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NEURAL MICROPROBES FOR MULTISITE RECORDING

Microelectronic technology has been used to construct passive, multisite microprobes for investigating the electrical activity of neurons in live brain tissue. The probes are built on a thin molybdenum support, with gold electrodes sandwiched between two polyimide dielectric layers. Windows in the top insulating layer expose the electrode sites and bonding pads. Microprobes with different numbers of sites have been constructed for use in various experimental situations. A critical consideration is the useful lifetime of the probes under *in vivo* experimental conditions. Probes have survived immersion in saline solution for more than 750 hours before site impedance degradation indicated failure. A four-site probe has been used to observe neural activity in the dorsal columns of anesthetized rats.

INTRODUCTION

For more than thirty-five years, fine-tipped microelectrodes have been inserted into living brains to record extracellular action potentials generated by individual neurons.[1] This method of observing single neurons in succession has contributed to significant advances in understanding the function of the brain. The application of microelectronic techniques has made possible the development of multielement microelectrodes that have many advantages over conventional single-site electrodes, some of which are as follows:

1. For single-site recordings, functional relationships between cells must be inferred from the population data obtained from successive experiments. Simultaneous recordings from many neurons allow functional relationships to be assessed directly.
2. For single-site recordings, a population of data is obtained by repeating the stimulus and/or behavioral paradigm while recording from different cells. This process is laborious for the experimenter and stressful for the animals. Multielectrode recordings can dramatically increase the number of neurons studied per experiment, thus reducing the number of experiments. Perhaps more importantly, the number of animals necessary to collect data is also reduced.
3. In many experiments, an evoked potential linked to a particular stimulus results in a noise signal that makes recording from single cells difficult. Differential recording from neighboring pairs of electrodes allows for rejection of a noise signal common to both elements.
4. The fixed spacing of the electrode elements facilitates studies of topographic organization and functional relationships in the neural system.
5. Prosthetic devices controlled by the central nervous system will require multiple recording and stimulating sites.

Two major approaches have been taken in the development of multielement electrodes. The first approach was to fabricate bundles of separate, single-wire microprobes with either a fixed three-dimensional configuration[2] or a fixed two-dimensional configuration with adjustable depth.[3] These arrays could record simultaneously from more than one neuron. The second approach, reported here, uses microelectronics technology to fabricate thin-film microprobes with a fixed geometrical arrangement of electrodes.[4,5] Using thin-film microelectronics technology to build microprobes with a linear array of electrode sites has several advantages: (1) a high degree of reproducibility, once the design and processing sequence have been developed; (2) a precise knowledge of the spatial distribution of electrode sites; (3) a high "packaging density" of electrode sites in a given implanted volume; (4) the possibility of incorporating the interface circuitry directly on the microprobe base; and (5) the possibility of arranging electrodes in a specifiable geometric pattern.

The multisite neuron probe is about 20 μm thick and has either four or six recording sites. The molybdenum–polyimide structure is constructed using standard microelectronic technology and does not require advanced semiconductor-processing techniques.

FOUR–SITE PROBE DESIGN AND FABRICATION

The four-site microprobe, which has been used for *in vivo* experiments, is constructed as a sandwich of four principal layers: (1) a rigid, supporting substrate of molybdenum (Mo); (2) an insulating layer of polyimide; (3) a conductor layer of patterned gold deposited on the polyimide layer; and (4) a top insulating layer of polyimide with windows that expose the site electrodes along the shank and with bonding pads at the head. Figure 1 shows the shape and dimensions of the four-site probe. In this probe, originally designed for superficial record-

