The history of optical microscope

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Abstract
This article describes the history of the optical microscope. The convex lens, an important component of the microscope, was already in use during the ancient era as a tool to create fire from sunlight. The quality of the lens improved in the 11th and 12th centuries and had begun to be used as a microscope component by the end of the 16th century. In the 17th century, innovation of the optical microscope accelerated the study of biological specimens. Robert Hooke designed a two-lens microscope and observed various microorganisms. He published “Micrographia” in 1665, in which the term “cell” first appeared. Another contributor to the microscope in the same period was Leeuwenhoek, who constructed a single lens microscope and discovered bacteria. In 1857, Zeiss, with the cooperation of Abbe and Schott, manufactured a stand type modern microscope that achieved a spatial resolution of 0.2 μm. Their microscope significantly contributed to subsequent discoveries in medical biology. Due to the wave nature of light, the spatial resolution of an ordinary microscope is limited to 0.2 μm. To overcome this limitation, various microscope configurations such as a two-photon laser-scanning microscope, a near-field scanning optical microscope, and a photoactivated localization microscope were developed. For the nonstaining visualization of biological specimens, optical microscopy utilizing a nonlinear optical phenomenon was proposed. Currently, the second harmonic generation microscope and the coherent Raman scattering microscope are widely used and several examples of these microscopic results are presented in this article.

Key words : Microscope, Lens, Nonlinear optical phenomenon, Fluorescence, Cell, Second harmonic generation, Coherent Raman scattering, Collagen

1. Introduction

During my days in the first grade of elementary school, 60 years ago, I found a magnifying glass with a price tag of 100 yen in the storefront of the local eyeglass shop. I was attracted to the cute, white frame with an elegant luster. I looked at it every day on the way home from school and finally bought it by saving my pocket money for 10 days. It magnified a small insect to the size of a gigantic monster and burned a dark paper by focusing sunlight. This was my first experience with the function of the lens.

A magnifying glass is a convex lens. The term “lens” originates from “lentil” (common name: flat beans, scientific name: Lens Culinaris). In the ancient Greek comedy “The Clouds” by Aristophanes (approximately 445 BC–385 BC), a lens appeared as a tool to create fire from sunlight. During the ancient era, lenses were used to make holes in parchment by focusing sunlight, to cauterize wounds for treatment, and would be applied for magic. The oldest example of a lens was excavated from the remains of Nineveh, an ancient city of Assyria in the north of what is currently Iraq.

Glass lenses were utilized as magnifying viewer after the 11th century, and the principle of the optical lens has been understood since, approximately, the 12th century. Because ancient lenses were made of quartz crystal, shaping the lens was quite difficult. Starting from the 13th century, high quality glass lenses came into production. Around this time, their use as eyeglasses became popular owing to the development of manufacturing and processing technology in Venice, Italy.
It is said that glasses were introduced in Japan when the missionary Francisco Xavier (Spain, 1506–1552) visited Japan in 1549. Ieyasu Tokugawa (Japan, 1542–1616), who was the first "Shogun" during the Edo period, regularly used glasses, and his glasses are now preserved in the Kunozan Toshogu shrine in Japan. He might have enjoyed the world of wonder of the modern lens.

2. Invention of microscope

Lenses had begun to be used as microscopes by the end of the 16th century. Hans Janssen and his son Zacharias Janssen (about 1580–1638) were Dutch eyeglass craftsmen who developed a two-lens microscope using an objective lens and an ocular (eye lens) in end of the 16th century. Robert Hooke (1635–1703), a British scientist, designed an elegant two-lens microscope, as shown in Fig. 1, with which he observed various microorganisms and published “Micrographia,” in 1665, a book describing observations made using his microscope. He observed small compartments in cork using his microscope and named them “cells” for describing biological organisms at microscopic scale. However, due to significant distortion of the observed image through his microscope and a magnification limit of 150× owing to aberration of the lens, the two-lens microscope did not gain popularity among scientists. Hooke is a known name in the field of mechanical engineering as well for the “Hooke’s law” stating, “the stress applied to a material is proportional to the strain on that material.”

