Electronic Supplementary Material (ESI) for Nanoscale.

Real-Time Visualization and Sub-Diffraction Limit Localization of Nanometer-Scale Pore Formation by Dielectric Breakdown

Adam Zrehen, Tal Gilboa and Amit Meller

Department of Biomedical Engineering,

The Technion – Israel Institute of Technology

Haifa, 32000, Israel

E-mail: ameller@technion.ac.il

Supplementary Information

Table of contents:

1. Circuit configuration

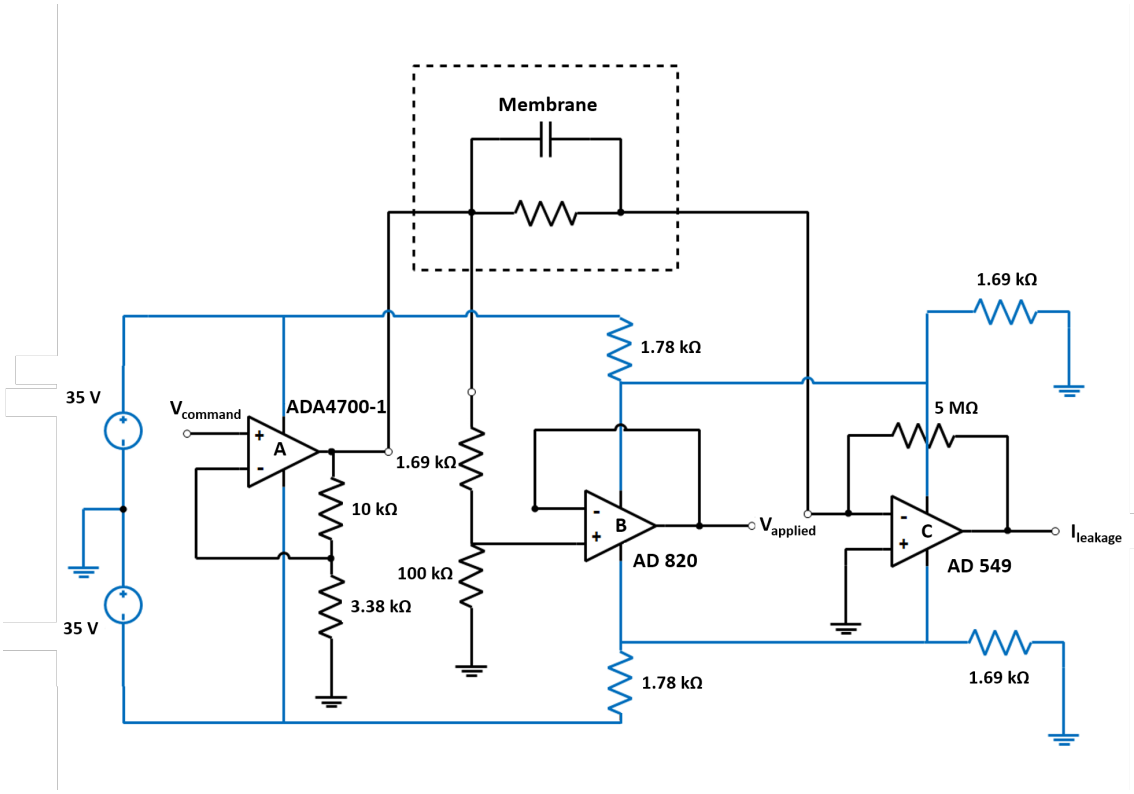

Figure S1. Circuit diagram for voltage/current amplifier assembled in-house.

Our circuit configuration (Figure S1) to output high voltages and measure pA-scale currents is based on the design of Tabard-Cossa et al[1]. In order to accelerate pore formation for our particular membranes, the circuit was modified to output voltages up to ± 35 V; however, typically voltages of ± 25 V are not exceeded. In order to maintain a single power supply (Agilent E3647A), the voltage inputs to op-amps B and C are scaled down with a resistor divider, although this is ordinarily best achieved using voltage regulators. The ADA4700-1 is capable of 30 mA output, so resistors on the order of 10 kΩ passing 35 V / 13,380 Ω ~2.6 mA are appropriate.

The load is being driven by a low-impedance source, the output of op-amp A, so a simple voltage divider consisting of a 100 kΩ and ~1 kΩ resistor (1.69 kΩ used for convenience) in series between the load and common produces a voltage across the 1.69 kΩ resistor approximately ~0.017 times the input voltage. The output of op-amp B is connected to the inverting input to implement a unity-gain buffer with high input impedance. The reduction in resistance lowers the Johnson noise injected into this buffer circuit by the 5 MΩ resistor. The circuit is assembled on a high-insulating Teflon breadboard to capitalize on the low input currents of the AD 549.

