Resistive-pulse sensing at the micro- and nanoscale

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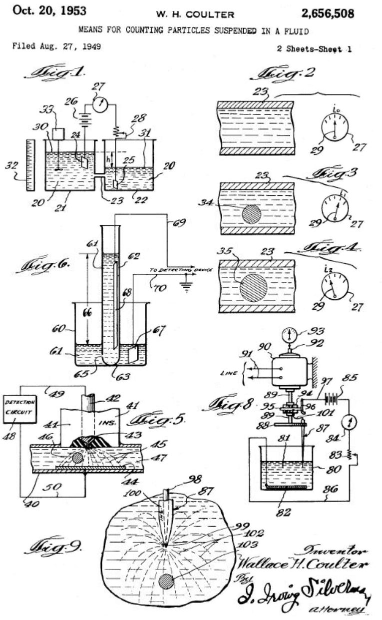
Outline

- 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



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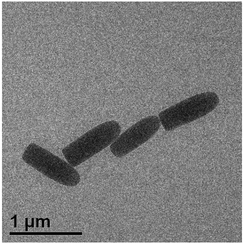

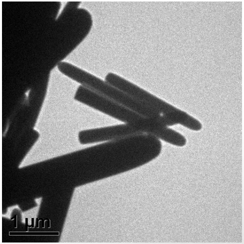

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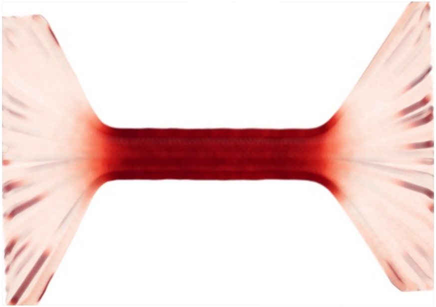

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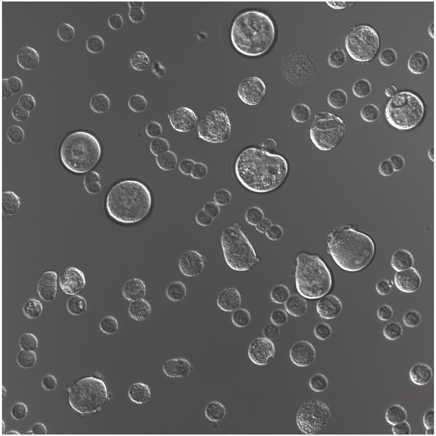
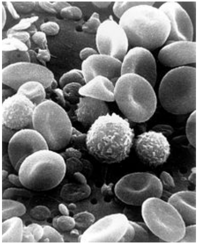


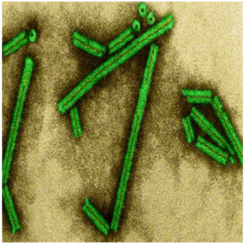
Resistive pulse sensing background

Resistive pulse sensing—description

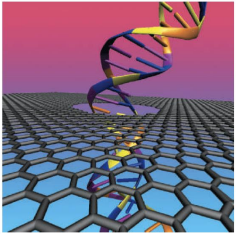
- Resistive pulse sensing (RP) is a method for single particle detection and characterization
- Works at any scale (nano, micro, milli, etc.) and in a diverse range of applications



Blood cell counting
($\sim$ several μm)



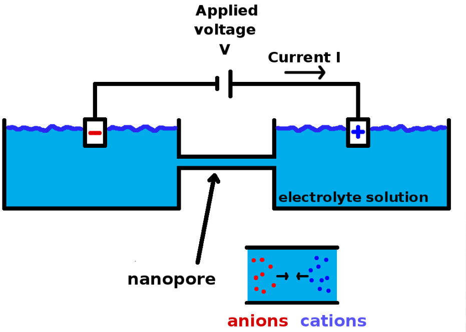
Virus detection
($\sim$ 10 nm)



DNA sequencing
(1 nm)

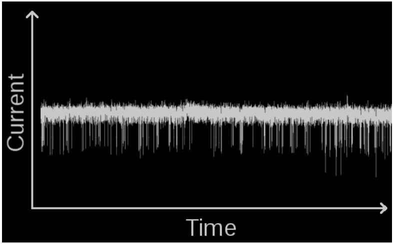
Resistive pulse sensing—how does it work?

- 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

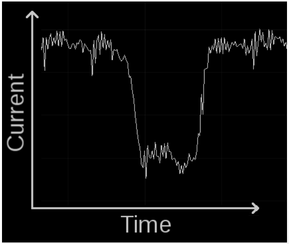


Resistive pulse sensing—how does it work?

