

GENERAL PHYSICS

I. MOLECULE MICROSCOPY

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1. DEVELOPING MOLECULE MICROSCOPY

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Health Sciences Fund

John G. King

a. Introduction

Measuring the variations in space and time of fluxes of neutral molecules from a sample can give information not otherwise obtainable concerning, for instance, biological systems. We call the group of techniques used molecule microscopy (MM) and we believe that when fully developed it will have the same kind of revolutionary impact on biology that electron microscopy and x-ray diffraction have had.

Why biology rather than, say, material science? Because in biology there are structures of interest – inhomogeneities – of various sizes, from microns to angstroms, and neutral molecules are a natural (if not very agile) conveyor of information about surfaces, binding properties, permeability, metabolism, and enzymatic processes. In materials science, angstrom resolution, which we do not yet have, is needed to reveal features of interest, and the refractory nature of the material (often made up of atoms with medium to high Z) permits the use of more energetic probes.

b. How Molecule Microscopy Works

Insofar as neutral molecules emitted by the sample are ionized, the resulting ions are selected by charge-to-mass ratio in a mass spectrometer, and counted by an electron multiplier, MM is a form of mass spectrometry. Spatial resolution is obtained in three ways:

(i) The sample emits molecules in all directions, but only those molecules from a given region on the sample pass (see Fig. I-1) through an aperture to strike the detector (the ionizer-mass spectrometer-multiplier combination). By moving the aperture back and forth in one direction and displacing it at right angles after each pass, one scans the entire sample. A synchronously moving cathode ray oscillator (CRO) spot is brightened

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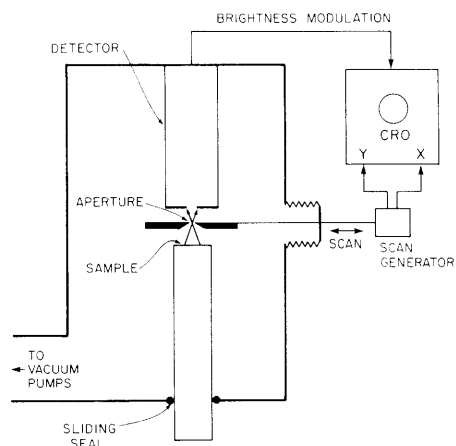


Fig. I-1. Scanning pinhole molecule microscope.

in proportion to the flux of neutral molecules at each point, thus producing a picture (a molecule micrograph) which can be photographed. We have built a crude form of such an instrument, the scanning pinhole molecule microscope (SPMM), and published some results.

(ii) The sample emits neutral molecules when locally heated (or otherwise stimulated) by either focused radiation or particles, or by heating from below with a matrix of addressable microheating elements (see Fig. I-2). A large aperture detector is used, and as the sample is scanned, i. e., one region after another on the sample is stimulated to emit, the picture of where molecules came from is built up by displaying the detector output on a CRO as before. We have not built such an instrument, a scanning desorption molecule microscope (SDMM), but have tried out various parts of it, and studied various aspects of the desorption and readsorption mechanisms.

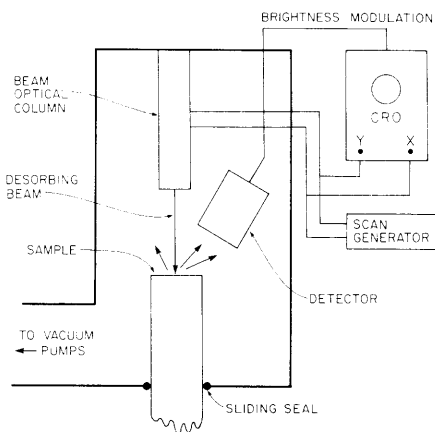


Fig. I-2. Scanning desorption molecule microscope.

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(iii) The two methods described require that the sample be in vacuum, which causes denaturation. Some samples must be studied *in vitro*, which we do by sampling the emitted molecules with a membrane-covered micropipette connected to the mass spectrometer (see Fig. I-3). Such a scanning micropipette molecule microscope (SMMM)

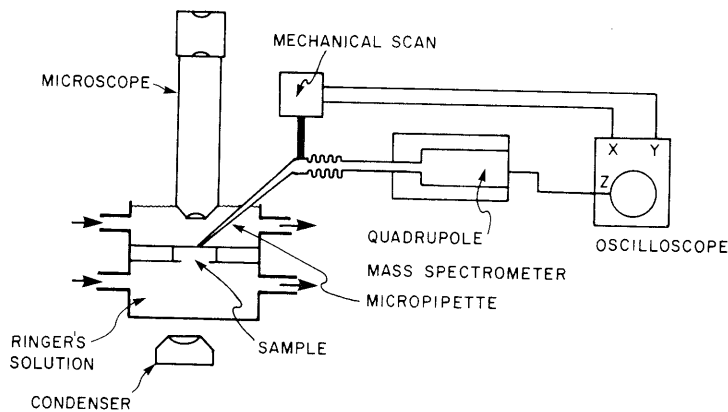


Fig. I-3.
Scanning micropipette molecule microscope.

is now under study and has readily detected pores in nucleopore filter, and in a first experiment, spatial variations in permeability in frog bladder, a commonly studied epithelium.

