Single Nanopores in Silicon Nitride Membranes with Applications to Viral Sensing

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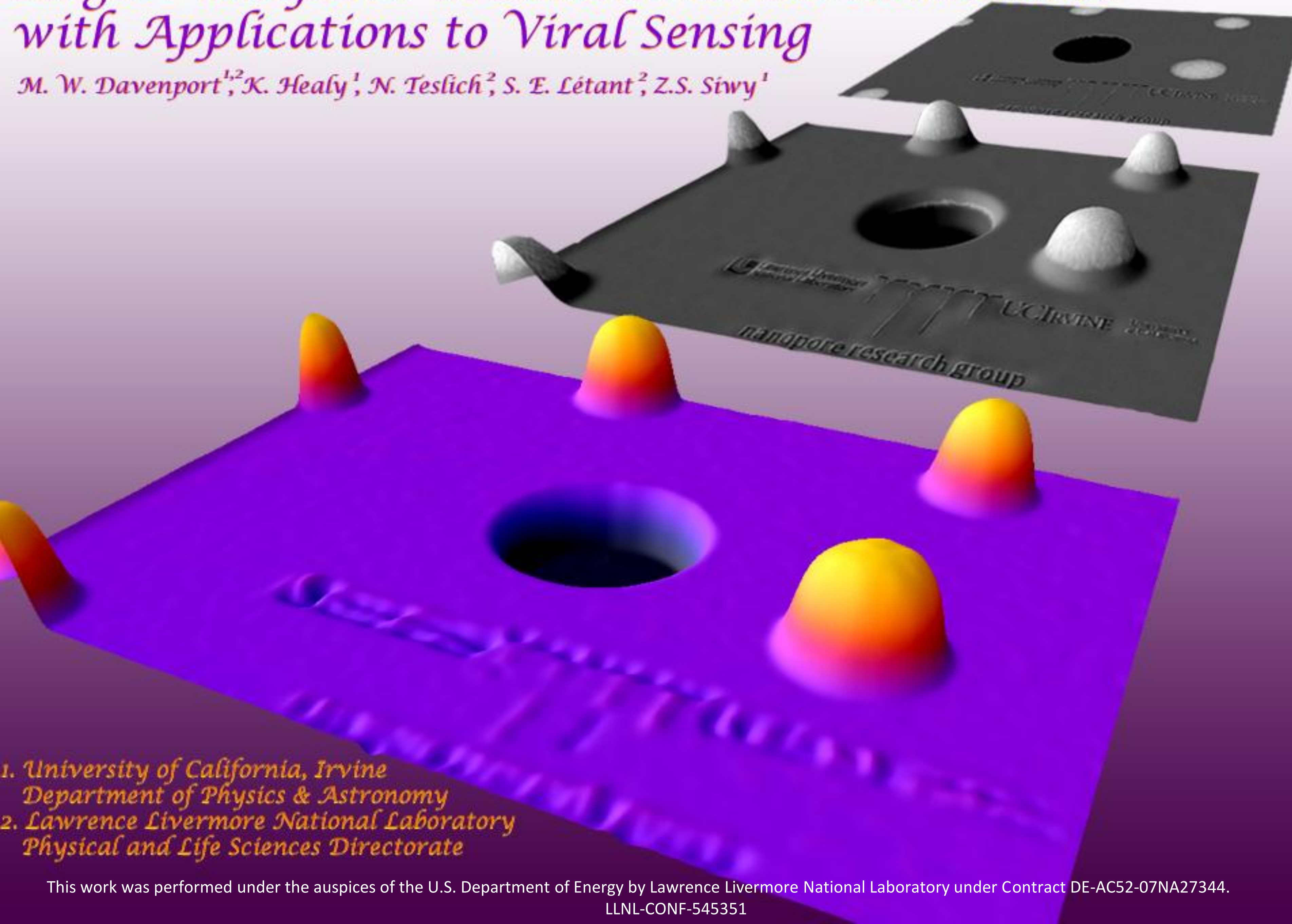

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2. Lawrence Livermore National Laboratory
Physical and Life Sciences Directorate

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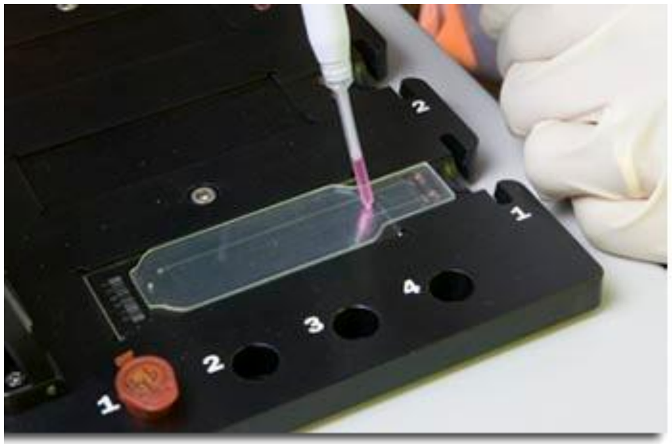
LLNL-CONF-545351

Motivation

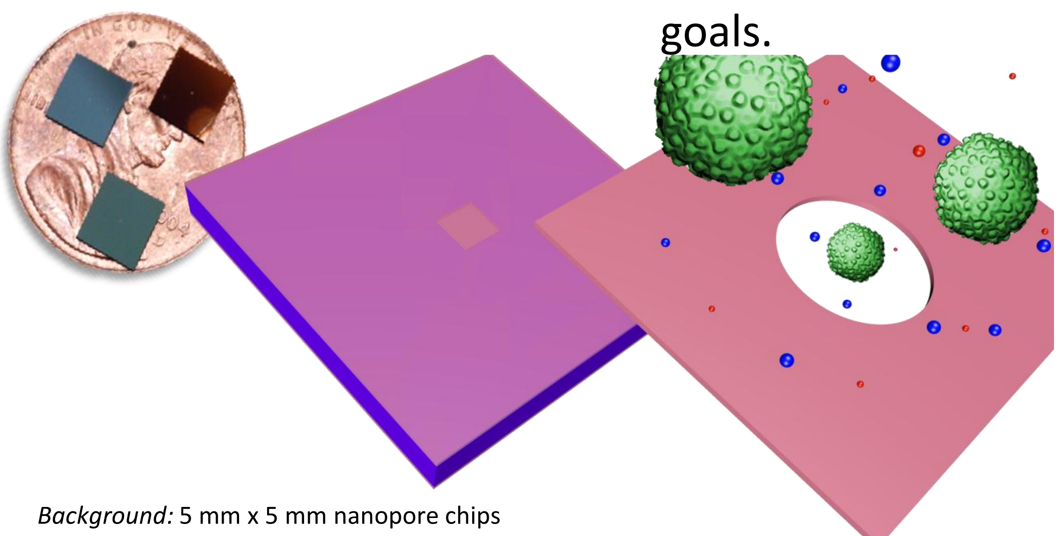


The Lawrence Livermore Microbial Detection Array contains 388,000 probes for viral and bacterial agents. *Photos by Jacqueline McBride/LLNL*

While current viral sensing methods are extremely sensitive, there is still a need for platforms capable of:

- Detecting engineered viruses
  - Cannot rely on chemical labels/markers
- Being integrated into device architectures for point-of-care assessments
  - Rapid detection
  - Portable
  - Inexpensive

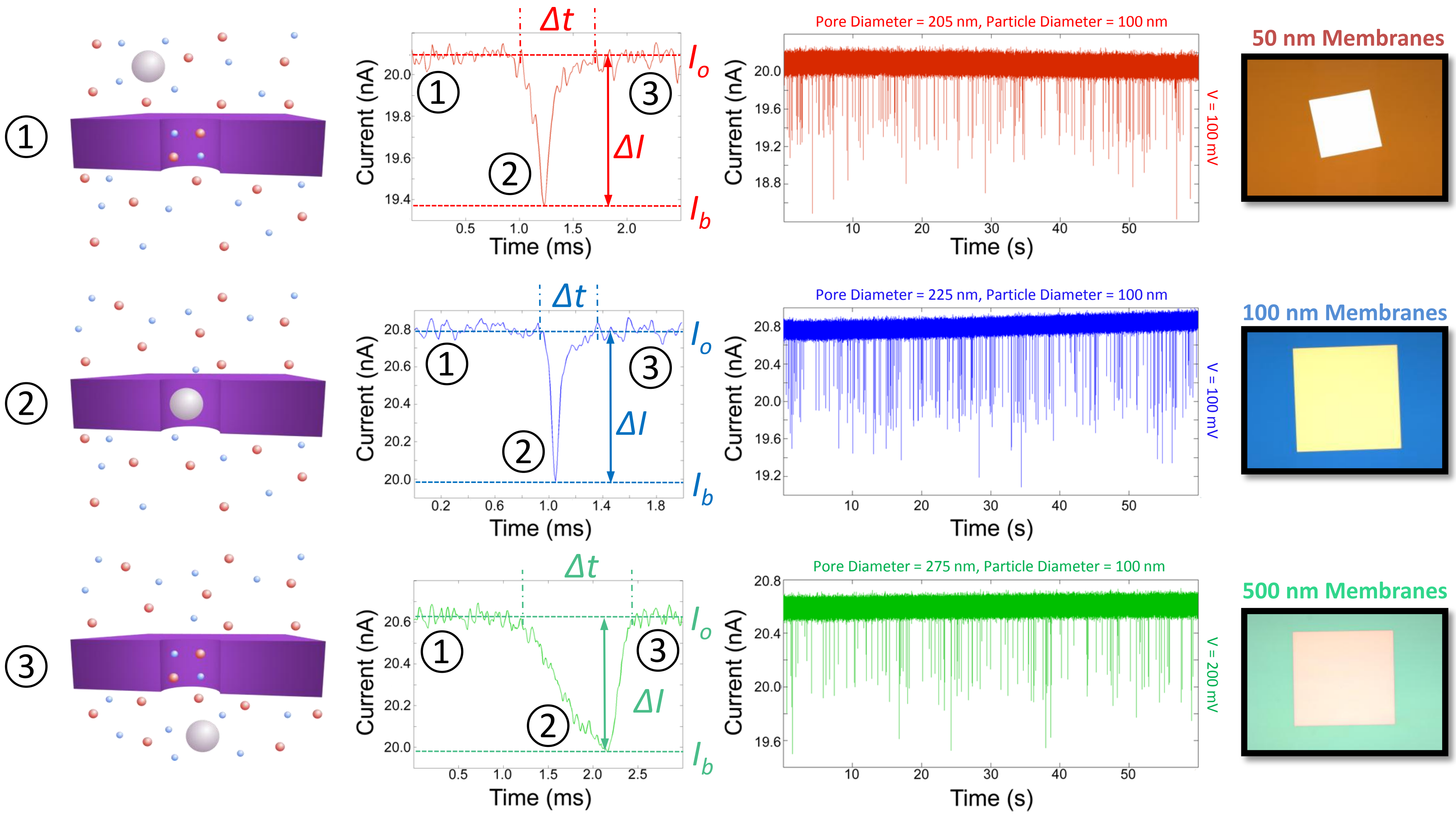
Nanopores could provide a single pathway to achieve these goals.



