

General Physics

1. Molecule Microscopy

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1.1 Research Objectives

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Molecule microscopy depends on the spatial variation of emissions of neutral molecules from the sample in vacuum. It produces images that reveal variations in the number of molecules present on the surface of (or, in some cases, coming through) the sample. These molecules can have been part of the sample, or may have been applied as a stain. There being no useful lens for neutral molecules, spatial resolution must be obtained as follows. An aperture can be scanned above a sample from which molecules are evaporating so that only molecules from one region of the sample can enter a detector, as determined by the geometry of the straight line molecular paths. Or the aperture can be at the end of a micropipette equipped with a permeable plug, useful when the sample is in liquid solution as with surviving tissue. Finally, the sample can be kept at such a temperature that negligible evaporation of molecules occurs except where local stimulation of evaporation by heating, electron stimulated desorption (ESD) or other means takes place in response to a localized scannable pulsed source of energy, such as a focused beam of electrons. We have done some work with all three systems: aperture or "pinhole,"¹ micropipette² and scanning desorption.^{3,4}

Status

We are not at present continuing work with the aperture or "pinhole" type instrument—it could have been developed beyond the initial stage described in reference 1 where the first pictures ever taken with evaporating water molecules are exhibited, but the other forms appeared to be more useful. Thus the scanning micropipette MM (SMM)² is being applied to studies of transport in tissue at M.I.T. (see 1.3 below) at Massachusetts General Hospital and at Boston University School of Medicine. We have worked on various aspects of the scanning desorption molecule microscope (SDMM). Thus, to establish the contrast mechanism for SDMM with thermal desorption as it might be used in biology, we have studied the desorption of water from representative samples of protein, carbohydrate and lipid.⁵ As a frozen thin sample is raised from 100 K to 800 K three kinds of peak in the rate of desorption of water are found—peaks around 120 K from the sublimation of ice in zero

order, sample specific BET peaks in the range from 220 K to 500 K and pyrolysis peaks above 500 K⁶. As long as the sample is not pyrolysed it can be restrained. We have also investigated the ESD of water and ethanol from the same samples³ and find cross sections around 10^{-16} cm². We have also studied a variety of thermal desorption techniques including heating by localized addressable microheaters, a project in microstructure construction limited at present to micrometer resolution; and heating by focused laser light.⁴

In summary, with our accumulated knowledge, we are now in a position to design an SDMM with either micrometer or nanometer resolution. Yorker⁴ exhibits pictures of water desorbed from a stearic acid droplet and from squamous cells with 5 μ m resolution and discusses in general how to get micrometer resolution. Such instruments are of interest in physiological studies, but do not help at the more fundamental molecular level where much higher resolution is needed. Recently we have figured out how to design a SDMM with nanometer resolution. Why is it important? Because SDMM with its surface specific and essentially chemical contrast mechanism has many applications in which the localization of small molecules on sample surfaces can be used to give otherwise unobtainable information. Studies of the distribution of water on biological surfaces can give information about the sites where it binds and help elucidate the various specific recognition processes that take place at these surfaces. Water plays a role in corrosion initiation and failure in metallurgy, and in catalytic reactions in chemistry. We expect the SDMM to be useful wherever the irregular nature of samples does not permit diffraction studies of the adsorbate.

1.2 Design of Nanometer SDMM

How big a signal do we have to work with? Consider a monolayer of water adsorbed on a surface—a 2 nm spot will have about 40 molecules and they must provide the signal that eventually generates one element of the picture produced by the SDMM. Initially we plan to have 10^4 elements in the picture each 2 nm by 2 nm with 3 to 5 shades of gray (attainable if the background is less than 3 counts per element) and requiring 10^2 seconds exposure.

