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The electron microscope on the eve of its first centenary

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SUMMARY

In a few years' time, the electron microscope will be 100 years old. The ideas leading to the invention of the instrument emerged in Berlin between 1928 and 1933. Ernst Ruska is the undisputed inventor of the transmission electron microscope. In the mid-1930s, scientists from several European countries and especially from the United States, France, Canada and Japan became interested in contributing to the new technology. Ernst Ruska was awarded the 1986 Nobel Prize in Physics for his invention.

Key words: History of electron microscope – Ernst Ruska – Electromagnetic lenses – Transmission electron microscope – Scanning electron microscope

PREAMBLE

In a few years' time, the first centenary of the birth of an instrument and the science associated with it, will be celebrated: the electron microscope and electron microscopy. As in the case of the optical microscope, invented by the Dutchman Zacharias Janssen in 1590 (the attribution of the invention of the optical microscope to Janssen is based on a letter from William Borelius, a Dutch physician and childhood friend of Janssen). According to this letter, Zacharias Janssen and his father, Hans Martens, invented the microscope in 1590 and shared its discovery with Albert VII, Archduke of Austria (https://www.mundomicroscopio.com/primer-microscopio/). The electron microscope has made it possible to visualize structures smaller than 200 nm, which is the resolution limit of a conventional optical microscope. The electron microscope has had an immense impact in many research fields, such as materials, chemistry, biology and medicine, geology, etc. – to name but a few.

This article is dedicated to the acknowledgement of the scientists who made this invention possible and to recall some of the circumstances under which it occurred.

INTRODUCTION

I would like to begin with some general aspects of the instrument: the electron microscope. The resolution of a microscope – the minimum separation between points distinguishable by observation - is limited by the wavelength of the radiation used to make the image. Thus, electron microscopes have a much higher resolution capacity than optical or photonic microscopes, because the wavelength of electrons is many

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times shorter than that of light. While the best conventional optical microscopes can reach about one-fifth of a micron (1 micron = 1 thousandth of a millimeter), however rarely approach this limit, electron microscopes easily achieve a resolution hundreds of times higher – to the order of a millimicron (one thousandth of a micron, equivalent to ten Angstrom units) - and that without reaching the theoretical limit.

Basically, in a transmission electron microscope, a beam of high-voltage electrons is directed from a cathode to an anode, through a column or tube under a very high vacuum, where the beam passes through the sample and various magnetic or electric fields which act as lenses, until they hit a luminescent screen where the image is formed. The final magnification obtained is a function of the focal lengths and the position of the lenses along the trajectory of the electron beam, in the same way as in an optical microscope. In electron microscopes, the focal lengths are modified electronically and the positions of the lenses are fixed, while in the light microscope the opposite is the case. However, the results correspond as both are based on the same optical theory. The invention was an almost natural further development of cathode ray technology and oscilloscopes.

CATHODE RAY TUBES AND OSCILLOSCOPES

Probably the first of these devices was the glass tube invented by Geissler in 1850, which contained a rarefied gas and in which an electrical current induced the emittance of characteristic lighting, as in the later-developed neon tubes. Throughout the second half of the 19th century, cathode ray tubes were further developed. For example, William Crookes' cathode ray tube (1875), where cathode rays were deflected by the influence of a magnetic field and produced images by impacting a phosphorescent screen (for Crookes ray tube see: https://global.britannica.com/biography/William-Crookes).

Later Ferdinand Braun (1897) built a tube in which cathode rays could be deflected both horizontally and vertically (Martin, 1986). In the same year, J.J. Thomson (Strutt, 1942) discovered the first subatomic particle, for which the Irish physicist George Johnston Stoney proposed the term "electron" (Encyclopaedia Britannica, 1910). At this point we must mention Emil Wiechert, a German physicist born in East Prussia who worked at the University of Königsberg. Wiechert discovered at the same time as J.J. Thomson (commonly known as the discoverer) the particle now called the "electron", which he named "Teilchen" (particle). In April 1896, at a conference at the Königsberg Physical and Economic Society, he reported the existence of a particle whose mass had to be significantly smaller than that of the hydrogen atom. On January 7, 1897, he reported at a conference in the same scientific society that he had detected the particle and determined that its mass was approximately 2,000 to 4,000 times smaller than that of the hydrogen atom. In September 1897 he announced the exact values of the particle mass. Thomson's lecture at the Royal Society took place on April 30, l 1897. The scientific activities at Königsberg were obviously unknown in London.

From the end of the 19th century until well into the 20th century, the search for cathode ray tubes and oscilloscopes continued, as these devices were very important in the electricity-producing industry. The Braun tube was improved by creating a vacuum inside to facilitate the circulation of lightning.

Undoubtedly, the development most relevant to our topic was made by Hans Busch, who constructed and precisely measured the parameters of the first electromagnetic lens (Busch 1912, 1926). Busch (1884-1973) studied physics in Berlin and Göttingen, where he received his doctorate. In 1922, he joined the University of Jena where he became a professor and where he developed electronic optics and electronic lenses.

Another important scientist in our context was the Hungarian Dennis Gabor (1900-1979). Early in his career, Gabor analyzed the properties of high-voltage power transmission lines using cathode ray oscilloscopes, which sparked his interest in electron optics. Gabor's research on the basic processes of the oscilloscope contributed to the development of other devices, such as television tubes. In 1921, he joined the Department of Electrotechnics at the Berlin-Charlottenburg Technical University (TU-Berlin), and, after completing his doctorate in 1927 (on the recording of activity in electrical circuits with the high-speed cathode ray oscilloscope), he joined the Siemens physics laboratory. He had to leave Germany in 1933 due to his Jewish origins. In 1971, he received the Nobel Prize in Physics for the invention of holography, with which he tried to correct electronic optical aberrations. Many of Gabor's discoveries were very useful to Knoll and Ruska in connection with the electron microscope.

As has become clear from the above, Berlin was leading in physics and electronics in the 1920s and – seen from today's perspective – it is surprising that the idea of conceiving and building a microscope did not spark earlier. This all the more so, in view of the fact that this was closely related to the work being carried out on oscillographs.

ERNST RUSKA AND THE ELECTRON MICROSCOPE

Ernst Ruska is indisputably considered to be the inventor and builder of the first electron microscope, even if that first instrument was nothing more than a proof of principle (Fig. 1). On March 5, 1931, the microscope was publicly presented by Ruska and his tutor, Max Knoll. The coverage in German press and radio was remarkable and extensive, even if it was incorrectly reported that Ruska came from Leipzig. He, in fact, was born in Heidelberg in 1906 (see Kalendarblatt Deutsche Welle 9. Mars 1931). The instrument was a prototype with little ability of magnifying the image projected. However, it was the basis for the development of the electron microscope as understood today.

