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2.3
Electron Microscopy in Belgium

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I. Introduction

Despite its small size, Belgium has played an important role, not only in the development of electron microscopy as a technique, but also in the dissemination of the use of electron microscopy in materials science as well as in biomedicine. The number of electron microscopes (and hence users) per capita still ranks among the highest in the world.

II. The Pioneer

As early as 1932, immediately after the first results were obtained by Knoll and Ruska, Ladislaus ("Bill") Laszlo Marton realized the potentialities of this new technique. Marton was born in Budapest (August 15, 1901). He obtained his Ph.D. in Zurich on X-ray spectrometry and in 1928 he began working in the small group of Prof. Henriot at the Faculty of Science of the Free University of Brussels (ULB). Professor Henriot (1855–1961) stimulated him to devote his research to the construction of an electron microscope, although the financial means were very limited. His only source of funds was a modest grant from the Institut National de Physique Solvay, whose scientific secretary was Prof. Henriot himself and who provided Marton with a fellowship for several years.

His first prototype with a horizontal column (Fig. 1) was ready by the end of 1932. His first paper about this subject was published in Flemish in 1933 together with Maurice Nuyens in the journal *Wis- en Natuurkundig Tijdschrift* under the title "Meetkundige Optiek der Elektronen" (Marton and Nuyens, 1933). It had only one electromagnetic lens, outside the vacuum. Originally Marton wanted to use the instrument to investigate photoelectricity, but in view of the fact that the AEG laboratory in Berlin had already started similar research with ample financial means, he abandoned this idea (Süsskind, 1986).

