

Novel techniques for refractive index determination of single nanoparticles in suspension

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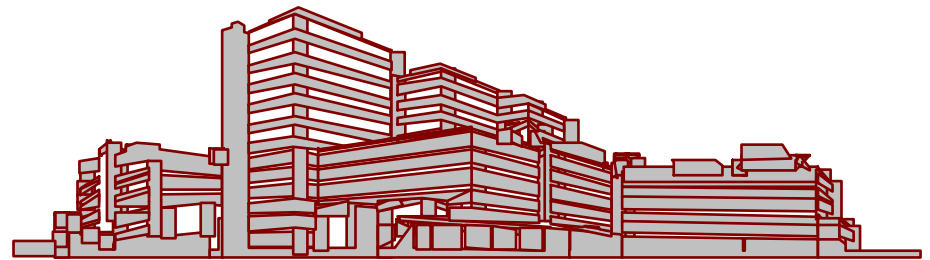
February 8th, 2015



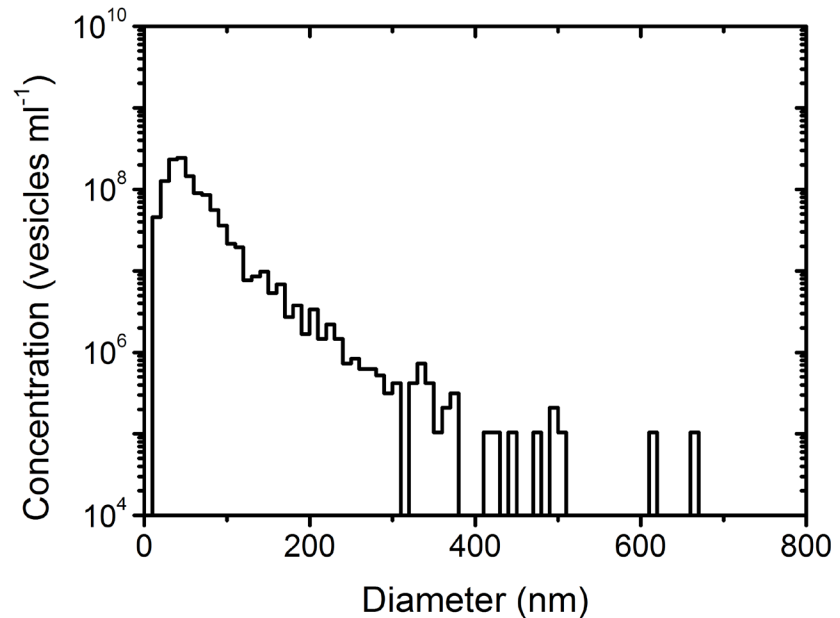
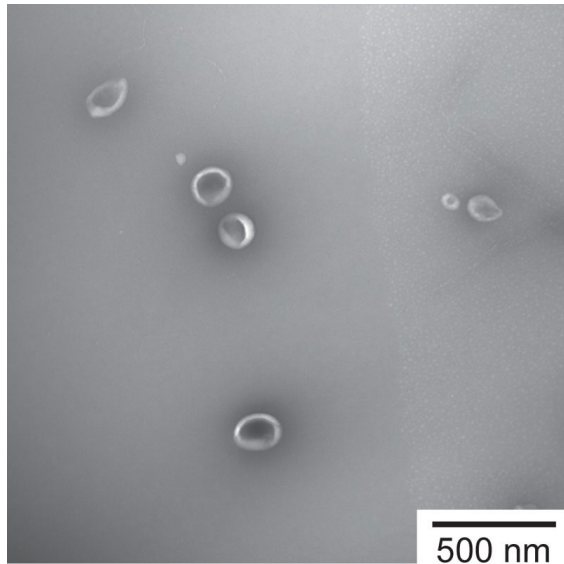
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 - Patrick Hole
 - Andrew Malloy
 - Jonathan Smith



Introduction to extracellular vesicles



- cells release vesicles (e.g. exosomes):
biological nanoparticles with receptors, DNA, RNA
- specialized functions
- clinically relevant

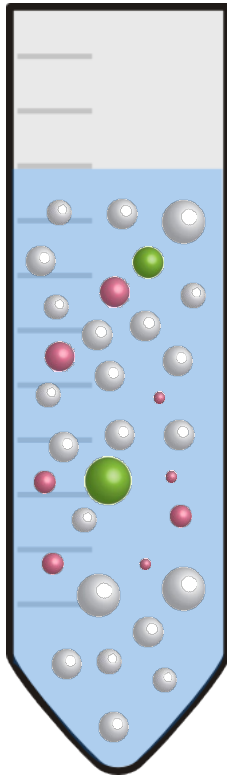
Add extracellular vesicle concentrations to hematology reference tables






Hematology parameter	Concentration (cells mL ⁻¹)
Platelet count	1.50 – 4.00 · 10 ⁸
Erythrocyte count	4.50 – 5.50 · 10 ⁹
Reticulocyte count	2.5 – 10.0 · 10 ⁷
Leukocyte count	4.0 – 10.5 · 10 ⁶
Total cell count	4.7 – 6.0 · 10 ⁹

Hematology parameter	Concentration (vesicles mL ⁻¹)
Platelet vesicle count	2.3 – 6.2 · 10 ⁹
Erythrocyte vesicle count	7.0 – 8.6 · 10 ¹⁰
Reticulocyte vesicle count	3.9 – 15.6 · 10 ⁸
Leukocyte vesicle count	6.2 – 16.4 · 10 ⁷
Total vesicle count	7.3 – 9.4 · 10 ¹⁰

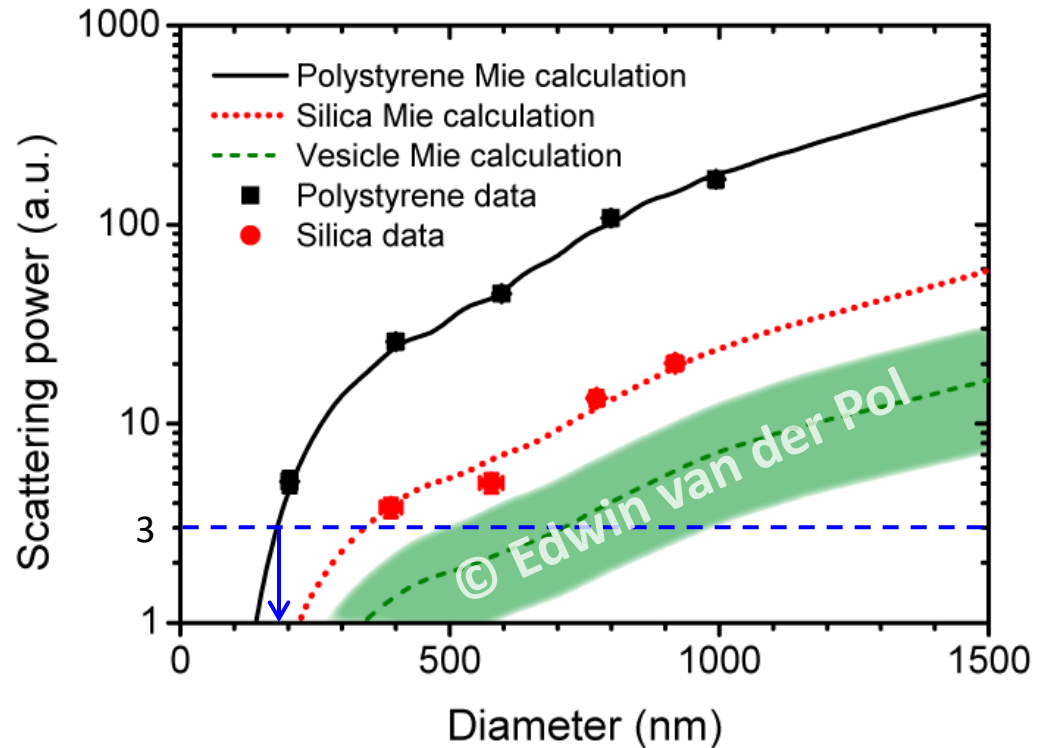
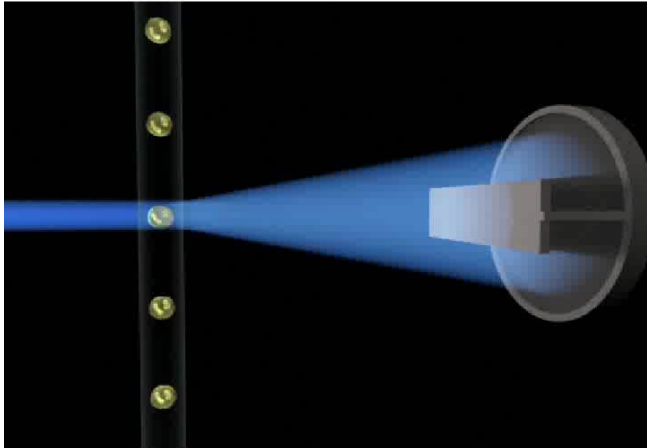
Determine refractive index to identify vesicles



