

Refractive index of extracellular vesicles by nanoparticle tracking analysis

Edwin van der Pol^{1,2}

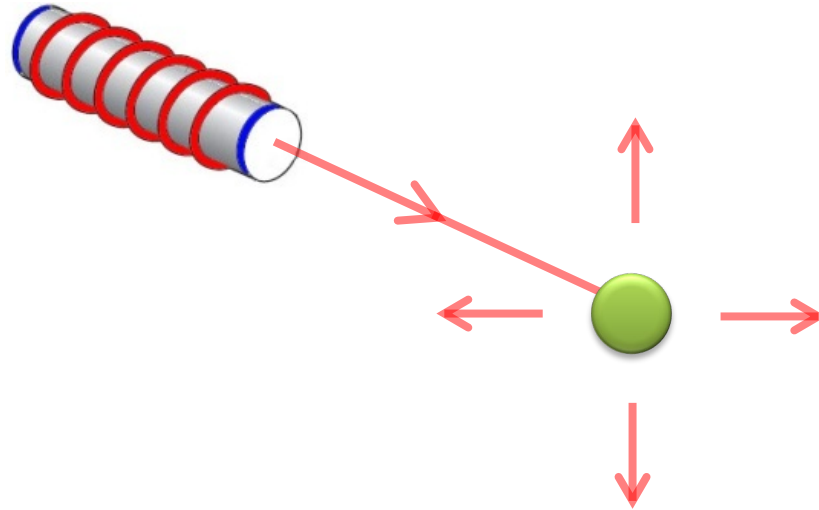
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April 30th, 2014



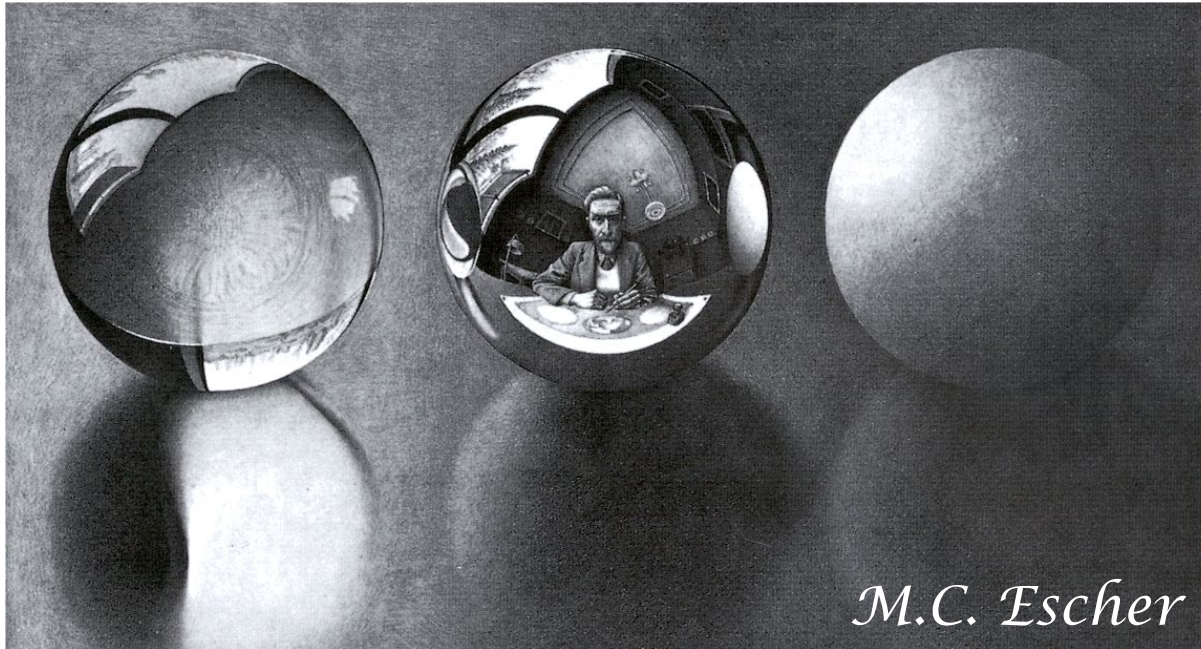
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Introduction to light scattering



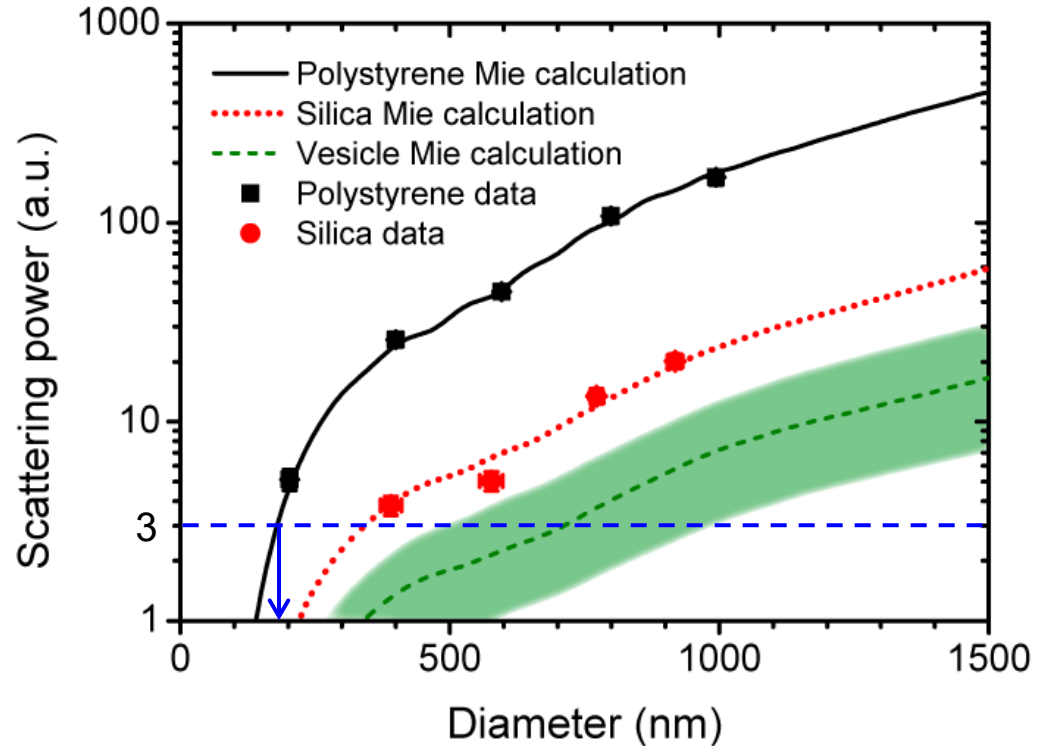
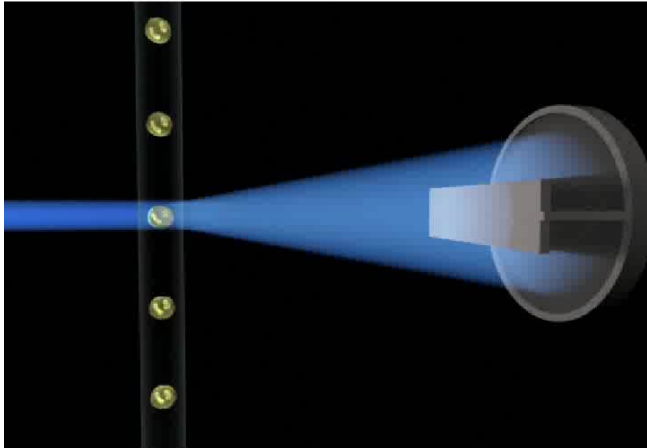
- light illuminating a vesicle is partly absorbed and partly scattered (deflected)
- light scattering depends on size and refractive index