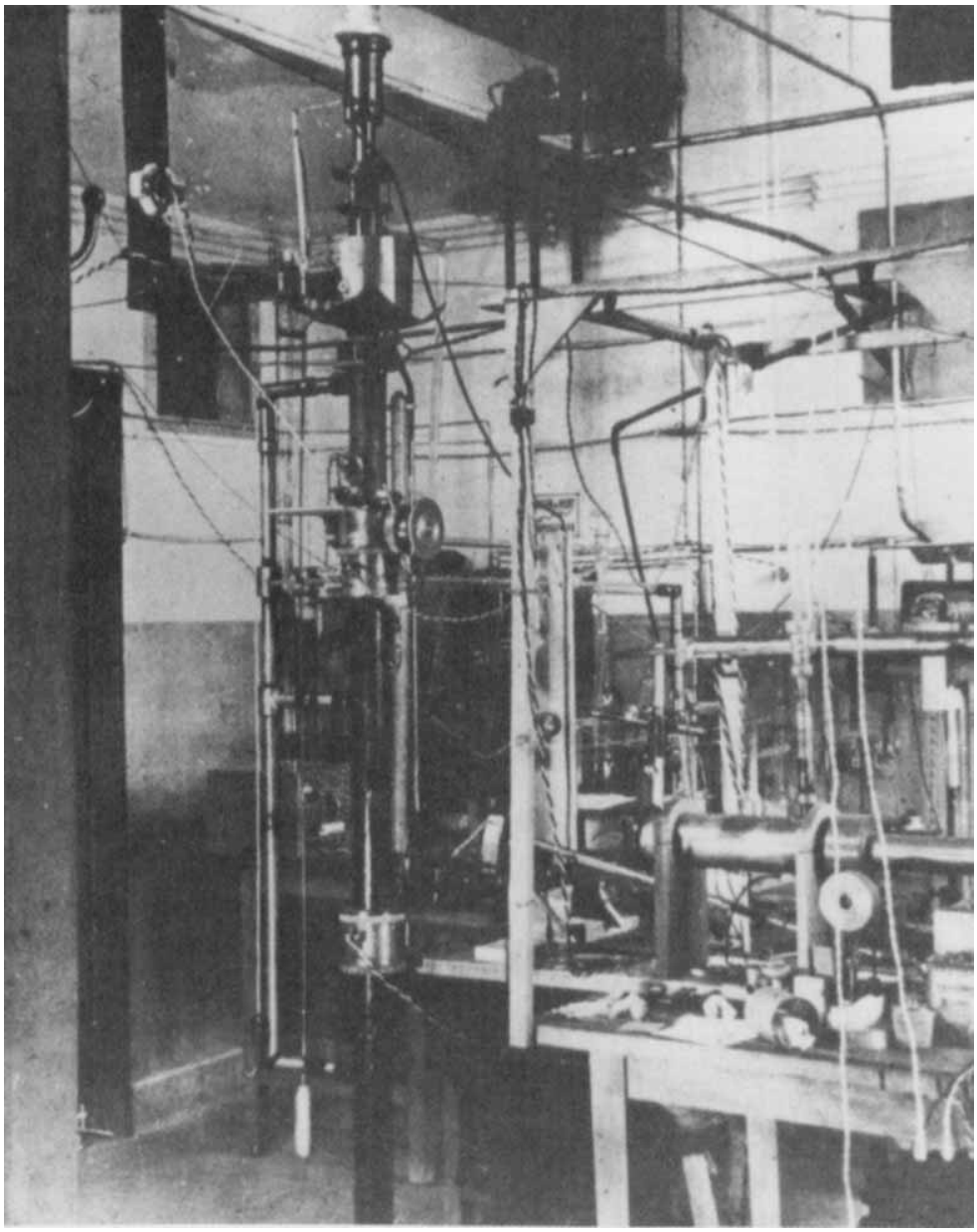

FIGURE 1. Photograph taken in 1935 of the two electron microscopes built by L. Marton and used for the earliest biological work. The horizontal instrument (right, in foreground) was built in 1932, the vertical one (left, in background) in 1934.

With this instrument, Marton was able to image the spiral cathode. The image was not homogeneous, which Marton attributed to different work functions of the different crystalline phases. This result encouraged him to construct a second instrument in 1933 with a vertical column consisting of a condenser and two lenses (Fig. 1). The instrument had an electron optical magnification of 1000 × in two stages over a total imaging length of 72 cm. Only the projector lens had polepieces. The objective focal length was

estimated to be about 10 mm and the projector focal length 12.5 mm. The goal of this project was twofold: to explore the possible application of electron microscopy, and to understand the mechanism of image formation. Before starting the investigation of biological samples in April 1934, Marton consulted his colleagues in the life sciences at the ULB, the zoologist Paul Brien and the botanist Marcel Homès (later president of the Royal Academical Society of Belgium). Homès provided him with microtome sections impregnated with osmium tetroxide. The thickness of the slices was still several micrometers. The object was then mounted on a copper grid. In this way Marton obtained the first micrographs of a sundew plant (Fig. 2). Although the organic material was destroyed by electron bombardment, the osmium skeleton remained unchanged to reveal the structure and provided sufficient contrast to obtain an interpretable image. Marton immediately realized that by staining biological objects with heavy metals, the structure can remain visible; and even without staining, biological specimens can be resistant to electron irridiation for a sufficient time to record the photographs, so as to reveal details beyond the resolution of the optical microscope. This opened enormous perspectives for the investigation of biological samples.

He presented his results at the meeting of the Belgian Academy on May 8, 1934, and published a short note in *Nature* (Marton, 1934a).

Marton then visited Ruska, Knoll, and Brüche in Berlin, who did not believe his results. In Belgium too, he was criticized, even by the eminent

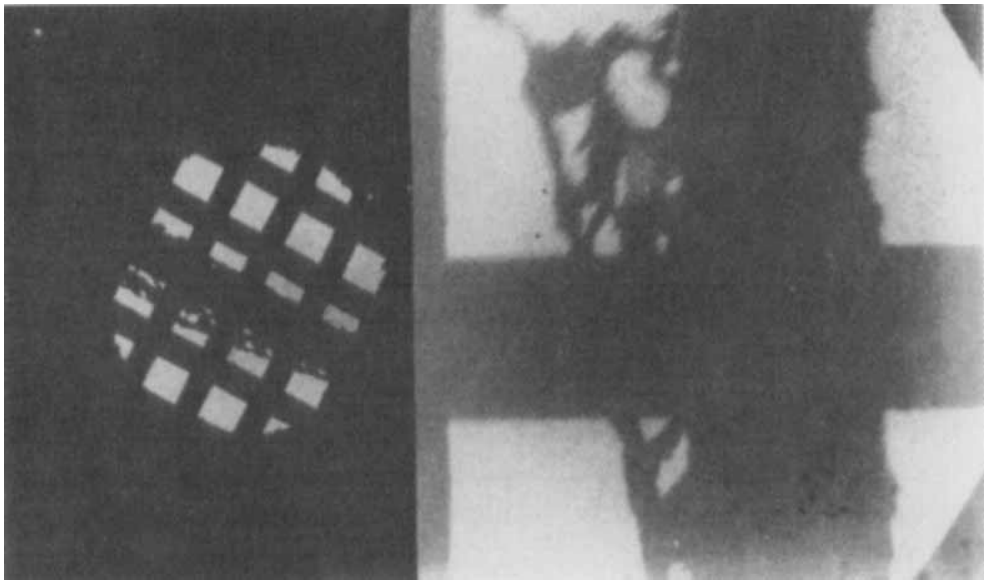

FIGURE 2. (left and right) First biological electron micrograph, obtained with the horizontal microscope; the specimen is a 15-μm-thick microtome section of *Drosera intermedia*, stained with osmium tetroxide (April 1934).

bacteriologist Jules Bordet (Nobel Prize for the discovery of immuno factors in blood serum), who, after Marton's lecture, said: "Oh no, not an electron microscope, we have enough trouble trying to interpret the images we get with a light microscope!"