At the same time as Hooke, Antonie van Leeuwenhoek (1632–1723), a Dutch scientist, developed an attractive microscope with a single lens, shown in Fig. 2, to observe insects and other specimens. He discovered the existence of erythrocytes in blood in 1673 and found bacteria and sperm. Though the construction of his microscope was quite simple, the image distortion was smaller than that of the two-lens microscope, even at magnifications over 200×. No one was able to exceed Leeuwenhoek's microscope design and technique for many years.

Fabricating a single lens microscope is an easy task. Let us prepare a coin with a hole at the center, e.g., a Japanese 5 yen coin, and then put a drop of water in the hole. The water-filled hole functions as a convex lens. Even such a cheap coin-lens magnifies objects by dozens of times. However, observations must be performed quickly because water gradually evaporates and the magnification reduces with time.

3. Modern microscope

Carl Zeiss (Germany, 1816–1888), who was a small factory owner in Jena, Germany, improved the composite lens of the microscope with the cooperation of Ernst Abbe (Germany, 1840–1905) and Otto Schott (Germany, 1851–1935) and finally produced a high-performance stand-type microscope in 1857, as shown in Fig. 3. Abbe invented the mathematical theory linking spatial resolution to light wavelength and designed a chromatic aberration corrected objective lens called “Apochromat.” This lens reduced the spatial resolution of the microscope down to 0.2 μm.
Although the spatial resolution of the optical microscope is proportional to the observed wavelength because of the wave nature of light, the minimal resolution is 0.2 μm even when observing the object with blue light. This means that the observation of nanometer scale objects such as viruses is impossible using an ordinary microscope. In order to observe ultrasmall objects, high-resolution electron microscopes or scanning probe microscopes, such as a scanning tunneling microscope (STM) and an atomic force microscope (AFM) should be used. However, this article is concerned with the optical microscope. Therefore, I will describe the history of ultrahigh-resolution microscopes at the next opportunity.

The wave nature of light causes diffraction, resulting in the formation of an orbicular (ring-shaped) zone around the blurred image. When the orbicular zone is cut with an adequate aperture, a sharp image is obtained. One attractive imaging technique to correct the orbicular zone is by using confocal microscope configuration, in which a pinhole is placed at the confocal plane of the lens to eliminate out-of-focus light, as shown in Fig. 4. Marvin Minsky (USA, 1927–2016) presented the principle of confocal imaging to overcome the limitations of the ordinary wide-field fluorescence microscope, and patented it in 1957. As only one point in the sample is illuminated at a time, scanning over a regular raster in the specimen is required for reconstruction of 2D or 3D images. The laser-scanning confocal microscope is currently the most widely used confocal variation and contributes to scientific and industrial fields such as life science, materials science, and semiconductor examination.

Recently, a variety of microscopes with improved spatial resolution exceeding the diffraction limit have been developed; for example, the two-photon fluorescence microscope (Denk et al., 1990), stimulated emission depletion (STED) microscope (Hell and Wichmann, 1994; Willig et al., 2006), near-field scanning optical microscope (NSOM) (Pohl, 1984; Inoue and Kawata, 1994), saturated excitation (SAX) microscope (Fujita et al., 2007), and photoactivated localization microscope (PALM) (Betzig et al., 2006). Eric Betzig (UK, 1960–), Stefan Hell (Germany, 1962–), and William Moerner (USA, 1953–) were awarded the Nobel Prize in chemistry in 2014 for the development of super-resolved fluorescence microscopy.