2. Current change threshold

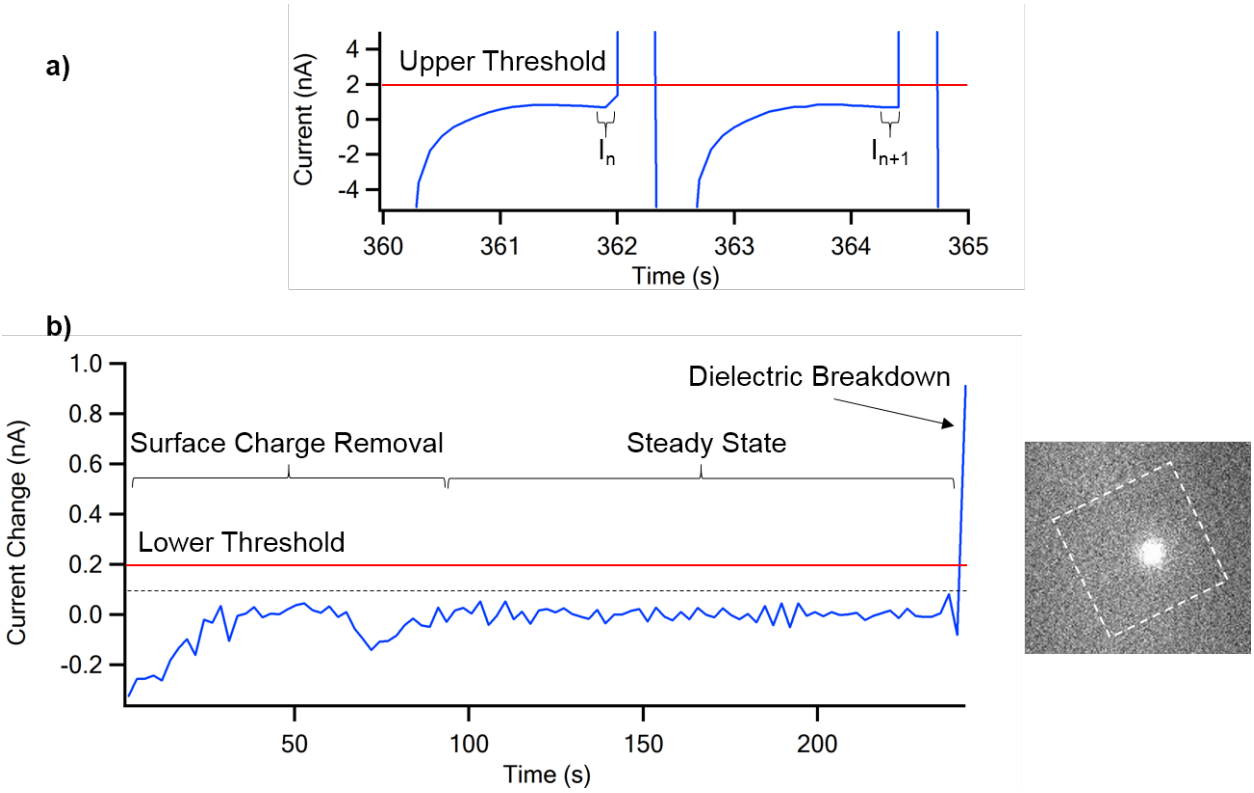

Figure S2. Thresholding motif for DB determination **(a)** Upper/static threshold. **(b)** Lower/moving threshold.

The average current value of the final 100-150 ms of the capacitance trace following a high voltage pulse (Figure S2a) is computed (I_{n+1}) and compared with the previous value (I_n). If $I_{n+1} - I_n > 0.2$ nA (above red line in Figure S2b), then pore formation is signaled, as confirmed optically. Typically, $I_{n+1} - I_n$ varies less than 100 pA (below dotted line) between voltage pulses following surface charge removal, and the system is considered to be in steady state. An additional static upper threshold (Figure S2a) is set to prevent over-enlargement of pores which may form gradually (< 200 nA per iteration), although this occurs very rarely. We observe cases where the end of the capacitance trace is at, below, or above 0 nA at steady state, and there is no correlation with the pore formation dynamics. The open pore current probed at 300 mV immediately following breakdown is a rough (~20%) approximation since the electrode potential difference must first be offset at 0 V. Note that initial current readings of several nanoamperes in magnitude typically precede DB experiments and represents a different phenomenon than initial defects[2], likely surface charge effects. As confirmed by real-time imaging, the surface charge can be removed following 250 ms 1-3 V pulses without the accidental creation of a nanopore.

3. DNA translocations

Figure S3 gives an example DNA translocation experiment with a DB-fabricated nanopore. Successful translocations as opposed to collisions exhibit a strong dwell time dependence on the applied voltage[3].

