

Tunable resistive pulse sensing analysis of extracellular vesicles

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A. Sturk, R. Nieuwland

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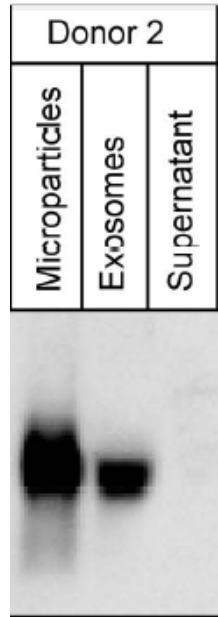
*Vesicle Observation Center, Academic Medical
Center, University of Amsterdam, The Netherlands*



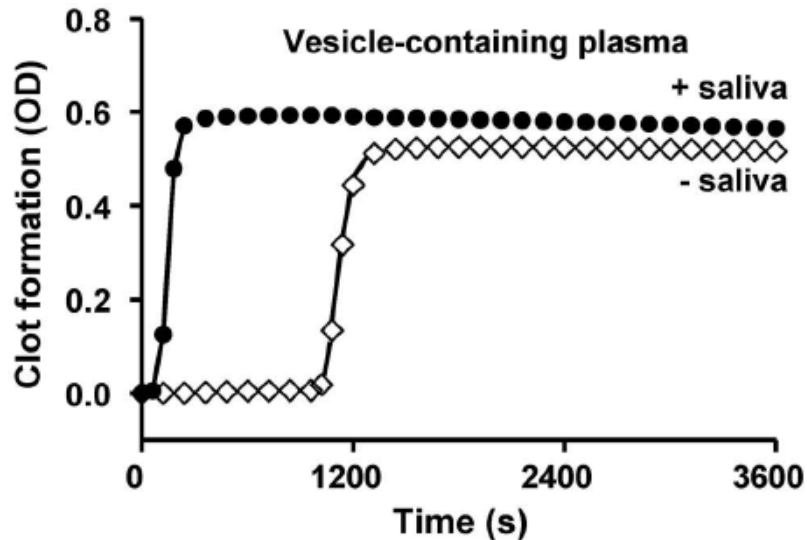
**Vesicle
Observation Center**



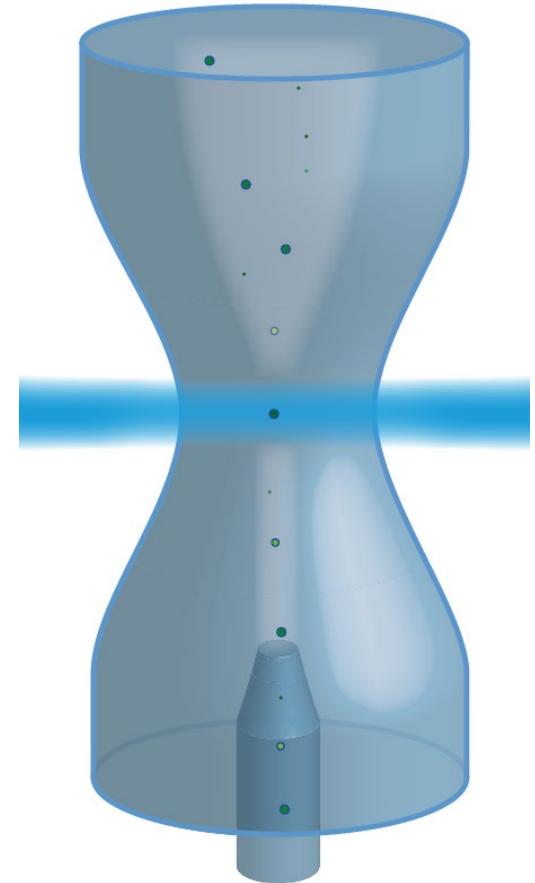
Common goal: information from extracellular vesicles (EV)



bulk methods



functional methods

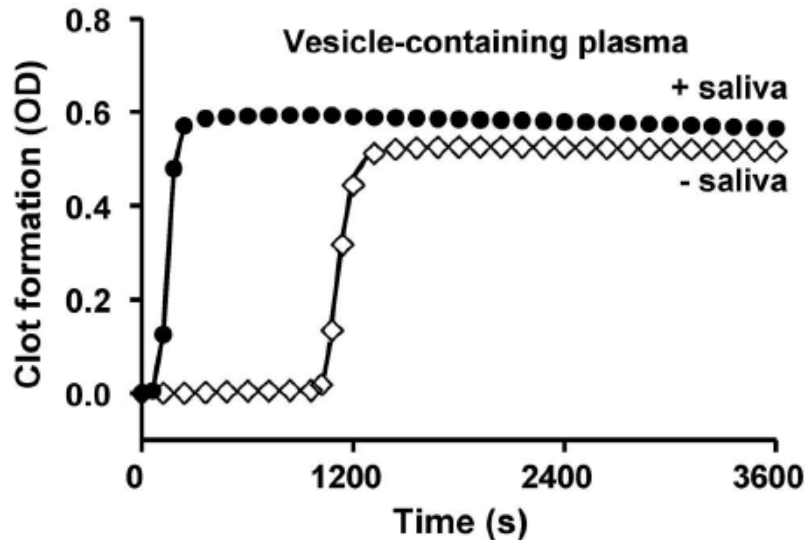


single particle methods

Common goal: information from extracellular vesicles (EV)



bulk methods



functional methods

Izon, qNano



single particle methods

Common goal: EV detection with single particle methods

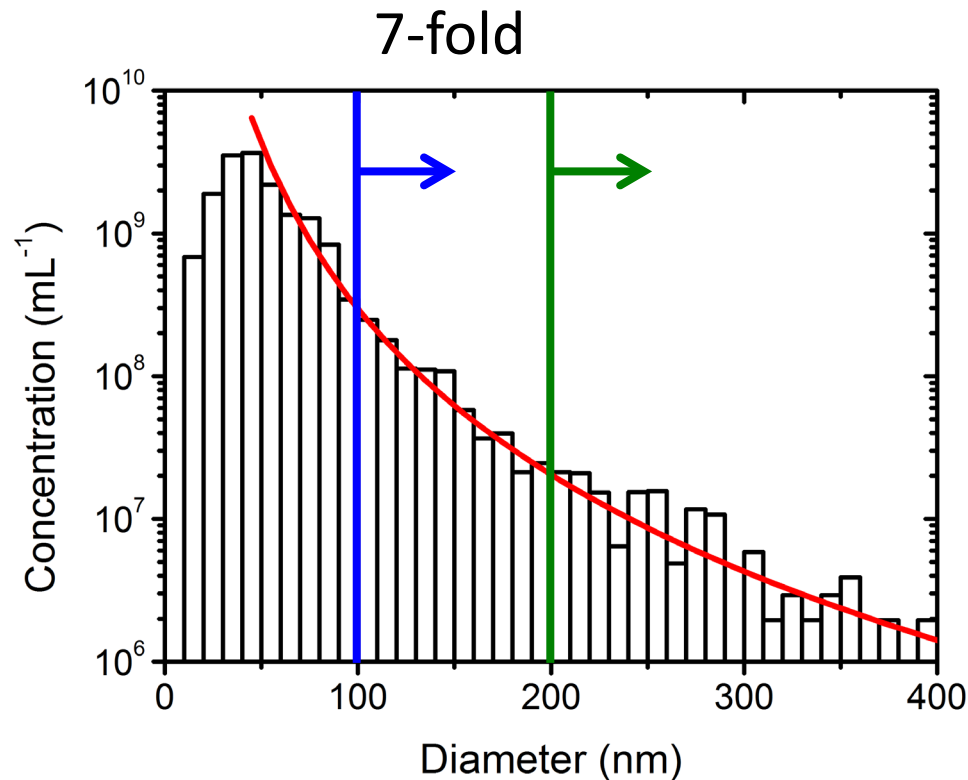
*if 1 / 1,000 EV is rare
you need to count 1,000,000 EV
(3% error)*

