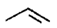
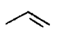
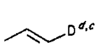
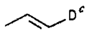
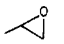
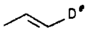
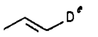
Table I. Microwave Data for Propylene Oxide Isotopomeric Mixtures

run	transition, peak intensity (trans-d_1/d_0)					$n_{t\text{-}d_1}/n_{d_0}$[a]
	$9_{2,7}$–$9_{1,8}$	$10_{2,8}$–$10_{1,9}$	$11_{2,9}$–$11_{1,10}$	$12_{2,10}$–$12_{1,11}$	$13_{2,11}$–$13_{1,12}$	
2	38/9	41/11	54/12	66/16	81/18	4.8 (0.3)
3	17/3	21/4.5	26.5/5	32/7	40/8	5.7 (0.5)
4	0.9/15	0.9/17.5	1.1/20	1.6/28	1.8/30	0.06 (0.01)
5	0.95/21	1.11/25	1.3/31	1.6/41	2.2/48	0.05 (0.01)
6	15.5/7	19/8	21.5/10	30/12	35/14	2.5 (0.2)
7	3.0/24	3.0/29	3.5/31	4/42	5/46	0.12 (0.01)
8	24/6.8	30/7.9	37/9.6	47/11.9	61/14	4.4 (0.2)
9[b]	17/13	24/14	30/18	39/23	48/29	1.6 (0.2)

[a] Average isotopomer ratio and standard deviation calculated following ref 5a; for each transition $n_a/n_b = R^{-1}(I_a/I_b)$ where I_a is the peak intensity for species a etc. and R converts intensity ratios into the mole ratio n_a/n_b. The values of R for the five transitions are respectively 0.86, 0.87, 0.88, 0.88, and 0.89 for $n_{t\text{-}d_1}/n_{d_0}$ and 1.0, 0.99, 0.97, 0.96, and 0.95 for $n_{t\text{-}d_1}/n_{c\text{-}d_1}$. [b] Peak intensities for trans-d_1/cis-d_1; d_0 not determined.

Table II. Epoxidation of Propylene by Cytochrome P-450$_{LM_2}$

run	substrate	conditions	epoxide yield, μmol	% d_0	% *trans*-d_1
1		P-450/reductase/H_2O, NADPH/O_2	1.6	100	
2		P-450/Reductase/D_2O, NADPH/O_2	2.2	17	83[b]
3			2.4[a]	15	85
4	 [d,c]	P-450/Reductase/H_2O, NADPH/O_2	2.3	94	6[b]
5			1.8	95	5[b]
6	 [c]	P-450/ϕIO/H_2O	2.0	27	73[b]
7		P-450/Reductase/D_2O, NADPH 3 days		89	11[b]
8	 [e]	Fe(TPP)Cl/Ph-IO	60	18	82[b]
9	 [e]	Mn(TPP)Cl/Ph-IO	50	38[f]	62

[a] Recovered propylene showed 4.7% *trans*-1-deuteriopropylene. [b] Less than 1% *cis*-1-deuteriopropylene oxide was detected. Loss of deuterium was confirmed by GC–mass spectrometry (*m*/*e* 58, 43). [c] Starting material was 72% *trans*-1-deuteriopropylene, 28% propylene. [d] Deuterium content of recovered propylene was unchanged. [e] Starting material was 80% *trans*-1-deuteriopropylene, 20% propylene. [f] *cis*-1-Deuteriopropylene oxide; propylene oxide not determined.

species is an attractive candidate for the "active oxygen species" in the cytochrome P-450 cycle. Although the detailed mechanism of olefin epoxidation by simple oxometal species is not yet clear, the interesting suggestion that chromyl reagents[8] and oxomanganese porphyrins[9] react with carbon–carbon double bonds to form an oxametallacycle may offer an opportunity for proton exchange. Thus, the cycloaddition of propylene with **1** could reasonably form **2**. There is ample precedent that metal alkyls can be deprotonated to form metal carbenes.[10] Loss of a proton from **2** would provide access to the iron carbene **3**. Metallocarbene species have been suggested to result from the reductive metabolism of halocarbons by cytochrome P-450.[11] Further, an iron carbene such as **3** would provide a path to the *N*-alkylporphyrins **4** which are known to derive from terminal alkenes.[12]

