Determination of the refractive index of vesicles using nanoparticle tracking analysis

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Disclosures of: Edwin van der Pol

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Patents	No conflict of interest to disclose
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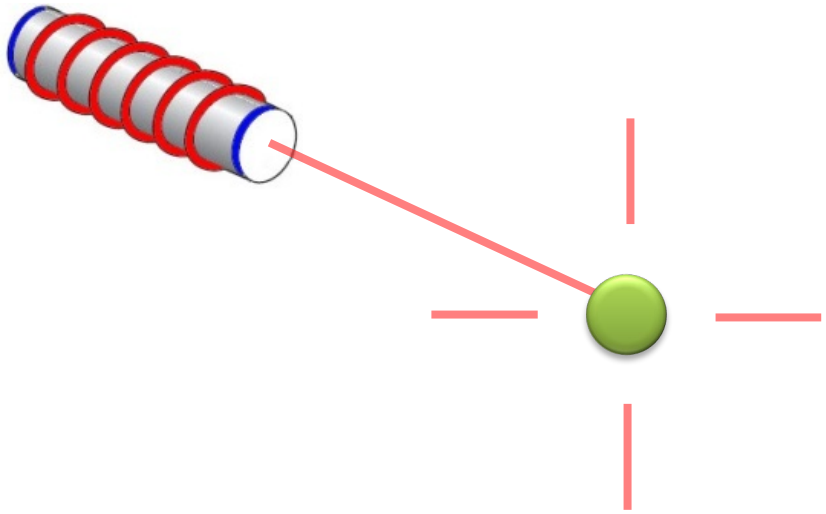
Other	No conflict of interest to disclose

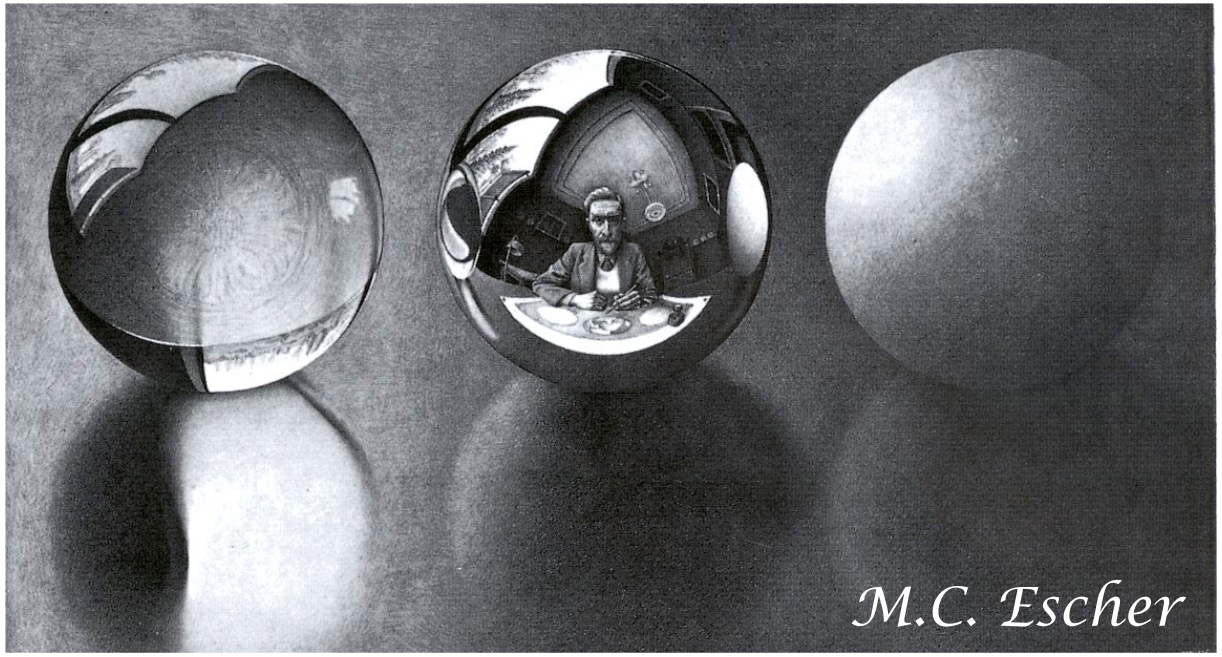
Presentation includes discussion of the following off-label use of a drug or medical device: N/A

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

Motives of studying the refractive index

water (n = 1.34)

