Refractive index of extracellular vesicles by nanoparticle tracking analysis

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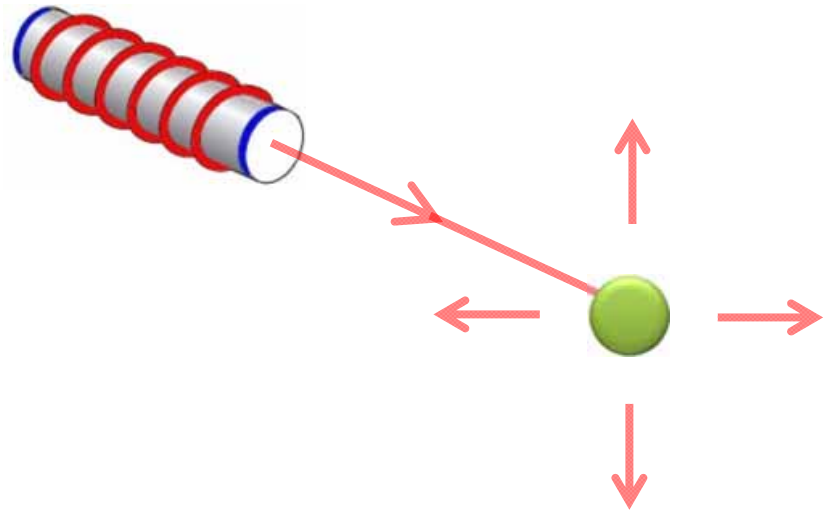
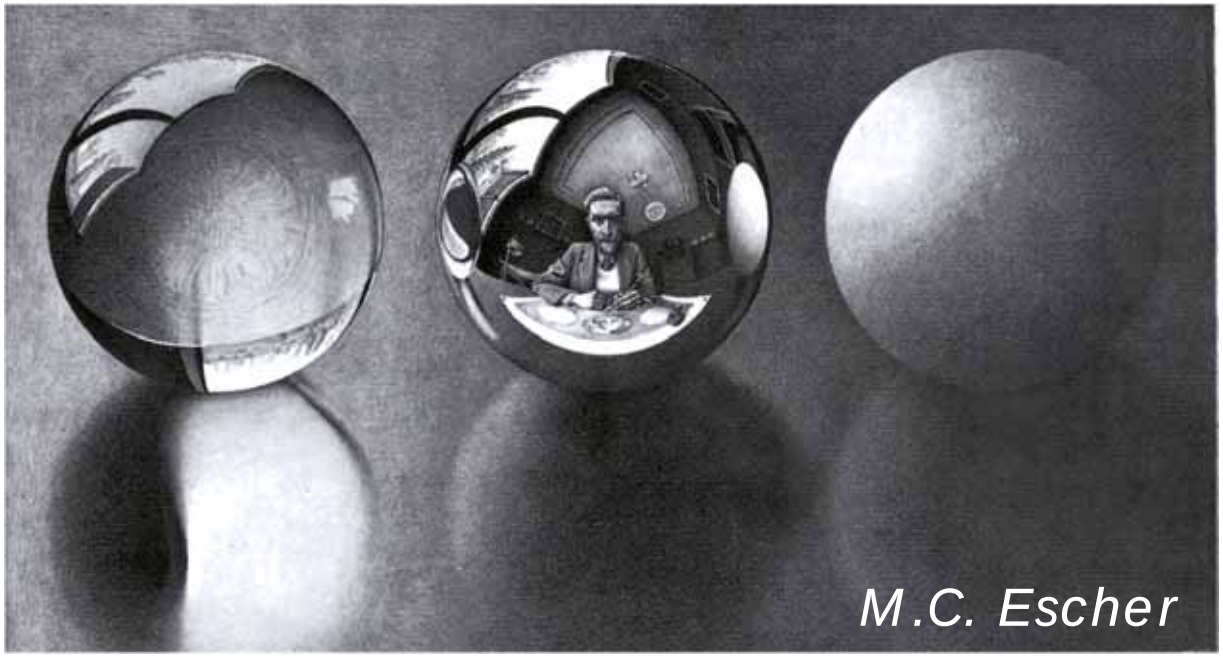
Introduction to light scattering



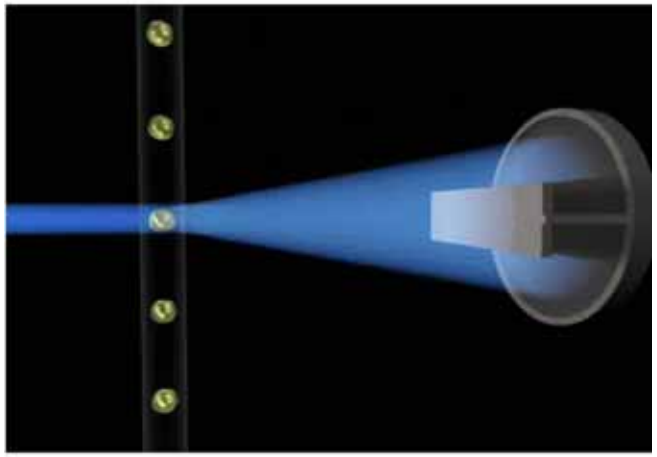
- light illuminating a vesicle is partly absorbed and partly scattered (deflected)
- light scattering depends on size and refractive index