-  vesicles ($1.36 \leq n \leq 1.45$ for $d > 500$ nm)*
-  lipoproteins ($n = 1.45-1.60$)
-  protein aggregates ($n = 1.53-1.60$)

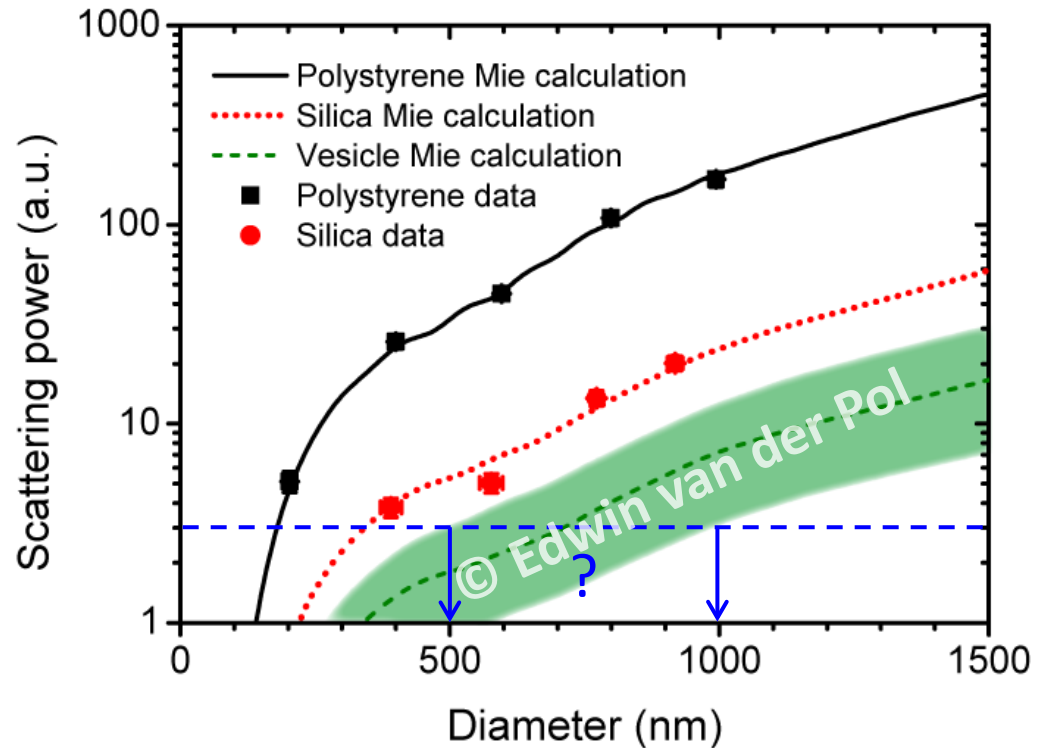
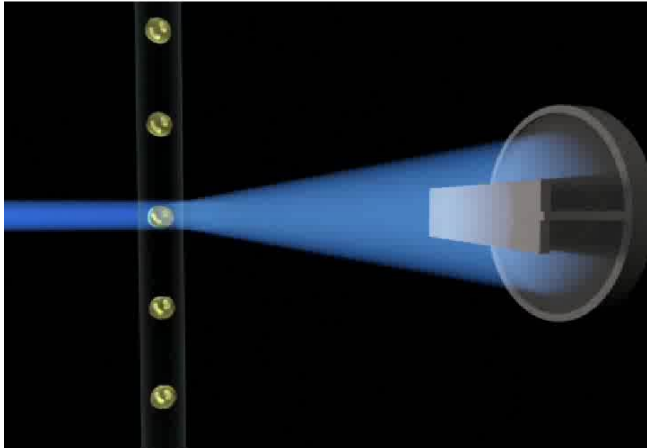
* Konokhova et al., J Biomed Opt (2012)

Refractive index to relate scatter to diameter



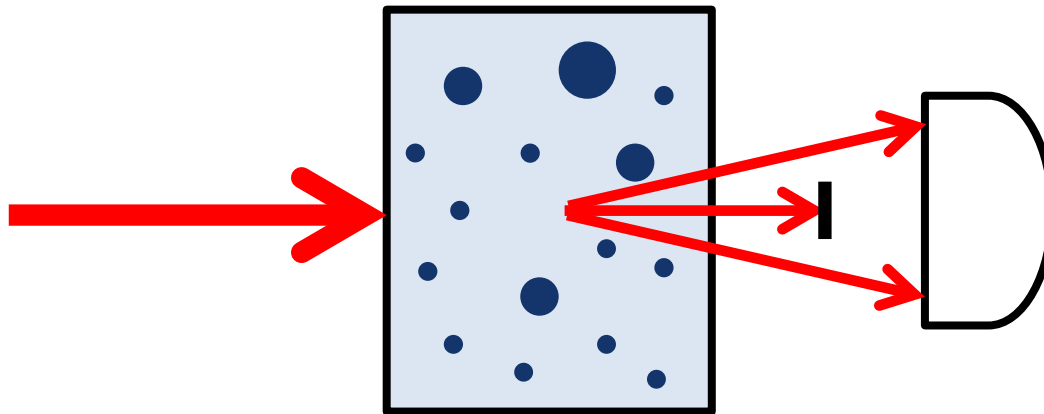
- flow cytometry is widely used to detect single vesicles
- refractive index provides scatter to diameter relation

Refractive index of vesicles is unknown



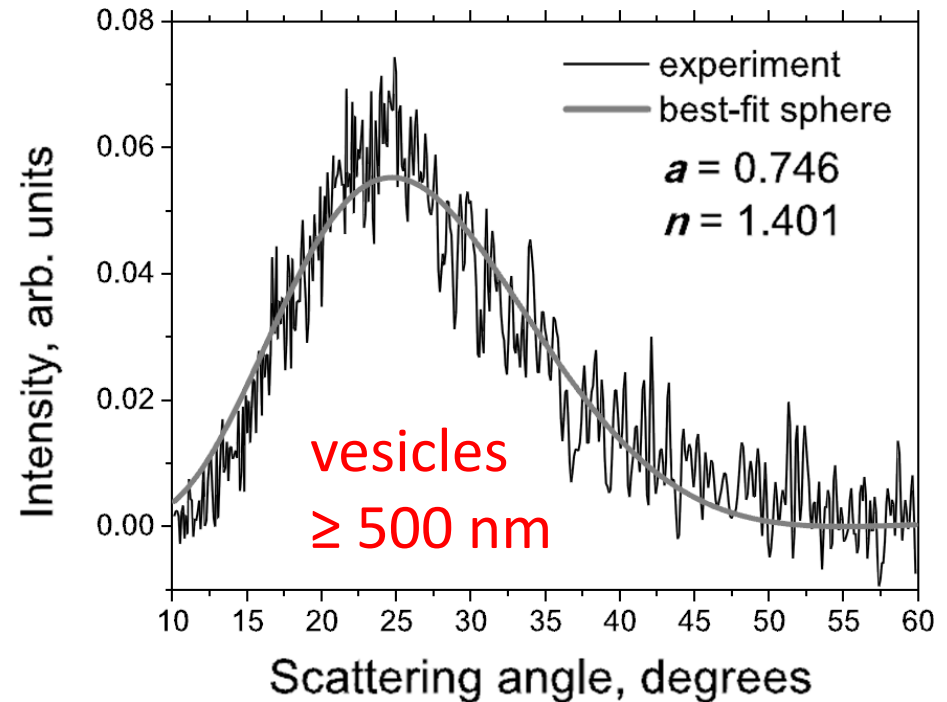
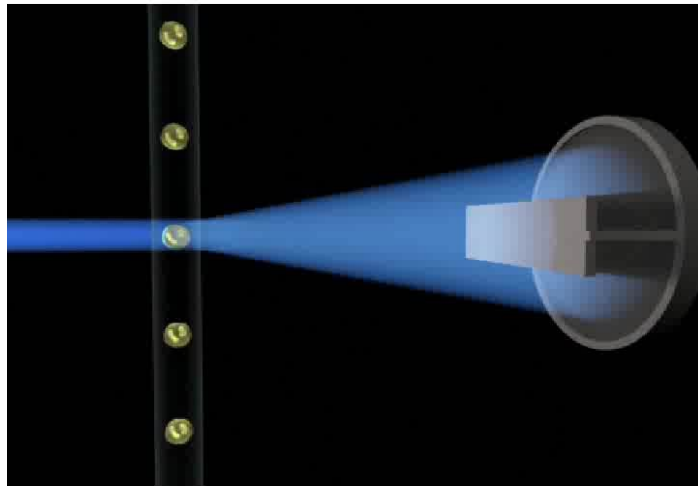
- refractive index of vesicles is unknown
- detection range is unknown

Methods for refractive index determination of single nanoparticles in suspension



- Refractive index matching (multiple particles)

Methods for refractive index determination of single nanoparticles in suspension



- Multi-angle light scattering* (single particles)
- Refractive index matching (multiple particles)

* graph adopted from: Konokhova et al., J Biomed Opt (2012)

