Resistive-pulse sensing at the micro- and nanoscale

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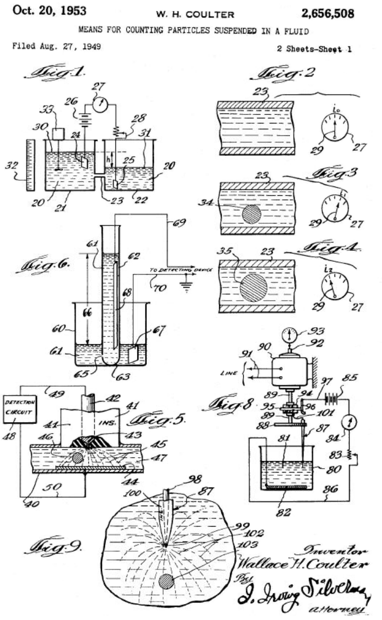
Outline

- 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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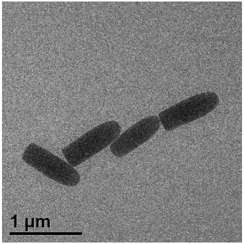

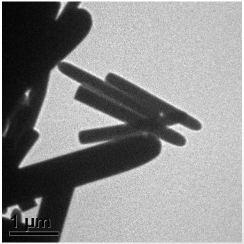

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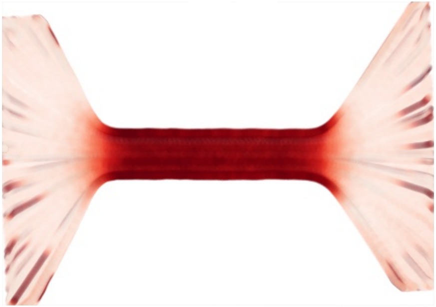

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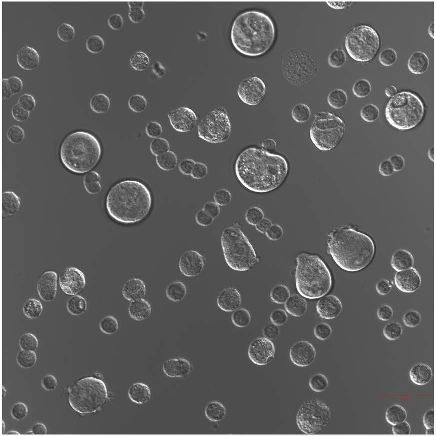
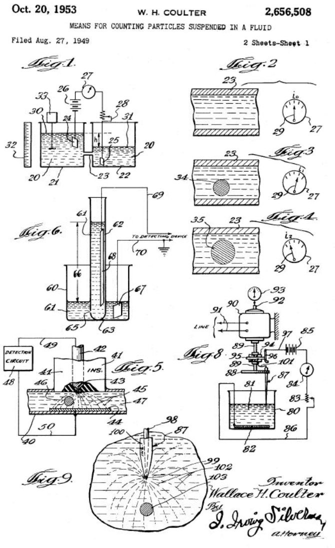


Resistive pulse sensing background

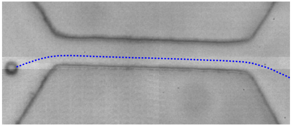
Resistive pulse sensing—description

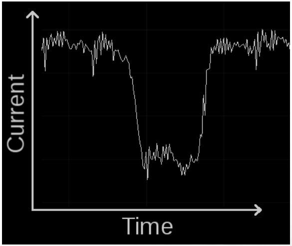
- Resistive pulse sensing (RP) is a method for single particle detection and characterization
- Works at any scale (nano, micro, milli, etc.)
- A diverse range of applications: red blood cell counting (several μm, virus detection $(10 - 100\,\mu$m, and DNA sequencing $(\sim 1\,$nm), among others

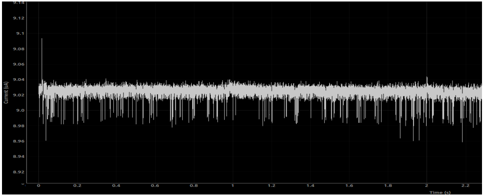


Resistive pulse sensing—how does it work?