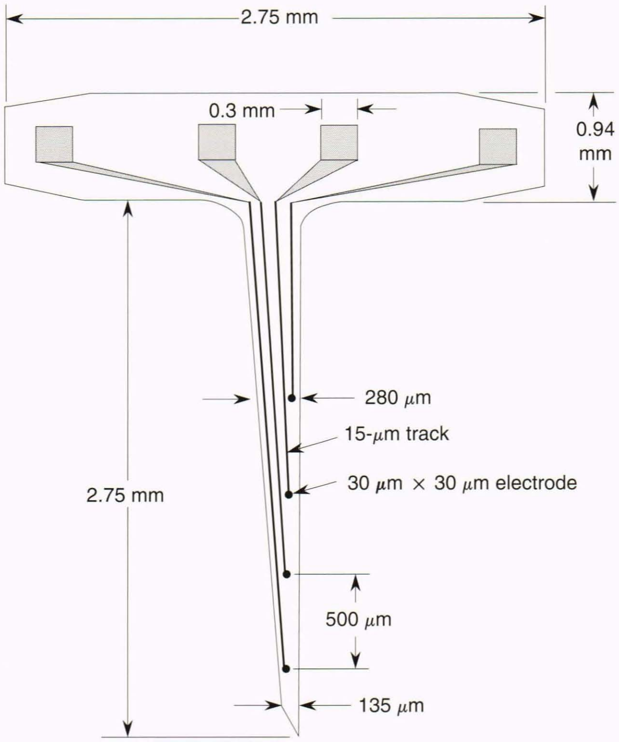

Figure 1. Plan view of the four-site probe (drawing not to scale).

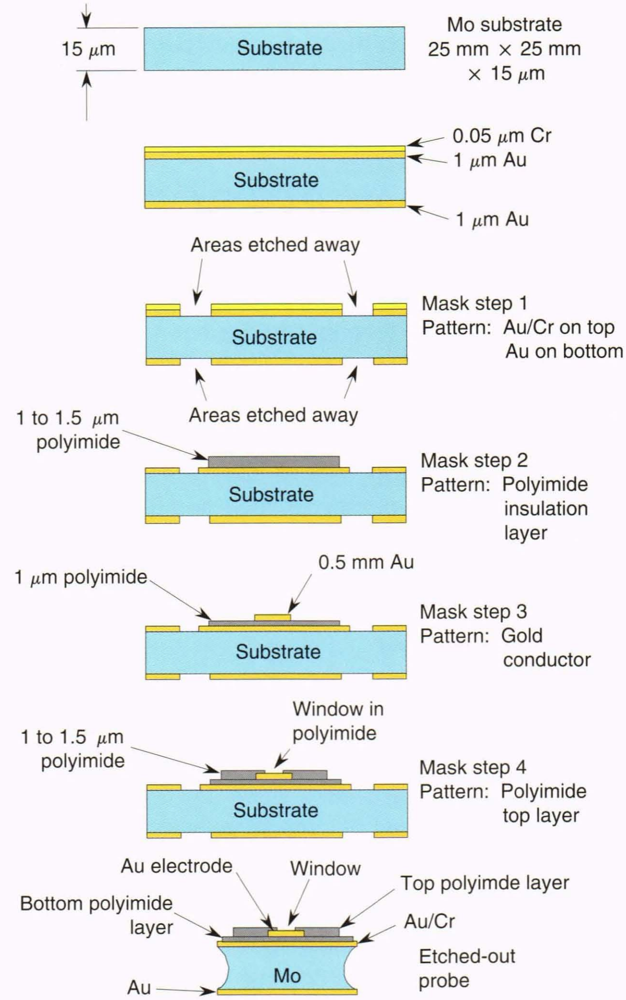

Figure 2. Major steps in the fabrication of the four-site probe.

ings in monkey cortex, the electrode sites are collinear and 500 μm apart.

A cross-sectional view of the fabrication sequence is shown in Figure 2. Four photolithographic masks are required to manufacture the probes, and there are four complete probes on each 15-μm-thick, 25 mm × 25 mm Mo substrate, which is of high quality; is pinhole-free; and has a smooth, rolled finish. The polyimide layer is formed from a liquid precursor material that allows it to be spin-coated onto the substrate. A thin chrome layer sputter-coated onto the Mo substrate provides an adhesion surface for the polyimide, which does not adhere well to gold. After heating, the precursor layer is cured (polymerized) and becomes a polyimide film, 1.0 to 1.5 μm thick. The gold electrodes and associated conductor structure are sputter-deposited to a thickness of 0.5 μm. Sputtered gold is chemically stable and is generally considered to be compatible with brain tissue. The probes are separated from the surrounding Mo substrate onto which they have been patterned by chemical etching. The initial layers of gold on the front and back of the substrate serve as etching masks. The probes, however, remain attached to the substrate by three narrow Mo tabs, cut just before mounting and assembly.