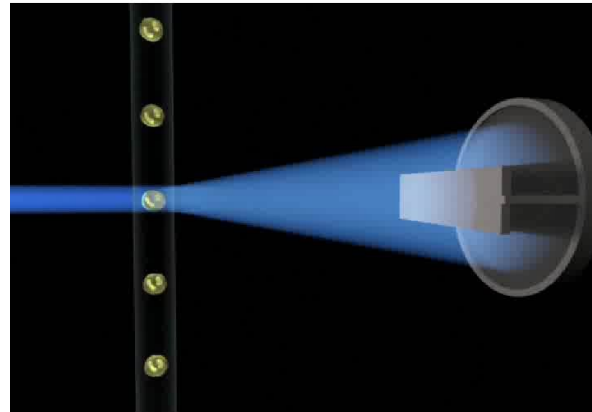
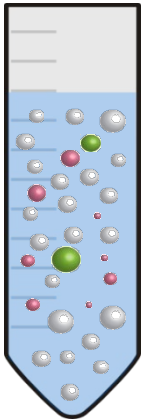
Problem

- no method to determine the refractive index of single nanoparticles (< 500 nm) in suspension

Method	Single particles	Size (nm)
Refractive index matching	–	All
Multi-angle light scattering flow cytometry	+	≥ 500

Goal

- determine the refractive index of single nanoparticles in suspension
 - identify vesicles in plasma
 - provide insight in vesicle detection by flow cytometry



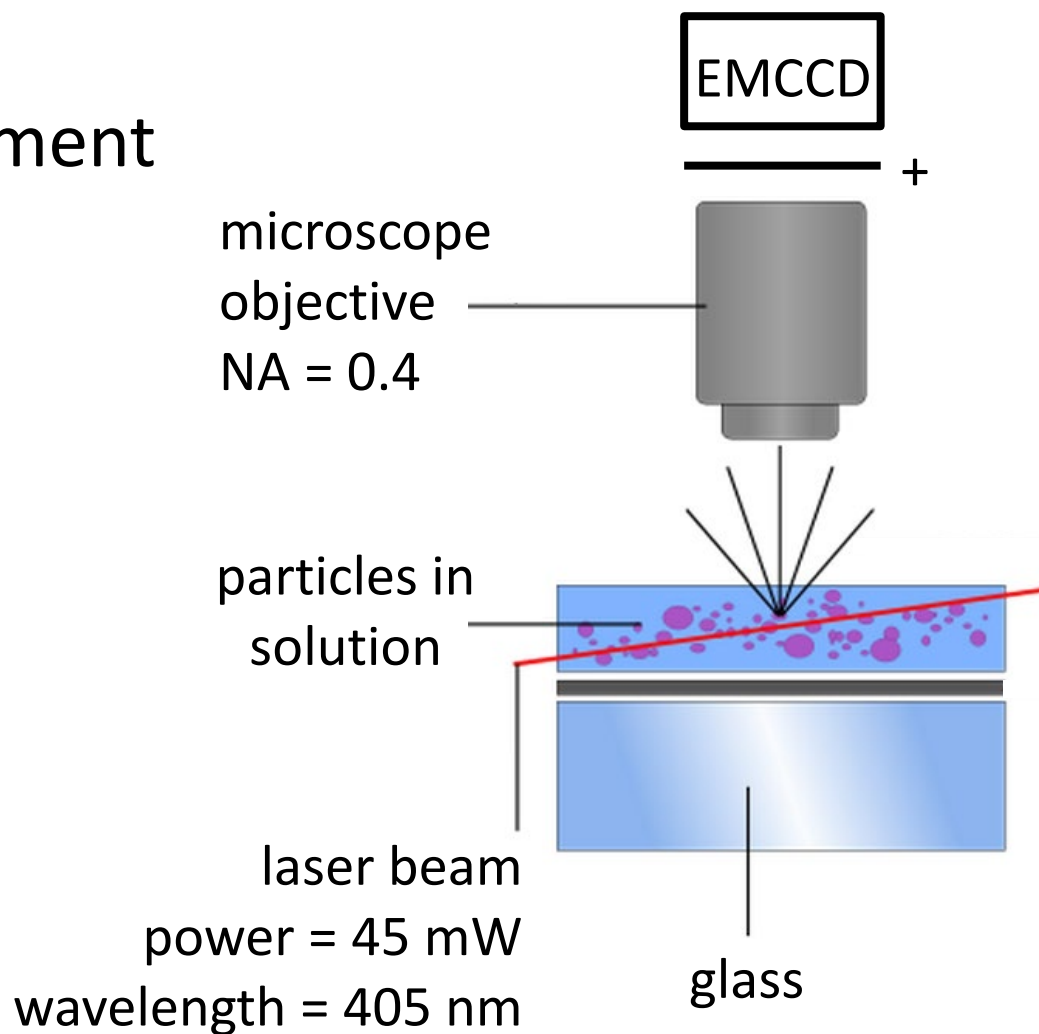
Methods - single particle tracking (SPT)



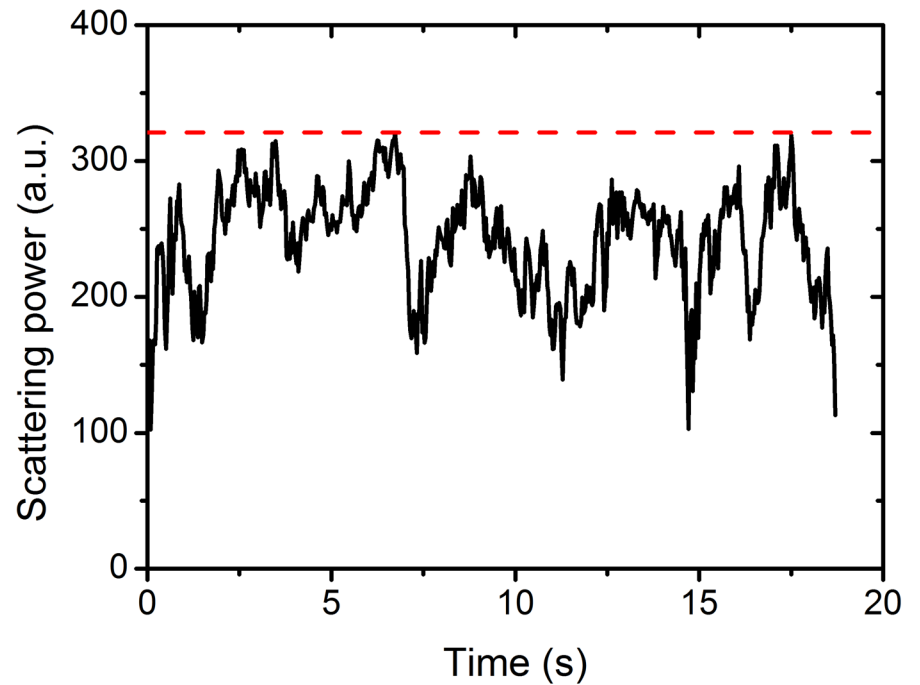
- obtain **particle diameter** d by tracking the Brownian motion of single particles (Stokes-Einstein equation)
- measure **scattering power** P
- derive particle **refractive index** $n(P, d)$ from Mie theory

Methods - setup

- Commercial instrument
 - Nanosight NS-500



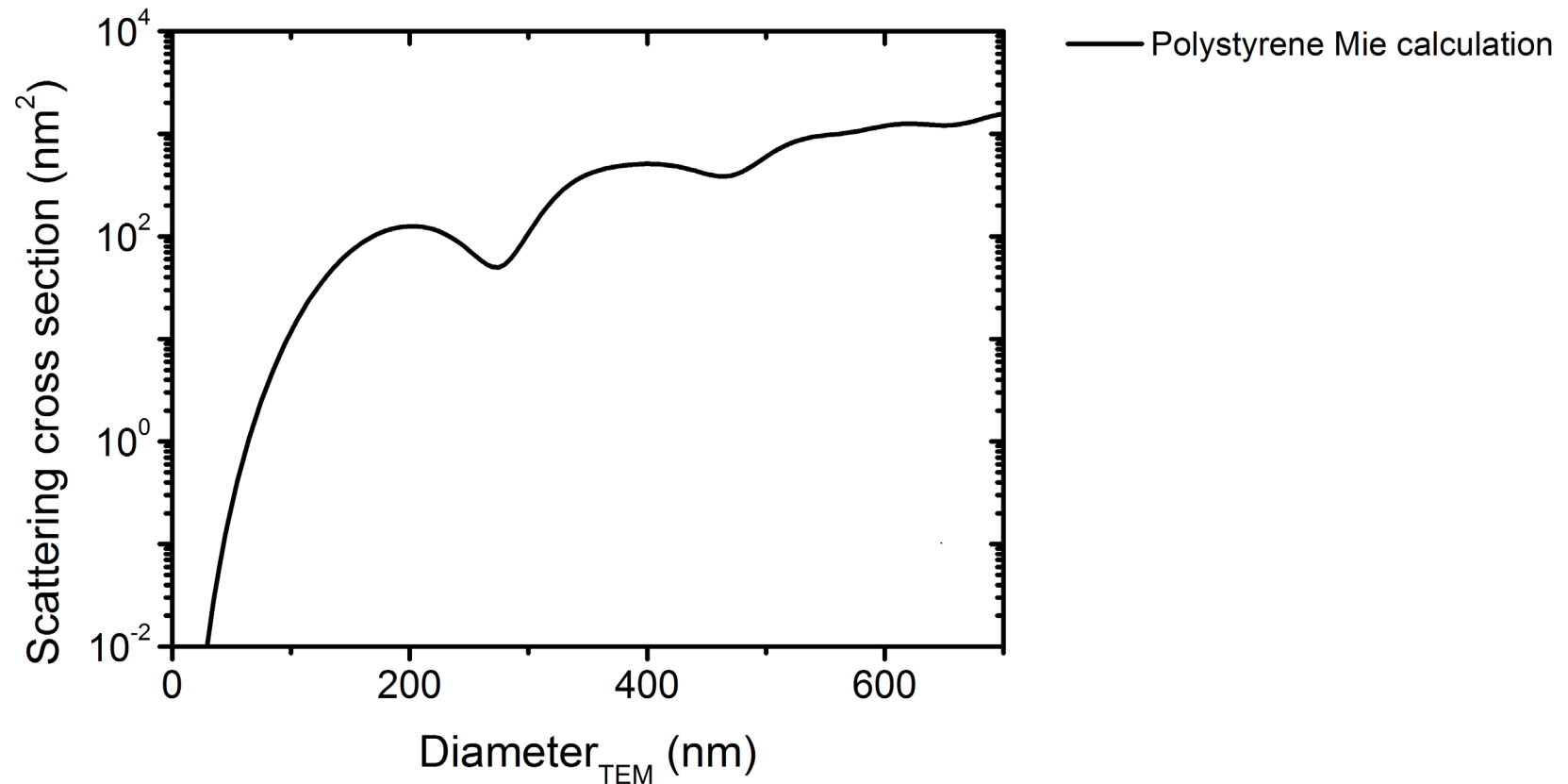
Methods - data acquisition and processing



