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Diatom-based Sensors

"Organic/Inorganic Interfaces and Health Science Applications"

iMAPS Device Packaging Conference

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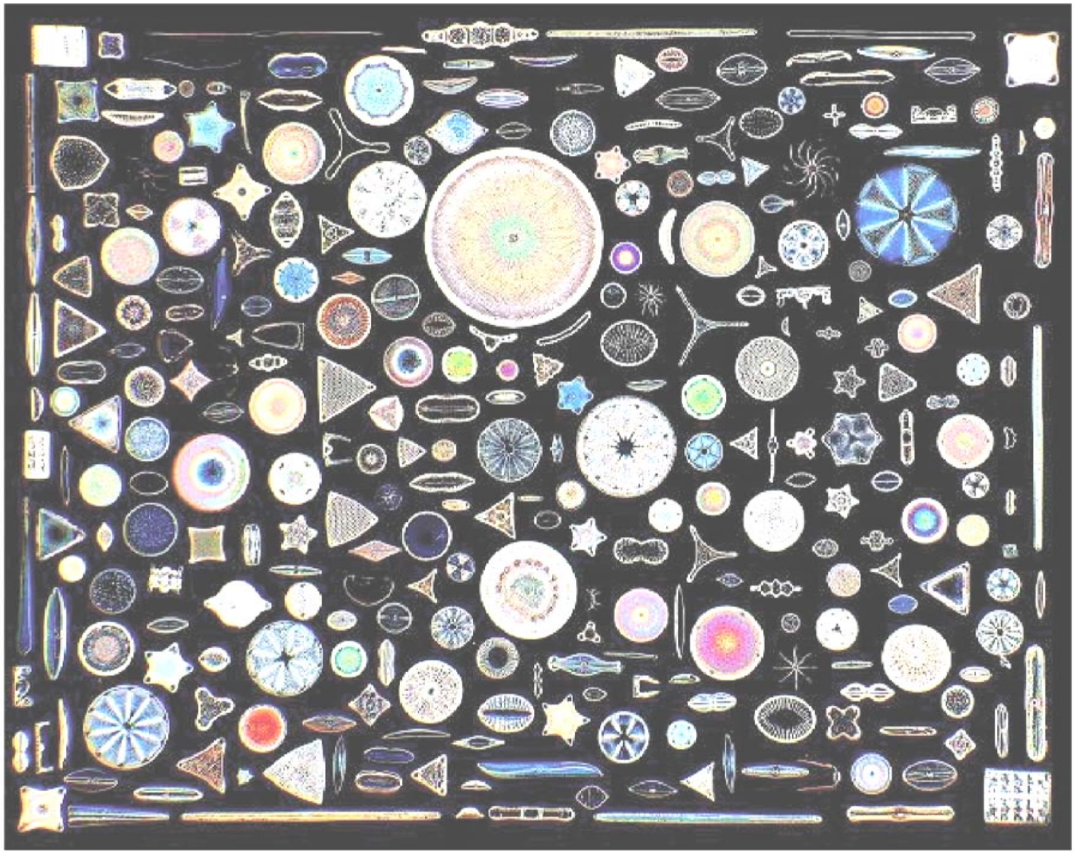
Biogenic Silica Nanostructures : Diatoms

- Unicellular, eukaryotic, *photosynthetic algae*
- There may be up to *100,000 different species* of diatoms
- Diatoms range most commonly from *1 μm to 500 μm in diameter*
- Cell wall is made of *silica* and called a *frustule*



R. Gordon et al., Trends in Biotechnology 27 (2009) 116-127

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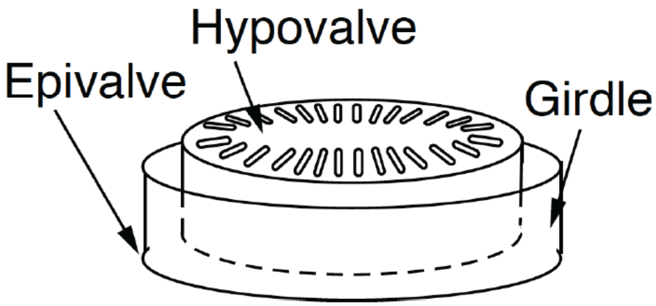
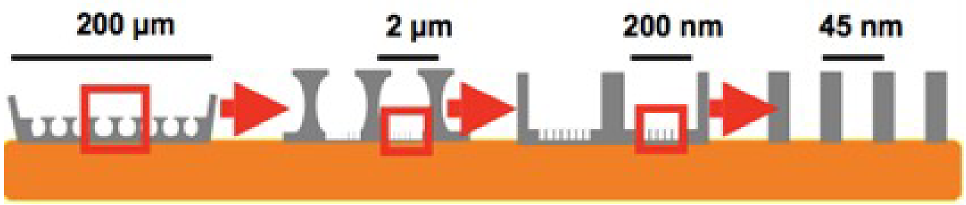
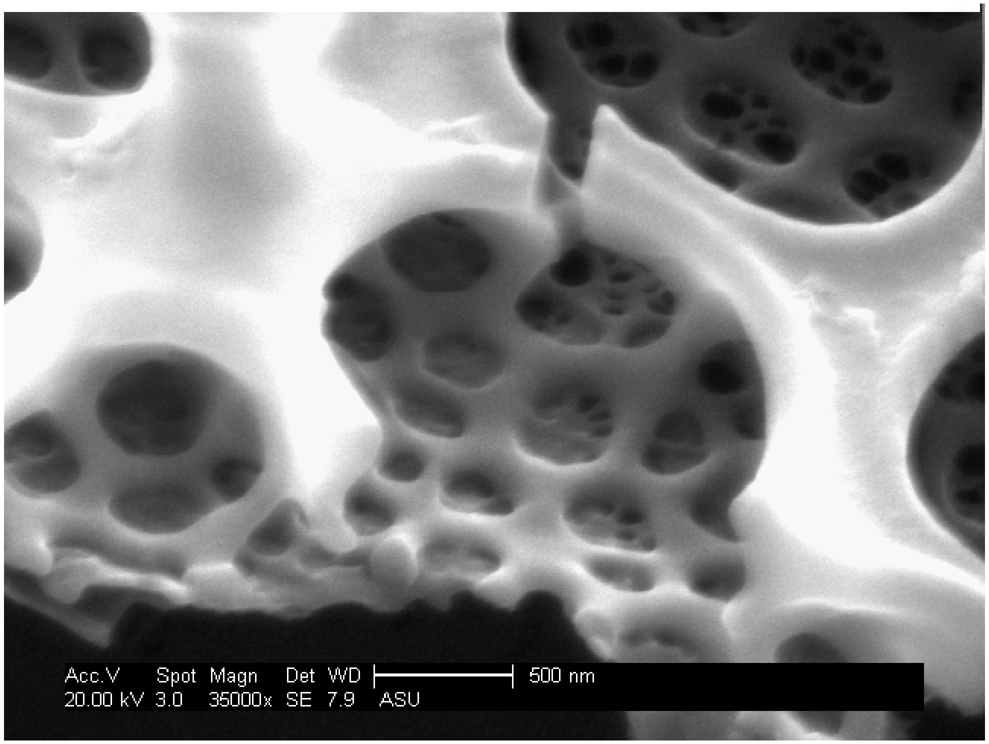
Natural Silica Diatom Nanopores







- Diatom *frustules* exhibit a *hierarchical pore structure* with pore sizes down to 40 nm
- Diatoms can be grown to reach *200 µm in diameter*
- The silica diatom shell can be *chemically modified*

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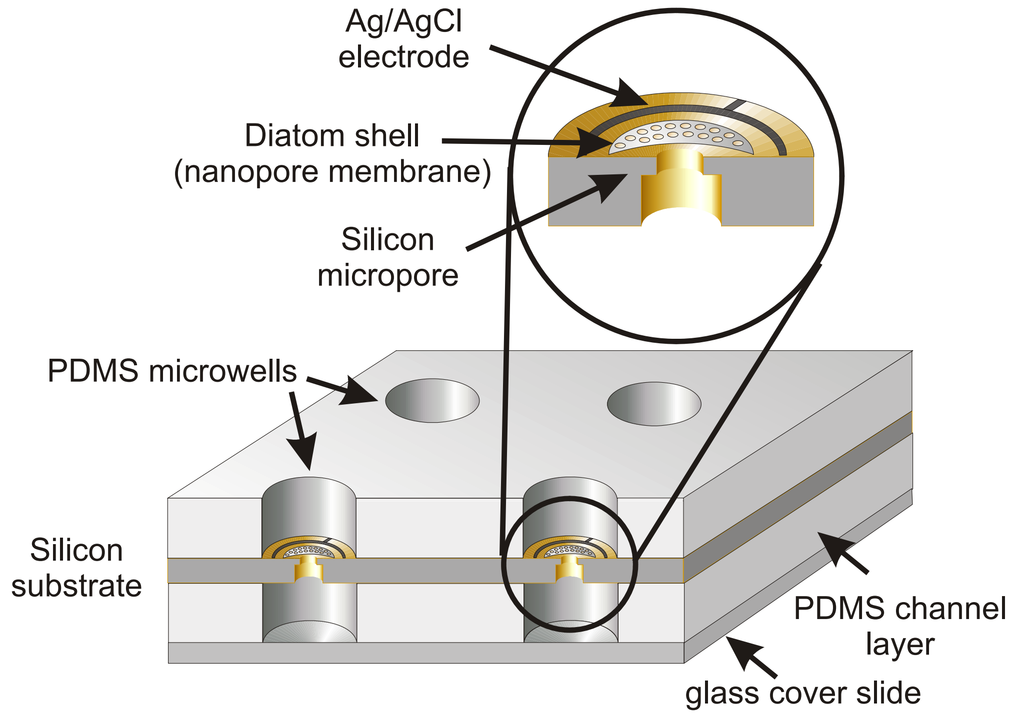
Diatom - Silicon Sensor Platform