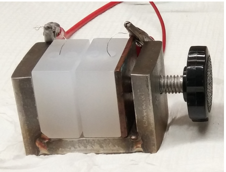
- A nanopore immersed in electrolyte solution acts as an ionic resistor
- Current-Voltage relationship follows Ohm's law $V = IR$
- When a particle enters the channel its resistance changes, yielding a pulse in the measured ionic current
- Pulse properties yield information on size, shape, charge, and concentration of particle



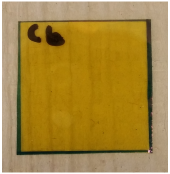




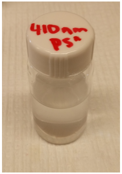
Resistive pulse sensing—system components



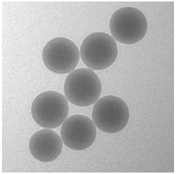
Conductivity cell



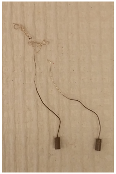
Pore membrane



Electrolyte



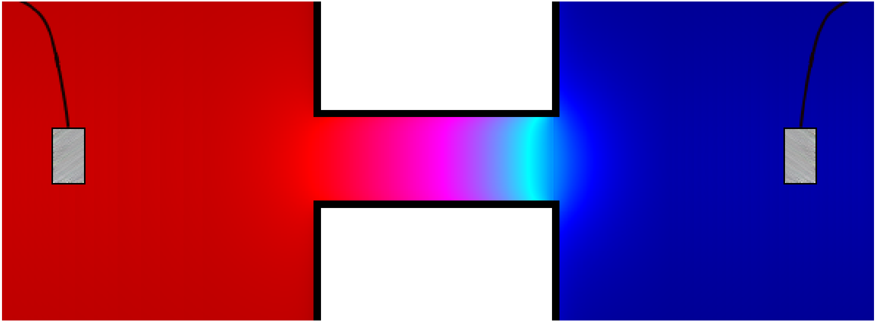
Particles



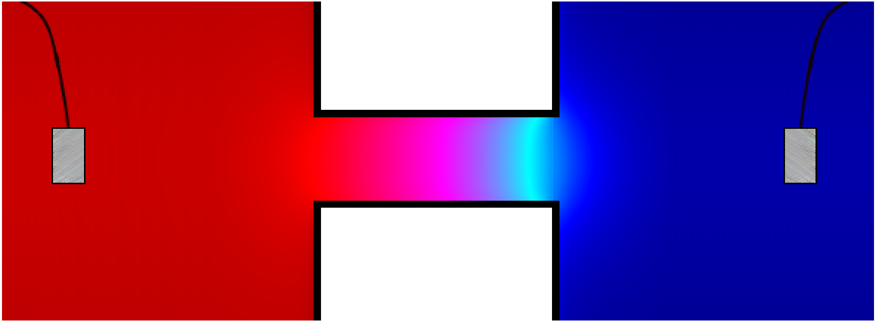
Ag-AgCl
electrodes

Voltage amplifier + current recorder

Resistive pulse sensing



Resistive pulse sensing—electrostatic boundary conditions



Resistive pulse sensing—ion transport

- Ion transport is driven by **diffusion**, **convection**, and **electric migration**
- Diffusion: Average flow of ions from high to low concentration
- Convection: Ions move with the fluid/solvent
- Electrical migration: Ions move in electric field

$$\vec{J}_i = \underbrace{z_i e D_i \nabla c_i}_{\text{diffusion}} + \overbrace{z_i e c_i \vec{u}}^{\text{convection}} + \underbrace{z_i e c_i \mu_i \vec{E}}_{\text{migration}}$$

$$I = \sum_i \iint_S \vec{J}_i \cdot \hat{n} dS$$

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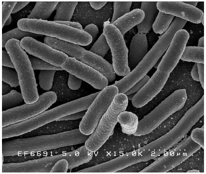
Resistive pulse sensing of high-aspect ratio particles

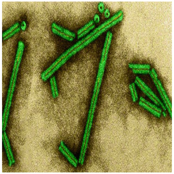
High-aspect ratio resistive pulse sensing—motivation

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



e. coli

$L \sim 2\,\mu$m



tobacco mosaic virus

$L \sim 300\,$nm

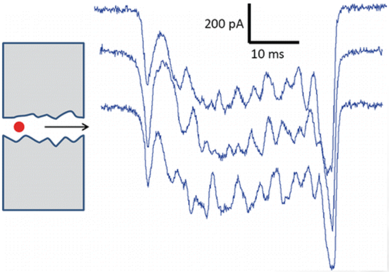
- The ability to measure particle shape is highly desirable for sensing applications
- How can we extend RP sensing to measure length in addition to volume?