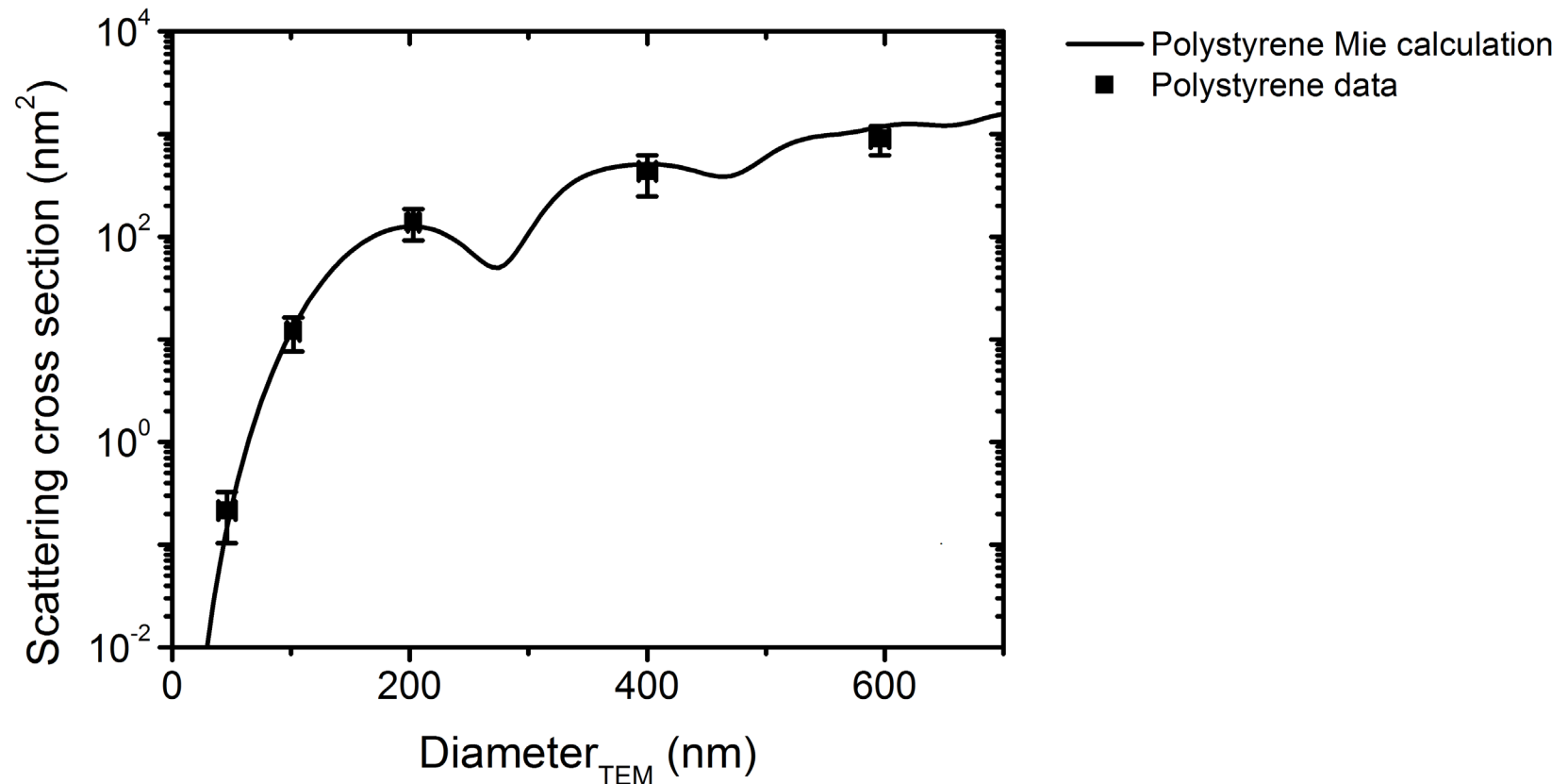
Methods - approach

- calibration
 - measure light scattering of beads
 - describe measurements by Mie theory
- validation
 - determine refractive index of beads mixture
- application
 - determine refractive index of vesicles

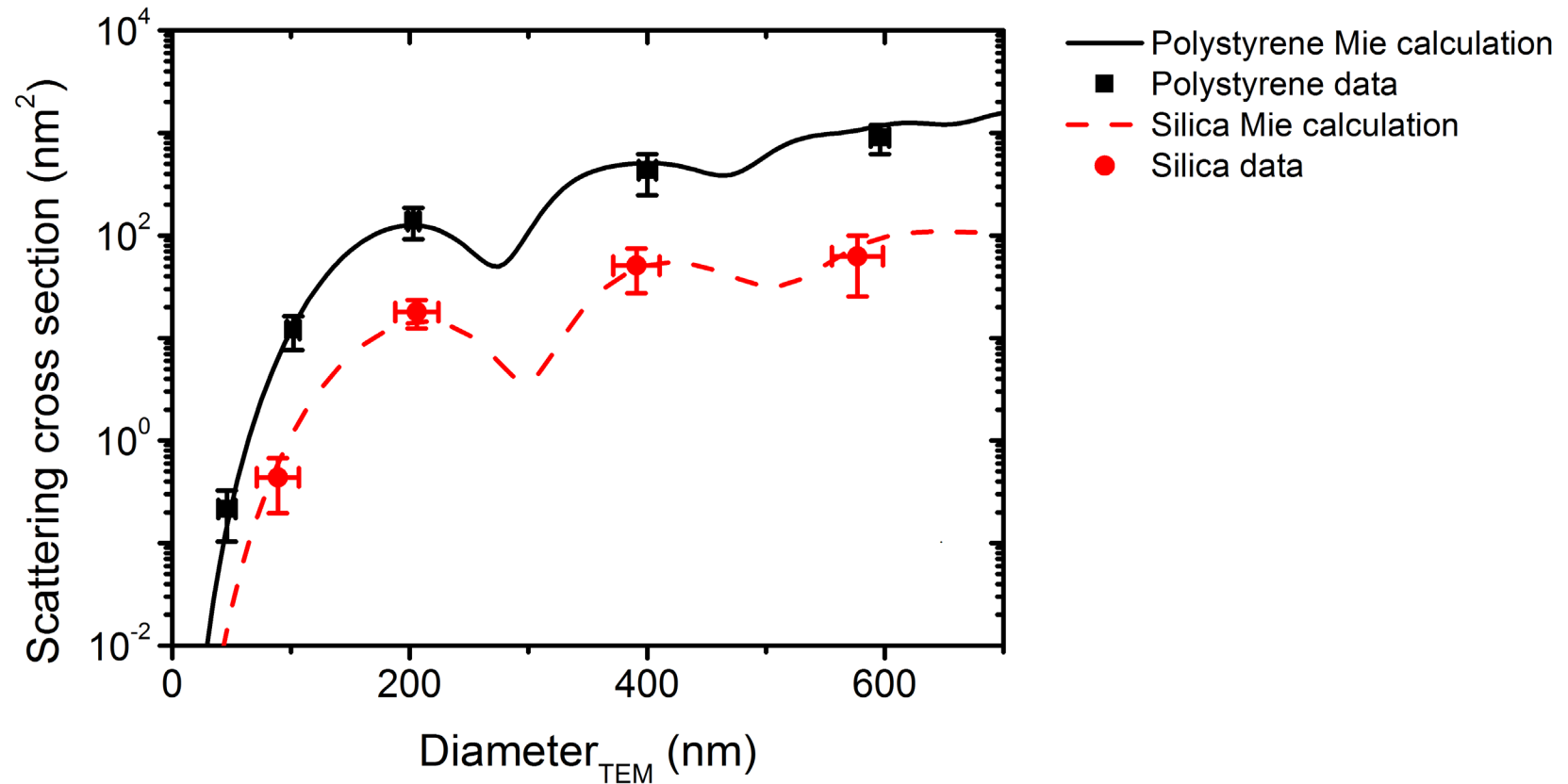
Results - scattering cross section vs. diameter of polystyrene beads by Mie theory



