MICROSCOPY TODAY

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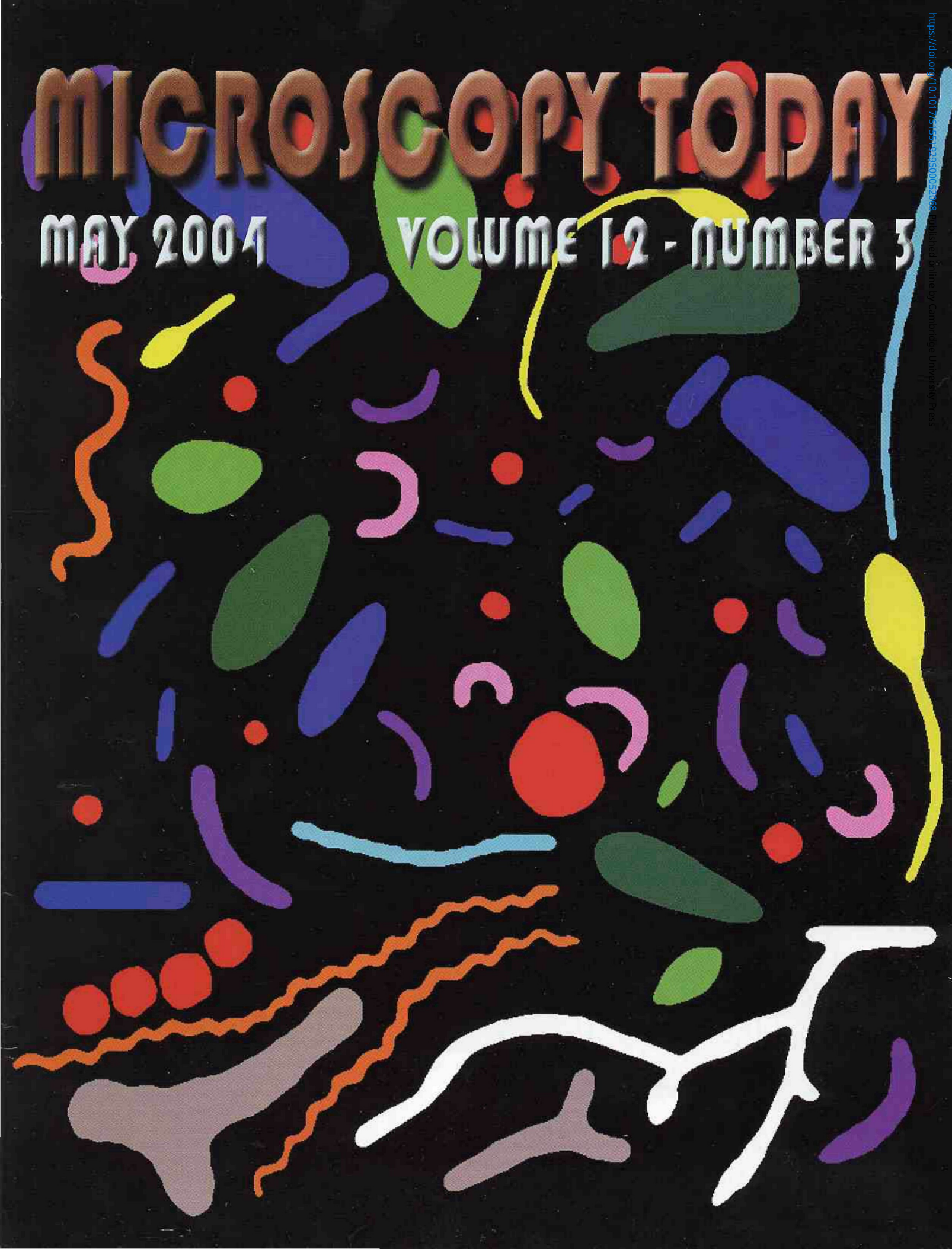