Resistive pulse in non-constant width pores

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

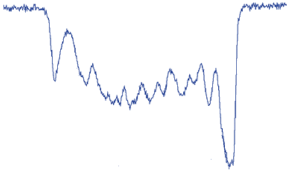
$$\Delta R\left(z'\right) = \frac{\rho}{\pi}\left[\int_{z=z'}^{z=z'+l_p}\left(\frac{1}{r_P^2\left(z\right) - s_p^2\left(z\right)} - \frac{1}{r_P\left(z\right)^2}\right) dz\right]$$

- 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!

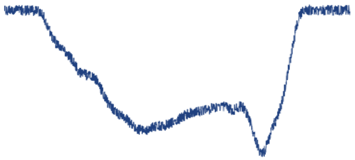


RP signal resolution

- 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



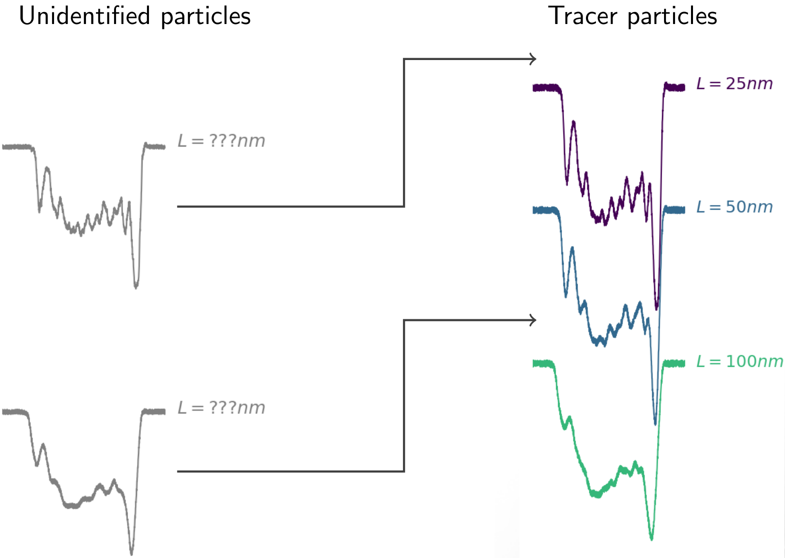
Short particle



Long particle (simulated)

Can we use this knowledge to measure particle length???

Qualitative length comparison



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

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

In terms of the measured RP signal, we relate resistance to current via

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

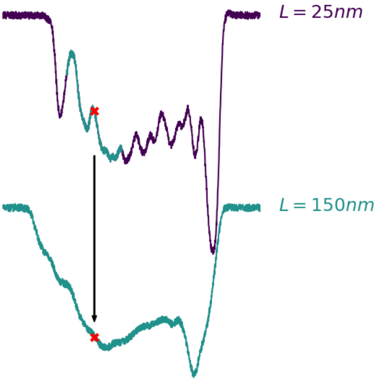
Quantitative length measurement

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

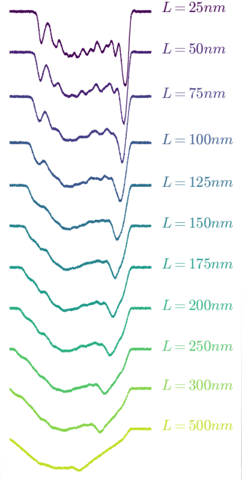
1. We transform the signals of short particles via a moving average to simulate the signals of longer particles
2. Then, we compare an unknown particle's signal with each of the simulated signals of the shorter particle
3. The comparison with the greatest similarity yields the length of the particle

