The microscope as a scientific tool

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The NIMROD LENS, a quartz artefact found by the archaeologist J LAYARD in the ruins of Nineveh and reported in 1853 (LAYARD J, 1853) is often believed to be the oldest optical device (dated between 750 and 710 BC). Yet, there is no evidence that the NIMROD LENS could be used for magnification; just possibly this quartz piece was an item of jewelry. We also do not exactly know whether other ancient artifacts from several centuries BC, found in the Mediterranian region, may have served as lenses for the observation of minute objects in the sense of a telescope or a microscope. Also, Chinese may have already used simple magnifying glasses by combining a lens and a water-filled tube to detect small objects at the time of Marco Polo's travels. In any case, one can argue that ancient cultures had knowledge of optical lenses. It is possible that they used such devices not only as burning-glasses.

Magnifying tools related to microscopy are technical equipments to produce images from structures which would be too small to be observed by the naked eye. The microscope itself can be seen as a further development of magnifying glasses, thus, as derivatives of lenses like burning-glasses, already known in ancient times. IBN AL HAITAM (965-1038, also named ALHAZEN [in Latin]) is adjudged to be the first to have the idea of using lenses (plano-convex) for the magnification of objects. In his work "Kitab-al-Manazir", in Latin *Opticae Thesaurus* translated from Arab in Latin in 1270 by ERAZM GOLEK VITELLO, later published by F RISNER (1572) IBN AL HAITAM referred to such invention by cutting glass in order to obtain magnifying glasses (so-called reading stones or reading glasses). Reading stones are probably the precursors of spectacles made from beryl. The first spectacles with convex lenses appeared in Italy at the end of the 13th century. ALHAZEN's *Opticae Thesaurus* influenced to a great extent the development of optics after its translation into Latin (first translations in the 12th or 13th century). Science of optics was closely related to the discovery of the telescope, the works and views of scientists like GALILEO and KEPLER paved the way for new sights of the cosmos.

The invention of the microscope is closely related with the invention of the telescope. Yet, the real invention of the microscope as a compound instrument as well as the beginning of microscopy is quite obscure and remains a matter of some dispute. In historical reviews, the manufacture of the (compound) microscope is credited to the brothers JANNSSEN and to C DREBBEL in Holland. In this context, both J KEPLER and G GALILEI who have developed astronomic telescopes, can also be mentioned. The term *microscope* (coined by F VON BAMBERG in analogy to telescope) goes back to members of the ACADEMIA DEI LINCEI in Rome (G GALILEI was one of its members). Apart from *microscopy* by classical microscopes, technical developments enable now *nanoscopy* with resolution in the order of 10^{-9} meter. It is supposed that *nano world* becomes a hot spot of physics, chemistry, biology, medicine and other sciences.

Stages of invention, evolution and manufacture of microscopes are compiled in the following list. This documentation is certainly not complete. In addition, some uncertainties may exist. Throughout the study of historical dates, authorship and timetable are sometimes imprecise,

essentially, because old sources and references are either not exact or not exactly cited. For those who are interested in historical details, I am referring to papers referenced below.

Microscope technology – steps of invention and development [1]

BC: The knowledge of *single lens magnifying glasses* dates back to ancient times. It is known for over 2000 years that glass bends light. The art of cutting rock crystals in the form of magnifying glasses is described in documents from the mediterranien area, but neither names nor dates of the discoverers are exactly mentioned. Some description of the *action of lenses* appears in the "Optics Thesaurus Alhazeni Arabius Basil".

c1000: IBN AL-HAITAM (965-1039), also known as ALHAZEN (in Latin) described in his book "Kitab-al-Manazir" the basics of *geometrical optics* and theorized the manufacture of lenses, f.e. cutting of glass in order to obtain *lenses for the magnification* of objects. IBN AL-HAITAM's work was later translated in Latin which significantly influenced the development of optics.

1538: FRACASTORO H (also named GIROLAMO FRACASTORO), was attributed the first notion on the effect of superimposing two lenses for the observation of objects in more detail and the first suggestion of the use of telescopes and microscopes (published in his *Homo-centrica, eiusdem de causis criticorum dierum per ea quae in nobis sunt*) The descriptions are brief and incomplete.

1595: The manufacture of the *first microscope* is credited to H JANSSEN and Z JANSSEN from Middelburg (Holland) even if the invention of the compound microscope is still a matter of dispute. The microscope was continuously improved during the following centuries. The same holds true for the *telescope*, invented by H LIPPERSHEY around 1608, according to most sources. In 1609, G GALILEI constructed his telescope. J KEPLER published in 1611 ("Dioptrice") drawings of his telescope using a convex lens ocular.

1609: G GALILEI was able to focus his telescope to view small objects. This can be named a *compound microscope* (convexe and concave lenses). He improved the technique in the following years and called it *Occhiolino*.

1611: J KEPLER is a key figure in the 17th century scientific revolution. His book "Dioptrice" is fundamental for *optics as science*.

1619: C DREBBEL presented a *compound microscope* with convex objective and convexe eyepiece. Some historians will see him as inventor of the compound microscope.

1645: AM SCHRYL DE RHEITA described in "Oculus Enoch et Aliae sive Radius Siderio Mysticus" a *binocular microscope*. E DIVINI constructed a microscope with sliding tubes for focussing and protracting tubes for variable magnifications.

1646: A KIRCHER collected in his "Ars magna lucis et umbrae" the *optical knowledge of the time*. The descriptions and illustrations demonstrated the use of the microscope with some relevance to medicine.