- Diatom *frustules* are *mechanically stable* and can be *mounted on a larger substrate* such as *a silicon micropore device*
- *Mounting* can be accomplished *using poly-L-lysine* or *epoxy resist*

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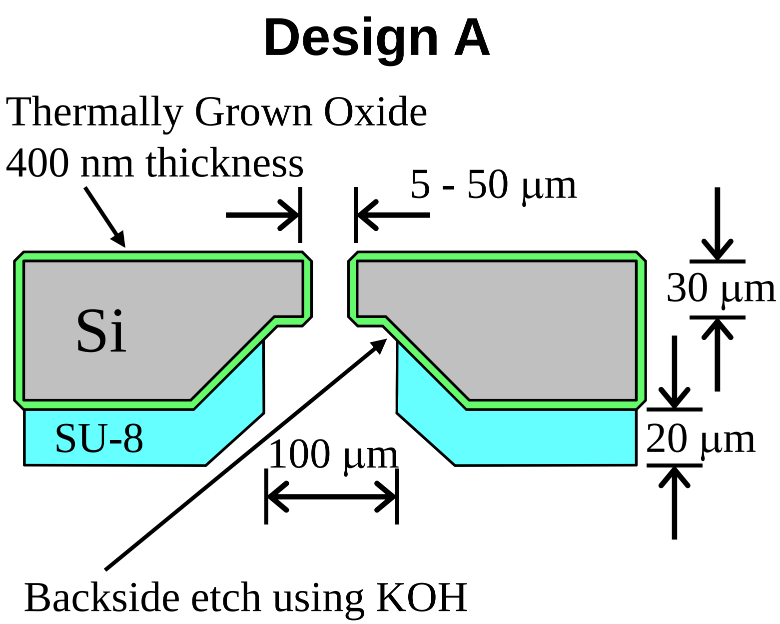
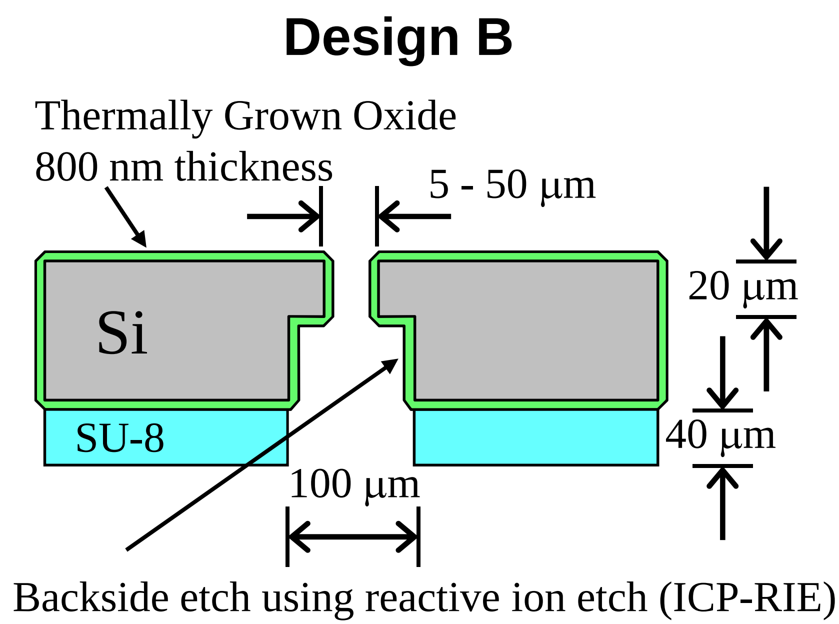
Silicon Micropore Substrate Design





- ✓ KOH etch is highly reproducible
- ✗ Tapered backside etch limits the spacing of the pores
- ✗ Taper results in thin SU-8 layer → capacitance increase !

- ✓ SU-8 does not have to cover the edge of the back side pore
- ✗ Dry backside etch shows a size dependent etch rate

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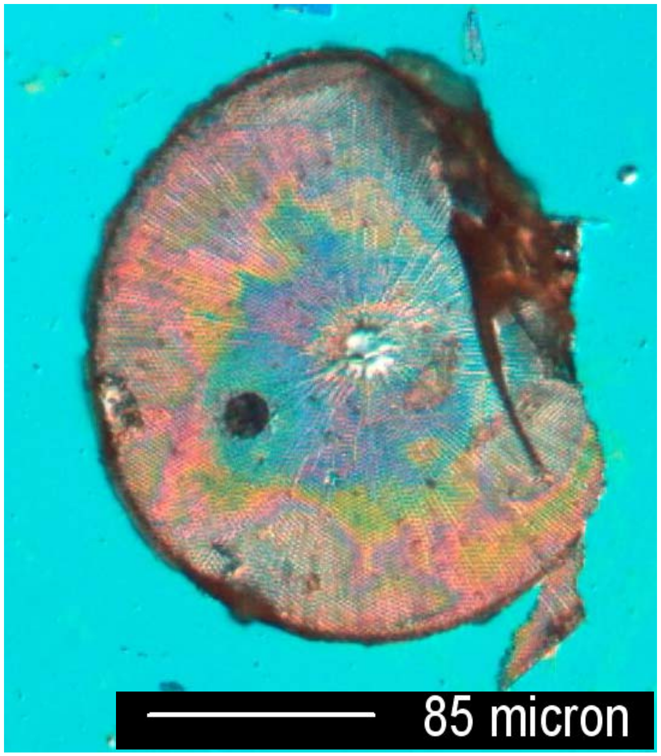
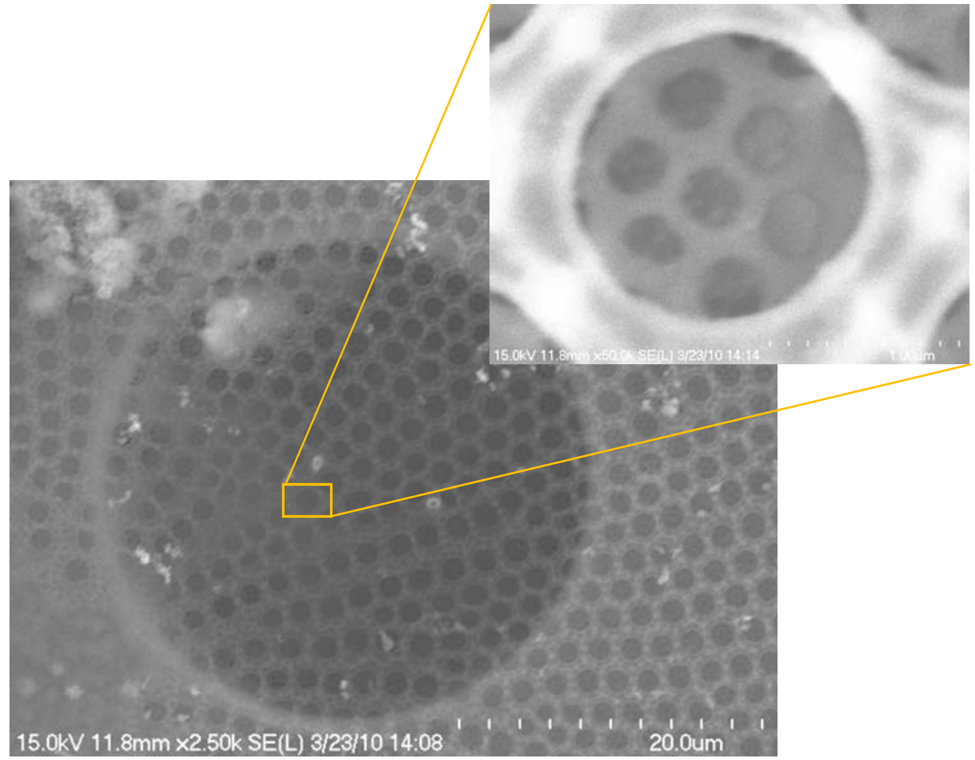
Diatoms on Silicon Micropores



Optical micrograph of Diatom mounted using Epoxy glue on a Si chip on top of a 20 µm pore



SEM Image of Diatom on Si chip

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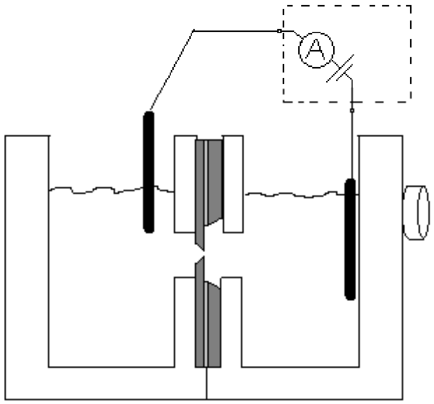
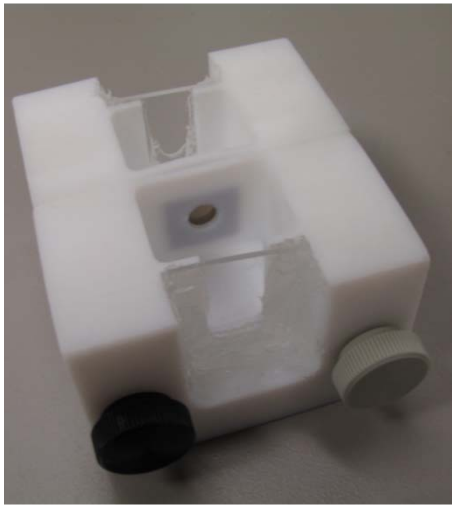
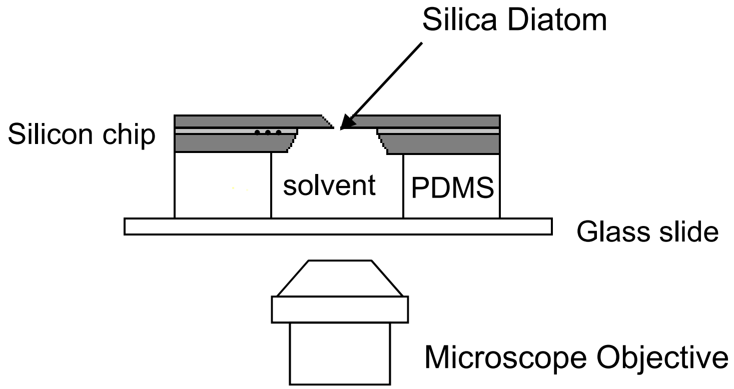
Experimental Setup







- For *electrochemical experiments* the silicon chip was mounted in a *Teflon holder*
- Electrodes connected to a *patch clamp amplifier* measured the *changes in current* when particles diffused through the nanopore.
- Molecular *diffusion assays* were done on a *fluorescence microscope* using a PDMS layer to create a well on the bottom side of the silicon chip and placing the PDMS on a glass slide.

