Experimental Studies of Resolution in Scanning Ion Conductance Microscopy

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Spatial resolution of images recorded with scanning ion conductance microscopy (SICM) was examined with well-defined samples prepared by focused-ion beam (FIB) milling. Fiduciary standards of controlled size, shape, and spacing were investigated as a function of imaging parameters and pipette tip size. Topographic images of the standards were acquired in ac feedback mode. We report that two features can be resolved when spaced apart at distances as small as 0.5x the probe inner radius, in agreement with recently reported models, and examine effects of probe-surface distance and feature geometry on SICM resolution.

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The scanning ion conductance microscope has developed into a sophisticated tool for fine probe control at the nanometer scale. SICM has proven useful in a number of applications, especially for imaging biological samples. With recent improvements in instrumental performance, detailed studies of image formation and resolution are of special interest. Lateral resolution has been reported in previously performed studies with contradictory conclusions. Elucidation of factors that influence SICM resolution is important for improved data collection, and becomes of even greater importance when quantitative measurements are of interest. Because SICM is a non-contact imaging method and a range of pipette dimensions, electrolyte concentrations and probe-surface distances (Dps) can be chosen, a sole experimental parameter that defines resolution is difficult to describe. An early report by Korchev and coworkers described the operating mechanism of SICM as “effectively a ‘spherical sensor’ of diameter d that can ‘roll’ over surface irregularities in the specimen without damaging it or the pipette tip.” The concept of a ‘spherical sensor’ provides a heuristic description of the probe-surface interaction in SICM that is ultimately responsible for resolution. Subsequent models of the probe-surface interaction suggest that the ‘spherical sensor’ at the tip of the pipette probe can be distorted by interactions with the sample through “current squeezing.” This phenomenon results in an effective “sensing zone” in the immediate vicinity of the pipette tip. The nature of this zone (e.g. dimensions, shape) is complex, variable and dependent on multiple experimental parameters. Here, we describe experimental efforts to determine resolution in SICM with pipettes and samples that have been well-characterized with electron microscopy.

A schematic of SICM is shown in Figure 1a, and physical parameters related to probe geometry and position are shown in Figure 1b. When an appropriate bias is applied between a Ag/AgCl electrode in the pipette probe and a second Ag/AgCl electrode in solution, ion current flows through the pipette tip. Ion current magnitude is dependent on probe geometry, electrolyte concentration, and electrolyte conductivity (κ) as described by equations 1–3. Ion current is a function of applied bias (U) and the sum of two experimentally determined resistances termed pipette resistance (Rp) and access resistance (RAC). Experimental parameters such as outer radius of the pipette tip (ri), inner radius of the pipette tip (ri), radius of pipette base (rb), pipette length (h), and Dps are important for determination of resistance terms. A plot which correlates Dps with dc ion current is shown in Figure 1c; as Dps decreases, Rac increases and ion current approaches a minimum value. Distance-dependence of ion current is the basis for feedback and control of probe position for non-contact SICM imaging. For practical applications, more complicated feedback methods (e.g. “ac mode,” or “hopping mode”) are employed for scanning.

\[ i_{DC} = \frac{U}{Rp + RAC} \]  
\[ Rp = \frac{h}{\pi \kappa \sigma r_2 r_1} \]  
\[ RAC = \frac{2 \ln \left( \frac{r_1}{r_2} \right)}{\pi \kappa \sigma D_{ps}} \]

Previous efforts to determine the effect of system parameters, especially ri, on SICM resolution have been performed both experimentally and with finite element method (FEM) simulations. Experimental studies have shown that features spaced apart at distances as small as 0.5ri can be resolved, and that image resolution can be qualitatively improved as Dps is decreased. Simulated SICM experiments have suggested the limit of lateral resolution is approximately 2–3ri, and that feature geometry can affect resolving power. Lateral resolution, defined as the smallest distance between features where both objects can be distinguished, has been described qualitatively, and as a function of advanced image processing. Table 1 summarizes resolution studies reported to date. Here, in an effort to compare our results to light microscopy, we have adapted a definition of lateral resolution from optical microscopy based on the Rayleigh criterion. Briefly, when two closely-spaced objects of similar height are imaged and a line profile is generated, the change in intensity between features can be examined to determine image contrast and resolution. If intensity in this region decreases ≥26% of object height, sufficient contrast is present for lateral resolution, parameterized as baseline return, BLR (Fig. 1d).