Quantitative length measurement—parametric signal transformation

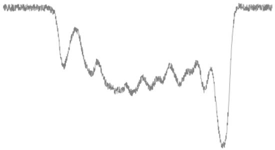
Single transformation

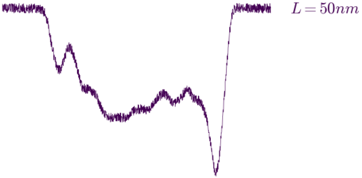


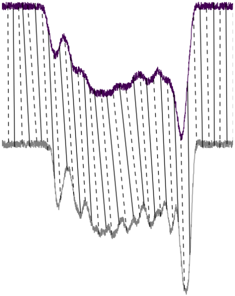
Multiple length transformations



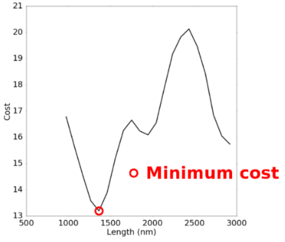
Quantitative length measurement—signal similarity measure







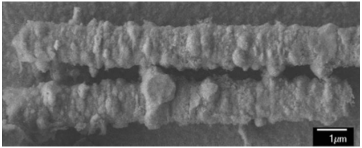
$$\text{Cost} = \sum_{(i,i')} \left[\left(\frac{\Delta I}{I_p} \right)_i - \left(\frac{\Delta I}{I_p} \right)_{i'} \right]$$



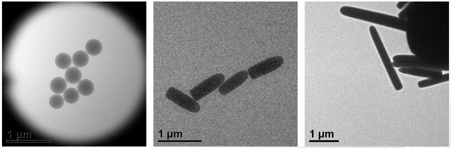
Length measurement experimental test

Can we experimentally 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 400 nm polystyrene beads ('spheres')
  - 590 nm rods ('short rods')
  - 1920 nm rods ('long rods')

