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HISTORIC MILESTONES ON THE PATH OF THE BIRTH OF MICROSCOPY FROM LIGHT TO ELECTRON MICROSCOPY, AN INTERDISCIPLINARY TECHNIQUE. PART1 LIGHT MICROSCOPY

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Microscopes drove theories by providing the tool needed to make advances in many fields of science. Observations through the microscope primarily determined what early scientists thought and advanced science to a great extend as we know it today across many fields of science, becoming a dominating technique. What is captured here are some of the major milestones on the path of the birth of microscopy. The following is covered: The beginning of the light microscope, Early Stereo Microscope development, The Ultra Microscope, Differential interference contrast (DIC). Inverted light Microscope, The Fluorescence microscope, Confocal Microscopy, Confocal Scanning Light Microscope (or 'CSLM'), Transmission Electron microscope, Scanning Electron Microscope (SEM) and the Environmental scanning electron microscopy (ESEM). With more older manuscripts becoming available digitally, this overview fills in many gaps that exist in some of the previous work, covering a wider history in microscopy. It is also the single most recognised technique, which in its development and application, has produced more Nobel prize laureates than any other technique. Quite a few laurates are born and imbedded in the history of microscopy, some are covered here.

Key Words: History, Microscopy, Electron Microscopy, Review, Nobel Prize, interdisciplinary technique

INTRODUCTION

It all started back in the 9th century, with the invention of the "read stone" [1]. The read stone´s purpose is yet not clearly known. It might have also been only used to ignite a fire, not for magnifying purposes. The read stone was replaced by the use of spectacles [2] both these were necessary precursors to the invention of the first "microscope" based on the telescope. Please note that a detailed history of these precursor inventions are not covered in this article, more detail can be found in [3, 4]. In nature, some of the most basic activities happen at the microscopic scale, far beyond the limits of what we can see by eye. New technology needed to be developed for us to observe these processes, not visible to the naked eye. This formed the drive force pushing microscopy into the small beyond. The first person to mention the actual use of lenses for a definite purpose was the English monk Roger Bacon (1214-1294) who was the initiator of experimental physics. In Opus Majus, written in 1276 for Pope Clement, he discusses the efficiency of a curved crystal lens in magnifying objects [5].

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THE BEGINNING OF THE LIGHT MICROSCOPE

Although the invention of the microscope remains in the dark, it is known that Zacharias Janssen, who was a spectacle maker in Middelburg Netherlands, in 1590 made a compound microscope. He discovered in 1590 how to combine convex lenses in a tube to make an instrument for magnifying minute objects, thus the discovery and invention of the microscope [6,7]. The most active early investigators with the microscope were members of the Academy of the Lynx (Accademia diLincei) in Rome. This scientific society was formed in 1603 under the inspiration of Federigo Cesi, Duke of Aquasparta and ceased to exist with Cesi's death at the age of 45 in 1630 [8]. In 1624, Galileo developed a compound microscope and called it the "Occhialino," the "Little Eye." He used two convex lenses (as in a Keplerian system) instead of concave and convex lenses, as he had used in his initial telescope design [9]. Pierre Borel, a physician at the Court of Louis xiv, whom was among the first to use the microscope Borel's data were published in 1655 in his "Dc vero telescopii invcntore". [10, 4]. Giovanni Faber was the first to use the term "microscope" which is derived from the Greek words for "small" and "to look at or see.". He also was a fellow member of the Academia Dei Lincei, [9]. Anthony van Leuwenhook, a Dutch scientist, produces a single lens microscope describing microorganisms since 1632. He is often seen as "The farther of Microscopy" and a replica of his microscope can be seen in Figure 1. A comprehensive review of the contributions made by Anthony van Leuwenhook can be found in the publication by Gillen&Oliver in 2012 [11].

Figure 1. Replica of microscope that was used by Anthony van Leeuwenhook. Zeiss Museum of Optics, Jena. Credit: Stephanus H Coetzee.

Joseph Giuseppe Campani (1635–1715), an Italian optician and astronomer was the first to use a microscope (called novum microscopium) to observe human wounds, according to descriptions

published in 1686 in *Acta Eruditorum*. At the time he was considered an expert on grinding and polishing lenses, [12]. The world of microscopy really got noticed with the works of an English physicist, Robert Hooke, who discovered micro organisms and described them in "Microgephia" [13] and the book is electronically available online from the Royal Socety [https://ttp.royalsociety.org/ttp/ttp.html?id=a9c4863d-db77-42d1-b294-fe66c85958b3&type=book]. In the book there is a folding out plate just before page 1. The microscope used is displayed in Figure 2. This can also be found in "Roberts Hooke's Microphagia of 1665 and 1667" [2010Don] and reference to the work in "The discovery of Microorganisms Revisited" [14].

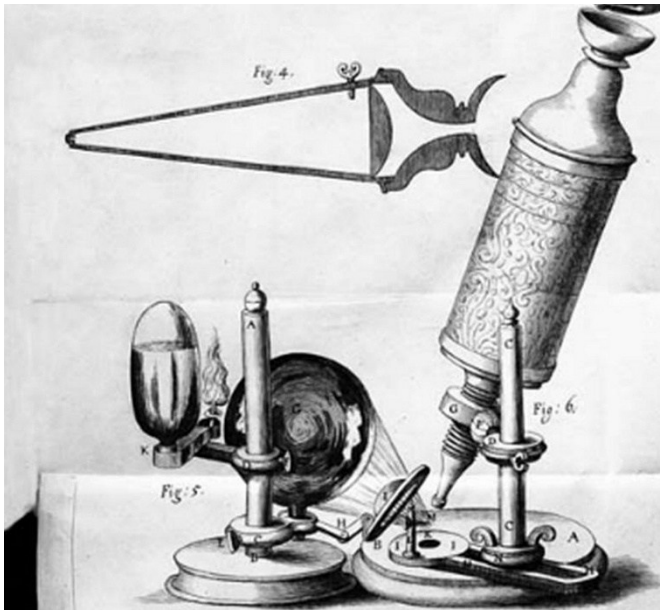

Figure 2. Hooke's new arrangements for artificial illumination of the specimen under the compound microscope and of the adjustable specimen holder. [13].

This new research field of microscopy, excited Carl Zeiss, a German mechanic, to dedicate his life to the building of simple microscopes. The first low power microscope was built in 1847 as stated in "Carl Zeiss in Jena 1945-1990" [15]. Many of these are preserved at the Zeiss Museum of optics, Jena. Some of the images produced here were from the Museum of optics in 2016. Below are some early versions of microscopes (Figure 3 and 4).

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Figure 3. An early simple microscope with a fixed magnification. Zeiss Museum of Optics, Jena. Credit: Stephanus H Coetzee.

Figure 4. An early "pocket screw "Microscope field kit with different magnification lenses. Zeiss Museum of Optics, Jena. Credit: Stephanus H Coetzee.

Microscopy really started developing with leaps and bounds each time problems with optics are solved. The invention of the achromat lens also remains in the dark, most probably it was invented several times and independently from each other. In 1730's we found a record Chester More Hall described the achromatic lens, which placed concave and convex lens elements together. This overcomes chromatic aberrations. These were made by trial and error, each being a matching pair, sparked the development of compact lenses [16]. The objective revolving nosepiece was already patented files as early 1928, the patent was granted in 1928 by Otto Henker, under the company Carl Zeiss, US patent 1,647,041 [17]. In 1830, Joseph Lister recognized the problem of spherical aberration, that was fully understood first by Prof Abbe in Jena [18]. During the mid-1800's Francis Wenham, and Edmund Wheeler have pioneered the use of the dark-field microscopy [19, 20]. Toward the 1880's, Lord Rayleigh gave us the equation to determine the resolution of light [21]. In 1834 Henry Fox Talbot equipped a microscope with polarizers and a different contrast method was born besides bright field. [22]. One of the main contributors to the design of microscope lenses was Ernst Abbe in 1873 [23], which gave us the Abbe number (Vd-number) which is the constringence of transparent material, as per equation below.

$$\nu = \frac{\eta D-1}{\eta F-\eta C}\eta \mathrm{d}$$

λ_F – 486.1 nm (blue Fraunhofer F line for hydrogen) [24]

λ_D – 589.2 nm (orange Fraunhofer D line for sodium)

λ_C – 589.2 nm (red Fraunhofer C line for hydrogen)

The need for different types of optical glass with different refractive behaviour was already seen by Fraunhofer. Abbe new of his ideas and with the collaboration with Otto Schott found a glass chemist who could develop and produce such optical matters. But Abbe was the first who clearly understood what was responsible for the resolving power of the light microscope (1872/ 73). Abbe opened the path to qualifying glass, determining the focal length and can be used to correct achromatic defects and determine the resolution limit of a lens. With this knowledge Carl Zeiss in Jena capitalized on the optimization based on this work. The first homogeneous oil immersion objective lens was developed on the suggestion of John Ware Stephenson; production began in early 1877 by Carl Zeiss. This can clearly be seen from Carl Zeiss and from their catalogue number 28 that was released in 1889. [25]. The complexity of the lenses already available at the time can be seen from the figure below from catalogue number 28 page 5 with Zeiss offering an immersion objective with a corrective adjustment for different coverslip thickness. Two articles reviewing modern objectives after 1970s, which were reported by Riesenberg in 1988 [26] and Broome in 1992 [27]. An excellent review was done lately by Zhang [28]. From here on, designs were based on sound laws of physics rather than trial and error. Abbe devised an Apertometer in 1876 for determining the numerical and angular aperture of objectives

(Figure 5). The full use is described in a publication in the Journal of the Royal Microscopical Society in 1877 [29].

