Estimating the geometry of scanning ion conductance microscope pipettes from resistance variation with breakage

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Introduction

Scanning ion conductance microscopy (SICM) produces high-resolution topographic images under near-physiological conditions [1]. The lateral resolution and non-contact working distance depend closely on the geometry of the fine glass micropipette used as a proximity detection probe, in particular the internal half cone angle (θ) and the inner tip radius (r_0) [2]. Direct measurement of these parameters requires the use of a different imaging modality, most commonly scanning electron microscopy (SEM). Such measurements are difficult and unreliable, and in practice crude estimates based on a single resistance measurement are usually used instead.

We have developed a more sophisticated estimation method that fits a model of the geometry to multiple resistance measurements recorded while breaking the pipette tip [3]. The method is simple to implement and allows significant improvement in quantification of SICM data.

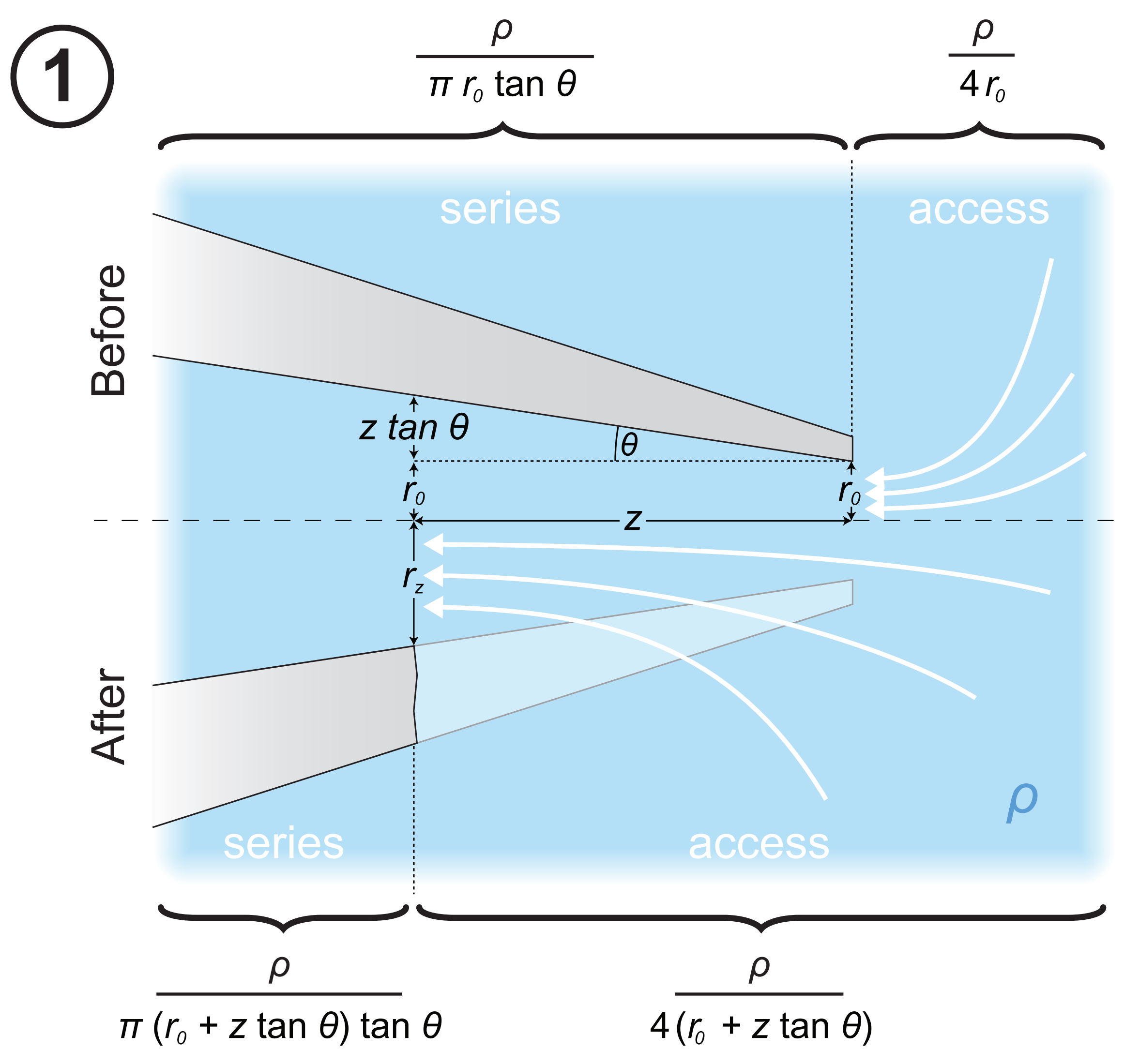
Model