Fig. 1.- Ernst Ruska standing next to the column of a Siemens model 1A electron microscope in the 1950s (https://www.biografiasyvidas.com/biografia/r/ruska.htm)

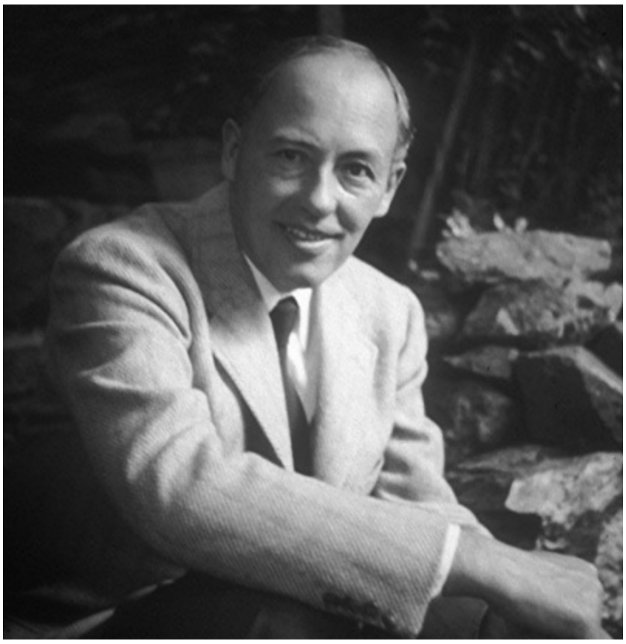

Fig. 2.- Max Knoll, Ruska's doctoral supervisor in Berlin (https://www.goodreads.com/author/show/4289817.Max_Knoll).

At the age of 19, Ruska began studying physics and electrical engineering in Munich. In 1927, the family moved to Berlin where he continued his studies, specializing in high voltage electrical devices and attending the course held by Professor Adolf Matthias at the TU Berlin. At the end of the summer semester of 1928, Professor Matthias set up a working group to develop a high-speed oscilloscope for measuring electrical processes in power plants and high-voltage lines. The management of the group was entrusted to Dr. Max Knoll (Fig. 2). The youngest students in the group were Bodo von Borries and Ernst Ruska. The working atmosphere within the group was excellent, as Max Knoll practiced the philosophy of teamwork, something uncommon at that time in Germany.

Ruska was commissioned to work in the field of high vacuum and related techniques, but also with examination of cathode ray tubes and their behavior under the influence of electromagnetic coils with the aim of making the electron beam as thin as possible. Hans Busch had already shown that the behaviour of a magnetic coil (lens) with electron beams was the same as a glass lens with photon beams (Busch 1927).

Based on Busch's studies, Ruska built an electromagnetic lens in the course of his work in the group with which he obtained images of the hole of an aperture disc ("Lochblende"). These were obviously the first images obtained with electrons (Ruska, 1929).

His diploma thesis (graduation paper) was devoted to electrostatic lenses, as an alternative to electromagnetic lenses, proving in the end that the latter were far superior to the former (Ruska, 1930). He presented his diploma thesis on December 23, 1930. Then the problem of finding a job arose.

The country's economic situation had badly deteriorated and there was practically no chance of finding a job either in industry or at university. Following his parents' wishes, Ernst Ruska decided to pursue a doctorate at the TU Berlin (Technical University): a job – but without a salary.

By 1929 Ruska had already shown that images could be perfectly focused with one coil. The next question was whether the image could be further enhanced by the addition of a second coil. He did indeed succeed in constructing something new: a prototype electron microscope, equipped with new lenses. In April 1931, he experimentally proved that electrons considerably surpassed the resolution provided by the optical microscope. Ruska received his doctorate from the TU-Berlin in August 1933, and the microscope he then built has to be regarded as the first electron microscope in the history (Fig. 3).

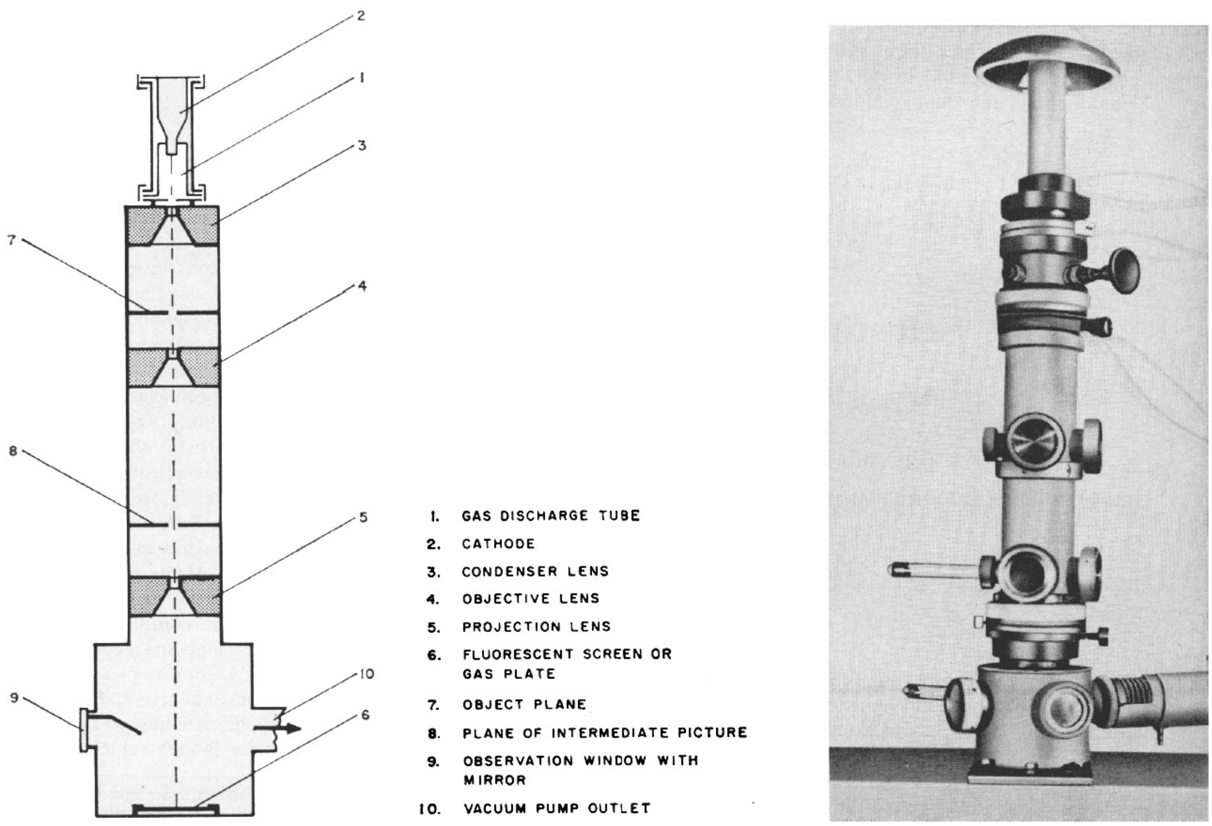

Fig. 3.- Transmission electron microscope designed by E. Ruska in the early 1930's. On the left is a technical drawing of the instrument. On the right an image produced by the instrument. Both images taken from an article by Freundlich (1963) with permission of the Science (AAAS) journal.

