Refractive index determination of single sub micrometer *vesicles* in suspension using dark-field microscopy

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  - Laboratory Experimental Clinical Chemistry
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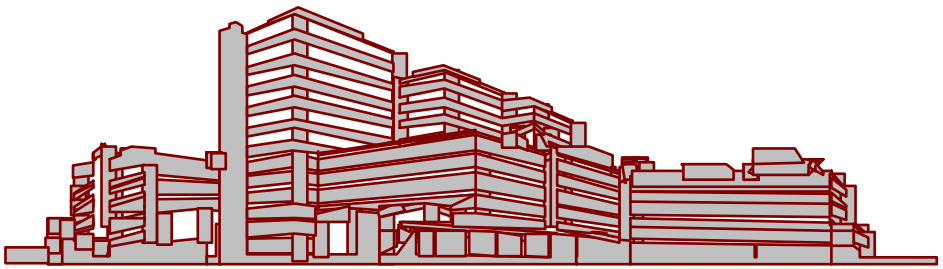
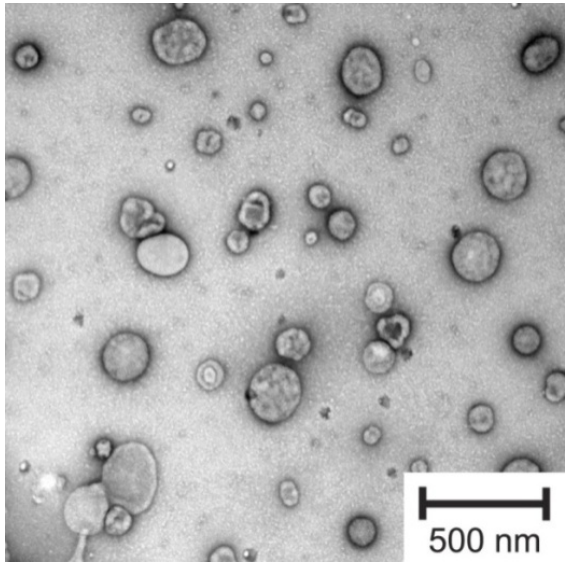
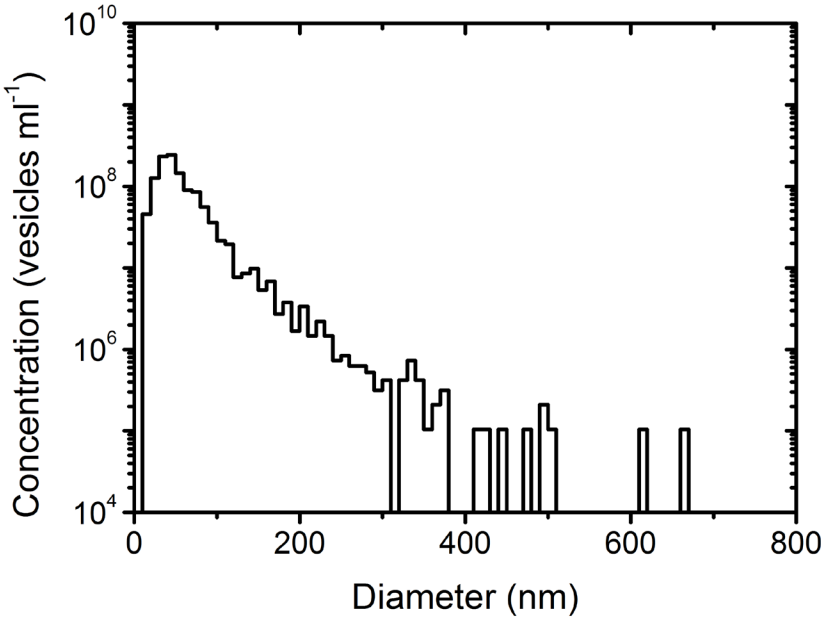


Introduction to extracellular vesicles

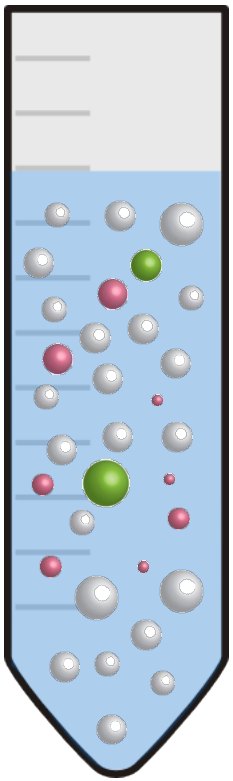




- cells release vesicles:
  spherical particles with phospholipid bilayer
- specialized functions
- clinically relevant

van der Pol et al., Pharmacol Rev (2012)

Determine refractive index to identify vesicles



vesicles ($1.36 \leq n \leq 1.45$ for $d > 500$ nm)*

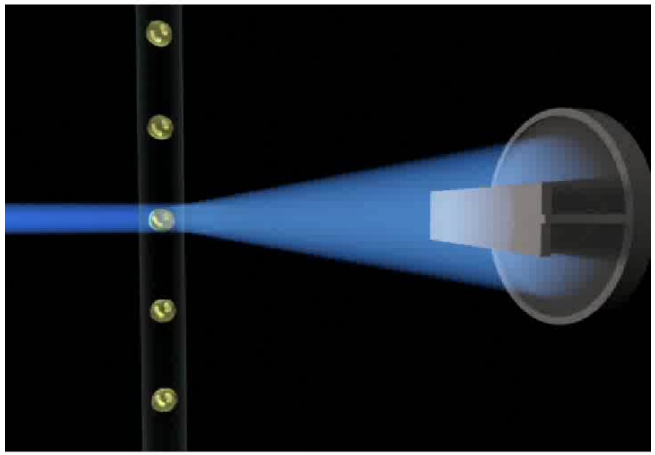
lipoproteins (n = 1.45-1.60)

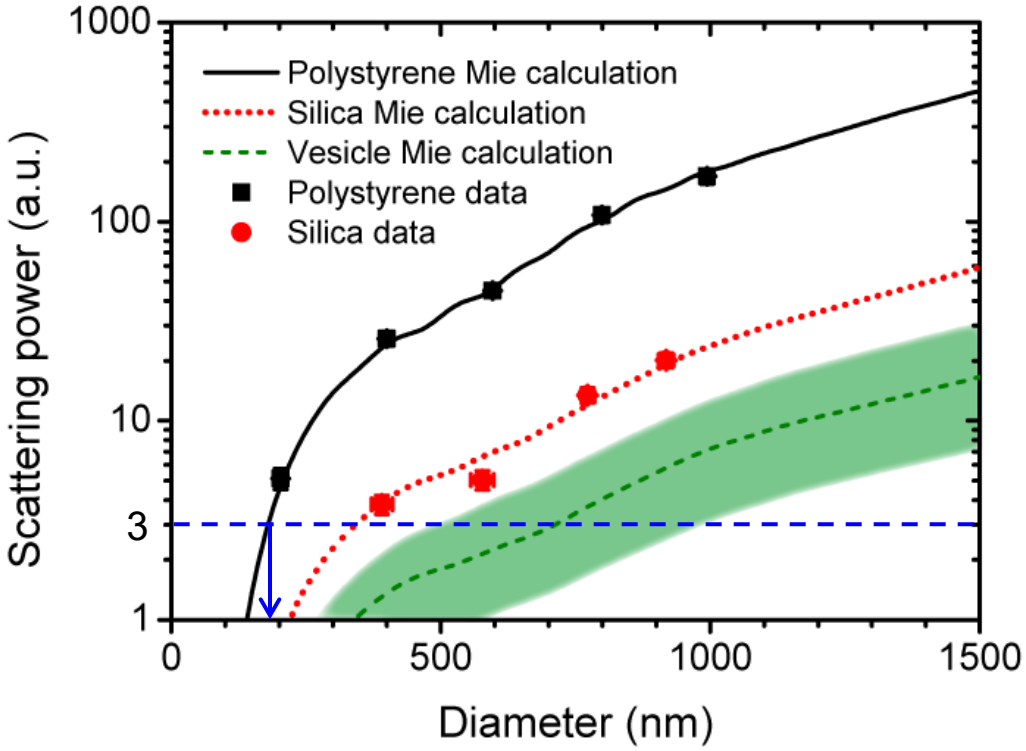
protein aggregates (n = 1.53-1.60)

- measure the refractive index to distinguish vesicles from lipoproteins and protein aggregates

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

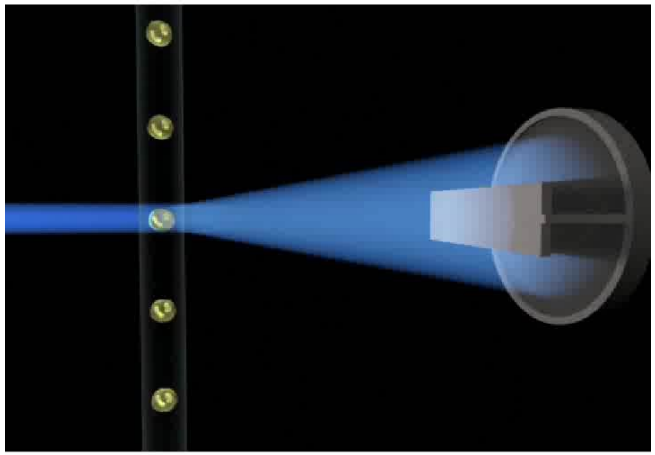
Refractive index to relate scatter to diameter