In the autumn of 1934, Marton started to build a third instrument, again vertical, at the cost of enormous effort. The university funding was now nearly nil. Furthermore, he could not persuade the biomedical sector to invest in this project. Marton had to buy parts in the flea market, usually with his own money. The instrument was equipped with airlocks for the specimen and the photographic plates and a beam shutter. The instrument operated at an accelerating voltage of 45–50 keV, and the exposure times were of the order of 0.1 s. The total imaging length was almost 1400 mm. Both objective and projector lenses had polepieces. In principle, a magnification of about 5000 could be reached, but he used a magnification of 700. In order to reduce the irradiation of the specimen, Marton used a very convenient method of focusing first on a radiation-resistant test specimen and then bringing the biological specimen to the same axial position by means of the specimen airlock. In order to focus the image, which was not visible on the screen, he calibrated the lens currents.

To improve the technique, he cooled the sample by supporting it on a very thin (0.5-μm) cooled supporting grid of aluminum. In this way he was able to improve the resolution to about 1 μm. He was able to visualize the cell wall and the nucleus of an orchid. Marton even succeeded in observing samples without fixation, by mounting them on thin (20-nm) supporting foils of collodion (nitrocellulose) (Fig. 3). He also contributed to the understanding of the image formation. He realized that the image contrast was not caused by absorption (as in optical microscopy) but by scattering and used the Bethe formula to calculate and tabulate mean scattering angles for multiple scattering as a function of thickness and electron velocity for different materials.

Marton also studied the resolving power of the electron microscope (Marton, 1936) and in particular he showed how the contrast decreases with increasing numerical aperture, so that the upper bounds for the resolution are limited by the spherical aberration. He also introduced the concept of depth resolving power.

Marton devoted most of his efforts toward improving the electron microscope for biomedical research and particularly to reducing the necessary electron dose. He introduced a number of improvements that are now in common use, such as:

- Airlocks for the specimen and photographic plate
- Movable photographic plates for multiple exposure

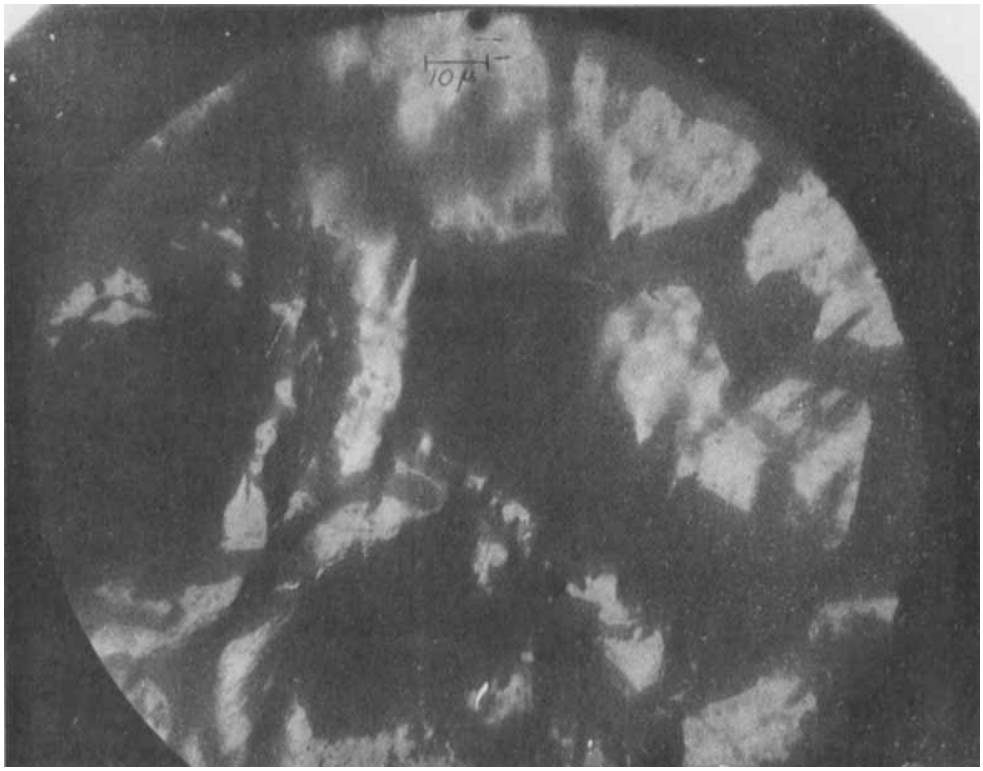

FIGURE 3. Root of *Neottia nidus avis* on collodion films (January 1936); micrograph obtained with the vertical microscope.