Results - scattering cross section vs. diameter of polystyrene beads



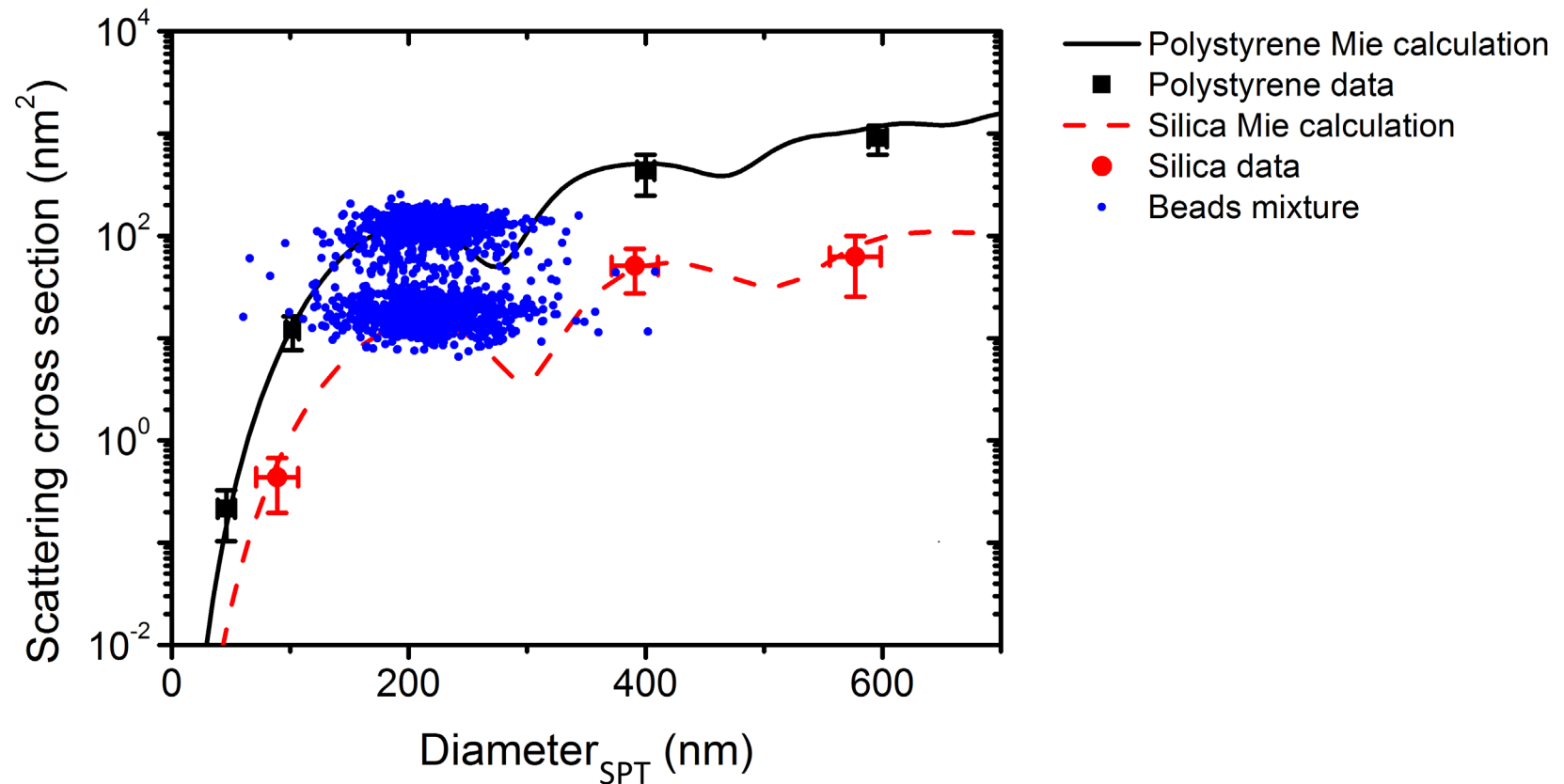
Results - scattering cross section vs. diameter of polystyrene and silica beads