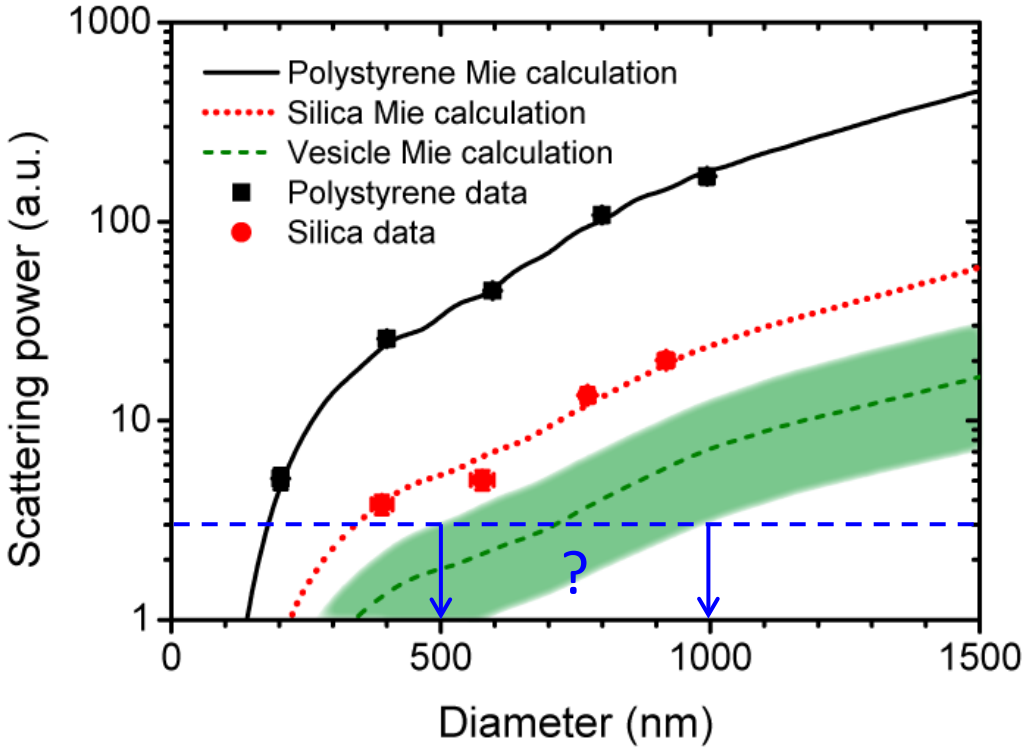




- flow cytometry is widely used to detect vesicles
- refractive index provides scatter to diameter relation

Refractive index of vesicles is unknown

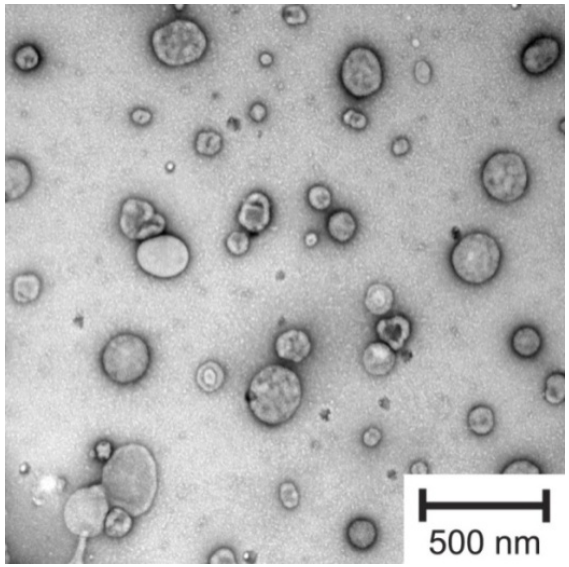




- refractive index of vesicles is unknown
- detection range is unknown

Goal

- determine the refractive index of sub micrometer vesicles in suspension
  - distinguish vesicles from lipoproteins and protein aggregates
  - provide insight in the vesicle detection range of flow cytometry



Methods - single particle tracking

- 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

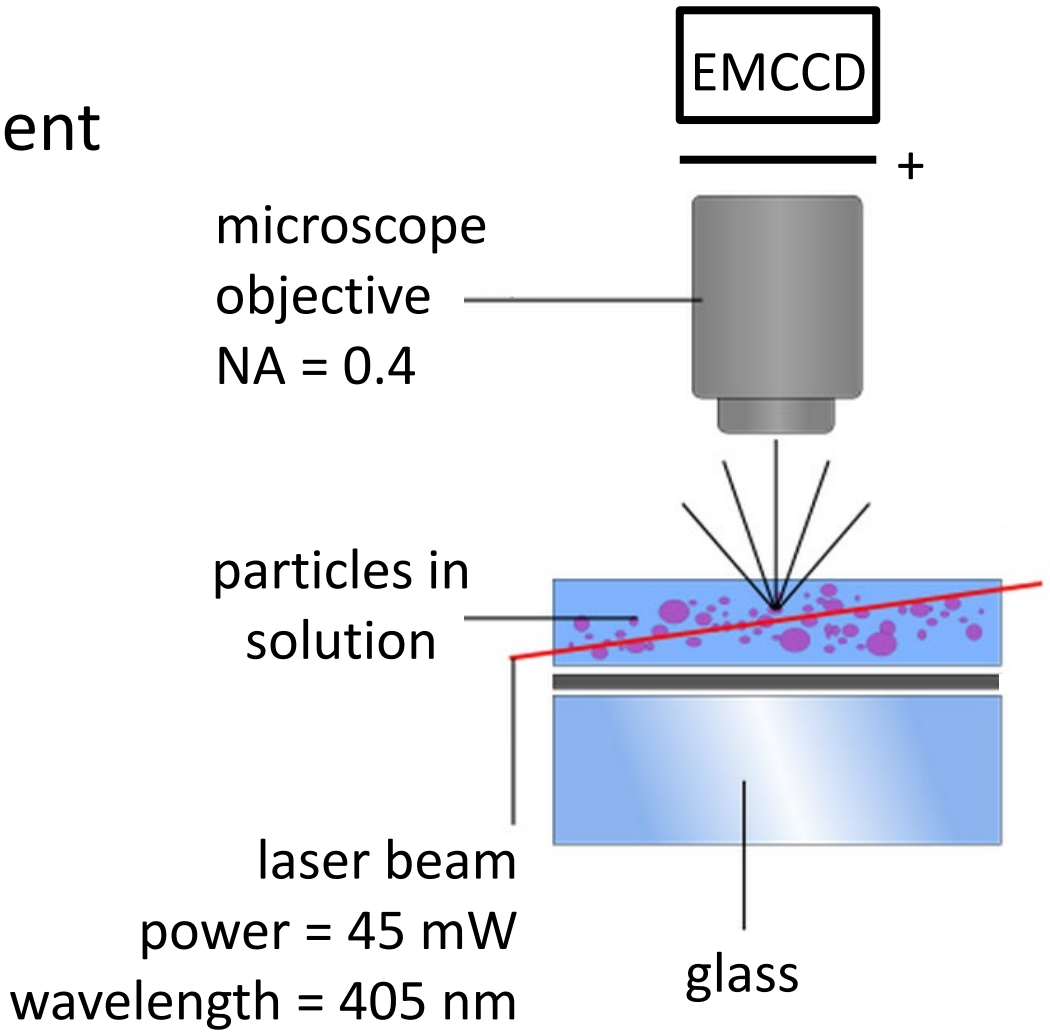
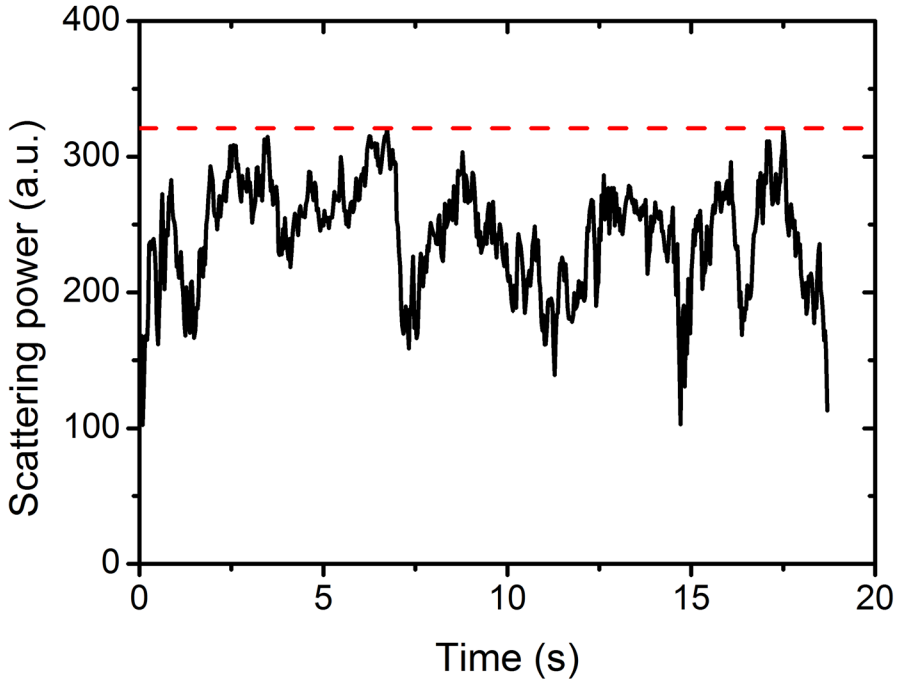


figure adapted from Nanosight Ltd, UK

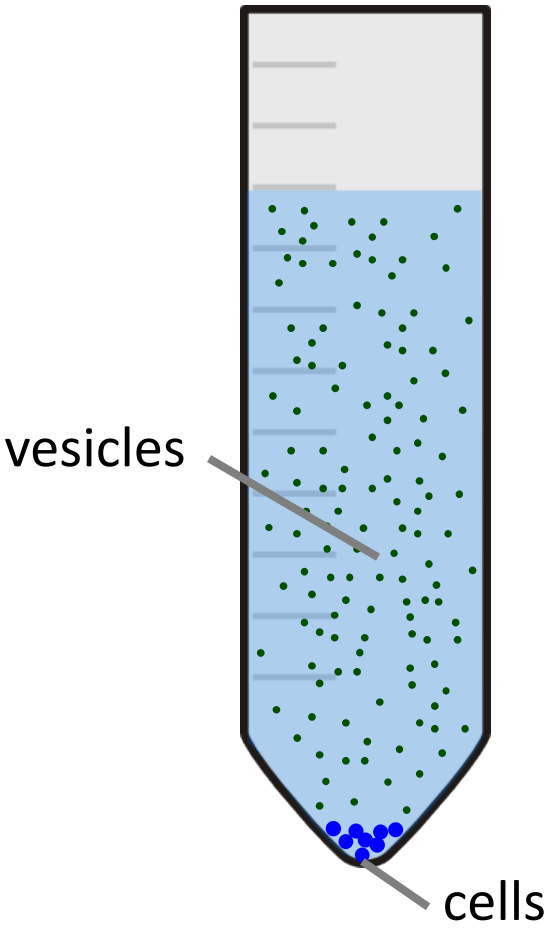
Methods - data acquisition and processing