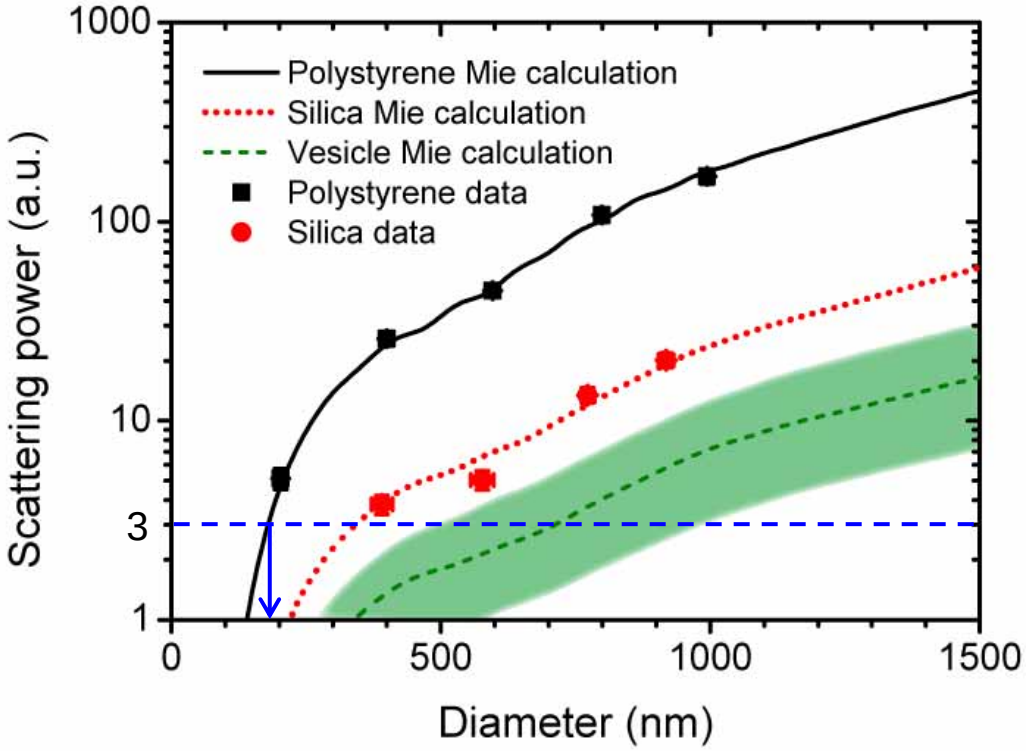
Introduction to the refractive index



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

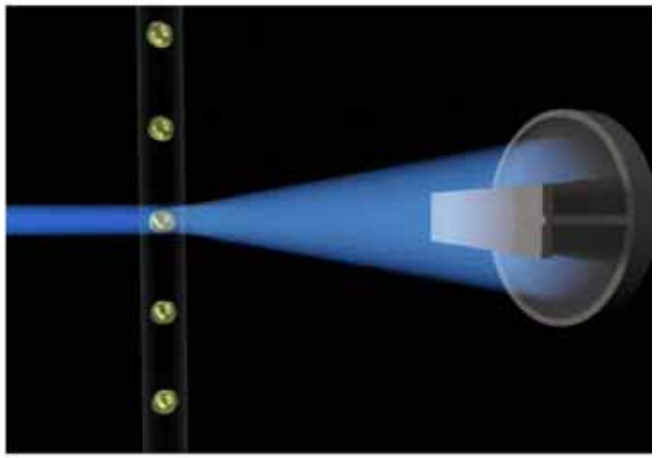
Refractive index to relate scatter to diameter

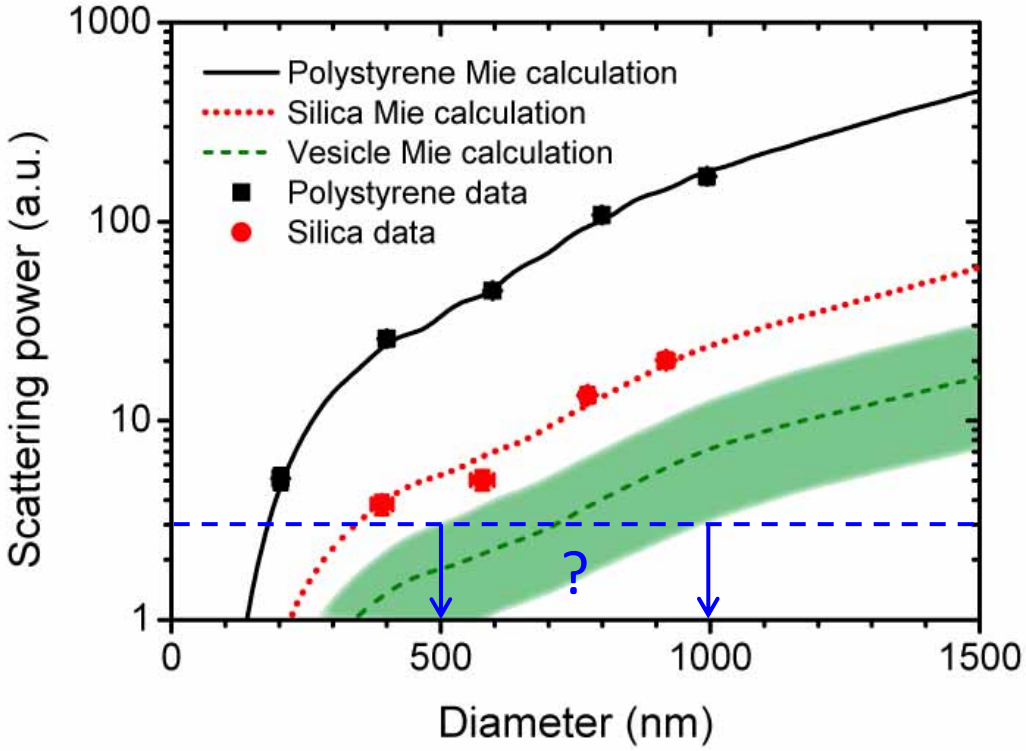




- flow cytometry is widely used to detect vesicles
- refractive index provides scatter to diameter relation

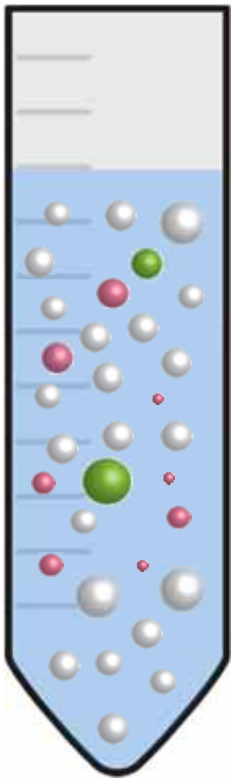
Refractive index of vesicles is unknown





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

Determine refractive index to identify vesicles

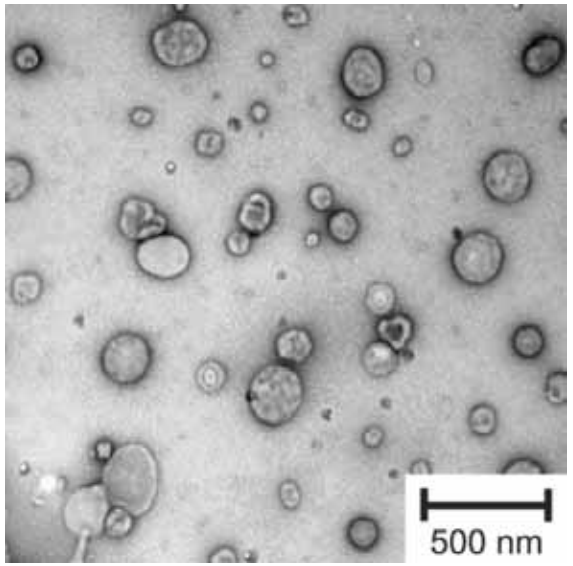


- lipoproteins (n = 1.45-1.60)
- protein aggregates (n = 1.53-1.60)
- vesicles ($d \geq 500$ nm → $n = 1.40$* ; $d < 500$ nm → n = ?)