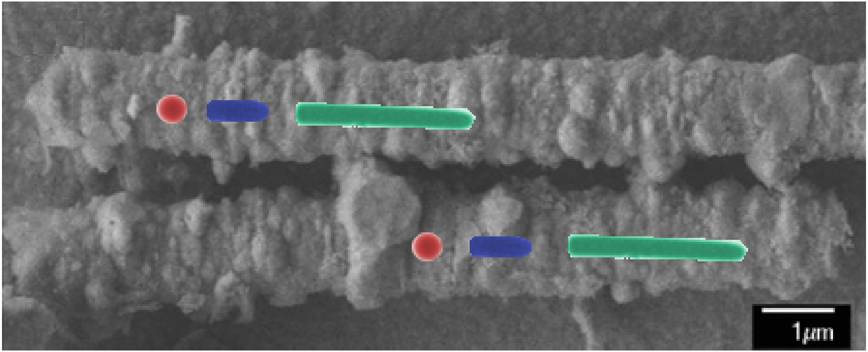


PET pore metal replica

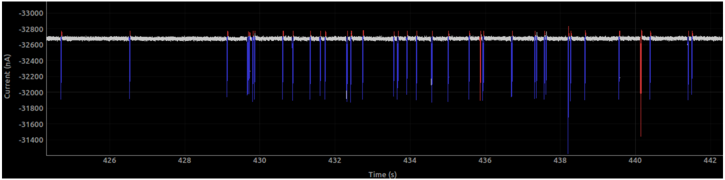


Nanoparticles

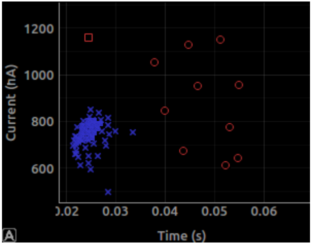
Particles to scale



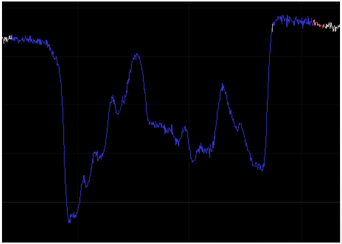
Results—raw data



Resistive pulse time series

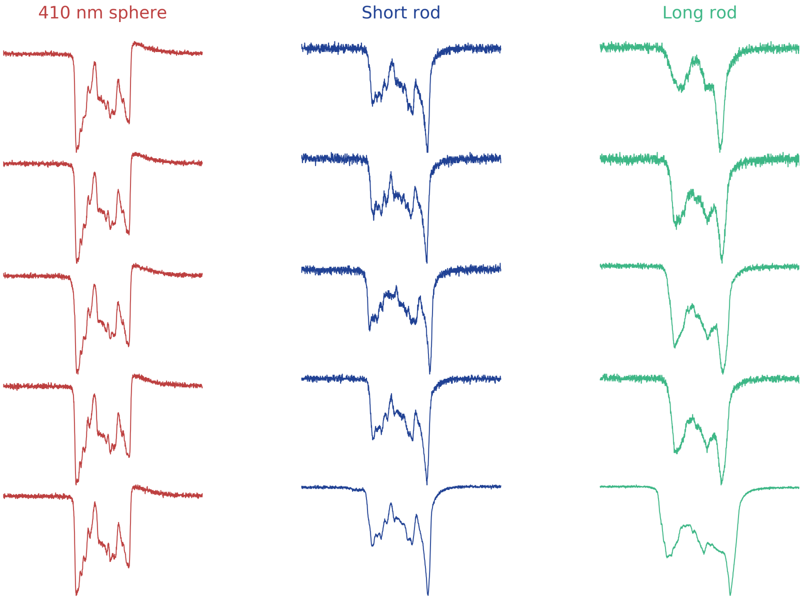


Amplitude-duration scatter

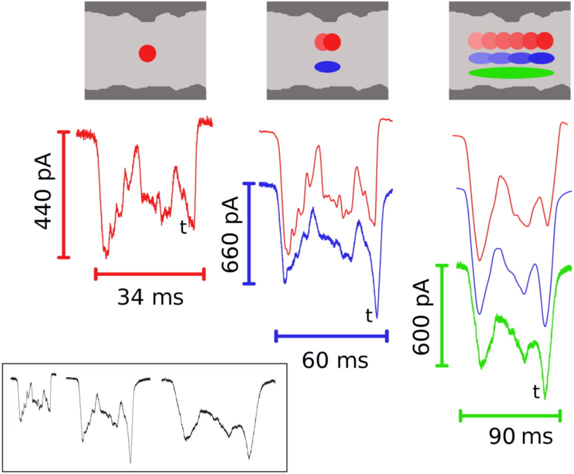


Raw event

Results—sphere, short rod, and long rod events

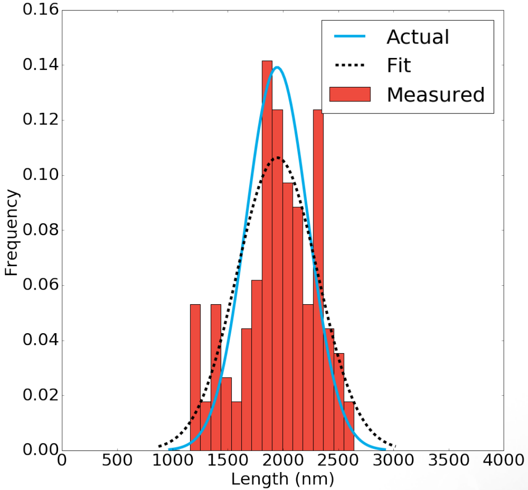


Results—Qualitative event comparison



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!

Future work

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 1

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 the physical observable are only accurate under restricting conditions that are seldom met in experimental systems

For instance, spheroids moving along the axis of an infinitely long cylinder

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 2

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 3

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 4

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

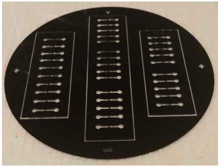
However, no such restriction is necessary at the *microscale*, above the optical diffraction limit!

The results should generalize to the nanoscale as well, since the confounding factors arise due to electrostatic boundary conditions that are scale independent (in the mean-field approximation)

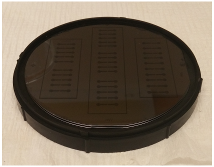
Objective: Create a hybrid resistive pulse-optical characterization platform

PDMS channel fabrication

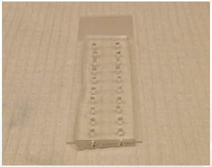
1. Channel design is printed onto a high-resolution phototransparency
2. SU8 photoresist is spun onto a silicon wafer
3. UV light shone through the transparency and onto the SU8, cross-linking the polymer bonds
4. Wafer developed with SU8 developer, leaving the mold for the channels
5. PDMS is poured onto the channel mold and cured
6. PDMS removed from wafer and bonded with glass slide



