History of the Optical Microscope in Cell Biology and Medicine

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Advances in our understanding of cell biology are intimately connected with new optical microscopes and specimen preparation techniques. From the seventeenth century onward these technical advances impacted on the fields of cell biology and medicine, and sometimes these advances occurred reciprocally.

Introductory article

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Introduction

The development of the microscope is tightly coupled with developments in cell biology and medicine. Microscopes provide the observer with enhanced resolution (ability to observe two nearby objects as distinct objects), contrast (ability to detect different regions of the specimen on the basis of intensity or colour) and magnification (ability to make small objects visible). The human eye can resolve objects of the order of 0.1 mm, while the light microscope can resolve objects on the order of 0.2 μm (200 nm) with a magnification of 1000×. The transmission electron microscope can resolve objects on the order of 0.1 nm (10 Å units). Note the diameter of a hydrogen atom is on the order of 1 Å unit. Cells and their components are typically very small; therefore, we require a microscope with appropriate resolution, contrast and magnification to see them. For example, their typical linear dimensions are: a cell (20–30 μm), a red blood cell (7.6 μm), a mitochondrion (2–5 μm), a nucleus (10 μm), microvilli (1 μm), a cell membrane (10 nm), a microfilament (8–10 nm), a bacterium (1–2 μm) and a virus (10–100 nm).

The Light Microscope

The simple microscope contains a single lens. The compound light microscope consists of several lenses: condenser lens (focuses the illumination), microscope objective (collects and focuses light from the specimen) and an ocular lens or eyepiece (forms an image on the retina). If the magnification of the objective is 100× and that of the eyepiece is 10×, then the total magnification of the compound microscope is 1000×. The most important factor is the resolution, which is a function of the NA (numerical aperture is a measure of the light collecting cone equal to $n\sin\theta$, where θ is the half angle of the light cone from the lens) of the objective lens, λ or the wavelength of illumination light, and n is the refractive index of the medium between the specimen and the objective. For example, with NA equal to 0.94, and n equal to 1.5 (oil) and λ equal to 450 nm (blue light), then the resolution limit is about 0.2 μm (200 nm). Electron microscopes can achieve a much higher resolution and magnification than light microscopes because electrons (focused with electromagnetic lenses) that are used to form the image have a wavelength of 0.005 nm. In 1931, Knoll and Ruska constructed the first electron microscope, and in 1933, Ruska constructed an electron microscope with a resolution of 0.05 μm, that surpassed the resolution of the light microscope.

Sources of Invention and Technical Innovation in Microscopy

The history of the microscope and its associated techniques is replete with examples of independent invention and development that spans many countries and a period of a few hundred years. What were the driving forces of technical innovation in microscopy? It is of note that van Leeuwenhoek (the inventor of the single lens microscope) was a draper; he devised his first single lens microscopes to be able to observe the fine threads in cloth; only at the age of 40 when he turned his microscope to living organisms did he make his monumental discoveries.

Typically technical innovations in instruments and techniques resulted in new discoveries in the life sciences; however, there are notable exceptions such as the invention of the confocal microscope as a solution to the problem of optically sectioning specimens. Another example is the invention of the multiphoton excitation microscope, which

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provided a solution to the problem of how to provide deep-tissue imaging with fluorescent dyes that absorb in the ultraviolet region without killing the cells.

The development of various types of optical microscopes incorporated the following components: microscope objectives that minimize chromatic and other optical aberrations (image distortion), stands that minimize mechanical vibrations and sources of illumination from sunlight to lasers. Additionally, there were new methods of fixing and cutting specimens (microtomes), specimen staining techniques (dyes, stains, molecular probes) that increase specimen contrast. Also important were the development of various optical methods that provide contrast (phase and differential interference microscopes, fluorescence microscopes) for live cells, techniques for imaging long-term live cell cultures (time-lapse and video-microscopy), optical techniques to provide optical sectioning of specimens (confocal microscopes), and nonlinear-optical imaging techniques (multiphoton, harmonic generation and coherent anti-Stokes Raman microscopes). The history of the microscope is grounded in physics, specifically optics, but it includes glasses – their optical properties, their mechanical-thermal properties and their manufacture and grinding techniques – to produce optical components.

Early Microscopes and Their Use

The invention of the microscope is credited to Zaccharias Janssen (1587–1638) and his son Hans Janssen (1534–1592), two Dutch eye glass makers who placed multiple lenses in a tube and observed the image was magnified.