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Nanopore Sensors

- **Nanopore membranes can act as selective filters**
  - ❑ An optically transparent *nanopore membrane* allows to *stimulate a cell locally* while observing an optical response
- **Nanopores can be used as conductimetric sensors**
  - ❑ In *conductimetric sensors* the conductance is influenced *by particles or biomolecules* that *reduce the cross-section area* of a *nanopore* through which an ionic current is passing.
- **Nanopores can host membranes and biomolecules**
  - ❑ Solid-state nanopores can *host biomembranes*, allowing the study of an *array of membranes* or an *array of biomolecules* inserted in these membranes

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Nanopore Membranes as Selective Filters

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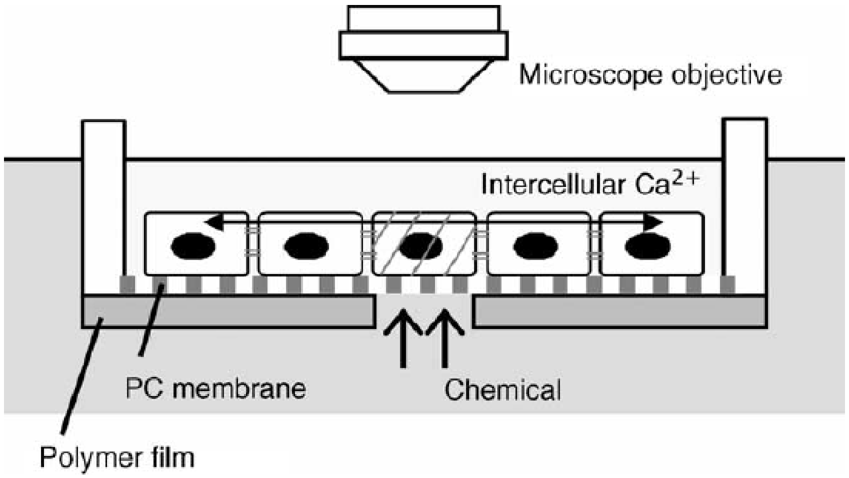
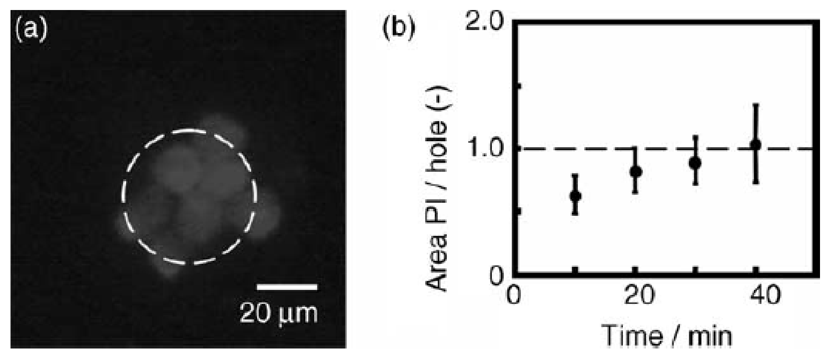
Localized Cell Stimulation





- Porous *polycarbonate membrane* used as *cell culture substrate*
- Polymer film *limits the area* between the upper and the lower chamber that is *permeable to chemicals*

- Local dose of *ethanol triggers* initial *fluorescence*
- The fluorescence *area increases* with time *due to cell-cell communication* rather than chemical stimulation

K. Takoh et al., Sensors and Actuators B 108 (2005) 683–687

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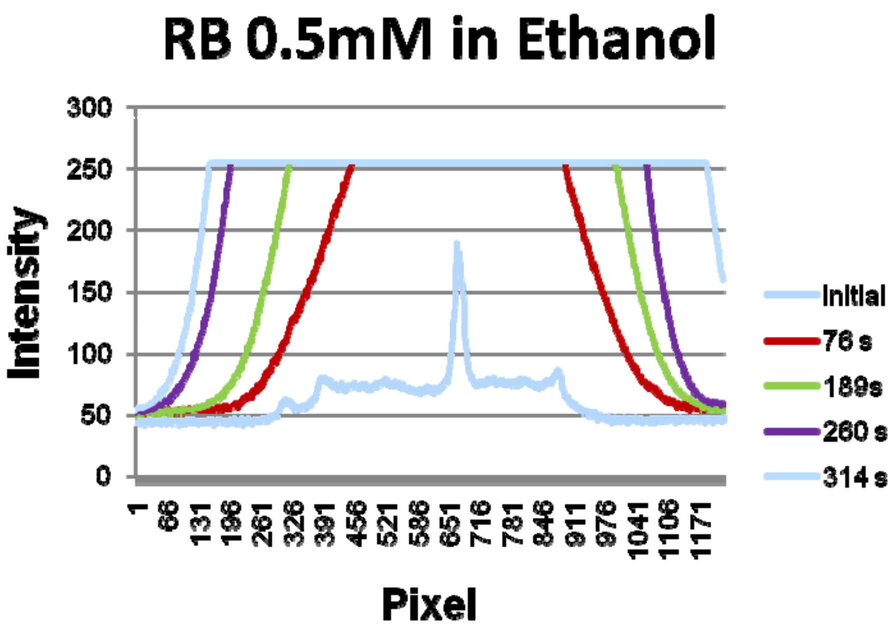
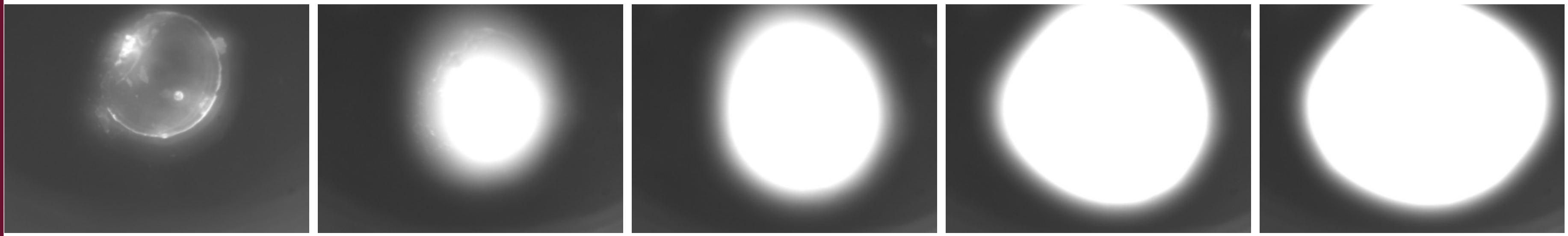
Fluorescence Assay: Rose Bengal in Ethanol





initial 76s 189s 260s 314s

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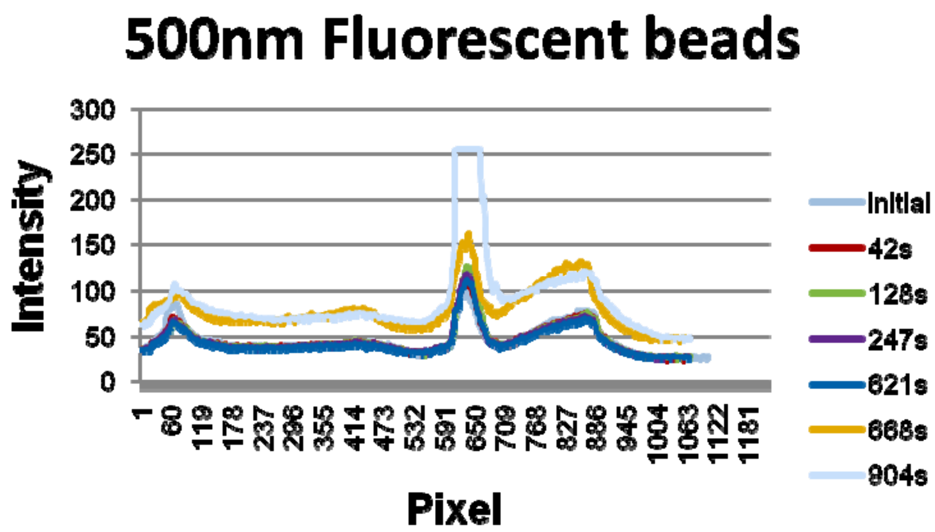
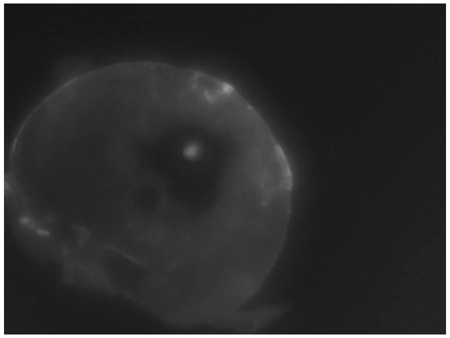
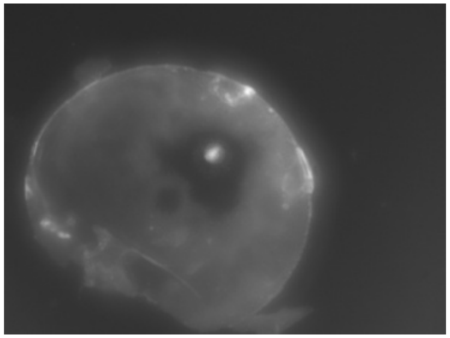
Fluorescence Assay: 500nm Polystyrene Beads

