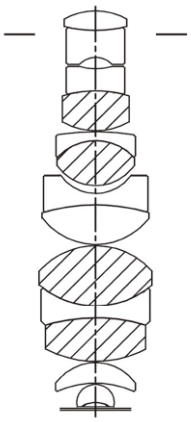


Fig. 7.40 Olympus Apo 100 ×oil HR [21)]

(5) Water Immersion Objective Lenses

Objective lenses have long been used with water as the immersion liquid (see 7.1.1). In recent years, this has again resurged for viewing cells in culture fluid. E. Neher and B. Sakmann, developers of one such electrophysiological technique, the patch clamp, were awarded the Nobel Prize in Physiology or Medicine in 1991, bringing neuroscience and electrophysiology into the spotlight. Water immersion objective lenses also played an important role in viewing and handling culture cells in new areas of technology, such as genetic analysis and monomolecular imaging. An upright microscope with a special stationary stage is used for this. Unlike ordinary objective lenses, the water immersion area is insulated, and the lens has longer working distance and an angled tip to make manipulation easier. High magnification objective lenses have a correction collar mechanism to correct aberration relative to the depth of the immersed cells. Since the objective lens is stationary, it has a relatively low magnification and high NA, and changes in magnification are carried out using a separate magnification changing device. Figure 7.41 shows the exterior view of a Nikon CFI75 LWD 16× W (NA 0.80, WD 3.0mm), with a unique parfocalizing distance of 75mm.

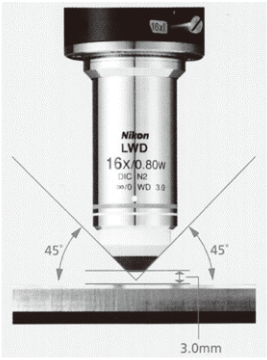


Fig. 7.41 Nikon CFI75 LWD 16× W [22)]

(6) Infrared Objective Lenses

The Si, GaAs and ceramic parts of semiconductors are transparent under infrared light with a wavelength over 1μm. This means that infrared light microscopy can be used for non-destructive viewing of IC patterns from the reverse side of chips (the reverse side of the silicon substrate needs to be ground), internal viewing of MEMS, inspecting ceramics pre-firing and various other applications, with the result shown on a TV monitor. Designed to take non-microscope viewing into account as well, infrared objective lenses correct aberrations in the range from visible to near-infrared (wavelength around 2μm) and ensure broad-spectrum transmittance. Infrared objective lenses are also used applications such as femtosecond lasers and laser repair of semiconductor circuits and liquid crystal substrates by YAG laser (1064nm). Products include the Olympus ULWDMIRPlan series (IC series) and LMPlan-IR series (UIS series), the Nikon LRPlanApo-NIR series and the Mitutoyo MPlanApo NIR series.

(7) Deep Ultraviolet Objective Lenses

Various companies have worked on developing ultraviolet objective lenses for viewing specimens in the ultraviolet range and for achieving higher resolution images. Ordinary optical glass, even fluoro-crown glass, with high ultraviolet transmittance, allows only wavelengths as low as 300nm. In deep ultraviolet, lower than 300nm, the optical materials are limited to fluorite, quartz etc. In 1959, Zeiss launched the Ultrafluar series, with high spectrum transmittance from around 240nm in the deep ultraviolet range through to near

infrared. Used in biological microscopes with glycerol as the immersion liquid (n_e=1.450, v_e=58), these were adopted for board wavelength range spectrum photometry and fluorescence microscopy. Deep ultraviolet objective lenses for high resolution industrial use include a special 100× lens (NA 0.9, WD 0.42mm, λ 266nm) in the LU2000-DUV laser microscope developed by Nikon in 1998 for use in a wafer inspection system, allowing contactless, non-destructive, real time inspection and evaluation at an ultra-high resolution of 0.1μm L/S. In 2001, Olympus developed the MApo 10× -248NC (NA 0.90, WD 0.2mm, λ 248nm, Figure 7.42) for DUV, as well as a DUV confocal unit. Both lenses were extremely complicated optical systems to manufacture, made up of groups of single lenses of synthetic fluorite and synthetic quartz without using cement lenses, requiring very high accuracy in processing and assembly.

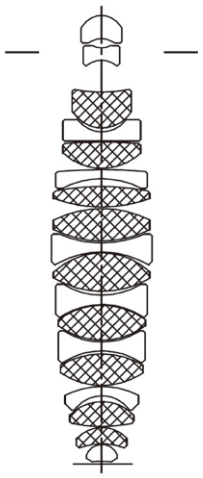


Fig. 7.42 Olympus MApo100× -248NC [21)]

(8) Other Lenses

There are many other microscope objective lenses besides the above, each designed for a different purpose. Other types of lenses include interference objective lenses that incorporate two-beam or multi-beam interferometers, reflection objective lenses, made up only of reflective surfaces and with no chromatic aberration, and measuring microscope objective lenses that fully correct distortion. The history and technological development of each of these has been omitted from this report due to space and time constraints.

7.5 Eyepiece Development in Japan

The eyepiece (see 2.6.3) is the lens that enlarges the image from the microscope, telescope or binocular objective lens. This is made up of two groups of lenses, with the front lens, or field lens, collecting the light rays from the objective lens and the back lens, or eye lens, enlarging the image. This two-lens system has long been used, as a single-lens system requires a larger lens diameter for a larger field. The Huygens eyepiece (Figure 7.43), invented by Dutch physicist C. Huygens in 1703, uses simple lenses for the field lens and eye lens, with the objective lens image position between the two lenses. The Ramsden eyepiece (Figure 7.44), invented by British J. Ramsden in 1783, has the image position on the objective lens side. The latter has the advantage of easily being able to insert graticules with crosshairs, graduation scales at the objective lens image position. In 1849, C. Kellner of Germany (see 3.4) invented the Kellner eyepiece (Figure 7.45) by replacing the eye lens in the Ramsden lens with an achromatic lens to improve chromatic aberration. As these eyepieces had relatively low field numbers, indicating a narrow field of view, and a low eyepoint height (the eye position of the viewer, shown in the figures as ●EP), they were used less frequently as microscopes became more advanced.

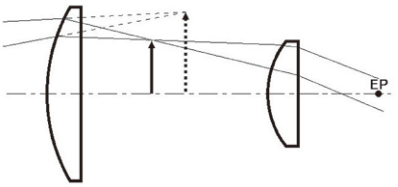


Fig. 7.43 Huygens Eyepiece

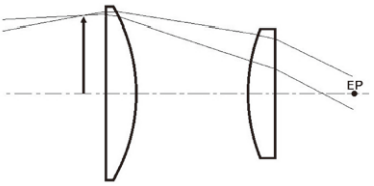


Fig. 7.44 Ramsden Eyepiece

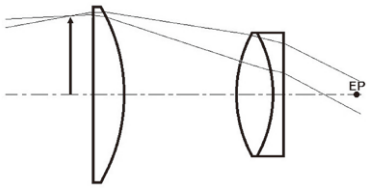


Fig. 7.45 Kellner Eyepiece

In 1860, Austrian optical instrument maker S. Plößl invented the Plößl eyepiece, an improved version of the Kellner eyepiece with four elements in two groups (Figure 7.46). In 1880, Abbe invented the Abbe eyepiece, made up of a triplet lens and a single lens (Figure 7.47). This eyepiece corrects aberration relatively well and is known as the orthoscopic eyepiece. It is still used today, as it can have a

high field number and be combined with high-end objective lenses, and has a high eyepoint, which makes it easy to use. In 1918, H. Erfle of Zeiss invented an eyepiece with a wide field of view for military binoculars (Figure 7.48). An improved version of the Plößl eyepiece with an additional single (or doublet) lens, this eyepiece has also been widely used in microscopes.

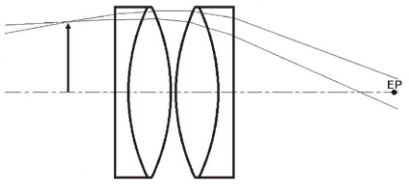


Fig. 7.46 Plößl Eyepiece

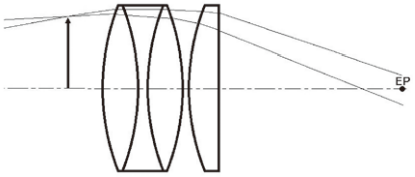


Fig. 7.47 Abbe Eyepiece

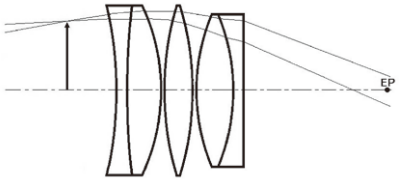


Fig. 7.48 Erfle Eyepiece

Before and after the war, most Japanese microscope eyepieces were the Huygens, Ramsden or Kellner types described above. As development progressed on Plan objective lenses, higher quality eyepieces with higher field numbers began to emerge. Standard 10× eyepieces included the Olympus WF10× (Abbe type), with a high eyepoint and field number of 18, and the Nippon Kogaku HKW10× (Plößl type), both of which appeared in the 1960s. In an ordinary viewing tube, the sleeve that held the eyepiece had a standard φ23.2 inner diameter. Olympus developed a new φ30 super-wide field viewing tube and used it in the EH and FH stands with the super-wide field eyepieces SW7× (field number 29) and SW10× (field number 26.5, Figure 7.49). In the late 1970s, improvements were made to eyepieces as new optical systems were adopted. Nikon developed the CF eyepieces, including the ultra-wide field UW10× with a field number of 26.5, while Olympus developed the WHK10× (Erfle type) with a field number of 20 for the new LB optical system. On completion of the UIS optical system, the company developed the WH10× with a field number of 22 (Figure 7.50) and the SWH10× with a field number of 26.5 (Figure 7.51). All of these eyepieces had quite complex lens structures to correct various aberrations.