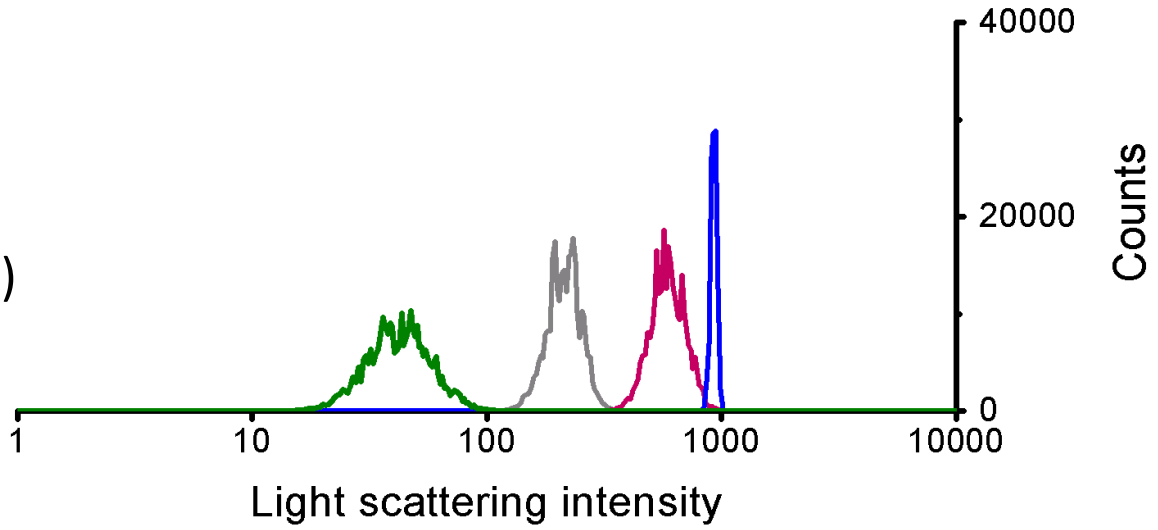
vesicle* (n = 1.4?)

lipid (n = 1.45-1.50)

protein (n = 1.53-1.60)

polystyrene (n = 1.61)

200 nm



- new label-free parameter
  - cellular origin
  - distinguish vesicles from contamination
- relate light scattering to vesicle diameter
- detection range

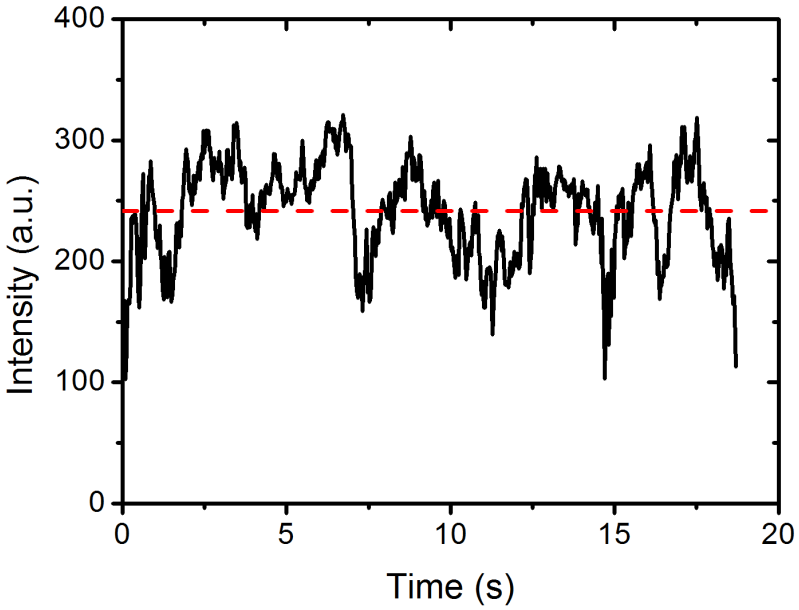
* Konokhova et al., J. Biomed. Opt. (2012)

Nanoparticle tracking analysis (NTA)

- determine size and concentration of vesicles
- additional parameters: light scattering or fluorescence

