

Resistive Pulse Sensing at the Micro- and Nanoscale

Preston Hinkle



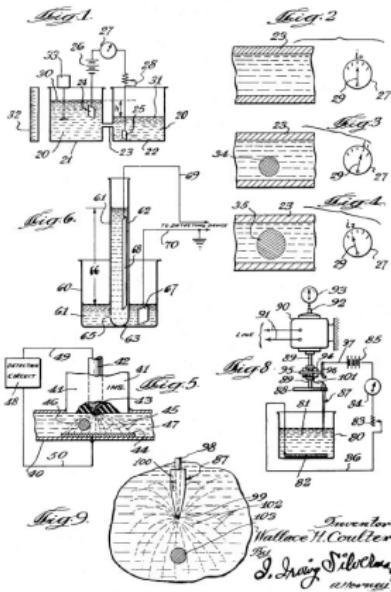
University of
California, Irvine

August 31, 2017

Outline

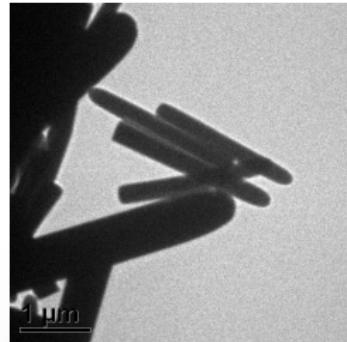
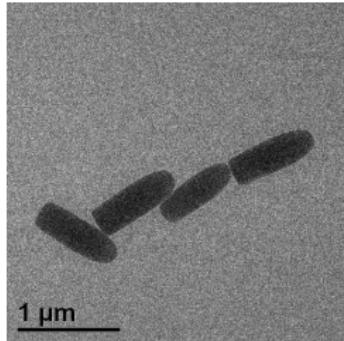
- Resistive pulse sensing background
- Resistive pulse sensing of high-aspect ratio particles
- Microscale resistive pulse sensing
 - Simultaneous imaging and resistive pulse studies
 - Cancer cell deformability cytometry

Oct. 20, 1953
W. H. COULTER
2,656,508
MEANS FOR COUNTING PARTICLES SUSPENDED IN A FLUID
Filed Aug. 27, 1949
2 Sheets-Sheet 1



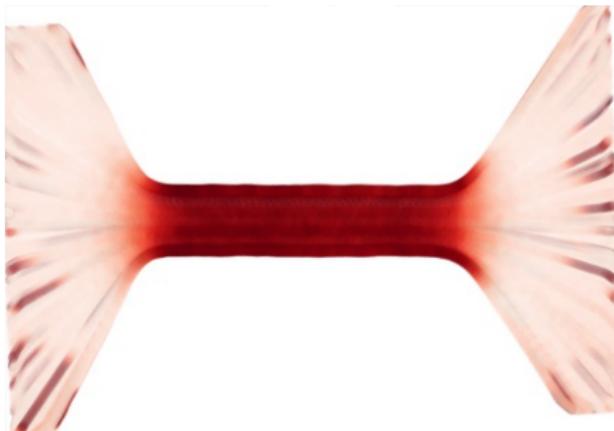
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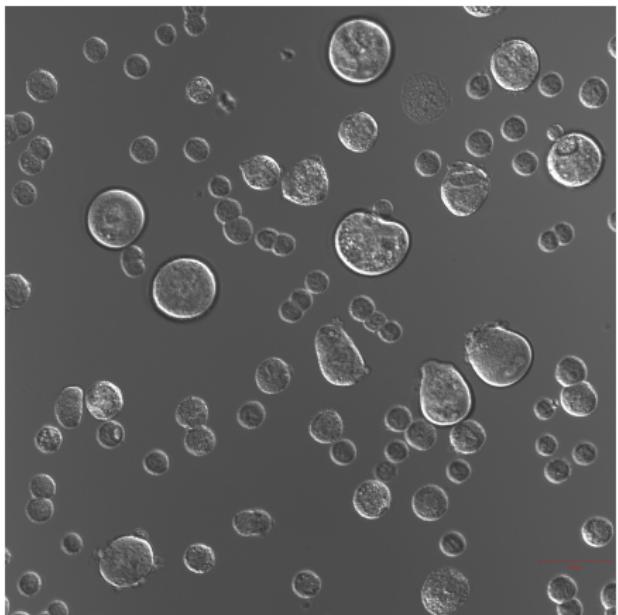
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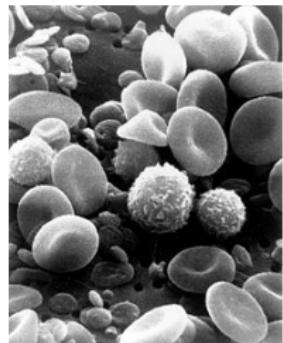
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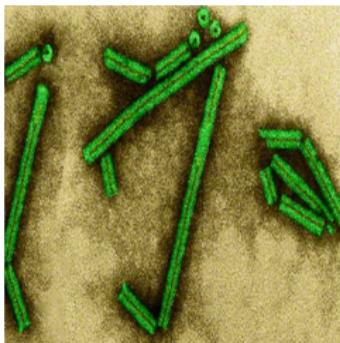
Resistive pulse sensing background

Resistive pulse sensing—description

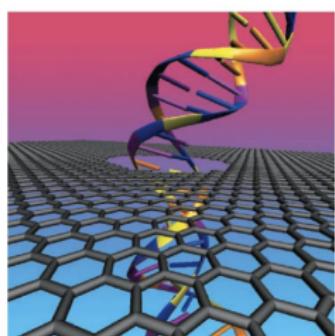
- Resistive pulse sensing (RP) is a method for single particle detection and characterization
- Works at any scale (nano, micro, milli, etc.) and in a diverse range of applications



Blood cell counting
(\sim several μm)



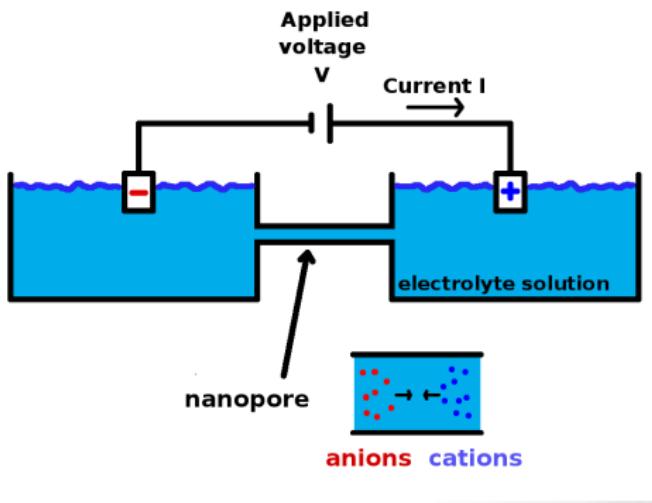
Virus detection
($\sim 50 \text{ nm}$)



DNA sequencing
(1 nm)

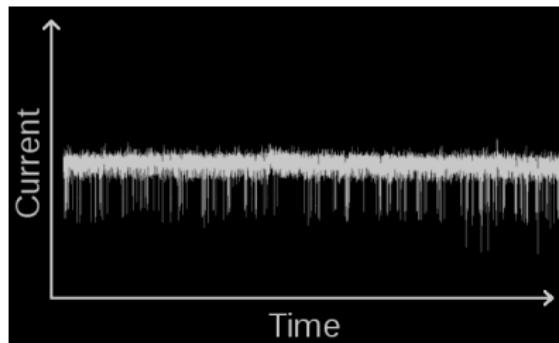
Resistive pulse sensing—how does it work?

- A channel is immersed in electrolyte solution
- An applied voltage induces an ionic current according to Ohm's law
 $I = V/R$
- The channel's resistance R is a function of the channel geometry and the conductivity of solution it is filled with

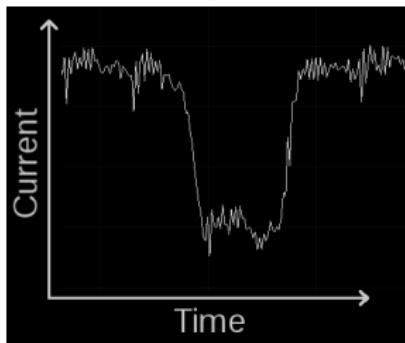


Resistive pulse sensing—how does it work?