The observed preference for trans protonation can be accommodated in Scheme I either by a stereoselective protonation of **3** to regenerate **2** or by ring closure to form the metallooxirane **5**. Epoxide production in the former process would result from reductive elimination from **2** while protolysis of the carbon metal bond would produce the epoxide from **5**. No definitive choice between these possibilities can be made with the data at hand, but mechanisms for propylene epoxidation which do not allow for proton exchange must be eliminated on the basis of these results. Efforts to detect intermediates such as **2**, **3**, and **5** in enzymic and model porphyrin-mediated epoxidations are currently under way.[13]

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Registry No. P-450, 9035-51-2; Fe(TPP)Cl, 16456-81-8; Mn(TPP)Cl, 32195-55-4; CH_2=$CHCH_3$, 115-07-1; (*F*)-CHD=$CHCH_3$, 1560-60-7; iodosulbenzene, 536-80-1; monooxygenase, 9038-14-6; propylene oxide, 75-56-9; *trans*-1-deuteriopropylene oxide, 34074-743.

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Scanning Electrochemical and Tunneling Ultramicroelectrode Microscope for High-Resolution Examination of Electrode Surfaces in Solution

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We describe here a new apparatus for examination of conductor (or semiconductor) surfaces immersed in solution by measurement of either the electrochemical or tunneling current that flows when an ultramicroelectrode tip is scanned above the surface. Results with a Pt-coated test structure yield a spacial resolution of about 30 nm. Most methods for examination of solid surfaces, e.g., scanning electron microscopy (SEM) or the various electron spectroscopies, require the sample to be in an ultrahigh vacuum (UHV) environment and are not suitable for in situ electrochemical studies. Optical techniques for in situ electrode surface studies exist (e.g., infrared and surface-enhanced Raman spectroscopy) but they are capable of only limited spacial resolution, as governed by diffraction limitations and source beam size. An alternative approach was suggested by recent investigations with scanning tunneling microscopy (STM) that demonstrate the possibility of direct determination of surface structure with atomic resolution.[1-5] In this method a very sharp metal tip is brought

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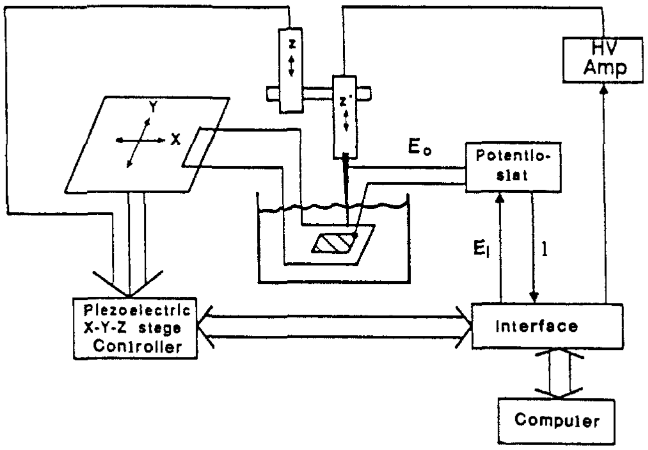

Figure 1. Block diagram of the electronic circuitry of the STM system. The *X–Y* stage, *Z* stage, and *Z'* piezoelectric pusher, data acquisition, and processing control are shown.

near (≤10 Å) the substrate surface, so that a tunneling current flows between the substrate and tip. The tip scans the surface in two dimensions, while its height is adjusted to maintain a constant tunneling current. This current is a function of electronic and geometric properties of the electrode surface. STM has been applied mostly to the study of the surface topography of solids (e.g., Si, Au) in UHV or, less frequently, at ambient air pressure. To our knowledge no reports exist for STM applied to samples immersed in solutions.

The utilization of methodology related to STM with substrate and tip immersed in solution as described here involves a number of differences compared to operation in UHV. In addition to the tunneling mode, when the tip is brought near (ca. 10 Å) the surface, an electrochemical mode is possible; here the tip is at a greater distance and the current is carried by faradic reactions at tip and substrate and ionic movement in solution. The use of a solution involves severe purity requirements and necessitates high chemical stability of probe tip but probably decreases susceptibility to vibration. In this paper, we demonstrate in initial studies that this technique can be applied to image an electrode surface in water with or without added redox couple. This microscope includes an *X–Y* stage with which the sample can be scanned at 10 nm per s and an ultramicro platinum tip electrode. A vertical resolution of ca. 30 nm and lateral resolution of the same order is achieved with the present device.

