Scanning ion conductance microscopy mapping of tunable nanopore membranes

Ankita Gangotra^{1,2} and Geoff R. Willmott^{1,2,a)}

¹*Departments of Physics and Chemistry, The University of Auckland, Auckland, New Zealand*

²*The MacDiarmid Institute for Advanced Materials and Nanotechnology, New Zealand*

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We report on the use of scanning ion conductance microscopy (SICM) for *in-situ* topographical mapping of single tunable nanopores, which are used for tunable resistive pulse sensing. A customised SICM system was used to map the elastomeric pore membranes repeatedly, using pipettes with tip opening diameters of approximately 50 nm and 1000 nm. The effect of variations on current threshold, scanning step size, and stretching has been studied. Lowering the current threshold increased the sensitivity of the pipette while scanning, up to the point where the tip contacted the surface. An increase in the pore area was observed as the step size was decreased, and with increased stretching. SICM reveals details of the electric field near the pore entrance, which is important for understanding measurements of submicron particles using resistive pulse sensing. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4999488>]

I. INTRODUCTION

Individual pores in thin membranes have become important tools in the fields of microfluidics and nanofluidics. Much research related to such pores has involved resistive pulse sensing, that is, detection and analysis of individual particles in aqueous solutions, based on the change in ionic resistance observed as a particle passes through a pore. One focus of this work has been the development of efficient technologies suitable to be used as benchtop sensors, using materials ranging from polymers^{1–12} to glass,^{13–17} carbon nanotubes,¹⁸ and silicon-based membranes.^{19,20} Such technologies typically target particles with length scales of tens of nanometers and greater, with emphasis on high throughput, ease of use, and ease of pore fabrication. These pores come in many shapes and sizes, and knowledge of pore geometry is often a factor which limits the precision of measurements. There is usually an underlying trade-off between precise characterization of individual pores, and the efficiency of fabrication and measurement.

Tunable resistive pulse sensing (TRPS) is a technique which exemplifies the broader field of resistive pulse sensing. Over the past decade, TRPS has been developed and applied to a wide variety of submicron particle types and measurement problems,¹ usually employing the protocols developed for particle concentration,² size,³ and charge⁴ measurement. TRPS differs from other resistive pulse techniques because the elastomeric membrane can be stretched in order to actuate the size of the sensing pore.⁵ The geometry of these tunable pores can be changed as measurements are taking place, with the membrane held within a fluid cell.

Typically, TRPS measurements have used the assumption that the pore geometry takes the form of a truncated cone. The limitations of this assumption have been revealed by imaging studies—for example, extensive scanning electron microscopy has shown that the pore openings are not typically circular,^{1,21} atomic force microscopy has shown that pores do not have sharp edges,⁶ and confocal microscopy has revealed the pore profile within the membrane.² However, the relevance of these conventional techniques can be limited by the difficulty of stretching the

^{a)}Electronic mail: g.willmott@auckland.ac.nz

membrane during imaging, and by the imaging physics, which is not related to ionic current measurements.

A second typical measurement assumption for TRPS is that the ion distribution is homogeneous, other than within an electrical double layer that is much smaller than pore opening radius. However, recent findings have highlighted the importance of variable ion distribution. Concentration polarization along the pore axis is most likely responsible for observations of ion current rectification⁷ (widely observed for other pore types^{17,22–25}) and biphasic pulses,⁸ while pulse distribution broadening⁹ indicates variations for particles following different off-axis trajectories through a pore. Therefore, there is an opportunity to improve the characterization of tunable pores using a 3D topographical mapping technique, which enables scanning as a function of stretch *in-situ*, and which measures ionic current so that the ion distribution and electric field local to a pore can be studied.

Scanning ion conductance microscopy (SICM) is a tool that has been in use for almost three decades,²⁶ and has been used to scan biological tissues since 1997.²⁷ SICM has found widespread application in contactless and *in-situ* mapping of biological surfaces such as living cells and proteins, as described in previous reviews.^{28–30} Recently, SICM has increasingly been used to probe materials in unconventional, multifunctional ways.^{31,32} An SICM system consists of two electrodes in an electrolyte, one inside a pipette and one outside in the bulk solution. When a potential bias is applied and the pipette approaches a sample surface, the flow of ions is restricted and the current drops. Hence, the surface can be topographically mapped without actually making contact with the pipette. The pipette tip diameter ranges from nanometer to micrometer scales, and the range of pipette sensitivity is approximately 1.5 times the diameter of the tip.³³ One of the main advantages of SICM is that it can be adapted for different types of experiments, as in this paper.

