MICROSCOPY TODAY

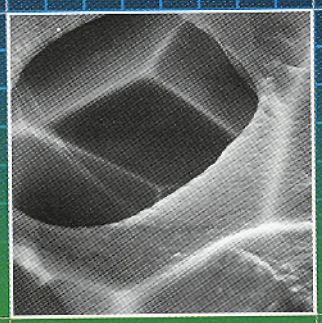
NOVEMBER 2000

ISSUE #00-9

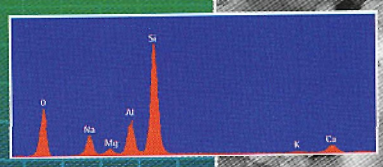
https://doi.org/10.1017/S1551929500059344 Published online by Cambridge University Press

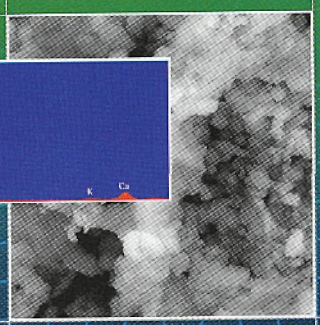
Ultra-high resolution FESEM Plus Variable Pressure?

Have it all – with LEO

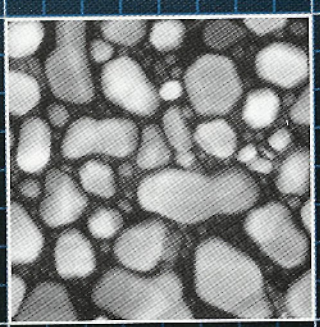


Styrofoam, uncoated, 30kV, 1,000x,
chamber pressure 35 Pa, VPSE detector





Lead oxide, uncoated, 21kV, 10,000x,
chamber pressure 34 Pa, VPSE detector



Gold on carbon, 30kV, 50,000x
chamber pressure 40 Pa, VPSE detector

Two unique innovations, one superb instrument! GEMINI and VPSE! Market leading ultra-high resolution electron optics and Variable Pressure Secondary Electron imaging brought together for the very first time with the new LEO 1530VP.

At last, it is possible to obtain ultra-high resolution imaging and analysis on specimens that are completely non-conducting, moist or outgassing for which a higher chamber pressure avoids time consuming specimen preparation. Specimens such as packaged semiconductor devices, glasses and ceramics, plastics, biological tissues and many more. Features include:

- Ultra-high resolution in high vacuum mode, at high and low beam energy, using the unique in-lens detector
- Ultra-high resolution in variable pressure mode, at high and low beam energy, using the patented VPSE detector

Why compromise? For supreme flexibility in ultra high resolution imaging and analysis, you can Have it all with the NEW LEO 1530VP.

USA: New York Tel (1) 914 747 7700 Fax (1) 914 681 7443 E-mail info@leo-usa.com
UK: Cambridge Tel (44) 1223 414166 Fax (44) 1223 412776 E-mail info@leo-em.co.uk
Germany: Oberkochen Tel (49) 73 64 94 6137 Fax (49) 73 64 94 4851 E-mail info@leo.de

Call Toll Free 800-356-1090

www.leo-usa.com

LEO

The power to resolve

https://doi.org/10.1017/S1551929500059144 Published online by Cambridge University Press

UNZIPPING A MEMBRANE

Stephen W. Carmichael and Julio M. Fernandez,[1] Mayo Clinic

The atomic force microscope (AFM) is well known for its outstanding spatial resolution, but it is becoming increasingly useful as the instrument for force spectroscopy. In the force spectroscopy mode, the AFM can measure tiny tension forces, in the piconewton (pN) range. Daniel Müller, Wolfgang Baumeister, and Andreas Engel have used the AFM in both the imaging and force spectroscopy modes to pull proteins out of membranes in a controlled fashion.[2]

Müller *et al.* used *Deinococcus radiodurans*, a bacterium best known for its high resistance to radiation (as its Genus name implies), as their test subject. They extracted a highly regular membrane from the bacterium, the hexagonally packed intermediate (HPI) layer. They mounted the HPI on mica, so that the hydrophilic outer surface of the HPI adsorbed strongly to the mica, exposing the hydrophobic inner surface to the silicon nitride AFM stylus. With the AFM in the contact imaging mode, they could clearly visualize what looked like a closely-packed group of circular pies, each pie cut into six equal pieces called protomers. At the center of the pie was a pore, whose diameter was controlled by the arrangement of the protomers.

After imaging the HPI with the AFM, Müller *et al.* brought the AFM stylus into contact with the specimen for one second, allowing the stylus to contact the protomers on the inner layer of the HPI. When the stylus stuck to a protomer on the inner surface, and the stylus was withdrawn, interesting measurements were made. In most cases, the force spectrograph showed a deflection of the stylus indicating a bond until the stylus was about 10 nm from the surface where the stylus sprang back indicating the bond had ruptured. To use our analogy, a piece of pie was picked up, but was dropped back into the pie before we got very far. In the remaining cases, three different spectrographs were seen. In the first case, a force peak of about 90 pN was seen after retracting the stylus about 15 nm, indicating that a molecular bridge was extended from the stylus to the surface. This is like a small thread was present that pulled our piece of pie back into the pie. In the second case, a force peak closer to 300 pN was measured, about three times higher than the first case when the protomer detached from the stylus and was pulled back into the hexagon by the molecular bridge. After this measurement, the follow up image made with the AFM showed a protomer was missing (follow up images after the other measurements did not show any changes). The thread connected to our piece of pie had been broken, and the piece was extracted from the pie. But the best is yet to come.

The third type of force spectrograph is the most amazing. There were up to six equally spaced peaks in the 200-300 pN range, with the peak-to-peak distance corresponding to about 7.3 nm. So we have picked up a piece of pie to find each piece of pie threaded together, occasionally allowing us to pick up the entire pie, piece by piece. Müller *et al.* referred to this as "controlled unzipping." This demonstrates the ability of the AFM to not only measure intermolecular forces, but to also directly visualize and correlate the resulting structural changes.

With current techniques it is difficult to study the structure of membrane proteins. The novel approach demonstrated by Müller *et al.* greatly increases our ability to examine the structural features of membrane proteins *in situ*. It will not be long until the single molecule AFM technique demonstrated in this article can monitor the conformational changes of ion channels and receptors during their physiological activity. ■

1. The authors gratefully acknowledge Dr. Andreas Engel for reviewing this article.

2. Müller, D.J., W. Baumeister, and A. Engel, Controlled unzipping of a bacterial surface layer with atomic force microscopy, Proc. Nat. Acad. Sci. USA 96:13170-13174, 1999.

INDEX OF ARTICLES

https://doi.org/10.1017/S1551929500059344 Published online by Cambridge University Press