# Imaging the Internal and External Pore Structure of Membranes in Fluid: TappingMode Scanning Ion Conductance Microscopy

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ABSTRACT We have constructed a combined TappingMode atomic force microscope and scanning ion conductance microscope. The design is based on a bent glass pipette that acts as both the force sensor and conductance probe. Measuring the pipette deflection allows more stable feedback than possible with previous versions of the scanning ion conductance microscope. Using this microscope, we have imaged synthetic membranes in both contact and tapping modes under fluid. Although contact mode operation is possible, we found that our microscope provided higher contrast and less apparent sample damage in the topographic and ionic conductance images in the tapping mode.

## INTRODUCTION

The atomic force microscope (AFM) has spawned a multitude of offspring since its invention (Binnig et al., 1986). The AFM is based on a sharp tip on the end of a flexible cantilever. The deflection of the cantilever is monitored as it is scanned over the sample surface. The AFM has proven especially useful for biological imaging because it is possible to operate in a liquid environment (Drake et al., 1989). In conventional dc-mode operation, the deflection can be recorded as a function of cantilever position. It also can be used as a feedback signal, where the deflection is kept constant by continuously adjusting the sample height. This is referred to as constant deflection mode. Recently, a new mode of AFM operation in fluids has been developed called TappingMode. In this case, the cantilever is oscillated at some set frequency. The amplitude of the cantilever response is monitored as a function of position with or without feedback. TappingMode is especially useful for imaging soft samples including biological specimens (Zhong et al., 1993). One exciting development is that this allows imaging of functioning biological samples under liquid (Hansma et al., 1994; Bezanilla et al., 1994; Fritz et al., 1995).

One of the earliest offspring of the AFM was the scanning ion conductance microscope (SICM) (Hansma et al., 1989; Prater et al., 1991). The first SICM was based on a pipette pulled down to a narrow aperture. An electrical system similar to that used by electrophysiologists was used to monitor the ionic currents flowing through the aperture at the end of the pipette as the pipette was scanned over the sample surface. The ionic signal was also used as a feedback signal. Although this was a workable arrangement, there were some problems with stability in the feedback. The microscope described in this work resolves these problems by using the pipette as a contact or tapping mode cantilever as well as a current sensitive probe.

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## EXPERIMENTAL SETUP

The combined SICM/AFM used in this work was based on a bent, hollow micropipette that is used as both the force and current detector. The pipettes were made of either pulled borosilicate glass tubing or quartz tubes. Although the borosilicate glass was convenient and easy to handle, we obtained the highest resolution and repeatability using pulled quartz tubes (Nanonics, Jerusalem, Israel). A typical pipette used in this work had an unpulled outer diameter of 1.0 mm, an inner diameter of 0.58 mm, a total length of 2–3 cm, and a shank of 5 mm. The bent portion of the pipette was typically 100 $\mu$m long. The apertures at the borosilicate tubes were difficult to get below 0.5 $\mu$m, whereas the quartz tubes could be pulled to below 50 nm. These dimensions were measured with high resolution electron microscopy.

The deflection of the pipette was measured using a conventional beam bounce technique (Meyer and Amer, 1989; Alexander et al., 1989). The pipette was mounted to a custom-built plexiglass fluid cell, which fit into our Multimode AFM (Digital Instruments, Santa Barbara, CA). Although the back of the pipette was rounded, sufficient light reached the AFM detector for it to operate well in both tapping and contact modes. The resonant frequency of the cantilever depended strongly on where the pipette was attached to the fluid cell. We observed a wide variation in the resonant frequencies; typically they were between 50 and 100 kHz. For the images shown in this work, the pipette was mounted to the fluid cell using heat-shrink tubing, leaving ~8 mm free. This resulted in a resonant frequency of 80 kHz. For TappingMode operation, a piezo stack (Tokin, Japan) was epoxied to the side of the fluid cell. Using this arrangement, a 0.5-V rms voltage was sufficient to excite a 10-nm rms free oscillation amplitude at the resonant frequency. During scanning in TappingMode, this oscillation amplitude was typically reduced by 50–65%. The quality factor of the pipettes was typically high compared with

