Updates on NASA Project

Detecting life in Ocean Worlds with low-capacitance solid-state nanopores

Biophysical Society 64th Annual Meeting
16 February 2020

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Nanopore Reader

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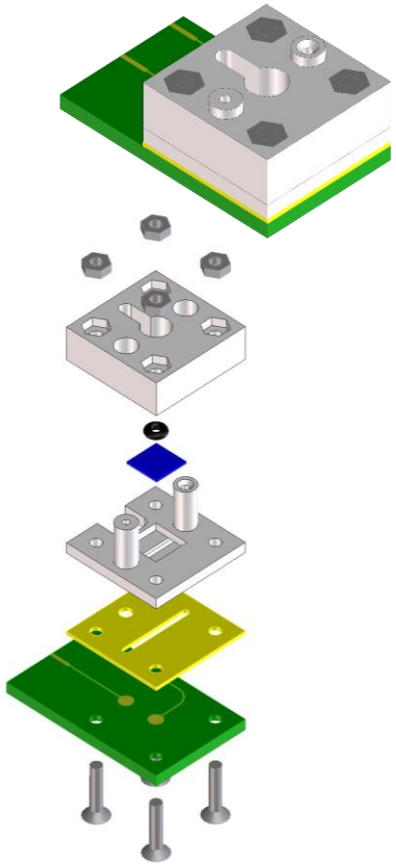
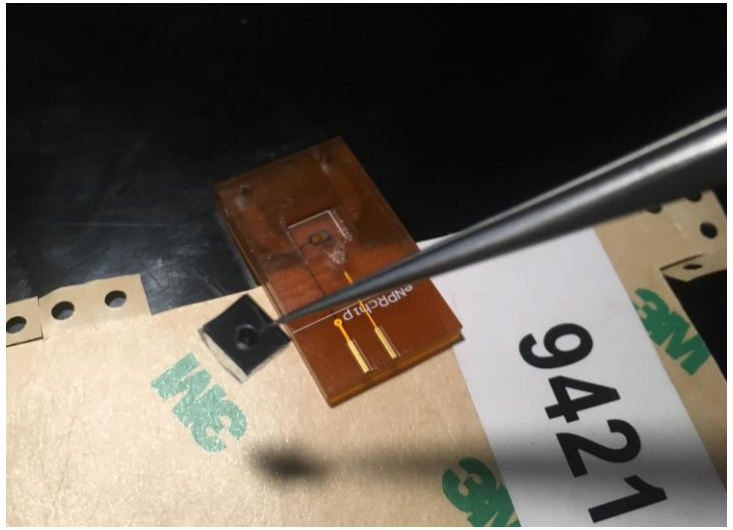
The miniaturized flow cell hosts round or squared nanopore chips

Features

- Open input (RMS) noise: 100 fA rms @ 1kHz; 380 fA rms @ 10 kHz; 3.2 pA rms @ 100 kHz
- Max sampling rate: 200 ksps
- Voltage control stimulus range of ± 2000 mV
- Auto electrodes voltage offset fine compensation
- Continuous C-membrane and R-seal estimation
- Zap pulse
- Digital filters: cutoff frequencies in the range between 62,5 Hz and 100 KHz
- USB powered
- Current ranges: ±200pA (Gain 2.25GΩ), ±2nA (Gain 225MΩ), ±20nA (Gain 22.5MΩ), ±200nA (Gain 2.25MΩ)

Flow Cells





- PCB (chlorinate electrodes)
- Nanopore chip secured with O-ring
- Low volume
    - 60 µL top solution
    - 30 µL bottom solution

- Rapidly change nanopore chip
- Load multiple flow cells for a series of experiments

Low noise fused silica chips

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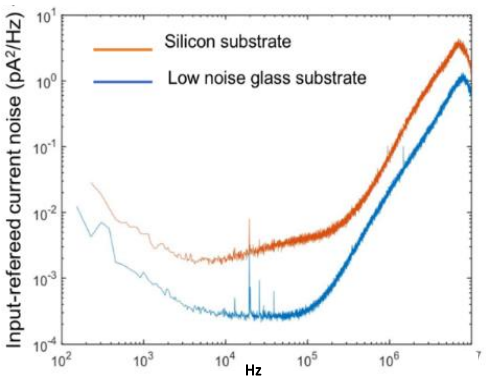
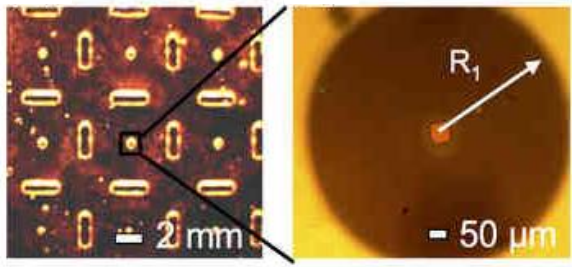
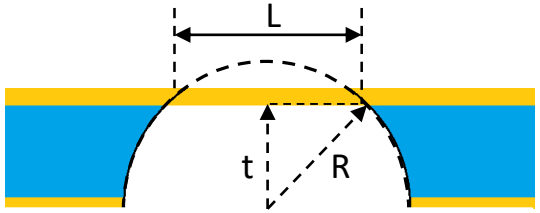
- Fused-silica supported silicon nitride membranes
- Chip capacitance < 2 pF
- 20 nm LPCVD low-stress silicon nitride
- These are now commercially available from Goeppert