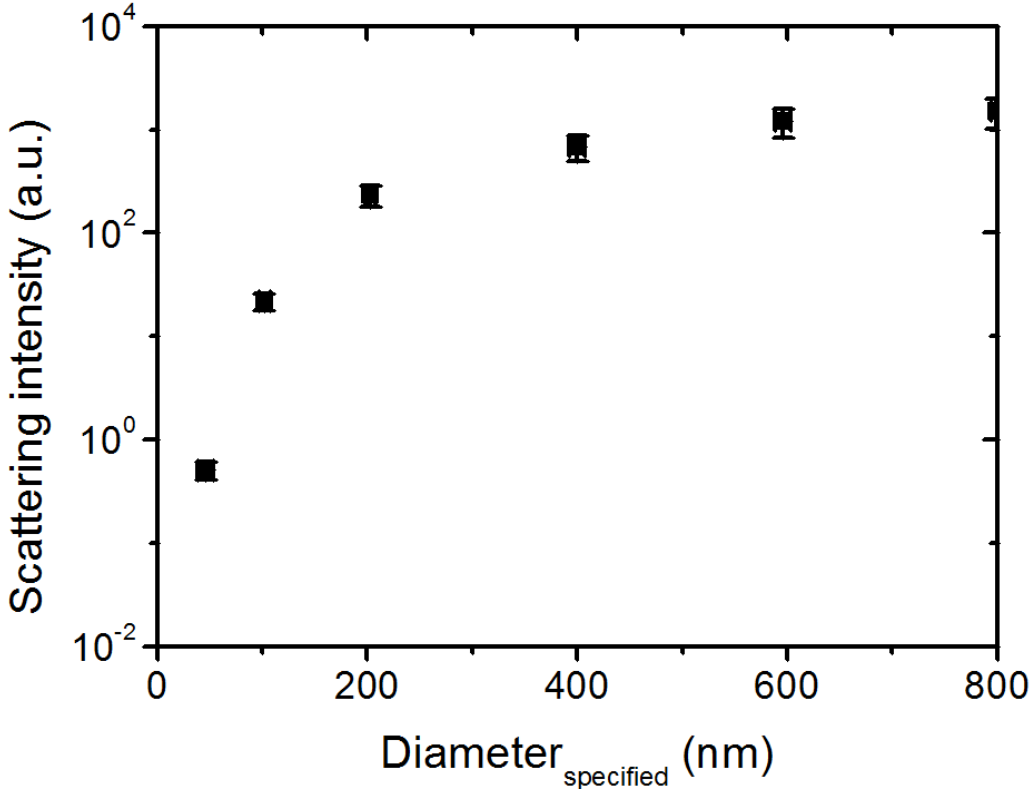
Video by Chris Gardiner

Method – measure light scattering by NTA

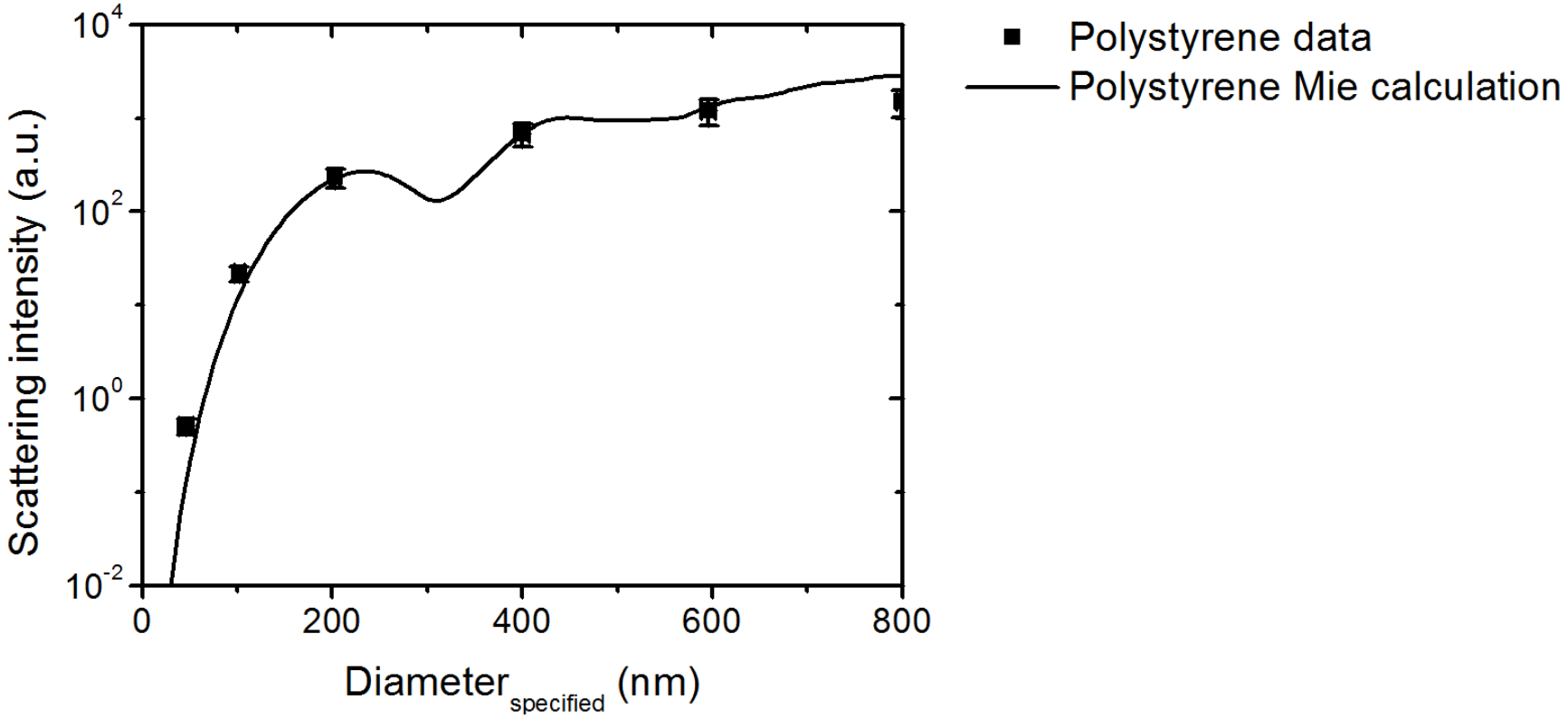


- no pixel saturation
- video processing by NanoSight NTA 2.3
  - intensity corrected for camera shutter time and gain