On the basis of studies by Busch (1927), Ruska and his instructor Knoll were convinced that – due to the thin mass of the electron – the electron microscope must provide images of an object or sample at much higher magnification than possible with an optical microscope. In his speech at the Nobel Prize (1986), Ruska explained that they had not known de Broglie's theories (1924) until well into 1931, despite the fact that they had been confirmed experimentally by Davisson, physics Nobel Prize winner of 1927. At that time, German universities were subject to severe economic constraints that affected, among other things, the acquisition of bibliography.

De Broglie's theories – not shared by everyone at first – worried Ruska at first because they included an unknown wave ("Materialwelle"). However, when he applied de Broglie's equations, he saw his own predictions widely confirmed, as these equations ultimately confirmed or agreed with the ideas and work hypothesis which Ruska and other scientists had formulated earlier.

Being unaware of the mutual bibliographies, de Broglie published in 1950 the work entitled "Optique eletronique et corpusculaire", in which he did not cite the fundamental works of Brüche and Scherzer (1934) or Busch and Brüche (1937) and von Ardenne (1940), nor of the English scientist Myers (1939). It is, however, interesting to note that all of them had been cited by the Frenchman Paul Chanson (1947) in his doctoral dissertation (for all these citations see Hawkes, 2004). In view of these bilateral omissions and unawareness, it is obvious that de Broglie and his ideas did not influence the development of geometric electronic optics, a concept elaborated by German scientists (Hawkes, 2004).

THE SERIAL CONSTRUCTION OF THE TRANSMISSION ELECTRON MICROSCOPE (TEM)

In order to achieve higher magnifications, electromagnetic lenses had to be improved. Ruska had already described solutions for this in his course work of 1929 and also dealt with the subject in his diploma work (1930) and doctoral thesis (1933). The solutions basically consisted of surrounding the coils with an iron cover, which considerably reduced the focal length of the lenses. Together with his colleague and friend Bodo von Borries, also a doctoral student as himself at the TU Berlin, they patented these solutions (Borries, B von, Ruska, E, 1932). It should be noted that von Borries (Fig. 4) was a key figure in the technical and industrial development of the electron microscope and that, in his younger years, he unfortunately did not receive the recognition he deserved (Gelderblom, 2020).

Fig. 4.- Bodo von Borries (1905-1956). DGE historical images (https://www.dge-homepage.de).

In the light of his new findings, Ruska decided to build a new electron microscope with greater magnification capacity. Unfortunately, funding was lacking; not only did he not have a salary but also the university was not able to finance either the building materials nor the necessary work. In April 1932, Max Knoll left the university and moved to the Telefunken company in Berlin, dedicating himself exclusively to the development of television. The vacant position was filled by Prof. Matthias with von Borries, who thus became the head of the laboratory and the work program of the group essentially remained unchanged.

Fig. 5.- Electron microscope built by E. Ruska in 1938 and rebuilt in 1980 (Source: Wikipedia)

In the fall of 1933, Ruska's situation improved markedly thanks to the personal recommendation of Max von Laue, the 1914 Nobel Laureate in Physics, who helped him to get a scholarship with resources for himself (salary) and the necessary materials. This enabled him to build a second electron microscope in just over three months, with construction completed in November 1933.

Nonetheless, Ruska's stay at the university could not be extended and he decided to accept a position at the company *Fernsehen* AG in Berlin. The 2nd device he had built, capable of 12.000-fold magnification, was left at the university where it was used with great success by students of the department (Fig. 5).

Although busy with industrial work, Ruska and von Borries continued to their search for funds to enable serial production of the microscope. Their unlucky streak ended thanks to the mediation of Helmut Ruska, Ernst's younger brother, who was an attending physician in the Department of Internal Medicine at the Charity Hospital in Berlin. Helmut had been an avid supporter of the electron microscope from the very beginning. He believed strongly in the future of the instrument, especially in the area of infectious diseases and biomedicine.

Helmut convinced his superior, Professor Dr. Richard Siebeck – a very influential and prestigious medical doctor and professor – of the value of the new instrument. Professor Siebeck wrote a document evaluating and supporting the scientific project of the electron microscope in which he openly motivated the companies Siemens (Berlin) and Carl Zeiss (Jena) to take an interest in the invention. However, the two companies could not reach a collaboration agreement and, ultimately Siemens took sole charge of the project. Bodo von Borries, Ruska and his brother Helmut negotiated with Siemens and the result was the creation of the "Laboratorium für Übermikroskopie" (Hyper-microscopy laboratory) in Berlin-Spandau, under the direction of von Borries, who had already been part of the Siemens workforce since 1934.

By the end of 1938, two other units of a third Ruska model were built, which were, however, still considered experimental. These instruments already had several lenses (condenser, lenses with pole pieces, projector, etc.) and important additional electronic equipment (vacuum lock to change the sample and photographic plates, etc.), and reached 30.000-fold magnification. The two microscopes were made available to Helmut Ruska for training and for the development of sample preparation techniques. Being a physician, he, of course, was mainly interested in bio-medical samples and was the first person world-wide to photograph virus particles (Fig. 6) (Ruska H, von Borries, Ruska E, 1940; Borries B von, 1949).

By the autumn of 1943, more than 40 microscopes had been built and put into service. One of these, with the production number 26, went to the renowned Professor Arne Tiselius' laboratory at Uppsala University (Gelderblom and Krüger, 2014). The chemical and metallurgical industries acquired many microscopes from Siemens. On the advice of Hitler's private doctors, the Central Government Chancellery in Berlin also acquired three of them for biological weapon research (Gelderblom, 2020).