- When a particle enters the channel its 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
- Electrostatics
- Particle transport mechanisms

Resistive pulse sensing—ion transport

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

$$\vec{J_i} = \underbrace{z_i e D_i \nabla c_i}_{\text{diffusion}} + \overbrace{z_i e c_i \vec{u}}^{\text{convection}} + \underbrace{z_i e c_i \mu_i \vec{E}}_{\text{migration}} \quad \text{(Nernst-Planck equation)}$$

$$\sigma \equiv \sum_{i}^{\text{species}} z_i e c_i \mu_i \qquad \text{(Conductivity)}$$

Resistive pulse sensing—electrostatics

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

$$\vec{J} = \sigma \vec{E} \qquad \text{(Ohm's law)}$$
$$\rightarrow \nabla^2 V = 0 \qquad \text{(Laplace equation)}$$
$$\left.\vec{J} \cdot \hat{n}\right|_{\text{channel}} = 0 \qquad \text{(Boundary conditions)}$$
$$\left.\vec{J} \cdot \hat{t}\right|_{\text{electrode}} = 0$$

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

$$I = \frac{V}{R} = V\frac{A}{\rho L} \qquad \text{(Ideal cylinder)}$$

Resistive pulse sensing—electrostatics

The presence of a 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

Both of these effects contribute to the transient reduction in current when the particle passes through the channel
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—electrostatics

The equations for the change in resistance in the occupied case have been solved analytically in some limiting geometries

$$\frac{\Delta R}{R_0} = \frac{d^3}{LD^2}\left[1 - 0.8\left(\frac{d}{D}\right)^3\right]^{-1}$$

(On-axis sphere through cylinder)

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

(On-axis ellipsoid of revolution)

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

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

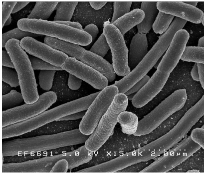
Resistive pulse sensing—single particle transport

- 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; usually only important in the absence of other forces
- Convection: Motion due to fluid flow, which is induced by external pressure or electroosmosis
- Electrophoresis: Effective force on charged particles in electric fields

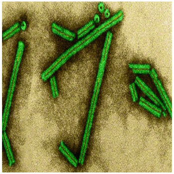
Resistive pulse sensing of high-aspect ratio particles

High-aspect ratio resistive pulse sensing—motivation

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



e. coli
$L \sim 2\,\mu$m



tobacco mosaic virus
$L \sim 300\,$nm

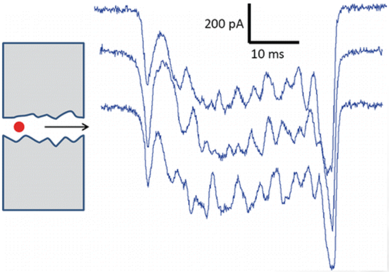
- The ability to measure particle shape is highly desirable for sensing applications
- How can we extend RP sensing to measure length in addition to volume?

Resistive pulse in non-constant width pores

- Consider the RP amplitude for translocation through non-uniform pores

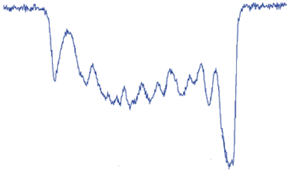
$$\Delta R\left(z'\right) = \frac{\rho}{\pi}\left[\int_{z=z'}^{z=z'+l_p}\left(\frac{1}{r_P^2\left(z\right) - s_p^2\left(z\right)} - \frac{1}{r_P\left(z\right)^2}\right)dz\right]$$

- 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!