Introduction to the refractive index



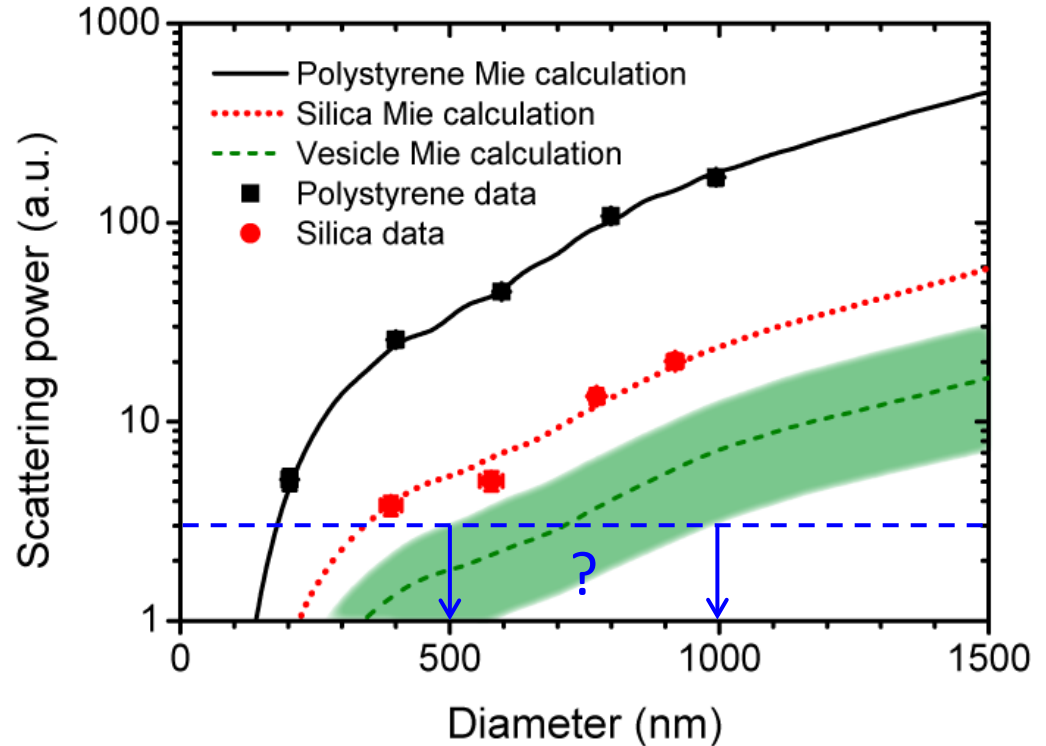
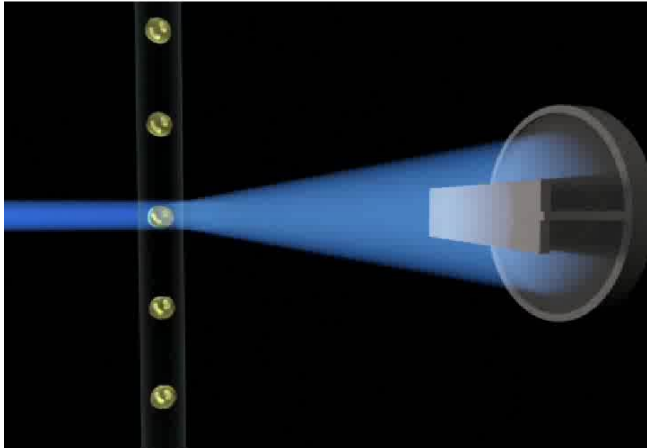
- the refractive index
 - is defined as $n = c_{vacuum} / v_{medium}$
 - affects refraction and reflection

Refractive index to relate scatter to diameter



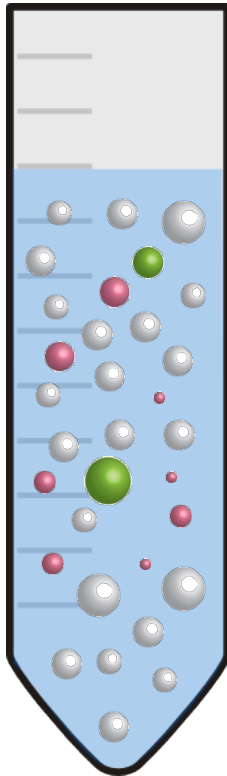
- flow cytometry is widely used to detect vesicles
- refractive index provides scatter to diameter relation

Refractive index of vesicles is unknown



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

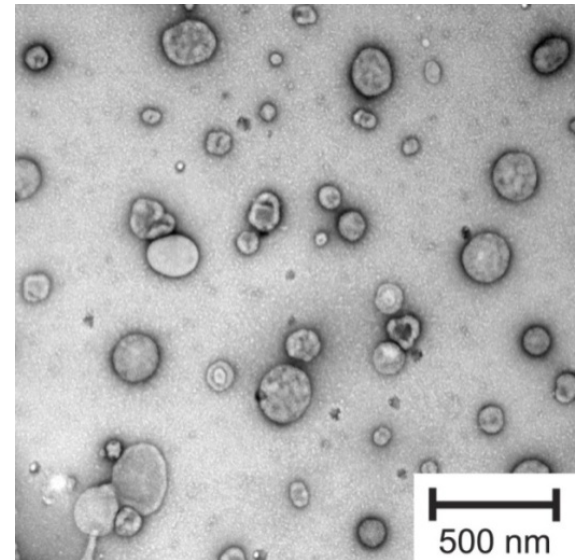
Determine refractive index to identify vesicles



- lipoproteins ($n = 1.45-1.60$)
- protein aggregates ($n = 1.53-1.60$)
- vesicles $\left(\begin{array}{l} d \geq 500 \text{ nm} \rightarrow n = 1.40^* \\ d < 500 \text{ nm} \rightarrow n = ? \end{array} \right)$

Problem

- hitherto no technique is capable of determining the refractive index of particles being
 - <500 nm
 - heterogeneous in size
 - heterogeneous in refractive index
 - in suspension