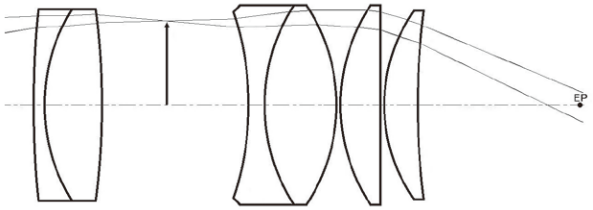


Fig. 7.49 SW 10× [21)]

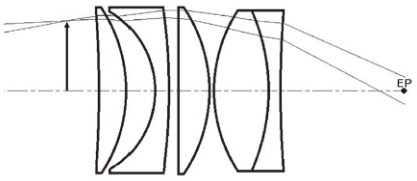


Fig. 7.50 WH 10× [21)]

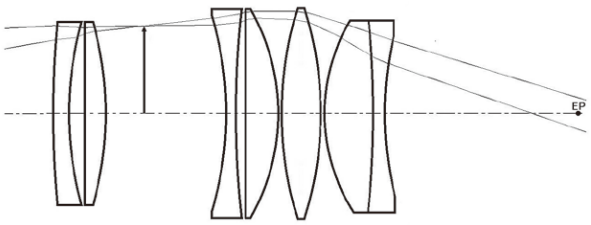


Fig. 7.51 SWH 10× [21)]

Improvements to eyepieces have continued in this manner and objective lenses continue to progress in keeping with new microscope technology. It can only be imagined what new developments will emerge in the future. While microscope images may never be visible to the naked eye, there are high hopes for new concept eyepieces, such as combining with digital display technology.

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8 The Latest Optical Microscopes

As discussed in previous chapters, long and tireless efforts have been made to improve the specifications and performance of optical microscopes through the mastery of increasingly advanced optical technology. This has resulted in extremely high-level optical instruments, and it was believed that it would be difficult to extend much further beyond the bounds of existing designs and production technology. Accordingly, little was expected in terms of progress on electron microscopes and various probe microscopes. However, in the late 1980s, the laser scanning microscope and other new types of optical microscopes began to emerge. These once again drew microscopes into the limelight, with the special characteristic of being able to view live specimens. This chapter discusses the rapid development and popularization of laser scanning microscopes, multiphoton excitation microscopes and super resolution microscopes. Each of these is still developing in terms of theoretical research, technological development and application. The hope is that these will be the subject of their own systematic survey of technology in the future.

8.1 The Laser Scanning Microscope (LSM) [1) 2)]

The laser, one of the greatest inventions of the 20th century, was invented in the 1960s. Due to its distinguishing characteristics of coherent phases, superior directionality and monochromaticity, it has been used in various fields, including imaging, information, communication, measuring, medicine and processing. Using a laser beam as a microscope illumination source on its own would only produce an inferior image glaring with interference noise. Instead, lasers are used in microscopes to form images by rapidly scanning a laser spot on the specimen. Figure 8.1 shows the basic optical system of a laser scanning microscope. The laser beam passes through a beam expander and expands in diameter, then converges again after passing through two scanners (such as a mirror galvanometer) in the X and Y directions. The light passes through the objective lens and forms a spot on the specimen. The light reflected or fluoresced from the specimen travels back through the incident light path of the laser and is transmitted into a detector system by a half mirror or dichroic mirror. The signal is processed, and an image is displayed on a monitor. The laser beam transmitted by the specimen passes through a condenser lens and into a detector. The combined use of a Nomarski prism can produce a differential interference contrast image for non-stained specimens.

A confocal optical system can be arranged by having a pinhole at a conjugated position with the spot on the specimen when focusing (Figure 8.2). Images created in these confocal optical systems have the following distinguishing characteristics compared to ordinary microscope images.

1) Since the light from outside of the focal position is excluded, it is possible to optically section thick specimens. Processing then overlapping these images can form a clear, three-dimensional image.
2) Light outside of the specimen spot, a cause of flares in ordinary microscopes, is cut out completely, allowing a very high contrast image to be obtained.
3) Making the pinhole very small can theoretically increase the resolving power by 1.4× that of ordinary microscopes.

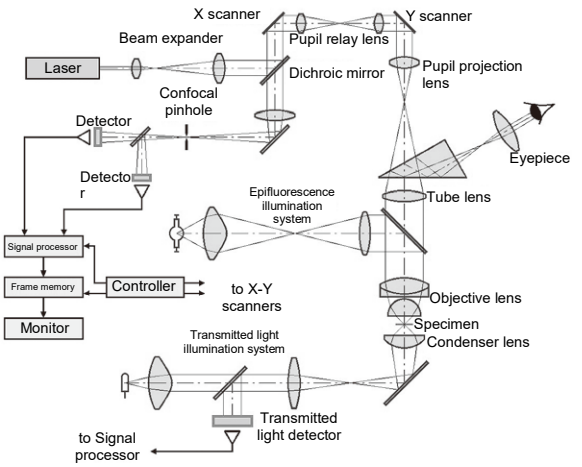

Fig. 8.1 Basic Optical System of a Laser Scanning Microscope [1)]

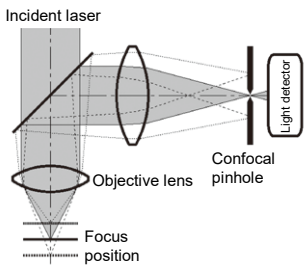

Fig. 8.2 Confocal Optical System [1)]

The basic principle of confocal microscopes that have pinholes on the illumination side and the detection side to manipulate the specimen was invented by MIT graduate M. Minsky in 1957. In 1966, Czech scientist M. Petráň invented

the multi-pinhole microscope. Using an extension of the Nipkow disk principle for mechanical television image scanning, this microscope makes it possible to view the confocal image with the naked eye or by CCD by rapid rotation. This idea was first applied to lasers for laser scanning microscopes by P. Davidovits and A. M. D. Egger in 1969. Theoretical research on LSMs and confocal optical systems was propelled by British scientists C. J. R. Sheppard and T. Wilson around 1977-1980. As the advantages of the confocal laser scanning microscope (CLSM) would be utilized best in fluorescence microscopes in medicine and biology, developments were concentrated on fluorescence microscopes, with various combinations of fluorochromes and lasers being developed. The industrial LSM had the same basic structure, but had a micro electro mechanical system (MEMS) built into the XY scanner to make it more compact. It has been widely used for high-resolution three-dimensional measuring instruments.

Commercialization of the LSM started in 1982 with Zeiss announcing an LSM with a He-Ne laser and galvanometer mirror scanning. In 1985, Bio-Rad started marketing the MRC-500, jointly developed with Cambridge University. The same year, NJS Corporation (now Lasertec Corporation) announced a color LSM, marketed from 1986 as the ILM series (industrial). Olympus released the biological LSM-GB (upright) and LSM-GI (inverted) in 1990. These machines had an image memory of 640×480 pixels. An improved model, the LSM-GB200, was launched in 1992 (Figure 8.3). Nikon released its first LSM in 1993, the RCM8000 (Figure 8.4). Yokogawa Electric entered the biotechnology arena in 1996 with the launch of the CSU series CLSM scanning unit, using a rapidly rotating multi-pinhole disk. The same year, Olympus brought out the new Fluoview (FV) LSM, followed by the FV300/500 in 1999, the FV1000 in 2004 (Figure 8.5), the box-shaped FV10i in 2008 (Figure 8.6) and the FV3000 in 2016. With each model, the company has continued to add improvements function and performance. Nikon launched the Digital Eclipse C1 confocal system in 2002, followed by the C1si real spectral imaging LSM system with an added spectral function in 2005, the functionally-improved A1/A1R CLSM system in 2008 and the C2+/C2si+ in 2011 (Figure 8.7). Major Japanese industrial laser scanning microscopes include the Olympus OLS (LEXT) series (Figure 8.8), the Keyence VK-X series and the Lasertec Optelics.

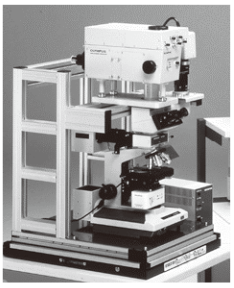

Fig. 8.3 Olympus LSM-GB200 [3)]

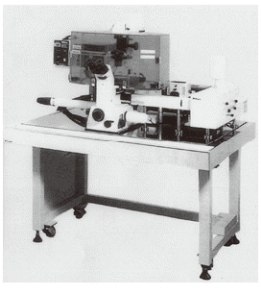

Fig. 8.4 Nikon RCM8000 [4)]

Fig. 8.5 Olympus FV1000 [3)]

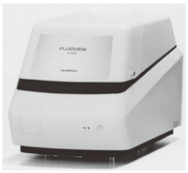

Fig. 8.6 Olympus FV10i [3)]

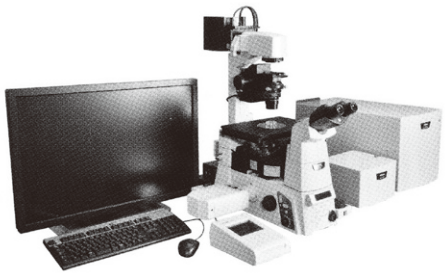

Fig. 8.7 Nikon C2+ [4)]

Fig. 8.8 Olympus LEXT4000 [3)]

8.2 The Multi Photon Excitation Laser Scanning Microscope (MPE LSM) [5) 6) 7)]

When fluorescent molecules are irradiated with an excitation light, if two excitation photons are absorbed at the same time (two-photon excitation), the energy doubles, a phenomenon similar to an excitation light with a half wavelength. Where this occurs with more than two photons, it is called multi photon excitation (MPE). This process only occurs very rarely in the natural world, although the probability can be increased by increasing the photon density to very high levels. Two-photon excitation microscopes use a femtosecond (10^{-15}) ultra-short pulse laser for an illumination source to prevent damage to the specimen from the high photon density. When a laser beam is concentrated onto the focal position of an objective lens, it automatically produces a confocal effect as two-photon excitation occurs in that position only. The image is formulated by scanning with X and Y scanners in the same way as a regular LSM, but there is no fluorescence loss as no confocal pinhole is required (Figure 8.9). Fluorescence detection near the image of the objective lens detects more fluorescence, including scattered fluorescence. Since the excitation wavelength can be twice as long, a near-infrared laser (Ti:sapphire) is used as it produces better biological tissues transparency than visible light or ultraviolet lasers. This allows microscope imaging to depths of several hundred μm to several mm below the surface of the tissue without causing damage. As a result, it is possible to view neural activity and blood flow in living animals. Two-photon excitation microscopes are often structured like a confocal LSM, although they have a broader wavelength range than one-photon excitation microscopes, so the objective lenses need to be high performance lenses with high transmittance across the wavelength range and have chromatic aberration properly corrected, as well as a long working distance to achieve deep specimen viewing. Instead of water, the immersion liquid is usually either silicone oil, as it has a closer refractive index to biological cells, or a special solution to make the tissue more transparent. Figure 8.10 shows an example of a special multi-photon excitation objective lens (Olympus XPlan N 25× SVMP, NA 1.0 WD 4mm, silicone immersion). Japanese MPE LSMs date back to 2006, with the launch of the FV1000-MPE by Olympus (Figure 8.11) and the A1R-MP by Nikon (Figure 8.12).