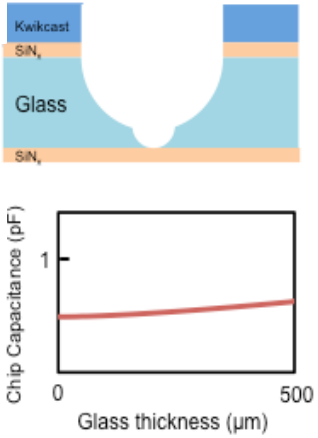
- Low capacitance improves noise performance

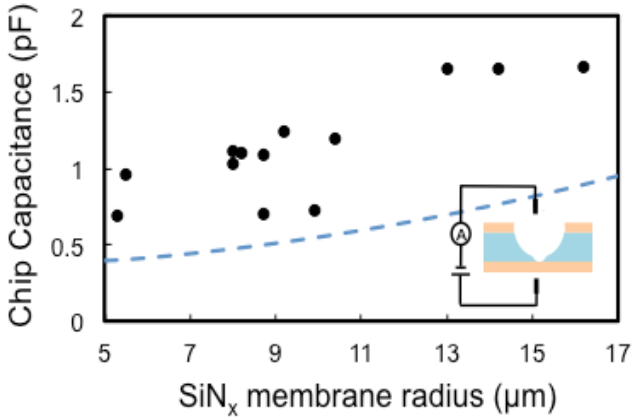


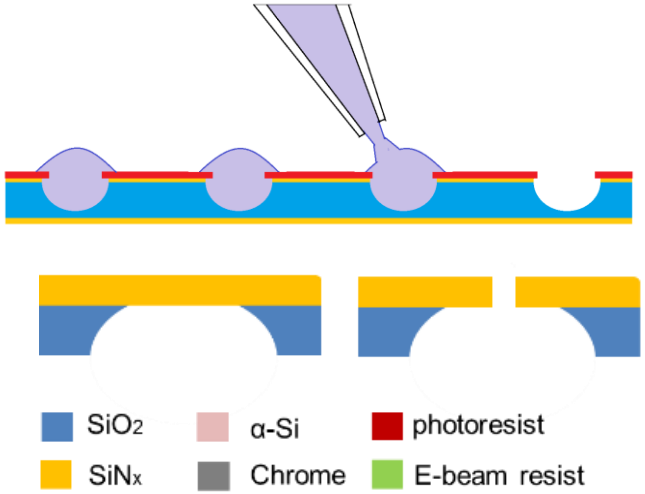




$$L = 2\sqrt{R^2 - t^2}$$







Detecting signs of extraterrestrial life

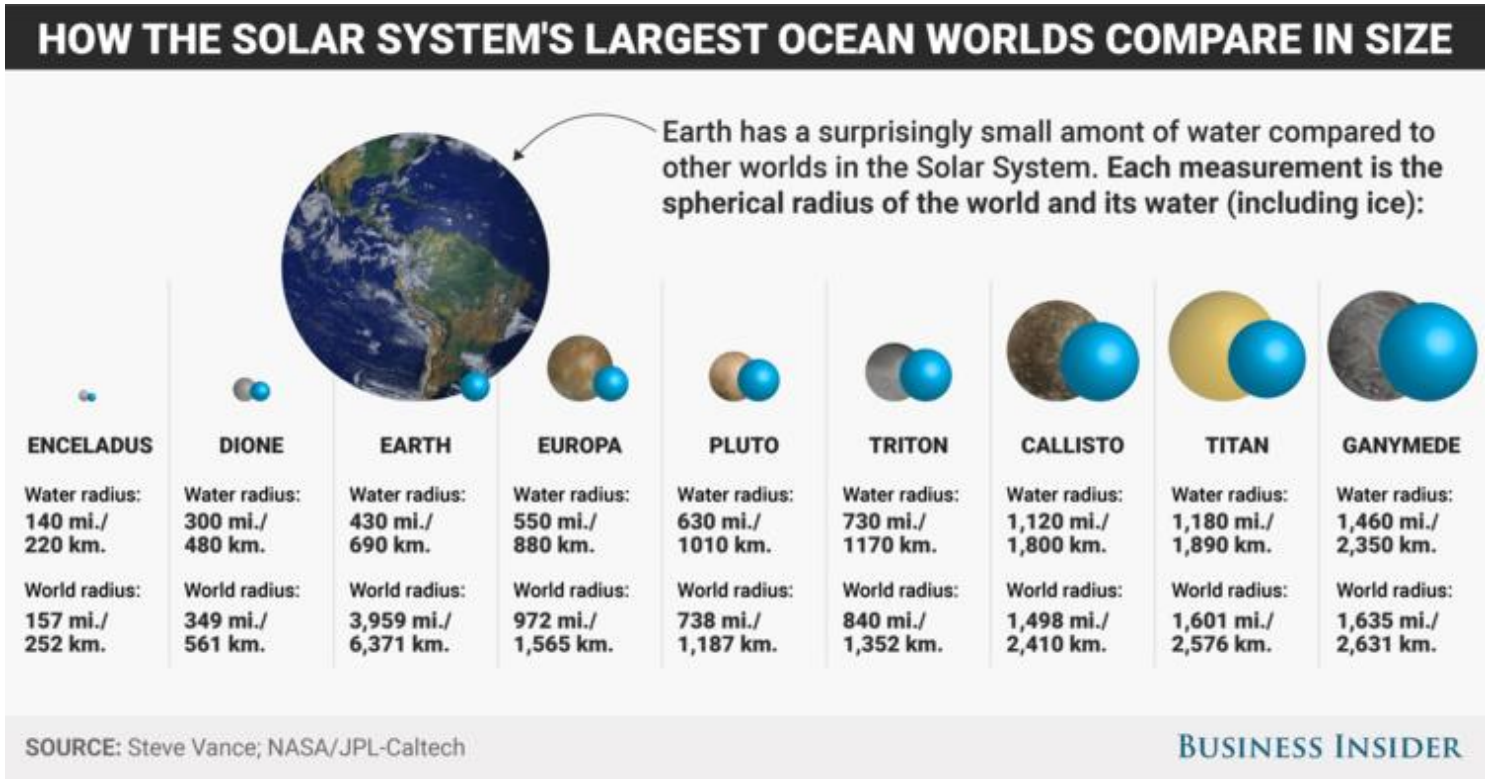
80NSSC18K1545

Is there life on other bodies of our solar system?

- Liquid water
- Look for molecules of life
  - Amino acids