Background: 5 mm x 5 mm nanopore chips
Foreground: Enlargement of pore region depicting translocating viruses (green) and ions (blue and red) .

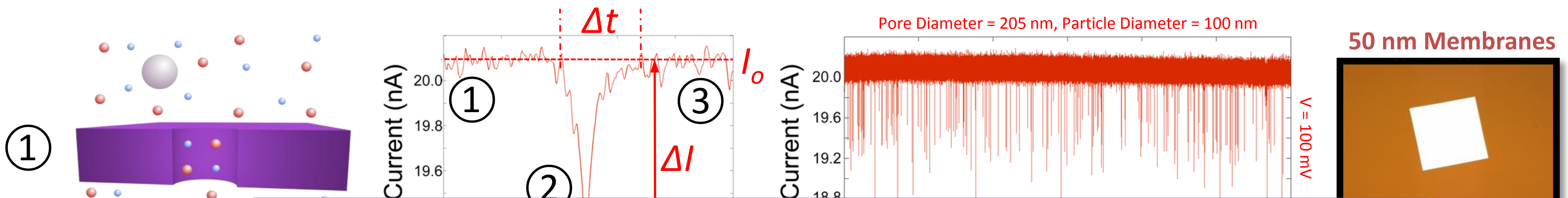
Point-of-care systems for analyzing hemoglobin, glucose & white blood cells *(HemoCue®, www.hemocue.com)*

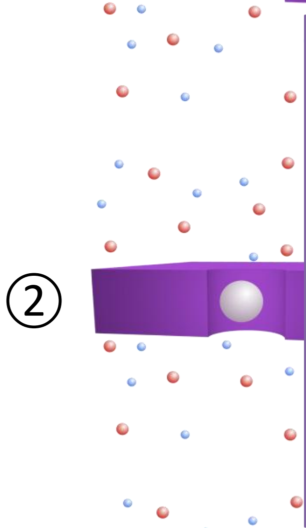
Resistive Pulse Sensing



We aim to develop our understanding of how best to design nanopore sensors for virus-sized analytes.

Resistive Pulse Sensing



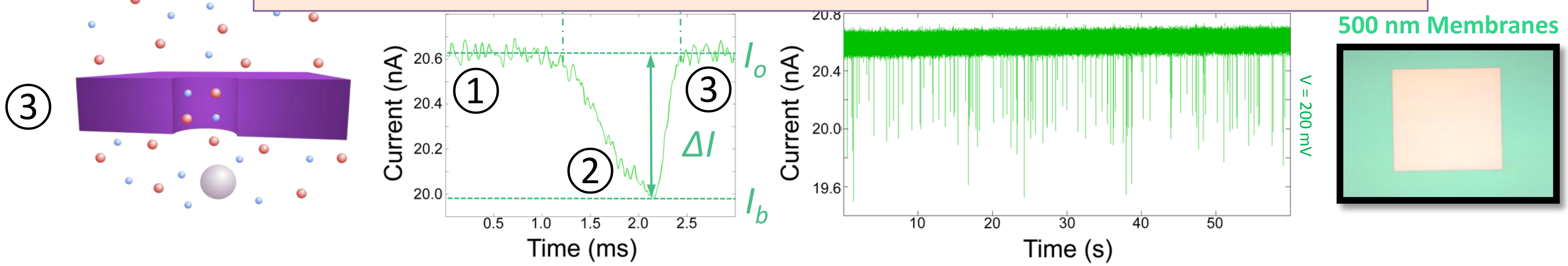


To that end, we have studied:

1. Relative Event Depth: $\alpha \equiv \Delta I / I_o$
2. Event Duration: Δt

in terms of 4 variables:

1. **Pore Length (L)**
2. Pore Diameter (D)
3. Particle Diameter (d)
4. Applied Voltage (V)



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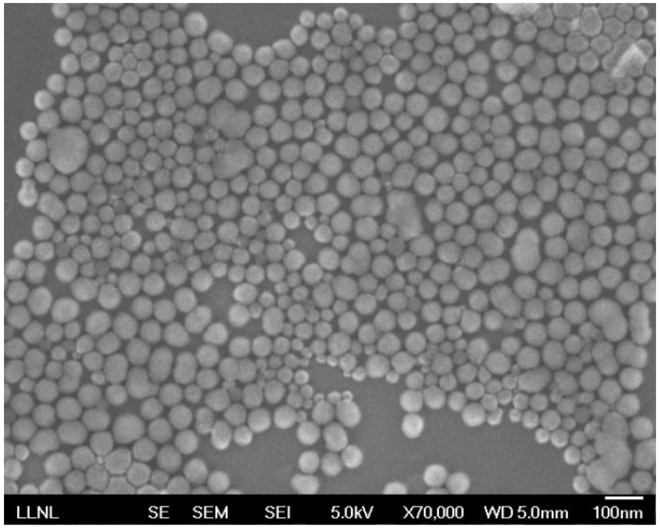
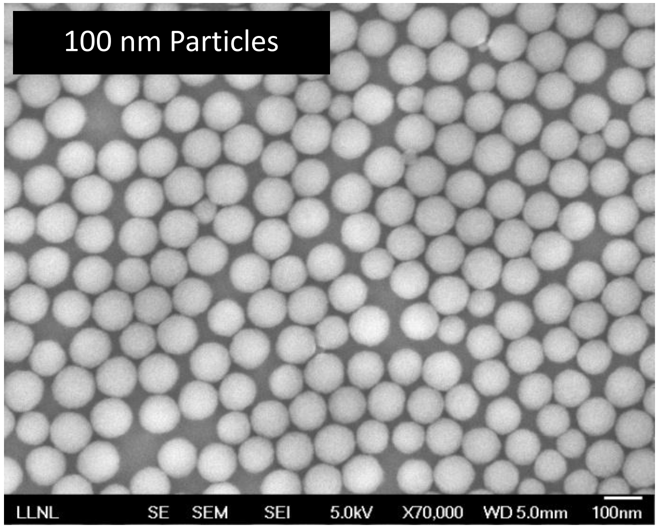
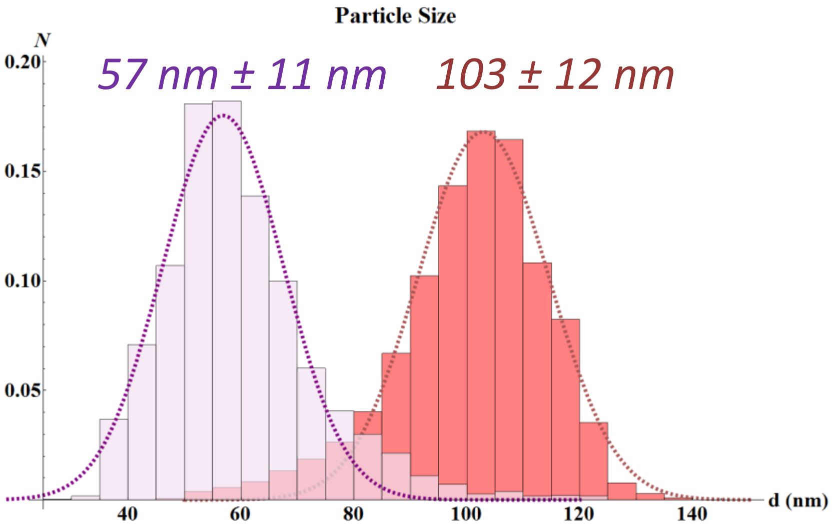
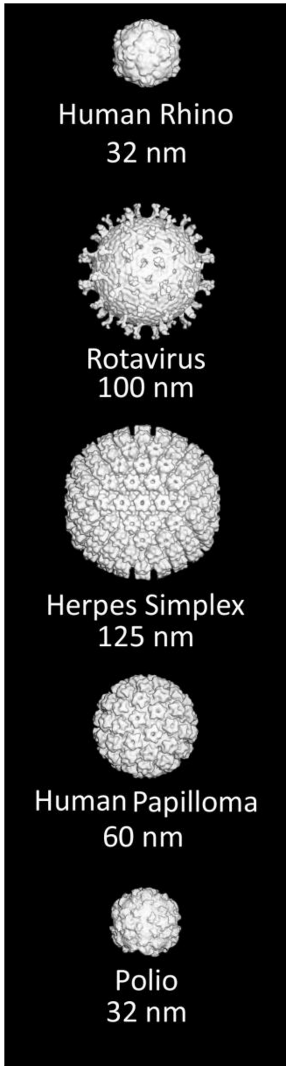
Viral Simulants



Silica nanoparticles were used as models for viruses in a size range representative of virions responsible for human infections: ~ 30 – 120 nm. Given the distribution of silica particle sized, we were able to simulate this range with two nominal diameters: 50 and 100 nm as reported by Polysciences, Inc.