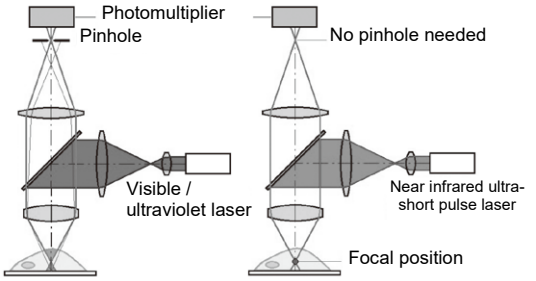

Fig. 8.9 Comparison of LSM and MPE LSM Structures [1)]

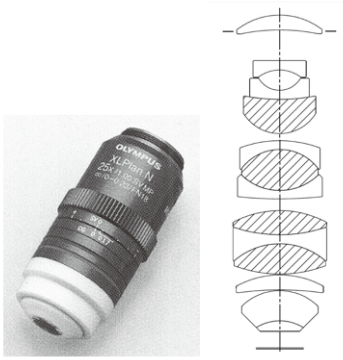

Fig 8.10 Olympus MPE Specialized Objective and Configuration [3)]

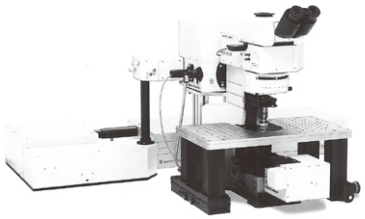

Fig. 8.11 Olympus FV1000-MPE [3)]

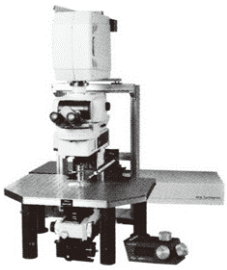

Fig. 8.12 Nikon AIR-MP [4)]

8.3 The Super Resolution Microscope (SRM) [8) 9) 10) 11)]

Super resolution in optical microscope is an optical method of producing a resolution higher than the resolution limit (around 200nm) given by the 2.7 formula (see 2.5) taken from Abbe's imaging theory. Electron microscopes use electron beams with wavelengths far shorter than light and produce higher resolutions than optical microscopes. However, there are many issues with their use in bioresearch, such as being unable to produce images of living cells and incapable of multi-staining. Accordingly, there has been a demand for a super resolution microscope with the technology to go beyond optical limits using light that is not harsh on organisms. New super resolution methods developed to resolve this include PALM, STED and SIM. Each of these targets fluorescence imaging. Developers E. Betzig (USA), S. W. Hell (Germany) and W. E. Moerner

(USA) were awarded the Nobel Prize in Chemistry in 2014. The main features of each system are outlined below.

(1) Localization

Photo-activated localization microscopy (PALM) uses strong excitation specific to certain fluorescent probes in a specimen to illuminate certain molecules separately and locate the center. Once the fluorescence fades out, excitation is carried out again to find the center of a different molecule. By repeating this process, it is possible to measure between different points of light emission with an accuracy of around 10nm, making it possible to record the location of each molecule in the specimen and build up a fluorescent image from their distribution. While the fluorescence from individual molecules is extremely faint, imaging is possible using total internal reflection fluorescence microscopy (see 5.7) to suppress the background image significantly. Stochastic optical reconstruction microscopy (STORM) is another method of super resolution microscopy that uses a similar optical system and analysis method.

(2) Stimulated Emission Depletion (STED)

Overlaying stimulated emission depletion laser beams (STED beams) with a toroidal intensity distribution at the excitation laser focal point causes the excited fluorescent molecules in the middle to emit light by natural emission and in the peripheral area by stimulated emission. The stimulated emission has the same wavelength as the STED beam. When the intensity is increased, the area of natural fluorescence emission decreases. Cutting out the stimulated emission wavelength can produce fluorescence emission with high resolution of several dozen nm.

(3) Structured Illumination Microscopy (SIM)

The specimen is illuminated with excitation light that has a fringe pattern (structured illumination). Images are captured while changing the direction and phase of the fringe. The images are then combined, processed and recompiled as a high-resolution image. The resolution can be twice as high as that of a regular microscope. This system has the advantage of being able to produce high-resolution images relatively quickly using conventional fluorescent probes.

Leica was the first to commercialize a super resolution microscope (STED) in 2007, followed by Zeiss (PALM) at the end of 2009. Nikon also launched the N-SIM (Figure 8.13) and the N-STORM (Figure 8.14) at the end of 2009, in a license agreement with the University of California and Harvard University, respectively. Olympus launched the FV-OSR software module for spinning disk super resolution microscopy (SDSRM), jointly developed in 2015 with RIKEN. The company integrated this software into the FV series confocal LSM.

Super resolution microscope technology is still under development, with the expectation that it will be possible to view live specimens at higher resolutions. There are high hopes that these new optical microscopes will lead to amazing discoveries in life science in the near future.

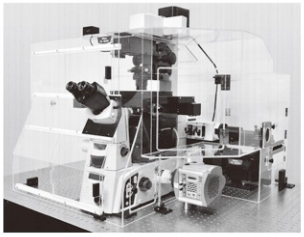

Fig. 8.13 Nikon N-SIM [4)]

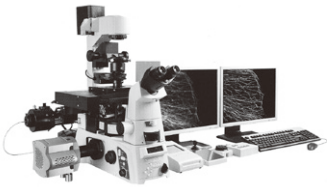

Fig. 8.14 Nikon N-STORM [4)]

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9 Conclusion and Discussion

It has been more than 420 years since the microscope was invented. Its development history includes the knowledge, passion and perseverance of pioneers wanting to explore the microscopic world. It has played a significant role in developments in medicine and other fields of science and industry. The history of microscope development can be divided into the following time periods, as shown in the flow chart of optical microscope development in Appendix 3.

1) Dawn Era: From the emergence of the first microscopes through to the early 19th century, scientific results from compound microscopes include R. Hooke's publication of "Micrographia" (1665) and A. Leeuwenhoek's discovery of microbes using a single-lens microscope (1673). Single-lens microscopes offered better magnification and resolving power.
2) Performance Improvement Era: In the mid-19th century, high-magnification objective lenses began to be produced using combinations of achromatic lenses. These compound microscopes far outdid single-lens microscopes in performance and operability. Research began on objective lens theory and design. Polarized light microscopes and reflected light microscopes emerged at this time.
3) Theory/Technology Establishment Era: In the late 19th century, E. Abbe of Germany established a microscope imaging theory and a method of objective lens design by ray tracing. Apochromatic lenses and the homogenous immersion method reached completion, significantly improving performance. Successive discoveries of disease pathogens were made using microscopes during this time, while German microscopes made by Zeiss and Leitz (now Leica) took a world-leading position. By the turn of the 20th century, German researchers had produced a succession of microscopes with various viewing methods still used today, such as stereomicroscopes, ultramicroscopes (dark field microscopes) and fluorescence microscopes.
4) Intense Pursuit Era: In the 20th century, impacted by two world wars, the microscope development environment changed drastically, with the development of lens design by computer and new types of glass, and the introduction of electronics technology. Japan started with zero experience in microscope production and steadily improved its products. With several new optical systems developed from the late 1970s onwards taking optical microscope performance close to its upper limits, Japan came to rival Germany for global dominance.
5) New Optical Microscope Era: The emergence of confocal laser scanning microscopes in the late 1980s drew attention to optical microscopes, once thought to be limited, as essential tools in bioimaging and other cutting-edge fields of research. Progress has been made on multi photon excitation laser scanning microscopes and super resolution microscopes, with further development expected.