- Specimen stage with mechanical x–y control
- Specimen carousel for multiple specimens
- Electronic exposure control
- Beam blanking, beam shift
- Better phosphor for increased contrast

Marton also succeeded in interesting André Callier, the director and founder of a Belgian manufacturing company (Société Belge d'Optique, Ghent) in the construction of an electron microscope according to his design, but unfortunately Callier died before the construction was started.

Probably owing to lack of funding, but also in view of the political situation in Europe, the electron microscopy project in Brussels was abandoned before World War II and Marton left Brussels for the United States. He later introduced techniques for determining the thickness of the object, stroboscopy for time-varying objects, stereoscopy (Marton, 1944), electron optical shadowing, electron interferometry, X-ray shadow microscopy, and electron energy loss studies. In 1945 he obtained the medal of the ULB, and in 1955 he became a member of the Royal Academy of Belgium.

III. The War Period

Immediately after Marton left Belgium, activity in the field became nearly nonexistent. Since commercial instruments were not available, activities were started at the universities of Brussels and Ghent to construct home-made diffraction instruments. At the University of Brussels, Dr. O. Goche developed an electron diffraction apparatus, based on the design introduced by Finch. This instrument was then used for the study of thin layers. At the University of Ghent, in 1943, A. Lagasse, in the framework of his Ph.D. research, constructed an electron diffraction instrument. The instrument is shown in Fig. 4. The quality of the diffraction patterns obtained was excellent, as can be judged from the example in Fig. 5, obtained from graphite. Lagasse used his instrument to investigate thin metal layers and the structural relation between deposits and the crystal substrate.

Immediately after the war, a "real" electron microscope facility, one of the first in the world, was set up at the University of Ghent; this provided services for biologists as well as for physicists. The first director was Van der Meersch, who was after a short period succeeded by Lagasse.

The first commercial TEM was installed in 1949 at the University of Liège. It was an RCA instrument (type EMU2) operating at 50 keV and used for materials science as well as biology. The first results in metallurgy using replica techniques were obtained by P. Cohen and L. Habraken and were presented at the Delft Conference (July 1949). The microscope remained in operation until 1970.

The first person to introduce electron microscopy in biomedical research was M. H. A. De Groodt-Lasseel, a former student of B. von Borries, who also purchased the first microtome and started a very productive scientific career. Another pioneer of the use of electron microscopy in biomedicine was Albert Claude (Bordet institut) (Porter *et al.*, 1945), the later Nobel Prize winner, who first observed the structure of tissue cells in the electron microscope, which, fortunately, were sufficiently thin and did not need microtomy.

In the early 1950s, other instruments were installed in Leuven (RCA), Ghent (Siemens), and ULB (RCA). The work in Leuven was carried out by Van Itterbeek and L. De Greve, who studied the structure of epitaxial films in relation to electron conductivity. The first JEOL microscope (JEM 100) was installed at the University of Namur.

In the 1950s and 1960s several electron microscopic groups were established by different scientists, all prominent in their research fields: in biomedicine, A. Claude (Brussels), Ch. De Duve (Leuven, Brussels), J. Drochmans and P. Dustin (Brussels), Firket (Liège), M. De Groodt