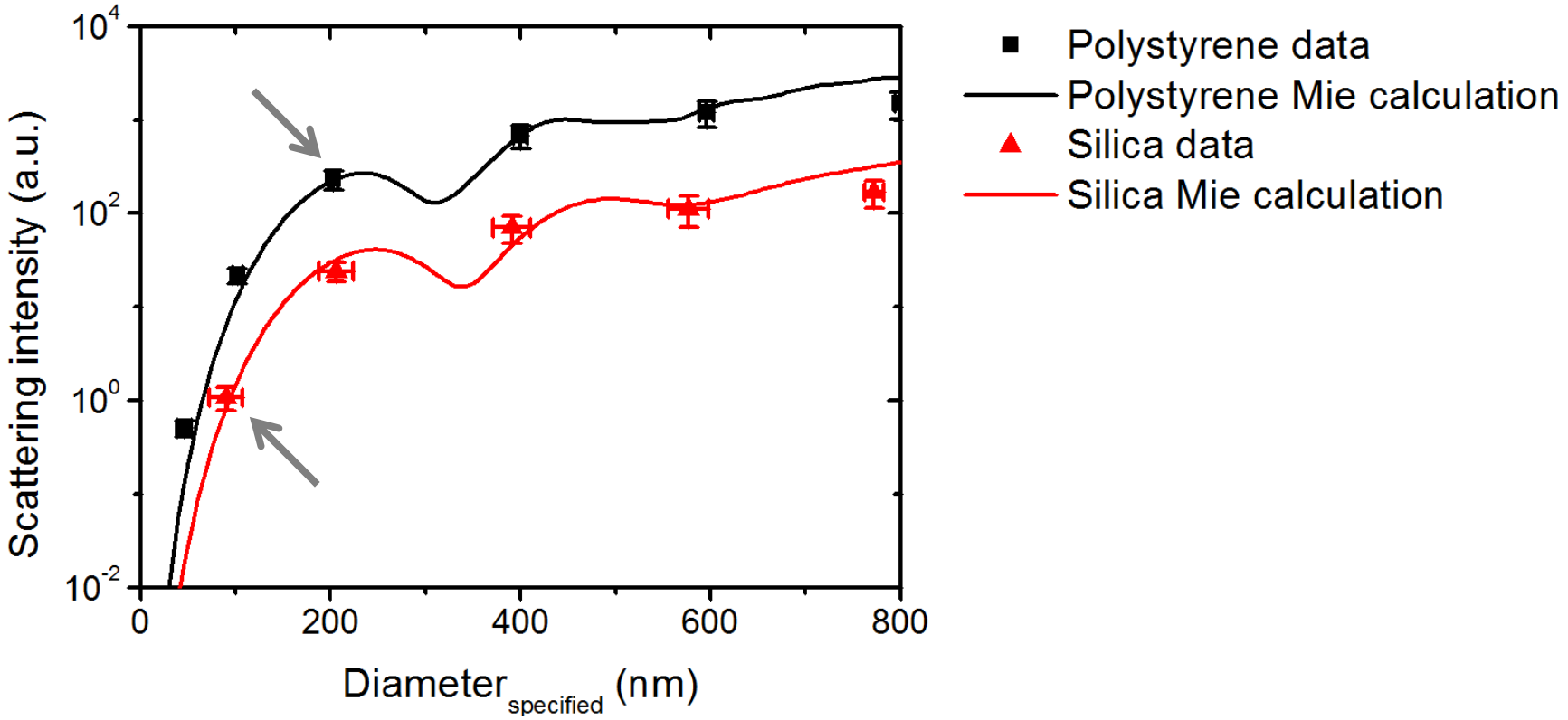
Scattering power versus diameter of beads