This report discusses Japanese microscope technology, especially optical technology, in the context of optical microscope history, in the light of above. In the late Meiji Period, the dawning era of the optical industry, the pioneers of Japanese microscope industry made earnest efforts to duplicate German microscopes by dismantling them, measuring them and producing their own. Replicating the performance of objective lenses was a near impossible task, requiring high-precision machining and assembly, while not knowing the type of glass used. These challenges were surmounted with ardor and true artisan spirit, and the "M & Katera" microscope was launched in 1914, laying the foundation for the Japanese microscope industry that followed. Takachiho Works (now Olympus) was established in 1919 and went on to complete the high-end apochromatic objective lens in 1934. This ground-breaking achievement demonstrates the level of growth in the Japanese optical industry at this time. In 1917, Nippon Kogaku (Japan Optical, now Nikon) had the best optical technology in Japan and was developing and producing all kinds of optical instruments. Thus we see that the basic technology in the Japanese optical industry was already at world standard before the war. After the war, as the industrial world transitioned to peacetime industries, the optical instruments industry came into full bloom. The postwar rebuild of the microscope industry began by reviving prewar microscope models, accompanied by research and standardization initiatives by industry, government and academia. Olympus and Tiyoda Optical (formerly M & Katera) completed their own phase contrast microscopes, prototyped by the Germans during the war. This was a particularly noteworthy achievement, given the poor development conditions following the war. Japanese microscopes were being produced by Tiyoda Optical, Olympus, Nikon and many other manufacturers. These companies were steadily and reliably growing more competent in their development and production capabilities, and the products being developed were becoming more advanced and higher quality. As a result, production increased to meet the steadily growing domestic demand as well as the growing number of exports to the West and other regions overseas. Japan's global reputation for quality, value and service was growing. In the 1950s, companies began launching system microscopes with different model units, such as the Nikon S series and the Olympus E series. The 1960s saw the development of high-end universal photography microscopes, such as the Tiyoda Polyphoto, the Olympus Photomax and Vanox and the Nikon Apophot. Differential interference contrast, epifluorescence, modulation contrast and other new viewing methods were incorporated, and systems began to catch up with those of the world-leading German microscopes. The quality of Japanese optical microscopes started taking great strides forward in the late 1970s. Central to this was the development of new objective lenses and optical systems, with overall microscope systems improving to match. In 1976, Nikon launched the

new CF series optical system and new V series microscope, followed by the X and Y series in 1978. Olympus brought out the new LB series optical system in 1978, followed by the BH2 series in 1980, the IC series industrial microscope optical system in 1981 and the New Vanox AH2 in 1983. In 1985, Nikon added the new, high-spec NCF series optical system to its lineup. German company Zeiss announced the new ICS infinity-corrected optical system and the new Axio series microscope in 1986, while Leitz (Leica) announcing the Delta optical system and new DM series microscope in 1992. Olympus responded with the new UIS infinity-corrected optical system and the BX, AX and IX in 1993-1994, and Nikon announced the new CFI_{60} optical system and Eclipse series in 1996. These four companies from Japan and Germany continue to compete for the top position in the global optical microscope industry.

Throughout the development history of optical microscopes, all kinds of optical theories and technologies have emerged, each limited by the resolution limits of light. Aware of this, some researchers believed that there was no further scope for optical microscope development, in contrast to electron microscopes or probe microscopes. However, as phase contrast and differential interference contrast made it possible to observe live cells directly and the emergence of new fluorochromes and fluorescent proteins meant fluorescence microscopes had a central role in immunology, DNA, neurology and molecular biology, interest in optical microscopes grew once more. This was spurred on all the more by the emergence of laser scanning microscopes in the late 1980s. Confocal laser scanning microscopy was particularly effective and combined with fluorescence microscopy to become an essential part of cutting-edge bioimaging research. With developments being made in multi photon excitation laser scanning microscopes and super resolution microscopes, the importance of optical microscopes can only be expected to increase in future.

Japan has thus come to rival Germany in leading the world in the field of optical microscopes. How has the Japanese optical microscope industry been able to develop to this point, having started out by replicating microscopes from overseas only 100 years before? This is undoubtedly due to the Japanese people's strong interest and adoration for optical instruments and our aptitude for precision machining, as well as official recognition of the importance of these optical devices through national policy. Domestic production of optical glass, independent development of lens design software, technology development and standardization through postwar collaboration between industry, government and academia, as well as various other concurrent developments in infrastructure, have been important elements as well. The urge to compete with Germany and challenge its revered position, as well as development rivalry between Tiyoda, Olympus, Nikon and other Japanese manufacturers, were essential to the development of the Japanese optical microscope. On top of this, forward progress in medical and bioscience research and all kinds of industry areas meant strong user demand, which provided feedback to microscope researchers and developers and a driving force to refine the product further.

Looking back at the history of optical microscopes in Japan, whether it be the emergence of new methods of microscopy or the adoption of infinity-corrected optical systems, it has all been to keep in step with inventions or technological innovations overseas, especially Germany. It has been a history of playing catchup, of working out how to develop and market products with better function, performance and cost performance than the preceding German counterparts as soon as possible. However, amidst all this, developers, engineers and manufacturers have put their original creative ideas together and devoted themselves to producing good products. This is Japan's strength, and has been the driving force in attaining to a world-leading position. By sheer persistence, Japan has been able to produce world-first products and technologies, such as the CF optical system by Nikon and the fully automated universal microscope by Olympus. Japan has finally drawn level to Germany and can be expected to produce new, original and important technologies and products in the future. Whether this happens or not only depends on how much is invested into technological development. This strongly depends on the magnanimity of forward-thinking senior managers.

As stated many times over, microscopes are an important tool in understanding the microscopic world. The aspiration of developers, or, to use an old turn of phrase, the "romance" of developing our own products to achieve the ideal of contributing to the world has been a major source of motivation. When the author was in charge of microscope lens design, he came across a translation of Scheffel's "Gläserne Wunder", or "the wonder of glass". Translated during the Pacific War, the book told the story of microscope development success by Zeiss, Abbe and Schott. The author of the present report recalls being profoundly impacted by Abbe talent as a physicist, his struggle to establish microscope theory and design, his leadership and guidance of young researchers and his competence as a business manager after Zeiss' death, establishing the Carl Zeiss Foundation and setting in a ground-breaking social security system that implemented eight-hour work days, paid leave, a pension system and freedom of ideology and background. The author resolved to aspire to becoming a developer like Abbe. The idea of taking over microscope development, brought thus far by the wisdom, passion and perseverance of one's forebears, including current superiors and seniors, and contributing to the advancement of science and industry by developing a global-performing product was indeed a "romantic" notion and it came with a sense of self-fulfillment. The author recalls with gratitude being given the opportunity to work on microscope development, and how deeply moving it was to rival the world-leading German microscopes in a fight to the top through the development of new LB, IC and UIS optical systems. Of course, there were many others working on this besides the author, and the sentiment is surely shared by all business-founding pioneers, superiors, seniors and juniors alike, and indeed anyone who has worked alongside Japanese microscope manufacturers. It is because of this vector of positivity, formed by so many involved in microscope development, that Japan has become a microscope superpower. Such is the emotional belief of the author.

Dr Yamanaka Shinya was awarded the Nobel Prize in Physiology or Medicine in 2012 for his research on iPS cells.

Dr Yoshinori Ōsumi received the same award in 2016 for his research on autophagy. Both were dependent on the optical microscope. In 2014, the Nobel Prize in Chemistry was awarded to Betzig (USA), Hell (Germany) and Moerner (USA) for their research on super resolution fluorescence microscopy. This indicates that the world is paying attention to new theoretical research using optical microscopes and new practical development of optical microscopes. It is an ongoing challenge to develop optical microscopes that go beyond previous limitations. The author would be delighted if this report were to be of some use to those working with Japanese microscopes, so that Japanese optical microscopes will continue to lead the world.

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Schott Japan Corporation:	Inoguchi Kazuyuki
Tanaka Scientific Limited:	Shimodaira Katsuhiko
Mitutoyo Corporation:	
	Public Relations Section, Sales Division

Appendix 1

1. List of JIS Related to Optical Microscopes

As at Dec 20, 2016

JIS code: year of enactment/revision	Title	Corresponding ISO, combined/abolished
B 7132: 1949 → 1998	Biological microscopes	abolished 2009 ⇒ MIS 1001: 2011
B 7132-1: 2009	Microscopes - Imaging distances related to mechanical reference planes - Part 1: Tube length 160 mm	ISO 9345-1
B 7132-2: 2009	Microscopes - Imaging distances related to mechanical reference planes - Part 2: Infinity-corrected optical systems	ISO 9345-2
B 7133: 1951	Biological microscopes for dry objectives	abolished 1998 ⇒ JIS B 7132: 1998
B 7134: 1951	Small size biological microscopes	abolished 1999 ⇒ MIS 8801: 2008
B 7135: 1957	Single objective binocular microscopes	abolished 1986 ⇒ JIS B 7132: 1986
B 7136: 1965	Medium size biological microscopes	abolished 1999 ⇒ MIS 9503: 2008
B 7137: 1990	Stage micrometer for biological microscope	abolished 1999 ⇒ MIS 9001: 2008
B 7138: 1990	Net Stage micrometer for biological microscope	abolished 1999 ⇒ MIS 9002: 2008
B 7139: 1951 → 1997	Stereo microscopes	abolished 2008 ⇒ reorganized into JIS B 7139-1 to 4:2008
B 7139-1: 2008	Stereomicroscopes - Part 1: Minimum requirements for stereomicroscopes	ISO 11884-1 & ISO 11884-2
B 7139-2: 2008	Stereomicroscopes - Part 2: Testing of stereomicroscopes	ISO 15227
B 7139-3: 2008	Stereomicroscopes - Part 3: Marking of stereomicroscopes	ISO 11883
B 7139-4: 2008	Stereomicroscopes - Part 4: Information provided to the user	ISO 15362
B 7140: 1951	Microscope test specimens	abolished 1999 ⇒ MIS 8602: 2008
B 7141: 2012	Microscopes - Screw threads for objectives and related nosepieces	ISO 8038
B 7142: 1951	Microscopes-Screw threads for objectives	abolished 1994 ⇒ JIS B 7141: 1994
B 7143: 1951 → 1977	Engagement between microscope eyepiece and eyepiece sleeves	ISO 10937
B 7144: 1951	Microscope - Connection of condensers for transmitted light with substage sleeves	abolished 1999 ⇒ MIS 9501: 2008
B 7145: 1951	Microscopes - The fitting of stage accessories	abolished 1999 ⇒ MIS 9502: 2016
B 7146: 1951	Fit of clips	abolished 1995 ⇒ JIS B 7145: 1995
B 7147: 1967	Biological microscope objectives	abolished 1999 ⇒ MIS 9301: 2011
B 7148: 1967	Microscope eyepieces	abolished 1999 ⇒ MIS 9201: 2007
B 7149: 1951	Microscopes - Eyepiece reticles	abolished 1999 ⇒ MIS 9505: 2007
B 7150: 1967	Micrometer microscopes	abolished 1999
B 7151: 1967	Micrometer eyepieces	abolished 1999
B 7152: 1967	Biological microscope objectives and eyepieces - Methods of measurement of performance	abolished 1999 ⇒ MIS 9101: 2016
B 7158-1: 2010	Designation of microscope objectives - Part 1: Flatness of field/Plan	ISO 19012-1
B 7158-2: 2011	Designation of microscope objectives - Part 2: Chromatic correction	ISO 19012-2
B 7158-3	Designation of microscope objectives - Part 3: Spectral transmittance	ISO 19012-3, 2017 enactment expected
B 7251: 2000	Reference system of polarized light microscopy	ISO 8576
B 7252: 2015	Marking of microscope objectives and eyepieces	ISO 8578
B 7254: 2007	Microscopes - Magnification	ISO 8039
B 7255: 2007	Microscopes - Interfacing connection type C	ISO 10935
B 7256: 2007	Microscopes - Information provided to the user	ISO 12853, 2017 revision expected
K 2400: 2015	Microscopes - Immersion liquids for light microscopy (Supplementary Issue)	ISO 8036
R 3702: 1957 → 1996	Cover glasses for microscopes	ISO 8255-1 & ISO 8255-2
R 3703: 1957 → 1996	Slide glasses for microscope	ISO 8037-1 & ISO 8037-2
T 4204:	Hemocytometer	abolished 2001
T 10936-1: 2014	Operation microscopes - Part 1: Requirements and test methods	ISO 10936-1