Here, SICM apparatus has been modified to enable scanning of the topography of tunable pores. The effects of three main parameters on the measured size and shape of the pores has been investigated: tip-sample separation (determined by a threshold current, or set point), scanning step size, and pore stretch. Although SICM has not been applied to tunable nanopores until now, analysis of other nanopore types has been reported over the past decade using scanning probe techniques such as SICM and scanning electrochemical microscopy (SECM).^{34–43} Most notably, Chen *et al.*³⁴ simultaneously scanned the topography and the ion current pathways emanating from 300 nm and 500 nm diameter, 25 μ m long cylindrical polyimide nanopores, in AC modulating mode. Increasing the difference in ionic concentration between upper and lower chambers resulted in an increase of ion current transport through the nanopores. Subsequently, the effect of varying tip-sample separation on ion current profiles was studied in polyimide nanopores ranging from 273 nm to 930 nm in diameter, with decreasing separation leading to better scanning resolution.³⁶ The voltage-current properties in the vicinity of such nanopores have also been studied.^{36,37}

II. MATERIALS AND METHODS

The experiments discussed, along with their corresponding parameters, are given in Table I. Nanopipettes were fabricated with a pipette puller (P-2000, Sutter Instruments) from quartz capillaries with 0.5 mm inner diameter and 1 mm outer diameter (QF100–50–7.5, Sutter Instruments). Two different nanopipettes (Pi50 and Pi1000) were used and characterised with TEM [Fig. 1(a)] and SEM [Fig. 1(b)]. The inner diameter of the tip opening of the Pi50 pipette was approximately 35–50 nm and that of the Pi1000 was 800–1000 nm.

SICM scans were performed on two types of tunable nanopore membranes, NP800 and NP2000 (Izon Science, Ltd.), where the number refers to an approximate ideal particle size in nm for resistive pulse sensing. Figures 1(c) and 1(d) are SEM images of typical NP800 and NP2000 pores, in which cracks are observed on the membrane surface due to Pt coating of the specimen prior to imaging. Because coating the nanopore membrane is a destructive process and SICM imaging could also damage the membrane (stress-softening and marking caused by pipettes), the SEM images presented do not show the pores scanned with SICM. Some salt residue can be noticed in these SEM images around the pore openings as a result of prior TRPS measurements.

TABLE I. Details of SICM experiments performed on tunable nanopores.

Investigated parameter	No.	Fig.	Pore	Stretch (mm)	Pipette	Step size (nm)	I_{th} (%)	Z_m (μ m)
I_{th}	1.1	3(a)	NP800	48	Pi50	500	99.5–98.5	10
	1.2	3(b)	NP2000	48	Pi50	2000–500	99.5–99.0	10
Step size	2.1	4(a)	NP800	48	Pi50	2000	98.5	10
						500	98.5	10
	2.2	4(b)	NP2000	48	Pi50	2000	99.0	7
						500	99.0	7
Pore stretch	3.1	6(a)	NP2000	48–52	Pi1000	1000	99.0	7
	3.2	6(b)	NP2000	46–52	Pi50	1000	99.0	7

Taking *in-situ* ionic current measurements is an important part of studying tunable nanopore systems. SEM imaging of tunable nanopores on its own, while useful for visualising topography, cannot be carried out *in-situ* to measure ionic current through the pore and changes caused by nanopore actuation i.e., stretching the membrane. AFM imaging has similar issues and it can also be a destructive process.

The nanopipette scanning apparatus was set up as in Fig. 2(a). Ag/AgCl electrodes were used; a wire was placed inside the pipette; and the second electrode was embedded within the fluid cell. A voltage of 0.5 V was applied, and the resulting current was measured by a low

FIG. 1. Nanopipettes and nanopore membranes used in the SICM scans. (a) TEM image of Pi50. (b) SEM image of Pi1000. (c) SEM image of the smaller entrance of an NP800 pore at 48 mm stretch. (d) SEM image of the smaller entrance of an NP2000 pore at 48 mm stretch.

FIG. 2. SICM set-up and mode of operation. (a) Schematic showing the apparatus and control connections. (b) Hopping mode SICM in which the pipette approaches the surface until the current falls to a certain threshold I_{th} , after which it is retracted to a certain height and moved to the next step. The process is then repeated for each step of the scan. The Z travel (height) is recorded when I_{th} is reached, with a minimum value defined as $Z = 0$ (Z_{th}). Z_m is the average membrane height.

noise potentiostat built in-house. The electrolyte used was $\times 10$ phosphate buffered saline (PBS, Sigma Aldrich). At such a high electrolyte concentration, the signal to noise ratio is high, and at low electrolyte concentration (e.g., $< \times 1$ PBS) current measurements could be affected by ion current rectification and the increasing electrical double layer size. A nano-piezo (Nanocube, Physik Instrument) was used for the pipette positioning. LabVIEW software (National Instruments) was used to interface between all the devices and record the data.