Herein we describe the utilization of a focused-ion beam (FIB) to produce well-defined features for resolution characterization. A FIB was used to enable quick, efficient, and reproducible production of resolution standards of various size, shape, and geometry at small (10s of nm) scales. Our studies suggest lateral resolution below simulated limits can be achieved, and factors besides probe size, such as Dps and feature geometry, are important experimental parameters that can ultimately have significant effects on SICM resolution.

Experimental

Chemicals and Materials.— Solutions were prepared with 18 MΩ-cm H2O from a Milli-Q water purification system (Millipore Corp., Danvers, MA, USA). Potassium chloride (Mallinckrodt, Phillipsburg, NJ, USA) solution of 0.1 M concentration was used as the electrolyte for SICM measurements. A 1 μM ethanolic solution of generation 5, (polyamido)amine (PAMAM) dendrimer with ethylenediamine core (Sigma-Aldrich, St. Louis, MO, USA) was prepared as described previously.

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Resolution standard fabrication and characterization.—Resolution standards were FIB-milled (Zeiss Auriga Workstation, Oberkochen, Germany) into gold-coated, glass coverslips. Two geometries with a variety of dimensions were prepared: raised bars (ca. 40 nm or 100 nm height; 750 nm width x 1 μm length or 1 μm width x 1 μm length; 30 nm – 150 nm spacing), and trenches (ca. 100 nm depth; 1 μm length; 200 nm – 3 μm width). Dendrimer functionalization of coverslips was performed as described previously.8 Briefly, clean coverslips were soaked in dendrimer solution for 3 hours. Slides were dried with nitrogen and immediately sputter-coated (Denton Desktop V, Moorestown, NJ, USA) with gold. The purpose of the gold coating was to mitigate charge for FIB-milling and scanning electron microscopy. Table 1. Compilation of previously performed resolution experiments. Mode indicates feedback mechanism used; Method describes whether study was performed under experimental or simulations conditions; Resolution indicates the type of resolution studied; Definition describes the criteria by which resolution was determined; Result/Resolution Limit indicates the resolution achieved, and Reference is the citation for the study.

| Mode | Method | Resolution | Definition | Result/Resolution Limit | Reference |
|------|--------|------------|------------|-------------------------|-----------|
| DC   | Exp    | Lateral    | ‘Dip’ in profile | 2ri                  | 1         |
| DC   | Exp    | Vertical   | Visual comparison with SEM image | 50 nm     | 7         |
| AC vs. DC | Exp | ion current | Approach curve comparison | AC more sensitive that DC | 9         |
| AC   | Exp    | Lateral    | 2D fast Fourier transform | 0.5ri     | 3         |
| AC with improved lock-in algorithm | Exp | Lateral | image and line profile comparison | Enhanced resolution with improved lock-in algorithm | 10        |
| Hopping | Exp | Lateral | Visual examination of image | ri     | 11        |
| AC   | Exp    | Vertical   | Line profile comparison | Improved resolution | 5         |
| DC   | Model  | Lateral    | Visual examination of image | 3ri | 4         |
| AC   | Exp    | Vertical   | AFM image comparison | 10’s of nm | 12        |
| Hopping | Model | Lateral | ‘Dip’ in line profile ≥ 0.05ri | 2ri  | 6         |
| Hopping | Model | Lateral | ‘Dip’ in line profile ≥ 0.05ri | Lateral resolution affected by sample geometry | 6         |