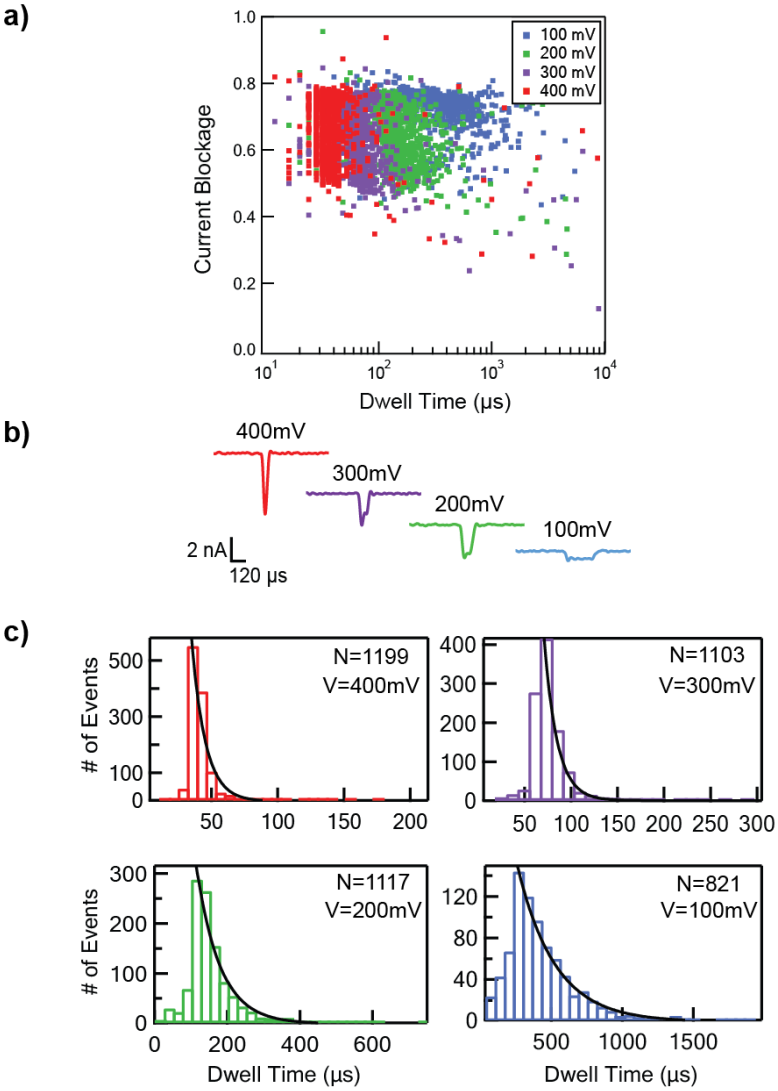

Figure S3. Translocation of 6 kbp DNA through a nanopore. The size of the pore is 5.1 ± 0.2 nm based on the conductance model and 4.5 ± 0.8 nm based on the current blockage. **(a)** Translocation event blockages and dwell times plotted at different applied voltages. **(b)** Sample translocation events at each voltage. **(c)** The time constants associated with the translocation dwell time histograms are 10.4 s, 14.1 s, 65.2 s, and 265.6 s from highest to lowest voltage.

4. Formation of multiple pores

We observed the formation of multiple nanopores in up to 20% of experiments performed for a particular batch of chips. Figures S4 and S5 show examples of multiple nanopores forming by a single voltage pulse and by two voltage pulses, respectively. In Figure S4b, the pore forms close to the edge (< 1 µm), but not precisely at the edge, as confirmed by a Gauss fitting. We fabricated a 3 x 3 structure of thin regions to test the possibility of intentionally forming an array of nanopores (Figure S6). We found that while we were able to create multiple pores in different wells, the continued presence of an electric field drove growth of previously formed pores, as suggested by the increase in fluorescent intensities emitting from the existing pores.