Antoni van Leeuwenhoek (1632–1723) used his single lens microscope to observe single bacterial cells and other microorganisms that he called 'wee animalcules'. He studied many specimens including liver, brain, fat and muscle tissues (stained with a dye). Additionally, he observed the cornea of the eye, the lens, the retina, the optic nerve, as well as biological fluids such as blood, urine, sweat and tears. van Leeuwenhoek observed the flow of blood globules (blood cells) in the capillaries in the gills of the tadpole; he noted that the flow was simultaneous with the heartbeat of the tadpole. He was not aware of Malpighi's discovery of blood capillaries (1660) and red blood corpuscles (1674).

In 1664 Robert Hooke (1635–1703), a contemporary of Newton, published *Micrographia*, a book which attracted widespread attention to the microscope and its marvelous capabilities. Hooke wrote in the preface of *Micrographia* that his motivation for the book was to promote the use of instruments in science. With his compound microscope and an oil lamp as a light source, he observed the eye and the morphology of common fly and he described the fruiting structures of molds. Later in 1665, Hooke observed cells (similar to monk's cells) in slices of cork and made similar observations of plant cells. Most importantly, he noted that the single lens microscope is superior to the compound microscope since the former had minimal chromatic aberrations (different colours of light focus at different points).

A major advance in the design of microscope objectives occurred when Joseph Jackson Lister (1786–1869) developed his version of the achromatic objective with no spherical aberration. He used his new microscope objective (achromatic doublets and triplets that did not multiply the spherical aberration and coma of each lens) to observe red blood cells and striated muscle. This was an important development because until the 1830s the compound microscope yielded low-quality images; on the contrary, the single lens microscope did not suffer from these optical aberrations.

Giaovanni Battista Amici (1786–1863) was another innovator and developer of microscopes. He was the first to use an immersion objective to increase the resolution (earlier suggested by Brewster). He used an ellipsoidal mirror objective to solve the problem of chromatic aberrations. This idea was first proposed by Christiaan Huygens as a method to avoid chromatic aberrations; earlier Newton made a similar proposal. Amici made three major improvements of the microscope: he developed a semispherical front lens achromatic microscope objective, he described the effect of coverslip thickness on image quality, and he introduced the use of the water immersion microscope objectives.

How were linear dimensions and resolution measured in microscopes? The magnification of van Leeuwenhoek's various microscopes ranged from 3× to 266× and the resolution ranged from 1.3 to 8 μm. He measured size in his specimens by comparison with a grain of sand or the diameter of a hair. Later, in the seventeenth and eighteenth centuries the resolving power of an objective was tested with the wings of insects or the feathers of birds; and for higher resolution objectives, diatoms were the test objects.

Over a period of decades the compound microscope began to attain the look of the modern optical microscope. Christian Hertel (1683–1725) in 1712 developed the mechanical x–y stage to move the specimen with fine mechanical control. In 1854, Camille Sebastien Nachet (1799–1881) invented his version of the stereomicroscope that contained two eyepieces. By 1897, the firm Zeiss manufactured its stereomicroscope that provided three-dimensional views of the specimen.

Advances in Microscope Design

The credit for the first theoretical advances in microscope design based on physics is given to Ernst Abbe (1840–1906). In 1866, Abbe met Carl Zeiss, who proposed that Abbe establish a scientific foundation for the manufacture of optical microscopes. Abbe made theoretical contributions to the theories of the resolving power of microscope, the role of diffraction in image formation, and the formation of aberrations. In 1872, Abbe formulated the 'Abbe Sine Condition' that permitted the design of objectives with maximum resolution and minimal aberrations. He invented the Abbe condenser in 1872 which illuminated the full aperture of the microscope objective and thus provided

maximum resolution. In 1873, Abbe formulated the limit of optical resolution of a microscope in terms of the numerical aperture and the wavelength of the incident light. Furthermore, Abbe invented apochromatized objectives (multiple colours of incident light focus at the same point) in 1886; these achromatic objectives provided a resolution of 1 μm, and this advance, together with oil-immersion objectives, permitted the subsequent major discoveries in bacteriology and cell biology. The concept of the numerical aperture of an objective is credited to Abbe.