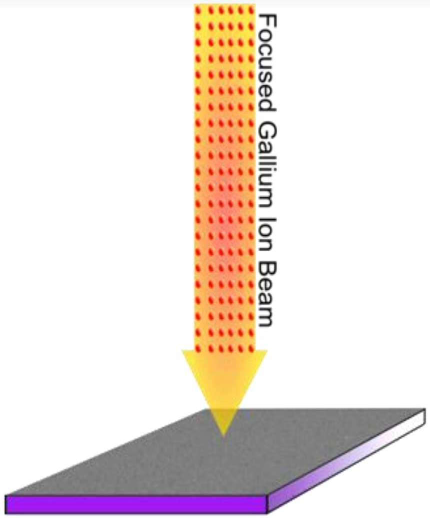


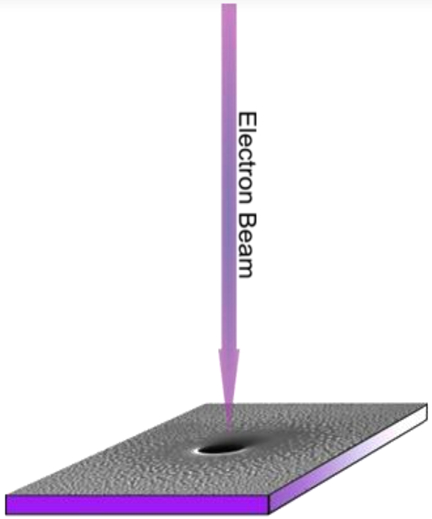


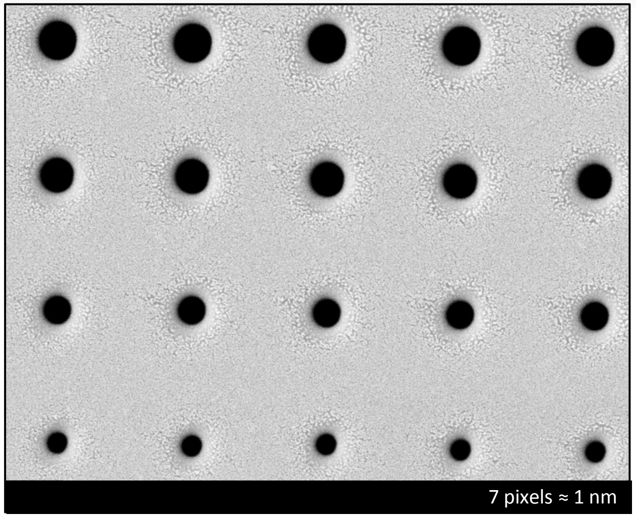


Viral images made by T.S. Baker, N.H. Olson & S.D. Fuller using cryo-EM and 3D image reconstruction. *Microbio and Mol. Bio. Rev.* **63** (1999)

Pore Fabrication







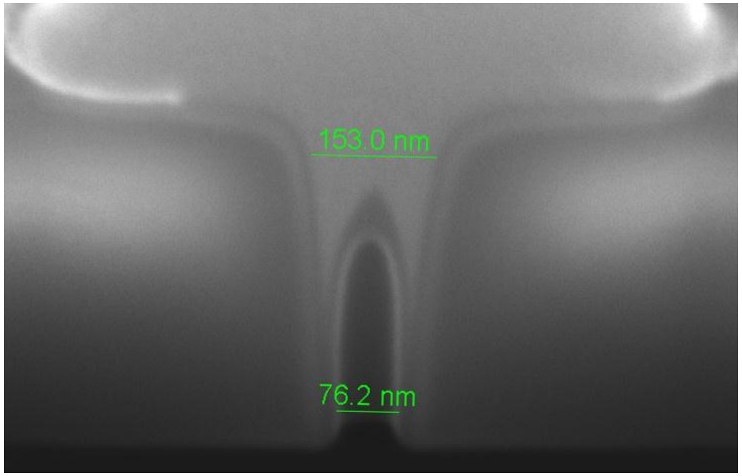
① D = 324 nm ± 4 pixels

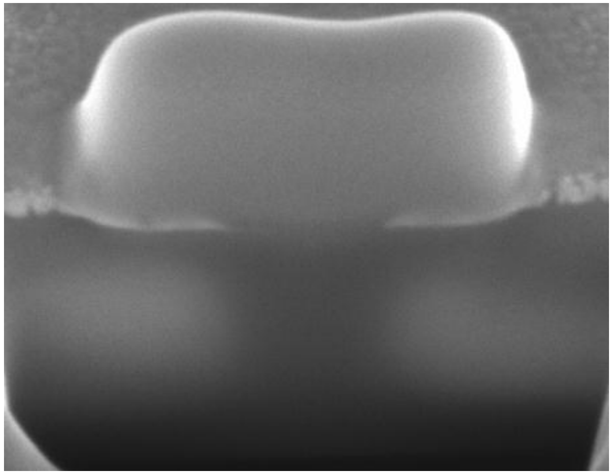
② D = 293 nm ± 5 pixels

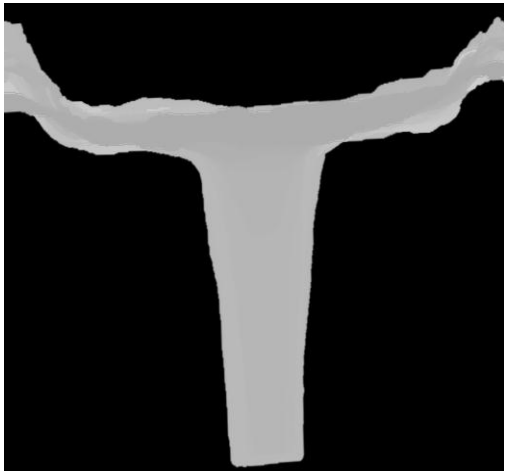
③ D = 242 nm ± 6 pixels

④ D = 184 nm ± 5 pixels

By milling multiple pores, we are able to establish the milling parameters required to drill *single pores* of a given size. We can also use the FIB to create slice-and-view reconstructions of a pore's interior:



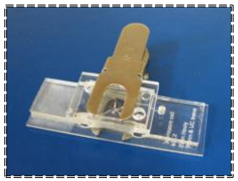




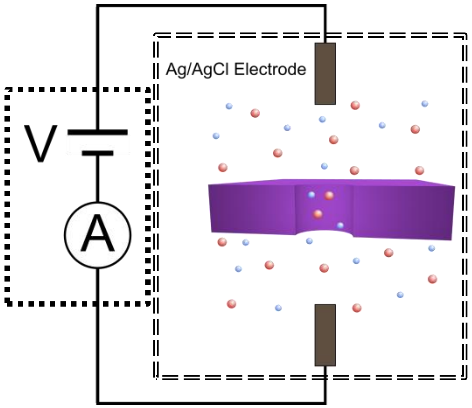
Pore Resistance

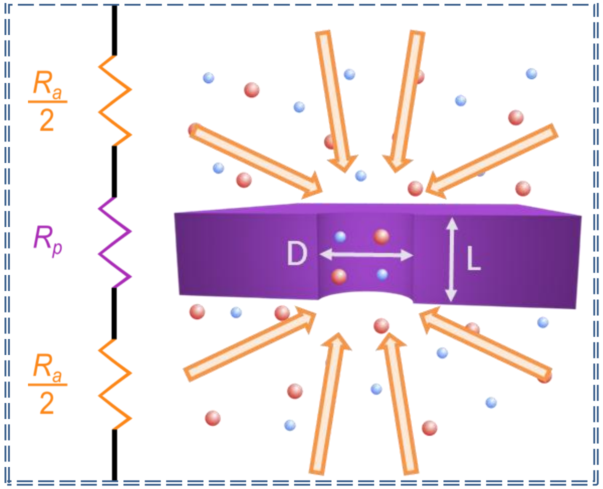
HEKA EPC-10

PDMS Conductivity Cell



K. Healy, UPenn/UCI





$$R = R_p + R_a$$

Traversing pore (R_p) — Accessing pore (R_a)