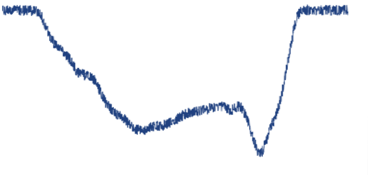


RP signal resolution

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



Short particle



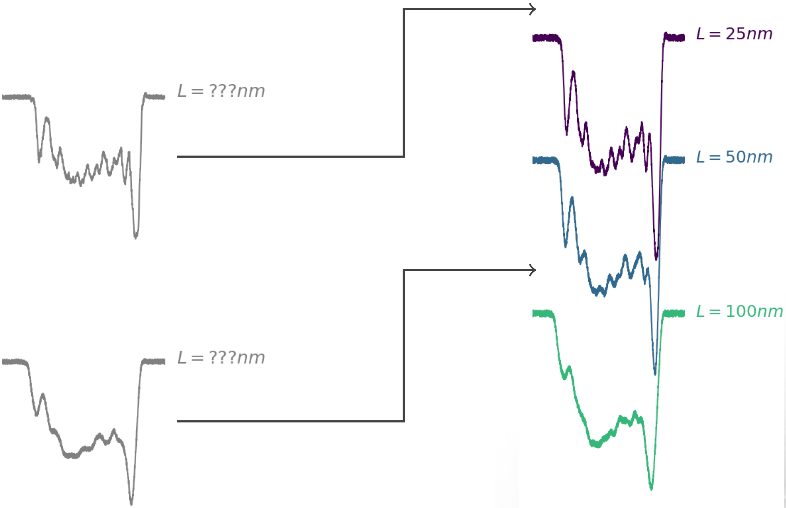
Long particle (simulated)

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

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

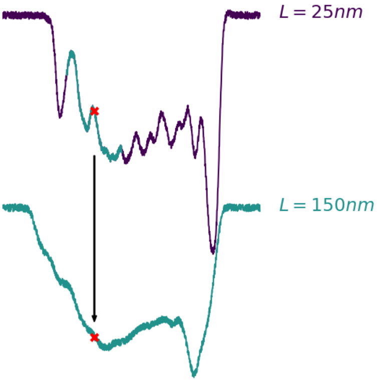
Quantitative length measurement

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

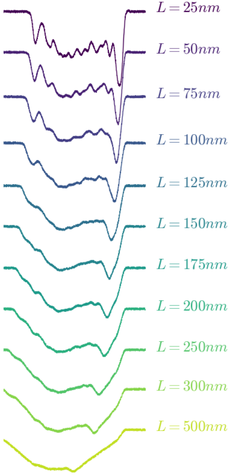
1. We perform a moving average transformation on the signals of shorter particles to simulate the signals of longer particles
2. Then, we compare an unknown particle's signal with each of the simulated signals of the shorter particle
3. The comparison with the greatest similarity yields the length of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

Quantitative length measurement—parametric signal transformation

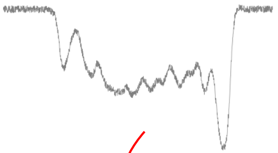
Single transformation



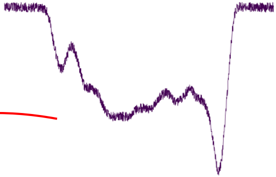
Multiple length transformations



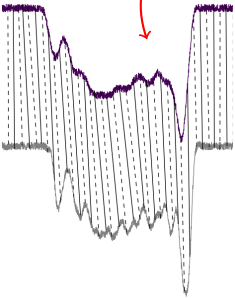
Quantitative length measurement—signal similarity measure



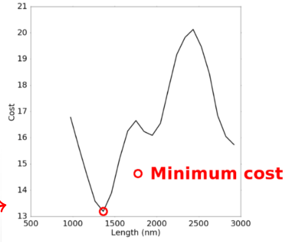
$L = ???nm$



$L = 50nm$



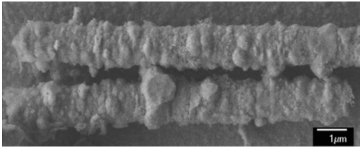
$$\text{Cost} = \sum_{(i,i')} \left[\left(\frac{\Delta I}{I_p} \right)_i - \left(\frac{\Delta I}{I_p} \right)_{i'} \right]$$



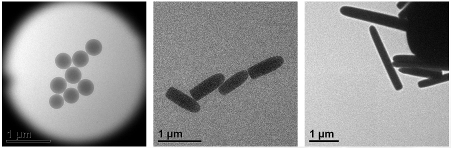
Length measurement experimental test

Can we implement and test the length measurement protocol?

- Experiments were conducted with single pores etched into PET membranes ($D \sim 750\,\mathrm{nm}$, $L = 12\,\mu\mathrm{m}$)
- Three types of particles were tested
  - 280 and 400 nm polystyrene beads (‘spheres’)
  - 590 nm rods (‘short rods’)
  - 1920 nm rods (‘long rods’)