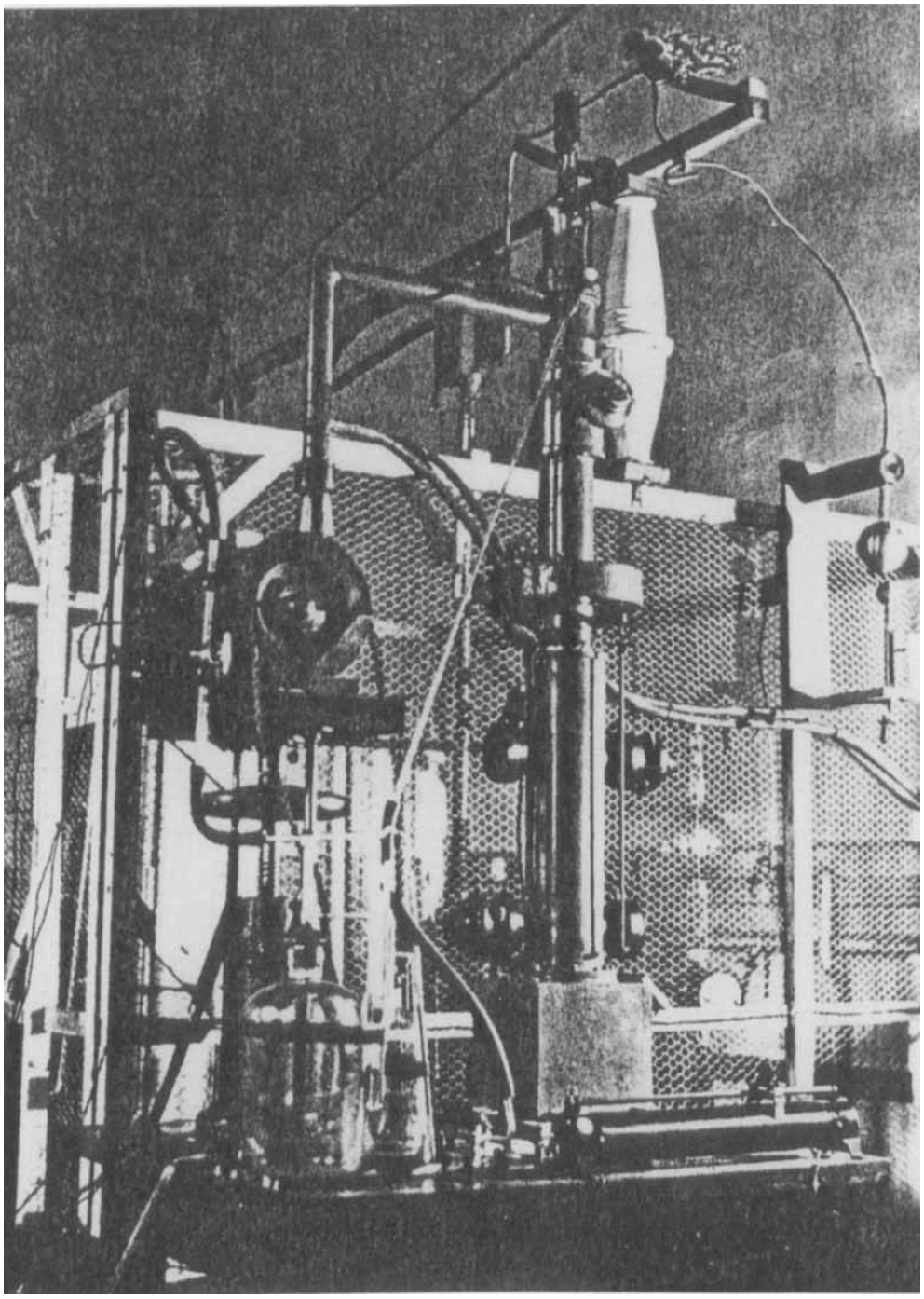

FIGURE 4. Electron diffraction instrument built by P. Lagasse. It was constructed and located at the Faculty of Science of the University of Ghent and operated at 70 kV. The accelerating voltage was generated with a Siemens & Halske 220-V/80-kV transformer. The vacuum was obtained by means of a Holweck pump and an oil diffusion pump.

and D. Scheuermann (Antwerp), pioneering freeze-fracturing and X-ray microscopy; in materials science, Amelinckx (Antwerp), Habraken, Greday (Liège), De Berghezan, Deruyttere, and Delaey (Leuven).