4. Observation of vital specimens

It is essential to observe vital specimens under the microscope in life sciences. As most of the biological sample is transparent, staining the sample with additional preparation is required to visualize the specimen during the examination with an optical microscope. However, such staining procedures change the cell physiology and may kill the cell. In 1933, Fritz Zernike (Netherlands, 1888–1966) proposed a phase-contrast microscope that converts phase shifts in light due to the difference in refractive indexes between the unstained cells and their surrounding aqueous medium to the brightness of the image. He was awarded the Nobel Prize in physics in 1953 for his invention of the phase contrast microscope. Although the phase contrast microscope permits visualization of the internal cell structure without staining, it does not provide information regarding the material of the specimen.
In order to visualize the physiological processes and protein localization in vivo, nontoxic staining that allows specific targeting of subcellular structures is required. Among the various materials used for specific staining, green fluorescent protein (GFP) (Shimomura et al., 1962) or its derivatives are widely used. Osamu Shimomura (Japan, 1928–) was awarded the Nobel Prize in chemistry in 2008 for the discovery and development of the GFP along with two other scientists: Martin Chalfie (USA, 1947–) and Roger Tsien (USA, 1952–).

Luminescent inorganic compounds are attractive alternatives for the organic probes because of their low-toxicity in biological specimens. A high-spatial-resolution multicolor-imaging technique was proposed by utilizing cathodoluminescence from rare-earth-doped nanophosphors in combination with a scanning electron microscope (Niioka et al., 2011; Furukawa et al., 2015; Fukushima et al., 2016).

However, pretreatment, such as gene transfer and introduction of the luminescent probe into the living cell, can influence the cellular physiology. In order to realize “live viewing,” further development of noninvasive imaging technology that allows observation and identification of the living tissue and cells at the molecular level is required.

5. Nonlinear optical microscope

For the nonstaining visualization of the biological specimens, optical microscopy utilizing a nonlinear optical phenomenon (NLOP) has been developed. I will introduce the second harmonic generation (SHG) microscope and coherent Raman scattering (CRS) microscope in this chapter as examples of the NLOP microscopy technology.

5.1. Nonlinear optical phenomena

NLOP describes the behavior of light in the media in which the dielectric polarization responds nonlinearly to the electric field of light. Because the nonlinearity is only observed when light intensity is significantly high, the use of high peak power light sources, e.g., picosecond or femtosecond lasers, is essential to cause the NLOP. In spite of high peak power, the average power of the pulsed laser can be reduced to a considerably low value so as to not damage the cells and tissues. For example, the average power of the laser with a pulse width of 100 fs and a repetition frequency of 100 MHz is approximately 1 W; however, it attains a peak power of 100 kW! As NLOP occurs in a very small region around the focal point of the objective lens in the microscope configuration, this technique also provides unprecedented capabilities for high spatial resolution imaging.

The typical nonlinear optical microscope is a two-photon excitation laser scanning fluorescence microscope, as previously described. An energy diagram of the two-photon fluorescence is shown in Fig. 5 (a). This is a process called two-photon excitation. As shown in the figure, one of the fluorescent molecules enters a higher energy state by simultaneously absorbing two photons of frequency $\omega_1$. The energy is released as the fluorescence of frequency $\omega_2$ when returning from the higher state to the ground state ($S_0$).

![Fig. 5 Optical processes of two-photon fluorescence (a), second harmonic generation (SHG) (b) and coherent anti-Stokes Raman scattering (CARS) (c).](image)

As fluorophores are commonly used for biological specimens with excitation spectra in the range of 400–500 nm, the excitation wavelength lies in the range of 700–1000 nm (i.e., infrared). As the infrared light minimizes scattering in the
tissue and phototoxicity to the cell, fluorescence microscopy utilizing two-photon excitation can be an attractive alternative to ordinary confocal microscopy owing to its deeper tissue penetration and reduced phototoxicity. However, it should be noted that the tissues and cells require labeling with a fluorescent dye, except when observing autofluorescence.

