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MOLECULAR IMAGING IN HIGH ELECTRIC FIELDS*

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For the past thirty years, attempts have been made to use the field-electron emission microscope (FEEM), and the field-ion microscope (FIM) to image organic molecules.¹ These attempts were inspired by the simplicity of the techniques and the potential for achieving high image contrast, magnification, and resolution. Since both microscopies rely on direct point projection for imaging, there is no need for electron-lens systems or devices to minimize specimen vibration. As a result, both techniques offer the hope of achieving high quality molecular images with a minimum of effort.

In the early 1950's the FEEM was the only microscope which had demonstrated a magnification of $10^6$ at an image resolution better than 2nm. Since it had already been used to image the diffusion of monomolecular films on clean metal surfaces, there was growing optimism that individual organic molecules could be imaged as well. In 1951, striking FEEM patterns of flaventhrene and copper-phthalocyanine were obtained which displayed the known symmetry of each molecule (two-fold and four-fold, respectively). Unfortunately, the success of these experiments was relatively shortlived. By the mid 1950's, experiments conducted with a large number of structurally different molecules produced primarily two- and four-fold images. It became clear that FEEM patterns were reflecting some common feature of all the imaged species, but were not imaging the actual contour of each molecule. In 1956,

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the low temperature field-ion microscope (FIM) was introduced. It provided another opportunity for examining molecular structure, but with greatly improved image resolution (0.2 nm under optimum conditions). However, the higher field strength associated with field-ion imaging presented a problem. The average electric field, \( F \), required for field-ionization is quite large (\( F > 20 \text{ V/nm} \)). Associated with the field is an outward directed field-stress, or electrostatic pressure, \( -P = \varepsilon_0 F^2/2 \). Even at the lowest FIM imaging fields (obtainable with hydrogen) \( -P = 2 \times 10^9 \text{ joules/m}^3 = 128 \text{ tons/in}^2 \). Such stresses can be an advantage in conventional FIM imaging because contaminant species left by the tip etching process will tend to be removed as imaging conditions are reached. They are a disaster if one wishes to image a biomolecule because the molecule can be severely distorted, or even removed from the surface prior to the imaging event.

Several ingenious schemes were developed in an attempt to circumvent the high field-stress encountered during FIM imaging. These essentially involved embedding a molecule in a metallic matrix which could survive imaging,\(^1\) or observing an FIM pattern at the earliest stages of image formation where the field strength was a minimum.\(^3\) The resulting images were not convincing. Few, if any, exhibited recognizable structure. Most were transient, and reproducibility was very poor. Furthermore, there was never any assurance that a molecule had actually been deposited within the imaged area of the tip surface. Unlike the electron microscope, the FIM can examine only a small, fixed area of the specimen surface. There is simply no way to move from place to place over macroscopic distances in order to find a region where molecular deposition might be appropriate for imaging. The limited field-of-view of an FIM image is not a trivial matter because it requires one to place a few isolated molecules within a well defined region of the tip apex. In the past, the success of a molecular deposition procedure was based on the PEEM or FIM image which it produced. Since these were not particularly convincing, previous deposition procedures could be very unreliable. In order to examine the deposition problem in more detail, a direct method of determining molecular coverage on a field-emitter tip was developed.\(^4\) A transmission electron microscope
(TEM) is used to view a molecule coated tip in profile. Since a metallic field-emitter scatters electrons very effectively, it appears opaque in a TEM micrograph. Unfortunately, most organic molecules are weak electron scatters and are essentially invisible at submonolayer coverages. One notable exception is ferritin. Ferritin is a nearly spherical molecule approximately 11nm in diameter. It consists of a protein shell (apo-ferritin) which surrounds a core of ferric hydroxide micelles. These are arranged in a roughly tetrahedral structure measuring some 6nm in extent. Because the core contains about 5000 iron atoms it scatters electrons very well and can be easily seen on a field-emitter tip in the TEM.\textsuperscript{5}

Recently, we have been successful in imaging submonolayer coverages of ferritin on tungsten field-emitter tips with a new low-field shadowing technique.\textsuperscript{6} Unlike conventional shadowing methods used for TEM analysis, we remove the shadowing species in order to form an image. An electric field of a few volts per nanometer is used to create, and radially project ions formed from a layer of benzene condensed in vacuum onto the apex of a tip coated with ferritin molecules. To achieve an image resolution which is better than 3nm, the tip is cooled to 150 Kelvin. By increasing the electric field linearly with time from zero to approximately 4 V/nm, the benzene layer is gradually removed revealing the embedded molecules. A chevron CEMA detector images the arrival of the benzene ions. As ferritin molecules are exposed, dark regions appear in the benzene ion image. The shape of these regions reflect the morphology of the ferritin molecules at a fixed distance from the tip surface corresponding to the thickness of the remaining benzene layer. Sequential images can be recorded on film or video tape during the removal of the benzene layer. Four representative images (labeled L-0) are shown in Figure 1. By processing these images digitally, a three-dimensional reconstruction of the ferritin molecules can be obtained. A reconstructed image is shown in Figure 1B which corresponds to the region outlined in white in Figures 1A-10. In the reconstruction, the dark regions of each CEMA image were assigned a different grey level, and then superimposed. The lightest region (L) is furthest from the tungsten tip surface.
From the appearance of several reconstructed images, we have concluded that the ferritin molecule is often distorted. The distortion does not seem to be associated with imaging which has been shown to be nondestructive and reproducible, even after exposure of the tip to laboratory ambient. We cannot determine if the distortion results from some inherent interaction of apo ferritin with the tungsten surface, or from unavoidable denaturing of the protein prior to imaging. At this time we do not know how serious a problem the distortion represents. Attempts to assess its importance by imaging a more highly structured molecule (e.g., heavy meromyosin) have not yet been successful. Since other molecules cannot be observed on the tip surface in the TEM we do not know if the deposition procedure (developed for ferritin) is at fault, or some unknown aspect of the imaging technique. Until a more highly structured molecule is imaged, we will not know how much information can be extracted from the three-dimensional imaging capability of the present microscopy.

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References


Figure 1. Point-projection imaging of ferritin on a tungsten tip.