Later in the twentieth century there were many seminal developments in microscope design that improved the contrast of biological cells and tissues. Starting in 1936 with the development of a microscope photometer for quantitative microscopy Torbjörn Caspersson, working in Stockholm, studied the absorption of various proteins and subsequently developed the field of microscopic spectrophotometry of cells and tissues. He failed to construct a microscope-fluorometer because of the then insurmountable problem of photobleaching of fluorescent dyes during ultraviolet excitation. In 1943, Evgenii Brumberg developed a microscope that used three colours of incident light and produced images in full colour.

Several inventors independently solved the problems of low contrast in thin objects, e.g. cell in culture. In 1931, Linnik developed his interference microscope that provided increased contrast. The Dutch physicist Frits Zernike (1881–1966) in 1934 solved the problem of the low contrast of living cells in culture by inventing his phase contrast microscope (it converted differences in the phases of light from the different parts of the specimen into light and dark intensities that are easily observed). In 1943, researchers in the Zeiss factory in Jena produced a time-lapse film of meiotic divisions in the spermatogenesis of grasshoppers; this film stimulated commercial interest in phase contrast microscopy. Zernicke received the Nobel Prize in 1953 for his invention of the phase contrast microscope. In 1956, Georges Nomarski invented a differential-contrast microscope which also improved the contrast of live cells in culture.

The Fluorescence Microscope

The next problem to be solved was how to achieve improved specificity and contrast in optical microscopy; and the solution was the application of fluorescence (incident light of short wavelength caused a fluorescent molecule to emit light of a longer wavelength) to microscopy. The development of the fluorescence microscope followed a series of technical advances in microscope design as well as the use of intrinsic fluorescence molecules as well as synthetic fluorescence molecules. In the former category are new sources of ultraviolet light, the use of liquid filters to separate specific wavelengths or colours of illumination, the use of solid or liquid filters to separate the incident excitation light from the emitted light that occurred at longer wavelengths, the design of objectives and lenses that can transmit ultraviolet light, and photographic and solid-state detectors to record the images. In the latter category are organic dyes and stains as well as fluorescent dyes that are chemically bound to antibodies that can couple to a specific protein in a cell, and thereby provide contrast in microscopy that has very high specificity (single protein) and results in high contrast images.

The dye fluorescein that was first synthesized in 1871 has a long history connected with the cell staining. In 1881, the bacteriologist Paul Ehrlich (1854–1915) used fluorescein to observe the passage of aqueous humour in the eye. He also used various aniline fluorescent dyes to stain bacteria and thereby increase their contrast in the microscope.

August Köhler (1866–1948) working in the Jena Zeiss factory developed in 1893 a new system of microscope illumination (later named Köhler illumination) for photomicrographic purposes. He also made microscope observations of autofluorescence (self-fluorescence) of biological specimens excited with ultraviolet light. He observed the ultraviolet image of unstained chromatin in the cell nucleus with incident light of 275 nm.

In 1903, Henry Friedrich Wilhelm Siedentopf (1872–1940) and Richard Adolf Zsigmondy (1865–1929) invented the ultra-microscope to observe colloids. Henry Friedrich Wilhelm Siedentopf constructed a dark-field condenser that blocked the incident light from entering the microscope objective, thereby improving the specimen's contrast. Henry Friedrich Wilhelm Siedentopf first reported that the use of ultraviolet light produced specimen fluorescence, and that was a problem since it reduced the specimen's contrast!

Then in 1904, August Köhler invented the ultraviolet microscope that preceded the fluorescence microscope. A camera was required to detect the very weak image. Köhler used the quartz monochromatic ultraviolet objective previously developed by Moritz von Rohr. The motivation to use ultraviolet light was based on the fact that shorter wavelengths of incident light result in increased resolution of optical microscopes (from the work of Abbe).

By 1910, Lehmann of the Carl Zeiss factory in Jena used the light source developed by the American Robert Wood (1868–1955) to make a prototype fluorescence microscope. In 1913, the Zeiss firm introduced its luminescence microscope. Subsequently Max Haitinger (1868–1946) introduced the term 'fluorochroming' or fluorescent staining of specimens, and he developed many of the staining techniques for the observation of specimens in the fluorescence microscope.