The SHG light is obtained by the two-photon process shown in Fig. 5 (b), which results in a frequency twice (i.e., a wavelength half) that of the initial photons. As the SHG is an even-order NLOP, the SHG light is generated only from substances of noncentrosymmetric molecular structures. Biological specimens such as collagen, microtubules, and muscle myosin can emit SHG light signals. When the SHG light is generated, the material containing fluorophores simultaneously generates two-photon fluorescence.

CRS is a nonlinear Raman scattering phenomenon, which requires no staining. In particular, coherent anti-Stokes Raman scattering (CARS) (Duncan et al., 1982; Hashimoto et al., 2000) and stimulated Raman scattering (SRS) (Freudiger et al., 2008; Ozeki et al., 2012) are widely used for microscopic imaging. Resulting images display the contrast of the intrinsic vibrations of the objective molecules. The CRS employs two laser sources with different frequencies: a pump beam of frequency $\omega_1$, and a probe beam at frequency $\omega_2$, as shown in Fig. 5(c). When the frequency difference between the two lasers coincides with a specific molecular vibration ($\Omega$), resonantly enhanced CRS signals are obtained as light emissions at $\omega_3 = 2\omega_1 - \omega_2$ (CARS) or energy transfers from $\omega_1$ to $\omega_2$ that result in a decrease of pump light intensity and increase of probe light intensity (SRS).

5.2. Application of the SHG microscope for in vivo visualization of dermal collagen fiber

Collagen molecules are structural proteins, which play an important role in determining the morphology and functional properties of tissues and organs. Collagen makes up 40% of the human skin dermis. Therefore, there is a strong need for noninvasive methods that can inspect 3D structures and determine the amount of collagen in the dermis for diagnosis in the clinical field. Here, I will introduce our SHG microscope for the visualization of collagen fiber in the human skin. In most previous studies, a mode-locked Ti:Sapphire laser (central wavelength of approximately 800 nm) was used as the light source to generate the SHG light with a wavelength of approximately 400 nm from the collagen fibers (Roth and Freund, 1981; Cox et al., 2003). However, scattering and absorption of the skin at these wavelengths often limit in vivo monitoring of the dermal collagen fiber in a reflection optical setup. The use of a laser with a wavelength longer than 1200 nm effectively reduces the influence of scattering and absorption. Based on this concept, we have constructed an SHG light microscope equipped with a 1250 nm Cr:Forsterite femtosecond laser as the excitation light source, and demonstrated its potential to examine the dermal collagen for skin diagnosis (Yasui et al., 2009, 2013; Tanaka et al., 2013).

Details of the SHG microscope setup are described elsewhere (Yasui et al., 2013). The backscattered SHG light component in the sample was detected using a photomultiplier tube under photon counting operation. The measurement time was set to 2 sec/frame on a 600 $\mu$m × 600 $\mu$m area. A wider area SHG image was constructed by successively connecting multiple frames. The measurement of the human skin was conducted with the permission of the ethical committee for human experiments at Osaka University.

Fig. 6 SHG microscope for in situ human skin measurement and resultant image of dermal collagen in cheek.
Figures 6 shows a view of the measurement of in situ human cheek, and the resulting SHG images of the cheek skin (note: white indicates collagen rich areas). One can clearly detect the distribution of the dermal collagen fibers and hair follicles.

5.3. Multimodal imaging

We have developed two types of multimodal nonlinear optical microscope systems: the two-photon fluorescence/SHG microscope, and CRS/SHG microscope. For the fluorescence/SHG microscope, monitoring of an engineered cell is demonstrated in Fig. 7, where fibroblasts were embedded and cultured in type I collagen gel. A confocal laser microscope (Nikon C1) equipped with a Ti:Sapphire laser was used to simultaneously observe the two-photon fluorescence and SHG images. Because native fibroblasts did not emit fluorescence themselves, the cells were stained with fluorescent tracer. The cells were cultured in an incubator set on the sample stage of microscope, and a time series of multimodal images were acquired.

