TOWARDS ULTRA-FAST PARALLEL DNA ANALYSIS: SUB-WAVELENGTH METALLIC NANOPORE ARRAYS FOR HIGH-THROUGHPUT SINGLE MOLECULE SPECTROSCOPY

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ABSTRACT

We present a novel approach for optically detecting DNA translocation events through an array of solid state nanopores, with the potential to allow for parallelised spectroscopy for high-throughput single molecule DNA analysis.

Keywords: nanopore, translocation, zero-mode waveguide, fluorescence spectroscopy

1. INTRODUCTION

The creation of nanochannels or nanopores in thin membranes has attracted much interest due to the ability to isolate and sense single DNA molecules while they translocate through the highly confined channels [1]. Short translocation lengths make decontamination of the nanopores facile whilst maintaining effective spatial confinement for single molecule detection. Nanopores have already been fabricated in silicon dioxide [2] and silicon nitride [3] membranes and thus in principle may be integrated into monolithic analysis systems. Although the detection of translocation events can be performed electrically by measuring the ionic current [4], we propose to optically probe fluorescently labelled DNA molecules translocating through 50-300 nm wide pores within a thin aluminium/silicon nitride membrane. Such apertures in a metallic layer act as both nanofluidic channels and zero-mode waveguides (ZMW). Since the aperture diameter is much smaller than the excitation wavelength, an evanescent field is produced limiting the excitation regime to a highly localised area [5].

2. EXPERIMENTAL DETAILS

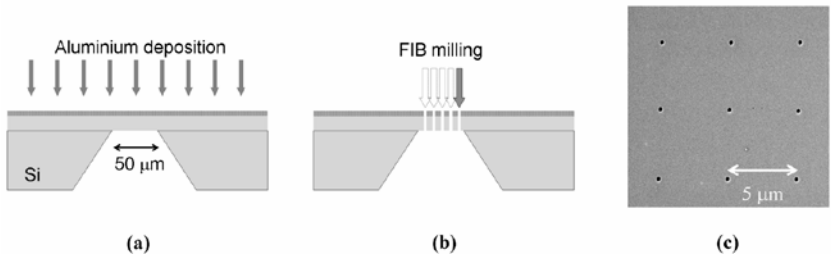

Figure 1. Device fabrication: (a) KOH etching followed by aluminium deposition; (b) Milling of channels by focused ion beam; (c) SEM image of 0.3 μm wide channels.

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Figure 1 illustrates the basic process for device fabrication. A free standing 200 nm thick silicon nitride membrane is fabricated by standard photolithography and KOH wet etching. The membrane is then deposited with a 100 nm thick aluminium layer. Subsequently, holes are milled sequentially using a focused ion beam (FIB). Figure 1c shows typical results after milling 0.3 μm wide pores in the membrane, giving an aspect ratio close to unity.

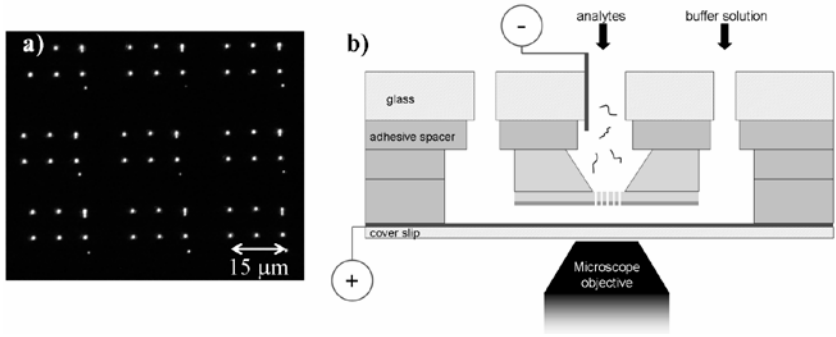

Figure 2. a) Optical image of a membrane: the aluminium layer is opaque to visible light. b) A schematic of the experimental setup for translocation detection.

Double stranded lambda-DNA molecules were labelled with YOYO-1 (Molecular Probes) at a ratio of five base pairs per dye molecule and the solution was then diluted to yield a concentration of 10 pM. Before injecting the analyte solution, the nanopore device was treated with oxygen plasma to ensure the inner surface of the channels is hydrophilic in nature. DNA detection was performed with a custom-built confocal microscope with imaging capabilities. Figure 2 shows the experimental setup. In this approach, the excitation light from a mercury lamp is focused onto the membrane surface and the fluorescence signal is collected by the same objective. The signal is then directed to an emCCD camera. A glass cover slip coated with indium tin oxide (ITO) is used as an electrode to apply a voltage across the buffer solution.

3. RESULTS AND DISCUSSION

Translocations of DNA molecules through the array of pores can be observed in real-time. Figure 3 shows a typical translocation event under an applied voltage of 2.0 V.

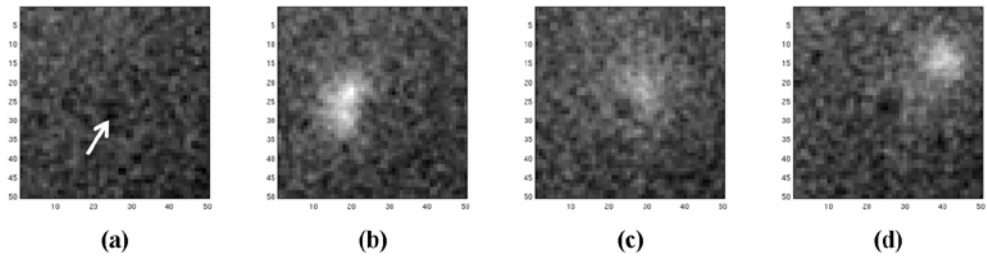

Figure 3. A single DNA molecule translocation imaged at t=0 (a), t=0.11 s (b), t=0.28 s (c) and t=0.50 s (d). Each pixel is 81 nm wide. The arrow points to the center of the pore.

By recording a sequence and selecting the areas of interest on the frames, it is possible to obtain a spectrograph for each of the pores, as shown in Figure 4.

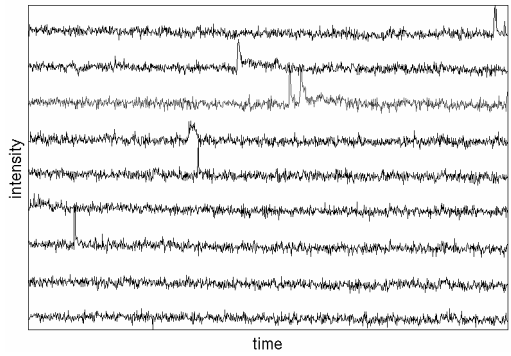

Figure 4. Signal intensities from nine different pores acquired simultaneously under an applied voltage of 4.5 V. The total acquisition time is 20 s. The signals have been shifted vertically for convenience. Each peak corresponds to a translocation.

It is found that the behaviour of the molecules within the channels greatly depends on the hydrophilic state of the surface. Because the oxygen plasma treatment has only a transitional effect, the nanopores start to lose the hydrophilic state after one or two hours.

4. CONCLUSION AND FUTURE WORK

These studies demonstrate that it is possible to obtain a high spatial resolution DNA analysis whilst independently controlling the applied voltage that drives the molecules into nanopore. Another feature of this approach is the possibility of parallelising the analysis of the molecules by probing an entire array of nanopores under uniform illumination. Operations such as fragment sizing of DNA molecules are potentially achievable on timescales significantly shorter than with single pore devices. We expect this work to have a high impact on the biomedical community and to open new routes to faster, parallel biomolecular analysis.

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