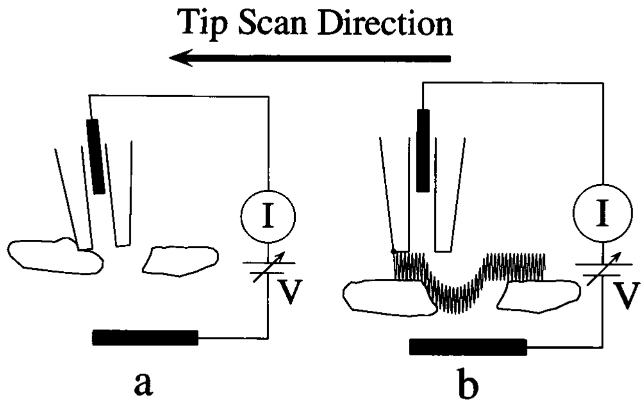


FIGURE 1 Diagram of the interactions between the cantilever and sample membrane. Electrodes were placed inside the pipette and the fluid cell. (*a*) Contact mode operation. Adhesive forces between the tip and sample result in lateral forces that tilt the scanning probe tip and stress the sample. (*b*) TappingMode operation where the reduction of lateral forces results in higher resolution and less sample perturbation. In tapping mode, the pipette was oscillated with an amplitude of ~10 nm over the surface.

conventional cantilevers operating in fluid, on the order of 10. A schematic diagram of the pipette tip interacting with a hypothetical membrane surface is shown in Fig. 1. Figure 1 *a* represents contact mode operation and Fig. 1 *b* shows TappingMode operation.

The ionic current was measured using a commercial patch clamp amplifier (Dagan Corp., Minneapolis, MN) operating in the constant voltage mode. In this work we measured the direct current component of the ionic current. The electrodes were standard Ag/AgCl half cells (World Precision Instruments, Sarasota, FL) submersed in a 100 mM KCl solution. For this work, the electrochemical circuit was biased at 50 mV and current levels were between 0 and 150 pA. The current noise was ~0.5 pA rms while operating in a 0- to 5-kHz bandwidth.

## RESULTS AND DISCUSSION

Contact and TappingMode images of the topography and ionic conductance of a synthetic polycarbonate membrane with pore size of 200 nm (Millipore, Bedford, MA) are