- When a particle enters the channel, the channel's resistance changes, yielding a pulse in the measured ionic current
- Pulse properties yield information on size, shape, charge, and concentration of particle



Resistive pulse time series



Series zoomed in on a single event

Resistive pulse sensing—physics

In order to understand resistive pulse sensing and the amplitudes of the event pulses, we need to consider the following domains of physics

- Ion transport mechanisms
- Electrodynamics
- Particle transport mechanisms

Resistive pulse sensing—ion transport

- Ion transport in general occurs via a combination of **diffusion**, **convection**, and **electrophoresis** (or electrophoresis)
- Diffusion: Average flow of ions from high to low concentration
- Convection: Ions are carried passively with the fluid/solvent
- Electrophoresis: Ions drift in an electric field

$$\vec{J} = \sum_i^{\text{species}} \left[\underbrace{-z_i e D_i \nabla c_i}_{\text{diffusion}} + \overbrace{z_i e c_i \vec{u}}^{\text{convection}} + \underbrace{z_i e c_i \mu_i^{ep} \vec{E}}_{\text{electrophoresis}} \right]$$

(Nernst-Planck equation)

$$\mu_i^{ep} = \frac{z_i e}{k_B T} D_i \quad (\text{Einstein-Smoluchowski relation})$$

Resistive pulse sensing—ion transport

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$$\vec{J} = \sum_i^{\text{species}} z_i e c_i \mu_i^{ep} \vec{E} = \sigma \vec{E} \quad (\text{Electrophoretic current})$$

$$\mu_i^{ep} = \frac{z_i e}{k_B T} D_i \quad (\text{Einstein-Smoluchowski relation})$$

Resistive pulse sensing—electrodynamics

To solve for the currents in the pore (empty and occupied), we treat the system as a classical electrodynamics problem

$$\vec{J} = \sigma \vec{E} \quad (\text{Ohm's law})$$

$$\rightarrow \nabla^2 V = 0 \quad (\text{Laplace equation})$$

$$\vec{J} \cdot \hat{n} \Big|_{\text{channel}} = 0 \quad (\text{Boundary conditions})$$

$$\vec{J} \cdot \hat{t} \Big|_{\text{electrode}} = 0$$

For an unoccupied cylindrical pore with electrodes exactly at the pore entrance and exit, we find

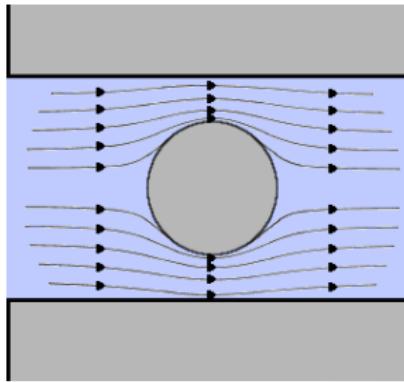
$$R = \rho \frac{L}{A} \quad (\text{Ideal cylinder})$$

Resistive pulse sensing—electrodynamics

The presence of an insulating particle increases the system resistance for two reasons

1. The volume occupied by the particle no longer contains conductive solution
2. Electric field lines in the vicinity of the particle are distorted, with reduced axial components

The result is a decrease in current when the particle is inside the channel



Resistive pulse sensing—electrodynamics

Ignoring the distortion of the electric field, a reasonable approximation for the resistance of the channel with or without the particle can be found *via*

$$\Delta R = \int \rho \frac{dz}{A(z)}$$

,

where $A(z)$ is area of the annular region of solution at position z

Resistive pulse sensing—electrodynamics

Analytic solutions to resistive pulse amplitude:

$$\frac{\Delta R}{R_0} = \frac{3}{2} \frac{v}{V}$$

(Sphere through cylinder)

v : Particle diameter

V : Channel diameter

f_{\perp}, f_{\parallel} : Electrical
shape factors

$$\frac{\Delta R}{R_0} = [f_{\perp} + (f_{\parallel} - f_{\perp}) \cos^2 \alpha] \frac{v}{V}$$

(Ellipsoid through cylinder)

α : Angle of rotation

Since we usually measure current in an experiment instead of resistance, it's conventional to replace R for I using Ohm's law:

$$\frac{\Delta R}{R_0} \Rightarrow \frac{\Delta I}{I_p}$$

Resistive pulse sensing—single particle transport

Similar to ions, single particle transport can occur via **thermal motion, convection, and electrophoresis**

- Thermal motion: Collisions with atoms and molecules causes random diffusive motion
- Convection: Motion due to fluid flow, usually induced by external pressure or electroosmosis
- Electrophoresis: Effective force on charged particles in electric fields

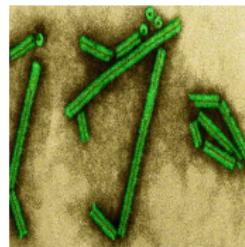
Resistive pulse sensing of high-aspect ratio particles

High-aspect ratio resistive pulse sensing—motivation

- Aspherical particles are ubiquitous in biology—e.g., many viruses and bacteria are approximately ellipsoidal



e. coli
 $L \sim 2\text{ }\mu\text{m}$



tobacco mosaic virus
 $L \sim 300\text{ nm}$

- The ability to measure particle shape is highly desirable for sensing applications
- How can we extend RP sensing to measure length in addition to volume?

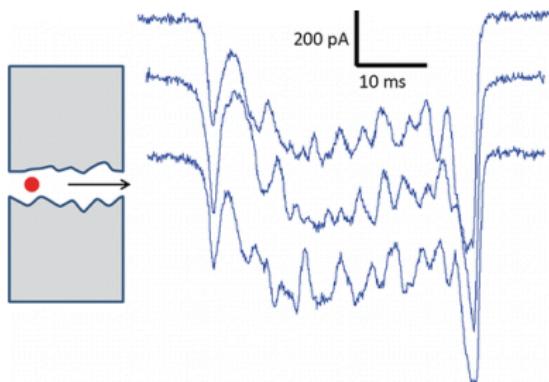
Resistive pulse in non-uniform pores

Consider the RP amplitude for translocation through non-uniform pores

$$\Delta R(z') = \frac{\rho}{\pi} \left[\int_{z=z'}^{z=z'+l_p} \left(\frac{1}{r_p^2(z) - s_p^2(z)} - \frac{1}{r_p(z)^2} \right) dz \right]$$

RP amplitude is a function of the pore geometry **local to the particle's position**

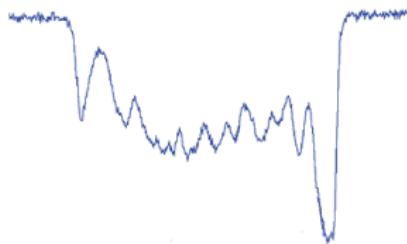
Particles map the interior of the pore during translocation with their RP signal!



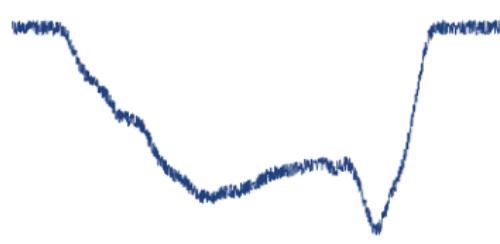
Pevarnik et al. ACS Nano. 6, 7295-7302 (2012).

RP signal resolution

- Particles map pore interiors with a length-dependent resolution
- If a particle has length less than the characteristic length scale of channel irregularities, the produced signal is a high-resolution mapping
- Particles with lengths greater than characteristic length scale of channel irregularities produce low-resolution mappings



Short particle

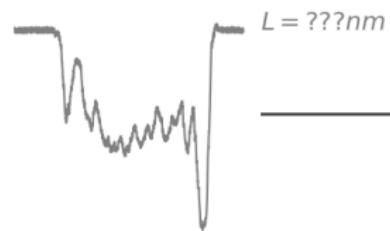


Long particle (simulated)

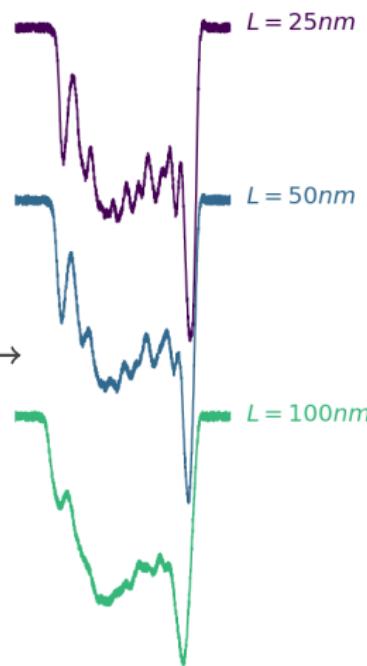
Can we use this knowledge to measure particle length?

Qualitative length comparison

Unidentified particles



Tracer particles



Reexpressing the RP amplitude of a long particle in terms of shorter particles

Because resistances add in series, we can express the RP amplitude of a long particle as a sum over the RP amplitudes of shorter particles

$$\begin{aligned}\frac{\Delta I}{I_p} &= \frac{\Delta R_I}{R_0} = \frac{\rho}{\pi} \left[\int_z^{z+l_p} \left(\frac{1}{r_P^2(z') - s_p^2(z')} - \frac{1}{r_P^2(z')} \right) dz' \right] / R_0 \\ &= \sum_{i=0}^{n-1} \frac{\rho}{\pi} \left[\int_{z+il_s}^{z+(i+1)l_s} \left(\frac{1}{r_P^2(z') - s_p^2(z')} - \frac{1}{r_P^2(z')} \right) dz' \right] / R_0 \\ &= \sum_{i=0}^{n-1} \Delta R_s(z + il_s) / R_0\end{aligned}$$