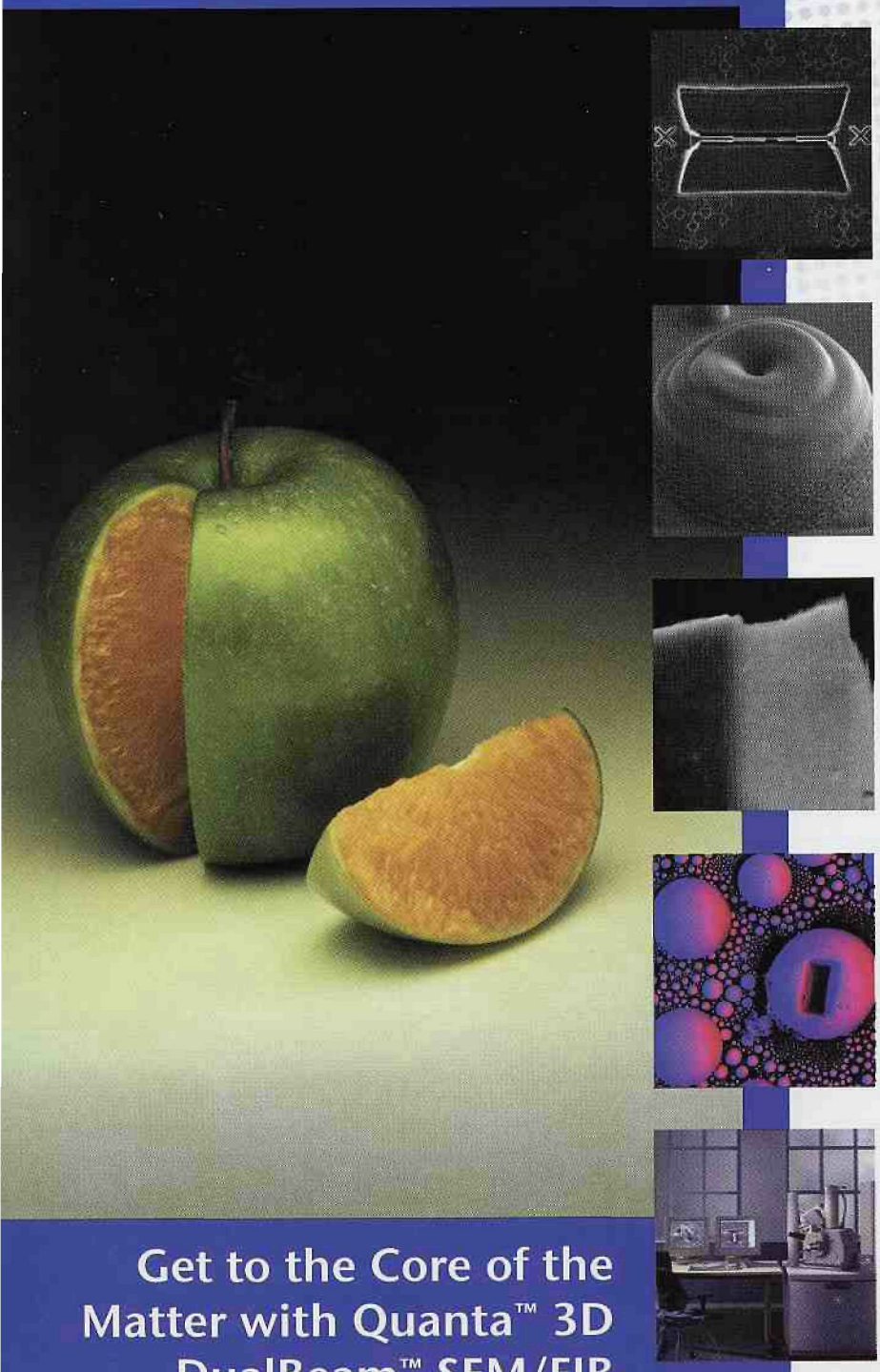


Is Your Sample Hiding Secrets from You?