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

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

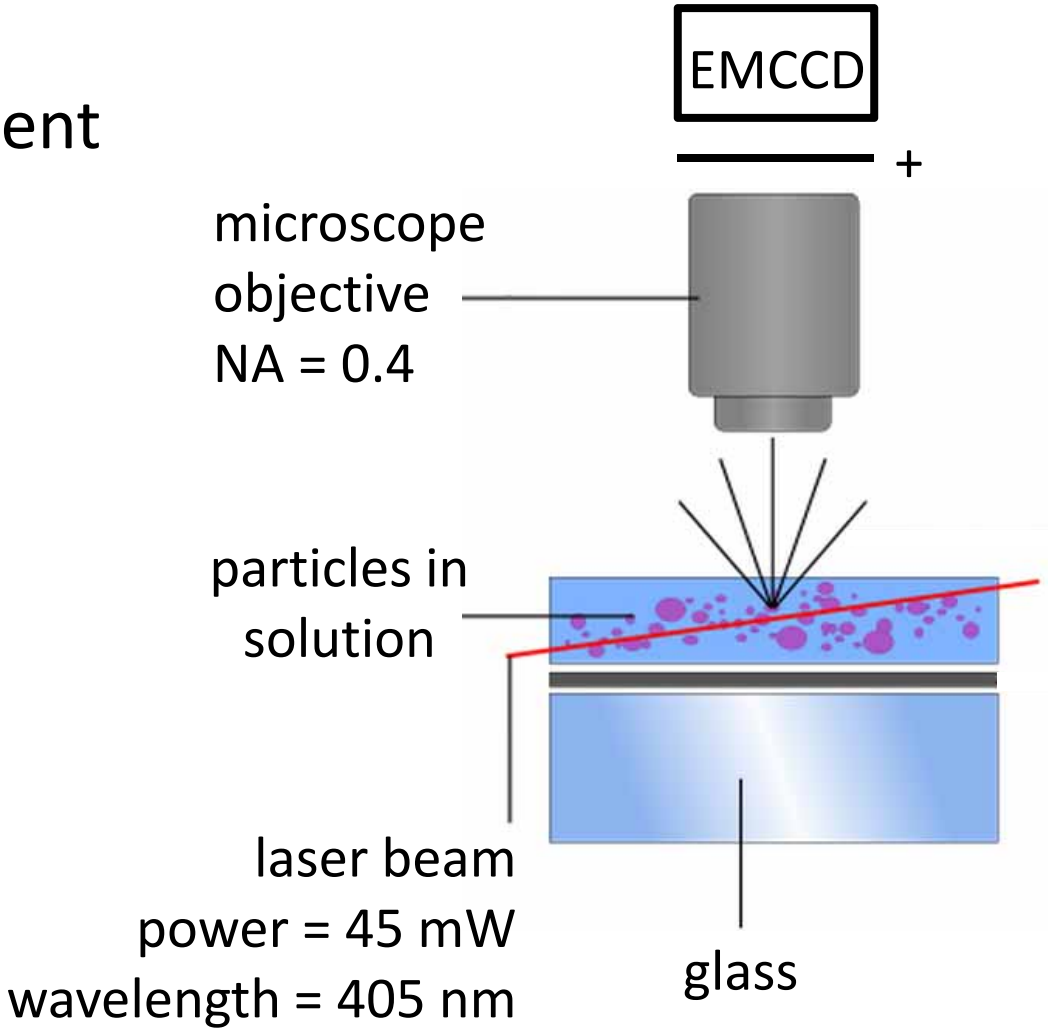
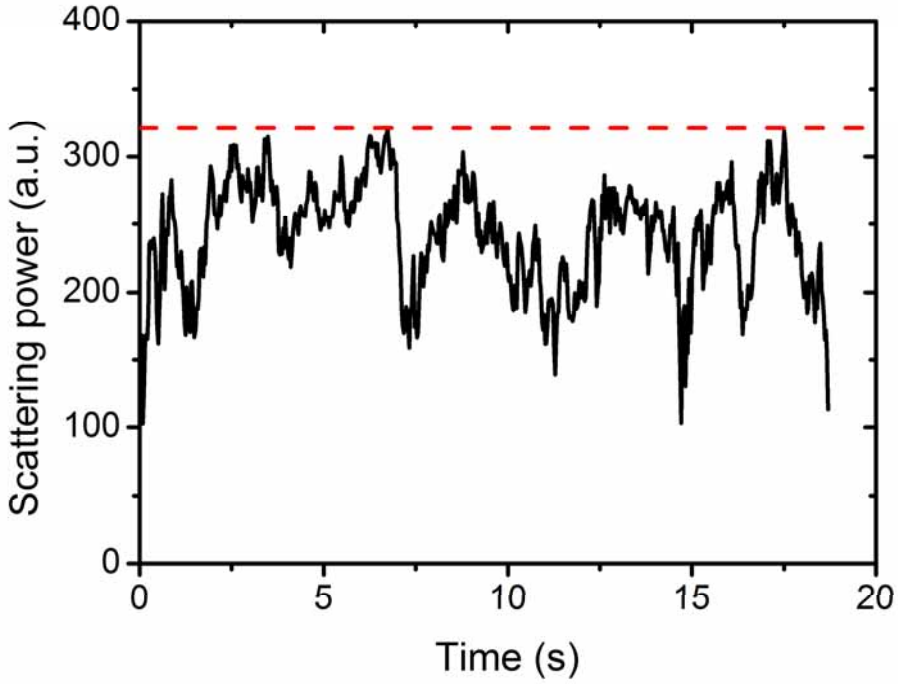


figure adapted from Nanosight Ltd, UK

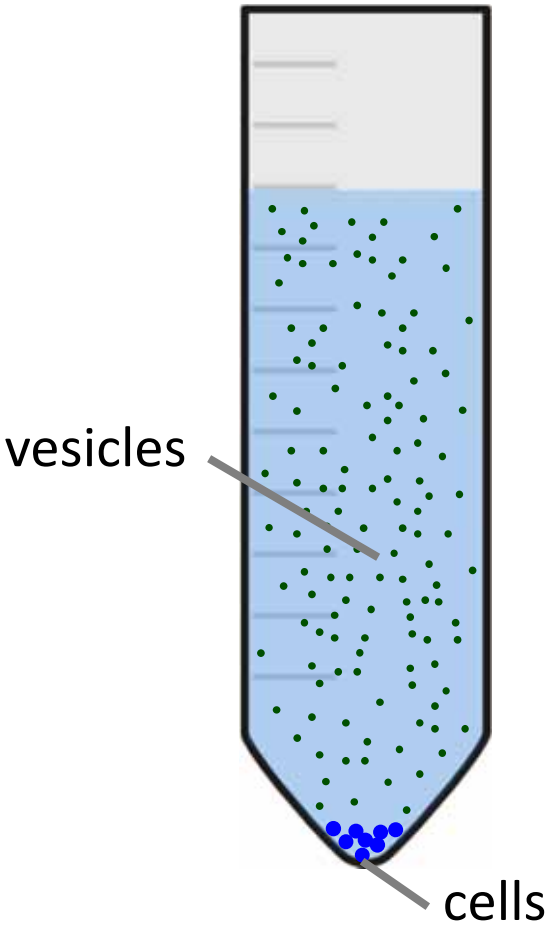
Methods - data acquisition and processing