- intensity corrected for camera shutter time and gain
- minimum tracklength 30 frames
- discard scatterers that saturate the camera

Methods - samples

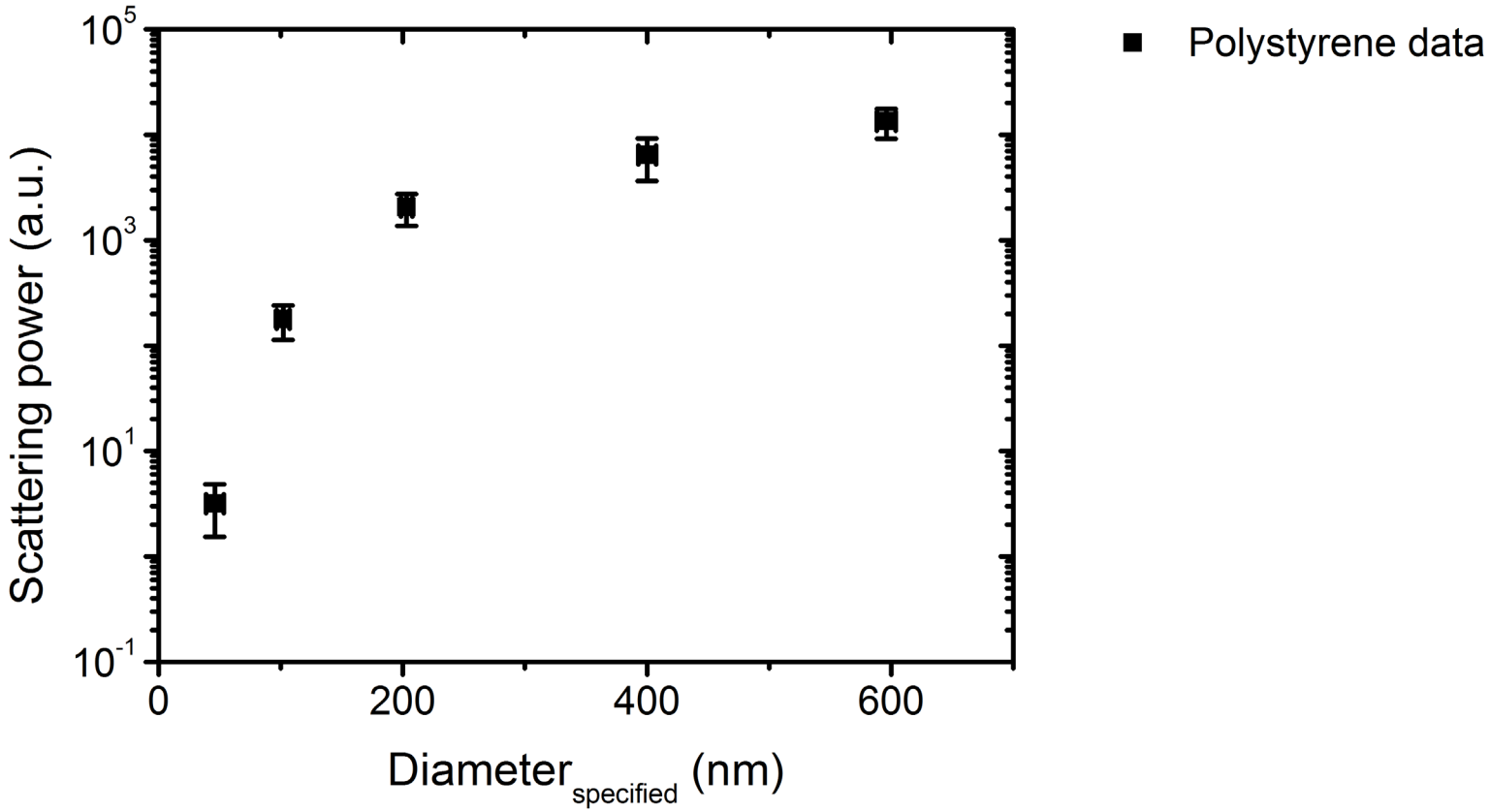
- Polystyrene beads (*n*=1.63)
  - Thermo Fisher Scientific, USA
- Silica beads (*n*=1.45)
  - Kisker Biotech, Germany
- Human urinary vesicles
  - differential centrifugation
  - protocol from metves.eu



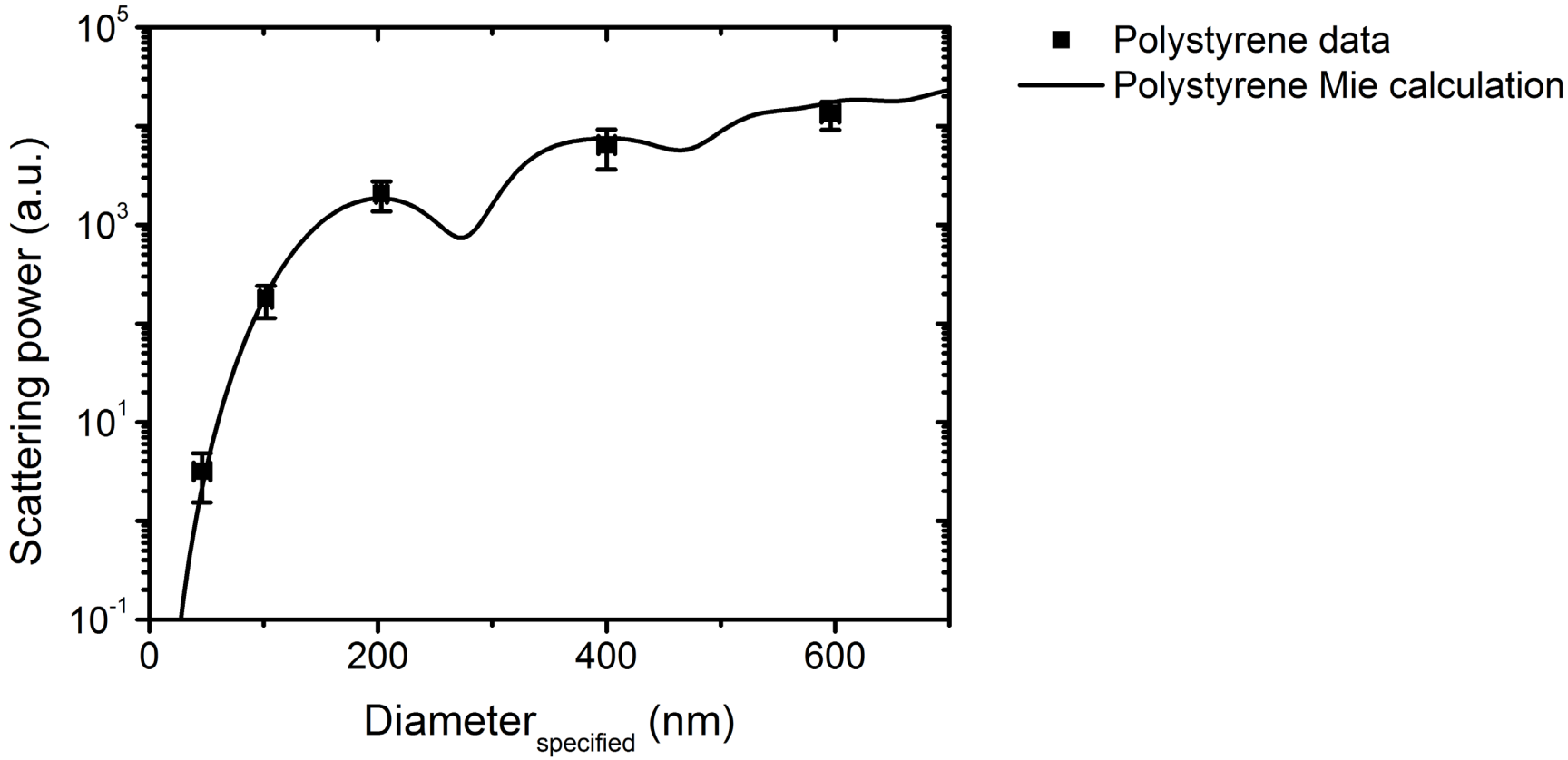
Methods - approach

- measure light scattering of beads
- describe measurements by Mie theory
- derive particle diameter from Brownian motion
- validate technique with a beads mixture
- determine the refractive index of vesicles

