

RESEARCH ARTICLE | NOVEMBER 01 1991

Improved scanning ion-conductance microscope using microfabricated probes

C. B. Prater; P. K. Hansma; M. Tortonese; C. F. Quate



Rev. Sci. Instrum. 62, 2634–2638 (1991)

<https://doi.org/10.1063/1.1142244>



Articles You May Be Interested In

Double micropipettes configuration method of scanning ion conductance microscopy

Rev. Sci. Instrum. (July 2016)

Scanning ion conductance microscopy mapping of tunable nanopore membranes

Biomechanics (September 2017)

Simultaneous scanning ion conductance and atomic force microscopy with a nanopore: Effect of the aperture edge on the ion current images

J. Appl. Phys. (November 2018)



Review of Scientific Instruments Special Topics Now Online

[Learn More](#)

Improved scanning ion-conductance microscope using microfabricated probes

C. B. Prater and P. K. Hansma

Department of Physics, University of California, Santa Barbara, California 93106

M. Tortonese and C. F. Quate

Department of Applied Physics, Stanford University, Stanford, California 94305

(Received 20 May 1991; accepted for publication 29 July 1991)

The scanning ion-conductance microscope (SICM) is a member of the growing family of scanning probe microscopes, which includes the scanning tunneling microscope and the atomic force microscope. The SICM scans a small aperture over a sample immersed in electrolyte and measures the ionic current flowing through the aperture. This current serves as the feedback signal for standard scanning probe microscope electronics, which can then provide images of the topography of a sample surface, or the distribution of ionic current flow through the pores in a porous sample. The resolution of this microscope is essentially set by the size of the aperture. We have developed silicon microfabricated probes for the SICM that greatly improve its performance. Previous SICMs used fragile glass micropipettes as probes. The new microfabricated probes are end caps for a 1.5 mm glass capillary. A hollow tip is fabricated in the center of a silicon membrane. An aperture is formed at the tip apex using microfabrication techniques including photolithography and etching, giving typical minimum diameters of 250 nm. Unlike the glass micropipettes used previously, the new tips are compact and hence mechanically robust. The tips are mounted on a flexible membrane that allows the tip to deflect away from the surface in case of a collision with the sample surface. In addition, the microfabricated probes have been designed to have a high mechanical resonant frequency, allowing scan speeds up to 50 times faster than used with glass micropipette probes. With these new probes we have imaged the surface topography of a plastic diffraction grating, and the ion flow through porous polycarbonate membrane filters. Finally, the new microfabricated probe was designed as a general probe that may be adapted for use in other scanning probe microscopes.

I. INTRODUCTION

In this article we present results from an improved scanning ion-conductance microscope (SICM).¹ The SICM is able to image both the topography of a sample under electrolyte, and any ion flow through that sample. The principal improvement of this SICM is to incorporate a microfabricated probe to replace fragile glass micropipettes used in previous versions of the microscope.¹

The microscopic study of the liquid-solid interface has many applications. The surface topography of a biological sample can give detailed information about the three-dimensional structure of a sample, and hence important clues about the specimen's function. Images of the surface of industrial samples provide information for research and development and quality control. The passage of ions through pores in biological membranes, such as electrical impulses carried through nerve cells, is a fundamental method of communication in the biological world. Flow through pores in artificial membranes forms the basis of technologies like reverse osmosis desalination of water, electrochemical fuel cells, and dialysis.

Scanning probe microscopes,² like the scanning tunneling microscope³ and the atomic force microscope,⁴ have made new inroads in the microscopic study of topography. Vibrating probe measurements^{5,6} and the scanning electro-

chemical microscope⁷ have been used to measure the flow of ionic currents near biological samples. This improved SICM, incorporating a microfabricated probe, offers a new high-resolution tool for studying both topography and ion-flow characteristics at the liquid-solid interface.