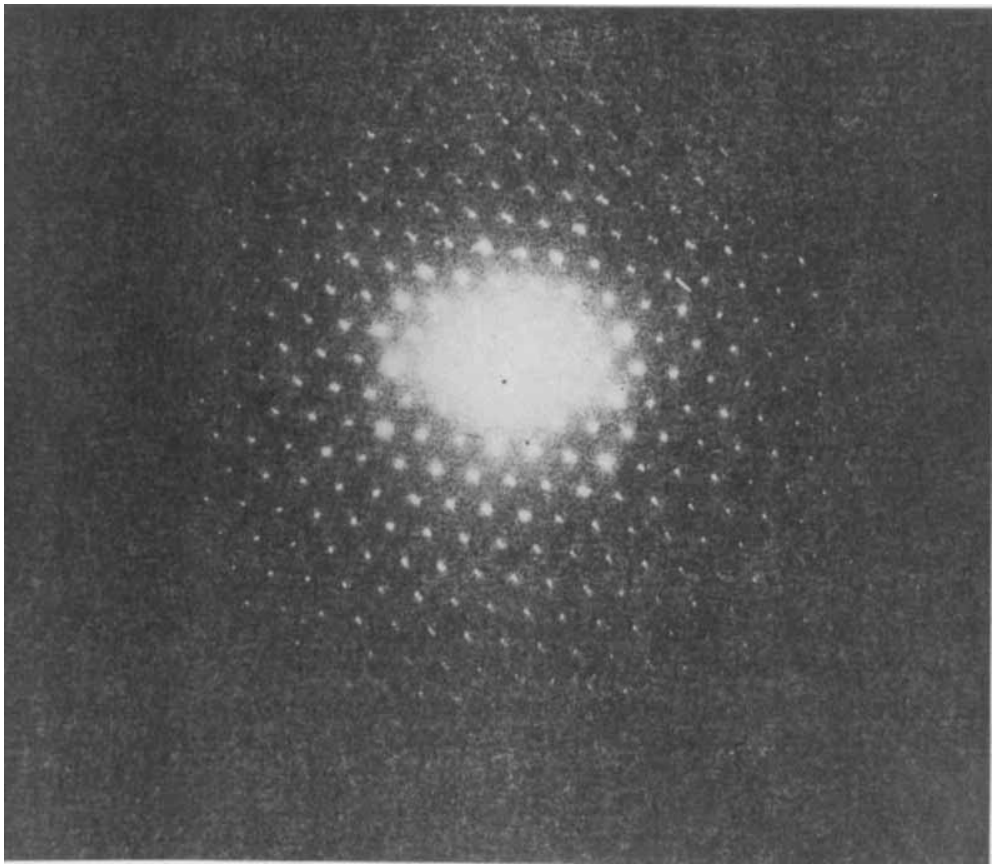

FIGURE 5. Electron diffraction pattern of graphite taken with the instrument of Lagasse.

In the 1980s more than 100 electron microscopes (TEM and SEM) were in operation in Belgium.

IV. SOCIETY FOR ELECTRON MICROSCOPY

A number of Belgian microscopists were present at the international conference in Delft (1949): A Claude (Bordet), P. Cohen, Ch. Gregoire, L. Habraken (Liège), L. De Greve (Leuven), A. Lagasse, J. Roose, and J. Voets (Ghent). At the international conference in Paris (September 1950), presentations were made in materials science (Habraken, Cohen) and biology (Gregoire and Florkin).

In the early 1950s, a first attempt had been made to found a Belgian Society for Electron Microscopy, with L. Habraken as President and Van der Meersch as Secretary. This society did not survive. In 1957, under the initiative of L. Habraken, A. De Gueldre, M. Desirant, A. Lagasse, M. De Groodt, J. Fripiat, and A. Bruaux, the Comité Belge de Microscopie Electronique—Belgische Comiteit voor Elektronenmicroscopie was

founded and officially recognized by the IFSEM in 1957–1958 at the international conference in Berlin (and the official fees were paid!). At the moment the committee consisted of 40 members, 8 from the biomedical sciences, 27 from physics, chemistry, and metallurgy, and 5 others. The President was A. Lagasse, and the Secretary/Treasurer was T. Greday. This committee remained in office until 1966. The present Society was established in 1966 with President A. Claude, Van Itterbeek (Vice President), Lagasse (Administrator), Firket (Treasurer), Baudhoin (Assistant Secretary), and Greday (Secretary). Greday retained this function until 1990. The society now has about 200 members.

V. International Meetings and Conferences

Liège (May 1956)	Journées internationales de microscopie electronique appliquée à l'industrie
Brussels (May 1967)	Joint Meeting of the BVEM/SBME with the SFME (French society)
Liège (May 1973)	Joint Meeting of the BVEM/SBME with the DGEM (German society) and NVEM (Dutch society)
Rotterdam (May 1974)	Joint Meeting of the BVEM/SBME and the NVEM
Antwerp (September 1970)	International Congress on High Voltage Electron Microscopy
Liège (May 1983)	Joint Meeting of the BVEM/SBME with the SFME
Antwerp (September 1983)	Joint Meeting of the BVEM/SBME with the DGEM (German society for electron microscopy)
Lille (May 1984)	Joint Meeting of the BVEM/SBME with the SFME
Antwerp (September 1985)	Joint Meeting of the BVEM/SBME and the Deutsche Anatomengesellschaft
Wageningen (December 1990)	Joint Meeting of the BVEM/SBME with the NVEM
Antwerp (December 1992)	Joint Meeting of the BVEM/SBME with the NVEM
Papendal (December 1994)	Joint Meeting of the BVEM/SBME with the NVEM