Figure 5. Abbe's Apertometer Credit, Zeiss archives.

The Abbe theory describes mathematically the special resolution limit of an optical system using light.

$$d\text{MIN} = \frac{0.612 * \lambda}{n.\sin(\alpha)}$$

d = resolution

λ = wavelength of imaging radiation

n = index of refraction of medium between point source and lens, relative to free space

α = half the angle of the cone of light from specimen plane accepted by the objective (half aperture angle in radians)

In Abbe's equation, n sin α is often expressed as numerical aperture (NA)

Thus the expression can rewritten as:

$$d\text{MIN} = \frac{0.612 * \lambda}{NA}$$

The effect of wavelength (color) on the resolution can be calculated with the equation above. For this comparison we will use the numerical aperture of lenses dated back to 1889. For the comparison wavelength calculation of imaging radiation, we will use the blue Fraunhofer F line for hydrogen of 486.1 nm and the red Fraunhofer C line for hydrogen of 589.2 nm of 1814 [23] 1873Abb]. Taking one of the Apochromatic apertures with a NA of 1.4 of Padge 10 catalogue number 28 of Carl Zeiss in 1889 [25] Zei1889] and comparing the maximum resolution achievable at the time, dependent of color of light used

λ_C – 589.2 nm (red Fraunhofer C line for hydrogen)

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$$\text{MIN} = \frac{0.612 * \lambda}{NA}$$

$$d\text{MIN} = \frac{0.612 * 589.2}{1.4}$$

$$d\text{MIN} = 257.56\text{nm}$$

Λ_F - 486.1 nm (blue Fraunhofer F line for hydrogen)

$$d\text{MIN} = \frac{0.612 * \lambda}{NA}$$

$$d\text{MIN} = \frac{0.612 * 486.1}{1.4}$$

$$d\text{MIN} = 212.49\text{nm}$$

Hence using blue light rather than red light gives a better resolution of 45.07nm at the time. This principle formed the basis of many microscopes being equipped with a color filter to be used with bright filed microscopy much later. Modern objective lenses have a much better resolution.

EARLY STEREO MICROSCOPE DEVELOPMENT

The publication of "Dioptrice seu demonstratio eorum quae visui & visibilibus propter Conspicilla ita pridem inventa accidunt" the work by Kepler in 1611 suggested that using convex lenses within the eyepiece and a convex lens in the objective would help improve the image. [30, 31]. Kepler circulated a manuscript for a Philosophie in Optics that would eventually be published (posthumously) as Somnium for a Philosophie in Optics, which makes this the first Thesis in Optics. Stereo microscopy started off with a single lens approach, the first attempt was in 1677, by French Capuchin Friar at Orleans. Capuchin 's microscope is thought to have reversed the eyes optically, giving pseudoscopic depth [32]. The first single lens stereoscopic microscope was designed in1851 by I. L. Riddell and described in1853. It uses a beam splitter and two oculars [09]. Wenham is credited with inventing the 'Wenham binocular' microscope, a binocular arrangement in 1853 [32]. The first comparison microscope (not a true stereo microscope) saw the light in 1913 and was build by Leitz, Germany and can be seen in the Figure 6 below from the article "125 years of comparison Microscopy" [33]. No records were found that it went commercial.

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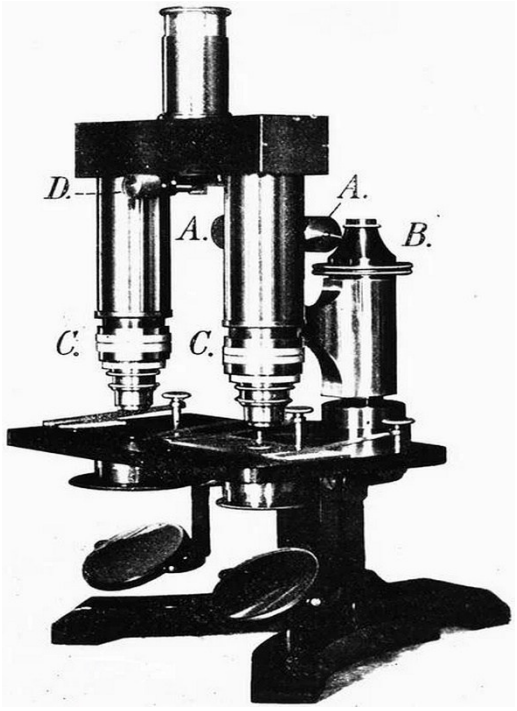

Figure 6. The first comparison light microscope build by Leitz [33].

Zeiss introduced their own stereo microscope in 1892 and became commercially available in 1896, produced by Carl Zeiss in Jena, Germany. A description in the catalogue of Carl Zeiss "Microscope and Microscope accessories. 32nd edition on Page 72 in 1902 read: "In the course of 1897 we introduced a new form of binocular microscope after the designs by Mr. Horatio Saltstall Greenough in which stereoscopic vision is obtained, but by a combination of microscopes, complete in themselves and combined with erecting prisms. This type of microscope is also adjustable by means of rack and pinion and can be seen in Figure 7 from Zeiss Catalogue No32 f 1902 [34].

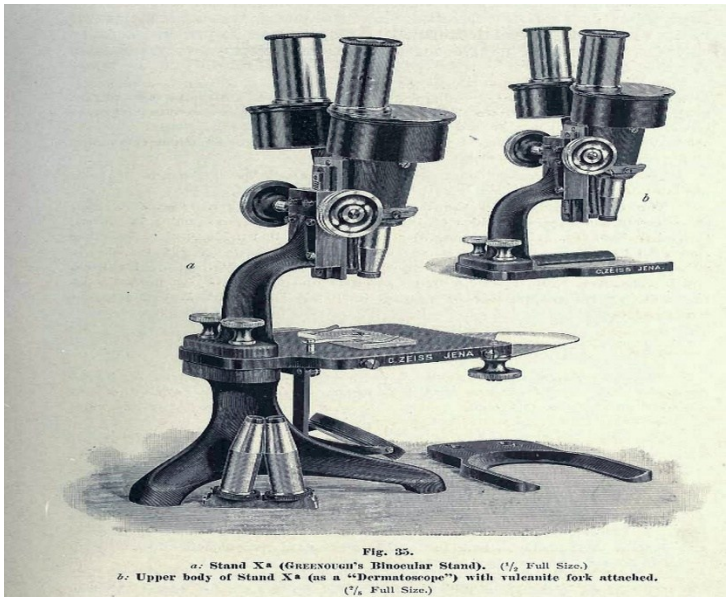

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Figure 7. The first commerially available stero light microscope. Zeiss Catalogue No32 of 1902 [34].

THE ULTRA MICROSCOPE

The Ultra Microscope was invented by Richard Zsigmondy in 1903, who was a professor in Inorganic Chemistry at the

University of Gottingen aimed at observing colloids as described in his book "Colloids and the Ultra Microscope published in 1909 [35] He was obsessed to observe directly particles the size of a crystalloid molecules directly .According to Zsigmondy "It must be here noted that the limits of microscopic resolvability (limit of visible ''separation'') determined by Abbe and Helmholtz, is often confounded with the limit of ''visibility." Those isolated particles, whose diameter is a fraction of a wave-length of light, can still be seen, are expressly stated by Abbe himself. He used side illumination of particles perpendicular to the viewing angle. As the bright light scatters off the particles, they appear as flashes against a dark background allowing the movement of particles to be visible. The Ultramicroscope was built in close collaboration with Zeiss. He received the Nobel prize in Chemistry in 1925. He received the prize in 1926 [36] In Figure 8 is a line diagram of the Ultramicroscope that was build by Zeiss in 1909.

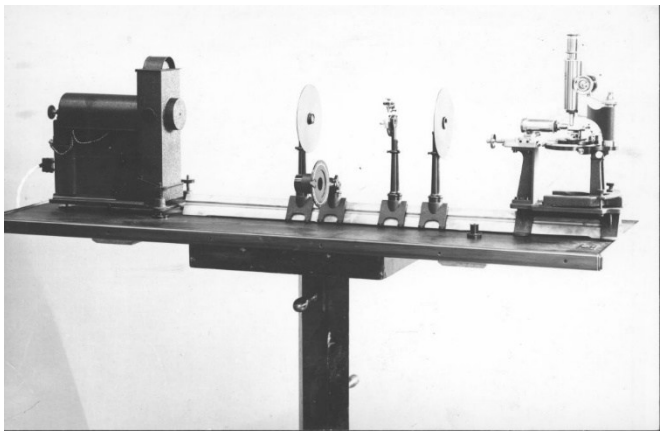

Figure 8. Ultramicroscope that was built in collaboration with Carl Zeiss, Jena. https://www.flickr.com/photos/zeissmicro/6892932822.

DIFFERENTIAL INTERFERENCE CONTRAST (DIC).

The DIC technique was invented by F. H. Smith in 1947 and patented: U.S. Patent, Number 2,601,175 [37]. According to Smith the principles is a follow: "The principles underlying the present invention may be understood by considering two mutually coherent light beams from the same source. In accordance with the usage in the art the term "mutually coherent' is applied herein to a plurality of beams having precise accordance between their luminous vibrations, so that if such beams are appropriately Superposed, e. g. by directing them along Substantially coincidental paths, the vibrational relationship between them is independent of the Sequence of random vibrational changes in the individual beams and visible interference effects may be produced. These interference effects enable a transparent or like object to be examined more easily than is possible using the usual form of visual

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microscope" It was patented (US Patent 2,601,175) in 1952. Figures 1 and 2 from the original patent describing interference contrast can be seen in Figure 9 [38].