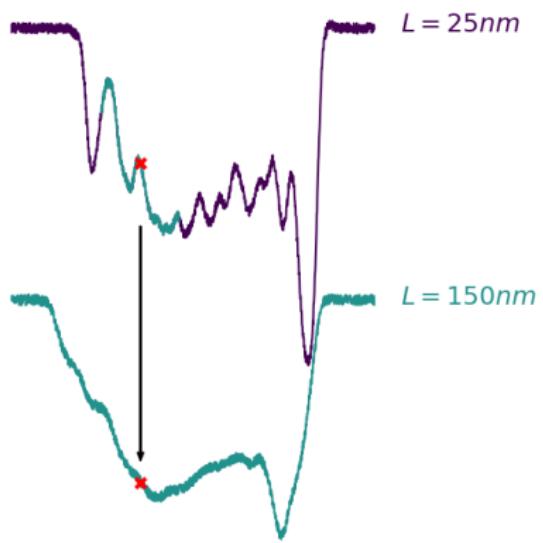
Quantitative length measurement

Reexpressing the amplitude of long particles in terms of amplitude of short particles suggests a protocol for measuring length

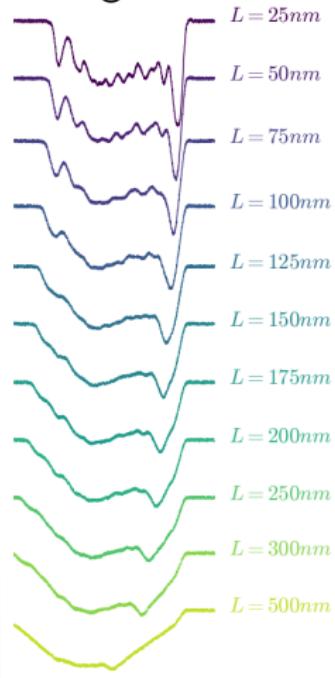
1. We perform a moving average transformation on the signals of shorter particles to simulate the signals of longer particles
2. Then, we calculate the 'distance' between an unknown particle's signal with each of the simulated signals of the shorter particle
3. The comparison with the greatest similarity yields the length of the particle

Quantitative length measurement—parametric signal transformation

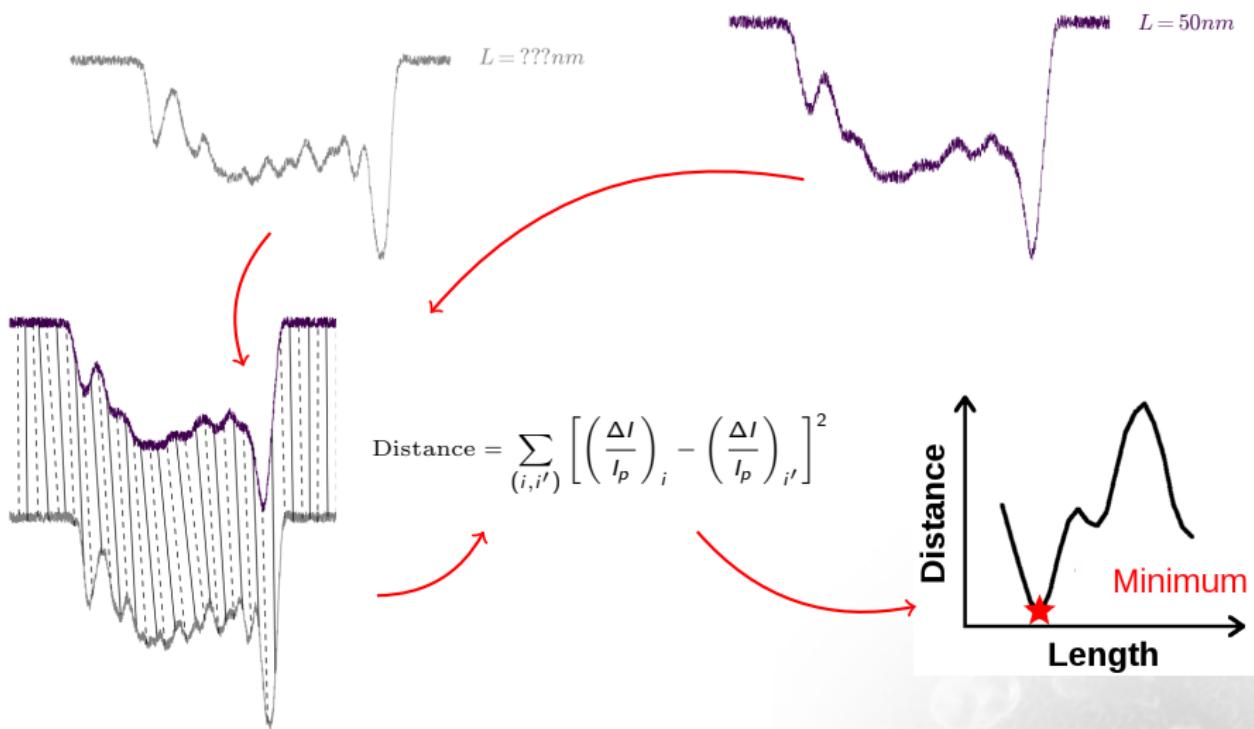
Single transformation



Multiple length transformations



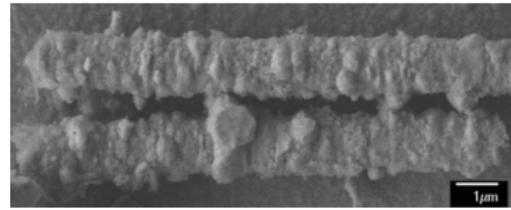
Quantitative length measurement—signal similarity measure



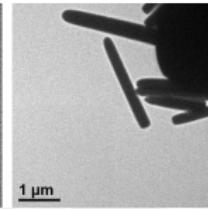
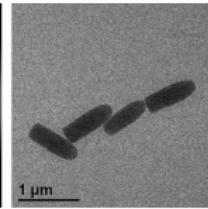
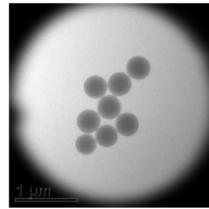
Length measurement experimental test

Can we implement and test the length measurement protocol?

- Experiments were conducted with single pores etched into PET membranes ($D \sim 750 \text{ nm}$, $L = 12 \mu\text{m}$)
- Three types of particles were tested
 - 280 and 410 nm polystyrene beads ('spheres')
 - 590 nm silica rods ('short rods')
 - 1920 nm silica rods ('long rods')

