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ELECTRON MICROSCOPY TO ELECTRON MICROPROBE ANALYSIS: THE EARLY DAYS

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The period I shall describe is the decade from 1935 to 1945. That is from 44 to 54 years ago and safely beyond the reach of most my readers' memories. Looking back, it was a most exciting, even spectacular, decade during which a remarkably small group of researchers scattered around Europe and North America took the magnetic transmission electron microscope (TEM) from a rather quiescent, theoretical concept to a reasonably reliable, practical instrument that was in serial production. By the end of the decade it had a routine resolving power of better than 10 nm, and in expert hands was reaching 1 nm. In that same period, the first attempt to build a scanning electron microscope was made and the basic principles of microprobe analysis were demonstrated. The fields of application of the TEM were expanded from essentially zero to the full range of the light microscope, with two exceptions. There was not yet any way of cutting sufficiently thin sections of biological materials. That came a few years later. Color, so valuable in light microscopy, seemed to have no counterpart in electron microscopy.

It seems almost incomprehensible in today's world that, in the middle 1930s, a few individuals were putting enormous effort into developing a high-resolution electron microscope when the only type of specimen we were sure we could examine was no more exotic than the edge of a razor blade. It was generally assumed that the electron intensity required to make a highly magnified image useful would immediately destroy any but the most refractory specimen. In 1937 Marton published his paper in which he demonstrated that a sufficiently thin suspended film of collodion could be the counterpart of the microscope slide. Immediately the entire field of very fine solid particles --paint pigments, colloids, bacteria, viruses, etc., was opened up, followed rapidly by replicas of metallurgical specimens and shadowing techniques. Although beam-induced contamination of the specimen was recognized early, it was several years before it was recognized that the electron beam did cause some radiation damage even in the thinnest specimens. It was also several years before it was recognized that specimens sufficiently thin to avoid beam heating couls still be too thick to permit the extraction of intelligence from the images because of a plethora of overlapping structures.

Why did the TEM come into being in that particular decade? My view is that the technological infrastructure of the electronics industry was coming together. Suddenly we had 50-liter/s oil-diffusion pumps instead of fractional liter/s mercury pumps. We had continuously reading electronic vacuum gauges instead of McLeod gauges; rapid demountability with synthetic-rubber O-rings and gaskets instead of ground grease joints; vacuum-tight, flexible metal bellows that gave us new opportunities for designing means of manipulating within a vacuum chamber. I am sure some high-vacuum technologists may blanch as I list these developments. I understand; but to us, in 1940, they were miracles. On the electronic circuitry side, we had regulated high-voltage supplies instead of expensive and dangerous capacitor-resistor filter chains, and regulated current supplies instead of water-cooled lens coils and banks of automobile batteries. These developments, and others, speeded our work by an order of magnitude or two. Such numbers practically guarantee the historical coincidence of the development of an enabling technology and the emergence of a major new development.

Unfortunately, although the technological infrastructure that supported the design and construction of TEMs was becoming available, the instrumentation needed to study their limiting performance did not exist. Before 1940 few of us appreciated how many external and internal factors were capable of disturbing the TEM image, or the sensitivity of the images to these factors. Ultimately, the instruments themselves, being the only devices with sufficient sensitivity, had to become the means for detecting and measuring the effects of the myriads of technical problems that existed in those early instruments. The read-out was, of course, the nature and degree of blurring of the recorded image. It was because of the low intensity of the visual images in those days that the sensitivity was achievable only in the recorded image and not in real time. Not only was the read-out very ambiguous, but it related only to events that occurred during the exposure interval. For example, the effect of a thermal drift of the specimen on the image was indistinguishable from the effect of an insulating particle, somewhere near the imaging beam, that was being charged by scattered electrons and was slowly deflecting the image. Similarly, the effect of a minute mechanical vibration of the column could be indistinguishable from the two just mentioned.

Thus, the development of the TEM became an unending repetitive series of proposed hypotheses, followed by the design of an experimental test and then by the actual test. Because of the variability of many of the defects and of their large number, the successful identification and removal of one defect did not necessarily lead to an observable improvement. However, as it turned out, it was that same variability that prevented the process from being

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completely discouraging. As we were struggling to achieve consistent results, at, say, 10 nm and making thousands of exposures, there was always the unexpected exposure that showed resolving power several times better. Those were the micrographs that were published but, more important, they were the teasers that persuaded the early workers to keep trying.

By 1943 the number of defects still present had been whittled down to the point where identifying and removing the remaining ones became relatively easy. As a result, some spectacular improvements in resolving power were made quickly and easily. It was this phenomenon that allowed the implicit astigmatism of the magnetic objective to emerge from the masking effect of other problems and to be corrected by the development of the stigmator.

If I have a message in this part of my account, it is to note that the great bulk of the work that brought the TEM into being as a most useful tool for science had nothing to do with the basic theory of the electron microscope. That also leads to a small complaint that, through some form of accepted snobbery (of which I was as guilty as anyone), no reports of any of this work were ever published. Although there was much word-of-mouth exchange of information among the early workers, I fear that our behavior condemned many newcomers here and overseas to repeating many of our mistakes.