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

Methods - samples

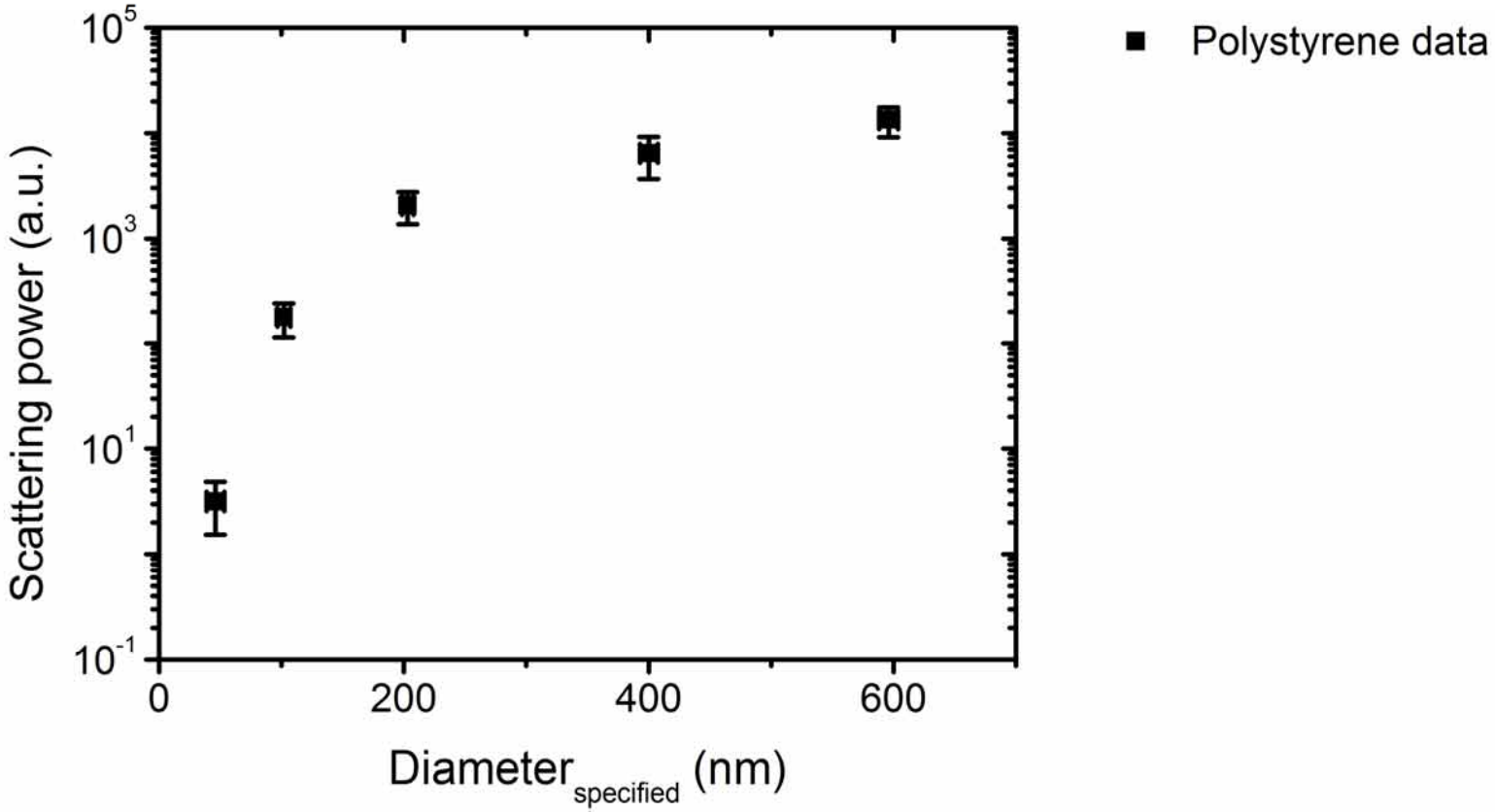
- Polystyrene beads (*n*=1.63)
  - Thermo Fisher Scientific, USA
- Silica beads (*n*=1.45)
  - Kisker Biotech, Germany
- Human urinary vesicles
  - differential centrifugation
  - protocol from metves.eu