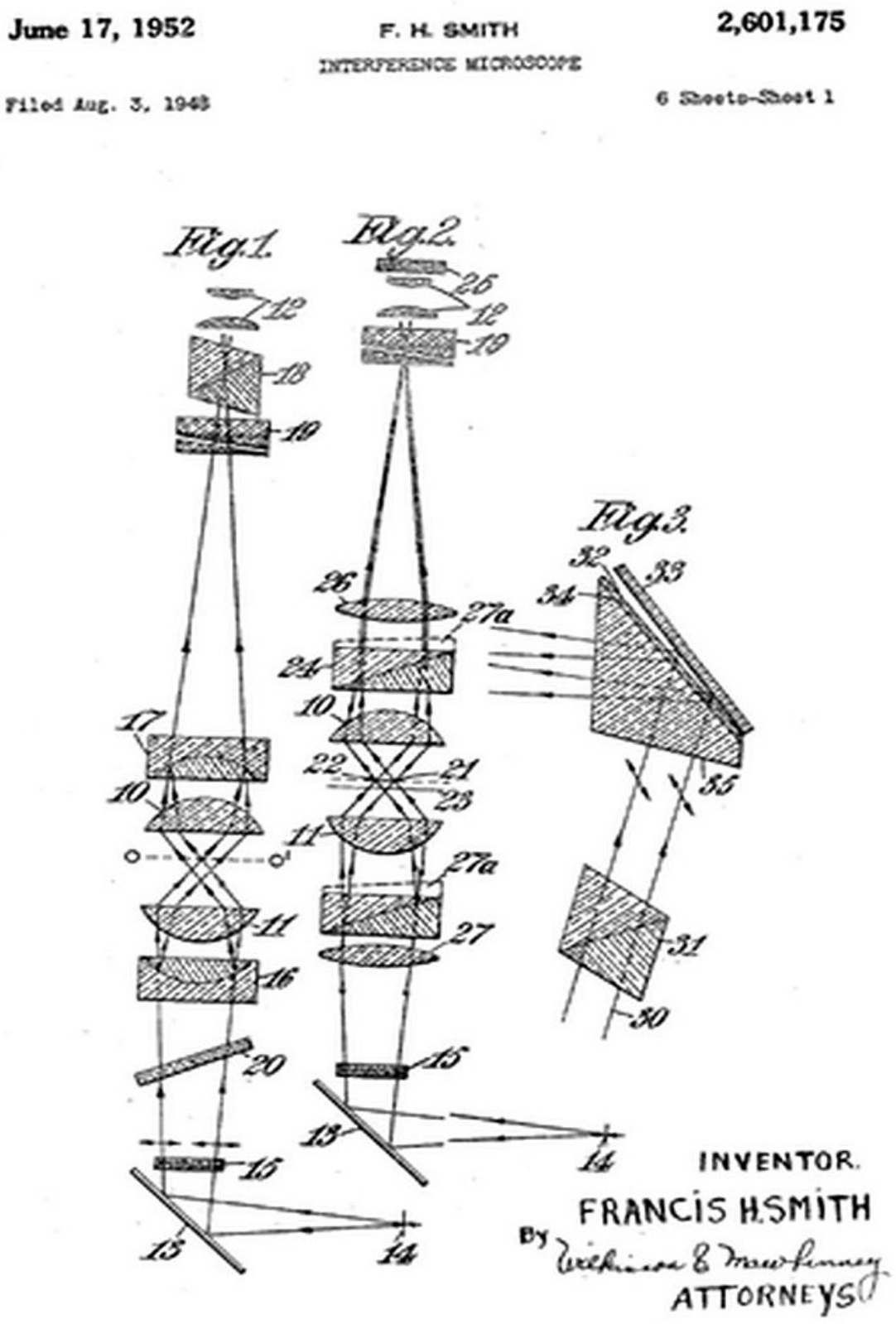

Figure 9. A line diagram from US Patent 2,601,175 filed in Aug 1948.

Smith published this new contrast method in 1955 [39]. In 1952 G. Nomarski proposed a special prism, the Nomarski prism that was a modified Wollaston- type prism, so that the DIC- prisms could be mounted outside the condenser front focal plane (here the aperture stop should be located) and the objective´s back focal plane (that is difficult to put a prism into) [37]. Nomarski was granted a patent on 1960 for the "Interferential polarizing device for study of phase objects" U.S. Patent, Number 2,924,142 and so the Nomarski prism is born [40]. Carl Zeiss of Oberkochen has been supplying interference-contrast equipment for transmitted light and since 1967 for reflected light, both based on suggestions by Professor G. Nomarski. This equipment was developed in close cooperation with Nomarski. A detailed theoretical and practical evaluation of the prototype was undertaken by Zeiss over a period of four years. The results were presented at the Centenary Conference of the Royal Microscopical Society in London in 1966 [41].

THE POLARISED MICROSCOPE

In 1808, Étienne Louis Malus discovered that light could be polarized (a term coined by Malus) by reflection as he observed sunlight reflected from the windows of the Luxemburg Palace in Paris through an Iceland spar crystal that he rotated. Through with additional experimentation, he determined that the capacity to polarize light was not limited to Iceland spar and other special types of crystals, but that the reflections from most ordinary substances could also produce the effect. Malus read a preliminary notice of his discovery on the 12th December, 1808 [42], and his essay presented in 1810 gained the prize offered by the Institute. The Royal Society of England was amongst the first to recognize the value of Malus' contribution to optical science Malus's work on polarization was published in 1809, and in 1810, [43]. William Nicol invented the polarizing prism in 1828, which was a key component for early polarizing microscopes [44]. In 1830, Giovanni Battista Amici designed and built the first polarizing microscope [45]. The first commercial polarized light microscope is not definitively attributed to a single inventor or company. Over time, the principles of polarized light microscopy were incorporated into commercially available microscopes, from various manufacturers.

PHASE CONTRAST MICROSCOPY

Frits Zernike experimenting with reflection gratings in 1930 and saw "Ghost lines" that occur to the left and right of each primary line in spectra created by means of a diffraction grating [46]. Together with ZEISS he developed the first phase-contrast microscope, the prototype of which was made in 1936 allowing the viewing of living cells without chemical staining. Frits Zenke received the Nobel prize for Physics in 1953 "for his demonstration of the phase contrast method, especially for his invention of the phase contrast microscope" [46]. Zernike produced the first phase contract microscope in the early 1930s. Together with Carl Zeiss the first prototype was build 1936 [47]. It took some time for the microscope to become commercially available. Carl Zeiss offered the phase contrast equipment (attachments) compatible with conventional type microscopes and Lumipan microscope in 1941 [48]

see Figure 10. The early application of Phase contrast in microscopy was well understood by Zernike, Köhler, Loos, Michel and Knoll [49, 50, 51, 52, 53]. This understanding and application propelled the use of Phase contrast microscopy.

Figure 10. Carl Zeiss phase contrast equipment (attachments) compatible with conventional type microscopes and Lumipan microscope.

Alexander Smakula invented and patented interference-based optical anti-reflective coatings in 1935 at Zeiss, improving light microscopy by minimizing reflections and maximizing light transmission through lenses [54]. These coatings, particularly single-layer and multi-layer designs, reduce unwanted reflections and enhance image clarity and contrast.

INVERTED LIGHT MICROSCOPE

John Lawrence Smith, Professor of Chemistry at the University of Louisiana in 1852 The new microscope was first presented at Societe de Biolcgie of Paris in September 1850 [55]. Additional improvements were made by Prof. Lawrence in the micrometre movement and was presented to the American Scientific Association in 1851. An translated work was then published in the American Journal of Arts in 1852 [56] Lawrence new inverted light microscope was manufactured by the Nachet firm. In Figure 11 is image of an inverted microscope that was by A Nachet in the “Cataloque Descriptif

des Instruments De Micrographie" 1886 [57]. A photo of the physical microscope can be seen in Figure 12 from the website http://www.antique-microscopes.com.

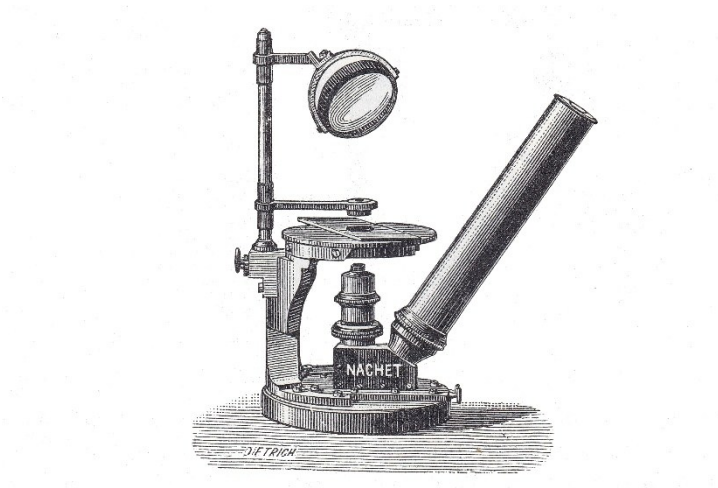

Figure 11. Lawrence Inverted microscopes offered by Maison Nachet & Fils in 1886 [57].

Figure 12. A photo of the Lawrence Inverted microscopes. http://www.antique-microscopes.com/photos/Nachet_Chemical_Inverted_microscope.htm.