To perform SICM on a tunable nanopore, the nanopore membrane was held by metal teeth on a rig over an open fluid cell filled with electrolyte. The pore stretch measurement used in Table I and elsewhere refers to the separation of these teeth, which could be moved to macroscopically stretch the membrane from a typical starting (relaxed) position of ~ 42 mm. The pipette was positioned in the electrolyte covering the upper surface of the membrane [Fig. 2(a)]. Hence, flow of ions between electrodes was only possible through the pore. To confirm wetting of the pore, the ion current was monitored as the electrolyte was loaded. As soon as a flow of ions through the pore was established, there was a sudden rise in ion current. In these experiments, hopping mode SICM raster scans [Fig. 2(b)] were carried out with a constant X and Y step size.

Two types of thresholds were used for hopping mode scans: current threshold (I_{th}) and height threshold (Z_{th}). I_{th} was defined as a percentage of bulk current (I_0) and was the usual threshold during scanning of the nanopore membrane, away from the pore. I_0 was measured at the start of each approach, which prevented drift due to evaporation. The approach begins at Z_0 , at least $10 \mu\text{m}$ above the average membrane surface height (Z_m), where there was no significant restriction to the flow of ions. While scanning directly over the pore, I_{th} would not be reached as there would not be any surface to restrict the current. The pore length (membrane thickness) is approximately $200 \mu\text{m}$, and the maximum travel on the piezo in the Z -direction is $100 \mu\text{m}$. Thus, Z_{th} was introduced, defining the maximum distance the pipette could travel into the pore and hence the minimum height of a scan. This minimum was set $7\text{--}10 \mu\text{m}$ below the

average membrane position Z_m , and the Z scale was set to zero at Z_{th} . Hence, Z_m was at a relative height of 7–10 μm . After scanning, the recorded Z height was plotted as a function of X and Y position to obtain a topography map of the surface. The area of the pore measured from such a map is defined as the region in which the pipette reaches Z_{th} , and is therefore a function of Z_{th} .

The main source of experimental uncertainty is the spatial resolution in the XY plane, which is determined by the size of each X and Y step that the pipette takes during a scan, in combination with the pipette resolution. The measured area may be underestimated because it cannot be determined within one step size whether the pipette would reach Z_{th} or the pore wall. Thus, the measured area of the nanopore is a lower bound (A_{LB}), and there is a higher bound (A_{HB}) given by

$$A_{HB} = A_{LB} + A_{Error}, \quad (1)$$

where the error in the measured area (A_{Error}) is approximately the step size times the measured pore perimeter. The observed nominal position uncertainty given by the instrument control system for the X , Y , and Z piezos was 100–150 nm, so data points are not always uniformly distributed and the topography scans have variations that appear to be smaller than the step size. Flat shading and interpolation methods were used to plot each topography scan. Each quadrilateral formed with four data points at the vertices defaults to a colour corresponding to the lowest Z height of the four points. Other uncertainties may be associated with piezo travel in the Z direction, which may overshoot I_{th} due to its frequency response, and electrical noise in the current.

III. RESULTS AND DISCUSSION

A. Varying I_{th}

For experiments 1.1 and 1.2, height profile scans with varying I_{th} are shown in Fig. 3. To visualise the data better, the topography maps were plotted with a black outline depicting the nanopore area, i.e., where Z_{th} was reached before I_{th} . It can be seen that as I_{th} decreases, the pipette becomes more sensitive to the features on the surface and the area of the pore becomes larger. However, I_{th} could only be decreased to a certain limit (around 98%–99% of I_0) before the pipette crashed into the surface, which could be determined by a sudden rise in the ion current.

For experiment 1.1 [Fig. 3(a)], an NP800 pore was scanned, Z_m was set at $Z = 10 \mu\text{m}$, and the step size was 500 nm for all three scans. During scans at $I_{th} = 99.5\%$ and 99% I_0 , the pipette was prevented from reaching Z_{th} , i.e., the dark blue region is absent.

For experiment 1.2 [Fig. 3(b)], an NP2000 pore was scanned, Z_m was set at $Z = 7 \mu\text{m}$, and the step size was 2000 nm, 1000 nm and 500 nm for I_{th} at 99.5%, 99.25% and 99% respectively. Unlike in the case of the NP800 pore, Z_{th} was reached even at high I_{th} . The dark blue region, where $Z = 0$, increased in area as I_{th} decreased. When I_{th} was at its lowest, at 99% I_0 , the pipette pressed into the surface, as indicated by the fragmentation of the topography map. This fragmentation may also have been caused by the smaller step size (500 nm), which is further investigated in Sec. III B.