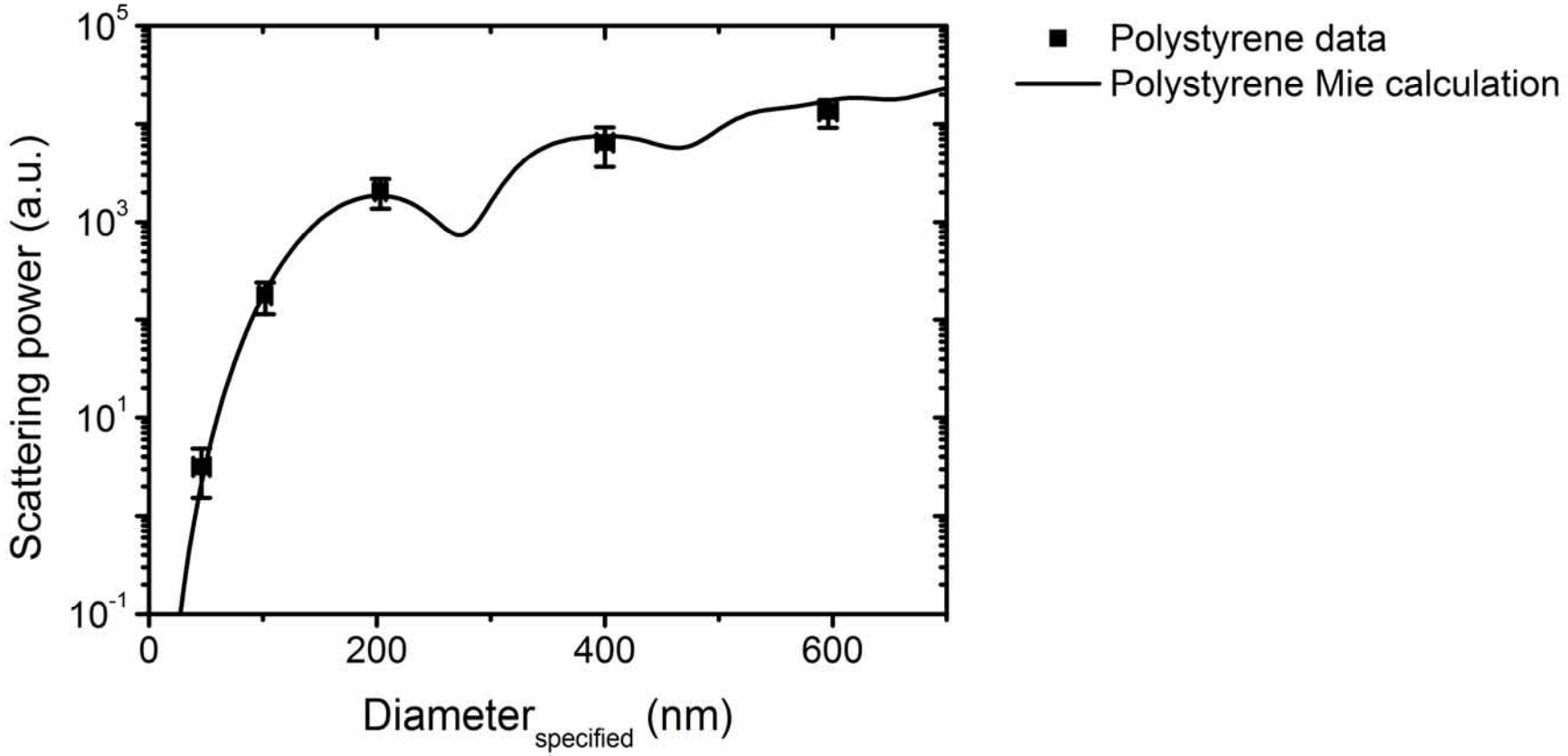
Methods - approach

- 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

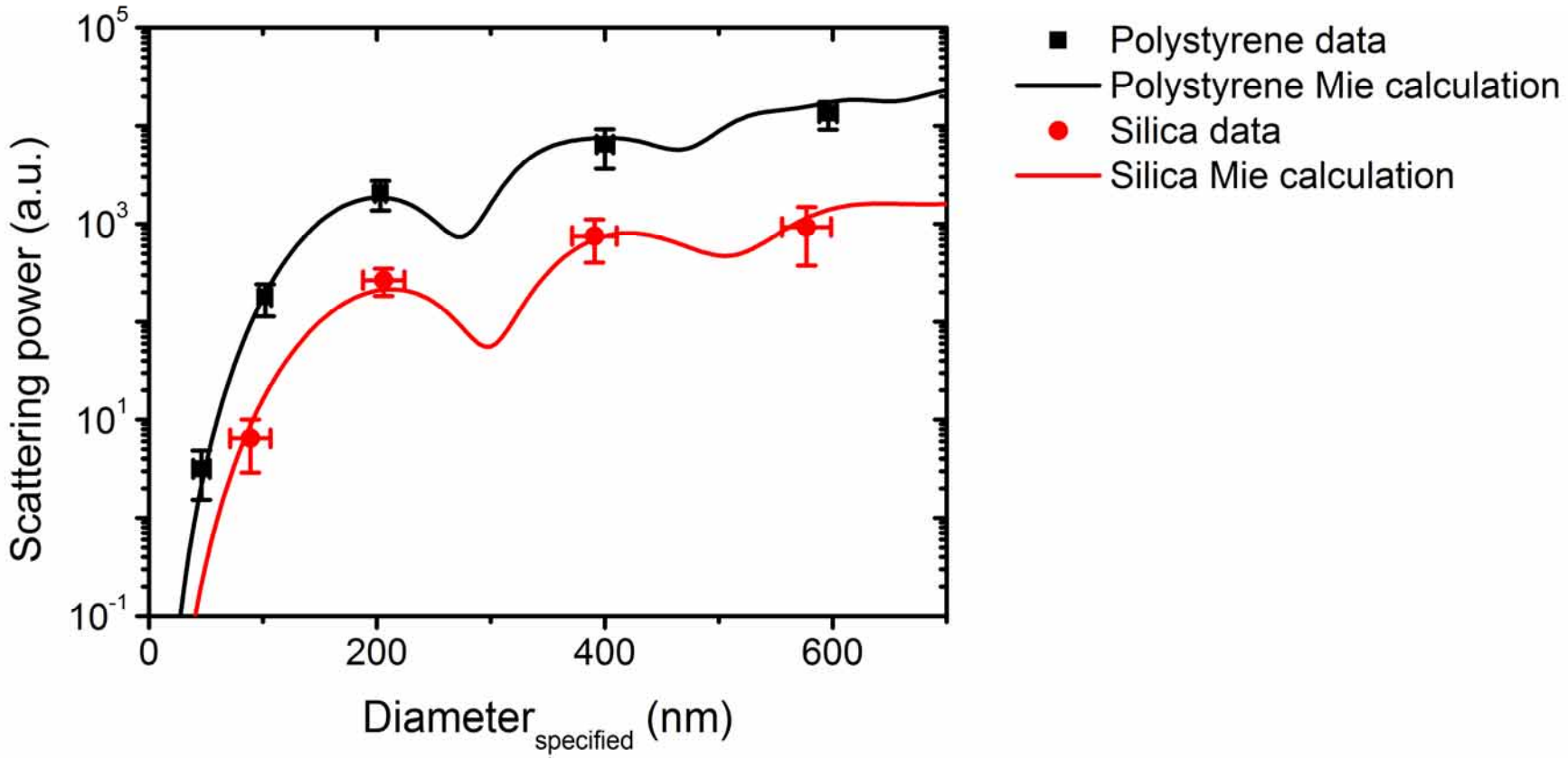
Results - scattering power versus diameter of polystyrene beads



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



