

GENERAL PHYSICS



## I. MOLECULE MICROSCOPY

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## 1. SCANNING DESORPTION MOLECULE MICROSCOPY (SDMM)

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John G. King, Jeffrey G. Yorker

The ideas of molecule microscopy were first discussed in our group around 1970 and were briefly presented in our 1973 paper.<sup>1</sup> They arose naturally from previous research on evaporation from liquid helium, which was abandoned only because the molecule microscope appeared compellingly interesting. It uses a new contrast mechanism which is surface-specific and gives information about the surface through the mapping of variations in the binding energy of adsorbed molecules. There are applications of these techniques in material science, but very high resolution is necessary, since the phenomena are on the scale of angstroms. In biology, however, important structures on the scale of microns exist, which is one reason that we are interested in applying the instrument to problems in biology, where surface properties of all kinds are being considered and are currently under intense study by many techniques.

Over the last few decades many new probes of bulk and surface properties of great value in the material sciences have evolved. Surface techniques have become especially significant, as the importance of the surface has been understood, as theoretical developments have taken place, and most of all (although at a technical level) as the vacuum in which the sample is placed has been improved to  $10^{-12}$  Torr and better, with monolayer times of  $\sim 10^7$  seconds. The variety of microprobes using electrons, ions, and x-rays for both stimulation and detection has multiplied

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greatly as well, and many of them are being used in the biological sciences, where it is desired to determine, element by element, what is present in a small sample of tissue or other bio-material. The classical imaging instruments — the optical microscope, with refinements which variously use interference techniques, television imaging, and computer-assisted methods; and the electron microscope in its many forms — have all undergone continued development and improvement. It is unnecessary to emphasize the importance of all of these instruments in all of the sciences. We believe that the molecule microscope, with its quite different mechanism of contrast, chemical in nature and surface-specific, will play a role on a par with x-ray diffraction and electron microscopy in the future of biology. The fact that it may also be useful in material science does not seem as compellingly interesting, which is another reason why we have increasingly involved ourselves in biological work, and identified collaborators who are interested in joining with us in the exploitation of this new instrument.

We are continuing the development of the Scanning Desorption Molecule Microscope (SDMM) with the aim of establishing its usefulness in biological research. Before discussing our goals, some description of the SDMM, an instrument based on well-known principles not hitherto applied in this way, is appropriate.

An optical or electron microscope produces an enlarged image with contrasting light and dark regions that reveal differences in the way the sample transmits or reflects light or electrons. In the SDMM, contrast comes from differences in the number of molecules that evaporate (desorb) when different small parts of the surface of the sample are heated (or otherwise stimulated), one after the other. These molecules can have been part of the sample, or can have been applied beforehand as a stain, or can come through it. In the case of a stain, the number of molecules evaporated from a part of the sample at different temperatures will reveal the strength of binding of the molecules to the surface, thus making it possible, in the case of water, for example, to directly visualize hydrophobic regions where water binds weakly and hydrophilic regions where water binds strongly. The SDMM produces an image by heating each small part of the sample in turn and brightening the spot of a Cathode Ray Tube (CRT) in proportion to the number of molecules that have evaporated. The spot moves so that it is always in the same relative

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place on the CRT screen as the part of the sample being heated, and the magnification is therefore approximately the ratio of the screen-to-sample dimensions.

To make a working instrument we must develop the following apparatus and techniques.

1. A vacuum system with adequate performance.
2. A vacuum lock for easy introduction and removal of samples.
3. Methods for maintaining the sample at a desired temperature and for evaporating molecules from it in a localized way.
4. Methods for applying staining molecules to the sample.
5. Methods for etching away undesired surface layers of the sample in a controlled way.
6. Devices for detecting and selecting species of molecules that evaporate from the sample.
7. Apparatus for processing the resulting signals and for forming images.
8. Suitable test samples, and studies in collaboration with interested biologists.
9. A catalog of staining molecules and their behavior relative to various substrates of biological interest with various degrees of denaturation.

It is our goal in 1981 to obtain adequate funds to continue work in each of the nine categories listed above.

### References

1. J.C. Weaver and J.G. King, "The Molecule Microscope: A New Instrument for Biological and Biomedical Research," Proc. Nat. Acad. Sci. USA 70, 2781-2784 (1973).

### 2. SCANNING MICROPIPETTE MOLECULE MICROSCOPE (SMMM)

Health Sciences Fund

Joseph A. Jarrell, John G. King

Work supported by the Harvard-M.I.T. Program in Health Sciences and Technology led to interesting collaborations with Dr. Alvin Essig of the Boston University

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Medical School and Dr. John Mills at the Massachusetts General Hospital to exploit the so-called Scanning Micropipette Molecule Microscope (SMMM), which differs significantly from the SDMM in that the sample can be surviving tissue in vitro instead of being in vacuo.