Goal

- determine the refractive index of extracellular vesicles <500 nm in suspension

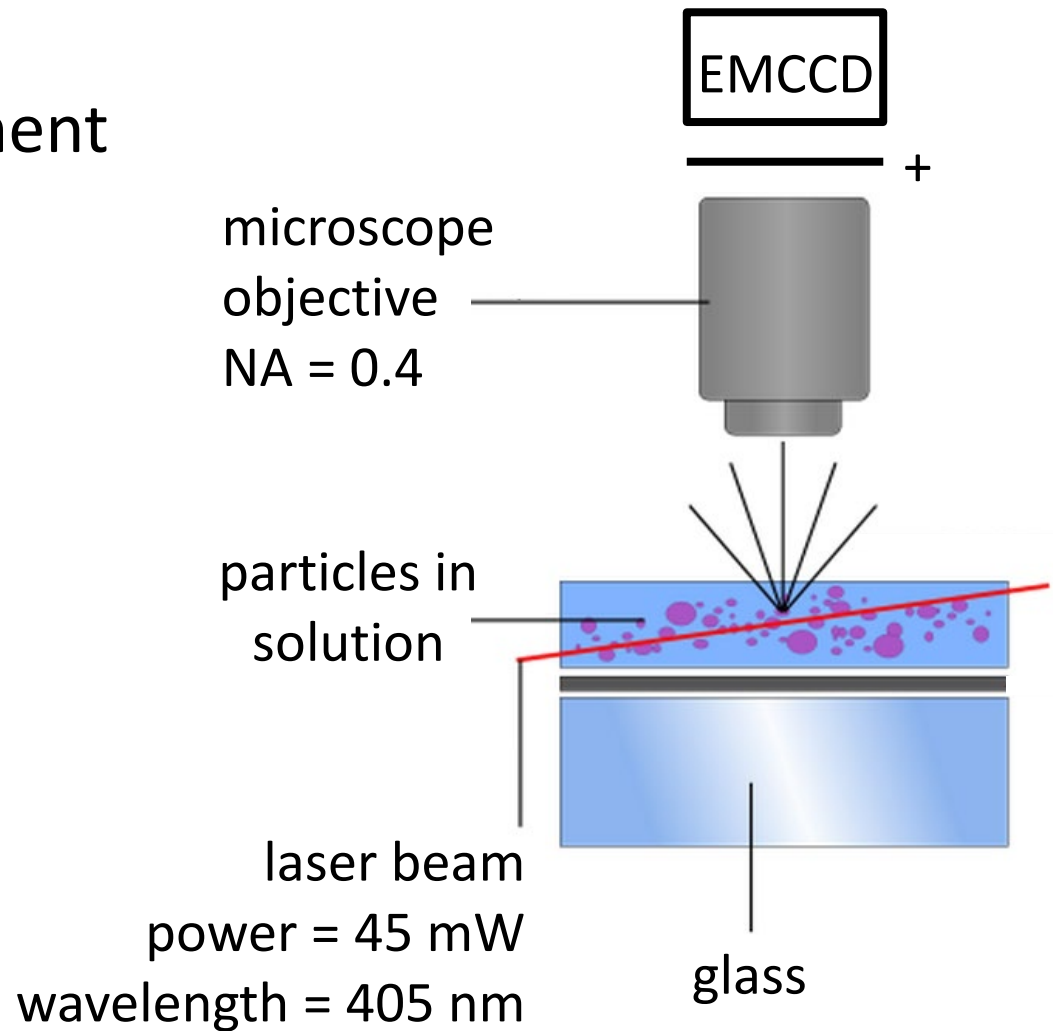
Methods – nanoparticle tracking analysis



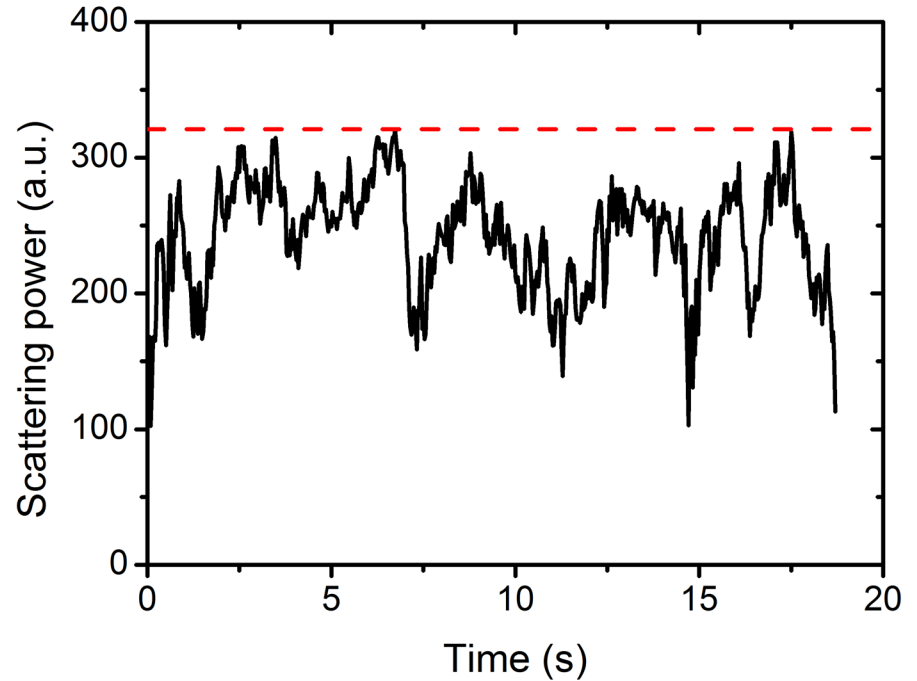
- obtain **particle diameter** d by tracking the Brownian motion of single particles (Stokes-Einstein equation)
- measure **scattering power** P
- derive particle **refractive index** $n(P,d)$ from Mie theory

Methods - setup

- Commercial instrument
 - Nanosight NS-500



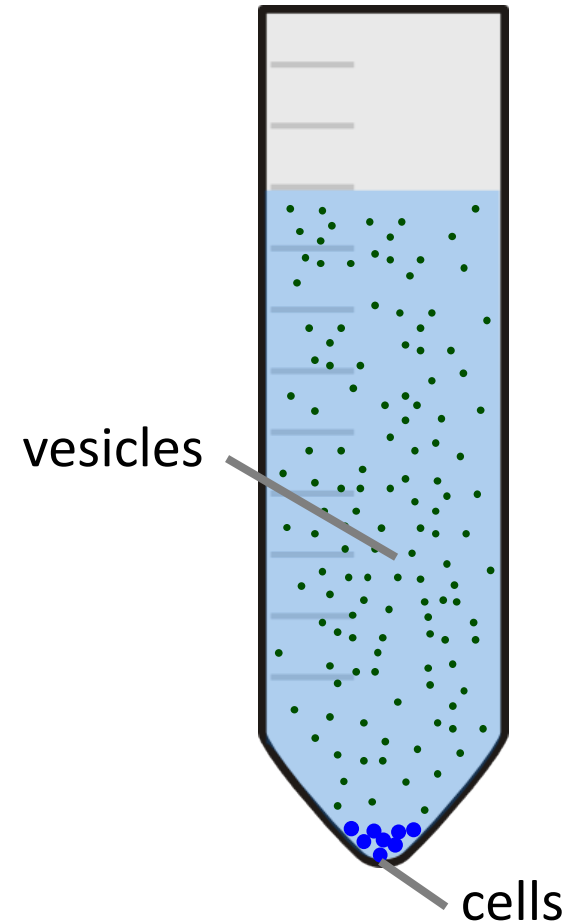
Methods - data acquisition and processing