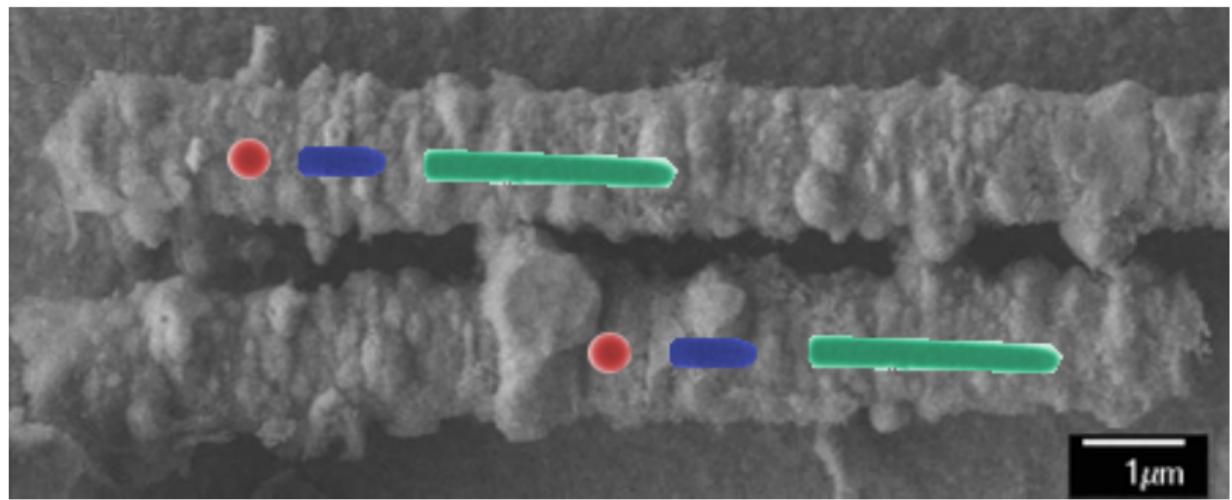


PET pore metal replica

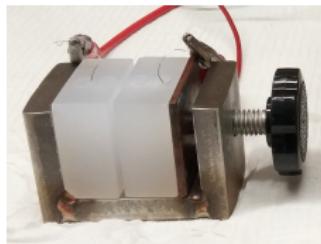


Nanoparticles

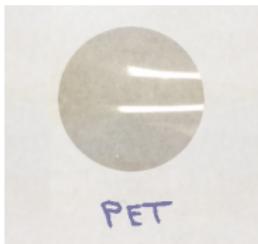
Particles to scale



Polymer nanopore experiment components



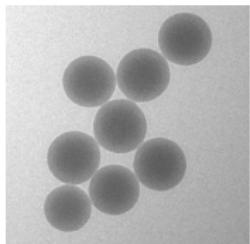
Conductivity cell



Pore membrane



Electrolyte



Particles

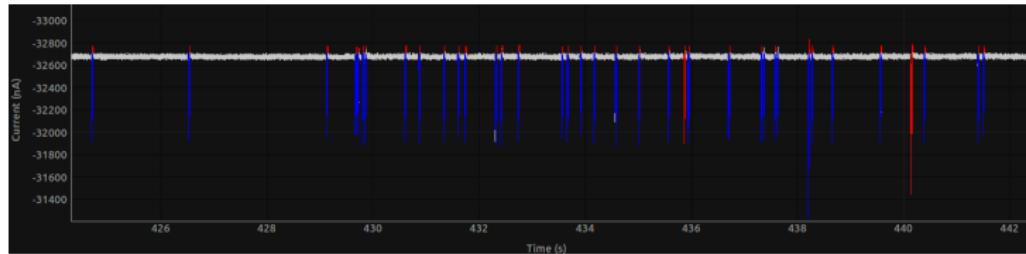


Ag-AgCl
electrodes

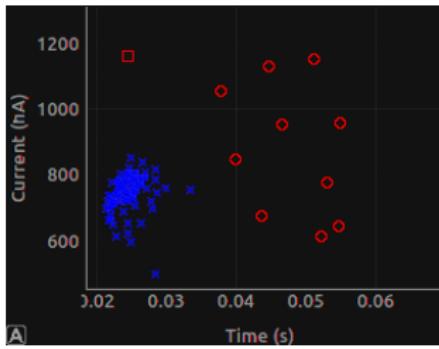


Voltage amplifier + current recorder

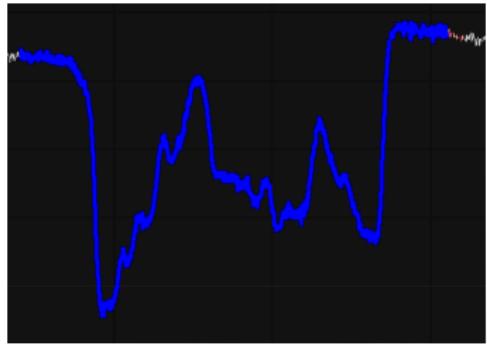
Results—raw data



Resistive pulse time series



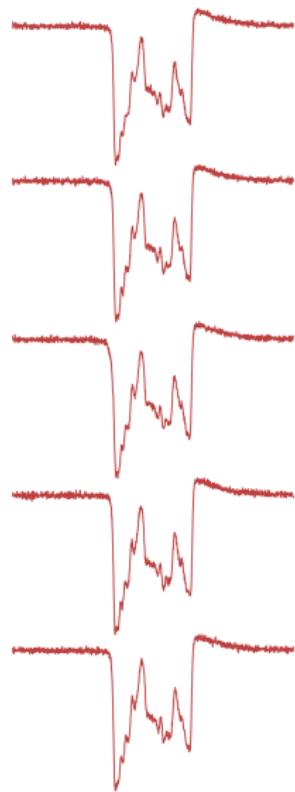
Amplitude-duration scatter



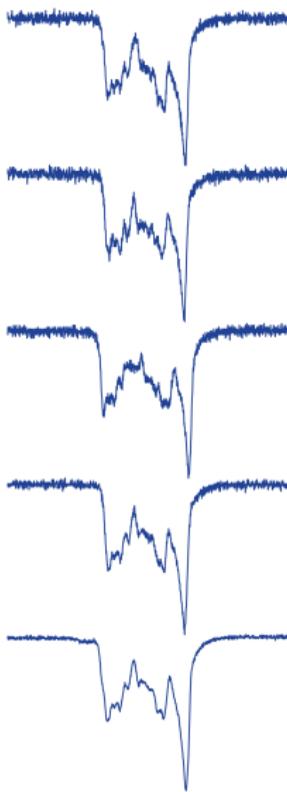
Raw event

Results—sphere, short rod, and long rod events

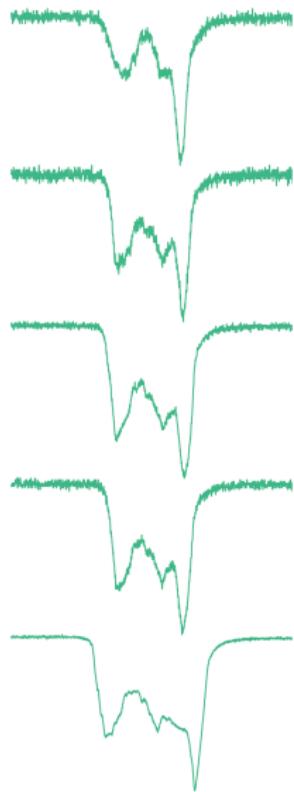
410 nm sphere



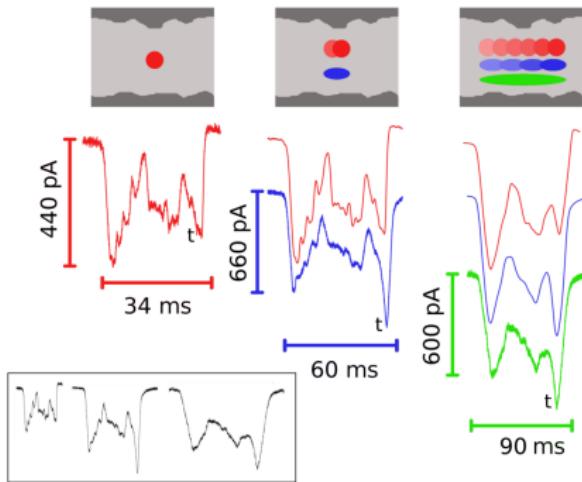
Short rod



Long rod



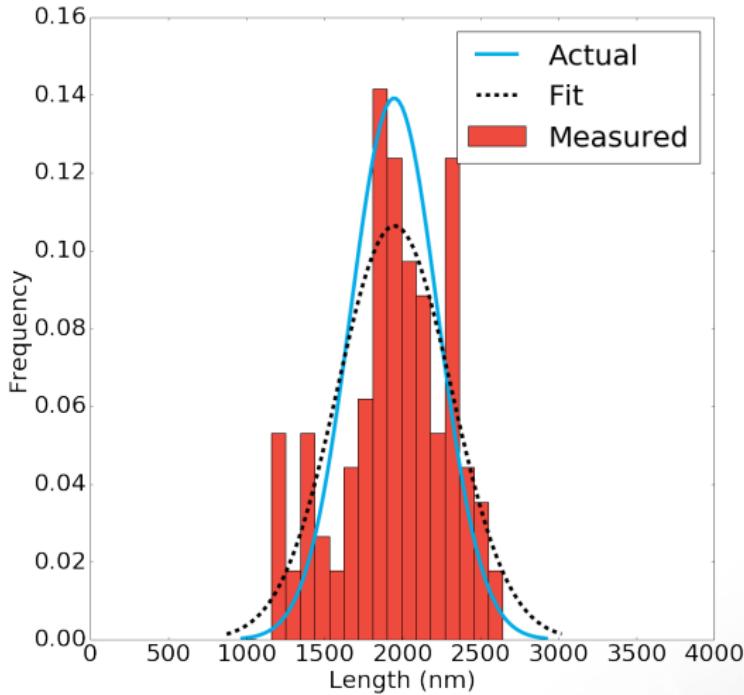
Results—Qualitative event comparison



Qiu et al. ACS Nano 9, 4390-4397 (2015).

The averaging process produces signals that are qualitatively similar to the observed signals of longer particles

Results—Quantitative event comparison



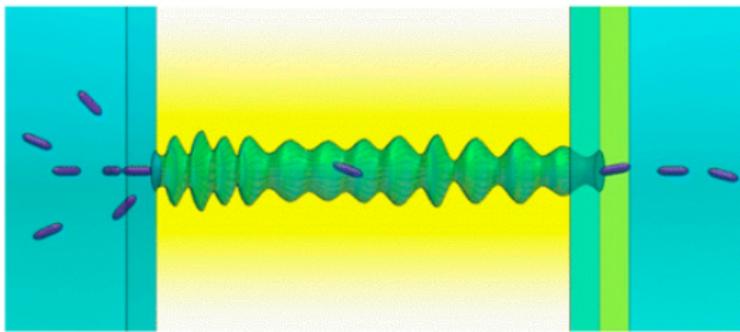
Quantitative measurement of particle length yields a distribution closely matching the actual distribution of lengths!

Conclusions & Future work



Pores with Longitudinal Irregularities Distinguish Objects by Shape

Yinghua Qiu,^{1,3,4} Preston Hinkle,^{1,2†} Crystal Yang,^{4,5} Henriette E. Bakker,² Matthew Schiel,¹ Hong Wang,¹ Dmitriy Melnikov,⁶ Maria Gracheva,⁶ Maria Eugenia Toimil-Molares,^{7,8} Arnout Imhof,¹ and Zuzanna S. Siwy^{*,1,5,9}



Conclusions & Future work

It's apparent that the model for how particles map the interiors of pores is accurate

Some things left for the future:

1. Test robustness of quantitative length measurement
2. Run length measurement protocol on particles of various unknown lengths, test results
3. Reduce system scale: Test length measurement protocol with fabricated nanochannels with controlled geometries

Hybrid imaging-resistive pulse measurements in microfluidic channels

Motivation—Why introduce optics?

In an RP experiment, the usual parameters measured are amplitude and duration, which can relate to particle's volume, charge, etc.

But, the equations which relate the RP signal to physical observables are only accurate under sterile conditions

In reality, the experimental setup can seldom be constrained to this degree

Motivation—Why introduce optics?