1654: J WIESEL (Augustinus Opticus), an optician and telescope maker in Augsburg introduced the *field lens* (also called collective lens) for the microscope.

1665: R HOOKE (wave theory of light) optimized the *illumination* by use of a water filled bowl being placed in front of a oil lamp. In his work "Micrographia" he also described details about the manufacture of his compound (double-lens) microscope; *Microscopium compositum*. For the first time the term "cell" was coined.

[1] Data obtained from scientific literature, historical reviews, and from publications of manufacturers of optical devices

1670: C HUYGENS (wave theory of light) constructed a *double-lens ocular* with the field lens near the interim image and an ocular lens. A field diaphragma within the interim image was used to reduce chromatic abberations. C HUYGENS formulated the undulation theory of light.

1671: CHÉRUBIN D'ORLÉANS described in his book „La Dioptrique Oculaire" lenses, telescopes and microscopes including descriptions of the tools and techniques in their manufacture. He applied the principle of a binocular telescope to the design of a microscope – *binocular microscope* – in order to view objects with both eyes. The binocular microscope consisted of two separate microscopes, one for each eye, with independent optical systems (illustrated later in „La Vision Parfaite", dated 1677).

1672: J C STURM developed *double-lens* objectives.

1673: A VAN LEEUWENHOEK presented in London (Royal Society) his famous *microscopical observations* which included illustrations from bacteria in human saliva, red blood cells, sperma etc. He used different types of microscopes for different preparations (*one-lens microscopes*; Microscopium simplex). His lenses could reach 270-fold magnification, see also A VAN LEEUWENHOEK, *Arcana naturae detecta* (Delft 1695).

1675: MALPIGHI used a microscope for numerous anatomical studies including plant anatomy. He described the capillary system in the lung of frogs. The studies were published in several books (*Opera omnia* 1686/1687/1699).

1688: E DIVINI combined several lenses in a fixed system for a compound microscope.

1690: C HUYGENS provided foundations for the wave theory of light.

1691: F BONANNI (F BUONANNI) designed a compound microscope with three lenses and published his microscopical works in *Observationes circa viventia, quae in rebus non viventibus reperiuntur. Cum micrographia curiosa* (1691).

1704: I NEWTON described in "Opticks" (Opticks: Or a treatise of the reflexions, refractions, inflexions and colours of light) *discoveries and theories concerning light and color*.

1716: CG HERTEL published *instructions for glass grinding*, making optical lenses and for the manufacture of the relevant machines.

c1725: E CULPEPER created the *Culpeper-type microscope*, compound microscope with a sliding focus (moving the tube within its sleeve for focusing). The concave mirror below the stage allowed illumination from below. Culpeper-type microscopes were produced by many microscope makers with some modifications for the next 100 years.

1733: Documents dealing with the invention of *achromatic lenses* support the opinion that CM HALL realized c1733 how to correct chromatic and spherical aberration. He charged two different manufactures (E SCARLETT and J MANN) to make a lens from crown and flint glass for an achromatic telescope. They in turn sub-contracted the lens finishing to one and the same optician, namely G BASS, and he realized the achromatic properties after fitting the two parts together. It is assumed that CM BASS let know other competitors from the lens's properties and the method of making an *achromatic doublet*.

1744: J CUFF came up with a unique design which was known as CUFF's *new constructed double microscope*. The stage was freely accessible to the hands of the user, it incorporated a fine adjustment nechanism for precise focusing.

1747: L EULER described in "Sur la perfection des verres objectifs des lunettes" the theory how to obtain *achromatic lens systems*. The theory of I NEWTON on refraction (in *Opticks*, [1704] was denied by L EULER. The original refraction theory asserted that it was theoretically impossible to correct chromatic aberration using a colour dispersion-free (so-called achromatic) telescope. L EULER named as example the human eye as apparently achromatic lens system and proposed to conduct new experiments. In his theoretical work ("Constructio lentium objectivarum" 1762), L EULER described the basics of *achromatic lens* systems and concluded that chromatic aberration can be indeed corrected by combining lenses with differing refractive indices and, moreover, having a cavity between them filled with water.

1754: S KLINGENSTIERNA's publication "Anmerkung über das Gesetz der Brechung bey Lichtstrahlen von verschiedener Art, wenn sie durch ein durchsichtiges Mittel in verschiedene andere gehen" (Königliche Schwedische Akademie der Wissenschaften, Abhandlungen aus der Naturlehre, 16, 300-309, 1754) gave a proof that NEWTON's statement disagrees with the law of refraction. J DOLLOND, a defender of I Newton's theorem, became informed and subsequently investigated himself the problem. Finally, his findings were in agreement with the results of S KLINGENSTIERNA.

1758: Quite independently from CM HALL's conceptions to correct *chromatic and spherical aberration*, J DOLLOND was ambitious in making achromatic lenses. He published in 1758 "An account of some experiments concerning the different refrangibility of light" (Phil Trans 50, 741-742, 1758) and designed the construction of achromatic lenses; J DOLLOND obtained the patent for the construction of *achromatic lenses*. In the story of authorship it is worthwhile to note that J DOLLOND had knowledge of L EULER's arguments on how to construct achromatic lenses and knowledge of S KLINGENSTIERNA's studies on *achromatic and aplanatic optical systems*. However, these works were not appreciated in J DOLLOND's account of his own investigations. The paper of J RAMSDEN addressed to the Royal Society, "Some observations on the invention of achromatic telescopes" (Roy Soc Letters and Papers, IX, item 138, 1789), raised questions on the true history of J DOLLOND's invention. The story of invention of *achromatic object glasses* triggered a dispute in the later part of the 19th century. For more details see f.e. I NESTERENKO (2018) and R SORRENSON (2001).

