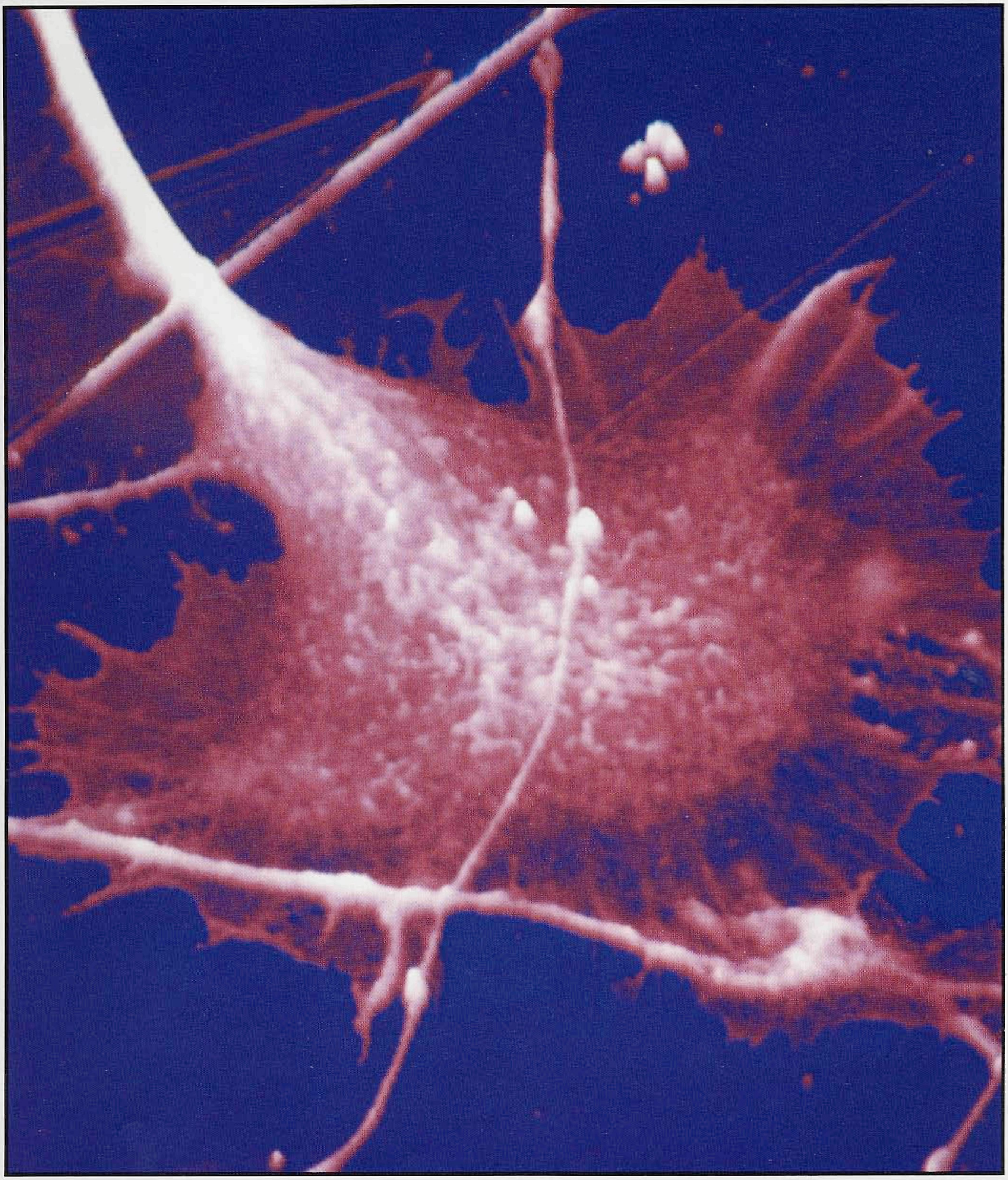
MICROSCOPY TODAY



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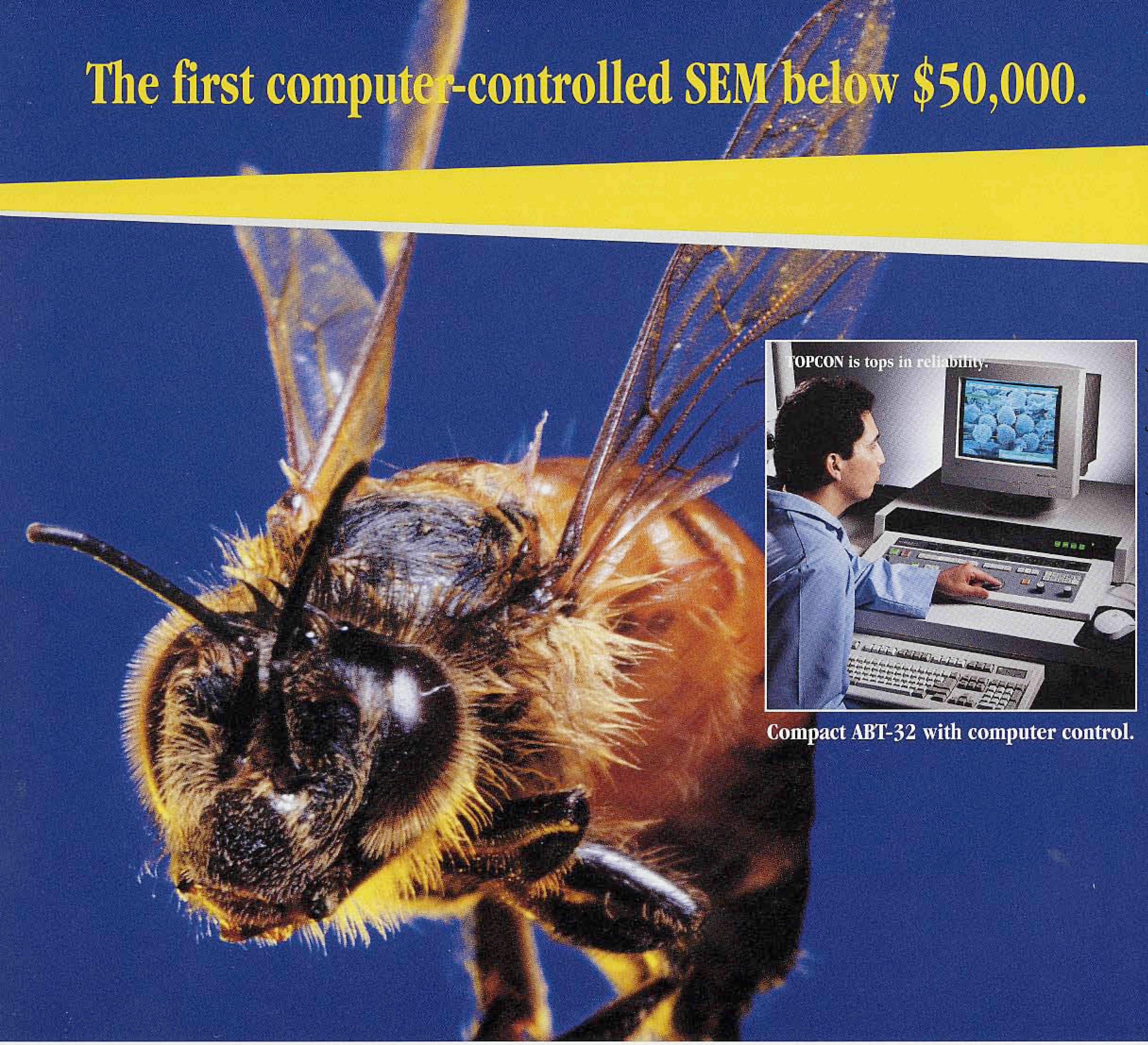

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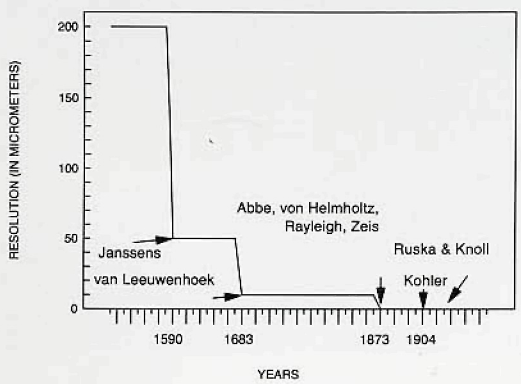
THE RESOLUTION REVOLUTION