2. List of ISO Standards Related to Optical Microscopes

As at Dec 20, 2016

Code: year of enactment/revision	Title	Corresponding JIS
ISO 8036: 2015	Immersion liquids for light microscopy	K 2400
ISO 8037-1: 1986	Slides – Part 1: Dimensions, optical properties and marking	R 3703
ISO 8037-2: 1997	Slides – Part 2: Quality of material, standards of finish and mode of packaging	
ISO 8038: 2013	Screw threads for objectives and related nosepieces	B 7141
ISO 8039: 2014	Values, tolerances and symbols for magnification	B 7254
ISO 8040: 2001	Dimension of tube slide and tube slot connections	—
ISO 8255-1: 2011	Cover glasses – Part 1: Dimensional tolerances, thickness and optical properties	R 3702
ISO 8255-2: 2013	Cover glasses – Part 2: Quality of material, standards of finish and mode of packaging	
ISO 8576: 1996	Reference system of polarized light microscopy	B 7251
ISO 8578: 2012	Marking of objectives and eyepieces	B 7252
ISO 9344: 2016	Graticules for eyepieces	(B 7149)
ISO 9345-1: 2012	Imaging distances related to mechanical reference planes – Part 1: Tube length 160 mm	B 7132-1
ISO 9345-2: 2014	Imaging distances related to mechanical reference planes – Part 2: Infinity- corrected optical systems	B 7132-2
ISO 10934-1: 2002	Vocabulary for microscopy – Part 1: Light microscopy	—
ISO 10934-2: 2007	Vocabulary for microscopy – Part 2: Advanced techniques in light microscopy	—
ISO 10935: 2009	Interfacing connection type C	B 7255
ISO 10936-1: 2000	Operation microscopes – Part 1: Requirements and test methods	T 10936-1
ISO 10937: 2000	Diameter of interchangeable eyepieces	B 7143
ISO 11882: 1997	Interfacing connections for 35 mm SLR photo camera (T – thread adaptation)	—
ISO 11883: 1997	Marking of stereomicroscopes	B 7139-3
ISO 11884-1:2006	Minimum requirements for stereomicroscopes – Part 1: Stereomicroscopes for general use	B 7139-1
ISO 11884-2:2007	Minimum requirements for stereomicroscopes – Part 2: High performance microscopes	
ISO 12853: 2015	Information provided to the user	B 7256
ISO 15227: 2000	Testing of stereomicroscopes	B 7139-2
ISO 15362: 2014	Stereomicroscopes – Information provided to the user	B 7139-4
ISO 18221: 2016	Microscopes with digital imaging displays - Information provided to the user regarding imaging performance	—
ISO 19012-1: 2013	Designation of microscope objectives – Part 1: Flatness of field/Plan	B 7158-1
ISO 19012-2: 2013	Designation of microscope objectives – Part 2: Chromatic correction	B 7158-2
ISO 19055: 2015	Minimum requirements for binocular tubes	—
ISO 19056-1: 2015	Definition and measurement of illumination properties - Image brightness and uniformity in bright field microscopy	—

3. List of MIS (Microscope Industrial Standards of Japan Microscope Manufacturers' Association)

As at Dec 20, 2016

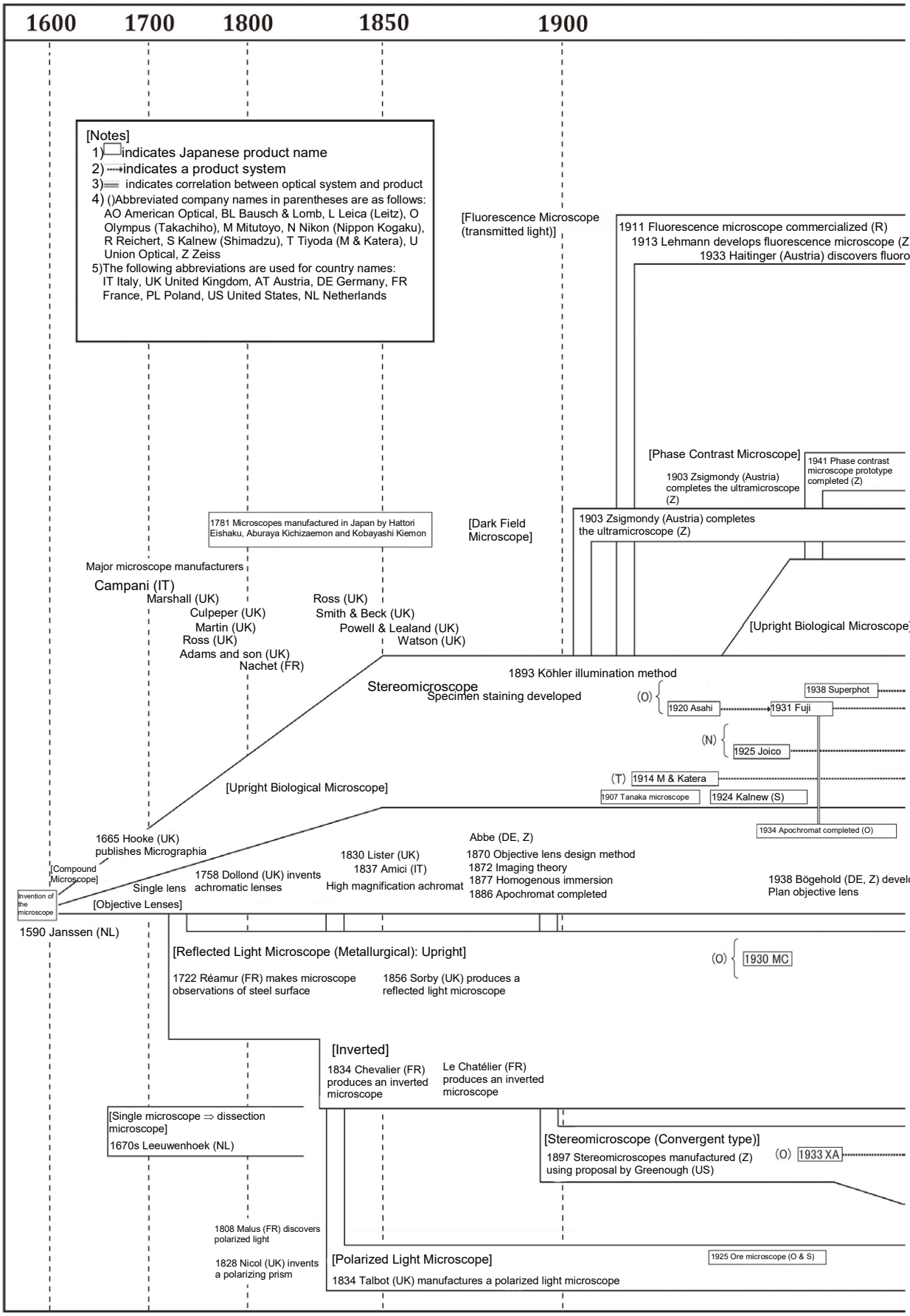
Code: year of enactment/revision	Title	Corresponding JIS
MIS 7801: 2016	Enactment methods for industrial standards of Japan Microscope Manufacturers' Association	—
MIS 7804: 2007	Test Specimens for total parcentricity of small biological microscopes	—
MIS 8602: 2008	Microscope test specimens	JIS B 7140 (abolished Oct 1999)
MIS 8801: 2008	Small size biological microscopes	JIS B 7134 (abolished Mar 1999)
MIS 9001: 2008	Stage micrometer for biological microscope	JIS B 7137 (abolished Oct 1999)
MIS 9002: 2008	Net Stage micrometer for biological microscope	JIS B 7138 (abolished Oct 1999)
MIS 9101: 2016	Biological microscope objectives and eyepieces - Methods of measurement of performance	JIS B 7152 (abolished Oct 1999)
MIS 9201: 2007	Microscope eyepieces	JIS B 7148 (abolished Oct 1999)
MIS 9301: 2011	Biological microscope objectives	JIS B 7147 (abolished Oct 1999)
MIS 9501: 2008	Microscopes - Connection of condensers for transmitted light with substage sleeves	JIS B 7144 (abolished Oct 1999)
MIS 9502: 2016	Microscopes - The fittings of stage accessories	JIS B 7145 (abolished Oct 1999)
MIS 9503: 2008	Medium size biological microscopes	JIS B 7136 (abolished Mar 1999)
MIS 9001: 2008	Microscopes - Eyepiece reticles	JIS B 7149 (abolished Oct 1999)
MIS 1001: 2011	Biological microscopes	JIS B 7132 (abolished Oct 2009)