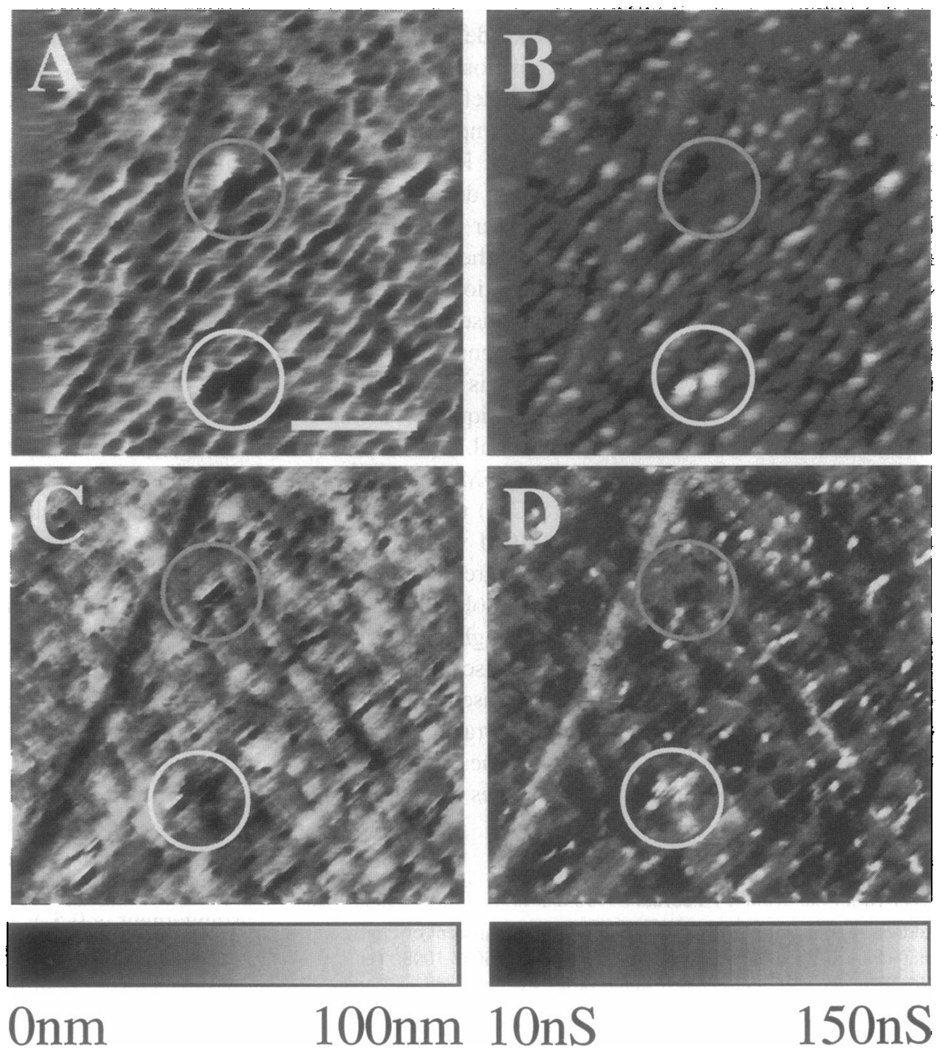


FIGURE 2 Nucleopore membrane showing the difference between tapping and contact mode operation of the AFM/SICM over the same area of a synthetic membrane. *A* is the contact mode topographic image, *B* is the contact mode conductance, *C* is the TappingMode topographic, and *D* is the TappingMode conductance image. Note that in both the contact and TappingMode images there are differences between the sizes of the pores in the topographic images and the ionic conductance images. The increase in resolution in TappingMode is readily apparent in both the topographic and ionic conductance images. Both the contact and TappingMode images show differences between the topography of pores and the ionic conductance. As an example of this, the reader is referred to the circled areas on the images. In both operating modes, the area circled in white shows a group of pores that appear to be deep on the AFM image and highly conductive on the SICM image. The area circled in gray contains a large pore which appears deep in the AFM image, but is nonconductive on the SICM images.

shown in Fig. 2. Both contact and TappingMode images were made over the same area of the sample surface. This is apparent from the scratch running down the left side of the images. There are also a number of pores circled on both images that are coincident in the two images.

Fig. 2 *A* shows the contact mode topography image measured with feedback. There is a band on the left edge of the image resulting from lateral forces on the tip as the scan direction is reversed. Fig. 2 *B* shows the corresponding ionic conductance image. The complementary nature of the topographic and ionic conductance images are apparent from the two features circled in the images. The circles in the topographic image are centered on two apparent pores. The ionic conductance image, however, shows that the pore centered in the gray circle had a low conductance whereas the pore centered in the white circle had a relatively high conductance.

The topographic image for TappingMode is shown in Fig. 2 *C* and the associated ionic conductance image is shown in Fig. 2 *D*. As in the contact mode images, there are differences in the conductance of the pores circled in gray and white on the images. There are also a number of interesting differences between the contact and TappingMode images. The resolution in Fig. 2, *C* and *D* is significantly better than that of Fig. 2, *A* and *B*. This increase in both the topographic and conductance resolution is particularly evident for the pores in the center of the white circle. In the contact mode images, there appear to be two large, closely spaced pores with high conductances. In the TappingMode images the two pores are shown actually to be four to five very closely spaced pores. One reason for the enhanced resolution may be the smaller lateral forces associated with TappingMode AFM. This would reduce sample deformation by the tip and effectively "stretch" the pore openings less than in contact mode scanning. The reduction of lateral forces in TappingMode is also supported by the absence of any sort of band on the edge of the TappingMode images. This reduction of lateral forces will be an important improvement when using the combined AFM/SICM to image biological membranes and macromolecular sheets.

## CONCLUSIONS

We have constructed a combined scanning ionic conductance/atomic force microscope that is sensitive to the surface topography and ionic conductance of synthetic membranes in an ionic solution. The microscope is based on a bent glass pipette. Although the microscope can be operated in contact mode, we found that it provided improved resolution of both topography and ionic conductance in tapping mode due in part to less lateral forces. This is important for future applications of this microscopy to biological membranes.

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