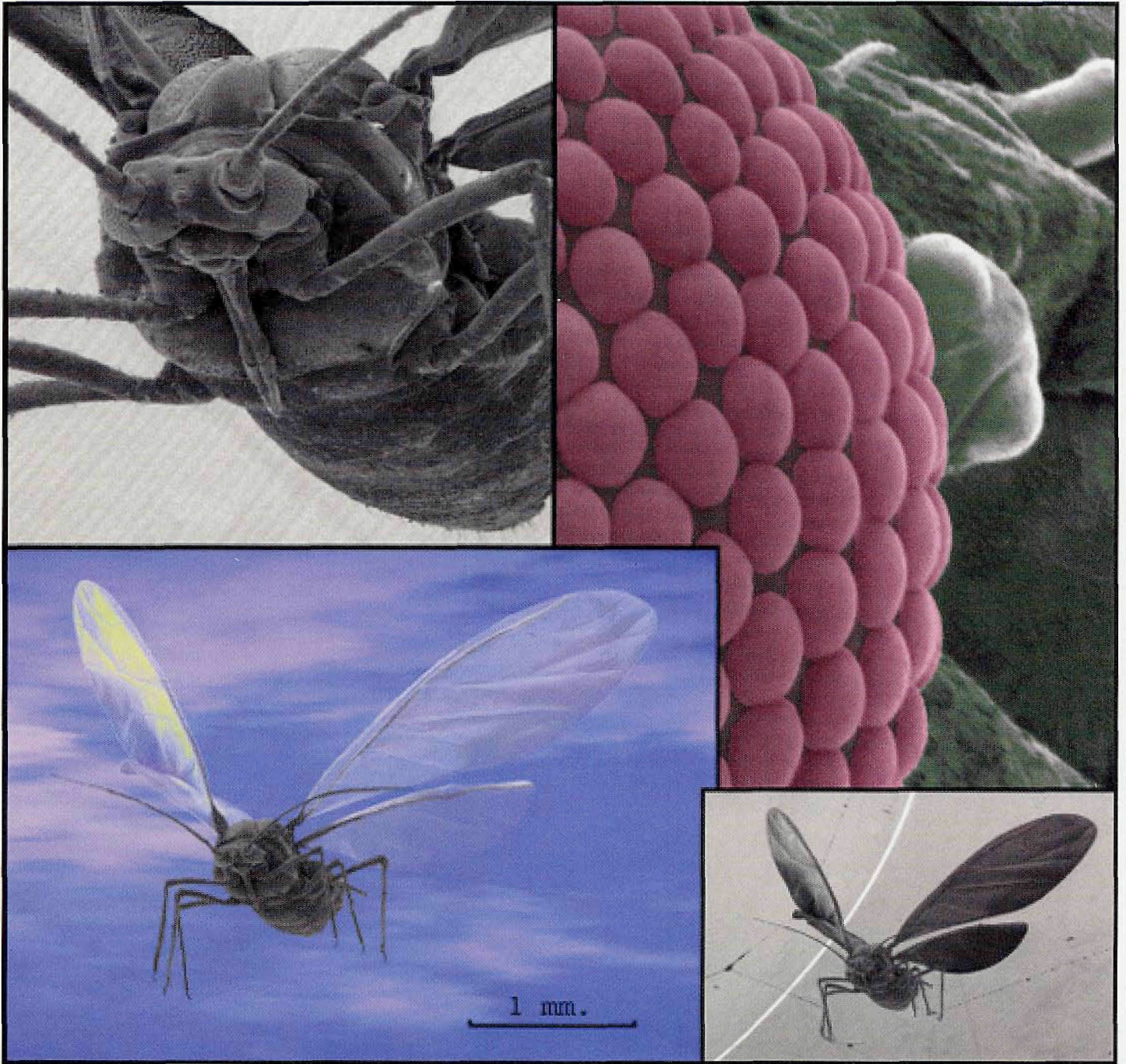


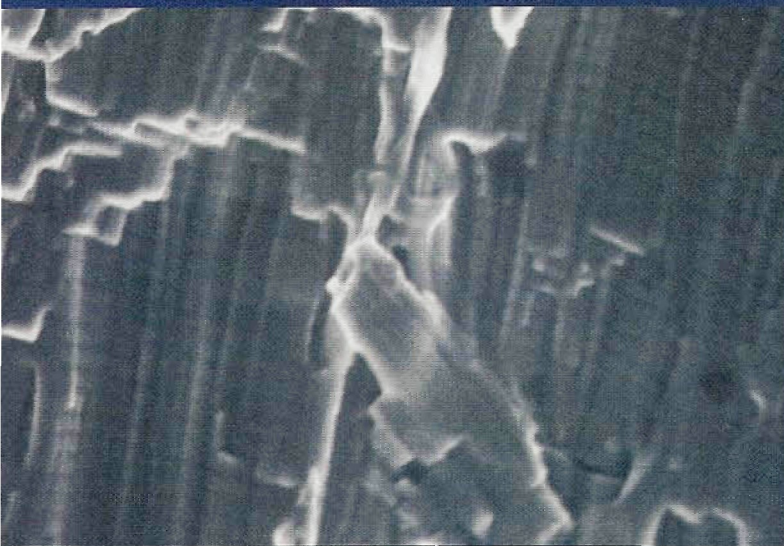
MICROSCOPY TODAY

MAY 1997

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Rat skin muscle fabric -
in it's original - hydrated - form.



