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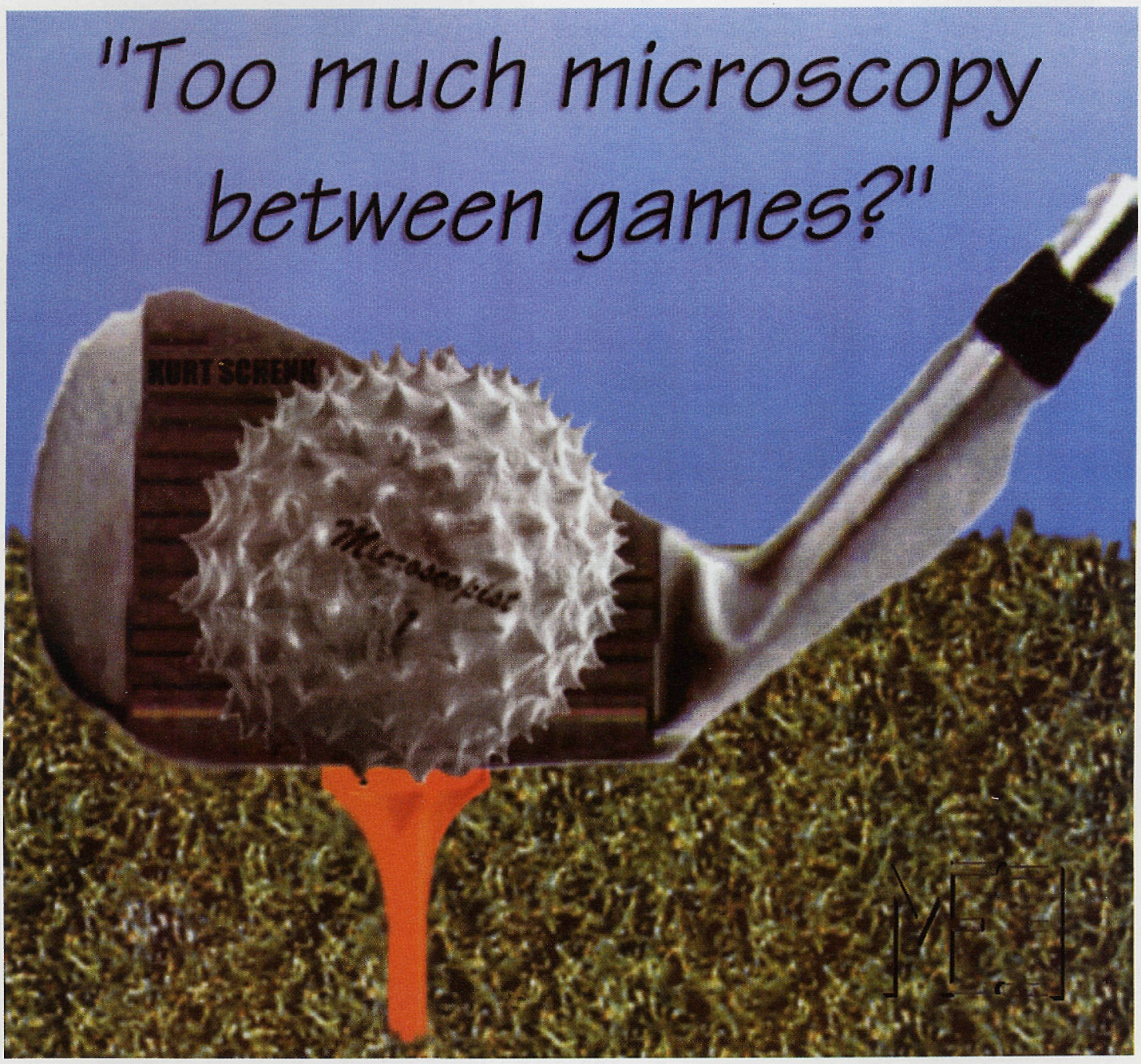

With the New LEO VPSE the difference is clear...

Take a look at the two micrographs of pollen on the left. Both were taken in variable pressure mode, one using a backscattered electron detector, the other using LEO's new VPSE (Variable Pressure Secondary Electron) detector. For low atomic number material such as this, the difference is clear-and so is the image taken with the VPSE!

