DNA FRAGMENT TRANSLOCATION IN ARTIFICIAL SEA WATER THROUGH NANOPORES USING A PORTABLE MINI READER AND FLOW-CELL

LB109

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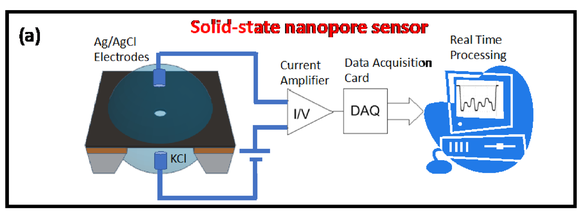
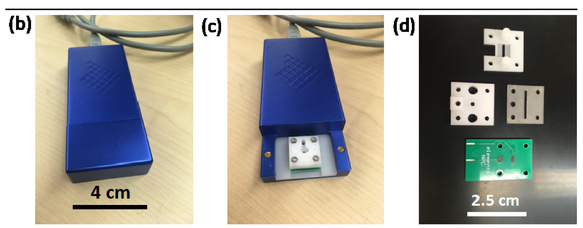
Abstract

Nanopore sensing is a powerful tool for the detection and characterization of biomolecules, such as nucleic acids. Solid-state nanopores act as single-molecule sensors that can function in a variety of harsh conditions, such as pH and temperature. The expected, resilient nature of solid-state nanopores makes them attractive potential candidates for taking this technology into the field to measure environmental samples for life detection applications and water quality monitoring. Towards this goal, here we measure artificial (synthetic) sea water samples spiked with DNA fragments at different concentrations to establish the limits of nanopore sensitivity in candidate environmental conditions.

We use a compact, portable read-out device with miniaturized flow-cells, and obtain data with nanopores in 20-nm-thick, low-stress silicon nitride suspended on low-capacitance fused-silica (glass) chips at bandwidths up to 100 kHz. We measure 400 bp, 1000 bp, and 15000 bp DNA fragments, both mixed together and separately. We show that the fragments can be discriminated by analysis of current signal and dwell-time magnitudes.

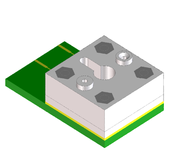
Nanopore Mini Reader and Flow Cell

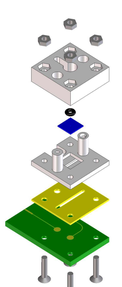


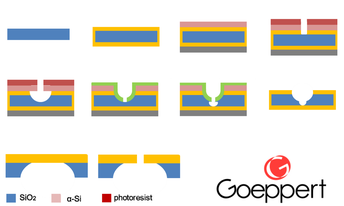


eNPR amplifier has the following features:

- very small footprint (30 x 15 x 74 mm);
- Low noise acquisition: ≈**100 fA rms @ 1 KHz** - ≈**380 fArms @ 10 KHz** - ≈**3,2 pA rms @ 100 KHz** (open circuit);
- 8 selectable sampling rates in the range 1.25 kHz - 200 kHz;
- 4 input current rages: ±200 pA (gain 2.25 GΩ), ±2 nA (gain 225 MΩ); ±20 nA (gain 22.5 MΩ); ±200 nA (gain 2.25 MΩ);
- Voltage stimulus range between ±2000 mV, 1 mV minimum step;
- a fine automatic voltage offset compensation with 62,5 µV step resolution;
- simple EDR software interface (also available for Mac OS) and data output format compatible with commercially available software (pClamp®, Matlab®, text);
- an online analysis module for real time analysis of data, as current histogram, Dwell time analysis, I/V graph.







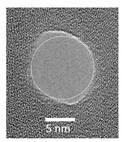
Low-noise fused silica chips

- Chip capacitance < 2 pF
- Fused silica substrate
- 20 nm LPCVD low-stress silicon nitride

- Low capacitance improves noise performance at high bandwidths [**1**].
- Provided by Goeppert, LLC.