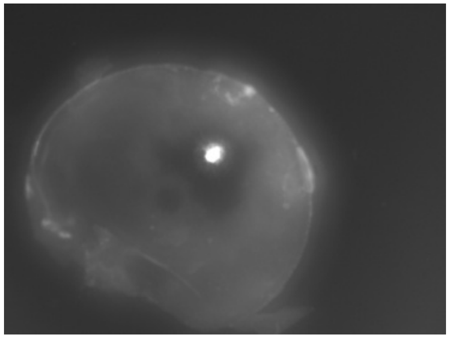




initial



668s



904s

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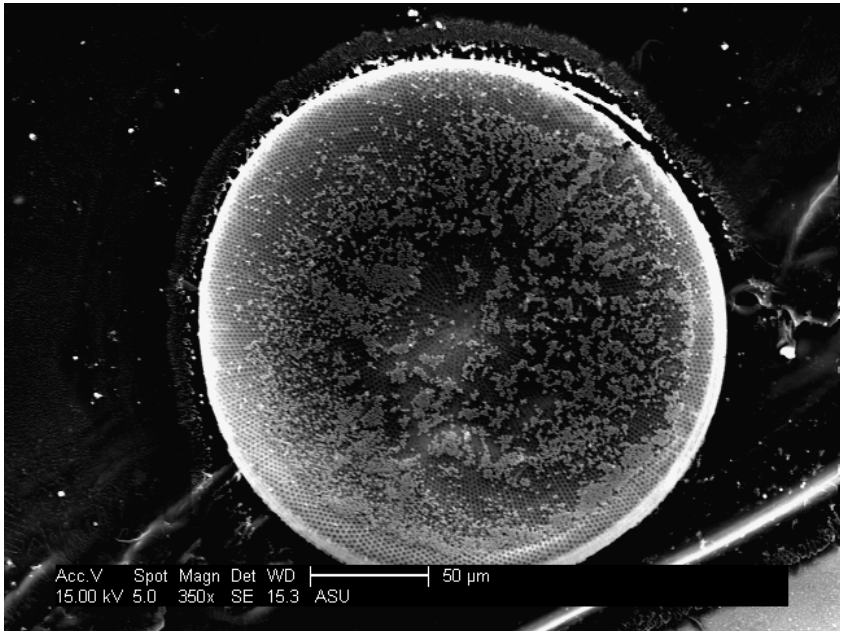
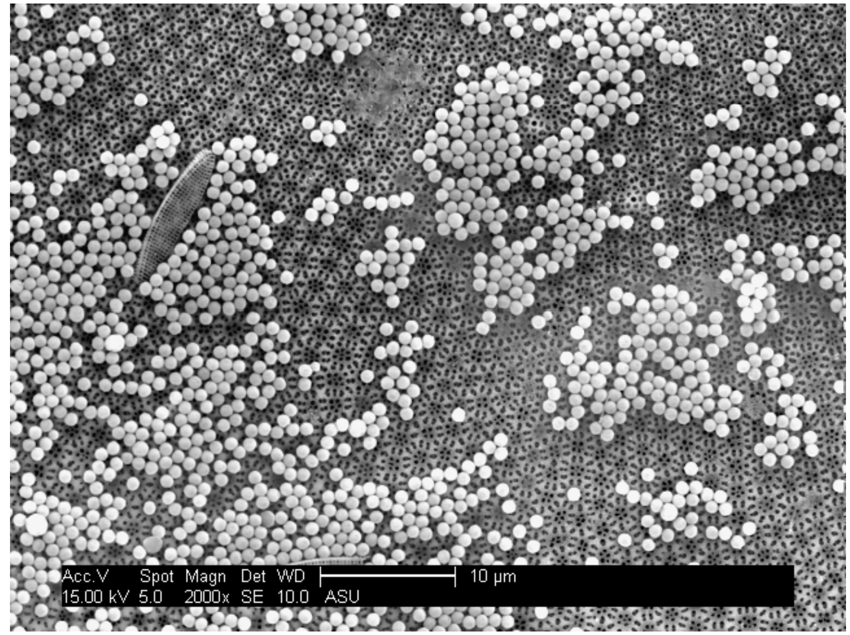
Particle Retention





- *1 µm Polystyrene particles* do not migrate through the diatom pores and *are efficiently retained*

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Nanopores as Conductimetric Sensors

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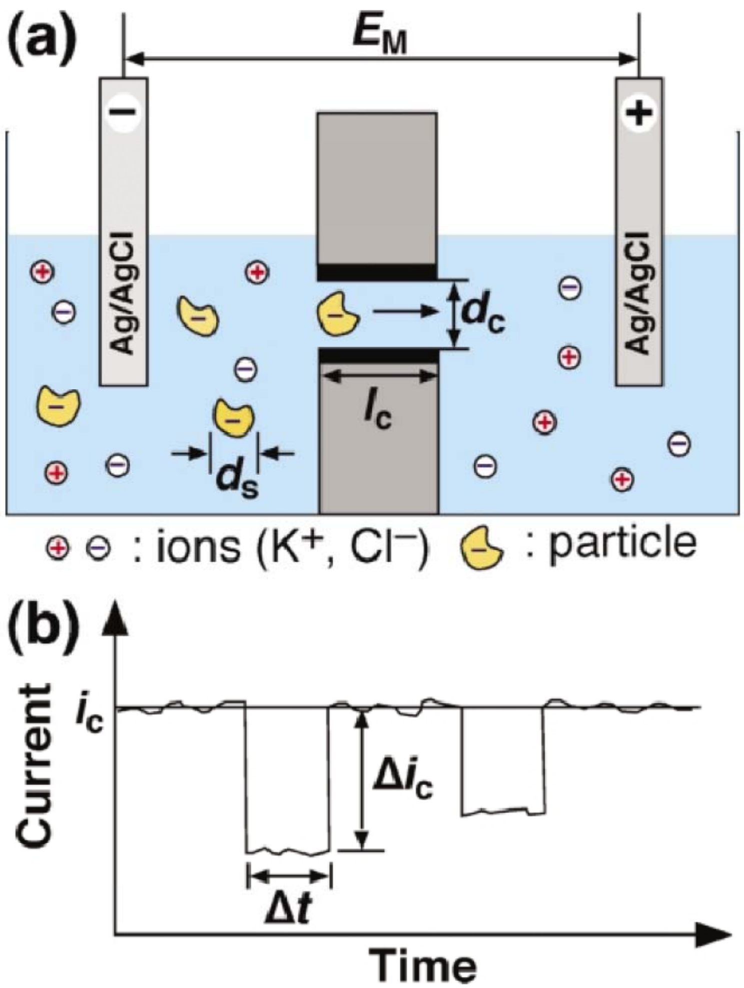
Coulter Counter



R.R. Henriquez et al., Analyst , **129**, 478-482, 2004

Electric field-driven Coulter counter

- A membrane divides two chambers containing an electrolyte solution.
- *Particles* of appropriate size and charge will *enter the channel* and *reduce the ion current*.

Coulter counter data

- Consist of a *series of current pulses.*
- The *height of the pulse*, Δi_c, is related to *particle size* and the *width, Δt*, corresponds to the *particle transit time*.