Figure 1. (a) Schematic of SICM set-up. Application of bias between a Ag/AgCl electrode in an electrolyte-filled pipette and an external electrode in bath solution generates ion current through the pipette tip. (b) Scale illustration of important experimental parameters when probe is brought in close proximity to the sample: h: tip length; ri: outer probe radius; ri: inner probe radius; Dps: probe-surface distance; red gradient: sensing zone. (c) Approach curve which describes the distance dependence of ion current. As Dps decreases, ion current decreases. Inset equation represents distance-dependence relationship; ri: inner radius of pipette base. Equation is from Reference 8. (d) Depiction of resolution measurement; BLR is baseline return, which is a percentage of probe travel in the z-dimension with respect to feature height.
microscopy (SEM) imaging, and also to provide optical contrast for positioning of SICM probes over milled features. Without dendrimer functionalization, glass-to-gold adhesion was poor, and free-floating gold clogged the SICM probe during experiments. Bar geometries spaced 50 - 150 nm apart were milled with a 50 pA gallium ion beam of 1 μs dwell time. Bar geometries spaced 30 nm apart were milled with 20 pA current. Characterization of milled features was performed by SEM (Zeiss Auriga Workstation, Oberkochen, Germany) with a 5 kV electron beam. An SEM image of 30 nm spaced bars is shown in SI 1a. Averaged consecutive line profiles (n = 128) taken across the feature are shown in SI 1b. A description of each resolution standard used for these studies is shown in SI 2.

**Instrumentation.—** Data was acquired via a ScanIC scanning ion conductance microscope (ionscope, London, U.K.). To perform an experiment, a milled sample was mounted in a petri dish and submerged in 0.1 M KCl. A potential difference (100 mV) was applied between the SICM electrodes. Vertical position of the probe was modulated at 200 nm at 800 Hz to produce a distance-modulated ion current (ac mode),9 which was used as feedback for non-contact imaging.

**Nanopipette fabrication.—** Nanopipettes were fabricated from quartz capillaries (inner diameter 0.7 mm and outer diameter 1 mm) with a P-2000 puller (Sutter, Novato, CA, USA). Pipettes were routinely fabricated to have inner diameters of 50 - 140 nm. The following program was used for fabrication: Heat = 650, Filament = 4, Velocity = 45, Delay = 160, Pull = 190. Characterization of pipettes was performed by scanning transmission electron microscopy (STEM and FE-SEM, FEI Quanta-FEG). A STEM image of a typical pipette used in these studies is shown in SI 3.

**Image collection.—** A number of parameters were optimized to obtain topographic images with SICM. Nanopipette probes were scanned 10 μm in the x-dimension. Each line contained 512 pixels to result in a nominal pixel size of 20 nm. Images were acquired at a rate of 30 seconds per line in the forward scan direction and were subsequently processed with Gwyddion.15 Each image was subjected to mean plane subtraction and height median matching in the fast scan direction. Consecutive line profiles taken of topographic images were averaged to decrease noise. For analysis, 128 line profiles were averaged. Great care was taken to ensure each line profile was drawn perpendicular to the gap between features, and that each line profile was drawn with the same dimensions, with no two identical lines. Trace and retrace line profiles were compared to optimize minimization of artifacts; an example of typical trace/retrace scans is shown in SI 4. Table SI 3 indicates the full-width-at-half-max (FWHM) of a single bar, the gap between the bar, and both bars for each feature, as well as the error associated with each BLR measurement.

**Results**

**Lateral resolution.—** To examine general characteristics of SICM lateral resolution under experimental conditions, three sets of closely spaced bars (50, 100, and 150 nm spacing; 100 nm height; 750 nm width; 1 μm length) were imaged with a 40 nm ri, 70 nm ri probe under identical imaging conditions. A topographic image recorded for 100 nm spaced bars is shown in Figure 2 (images for bars spaced 50 and 150 nm apart are shown in SI 5). Line profiles (vertically offset for clarity) taken across each image are shown in Figure 2b with BLR indicated, data were processed manually to determine the minimum point for each gap. When bars were spaced 150 nm apart (Figure SI 5a), features appeared separate and distinct in the topographic image, as signified by an obvious demarcation between bars. Line profiles (Figure 2b, solid trace) indicate BLR of 84%. As feature spacing decreased, BLR decreased, as well. For bars spaced 100 nm apart, (Figure 2a) the two bars appear distinct; however, the feature demarcation in the SICM image visibly decreased. Line profiles (Figure 2b, dashed trace) indicate BLR decreased. For bars spaced 50 nm apart (SI 5b), the demarcation between features is more difficult to distinguish. Line profiles (Figure 2b, dotted trace) indicate BLR decreased to 32%.