At the same time Carl Reichert of Vienna developed a competing fluorescence microscope with a new dark-field quartz condenser (the ultraviolet light did not enter the microscope objective, it only excited the specimen to fluoresce). In 1911, Hans Stübel observed autofluorescence (naturally occurring fluorescence without the application of a fluorescence dye to the specimen) during his microscopic studies of cells and tissues. Subsequent observations by Herwig Hamperl (University of Vienna) who initiated the use of the fluorescence microscope in pathology

confirmed the autofluorescence of the ocular lens of the eye, plant tissues, chlorophyll, amyloid, collagen, fibrin and elastic fibers. von Prowazek (1875–1915) was the first to introduce cellular staining into fluorescence microscope, i.e. his staining of living protozoa.

Philipp Ellinger in the 1920s was instrumental in the development of the intravital fluorescence microscope. Ellinger collorborated with August Hirt on its development. In 1933, Ellinger who was Jewish had to leave his position and subsequently Hirt who was a member of the Nazi SS claimed that he alone was the inventor of the intravital fluorescence microscope.

In 1929, the firm Carl Zeiss in Jena produced an intravital microscope with vertical illumination, a water-immersion microscope objective and an ultraviolet light source for the investigations of the distribution of fluorescent dyes in the kidneys and the liver of frogs and mice. This microscope was subsequently used for intravital observations of living skin, liver and kidney, and to study the microcirculation of organs and glands in the living animal. By 1932 various firms manufactured intravital microscopes and they were used to study the microvasculature, living skin, liver and kidney.

Several later developments improved the fluorescence microscope and resulted in its widespread use by cell biologists. In 1947 Siegfried Strugger (Münster), who previously developed the vital dye neutral red to stain plant cells, used acridine orange (a cellular stain) to differentiate live cells from dead cells. Strugger wrote a book on these studies: *Fluorescence Microscopy and Microbiology*. A major advance in the incident-light fluorescence microscope was the 1948 invention by Evgenii Brumberg and later developed by Ploem (1967) of the dichromatic beam-splitting plate that is used to separate the excitation light from the longer wavelength fluorescence. Albert Coons (1912–1978) and Melvin Kaplan are the inventors of immunofluorescence (1950) in which a fluorescent dye is chemically attached to an antibody; this technique resulted in an enormous increase in specificity (due to the antigen–antibody specificity) and resulted in many advances in cell biology. For example, in 1982 Mary Osborne and Klaus Weber used the fluorescence microscope together with fluorescent monoclonal antibodies to visualize the cytoskeleton protein tubulin in cells.

The Enduring Symbiotic Relationship between the Microscope and Cell Biology

During the second half of the seventeenth century humans for the first time observed the microscopic world: unicellular organisms, plant cells, spermatozoa, the fine structure of insect wings and compound eyes, and capillaries of the vascular system. Microscopic observations such as Leeuwenhoek's observation of spermatozoa stimulated new theories of generation; similarly the observation of capillaries in an organism provoked new directions in the concept of glandular action. The microscope proved not only to be a useful tool to facilitate the observation of microorganisms, but it became a critical tool in determining how biologists conceptualized cells and life itself. Two examples of its use in conceptualizing life are the theory of the cell as a unit of living organisms, and use in overturning the theory of spontaneous generation (in which living organisms were thought to be formed in putrefied organic matter).

Jan Swammerdam (1637–1680) observed erythrocytes (red blood cells) with a single lens microscope. He developed many techniques to improve the contrast of his specimens, such as the injection of coloured liquids into vessels and the use of coloured stains. His main work involved the use of the microscope to study the structure of the body of insects during different states of development, their respiratory and circulatory systems, their alimentary tracts and their reproductive organs. Additionally, he observed the compound eye of the bee. Swammerdam's microscopic observations were motivated by his deep reverence for God's works.

Marcello Malpighi (1628–1694) established the field of microscopic anatomy in Bologna where he discovered blood capillaries, observed the brain, the tongue and the retina, and the development of the chicken embryo. He studied the lungs, the lymph nodes and glands, and the brain. His research during 30 years at the University of Bologna focused on the physiological function of the human body, but he also studied a range of other animals such as fish, fowl, frogs and domestic animals. He is honoured by having his name associated with the following structures: the Malpighi layer in skin, to Malpighian tubules in insects, and the Malpighian tubules in the kidneys and the spleen.

