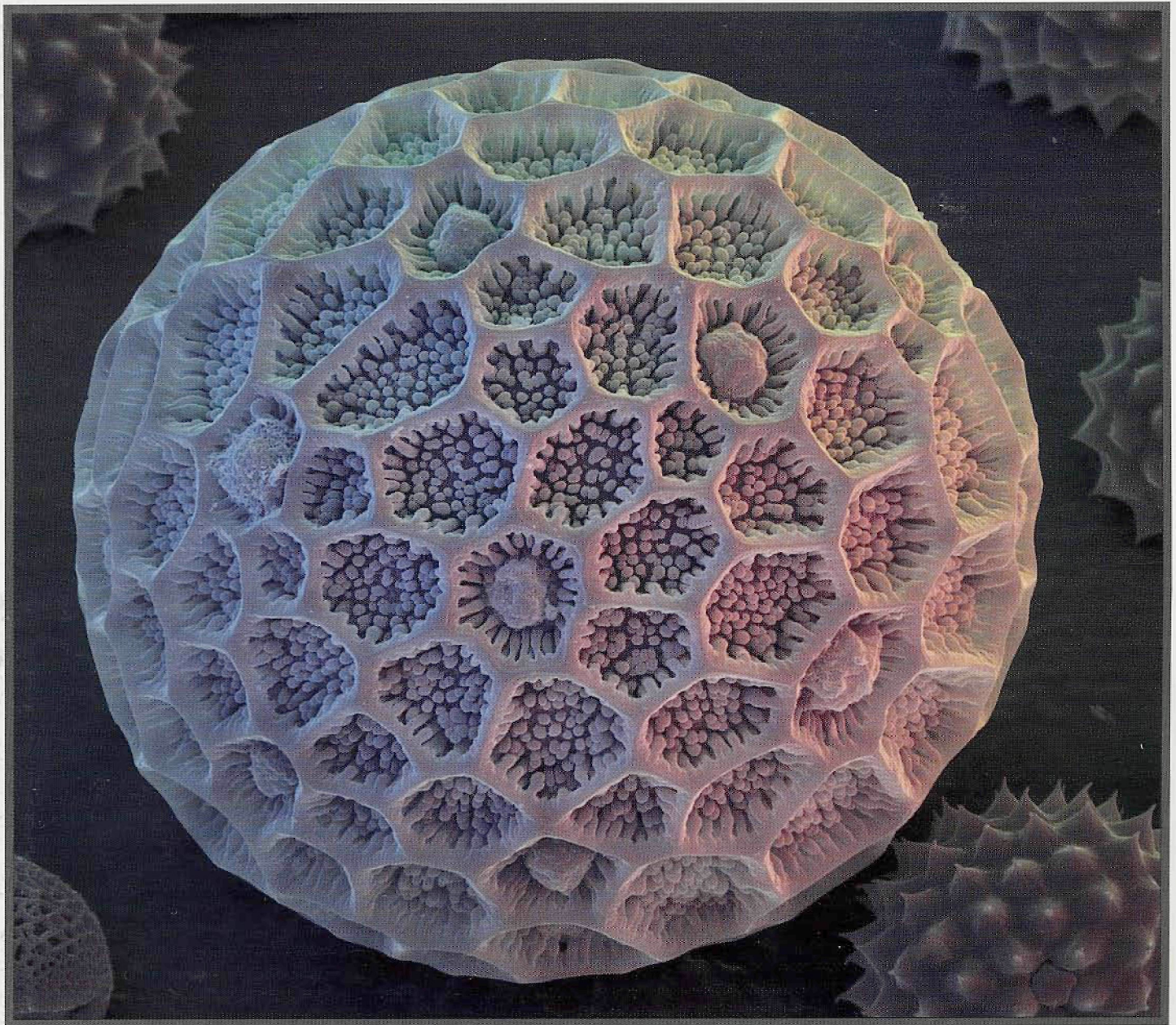


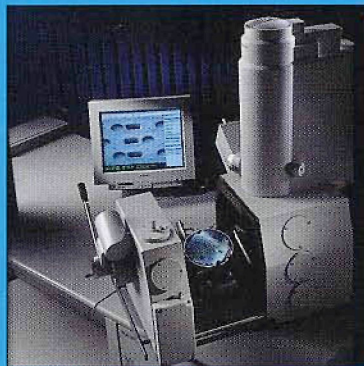
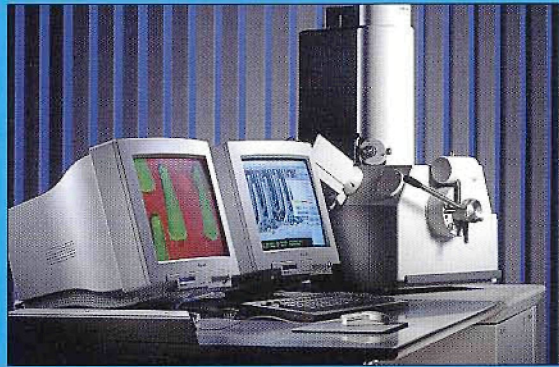
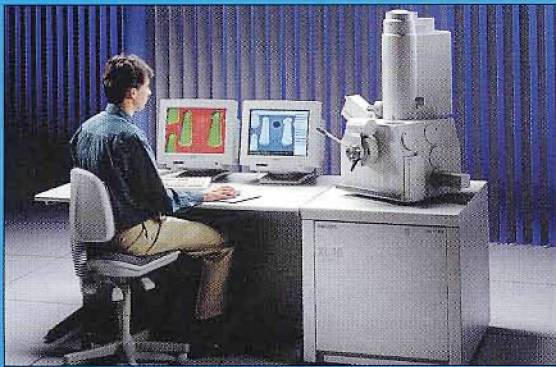
# MICROSCOPY TODAY