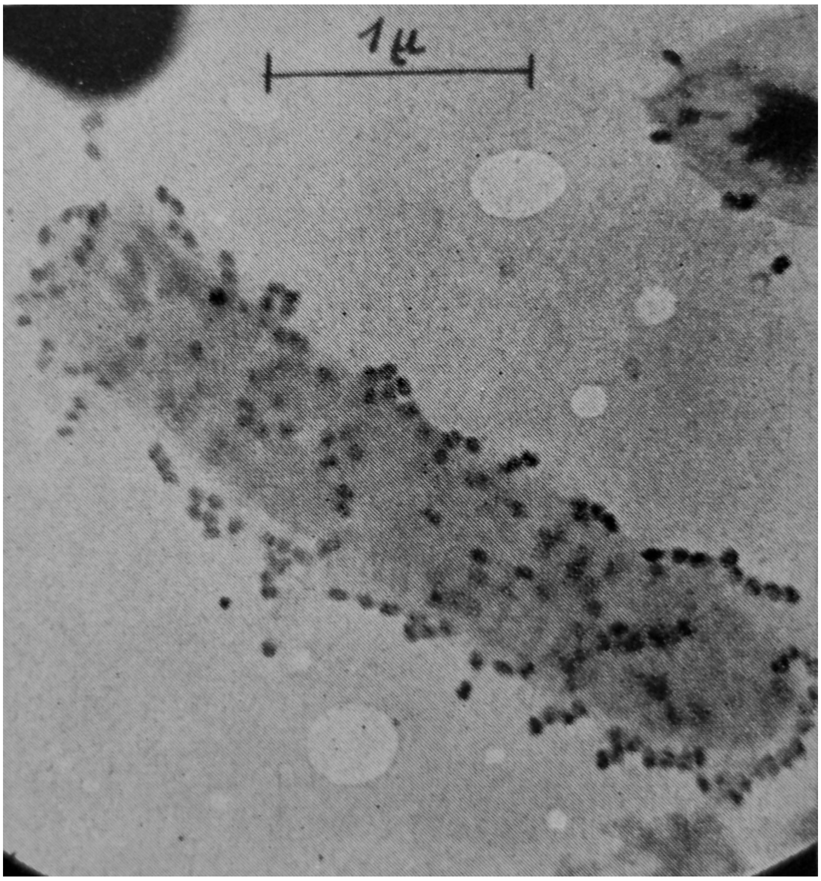

Fig. 6.- Bacteriophages (black dots) on the outer surface of a Proteus bacterium (in grey). Image (Figure 191) from "Die Übermikroskopie" by Bodo von Borries (1949) from a paper by Helmut Ruska (1941) entitled "Über ein neues, bei der Bakteriophagen Lyse auftretendes Formelement" Naturwiss, 29: 367-368.

Siemens launched an "Application and Teaching Laboratory", equipped with 4 microscopes and led by Helmut Ruska, where external scientists and potential clients could do internships. Studies with biological and medical objects were very difficult at that time, as there were no appropriate preparation techniques available to prepare objects for examination under the extreme conditions inside an electron microscope (vacuum, electronic rays, heating of the sample, etc.).

Towards the end of the war, the Siemens microscope factory was destroyed in a bombing raid and the three architects of the electron microscope, Ernst and Helmut Ruska and Bodo von Borries separated, ending a cooperation that had been so fruitful.

After the war, Ernst Ruska remained in West Berlin and immediately set about rebuilding the Siemens' laboratory and workshops to resume the manufacturing of electron microscopes. Thousands of devices were produced and ones such as those in the Elmiskop 1A and 101 series, became legendary as they enjoyed a well-deserved reputation for excellent performance and high reliability (Fig. 7). Siemens surprisingly stopped the production of electron microscopes in the late 1960s – an incomprehensible step in view of the fact that Siemens was a veritable leader in the world market.

OTHER RESEARCH AND MANUFACTURING LOCATIONS OF ELECTRON MICROSCOPES IN BERLIN

Apart from the TU Berlin and Siemens there were two further electron microscopy research and development sites in Berlin:

1) AEG (Allgemeine Elektrizität Gesellschaft = General Electricity Company, since 1929 with the participation of General Electric Company, United States)

and

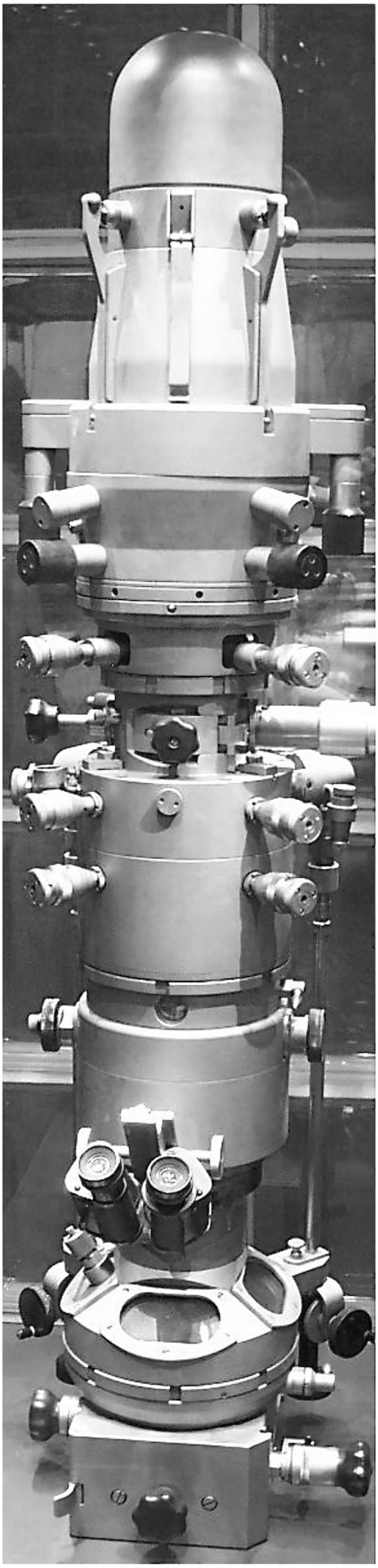

Fig. 7.- Image of the column of an electron microscope Siemens model Elmiskop 1 A (Source: Wikipedia).

2) "Forschungslaboratorium für Elektrophysik" (Electro-Physics Research Laboratory), privately owned by Baron Manfred von Ardenne, who was already the founder and director at the age of 21.