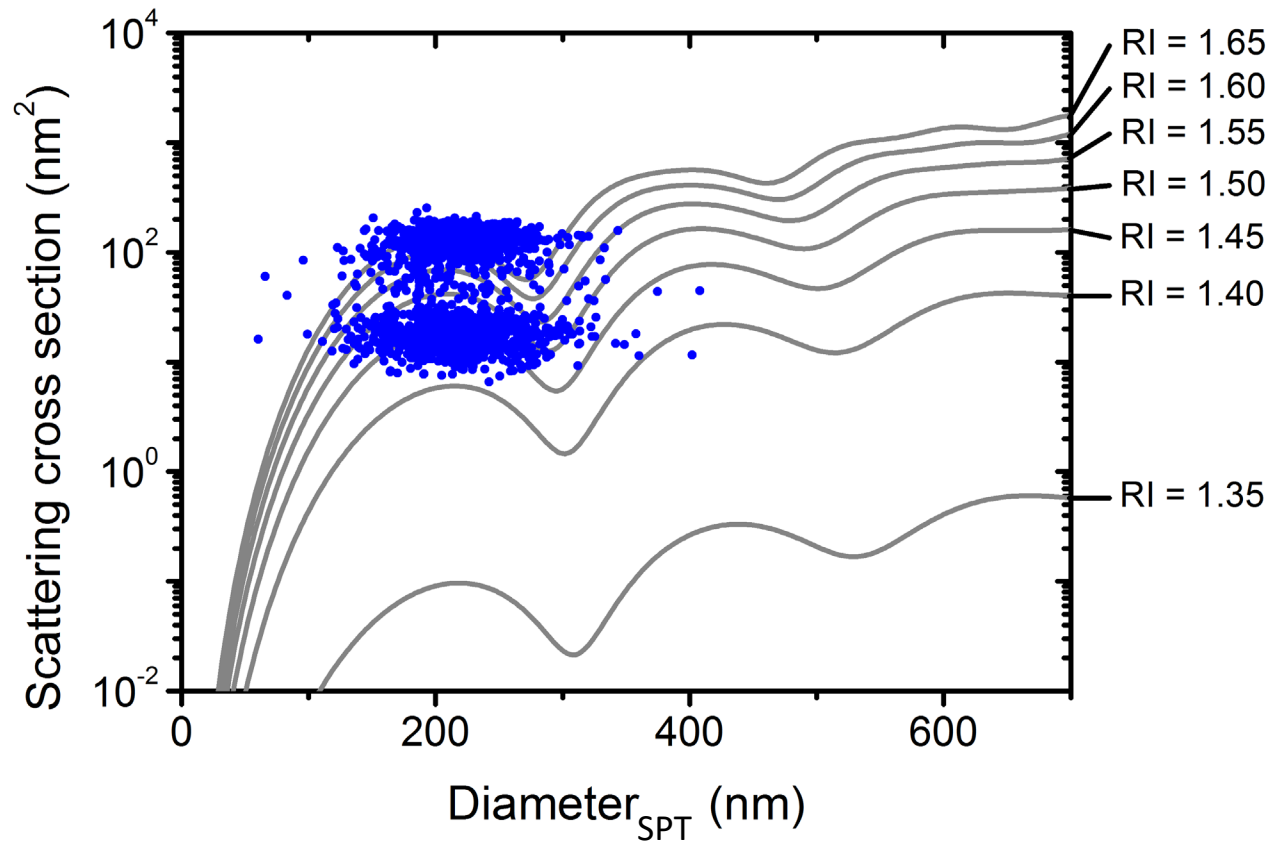
Methods - approach

- ✓ calibration
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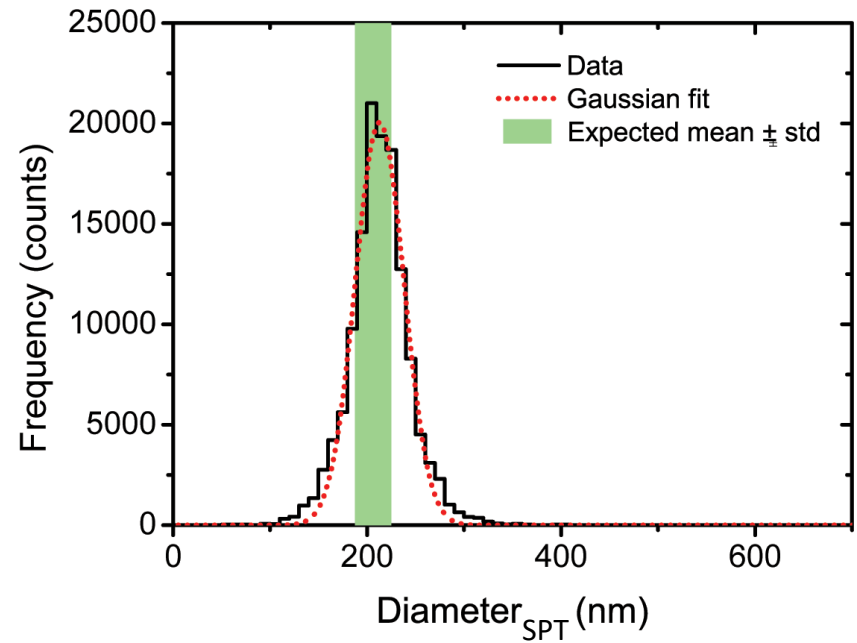
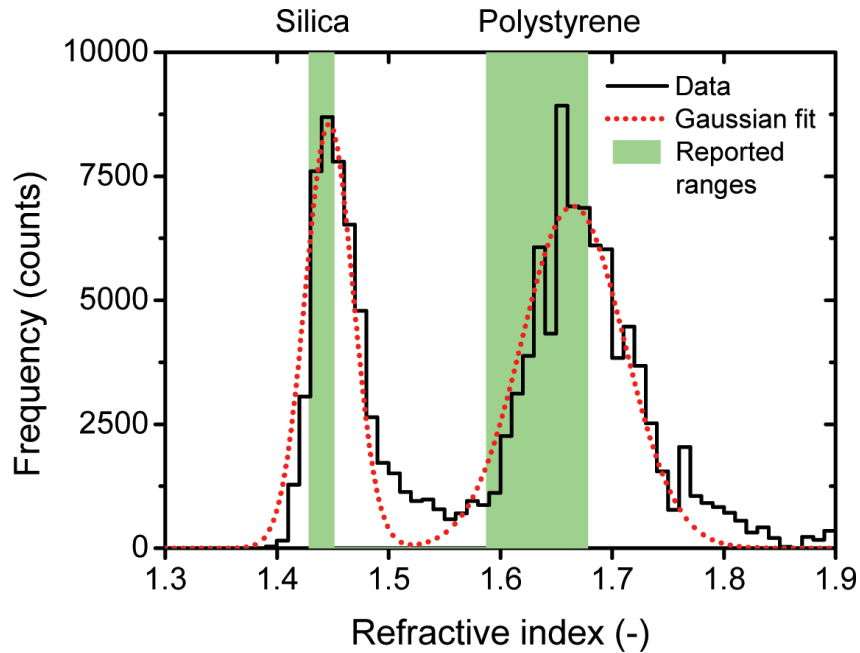
Results - scattering cross section vs. diameter of a mixture of polystyrene and silica beads



Results - scattering cross section vs. diameter of a mixture of polystyrene and silica beads



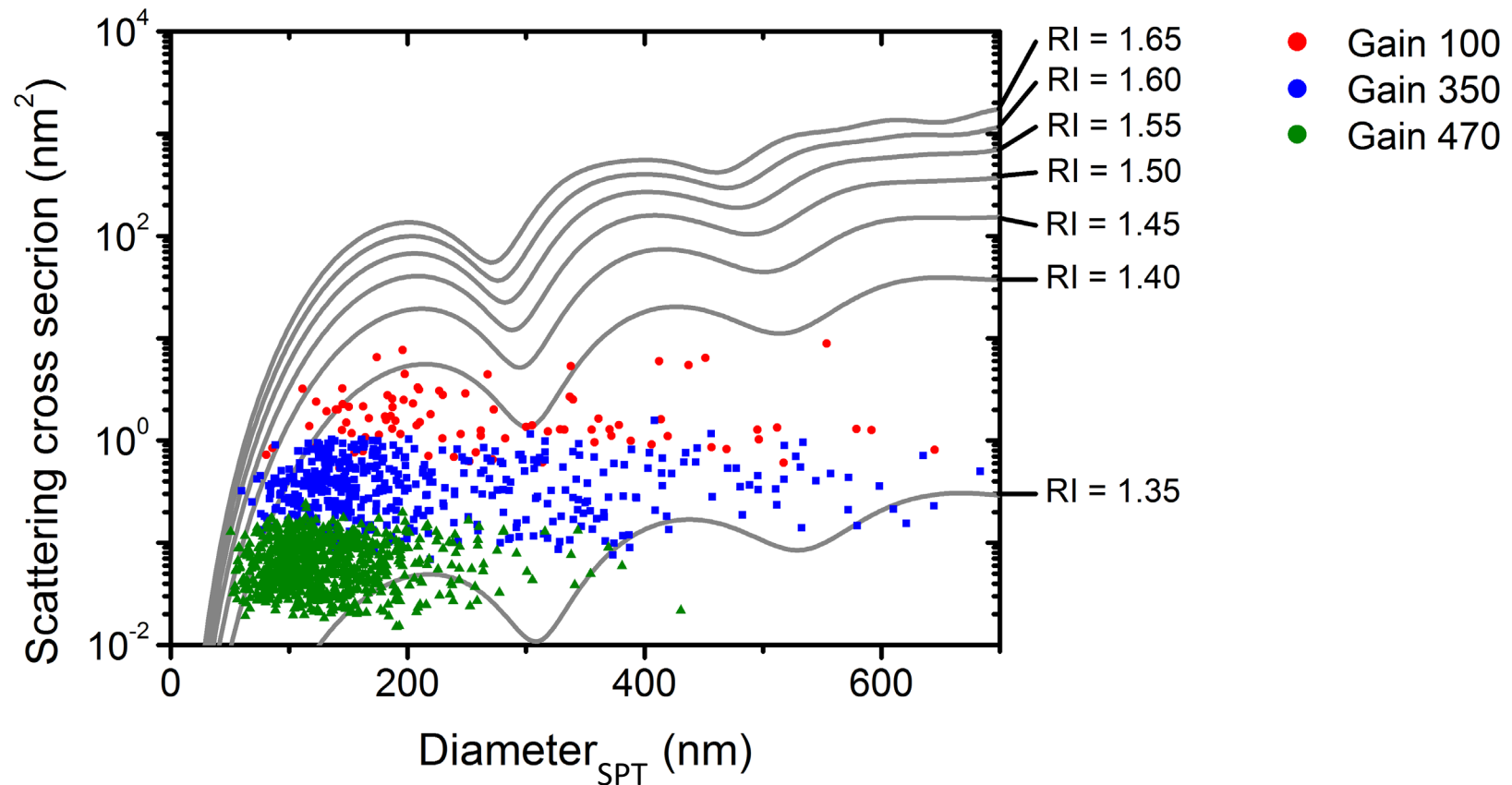
Results - refractive index and size distribution of a mixture of polystyrene and silica beads