VI. Nobel Prizes

Albert Claude, who worked at the Rockefeller Institute (New York) and afterwards at the Bordet Institute (Brussels), was the first to visualize tissue culture cells in the electron microscope. His paper together with K. Porter and E. Fulham was a real milestone in the use of the electron microscope in cell research. Fortunately, the cells he investigated were so thin in natural form that they did not need to be sectioned and could reveal the intrinsic potentialities of the technique. Albert Claude continued his research on the structural and functional organization of the cell mainly using electron microscopy. He improved the technique almost without help, and also developed the differential centrifugal method for fractionating cells. In this way he paved the way for morphologists as well as for the biochemists to enter and discover the unexplored domain of the interior of the cell, and he witnessed the success that resulted from this collaboration. His work lies directly at the origin of the discovery and study of the subcellular organelles. In 1974 he received the Nobel Prize for his work together with

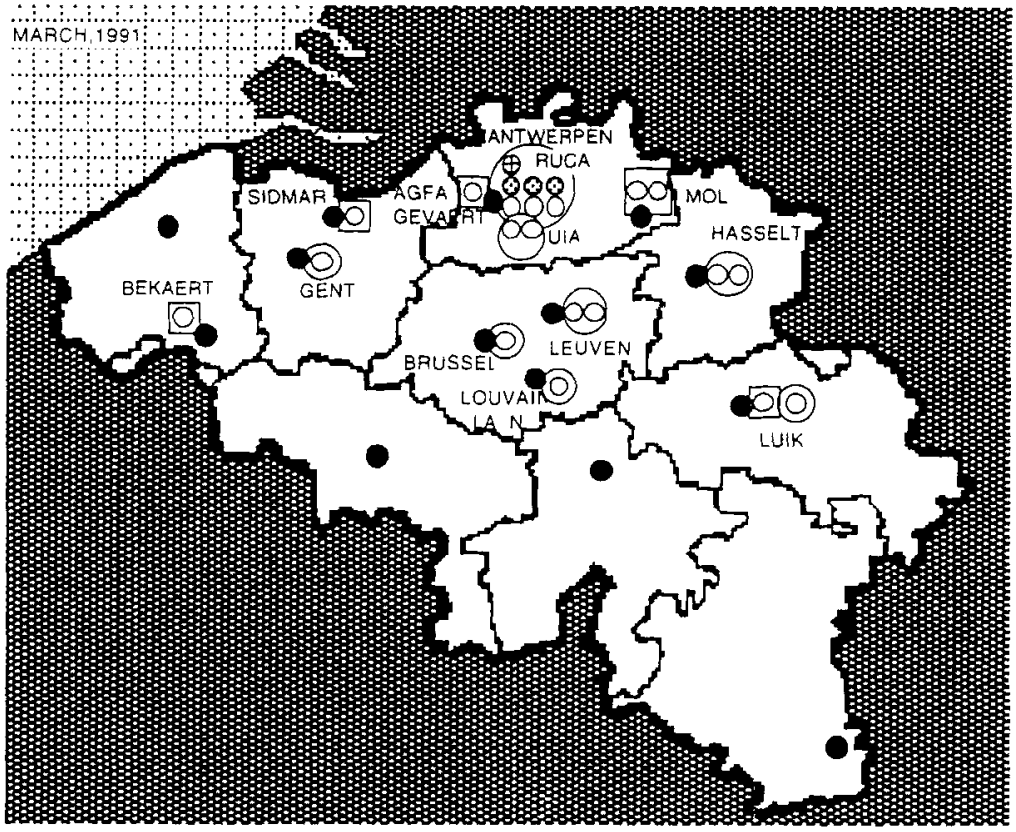

FIGURE 6. Distribution of transmission electron microscopes for materials research in Belgium. Instruments dedicated to: ⊕, high voltage; ⊛, high resolution; ○, analytical applications or general purposes.

Ch. De Duve (Univ. Leuven and Brussels) and George Palade. Albert Claude died in 1983.

VII. Antwerp and the Brite-Euram Project

The center of mass of electron microscopy in materials research in Belgium is the University of Antwerp. Due to the pioneering work of S. Amelinckx, first in Ghent (1950–), then in Mol (1960–), and afterwards in Antwerp (1965–), electron microscopy became recognized in Belgium as a major research technique and Antwerp became the center of mass of electron microscopy in materials science in Belgium (Fig. 6).