① The pipette is modelled as a truncated cone. There are two major components to its resistance: the internal ***series resistance*** of the volume of electrolyte inside the glass; and the external ***access resistance*** of the convergent paths through the bath solution to the circular pore at the pipette's tip [4].

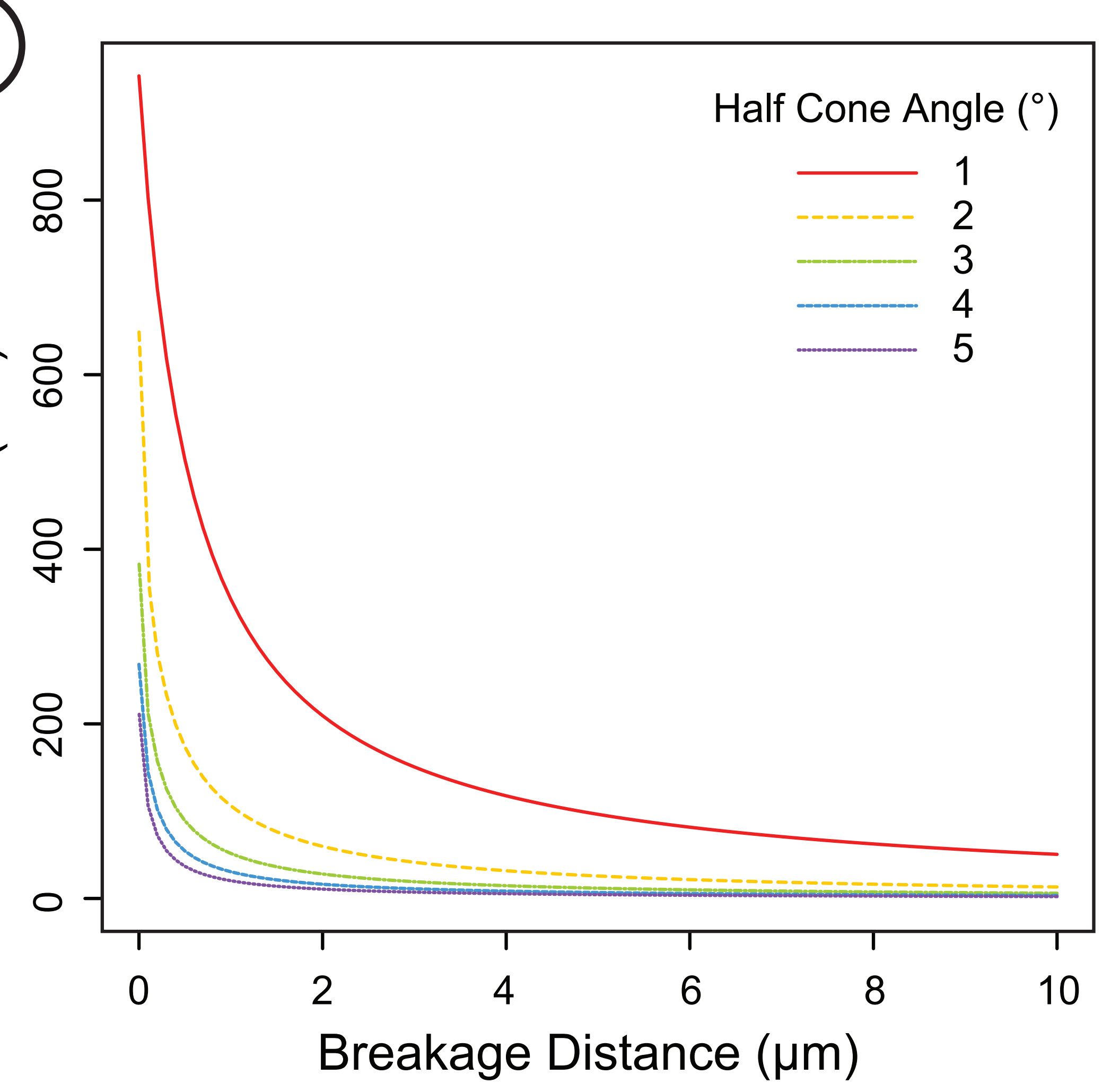
If a length z is broken from the tip, the pore radius is increased by $z \tan\theta$, reducing both resistance components. (We assume that the break is symmetric and z is much less than the overall length of the pipette.)