II. MATERIAL AND METHODS

The SICM works in the following way (refer to Fig. 1): An aperture mounted on a glass capillary is filled with electrolyte. The assembly is placed in a bath of electrolyte and positioned over the sample. A bias voltage is applied to the electrode inside the glass capillary and ions flow through the aperture to one of two electrodes in the electrolyte bath. The ionic current is measured and used as the feedback signal for standard scanning probe microscope control electronics. The two electrodes in the bath allow the SICM to function in one of two modes: one that is sensitive only to topography and the second mode sensitive to topography and to the flow of ions through pores in a sample. In the first mode, the electrode to the side of the sample is grounded and ions flow from the aperture, over the sample, and to that grounded electrode. If the aperture is positioned so that its distance from the surface is on the order of its diameter, the amount of current flowing from the aperture is reduced as surface features block the cur-

SICM

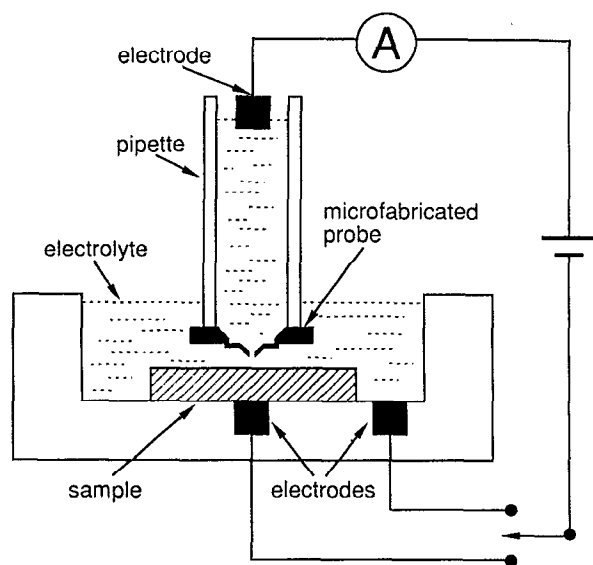


FIG. 1. SICM schematic.

rent flow. The sample is moved up and down on a piezoelectric translator under feedback control to keep the current constant while the sample is scanned under the tip. The vertical motion of the piezoelectric translator follows the topography of the sample. The voltage applied to the vertical axis of the piezoelectric translator is plotted as a function of position to form an image. This is analogous to the operation of the scanning tunneling microscope in constant current mode, or the atomic force microscope in constant force mode.

The second mode of operation is sensitive to ionic flow through pores in the sample. In this case the electrode directly under the sample is grounded and the ionic current flows through the aperture into the pores of the sample. The aperture is scanned across the sample without adjusting its vertical position and the current flowing through the aperture is measured as a function of position to form an image. The amount of current will increase as the aperture is brought directly over a pore. Thus an image obtained in this mode is a map of the pores that carry current through the sample. If the sample surface is rough, its topography also modulates the current, making the resulting image a combination of information about topography and ionic current flow through pores. In critical applications an image formed in this mode could have topographic contributions removed by comparing it to an image taken in the topographic mode.

Previously, apertures were formed by drawing down 1–2 mm glass capillaries into micropipettes on a commercial micropipette puller.¹ This procedure resulted in typical aperture diameters of 200 nm. Micropipette tip diameters as small as 10 nm have been reported.⁸ Unfortunately, the glass micropipettes were long and thin, giving them low lateral resonant frequencies, and thus limiting the maximum scan rate. The micropipettes were also very fragile,

often breaking upon contact with a sample or during high-speed scans.

Because of the many advantages of microfabricated probes already in use in other scanning probe microscopy techniques, mainly in atomic force microscopy (AFM),⁹ we designed a microfabricated silicon probe for the SICM that could solve some of the problems encountered with the glass micropipettes. The geometry of the device consists of a square silicon membrane about 0.25 μm thick and 350 μm wide, which is in turn mounted on a square silicon post, 25 μm tall. The aperture is typically a square, 0.25 μm wide. The height of the tip and post are designed to allow clearance during the approach of the tip to the sample. The membrane has two purposes: first it can flex like a drum head to allow the tip to deflect away from any collision with the sample, minimizing damage to the tip and sample. Second, it has a high resonant frequency that allows fast scan speeds for imaging. The presence of the membrane thus removes two limitations inherent in the previous glass pipette probes: the breaking of the tip during its approach to the sample and the limited scan speed. The membrane has a theoretical resonant frequency¹⁰ of