Johannes Evangelista Purkinje (1787–1869) who coined the word protoplasm for the inside of cells was an experimental physiologist, embryologist and histologist. He used a compound microscope in his research and was the first to use a microtome to cut thin sections of his specimens. He discovered Purkinje neurons in the cortex of the cerebellum and the sweat glands in the skin. In his embryological research he coined the term 'protoplasm'. In 1839, Purkinje discovered excitatory fibres in the heart that are named after him; the Purkinje fibres are situated in the inner walls of the ventricles of the heart.

Robert Brown (1773–1858) used single lens microscope in his research. He discovered the nucleus of cells in plants, and whereas observing solutions of pollen grains he discovered 'Brownian motion'. He also observed cytoplasmic streaming. Leeuwenhoek first observed what was later named the nucleus, but Brown in 1833 named the nucleus as the large object in the cell.

The German physiologist, Theodor Schwann (1810–1882), and the German botanist, Matthias Jakob Schleiden (1804–1881) who encouraged Carl Zeiss to develop new and improved microscopes, collaborated and developed their cell theory, in which cells are the basic unit of

organisms. Schleiden's most important scientific works are Beiträge zur Phytogenesis (1838) and Grundzüge der Wissenschaftlichen Botanik (1842). In 1858, Schleiden published his popular book *The Plants and Their Lives*. Schwann used the microscope to observe the notochord, cartilage, lens of the eye, ganglion and pigment cells and confirmed their cellular composition. In 1839, Schwann proposed that both animals and plants are composed of cells. Schwann and Schleiden held the incorrect concept that cell develop from outside of cells; their concept of cell formation was eventually discredited and shown to be false.

The German botanist Ferdinand Cohn (1828–1898) is the founder of bacteriology. He used a microscope to investigate growth and division of plant cells. Additionally, he studied the life cycle of Bacillus as an example of the heat-resistant forms of bacteria. He demonstrated that when bacteria form endospores they can not be killed by heat sterilization.

The father of cellular pathology is Rudolf Virchow (1821–1902) who advocated the use of the microscope in pathology. Virchow, in 1858, wrote 'all cells derive from cells'. In this statement Virchow refuted the incorrect claims on cell formation made by Schwann and Schleiden. His seminal book *Cellular Pathology* demonstrated that diseases are the consequence of a disruption of normal cellular processes (**Figure 1**).

Figure 1 Microscope stand IV, No. 3207 manufactured by Oberhaeuser and Hartnack in Paris, France, in 1858. This microscope has a drum stand and polarizing equipment. This type of microscope was used by Rudolf Virchow in Berlin, Germany. All photos © by Timo Mappes, Karlsruhe (www.musoptin.com) and reproduced by permission.

The French chemist Louis Pasteur (1882–1895) performed experiments that resulted in the overthrow of the theory of spontaneous generation. From 1880 to 1890 he developed vaccines for the diseases anthrax, cholera and rabies, and helped to support the germ theory of diseases that was being developed by his contemporary Robert Koch. Louis Pasteur demonstrated that microorganisms are responsible for the purification of organic matter. Pasteur is also credited with the discovery of pasteurization to preserved food products (**Figure 2**).

The Russian biologist Ilya Metchnikoff (1845–1916), who worked with Pasteur in Paris, discovered the phagocytosis of bacteria by leucocytes and also investigated the pathogenesis of cholera. In 1908, Metchnikoff and Paul Ehrlich (1854–1915) who developed the humoral theory of immunity shared the Nobel Prize.

Robert Koch (1843–1919) is the founder of a seminal school of bacteriology; his students were among the important leaders in European and American bacteriology. In 1881, Koch developed experimental techniques, i.e. microscopy, staining of tissues, growth of bacteria on solid culture media, pure culture isolation and animal inoculation to understand the role of *Mycobacterium tuberculosis* in the transmission and progression of tuberculosis. Koch is credited with methods to study bacteria in pure culture.

Koch conceptualized the germ theory of disease and provided experimental evidence in its support. He devised Koch's postulates for the experimental study of infectious diseases. He made the first photomicrographs of bacteria (microscope and camera), developed microscopic pathology, and the methodology for disinfection and sterilization. He was awarded the 1905 Nobel Prize for Physiology and Medicine. Koch was an innovator in microscopy: he was the first to use an oil immersion objective and the Abbe condenser (with a lens system to fill the entire aperture of the microscope objective and thus achieve maximum resolution), the first to publish photomicrographs of bacteria, and he devised staining methods for bacteria to increase their contrast in the microscope.