$$R_p = \frac{4L}{\pi \kappa D^2} \qquad R_a = 2 \cdot \frac{1}{2\kappa D}$$

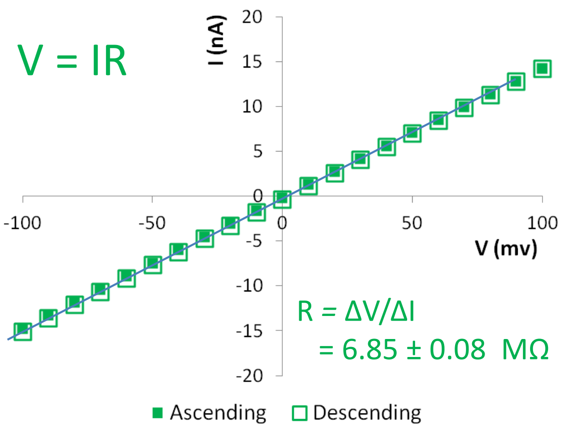
κ = electrolyte conductivity

$$R = \frac{4L + \pi D}{\pi K D^2}$$

$$L' = L + \frac{\pi}{4} D$$

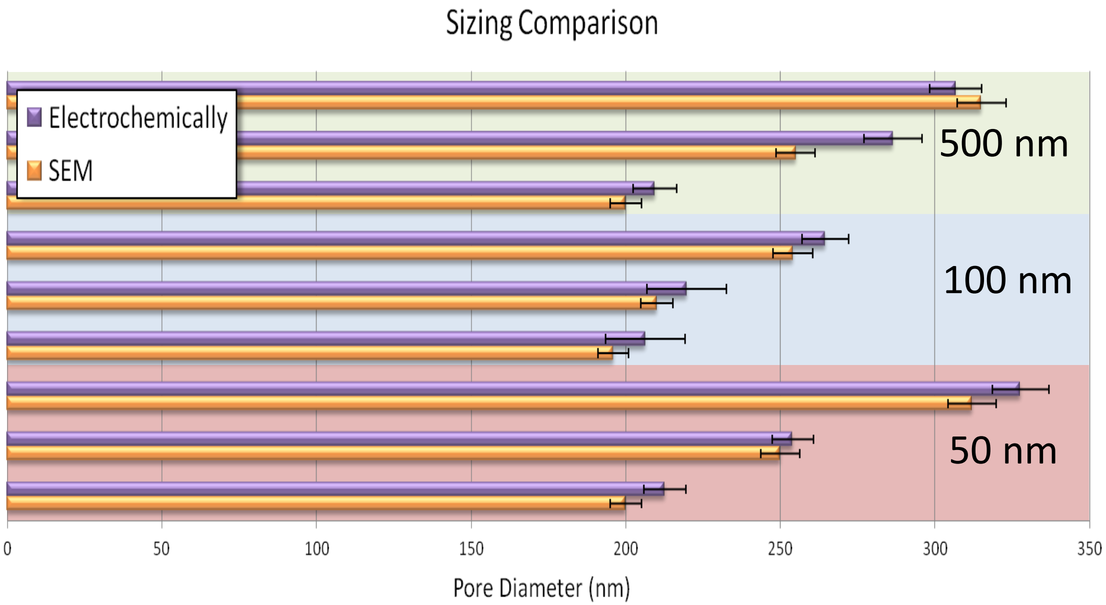
Sample Current – Voltage Curve

V = IR



L = 500 nm ⟹ D = 307 ± 8 nm

D_{SEM} = 315 nm

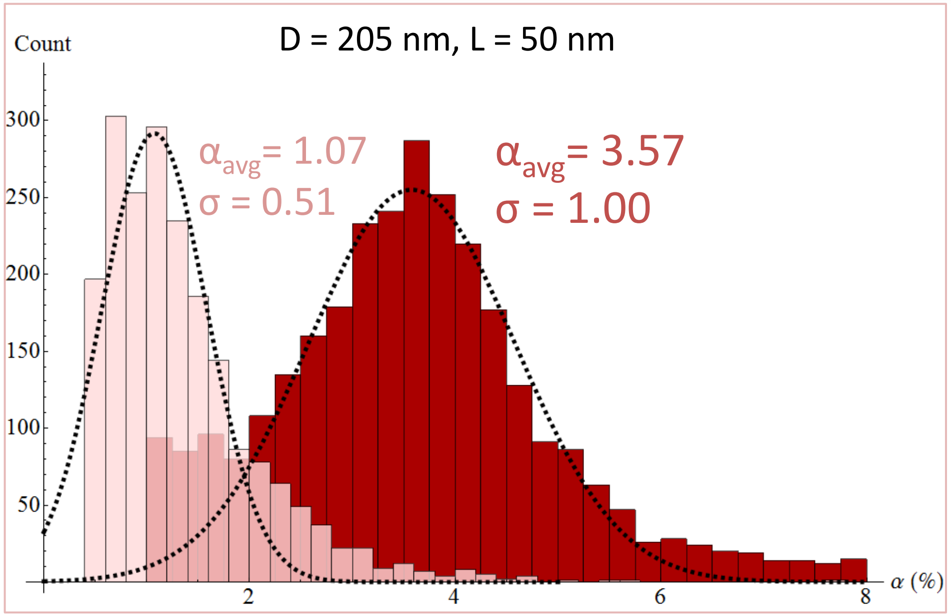


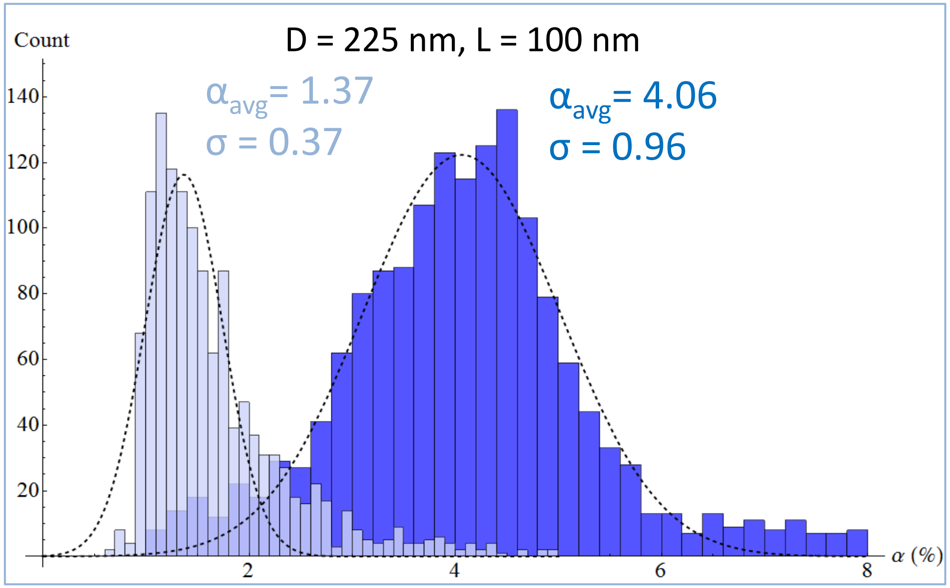
α: The Relative Event Depth

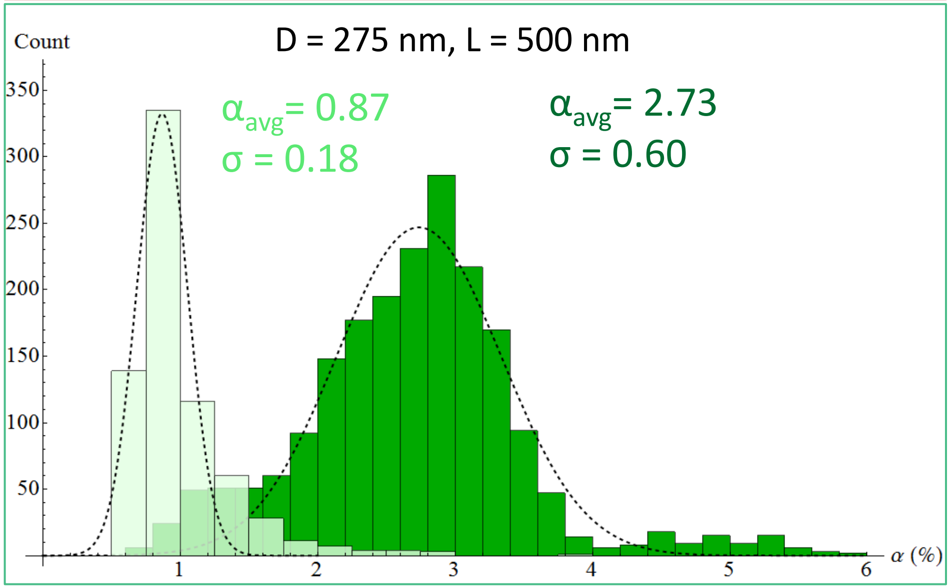
Histograms of relative event depths for each pore can be fit with a Gaussian function:

$$Ae^{-(\alpha-\alpha_{avg})^2 / 2\sigma^2}$$

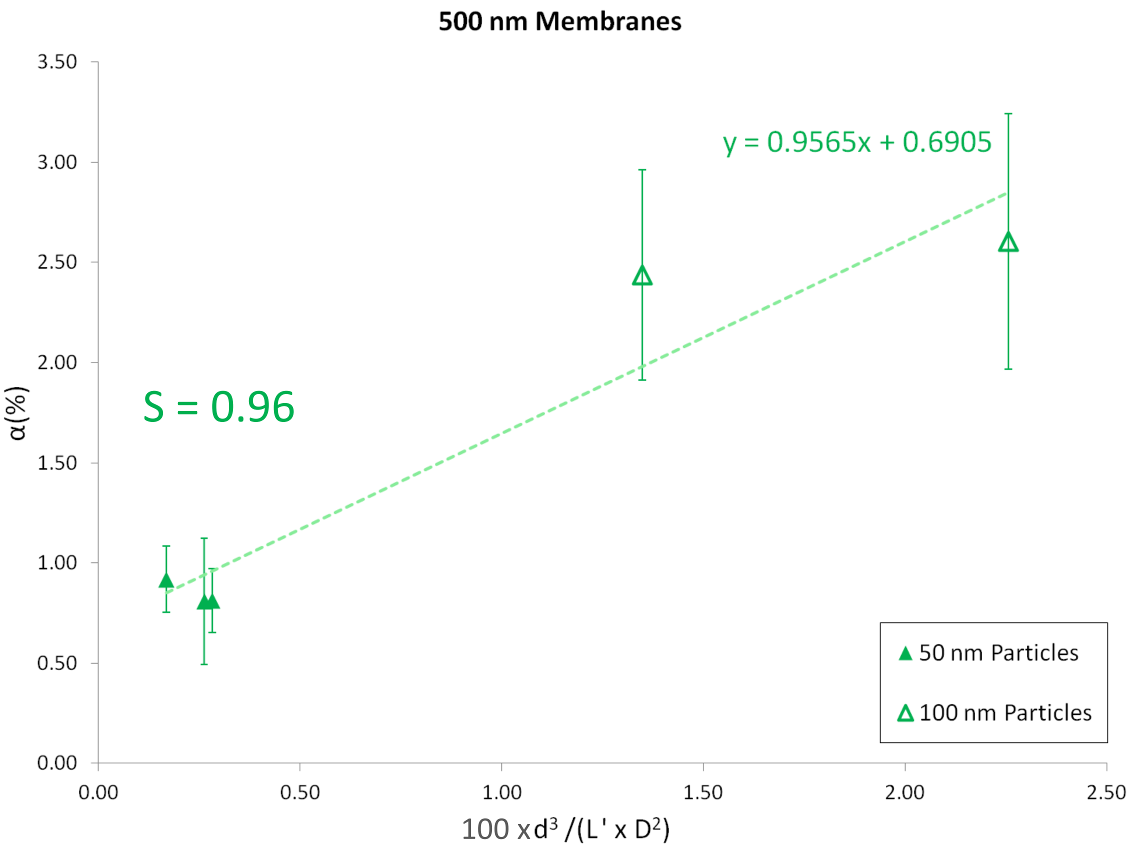
with its peak centered at α_{avg} with a standard deviation of σ. Histograms are shown for 50 nm (light bars) and 100 nm particles (dark bars) measured *separately*.