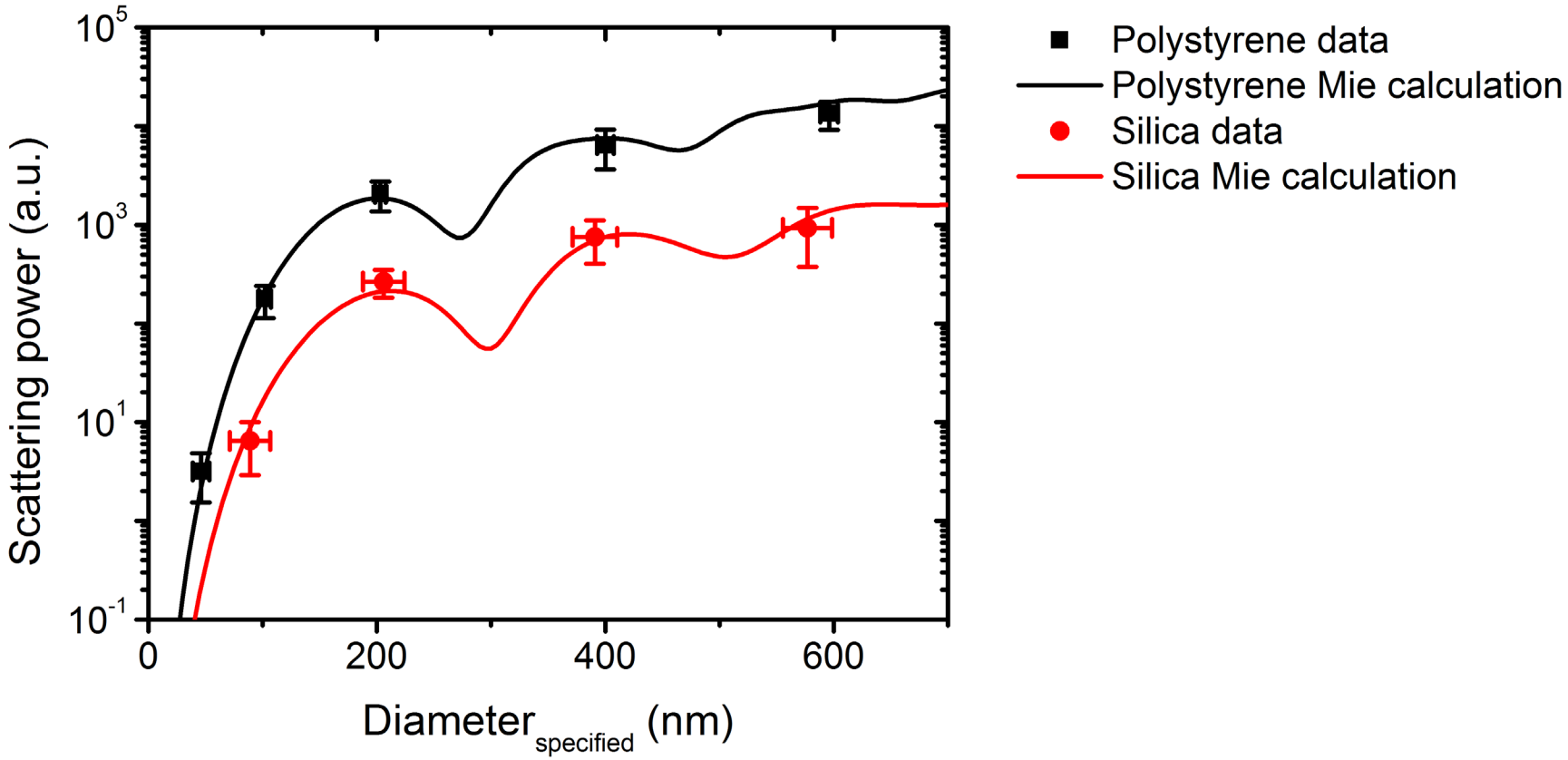
Results - scattering power versus diameter of polystyrene beads



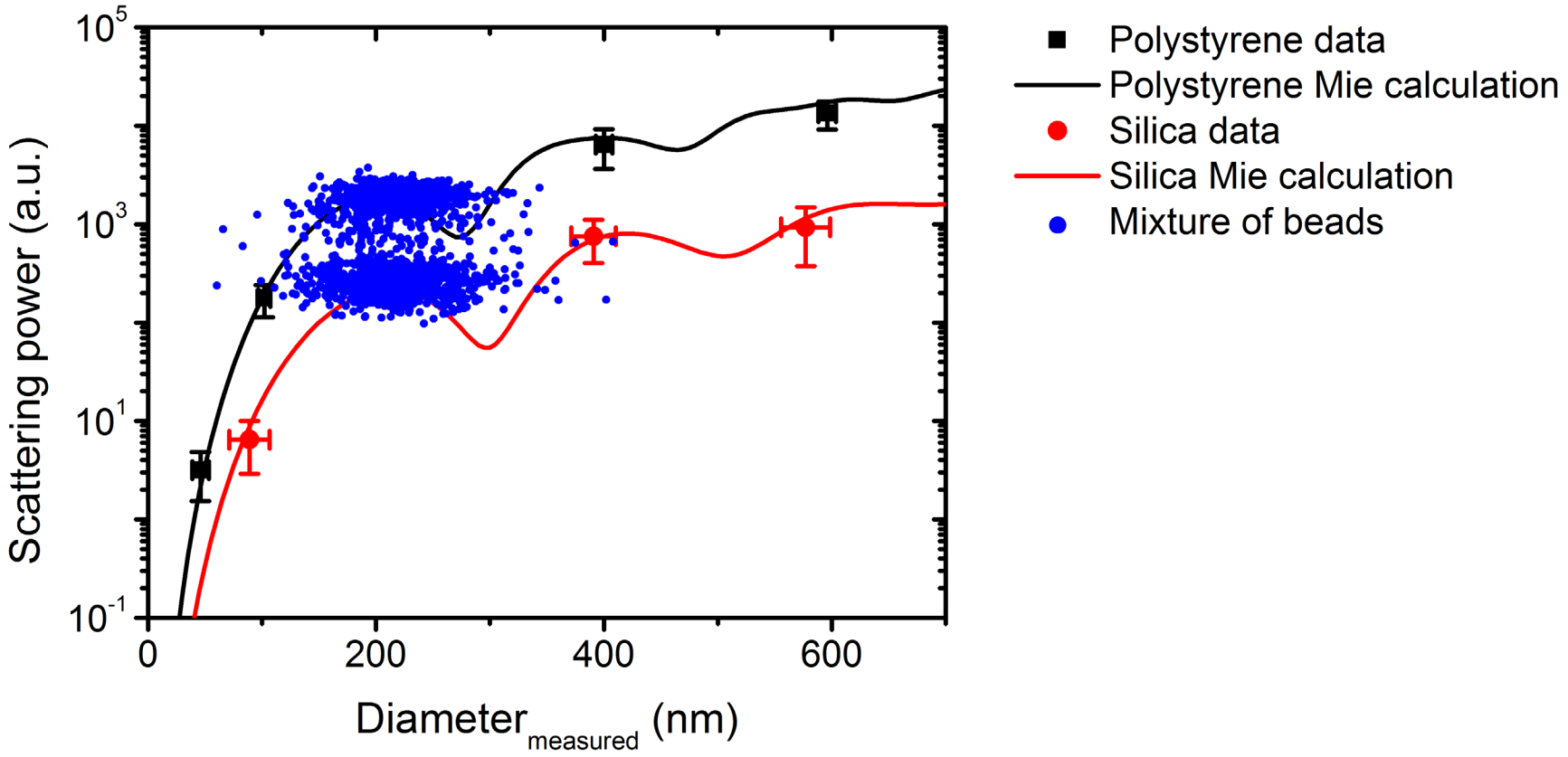
Results - scattering power versus diameter of polystyrene beads described by Mie theory



Results - scattering power versus diameter of polystyrene and silica beads



Results - scattering power versus diameter of a mixture of polystyrene and silica beads