**Probe size.—** FIB-milled features were also imaged as a function of probe size. Three different sized probes (25 nm ri, 42 nm ri, 60 nm ri, 76 nm ri, and 70 nm ri, 98 nm ri, respectively) were used to generate topographic images of 30 nm spaced bars (40 nm height; 1 μm width; 1 μm length). A topographic image obtained with the 25 nm ri probe is shown in Figure 3a, and a topographic image obtained with the 70 nm ri probe is shown in Figure SI 6. Deviations in image orientation between Figures 3a and SI 6 occur from slight discrepancies in sample and probe positioning. Comparison of the two images indicates the topographic image obtained with the 25 nm ri probe (Figure 3a) is more clearly resolved than the image obtained with the 70 nm ri probe (Figure SI 6). In the former image, a demarcation between features is apparent. The topographic image obtained with the 70 nm ri probe appears blurred, and a boundary between bars is not easily identified. Vertically offset line profiles generated over each image (25 nm ri probe: dotted trace; 60 nm ri probe: dashed trace; 70 nm ri probe: solid trace) are shown in Figure 3b. As probe size increased, BLR decreased. BLR percentages for the 25 nm ri, 60 nm ri, and 70 nm ri probes were 27%, 26%, and 20%, respectively. To maintain consistency, each image was obtained with the same set point, i.e. the same percentage of ion current was maintained between probe and surface.

**Probe-surface distance.—** The same set of 30 nm spaced bars used in the probe size study was imaged as a function of Dps. A 60 nm ri, 76 nm ri probe was used to obtain topographic images at Dps values of 68 nm, 57 nm, and 51 nm. A topographic image obtained with Dps of 51 nm is shown in SI 7a, and an image obtained with Dps of 68 nm is shown in SI 7b. A demarcation between features is observed, and each bar appears distinct in the topographic image taken with Dps of 51 nm (SI 7a). However, when larger Dps values were used, such as Dps of 68 nm, the two bars were not as easily distinguishable (SI 7b). Vertically offset line profiles taken across each topographic image are shown in Figure 3c. When Dps was larger (68 nm; dotted trace), BLR...
above 26% (resolved). The line profiles (Figure 2b) indicated each set of bars was resolved, with BLR of 32%, 74%, and 84%, respectively. Additionally, the magnitude of BLR change was not linear. For instance, a decrease in bar spacing of 50 nm between the 150 and 100 nm spaced bars resulted in BLR decrease of 10%, while a second 50 nm decrease between the 100 nm and 50 nm spaced bars resulted in BLR decrease of 42%. Consideration of the approach curve equation, shown in Figure 1c, explains this discrepancy. As bar spacing decreases, the maximum ion current over the feature decreases, as a result of a smaller conductance drop generated by the smaller spacing relative to the area of the sensing zone.

To compare BLR with previously used descriptions, we examined whether 100 nm (2.5\(r_i\)) spaced bars would have been considered resolved per previous studies. In the topographic image of 100 nm spaced bars (Figure 2a), each bar appears distinct, which satisfies the criterion set forth by Rheinlaender et al.\(^4\) Additionally, 50 nm (1.2\(r_i\)) spaced bars (SI 5b) also satisfy this condition. Line profiles of the 2.5\(r_i\) and 1.2\(r_i\) spaced features (Figure 2b, dashed trace) indicate that BLR observed is greater than 0.05\(r_i\) (2 nm for the conditions used here), which satisfies Del Linz et al.\(^5\) Use of BLR provides an easily applicable definition for lateral resolution that also corroborates well with previous descriptions to indicate resolution better than 2–3\(r_i\) can be achieved. Application of a threshold for resolution on the basis of imaging characteristics (i.e., relative probe travel) decouples resolution measurements from factors such as probe size or instrument noise, and provides a quantitative measurement that can be compared to other imaging methods.