To be acceptable for mounting, probes must pass a microscopic inspection and electrical testing to assure isolation of the recording sites from the substrate and from each other. Failures are usually caused by open tracks or connections within the mounting assembly (impedance is too high), or by over-etched or delaminated dielectric layers (impedance is too low). Sometimes, failure to clear the electrode windows completely may cause an impedance that is too high, or pinholes in the polyimide layers may cause an impedance that is too low. Acceptable probes are separated from the substrate frames, and 0.2-mm-diameter gold wires are manually attached to the bonding pads using gold conductive epoxy. The probes are then partially inserted into an easily handled tubular, polycarbonate fixture, which has a plug at the end opposite the probe tip for external electrical connection to the recording apparatus. The region around the bonding pads, within the tube, is encapsulated with a silicone compound and sealed with epoxy to ensure good insulation. A micrograph of the four-site probe is shown in Figure 3.

ELECTRICAL TESTING

For *in vitro* testing, each assembled probe is mounted in a test fixture within an electromagnetically screened box. The probe is immersed in a 0.9% saline aqueous solution to a level beyond the uppermost electrode site. When an alternating 10-nA peak-to-peak test current is passed between one of the probe electrodes and a reference electrode is immersed in the saline solution, a potential develops at the electrode–electrolyte interface. The potential is used to determine the probe site impedance.

A customized, computer-assisted test system has been constructed to measure impedance versus frequency for each recording site on the probe. It can measure all recording sites at a single frequency in a scan mode, or all recording sites at multiple frequencies at preselected time intervals.

A low-distortion signal generator provides precision frequency and amplitude test signals to each of the stimulus/measurement units (SMU's) in the system. Each SMU contains a voltage-controlled current source, a high-impedance buffer, and an amplifier with a filtered gain of 5. The current sources have a transconductance of 2×10^{-6} S, providing a 10-nA peak-to-peak output current for a 5-mV peak-to-peak input signal. High-impedance buffers (1000 MΩ) isolate each probe site from loading effects and provide input signal isolation to minimize cable capacitances and to increase measurement bandwidth. The output of each SMU is multiplexed to a single amplifier that provides an additional 26 dB of gain and high-frequency filtering. The final ac signal is measured with a voltmeter. Multiplexing control and system power are provided through the switch/control unit.

The signal generator, voltmeter, switch/control unit, and ±15-V power supplies are connected to a desktop computer via an IEEE 488 bus. The computer is programmed for completely automatic control of test signals, SMU multiplexing and probe electrode selection, and data collection and analysis. The system can run unattended for long periods, such as during life tests.

Calibration of the system is accomplished by substituting precision 1% resistors for the probe and correlating the measured potentials with a resistor value. The system is calibrated from 100 kΩ to 20 MΩ, with a sensitivity of 1 mV/kΩ. Within the calibration range, measurement accuracy is 1% to 2%, repeatability is 1% to 2%, and the resolutions are 10 kΩ and 1 kΩ for resistances above and below 1 MΩ, respectively. System noise limits the smallest measurable resistance to 10 kΩ.

Although calibrated using purely resistive elements, the probe sites are characterized by their ac impedances, which include both capacitive and resistive components. The impedances at each site are recorded at frequencies between 100 Hz and 10 kHz in a 1, 2, 5 sequence for at least sixty minutes to identify stabilized probe site impedances. From each production batch of sixteen probes, a probe is selected for further tests. The probe is immersed in the saline solution until at least one site shows signs of failure, denoted by a rapidly decreasing impedance. Results of these tests have proved that the performance of these probes is representative of the performance of other probes in the same batch that have site impedances in the 2- to 4-MΩ range after sixty minutes of testing. Initial impedance versus frequency measurements for a probe with 25 μm × 25 μm recording sites are shown in Figure 4. Figure 5 shows impedance measurements made on this probe during a 950-hour immersion in a physiological saline bath. Impedances of three sites on a single probe were stable for over 600 hours, and declined to half their initial impedance values after about 750 hours. Postfailure electron-micrographic studies of long-lived probes appear to rule out pinholes or delamination of the structure as explanations for the changes in impedance, which are believed to be caused by the effects of water absorption in the dielectric layers.