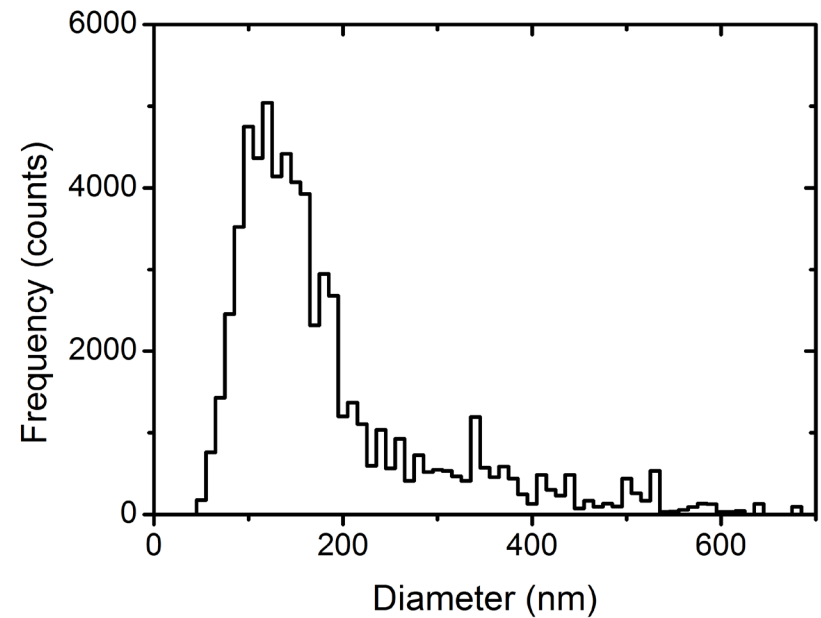
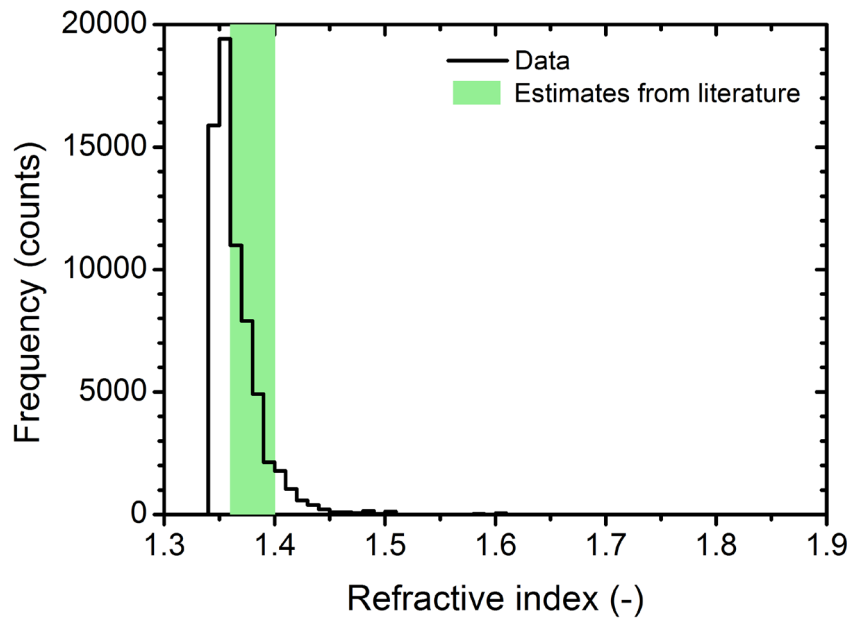
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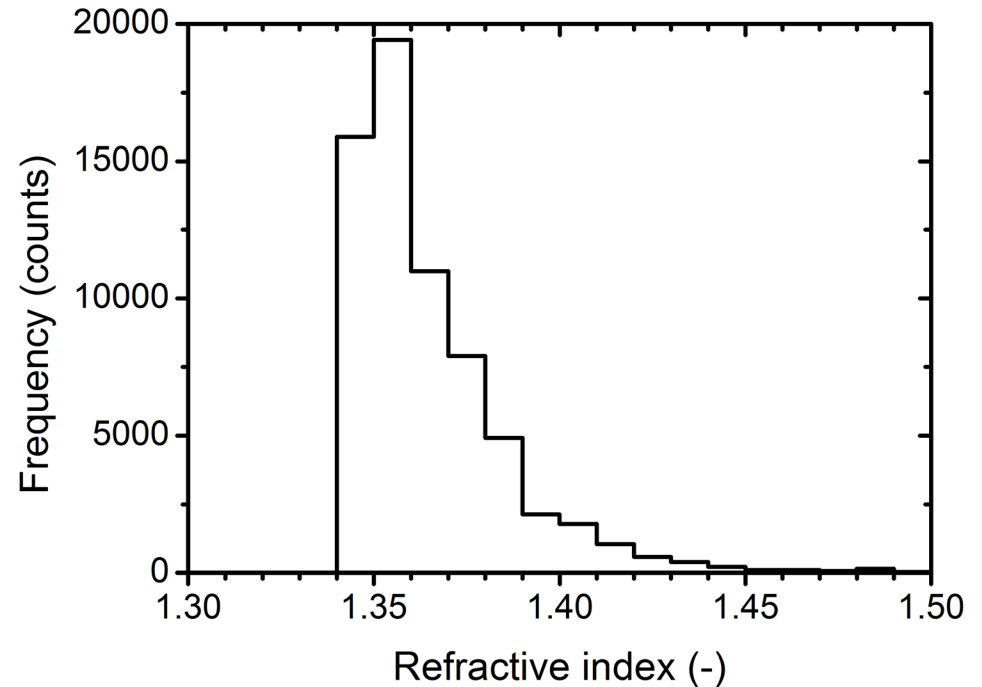
Results - scattering power versus diameter of urinary vesicles



Results - size and refractive index distribution of urinary vesicles

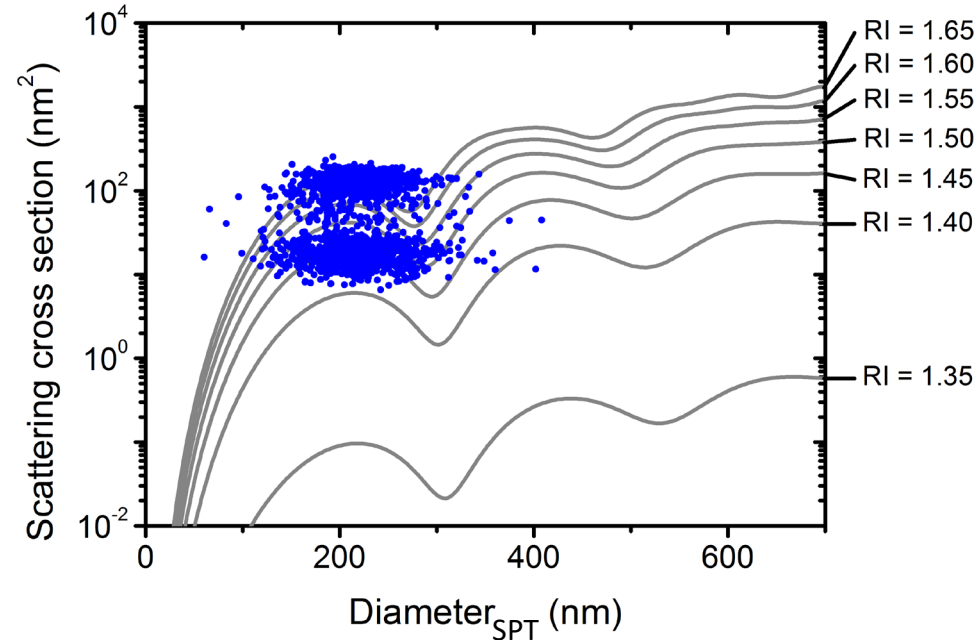
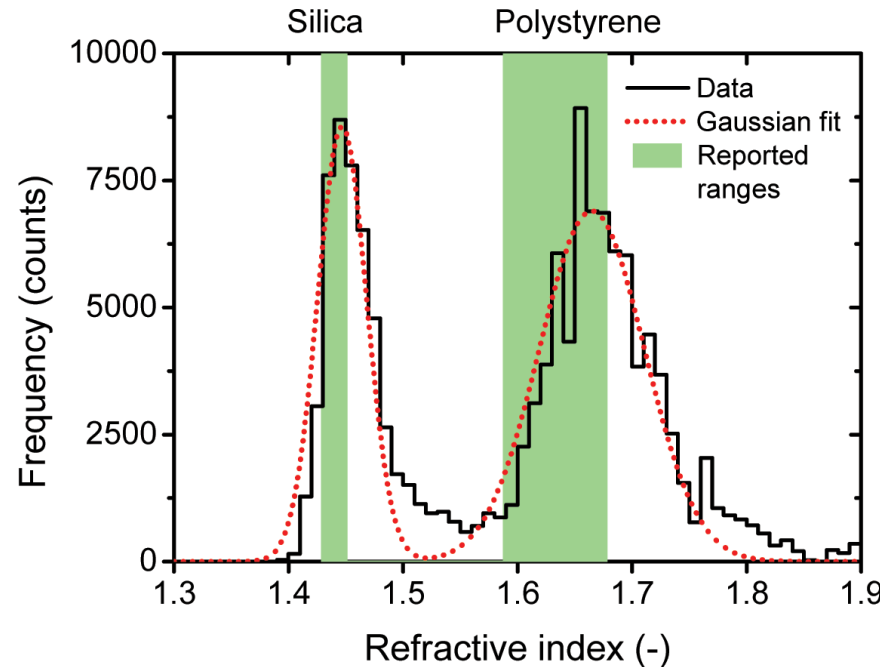