  - Proteins
  - “Benner-like molecules”

Candidate worlds

- **Enceladus (Saturn)**
- Europa (Jupiter)
- Titan (Saturn)
- Mars



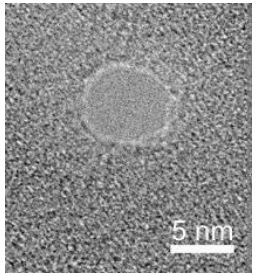
Motivation for using solid state nanopores

Single molecule detector

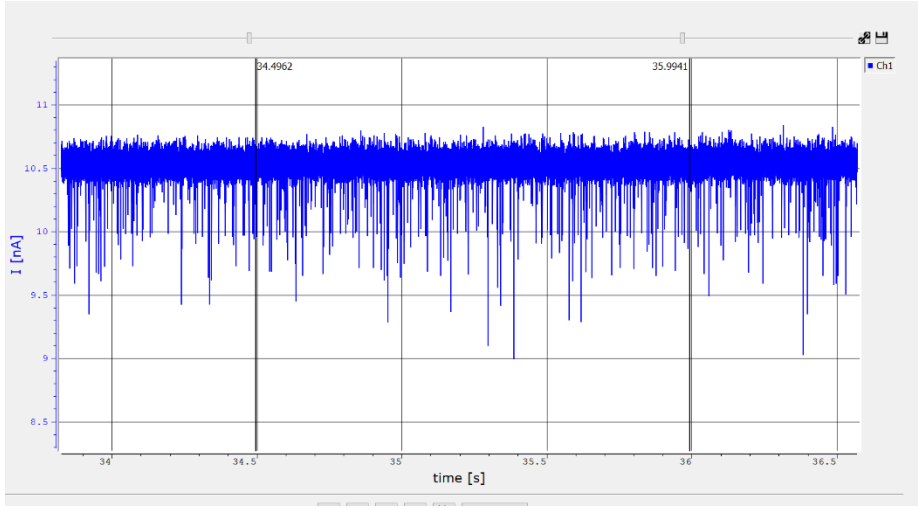
DNA or DNA like molecules (Benner-like) molecules are long, charged, contain different subregions

Can we use it for space flight?