NEURAL RECORDING RESULTS

For neurophysiological recordings, a low-noise preamplifier with a guarded input shield was constructed for each of the sites on the probe. In addition, injection of a

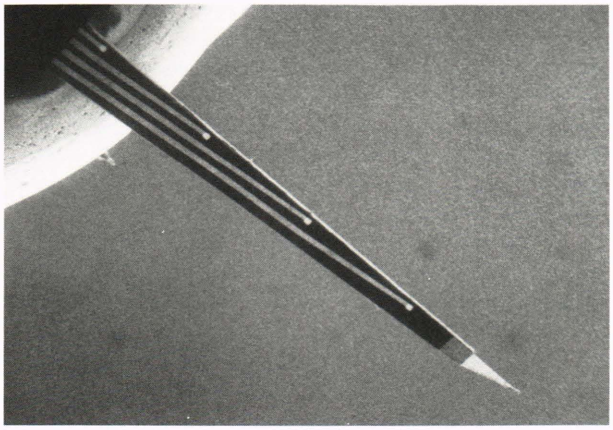

Figure 3. Electron micrograph (35×) of the four-site probe. (Reprinted, with permission, from Ref. 6, p. 70; © 1991 by IEEE.)

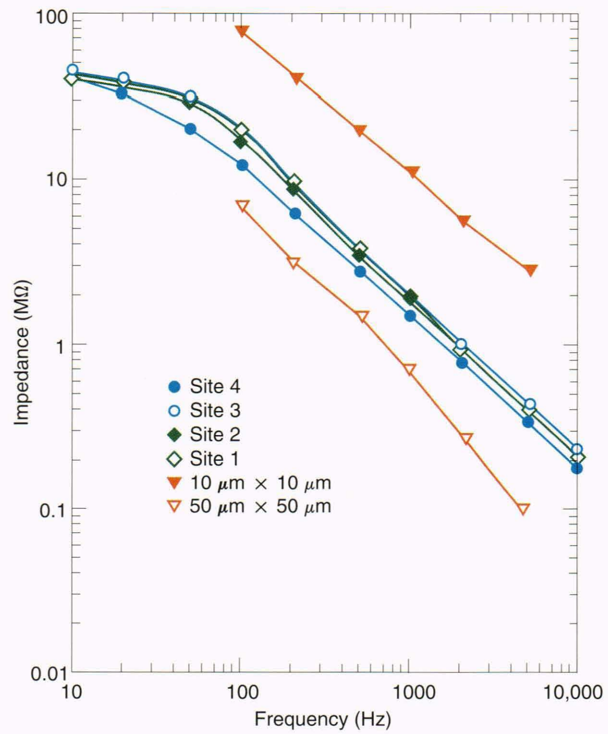

Figure 4. Initial impedance values at different frequencies for each 25 μm × 25 μm site of a four-site probe (the 10 μm × 10 μm and 50 μm × 50 μm data are from Ref. 4).

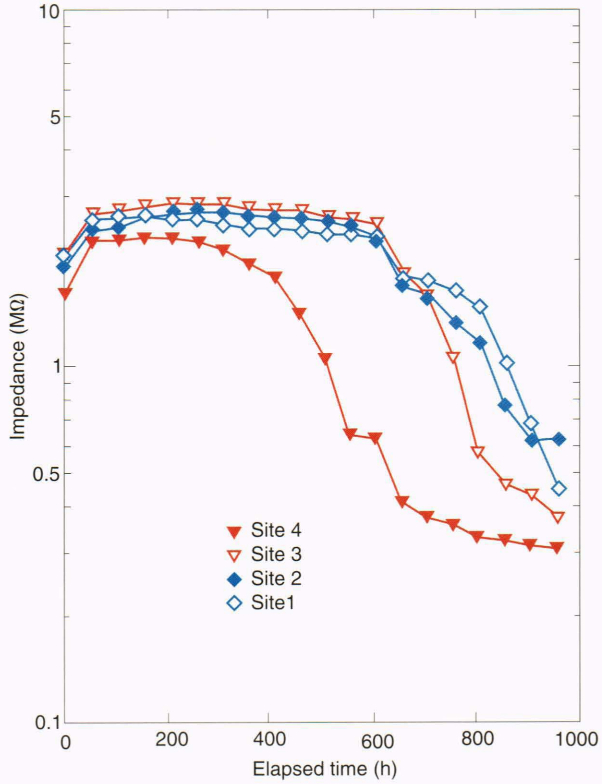

Figure 5. Impedance at 1.0 kHz for each site of a four-site probe during a 950-hour immersion in physiological saline solution.