Phototransparency



Silicon/SU8 wafer



PDMS channels

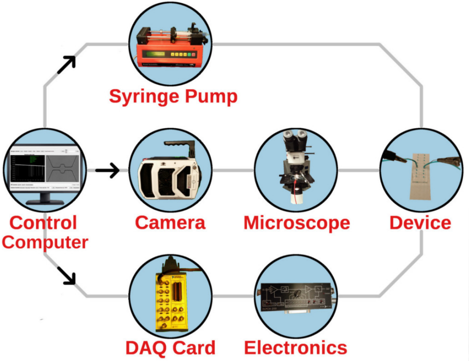
Hardware configuration

Device is placed on the stage of a microscope, which has a high-speed camera attached for capturing the images

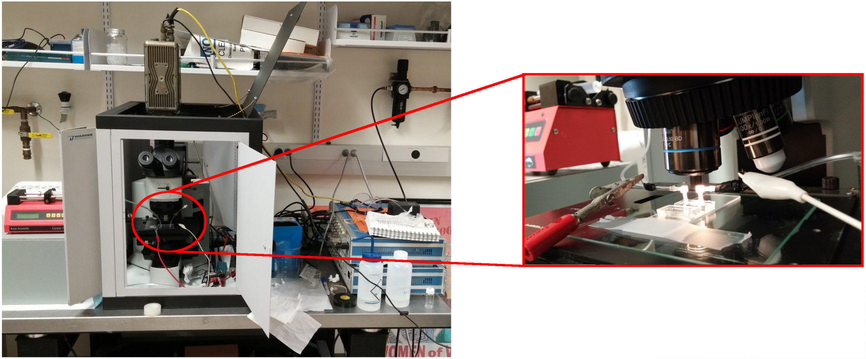
Electrodes are attached at the channel access ports for recording the RP signal

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

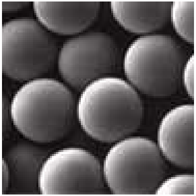
The camera and resistive pulse data are simultaneously recorded



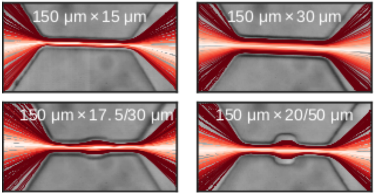
Hardware configuration



Channels and particles

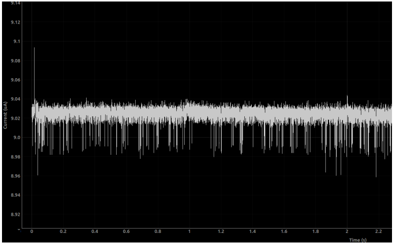


10 μm polystyrene beads



PDMS channels
Top row: straight
Bottom row: with cavity

Raw data—resistive pulse and optics



Raw RP series

data $= I(t)$

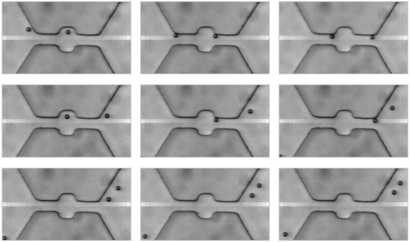


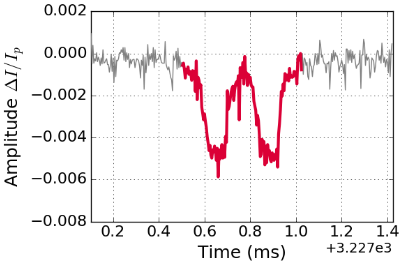
Image stills

data $= \{\mathrm{frame1}, \mathrm{frame2}, \ldots\}$

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

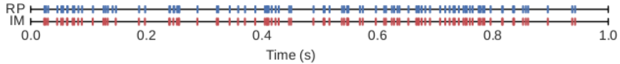
- RP events are detected via a threshold algorithm
- Individual particles are detected via image processing techniques and tracked across frames

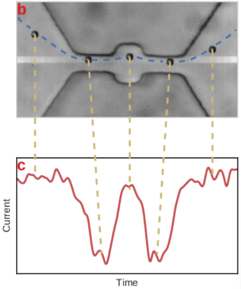
Synchronizing the two data sets

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

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





Resistance maps—video

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