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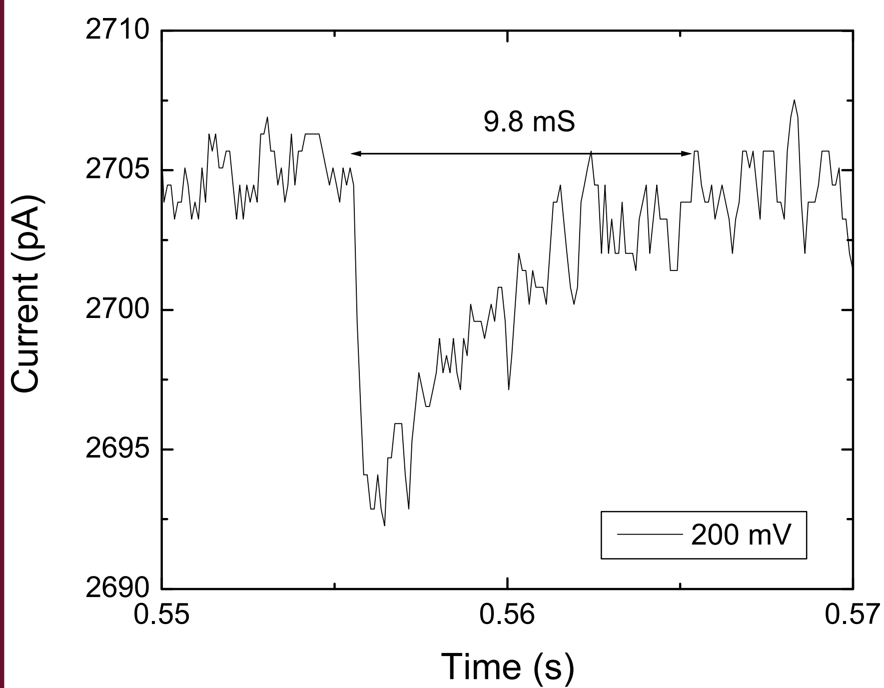
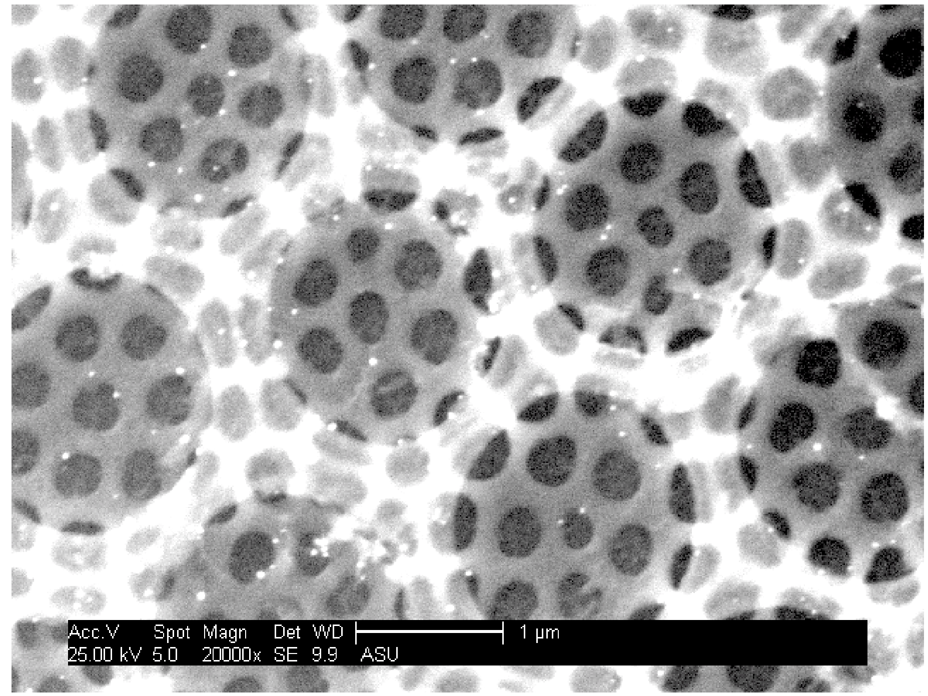
Coulter Counting: 25nm Gold Nanoparticles





- Deionized water (zeta potential: -70mV), σ ~ 23 μS/cm
- *positively charged* nanoparticles
- *long translocation time* = small mobility

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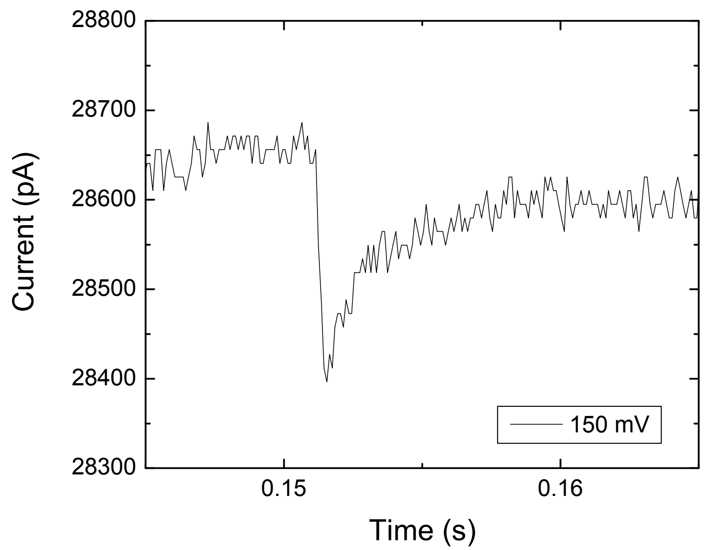
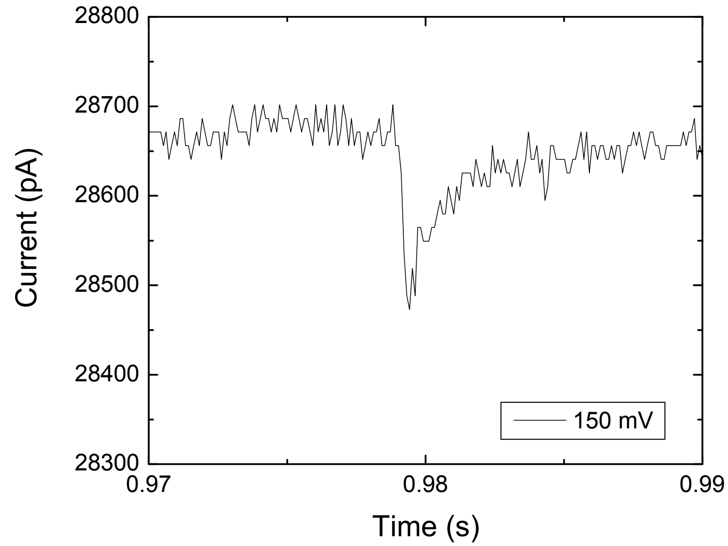
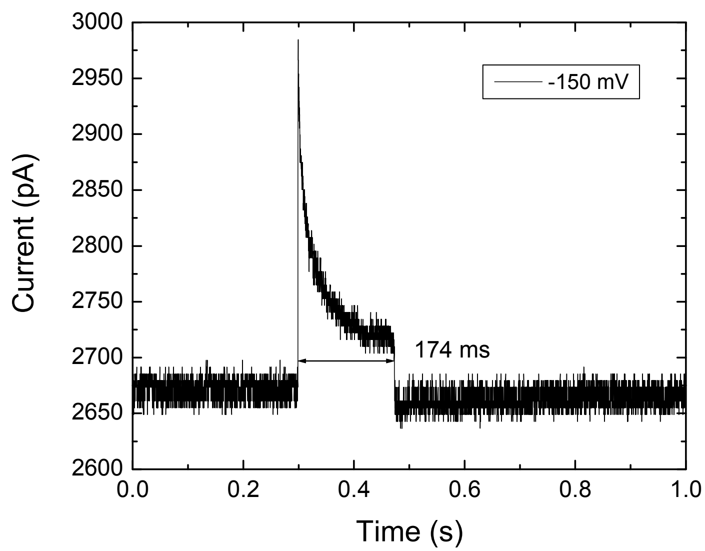
Coulter Counting: 50nm Length Gold Nanorods







- *varying* translocation *times*
- *signal shape* depends on how the *elongated rods* move through the nanopore

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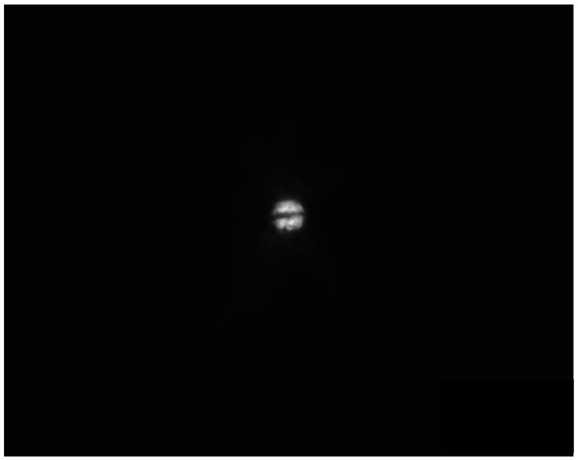
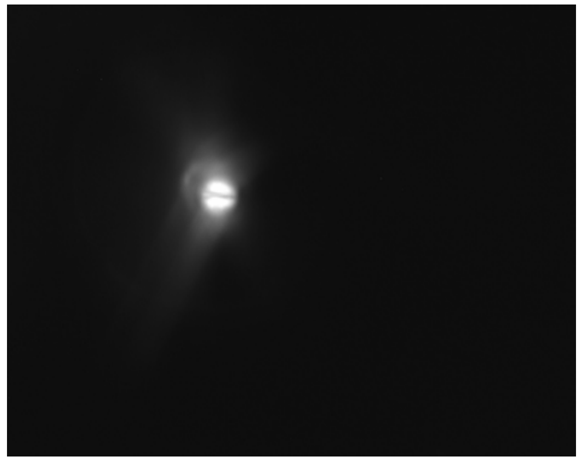
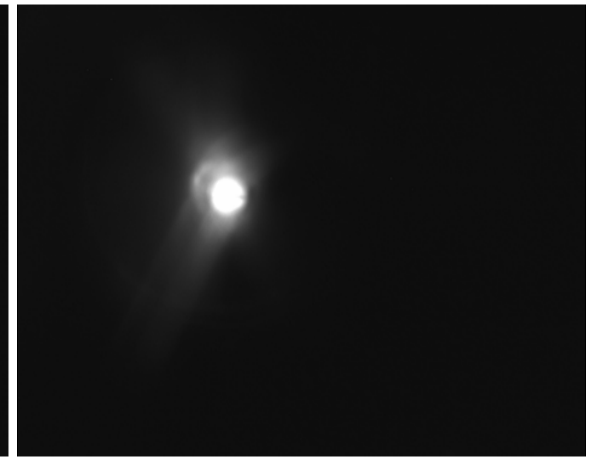
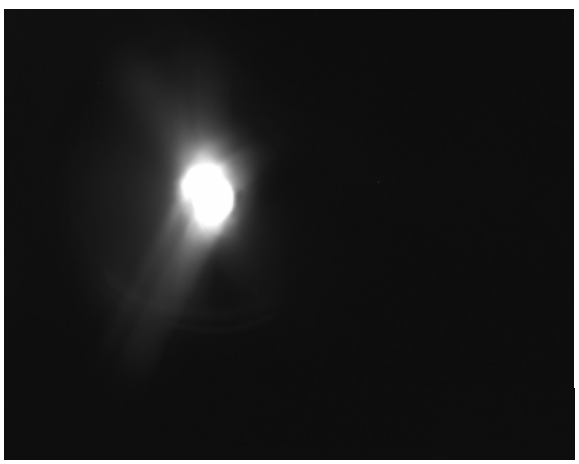
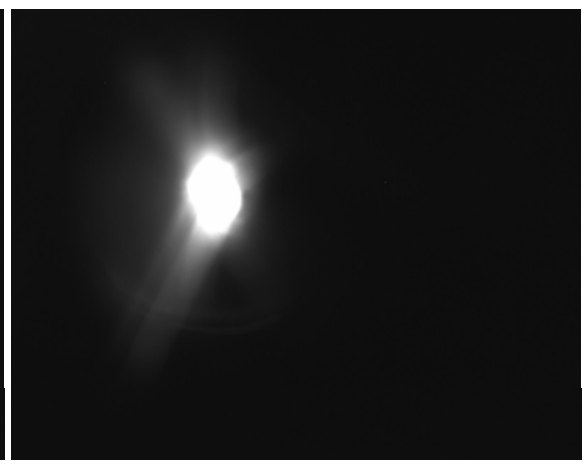
Combined Assay: 25nm ZnSe Quantum Dots