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# Get Down to the Fine Details



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# PHILIPS

# IMAGING WITH PHOTOELECTRONS

Stephen W. Carmichael,<sup>1</sup> Mayo Clinic

Most of us have used fluorescence microscopy and are all too familiar with the advantages and disadvantages of this tool: easy identification of one or more labels that fluoresce at different wavelengths (color) against the relatively dark background of the biologic substrate, but we can't always get the spatial resolution that is needed to answer our questions. Electron microscopy, both transmission and scanning modes, offer much better spatial resolution, but present problems in localizing a label, particularly on the surface of a cell. Wouldn't it be great if an imaging technique were available that gave us the possibility of localizing one or more labels on the surface of a cell with resolution approaching electron microscopy. Karen Hedberg, Bruce Birrel, Douglas Habliston, and Hayes Griffith at the University of Oregon<sup>2</sup> describe photoelectron imaging as the electron optical analogue of fluorescence microscopy, offering some of the advantages of both methods.

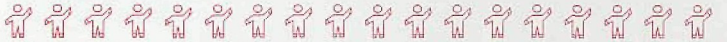
The photoelectron microscope relies on the photoelectric effect to create an image. In brief, the photoelectric effect describes the release of electrons from near the surface of a specimen caused by an exciting light, from an ultraviolet (UV) source in this example. The depth of the surface that participates in this phenomenon approximates the thickness of a plasma membrane, so the photoelectron microscope is ideal for visualizing this organelle. The electrons that leave the surface are of very low energy ( $\leq 1$  eV), so the specimen must be examined in a vacuum. This is the unfortunate part, since this requires fixation of the specimen, either by chemical or cryo methods, imposing some of the same limitations as electron microscopy.

But the good news is that an image can be restricted to the surface and one or more labels can be made to stand out clearly on a dark background, with much better resolution than with light optical methods. How does this work? The rate of emission of electrons (the emission current) varies with the nature of the material and the wavelength of the exciting light. Generally, at shorter wavelengths the emission currents of a cell surface and a silver-enhanced colloidal gold label tend to converge, meaning that the amount of electrons from the cell and the label are nearly the same and cannot be distinguished from one another. Happily, at longer wavelengths, the emission currents from these two materials differ rather widely, the result being that the gold labels light up against the darker cell surface. The micrographs published by Hedberg *et al.* resemble those taken with a

scanning electron microscope, except that particles on the surface are much more visible.

The exciting light source can be manipulated, either with filters or a tunable UV laser. With different labels having different emission currents, more than one label could be employed. Hedberg *et al.* present the thrilling prospect of an imaging technique that can localize several different moieties on the cell surface with the resolution of the electron microscope. They present several interesting examples, but you can appreciate that the possibilities are endless!

- 1 The author gratefully acknowledges O. Hayes Griffith for reviewing this article.
- 2 Hedberg, K.K., G.B. Birrell, D.L. Habliston, and O. Hayes Griffith, Tunable label contrast on the cell surface: Photoelectron imaging with multiple wavelength excitation, *J. Micros. Soc. Am.* 1(6): 253-261, 1995.



## Microscopy Images & Prints

*The image on the front cover of this issue, as well as on previous issues, is a small, low resolution, reproduction from a series of sixteen high quality, poster sized, lithographic prints of scanning electron microscope images by scientist/photographer David Scharf.*

*Mr. Scharf's images have earned critical acclaim for their artistic content and technical perfection and have been featured in Life, Time, National Geographic, Scientific American, Discover, Natural History, Harpers, The Saturday Evening Post, etc.*

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*All sixteen prints are illustrated in the January/February issue of this publication. And one may receive a no cost copy of this image summary by contacting Microscopy Today.*

- - - Ed.

### Front Page Image

#### SEM Photograph of a Polygonum Pollen Grain

Imaged on an ETEC SEM at 5 kV using the SEM Wideband Multi-Detector Color Synthesizer (designed, built and patented by David Scharf). Then acquired digitally at 2,048 X 1,536 pixels directly into a Macintosh Power PC as a TIFF file, using Digital Micrograph software and Digiscan hardware. Then output to a CELCO film recorder, using Ektachrome 100+ film, to produce a 4x5 transparency.

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Don Grimes, Editor