1760: S KLINGENSTIERNA published a comprehensive *theory for lens systems and achromatism*.

c1760: LF DELLEBARRE microscopes were devised with a five-component ocular and a single lens objective. Magnifications were achieved by different combinations of the lenses together with the use of a draw-tube.

1770: J VAN DEYL presented his first *achromatic microscope objective*, but production at large scale was not possible at that time. Other known pioneers in achromatic objectives were N FUESS (1784) and FG BEELDSNYDER (1791), GB AMICI (1814) and J VON FRAUNHOFER (1815).

1782: J RAMSDEN described in his paper "A description of a new construction of eye-glasses for such telescopes as may be applied to mathematical instruments" (read December 1782 and published in Phil Trans Roy Soc 73, 94-99, 1783) procedures by which the aberrations of color and sphericity were much diminished.

1812: WH WOLLASTON introduced a new combination of lenses (*Wollaston doublets*, i.e. two plano-concave lenses with an intervening aperture.

1813: D BREWSTER proposed *oil for achromatization* of a compound lens; the front lens of the objective is immersed in the liquid. With the development of glasses of differing powers, this system passed into disuse.

1814: GB AMICI presented the *camera lucida*, an even today used drawing equipment. Quite independently from D BREWSTER he made experiments with *immersion lenses* to diminuish the loss of light and to improve the image. He also continued to develop achromatic lens systems. Not before the 1830's the developed *achromatic lenses* by J LISTER solved some problems of spherical aberration.

1815: J VON FRAUNHOFER (spectral analysis, Frauenhofer'sche Linien in solar spectrum, diffraction gratings) developed *achromatic lenses* for microscopes (Utzschneider & Frauenhofer microscopes) and telescopes in the glassworks of REICHENBACH-LIEBHERR-UTZSCHNEIDER in Benediktbeuren. Experimental observations of the solar spectrum and the discovery of absorption lines were of great matter for the manufacture of *special glass types*. J VON FRAUNHOFER developed precise methods to determine optical constants for his new lenses.

1823: M SELLIGUE combined up to *four achromatic puttying parts* (plano-concave plus bi-convex lenses) for the construction of objectives. They were successfully used in microscopes being produced in JLV CHEVALIER's manufacture.

1829: W NICOL introduced prisms for *polarisation* microscopy.

1834: CS NACHET began to make lenses for C Chevalier. In 1840, CS NACHET set up his own business (Maison Nachet & Fils) and produced his own microscopes until the middles of the 20th century. The first microscopes based on the drum-type base models previously made by other manufacturers. NACHET's *microscope grand modèle* (c1887) was one of the best at that time.

1839: C CHEVALIER (the son of LV CHEVALIER) described a compound microscope with achromatic objectives. CHEVALIER adopted the idea of L EULER that achromates may be made by combination of of two lenses having a cavity between them filled with water. One of the earliest microscopes is illustrated in the book "Des microscopes et de leur usage" together with microscopical applications. Also, CHEVALIER made *daguerreotypic* experiments (the basis of future microphotography which was shortly thereafter realized with a microphotographic apparatus according to MAYER (R NEUHAUSS 1898, T PÉTERFI 1933).

1846: C ZEISS opened his manufacture in Jena; his merits are the consequent implementation of mathematically calculated optics done in collaboration with E ABBE und O SCHOTT (1879). The entry of *new glass types* opened an era of new *apochromatic objectives* which enabled excellent optics capable of high resolution.

1847: C KELLNER presented his *orthoscopic ocular* and founded in 1849 (with M HENSOLDT) the Optical Institute in Wetzlar. Later on, E LEITZ became partner of the company. He managed the Company from 1869 under his name.

1853: JL RIDDELL invented the first practical *binocular microscope* to enable binocular viewing of objects through a single objective lens and a prism system. The arrangement permitted the right eye to receive only light form the right half of the objective and vice versa.

1857: R WINKEL founded in Göttingen his manufacture "Winkel'sche Werkstatt in Göttingen" and produced microscopes. In 1911 ZEISS Company becomes main partner, R WINKEL GmbH is merging (1957) into the CARL-ZEISS-Foundation.

1859: E HARTNACK presented his *water immersion objectives*. They were suggested as the best immersion objectives at that time.

1869: E LEITZ Company was launched in 1869 by E LEITZ, the successor of C KELLNER's Optical Institute; C KELLNER died in 1855.

1869: JW STRUTT (Baron Rayleigh) published disvoveries in the fields of acoustics and optics covering among other things *wave theory and light scattering*. His extensive studies were reported in several book volumes (JW STRUTT [Baron Rayleigh], Scientific Papers, 1869-1881).

1873: E ABBE's theories brought the light microscope to perfection. He developed the new *theory of microscope image formation*, based on wave optics, the *diffraction theory*. He studied the nature of how microscope images are formed and formulated the theoretically possible resolution in light microscopy (approx. 0.20 µm). The resolving power of an objective is defined by the formula: $d = \frac{\lambda}{2\, n \sin \alpha}$ ($d = \frac{\lambda}{2 \times A}$)

[d = distance between two points, λ = wave length of light, *n* = fraction number of the medium between cover glass of specimen and front of objective lens and A = numerical aperture].

The numerical aperture of an objective lens is defined: $A = n \times \sin \alpha$

[*n* = fractive index of the medium between cover glass of specimen and front of objective lens]

The theories were published in 1873. Finally, ABBE's inventions allowed the construction of perfect *apochromatic objectives* (1886), *oil immersion lenses* (1878), *substage condensers* and *compensating eye pieces*.