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BREAKING OLD RULES

Stephen W. Carmichael,[1] Mayo Clinic

It has been dogma for more than a hundred years that the light microscope cannot resolve structures that are much closer together than the length of the wavelength of light. This limit is on the order of a few hundred nanometers. This rule was "broken" by using the electron beam as the illumination source. The shorter wavelength of the electron beam gave correspondingly better resolution, down in the nanometer range. The rule was "broken" again when non-optical scanning probe microscopes (such as the scanning tunneling and atomic force microscopes) were developed. This technology gave us resolution in the sub-nanometer range, imaging individual atoms and molecules. However, scanning probe microscopes scan the surface of the specimen, excluding what's underneath from our view. Wouldn't it be great if another rule could be "broken" so that we could look at individual molecules beneath the surface? As you have already guessed, this has been accomplished. Antoine van Oijen, Jürgen Köhler, Jan Schmidt, Michiel Müller, and Fred Brakenhoff have achieved super-resolution using visible light.[2]

They used a technique called Spectrally Selective Imaging to determine the position of single molecules within a crystal matrix. This technique takes advantage of the fact that a fluorescent molecule will behave slightly differently to a selected wavelength of light, depending on its immediate surroundings. van Oijen *et al.* used individual pentacene (an aromatic hydrocarbon) molecules embedded in p-terphenyl host crystal. For seven of these molecules, they found that the relative excitation frequency varied over a range of two to three thousand megahertz. Selecting one of these molecules they tune the excitation laser burn to achieve maximum emission from this molecule. Under cold (liquid helium) conditions, the centroid of the fluorescing molecule can be calculated to within 40 nm in the xy plane. The axial (z) position is obtained by measuring the radial size of the photon distribution as a function of the detector position along the optical axis This yielded a resolution of approximately 100 nm. This improvement in resolution represents an enhancement of about 20- and 65-fold in the xy and axial planes, respectively. This was repeated for the other molecules, each at its respective optimal excitation wavelength, resulting in a 3 dimensional map of the position of each pentacene molecule within the volume studied.

Interestingly, van Oijen *et al.* observed that the distribution of the pentacene molecules within the crystal was non-random. In three different experiments, the molecules were in a much more narrow axial portion of the crystal than would have been predicted by random distribution (within a plane of 1.3 μm rather than the expected 7 μm). They theorized that this resulted from local structural properties of the crystal matrix. On this length scale, this would be undetectable by other optical techniques

This is another demonstration of the old rules being "broken." By combining single-molecule spectroscopy with position-sensitive imaging, van Oijen *et al.* enhanced the limit of resolution using light by an order of magnitude. It was demonstrated that the method of Spectrally Selective Imaging may be used for molecules that undergo photobleaching or spectral diffusion, even if this occurs within a few seconds. It certainly seems possible to now determine the position of fluorescent molecules within structures other than a crystal, possibly within a cell, or within the nucleus. It will be exciting to see how this breakthrough will be utilized to map the position of molecules with greater precision than has ever been possible!

1. The author gratefully acknowledges Dr. Jürgen Köhler for reviewing this article.

2. Van Oijen, A.M., J. Köhler, J. Schmidt, M. Müller, and G.J. Brakenhoff, 3-Dimensional super-resolution by spectrally selective imaging, *Chemical Physics Letters* 292:183-187, 1998. See also van den Berg, R., Molecular imaging beats limits of light, *Science* 281:629, 1998.

Front Page Image
Third Prize - Just For Fun Micrograph Contest

At the recent Microscopy & Microanalysis '98 Conference in Atlanta, Microscopy Today held a "For Fun" contest - with micrograph entries being a composite of two or more images, at least one being microscopical in nature. With fourteen entries and hundreds of "votes", Kurt Schenk, from Material Evaluation and Engineering, Inc. won the third prize. His description of this third prize image is, "This image is a reflection of one possible result from too much microscopy. The "Golf Ball" is actually an ambrosia (ragweed) pollen grain imaged using a scanning electron microscope".