$$f = \frac{10.4}{2\pi} \left(\frac{Et^2}{\rho d^4} \right)^{1/2} = 31 \text{ kHz},$$

and a theoretical spring constant¹¹ of

$$k = 16.0(Et^3/d^2) = 0.4 \text{ N/m},$$

where $E = 1.9 \times 10^{11} \text{ N/m}^2$ and $\rho = 2.3 \times 10^3 \text{ kg/m}^3$ are the modulus of elasticity and density of the silicon, and $t = 0.25 \mu\text{m}$ and $d = 350 \mu\text{m}$ are the thickness and width of the membrane, respectively. Both the resonant frequency and the spring constant can be changed by adjusting the fabrication process parameters.

Three photolithographic steps are required to make the device. The fabrication process is outlined in Fig. 2. The starting material is a $\langle 100 \rangle$ silicon wafer. The first step of the process is to grow a 1- μm -thick oxide layer and to pattern it so that small circles are left on the frontside, centered in the middle of large square windows opened in the backside oxide [Fig. 2(a)]. The diameter of the oxide circle is one of the parameters that determines the final size of the aperture. Therefore, to allow for variation in subsequent steps in the process, the diameter of the oxide circles is varied systematically on each die on the wafer from 18 to 22 μm in 0.25 μm increments. Then thick photoresist is spun and patterned on the frontside and used as a mask against silicon reactive ion etching. About 25 μm of silicon is etched. After removal of the photoresist [Fig. 2(b)], the oxide circle left on the frontside is used as a mask against another silicon reactive ion etching process. The new etch is more isotropic than the first one, so that considerable undercut of the silicon under the oxide mask occurs. At this point boron is diffused into the silicon, while the oxide masks the diffusion [Fig. 2(c)]. Then a protective protective low temperature oxide (LTO) layer is deposited on the frontside of the wafer, the boron-doped region is etched from the backside, and the wafer is immersed in ethylene

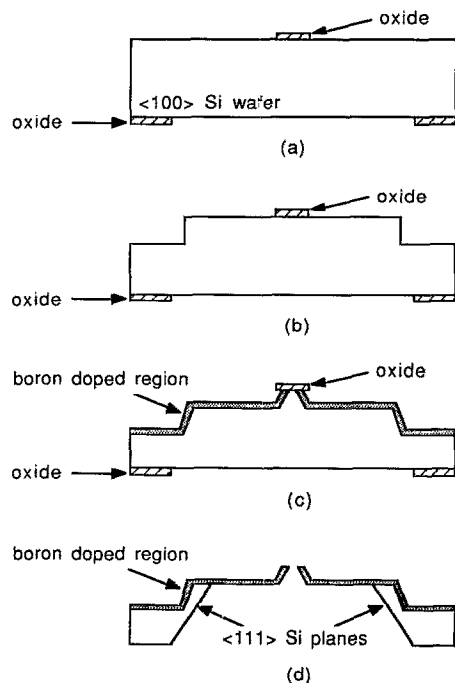


FIG. 2. Process for fabrication of the SICM probes. See the text for details.

diamine, pyrocatechol and water (EDP). EDP etches silicon preferentially, stopping at the $\langle 111 \rangle$ silicon planes and at the highly boron-doped region. After etching the frontside LTO and the thermal oxide the final structure is obtained [Fig. 2(d)]. Finally, a thin oxide layer is grown to achieve electrical isolation from the electrolytic solution. Given that the diameters of the oxide masks used to produce the cones vary by $0.25 \mu\text{m}$ from device to device on the same die, it is possible to obtain holes with varying sizes on the same die, the smallest of which is not bigger than $0.25 \mu\text{m}$. Some apertures have been further closed down by evaporation of oxide at an angle or by direct imaging of the aperture in a scanning electron microscope, which deposits hydrocarbons around the aperture. Apertures as small as 25 nm have been formed in the electron microscope. Both methods provide an opportunity for further improving the lateral resolution of the SICM, but the modified apertures have not yet been used for imaging and it is not clear if the modifications would be stable in electrolytic solution.