1879: First contact of E ABBE with O SCHOTT, a private facility for the development of *new glass species* was established. In 1884 foundation of the Jenaer Glaswerk for the development and production of special glasses and lenses by O SCHOTT, E ABBE, C ZEISS and R ZEISS (Schott & Genossen).

1893: A KÖHLER developed the so-called *Köhler illumination* with separate regulation of field iris and condensor iris.

1903: H SIEDENTOPF and R ZSIGMONDY developed the *ultramicroscope* (variant of the dark field microscope) for the visualization of colloidal particles. The ultramicroscope is regarded as the forerunner and simple version of *light-sheet microscopy* and the *light sheet fluorescence microscope* (LSFM).

1904: A KÖHLER and M VON ROHR introduced the *UV-microscope* with high transmission of ultraviolet light, and they are quite likely the first to have done fluorescence microscopy.

1911: C REICHERT introduced the *luminescence microscope, fluorescence microscopes* were developed at C ZEISS (1913) by H LEHMANN, O HEIMSTÄDT and S VON PROWAZEK as an outgrowth of the UV-microscope for the study of autofluorescence of organisms and bioorganic substances.

1924: L DE BROGLIE proposed in his thesis *Recherches sur la théorie des quanta* (Thesis Paris, 1924) that electrons have properties of waves (L DE BROGLIE, Phil Mag 47, 446-458, 1924). The work served as basis for developing the general *theory of wave mechanics*.

1927: H BUSCH was the founder of the *geometric electron optics* and elaborated the basics of *electron optics*. The path of electrons can be deflected by magnetic lenses; magnetic or electric fields act as lenses for electrons (BUSCH, H 1927, BUSCH H and BRÜCHE E 1937)

c1927: H STINTZING predicted the principle of scanning microscopy. In 1927, he applied for two patents (a) *Verfahren und Einrichtung zum automatischen Nachweis, Messung und Zählung von Einzelteilchen beliebiger Art, Form und Grösse*, German patent (DRP) No. 485155, filed 1927 and awarded 1929, and (b) *Einrichtung zum automatischen Nachweis, Messung und Zählung von Einzelteilchen beliebiger Art, Form und Grösse nach Patent 485155, insbesondere zur Erzeugung sehr enger Strahlenbündel*, German patent (DRP) No. 485156, filed 1927 and awarded 1929.

1928: EH SYNGE published the theory underlying the *near-field scanning optical microscope* (NSOM/SNOM).

1931: The era of *electron microscopy* started with a series of works by M KNOLL, E RUSKA and B VON BORRIES (Technische Hochschule Berlin). In 1931, M KNOLL and E RUSKA finished the construction of the first *two-stage transmission electron microscope*. The senior clinician of the Charité in Berlin, R SIEBECK, was persuaded of the importance of electron microscopy not only in physics or other fundamental research areas but also in medicine and pushed the industry to produce the instrument. SIEMENS made the step and produced the instrument. The first prototypes went into action early in 1938 (VON BORRIES B and RUSKA E 1940, MÜLLER HO and RUSKA E 1941).

c1931: H RUSKA (brother of E RUSKA) demonstrated the importance of electron microscopy in life sciences, especially in virology. He was engaged in sample preparation and application studies. SIEMENS set up a "Laboratorium für Übermikroskopie" headed by H RUSKA which could also be used by guest scientists. In the following years, H RUSKA *et al*. demonstrated bacteria and viruses by electron optical means. This attempt marked the beginning of viral taxonomy by morphological criteria (1931-1944).

1932: Two other groups in Berlin, the AEG group led by CW RAMSAUER and the independent entrepreneur M VON ARDENNE, were also active in the development of *different types of electron microscopes*. In the following years, all groups were able to present electron microscopes and published their technical features. The applicability of electron optics was proven by *various examples including biological objects* (publications from 1932 through 1941 by BRÜCHE E and JOHANNSON E 1932, BRÜCHE E and HAAGEN E 1932, BRÜCHE E 1943, MAHL H 1939, JAKOB A and MAHL H 1940, VON ARDENNE M 1938-1941, BRÜCHE E 1943). In the following years, a number of modifications and new developments in electron microscopy became published worldwide. Within the AEG team (from 1935 to 1941), H BOERSCH collaborated successfully on *geometric electron optics* (H BOERSCH 1936, 1942) and, after the war at the Physical Institute in Berlin (from 1954 to 1974 as successor of CW RAMSAUER) on subjects like *interaction of electrons, ions and light with matter and electromagnetic fields*.

1934: L MARTON presented his *electron microscope* and his *first studies of biological objects* (published in Nature 133, 911, 1934 and in Bull Cl Sci Acad Roy Belg Series 5, 20, 439-446, 1934).

1935: M KNOLL described the feasibility of the *scanning transmission microscope*.

1935: F ZERNIKE introduced *phase contrast* in microscopy and demonstrated phase contrast methods.

1936: EW MÜLLER designed *field emission microcopy*. It has, however, taken several years until the method could work to view atomic structures.