instrument requirements

- differentiate EV
- count $\geq 10^6$ EV
- reproducible



Common goal: reproducible EV detection with single particle methods



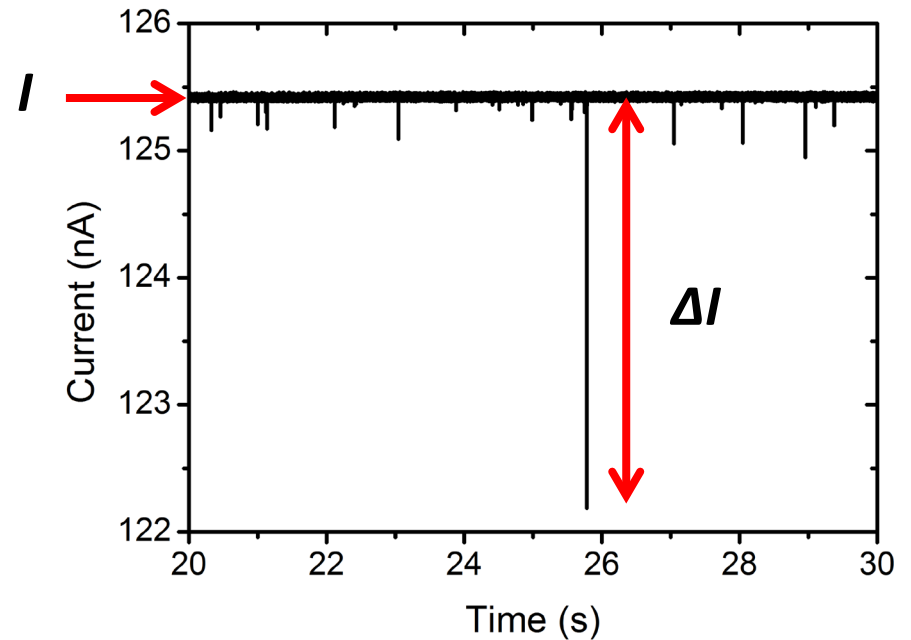
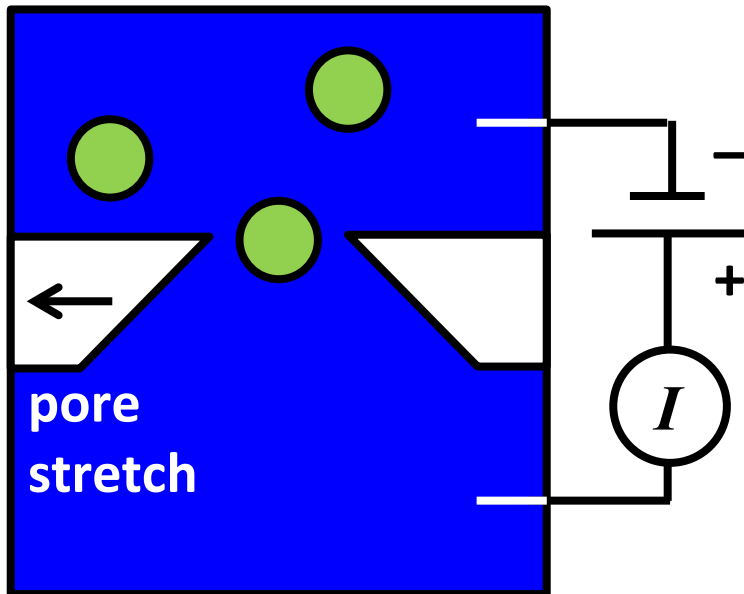
EV concentration

~~10^9 mL^{-1}~~

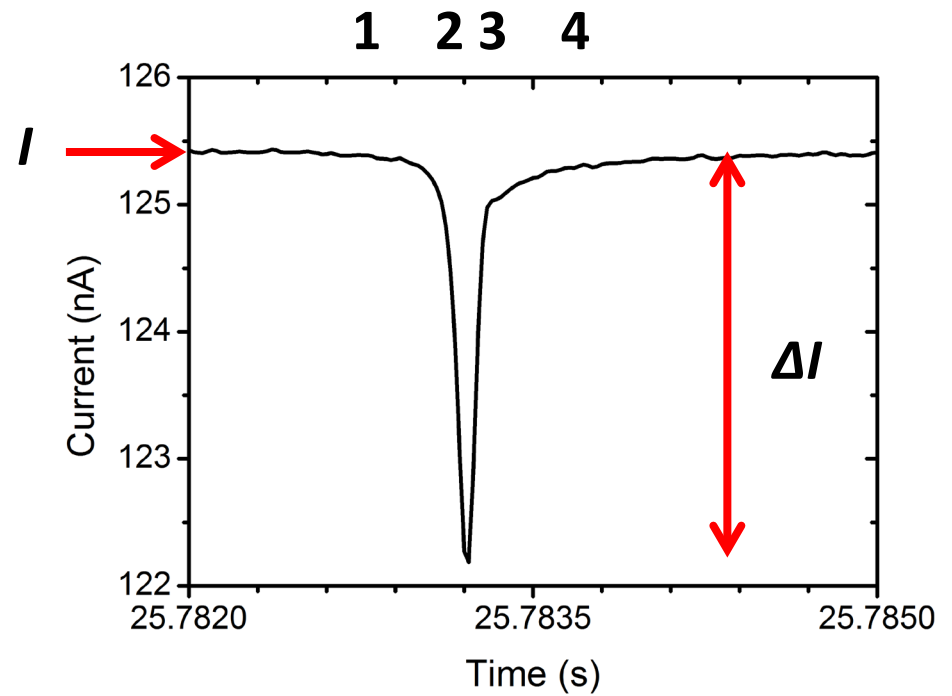
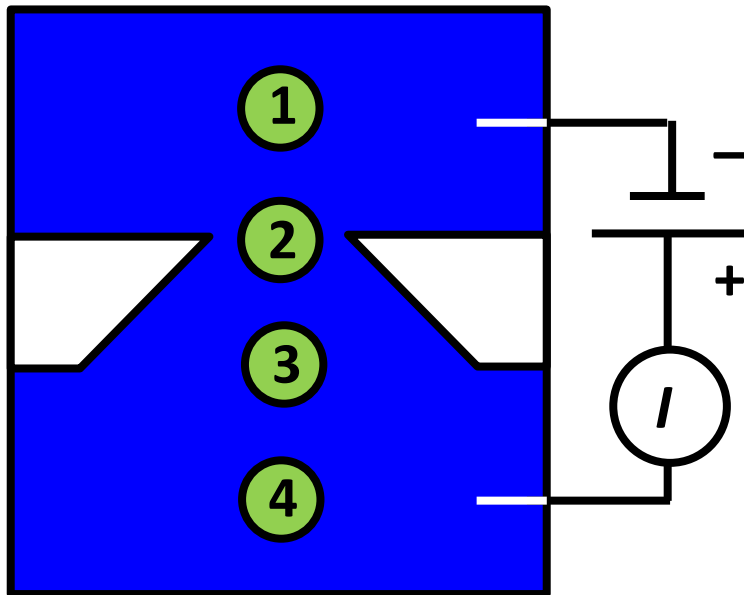
$10^9 \text{ mL}^{-1} (100 < d < 400 \text{ nm})$