- Radiation damage
- Temperature extremes
- Survive for many months
- Decontamination (sterilization)



Nanopore made in 20 nm thick low stress Silicon nitride by a TEM ablation technique.



Above: 15 kbp ds DNA translocating through a 15 nm diameter nanopore. 1M KCl, 10 mM Tris buffer, 1mM EDTA. 200 mV applied voltage.
100kHz bandwidth

Questions for this Phase II contract

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1) Quality control and performance

- What is the cost? Quality testing.
- How do we make them last a long time?
- How do we make them perform at specification? Success rate?

2) Specifications for DNA extraction from samples

- test commercial kits on analogue earth samples to extract DNA
- do different soil types require different treatments?
- feed DNA forward to nanopores for detection

Technical Objectives of the Contract

1,2,3: Optimize nanopore fabrication procedures in the low-noise chips with silicon nitride membrane.
4: Establish optimal pore cleaning, storage, and wetting procedure
5: Measure translocations through nanopores of pure analyte samples.
6: Establish optimal environmental sample preparation procedures.
7: Measure translocations through nanopores of environmental samples.
8: Create a database of all the above measurements including all parameters (pore parameters, sample type, conditions, translocation data).
9: Specify flight readiness parameters (pressure, temperature, radiation, vibration, etc.) needed for upcoming NASA missions

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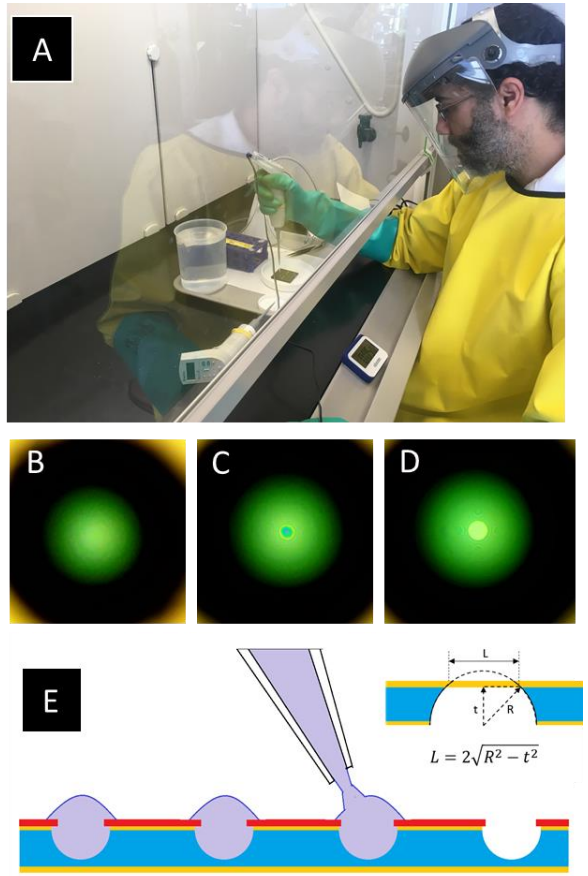
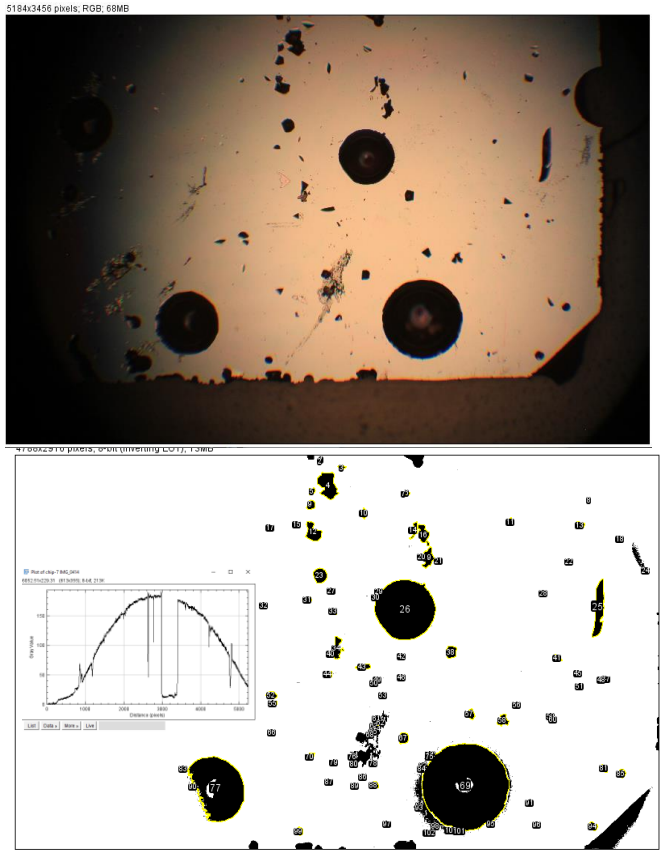
Quality control of low noise chips

Technical Objectives 1,2, & 3: Optimize nanopore fabrication procedures in the low-noise chips with silicon nitride membrane.