1938: M VON ARDENNE built the first functional *scanning electron microscope* (ARDENNE M VON, 1938).

c1940: In the early years of electron microscopy, the *design of appropriate lenses* (electromagnetic vs. electrostatic lenses) occupied the scientists. Physicists worldwide (f.e. England, France, Holland, Canada, USA, Switzerland, Japan and other countries) followed the example of the BERLIN groups and became engaged in further developments of electron microscopy including adequate equipment for physico-chemical and biological applications (VK ZWORYKIN et al. 1941, 1942, 1943, 1945; VE COSSLETT 1946, 1981, 1982; G DUPOUY et al. 1960). In these times, questions concerning *image formation* and *aberration* emerged soon and several approaches were proposed (O SCHERZER 1936, 1946, 1947; A NEßLINGER 1939; W GLASER 1940, 1956). Theoretical and practical aspects of electron microscopy were still subject of intensive studies as by G MÖLLENSTADT (1954, 1956, 1968). I must apologize, the great number of relevant papers published in the decades after the second world war cannot be given in this short tabulation, they are best reviewed by PW HAWKES (2001, 2004, 2009) and by H ROSE (2009).

1948: D GABOR studied on a new two-step method of optical imagery (microscopy by reconstructed wave-fronts) in order to circumvent spherical aberration in electron microscopy and presented a new microscopic principle, *holography* (GABOR D 1948, GABOR D 1949).

1951: H NAORA published his ideas of microphotometry. His studies were fundamental in the development of the confocal microscope.

1955: G NOMARSKI developed the *differential interference contrast (DIC) microscopy* by use of specialized prisms.

1961: ML MINSKY became patented the concept of *confocal microscopy*.

1967: EW MÜLLER added the time-of-flight spectroscopy to the field ion microscope, the *atom-probe field-ion microscope*, allowing the chemical identification of each individual atom.

1971: The concept of *4Pi microscopy* was formulated by C CREMER and T CREMER in their patent specification P 21 16 521.9 (Verfahren zur Darstellung bzw. Modifikation von Objekt-Details, deren Abmessungen ausserhalb der sichtbaren Wellenlängen liegen). The idea of a confocal laser scanning 4Pi microscope was published in 1978 (CREMER C and CREMER T, 1978). Confocal laser scanning 4Pi microscopy was realized and further developped by SW HELL (1992).

c1974: Acoustic microscopy (with scanning version) for biomedical applications was invented (LEMONS RA and QUATE CF, 1974; LEMONS RA and QUATE CF, 1975; RUGAR D et al. 1980).

1980s: Technical developments continued (computer control, advanced specimen stages, digital image recording etc.) and enabled *high resolution biological electron microscopy and tomography*. Low-temperature techniques combined with *cryo-electron microscopy* have gained some popularity together with electron tomography. Such methods proved to be important for the ultrastructural characterisation of multisubunit structures.

1982: G BINNIG, H ROHRER, C GERBER and E WEIBEL presented the *scanning tunnelling micrcospe* (STM); in the following years, the *scanning near-field optical microscope* (SNOM) was developed (POHL DW et al., 1984; DÜRIG U et al., 1986)

1986: G BINNIG, CF QUATE and C GERBER (1986) presented *atomic force microscopy* (AFM).

1988: K ITAYA invented the *electrochemical scanning tunnelling microscope* (ESTM/ECSTEM)

1992: SW HELL pioneered *STED (Stimulated Emission Depletion)-4Pi-fluorescence micro-scopy* (HELL SW and STELZER EHK, 1992; HELL SW et al., 1994; KLAR TA et al., 2000; DYBA M and HELL SW, 2002) With these techniques, resolutions of *30-50 nm* (about $\lambda/23$) can be achieved when operating at a wavelength of 750 nm. 4Pi microscoy has become an established nanoscopy method.

1993: VOIE AH et al. developed *light sheet fluorescence microscopy* (LSFM), called orthogonal-plane fluorescence optical sectioning (OPFOS). OPFOS features all the elements that are currently present in LSFM devices.

1995: Transmission electron microscopy experienced a new start owing to new developments of *aberration corrected electron optics* (HAIDER M *et al*., 1995, 1998).

Recommended readings on the development, and the use of microscopes

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Histology, biology, pathology

Optimized microscopes and histological microtechniques paved the way for discoveries in botany, cell biology, medicine and microbiology. Little by little the structure of cells, composition of tissues and special functions became identified.

X BICHAT (Marie François Xavier Bichat) may be considered as the founder of histology (BICHAT X, *Anatomie générale appliquée à la physiologie et à la médecine*, 1801) even if he worked macroscopically and never used a microscope. In the search for elementary parts of the organism he described substructures of organs ("membranes") with typical properties or functions (propriétés vitales). Hence the whole organism being the sum of "propriétés vitales" of tissues. Only much later, the perception of *histology* as a means of cell structure at the microscopical level became strengthened by C MAYER (August Franz Josef Carl Mayer) in his lecture notes *Histologie-Gewebelehre* (MAYER C, 1819).

The first steps of histotechnology arose from botanical studies (M RASPAIL 1825; GM SMITH 1915), while significant contributions to animal and human tissues started much later (FREY H, 1874 and 1886). Milestones were cell fixation (f.e. ethanol, salt solutions, aldehydes) and tissue cutting techniques. In the beginnings of microcopy, sections from plants and animal organs were prepared manually with razor blades. Data origins of microtomes are quite obscure, most probably because the first microtomes were simple devices and not widely documented. The precursor of "cutting instruments" is the dedicated *cutting engine* invented by G ADAMS/A CUMMINGS (HUMASON GL 1962) which was used and described by J HILL (1770) for the preparation of thick botanical specimens. The *cutting engine* was operated by hand: the sample held in a cylinder and sections cut from the top using a crank arm. Much later, real microtomes were developed with the aim to obtain tissue sections of reproducible thickness.