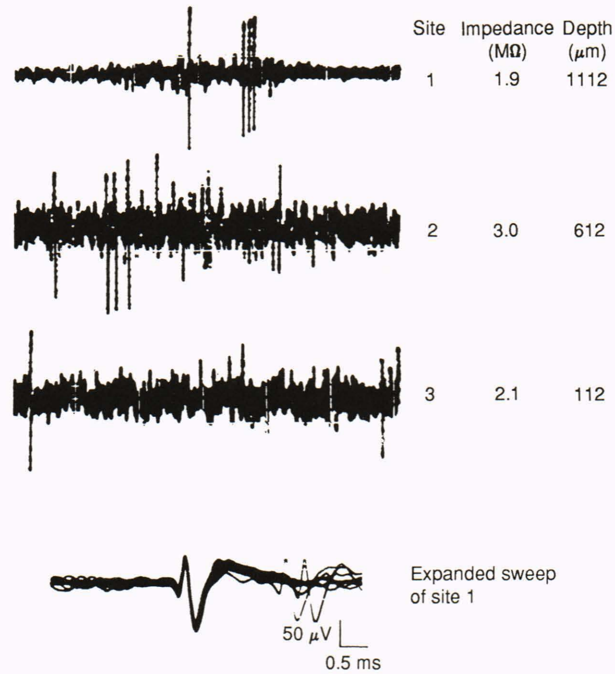

Figure 6. Simultaneous neural recordings from three sites of a four-site probe. These spinal cord recordings were made in the vicinity of the dorsal column nuclei of a chloralose-anesthetized rat. Impedance (at 1 kHz) and approximate depth of the recording sites are indicated; ten superimposed sweeps of a discriminated action potential from site 1 are shown at the bottom of the figure. (Reprinted, with permission, from Ref. 6, p. 72; © 1991 by IEEE.)

1-kHz constant-current signal (1 nA peak-to-peak) provided a means of monitoring probe impedance *in vivo*. Total system noise was equivalent to less than 5 μV rms at the input, over a 100 Hz to 10 kHz bandwidth.

Spinal cords in the vicinity of the dorsal column nuclei (DCN) of chloralose-anesthetized rats were used to evaluate the neural recording properties of the probes for three primary reasons:

1. A technique to stabilize the spinal cord for acute recordings free of motion artifacts had been established earlier.

2. The DCN are superficial structures reachable with the four-site probe design.

3. Dorsal column nuclei cells can be activated by electrical stimulation of the dorsal columns and physiologically identified by the presence of a cutaneous receptive field.

Simultaneous recordings from three different probe sites are shown in Figure 6. Action potential signals greater than 100 μV peak-to-peak were often recorded. In addition, the background noise was usually less than 20 μV. Signals from single cells were observed for several hours without any significant change in the action potential shape. The probe that has been used most often was reinserted into the spinal cord eight times for a total of twenty hours, and it was still able to record neural activity. Sometimes, cells that were presumably located in *nucleus gracilis* were identified on the basis of their superficial location, their response to electrical stimulation of the dorsal column, and the presence of a cutaneous receptive field on the ipsilateral (same-side) leg that responded to gentle touching of the skin.

SIX–SITE PROBE DESIGN AND FABRICATION

Another version of the microprobe has six electrode sites. Although conceptually the same as the four-site probe, this design differs from its predecessor both in configuration and in the details of construction. These changes reflect design modifications in the shank size and probe spacing that were required for acute neurophysiological experiments in the rat and the cat, and to make significant improvements in the probe-fabrication process. Figure 7 shows plan views of the six-site microprobe. This probe has a longer shaft and a narrower maximum width than the four-site microprobe, as well as more closely spaced recording sites, to allow neural recordings from deeper structures in the brain.

The six-site microprobe design incorporates several significant improvements in fabrication technique. The major processing steps are indicated in Figure 8. Again, four photolithographic masks are required to manufacture the probes, and there are twelve probes on each 15-μm-thick, 25-mm-square Mo substrate. All processing is performed on a single side of the substrate, eliminating the need for the front-to-back pattern alignment required in four-site versions of the probe. The up-