Results - scattering power versus diameter of polystyrene and silica beads



Methods - approach

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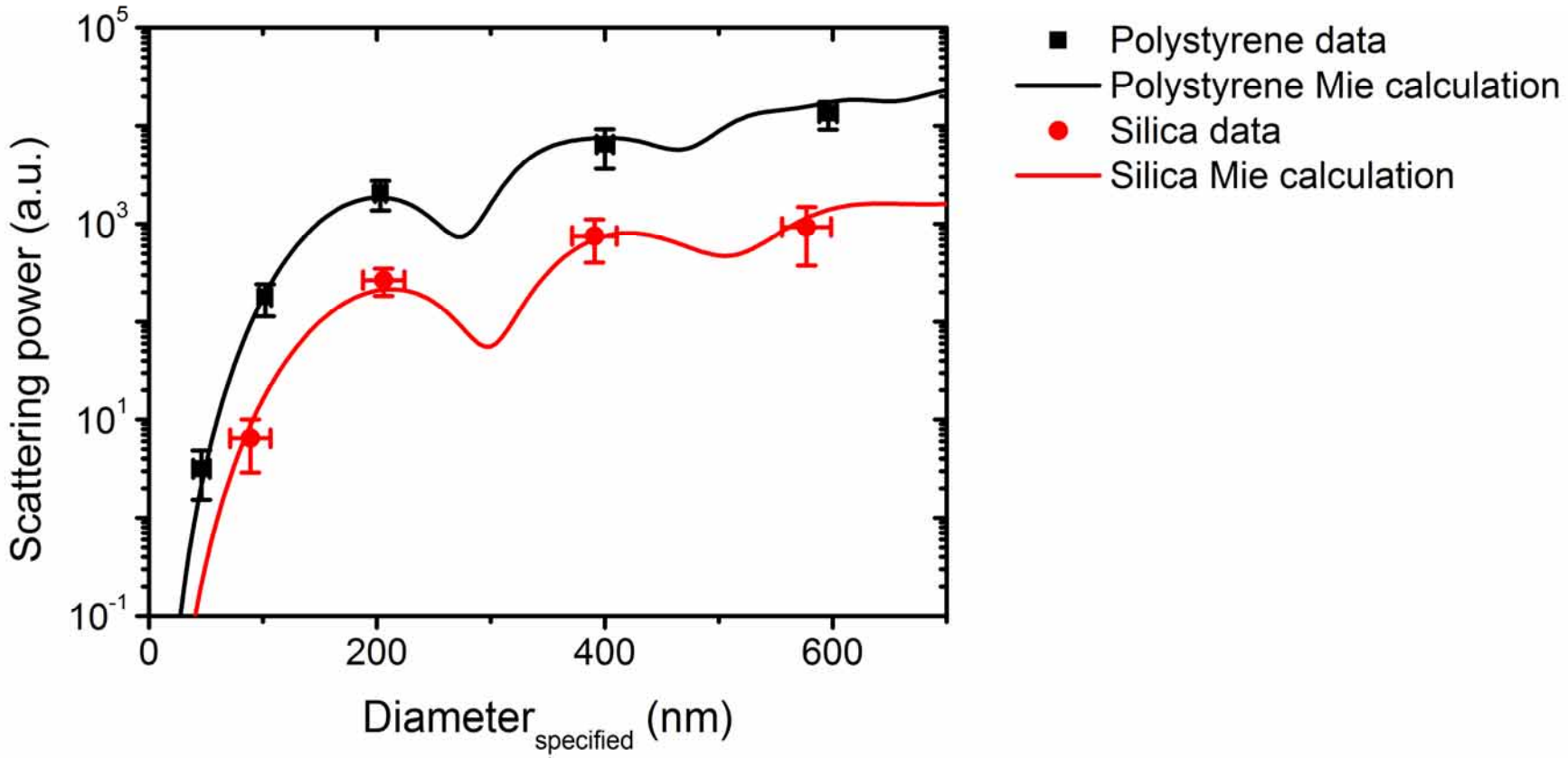
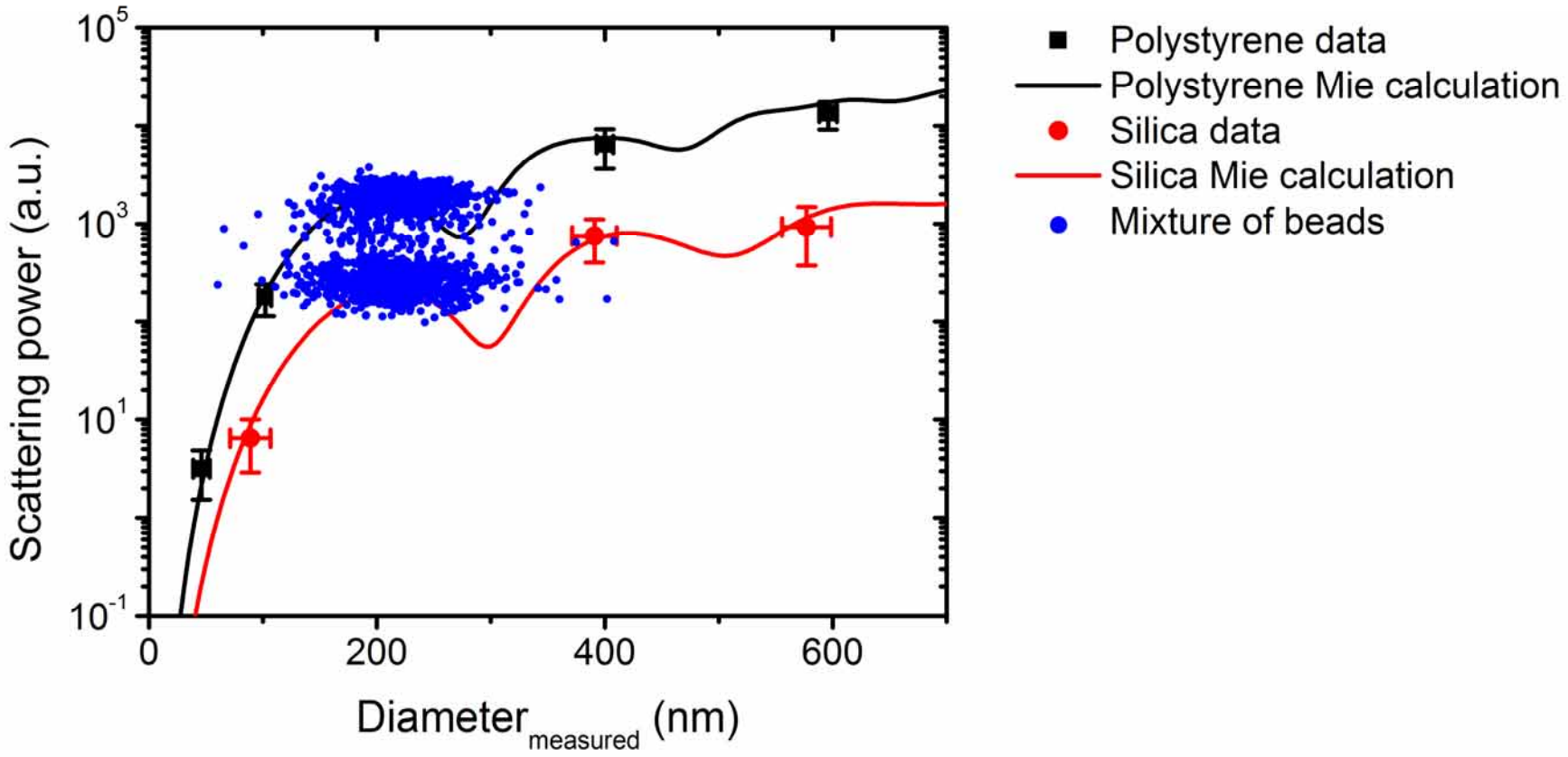