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

Methods - samples

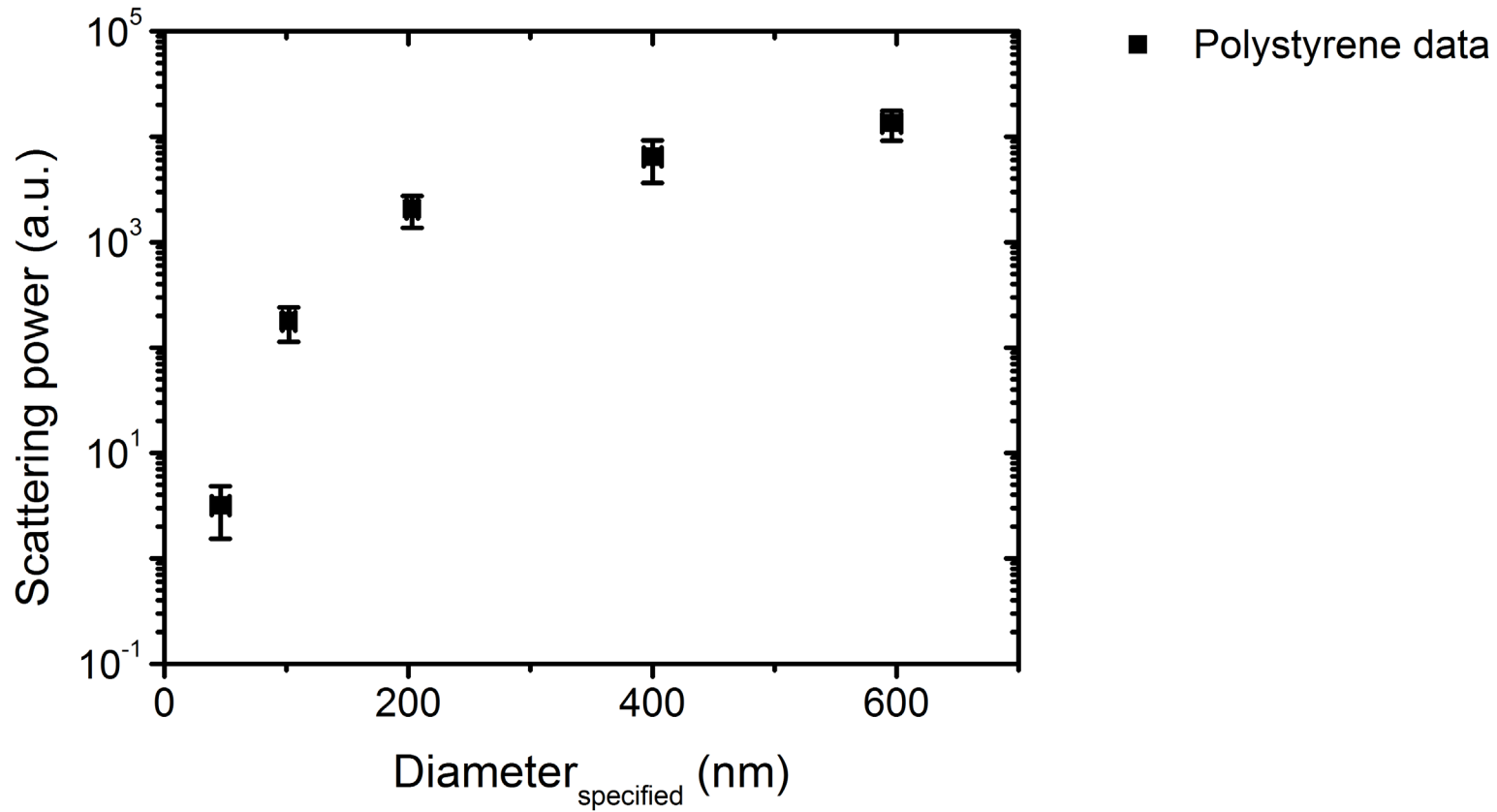
- Polystyrene beads ($n=1.63$)
 - Thermo Fisher Scientific, USA
- Silica beads ($n=1.45$)
 - Kisker Biotech, Germany
- Human urinary vesicles
 - differential centrifugation
 - protocol from metves.eu