At the end of 1800s, cutting techniques developed rapidly which allowed to cut consistently thin sections. Together with appropriate tissue fixation, tissue embedment (f.e. paraffin) and tinctorial methods (natural, synthetic dyes) fine structural studies of organs became better and better. The development of histological microtechniques is described by B BRACEGIRDLE in a fascinating review (BRACEGIRDLE B, 1978); see chapter *Microtomy of tissue specimens*, collection of sections [https://https://www.kuhlmann-biomed.de/wp-content/uploads/2020/11/IET_tissue_05.pdf].

The logical consequence of the development of microscopical microtechniques was the number of discoveries made in that time. For example, R BROWN published in 1831 a paper emphasizing the presence of nuclei in plant cells which he desrcibed as "opaque spots" in epidermis cells of orchids. J PURKINJE confirmed that animal tissues are composed of cells (see STUDNICKA FK [1936] *Joh. Ev. Purkinjes histologische Arbeiten*). J PURKINJE's studies covered a wide spectrum of microscopical and physiological experiments in interrelated biological sciences such as anatomy, histology and pharmacology.

The finding that plants as well as animals are composed of cells led to the evolution of the cell theory and the birth of cell biology. Both are strictly coupled with the fundamental works of M SCHLEIDEN (1838) and T SCHWANN (1839) who were the first to realize the significance of the cell as the basic unit of living organisms. SCHLEIDEN proposed that the nucleus was an elementary organ in plants being closely linked to their development. Then, SCHWANN stated "that there is one universal principle of development for the elementary part of organisms, however, different, and that this principle is the formation of cells". These conclusions are generally considered to mark the formulation of the "cell theory". Then, the papers of W WALDEYER (1888), W FLEMMING (1877, 1882, 1887) and T BOVERI (1909) shaped the perception of *chromosome* and *mitosis* for a solid foundation of cell biology.

In the case of histology, the contributions of J MÜLLER (1838), F HENLE (1840, 1855) and W WALDEYER (1863) were of great importance for subsequent pioneering histologists. It is also recommended to read in G VALENTIN's comprehensive anatomical works (1837-1843) as well as to read in the famous works of A KÖLLIKER (1852), OFC DEITERS (1865), H FREY (1859, 1863), S STRICKER (1871), L RANVIER (1875) and W FLEMMING (1882) to get an impression of the powerful research at that time. Genuine histological work began in the second half of the 19th century when T HARTIG (1854), C WEIGERT (1871, 1878), J GERLACH (1848, 1858, 1863) and P EHRLICH (1877, 1878, 1879, 1886) started with their systematic studies of natural and synthetic dyes (aniline derivatives).

The principle of histological staining relies on the treatment of tissue sections with dyes in solution which will react more or less specifically with defined cell and tissue structures. In its classical way, a uniform theory of tissue staining does not exist because the mechanisms of dye binding with the various cell components are quite heterogenous. Histological stainings are very complex. Conventional histological stainings are seen in connection with the chemical and morphological behaviour of cell structures. In a particular case, both natural and synthetic dye staings may be due to (a) chemical reactions; (b) physical adsorptions or absorption; and (c) physico-chemical processes. Usually, chemical and physical processes are running in parallel (BURCK HC, 1988). For details, see chapter *Dyes, stains, and special probes in histology* [link: https://www.kuhlmann-biomed.de/wp-content/uploads/2020/11/IET_introduct_05.pdf].

The step from histological science (evolution of the cell theory) to histopathology is connected with R VIRCHOW and his publication *Cellular-Pathologie* (1855, 1858). He is the founder of cellular pathology: "omnis cellula e cellula"; where a cell arises, there must have previously existed a cell. R VIRCHOW noted that lesions and diseases are found in the cells, a conception which is still actual.

Histochemistry

Defined chemical and biochemical reactions in histology are a progress in specific histological staining. One of the earliest microchemical reactions was the discovery by H F LINK (1807) in which he used iron sulfate for determining tannic acid in leaves. Then, M RASPAIL (1825) used iodine for the microchemical detection of starch. Fundamental work in histochemistry was done by R FEULGEN. The *Feulgen reaction* (FEULGEN R and ROSSENBECK H, 1924) is such an example. In its initial formulation, this stain was developed for the detection of DNA in the nucleus. It is important to note that the *Feulgen reaction* is an ordered series of chemical reactions and not simply a tissue stain. Since the demonstration that this technique is specific and stoichiometric for DNA, this reaction has become an important means for staining nuclear DNA and densitometric quantification. In his treatise, K ZEIGER (1938) has given an excellent overview of the physicochemical basics of histological and histochemical staining methods.

During further developments of microscopical stainings, significant progress was obtained with the formulation of defined substrates for the detection of *enzymes and isozymes* (enzyme histochemistry). G GOMORI (1939) and H TAKAMATSU (1939) described independently methods for the demonstration of phosphatases in tissue sections. A large number of histochemical reactions have appeared since then for the specific localization of enzymes at both light and electron microscopic levels. Enzymatic procedures have been collected in well known treatises, f.e. in the years 1952 and 1953 (G GOMORI, *Microscopic Histochemistry - Principles and Practice, 1952*; AGE PEARSE, *Histochemistry Theoretical and Applied*, 1968,

1980). For details, see chapter *Histological staining techniques* [link: https://www.kuhlmann-biomed.de/wp-content/uploads/2020/11/IET_introduct_04.pdf].