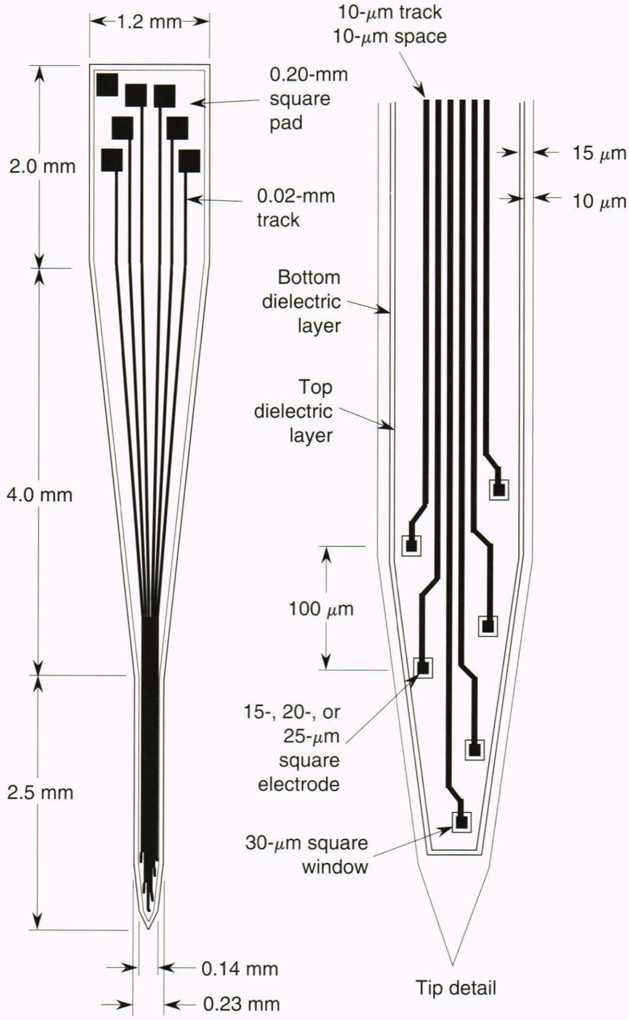

Figure 7. Plan views of the six-site probe (drawings not to scale).

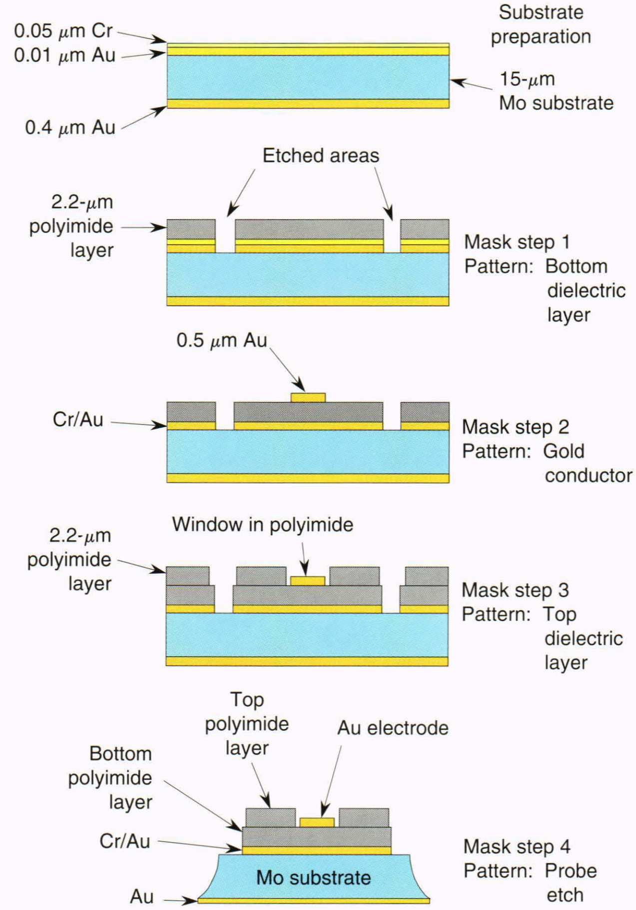

Figure 8. Major steps in the fabrication of the six-site probe.

per and lower dielectric layers are formed using a light-sensitive polyimide precursor. This material can be directly patterned by photolithography without the need for photoresist, which simplifies the processing required to form the layers. Conventional photoresist is used as the etching mask during the final Mo etch, which separates the probes from the substrate. Micrographs of the six-site probe are shown in Figure 9.

The mounting fixture for the six-site microprobe was modified to provide improved visibility during insertion of the probe into deep neural structures. The bonding-pad end of the probe is attached to a ceramic support that is inserted into a 25-mm-long, 1.6-m-diameter stainless steel tube. Gold wires, with a 0.13-mm diameter, are attached to the probe's bonding pads and passed through the tube to an external connector.