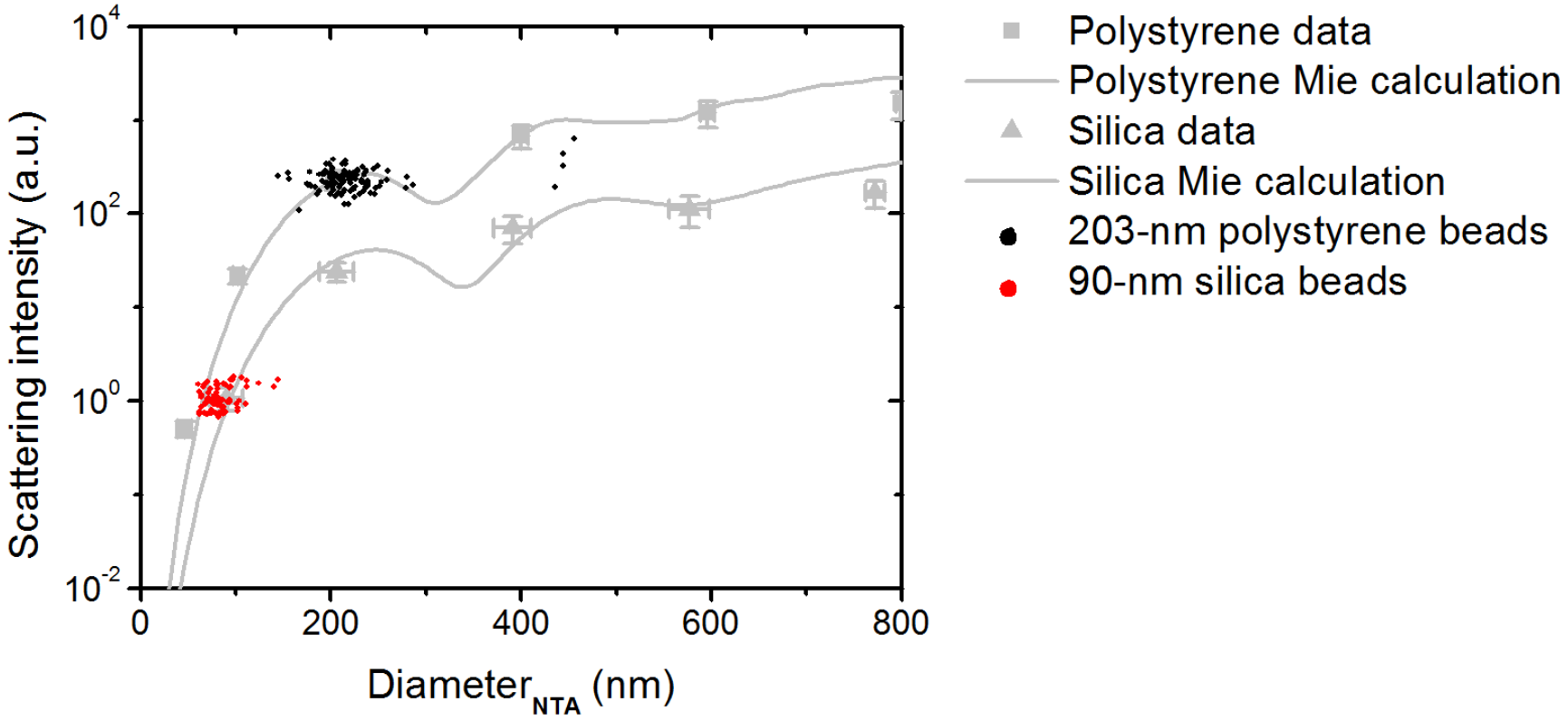
Scattering power versus diameter of beads



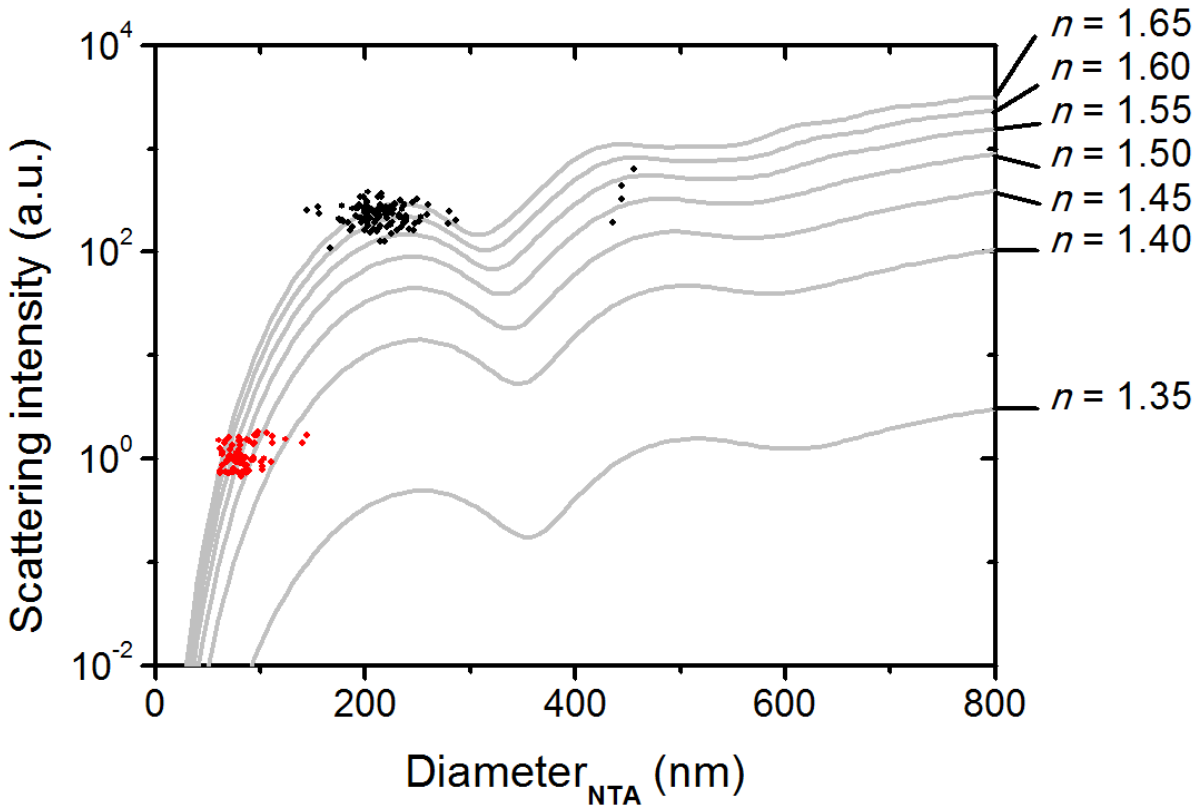
Scattering power versus diameter of beads