Thermal desorption is feasible for 10 nm resolution with thin samples on thin substrates, but because the spread in heat is comparable to the substrate thickness we choose to use ESD to attain 2 nm resolution. The sample will be on a thin (10 nm) substrate to minimize the effects of "bloom." For a desorbing pulse 10^{-6} seconds long and with ESD cross-sections of 10^{-16} cm² currents of 10^{-12} A must be delivered to a 2 nm spot, well within the current state of the art. The desorbed neutrals will impinge on and stick to a cold tungsten tip of 1 μ m radius. The tip oscillates at 100 Hz (eventually under servo control to ca 0.1 μ m) from a position 1 μ m above the sample to a position at the entrance of a time of flight (TOF) mass spectrometer. When the tip is above the sample it collects a pulse of desorbed molecules. When it is above the TOF tube a short (10^{-9} sec) positive high voltage (400 kV) pulse is applied to the tip which field desorbs and ionizes the molecules with high efficiency and sends the ions down the TOF tube for detection with appropriate fast electronics.

How low a pressure must be maintained in the vacuum system? If less than 1 molecule is to hit the detector tip during 1 cycle of the desorption–detection process, the pressure should be in the vicinity of 10^{-9} Pa, readily attainable—most easily by cryopumping in this case.

References

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1.3 Scanning Micropipette Molecule Microscopy (SMMM)

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The major goal of this research is the study of water transport at the cellular level across hormonally responsive tissues that model the distal tubules of the mammalian kidney, specifically amphibian urinary bladder epithelium.

During the past year we have successfully applied techniques for the microinjection of fluorescent dyes to this epithelium and demonstrated that the cells are coupled together and hence act as a syncytium. Previous attempts by others to demonstrate this coupling using electrophysiological techniques had produced conflicting data.^{1,2} Indeed, the validity of using such techniques in this epithelium has been disputed.³

These microinjection techniques, in conjunction with the scanning micropipette molecule microscope, will now enable us to examine the role of intercellular communication in the hormonal response (by the microinjection of cAMP) and to begin to elucidate the various intracellular biochemical events that occur between hormone binding on the basolateral cell membrane and the increase in water permeability that occurs at the apical cell membrane, by microinjecting various suspected intermediates such as the catalytic subunit of cAMP–dependent protein kinase.

References

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1.4 Electrical Neutrality of Molecules

Francis L. Friedman Chair

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The present experimental upper limit on any departure from neutrality of molecules is approximately 10^{-21} of one elementary charge. This can be interpreted as a limit on the proton–electron charge difference, or on the charge on the neutron, or on some combination of these. The occurrence of innumerable transformations involving both leptons and baryons, and our long–standing belief in the conservation of electric charge as a fundamental law of nature, certainly support the assumption of exact equality of charges of either sign on all elementary particles. Nevertheless, the possibility that there might be some minute difference between electron and proton charges is something that cannot be ruled out on the basis of any existing experimental evidence or theoretical scheme, and its existence would have profound implications.

The fundamental interest of this question has led to a number of experiments by various investigators. These experiments have involved three different approaches: (1) direct tests for any net charge in a volume of gas released from a container; (2) tests for a charge on an isolated body (analogous to the Millikan oil–drop experiment); (3) molecular–beam deflection experiments.

In 1973 King and Dylla¹ pioneered a new technique based on the fact that an electric field, periodic in time, applied to a homogeneous medium will excite acoustic waves at the same frequency if the molecules of the medium are not completely neutral. (This is separable from any induced polarization effects, which will occur at doubled frequency).

Using SF₆ gas in a spherical container, with microphones in the walls to detect acoustic waves set up by an oscillating voltage on a central electrode, King and Dylla were able to match the sensitivity (~ 1 part in 10^{21}) of neutrality determinations by the three previous methods. There is reason to believe that a well–designed experiment using a liquid medium instead of a gas might make it possible to gain as many as six orders of magnitude in sensitivity in such measurements. We have done some preliminary studies of matching of high–Q resonating microphones to cavities containing cryogenic liquids with encouraging results. We also have modest funds to continue the work during 1983.

References

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