Some confounding factors include

- Entrance effects in low or medium aspect ratio pores
- Non-spheroidal particles, rotational effects
- Off-axis translocation

The influence of each of these effects on the RP signal is difficult to measure

Motivation—Why introduce optics?

What if we could see what is happening during a resistive pulse experiment?

Then we could determine the influence of these confounding factors during the event translocation

For instance, we could directly observe the effect of off-axis translocation on the resistive pulse signal

The results would generalize to other resistive pulse experiments and lead to greater interpretability of the RP signals!

Motivation—Why introduce optics?

But, the trouble is that directly imaging nanoscale resistive pulse experiments is extremely difficult; need an electron microscope that can operate *in situ*

However, at the microscale we can use plain optics to image the experiments while simultaneously measuring the resistive pulses

The results should generalize to the nanoscale as well, since the confounding factors arise due to electrostatic boundary conditions that are scale independent

Experimental objective

Objective: Create a hybrid resistive pulse-optical characterization platform

Essentially, we want to devise a micro-sized resistive pulse system that we can also image

This requires adding a few elements to the standard resistive pulse set up

- Optically transparent, planar microchannels
- Pressure-induced flow, e.g. via a syringe pump (electrophoresis and electroosmosis are less pronounced at this scale)
- Microscope to magnify the image
- High-speed camera to capture images

PDMS channel fabrication

The channels were fabricated with PDMS, an optically transparent elastomer ideal for microfluidic experiments

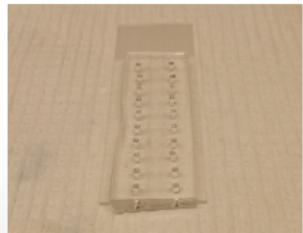
1. Channel design is printed onto a high-resolution phototransparency
2. Channel design is then transferred to a mold via standard soft photolithography
3. PDMS is poured over mold and removed after curing
4. PDMS bonded to a glass slide using an oxygen plasma



Phototransparency



Silicon/SU8 wafer



PDMS channels

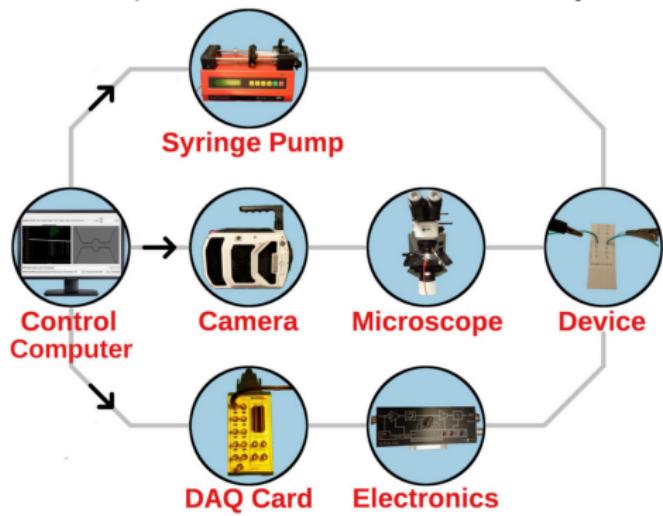
Hardware configuration

Device is placed on the stage of a microscope, which has a high-speed camera (> 100 kfps!) attached for capturing the images

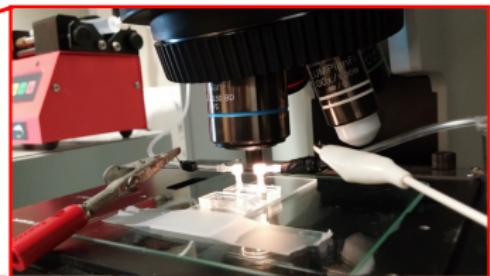
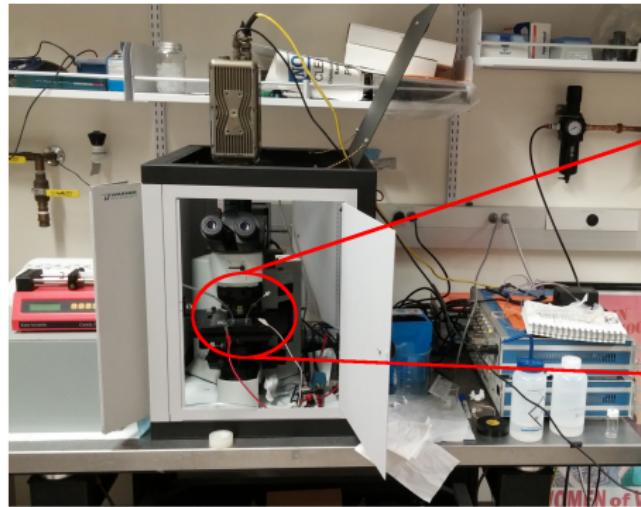
Electrodes are attached at the channel access ports for recording the RP signal

A particle suspension is driven through the channels via syringe pump

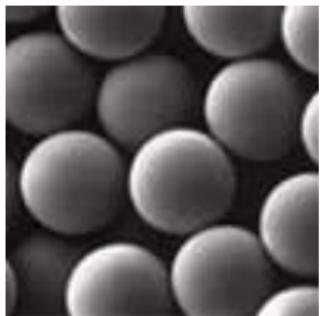
The camera and resistive pulse data are simultaneously recorded



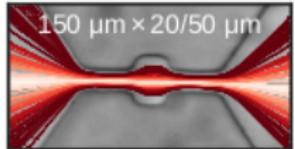
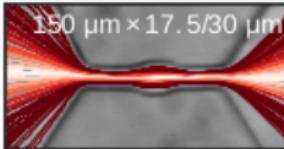
Hardware configuration



Channels and particles



10 μm polystyrene beads

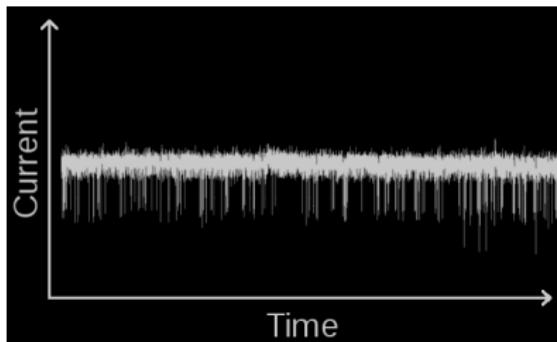


PDMS channels

Top row: straight

Bottom row: with cavity

Raw data—resistive pulse and optics



Raw RP series
 $\text{data} = I(t)$

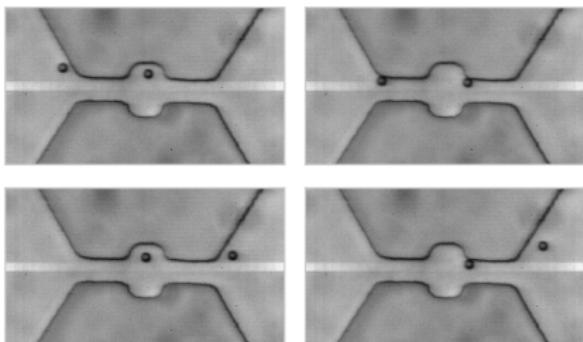
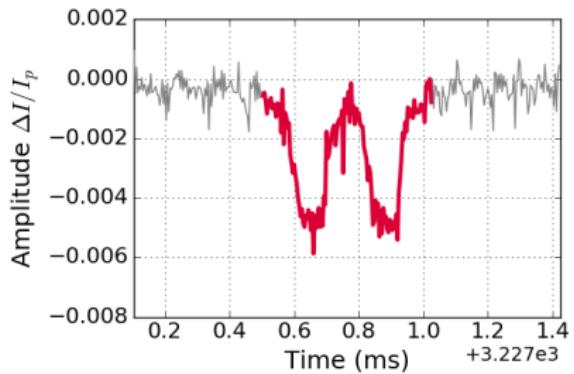


Image stills
 $\text{data} = \{\text{frame1}, \text{frame2}, \dots\}$

Start with two raw data streams recorded independently

- The objective is to connect the two data sets so that we know the instantaneous value of the current for each frame
- This will allow us to map the instantaneous state of the channel (occupancy, occupant position) to the current level

Tracked events



$$\text{data} = \frac{\Delta I}{I_p} (t_{RP})$$

$$\text{data} = \vec{x}_c (t_{IM})$$

Resistive pulse events and imaging events are independently detected in both data sets

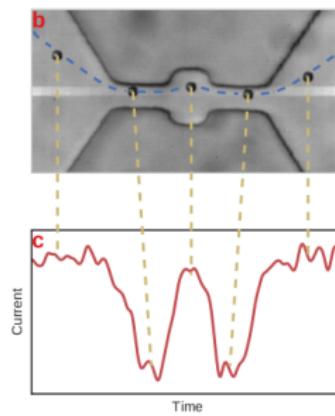
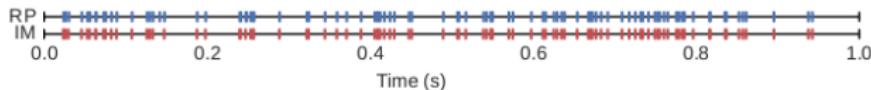
- RP events are detected via a threshold algorithm
- Individual particles are detected via image processing techniques and tracked across frames