Conclusions



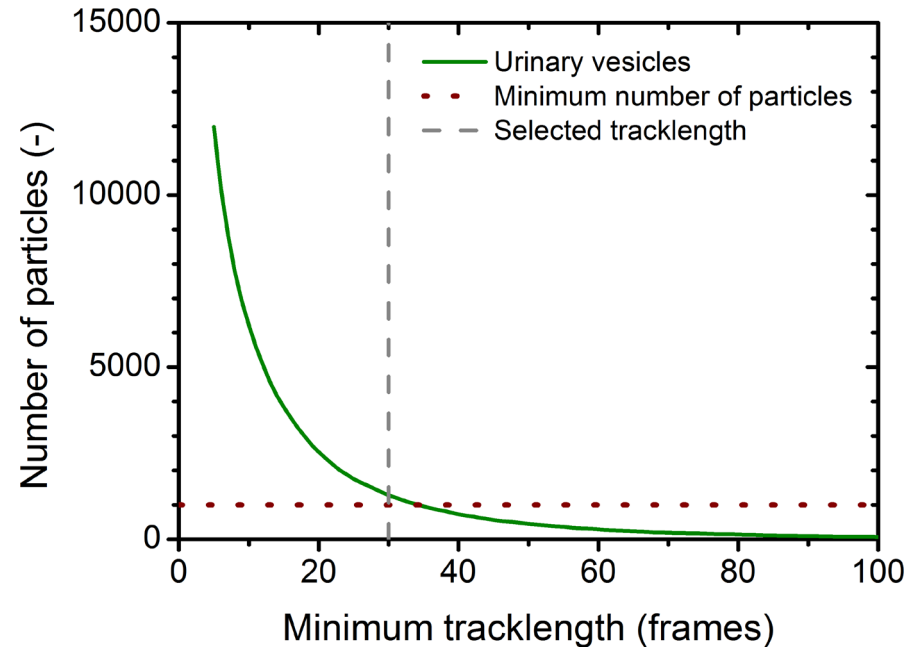
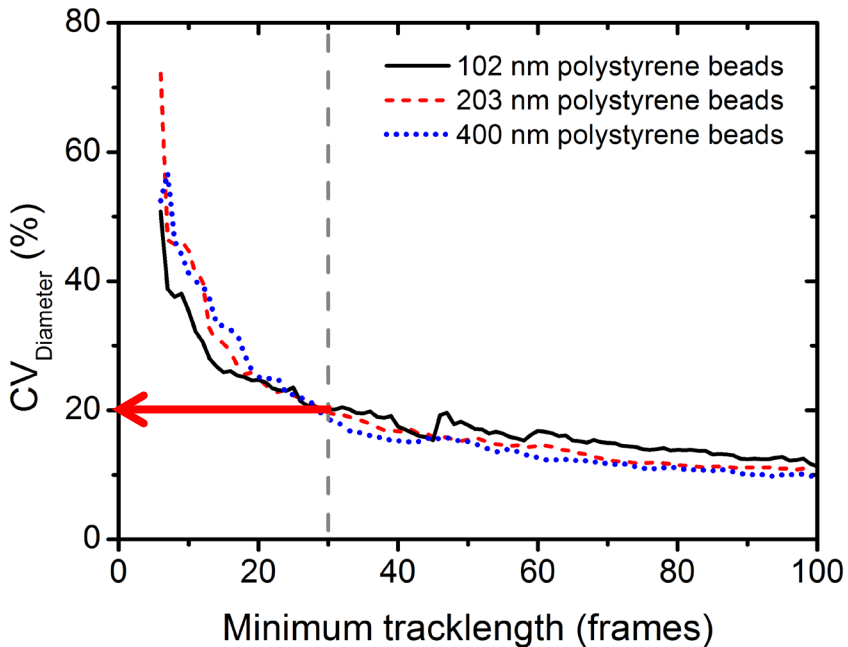
- single particle tracking can be used to determine the refractive index of nanoparticles in suspension
- mean refractive index of urinary vesicles is 1.37

Discussion



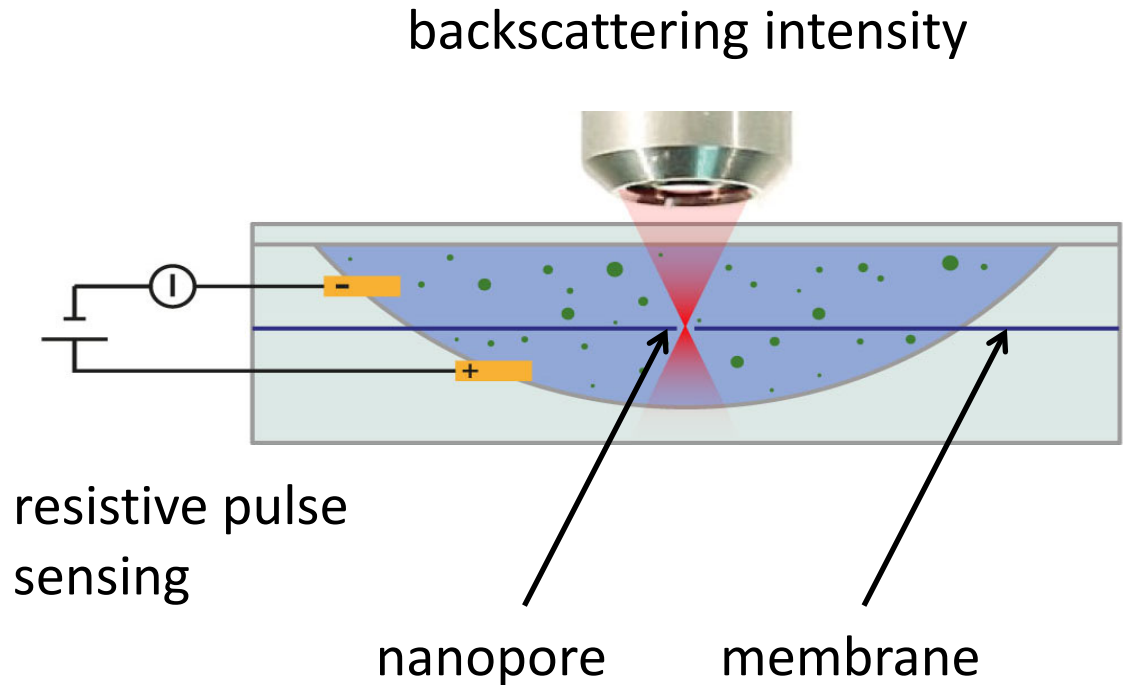
- accuracy: measurement error = 2.0 %
- precision: coefficient of variation (CV) = 2.8 %

Discussion

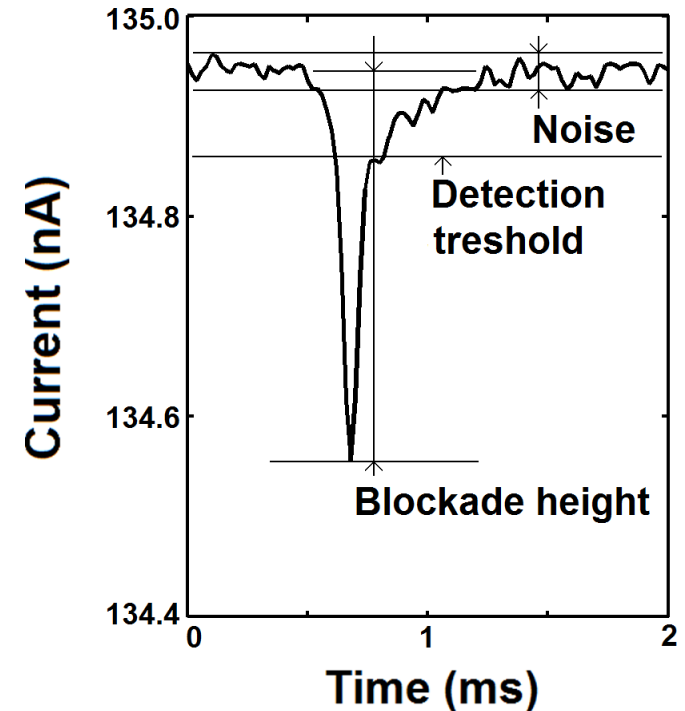


- increase precision by increasing minimum tracklength
 - consequence: number of tracked particles decreases

Outlook: hybrid backscattering – resistive pulse sensing for refractive index determination

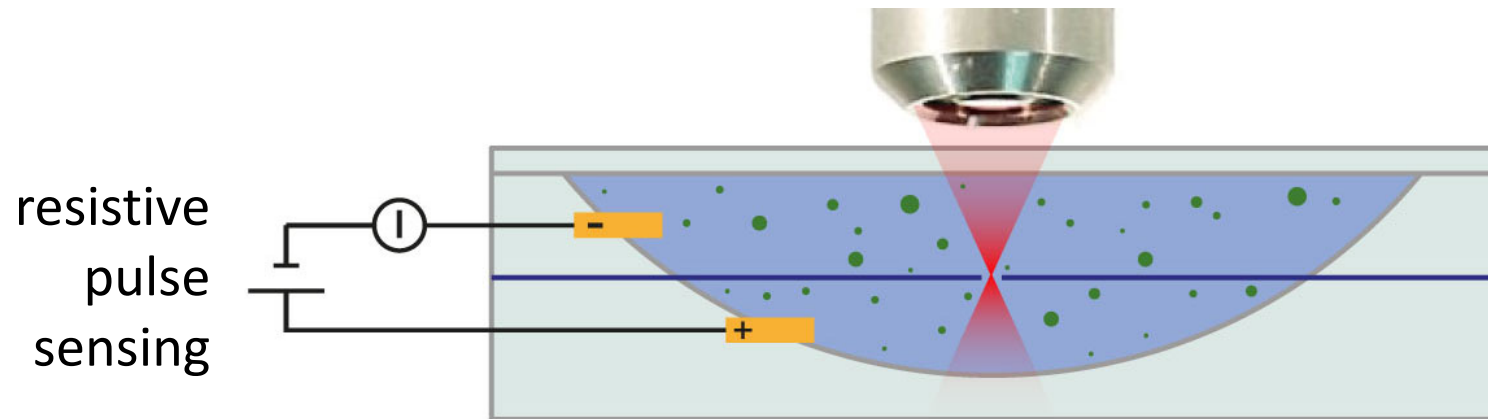


CV diameter = 7%



Towards vesicles as biomarkers for disease

backscattering intensity
fluorescence + Raman scattering*



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