Koch developed the science of microbiology. In 1873 he studied anthrax and published in 1877 the life cycle of *Bacillus anthracis*. Koch used air-dried films on cover slips that he stained with the aniline dyes that Ehrlich introduced into microbiology. In 1875, he discovered the tubercle bacilli and he discovered the pathogen of Cholera. In these seminal studies Koch obtained the first photomicrographs of bacteria in diseased tissue. Koch used a Zeiss microscope with a binocular eyepiece, a mechanical stage and an Abbe illuminator as well as an objective revolver. Koch also used a microscope produced by the firm Seibert and Krafft located in Wetzlar. In 1878, he published his book, *Aetiology of Tramatic Infective Diseases*.

It was common for researchers to use a variety of microscopes from different manufacturers in their work. The firm Reichert located in Vienna produced high-quality microscopes that were used by Louis Pasteur, Rudolf Virchow and Robert Koch. The firm Nachet located in

Figure 2 Microscope stand VIII, No. 4815 manufactured by Hartnack successor of Oberhaeuser in Paris, France, in 1864. This is the first microscope stand to show the horse-shoe base. All photos © by Timo Mappes, Karlsruhe (www.musoptin.com) and reproduced by permission.

Figure 3 Microscope stand II, No. 5272 manufactured by Carl Zeiss, Jena, Germany, in 1881. It is equipped with an Abbe condenser and a homogeneous immersion objective. Santiago Ramón y Cajal (Spain) and Robert Koch (Germany) used this type of microscope. All photos © by Timo Mappes, Karlsruhe (www.musoptin.com) and reproduced by permission.

Paris produced high-quality microscopes that were used by Pasteur. The Zeiss firm located in Jena used Koch's success in microbiology to market their new microscopes.

Santiago Ramón y Cajal (1852–1934) provides another example of how the microscope impacted on cell biology. Ramón y Cajal formulated the foundations of the neuron doctrine and laid the foundations of neuroscience. In 1889, Camillo Golgi discovered the Golgi apparatus with his development of silver and osmium staining of cells. Ramón y Cajal applied the Golgi method of staining to the brains of small animals, specifically fetal and neonatal mammals in which the myelin in either absent or less dense, and thus permitted his seminal microscopic observations of the connections between axons and dendrites (connections from other neurons). These microscopic observations culminated in his publication of the Spanish and French versions of the *Histology of the Nervous System of Man and Vertebrates*, and his Nobel Prize that he shared with Golgi in 1906 (**Figure 3**).

In 1885, during his tenure as Professor of Anatomy in the University of Valencia, Ramón y Cajal received a gift of a new Zeiss microscope with several objectives including the famous 1.18 numerical aperture for homogeneous immersion that represented the state-of-the-art in optics. As his work progressed Ramón y Cajal obtained newly developed microscopes. In 1900, he reported using Zeiss microscope objectives with numerical apertures of 1.30, 1.40 and 1.63. He also used microscopes manufactured by Reichert (Vienna) and Leitz (Wetzlar). He transformed his microscope observations into drawings with the aid of a camera lucida (drawing devices to simultaneously view the specimen in the microscope and the drawing paper); he used a version made by Nachet (Paris) and Abbe (Zeiss, Jena).

The development of the achromatic microscope permitted researchers to study the cell nucleus, especially its role in cell division, gamate formation and fertilization. Walther Flemming (1843–1905) discovered mitosis and the substance chromatin. Flemming showed in mitosis there is longitudinal subdivision of each chromosome and the two daughter chromosomes are pulled to each pole by fibres that he called 'asters'. He also described the nucleolus. In 1884 he used the term mitosis to describe the entire cycle. Between 1883 and 1885 August Weismann (1834–1914) one of the founders of genetics demonstrated the process of meiosis and in 1890 actually coined the term meiosis. Weismann is known for his theory that only germ cells (egg and sperm cells) and not somatic or body cells are the agents of heredity. Then in 1886, with the introduction of the Zeiss aprochromatic lenses, Flemming studied chromosome division in mitosis, as well as studies on

Figure 4 Microscope stand 2, No. 6194 manufactured by W&H Seibert, Wetzlar, Germany, in 1890. Robert Koch (Germany) used this type of microscope in his work. All photos © by Timo Mappes, Karlsruhe (www.musoptin.com) and reproduced by permission.