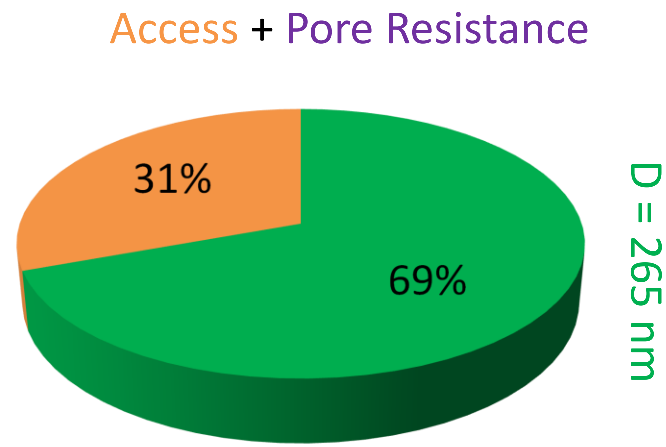






The S Factor



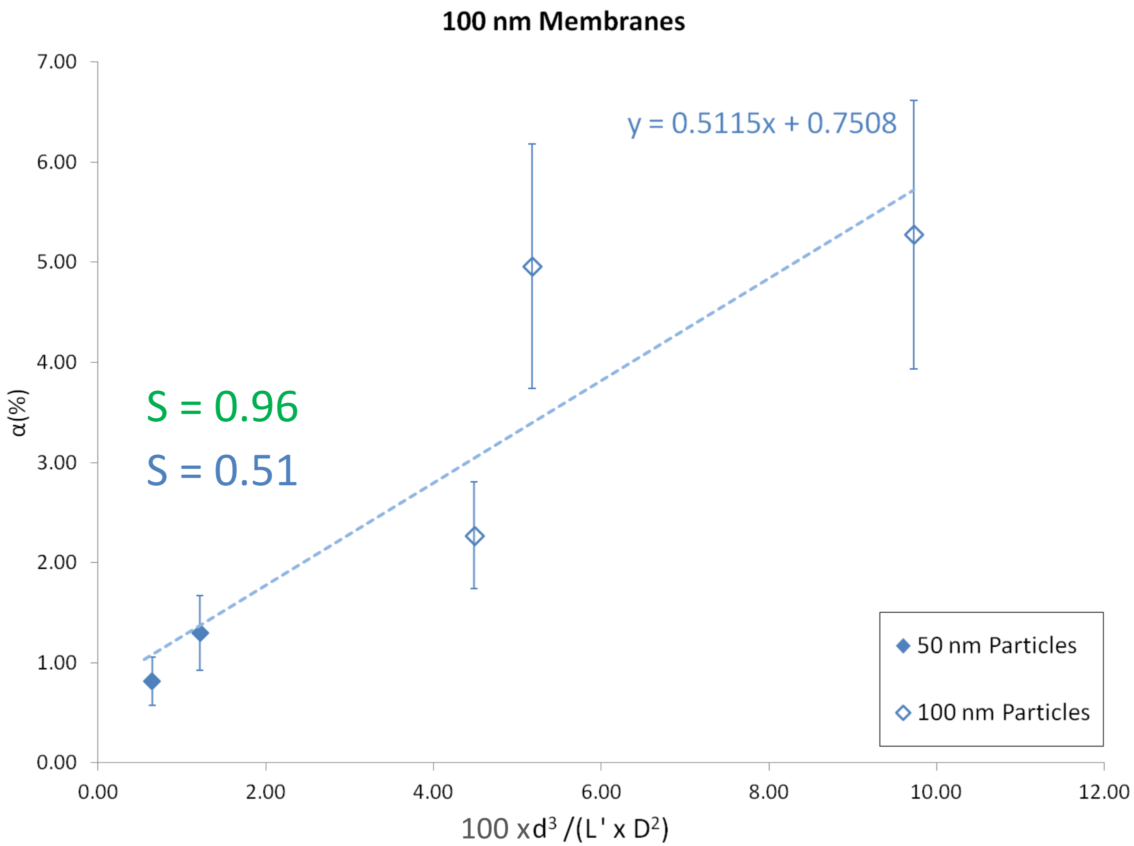


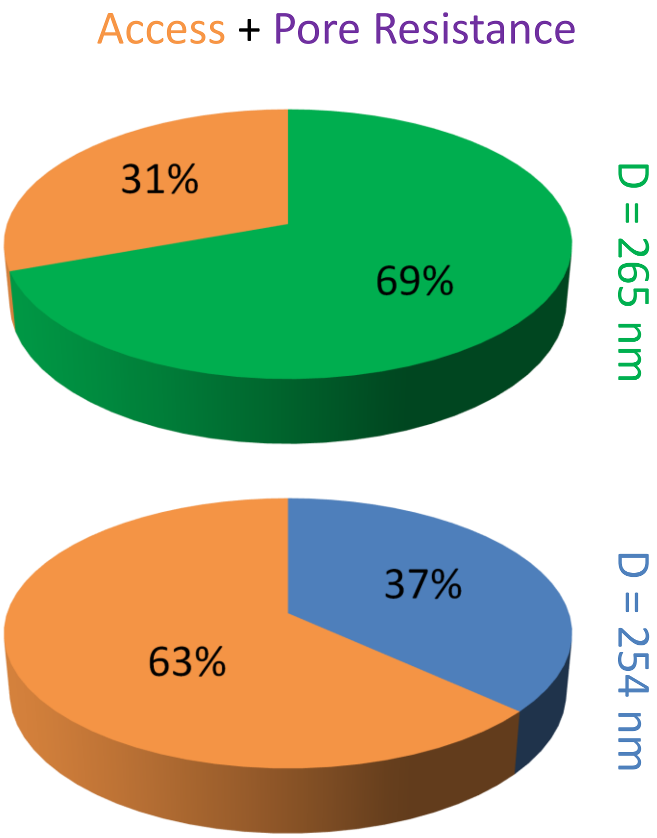
$$L' = L + \frac{\pi}{4} D$$

When L > d, it was shown that α is proportional to the volume ratio of the particle to pore, with a coefficient of proportionality, S , that was found to be ~ 1.[1]