Selective cell labeling, ligand binding assays

Histochemical methods and, particularly, selective cell labeling techniques using the principle of ligand binding assays (specific molecular probes) are prevailing conditions for the analysis of cells, organs and their functions in life science. A typical approach is immunohistology, i.e. the use of antibodies as molecular probes. Antibodies possess a high degree of specificity towards antigenic determinants. Because of the narrow range of specificity of an antibody molecule to bind with its antigenic determinant, immunochemical methods are very sensitive techniques. With respect to the definition of antigenic molecules (i.e. antigenic substances are those which initiate an immune response in a host followed by the formation of specific antibodies), immunological methods on the basis of antigen-antibody reactions are widely employed in qualitative and quantitative studies since the early years of the last century. Pioneers are f.e. by EHRLICH, LANDSTEINER, WITEBSKY, HEIDELBERGER, MARRACK, KABAT, OUDIN, GRABAR. From their schools developed subsequent generations of scientists in immunology, immunogenetics and molecular biology.

The concept of cellular antigen staining in tissue sections by labeled antibodies dates back to the early work of A H COONS and co-workers in 1941 describing immunofluorescence techniques for the microscope. In the beginning, simple methods were used which lacked sensitivity and specificity. Since then, improvements were made in conjugation techniques, tissue fixation, selection of labels and microscopes. In the meantime, immunohistochemistry has become part of molecular tissue pathology. The technique is a powerful but very complex tool with high sensitivity. There exist a large number of procedures to detect one or multiple antigens in the same cell or tissue preparation. For details, see chapter *Fluorescence microscopy* [link: https://www.kuhlmann-biomed.de/wp-content/uploads/2020/08/IET_introduct_03.pdf].

Antibodies have applications in research, in diagnostics and in other specialized areas which include pathology, forensic medicine, biochemistry and biology. Together with the modern concepts of molecular biology (notably nucleic acid probing, specific interaction of nucleic acid sequences with complementary probes and the polymerase chain reaction), antibody techniques are part of the most important tools in the study of the molecular and structural composition of cells. The extraordinary potential of immunocytochemistry can bridge molecular, cellular, organismal, and clinical investigations. Embedded in current proteomic technologies including cancer proteomics, immunocytochemistry contributes to the identification and understanding of complex changes in protein profiles.

Because the principles of immunohistology hold also true for other molecular probes such as lectins and nucleic acid staining by DNA and RNA hybridization techniques, those techniques are major candidates in the study of life processes. Studies of gene expression together with analysis of proteomics give useful information about presence of characteristic proteins in defined conditions. For details, see chapter *Cell staining with direct and indirect assay formats* [link: https://www.kuhlmann-biomed.de/wp-content/uploads/2020/11/IET_immustain_01.pdf], and chapter *Lectins and nucleic acids as molecular probes* [link: https://www.kuhlmann-biomed.de/wp-content/uploads/2020/11/IET_immustain_06.pdf].

Advanced microscopy, nanoscopy

The first electron microscope prototype was built in 1931 by the engineers E RUSKA and M KNOLL (1931). Since then electron optics were refined in a continuous pathway towards better resolution for the study of molecular structures. The beginnings of biological electron microscopy were strongly tied with improvements in tissue preservation, embedding and ultrathin sectioning. The introduction of new technologies, especially transistors, computer science and, in biology, the development of specific molecular probes, promoted sophisticated microscopes for all types of application in both biological and material sciences.

Today, microscopes with computer control, improved specimen stages, digital image recording and energy filtering enable applications of TEM for 3D structure determination, high resolution microscopy and electron tomography (MANNELLA CA et al., 1994; MCEWEN BF et al., 1995; MCEWEN BF and MARKO M, 1999; GRIMM R et al., 1998; STOWELL MHB et al., 1998; MCEWEN BF and MARKO M, 2001).

Increased resolution of electron microscopes and the development of thin-section methods (ultramicrotomes) opened in the 1950s a new era in cell biology. The potential of cryo-electron microscopy became apparent with GLAESER's work (GLAESER RM, 1971; TAYLOR KA and GLAESER RM, 1976); the way to obtain high resolution images from unstained frozen-hydrated specimens. Since the 1980s, low-temperature techniques combined with cryo-electron microscopy have gained some popularity, and, together with electron tomography, these methods have become important for the ultrastructural characterization of multisubunit structures such as membrane-associated proteins, ribosomes etc. (DUBOCHET J et al., 1988; DIERKSEN K et al., 1995; BAUMEISTER W and STEVEN AC, 2000; BAUMEISTER W, 2002; AL-AMOUDI A et al., 2004; DUBOCHET J, 2007; WAGENKNECHT T et al., 2002; BAUMEISTER W, 2004). Cryo-techniques in general will be beneficial to selective cell labeling in the sense of immunohistology (HUNZIKER EB and HERRMANN W, 1987; SWAGUCHI A et al., 2004).

New techniques in microscopy were introduced with the confocal microscope (EGGER MD and PETRAN M, 1967). The concept of confocal microscopy was originally invented by M MINSKY (patented in 1961) when studying neural networks in preparations of brain tissue to image biological events as they occur in living systems. Due to lack of intense light sources and computer capacity to handle large amounts of data, the invention of MINSKY remained for a long time unnoticed. Then, in the late 1970s and 1980s, advances in laser and computer technology led to the development of practical laser scanning confocal microscopes.

Confocal microscopy offers several advantages over conventional widefield optical epi-fluorescence microscopes, for example the ability to to exclude secondary fluorescence in areas removed from the focal plane (the elimination or reduction of background information away from the focal plane), and the capability to collect serial optical sections from thick specimens. It is achieved with an optical microscope as central part and integrated electronic detectors, several laser systems combined with wavelength selection devices, a beam scanning assembly and a computer for image display, processing and storage. The main source of this approach is the use of laser beams and spatial filtering techniques to eliminate out-of-focus light in specimens whose thickness exceeds the current plane of focus.