Figure 5 Microscope stand I^{c}, No. 51612 manufactured by Carl Zeiss, Jena, Germany, in 1910. The microscope stand is called the 'jug handle' stand and was made from 1898 to 1920 and was copied by other microscope manufacturers. The particular microscope shown is equipped with Zeiss apochromats and is the personal instrument of Raymond-Joseph Weissenbach (1885–1963), who gave the name to the Thibièrge-Weissenbach syndrome. All photos © by Timo Mappes, Karlsruhe (www.musoptin.com) and reproduced by permission.

fertilization, and meiosis, that is the reduction of the number of chromosomes in the formation of gametes.

The German zoologist Theodor Boveri studied the process of meiosis in the oocyte of the Ascaris, a nematode worm. Boveri used the microscope to observe how chromosomes redistributed their parts in cell division, halved the chromosomes in germ cell (sperm and egg) formation, and finally paired their chromosomes in the process of fertilization. Independently, between 1902 and 1903 Walter Sutton who worked in the United States found similar results; he demonstrated that the chromosomes contain the inheritable material of cells – the genes (**Figure 4**).

Not only advances in microscope design impacted on cell biology, but also there were important developments in specimen preparation, tissue sectioning and staining techniques. From about 1820 microscopists used glass slides for their specimens. In 1875 Paul Mayer developed the microtome, an instrument that can cut very thin sections of a specimen. By 1880 specimens were prepared as follows: fixation, dehydrating, staining with aniline dyes, embedding in paraffin and sectioning to produce thin sections with a microtome. At the end of the nineteenth century a larger number of synthetic dyes became available. Paul Ehrlich, formerly a co-worker of Robert Koch, used basic nuclear dyes and acidic dyes to stain the protoplasm (**Figure 5**).

New Developments in Light Microscopy: Live Cell Imaging

Marvin Minsky invented the confocal microscope (with the capability to optically section specimens) in 1957. The development of several types of confocal microscopes (a linear optical microscope in which the image intensity is proportional to the intensity of the incident light) during the last decades provided superb optical sectioning capability to observed cell structure and function. Its development was motivated by the need of cell biologists to image thick specimens without the need for mechanical (destructive) sectioning.

Modern biologists use new types of nonlinear microscopes (the brightness of the image is a function of the square of the intensity of the incident light) that were not available 15 years ago. All of these new types of microscopes provide optical sectioning capability and are compatible with long-term imaging of live cells, tissues and organisms. Some of these new nonlinear optical microscopes such as the multiphoton excitation microscope (the theory for which

was published in the 1931 doctoral thesis of Maria Göppert-Mayer) use near-infrared light from a pulsed laser to excite fluorescence from either naturally occurring cellular components or genetically induced production of fluorescent proteins. The use of near-infrared light permits deeper penetration into cells than ultraviolet light and is considerably less damaging to living cells. Other examples of these nonlinear microscopes include the following: second-harmonic generation microscopy, third-harmonic generation microscopy and coherent anti-Stokes Raman spectroscopy microscopy. The latter technique provides the additional advantage of chemical specificity. In addition to the development of new types of microscopes there are concomitant developments in laser sources (turn-key, diode-pumped femtosecond lasers), microscope objectives (long working distances, high numerical aperture and high transmittance of the near-infrared incident light and the longer fluorescence emission), and new molecular probe developments (coloured varieties of genetically encoded molecular fluorescent probes, probes with increased resistance to photo damage, and inorganic quantum dot probes).

Each of these new technologies improved the resolution and the contrast and provided the capability to 'optically section' cells, tissues and organisms and thus decipher complex processes such as cell signalling, proliferation, differentiation, development and programmed cell death or apoptosis. These new microscopes have been applied to study the structure and the function of the nervous system, the functioning of the immune system, protein trafficking within the cell, the biology of cancer, tumour growth and formation, embryonic growth and development, vascular development and its regulation, the function and manipulation of stem cells, as well as a variety of degenerative neurological diseases.

Note that microscopy began with Anthony van Leeuwenhoek's single lens microscope that he used to observe live cells, tissues and organisms. Today, live cell imaging and the imaging of living organisms is an emerging area of cell biological research; exciting new developments in light microscopy and ancillary staining techniques are driving this research.

Acknowledgement

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