Scattering power versus diameter of beads

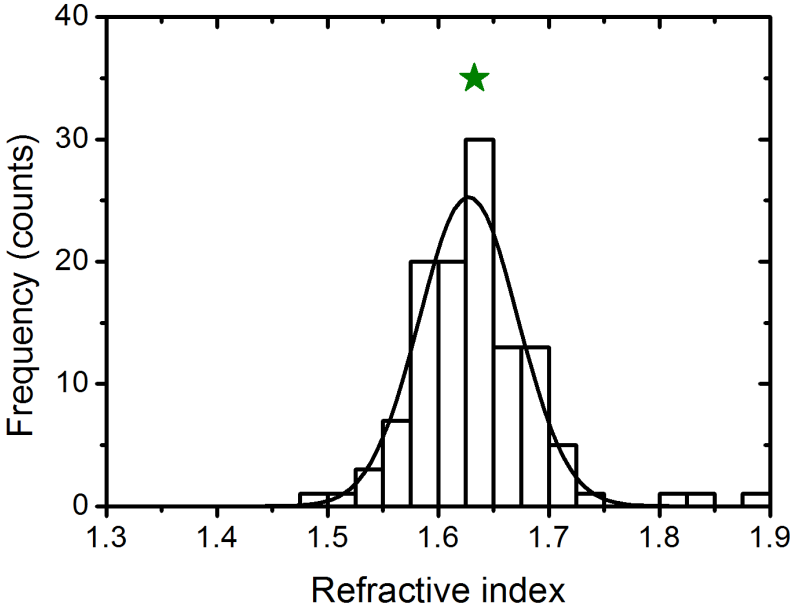


Scattering power versus diameter of beads



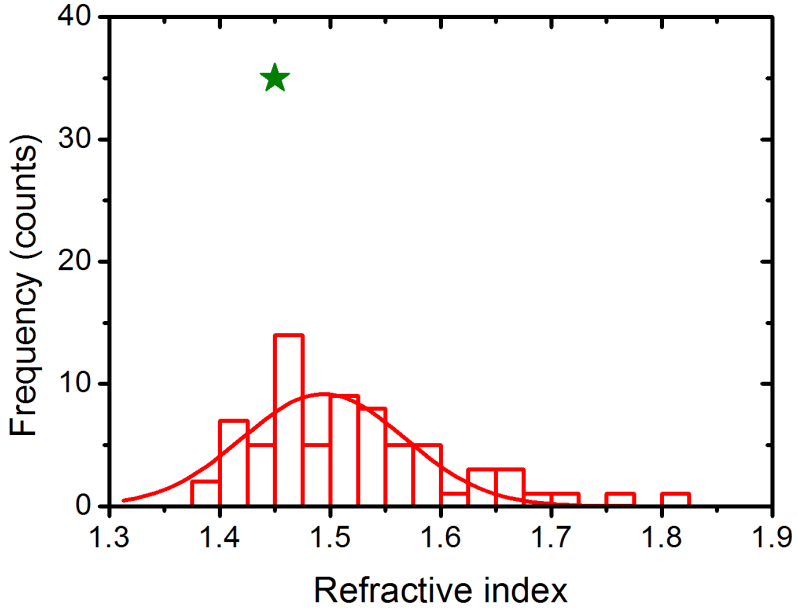