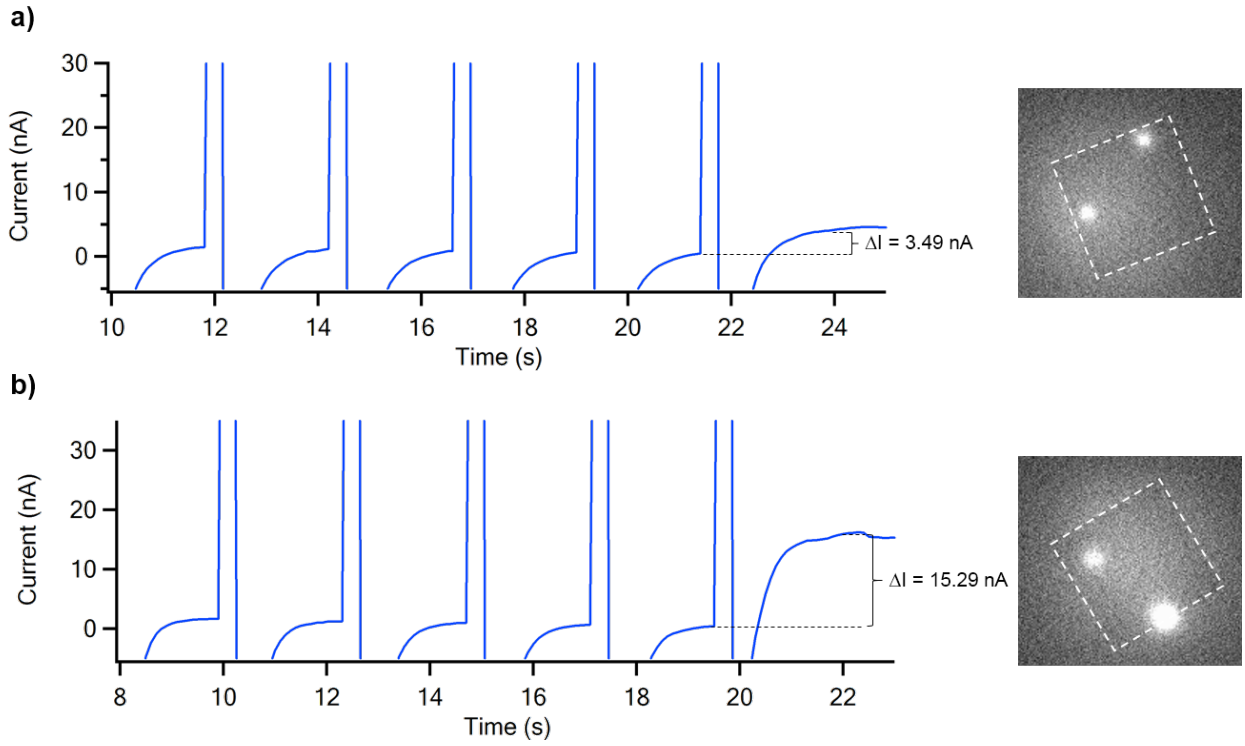

Figure S4. Pulse-voltage experiments leading to the formation of two pores after a single 225 ms 25 V pulse across a 22 nm thick, 20 x 20 μm^2 membrane. Pores form after a jump in current of 3.49 nA **(a)** and 15.29 nA **(b)**, respectively.

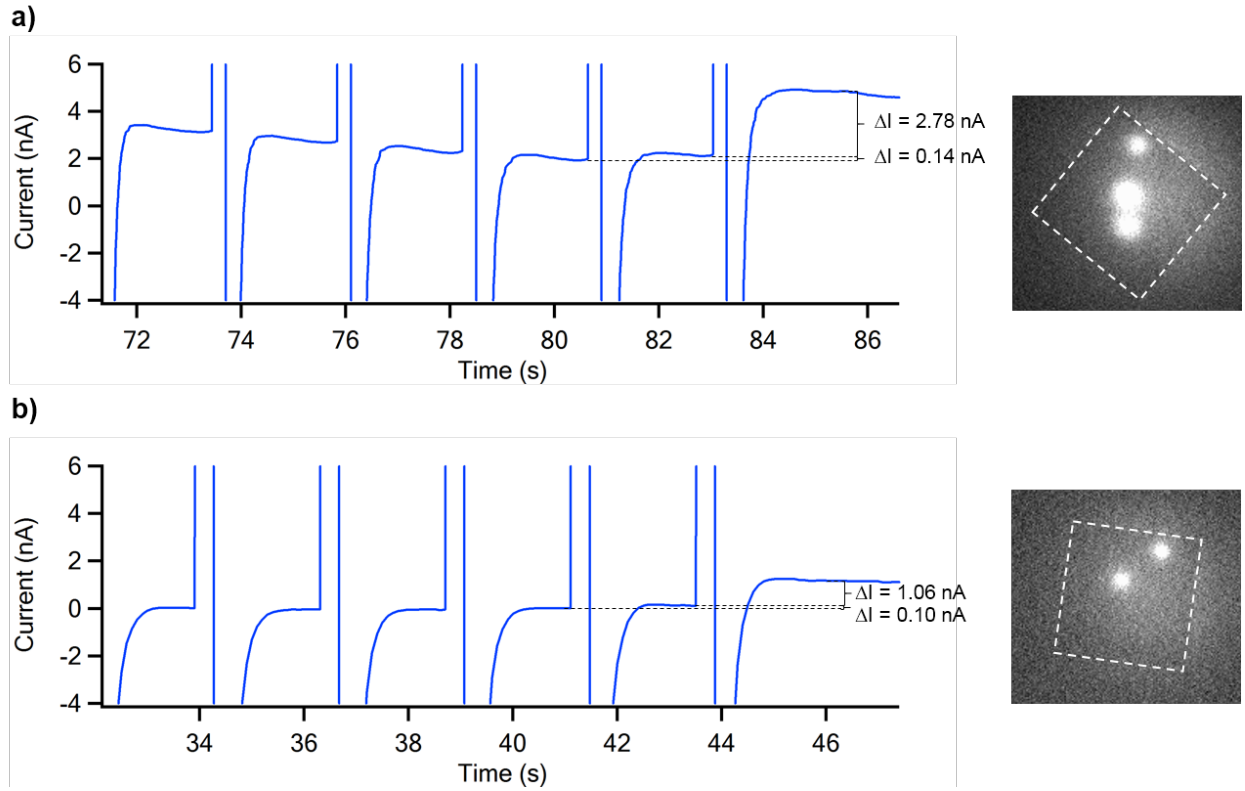

Figure S5. Pulse-voltage experiments leading to the formation of two or three pores after two 225 ms 25 V pulses across a 22 nm thick, 20 x 20 μm^2 membrane. **(a)** The formation of three pores follow closely with surface charge removal. **(b)** Two pores are formed. In both examples,