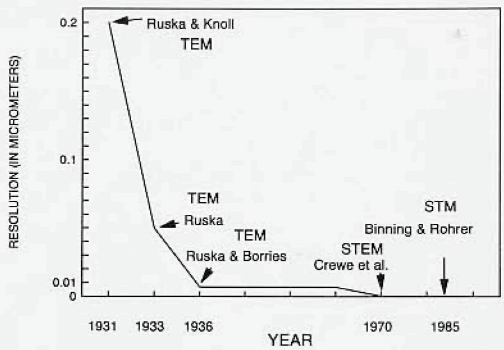
Stephen W. Carmichael, Mayo Clinic

The history of microscopy has focused on better and better resolution, as has been documented on these pages (*e.g.*, J.-P. Revel[1]). As one can see in the first graph, the early improvement in resolution was agonizingly slow, as it was dependent on technological improvements in optics and methods for preparing specimens. Hans and Zacharias Jensenn are credited with being the first to put two lenses in the same optical path to create a primitive microscope in 1590. Nearly a century later Antoni van Leeuwenhoek was impaling specimens on needles. Whereas van Leeuwenhoek and other observers were able to make significant observations to change fundamental concepts of their day, microscopists such as Ernst Abbe, Hermann Ludwig Ferdinand von Helmholtz, Lord John Rayleigh, and Carl Zeiss brought us to the brink of optimal performance of the light microscope. Abbé may have been the first to realize that the wavelength of light was a physical limitation to resolution. In 1876 he wrote "After all what we know from the science of our time, there is a limit to our vision which we cannot exceed. This limit consists of the nature of light itself... But we still have the consolation that there are lots of things between heaven and earth which we cannot imagine at this moment. Perhaps one day the human genius will find a way to transcend those limits which we cannot exceed. In my opinion, however, the instruments which one day will serve to observe the ultimate details of nature will only share the term *microscope* with the instruments of our time."[2] Ernst Abbé's remarks were remarkably prophetic and he would be delighted with the progress that has been made.

By the end of the 19th century apochromatic lenses, oil immersion, and other corrective measures had allowed August Köhler, using near ultraviolet, to reach the theoretical limit of resolution of the standard light microscope.



In the early 20th century, physicists were experimenting with new forms of irradiation having wavelengths considerably shorter than light. While there was fierce competition to find workable applications to microscopy, it was Ernst Ruska and Max Knoll who put two electromagnetic coils in the path of an electron beam. They first tested their instrument by using each coil separately. On 7 April 1931, they used the second coil to magnify the image produced by the first, just as the Jensenns had put a second lens in an optical path. The magnification achieved on that day was only 17.4X, but the transmission electron microscopes in routine use today are only technical refinements of this first instrument. Ruska by himself and working with Bodo von Borries built electron microscopes that surpassed the resolution of the light microscope.



As with the light microscope 300 years earlier, the transmission electron microscope did not become useful to biologists until details of specimen preparation were worked out. It was not until the 1950's, with the introduction of proper fixatives for preservation and epoxy resins for embedding that the research potential of the electron microscope was realized. Later, a breakthrough to very high resolution was made by Albert Crewe and co-workers using a scanning transmission electron microscope. They achieved atomic resolution using the Z-contrast method.

The next technological leap came unexpectedly (at least to me): the new generation of high resolution microscopes are not optical instruments, but rather sensitive electronic devices. Binning and Rohrer took advantage of the fact that the probability of finding an electron beyond the surface of a conductor falls exponentially with distance beyond the surface boundary; since electrons act as though they are digging tunnels, the effect is known as tunneling." They exploited this phenomenon by placing a very sharp electrode tip in contact with the "electron cloud" of the specimen. The application of a voltage and the sample causes electrons to flow in a narrow channel in the electron cloud; this is the

- Continued on Last Page -

Front Cover Image

On the cover is an atomic force image of axonal projections from a neuron isolated from the snail Helisoma and imaged using Digital Instrument's new BioScope Atomic Force Microscope (AFM). The BioScope AFM combines optical and atomic force microscopy to provide an effective magnification range of 25 to 10,000,000X. Its ability to operate in liquids and its highly rigid design provide superior stability and image resolution.

The spatulate feature in the image is the neuron's growth cone, a specialized structure that senses the environment as the axon grows. This structure was identified optically and then imaged with the AFM to reveal features which can not be seen with other techniques. Digital's proprietary TappingMode™ technique was used to prevent damage to the sample. Digital Instruments, Inc. Tel.: (800)873-9750 or (805)899-3380.

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Don Grimes, Editor