Validate method using beads

203-nm polystyrene beads



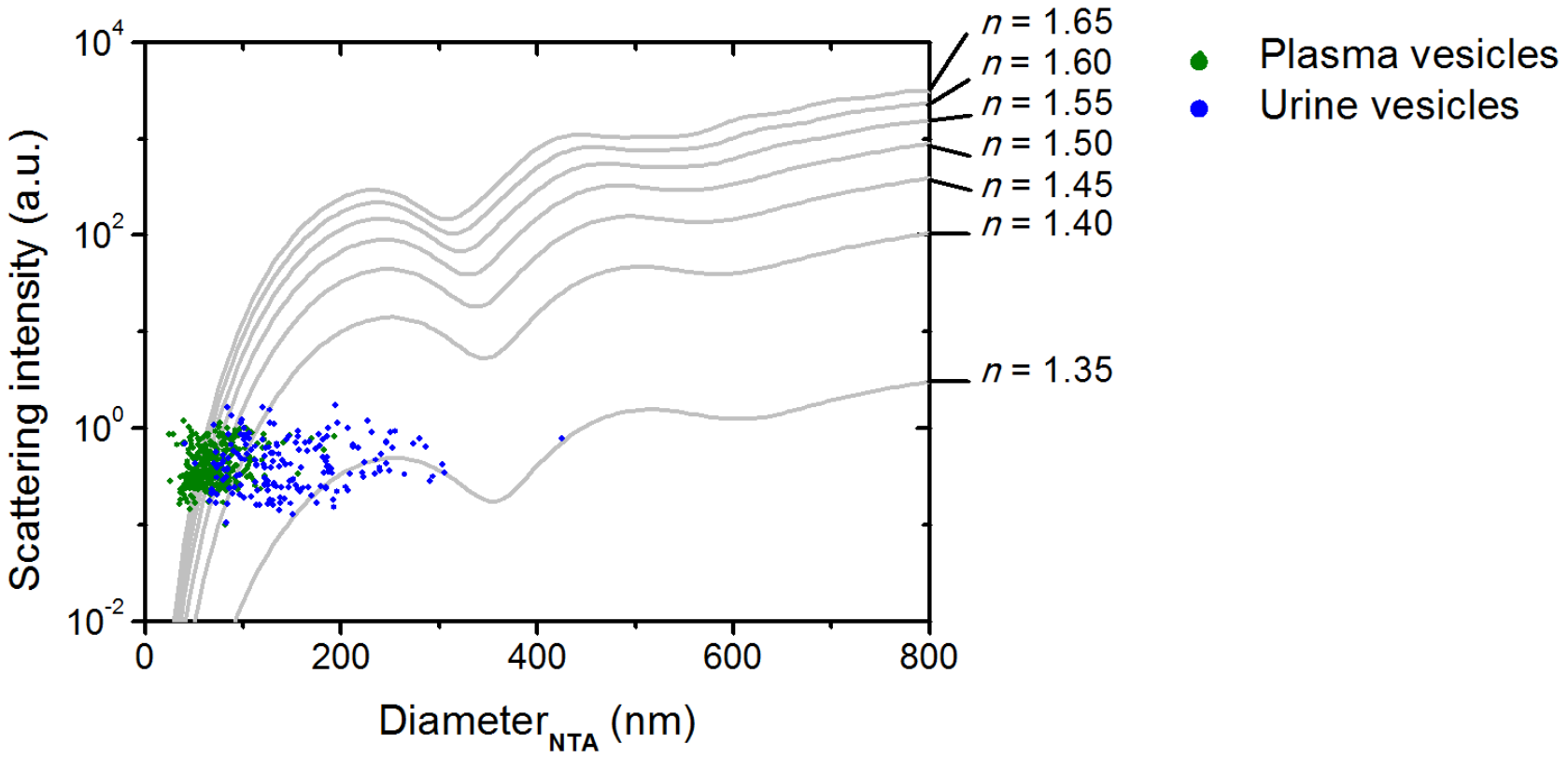
Accuracy: 1%
Coefficient of variation: 3%