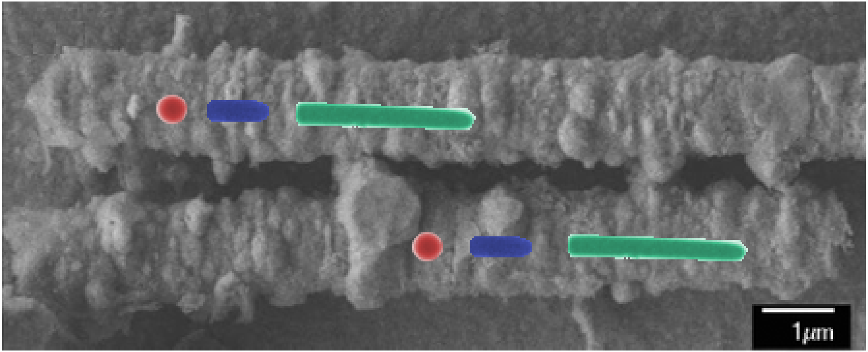


PET pore metal replica

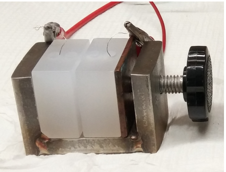


Nanoparticles

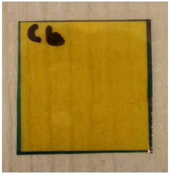
Particles to scale



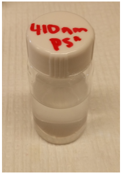
Polymer nanopore experiment components



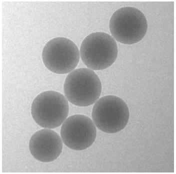
Conductivity cell



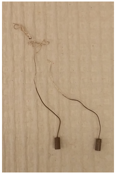
Pore membrane



Electrolyte



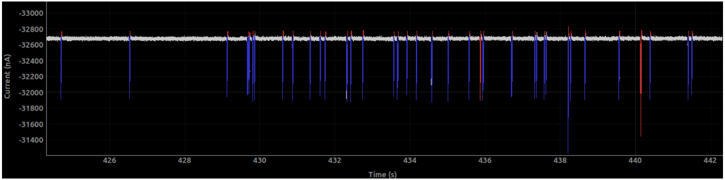
Particles



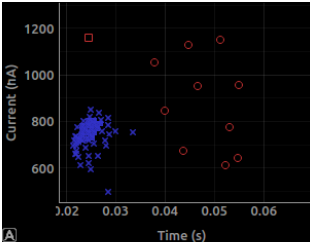
Ag-AgCl electrodes

Voltage amplifier + current recorder

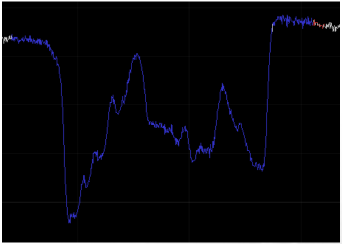
Results—raw data



Resistive pulse time series

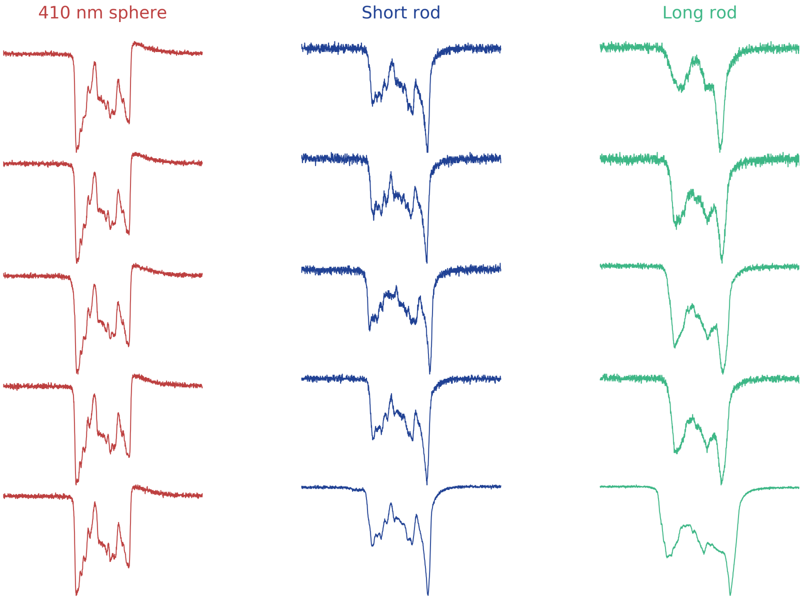


Amplitude-duration scatter

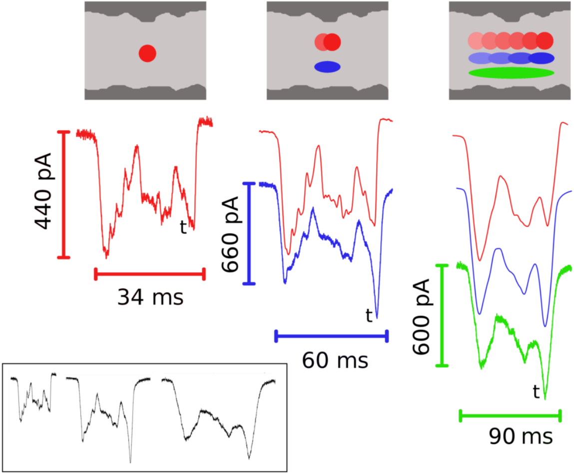


Raw event

Results—sphere, short rod, and long rod events

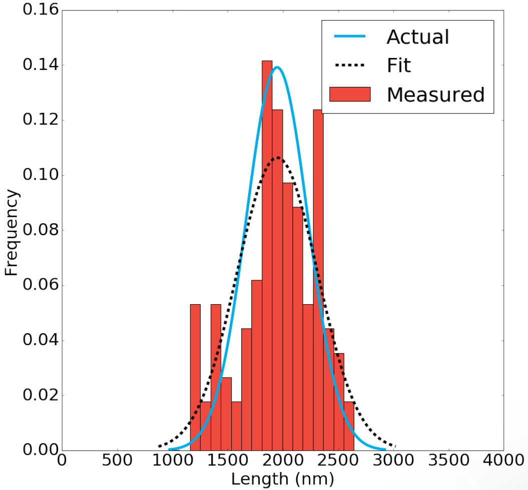


Results—Qualitative event comparison



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

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

This suggests the technique could be used to accurately particle length

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 planar 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 restrictive conditions or approximations that are seldom met in experimental systems

Sphere $$\frac{\Delta I}{I_p} = \frac{d^3}{D^2 L}\left[1 - 0.8\left(\frac{d}{D}\right)^3\right]^{-1}$$