[1] Ito, Sun and Crooks. *Anal Chem*, **2003**, *75,* 2399-2406

The S Factor

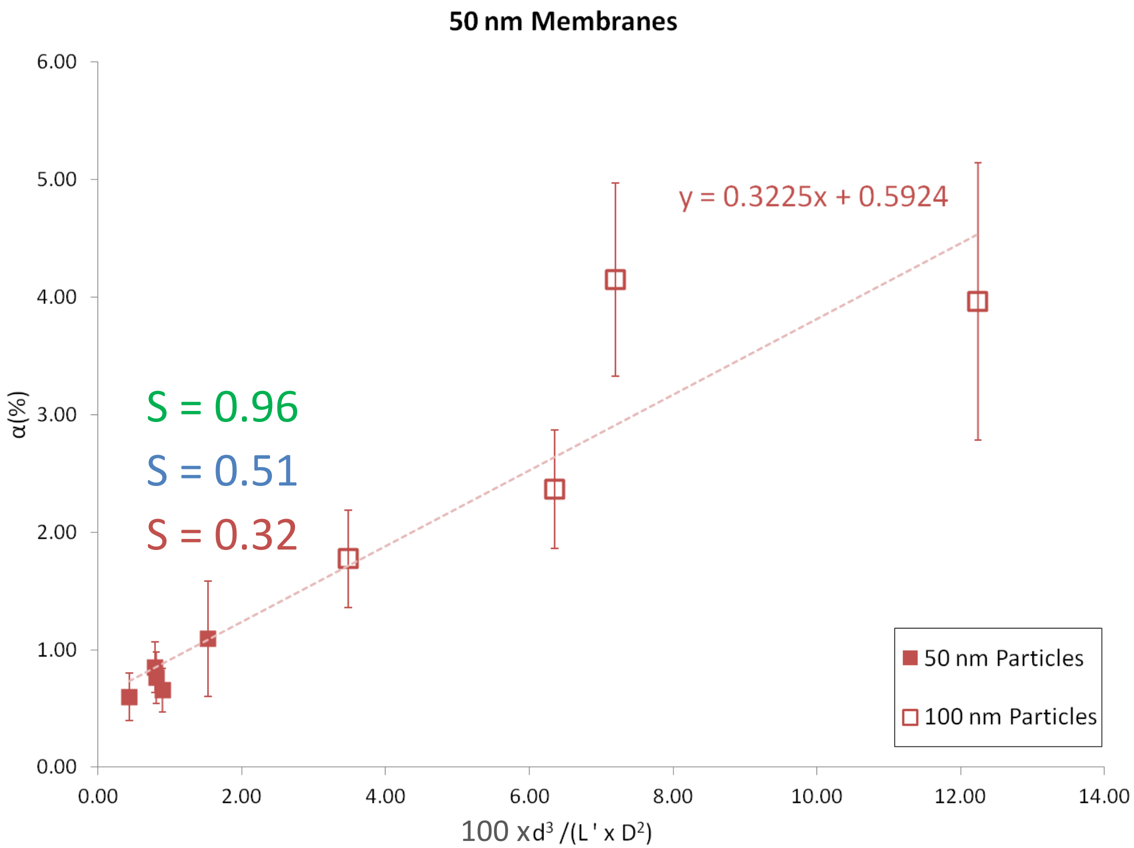


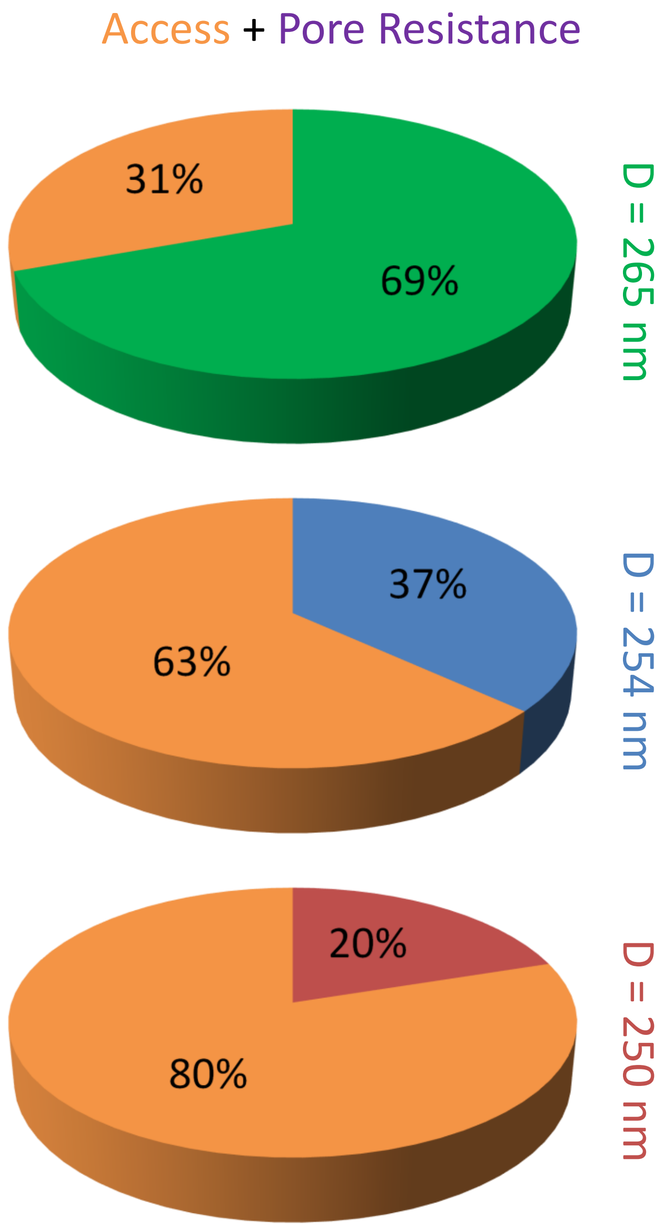


Earlier works had anticipated S becoming greater than unity as the particle occupied more of the pore's volume, but again for L > d. As d -> L, we see this is not the case.[2]

[2] Debois and Bean. *Rev. Sci. Instr.* **1970**, *41*, 909-916

The S Factor



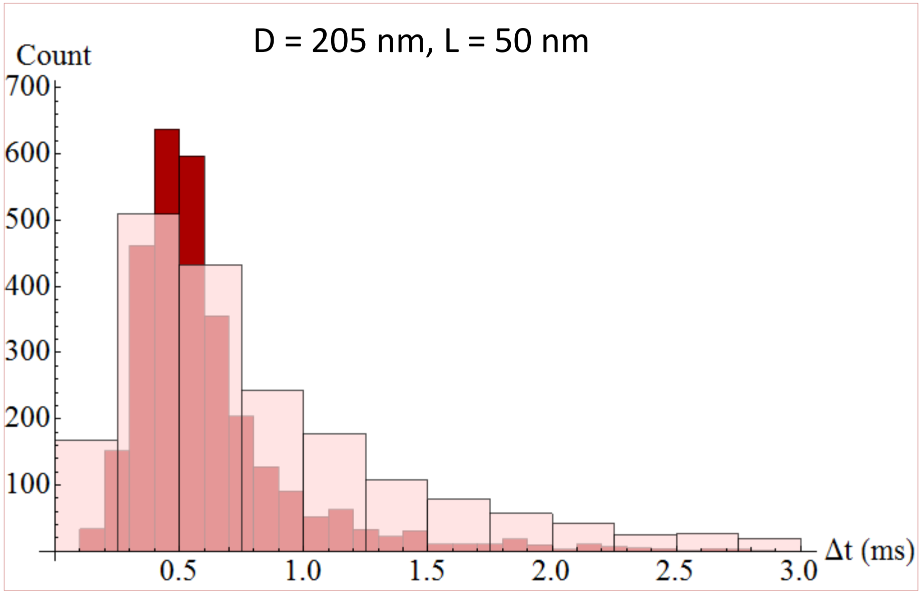


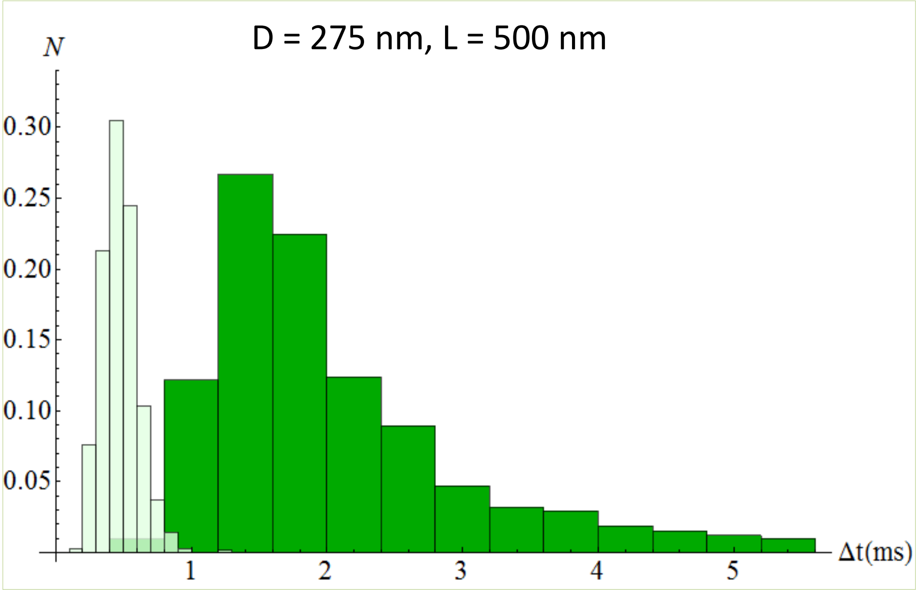
Further reducing L diminishes S. It may be that the translocating particle does not affect access resistance, which has been proposed previously.[3]

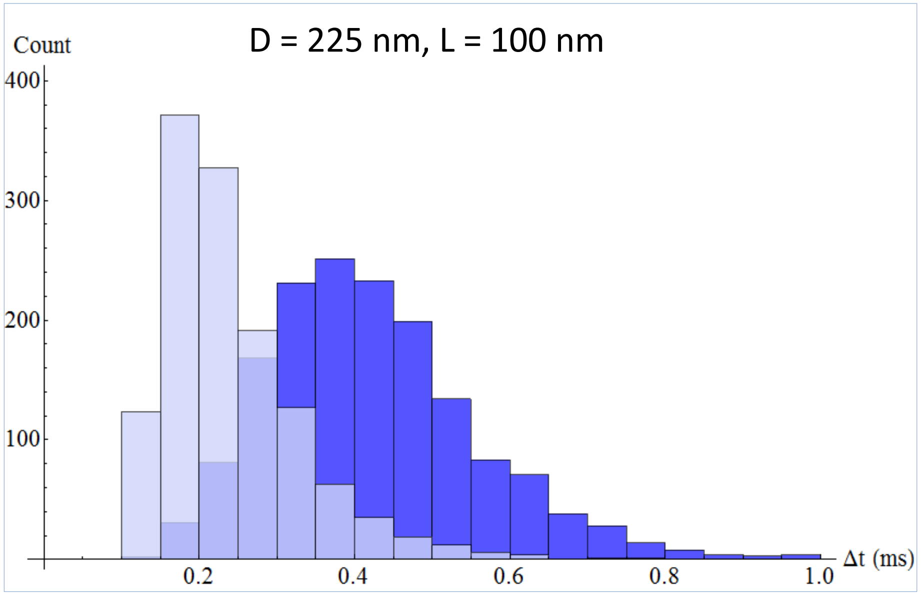
[3] Tsutsui et al. *ACS Nano,* **2012**, Articles ASAP (March 18th)

Event Duration

Histograms for event duration reveal that thicker pores with larger diameters are more effective at distinguishing between particles. Light bars represent 50 nm particle events and darker bars indicate events for 100 nm particles. Again, particles were measured separately.