However, the Nachet firm did not honor Prof John Lawrence Smith as the inventor while exhibiting it at the World's Fair in London in 1862 and he then had to defend/declare that it is his invention [58]. This can be read in the book published by JL Smith in 1873 [59]. Henry Louis Le Châtelier, the farther of Metallurgy, born in 1850 as early as 1900 designed the inverted type of microscope, which needs no mounting, the specimen being laid with its plane face downwards over an opening of the stage, with the objective immediately below and directed upwards, and the collimator and microscope tube horizontal, right-angled prisms being employed to deflect the incident and reflected beams [60] as shown in Figure 13 below.

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Figure 13. The Inverted Le Chattelier metallurgical Light Microscope.

The Zeiss Neophot is another milestone in birth of inverted microscopy since it allows to take micrographs of inverted samples. In Figure 14 a commercial inverted microscope the Zeiss Neophot from catalogue of 1953 can be seen [61].

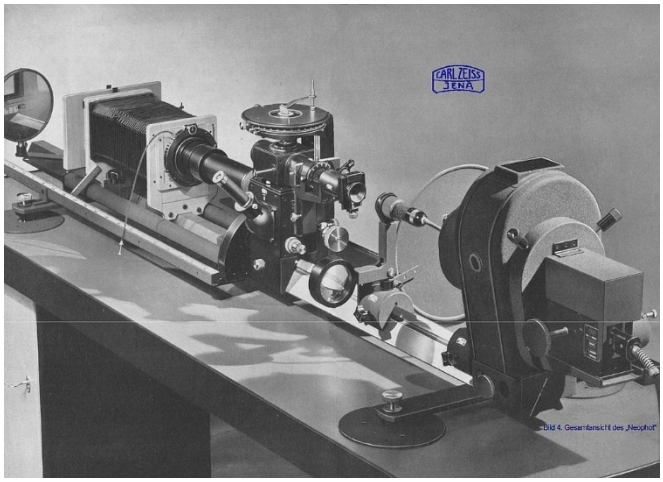

Figure 14. Zeiss Neophot from catalogue of 1953 [61].

Below, Figure 15, is a beautiful image of Pearlite, a structure in steel showing joint arrangement of thin layers of ferrite and cementite (Fe_3C), formed by a eutectoid reaction from austenite that was taken on the Neophot, showcasing the excellent resolution of the microscope.

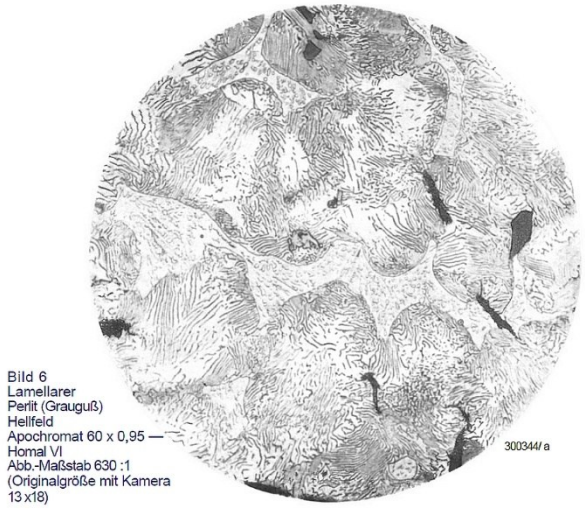

Figure 15. An image of Pearlite showing alternating layers of Ferrite and Cementite (Fe_3C) from catalogue of 1953 [61].

THE FLUORESCENCE MICROSCOPE

Fluorescence microscopy could only be born once fluorescence itself is discovered. In the 16th century by a Franciscan missionary that observed that *Lignum nephriticum* (Latin for "kidney wood") as capable of inferring fascinating opalescent hues to the water it came in contact with it. This observation was never pursued further scientifically at the time [62]. In 1845, Sir Frederik William Herschel noted that a quinine solution, although itself colourless and transparent, exhibits a "vivid and beautiful celestial blue colour," when illuminated and observed under certain incidences of sunlight [63]. Stokes, G. G. repeated some experiments by Sir David Brewster and Sir John Herschel in 1852. He discovered that fluorescence emission from an object represents a longer wavelength than the UV light that originally excited the object that phenomenon bears his name (known as the Stokes Shift) [64]. In 1856, William Henry Perkin only 18, set out with idea of making quinine by oxidizing allytoluidine, he accidentally produced the synthetic dye, mauveine, a purple dye, the first synthetic dye. This took the fashion industry by storm at the time. Although it was not used in Fluorescence, it is the first synthetic that was produced on a commercial scale due to the fashion industry. [65]. Helmhottz in 1874 suggested that, if an object itself emits light, fine structures can be better described. [66]. One of the oldest fluorescent dyes is Fluorescein dye was introduced into the field of ophthalmology in 1882 by Paul Ehrlich, who injected it intravenously in rabbits to observe the dynamics of aqueous tumour [67]. Another fluorescent dye used was by Hagemann 1938 that used Berberenu sulphate and then swapped to Auramine O a yellow, fluorescent dye [68]. In 1961 H.R. Novotny and D.L. Alvis, published a paper which described retinal angiography [69, 70]. August Köhler, while working at Carl Zeiss, developed Köhler illumination in 1893, Köhler illumination, is a method of microscope illumination that optimizes image quality [71]. It still used today and was crucial to the development of Fluorescent microscopy. The theory of fluorescence was also pursued in the field of Physics and Professor Eugen Cornelius Joseph

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von Lommel in 1875 said that hat a body only fluoresces by virtue of those rays which it absorbs, just as a photochemical reaction is only possible as a result of absorption of certain wavelengths. he posited that a substance must absorb light before it can emit the light as fluorescence. [72, 73].

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 [74]. Fluorescence microscopes were developed between 1911 and 1913 by German physicists Otto Heimstaedt and Heinrich Lehmann as a spin-off from the ultraviolet instrument. These microscopes were employed to observe autofluorescence in bacteria, animal, and plant tissues. [75, 76, 77]. In 1913, the Zeiss firm introduced its luminescence microscope. The first commercial fluorescence microscopes came from Carl Zeiss and Carl Reichert in 1911 [75, 78]. The microscope can be seen in Figure 16. Fluorescent antibody labelling was developed by Coons et. al. [79]. To improve the resolution of fluorescence images.

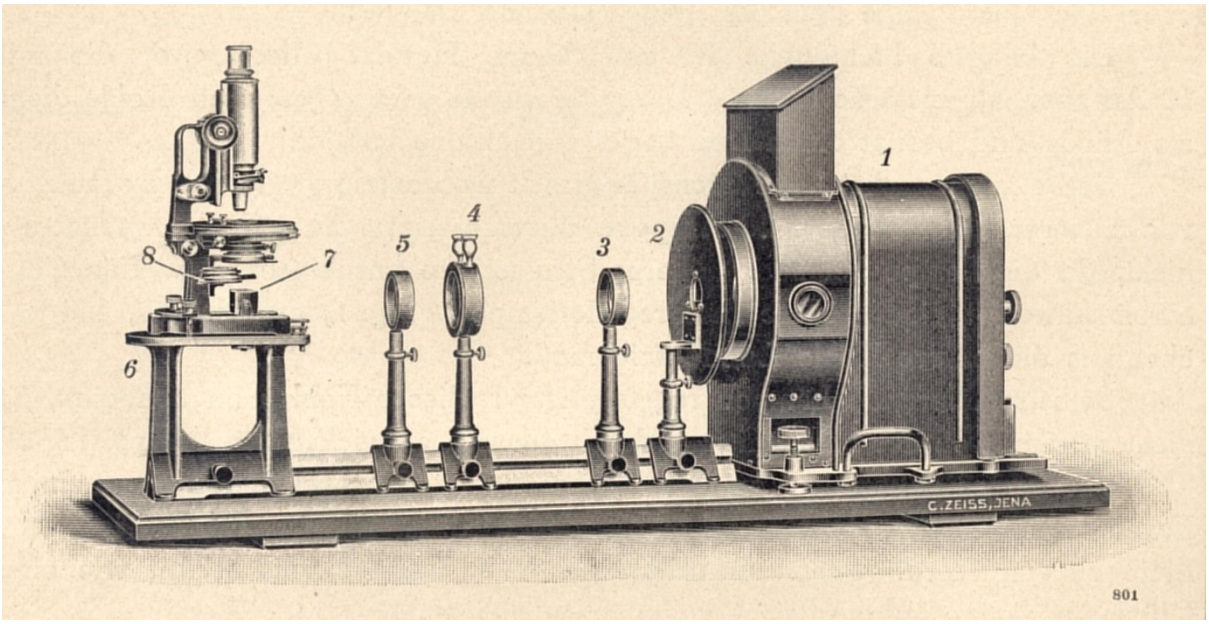

Figure 16. The first commercial fluorescent microscope 1911 [80, 81]. Curtesy of Carl Zeiss archives.