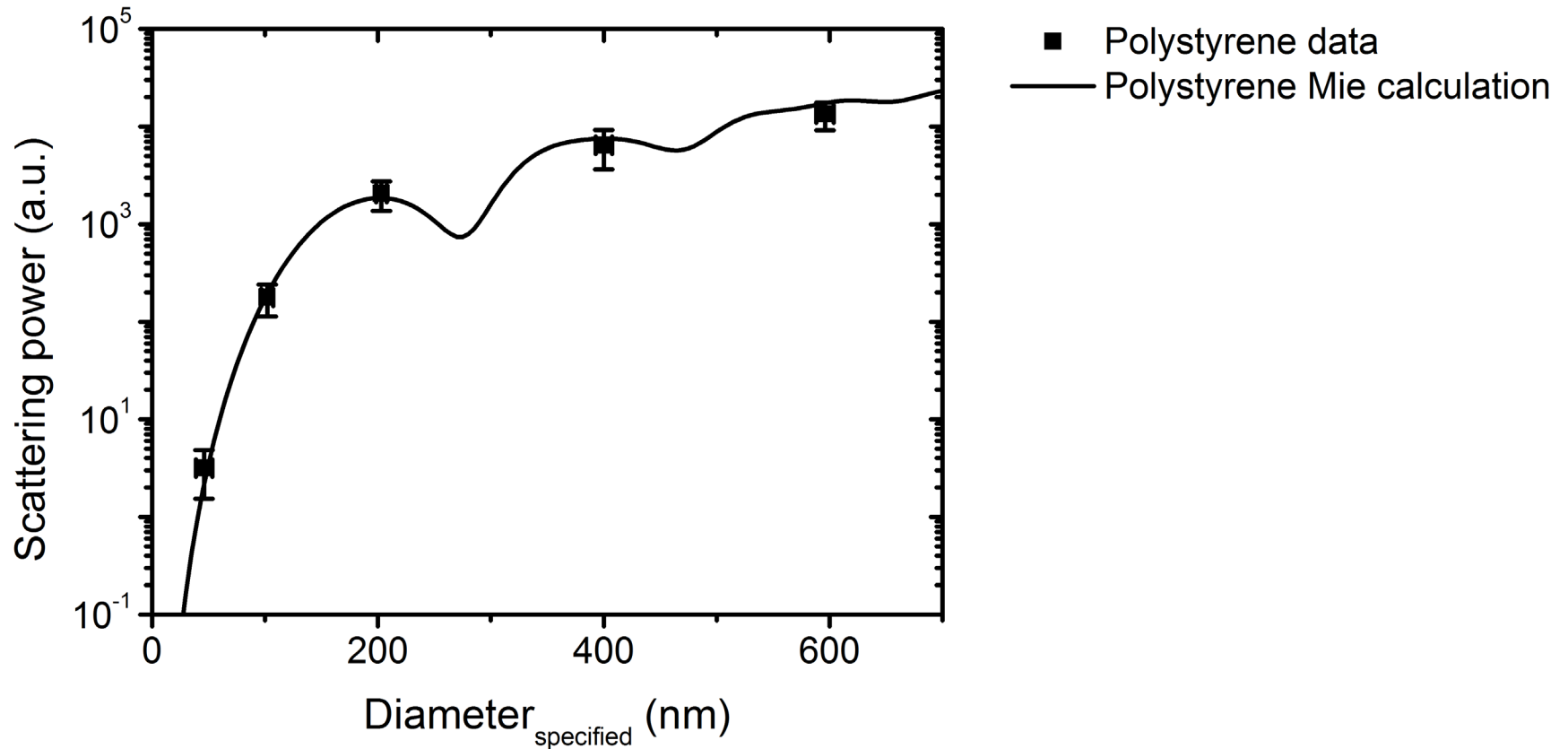
Methods - approach

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

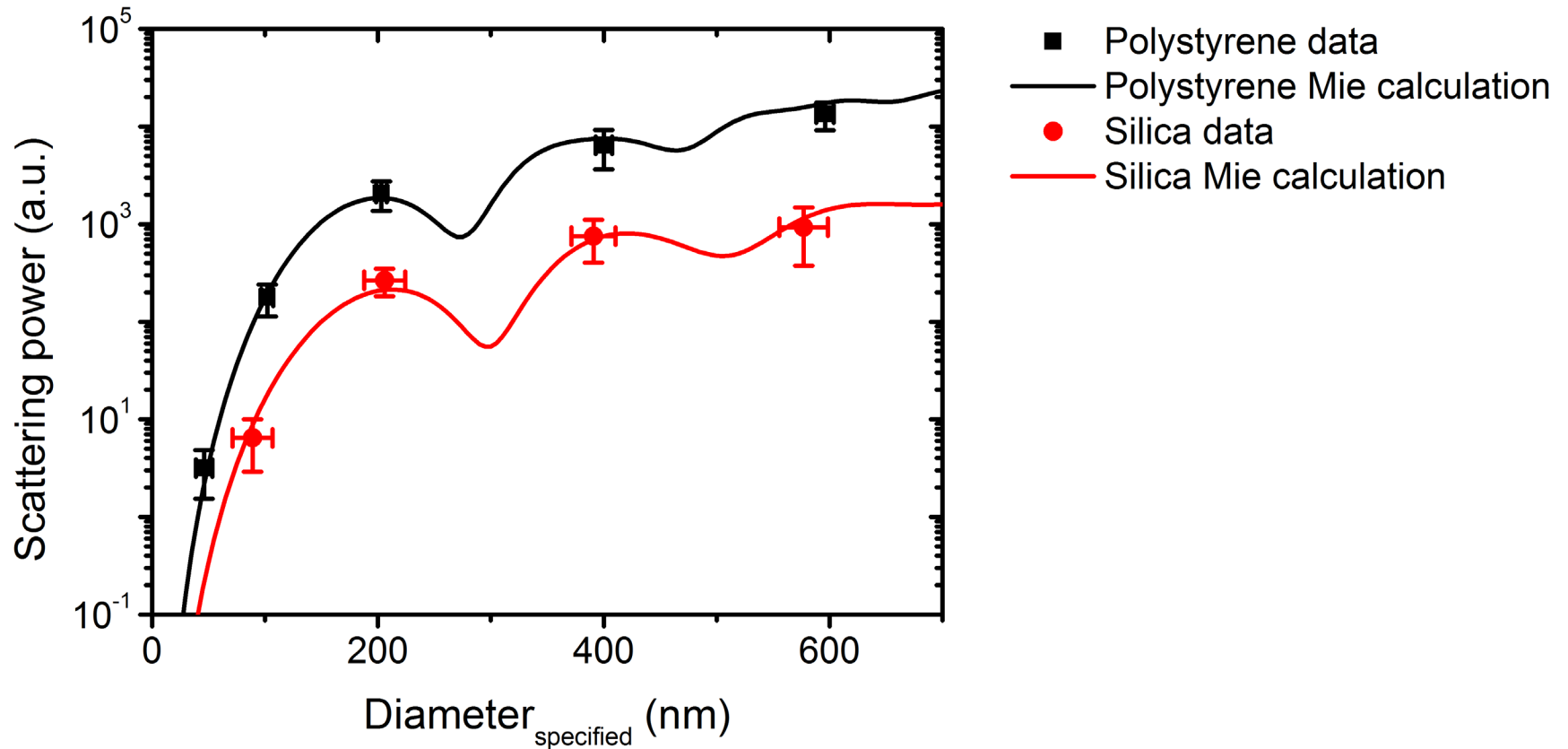
Results - scattering power versus diameter of polystyrene beads