1) On April 1, 1928, AEG set up a research center ("AEG-Forschungsinstitut") in Berlin-Reinickendorf, under the direction of the physicist Carl Ramsauer (1879-1955), which operated until mid-1945. Ramsauer, a professor of physics at the Technical University of Danzig, arrived in Berlin accompanied by some of his assistants. One of them, Ernst Brüche (1900-1985), made a very important contribution to the development of the electron microscope at AEG. In 1940 AEG donated its first transmission electron microscope to the Robert Koch Institute; it had been built by Hans Mahl (1909-1988) and Hans Boersch (1909-1986), Brüche's two assistants.

From the very beginning, the two major companies, Siemens and AEG, were embroiled in a fierce struggle for dominance in the field of electron microscopy. Siemens gave its scientists almost unlimited support to win the fight for prestige; this fight included struggling for patents, product promotion strategies and marketing, in a free-market framework rather than under a totalitarian wartime regiment (Müller, 2009).

After the war, Brüche succeeded Ramsauer in the management of the laboratory and, in 1947, with the support of AEG and Carl Zeiss (Jena), he moved to the city of Mosbach (Baden). The company name was changed to "*Süddeutschen Laboratorien zur Entwicklung und Herstellung von Elektronenmikroskope*", abbreviated SDL (English: Laboratories of South Germany for the development and manufacture of electron microscopes). AEG dropped out of the company in 1953, leaving only Carl Zeiss, who then decided to relocate the production of electron microscopes to its headquarters in Oberkochen, about 160 km southeast of Mosbach.

In 1949, Carl Zeiss launched a new model of electron microscope, the EM8, equipped with electromagnetic lenses. This was followed in 1956 by the model EM9, the first electron microscope in the world equipped with a photographic device with automatic control for photographic exposition time; a true novelty.

At the end of the Second World War, the activities in electron microscopy in Germany, both in academia and industry, were soon revived and a framework for a dialogue between the various parties involved had to be created. In February 1949 von Borries took the initiative by organizing a meeting in Düsseldorf (Germany) which, at the same time, was the founding act of the *Deutsche Gesellschaft für Elektronenmikroskopie* (German Society for Electron Microscopy), known by the abbreviation DGE and of which Ernst Ruska was the first president and von Borries its first secretary (Schimmel, 1996). In 1999, the 50th anniversary of the DGE was celebrated in Dortmund (Germany); I had the honor to be the president of the society at the time. On this occasion, Professor Lenz (Tübingen) published a brilliant article on the scientific activities of the congresses organized by the DGE during the first 50 years of its existence, documenting the scientific activities and progress in electron microscopy that took place in the divided, and later reunified, Germany (Lenz, 1999).

Prof. Brüche, on the other hand, left Zeiss, but remained in Mosbach, where in 1951 he founded the Arbeitsgemeinschaft für Elektronenoptik e.V. (AEO = Working Group on Electron Optics), an organization dedicated to teaching and advising scientists and technicians at university and in industry. From 1988 to 1998 the author of this article was president of this organization, which still exists today.

2) The other center in Berlin where electron microscopes were developed was the laboratory of Baron Manfred von Ardenne, set up in 1928 on his estate in Berlin-Lichterfelde. The von Ardennes belonged to a noble family originally from Hamburg. His grandfather Armand von Ardenne and his grandmother Elisabeth von Plotho divorced, which caused a great scandal throughout the country. The novelist Theodor Fontane knew Manfred von Ardenne's grandmother personally and this family history inspired him to write his famous novel Effi Briest (1896). The house of von Ardenne in Berlin, now known as Villa-Folke-Bernadotte, was – and still is – surrounded by a large park with room for additional buildings. Von Ardenne funded his research privately, as at a very young age he already had more than a hundred patents in the field of radio, telecommunications, and television, which yielded him invaluable profits.

Siemens hired von Ardenne in 1936 to investigate whether electron beams in scanning mode could correct the chromatic aberration of the lenses in the transmission electron microscope. Von Ardenne fulfilled the contract within two years by constructing the first scanning transmission electron microscope (STEM) in history. He also implemented it as a conventional scanning microscope, creating an electron beam of approx. 4 nm in diameter: a quite extraordinary achievement for the period (von Ardenne, 1938a). In 1937 he – curiously – patented the instrument in England (von Ardennne, 1938b). In 1941 the Prussian Academy of Sciences awarded him the Silver Leibnitz Medal for these discoveries, together with Ernst Ruska, von Borries, Brüche, Boersch and Mahl, who had also contributed very important work in the field of electron microscopy (Müller, 2009). Unfortunately, von Ardenne's instruments and devices were destroyed in an air raid in 1944.

After the war, von Ardenne accepted an offer from the Soviet Union; here he made a very important contribution to the nuclear program but did not continue his research in the field of electron microscopy. The scanning electron microscope was forgotten for a few years, until research was resumed in the 1940s, then however outside Germany.

SIEMENS PATENTS AND CONFLICTS OVER PRIORITY IN THE INVENTION OF THE ELECTRON MICROSCOPE

Max Knoll, Ernst Ruska and Bodo von Borries had worked at the TU Berlin since 1928, but, at that time, Siemens had had no industrial or commercial interest in electron microscopy; it even seems that there were agreements to transfer commercial space in this field to AEG - something of dubious legality. However, the head of Siemens' electronics department, the physicist Reinhold Rüdenberg, was apparently well informed of all activities related to electron microscopy in other laboratories in Berlin.

At that time, members of universities very rarely patented ideas or even new constructions. So, when, on June 4, 1931, in Prof. Carl Cranz's famous colloquium in Berlin, Max Knoll presented Ruska's research and the experimental demonstration of Busch's theories, those innovations were not protected by patents (Gorkom et al., 2018a). The intellectual satisfaction of the scientist was considered paramount to possible industrial and commercial interests.

Rüdenberg's assistant, Max Steenbeck, is known to have attended the conference and subsequently informed his superior (Gorkom et al., 2018a). Rüdenberg was a highly skilled physicist and understood the significance of Knoll's presentation. In the same year, 1931, he inconspicuously patented the idea of the electron microscope

Siemens allowed ideas to be patented even if they were in a very early stage and without any experimental verification or prototype device supporting them. In the following months Rüdenberg filed up to 8 more patents on the electron microscope, a term or name he did not invent. Obviously, since Rüdenberg was an employee, Rüdenberg's patents belonged to Siemens.

From 1937 onwards, laws were enacted in Germany to prevent Jews from gaining access to public administration jobs, universities, and large corporations. Rüdenberg, who was Jewish, was able to emigrate to the United States with the generous help of Siemens.