Effect of probe size.— Probe size was experimentally varied to examine the resultant effect on image resolution. Topographic images were generated of a set of 30 nm spaced bars with three different sized probes (25 nm \(r_i\), 60 nm \(r_i\), and 70 nm \(r_i\)), which was equivalent to bars spaced 1.2\(r_i\), 0.5\(r_i\), and 0.4\(r_i\), respectively under the imaging conditions used here. BLR percentages, obtained from line profiles taken across topographic images, were 27%, 26%, and 20%, respectively. These BLR percentages indicate that the 25 nm \(r_i\) probe and 60 nm \(r_i\) probe resolved the features, while the 70 nm \(r_i\) probe did not. These results further confirm that lateral resolution better than 2–3\(r_i\) can be achieved, and that a lower lateral resolution limit of 0.5\(r_i\) is apparent.

To understand the enhanced limit of lateral resolution observed, as opposed to that observed in simulations, we consider SICM operation mechanism in conjunction with sigmoidal fitting of experimentally obtained line profiles across the dip between features with a Boltzmann equation of the of the following form:\(^6\)

\[
y = A_2 + \frac{(A_1 - A_2)}{1 + e^{-\frac{x-x_0}{\alpha}}}
\]

where \(A_1\) is the initial position of the probe in the \(x\)-dimension, as the probe scanned left-to-right, \(A_2\) is the \(x\)-position of the probe which correlates to the minimum \(Z\)-position of the probe between features, \(x_0\) is the value of inflection as the probe descended between features, and \(dx\) refers to the change in \(x\) that corresponds to the change in \(Z\)-position values. A sigmoidal fit was used for these studies because of the sigmoidal-shaped probe travel across the FIB-milled resolution standards. The probe first follows a flat, horizontal line across the 'table top' of the bar feature, followed by sloped probe descent which reaches a minimum value before the probe begins to retract as the next feature wall is encountered (retraction point is represented by the last data point in each line scan). Figure 5a shows probe travel observed as the probe scans over this gap as related to the probe size. This data can be considered relative to the concept of a sensing zone for the probe/sample interaction. For a common set point (as is the case here), size of the sensing zone is dependent on probe radius, which determines both the height and width of the sensing zone (Figure 1d). A small \(r_i\) probe travels farther laterally before a drop in conductance is experienced, which results in a slower onset of descent into the feature gap. Further, because the sensing zone is smaller, steeper descent (and ascent) are observed for vertical probe

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**Figure 3.** (a) Topographic image of 30 nm spaced bars (1 \(\mu m\) width x 1 \(\mu m\) length; 40 nm height) obtained with 25 nm \(r_i\) probe. (b) Vertically offset line profiles taken across 30 nm spaced bars with 25 nm \(r_i\) probe (dotted trace), 60 nm \(r_i\) probe (dashed trace), and 70 nm \(r_i\) probe (solid trace). Baseline return percentages for each line profile are indicated. (c) Vertically offset line profiles taken across topographic images obtained with 60 nm \(r_i\) probe of \(D_{ps}\) 68 nm (dotted trace), \(D_{ps}\) 57 nm (dashed trace), and \(D_{ps}\) 51 nm (solid trace). Baseline return percentages for each image are indicated.

**Discussion**

Lateral resolution characterization.— To evaluate lateral resolution under real imaging conditions, experimental parameters (i.e., probe size and feature spacing) were chosen in accordance with previously reported lateral resolution models.\(^4\) These simulations have suggested that a given probe is limited to resolving two features spaced 2–3\(r_i\) apart. To compare to resolution limits of 2–3\(r_i\), FIB-milled bars spaced 50 nm, 100 nm, and 150 nm apart were imaged with a 40 nm \(r_i\), 70 nm \(r_i\) probe, which correlated to feature spacing below (1.2\(r_i\), at (2.5\(r_i\), and above (4\(r_i\)) the simulated resolution limit. Considering previous simulations, we would expect the 50 nm spaced bars to have a BLR below 26% (not resolved); the 100 nm spaced bars to have a BLR at 26% (resolved); and the 150 nm spaced bars a BLR was only 17%. For \(D_{ps}\) 57 nm and \(D_{ps}\) 51 nm, BLR was 22% and 26%, respectively.