4Pi-confocal fluorescence microscope and *STED* method (Stimulated Emmission Depletion) (CREMER C and CREMER T (1978); HELL SW and STELZER EHK, 1992; HELL SW et al., 1994; KLAR TA et al., 2000; DYBA M and HELL SW, 2002) represent significant advances in fluorescence microscopy with great potential in biomolecular cell research. Spatial resolutions of 30-50 nm (about $\lambda/23$) are achievable. This extraordinary gain is really striking because

limitations in resolution by diffraction effects of the light (about half of the wavelength of the light used) usually applies to all farfield methods.

Breaking the diffraction barrier in microscopy has interesting aspects, thus, other attempts which have been proposed in the past, must be mentioned. The invention of scanning tunneling (STM) and atomic force microscopy (AFM) marked a new type of microscopy (BINNIG G et al., 1982; BINNIG G et al., 1986) in which an atomically sharp probe attached to a cantilever is scanned over the surface of a specimen. This has made nanometer resolution available, but this technique lacks molecular specificity. Hence, molecules may be observed, but their identity cannot be defined. This limits the usefulness of AFM. Yet, promising ways were tried which include f.e. immunogold-labeling and the modification of the probe by fluorescent molecules. The combination of high-resolution scanning probe and fluorescence has led to the development of the scanning near-field optical microscopy (SNOM) by A LEWIS et al. (1984), DW POHL et al. (1984) and U DÜRIG et al. (1986).

With the introduction of SNOM (scanning near-field optical microscopy, also known as NSOM, near-field scanning optical microscopy), optical resolutions of <50 nm were achievable. The basic principle of near-field optics is that light passes through a sub-wavelength diameter aperture and illuminates a sample placed within its near field (at a distance much less than the wavelength of the light).

EH SYNGE (1928) proposed the idea of using a small aperture to image a surface with sub-wavelength resolution using optical light. This very early idea to extend the microscopic resolution into the ultramicroscopic region was of theoretical nature. Even if the technical possibilities at that time impeded its realization, this idea led to the concept of optical near-field. Many years later, several authors reinvented this idea and published works on near-field optics (BETHE HA, 1944; BOUWKAMP CJ, 1950; O'KEEFE JA, 1956; MCCUTCHEN CW, 1967; ASH EA and NICHOLLS G, 1972). Optical resolutions in the order of $\lambda/60$ could be demonstrated.

In SNOM (as in the case of AFM), a sharp probe is used to map the topographic features on the sample surface. Besides topography, NSOM also generates optical images. The SNOM probe is a tapered optical fiber. Laser light is coupled into the fiber and used to excite fluorophores while the probe scans the sample surface. The optical fiber with a final diameter of 20-120 nm is metal-coated and serves to confine the light to the tip region. The tip is finally processed by an etching step to give a flat and circular endpoint and aperture which functions as the light source. Its diameter determines the optical resolution of the microscope. In fluorescence mode, the probe funnels the incident light wave to dimensions that are below the diffraction limit. Typically, the optical near-field generated at the aperture has significant intensity only in a layer of <100 nm from the aperture because the intensity of the evanescent light decays exponentially. Thus, the probe can excite fluorophores only within a layer of <100 nm from the probe (i.e. the near-field region). The sample fluorescence is subsequently collected by optics and transformed into an optical image.

Optical images of a sample are dierectly viewed or are collected with a camera. The imaging device should be selected f.e. with respect to high resolution, sensitivity and variable exposure times. Digital cameras are useful due to its digital, linear and quantitative properties. Cameras should at least offer high signal sensitivity, low noise and the ability to quantify the intensity distribution. With fluorescence filters correctly chosen, they image the fluorescence intensity of each fluorochrome separately. One can handle later the respective images within a specific color space by use of appropriate software. The resulting images can be analysed or further processed. Postprocessing of images helps to uncover details. Deconvolution algorythms, for example, will provide informations about the distortion of a small light spot (refraction

limited) by the operating microscope and can be used to equalize images. In conventional microscopy, images of the sample are captured by hardware centered around the objective lens. Some new unconventional methods have been developed recently which allow to avoid the limitations inherent in the classical design of microscopy (MCLEOD E and OZCAN A, 2016; OZCAN A and MCLEOD E, 2016).

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Ibn al-Haytam A (translated by F Risner 1572)
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Leeuwenhoek A. van (1673)
Malpighi M (1675-1679)
Malpighi M (1687)
Huygens C (1690)
Bonanni P (1691)
Leeuwenhoek A. van (1695)
Newton I (1704)
Hertel CG (1716)
Euler L (1747)
Klingenstierna S (1754a, 1754b)
Dollond J (1758)
Martin B (1759)
Klingenstierna S (1760)
Morgagni GB (1761)
Euler L (1762)
Hill J (1770)
Ramsden J (1783)
Ramsden J (1789)
Bichat X (1801)
Link HF (1807)
Fraunhofer J (1814-1815)
Mayer C (1819)
Fraunhofer J (1821)
Fraunhofer J (1823)
Raspail M (1825)
Brown R (1831)
Valentin G (1837-1843)
Müller J (1838)
Schleiden M (1838)
Chevalier C (1839)
Schwann T (1839)
Henle F (1840)
Gerlach J (1848)
Kölliker A (1852)
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Hartig T (1854a, 1854b)
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