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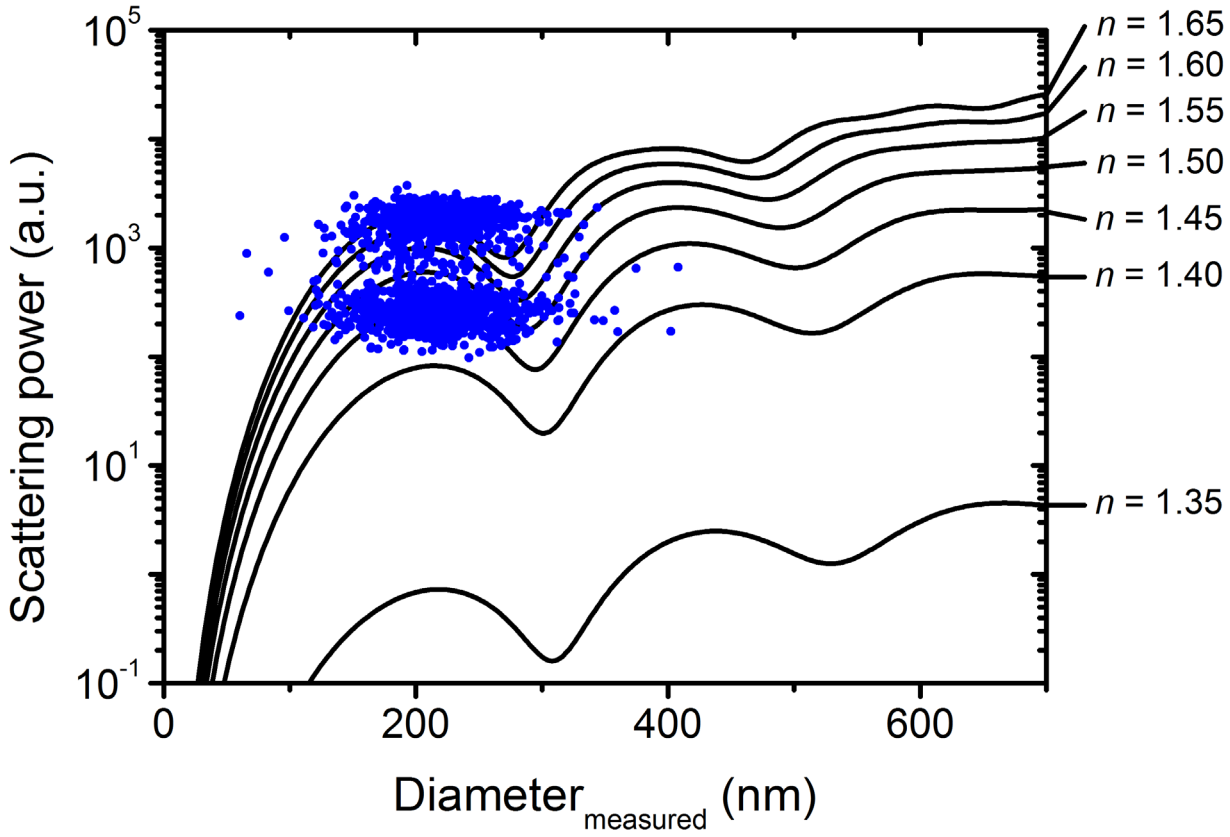
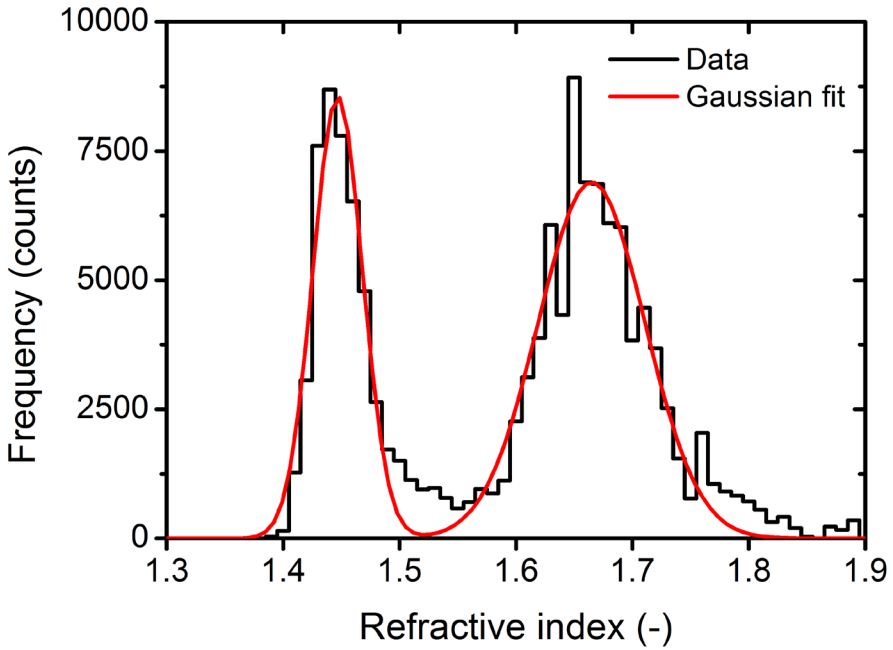
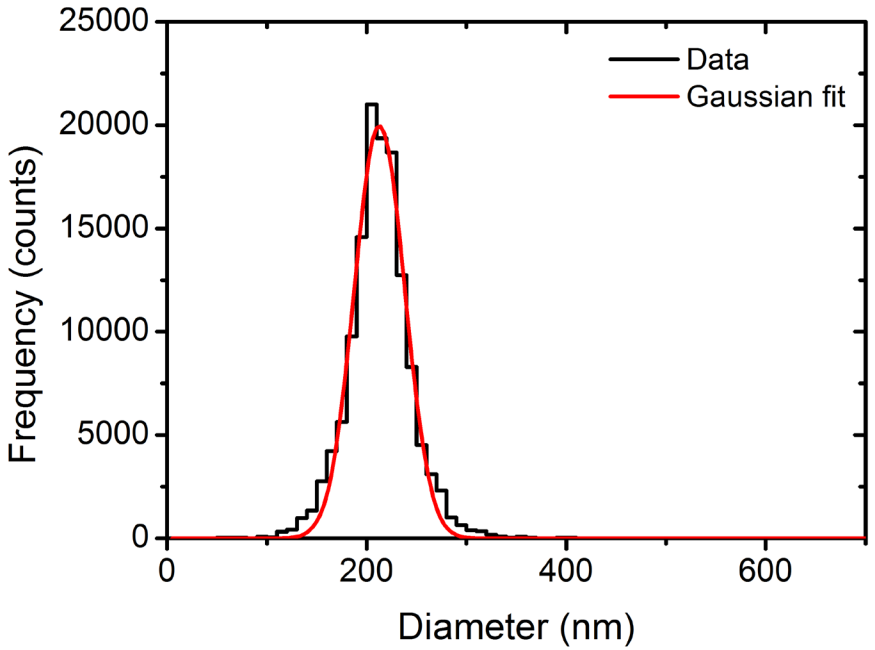


Results - refractive index and size distribution of a mixture of polystyrene and silica beads





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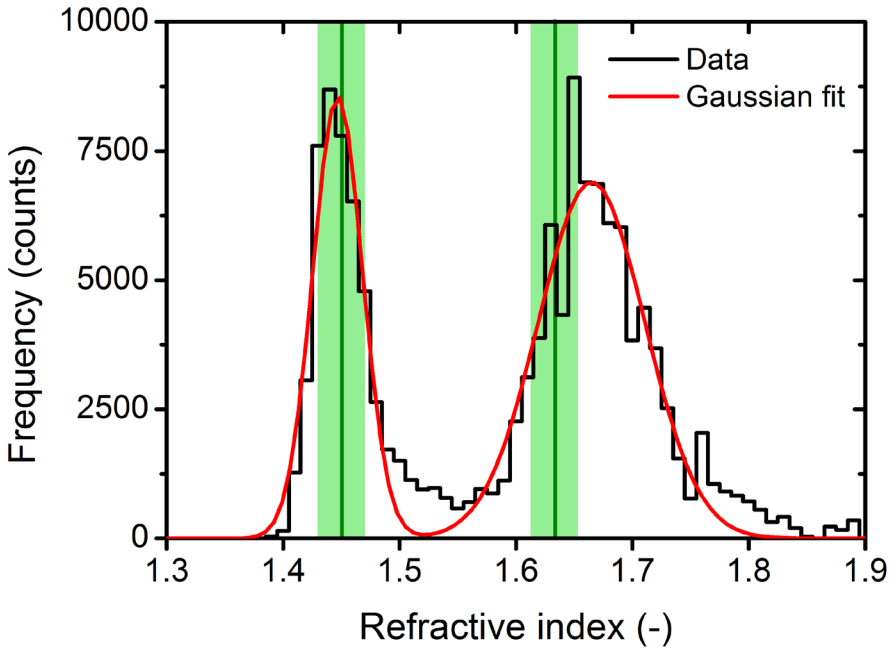
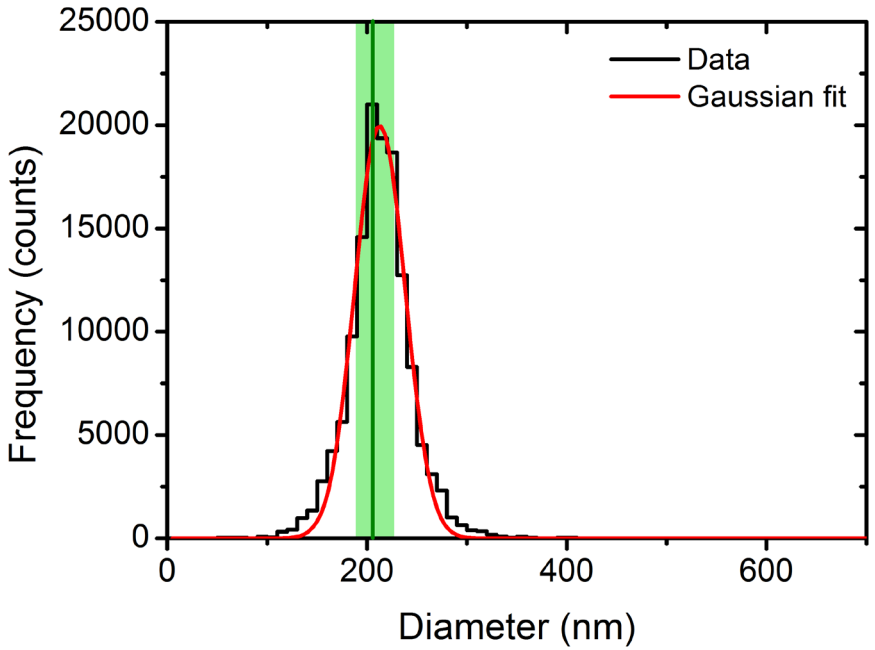


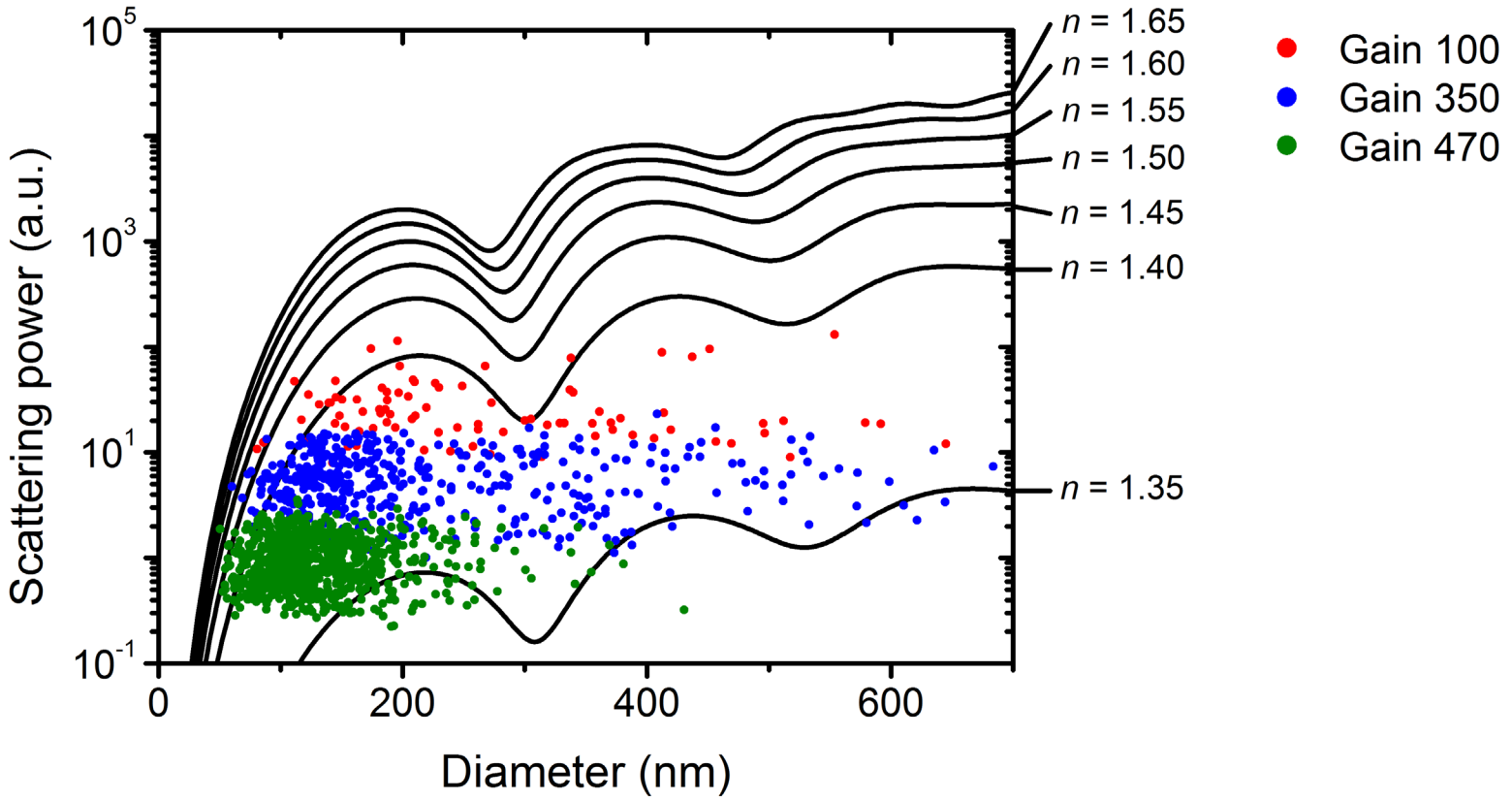


	n silica	*n* polystyrene	diameter (nm)
Expected	1.45±0.02	1.63±0.02	206±18
Measured	1.45±0.02	1.67±0.04	213±25