Synchronizing the two data sets

After the events are detected independently, we plot a sequence of the time at which each event occurs in its own data stream

Then, we align the two sequences, resulting in a synchronized data set

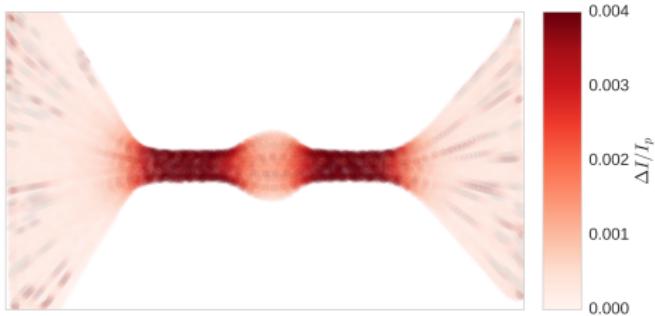
$$\text{data} = \frac{\Delta I}{I_p} (t, x_c, y_c)$$



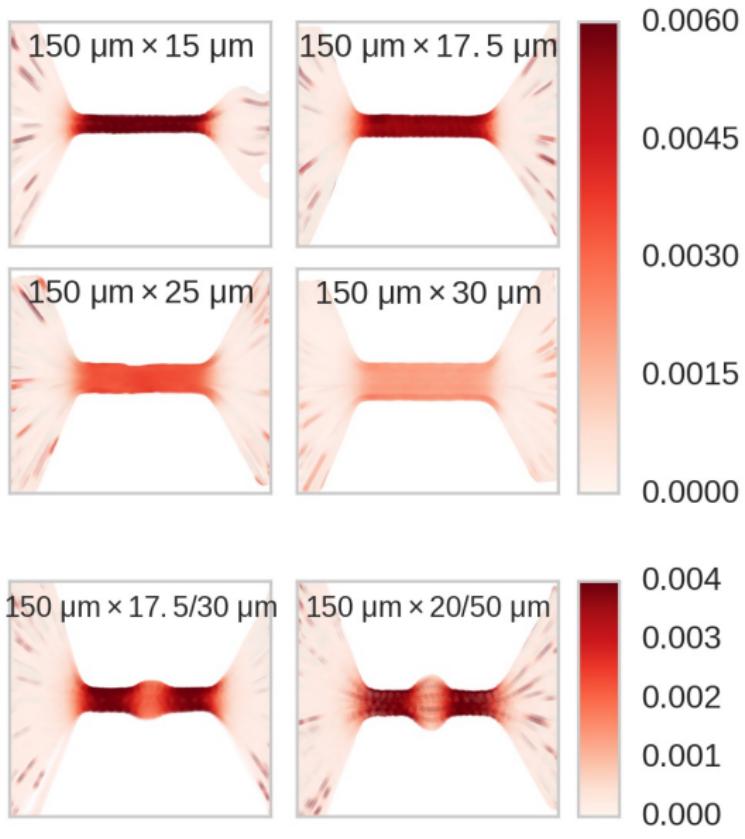
Resistance maps

Synchronizing the two data streams allows us to create ‘resistance maps’ of the channel, plots where each particle position is mapped onto the instantaneous value of the RP amplitude

Resistance maps



Resistance maps



Key scientific questions

The hybrid resistive pulse-imaging platform is a general tool for enhancing the interpretability of resistive pulse experiments

We were interested in answering the following:

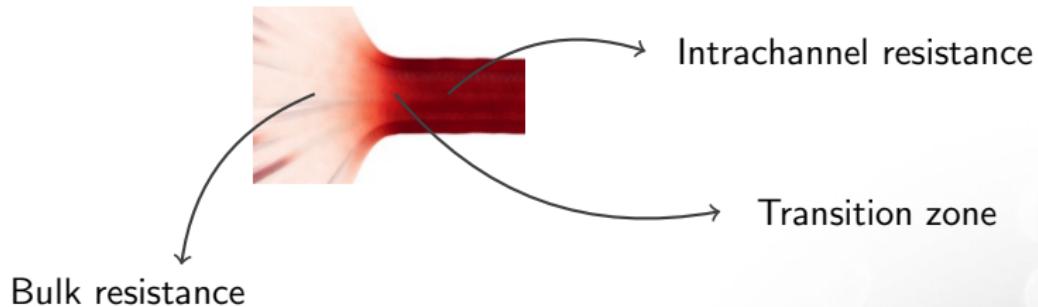
- How far into a channel must a particle travel before the RP amplitude plateaus?
- How does off-axis translocation effect the RP amplitude in constant width and non-constant width channels?
- How is the resistance distributed in channels with varying widths?

Channel entrance effects

In RP experiments, event duration can be used to measure the ζ -potential of the particle or pore

In order to measure duration accurately, the exact time corresponding to particle entrance and exit must be known

There is no standard point in RP signal at which to mark the event start and stop

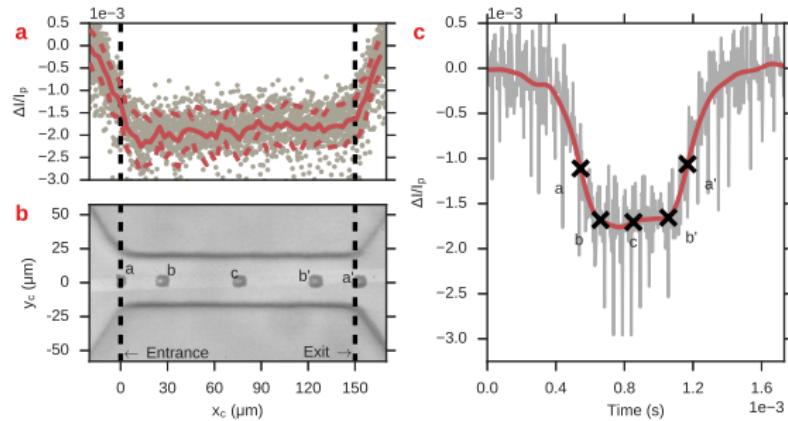


Channel entrance effects

Plotting current amplitude $\Delta I/I_p$ versus axial position x_c shows how the current transitions to its full amplitude as the particle enters the channel

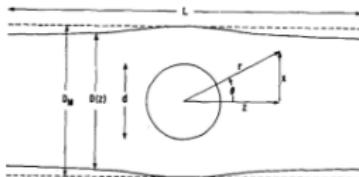
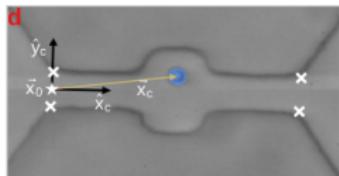
We found the full amplitude was not attained until the particle was well-within the channel, even as much as $\sim 10 \mu\text{m}$ (7% of the total channel length)

The channel-crossing threshold most closely coincides with the FWHM of the RP signal, suggesting that current value may be the most appropriate to choose for the channel entrance and exit positions



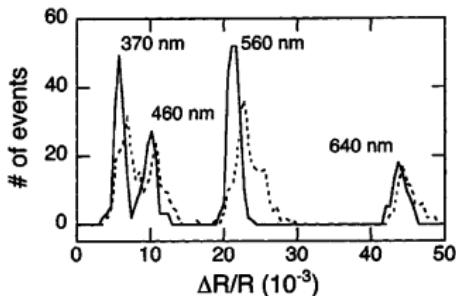
Effect of off-axis translocations

Electrostatic boundary conditions at the surface of the insulating particle leads to distortion of the electric field in its vicinity



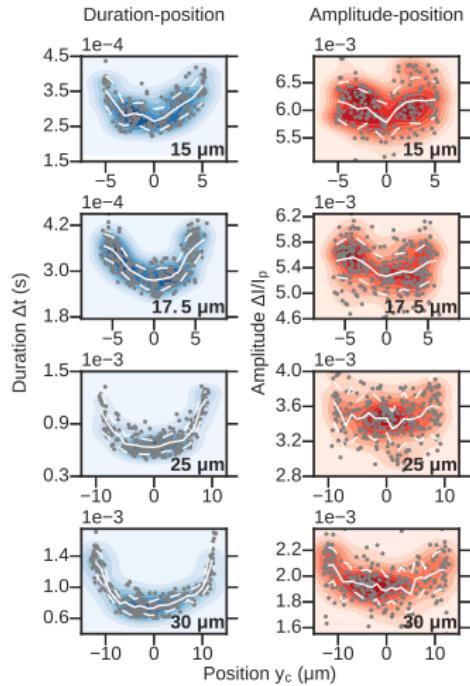
When the particle travels off-axis, this distortion couples with the distortion of the \vec{E} field in the vicinity of the channel, increasing the total system resistance

This off-axis effect leads to a dispersion in the amplitudes produced by particles of the same size, ultimately resulting in larger uncertainties in their measured volumes



