Electrochemical Lasso for Trapping Biomolecules inside Zero-Mode Waveguides

CNF Project Number: 2214-13

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Primary Source(s) of Research Funding: NIH/National Human Genome Research Institute award no. HG009186

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Primary CNF Tools Used: Electron-beam lithography, e-beam evaporation and lift-off

Abstract:

Single molecule real time (SMRT) sequencing technology developed by Pacific Biosciences is a robust single molecule DNA sequencing method in which DNA strand replication by an individual DNA polymerase is imaged using fluorescently labeled nucleotides [1-2]. SMRT sequencing, however, suffers from inefficient loading of DNA molecules into the zero-mode waveguides (ZMWs), a sequencing unit that provides the smallest available volume for light detection. In addition, ZMWs are biased towards diffusion-based entry of short DNA templates. To overcome these challenges, our lab introduced two powerful tools, i.e., nanopore ZMW (NZMW) [3] and porous ZMW (PZMW) [4], to electrokinetically capture DNA fragments inside ZMWs. The efficiency of voltage-induced DNA loading into these waveguides is length-independent and is 6-7 orders of magnitude larger than diffusion-based SMRT sequencing. Although NZMW and PZMW are effective tools for capturing long DNAs in picogram levels, they are fabricated on top of free-standing ultrathin membranes, which makes them fragile and difficult to scale up. In this work, we introduce a new technique in order to draw DNA fragments inside ZMWs fabricated on fused silica substrates.

Summary of Research:

The working principle of our design relies on embedding metallic electrodes (platinum, Pt) under the waveguides to create an electric field. This electrode film is separated from the ZMWs made of aluminum, by a dielectric layer. Application of a voltage to the electrode layer with the use of proper electrolyte allows efficient electrophoretic DNA capture at picogram levels. Electron-beam lithography, e-beam evaporation, and lift-off are used to fabricate 100 nm ZMW arrays in wafer scale. Our new device eliminates the need for free-standing membranes and enables scaled-up fabrication, reduces the background optical noise, and improves the DNA loading efficiency by several orders of magnitude.

We have continued to fabricate these devices for DNA sequencing experiments. Figure 1 shows PtZMWs' fabrication process and Figure 2 is scanning electron microscope image of the top view and cross-section of an array of ZMWs.

References:

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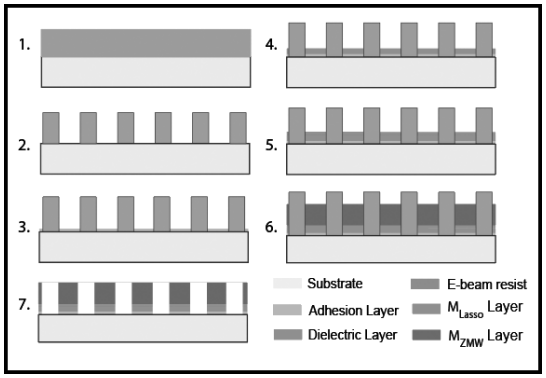

Figure 1: Schematic representation of ZMW fabrication process: 1. Spincoat negative e-beam resist and bake. 2. Expose using e-beam lithography and develop resist. 3. E-beam evaporation of an adhesion layer on the fused silica substrate, followed by deposition of Pt layer (electrode), alumina (dielectric layer), and 100 nm aluminum (cladding layer of ZMWs). 4. Lift-of-resist using 1165 stripper.

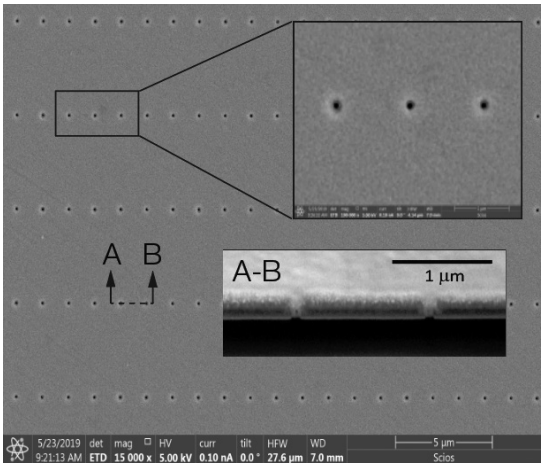

Figure 2: SEM of an array of 100 nm diameter ZMWs. Cross-section of the ZMWs shown in the bottom inset demonstrates that the cladding layer (aluminum) is separated from the electrode (Pt) by a dielectric layer (Al_2O_3).