Ellipsoid $$\frac{\Delta I}{I_p} = \left[f_\perp + \left(f_\parallel - f_\perp\right)\cos^2\alpha\right]\frac{v}{V}$$

Motivation—Why introduce optics?

In reality, the experimental set up can never be constrained to this degree

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 better interpretability of the resistive pulse 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, no such restriction is necessary at the *microscale*, above the optical diffraction limit!

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

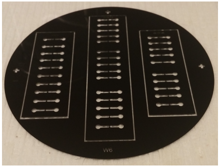
This requires adding a few elements to the standard resistive pulse set up

- 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

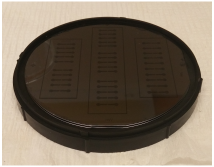
PDMS channel fabrication

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

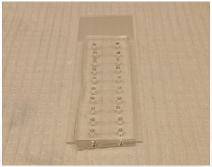
1. Channel design is printed onto a high-resolution phototransparency
2. Channel design is then transferred to a mold via standard soft photolithography
3. PDMS is poured over mold and removed after curing
4. PDMS bonded to a glass slide using an oxygen plasma



Phototransparency



Silicon/SU8 wafer



PDMS channels

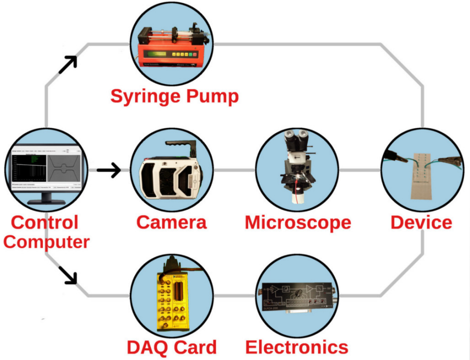
Hardware configuration

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

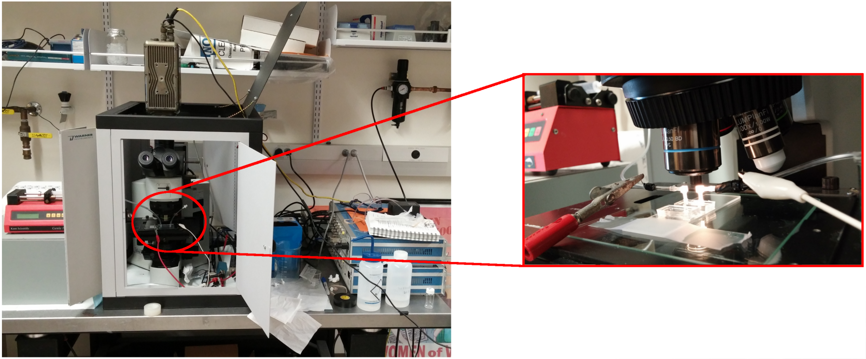
Electrodes are attached at the channel access ports for recording the RP signal