Nonstaining visualization of the cellular configuration can be achieved by the detection of third harmonic generation (THG) optical signal. This is the third order NLOP, which results in a signal at one-third wavelength of the irradiating light. Various conditions in the specimen can cause THG. One possibility of the cause is a refraction index transition in the specimen that provides contrast in the cell shape. Although the efficiency of the THG is quite low, a combined SHG and THG microscopy was successfully utilized for the in vivo optical inspection of human oral mucosa (Tsai et al., 2011).

Imaging of lipid and fibrous components is important for the diagnosis and research of atherosclerosis. The CRS is highly sensitive to the lipids, while the SHG informs about the existence of collagen, which is the main fibrous component in atherosclerotic lesions. To demonstrate the atherosclerosis imaging, tissue sections obtained from a left...
common carotid artery of a 32-week-old mouse fed with lipid-rich diets were examined. Figure 8(a) shows the control tissue images of the section stained with Oil-red-O that labels the lipids (Hashimoto, 2014). The serially sectioned nonstaining tissue sample was successively observed with the SHG (blue) and SRS (green) (Fig. 8(b)). The Oil-red-O positive region provided a large SRS signal, and the adventitia showed an intense SHG signal.

6. Conclusion

The history of the optical microscope has been described in this article. Recent advances in nonlinear optical microscopy permit the acquisition of morphological and physiological cell information. As the efficiency of the NLOP is not significantly large to always provide a practical microscope image with satisfactory signal-to-noise ratio, high-power irradiation of the sample with laser light is often required to increase the output signal level. However the high-power irradiation may result in the damage of the objective cell. For further contribution of microscopy to cell biology, we expect further innovation of high spatial resolution optical microscopy, which permits real-time imaging without unwanted influence on the cellular physiology.

References

Betzig, E., Patterson, G. H., Sougrat, R., Lindwasser, O. W., Olenych, S., Bonifacino, J. S., Davidson, M. W., Lippincott-Schwartz, J. and Hess, H. F., Imaging intracellular fluorescent proteins at nanometer resolution, Science, Vol. 313 (5793), pp. 1642–1645 (2006).

Cox, G., Kable, E., Jones, A., Fraser, I., Manconi, F. and Gorrell, M. D., 3-dimensional imaging of collagen using second harmonic generation, J. Structural Biol., Vol. 141(1), pp. 53–63 (2003).

Denk, W., Strickler, J. H. and Webb, W. W., Two-photon laser scanning fluorescence microscopy, Science, Vol. 248 (4951), pp. 73–76 (1990).

Duncan, M. D., Reintjes, M. D. and Manuccia, T. J., Scanning coherent anti-Stokes Raman microscope, Opt. Lett., Vol. 7(8), pp. 350–352 (1982).

Freudiger, C. W., Min, W., Saar, B. G., Lu, S., Holtom, G. R., He, C., Tsai, J. C., Kang, J. X., Xie, X. S., Label-free biomedical imaging with high sensitivity by stimulated Raman scattering microscopy, Science, Vol. 322 (5909), pp. 1857–1861 (2008).

Fujita, K., Kobayashi, M., Kawano, S., Yamanaka, M. and Kawata, S., High-resolution confocal microscopy by saturated excitation of fluorescence, Phys. Rev. Lett., Vol. 99(22), 228105 (2007).

Fukushima, S., Furukawa, T., Niioka, H., Ichimiya, M., Sannomiya, T., Tanaka, N., Onoshima, D., Yukawa, H., Baba, Y., Ashida, M., Miyake, J., Araki, T. and Hashimoto, M., Correlative near-infrared light and cathodoluminescence microscopy using Y2O3:Ln, Yb (Ln = Tm, Er) nanophosphors for multiscale, multicolour bioimaging, Scientific Reports, Vol. 6, 25950 (2016).

Furukawa, T., Fukushima, S., Niioka, H., Yamamoto, N. Miyake, J., Araki, T. and Hashimoto, M., Rare-earth-doped nanophosphors for multi-color cathodoluminescence nano-bioimaging using scanning transmission electron microscopy, J. Biomed. Opt., Vol. 20(5), 056007 (2015).