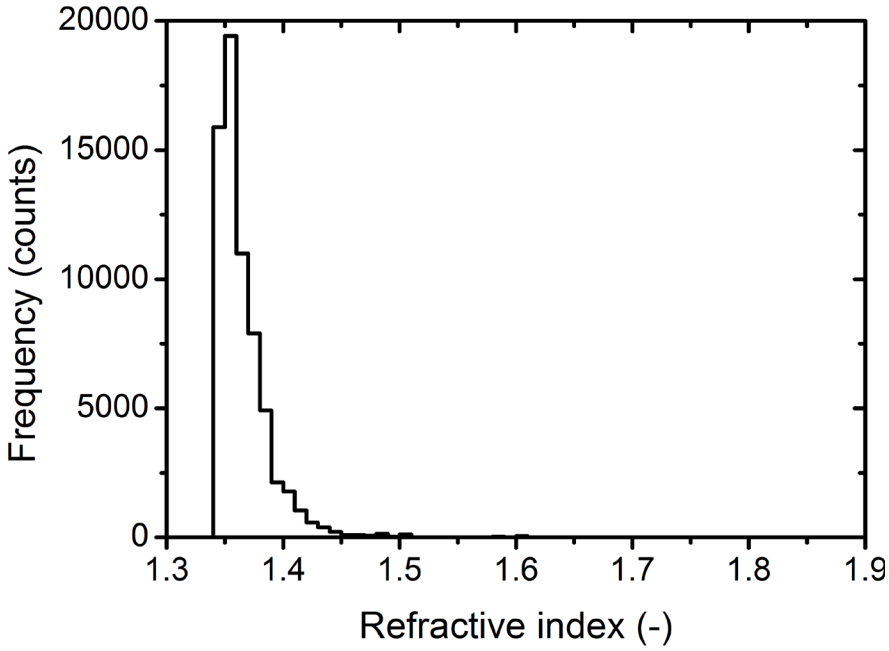
Methods - approach

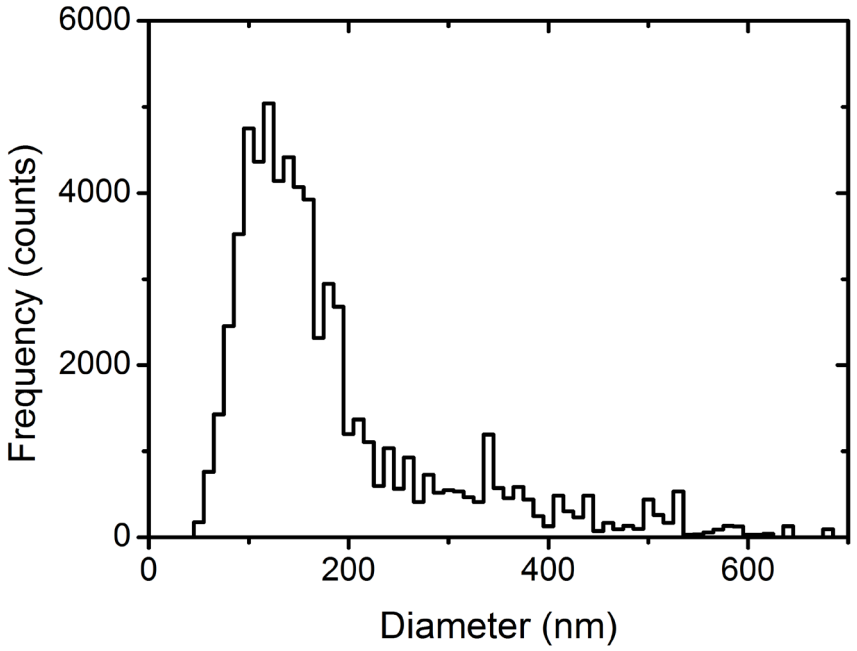
- measure light scattering of beads
- describe measurements by Mie theory
- derive particle diameter from Brownian motion
- validate technique with a beads mixture
- determine the refractive index of vesicles