Measurements

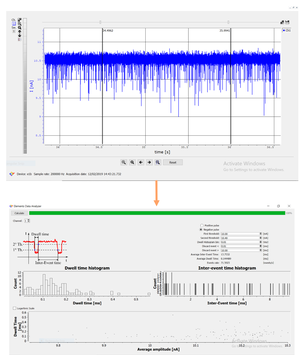
Solid-state Nanopore



Nanopores in silicon nitride:

- Nanopores formed by ablation using a JEOL 2010F Transmission Electron Microscope
- Diameters range from 5 to 20 nm
- 20 nm thick silicon nitride
- Low-noise fused silica substrate

Real-time statistics

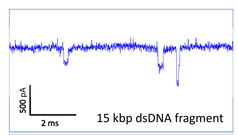


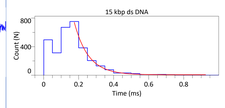
Above: 15 kbp ds DNA translocating through a 15 nm diameter nanopore. 1M KCl, 10 mM Tris buffer, 1mM EDTA. 200 mV applied voltage.

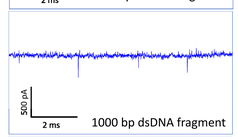
Elements Data Analyzer software:

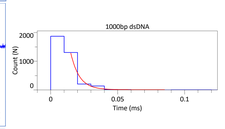
- Dwell-time histogram
- Interevent time histogram
- Scatter Plot of Dwell-time vs. Average Amplitude
- Event rate

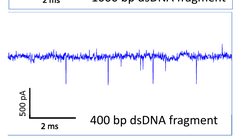
Exported and analysis

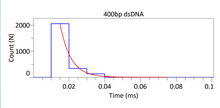


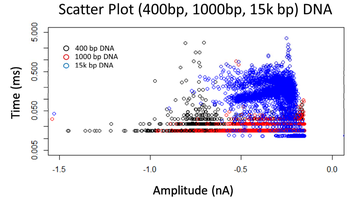












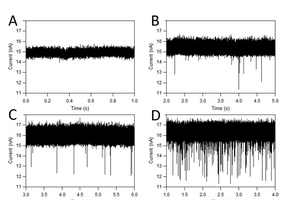
Translocation of DNA fragments

- DNA fragment translocations on a 17 nm diameter nanopore. 1M KCl, 10mM Tris, 1mM EDTA, pH 8.0.
- Applied voltage +200 mV. Chip was flushed with IPA and water between introduction of new analytes.
- Scatter-plot of DNA dwell-time vs event amplitude for 400, 1000, and 15,000 bp dsDNA fragments, showing separation of DNA fragments.

Dwell-time histograms fit with a single-term exponential.

- 15kbp DNA – τ = 94 ± 7µs
- 1000bp DNA – τ = 29 ± 9µs
- 400bp DNA – τ = 9 ± 6µs

Analyte Detection in Artificial Sea Water detection



Ionic current through a nanopore using Artificial Sea Water

(A) Trace showing artificial sea water without any added analytes at +1V transmembrane bias voltage. (B) with 1nM 1500bp dsDNA. (C) with 10nM added 1500bp dsDNA. (D) with 100nM added 1500bp dsDNA.

Cis side of solution was RICCA® Synthetic Seawater, ASTM D 1141 (artificial sea water)
Trans side was 1M NaCl. [**2**]
Nanopore diameter was 11 nm.

Conclusions

- The combination of low-noise solid-state nanopores and the compact eNPR amplifier allowed for rapid characterization of DNA fragments.
- Analysis of DNA fragment events showed a separation of different fragment types in a Dwell-time vs. Current Amplitude scatter plot.
- Dwell-time of DNA fragments in the nanopore depended on DNA length.
- Spiking a sample of synthetic seawater with a DNA fragment showed that the seawater can be used as a detection medium without the need further buffering.
- Using different concentrations of a DNA spike demonstrates that detection in seawater is possible at picoMolar concentrations.

Acknowledgments

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