Developed a new protocol for low-noise chip manufacture with better success rate, fewer defects, and lower cost

Developed automated characterization tool using ImageJ software to reliably sort chips by quality





Testing and optimizing nanopores for long term use (2000 nanopores)

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Technical Objective #4

"Wetting treatment"

- Oxygen plasma vs. piranha vs. isopropanol

Storage conditions

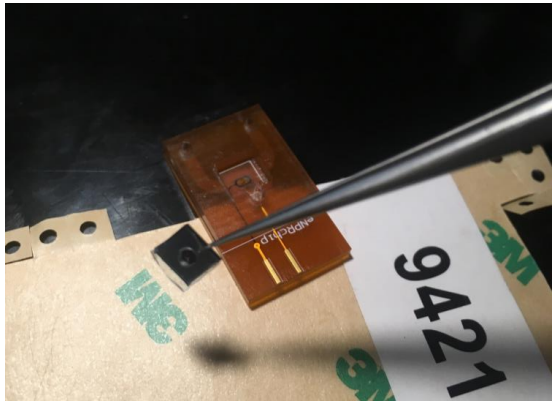
- Vacuum, air, isopropanol, 1M KCl

Coatings for better performance/robustness

- Chemical modification
  - APTS & SA
  - PEG
- Atomic Layer Deposition
  - Aluminum oxide, Hafnium oxide, silicon oxide

$$G_0 = \sigma\left[\frac{4t}{2d^2} - \frac{1}{d}\right]^{-1}$$

We assume the open conductance, G_0 , can be modeled by the above equation, where σ is the solution conductivity, t is the membrane thickness, and d is the nanopore diameter



Nanopore stability

Oxygen Plasma vs. Piranha cleaning

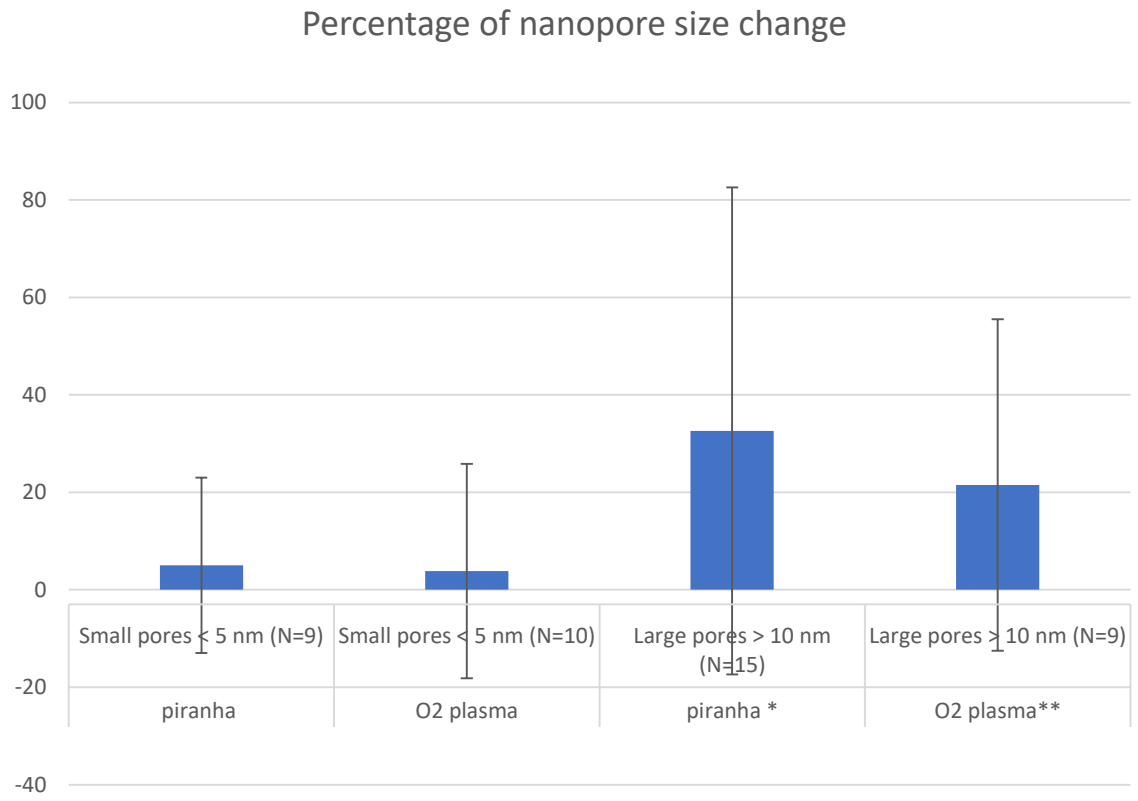
Total of 176 nanopores tested (some at NASA)