Results - scattering power versus diameter of urinary vesicles

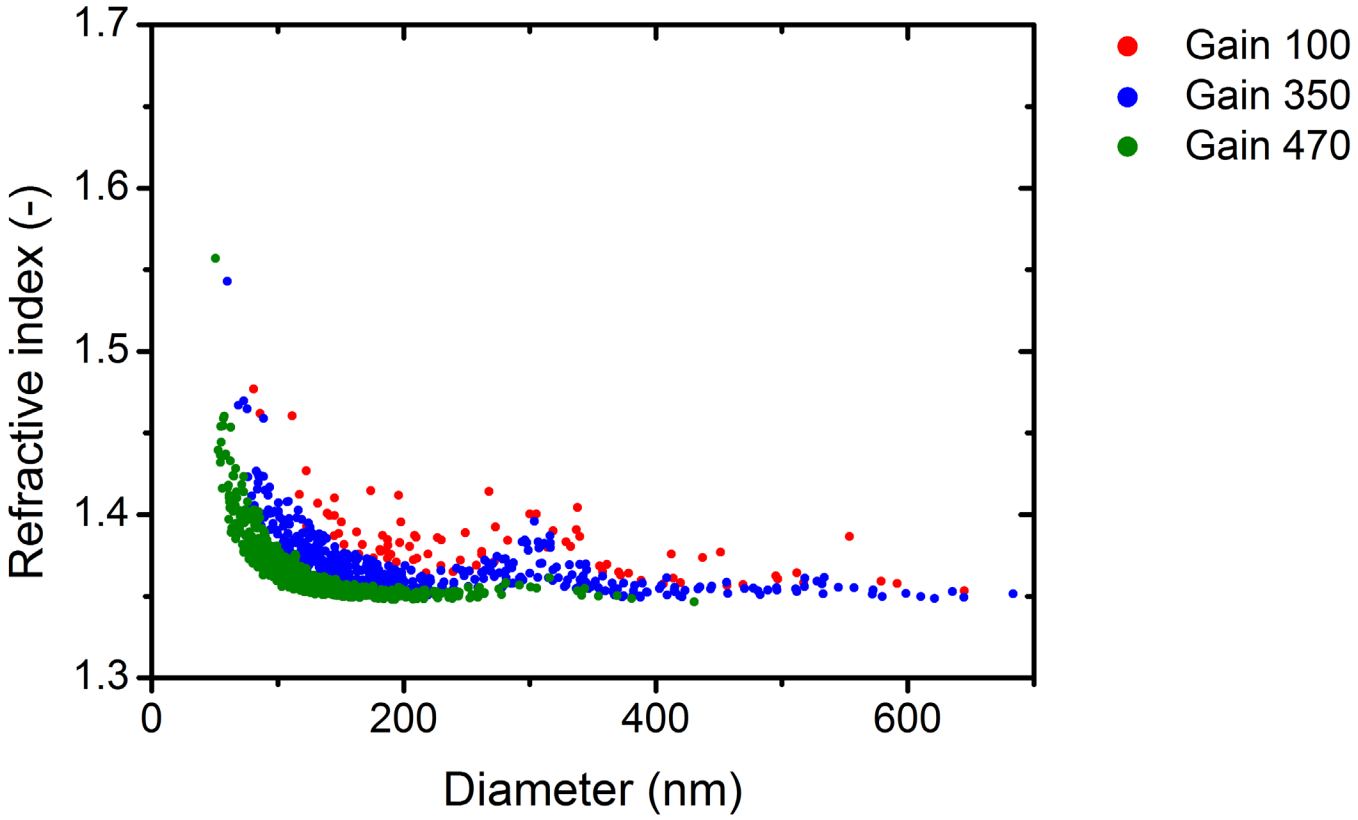


Results - size and refractive index distribution of urinary vesicles

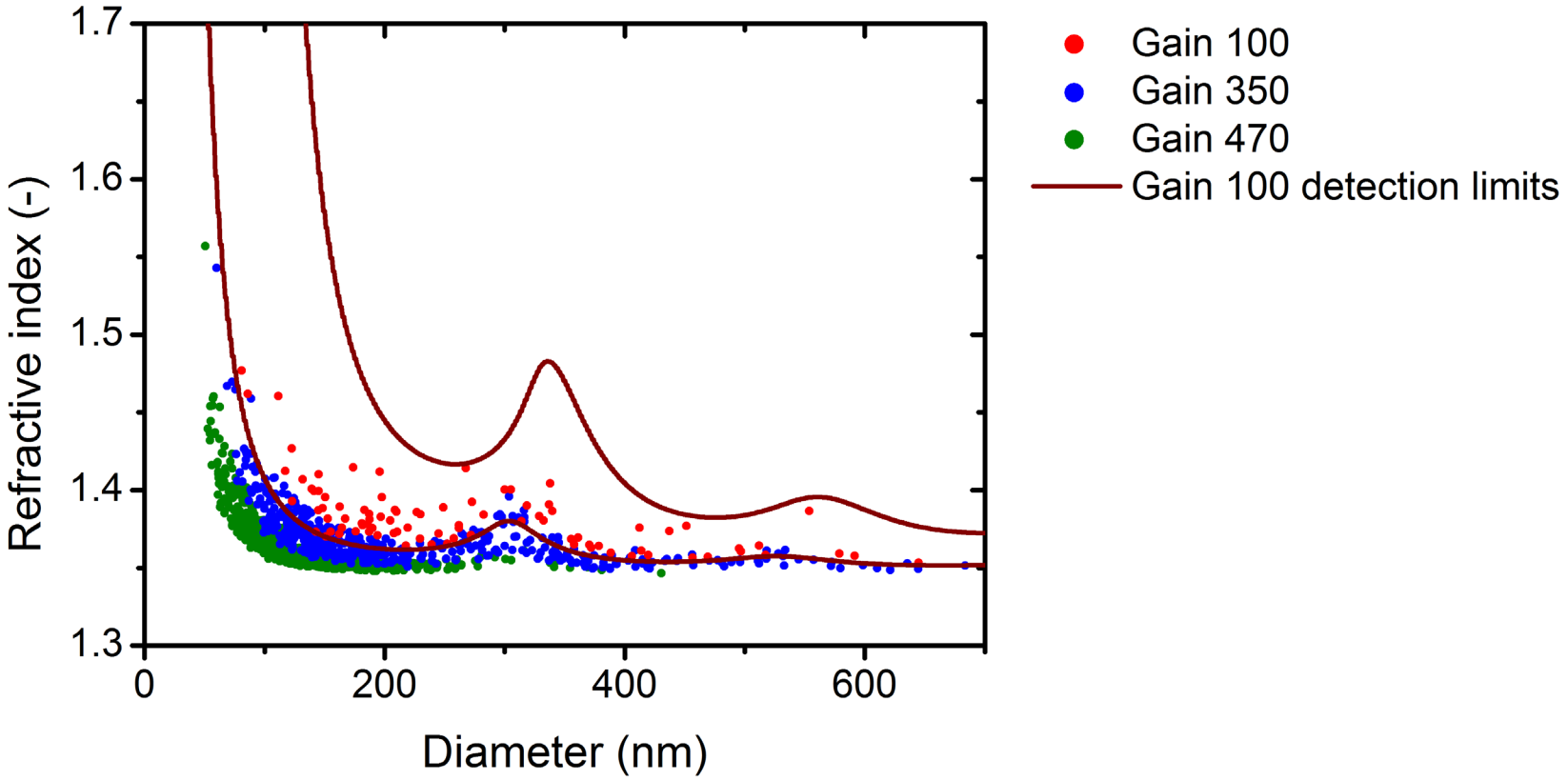




Results - refractive index versus diameter for urinary vesicles



Results - refractive index versus diameter detection limits for urinary vesicles



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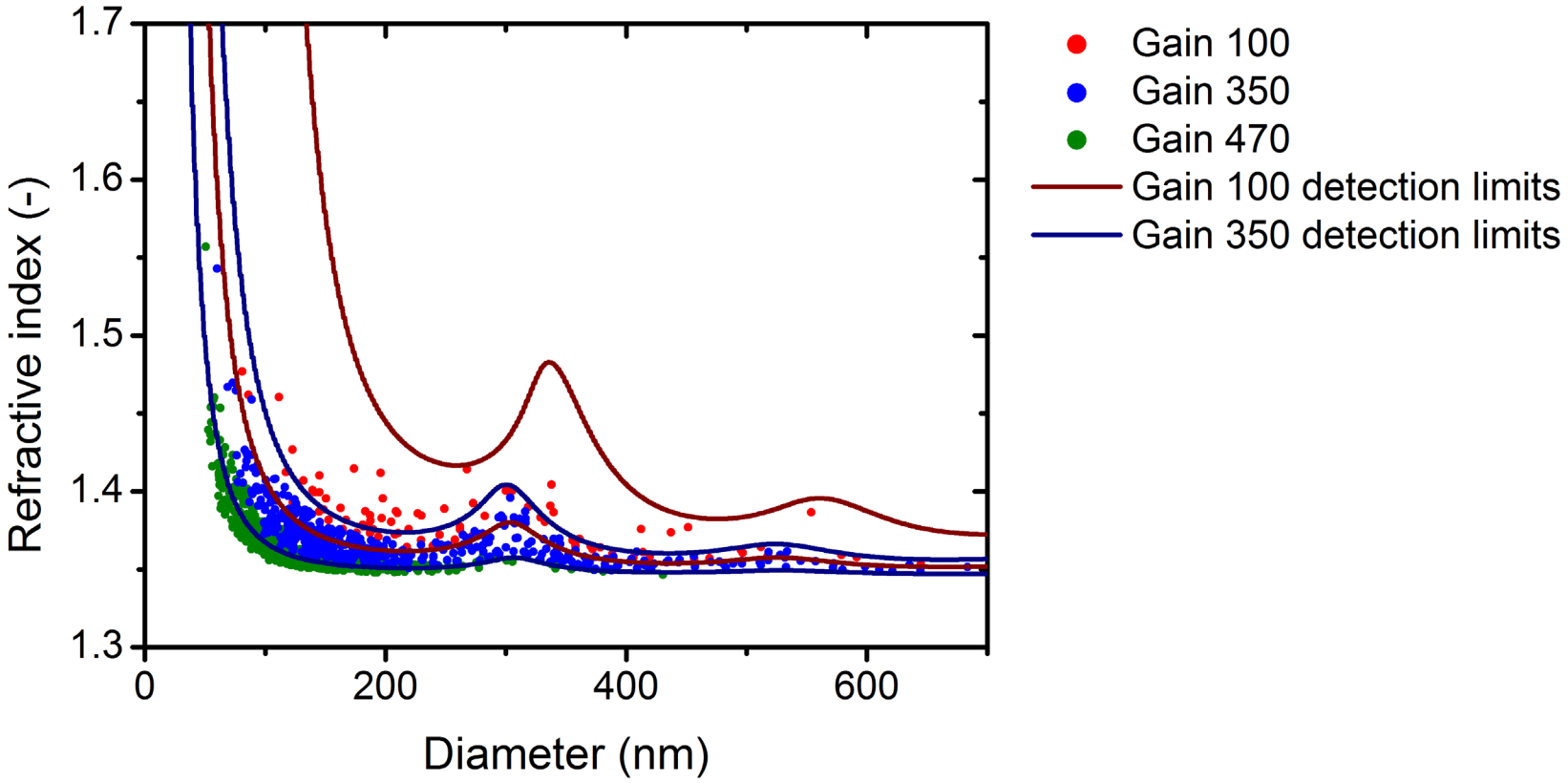

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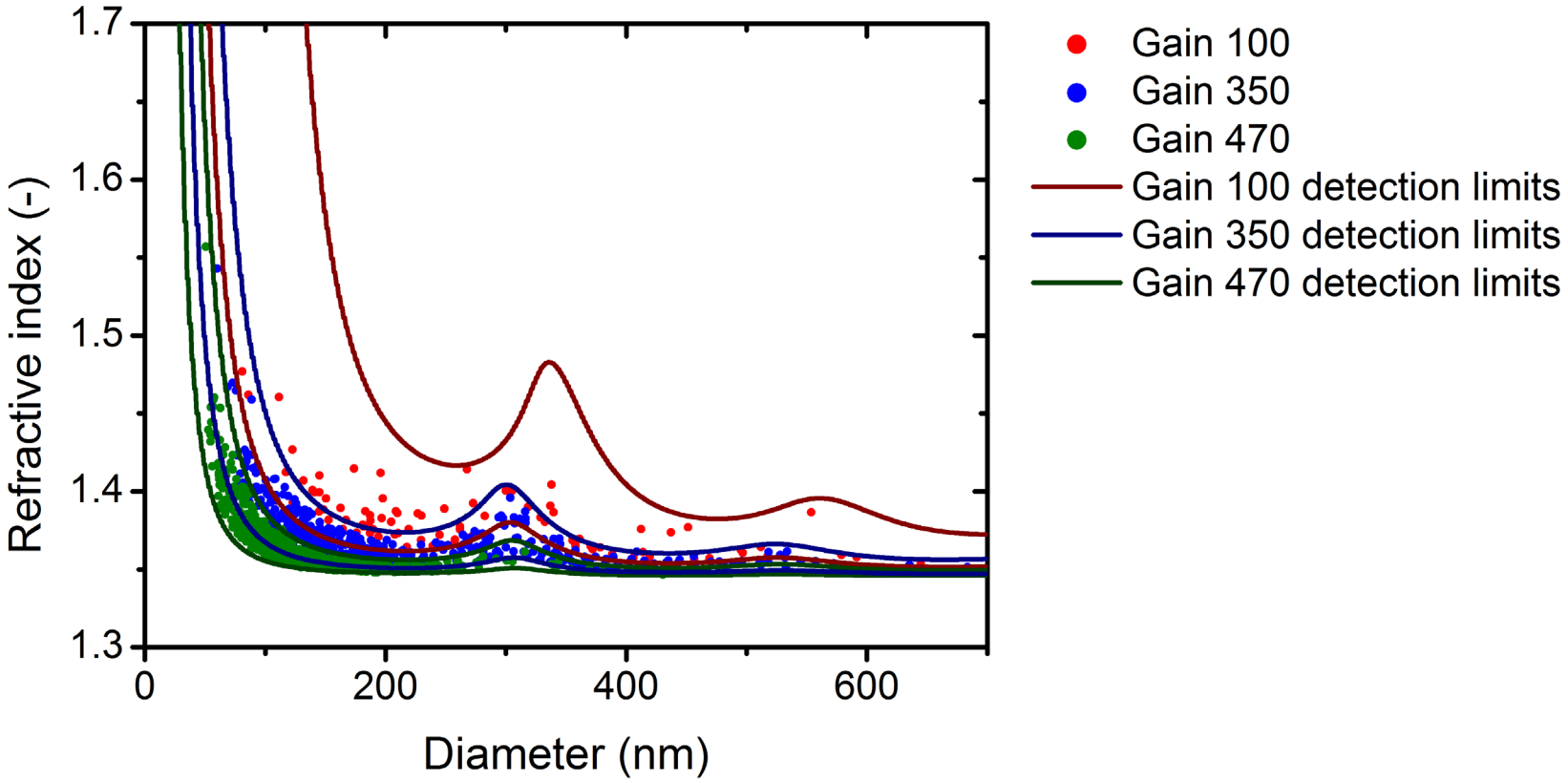