A particle suspension is driven through the channels *via* syringe pump

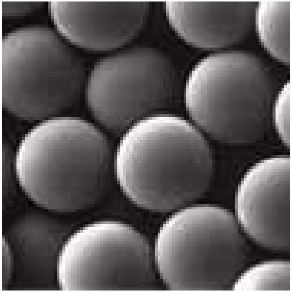
The camera and resistive pulse data are simultaneously recorded



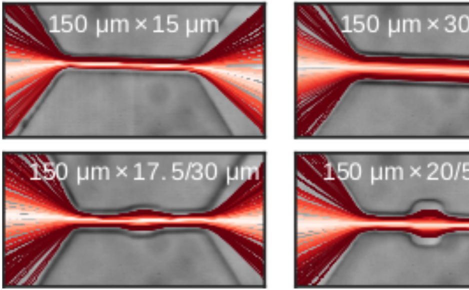
Hardware configuration



Channels and particles

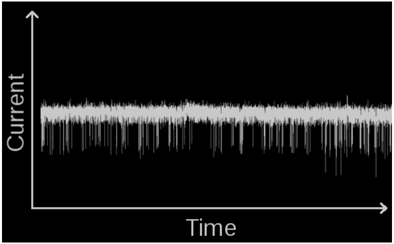


10 μm polystyrene beads



PDMS channels
Top row: straight
Bottom row: with cavity

Raw data—resistive pulse and optics



Raw RP series
data $= I(t)$

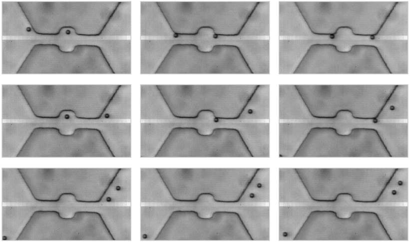
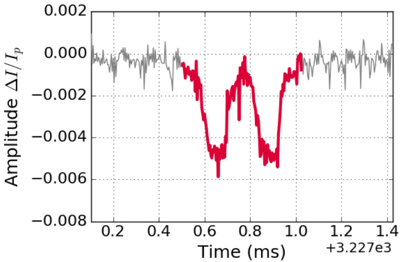


Image stills
data $= \{\text{frame1}, \text{frame2}, \ldots\}$

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 instanteous state of the channel (occupancy, occupant position) to the current level

Tracked events



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

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

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

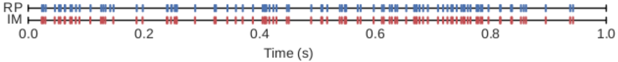
- RP events are detected via a threshold algorithm
- Individual particles are detected via image processing techniques and tracked across frames

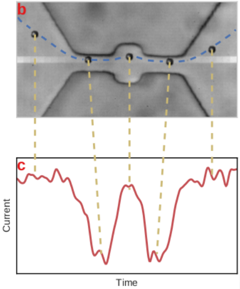
Synchronizing the two data sets

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 map

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

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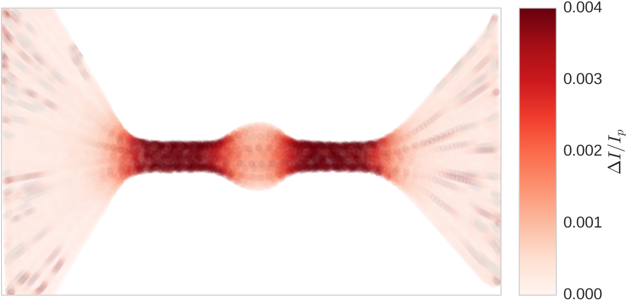

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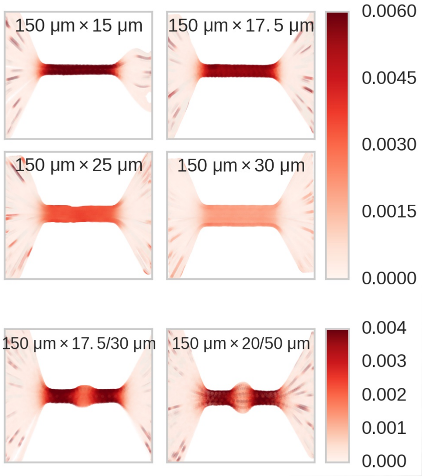


Key scientific questions

The hybrid resistive pulse-imaging platform, along with the resistance maps, 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?