CONFOCAL MICROSCOPY AND CONFOCAL SCANNING LIGHT MICROSCOPE (CSLM)

The development of the incident light (epi)-fluorescence microscope was the beginning of fluorescence microscopy and formed the basics to confocal microscopy. The prototype of the epi-fluorescence microscope was designed in 1929 by Philipp Ellinger and August Hirt. They referred to it as 'intravital microscope' [82]. The excitation light passed through a series of filters get the required wavelength to pass through the objective lens to the observed tissue. The excitation wavelength cause emission of the fluorescent stain to emit fluorescent light for observation and recording. Two researchers from the Soviet Union E.M. Brumberg and T.N. Krylova had developed a so-called dichroic beam splitter for UV excitation with incident light. This development was an important input for fluorescence epi-

illumination microscopy [83]. A dichroic material can let light of a certain wavelength range pass through, whereas light of other wavelengths is reflected. This was followed by the work of J.S Ploem in who worked on the use of a vertical illuminator with interchangeable dichromatic mirrors for fluorescence microscopy with reflected light. His research was published in 1967 [84]. This led to the development of the fluorescence microscope, the Wild-Leitz Ploem Opak in the late 1960's. Minsky, while being primary a mathematical student additionally also studied biology, neurophysiology and neuroanatomy. One of Minsky frustrations is that he was unable to see the brain neuron network neither while being at Harvard nor Princeton. This is understandable due to the high density of the brain. The multiple scattering of light through such a high-density material made it impossible to distinguish the neural network. Minsky knew that without an instrument capable of optical sectioning to eliminate the out of focus light, this will never be possible. Minsky designed his symmetrical microscope with an objective lens and a pinhole at either side of the specimen to eliminate the scattered and out-of-focus light. Zirconium arcs was used as an illumination source to get the right intensity of illumination. The initial process was very tedious to produce to obtain an image (each point scan taking up to 10 seconds). Minsky filed his patent on the 7th of November 1957 and it was granted on the 19 December 1961 US patent 3,013,467 [85, 86]. Minsky's invention was ahead of its time and could not take off since the computer power to handle large stacks of data was not developed yet and possible also a lack of an intense enough Lightsource [87]. He was also the father of Artificial intelligence [88].

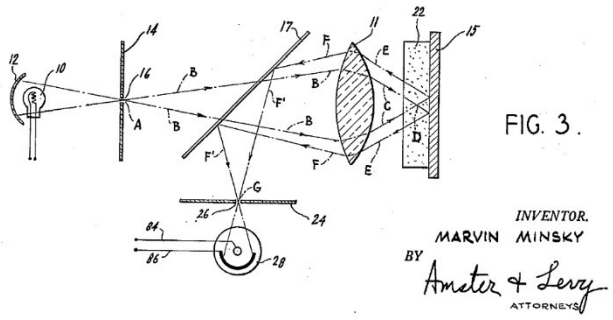

Figure 17. Diagrammatic view of an optical system arranged in accordance with Minsky's invention [85].

To get to the next leap, it all has a beginning, a catalyst, it started with the Airy disc [89]. In some cases the resolving power can be improved by apodization of objectives according to French authors, where the Airy disc is replaced by a disc with Gaussian distribution of light intensity around the centre. Lau's method (German Pat. No. 26,832) [90]. The first improvement was done by Mojmir Petrán, Plzen; Milan Hadravsky where the Nipkow disc was born as in United States Patent 3,517,980 filed on the 4 December 1967 and the patent was granted 30 June 1970 titled "Method and arrangement for improving the resolving power and contrast" [90]. Nipkow disc, is a mechanical spinning disc which has a spiral pattern of thousands of individual pinholes drilled in it. When intense light is passes through a Nipkow

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spinning disc, thousands of points on the specimen are illuminated simultaneously. The arrangement and light path can be seen in Figure 18 from United States Patent 3,517,980.

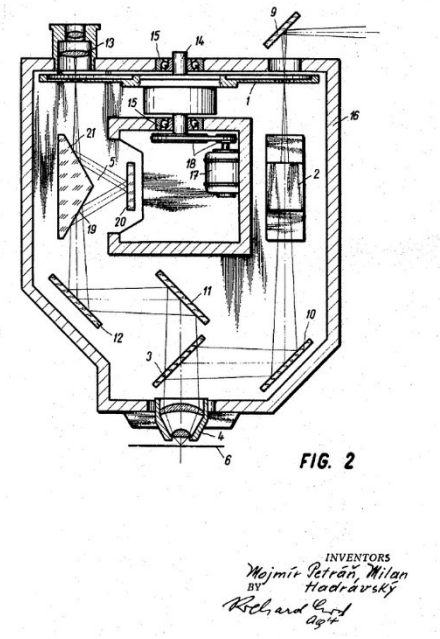

Figure 18. a partial sectional view of an arrangement for improving the resolving power and contrast [90].

This was followed by another patent by Mojmir Petrán, Plzen; Milan Hadravsky, Plzeri; Miroslav Maly, Prague, all from Czechoslovakia "United States Patent 4917478 filed on the 27th June 1988 and granted on the 17 April 1990 for "Arrangement for illumination and scanning of an object by means of a scanning disk similar to a Nipkow disc." (Figure 19) Mojmir Petrán, Plzen; Milan Hadravsky, Plzeri; Miroslav Maly, Prague, all of Czechoslovakia [91].

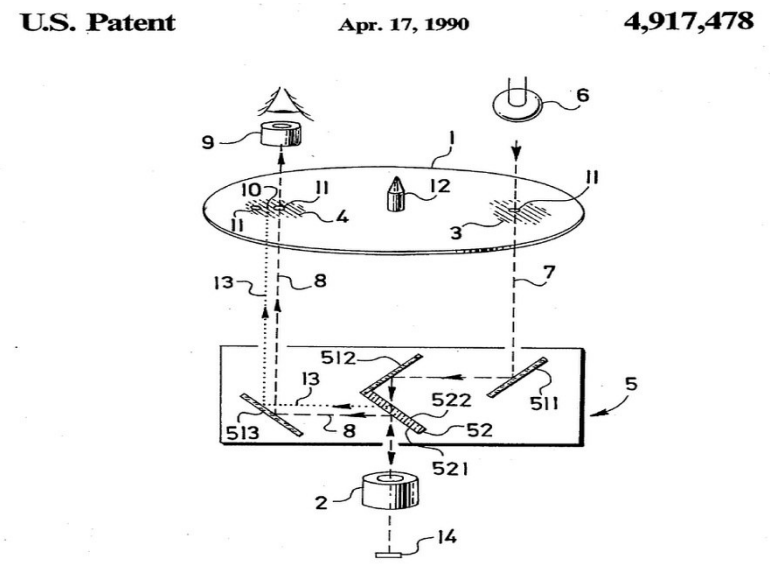

Figure 19. A drawing showing the arrangement of the scanning disc as in United States Patent 4917478 [91].

The first scientific paper describing the this new type of reflected-light microscope was published by D Egger in 1967 [92] titles " New Reflected-Light Microscope for Viewing Unstained Brain and Ganglion Cells" Fluorescence emissions occupy a broad band of AS longer than the excitation, so they cannot be descanned by the acousto-optical scanner. One innovative but difficult solution to this problem was patented by Goldtein U.S. Patent No. 4,827,125 [93]. This solution was abandoned due to its difficulty. The first true Scanning Laser Microscope (SLM) was designed and built by M. David Egger and Paul Davidovits whom designed and built a prototype of a scanning microscope for thick objects of low reflectivity and low optical contrast. As light source, a 5mW He-Ne continuous wave laser (Spectra-Physics Model 120) was used. This was published in nature in 1969 [94]. The term 'confocal' coined by Colin J. R. Sheppard and A. Choudhury in 1977 in a publication "Image formation in a scanning microscope" [95]. The term 'confocal' means 'having the same or common focus'. G. Fred Brakenhoff developed a laser scanning microscope in 1979 [96]. He published his findings in the Journal of Microscopy and it seems to be the first time the term Confocal Scanning Light Microscope (CSLM) was used. In 1982, a company called 'Oxford Optoelectronics' (since acquired by Bio-Rad) offered the first commercially available stage scanning CSLM which was connected to a computer (the 'SOM-25'). This was described by C.J Cox in 1983 and the first simple digital image processing algorithms were applied. Zeiss launched the LSM 44 in 1982 and the MRC 500 confocal scanning microscope was commercialized by Bio-Rad was launched in 1987 [97].

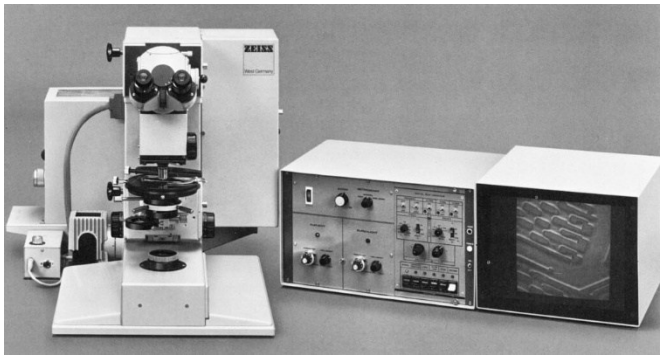

Figure 20. Zeiss LSM 44 that was launched in 1982 https://www.flickr.com/photos/zeissmicro/7039029735/].