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Scanning Wet Specimens

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Would it be useful if you could examine a wet, perhaps even a living, specimen in the scanning electron microscope? Of course we think that this would be impossible, given the vacuum the specimen would be subjected to in the microscope. However, in the realm of materials research, the Environmental Scanning Electron Microscope (ESEM) is being developed. Apparently this instrument has not been fully appreciated and utilized by biologists.

As reviewed by Athene Donald,2 a pioneer in developing the ESEM, this instrument has been commercially available for a decade. It is still evolving as a useful tool in looking at materials such as cement, natural fibers, and aqueous dispersions. Applications of the ESEM to biological studies are extremely limited. However, studies on biological samples, even living cells, are possible (although not without problems and limitations).

One of the keys to the design of the ESEM is a column that allows the gun to discharge electrons in a high vacuum (~10-6 torr) yet still allow the sample to exist in an atmosphere of several torr. This is accomplished with a system of differential pumping and pressure-limiting apertures that allow an approximately 107 difference in pressure surrounding the gun versus the specimen. Nevertheless, the temperature of the specimen chamber turns out to be critical. Although the pressure surrounding the specimen is high in some respects, it is low in a physiological sense. The temperature needs to be around or below10° C or else evaporation from the specimen would occur rapidly, thereby changing the sample. However, slight changes in temperature can lead to changes in hydration of the specimen, and resultant morphological changes can be observed in real time. Even the results of chemical reactions may be observed.

The other key to the ESEM is how the electron beam interacts with the gas (which could be water) in the specimen chamber. Gas molecules are ionized by the electrons (designated as secondary electrons if their energy is below 50 eV, or backscattered electrons if their energy is above 50 eV) emitted from the sample when it is struck by the focused narrow beam of electrons from the gun. Each such ionizing collision generates one or more additional "daughter" electrons resulting in a "cascade amplification." All of these electrons are attracted to the positively charged detector. The detector is a specially designed component, because the detector in a conventional SEM would not work at these pressures. The positive ions drift down toward the surface of the sample. In this manner, the gas in the specimen chamber is not a passive participant in formation of the image, but actually plays a role in signal detection.

There are some important obstacles to be overcome before the ESEM could be a routine instrument for biologists. The first one, as already alluded to, is the design of the detector. According to Donald, we can expect significant progress from the manufacturers (FEI/Philips has the trademark for ESEM, but other manufacturers are selling "variable pressure" instruments) in designing detectors that work better at higher pressures. The other obstacle is interpreting contrast in the ESEM. In many cases, contrast may be no different than under conventional circumstances, but fully understanding contrast in the ESEM is still far from complete.

It is already clear that the ESEM has made important contributions to materials science, particularly in examining the morphology of hydrated specimens and insulators (because a conductive coating is not needed). However, what interests me the most is the potential to use the ESEM to examine biologic specimens where only minimal preparation of the specimen is required. There is some question if living cells could survive the probing beam. Examination at low magnifications, requiring a low dose of electrons, would increase survivability, but offers little advantage over light microscopy. It is an exciting possibility that more sensitive detectors will be developed that would allow low doses of electrons to give us high-resolution images of the surfaces of living cells, perhaps even in real time! ■

References:

[1] The author gratefully acknowledges Dr. Athene Donald for reviewing this article.

[2] Donald, A.M., The use of environmental scanning electron microscopy for imaging wet and insulating materials, Nature materials 2:511-516, 2003.

INDEX OF ARTICLES

ABOUT THE COVER

Classification of Microbial Morphotypes by CMEIAS

This issue of **Microscopy Today** features the first free software release version of the Center for Microbial Ecology Image Analysis System (CMEIAS), which analyzes digital images of microorganisms and classifies their morphotypes automatically. The figure shown illustrates the classification output image whereby each different microbial cell is uniquely pseudocolored according to its assigned morphotype. Image provided by Frank Dazzo. See pages 18-23 for details.