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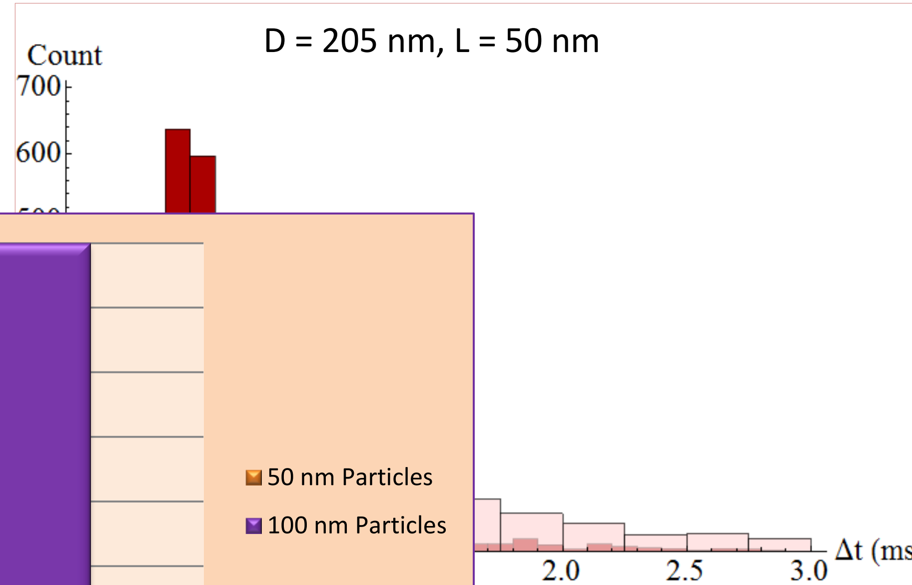
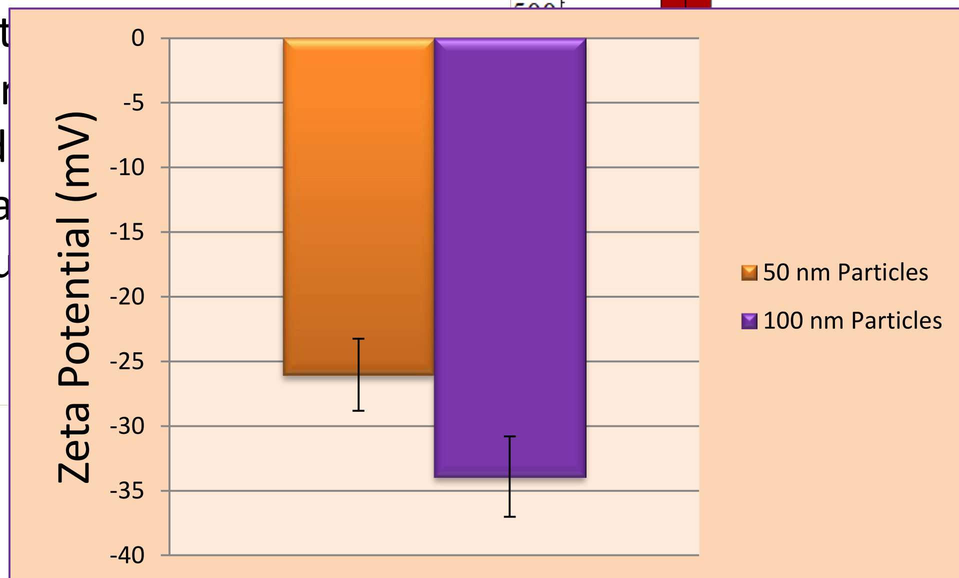
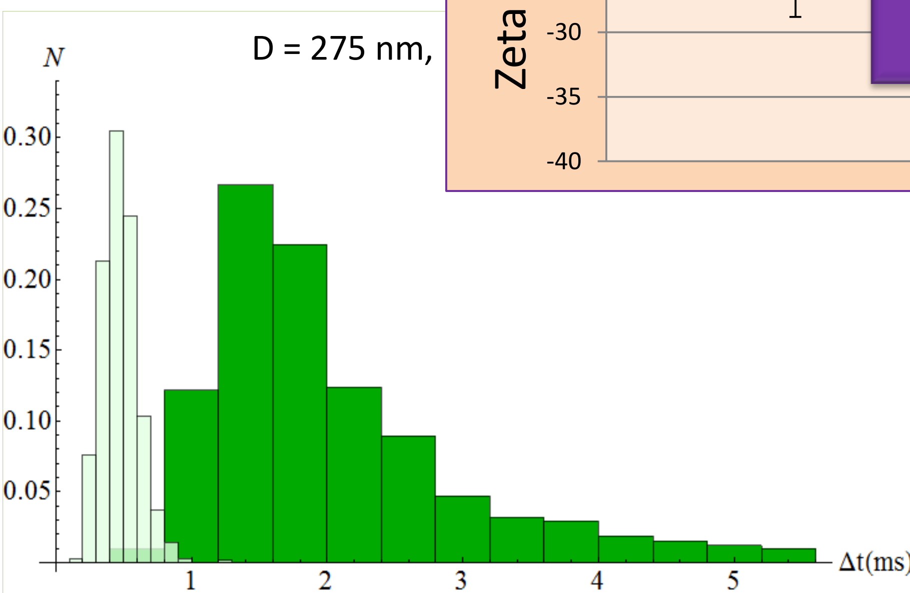
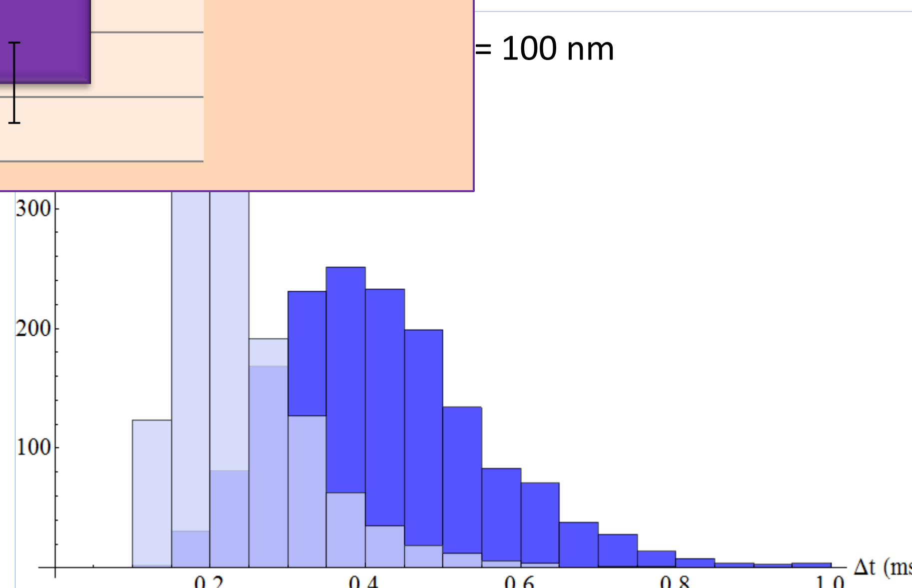






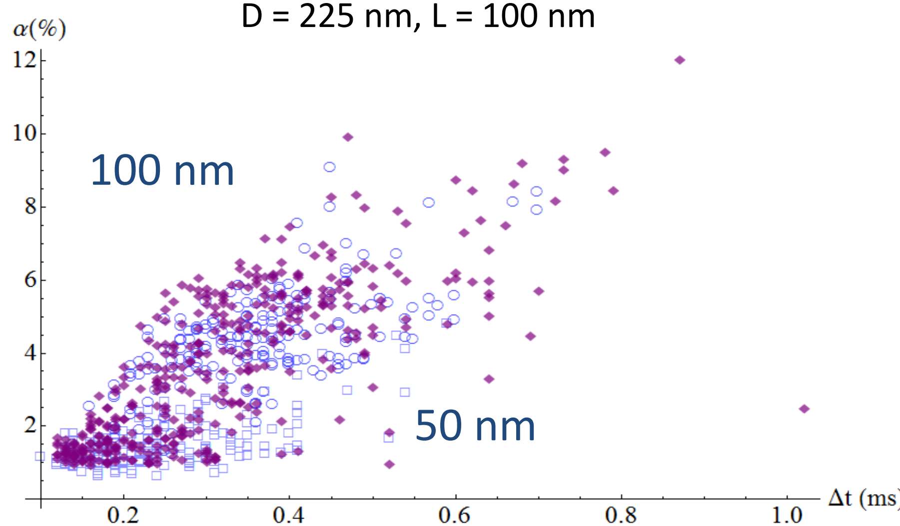


Particle Distinction

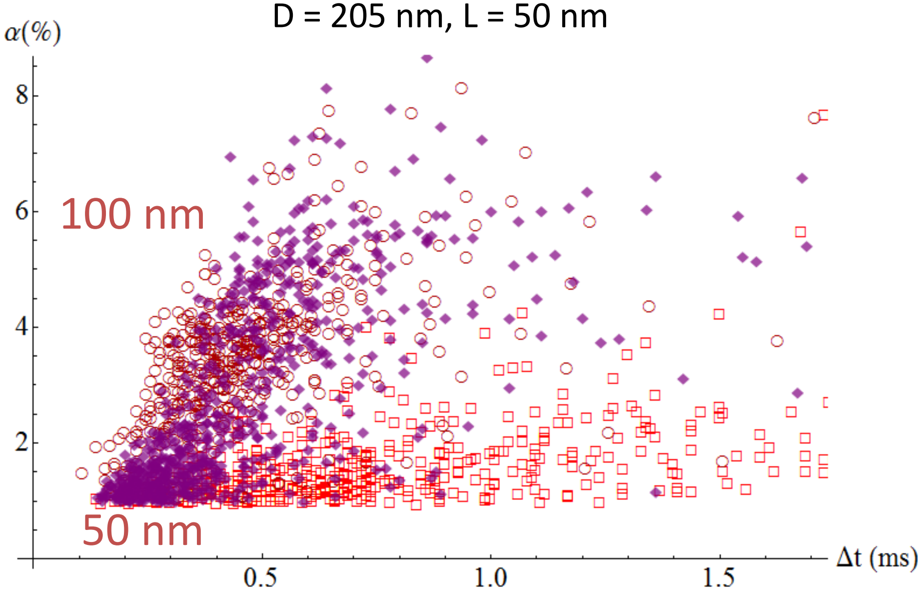
All the plots shown thus far have been for particles measured separately. Can these pores distinguish between different sized particles in the same suspension?