56 surviving nanopores piranha treated

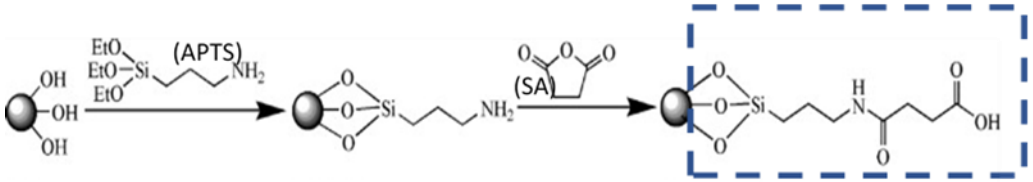
- 30% break rate with 10 minute heated piranha

40 surviving nanopores O2

- 3% break rate at 25W forward power O2 plasma
- 100% break rate with plasma wand treatment (n=13)



Does Modification of surface help?

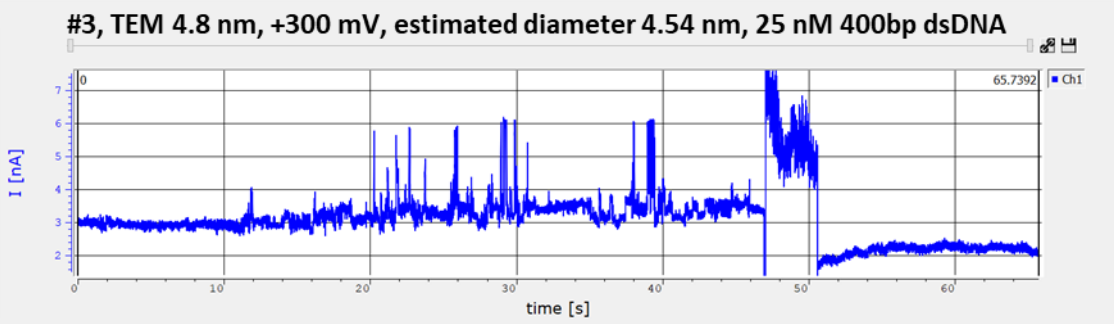


Preliminary Results

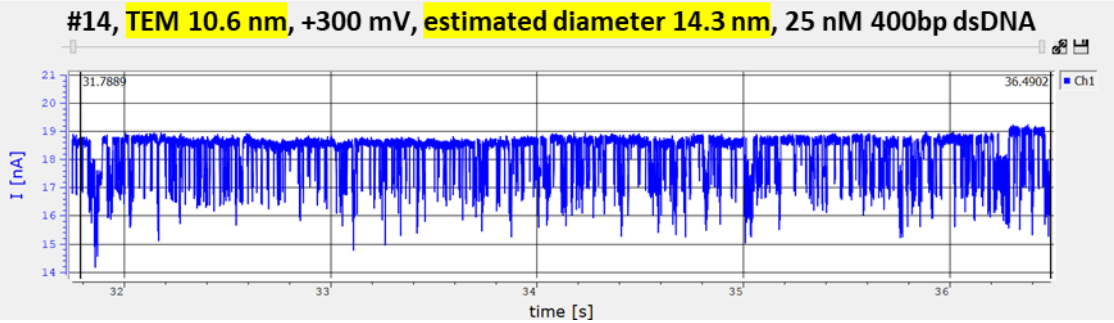
An APTS-SA modification improves the ability of nanopores to translocate DNA up to several weeks after first treatment

Atomic Layer Deposition with Hafnium Oxide inhibits nanopore expansion both during piranha treatment and with prolonged soaking in 1M KCl.

Atomic Layer Deposition with Aluminum Oxide temporarily improves probability of good translocation data, but later has no effect.



Current traces during the translocation of 25 nM 400bp dsDNA through one SiN nanopores as a function of time at *Day=15* after cleaning. The pore was non-coated and stored in IPA. The measurement was performed in 1 M KCl, 10 mM Tris and 1 mM EDTA, pH 7.5 at +300 mV.



Current traces during the translocation of 25 nM 400bp dsDNA through one SiN nanopores as a function of time at *Day=15* after cleaning. The pore was surface modified and stored in air. The measurement was performed in 1 M KCl, 10 mM Tris and 1 mM EDTA, pH 7.5 at +300 mV.