- a concentration statement includes a size range

Tunable resistive pulse sensing (TRPS) principle



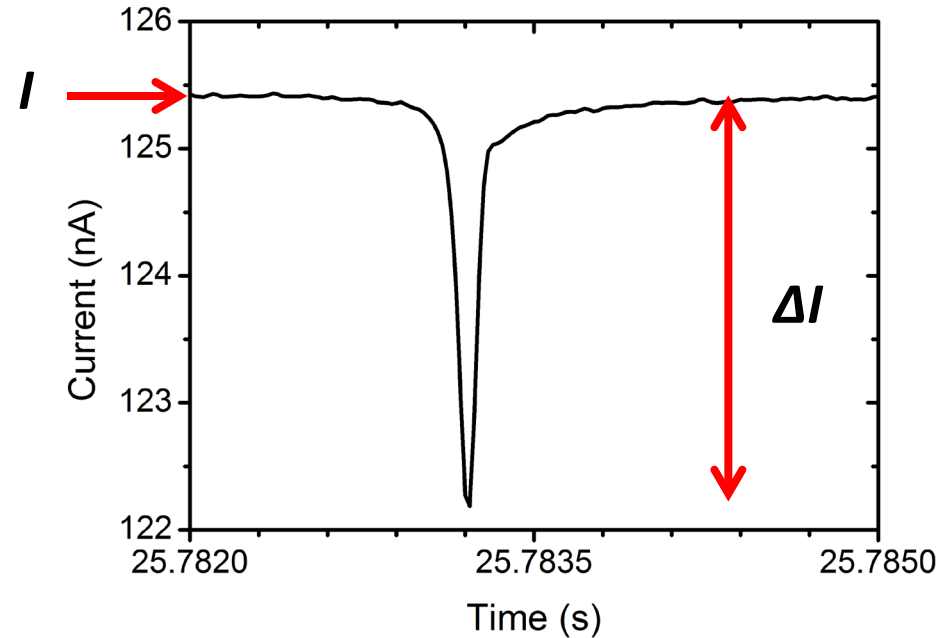
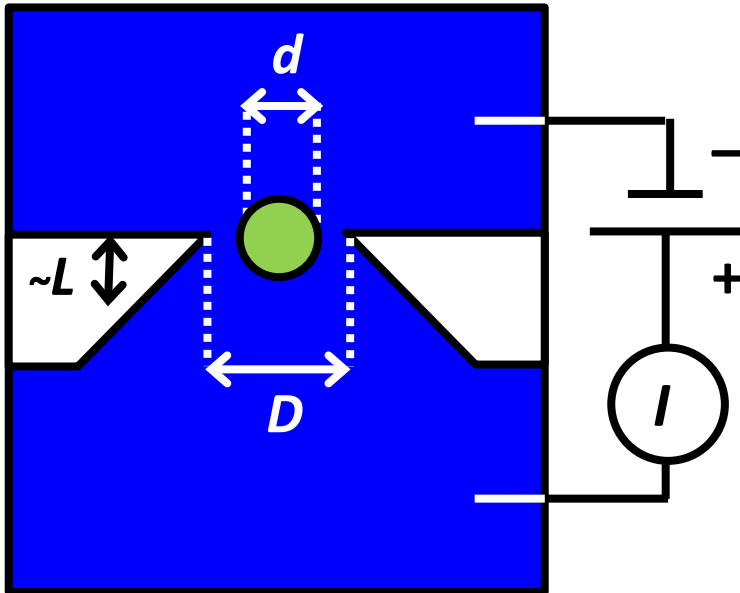
Size determination



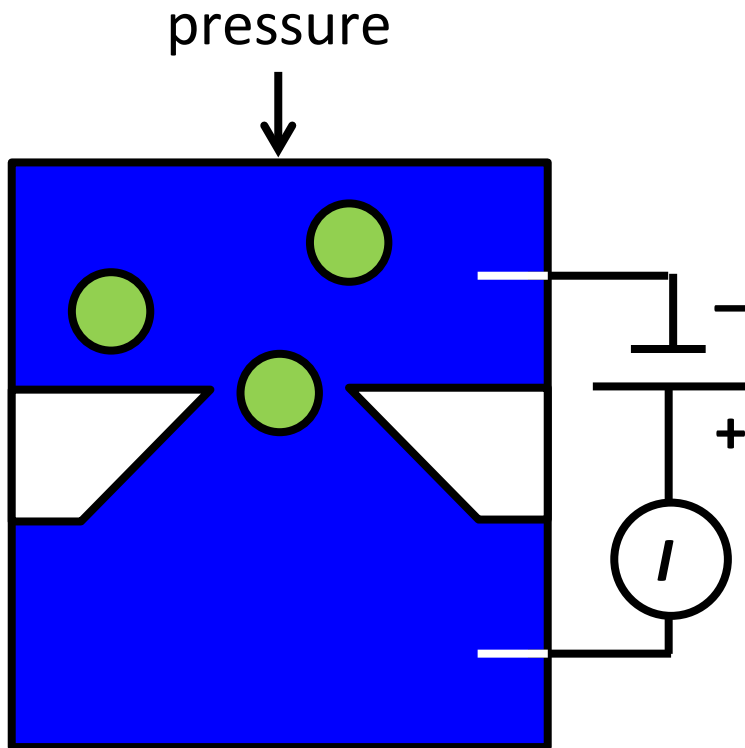
Size determination

$$\frac{\Delta I}{I} = \frac{d^3}{cLD^2}$$

c scaling factor
 cLD^2 obtain by calibration
with reference beads



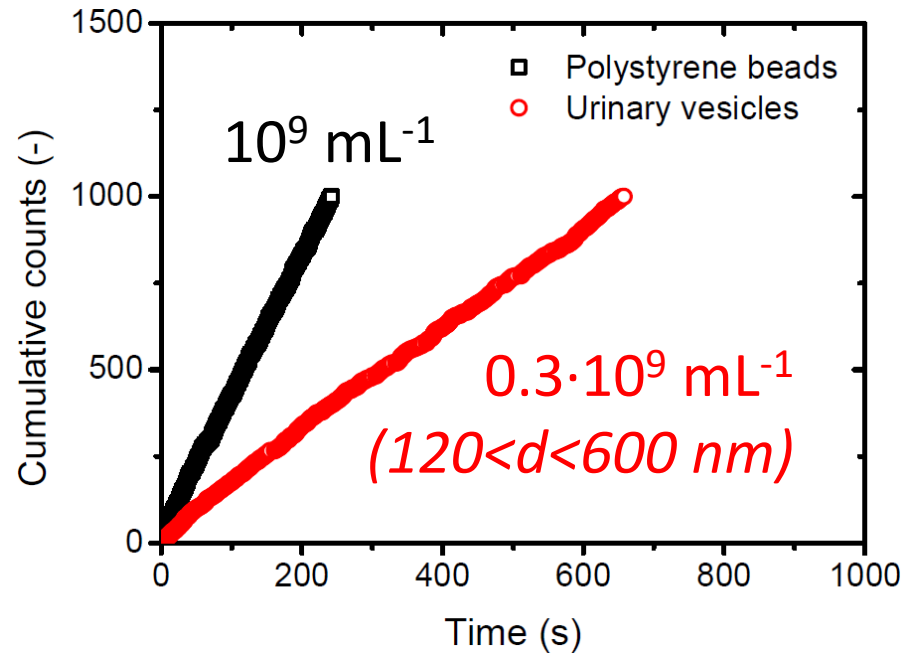
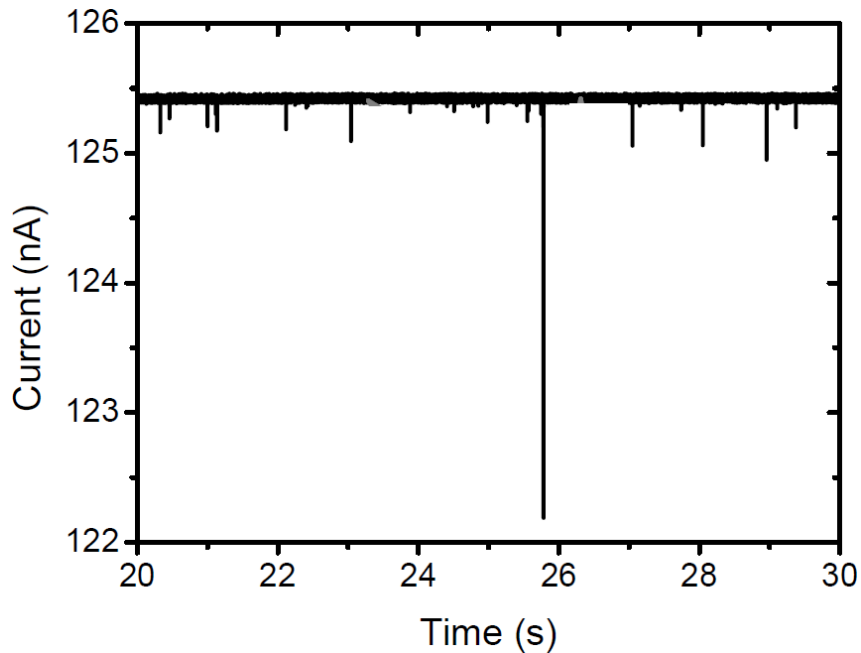
Why particles move through the pore