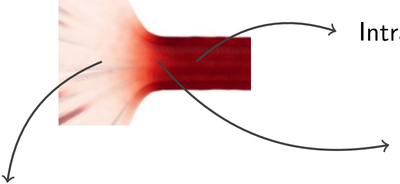
The resistance map allows these questions to be answered

Channel entrance effects

In RP experiments, event duration can be used to measure the $\zeta-$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

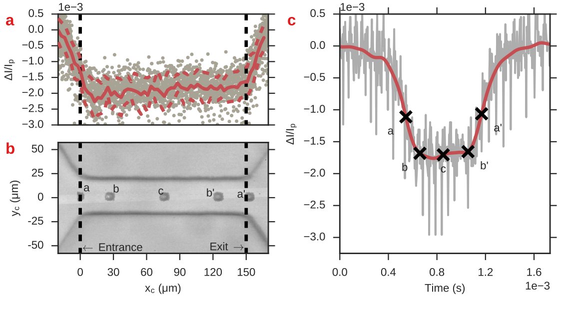


Channel entrance effects

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

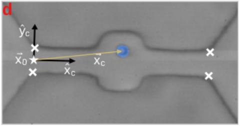
We found the full amplitude was not attained until the particle was well-within the channel, even as much as $\sim 10\,\mu$m (7% of the total channel length)

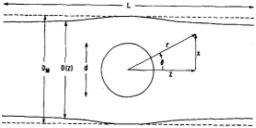
The channel-crossing threshold most closely coincides with the FWHM of the RP signal, suggesting that current value may be the most appropriate to choose for the channel entrance and exit positions



Effect of off-axis translocations

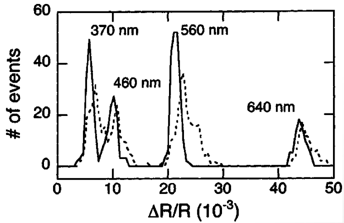
Electrostatic boundary conditions at the surface of the insulating particle leads to distortion of the electric field in its vicinity



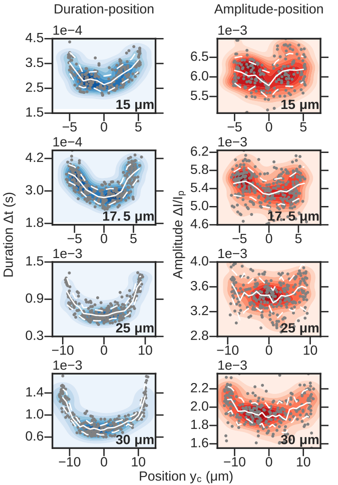


When the particle travels off-axis, this distortion couples with the distortion of the $\vec{E}$ field in the vicinity of the channel, increasing the total system resistance

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

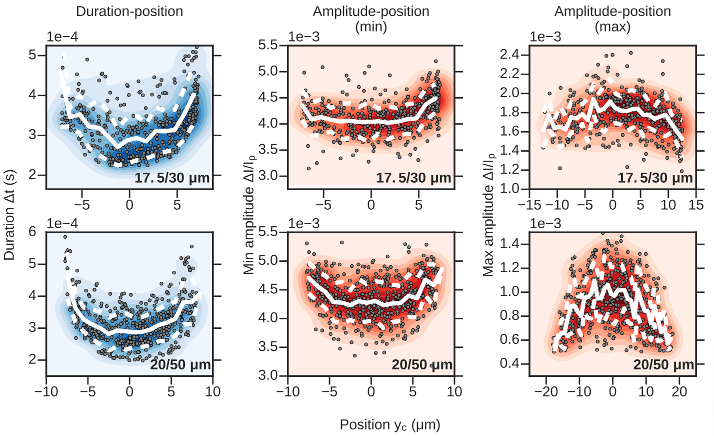


Off-axis translocation in straight channels



The scatter plots clearly show an increase in event amplitude with transverse displacement

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 future work

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

In the future, we could look at translocations for aspherical particles

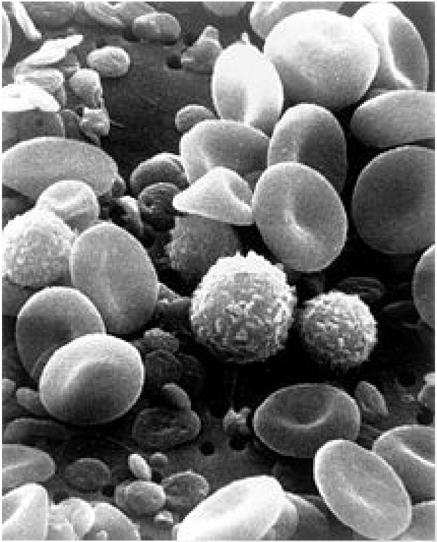
Particle rotation dynamics can affect the amplitude of the resistive pulse signal, and the hybrid approach could be used to better understand this effect