There are several reasons for the pipette not reaching $Z = 0$ in experiment 1.1. First, I_0 for an NP800 with a Pi50 pipette was comparatively small at 99 nA. So, the change in current between I_0 and I_{th} for 99.5% and 99% I_{th} was comparable to the noise level, which was ~ 1 nA peak-to-peak. Additionally, the larger step size could result in spatial sampling effects, i.e., not enough sampling points near the middle of the pore. These data are also consistent with reduction in the current as the pipette approaches the pore walls in the XY plane, preventing the pipette from reaching Z_{th} . This may demonstrate the importance of the lateral part of the volumetric region over which the pipette is sensitive,³³ and is also consistent with the traditional model of electro-osmotic effects, in which current flow is reversed near the walls of a long thin cylinder.⁴⁴ However, the electrical double layer for $\times 10$ PBS (< 1 nm) is much smaller than the step size, and recent finite element modelling of resistive pulse sensing near pore openings has

FIG. 3. Local profile scans on a tunable nanopore, with I_{th} varied, where the black outline encloses the nanopore area i.e., where Z_{th} was reached before I_{th} . (a) Experiment 1.1: Scans of NP800 with Pi50 at 500 nm step size. (b) Experiment 1.2: Scans of NP2000 with Pi1000 at 2000 nm, 1000 nm, and 500 nm step size, respectively.

shown that larger resistive pulses are observed for particles passing near to the pore walls, due to a greater electric field in that region.^{9,45} The nature of these competing effects, and the well-resolved geometry of the electric field around the pore entrance, could be probed in future SICM experiments.

The current threshold experiments helped to optimise I_{th} for the subsequent experiments, in which it was held at values between 98.5% and 99%. Any value higher would render the pipette less sensitive, and any value lower would lead to the pipette crashing into the surface.

B. Varying step size

Experiments 2.1 and 2.2, which were scans of NP800 and NP2000 pores, respectively, gave topography maps shown in Fig. 4. First, rough scans of the surface were carried out at 2000 nm step size to identify the region of interest, shown in Figs. 4(a-i) and 4(b-i). Then, the step size was changed, and finer scans were carried out at 500 nm step size over the identified pore, shown in Figs. 4(a-ii) and 4(b-ii). Some features seen in the rougher scans are likely due to defects on the nanopore membrane sustained over repeated experiments.

In Fig. 4(a), the NP800 pore has $A_{LB} = 8.75 \mu\text{m}^2$ and $A_{HB} = 13.4 \mu\text{m}^2$. The nanopore is clearly visible and has edges comprised of straight lines, rather than rounded curves. This is likely to be an artefact of scanning, because the size of the pore compared to the step size is relatively small in the X and Y directions, causing a “pixelation” effect. The scans proceed in a straight line raster, consistent with the outcome. SEM images of the pore after SICM scans found the pore area to lie between $20 \mu\text{m}^2$ and $50 \mu\text{m}^2$.

In Fig. 4(b), the NP2000 pore has $A_{LB} = 65.25 \mu\text{m}^2$ and $A_{HB} = 81.84 \mu\text{m}^2$. While the shape of the pore is near-circular, the raster scan does produce some artefacts along the scanning direction, i.e., parallel to the X axis. From SEM imaging of the NP2000 pore after SICM scanning, the pore area was found to lie between $75 \mu\text{m}^2$ and $150 \mu\text{m}^2$.

It is important to note that unlike in previous studies^{34,36} where the SEM measured values of pore diameter were smaller than the SICM measured values, here the pore area derived from SICM was smaller due to the pore area definition discussed in the experimental section. Also after multiple SICM scans, the pore areas most likely became larger due to inelastic fatigue. It

FIG. 4. Rough and fine topography maps of nanopores. (a) Experiment 2.1: NP800 scanned at I_{th} of 98.5% with Pi50 at 2000 nm step size in (i) and 500 nm step size in (ii). (b) Experiment 2.2: NP2000 scanned at I_{th} of 99% with Pi50 at 2000 nm step size in (i) and 500 nm step size in (ii).

is not expected that pore entries are perfectly rounded, based on SEM results obtained before SICM scans, as shown in Figs. 1(c) and 1(d). From these images, the pore area at 48 mm stretch for a typical NP800 was found to be $13.5 \pm 2.5 \mu\text{m}^2$ and for NP2000 was $54.2 \pm 8 \mu\text{m}^2$. These values are comparable to the areas of the pores measured with SICM.