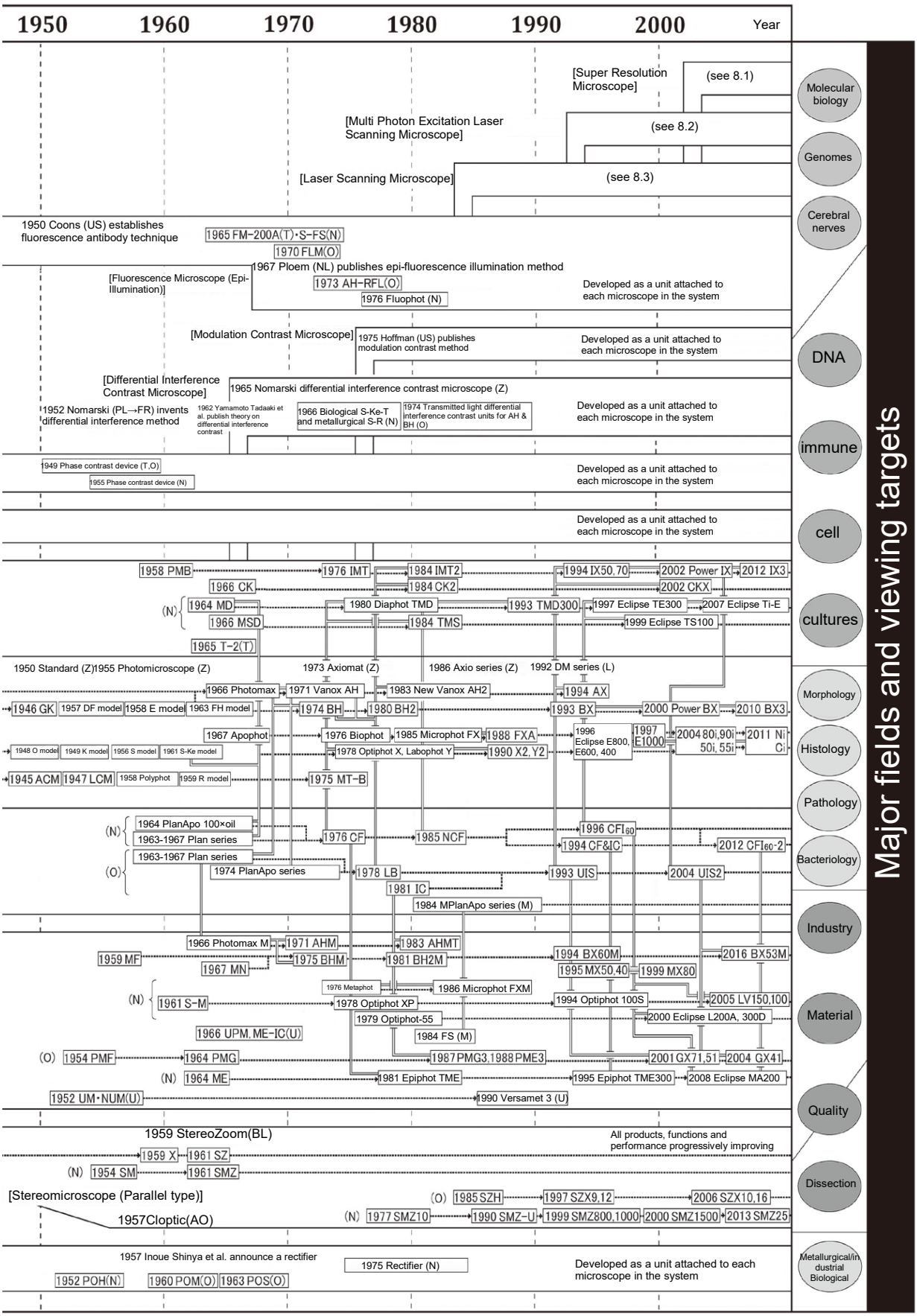
Appendix 2

Establishments of Japanese Microscope Manufacturers (Members of JMMA and others)

Year		Comment
1875	Founder Shimadzu Genzō commences production of scientific instruments (Kiyamachi, Kyoto)	Now Shimadzu Corporation
1892	Tanaka Mokujirō establishes Tanaka Merchants, importing medical instruments and chemicals (Nihonbashi, Tokyo)	Now Tanaka Scientific Ltd.
1906	Sugita Tōtarō establishes Sugita Merchants (Nihonbashi)	Now Sugitoh Co., Ltd.
1910	Uchida Kotarō establishes Suitaigō as a business for the South Manchurian Railway (Dalian)	Now Uchida Yoko Co., Ltd.
1914	Suzuki Tamotsu opens business Suzuki Tamotsu Merchants, importing optical products (Yokohama)	Now Tohkai Sangyo Co., Ltd.
〃	Matsumoto Fukumatsu establishes M & Katera Optical Works (Nihonbashihoncho)	Now Sakura Finetek Japan Co., Ltd.
1917	Tokyo Keiki, Iwaki Glass and Fuji Lens Factory merge to form Nippon Kogaku K.K. (Japan Optical Industries Company)	Now Nikon Corporation
1919	Yamashita Takeshi establishes Takachiho Works (Hatagaya, Shibuya)	Now Olympus Corporation
1921	Katō Kakitsu and Shintō Shinkichi establish Kalnew Optical Industries (Yotsuya, Shinjuku)	Now Shimadzu Device Corporation
1927	Suzuki Taiichi goes independent from Takachiho Works and establishes Toyo Optical Company (Eliza)	Ceased operation in 2002
1930	Spectacles dealer Katō Rokujirō Merchants established (Takecho, Taito)	Now Carton Optical Industries Ltd.
1932	Tokyo Optical established from the surveying instruments division of K. Hattori Seikosha	Now Topcon Corporation
1935	Nishino Kunisaburō and others go independent from Takachiho Works and establish Yashima Optical Co., Ltd.	
〃	Ima Koshichi establishes Ima Optical Manufacturing Company (Ima Precision Instruments established the same year, Minamidai, Nakano)	Now Ima Optical Manufacturing Co., Ltd.
1939	Yamashita Takeshi establishes Nisshin Optical and Precision Instruments (sump microscope)	Ceased operation in 1950
1940	Ishii Harukichi establishes Kyowa Optical and Precision Instruments Works (Honan, Suginami)	Now Kyowa Optical Co., Ltd.
1947	Suzuki Seitarō establishes Suzuki Works (Yayoicho, Nakano)	Now Seiwa Optical Co., Ltd.
〃	Anno Kuniji establishes Nikken Nihon Microscope Works (Minamiaoyama, Minato)	
1948	Hiruma Teruji establishes Besta Optical Inc. (Minamidai, Nakano)	
〃	Yashima Denki optical division established independently as Union Optical (founding president: Yanagawa Satoru)	Company went bankrupt in 2010
〃	Ishii Hisayoshi and others establish lens design and manufacturing company Sankeisha Co., Ltd. (Kamiuma, Setagaya)	
1950	Shimoyama Kazuyoshi establishes Krea Seiko Co., Ltd. (Hatsudai, Shibuya)	
1954	Konno Iseo establishes Shin Nihon Tsusho Co., Ltd. (Yotsuya, Shinjuku)	
1955	Hotta Fushio establishes Chuo Precision Industrial Co., Ltd. (Kanda Ogawamachi, Chiyoda)	
1956	Kitagawa Kiyosuke establishes Asahi Optical Works (Koenji, Nakano)	
1959	Minato Optical Industry Co., Ltd. established through funding from the city of Iida, Nagano	
1960	Uchiyama Yasushi establishes Tokyo ITV Laboratory (later NJS Corporation)	Now Lasertec Corporation
1963	Yomoda Kazuo establishes NSK Ltd. (Aoyama, Minato)	Now Nissho Optical Co., Ltd.
〃	Shimodaira Makoto establishes Shibuya Optical Works (Hatsudai, Shibuya)	Now Shibuya Optical Co., Ltd.
1975	Satō Zensuke establishes Meiji Labax Co., Ltd. (Higashiikebukuro, Toshima)	Now Meiji Techno Co., Ltd.
1978	Kajiro Michio establishes Hirox Co., Ltd. (Akasaka, Minato)	
1985	Yamamoto Masao establishes Scalar Corporation (Wakaba, Shinkjuku)	
1987	Fujiyama Kazuo establishes Optart Corporation (Kōtōbashi, Sumida)	
2011	Yoshimine Takashi establishes Micronet Corporation (Kawaguchi, Saitama)	