Figure 3 shows two electron micrographs of a completed device. Figure 3(a) shows the square post, $25 \mu\text{m}$ tall and $450 \mu\text{m}$ wide, with the tip visible in its center as a small bright spot. Figure 3(b) shows a cone, $15 \mu\text{m}$ tall, with a $0.4 \mu\text{m}$ aperture at its apex.

The finished probes were then glued with Devcon 2-Ton epoxy onto 1-cm-long pieces of 1.5-mm-diam glass capillary. The glass pipette provides a rigid support for the probe that fits into commercial microelectrode holders containing a Ag/AgCl electrode.¹² The sample was mechanically clamped into a plastic sample holder that contained two more Ag/AgCl electrodes, one for imaging to

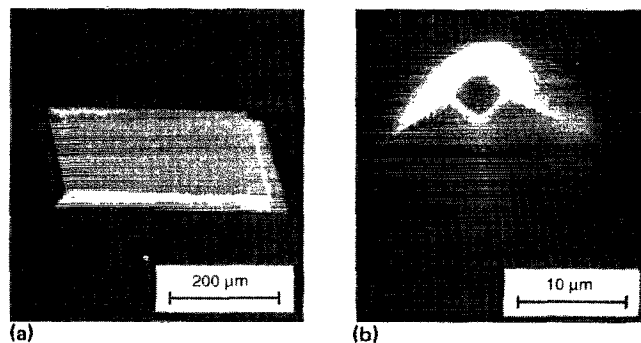


FIG. 3. SEM micrographs of SICM probes. (a) A view of the post that holds the SICM tip. The center of the posts is thinned to form a flexible support for the tip. (b) A closeup view of the SICM tip. The tip is hollow with aperture typical minimum sizes of 250 nm .

pography and the other for imaging ionic current flow. The samples were imaged under 0.1 M NaCl or 0.1 M KCl . The pipette and probe assembly had a typical resistance of a few megaohms.

Bias voltages of $0.05\text{--}5 \text{ V}$ were applied to the electrode in the pipette and one of the electrodes in the sample holder was grounded. The current was amplified using a home-built current-to-voltage converter. The feedback resistor was varied between $1 \text{ M}\Omega$ and $1 \text{ G}\Omega$, to give a signal voltage around 1 V . This signal was then input into standard Nanoscope II (Ref. 13) electronics which handled data acquisition and feedback control.

Mechanically, the rest of the SICM is very similar to commercial AFMs. The sample was scanned under a fixed probe using an xyz piezoelectric translator from a commercial AFM.¹³ The translator used had a range of $13.5 \mu\text{m}$, but translators with larger ranges could be used in principle. The SICM probe was held over the sample in a home-built head similar to the head that holds the probe for the AFM.

III. RESULTS AND DISCUSSION

Figure 4 shows an example of the SICM's ability to image surface topography. The sample is a plastic diffraction grating with a pitch of $1.9 \mu\text{m}$.

Images of ionic current flow through pores in polycarbonate membranes are shown in Fig. 5. In these images, bright areas correspond to areas of higher current density, and darker areas to lower current density. Figure 5(a) shows the current flow through a $0.8 \mu\text{m}$ Nuclepore¹⁴ filter. The ions flowing through the pores are clearly seen as bright circles in the image. These circles have peak currents roughly $5\text{--}20 \text{ nA}$ above an 800 nA background. Figure 5(b) is a similar image of a $0.1 \mu\text{m}$ Poretics¹⁵ filter. Bright spots, roughly $0.1 \mu\text{m}$ in diameter, with peak currents of $5\text{--}15 \text{ nA}$ above a 140 nA background are visible. In both images there is some structure to the background, coming from modulation of the current by the surface roughness. Atomic force microscope studies of these membranes have shown similar surface roughness.¹⁶

These images were acquired at scan rates of up to $700 \mu\text{m/s}$, almost 50 times faster than typical rates used suc-

cessfully with glass micropipettes. Further, none of the probes used in these experiments broke while scanning the sample, while the glass micropipette probes used previously were often damaged while scanning.