CONCLUSIONS AND FUTURE DEVELOPMENT

Four- and six-site microprobes constructed on Mo substrates have been used successfully to measure single-unit neural activity. The probes are constructed using standard microcircuit techniques. Failure of the four-site probes after 500 to 600 hours in a saline bath appears to be caused by water permeation of the polyimide insulating layer. Significant improvement in probe longevity was achieved by increasing the thickness of the polyimide layers in the four-site probe from 1 to 1.3 μm, and it is anticipated that the 2.2-μm-thick layers of the six-site probes will further increase the immersion lifetime.

Another method of applying and patterning much thicker dielectric layers has been developed recently and offers promise of still greater longevity. The present probes use chemical etching to delineate the electrode windows in the polyimide layer. Undercuts at the polyimide window edge and pinhole formation tend to degrade probe performance, especially over time, as aggressive biological fluids (or saline solution for the test structures) permeate the layer. A newer technology, which has not yet been applied to the microprobes, has been developed in connection with a program to fabricate multilayer, thin-film, microelectronic circuit boards. This technology permits the deposition of substantially thicker polyimide layers (up to about 15 μm), with an anticipated reduction in moisture transmission through the layers. Small windows have been etched in polyimide using an anisotropic plasma etch that preserves

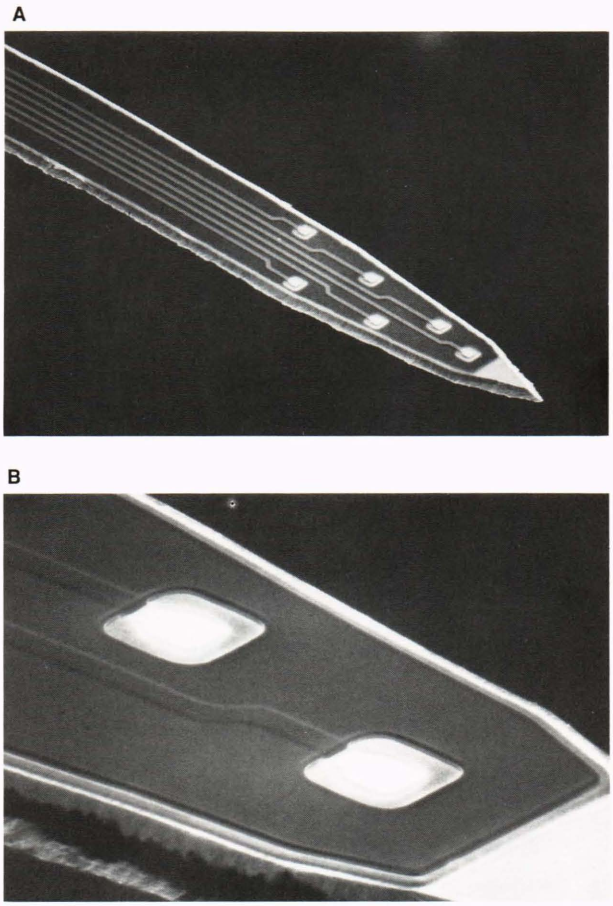

Figure 9. Electron micrographs. **A**. Lower portion of the six-site probe shank (73×). **B**. Lowest two electrode sites (511×). (Reprinted, with permission, from Ref. 6, p. 73; © 1991 by IEEE.)