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



Results - scattering power versus diameter of polystyrene and silica beads



Methods - approach

✓ calibration

- ✓ measure light scattering of beads
- ✓ describe measurements by Mie theory

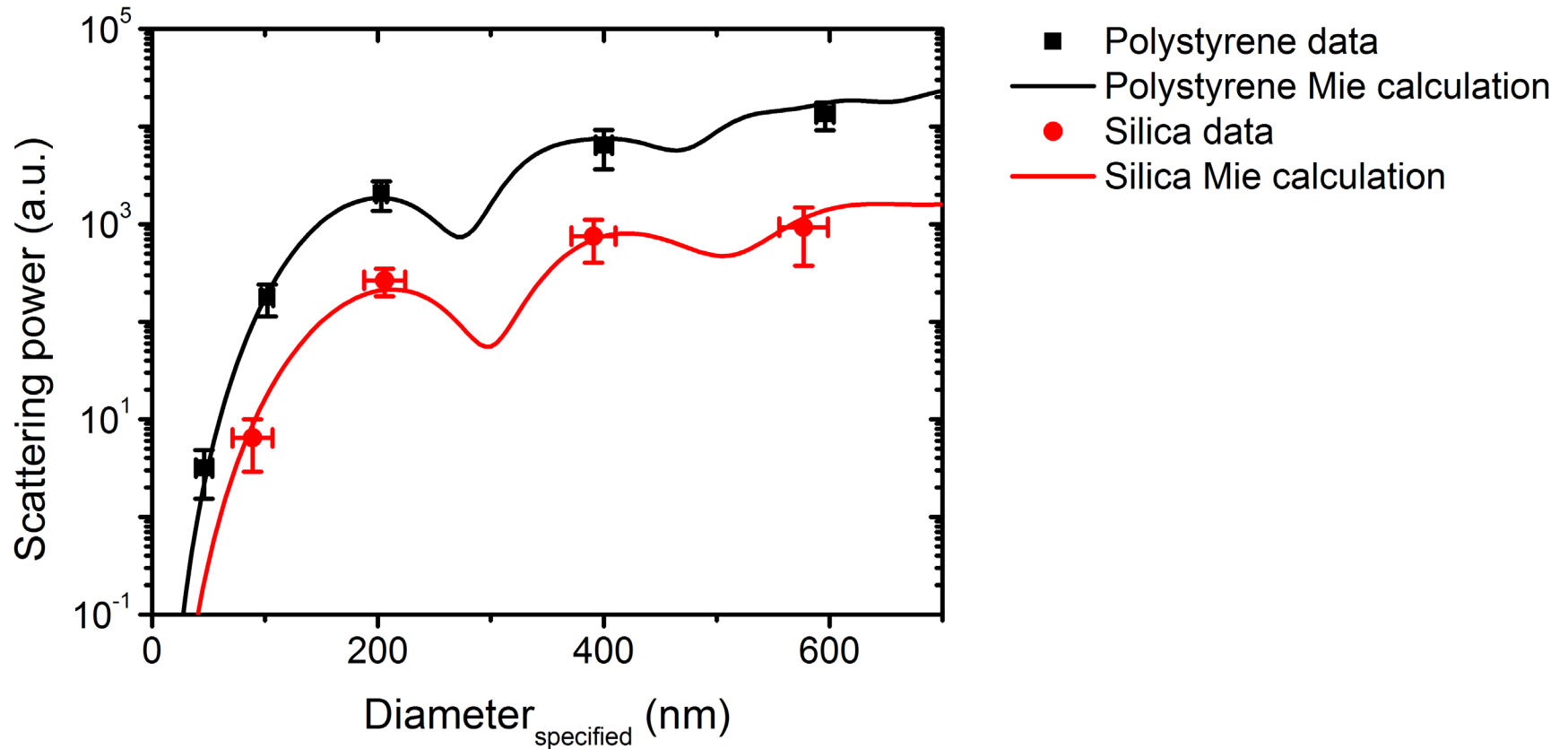
● validation

- measure light scattering and diameter of beads mixture

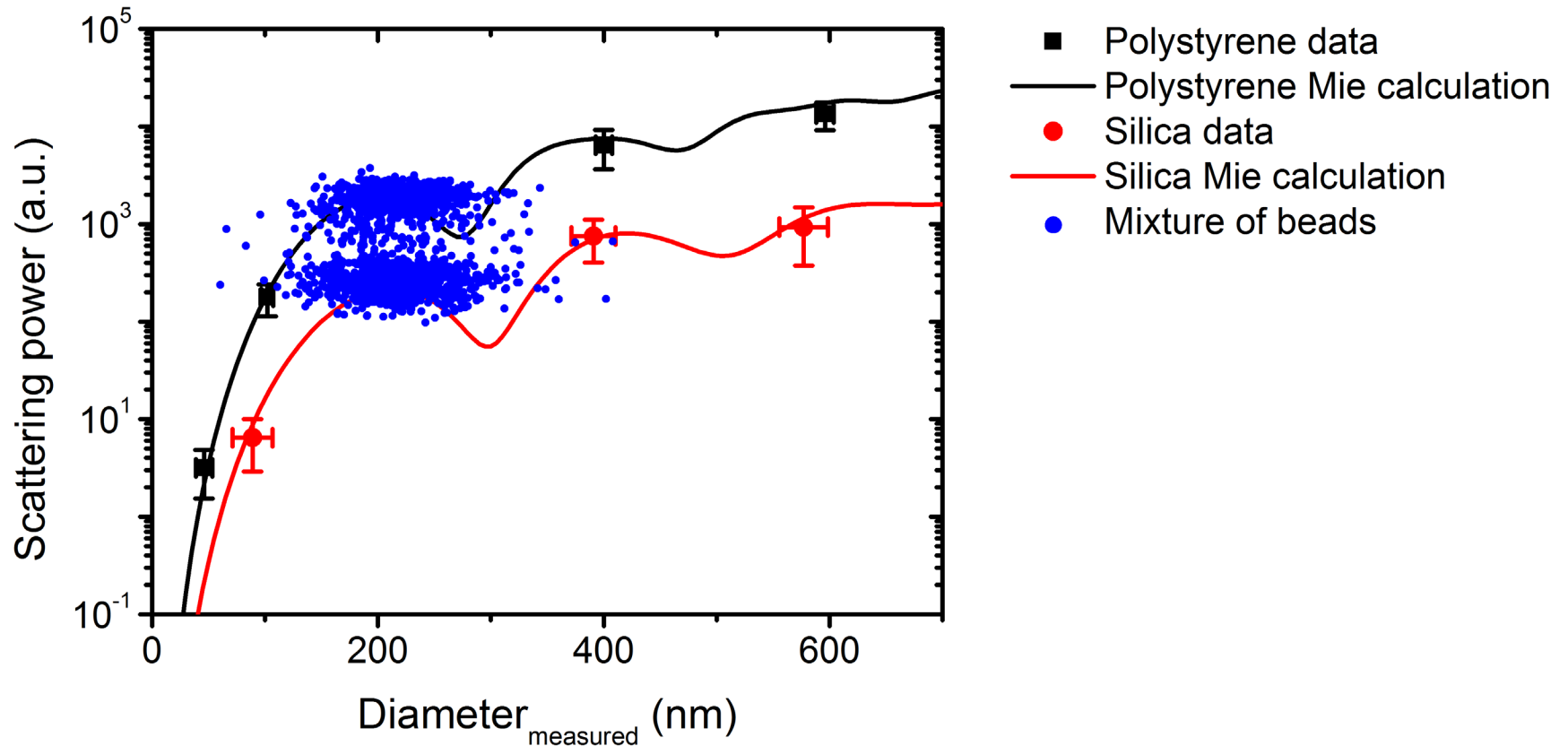
● application

- determine the refractive index of vesicles

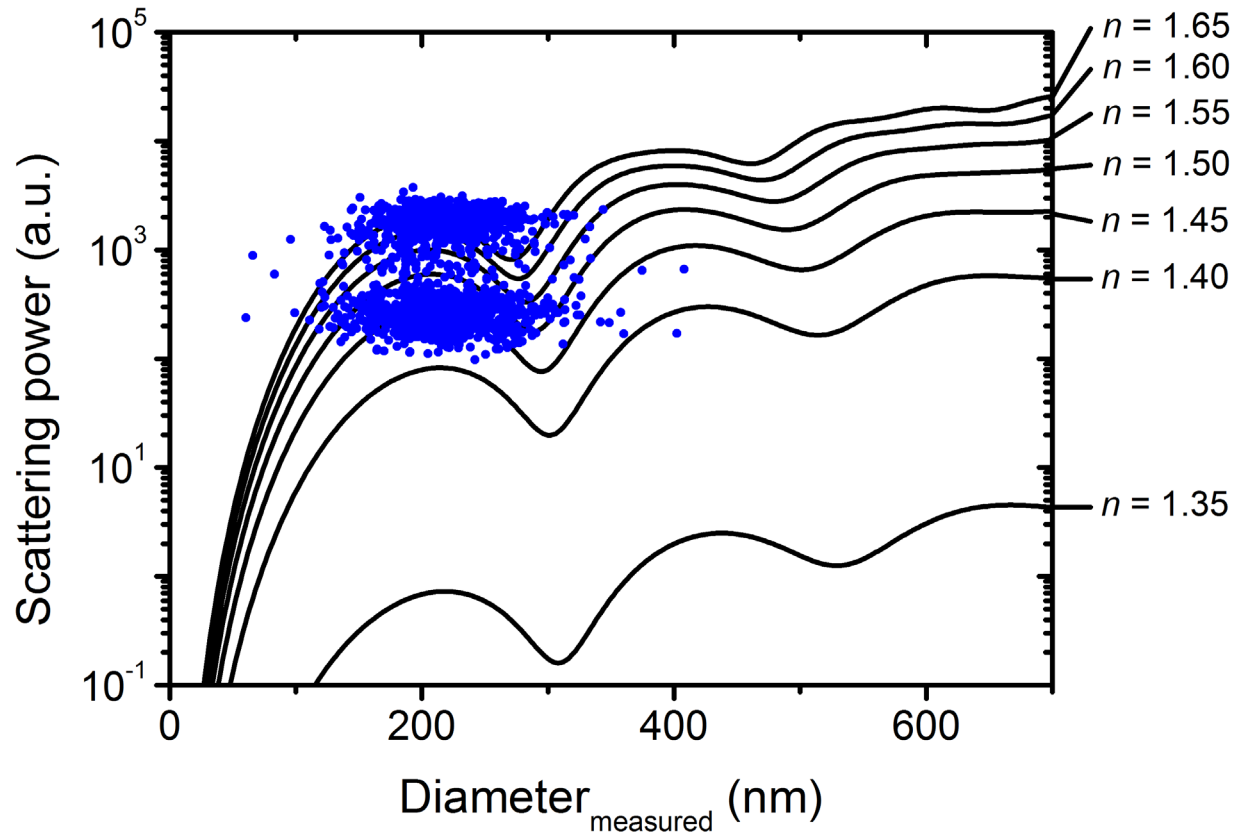
Results - scattering power versus diameter of polystyrene and silica beads