The major goal of this effort has been to develop an instrument with spatial resolution of 1  $\mu\text{m}$ , to study in vitro transport across epithelia (biological tissues). Light and electron microscopy have been enormously useful in revealing the structure of biological materials. Radioisotopes and other techniques have provided information about average transport rates across living tissues. SMMM provides a link between our knowledge of transport and our knowledge of structure by allowing the direct mapping of transport onto microscopic structure.

A simplified diagram of this instrument is shown in Fig. I-1. There is a perfusion chamber that will allow both simultaneous observation of the sample with a light microscope and the introduction into the field of view of a glass micropipette with a tip diameter of 1-10  $\mu\text{m}$ . The tip of the micropipette is sealed with a small plug of a permeable material such as dimethyl silicone rubber or cellulose acetate. The inside of the micropipette is connected via a flexible vacuum coupling to the inlet of the ionizer of a quadrupole mass spectrometer, and hence is under vacuum. The micropipette is inserted in solution and scanned over (or placed at various points on) the surface of the sample, much as in the experiments of Frömter and Diamond,<sup>1</sup> in which a microelectrode was used to map out pathways of high ionic conductance across a living epithelium. Whatever molecules are present that will permeate the plug in sufficient amount can be detected by the quadrupole mass spectrometer. Thus spatial variations in the concentrations of the permeating molecules can be mapped out and correlated with the in vitro morphology as observed with the light microscope.

The instrument was first used to detect spatial variations in fluxes of dissolved helium. Under these conditions, the resolution of the apparatus was demonstrated by detecting the flow of helium-saturated water through 5  $\mu\text{m}$ -diameter holes in a 10  $\mu\text{m}$ -thick polycarbonate sheet with a 3  $\mu\text{m}$ -diameter probe. It was hoped that the dissolved helium would prove to be a suitable tracer for transepithelial water flow because, for technical reasons involving the nature of vacuum systems, helium is much easier to detect than water. This did not prove to be the case,

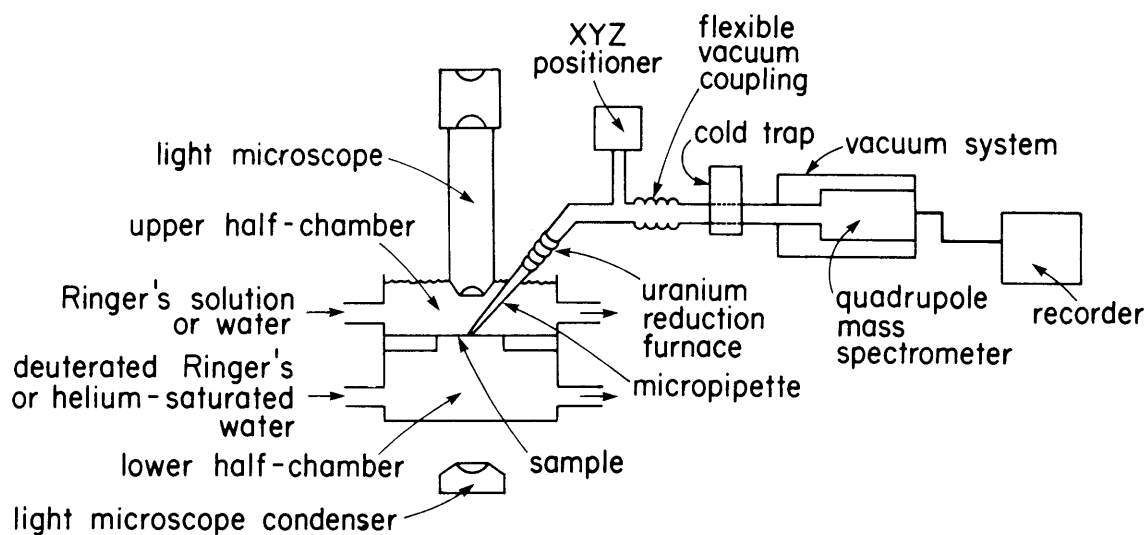


Fig. I-1. Schematic of the Scanning Micropipette Molecule Microscope (SMMM).

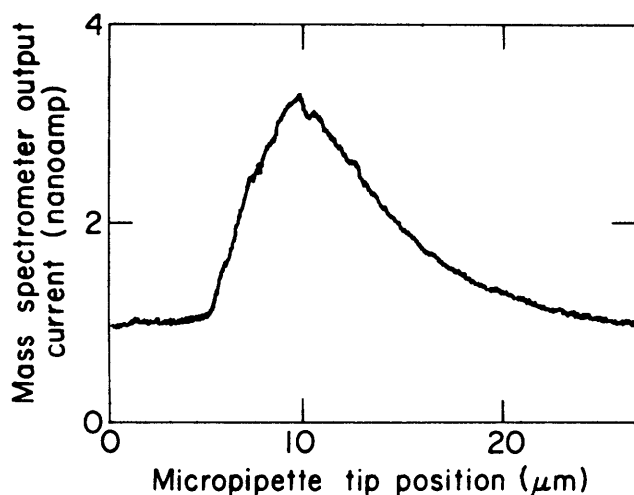


Fig. I-2. Trace of mass spectrometer output current as micropipette tip was scanned over a 3  $\mu\text{m}$ -diameter hole in polycarbonate film. The upper half-chamber was perfused with normal Ringer's, the lower half-chamber with 32% HDO Ringer's. Scan rate, 0.033  $\mu\text{m}$ . Electrometer time constant, 1 second. The plug material was cellulose acetate. The vacuum-system background current has been subtracted so that the current plotted is proportional to the local HDO concentration. The proportionality constant is roughly 2 nanoamp/10% HDO.