When Knoll and Ruska first published their work on the electron microscope, Rüdenberg had already filed his patent, which is why he claimed recognition as the inventor of the device. Rüdenberg brought the dispute before the US courts. A Boston court ruled against him. During the hearing of the case, Judge Charles Wyzanski of the District Court in Boston (Massachusetts) asked Rüdenberg if he could present any kind of apparatus or construction in support of his theory or idea, to which Rüdenberg replied with a laconic: "No". In his ruling, Judge Wyzanski even took Rüdenberg's family circumstances into account, which gave another dimension to the problem. Here is part of the verdict:

"What happened in 1930 and 1931 can now be told. In the fall of 1930 Professor Rudenberg's younger son was stricken with infantile paralysis. The father, deeply concerned about that illness, learned that doctors knew that it was carried by a virus of poliomyelitis, but a virus so small that it could not be studied under the lens of any existing microscope or any microscope which would depend for its operation upon light waves. The trough between light waves was so much larger than the virus that observations were impossible. Thereupon Professor Rudenberg set himself to devise a new type of microscope. First, he thought of an X-ray microscope. This proved impractical. And finally, he hit upon a microscope based upon electronic principles. (...). In accordance with what was his usual practice, Professor Rudenberg merely drew certain sketches of this invention. He did not embody it in any material apparatus".

Nevertheless, the issue was pursued further; Rüdenberg's descendants continued to sue and make their claims public years after his death.

As already mentioned, Siemens was not interested in the new invention until, among other reasons, it received support from the famous Professor Siebeck. Siemens then unilaterally annulled the previous agreements with AEG, opening a new front of conflict between the two companies.

DEVELOPMENT AND CONSTRUCTION OF ELECTRON MICROSCOPES OUTSIDE GERMANY

It was probably Brussels where the work of Ruska and Knoll first found immediate resonance. A Hungarian scientist, Ladislaus Laszlo Marton, who was a member of a research group led by Professor Henriot of the Faculty of Science at the Free University of Brussels (ULB), was encouraged by his superior to build an electron microscope like Ruska's. Construction was completed by the end of 1932 and the results published in

Flemish in 1933. The following year he built a second, improved, microscope. Marton contacted biologists at his university who provided him with plant samples (*Intermediate Drosera*) from which he obtained images with the electron microscope, which was a world premiere. In addition, he introduced for the first time the fixation of a biological sample for electron microscopy with osmic acid, publishing it in a short note in the journal Nature (Marton, 1934). In the fall of 1934, he built a third microscope (Marton, 1934, 1935, Van Dyck, 1996).

Marton corresponded with Ruska and even visited him in Berlin in late June 1934, on his return journey from a visit to his homeland, Hungary, with his wife Claire. During this visit, he also met Professor Brüche (AEG) and Max Knoll (*Fernsehen* AG) and showed the three of them his images of biological objects, which was a source of surprise and admiration (Gorkom et al., 2018). With the publication of in Physical Review (1934), Marton became known in American scientific circles, demonstrating, for the first time, the possibility of examining biological specimens with an electron microscope.

Significant changes were taking place in neighbouring Germany in those years, which is why Marton decided to emigrate to the United States.

The development of electron microscopy received a major boost in the mid-1930s in the United States when Radio Corporation of America (RCA) decided to develop the instrument and make it a commercially-viable product. In 1938 Vladimir Zworykin, head of RCA's electronic research, hired Marton, who built the RCA Model A, an extremely costly device and difficult to operate. Zworykin could not convince Marton to modify and simplify the microscope.

Zworykin then brought in physics students from the University of Toronto who had built a microscope in the course of a doctoral dissertation (Watson, 1993). In 1935 Eli F. Burton was the director of the Physics Department at the University of Toronto. He became interested in electron optics, probably influenced in this by his friend W.H. Kohl, an emigrated German physicist. Under Burton's leadership, the undergraduates James Hiller and Albert Probus built the first TEM in North America in 1938, equipping it with electromagnetic lenses. The device they built at RCA, called "B", was far superior to model "A" and became the workhorse of the pioneers in the field of electron microscopy in the USA, especially in biological applications.

As, during World War II, scientists on both sides of the Atlantic were completely isolated from each other, electron microscopy in the USA evolved with different characteristics to those in Europe. One such aspect is related to biological and medical research.

The RCA company employed a fellow of the National Research Council (NRC), Thomas Anderson, who established many of the methods for sample preparation (mostly biological) and image interpretation. He maintained close relationships with the University of Pennsylvania and the Institute of the Rockefeller Foundation. It was precisely in the latter that renowned researchers in the field of biology worked, such as Albert Claude, George Palade, Christian de Duve, Keith Porter, among many others. They recognised the exceptional potential of the new technique. In view of these activities, the Rockefeller Foundation strongly supported RCA in the ongoing development and construction of electron microscopes (Rasmussen, 1997).

The problem of preserving samples, especially biological ones, which at the time was only partially resolved in Germany, remained a matter of central importance, especially in the United States (von Borries, 1949; Pease, 1960). On the one hand, there was an urgent need to obtain exquisite preservation of the biological samples, and on the other hand, to cut them as thinly as possible so that the electrons could pass through them, allowing the formation of an interpretable image.

During World War II and well into the 1950s, considerable progress was made in the United States in terms of preparation techniques, which led to fundamental discoveries in cell biology; the basis of what we know today as molecular biology (see attached Table 1).

Table 1. Fundamental discoveries in cell biology.

Decade	Discoveries and biomedical applications	Investigators
1930	Invention and construction of the first TEM First STEM/SEM First images of bacteria and virus (TEM)	Ruska and Knoll von Ardenne Helmut Ruska
1940	First images of whole cells (uncut) TEM Treatise on electron microscopy (*Übermikroskopie*)	Porter, Claude and Fuhrman von Borries
1950	Inner membrane of mitochondria (TEM) Endoplasmic reticulum (TEM) First images of chemical synapses (TEM) Golgi apparatus (TEM) Lysosome (cell fractionation and TEM) Muscle sliding filament theory	Palade Palade and Palay Palade and Palay Dalton and Felix de Duve Huxley
1960	First description of the centrosome (TEM) Animal chromosomes (TEM) First immunohistochemical labelling with ferritin (TEM) Low temperature preparation of biological specimens (TEM) New scanning electron microscopes (SEM)	Bernard and de Harven Ris Singer and Schick Fernández-Morán Oatley and his group at the Cambridge University (UK)
1970	SEM wide application of in cytology, embryology, neuroanatomy, etc.	Revel, Boyde, Meller, Mestres, and others
1980--	Cryo-electron microscopy Cryo-electron tomography	Dubochet, Studer and others Baumeister, Briggs, and others

TEM: Transmission Electron Microscope, STEM: Scanning Transmission Electron Microscope, SEM: Scanning Electron Microscope.