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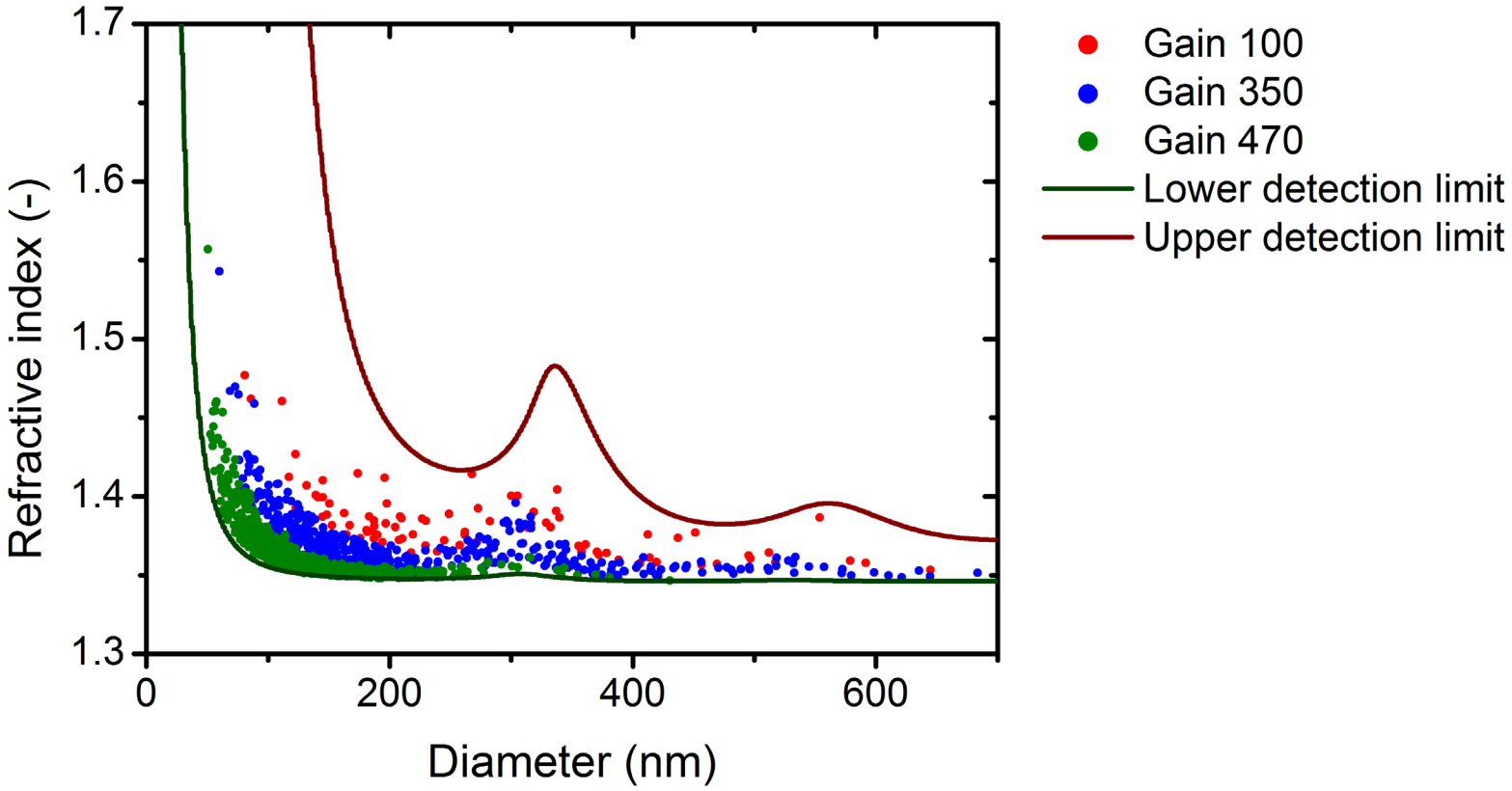
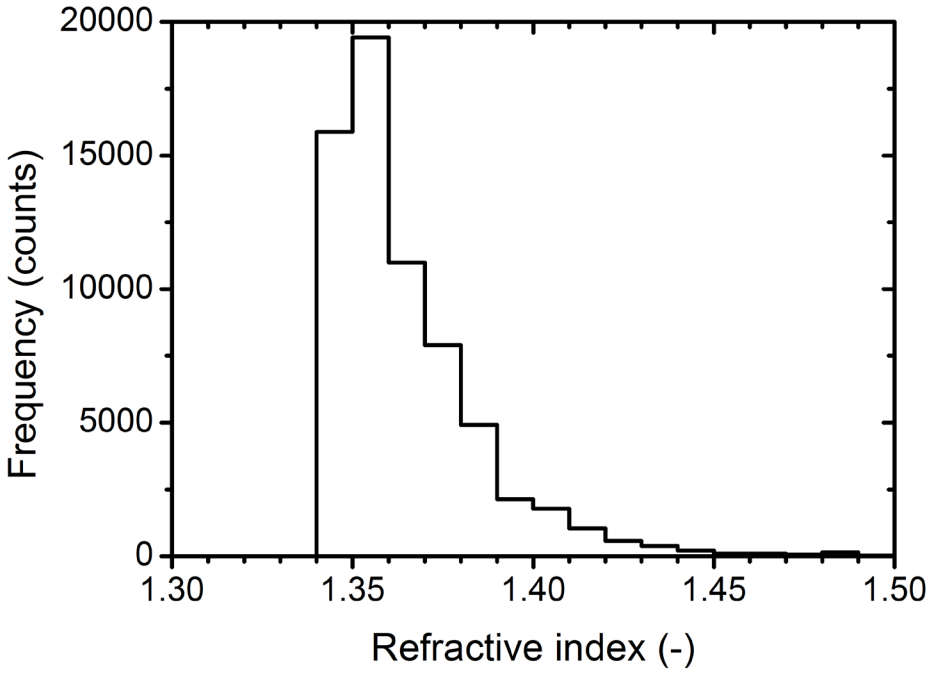
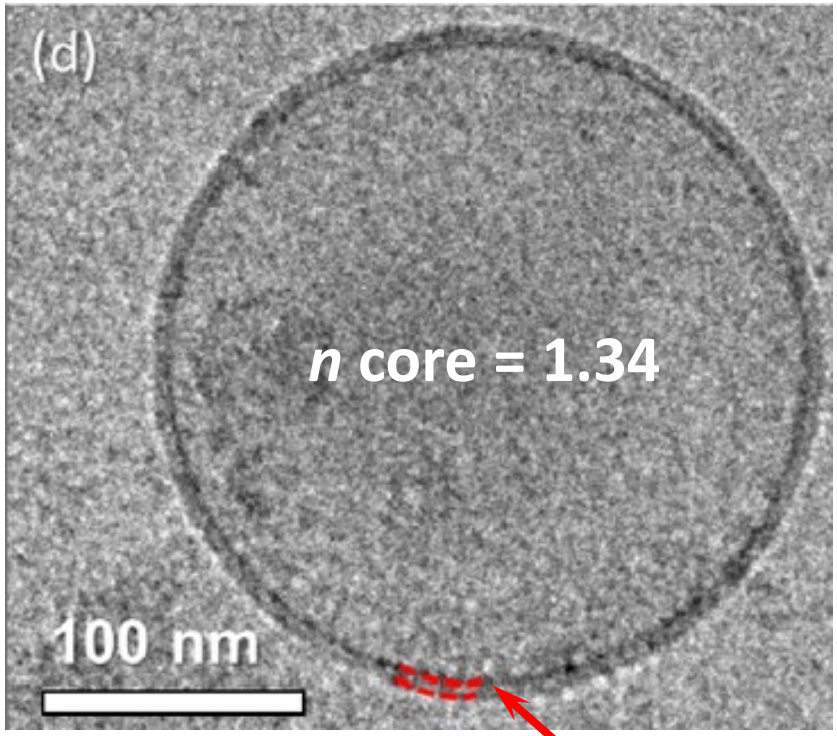


Conclusions



- single particle tracking can be used to determine the refractive index of single sub micrometer particles
- median refractive index of urinary vesicles is 1.36 with 90% of the vesicles between 1.35 and 1.41

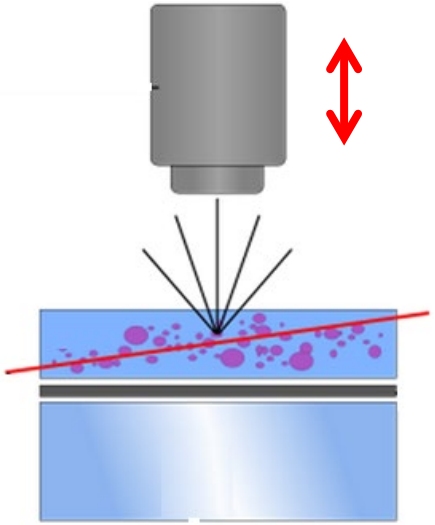
Discussion - urinary vesicles contain mainly water



thickness = 5 nm
n membrane = 1.46 *

image courtesy of Issman et al., PLoS ONE (2013)
* van Manen et al., Biophys. J. (2007)

Improvements



- scanning objective along optical axis
  - measure scattering exactly in the focal plane
  - increase tracklength and diameter accuracy
- more homogeneous illumination

- 8939-2: *Distinction of tumor-derived vesicles from normal vesicles by Raman microspectroscopy*
  - today at 13:20 in room 202 (Mezzanine)
- more on vesicle detection: edwinvanderpol.com