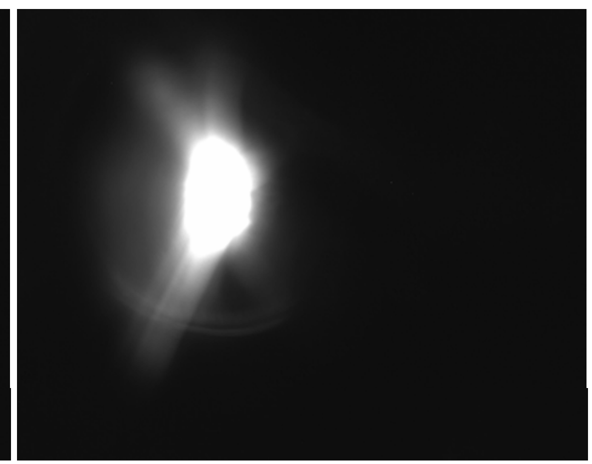

initial


28m


30m


33m


35m


43m

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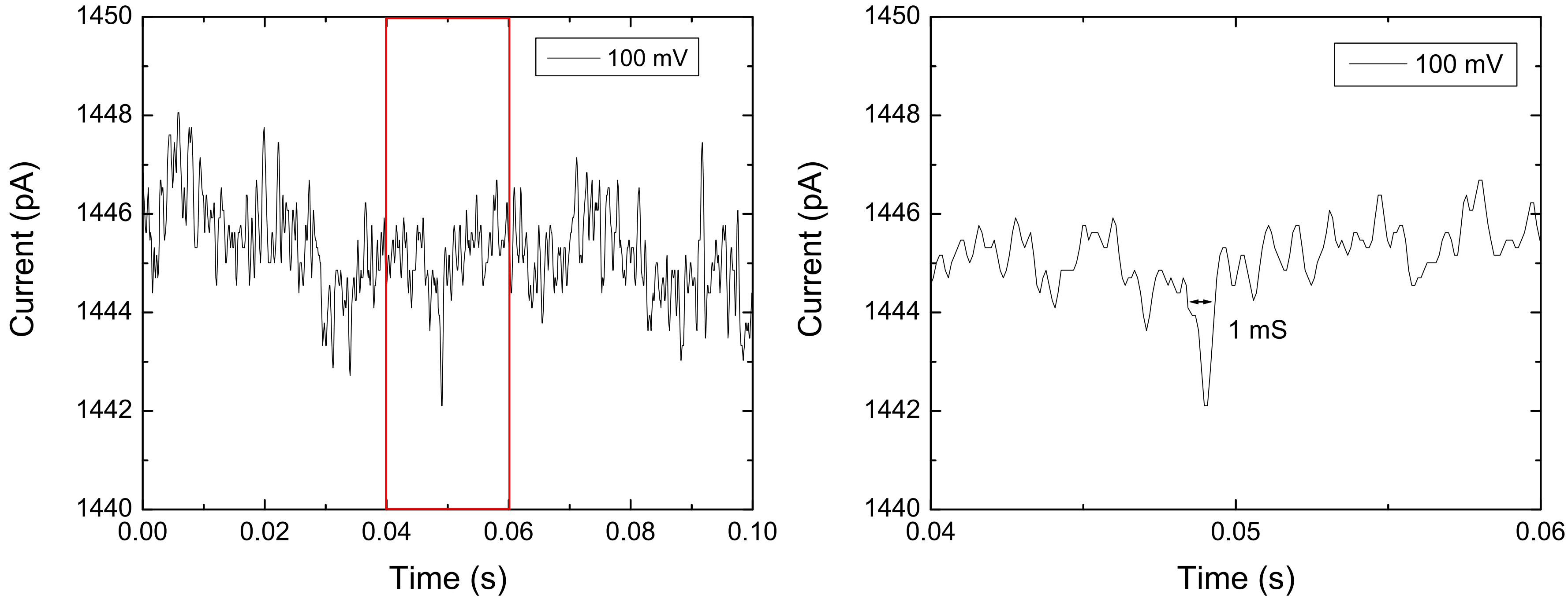
Combined Assay: 25nm ZnSe Quantum Dots



- Deionized water, σ ~ 23 μS/cm
- approx. *200 nanopores conducting* (~ 7.2 pA/pore)
- ΔI = 2.2 pA expected, ΔI = 2.6 pA measured
- *Custom-built transimpedance amplifier*, Gain = 1mV/pA
- *Measurements performed while sample was illuminated*

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Nanopores as Biomembrane Hosts

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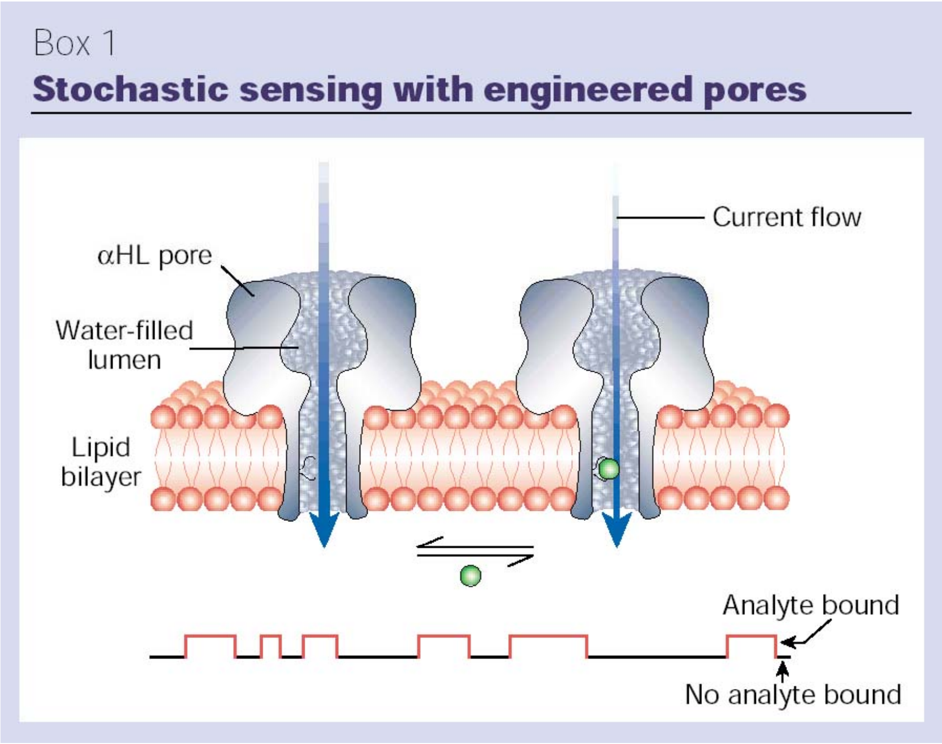
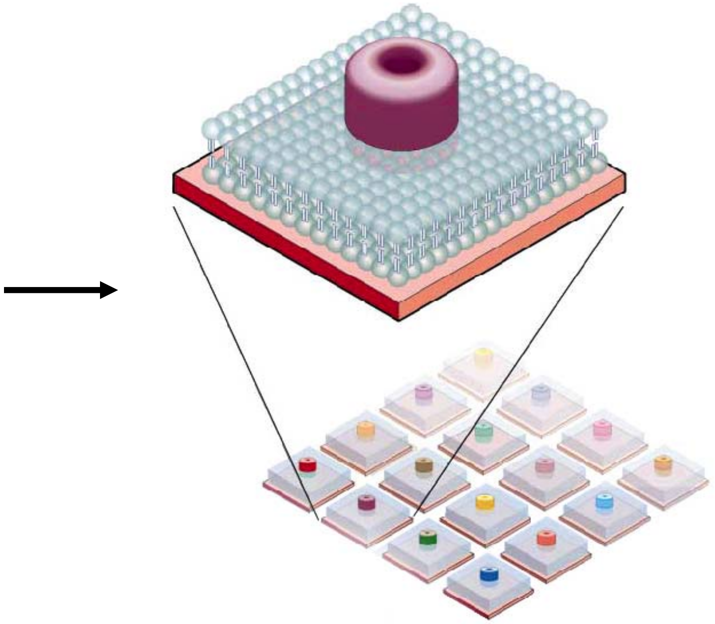
Ion Channel Array





- *frequency* of binding event reveals *concentration* of analyte
- *duration* and *amplitude* reveal *identity* of analyte