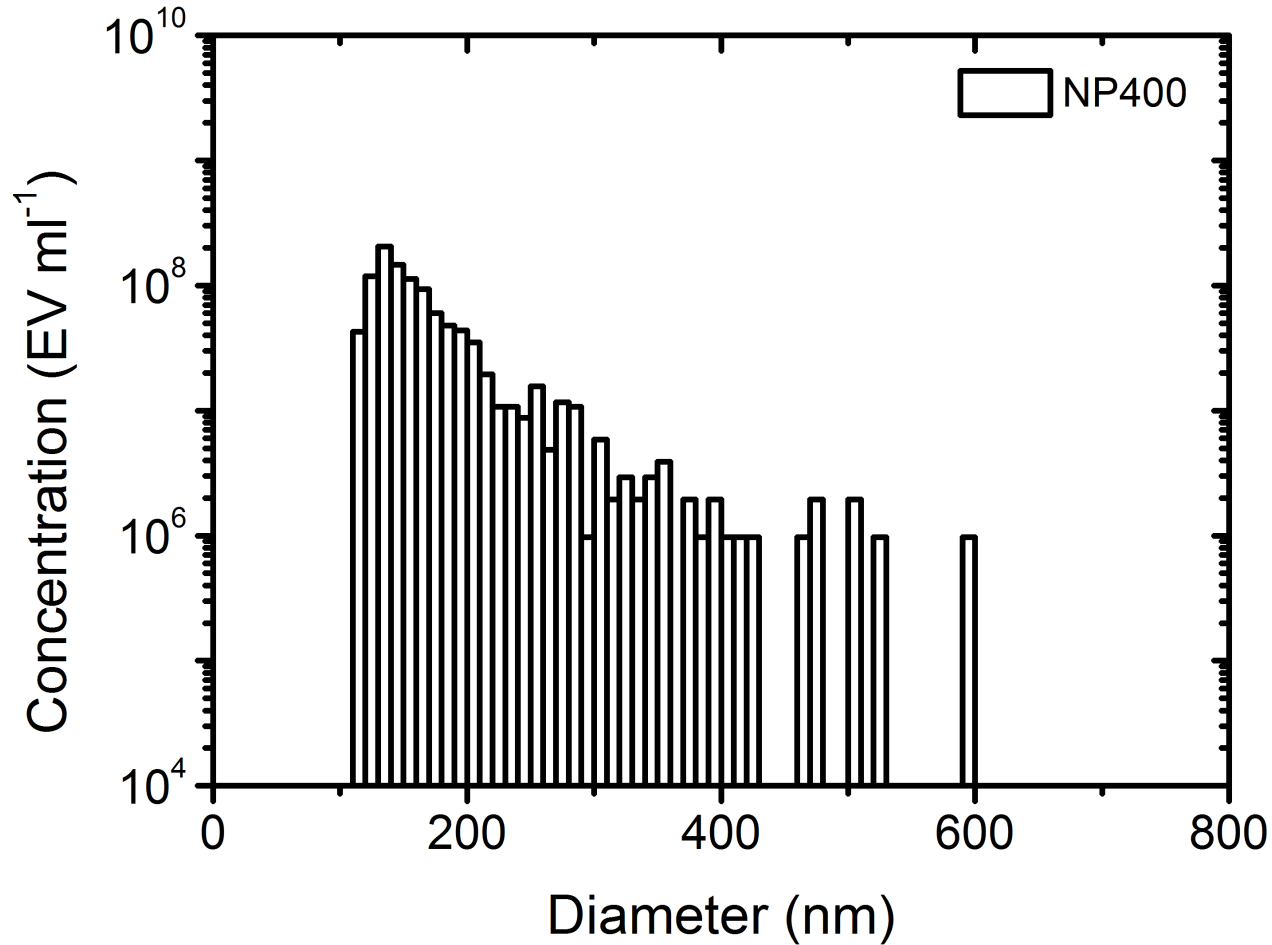
- Brownian motion
- electroosmosis
- electrophoresis
- external pressure

Concentration determination

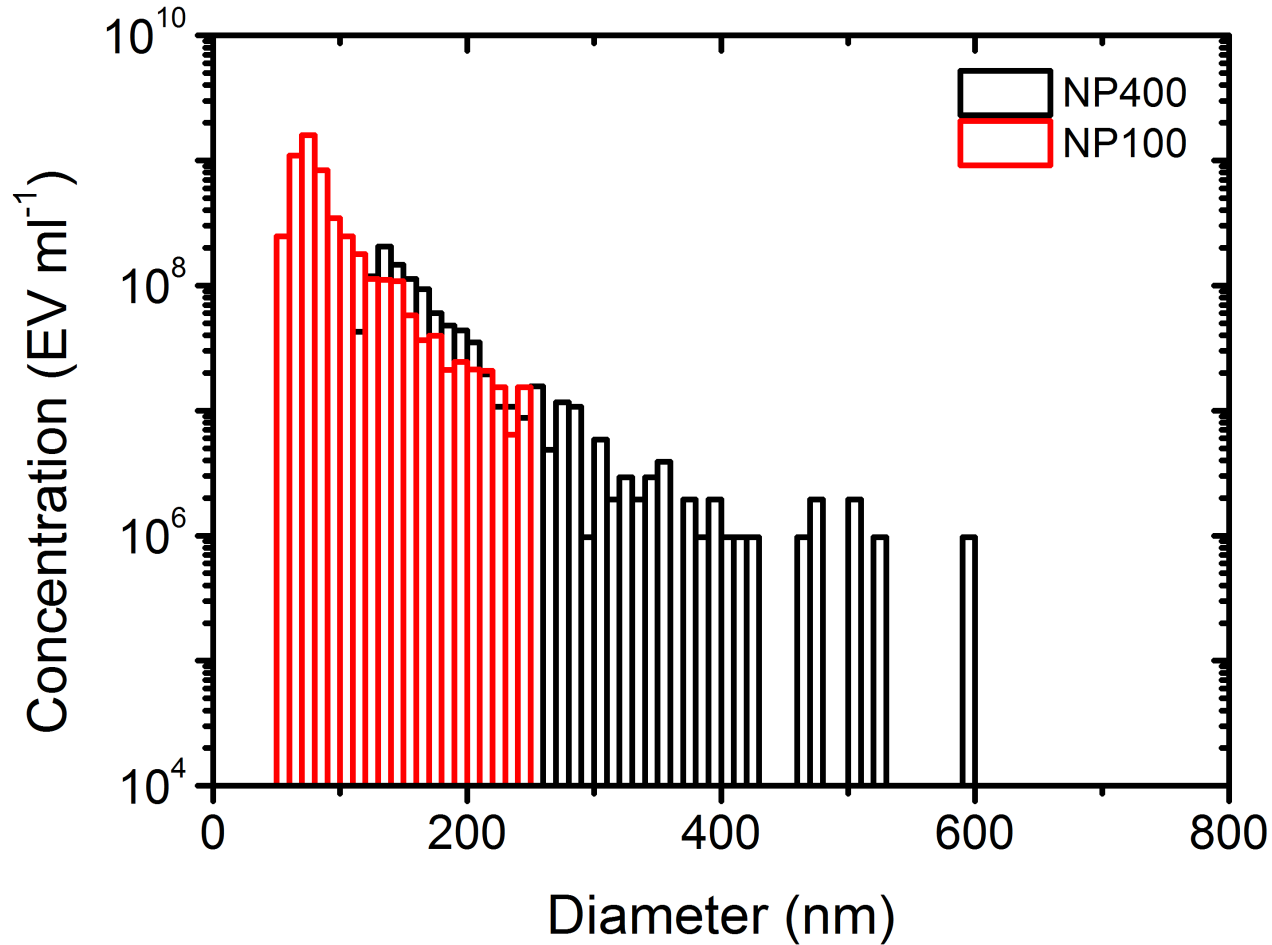
external pressure dominates flow:



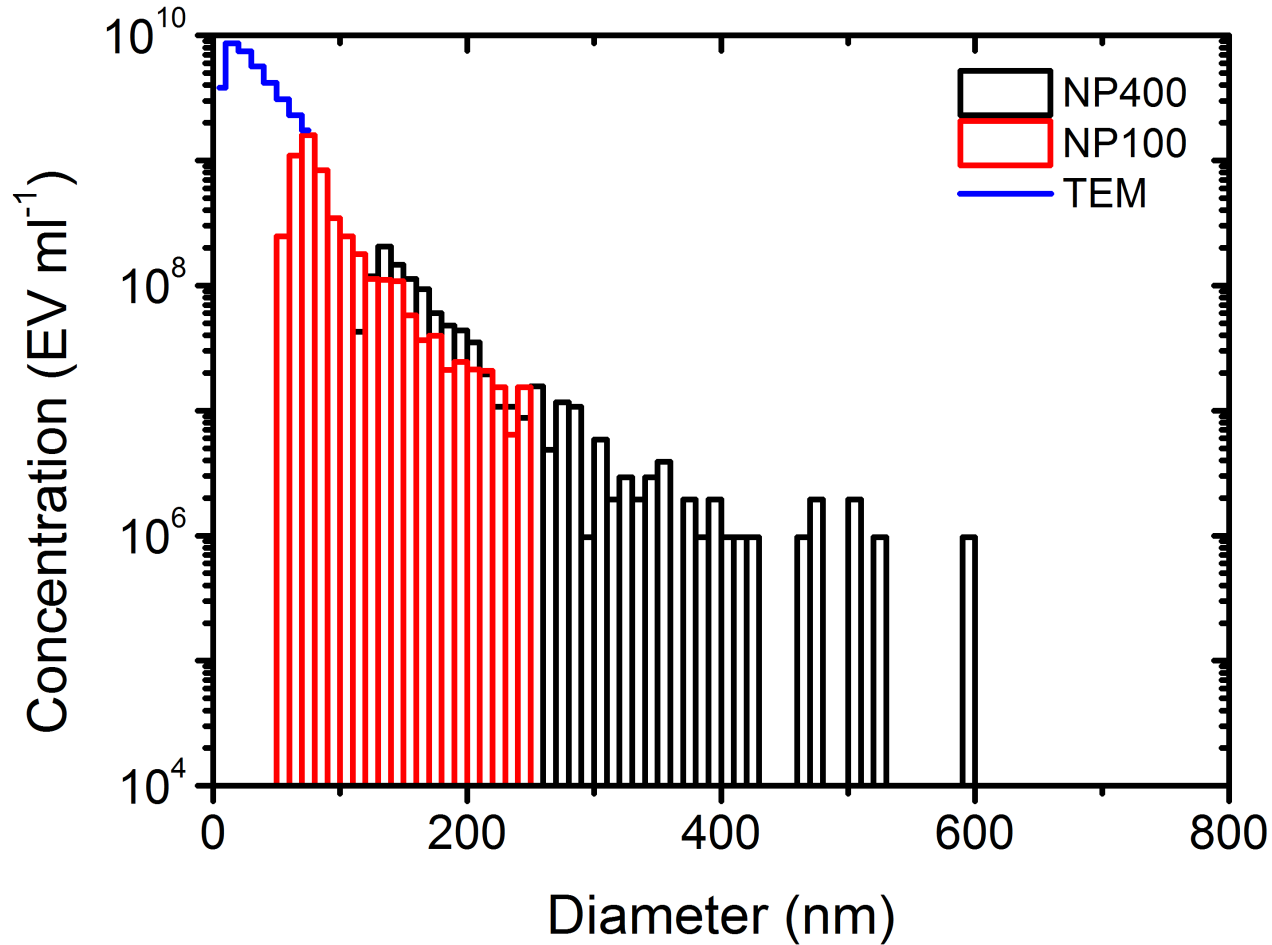
TRPS: size and concentration example



TRPS: size and concentration example



TRPS: size and concentration example



Common goal: EV detection with single particle methods

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(3% error)*

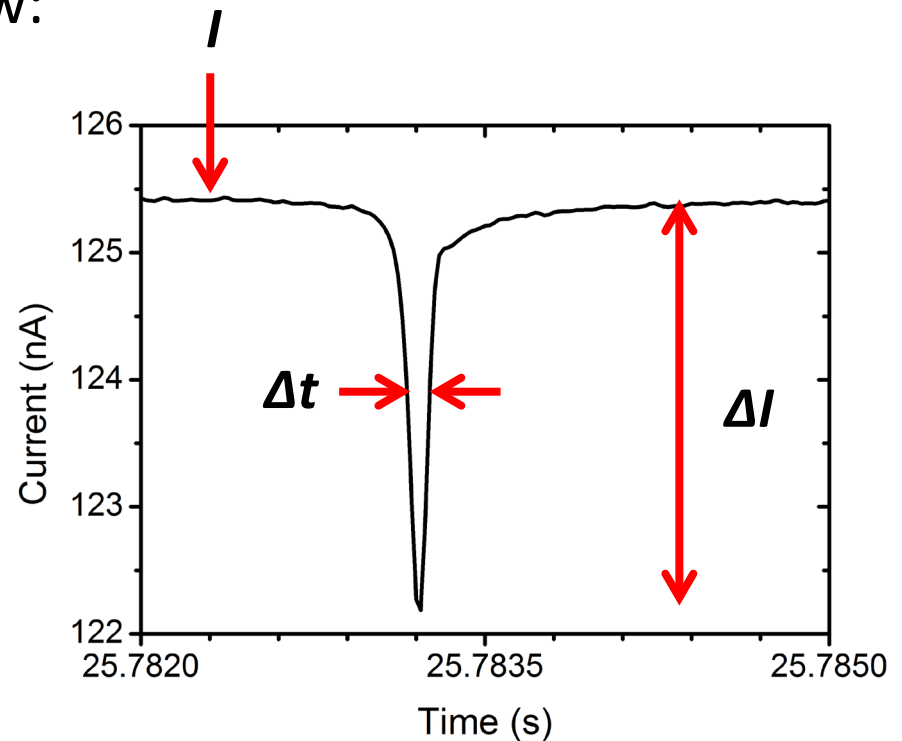
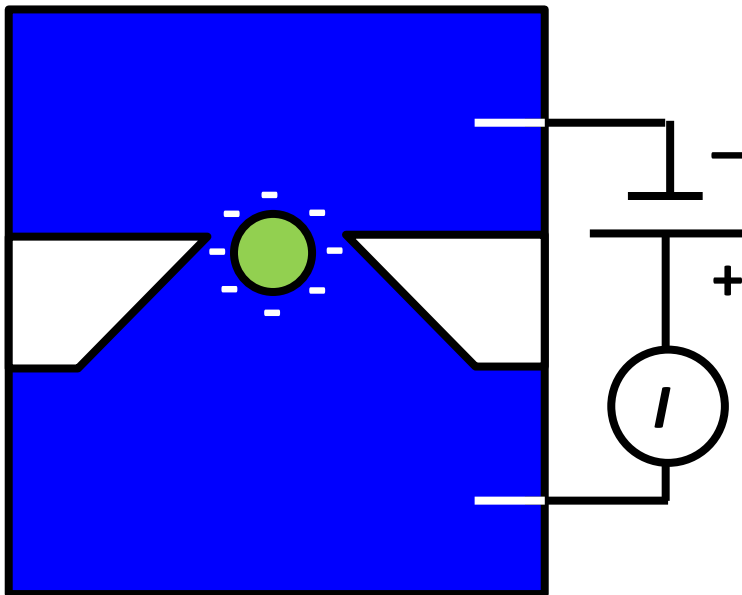
instrument requirements

- differentiate EV
- count $\geq 10^6$ EV
- reproducible



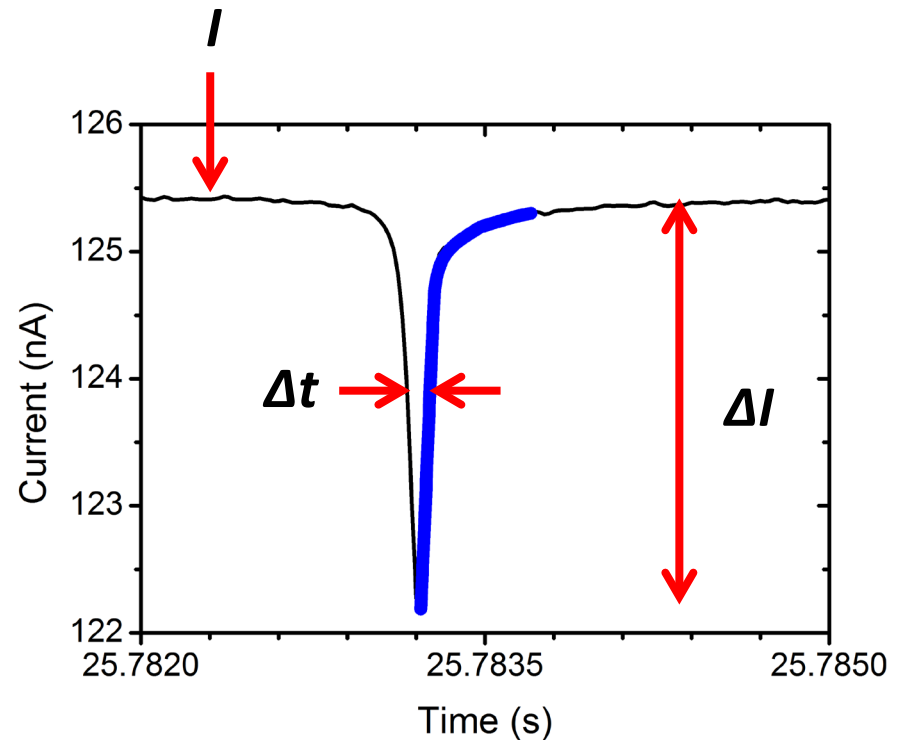
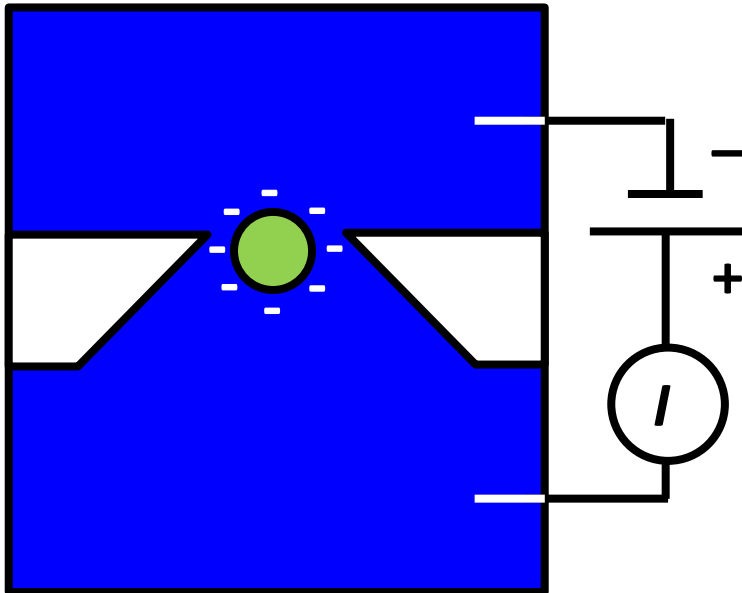
ζ -potential (surface charge) determination