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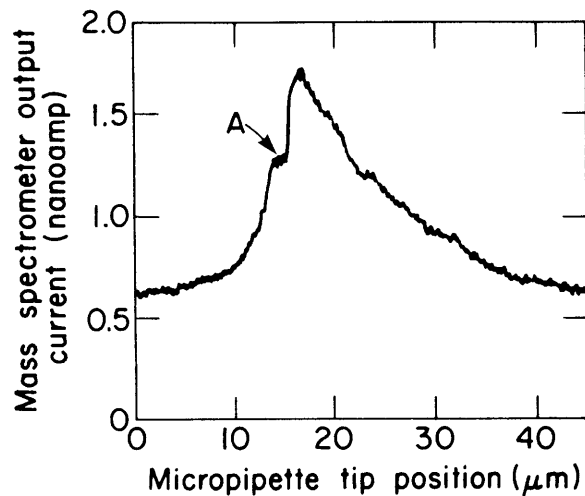


Fig. I-3. Trace of mass spectrometer output current as micropipette tip was scanned over the opening of an unstimulated mucus-secreting duct in the abdominal skin of the frog. All experimental conditions the same as those used in recording Fig. I-2. The period of no change followed by a sharp rise, marked at A, probably resulted from the micropipette tip momentarily catching, and then breaking free from the epithelial surface.

however, primarily due to the high permeability of helium through lipid. Consequently, we chose to solve the technical problems involved in detecting small fluxes of water. This involved the development of an in-line uranium reduction furnace in which water is reduced to hydrogen which is then detected by the mass spectrometer. Typical results demonstrating current capabilities are shown in Figs. I-2 and I-3. Figure I-2 is a scan with a 2.5  $\mu\text{m}$ -tip micropipette over a 3  $\mu\text{m}$ -diameter hole in a piece of Nuclepore. The probe side of the chamber was perfused with 32% HDO Ringer's.

Figure I-3 is a scan under similar conditions except that here the sample was a piece of frog abdominal skin and the micropipette tip was scanned over the opening of an unstimulated mucus-secreting duct.

The first biological problem we have chosen to study with our prototype instrument is the localization of water transport across toad urinary bladder. The toad urinary bladder duplicates the function of the human kidney distal tubules and collecting ducts as the primary site of water reabsorption from the glomerular



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filtrate and this reabsorption is controlled by the same hormone in both tissues. The importance of these reabsorptive processes may be appreciated by realizing that, without them, human water loss from urine production and excretion would be about 7 liters/hr versus a normal urine production rate of 0.06 liter/hr.

Specifically, we intend to study which cell types in these tissues are responsible for water transport and, furthermore, within a given cell type, whether the modulation of water transport by hormones occurs by "turning on and off" different numbers of cells or whether the permeabilities of all cells are varied uniformly. There is some morphological evidence in favor of the former hypothesis.<sup>2</sup>

An additional experiment will be to inject into a given cell, by micropuncture, different biochemical intermediates that are part of the hormone response chain, and then look for an increase in water permeability of adjacent cells. A positive result would be the first direct evidence of cell-cell communication in the modulation of a transcellular physiological parameter.

Another area of interest concerns cerebral arteriolar damage as a result of acute hypertension. There is morphological evidence<sup>3</sup> that this results in the formation of what appear to be small (1-2  $\mu\text{m}$ -diameter) "holes" in the junctions of cells lining the cerebral arteries. With our instrument we are in a unique position to determine whether these "holes" constitute real leakage pathways across the arteries. We have recently established a collaborative effort with Dr. John Peterson of the Department of Neurosurgery at Mass. General Hospital to pursue this problem.

The SMMM can also be used to study the transport of other species. Some volatile atoms and molecules, e.g., He, O<sub>2</sub>, CO<sub>2</sub>, and lactic acid will permeate the micropipette tip plug. Others such as urea which cannot do so may be converted to volatile molecules, CO<sub>2</sub> and NH<sub>3</sub>, by immobilizing the appropriate enzyme, in this case urease, on the micropipette tip. We have done preliminary work demonstrating the feasibility of this approach.<sup>4</sup>

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References

1. E. Frömter and J. Diamond, *Nature (New Biology)* 235, 9 (1972).
2. D.R. DiBona, Hormonal Control of Epithelial Transport (Inserm, Paris, 1980).
3. H.A. Kontos et al., *Science* 209, 1242 (1980).
4. J.C. Weaver et al., *Biochim. Biophys. Acta* 438, 296 (1976).