Despite our problems, the TEM did begin to be useful and started providing essential information to an expanding universe of research, sometimes in spectacular and unexpected ways. One such incident sticks in my memory as a particularly significant demonstration of the role of the early TEM and as a cornerstone of my own understanding.

The time was the fall of 1940. We had the first developmental model of the RCA EMB microscope working in my laboratory in Camden, N.J. We received a call from Dr. Wendell Stanley asking if it would be possible to take a look at one of his tobacco mosaic virus (TMV) preparations. Dr. Stanley was working at the Rockefeller Institute Laboratories in Princeton, N.J. (In 1946 he received the Nobel Prize in chemistry for his work on plant viruses.) We made a date and within an hour after he arrived, we had a spectacular picture of the characteristic rods of his virus. In minutes, we had confirmed what had taken Dr. Stanley's group many years to determine, indirectly, by means of such techniques as low-angle x-ray scattering, the ultracentrifuge, birefringence optical studies, plus all the necessary biological tests. Note that I used the word "confirmed."

It is illuminating to consider what the scenario might have been if the TEM had been available when Dr. Stanley started his work. He would have doubtless compared micrographs of juices from healthy and from the infected plants, and his attention would have been attracted by the presence of the TMV rods as alien structures present only in the juices from the infected plants. Please note that the presence of the rods would have been strongly suggestive but would have proved nothing. However, supported by the TEM results, showing that the rods were indeed the infectious agent, would have been a rather straightforward and relative simple procedure.

The spectacular confirmation of Dr. Stanley's work is only one indicator of the value of the TEM. My alternative scenario presents another measure and shows that a more important, but less appreciated, value of the TEM was in the enormous improvement in the efficiency that would have occurred in Dr. Stanley's research on the basis of what the TEM did *not* show! Without the TEM, the possible presence of each of all the particle sizes and shapes that the virus could have had would have had to be postulated and tested.

This little anecdote shows that, in those days, the TEM had two functions. The first was to illuminate "submicroscopic" geometric structures relevant to ongoing research programs. The second was to increase the efficiency of research by greatly reducing the number of possible geometric structures and thus eliminating the need to test the relevant hypotheses.

As we struggled to appreciate the significance of the electron microscope images in research, it became quite clear that geometric information, in isolation, had relatively little value. It did assume importance when taken in conjunction with information from related research. Around 1940 most of the early workers recognized how valuable it would be to be able to identify the chemistry of the structures that the TEM was making visible. Staining with heavy-metal compounds became useful for enhancing the contrast in images of organic materials, but that was a very blunt probe for identifying the chemistry of the specimen.

There was a flurry of activity in our laboratory to explore the potential of electron diffraction as a means of identifying the chemical structure of small particles and structures. The effort fizzled for a number of reasons. It was essentially worthless for small organic sturctures. Very small inorganic particles tended to be single or near single crystal structures with indeterminate orientation. Given the cumbersome facilities for image intensity measurements available to us at that period, it became clear that the limited results obtained did not justify the effort required. Nevertheless, the research had a payoff. We obtained invaluable experience in the design and operation of an electron-probe type of instrumentation.

The probe system used in the electron-diffraction experiments was a stationary probe that produced a transmission shadow image of the specimen. As the probe was focused closer to the specimen, the magnification in the shadow image increased. Ultimately, the part of

the specimen that covered the entire field was the area being bombarded by the probe, and therefore the area subject to analysis. Moving the specimen enabled the operator to select any point for examination.

In 1941 we had tried to build a scanning microscope. It was primarily an effort to apply high-resolution electron microscopy to metallurgical and other solid specimens. (This was prior to the development of replica techniques.) We did succeed in building such an instrument and obtaining a few pictures. However, it became painfully clear that the technology of 1940 was not up to our objectives and that we could do much more good by continuing the development of the TEM.

Early in 1943 I was browsing in the library and ran across an article by G. Ruthemann in *Naturwissenschaften*. Ruthemann had investigated the electron velocity distribution in the electrons transmitted by thin films of collodion. He showed that the discrete energy losses caused by the excitation of the K-levels of C, N, and O produced observable peaks in the distribution of electrons that had suffered energy losses due to inelastic collisions. Reading that article was an interesting experience in how the human mind works. In seconds my mind recognized the possibility of using the phenomenon for microanalysis and provided me with essentially the complete design of our first instrument! I finally truly understood what the patent attorneys meant when they talked about the "flash of inspiration."

We quickly modified one of our probe instruments and confirmed Ruthemann's results. We also extended them to include levels for some other light elements. We even did some work on the detection of the x rays emitted from a very small area of the specimen. All of this was very good research that we pursued very enthusiastically. Unfortunately, our enthusiasm began to turn to discouragement as we began to realize that, once again, our concepts were running far ahead of the technologies needed for their routine application. It was almost three decades before all the technologies needed to make microprobe analysis reasonably practical came together. Among them were some very major developments, such as very-high-resolution scanning transmission electron microscopes and very powerful small computers, as well as a broad range of very sensitive sensors. The stories of those developments will have to be told by the individuals involved.

For my part, the exercise of reexamining our activities from a vantage point of a half a century later has been a most interesting one and I appreciate the opportunity to do it. It also presents the opportunity to appreciate once again how valuable the inexperience and naivety of youth can be.