H. Bayley and P. Cremer, Nature 413, 226-230 (2001)

- *array platform*: engineered pores with *different sensitivity* sensing in parallel, ionic current signal pattern *processed* using *DSPs*

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Nano - Bilayer Lipid Membranes

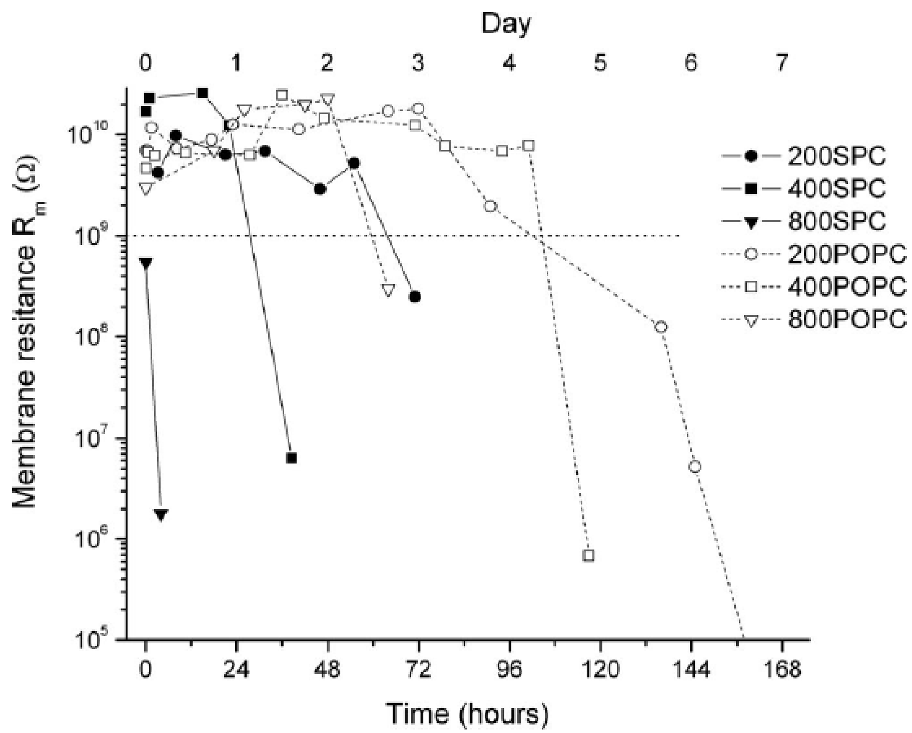

FIG. 4. Stability of bilayers of soy PC (SPC, ●, ■, ▼) and POPC (○, □, ▽) on nanopore arrays of different pore diameters (200 nm ●, ○, 400 ■, □, and 800 nm ▽, ▼). The dotted line indicates the very high threshold of 1 GΩ above which bilayers are present in all pores of the support. If only one pore opens, the membrane resistance drops to 200 MΩ (data not shown). Bilayers consisting of POPC are more stable than those of SPC, which need a pore diameter of 400 nm (■) or smaller to maintain a sealing above 1 GΩ for 1 day.

L. X. Tiefenauer and A. Studer, Biointerphases **3**(2),FA74-FA79, 2008.

- lipid bilayer membranes show *higher seal resistance* and *longer lifetime* (approx. 4 days) when formed on *200 nm* diameter *silicon nitride nanopore membranes*

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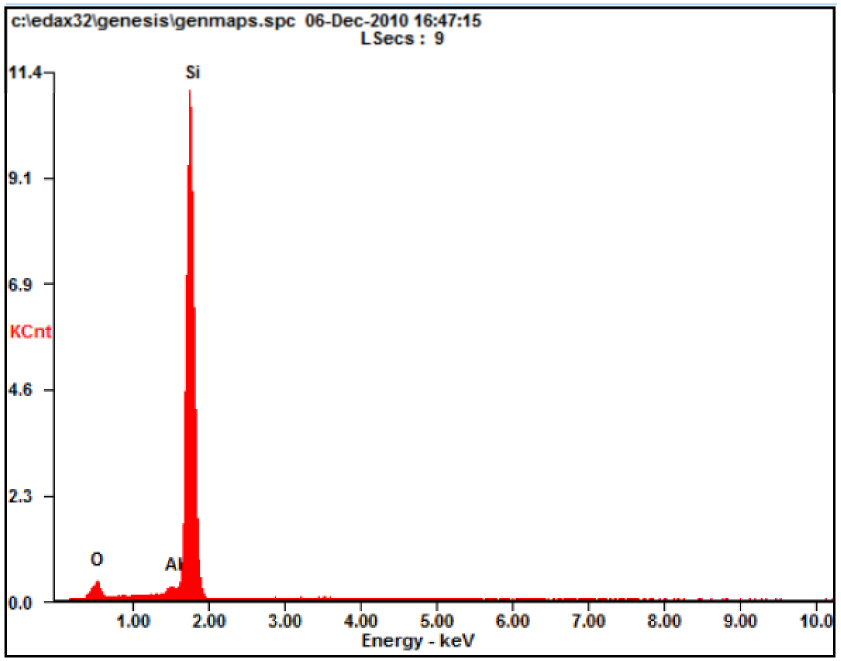
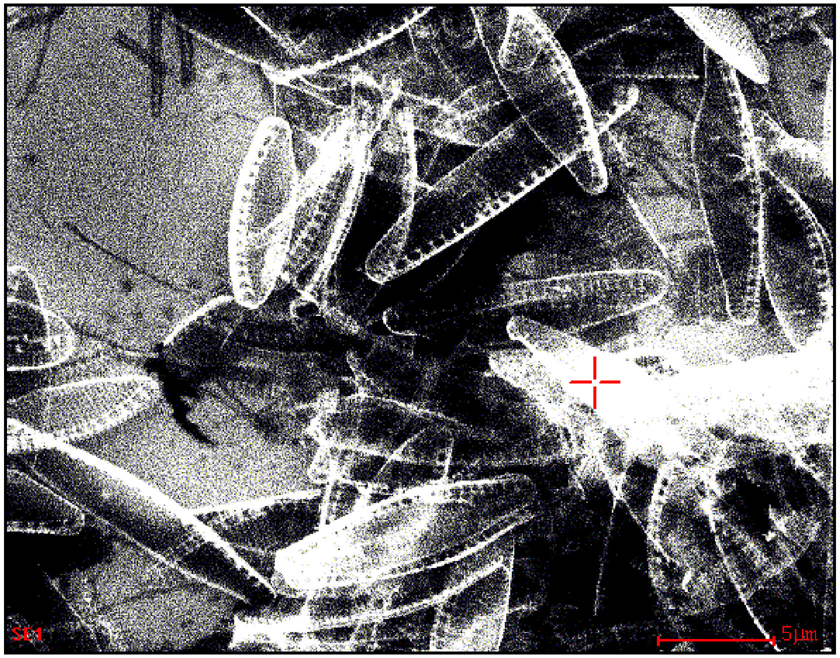
Alumina ALD on Diatoms





- Atomic Layer Deposition of alumina to *modify the surface properties*
- alternative to porous alumina membranes with *reduced pore length*
- *high mechanical stability* of diatoms due to their hierarchical pore structure

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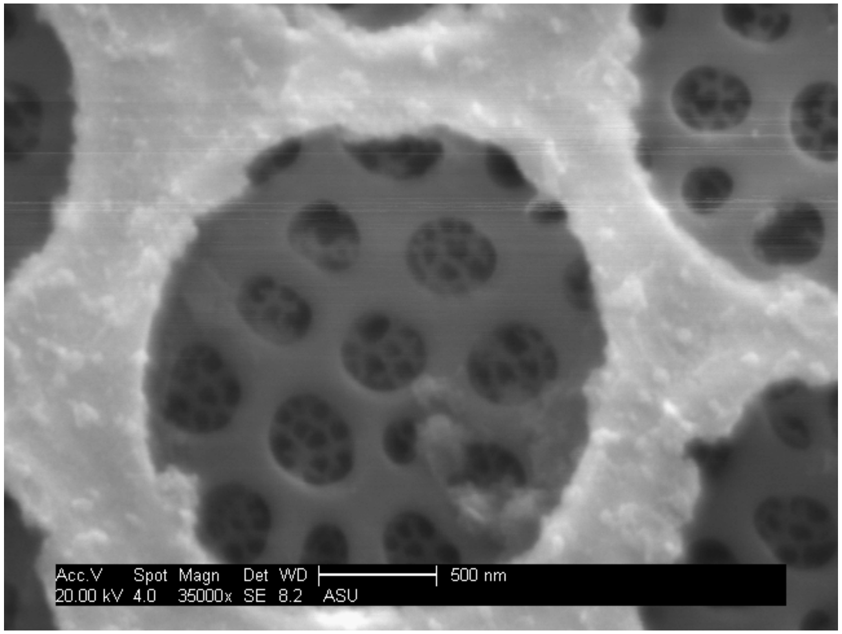
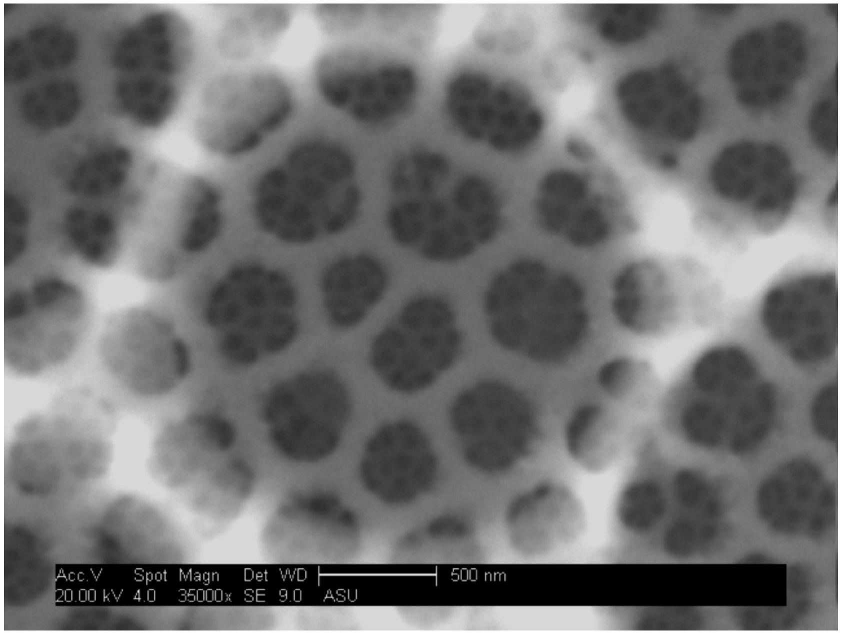
NaOH Etching