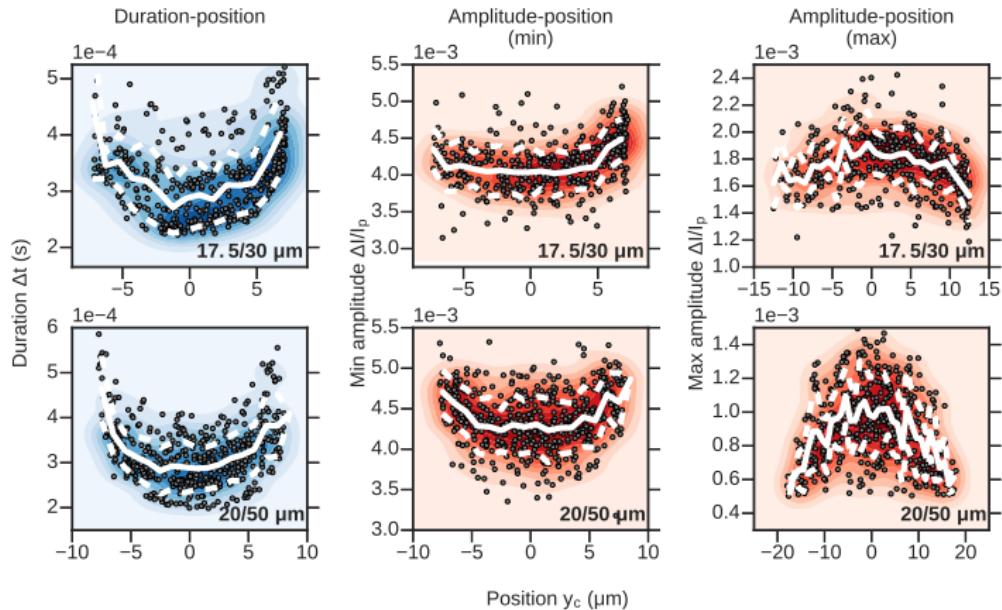
Saleh, O.A. and Sohn, L.L. *Rev. Sci. Instrum.*
73, 4396–4398 (2002).

Off-axis translocation in straight channels



The scatter plots show an increase in event amplitude of close to 10%, in agreement with theoretical expectations.

Off-axis translocation in cavitated channels



In channels with a central cavity, we observe the opposite effect of lateral displacement
when the particle is inside the cavity.

Conclusions and ongoing work



OPEN

A hybrid resistive pulse-optical detection platform for microfluidic experiments

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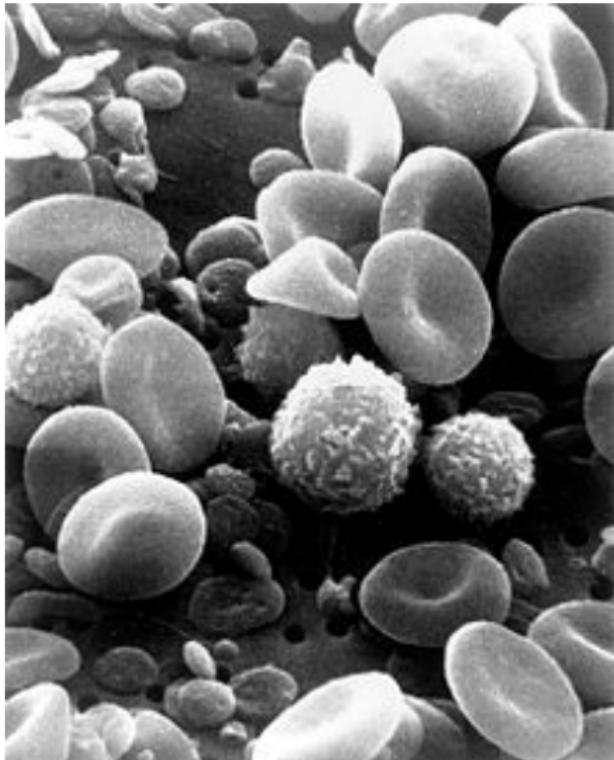
The hybrid resistive pulse-optical detection platform allows one to explore position-related effects on the resistive pulse amplitude

We are currently interested in using the hybrid approach to study particle deformation dynamics, and how deformation affects the resistive pulse amplitudes

Resistive pulse sensing of biological cells

Cell mechanical properties

- Traditional cell characterization platforms have been primarily chemical, e.g. fluorescence microscopy
- Recently, researchers have recognized the importance of measuring mechanical properties of cells
- For instance, size and shape can be predictive of cell type

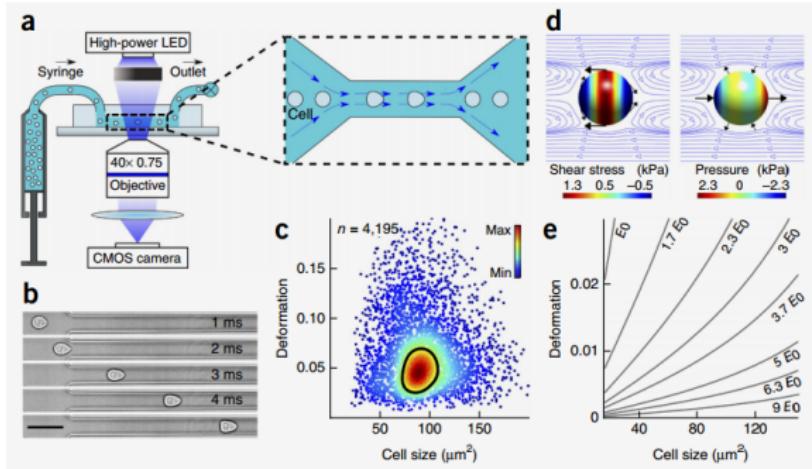


Cell mechanical properties

- Another important mechanical feature is stiffness—cells are elastic and deformable objects
- Stiffness depends on physiological properties in the membrane and body, such as cytoskeletal strength
- Stiffness varies across cell lines, and has shown to be predictive in the same way as size and shape are—for instance, cancer cells are generally more elastic than non-cancerous cells

Stiffness detection

- Is it possible to build a sensor that measures cell deformability?
- One way to measure deformability is to drive cells through ultra fast fluidic flows
- Hydrodynamic forces act on the particle, inducing a measurable deformation response



Otto et al. *Biophys. J.* **109**, 2023–2036 (2015).

Optical measurement of deformation

- The cell's deformation response is usually determined via high-speed microscopy
- High-speed imaging is computationally expensive—many images must be rapidly recorded and undergo complex transformations in order to fit the shape of each cell
- Real-time analysis is bottlenecked at the CPU; current throughputs are only ~ 100 cells/second
- Can we replace imaging with resistive pulse, which is less computationally demanding to analyze?

Imaging bandwidth ~ 1 GB/s

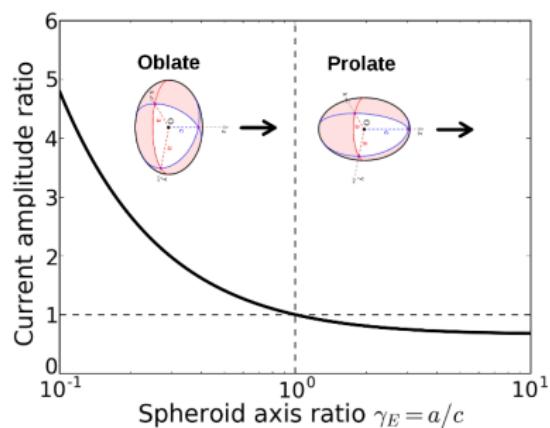
Resistive pulse bandwidth ~ 1 MB/s

Optical measurement of deformation

We can approximate deformed cell configurations as ellipsoids, which have resistive pulse amplitude described by

$$\frac{\Delta I}{I_p} = f_{\parallel} \frac{V}{V}$$

f_{\parallel} : 'electrical shape factor', related to aspect ratio of the ellipse



Particles of the same volume but different aspect ratio have different resistive pulse amplitudes $\Delta I / I_p$!

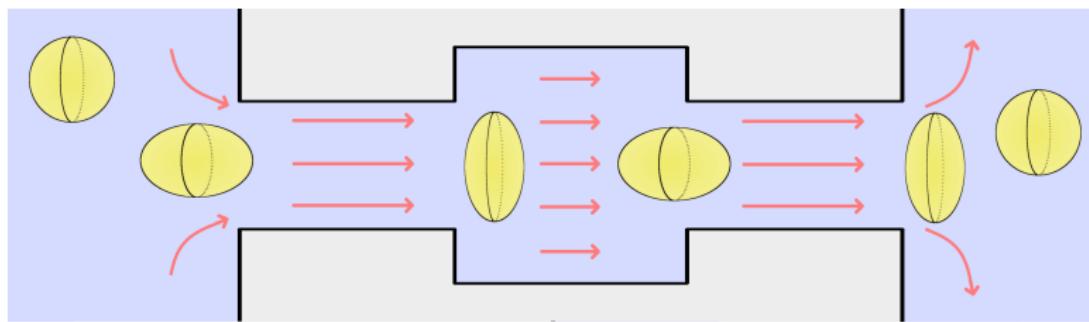
Proposed channel design for inducing deformation