Results - scattering power versus diameter of a mixture of polystyrene and silica beads



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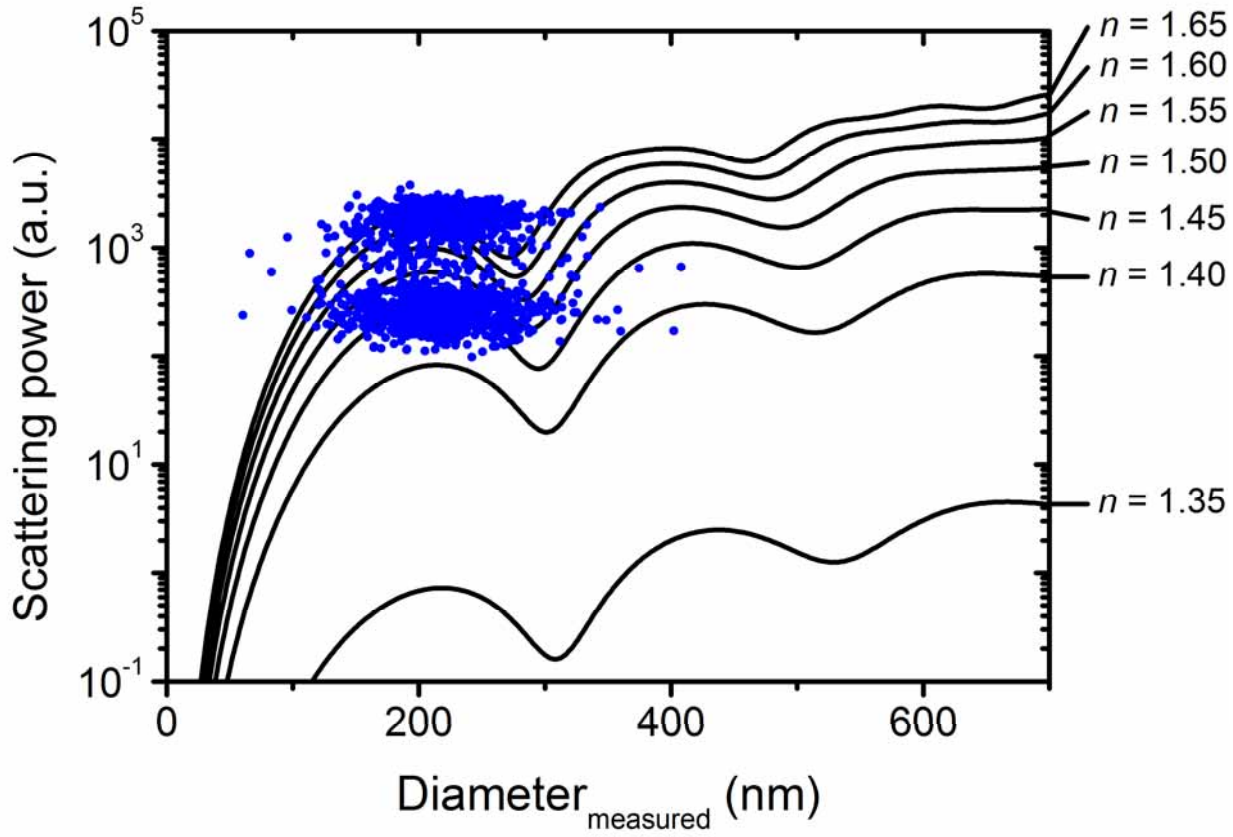
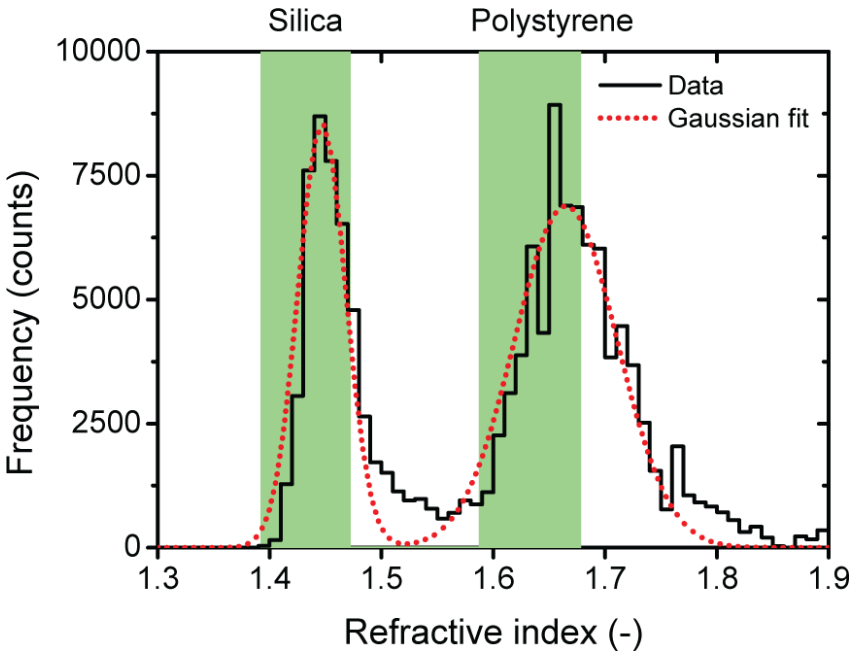
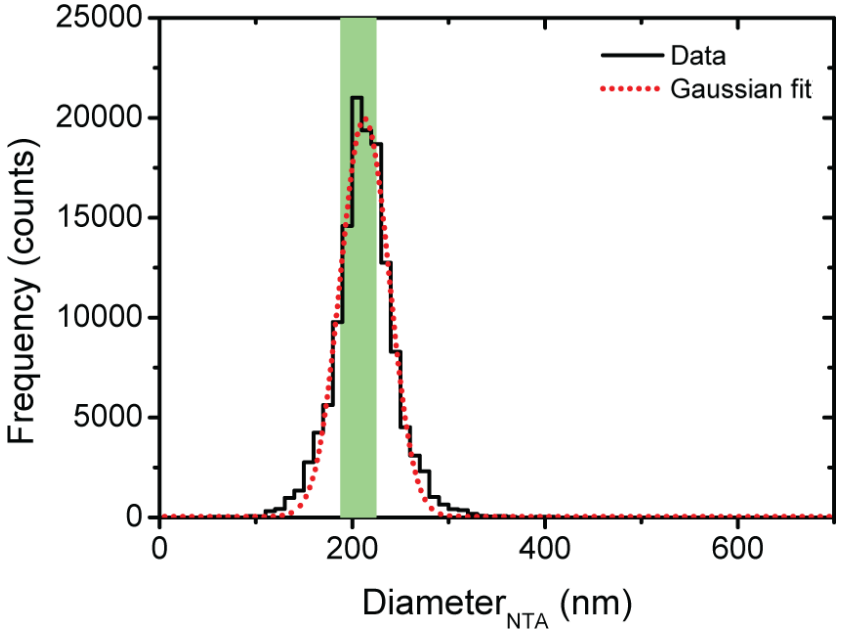