the initial 0.14 nA and 0.10 nA increment fall below the lower/moving threshold. Since the membranes were imaged following DB, it is uncertain whether multiple pores formed just by the first pulse.

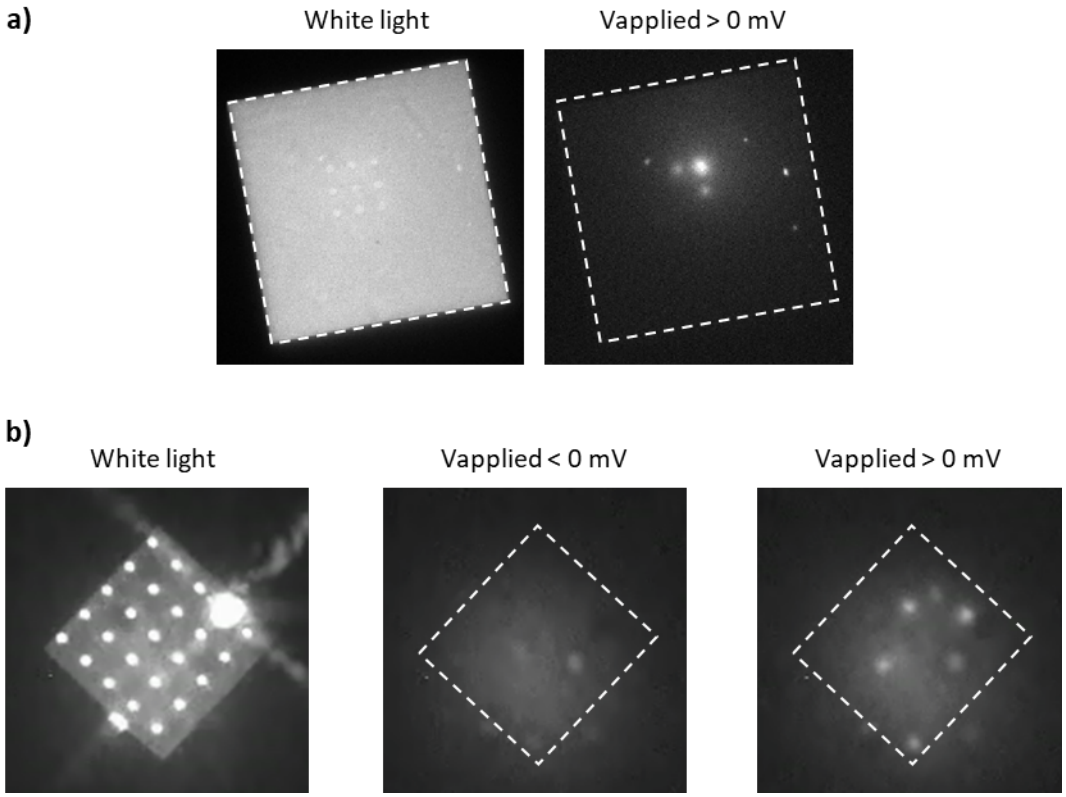

Figure S6. (a) Formation of multiple pores in an array of thin regions following additional voltage pulses after a first DB event. White light illumination reveals an array of 3 x 3 wells ~2 μm in diameter. The 3 pores formed likely differ in size as suggested by the dissimilar fluorescent intensities. (b) Formation of multiple pores in 120 nm in diameter nanowells fabricated in 130 nm thick polycrystalline Au, as described previously[4]. Compared to (a), the fluorescent intensities are similar, suggesting uniform pore sizes.

5. Discriminating false DB events

Figures S7 and S8 show examples of characteristic DB events which do not correlate with actual pore formation. More specifically, in S7 (a) the relative increase of 0.38 nA measured at ~67.5 s relative to ~65.3 s activates the threshold but then the current immediately drops. Actual pore formation follows another several hundred seconds of pulsing by a larger 3.49 nA increase as confirmed optically (b). In S8, the current fluctuates following a characteristic breakdown event (a), and then drops. Pore formation occurs after significant additional pulsing (b).