- □ 50 nm Particle Events (Separate)
- ○ 100 nm Particle Events (Separate)
- ◆ Bead Mixture Events

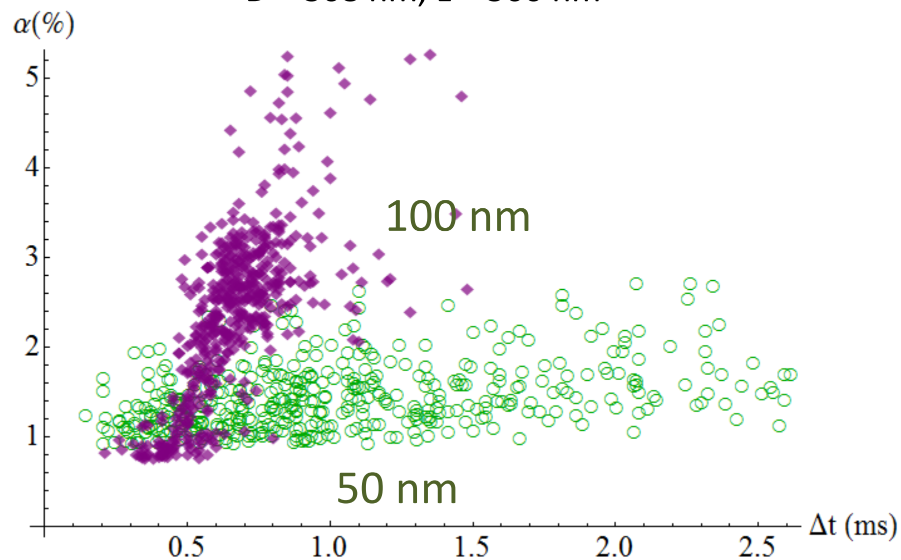
D = 225 nm, L = 100 nm



D = 205 nm, L = 50 nm

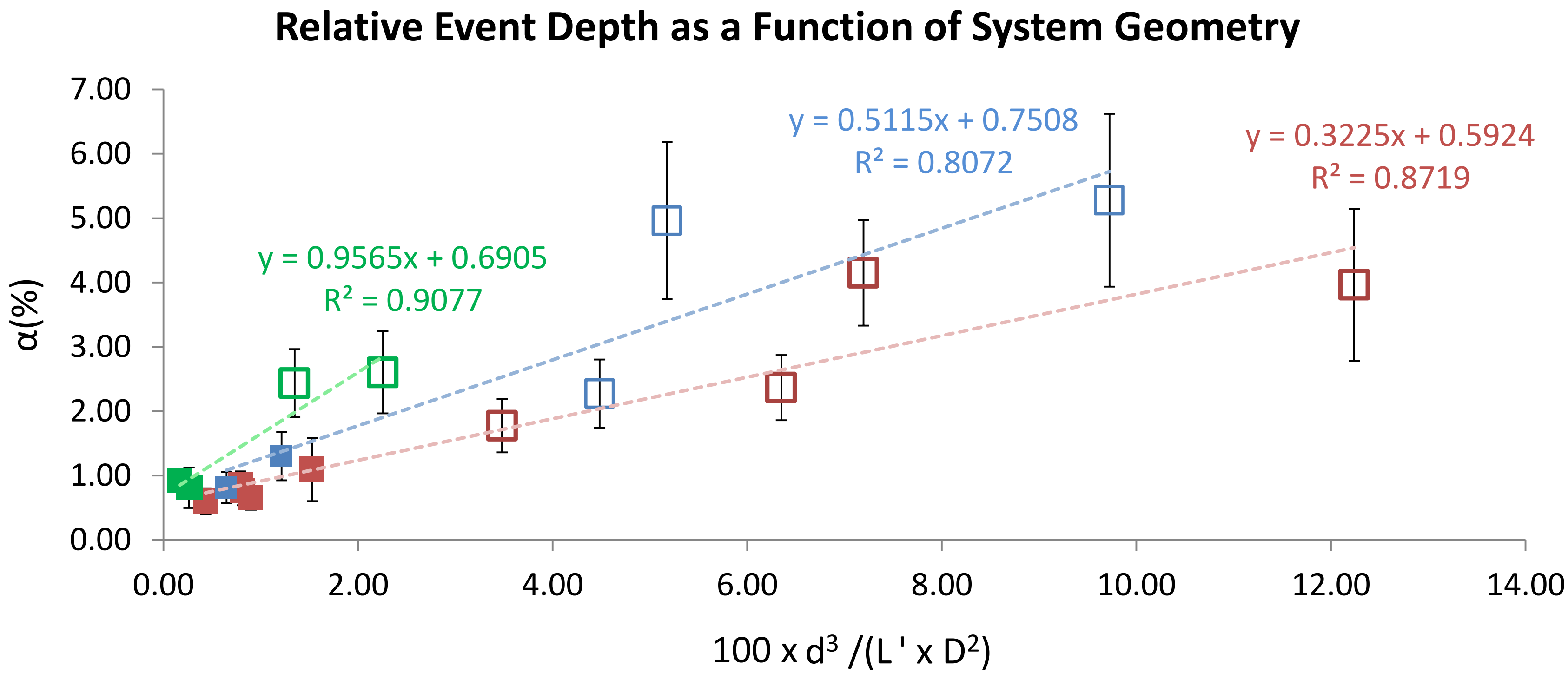


D = 308 nm, L = 500 nm



Conclusions

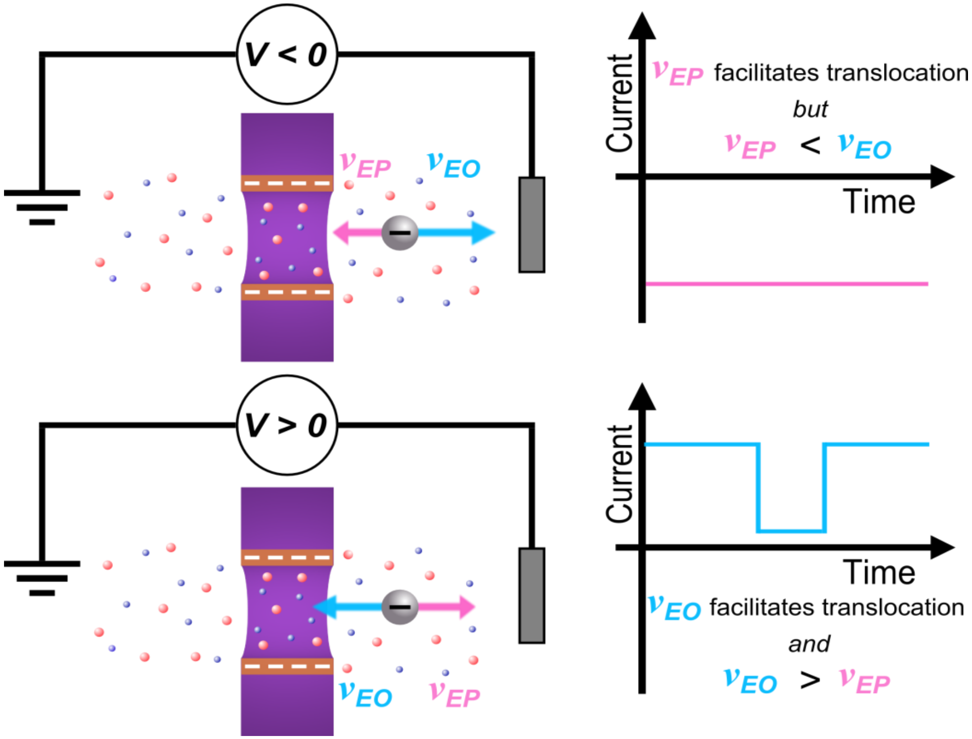
•Working with the shortest pores does not necessarily guarantee the best response.



A more complete, quantitative model for α may allow us to calculate optimal pore structure for analytes of given dimensions.

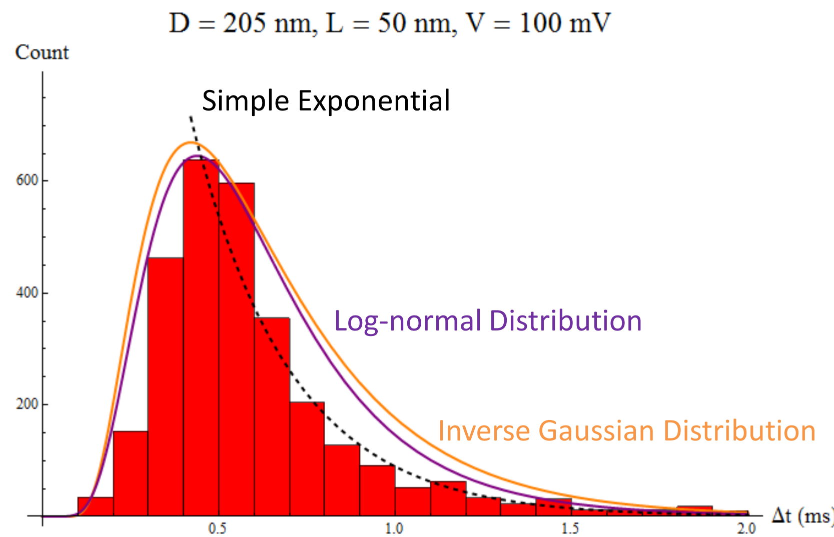
Conclusions

- While electrophoresis (EP) is usually employed in nanopore sensing, electroosmosis (EO) is also a suitable basis for mass transport in this detection scheme.



$v_{EP} \sim \zeta_{particle}\mathbf{E}$

$v_{EO} \sim -\zeta_{pore}\mathbf{E}$



Both $\zeta_{particle}$ and ζ_{pore} are < 0, thus the two velocities are always opposed. By fitting the event duration distributions, we hope to better understand the nature of that competition.

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