Original Diatom



Diatom after 30 min etch in 1N NaOH



- NaOH etching allows *tailoring of the nanopore size*
- long etching results in fragile shells
- shells were *suspended on 5 μm diameter Si* apertures

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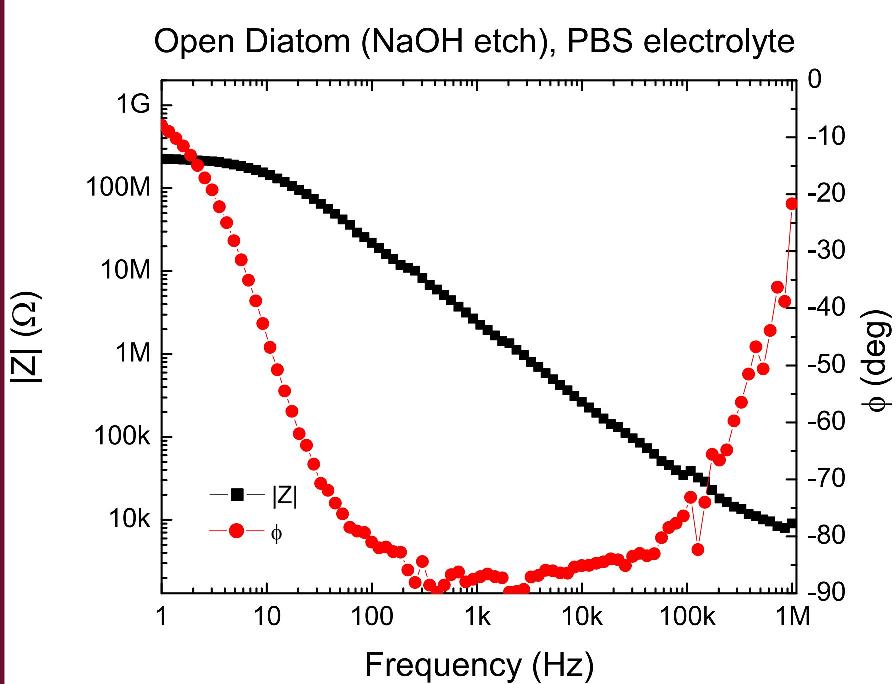
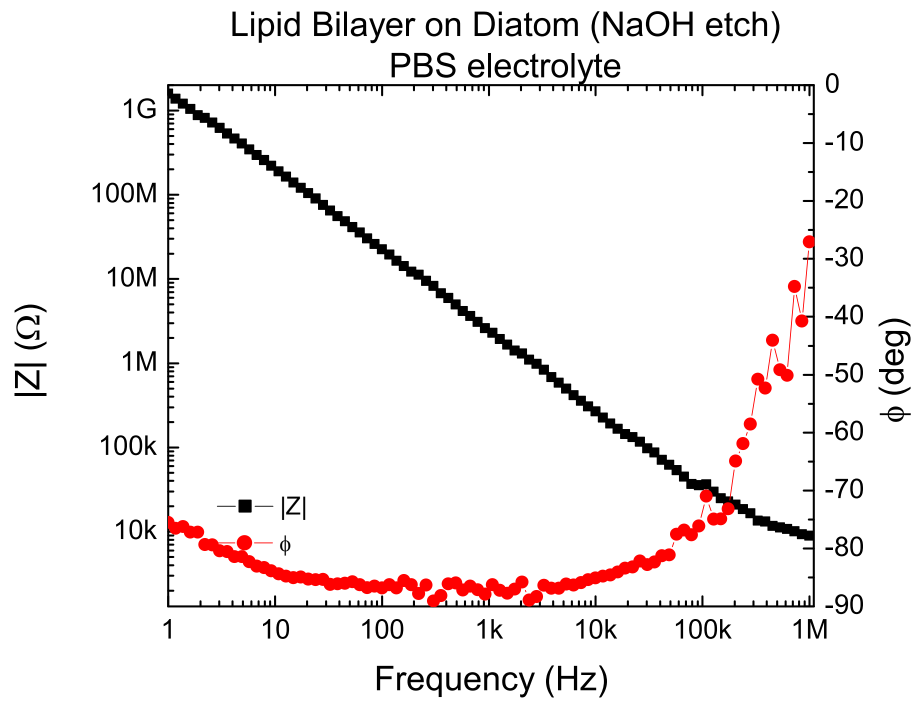
NaOH Etching





- larger pore size allows *Gigaseal formation*
- *higher open pore resistance* due to *5 µm diameter Si* aperture
- clear *change* from *resistive* to *capacitive* behavior *at low frequencies*

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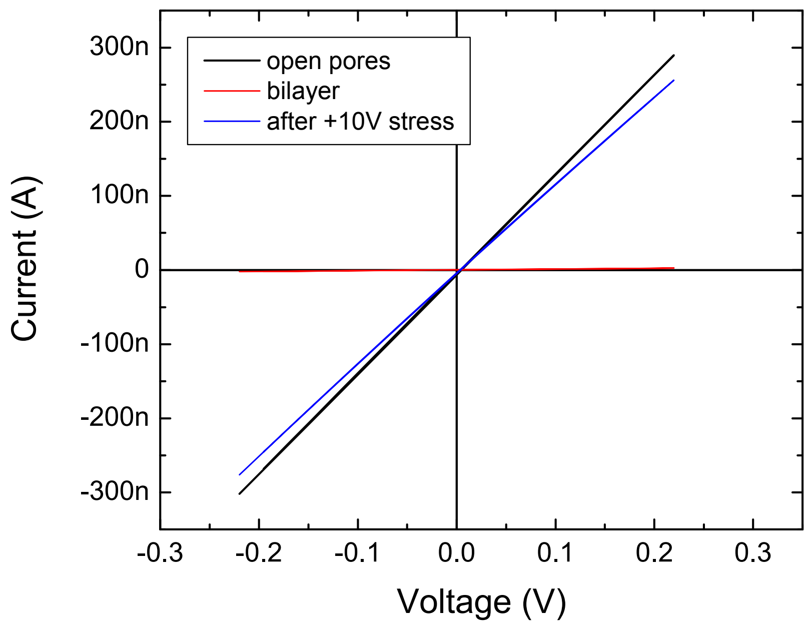
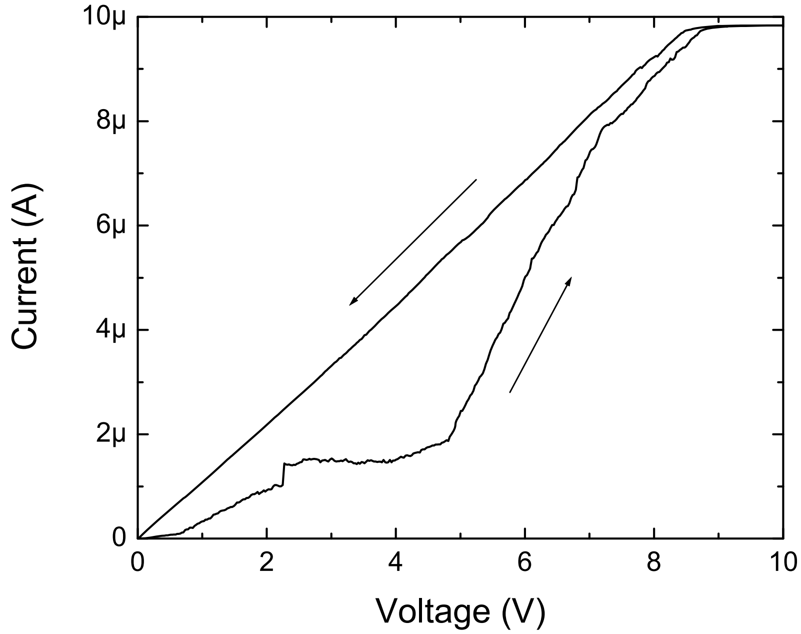
Lipid Bilayer Formation on Diatom





- lipid bilayers show a *step-wise breakdown*
- *no catastrophic failure* as observed for micron-sized apertures
- *higher breakdown voltage* compared to bilayers on micropores
- bilayers can be destroyed by a large enough voltage (zap)

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Future Perspectives

- *directed self-assembly* of diatoms on planar silicon surfaces
- *permanent mounting* of frustrules
- SU-8 based *microfluidics*

- **universal nanopore sensor platform**
- **lab-on-a-chip**

- *surface modification (high-k)* on the diatom surface to modulate its (electro-)chemical potential

- **Local chemical stimulation of a cell sheet through a diatom shell**

- *ion channel incorporation* into nano BLMs

- **Long-time stable bilayer membranes for ion-channel sensor arrays**

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Acknowledgements

- Kai-Chun Lin (Diatom sample preparation)
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- Dr. Trevor Thornton
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Thank You !