ELECTRON MICROSCOPY HISTORY AND THE DEVELOPMENT OF THE TRANSMISSION ELECTRON MICROSCOPE (TEM)

In 1897 J.J. Thomson put forward the idea that the rays were in fact streams of small subatomic particles. He called them "corpuscles". Thomson discovered Electron in 1897 [98]. Thomson received the Noble prize in 1906 for in recognition of the great merits of his theoretical and experimental investigations on the conduction of electricity by gases" [99, 100]. In 1913 Bohr introduced the model of an atom that

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postulated that the atoms consist of a positively charged nucleus surrounded by a system of electrons kept together by attractive forces from tile nucleus; the total negative charge of the electrons [101]. Louis de Broglie theorised that particles, such as electrons, could be described not only as particles but also as waves. He also suggested that electrons have very short wavelengths. This was published in 1924. [102]. Louis de Broglie received the Nobel prize for Physics in 1929 for the "wave nature of electrons" [103]. Davison and Germer in 1927 used the position of Laue beams after diffraction from a Nickel crystal to illustrate that electrons have a short wavelength [104, 105]. C.J. Davison and G.P Thompson was awarded jointly the Nobel Prize in Physics "for their experimental discovery of the diffraction of electrons by crystals" in 1937 [106]. Hans Busch made a phenomenal statement in 1925: "Eine kurze Spule hat also die Eigenschaft, die Kathodenstrahlen nach der Achse zu um einen Winkel abzulenken, der proportional der Achsenentfernung des Strahles ist. Genau die gleiche Eigenschaft besitzt aber fuer Lichtstrahlen eine Sammellinse" which means that the collective movement of an axially symmetric coil on electrons can be described in the mathematical language of geometrical optics, in terms of a focal length [107]. De Broglie's paper "Ondes et quanta" in 1923 inspired experiments on electron diffraction [108] in German. The paper was discussed in 1974 by A.F. by Kracklauer [109]. One of the people that was inspired was Dennis Gabor which were well known for his experiment in electron optics. These experiments also led to the invention of Holography for which he received the Nobel prize in 1971 [110]. Hans Busch, build the first electron magnetic lens in 1926 [107]. Ruska proved already in 1929 in his thesis titled "Ueber eine Berechnungsmethode des Kathodenstrahloszillographen auf Grund der experimentell gefundenen Abhaengigkeit des Schreibfleckdurchmessers von der Stellung der Konzentrierspule " that a cathode ray tube can produce magnified sharp images. A drawing from his thesis can be seen in Figure 21 below.

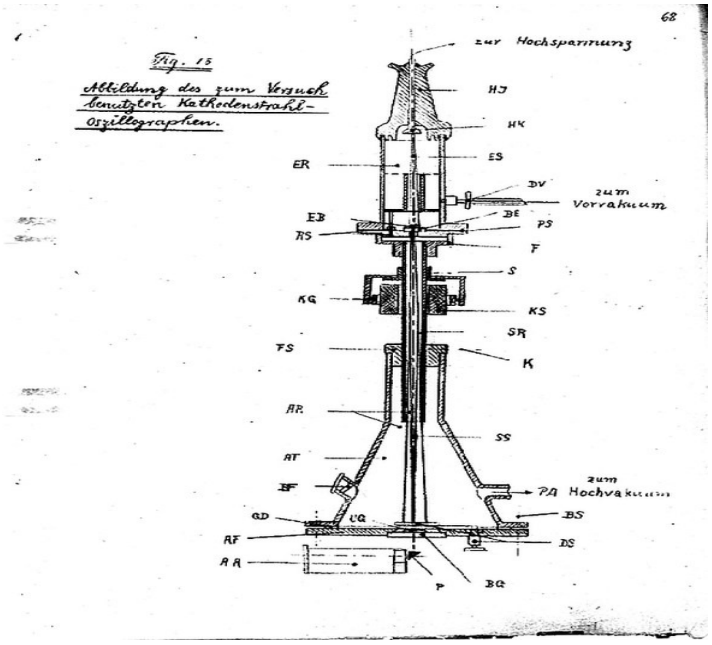

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Figure 21. Sketch by Ruska (1929) of the cathode ray tube for testing the imaging properties of the non-uniform magnetic field of a short coil. [111, 112].

Ernest Ruska received the Nobel Prize in physics in 1986 [113]. German Patent No. 690809 was the first patent in related to Electron Microscopy was by Marks Knoll on lenses in 1929 [112, 114]. The work on the lens was published in 1931 by Ruska and Knoll [115]. The development of the electron microscope was pursued, and the first Electron Microscope was built in 1931 with a magnification of 400 times. The work was published in 1932 [116]. A technical sketch and a copy of the first Electron Microscope can be seen in the Figures 22 and 23 below. Hans Gederblom wrote a good overview on the role Ruska played in the development of the Electron Microscope [117].

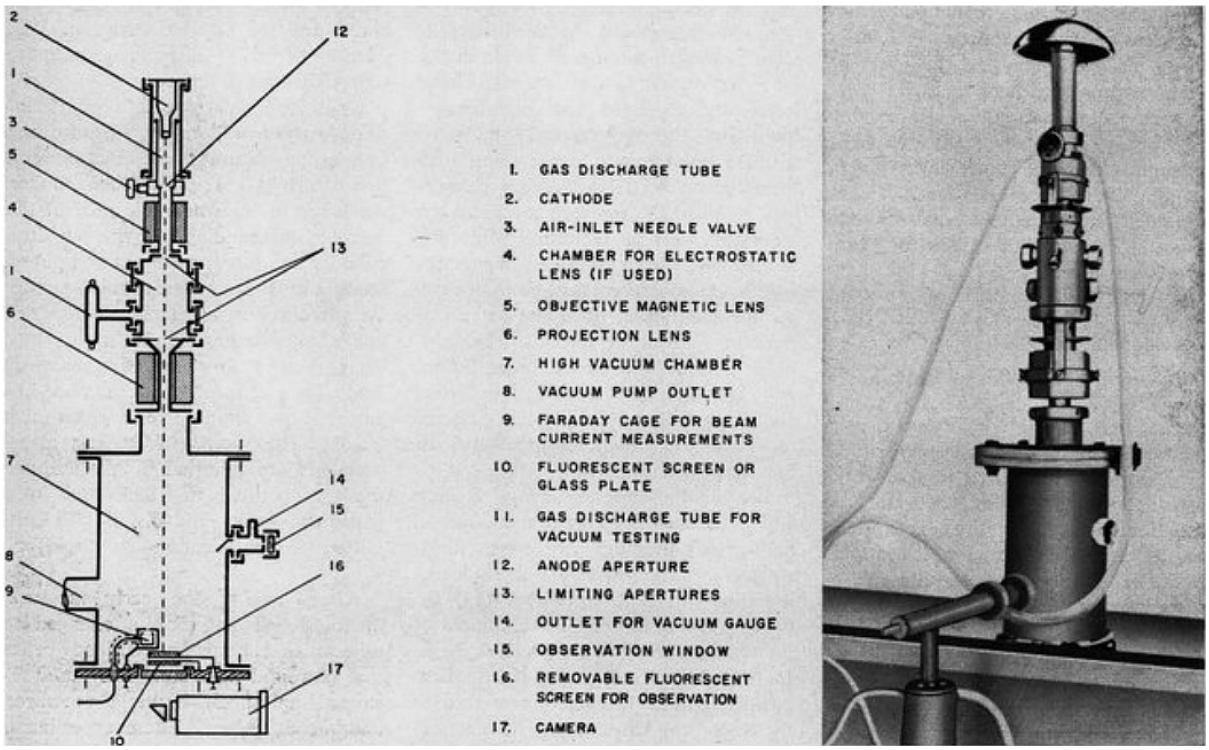

Figure 22. A technical sketch of the first electron microscope build by Max Knoll and Ernst Ruska in 1931 at the Technical University of Berlin. [118].

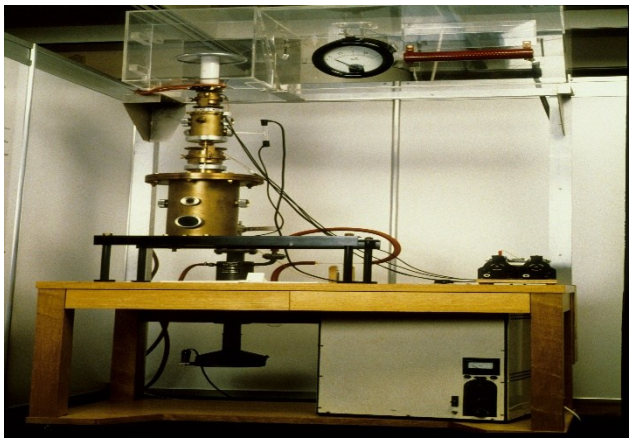

Figure 23. A replica of the first electron microscope build by Max Knoll and Ernst Ruska in 1931 at the Technical University of Berlin.
https://www.dge-homepage.de/historische_dias/images_historic_electron_microscopes.html

With commercially available Electron Microscopes on the market, the next logic step was to form the first society from Electron Microscopy in German Prof. Bodo von Borris formed the society on the 16 Feb 1949 in Dusseldorf the "(Deutsche Gesellschaft für Elektronenmikroskopie) DGE" of which Ernest Ruska was a founding member and was elected as the President of the newly founded society. [119]. They are currently still an active society more than 75 years later. Finally Siemens was chosen as an industrial partner to develop the first commercially available Electron Microscope. This was completed in 1939 [120]. The first commercially available Electron Microscope the "Siemens Super Microscope" with a magnification of 30 000 times can be seen in In Figure 24.

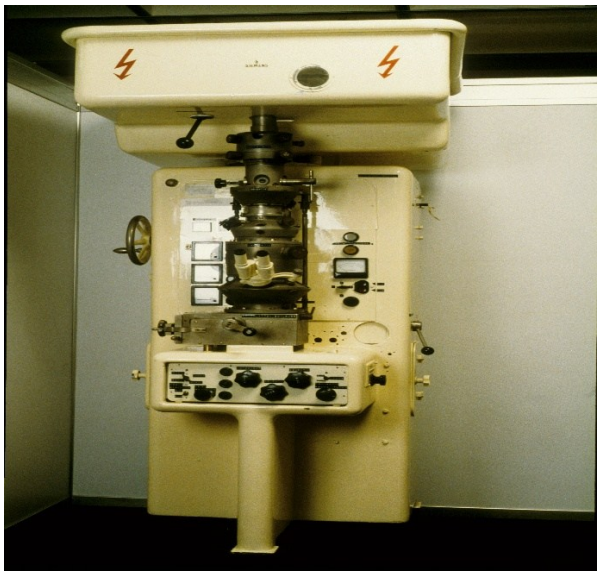

Figure 24. The first commercially available Electron Microscope by Siemens the Siemens Super Microscope in 1939. https://www.dge-homepage.de/historische_dias/images_historic_electron_microscopes.html

With the interest in electron microscopy more manufacturers developed their own electron microscopes. Their first Philips electron microscope was the well-designed EM 100, that was launched in 1949 as seen in Figure 25 [121].