It should be possible to further improve the resolution of the SICM by fabricating smaller apertures as higher resolution lithographic techniques become available. Perhaps the most promising technique at present would be to use focused electron or ion beams to either etch the holes, or mark areas to be etched. Nanometer-size holes have been formed in MgO crystals using direct electron beam etching.¹⁷

Measuring very small currents with the SICM should not be a problem. The SICM has previously imaged current flow as low as 40 pA. Further, patch clamp preamplifiers¹⁸ are used routinely to measure small ionic currents, including currents through single ion channels in biological systems (few pA), and at rates up to several kHz. Low noise scanning tunneling microscopes have already been constructed using such preamplifiers.¹⁹

The new microfabricated probes have been used first in the SICM, but they were designed as a general probe for a variety of scanning probe microscopes. It may be possible to use these probes, as the scanning aperture in a near-field optical microscope,^{20,21} after coating them with a suitable metal.

The probes are hollow and could be filled with a material to customize them for a particular application. For example, they could be filled with a metal to be used as an insulated tip in electrolyte for a scanning tunneling microscope²² or a scanning electrochemical microscope.⁷ The electrochemical microscope is similar to the SICM except that it scans a small electrode, rather than an aperture. The microfabricated probes could be adapted for the electrochemical microscope by evaporating a metal-like platinum to fill up the hollow tip to form a microelectrode. Finally, since the probe tip is mounted on a flexible membrane, the SICM, or one of these techniques mentioned above, could be combined with force microscopy by measuring the deflection of the membrane as the tip is scanned over a surface.

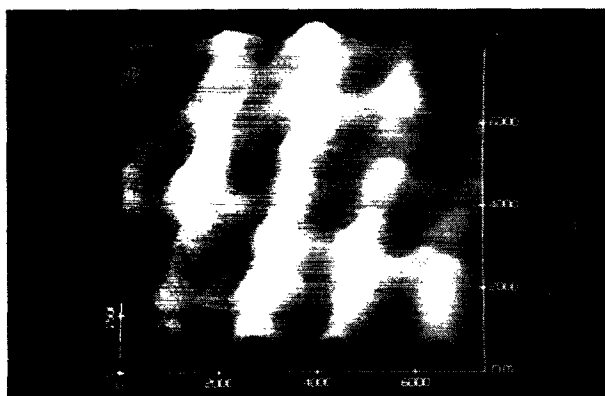
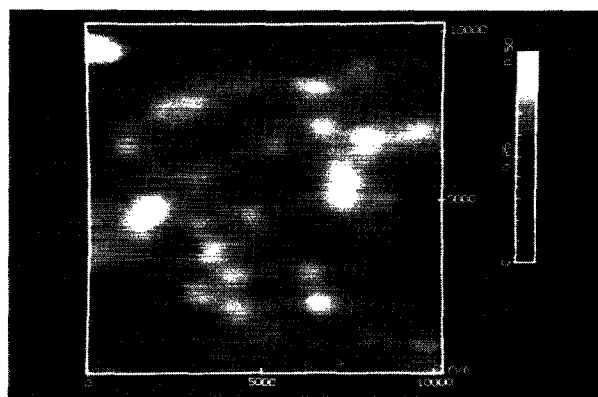
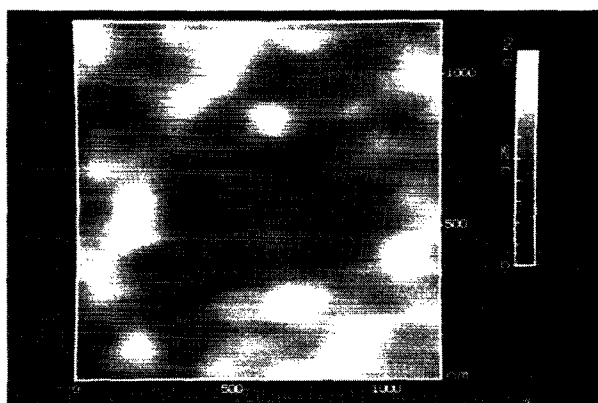


FIG. 4. SICM image of the surface topography of a plastic diffraction grating. The pitch of this grating is $1.9 \mu\text{m}$.