In those years, interest in electron microscopy also emerged strongly in other countries, such as England, France, the Netherlands, Sweden, Hungary, the former Czechoslovakia, Japan (Gorkom et al. 2018a, 2018b), and Switzerland (Günter, 1990). Unfortunately, this topic is beyond the scope of this article.

Before the course of the war turned against Germany, Siemens promoted the use of the electron microscope among researchers and users, following similar business strategies to those of the RCA in the United States. But, as already mentioned, scientific communication was very restricted, so that developments in Germany on the one hand, and in the allied countries, United States and England on the other, took place independently of each other. In fact, from the very beginning, the concepts developed along completely different lines: in Berlin research was based on the cathode ray tube technology and Hans Busch's lenses theory while the American and English physicists' work took a different direction. After de Broglie had formulated his corpuscular-wave theory and it had been confirmed by American and English scientists in 1927, they recognized the possibility of developing some kind of electronic lens. Although they approached the challenge from a different angle, they, in the end, reached the same conclusions as Hans Busch four years previously. The "North Atlantic" connection, as it were, explains the reason for the widespread belief that the design of the electron microscope began with de Broglie, when in fact this was not so.

THE SCANNING ELECTRON MICROSCOPE

The scanning electron microscope works on a different principle from that of the transmission one. With the scanning electron microscope, the electron beam's impact on the sample induces the emission of secondary electrons, retro-dispersed electrons and X-rays, among other signals, which can be captured and processed into images using specific detectors. For example, secondary electrons originate very close to the surface of the sample, producing images of the object with topographical and 3D information.

Max Knoll published a paper in 1935 in which he described how an electron beam could be used to obtain an image of the surface of electronic components (Knoll was researching in the field of television). This first device does not qualify as a microscope, as it was not equipped with lenses. However, Knoll developed the idea of obtaining images of a surface point-by-point (magnetic deflection of the electron beam = scanning), which were then combined to a single image. Years later, Siemens commissioned von Ardenne to build the first "Raster-Elektronenmikroskop" (scanning electron microscope), but the seminal idea for such an instrument was obviously Knoll's.

At the end of the war, this line of research was not continued in Germany, but in the USA the opposite happened. In the early 1940s, Zworykin, working at the RCA laboratories, built a scanning electron microscope based on the principles of Knoll's device, but with the addition of lenses and a scanning unit, such as those used in television. Zworykin's device had shortcomings, for example in terms of the vacuum system, and RCA decided not to continue with the project. The idea of a scanning microscope was discredited, and the scientific community was very sceptical that a useful instrument could ever be built.

In the late 1940s, in spite of the prevailing skepticism among scientists, Sir Charles Oatley (Fig. 8), of the Engineering Laboratories at Cambridge University, considered it worthwhile to undertake a further attempt at constructing a fully-functioning scanning electron microscope (McMullan, 1995). Many technical components developed in Oatley's laboratory during the years that followed (new lenses, new vacuum systems, other electron sources, new detectors for each of the signals emitted by the samples, etc.) have enabled the construction of a scanning microscope accepted by the scientific community. Oatley went to great lengths to convince microscope manufacturers and, in 1962 the Cambridge Instruments Company built the famous "Stereoscan" (Fig. 9), based on the prototypes designed by Oatley and his co-workers (Stewart and Snelling, 1965).

Fig. 8.- Sir Charles Oatley (1904-1996) (Source: Wikipedia).

The first Cambridge device (1964) was sent to the United States and was delivered to the company Dupont. The two instruments of the following year remained in England (North Wales and Leeds Universities) and a third went to the *Institut für Medizinische Physik* (Medical Physic) of the University of Münster-NRW (Germany), led by Professor Gerhard Pfefferkorn (Fig. 10). He had been von Ardenne's assistant in younger years and had just experienced the birth of the scanning microscope in Berlin (McMullan, 1995). The fact that one of the first Cambridge microscopes was delivered to Pfefferkorn's institute can be interpreted as a gesture of recognition of both the person and the research work done years earlier in Berlin, which had been continued post-war in Münster (Westphalia) together with Professor Ludwig Reimer (Reimer and Pfefferkorn, 1977).

The new instrument had an extraordinary impact not only on the materials sciences, but also on biology and medicine. Anatomists, embryologists, cytologists, etc. were stunned to see the images that could be obtained with the different signals. The two decades of the 70's and

80's can be considered as the golden age of scanning microscopy in the field of bio-medicine, especially in the United States, where, accompanied by specialized conferences and symposia, most of the world-wide activity in this field was concentrated. Since 1968, annual symposia have been held by Dr. Om Johari, a scientist at IITRI (Illinois Institute of Technology and Research Institute, Chicago), who has published a series of annual volumes entitled "Scanning Electron Microscopy". Similar symposia were organized in Germany and Austria by Prof. Pfefferkorn as chairman of the group EDO (Elektronenmikroskopische Direktabbildung und Analyse von Oberflächen = Electron microscopic direct imaging and analysis of surfaces) of the DGE. The scientific contributions presented were published in the series "*Beiträge zur Direktabbildung von Oberflächen* (BEDO)" (1968-69 till 1996).

Concurrent to these advances, specimen preparation techniques also progressed considerably. This was of fundamental importance, particularly in the fields of biology and medicine (Mestres and Stumpf, 1978; Meller, 1981).

Fig. 10.- Professor G. Pfefferkorn, Director of the Institute of Medical Physics at the University of Münster NRW (Source: private photo).

At one point the relevance of scanning electron microscopy seemed to be diminishing. However, two innovations changed the situation. The first was the invention of the environmental scanning