② The model predicts how the resistance should vary with breakage distance. Fitting this to experimental data showing how the resistance actually *did* vary for a given pipette allows us to estimate the unknown parameters r_0 and θ. (The third model parameter, the solution resistivity ρ, can be measured directly.)

①



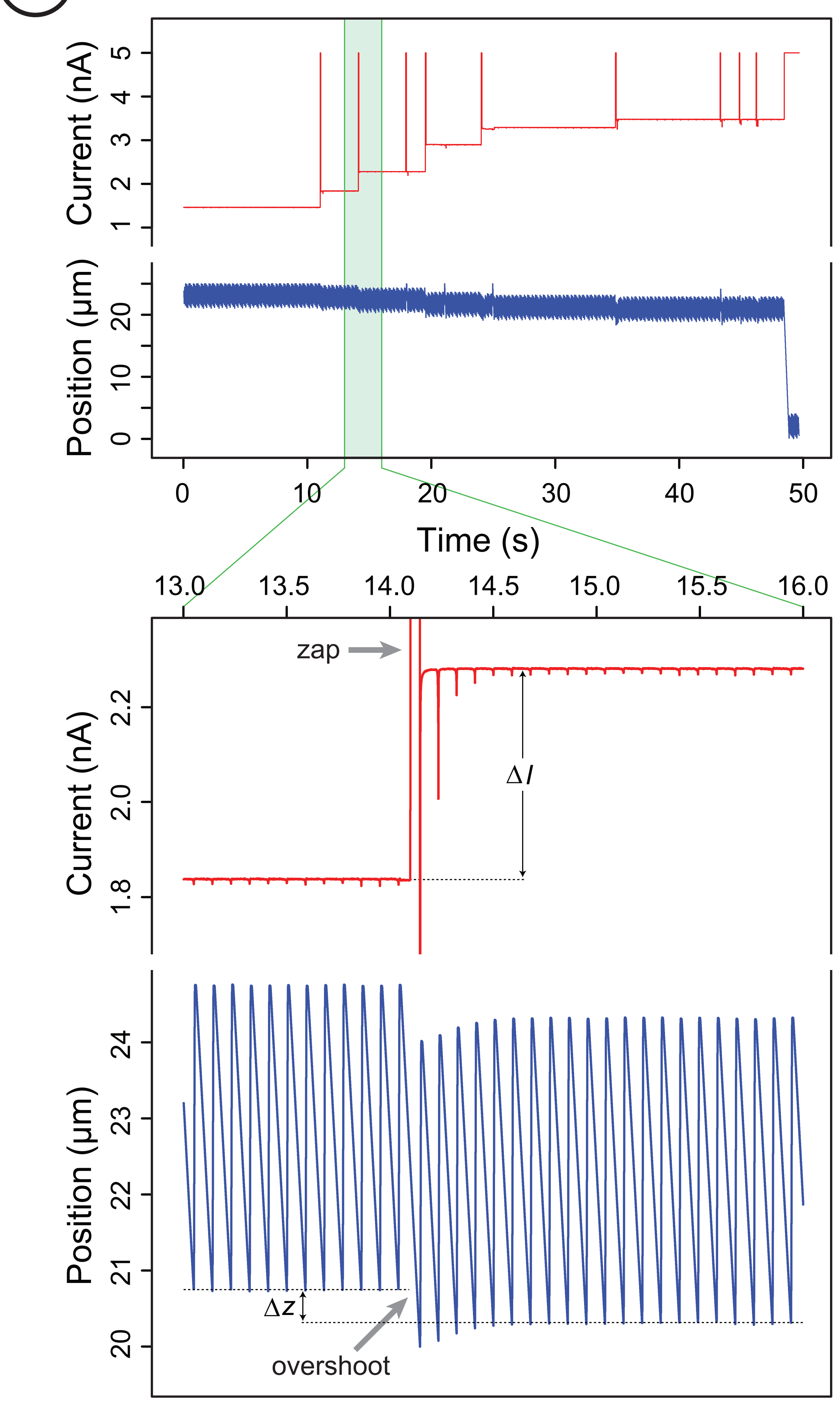
②



Breaking the tip

③ In ***hopping mode*** SICM, the pipette is repeatedly brought very close to the sample, retracting when the surface is detected [5]. By briefly overloading the detection process with a voltage pulse or ***zap***, we allow the approach to overshoot slightly. The resulting collision with the surface causes the fragile glass tip to break. The decrease in pipette length, Δz, manifests as a shift in the apparent surface position, measured via the SICM piezo capacitative sensors. A corresponding change occurs in the current passing through the tip, ΔI. From this we calculate the change in total resistance. By recording the positional and current changes across a series of breaks, we obtain the resistance and breakage distance data required to fit the model.