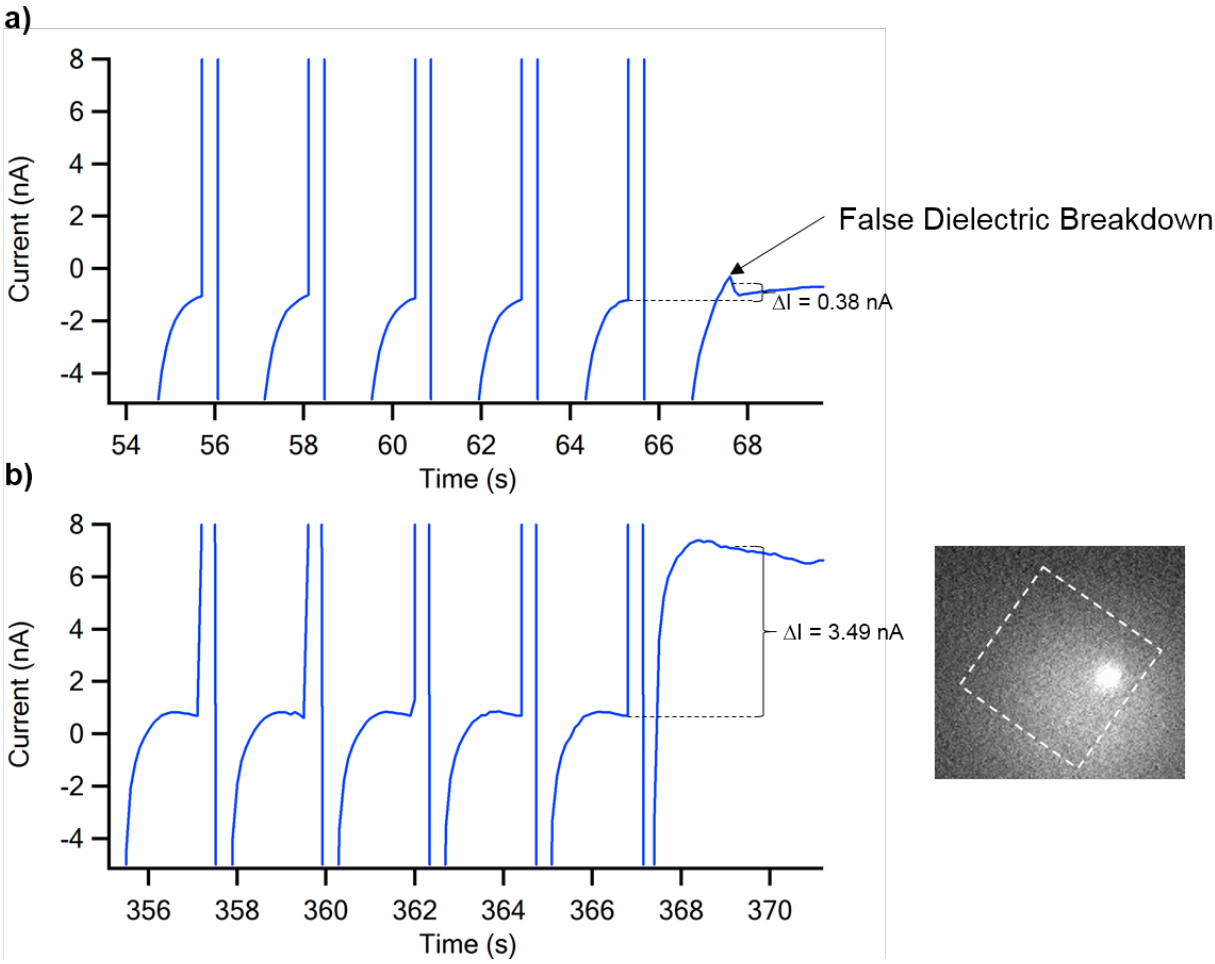

Figure S7. Pulse-voltage experiment resulting in a "false" DB event **(a)**, followed by a "true" event as confirmed optically **(b)**.

