Zero-Mode Waveguides on Thin Silicon Nitride Membranes for Efficient Single-Molecule Sequencing

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Primary CNF Tools Used: LPCVD CMOS Nitride – E4, JEOL 6300, SC4500 odd-hour evaporator, Zeiss Ultra SEM

Abstract:

Nanopores can generate localized electric fields to focus molecules to 3D positions with high precision. Previously, we have shown that the electrophoretic loading of nanopore zero-mode waveguides (NZMWs) is orders of magnitude more efficient than either diffusion or magnetic bead-based loading techniques. However, serial fabrication of uniform solid-state nanopores arrays is not currently feasible on a large scale. In this work, we demonstrate wafer-scale fabrication of porous membranes containing solid-state nanopore networks for large scale positioning of macromolecules using fabrication techniques derived from molecular layer deposition. These porous membranes will be used to fabricate porous zero-mode wave guides (PZMWs) as large-scale parallel replacements to NZMWs.

Summary of Research:

Zero-mode waveguides (ZMWs) are wavelength-scale apertures in aluminum films, used for single-molecule detection [1]. By immobilizing the DNA-polymerase template at the base of the ZMWs and imaging the incorporation of fluorescently-labeled nucleotide by polymerase a sequence of a single DNAs can be read [2]. Previously, it has been be demonstrated that by fabricating ZMWs on 50-nm-thick silicon nitride membranes, and drilling a 3-5 nm pore at the ZMW base, the efficiency of molecular loading is enhanced by orders of magnitude [3]. These ZMW structures were fabricated with e-beam lithography (JEOL 6300) at the CNF.

We continue to fabricate these ZMW devices for our DNA sequencing experiments. Figure 1 shows a darkfield microscope image of a device. The base of the ZMWs is modified by depositing molecule layer deposition to form a porous layer to facilitate more efficient capture of large DNA molecules. The ZMW surface is passivated using by SiO_2 deposited using atomic layer deposition (ALD) to protect it from electrochemistry with chloride buffer that may occur during voltage bias experiments.

These structures may be used for capturing DNA-polymerase complexes. In Figure 2, we show the SEM image (ZEISS Ultra) of Al pillars before lift-off process. In Figure 3, we show ZMW structure after the lift-off process.

References:

[1] Larkin, J., et al., Reversible Positioning of Single Molecules inside Zero-Mode Waveguides. Nano Letters, 2014. 14(10): p. 6023-6029.

[2] Eid, J., et al., Real-time DNA sequencing from single polymerase molecules. Science, 2009. 323(5910): p. 133-138.

[3] Larkin, J., Henley, R. Y., Jadhav, V., Korlach, J., and Wanunu, M. Length-independent DNA packing into nanopore zero-mode waveguides for low-input DNA sequencing. Nature Nanotechnol, 2017. 12, 1169-1175.

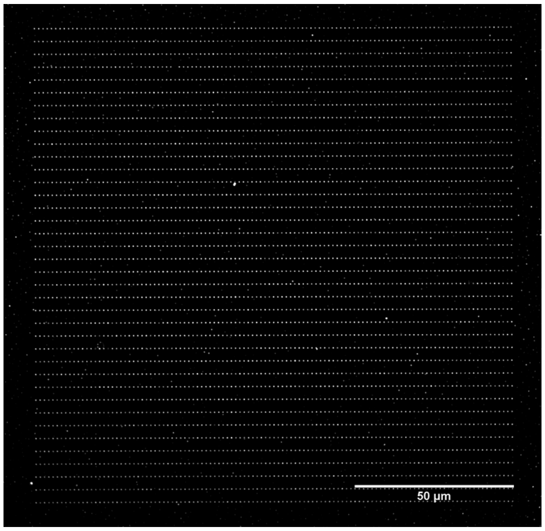

Figure 1: Conventional dark-field microscopy image of ZMWs on a thin SiN membrane after EBL.

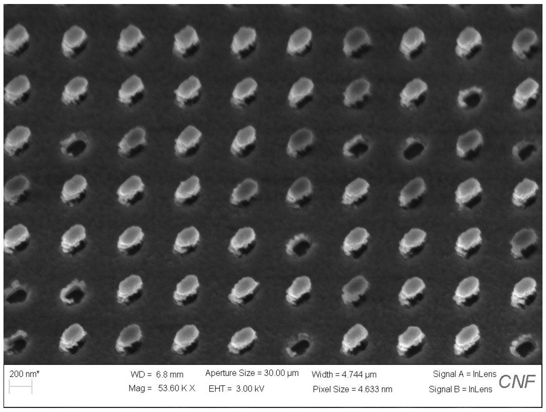

Figure 2: Scanning electron microscope image of Al pillars before lift-off process.

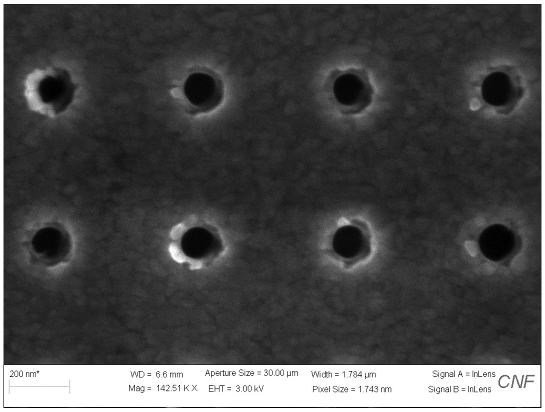

Figure 3: ZMW structure after the lift-off process.