Appendix 3 Flow Chart of Japanese Optical Microscope Development




1950
1960
1970
1980
1990
2000
Year
[Super Resolution Microscope]
(see 8.1)
[Multi Photon Excitation Laser Scanning Microscope]
(see 8.2)
[Laser Scanning Microscope]
(see 8.3)
1950 Coons (US) establishes fluorescence antibody technique
1965 FM-200A(T)・S-FS(N)
1970 FLM(O)
1967 Ploem (NL) publishes epi-fluorescence illumination method
[Fluorescence Microscope (Epi-Illumination)]
1973 AH-RFL(O)
1976 Fluophot (N)
Developed as a unit attached to each microscope in the system
[Modulation Contrast Microscope]
1975 Hoffman (US) publishes modulation contrast method
[Differential Interference Contrast Microscope]
1965 Nomarski differential interference contrast microscope (Z)
1952 Nomarski (PL→FR) invents differential interference method
1962 Yamamoto Tadaaki et al. publish theory on differential interference contrast
1966 Biological S-Ke-T and metallurgical S-R (N)
1974 Transmitted light differential interference contrast units for AH & BH (O)
1949 Phase contrast device (T,O)
1955 Phase contrast device (N)
1958 PMB
1976 IMT
1984 IMT2
1994 IX50, 70
2002 Power IX
2012 IX3
1966 CK
1984 CK2
2002 CKX
(N)
1964 MD
1980 Diaphot TMD
1993 TMD300
1997 Eclipse TE300
2007 Eclipse Ti-E
1966 MSD
1984 TMS
1999 Eclipse TS100
1965 T-2(T)
1950 Standard (Z)1955 Photomicroscope (Z)
1973 Axiomat (Z)
1986 Axio series (Z)
1992 DM series (L)
1966 Photomax
1971 Vanox AH
1983 New Vanox AH2
1994 AX
1946 GK
1957 DF model
1958 E model
1963 FH model
1974 BH
1980 BH2
1993 BX
2000 Power BX
2010 BX3
1967 Apophot
1976 Biophot
1985 Microphot FX
1988 FXA
1996 Eclipse E800, E600, 400
1997 E1000
2004 80i,90i 50i, 55i
2011 Ni Ci
1948 O model
1949 K model
1956 S model
1961 S-Ke model
1978 Optiphot X, Labophot Y
1990 X2, Y2
1945 ACM
1947 LCM
1958 Polyphot
1959 R model
1975 MT-B
1964 PlanApo 100×oil
1963-1967 Plan series
1976 CF
1985 NCF
1996 CFI60
1994 CF&IC
2012 CFI60-2
1963-1967 Plan series
1974 PlanApo series
1978 LB
1993 UIS
2004 UIS2
1981 IC
1984 MPlanApo series (M)
1966 Photomax M
1971 AHM
1983 AHMT
1959 MF
1975 BHM
1981 BH2M
1994 BX60M
2016 BX53M
1967 MN
1995 MX50,40
1999 MX80
1976 Metaphot
1986 Microphot FXM
1961 S-M
1978 Optiphot XP
1994 Optiphot 100S
2005 LV150,100
1979 Optiphot-55
2000 Eclipse L200A, 300D
1966 UPM, ME-IC(U)
1984 FS (M)
1954 PMF
1964 PMG
1987 PMG3,1988 PME3
2001 GX71,51
2004 GX41
1964 ME
1981 Epiphot TME
1995 Epiphot TME300
2008 Eclipse MA200
1952 UM・NUM(U)
1990 Versamet 3 (U)
1959 StereoZoom(BL)
All products, functions and performance progressively improving
1959 X
1961 SZ
1954 SM
1961 SMZ
[Stereomicroscope (Parallel type)]
1957Cloptic(AO)
1985 SZH
1997 SZX9,12
2006 SZX10,16
1977 SMZ10
1990 SMZ-U
1999 SMZ800,1000
2000 SMZ1500
2013 SMZ25
1957 Inoue Shinya et al. announce a rectifier
1975 Rectifier (N)
1952 POH(N)
1960 POM(O)
1963 POS(O)
Molecular biology
Genomes
Cerebral nerves
DNA
immune
cell
cultures
Morphology
Histology
Pathology
Bacteriology
Industry
Material
Quality
Dissection
Metallurgical/industrial Biological
Major fields and viewing targets

Appendix 4

Chronology of Optical Microscopes (focus on Japanese biological microscopes)

Year	Microscope Chronology	Related Items
c. 1590	Janssen father & son (Netherlands) produce a compound microscope	
1665	Hooke (UK) publishes “Micrographia” using a compound microscope	1608 Lipperhey (Netherlands) produces a telescope
1667-1670s	Leeuwenhoek (Netherlands) discovers microbes using a single-lens microscope	1609 Galileo (Italy) makes astronomical observations by telescope
1670s	Campani (Italy) produces a microscope	1613 Saris (UK) presents a telescope to Tokugawa Ieyasu
1690s	Marshall (UK) produces a microscope	
1700s	Culpeper (UK) produces a microscope	
1740s	Cuff (UK), Martin (UK), Adams (UK) and others produce microscopes	
1750s	Microscopes brought to Japan around this time	1758 Dollond (UK) designs an achromatic lens
1765	Gotō Rishun introduces the microscope in “Oranda Banashi”	
1781	Hattori Eishaku and Kobayashi Kiemon manufacture wooden microscopes	
1787	Morishima Chūryō describes a “micrascoben” and has an image of a microscope in “Kōmō Zatsuwa”	
1813	Doi Toshitsura, feudal lord of Koga Domain, starts observing snowflake crystals under microscope	
1827	Amici (Italy) announces a water immersion objective lens	1822 Daguerre (France) invents the daguerreotype photographic process
1828	Nicol (UK) invents a polarizing prism	
1829	Lister (UK) publishes an achromatic objective lens for microscope	
1833	Udagawa Yōan publishes “Shokubutsu Keigen”	
1834	Talbot (UK) invents the polarized light microscope	
1838	W. Watson & Son optical instrument business established (UK)	
1840	Spencer (USA) starts producing microscopes (later American Optical)	
1846	Zeiss starts producing optical instruments (Jena, Germany)	
1849	Kellner starts producing microscopes (Germany, Ernst Leitz from 1869)	
1850	Nachet (France) invents a binocular microscope using light splitting prisms	
1853	Bausch & Lomb (USA) established, start optical instrument production	
1861	Pasteur (France) disproves the theory of spontaneous generation	
1866	Royal Microscopical Society in London (RMS) established	
″	Abbe starts working at Zeiss, begins researching microscope theory	1868 Meiji Restoration
1873	Abbe publishes microscope imaging theory	
1876	Reichert (Austria) microscope company established	
1877	Abbe completes homogenous immersion method	
1879	Hansen (Norway) discovers leprosy bacillus	1879 Edison successfully lights an incandescent bulb
1882	Koch (Germany) discovers mycobacterium tuberculosis, and the cholera bacterium the following year	
1884	Abbe and Schott jointly establish an optical glass research laboratory	
1886	Abbe completes the apochromatic objective lens	
1890	Kitasato Shibasaburō and Behring (Germany) discover serum therapy (tetanus/diphtheria)	
1893	Köhler (Germany) publishes a microscope illumination method	
1894	Kitasato Shibasaburō and Yersin (France) discover the plague bacillus respectively	

Year	Microscope Chronology	Related Items
1897	Greenough and Zeiss jointly complete a stereomicroscope	
〃	Shiga Kiyoshi discovers dysentery bacillus	
1903	Siedentopf (Zeiss) and Zsigmondy develop the ultramicroscope	1901 first Nobel Prize awards ceremony
1904	Köhler (Zeiss) develops the ultraviolet microscope	1904 Russo-Japanese War breaks out (to 1905)
1907	Tanaka Mokujirō (Tanaka Partnership) manufactures microscopes	
1910	Katō Kakitsu and Shintō Shinkichi start prototyping microscopes, joined by Terada Shintarō	
1913	Lehmann (Zeiss) develops the fluorescence microscope	
〃	Noguchi Hideyo discovers syphilis spirochete in the brains of mental patients	
1914	Tanaka microscope and M & Katera are prize-winning exhibits at the Tokyo Taisho Exhibition	1914 First World War breaks out (to 1918)
〃	Matsumoto Fukumatsu of Iwashiya establishes M & Katera Optical Works and begins selling	
1915	M & Katera V/III released	
1917	Nippon Kogaku K.K. (Japan Optical Industries Company) established by a merger of Tokyo Keiki, Iwasaki Glass and Fujii Lens Factory	
1919	Yamashita Takeshi establishes Takachiho Works (now Olympus), releases the Asahi the following year	
1920	Takachiho Works registers the “Olympus” trademark	
1922	Olympus releases the Sakura student microscope (SA)	1923 Great Kanto Earthquake
1924	Katō Kakitsu and Shintō Shinkichi establish Kalnew Optical Industries (now Shimadzu Device Corporation)	
1925	Nippon Kogaku releases its first microscope, the Joico	
〃	Olympus and Shimadzu jointly develop a ore microscope	
1927	Suzuki Taiichi (former apprentice of Katō Kakitsu) establishes Toyo Optical Company (Eliza microscope)	
〃	Olympus releases the Showa oil-immersion microscope (GK)	
1928	Olympus Seika is awarded an Excellent Domestic Product Award at the Enthronement Commemoration Exhibition and is gifted to His Majesty the Emperor	
1929	Suzuki Junichi invents the SUMP method of microscope specimen preparation	
1932	Zernike (Netherlands) announces the phase contrast microscope (awarded the Nobel Prize in 1953)	
〃	Ruska (Zeiss) announces the magnetic field electron microscope (awarded the Nobel Prize in 1986)	
1933	Olympus releases the XA stereomicroscope	
1934	Olympus completes the Apochromat objective lens	
〃	M & Katera Optical Works changes its name to Tiyoda Optical	
1935	Yashima Optical Company established	
1938	Bögehold (Zeiss) designs a Plan objective lens with a flat image surface	
〃	Olympus completes the SuperPhoto universal photography microscope	1939 Second World War breaks out (to 1945)
1941	Zeiss prototypes and announces a phase contrast microscope	1941 Pacific War breaks out (to 1945)
1945	Optical instrument manufacturers start shifting to peacetime industries at the end of the war	
〃	Tiyoda Optical resumes microscope production	
1946	Japan Optical and Precision Instrument Manufacturers’ Association launched (microscope division launched)	1946 World’s first electronic computer ENIAC developed
〃	Olympus resumes production of the GK microscope at its plant in Ina	
1948	Nippon Kogaku releases the O model, the first postwar microscope, followed by the K model research microscope the following year	
1949	Tiyoda Optical and Olympus complete successive phase contrast microscopes respectively	