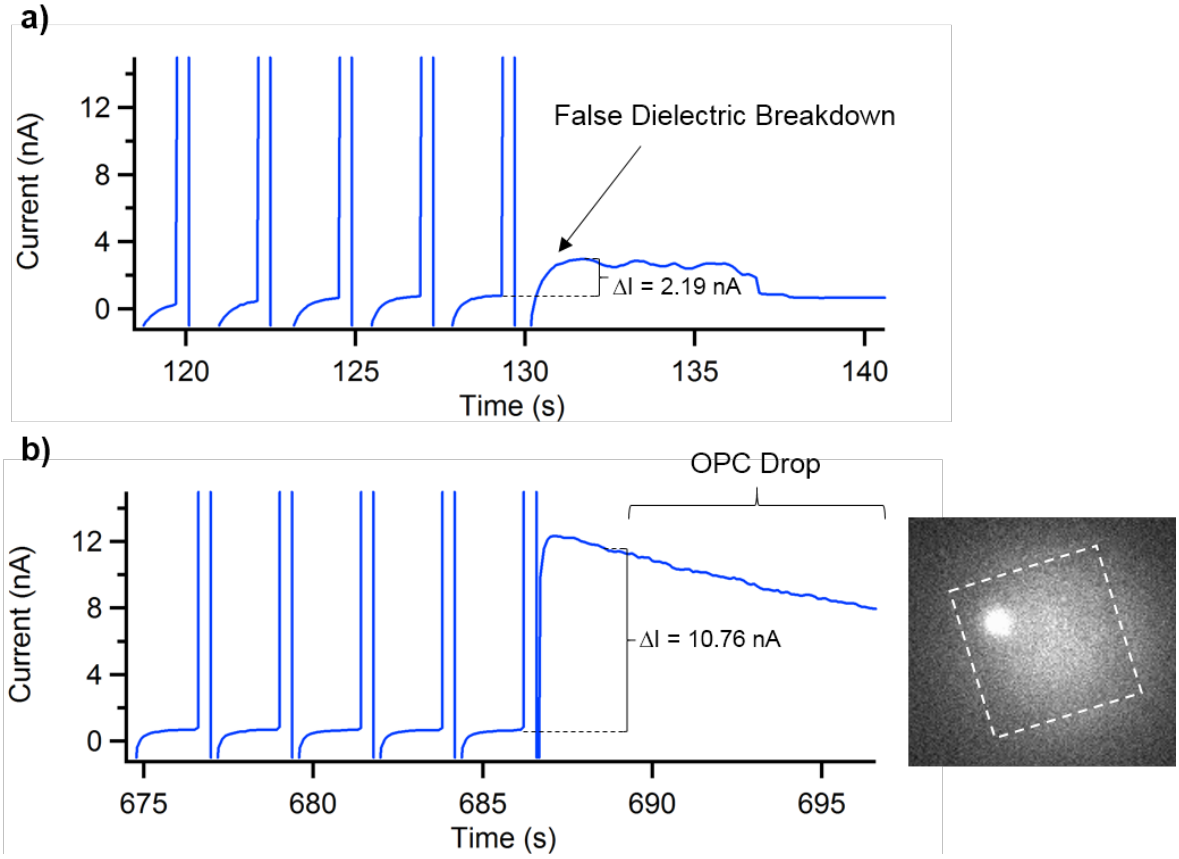

Figure S8. Pulse-voltage experiment resulting in a "false" DB event **(a)**, followed by a "true" event as confirmed optically **(b)**. The open pore current (OPC) drops by an additional ~7 nA over 30 s following breakdown (not shown in graph b).

6. Thin region membranes

Figure S9 gives additional examples of nanopore localization to selectively thinned regions. The membranes were fabricated as described previously[4]. Several batches with different membranes architectures were prepared. For one batch, arrays of thin regions (a) were exposed to increase the probability of alignment of the especially small features to the membrane. The thin region is clearly visible in the fluorescence microscopy setup by window-side illumination with a tungsten halogen light source (Fiber-Lite Model 190) as shown in (b) and (c). Colored images were taken with a DeltaPix camera mounted to a Nikon Eclipse microscope, illuminated from the membrane-side. Figure S10 shows a nanopore fabricated in a plasmonic nanowell device architecture used for enhanced single molecule fluorescence detection[4].