no external pressure, electrophoresis dominates
flow:

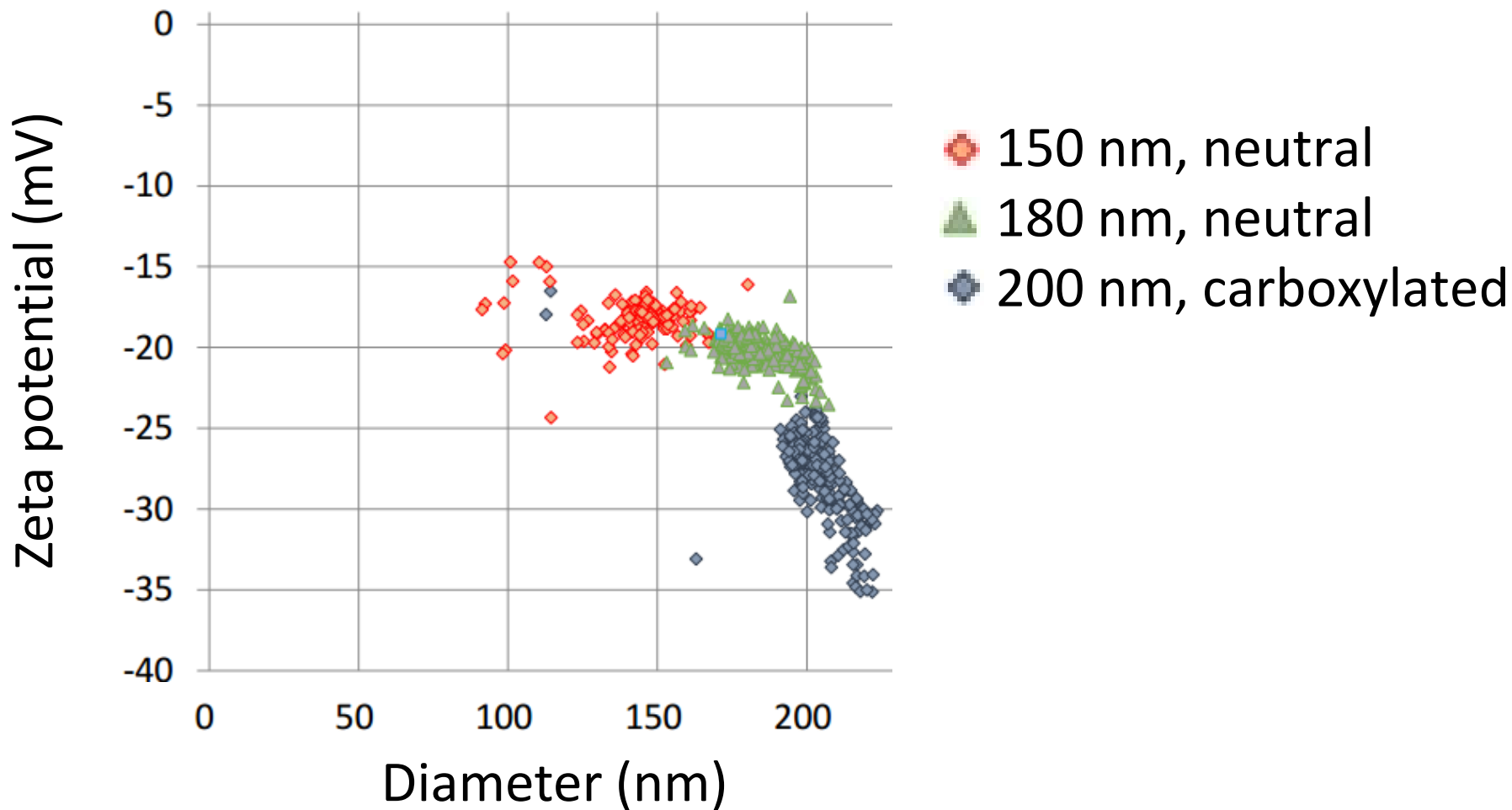


ζ -potential (surface charge) determination

no external pressure, electrophoresis dominates flow:

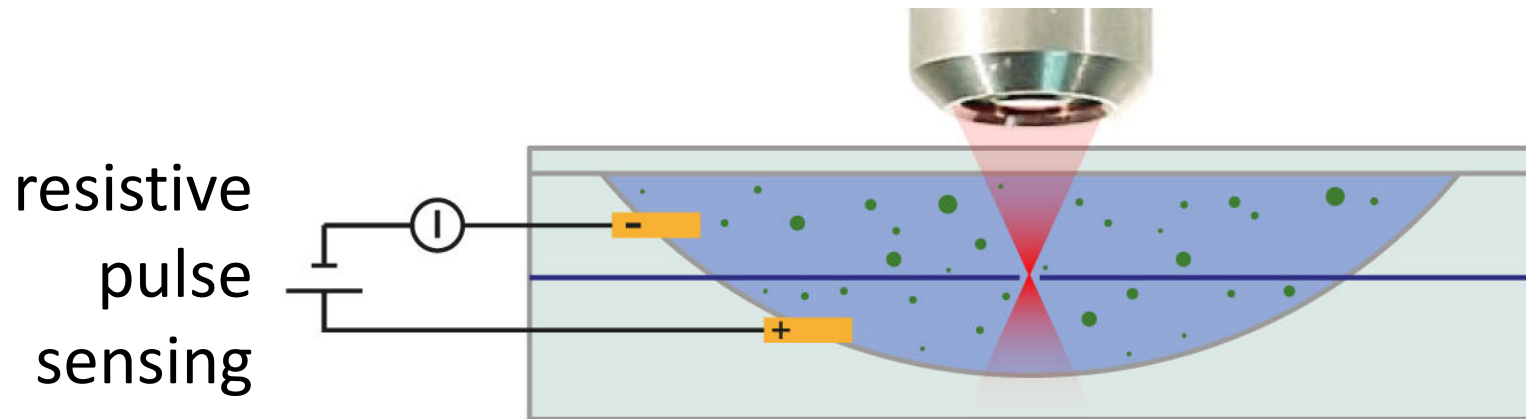


Example ζ -potential determination

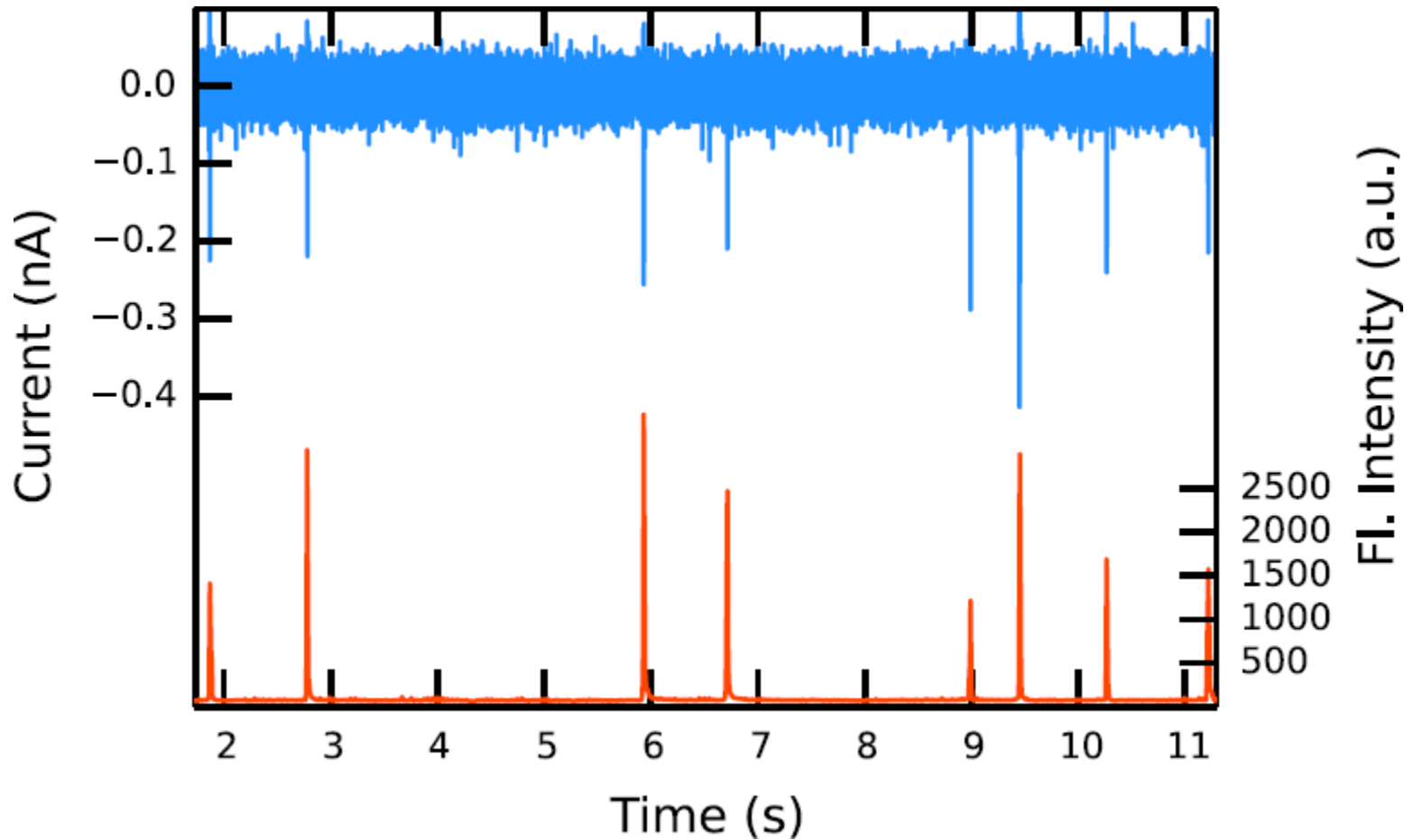


Determination of optical properties

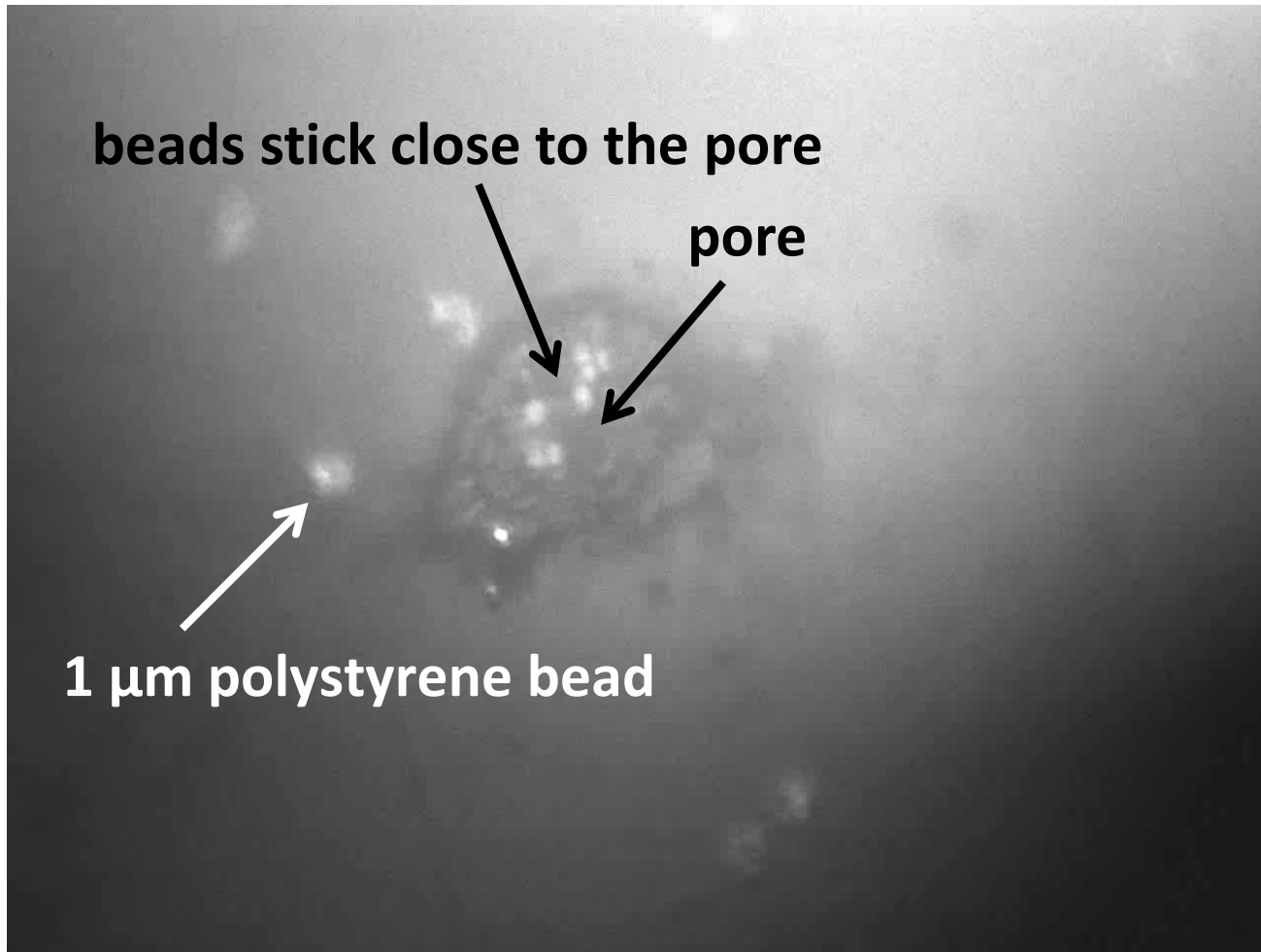
optical setup
scattering
fluorescence
Raman