Hashimoto, M., Araki, T. and Kawata, S., Molecular vibration imaging in the fingerprint region by use of coherent anti-Stokes Raman scattering microscopy with a collinear configuration, Opt. Lett., Vol. 25(24), pp. 1768–1770 (2000).

Hashimoto, M. and Araki T., Nonlinear Raman imaging using fast tunable picosecond laser, 32nd Physics Congress of the Samahang Pisikang Pilipinas 2014, Quezon City, Philippines, SPP2014-1C-01-1 (2014).

Hell, S. W. and Wichmann, J., Breaking the diffraction resolution limit by stimulated-emission: stimulated-emission-depletion fluorescence microscopy, Opt. Lett., Vol. 19(11), pp. 780–782 (1994).

Inouye, Y. and Kawata, S., Near-field scanning optical microscope with a metallic probe tip, Opt. Lett., Vol. 19(3), pp. 159–161 (1994).

Niioka, H., Furukawa, T., Ichimiya, M., Ashida, M., Araki, T. and Hashimoto, M., Multicolor cathodoluminescence microscopy for biological imaging with nanophosphors, Appl. Phys. Express, Vol. 4(11), 112402 (2011).

Ozeki, Y., Umemura, W., Otsuka, Y., Satoh, S., Hashimoto, H., Sumimura, K., Nishizawa, N., Fukui, K. and Itoh, K.,
High-speed molecular spectral imaging of tissue with stimulated Raman scattering, Nat. Photon., Vol. 6(12), pp. 845–851 (2012).

Pohl, D. W., Denk, W. and Lanz, M., Optical stethoscopy: Image recording with resolution $\lambda/20$ Appl. Phys. Lett., Vol. 44(7), pp. 651–653 (1984).

Roth, S. and Freund, I., Optical second-harmonic scattering in rat-tail tendon, Biopolymers, Vol. 20(6), pp. 1271–1290 (1981).

Shimomura, O., Johnson, F. H., Saiga, Y., Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea, J. Cell. Comp. Physiol., Vol. 59, pp. 223–239 (1962).

Tanaka, R., Fukushima, S., Sasaki, K., Tanaka, Y., Murota, H., Matsumoto, T., Araki, T. and Yasui, T., In vivo visualization of dermal collagen fiber in skin burn by collagen-sensitive second-harmonic-generation microscopy, J. Biomed. Opt., Vol. 18(6), 061231 (2013).

Tsai, M. R., Chen, S. Y., Shieh, D. B., Lou, P. J. and Sun, C. K., In vivo optical virtual biopsy of human oral mucosa with harmonic generation microscopy, Biomed. Opt. Express, Vol. 2(8) pp. 2317–2328 (2011).

Willig, K. I., Rizzoli, S. O., Westphal, V., Jahn, R. and Hell, S. W., STED microscopy reveals that synaptotagmin remains clustered after synaptic vesicle exocytosis, Nature, Vol. 440(7086), pp. 935–939 (2006).

Yasui, T., Takahashi, Y., Ito, M., Fukushima, S. and Araki, T., Ex vivo and in vivo second-harmonic-generation imaging of dermal collagen fiber in skin: comparison of imaging characteristics between mode-locked Cr:Forsterite and Ti:Sapphire lasers, Appl. Opt., Vol. 48(10), D88–D95 (2009).

Yasui, T., Yonetsu, M., Tanaka, R., Tanaka, Y., Fukushima, S., Yamashita, T., Ogura, Y., Hirao, T., Murota, H. and Araki, T., In vivo observation of age-related structural changes of dermal collagen in human facial skin using collagen-sensitive second harmonic generation microscope equipped with 1250-nm mode-locked Cr:Forsterite laser, J. Biomed. Opt., Vol. 18(3), 031108 (2013).