Extracting DNA from environmental analogues

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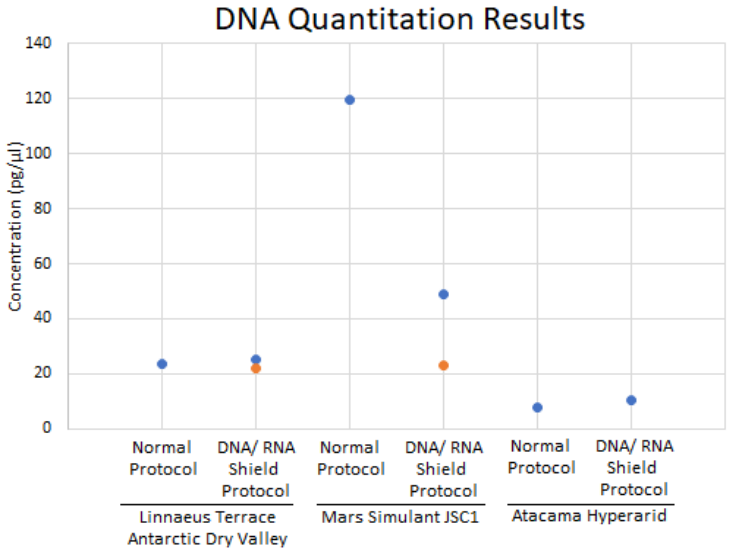
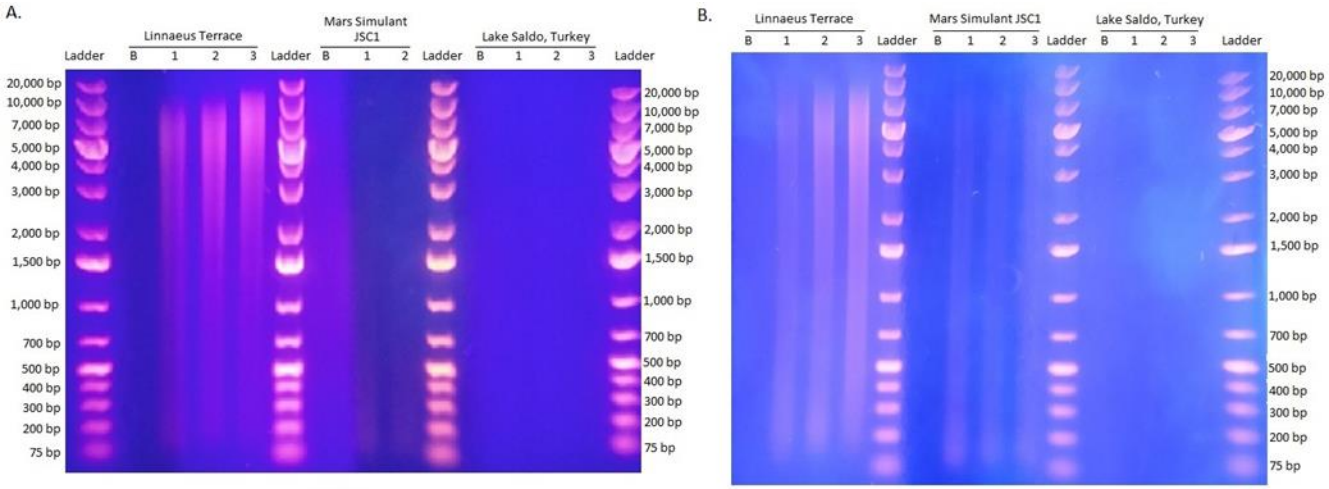
Commercial Kit Extraction

Qiagen DNeasy Powersoil Kit
Qiagen DNeasy Powersoil Pro Kit
Zymo Research Quick DNA Fecal/ Soil Microbe Microprep Kit
Zymo Research ZymoBiomics Microprep Kit