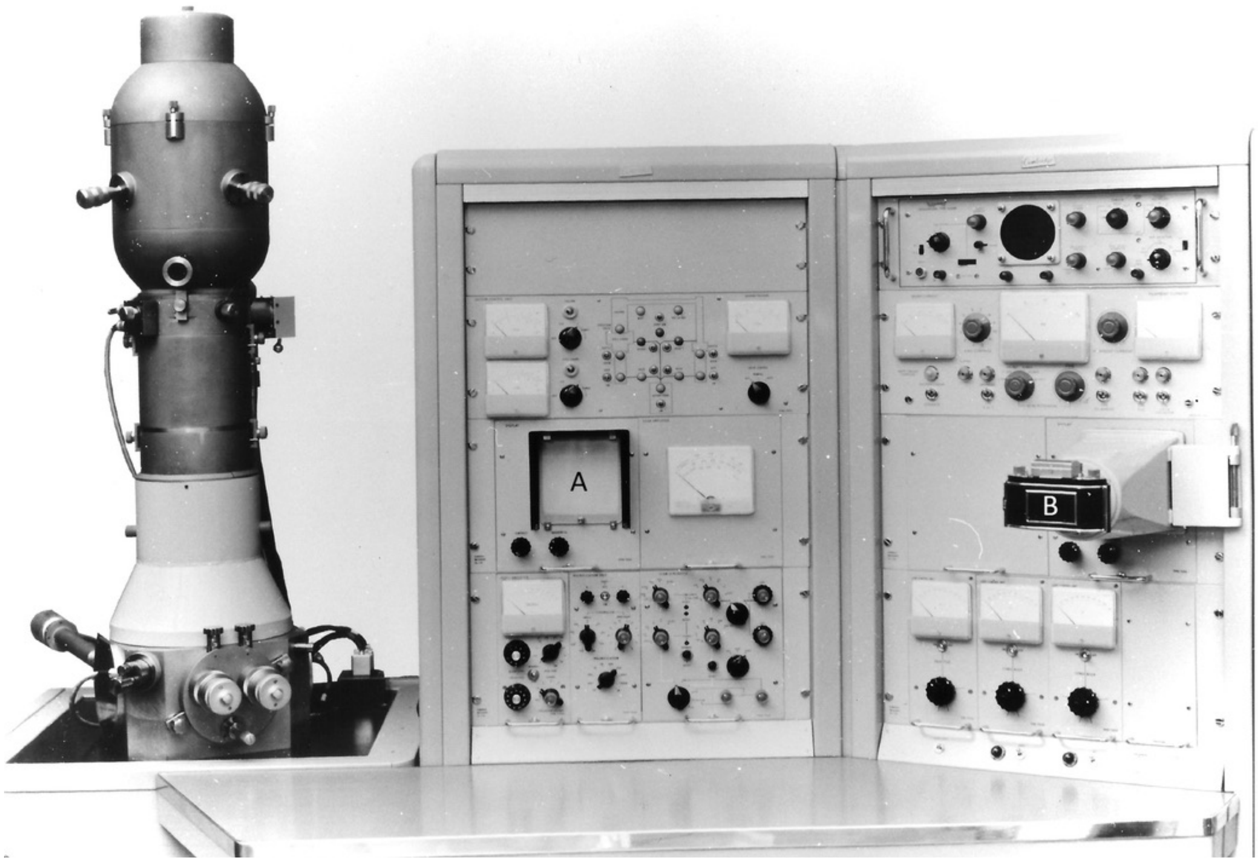

Fig. 9.- "Stereoscan MK1" scanning electron microscope from Cambridge Instruments. A: Screen, B: Camera (Source: Wikimedia Commons archive)

electron microscope, known as the ESEM (environmental scanning electron microscope) (Danilatos, 1988, Mestres et al., 2011), and the second is known as the SEM block-face (Denk and Horstmann, 2004).

Among many other applications, ESEM permits the examination of hydrated samples as well as samples that do not conduct electricity.

Block-face microscopy mainly uses the back-scattered electron (BSE) signal to obtain images of the surface of the block where the tissues are embedded. After each cut, the surface of the block - not the section - is photographed. High resolution 3D reconstructions can then be obtained by combining the images (Kubota et al., 2018). In addition, in the case of biopsies and clinical samples, not only are reconstructions possible but also large areas of the samples can be examined (Núñez-López et al., 2018).

THE ELECTRON MICROSCOPE AND THE NOBEL PRIZES

In 1974, Albert Claude, George E. Palade, and Christian de Duve were awarded the Nobel Prize in Physiology or Medicine for their development of cell fractionation and the application of electron microscopy to biology. It was the first major recognition that electron microscopy received.

Surprisingly enough, neither the invention of the device nor the achievements of those involved in its development had been acknowledged previously. As already implied above, the conflicts between the various research groups in Berlin and the instrument manufacturers were very noisy and unpleasant. It can be assumed that, in this environment of conflict, the Nobel Committee was unwilling – or even unable – to act.

The passage of time seems to have made things easier and in 1986 came a more favorable time: the Nobel Prize in Physics was awarded to Ernst Ruska (1/2) for the invention of the transmission electron microscope and to Gerd Binning (1 / 4) and Henrich Rohrer (1/4), for the discovery of the tunnel effect. The latter is of great importance in the so-called high-resolution microscopy in which the tunnel and atomic force microscopes find wide application. The Swedish Academy of Sciences said the award was given to Ruska: “for his fundamental work in electron optics, and for the design of the first electron microscope” (1986).

Many are of the opinion that other scientists such as Bodo von Borries, Martin Freundlich and even Helmut Ruska deserved recognition for their important contribution to the development of electron microscopy. They were indeed deeply involved in the process but, unfortunately, the evaluation of scientific merits is a difficult task which can leave individuals bereft of their rightful acknowledgement. In this sense it is worth remembering Newton’s phrase: “If I have seen further, it is by standing on the shoulders of giants” (Newton, 1675, quoted by Gelderblom, 2020).

Fig. 11.- Humberto Fernández Morán (https://www.biografiasyvidas.com/biografia/f/fernandez_moran.htm)

More recently, the electron microscopy has been recognized by the Royal Swedish Academy of Sciences. The Nobel Prizes Committee awarded the 2017 Nobel Prize in Chemistry to Jacques Dubochet, Joachim Frank and Richard Henderson, the main pioneers of Cryo-Electron Microscopy (Brzezinski, 2017).

The term “Cryo” refers to the conservation of biological samples using cold. In the early 1950’s, it was clear that chemical fixation, the most widely used method, caused artifacts, with the result that cells fixed with chemical agents did not exactly match living cells. For this reason, and parallel to improvements in chemical fixation techniques, there has been intense research on cryo-techniques since the early 1950s. Humberto Fernández-Morán, who was born in Venezuela 1924 and died in Sweden in 1999 (Fig. 11), was probably the first to propose the freezing of cells at low temperatures, thus avoiding artifacts produced by chemical fixation, leaving the cellular and molecular structures immobilized by the cold in their last moment of vitality (Fernandez-Morán, 1960, 1972; Padrón, 2001). Since then, this type of preparation has come a long way, as has the capability of electron microscopes to examine frozen sections. This is the technological frame in which the methods of cryo electron microscopy (cryo-EM) (Dubochet, 1988 and others) and cryo-electron tomography (cryo-ET) have been developed to the current level of perfection (Baumeister, 2016, 2021; Briggs, 2013 and others).

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