Consider channels containing a central cavity

Incompressibility of the fluid means that it must slow down in the cavity

Accelerating extensional flows pull the particle into an elongated geometry

Decelerating flows compress the particle axially

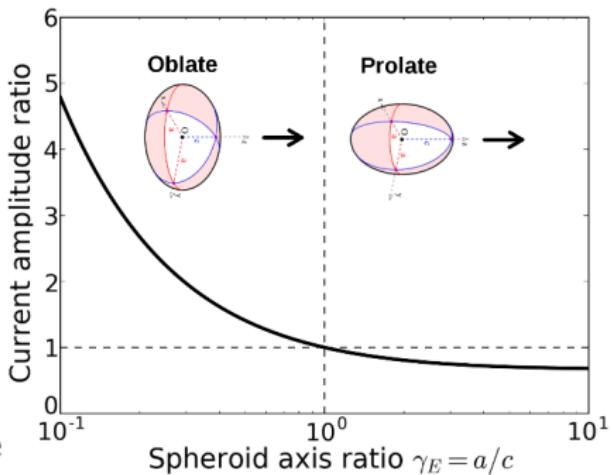


Deformation motif in channels with central cavities

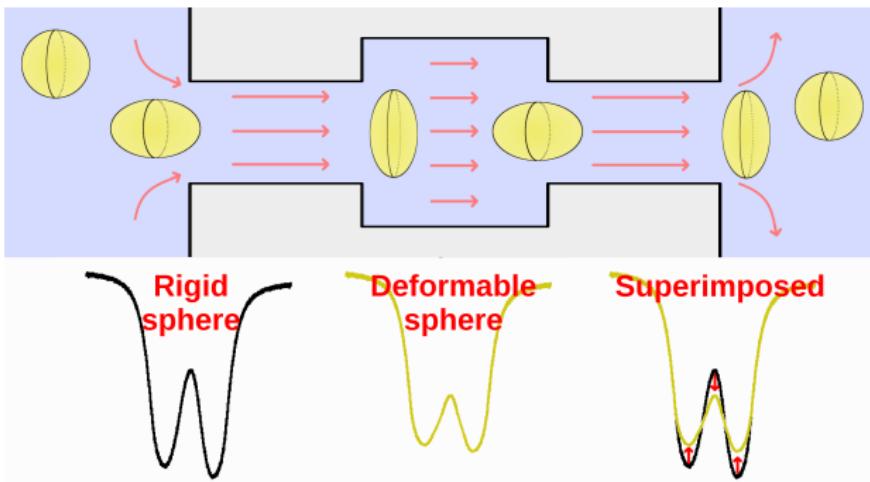
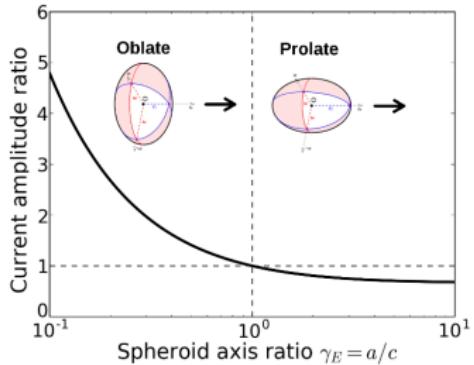
According to the deformation motif presented in the previous slide, deformations should adhere to the following pattern as the particle translocates

$s \rightarrow p \rightarrow o \rightarrow p \rightarrow o \rightarrow s$

s : spherical, p : prolate, o : oblate



Resistive pulse distortion



Key scientific questions

Experiments must be conducted to answer the following questions

1. Do the cells deform according to the presented motif?
2. Is the deformation observable in the resistive pulse signal?

The hybrid RP-IM system is employed to characterize the device and the cells' resistive pulses

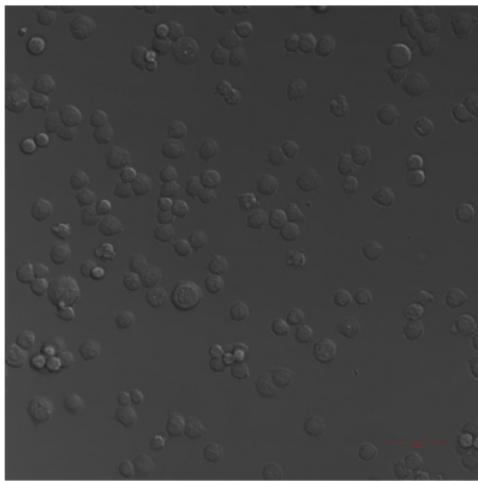
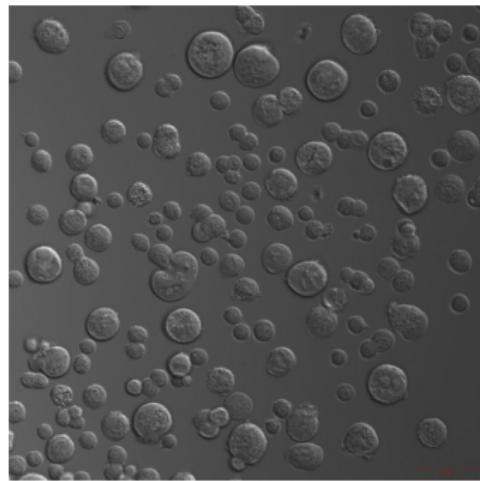
Ultimately, the goal is to remove the camera from the set up and measure deformability with resistive pulse alone

Experimental set up

Experiments were conducted with the hybrid resistive pulse-optical characterization platform

Two cell lines are currently being tested

1. HCT-116, colorectal cancer cells (left)
2. 293-T, embryonic kidney cells (right)

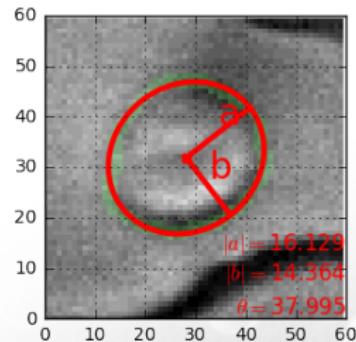
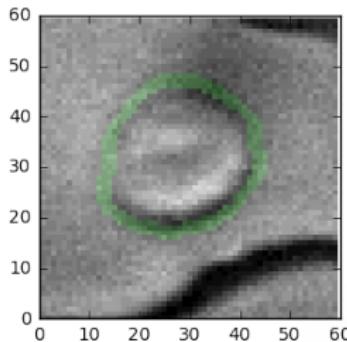
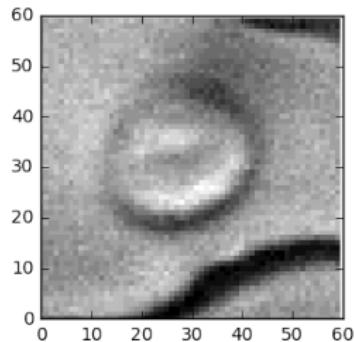


Data analysis

Individual cells were detected and tracked as they passed through the camera's field of view

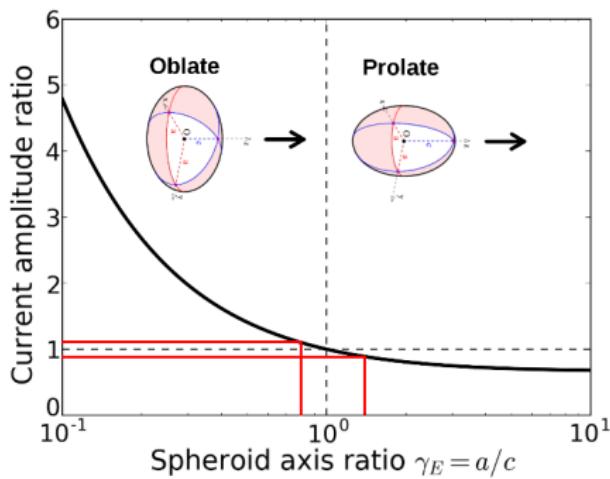
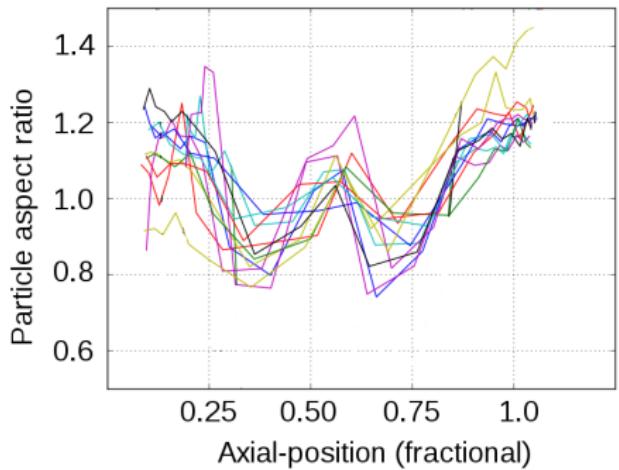
To determine whether particles deform, we fit ellipses to their borders at every frame in the video

After fitting the ellipses, we have a time-series of position \vec{x}_c , and ellipse axes \vec{a} and \vec{b} for each frame and each particle



Do the cells deform?

Axial position versus aspect ratio



The change in the aspect ratios agrees with the motif presented for the cavitated channels

Conclusions and future work

We've convincingly shown that our channel configuration is inducing two modes of deformations in cells

Current efforts are focused on improving our optical measurement set up

The magnitude of deformation observed implies that cell shape changes may be detectable with resistive pulse sensing after improving the signal-to-noise of our measurements

Thank you for your attention!

The Siwy Lab



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Dr. Timothy Plett



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James Boyd



Joseph Martinez



Cody Combs



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