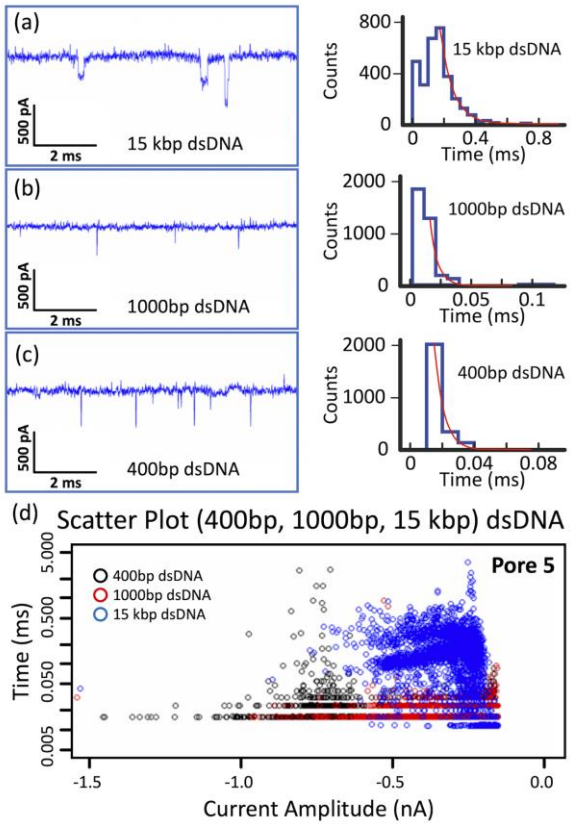
Analogue samples

Atacama Hyperarid
Mars Simulant JSC1
Linnaeus Terrace Antarctic Dry Valley
Pacific Sea water (TBT)

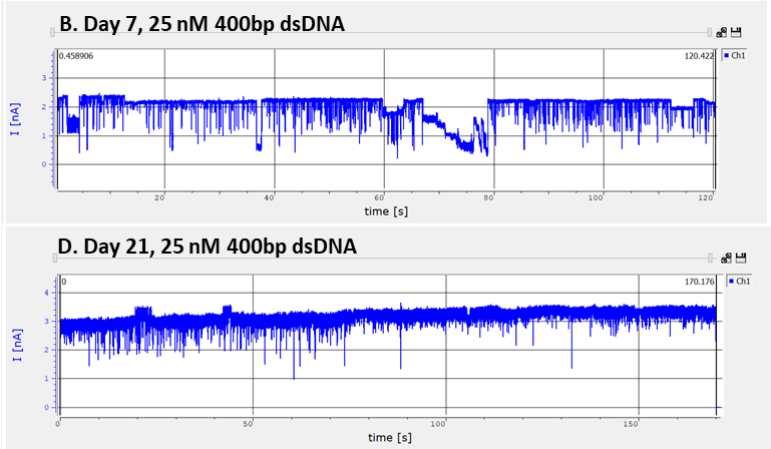


Agarose gel electrophoresis depiction of samples extracted using Qiagen DNeasy Powersoil Pro Kt. Soil samples from Linnaeus Terrace, Antarctic Dry Valley, Mars Simulant JSC1 soil, and Lake Salda, Turkey were spiked with 100 µL of a liquid culture of (A) *E.coli* and (B) *B. subtillus.*

Measuring DNA Translocations

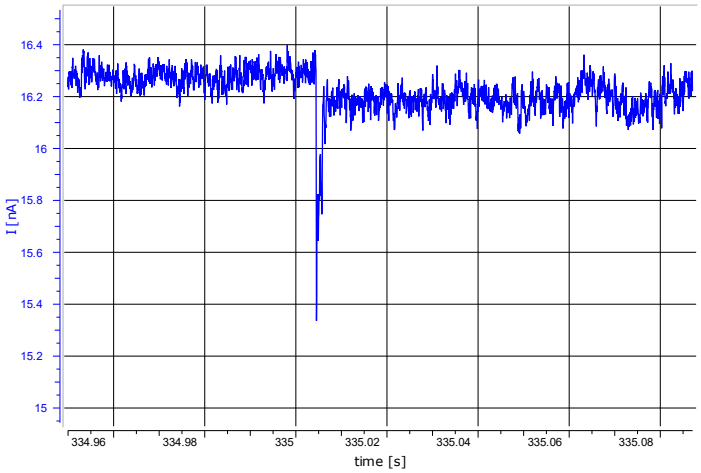


Measure translocations through nanopores of pure analyte samples. (**Technical Objective #5**)



Same nanopore measured two weeks apart. 1M KCl, 10mM Tris +300mV.

Measure translocations through nanopores of environmental samples. (**Technical Objective #7**)



DNA purified by ZymolLST kit. Event rate <1 per minute.
1M KCl,10mM Tris +300mV.

Future work

We have tested approximately 200 at this time. 1800 more to go.

Use DNA from extraction kits in our nanopores (started).

Testing Sea water samples

Long-term function of nanopores

Continue measuring modified nanopores

Exploring alternative polymers

Acknowledgments

Funding

SBIR Phase II contract
80NSSC18K1545

Institutions

Singh Center for Nanotechnology, Penn
Pennovation Works, Penn

People

- Federico Thei, Elements SRL
- Michele Rossi, Elements SRL
- Serge Kaddoura, Elements SRL
- **Zehui Xia**, Goeppert LLC
- **Margret Patchin**, Goeppert LLC
- Andre Scott, Goeppert LLC
- Vanya Buvac, Goeppert LLC
- Chris McKay, NASA
- Jennifer C. Xu, NASA
- Gary W. Hunter, NASA
- Marija Drndic, Penn