Figure 25. The Philips EM100 launched in 1949. https://www.dge-homepage.de/historische_dias/images_historic_electron_microscopes.html

JEOL, or Japan Electron Optics Laboratory Company, Limited launched the JEM-1 also in 1949 [122]. Lacking was the right sample preparation for biological samples for the newly found instruments, limiting the optimal applied progress. In 1953, Keith Porter and mechanical engineer Joseph Blum, from the Rockefeller Institute, invented as well as developed the first successful ultramicrotome. The first ones were made at the Rockefeller Institute [123].

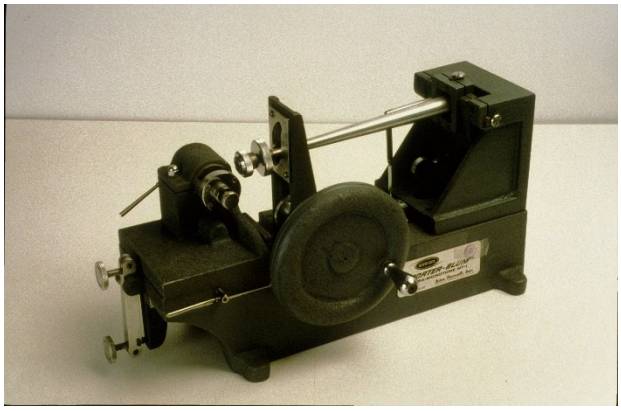

Figure 26. A Side view of the first commercial microtome the Porter-Blum MT1. https://www.dge-homepage.de/historische_dias/images_historic_ultra_microtomes.html

Electron microscopy and the advances made in sample preparation, together had a phenomenal impact in biological studies, especially in the understanding of the cell function. One of the early pioneers was George E Palade which made numerous contributions towards biological field. His first contribution was the use of a buffer with OsO4 and was known as Palade's fixative [124]. He jointly also received The Nobel Prize in Physiology or Medicine 1974 for his contributions [125].

THE DEVELOPMENT OF THE SCANNING ELECTRON MICROSCOPY (SEM)

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The origin of a scanning electron microscope can be traced back to 1929 with the idea put forward by Stintzing, et. al. as presented in German patent applications. No drawings accompanied the applications, and no evidence was found of the construction of an instrument based on the two patents. [126, 127]. Knoll in 1935 published the first images from solid samples obtained by scanning an electron beam [128]. A schematic diagram of Knoll's instrument can be seen in Figure 27.

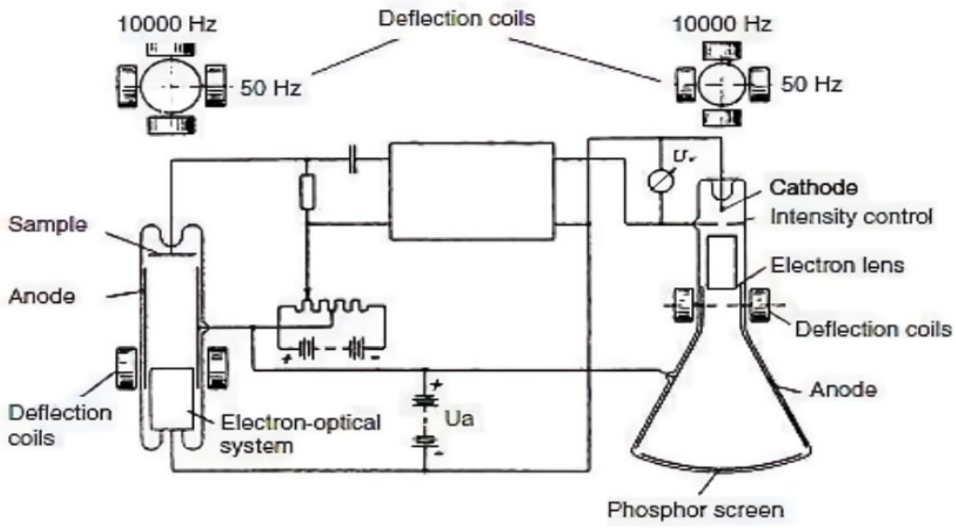

Figure 27. Schematic diagram on Knoll's electron Beam Scanner [128].

Manfred von Ardenne patented the Improvements in electron microscopes in 1937. British patent no. 511205 [129]. He published two papers in 1938 [130, 131] von Ardenne clearly stated the theoretical principles underlying samples to be viewed in the scanning microscope. His lab was destroyed in World War II. In 1942 another early instrument was constructed in the United States by Zworykin had a magnification above 10 000 times and a resolution better than 50nm. In Figure 28 he can be seen with the early Scanning electron Microscope

Figure 28. Dr. J Hillier in the foreground, Dr. VK Zworykin seated as well as R.L. Snyder with the early SEM, as published in the magazine Radio-Craft [132].

Charles Oatley was appointed lecturer at the Cambridge University in 1945 and started to build a scanning electron microscope together with D. MacMullan as one of his students. One of the important components was an electron multiplier with beryllium copper dynodes that was developed by A.S. Baxter [133, 134]. It started off as a 40kV TEM by McMullan [135], and when he left Cambridge a new group was formed under Smith which then converted into a STEM equipped with Baxter's detector [136]. The first micrographs taken was of etched Aluminium in 1953 (Figure 29) and the SEM at the time can be seen in the Figure 30.

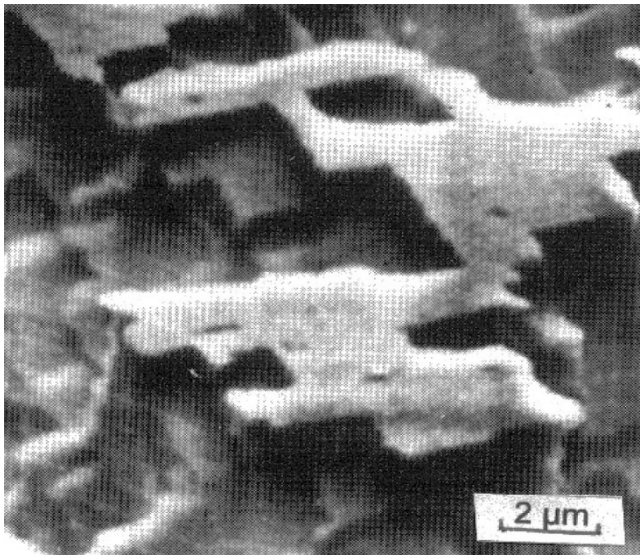

Figure 29. The first SEM micrograph of etched Aluminium at 16keV with a clear three-dimensional impression [136, 137]

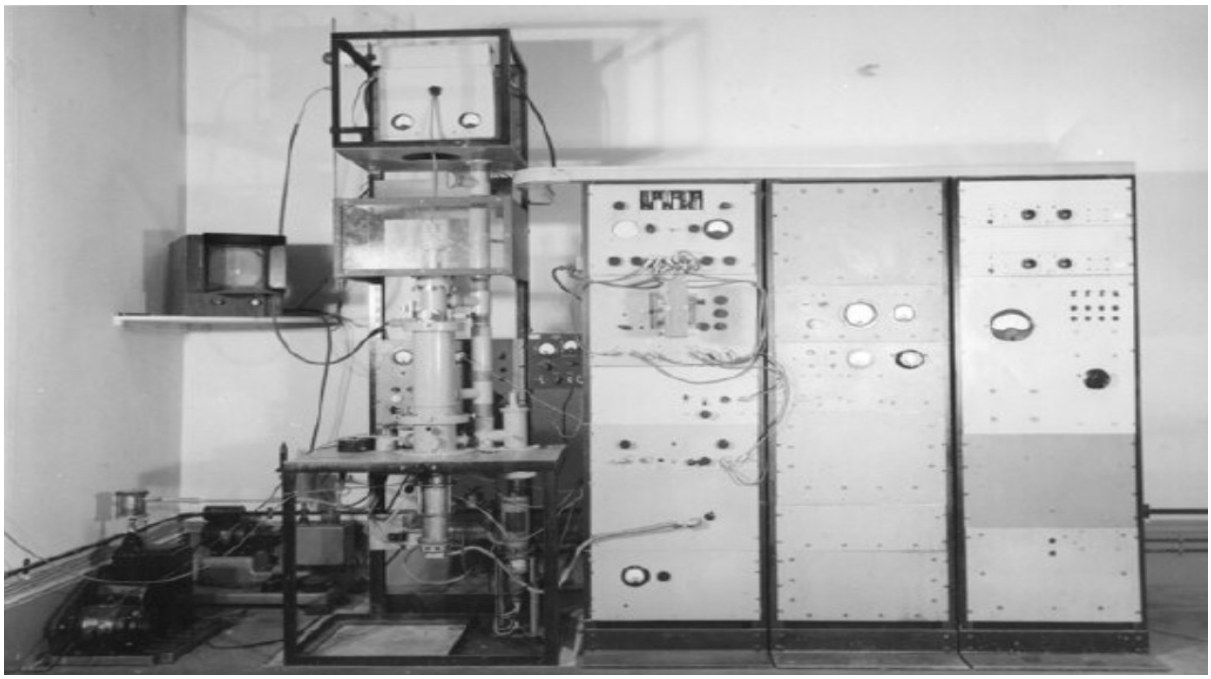

Figure 30. The Sem1 at Cabridge in 1953. [138].