Results - refractive index and size distribution of a mixture of polystyrene and silica beads

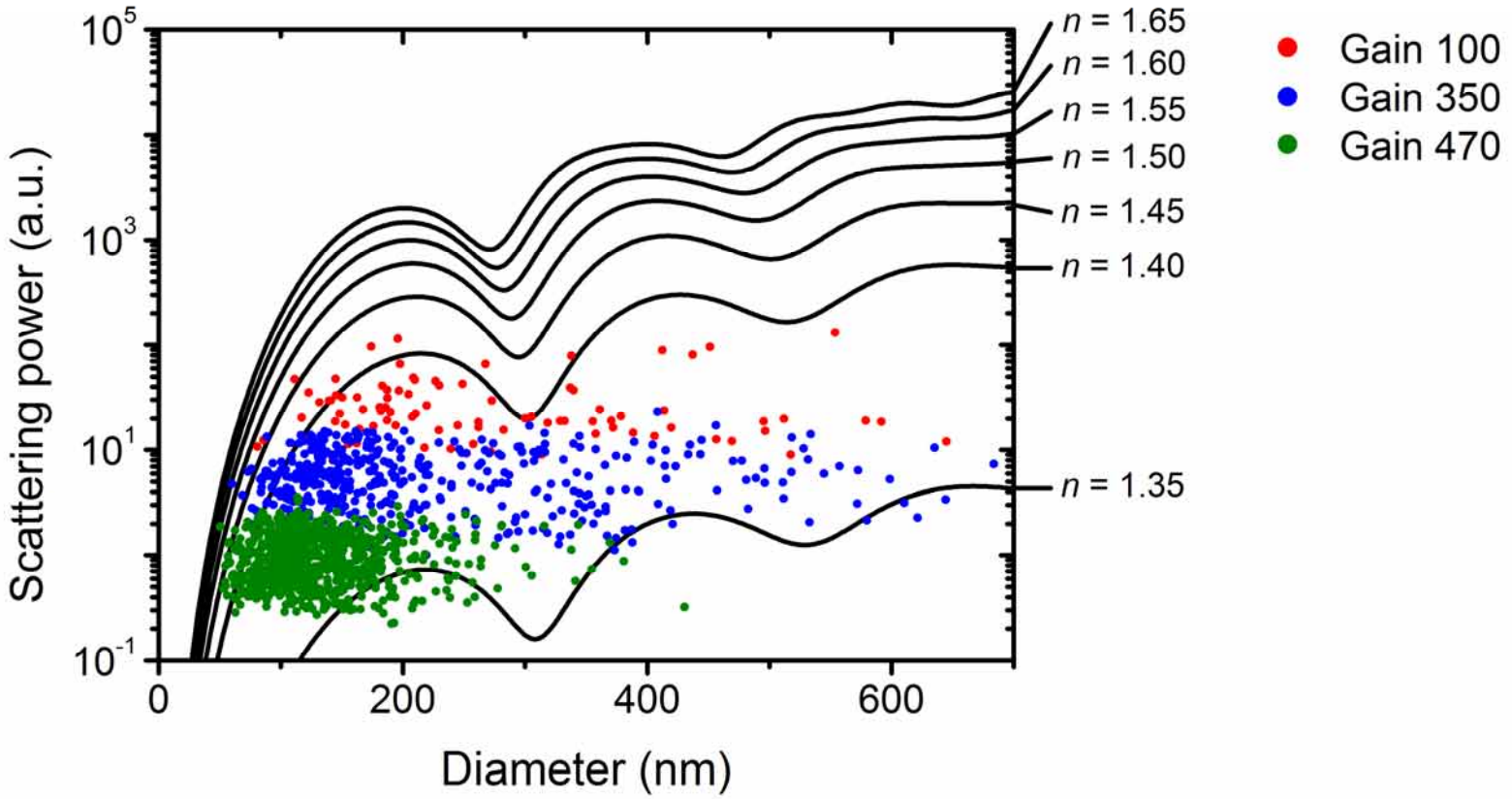




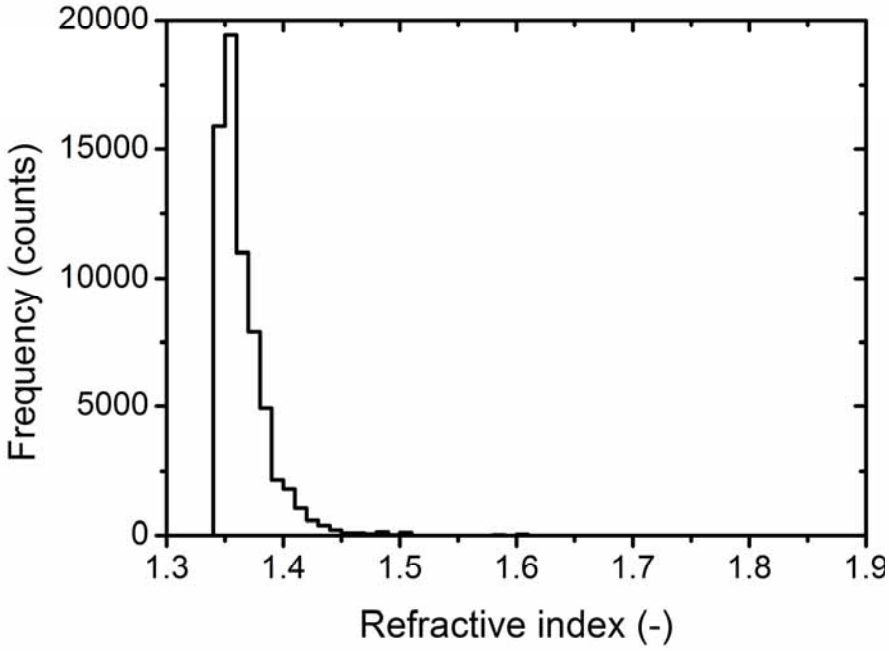
Methods - approach

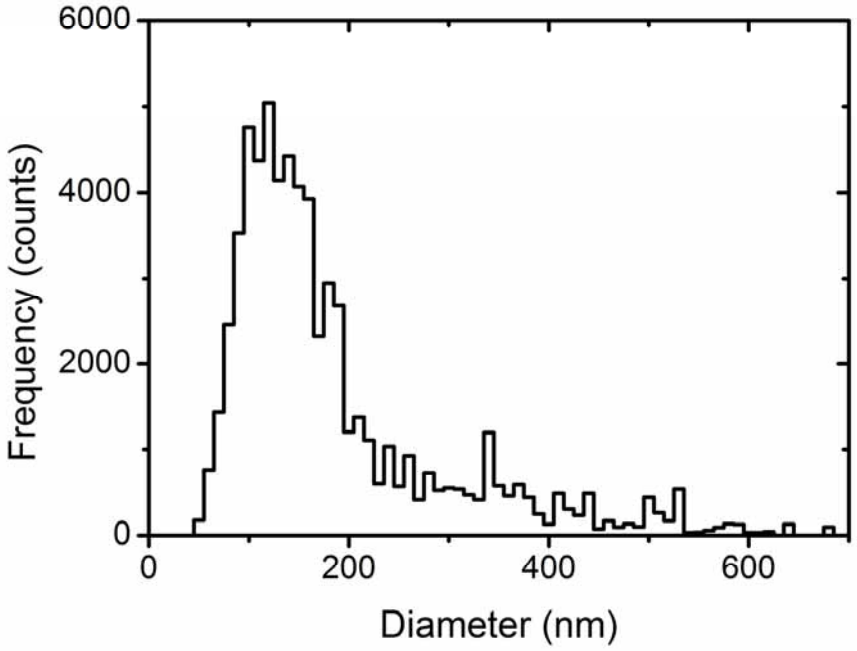
- calibration
  - measure light scattering of beads
  - describe measurements by Mie theory
- validation
  - measure light scattering and diameter of beads mixture
- application
  - determine the refractive index of vesicles

Results - scattering power versus diameter of urinary vesicles

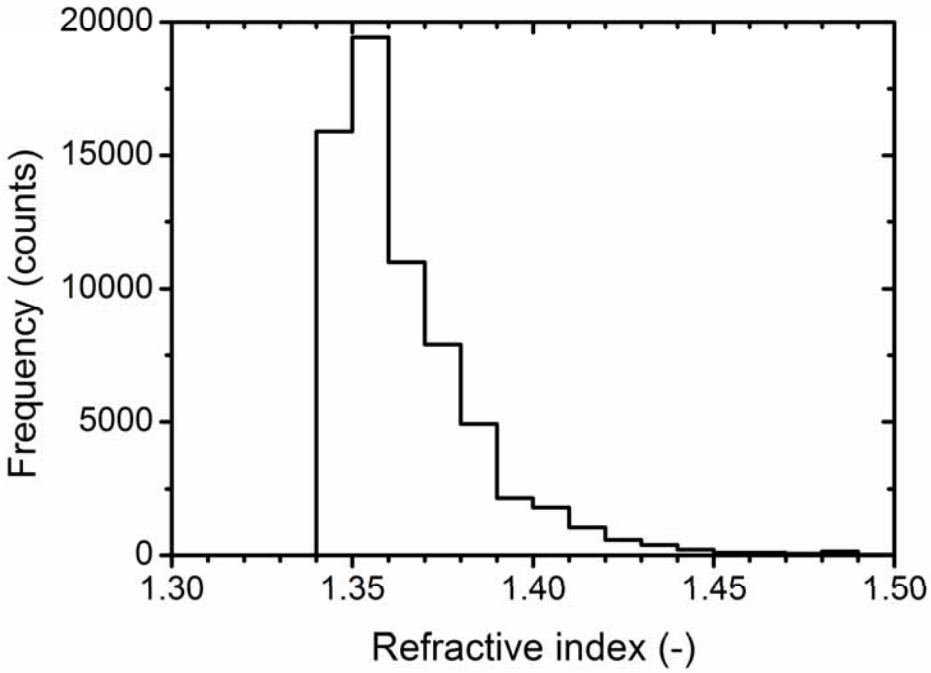


Results - size and refractive index distribution of urinary vesicles



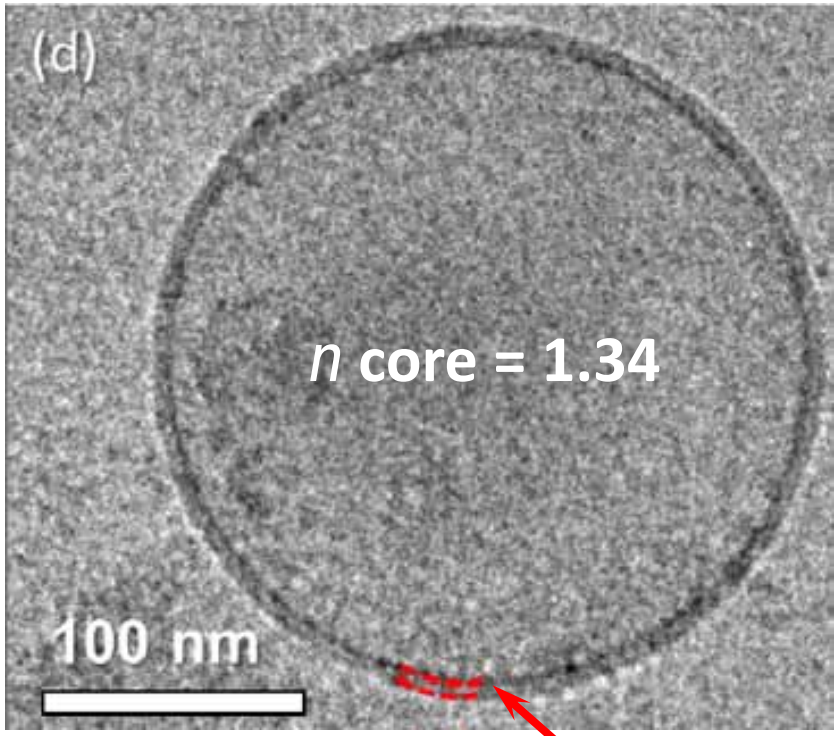


Conclusions



- 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



thickness = 5 nm
n membrane = 1.46 *

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

Acknowledgements

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  - Patrick Hole
  - Andrew Malloy
  - Jonathan Smith

More on vesicle detection:
edwinvanderpol.com