A schematic diagram of the scanning microscope developed in this laboratory is shown in Figure 1. The sample holder is scanned by a piezoelectric driver *X–Y* stage (IW-501/TS-100, Burleigh Instruments, Fishers, NY). The "coarse" movement of the tip is controlled by the same type of piezoelectric drive stage. The slowest scan rate of the *X–Y–Z* stage was 10 nm/s with a total working distance of 2.5 cm at each axis, which is controlled by a programmable controller (CE-1000). Therefore, with this arrangement, a large portion of sample can be continuously monitored. The fine adjustment of the tip is controlled by a second piezoelectric pusher (*Z'*), driven by a high-voltage amplifier (PZ-70M) at 50 Å/V. The submicron tip was constructed from 10-μm platinum wire sealed in a small glass capillary. This was further trimmed by electrochemical etching in aqueous solution containing saturated $CaCl_2$:H_2O:concentrated HCl = 60:36:4 (v/v). The Pt tip diameter was estimated by SEM to be less than 0.1 μm. A bias voltage of 90 mV was applied between the tip and substrate with a PAR 173/175 (Princeton Applied Research, Princeton, NJ) potentiostat and the tip lowered until a current of 5–25 nA flowed. The height of the tip was controlled by a computer (Apple IIe via an Isaac 91A interface) to maintain the current constant. The *X* and *Y* scanning was similarly controlled by the computer and the *X–Y–Z* positional data were stored on a floppy disk. The whole assembly rested on a vibration-free table (RS-510-12/XL 4B, Newport Corp., Fountain Valley, CA).

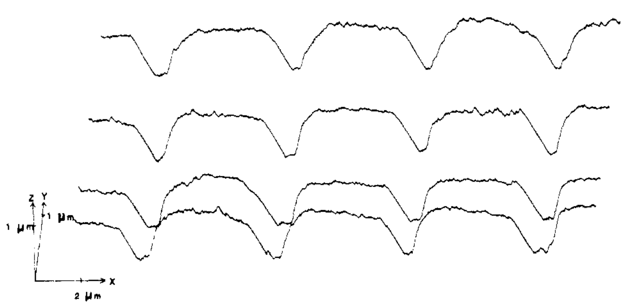

Figure 2. Several scanning surface contour lines of a test integrated circuit overlaid by 1000 Å of platinum. Monitoring current, 15 ± 10 nA. Bias voltage, 90 mV. Distance scale, 1 μm/div.

To test this apparatus, an integrated circuit (IC) test pattern was employed. This consisted of interdigitated Al structures, about 0.8 μm high with several different width and spacing patterns, deposited on an SiO_2 layer on single-crystal Si. Before mounting to the sample holder, the IC pattern was covered with about 1000-Å Pt by sputtering to yield a conductive substrate and prevent corrosion reactions of the Al patterns. A typical scan of a 10 μm × 10 μm section of the pattern with nominally 3-μm lines spaced 3 μm apart is shown in Figure 2. Each scan as shown was taken only once (not averaged). The observed features (line height, width, and spacing) agree well with the dimensions found by SEM of this pattern. Reproducibility was demonstrated by scanning repeatedly in the *Y* direction over the same section, without scanning in the *X* direction, and noting the appearance of the same submicron features (probably representing corrosion sites, uneven Pt deposition, and covered dust particles). The microtip could be moved to different sections of the IC test pattern to scan regions of different geometry. When *X* and *Y* scans were not carried out and the *Z* position was monitored, the observed variation (peak to peak) corresponded to about 30 nm. The scan shown was obtained with pure (ca. 3 MΩ) water obtained with a Millipore system, demonstrating that the observed currents are tunneling ones. The results were essentially the same at tunneling distances when the solution was made 1 mM in $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ by addition of the potassium salts. In the presence of redox couple, however, smaller faradic currents could be recorded when the tip was removed to larger distances from substrate.

Although there have been other reports of scanning electrode surfaces, e.g., by measuring the potential as a refrence electrode tip is moved[6a] or by injection of ions iontophoretically,[6b] the resolution attained was much lower than that reported here. While we have not attained the reported resolution of UHV STM, this still represents, to our knowledge, the highest resolution for a surface totally immersed in liquid. Further improvements should be attainable by improvement of the tip, decreasing the extent of vibrations and thermal drift, and better piezoelectric drives and controllers. Some special advantages in the electrochemical system are the possibilities of etching, deposition, or other modifications of the substrate during or between scans.

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