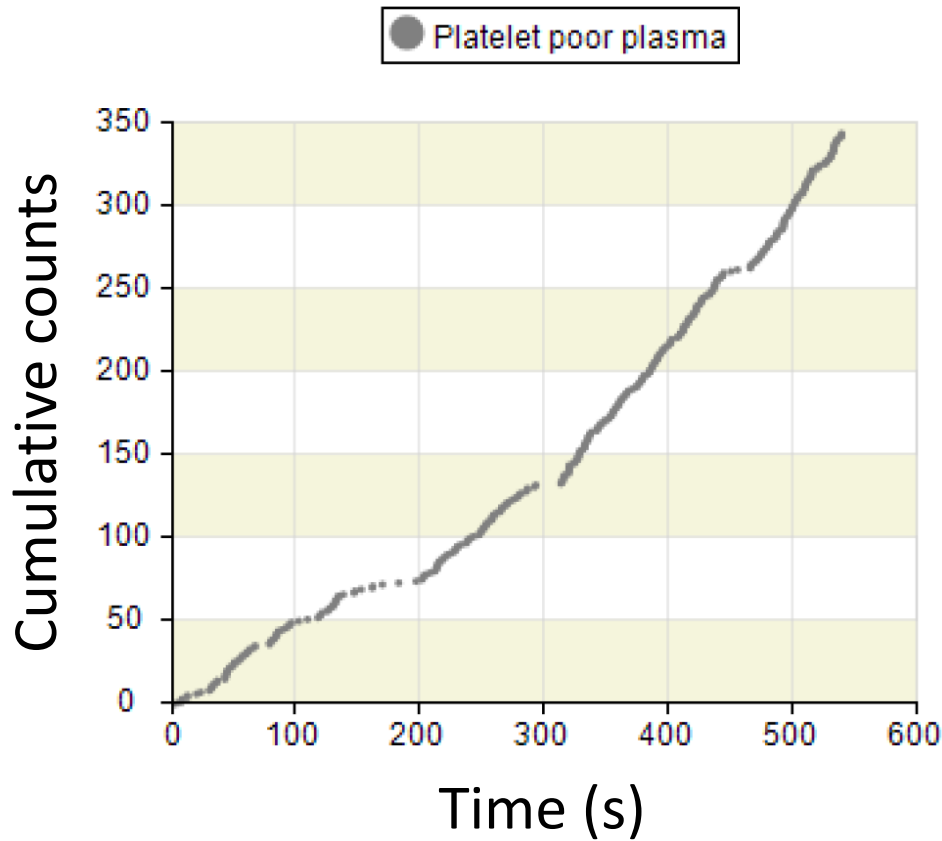
TRPS and fluorescence



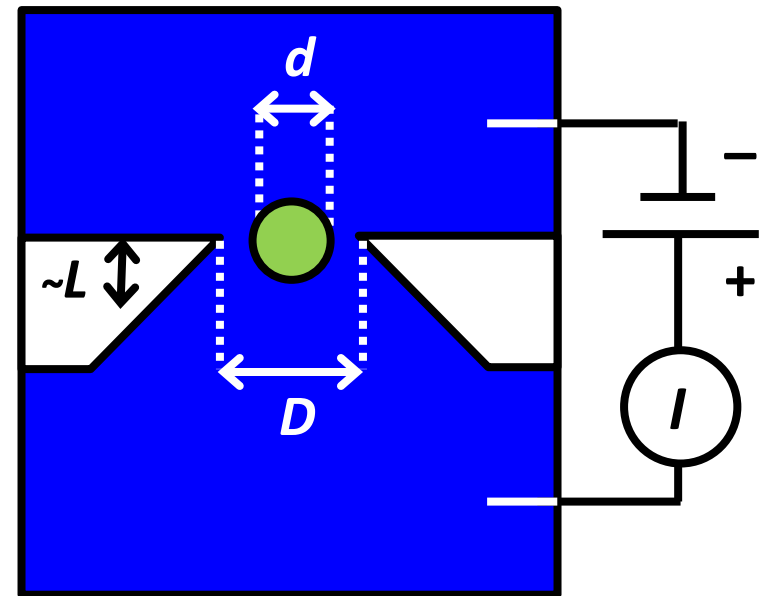
1 μm beads passing through the pore



Pore clogging



$$\frac{\Delta I}{I} = \frac{d^3}{cLD^2}$$

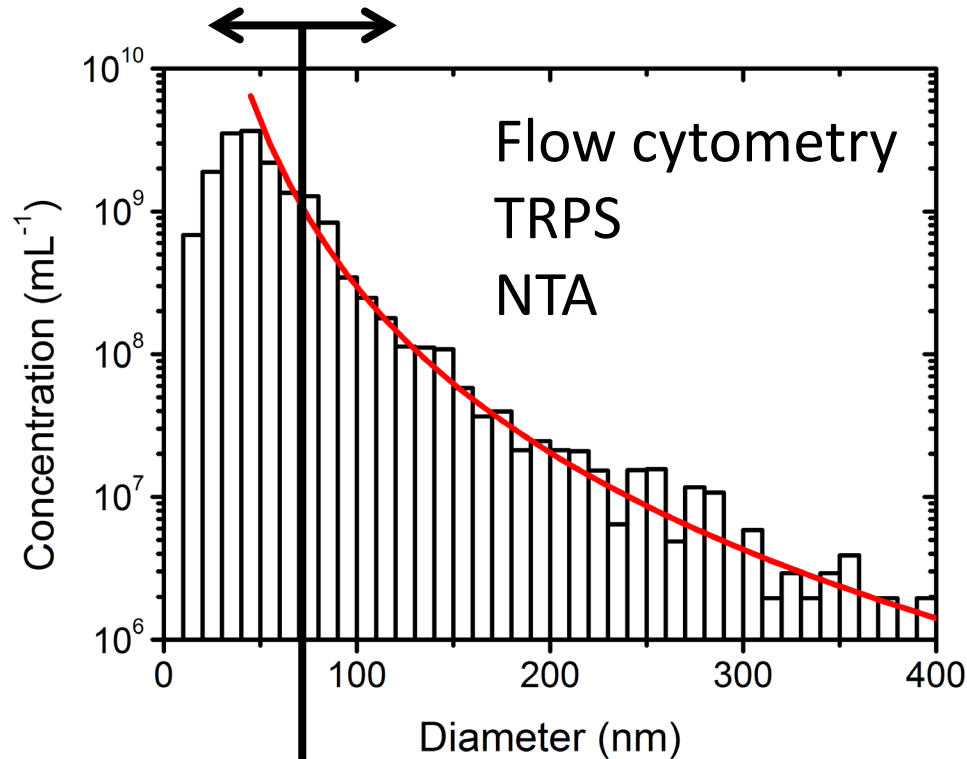


Size exclusion chromatography



Size exclusion chromatography

size exclusion threshold



lipoprotein particles,
soluble proteins

EV, chylomicrons,
high-molecular weight proteins

High throughput analysis?

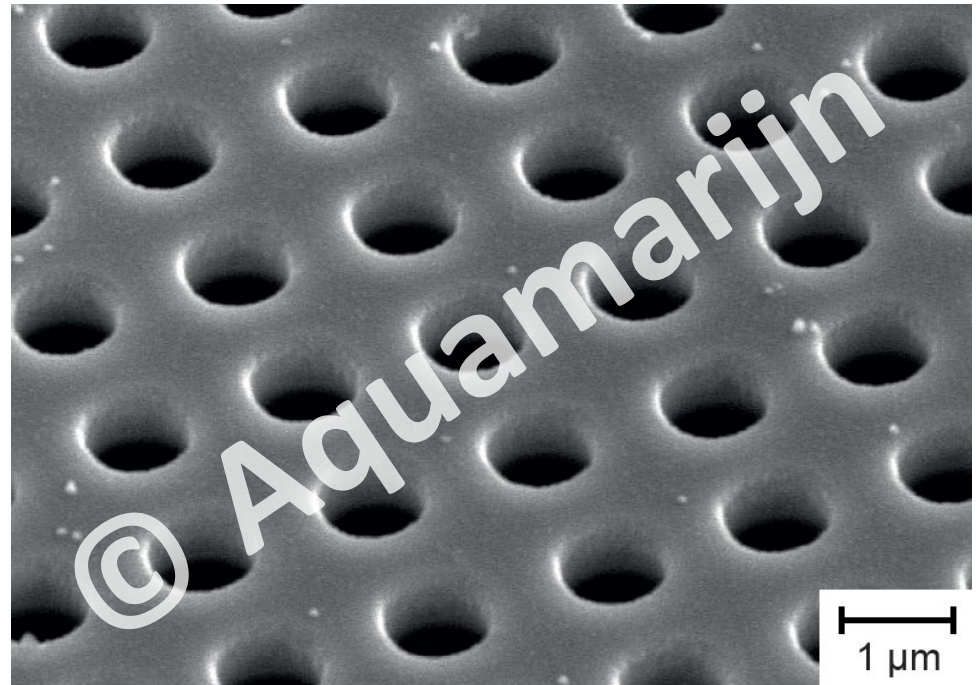
Method	Time to acquire signal of 1 EV (μs)	Time to detect 10^6 EV in practice (min)
Tunable resistive pulse sensing	1,000	10,000
Nanoparticle tracking analysis	1,000,000	10,000
Flow cytometry	1	10

numbers indicate order of magnitude

Summary TRPS analysis of EV

- size distribution
- concentration
- ζ -potential

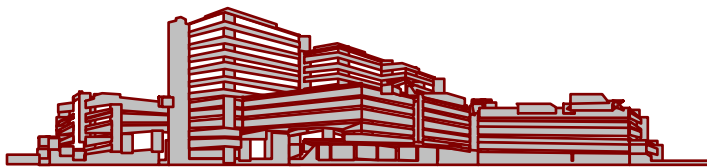
- pore clogging
 - size exclusion chromatography
- limited throughput
 - parallel solid pores



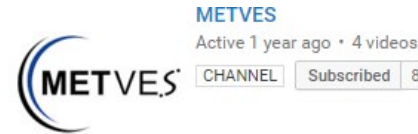
Acknowledgements

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