

## **Real-Time Visualization of Nanopore Formation by Dielectric Breakdown**

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Solid-state nanopores are extremely powerful label-free single-molecule sensors. One of the main bottlenecks of the technology is the precise and reproducible fabrication of the nanometer scale feature. The well-established electron and ion beam-based fabrication methods are inherently low-throughput, expensive, and inefficient. An attractive alternative utilizing controlled dielectric breakdown (CBD) was recently proposed<sup>1</sup>; however, unlike the microscopy techniques, the method provides practically no real-time visual feedback of the fabrication process, meaning that a number of fundamental details have remained hidden, hence limiting its broad use.

Herein, we introduce synchronous, real-time, electro-optical monitoring of nanopore formation by CBD. Using the same principle as sub-diffraction microscopy, our nanopore localization platform based on wide-field microscopy and calcium indicators provides nanoscale sensitivity. Our method enables us to establish critical limitations of the fabrication process. In particular, we find that under certain conditions multiple nanopores may form and that nanopores may preferentially localize at the membrane juncture, rendering nanopore sensing less effective. As the breakdown parameters of silicon materials are highly manufacturer-specific, we anticipate that our visualization platform will allow users to easily optimize and tailor CBD fabrication according to their needs. Furthermore, our technique extends the applicability of CBD to more complicated architectures, such as membranes with selectively thinned regions where localization to these regions must be verified.

### References

[1] H. Kwok et al., PLOS ONE **9**, e92880 (2014)