③

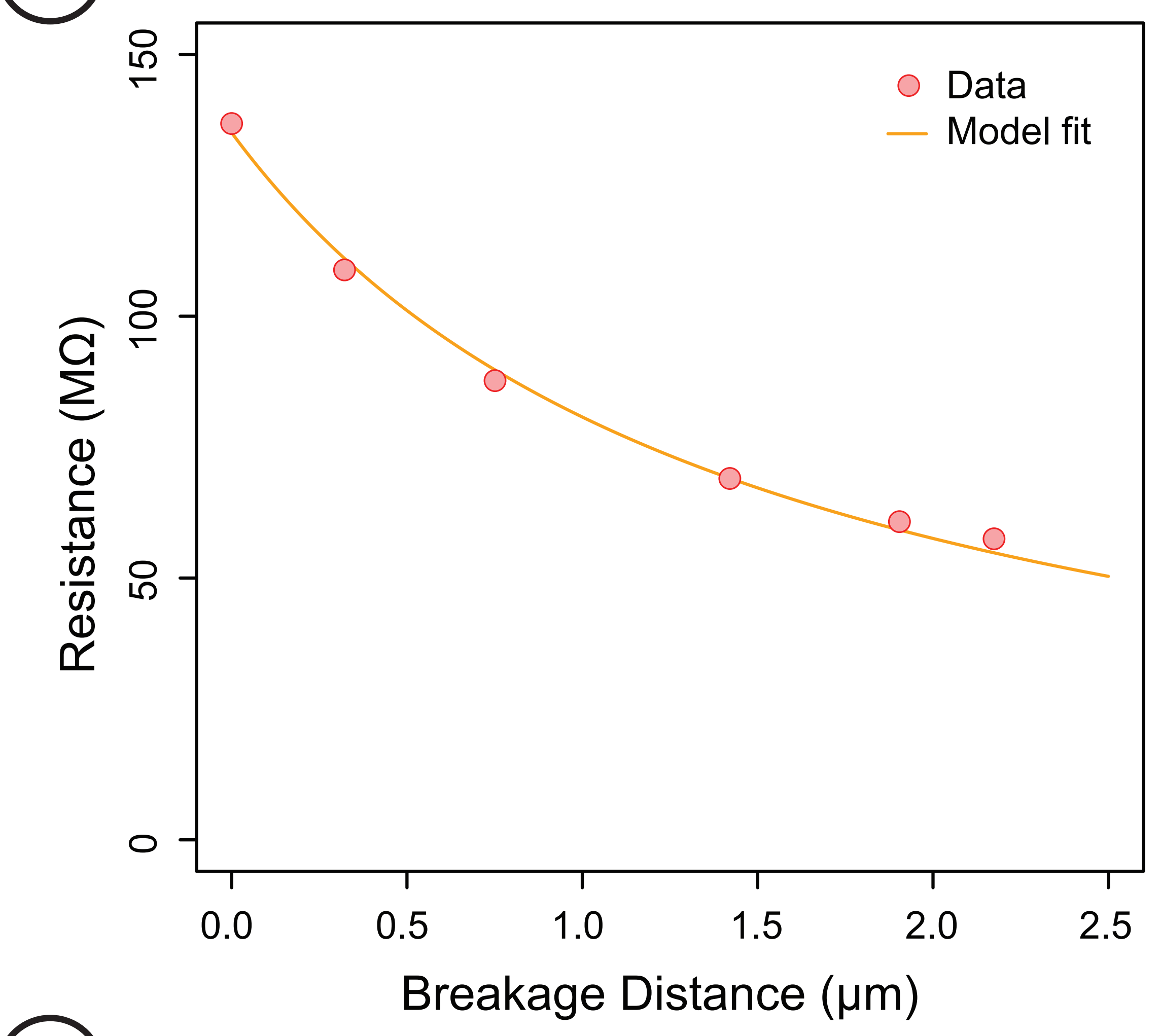


Results

④ Breakage data were recorded from pipettes pulled on a Sutter Instrument P2000 laser puller from 1.0 mm outer diameter, 0.5 mm inner diameter, borosilicate capillary glass. The fit between the model and experimental data was typically very close. A representative example is shown, corresponding to the breakage sequence depicted in panel ③.

⑤ To validate the results, tip geometry measurements were also made for the same kind of pipettes using SEM. In order to do so, the pipettes had to be coated with a layer of gold. The precise thickness of this layer was not known, but the nominal range of the sputter coater was 5–20 nm. Estimates were obtained for both ends of this range. The results from fitting breakage data were consistent with those from SEM, and indeed were bracketed by the estimates at the bounds of gold thickness, suggesting that the true thickness was somewhere in the middle of the range.