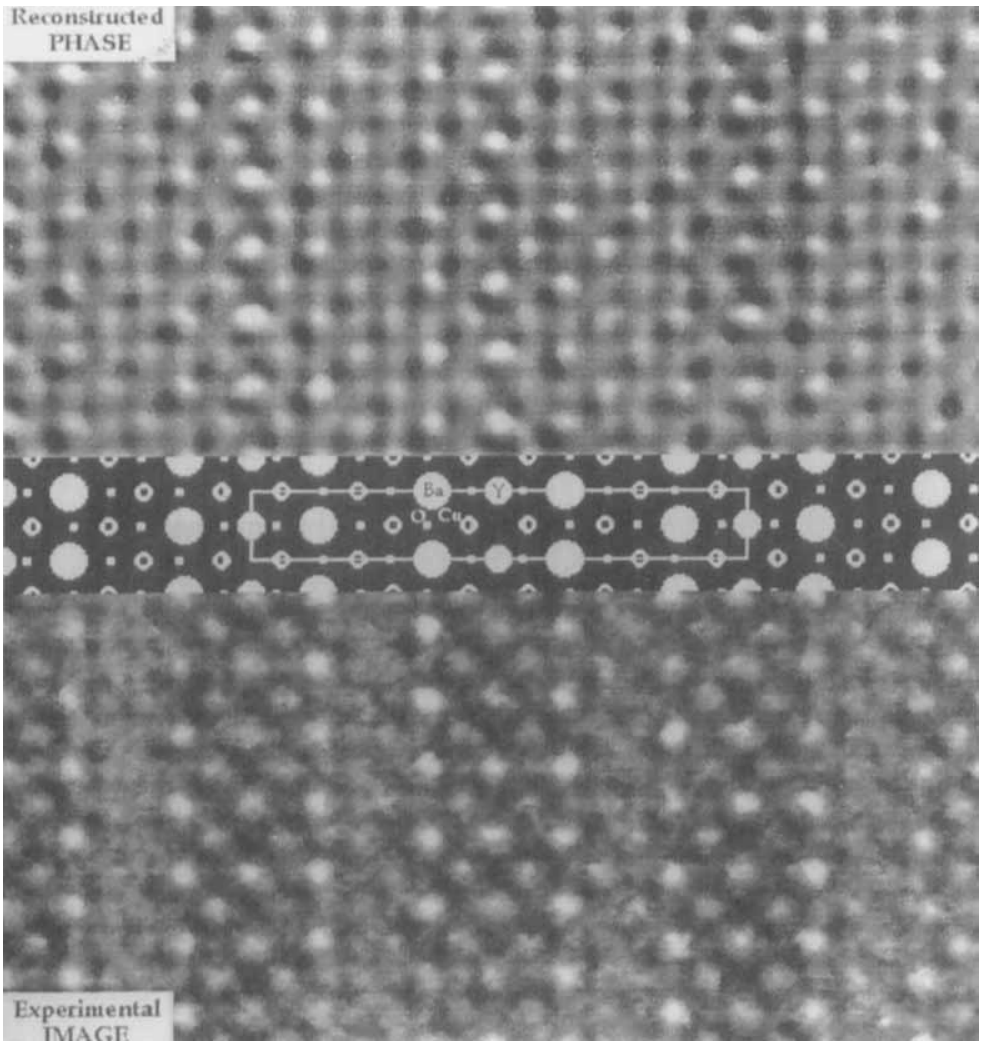

FIGURE 7. Reconstructed object wavefunction for $Y_1Ba_2Cu_4O_8$ using the method developed in Antwerp. Below: normal high-resolution image with a resolution of about 0.2 nm taken with a Philips CM20 ST FEG. Middle: structure model. Above: Reconstructed object phase, showing all atom columns with a resolution better than 0.15 nm.

Under the stimulus of Amelinckx and the guidance of Van Landuyt, Van Tendeloo, and Van Dyck, the EMAT group (Electron Microscopy in Materials) in Antwerp has become one of the leading centers in the world, with 7 electron microscopes and a team of 20 researchers. Over a period of about 25 years, it has welcomed more than 200 visiting scientists and published more than 1000 papers. The group is particularly known for its work in defects, alloys, semiconductors, superconductors, and fullerenes.

A culminating point is the construction of a new electron microscope, the working principle of which is based on ideas developed in Antwerp. It has been shown that by combining images taken at different focus values it is in principle possible, using image processing techniques, to reconstruct the structure of the object without prior knowledge about this structure and with a resolution well beyond the point resolution of the microscope (Fig. 7). In the framework of a Brite-Euram project, this instrument (a 300-keV FEG microscope) is now being constructed by Philips in collaboration with the Universities of Antwerp, Delft, and Tübingen. The microscope is a 300-keV instrument, equipped with a field emission gun, a $(1024)^2$ slow-scan CCD camera, a powerful image processing system (100 Mflops) and complete computer control and alignment of the instrument. The first prototype has a resolution of 0.1 nm and will be installed at the EMAT laboratory in Antwerp, where it will be evaluated on a variety of materials.

Acknowledgments

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