In particular, we are 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 are very predictive of cell type

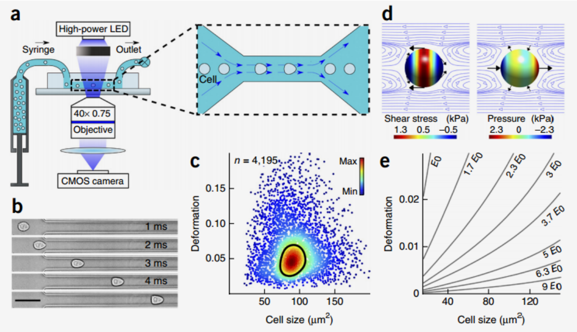


Cell mechanical properties

- Another important mechanical feature is stiffness—cells behave like elastic deforming 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.* 2015. Biophys. J. 109:2023-2036.

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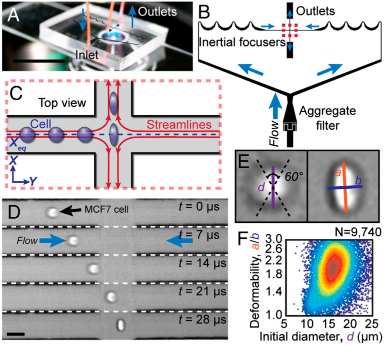


Gossett *et al.* 2012. PNAS 109:7630-7635.

Optical measurement of deformation

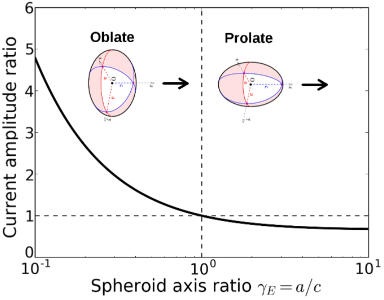
- Currently, cell deformation response is primarily determined via high-speed microscopy, which comes with a few disadvantages, including
  - High cost; high-speed camera can cost upwards of $100k
  - Computationally expensive—current cell throughput is limited to $\sim 100\,\mathrm{cells/second}$ for online analysis
- Can we replace imaging with resistive pulse, which doesn't suffer these drawbacks?

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



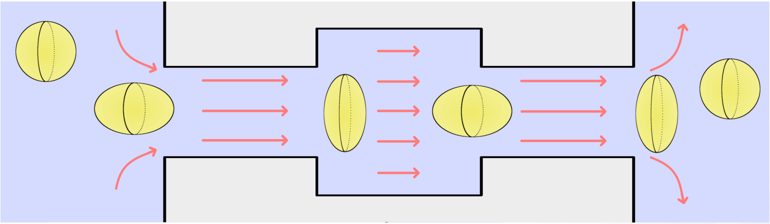
Proposed channel design for inducing deformation

Consider channels containing a central cavity

Mass continuity dictates that the fluid flow slows in the cavity

Accelerating extensional flows pull the particle into an elongated geometry

Decelerating flows compress the particle axially

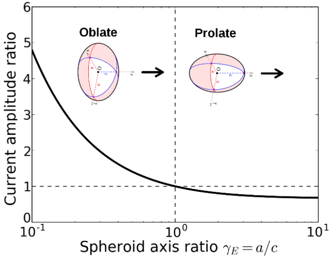


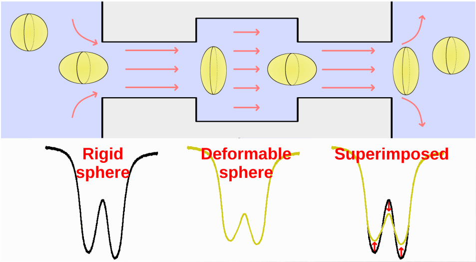
Resistive pulse distortion

According to the deformation motif presented in the previous slide, deformations should follow the following pattern

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

$$s : \text{spherical}, \; p : \text{prolate}, \; o : \text{oblate}$$





Key scientific 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

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1. Do the cells deform?

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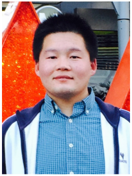
Dr. Elif Türker Acar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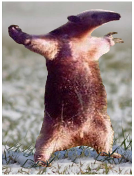
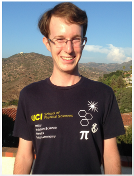
James Boyd


Joseph Martinez


Cody Combs


Rachel Lucas


Freida Rivera


Aaron Ellis

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