c. Applications of Molecule Microscopy to Biology

As the instruments are developed, characterized, and simplified, we believe that the number of applications will grow until the whole thing is taken for granted (as in electron microscopy). To get started we have been working on several collaborative projects:

(i) Cell Surface Studies will lead eventually to the application of SDMM to the identification of patches on erythrocyte ghosts and later to the identification of various regions in freeze-fracture preparations. At present Dr. Dusan G. Lysy is continuing his study of the contrast mechanism, based on the common experience that oil and water don't mix or, rather, that water does not stick to lipide surfaces. Besides the preparation of a compendium of staining molecules (any volatile species) and surfaces to be stained, he is about to study the desorption properties of cells – normal and transformed, as supplied by our collaborator, Dr. Phillips W. Robbins of the Department of Biology, M. I. T. This work is being supported for one year by a grant from the M. I. T. Health Sciences Fund.

(ii) Molecule Fluxes in Tissue is a collaborative project with Dr. Alvin Essig of the Department of Physiology at Boston University Medical Center. Dr. Stanley J. Rosenthal has built the molecule flux apparatus which makes possible the simultaneous

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measurement of CO_2 production and O_2 uptake by surviving epithelia mounted in Ussing chambers. We are able to relate our results to previous measurements by other methods, and new phenomena are now being revealed. Further studies will be made by Dr. Ying-Tung Lau. This work is being supported by NIH training grants and the Francis Friedman Chair of Physics at M. I. T. We expect also to apply to the M. I. T. Health Sciences Fund for support of this B. U. - M. I. T. collaboration. In a closely related project, Mr. Joseph A. Jarrell at M. I. T. has built an SMMM which is nearly ready to be put into operation. This project, which can answer such questions as where the CO_2 emitted by the tissue is coming from, and what the relative permeabilities of cells and junctions are, also has the collaboration of Dr. Essig, and is complementary to the optical microscopy studies of similar preparations carried out by Dr. D. DiBona at Massachusetts General Hospital.

(iii) Finally, the Volatile Enzyme Product technique (VEP) invented by Dr. James C. Weaver and being developed by him as an assay technique with collaboration from Drs. Charles L. Cooney and Anthony J. Sinskey of the Department of Nutrition and Food Sciences, M. I. T., can eventually be applied to MM. It removes the restriction that the molecules be volatile and makes possible the detection of either enzymes or substrates as long as the reaction yields volatile products. The VEP project has NIH support, and is described more fully in Section I-2.

(iv) Problems. The fact that the first SMMM produced pictures with the triple disadvantages of the loss imposed by the duty cycle of scanning (10^{-3}), the optical inefficiency of a pinhole (10^{-5}), and the mismatch of the detector size (i. e., inefficiency) (10^{-4}) shows that much higher performance in terms of resolution and contrast is possible. We have recently invented better detectors which are expected to have high efficiency, the right size aperture, and low background. For SDMM some thought has been given to matrix heaters to produce localized thermal desorption. These can be constructed by using modern electron beam etching techniques which provide spatial resolution of 10's of Å. Molecular photography, in which distributions of molecules captured on a surface are made visible by chemical amplification would be useful, as one could then use the shadow of Fresnel zone plates instead of pinholes to obtain greater molecule collecting power, as well as spatial resolution.

2. VOLATILE ENZYME PRODUCT ASSAY

National Institutes of Health (Grant 5 RO1 GM22633-03)

James C. Weaver

Configurations for the assay of either enzyme activity or substrate concentration have been explored during the past year. Most experiments utilized either urease or catalase; acetylcholinesterase was used occasionally. Major problems that continue to

be dealt with include continuous degassing of preexisting volatile contaminants (mainly a problem with oxygen and carbon dioxide), and reduction of background associated noise by use of larger liquid-nitrogen-cooled traps.

Recently, work has focused on the use of conveniently and temporarily immobilized microorganisms, particularly Saccharomyces cerevisiae. In experiments related to those reported by Mattiasson et al.,¹ we have observed the transient response of the microorganisms to pulses of either glucose or fructose, following either carbon dioxide or ethanol production. Since many microorganisms are involved with the utilization and production of a variety of low molecular weight volatile compounds (e. g., hydrogen, methane, formaldehyde, methanol, acetone, acetic acid, propionic acid, etc.), this approach should provide considerable utility in studying many microorganisms in real time.

References

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3. THERMAL ENZYME PROBE

National Institutes of Health (Grant 5 SO5 RR07047-11)

James C. Weaver

During the past year a flow-through version of the thermal enzyme probe (TEP) was successfully used with immobilized urease (EC 3. 5. 1. 5) to assay urea concentrations with a resolution of approximately 10^{-4} M, corresponding to a temperature difference, ΔT , resolution of less than 10^{-5} °C.¹ In order to achieve this performance it was necessary both to continuously degas the sample stream, and also to provide a very steady flow by means of a gas-pressure-driven pump and reservoir. By use of better matched, commercially available thermistors, there is reason to believe that a ΔT resolution of 10^{-6} °C is realistic. This capability, in combination with a microprocessor-controlled miniaturized version of flow-through devices^{2, 3} which carry the enzyme-catalyzed reaction to completion (and thereby provide two orders of magnitude more sensitivity), should allow one to perform rapid assays on 100-microliter samples at the 10^{-7} M level.

References

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