④



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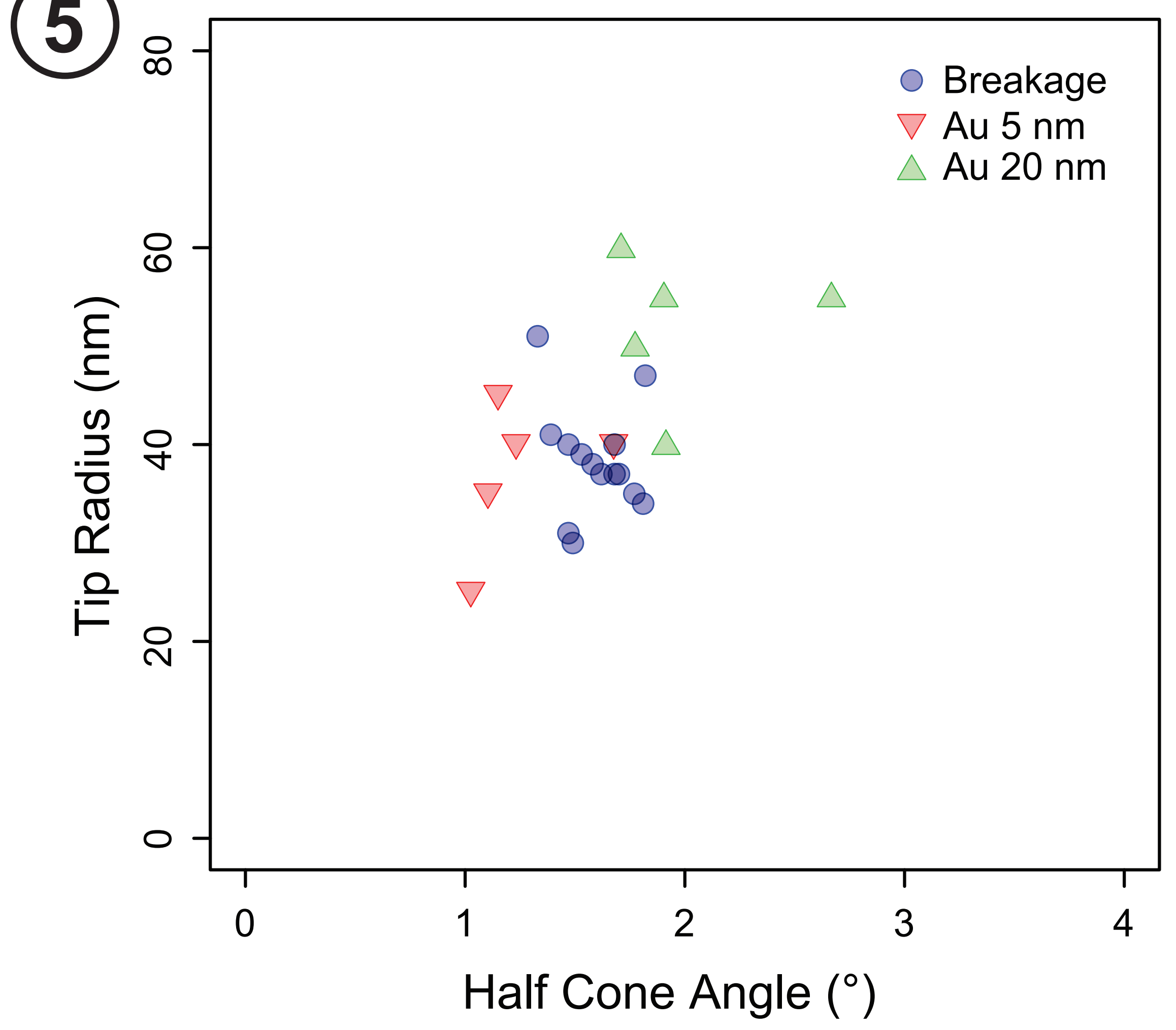

Conclusions

The fragile tip of a SICM pipette can be easily broken in a quasi-controlled fashion by allowing it to make brief contact with a rigid sample surface such as a microscope cover slip. The changes in resistance that occur with such breakage appear to be well modelled by a simple analytical description of the pipette tip. Fitting such a model to experimental breakage data allows the original geometry to be estimated.

The results produced by this method are consistent with those obtained by the more costly and difficult SEM approach, and avoid the uncertainty associated with the thickness of the gold coating required for SEM imaging.

Given the importance of pipette tip geometry to the interpretation of SICM data, the ability to estimate this geometry routinely within the context of a standard SICM imaging workflow should be of significant benefit to many users of the technology.

Acknowledgements

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References

[1] Hansma, P. K., et al, *Science*, 243(4891), 641–643, 1989
[2] Rheinlaender, J., & Schaffer, T. E., *J. Appl. Phys.*, 105(9), 094905, 2009
[3] Caldwell, M., et al, *Anal. Chem.*, 84(21) 8980–8984, 2012
[4] Hall, J. E., *J. Gen. Physiol.*, 66(4), 531–532, 1975
[5] Novak, P., et al, *Nat. Methods*, 6(4), 279–281, 2009