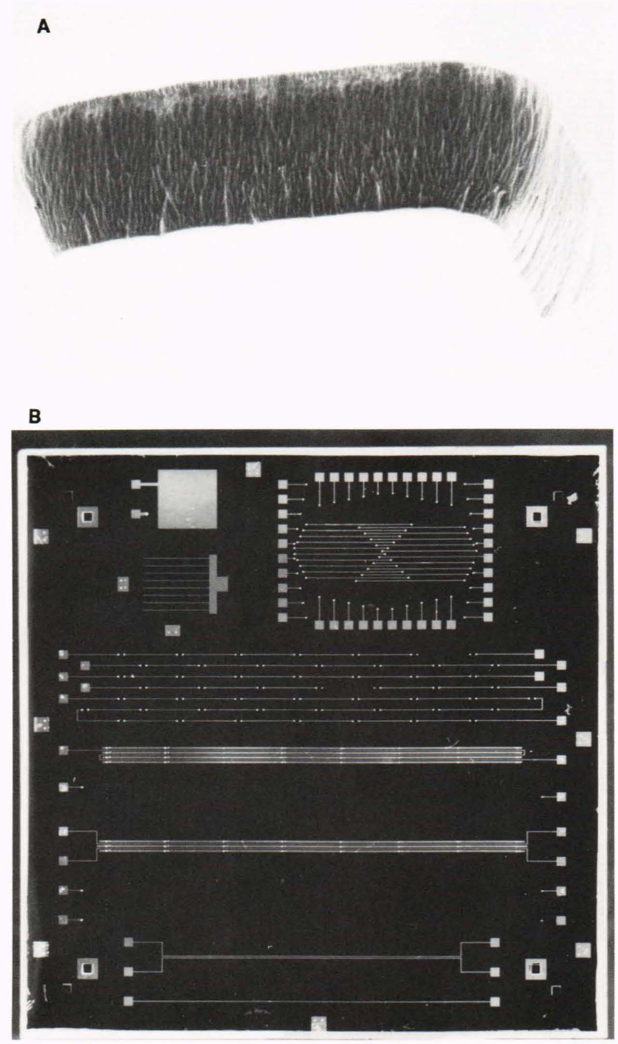

Figure 10. Patterned polyimide layers. **A**. Scanning electron micrograph view (1800×) of a via hole in a polyimide layer. **B**. Test pattern for multichip module with three patterned polymide dielectric layers (shown at about 1.5 times original size). Pattern is used to verify fabrication processes used to construct a multichip module.

straight sidewalls and minimizes undercutting of the window edge in contact with the underlying layer.

To fabricate these microscopic structures, a polyimide precursor liquid is spin-coated onto the substrate and then cured on a programmable hot plate whose temperature is gradually increased to a peak between 350°C and 400°C. Titanium is then sputtered on top of the polyimide film, and the metal layer is patterned using conventional methods to provide a nonerodible (in the plasma) etch mask for the polyimide. The pattern for the via hole (a conducting connection, or pathway, between one layer and another) is then etched into the polyimide using an oxygen-reactive–ion-plasma etching system. A view of a typical via profile is displayed in Figure 10A. The titanium is then removed by an acid etch, leaving the patterned polyimide layer. Low-stress polyimide layers processed in this manner were used to make the test pattern for the multichip test module shown in Figure 10B. A test pattern is used to verify the fabrication processes used in the construction of a multichip module.

Finding a way to increase probe longevity by improving the insulation of the bonding-pad encapsulation and connecting leads, to provide added strength in an environment hostile to the fragile microprobe structures, is a problem that still remains to be solved.

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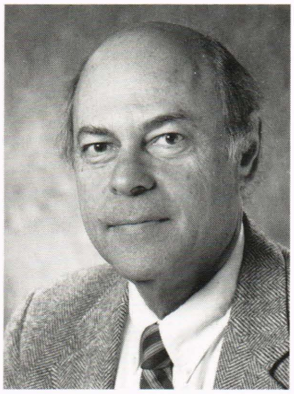

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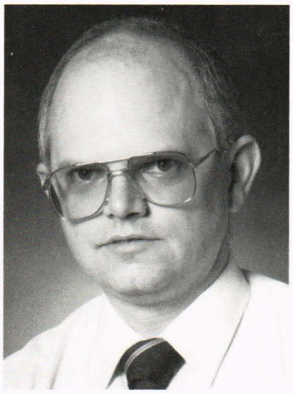

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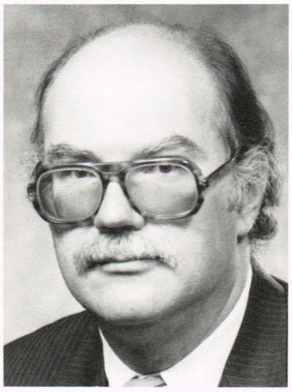

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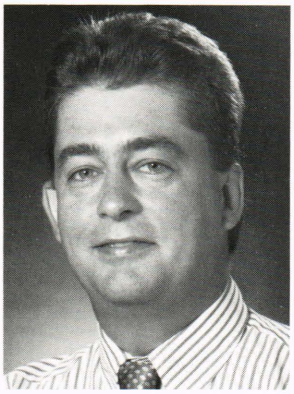

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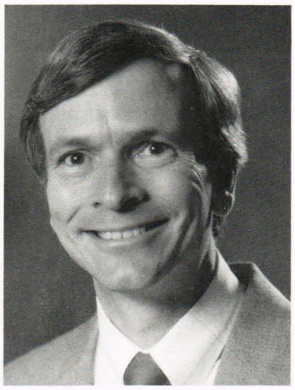

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