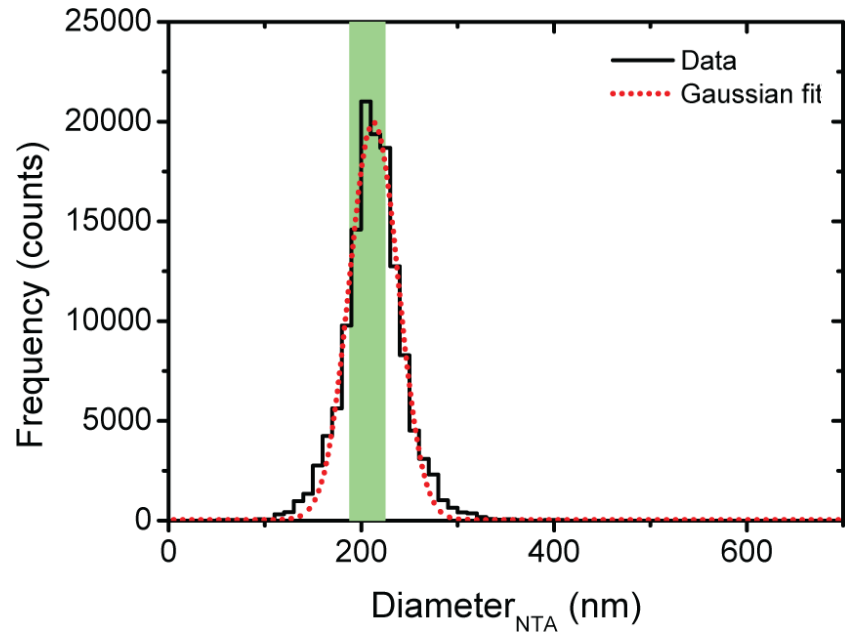
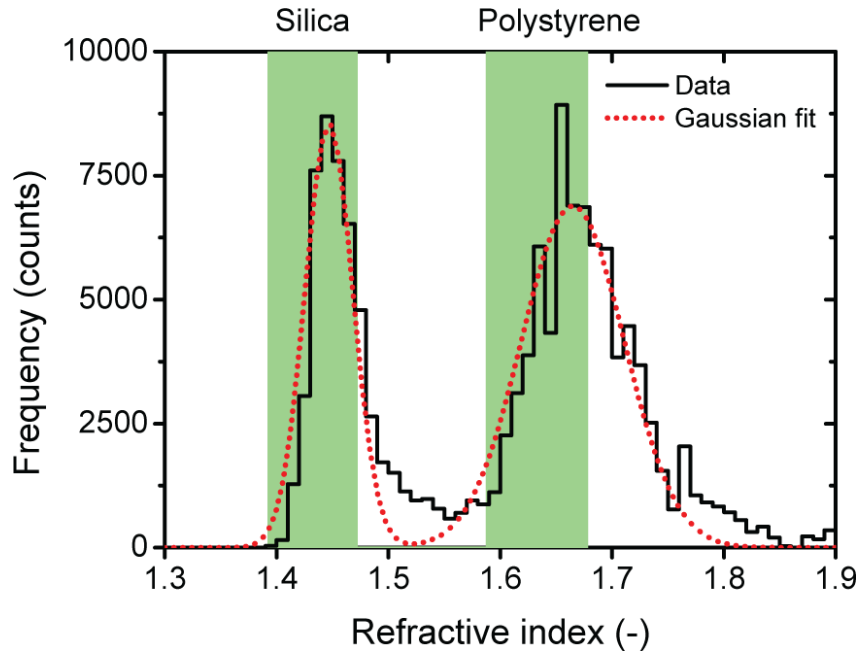
Results - scattering power versus diameter of a mixture of polystyrene and silica beads