Trenches.— Trenches of the same depth, but different width (200 nm vs. 3 \(\mu m\), both 100 nm deep), were examined under identical imaging conditions. Line profiles taken across both trenches are shown in Figure 4. When the 200 nm wide trench was imaged, the probe descended 20 nm less into the feature than when the 3 \(\mu m\) wide trench was imaged.
travel. When the 25 nm ri probe (dotted trace) was used to image 30 nm spaced bars, feedback was maintained with the sample while the probe descended steeply between the features. When probe size increased to 60 nm ri (dashed trace), the sensing zone area increased, which led to delayed probe descent. To maintain feedback, the 60 nm ri probe did not descend as steeply as the 25 nm ri probe. As probe size increased to 70 nm ri (solid trace), probe descent was further delayed, and occurred over a broader range in the x-dimension. The results observed in the probe size study are interesting because when 30 nm bars were imaged under identical conditions, except for a 35 nm increase in ri, BLR decreased only 1% and resolution only slightly improved. However, an additional increase in ri of 10 nm resulted in a 6% BLR decrease which suggest subtleties exist in direct comparison of resolution as a function of probe size.

**Effect of probe-surface distance.**—Topographic images were also obtained as a function Dps. Since the limit of lateral resolution from the probe size study was found to be ∼0.5ri, a 60 nm ri probe was used to examine whether changes in Dps could affect lateral resolution measurements. Despite a relatively small difference between three Dps values (68 nm, 57 nm, and 51 nm), lateral resolution significantly increased as Dps decreased, with BLR measured at 17%, 22%, and 26%, respectively. This result suggests that for larger tips with higher currents, resolution can be enhanced by lowering Dps. This conclusion represents an important trade-off to be considered for image resolution. Small ri probes are often more difficult to operate at low currents and smaller Dps, where larger ri probes operated at smaller than typical Dps may provide adequate resolution. This trade-off, speed of image collection, and the region of the approach curve in which feedback is maintained should be considered carefully.

**Trench features.**—Trenches of equal depth, but different width (200 nm vs. 3 μm, both 100 nm deep), were examined under identical imaging conditions. Line profiles taken across both trenches are shown in Figure 4. When the 200 nm wide feature was imaged, the probe descended into the trench 20 nm less than when the 3 μm wide trench was imaged. Sigmoidal fits to probe travel are shown in Figure 5b. When the 200 nm trench was imaged, the probe could not descend the entire 100 nm depth before the second wall of the trench was detected. However, when the 3 μm wide trench was imaged, the probe could travel the full depth of the feature because the trench was significantly wider than the probe dimensions. These observations, and those described for bar samples, are consistent with Del Linz et al., who suggested that samples with different conductance drops would be imaged with different resolution. In their study, ‘omega’ shaped samples, which had void areas at the base of the feature that led to a greater conductance drop than straight-walled features, were imaged with increased resolution. We show here that feature geometry does play an important role in lateral resolution, and that resolution is much more complex than an estimation based on probe size alone.

**Figure 4.** Overlaid line profiles taken across 200 nm trench (solid trace) and 3 μm trench (dashed trace). Imaging conditions (i.e. probe size and Dps) were identical.

**Figure 5.** (a) Overlaid sigmoidal fits applied to probe descent between features for topographic images obtained with 25 nm ri probe (dotted trace), 60 nm ri probe (dashed trace), and 70 nm ri probe (solid trace). (b) Overlaid sigmoidal fits applied to probe descent into trench features for 200 nm wide trench (dashed trace) and 3 μm wide trench (dotted trace).

**Conclusions**

We have described experimental measurement of FIB-milled features with SICM, and considered resolution relative to extent of probe travel (BLR). Results demonstrate lateral resolution is not limited to 2–3ri, and resolution as low as 0.5ri can be achieved. The importance of Dps is underscored. The effect of feature geometry was examined, as well. These results suggest a definition of lateral resolution, decoupled from experimental parameters, is useful in assessing relative lateral resolution when experimental parameters are varied. Probe size, Dps, and feature geometry are all important considerations for high-resolution imaging, and development of an understanding of the factors that affect resolution can enable more quantitative measurements to be made. Additional considerations, which include outer probe dimension (as opposed to only the dimension of the inner channel of the pipette) and to the surface charge of the substrate remain important parameters for experimental investigation.

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