To test the topography scans for repeatability, different scans were performed on each nanopore membrane with identical parameters (Fig. 5). For the NP800 pore, all 4 scans were performed using the same experimental set-up on the same day with the same pipette, with scan 4 the same scan as in Fig. 4(a). For NP2000, the first 4 scans were performed on the same day with the same pipette, while the last 2 were performed on different days with different pipettes, with scan 6 the scan shown in Fig. 4(b). While the pipettes were different on different days, the size of the tips was found to be approximately the same by measuring I_0 for each pipette. The scans done on the same day are repeatable, whereas those from different days clearly give different values. Therefore, mounting, stretching and dismounting the pore membrane on the stretching stage between experiments introduced an uncertainty in the pore area measurements. Partly, this may be systematic error attributed to inelastic deformation to the membrane, either through repeated stretching or impressions caused by pipettes.

C. Varying pore stretch

In experiments 3.1 and 3.2, profiles of the NP2000 tunable pore were investigated *in-situ* as a function of applied stretch. Figure 6 shows that, as expected, the more a pore is stretched, the more it expands.

FIG. 5. SICM area measurements for different scans with the same experimental parameters. (a) For the NP800 pore on the same day with Pi50. (b) For the NP2000 pore with Pi1000, with the pore remounted on different days and different pipette on each day. The range plotted for each scan is between A_{LB} and A_{HB} .

For experiment 3.1, the Pi1000 pipette was used at 1000 nm step size. The measured area increased between each successive stretch, shown in Fig. 6(a). Artefacts of the scanning (X) direction are visible in these profiles. This is a result of the pixelation effect described previously, although in this case reducing step size would not lead to increased resolution due to the large pipette size.

For experiment 3.2, the Pi50 pipette was used at 1000 nm step size. The measured area again increased between each successive stretch [Fig. 6(b)], apart from at 52 mm stretch. This could be due to random uncertainties, or the pore lacking mechanical response in this range of stretching. Artefacts of the scanning direction are less visible because of the pipette size used during these scans, rendering the system more sensitive to the change in current between each step.

In Fig. 6(c), an inconsistency in the areas was observed between the scans with Pi1000 and Pi50 pipettes, at the same stretch (48–50 mm). This is probably caused by the difference in the size of the pipettes. A large pipette, such as Pi1000, is sensitive to change over a greater area in the XY plane because for some XY points near the edge of the pore, Pi1000 is likely to recognise the edge whereas Pi50 might reach $Z=0$. This explains why the pore area may be underestimated when using a large pipette aperture, as observed here at 48 and 50 mm stretch. At 52 mm, measurements are within the error bars determined by the XY step size.

IV. CONCLUSION

Tunable nanopore membranes were scanned and mapped *in-situ* using SICM for the first time. As the threshold current (I_{th}) was decreased, the resolution of the scanning increased,

FIG. 6. Profiles of A_{LB} for the NP2000 pore as a function of stretch. (a) Using Pi1000 at 1000 nm step size. (b) Using Pi50 at 1000 nm step size. (c) Area of the NP2000 pore with increase in stretch for scans with Pi1000 and Pi50. The range plotted at each stretch is between A_{LB} and A_{HB} , and lines are drawn to guide the eye.

revealing features on the surface in greater detail and enabling the pipette to reach a threshold height within a pore. If I_{th} was lowered below a certain point, the pipette would crash into the surface. The influences of electrical noise and proximity of the pipette to the pore walls become more important for relatively small pores. Further scans were carried out while varying the step size of the raster scan, allowing identification of scan artefacts that were attributed to the step size, pipette resolution, and raster scanning method. Finally, the effect of stretch on the measured size of a pore was investigated. There was a definite increase in pore area as the stretch was increased, while the effect of scanning with a relatively small pipette was noticeable, with less pixelation observed in the outline of the pore area.

This work represents the preliminary findings of the use of SICM when scanning tunable nanopores. Although data from relatively large pore sizes have been presented, this paper presents considerable evidence that SICM has potential in scanning smaller nanopores. The most important direction for developing this work is to increase the spatial resolution allowed by the combination of the scanning controls and the pipette sensing. Such improvements would also allow details of the electric field around a pore to be observed, and mapping of ion currents to further elucidate the transport mechanisms acting on ions and particles moving through a pore. Experiments presented here have provided some initial evidence regarding ion currents near to the pore walls. The outcomes of such studies are broadly relevant to interpretation and precision of measurements made using resistive pulse sensors.

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