Acc.V	Spot	Magn	Det	WD	Exp	-----		20 µm
10.0 kV	3.0	1500x	GSE	7.3	13	WET	5.8 TORR	ESEM

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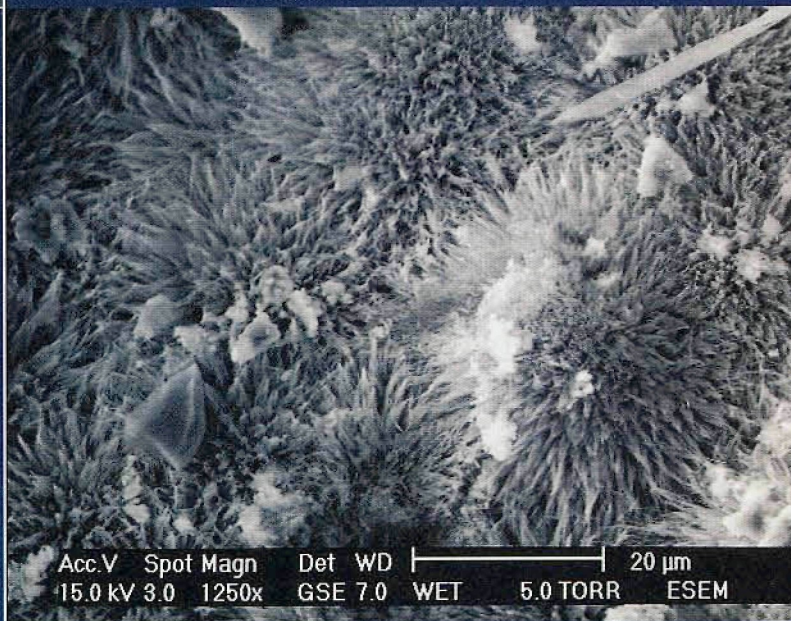
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Acc.V	Spot	Magn	Det	WD	-----		20 µm
15.0 kV	3.0	1250x	GSE	7.0	WET	5.0 TORR	ESEM

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EXOCYTOSIS AT THE ATOMIC LEVEL

Stephen W. Carmichael,¹ Mayo Clinic

As reviewed in this column on previous occasions, the atomic force microscope (AFM) is steadily making headway as an instrument that can make important contributions to biologic observations. Although the AFM is capable of operating in an aqueous environment, relatively little use has been made of this property to examine cellular structures under conditions that resemble those *in vivo*. A breakthrough in this regard was recently made by Stefan Schneider, Kumudesh Sritharan, John Geibel, Hans Oberleithner, and Bhanu Jena.² of Yale University and the University of Würzburg.

They examined the surface of isolated pancreatic acinar cells that could be stimulated to secrete amylase. The time course of this secretion is known to be minutes (rather than seconds or less with neuroendocrine or neuronal cells) which is compatible with the scan time of the AFM. They observed the cells with an optical microscope while probing in the "contact" mode, using a very low vertical imaging force (<1 to 3 nN). Silicone and silicon nitride tips were used with spring constants of 0.25 and 0.06 N/m, respectively. Stimulation of the cells resulted in a change at the apical surface of the cells, and not in other regions. "Pits" measuring 500 to 2,000 nm and containing 3 to 20 depressions measuring 100 to 180 nm in diameter were observed. The time course of changes in the "depression" size correlated positively with changes in amylase secretion from the living cells. Schneider *et al.* postulated that secretory vesicles transiently fuse at the plasma membrane depressions and release their contents at the apical surface. Results from this study also suggest that the cytoskeletal protein actin may influence the structure of these depression.

In a commentary in the same issue of *PNAS*, Julio Fernandez³ applauds the efforts of Schneider *et al.* as an important breakthrough, and points out a potential limitation of applying AFM to image exocytosis. The force needed to deflect the probe of the AFM may exceed the "stiffness" of the plasma membrane and what may be imaged instead is the underlying cytoskeleton. I imagine that a blind person can build an image in their mind of a structure by tapping (probing) the structure with a cane or similar physical extension of their tactile sense. If they are tapping a relatively soft

drape over a stiffer framework, they might logically build an image of the frame, not the overlying drape. This is not necessarily bad, as the frame may be even more interesting than the drape. Without proper correlative studies, Fernandez points out that we cannot assume that the AFM images in the present study are necessarily an accurate topographical map of the plasma membrane undergoing exocytosis.

Whether or not Schneider *et al.* have imaged the plasma membrane or the subcortical cytoskeleton during exocytosis remains to be determined. However, this stands as an important study for demonstrating the application of AFM to a biologic function. ■

1. The author gratefully acknowledges Drs. Bhanu Jena and Julio Fernandez for reviewing this article.
2. Schneider, S.C., K.C. Sritharan, J.P. Geibel, H. Oberleithner, and B.P. Jena, Surface dynamics in living acinar cells imaged by atomic force microscopy: Identification of plasma membrane structures involved in exocytosis, *Proc. Natl. Acad. Sci. USA* 94:316-321, 1997.
3. Fernandez, K.M., Cellular and molecular mechanics by atomic force microscopy: Capturing the exocytotic fusion pore *in vivo?* *Ibid.* 94:9-10, 1997.



Front Page Image

SEM Photo of a Male Fly

The insect was still connected to a spiders web when placed in a Philips XL40FEG to be imaged with 500 volts emission.

Digital images were made in greyscale as tiff files and exported from the microscope via network into a Macintosh Power PC.

The greyscale tiff files were processed into colour images by Mr. M. Biemans using software specific for this purpose. Lay-out also by Mr. Biemans.

More interesting images can be viewed at: <http://www.peo.philips.com>

Courtesy of Philips Electron Optics.

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Don Grimes, Editor