Smith continued the development under Oatley and substantially improved the SEM by adding lens stigmators, double-deflection scanning coils, scanning rotating coils and tilting stage, and developed better detectors. [139]. All these features are still in use in modern SEM's In 1960, Everhart and Thornley greatly improved the secondary electron detection. A new detector was created with a positively biased grid to collect electrons, a scintillator to convert them to light, and a light-pipe to transfer the light directly to a photomultiplier tube, this is still in use today. [140, 141]. The Cambridge Instrument Company marketed the first commercial SEM, the Stereoscan Mk1 in 1965 (Figure 31) [138]. Jeol launched JSM-1 in 1966. Hitachi launched the SEM HSM-2 in 1969 [142] and Philips entered the market later in 1972 with the PSEM500 [121].

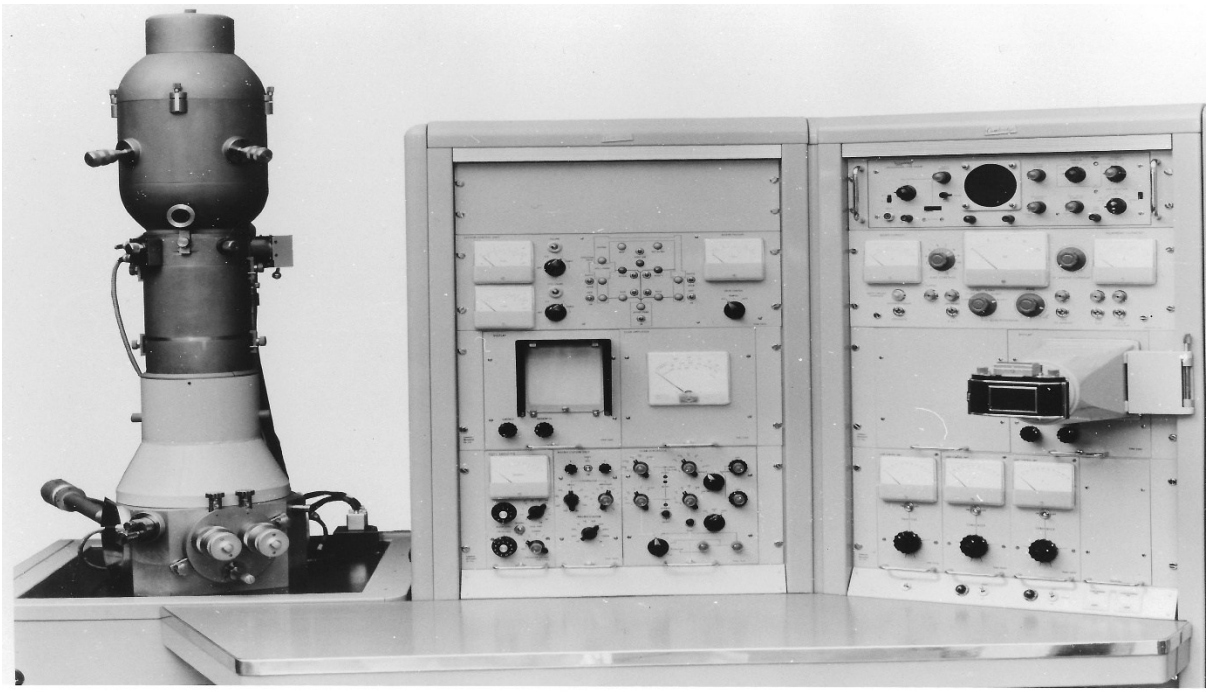

Figure 31. The by Stereoscan Mk1 produced by Cambridge Instrument Company https://commons.wikimedia.org/wiki/File:Stereoscan_MK1_(19515061054).jpg.

1. ENERGY DISPERSIVE SPECTROSCOPY (EDS)

Although Energy Dispersive Spectroscopy (EDS) it is not a "microscope" it is one of the most used attachments on Electron Microscopes and deserve a brief early historic development attention. It started with the first electron probe microanalyzer (EPMA) using an X-ray spectrometer, was built in 1951 by Casting. It could only analyse a single "spot" at a time. [143]. The first X-ray "dot map" compositional image was created by scanning the EPMA beam across the specimen surface to generate characteristic X-ray signals linked to beam position. [144, 145]. In 1964, liquid nitrogen cooling was added to detectors [146]. Another major development was the improved lithium-drifted silicon solid-state

detectors by Fitzgerald et.al.in 1968 [147]. The first EDS detector "Nuclear Diosdes 505" was in launched in 1969 by the company Nuclear Diodes Inc, later known as EDAX and currently known as AMETEK [146]. A more comprehensive writeup is by Friel and Lyman [148]

ENVIRONMENTAL SCANNING ELECTRON MICROSCOPY (ESEM)

To me personally the next historic development was the environmental SEM or ESEM as it is commonly know today. In a normal SEM a high vacuum environment is needed, which has an negative effect mainly on Biological samples [149]. The environmental SEM is a SEM that can maintain a minimum water pressure of at least 609Pa in the specimen chamber at 273K. It was know water vapour reduce charging on samples and was studied by Pfefferkorn and the research was published 1972 [150]. Robinson showed that viewing specimens in a high vapour pressure in a SEM is suitable for many applications [151, 152]. Danilatos and Robinson published a paper in 1979 describing the principles of Scanning Electron Microscopy at High Specimen Chamber Pressures [153]. Danilatos continued developing the ESEM and developed a gaseous detection device (GDD) for detection the ionized ions from the electron-water vapour interaction. This research was officially disclosed, Research Disclosure No. 23311:284 [154]. The breakthrough was published in the same year in Micron and Microscopedia Acta [155]. Also two US Patents was granted in 1986 US. Patent 4,596,928 patenting the atmospheric SEM [156]. and US Patent 4,992,662 for developing the multipurpose Gasius Detector [157]. The first commercial ESEM was exhibited in New Orleans by ElectroScan Corporation, with the aim of commercialisation Figure 32. Danilatos published the "Introduction to the ESEM" in 1992 [158] and fathered a total of 12 US patents and more details on the patents can be found at http://www.danilatos.com/patents.htm. Bogner et. al. has cone a comprehensive overview of the development of "wet SEM" [159].

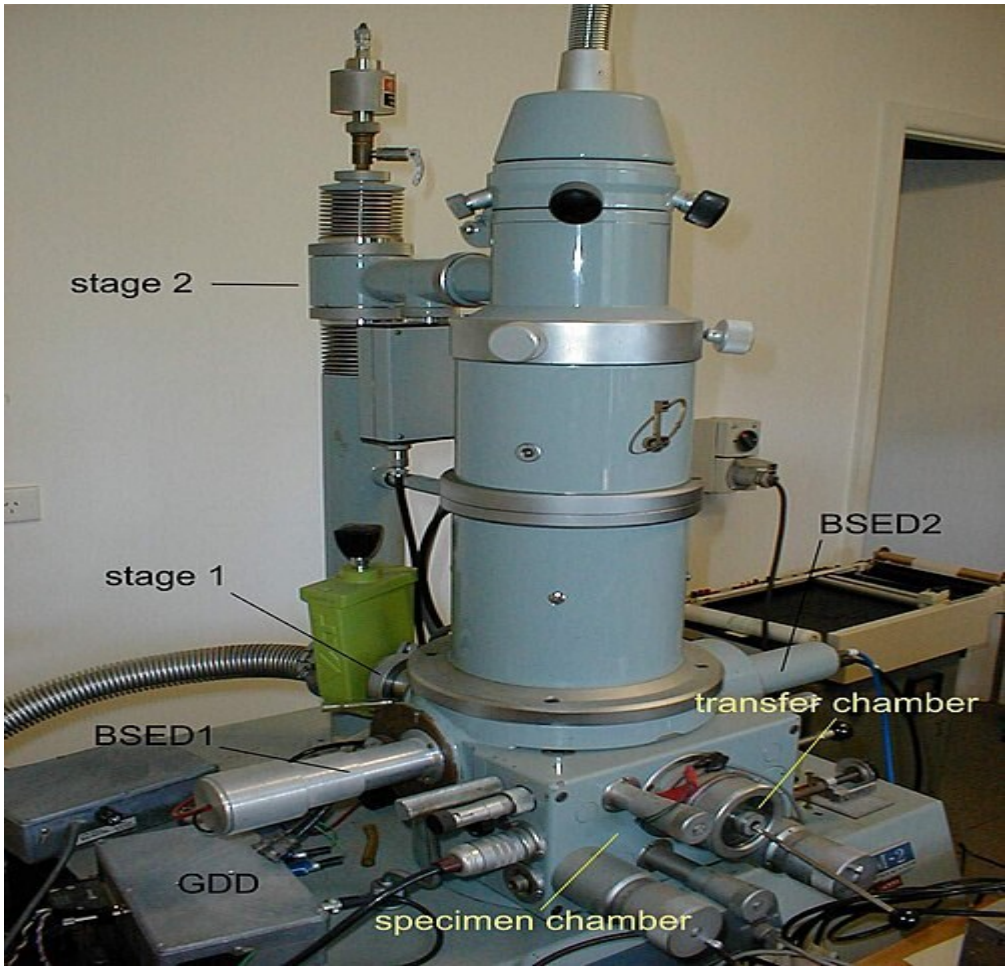

Figure32. The first commercial ESEM was exhibited in New Orleans by ElectroScan Corporation https://www.wikiwand.com/en/articles/Environmental_scanning_electron_microscope

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