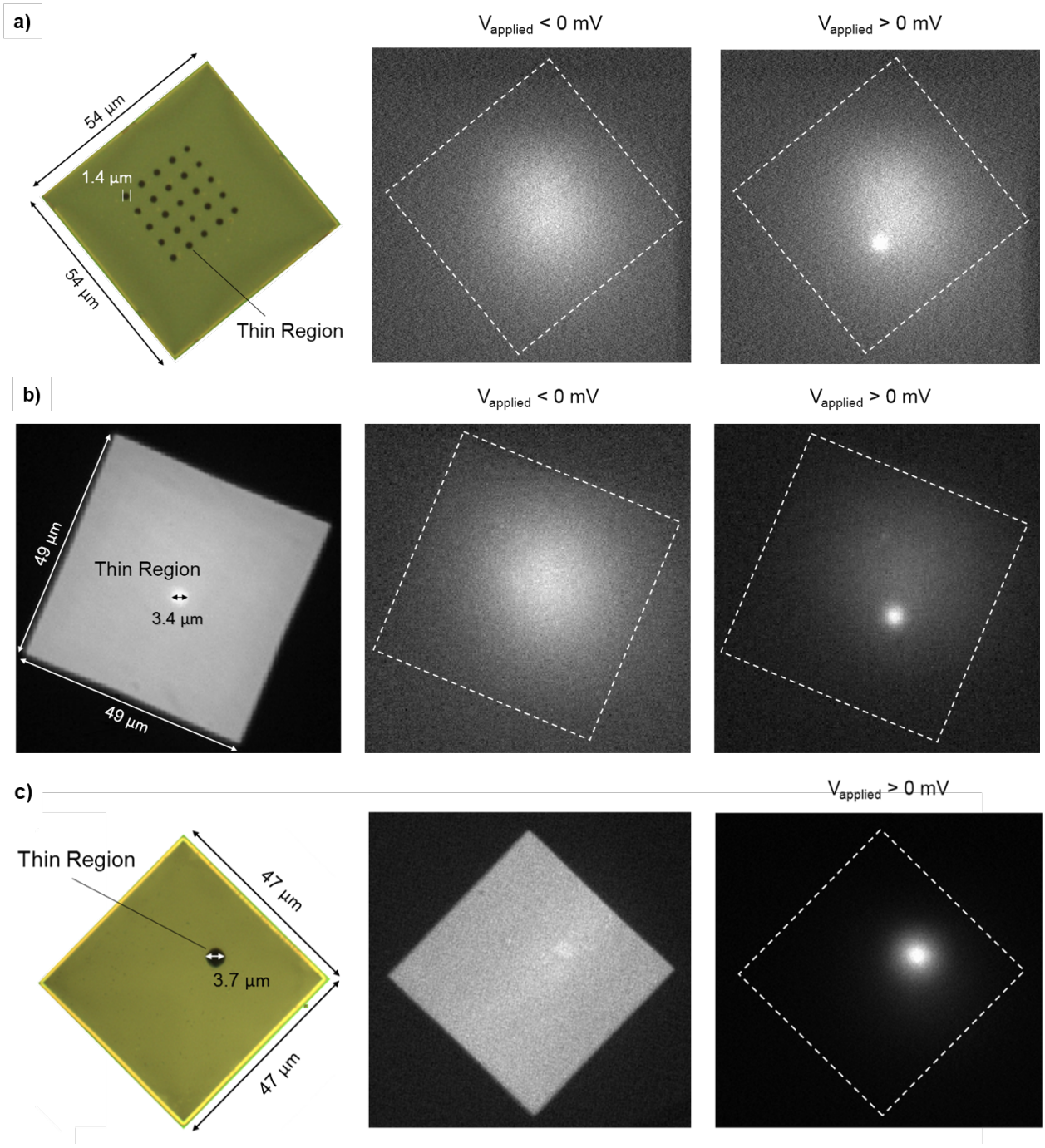

Figure S9. Examples of nanopores fabricated in thin regions. In all membranes, the global thickness is ~45 nm, whereas the thinned region thickness is 15-22 nm. **(a)** A nanopore is fabricated in 1 of 25 thin regions occupying 1.3 % of the total membrane surface area, **(b)** in a single thin region occupying 0.37 % of the total membrane surface area, and **(c)** in a single thin region occupying 0.5 % of the total membrane surface area.

7. Nanowells

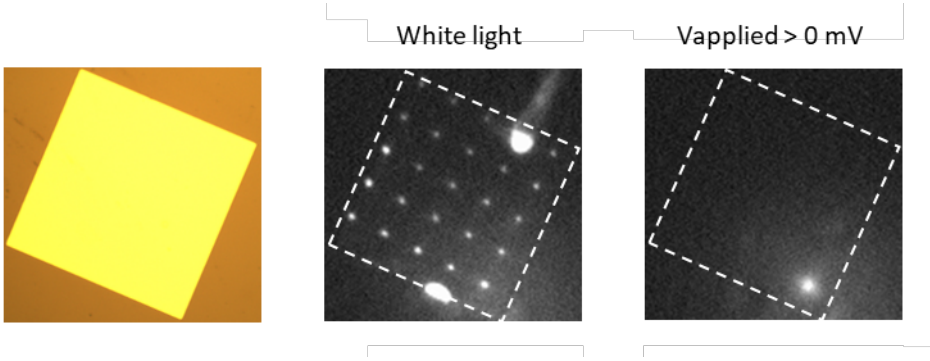

Figure S10. A nanopore fabricated in a single 120 nm in diameter nanowell of a 5 x 5 array, fabricated in 130 nm thick polycrystalline Au, as described previously[4]. The nanopore is identifiable by a single fluorescent spot at positive voltage. The nanowell array is visible by white light illumination (the gold blocks light through the rest of the membrane). Note that the two bright spots are orientation markers to facilitate nanowell identification.

References:

[1] H. Kwok, K. Briggs and V. Tabard-Cossa, *PLoS One,* 2014, **9**, DOI: 10.1371/journal.pone.0092880.
[2] K. Matsui, I. Yanagi, Y. Goto and K. Takeda, *Sci. Rep.,* 2015, **5**, 17819.
[3] M. Wanunu, J. Sutin, B. McNally, A. Chow and A. Meller, *Biophys. J.,* 2008, **95**, 4716.
[4] O. N. Assad, T. Gilboa, J. Spitzberg, M. Juhasz, E. Weinhold and A. Meller, *Adv. Mater*., 2016, **29**, 1605442.