90-nm silica beads



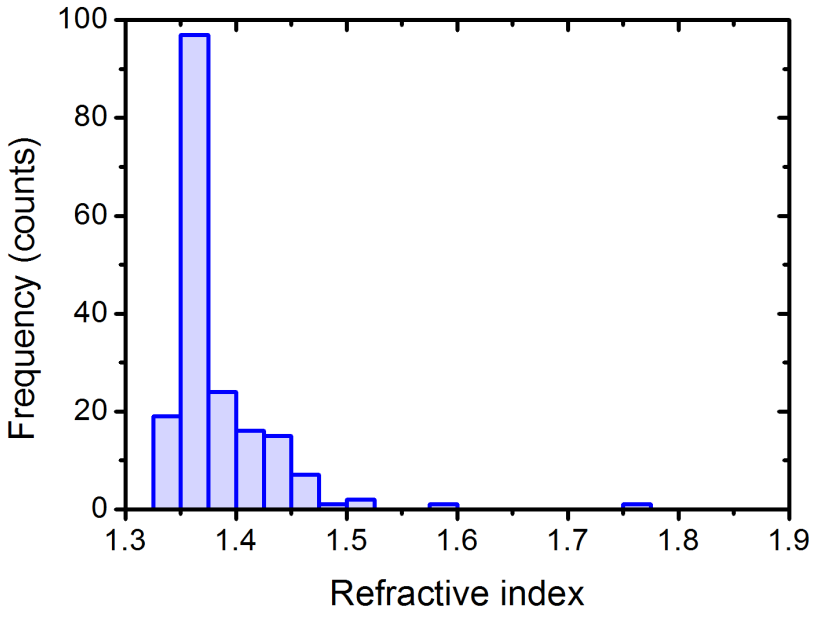
Accuracy: 3%
Coefficient of variation: 5%

Scattering power versus diameter of vesicles



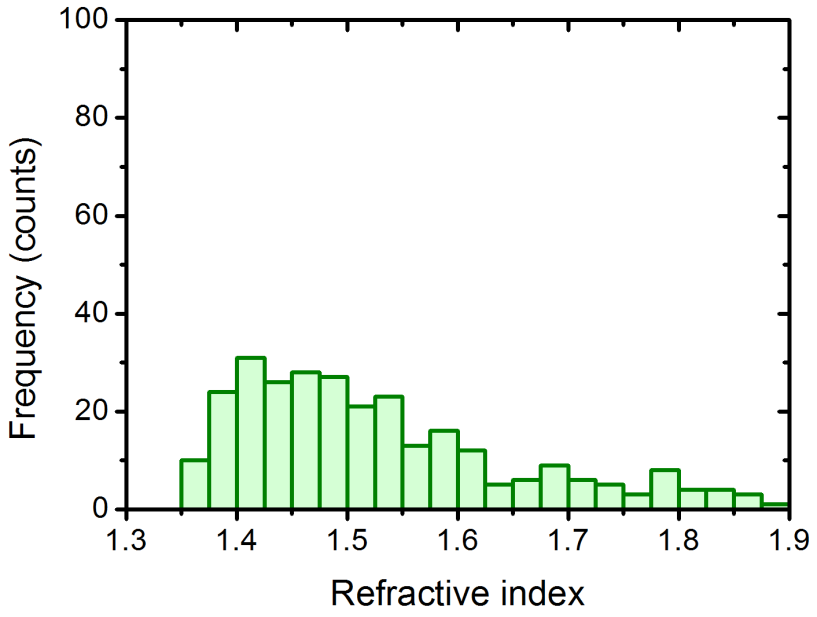
Refractive index distribution of vesicles by NTA

Urine vesicles



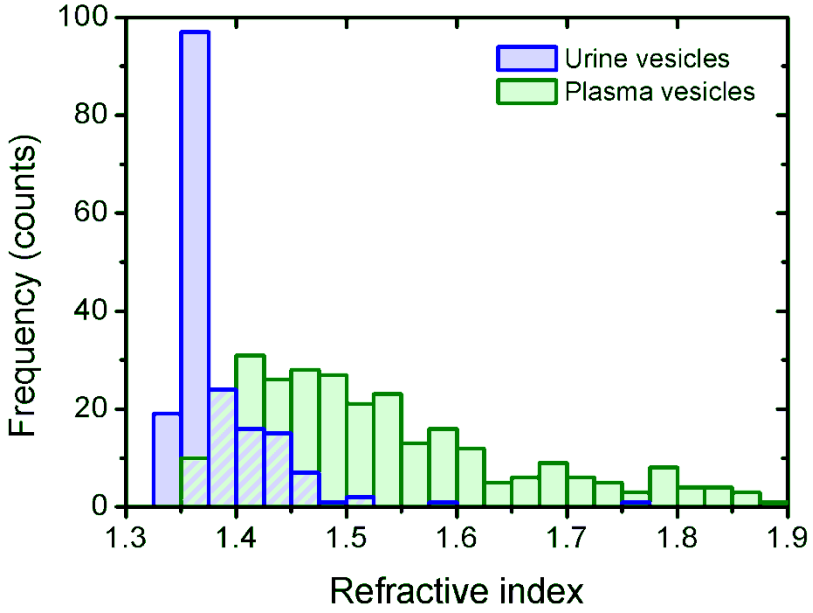
n = 1.36

Plasma vesicles



n = 1.49

Conclusions



- NTA can be used to assess the refractive index
- new reference materials have to be developed to calibrate optical instruments for vesicle detection

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More on microparticle detection:
edwinvanderpol.com