(a)



(b)

FIG. 5. A SICM image of current flow through polycarbonate membrane filters. Brighter regions correspond to areas of higher current flow. (a) Nuclepore $0.8 \mu\text{m}$ filter. The white spots are peaks 5–20 nA above an 800 nA background. (b) Poretics $0.1 \mu\text{m}$ filter. The white spots are 5–15 nA above a 140 nA background.

ACKNOWLEDGMENTS

We gratefully acknowledge the help of T. R. Albrecht for help in the design of the microfabricated probe and S. Akamine for suggesting the fabrication process. We also thank K. Cetera, G. Kelderman, P. Maivald, S. Manne, and M. R. Wilson for their help. This work was funded by the National Science Foundation Solid State Physics Grant DMR89-17164 (C.B.P.), National Science Foundation Grant ECS-89 17552 (M.T., C.F.Q.), the Office of Naval Research (P.K.H.) and Digital Instruments, Inc.

¹ P. K. Hansma, B. Drake, O. Marti, S. A. C. Gould, and C. B. Prater, *Science* **243**, 641 (1989).

² H. K. Wickramasinghe, *Scientific American* **260**, 98, 1989.

³ G. Binnig, H. Rohrer, Ch. Gerber, and E. Weibel, *Appl. Phys. Lett.* **40**, 178 (1982).

⁴ G. Binnig, C. F. Quate, and Ch. Gerber, *Phys. Rev. Lett.* **56**, 930 (1986).

⁵ L. Jaffe, *Trends Neurosci.* **8**, 517 (1985).

⁶ J. A. Freeman, P. B. Manis, G. J. Snipes, B. N. Mayes, P. C. Samson, J. P. Wikswo, and D. B. Freeman, *J. Neurosci. Res.* **13**, 257 (1985).

⁷ Scanning electrochemical microscope, A. J. Bard, F.-R. F. Fan, J. Kwak, and O. Lev, *Anal. Chem.* **61**, 132 (1989).

⁸ K. T. Brown and D. G. Flaming, *Advanced Micropipette Techniques for Cell Physiology* (Wiley, New York, 1986).

- ⁹T. R. Albrecht, S. Akamine, T. E. Carver, and C. F. Quate, *J. Vac. Sci. Technol. A* **8**, 3386 (1990).
- ¹⁰C. M. Harris and C. E. Crede, *Shock and Vibration Handbook* (McGraw-Hill, New York, 1961).
- ¹¹R. J. Roark, *Formulas for Stress and Strain* (McGraw-Hill, New York, 1965).
- ¹²Kwik-fil glass pipettes, microelectrode holders and Ag/AgCl electrodes were obtained from World Precision Instruments, Sarasota, Florida.
- ¹³Digital Instruments, Inc., 6780 Cortona Drive, Santa Barbara, CA 93117.
- ¹⁴Nuclepore Corporation, Pleasanton, California.
- ¹⁵Poretics, Inc., Livermore, California.
- ¹⁶P. Dietz, P. K. Hansma, O. Inacker, H.-D. Lehmann, and K.-H. Herrmann, *J. Membr. Sci.* (in press).
- ¹⁷P. S. Turner, T. J. Bullough, R. W. Devenish, D. M. Maher, and C. J. Humphreys, *Philos. Mag. Lett.* **61**, 181 (1990).
- ¹⁸B. Sakman and E. Neher, Eds., *Single Channel Recording* (Plenum, New York, 1983).
- ¹⁹R. Guckenberger, W. Wiegräbe, A. Hillebrand, T. Hartmann, Z. Wang, and W. Baumeister, *Ultramicroscopy* **31**, 327 (1989).
- ²⁰A. Lewis, M. Isaacson, A. Harootunian, and A. Muray, *Ultramicroscopy* **13**, 227 (1984).
- ²¹E. Betzig, J. K. Trautman, T. D. Harris, J. S. Weiner, and R. L. Kostelak, *Science* **251**, 1468 (1991).
- ²²R. Sonnenfeld and P. K. Hansma, *Science* **233**, 211 (1986).