Year	Microscope Chronology	Related Items
1950	Coons publishes an antibody antigen test method using fluorescence microscopy	
1952	Nomarski (France) announces a reflected light differential interference contrast microscope	
〃	Nippon Kogaku releases the POH polarized light microscope	
1953	Science Education Promotion Act established	1953 Watson and Crick propose a structure for DNA
1954	Japan Optical Industry Association established (Japan Microscope Manufacturers' Association established)	
1955	Nomarski announces a transmitted light differential interference contrast microscope	
1956	Nippon Kogaku releases the S model high-grade system microscope	
1957	Olympus develops the DF high-grade microscope with Japan's first vertically moving stage	
〃	Inoue Shinya announces a rectifier with better light polarization performance	
1958	Olympus develops the E model biological research microscope	
1959	Nippon Kogaku develops the S model Köhler illumination system	
〃	Olympus releases the MK educational microscope	
1960	Olympus releases the POM polarized light microscope	1960 Maiman succeeds with ruby laser oscillation
〃	Kalnew releases the SGL-600 educational microscope	
〃	Nippon Kogaku releases the E educational microscope	
1961	Nippon Kogaku and Olympus release the SMZ and SZ zoom-type stereomicroscopes	1961 Gagarin (USSR) becomes the first person in space
1962	Yamamoto Tadaaki (Nippon Kogaku) and Françon jointly announce a differential interference contrast microscope	
〃	Shimomura Osamu discovers green fluorescent protein (GFP) (awarded the Nobel Prize in 2008)	
1963	Olympus releases the EH & FH, Japan's first high-grade microscopes with built-in illumination	
〃	"History of Japanese Microscopes" exhibit held at the National Museum of Nature and Science	
1964	Nippon Kogaku releases the MD inverted microscope	1964 Tokyo Olympics
1965	Tiyoda releases the FM200 transmitted light fluorescence microscope	
1966	Olympus releases the Photomax universal photography microscope	
〃	Nippon Kogaku releases the T (transmitted light) and R (reflected light) differential interference systems	
〃	Olympus releases the CK inverted culture microscope	
1967	Leitz releases an epi-fluorescence system invented by Professor Ploem	
〃	Nippon Kogaku releases the Apophot universal research microscope	1969 Apollo 11 moon landing, first humans on the moon
1970	Olympus releases the FLM transmitted light fluorescence microscope	1970 Osaka World Expo
1971	Union Optical releases a Nomarski reflected light differential interference system	
〃	Olympus releases the AH (Vanox) high-end universal microscope	1972 Sapporo Winter Olympics
1973	Olympus releases the AH-RFL epi-fluorescence system	
1974	Olympus releases the BH high-grade system microscope series	
1975	Hoffman (USA) announces the modulation contrast method	
〃	Tiyoda releases the MT-B high-grade system microscope, closes the following year	
〃	Olympus completes the Plan Apochromat objective lens series	
〃	Olympus releases a transmitted light Nomarski differential interference device for the AH & BH	
〃	Nippon Kogaku releases a high-performance polarized light rectifier	

Year	Microscope Chronology	Related Items
1976	Nippon Kogaku releases the new CF optical system and the Biophot high-end microscope	
1978	Nippon Kogaku releases the Optiphot and Labophot high-grade system microscopes	
〃	Olympus releases the new optical system LB series	
1980	Olympus releases the BH2 high-grade system microscope series	
1981	Nippon Kogaku releases the TMD (Diaphot) inverted microscope	
1983	Olympus releases the AH2 (New Vanox) high-end universal photography microscope	
1984	Olympus releases the SZH high-end stereomicroscope	
1985	Nippon Kogaku releases the FX high-end universal photography microscope, progresses to FXA in 1988	
〃	NJS Corporation (now Lasertec Corporation) releases a laser scanning microscope	
1988	Nippon Kogaku K.K. changes its name to Nikon Corporation	1989 Berlin Wall comes down, East and West Germany united the following year
1990	Olympus releases the LSM-GB biological laser scanning microscope	
1993	Olympus announces the new UIS optical system and releases the BX high-grade system microscope	1995 Windows 95 released, personal computers grow in popularity
1996	Nikon announces the new CFI optical system and releases the Eclipse high-end system microscope series	〃 Hanshin-Awaji Earthquake
〃	Nikon releases the Fabre outdoor microscope	1998 Nagano Winter Olympics
2002	Olympus releases the CKX inverted culture microscope	2001 Synchronized terror attacks on the USA
2003	Olympus Optical Co., Ltd. changes its name to Olympus Corporation	2002 FIFA World Cup held in Japan & Korea
〃	Olympus releases the IX-DSU disk scanning confocal microscope	
2006	Yamanaka Shinya publishes on induced pluripotent stem cells (iPS) (awarded the Nobel Prize in 2012)	
2007	Nikon releases the Eclipse Ti inverted research microscope	
2008	Sakura Finetek Japan releases the TTM-200 microtome	
	Olympus releases the FV10i confocal laser scanning microscope	
〃	Nikon releases the A1 & A1+ laser scanning microscopes	
2010	Olympus releases the BX63 & 53 high-grade system microscopes	2010 Asteroid exploring spacecraft Hayabusa returns to Earth
〃	Nikon releases the A1R MP+ multi photon laser scanning microscope	
〃	Nikon releases the N-SIM and N-STORM super resolution microscopes	2011 Great East Japan Earthquake
2012	Shimadzu Rika releases the BA210EINT digital microscope	
2013	Nikon releases the SMZ2 research system stereomicroscope	
〃	Olympus releases the FVMPE-RS multi photon excitation laser scanning microscope	
2014	Uchida Yoko releases the D-EL4N digital microscope	
〃	Olympus announces the FV-OSR super resolution microscope	
〃	3 researchers from the USA and Germany who developed super resolution microscopy are awarded the Nobel Prize in Chemistry	
2015	“A Century of Japanese Microscopes” exhibition held at the National Museum of Nature and Science	
2016	Ōsumi Yoshinori awarded the Nobel Prize in Physiology or Medicine for clarifying the mechanism of autophagy	

Appendix 5

Location Confirmation Findings for Historical Resources Related to Optical Microscope Manufacturing Technology

No.	Name	Year manufactured	Manufacturer	Status of resource	Location	Reason for selection
1	Japan's oldest existing wooden microscope	1781	Kobayashi Kiemon	On display	Shimadzu Foundation Memorial Hall, Kyoto	This is a valuable resource that reveals the year of manufacture and the manner of use. The authentication certificate on the box reads, "Made by Kobayashi Kiemon, 1781". The accompanying "microscope viewing instructions" also include descriptions of how to use interchangeable objective lenses. (4.1, Fig. 4.1)
2	Tanaka microscope	1907	Tanaka Mokujirō (Tanaka Partnership)	In storage, not open to the public	Inoue Ryo collection, Osaka University, Kyoto	Japan's earliest mass-produced microscope, marketed in 1907. Modeled on the Leitz microscope with magnification from 25× to 600×. Won many awards at national expositions and similar. (4.2, Fig. 4.5)
3	M & Katera IV	1914	Terada Shintarō, Katō Kakitsu, Shintō Shinkichi, Matsumoto Fukumatsu	On display	Sakura Finetek Japan Co., Ltd., Chūō Ward, Tokyo	The M & Katera was an early mass-produced microscope that drove the Japanese microscope market before and after the war. The company later changed its name to Tiyoda Optical. The developers and the technology laid the foundation for the Japanese microscope industry. (4.2, Fig. 4.6). Registered as an Essential Historical Material for Science and Technology (No. 184) in 2014.
4	Apochromat objective lenses	1934	Takachiho Works (now Olympus)	On display	"Zuikodo" Olympus Museum of Technology and History, Hachioji, Tokyo	Apochromat objective lenses correct chromatic aberration as much as possible and are made from special lens material such as fluorite or alum, requiring advanced processing and assembling technology. For Japan to have achieved this before the war is a ground-breaking achievement that indicates a high level of optical technology.
5	Super Photo universal microscope with photographic equipment (Universal)	1938	Takachiho Works (now Olympus)	On display	"Zuikodo" Olympus Museum of Technology and History, Hachioji, Tokyo	A universal microscope that combines all the prewar optical technology. Capable of transmitted and reflected light, bright field and dark field microscopy and with integrated projection, photography and filming devices, this was an epoch-making product that could rival overseas products with every possible function for biological and metallurgical microscopes. (4.2, Fig. 4.10)
6	MD inverted microscope	1964	Nippon Kogaku (now Nikon)	On display	Nikon Museum, Shinagawa, Tokyo	Japan's first proper inverted biological microscope. Used for tissue cell research, it has a fixed stage and a focusing mechanism that raises and lowers the objective lens. The superior systematicity, which included a sideboard for filming equipment, has become standard in the high-grade inverted microscopes of today. (6.1, Fig. 6.2)
7	High sensitivity polarized light rectifier (Apophot universal research microscope)	1975	Nippon Kogaku (now Nikon)	In storage	100th Anniversary Project Room, Nikon Kumagaya, Kumagaya, Saitama	The rectifier was invented by Inoue Shinya et al. (USA) and uses a special optical system to dramatically increase polarized light characteristics, making it possible to view faint birefringence and similar during biological development. This Apophot went on the market in 1967 and was one of the highest quality microscopes of the day, with various types of viewing possible using transmitted and reflected light illumination at the same time. (5.4, Fig. 5.18)
8	CF optical system and Biophot biological research microscope	1976	Nippon Kogaku (now Nikon)	In storage	100th Anniversary Project Room, Nikon Kumagaya, Kumagaya, Saitama	This optical system marked a turning point for rapid improvement in Japanese microscope objective lens performance. This was the first system in the world to have the eyepiece and objective lens correct aberration independently of each other, whereas the prevalent conventional systems used the objective lens and eyepiece together to correct lateral chromatic aberration. The microscope itself had completely new functions and design. (7.4.2, Figs. 7.21 & 7.22, 4.6, Fig. 4.31)
9	New Vanox AHBS high quality photography microscope	1983	Olympus Optical (now Olympus)	On display	"Zuikodo" Olympus Museum of Technology and History, Hachioji, Tokyo	This microscope has a fully automated photography device, including the world's first autofocus for microscope. When the objective lens is changed electronically, the illumination system automatically adjusts to the best setting. With one stroke, this ground-breaking microscope system removed the labor intensity and provided the expertise for good quality photomicrography. (4.6, Figs. 4.38-40)