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



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



Methods - approach

✓ calibration

- ✓ measure light scattering of beads
- ✓ describe measurements by Mie theory

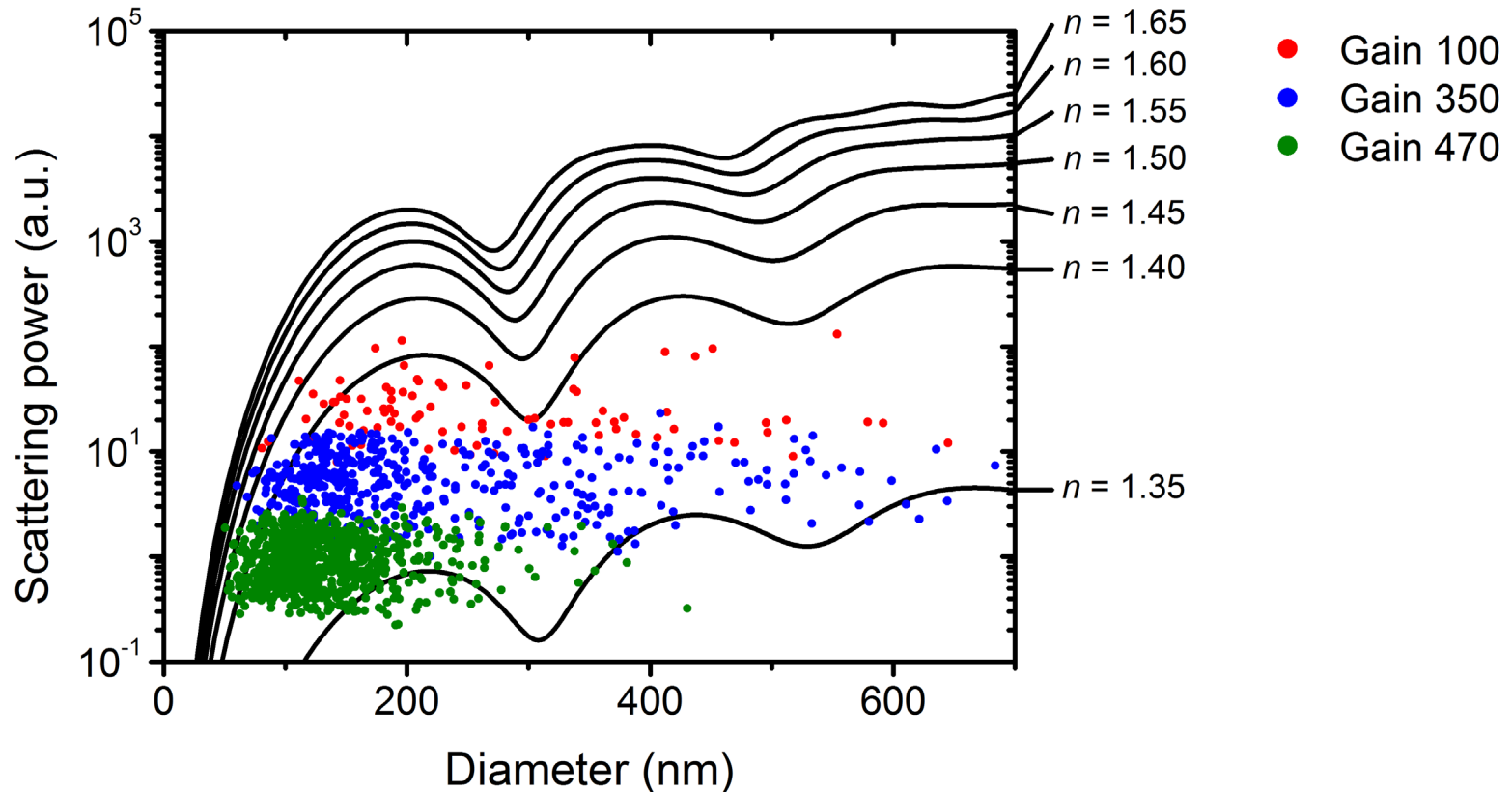
✓ validation

- ✓ measure light scattering and diameter of beads mixture

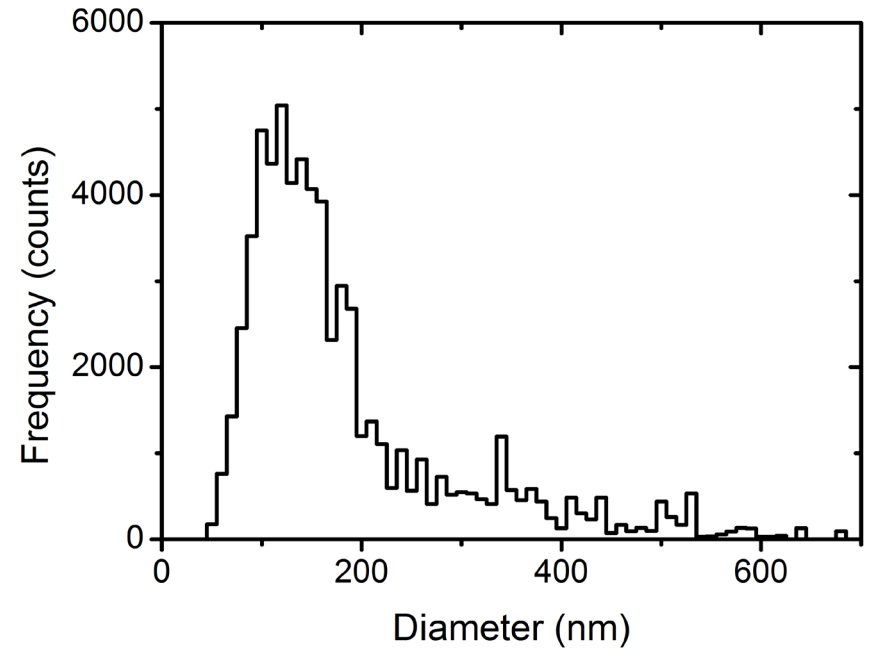
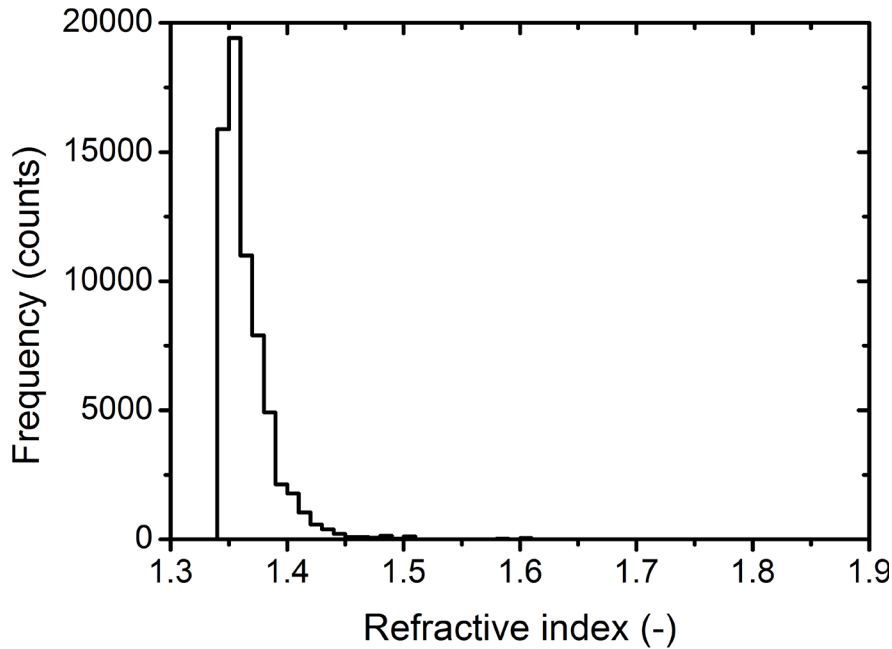
● application

- determine the refractive index of vesicles

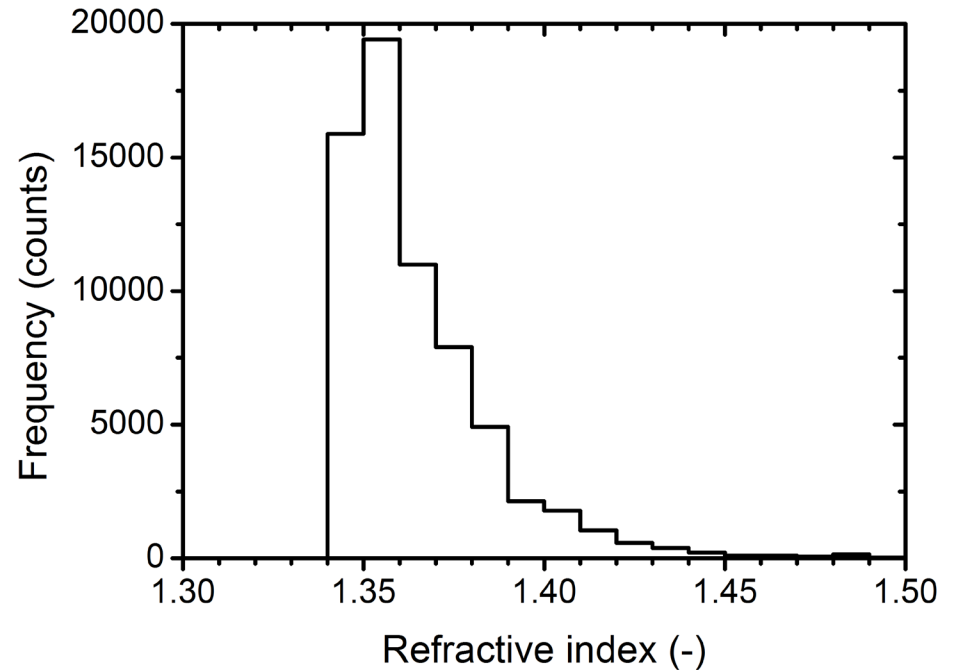
Results - scattering power versus diameter of urinary vesicles



Results - size and refractive index distribution of urinary vesicles



Conclusions



- nanoparticle tracking analysis can be used to determine the refractive index of single vesicles
- mean refractive index of urinary vesicles is 1.37

Discussion - urinary vesicles contain mainly water

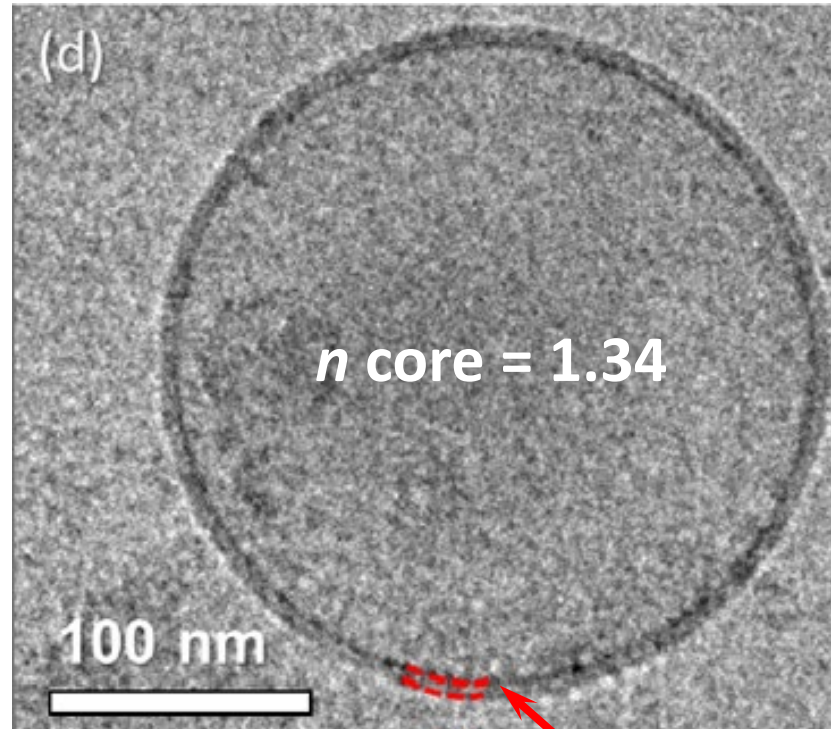


image courtesy of Issman et al., PLoS ONE (2013)

* van Manen et al., Biophys. J. (2007)

Acknowledgements

- Academic Medical Center
 - Laboratory Experimental Clinical Chemistry
 - Biomedical Engineering and Physics
- European Association of National Metrology Institutes (EURAMET)
 - The European Metrology Research Programme (EMRP) is jointly funded by the EMRP participating countries within EURAMET and the European Union
- University of Oxford
 - Chris Gardiner
- University of Birmingham
 - Paul Harrison
- NanoSight Ltd.
 - Patrick Hole
 - Andrew Malloy
 - Jonathan Smith

More on vesicle detection:
edwinvanderpol.com

