

Determination of the refractive index of vesicles using nanoparticle tracking analysis

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Biomedical Engineering and Physics

Laboratory Experimental Clinical Chemistry

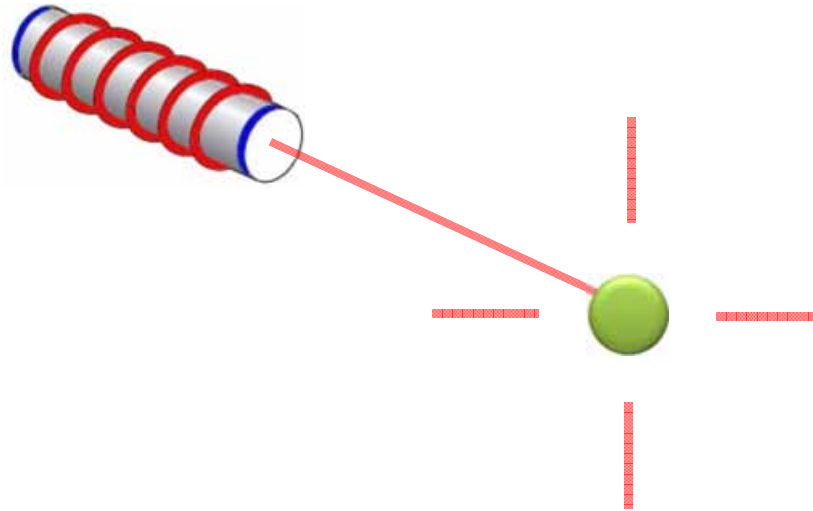
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Disclosures of: Edwin van der Pol

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Consultancy	No conflict of interest to disclose
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Major stockholder	No conflict of interest to disclose
Patents	No conflict of interest to disclose
Honoraria	No conflict of interest to disclose
Travel support	No conflict of interest to disclose
Other	No conflict of interest to disclose

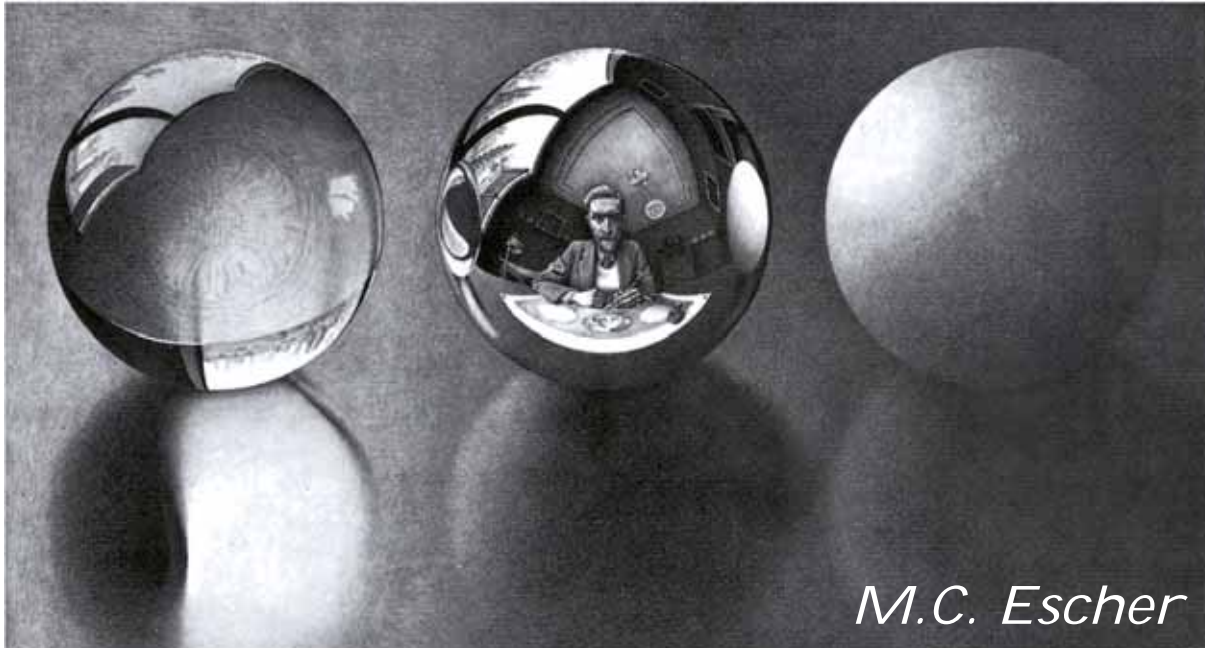
Presentation includes discussion of the following off-label use of a drug or medical device: N/A

Introduction to light scattering



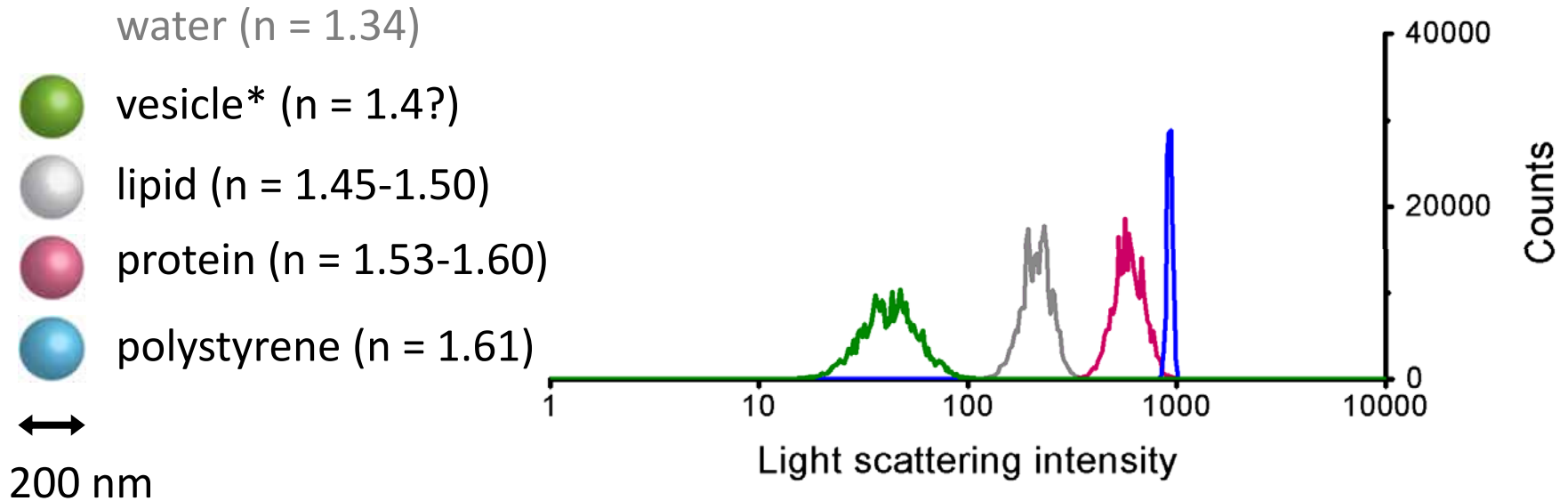
- light illuminating a vesicle is partly absorbed and partly scattered (deflected)
- light scattering depends on size and refractive index

Introduction to the refractive index



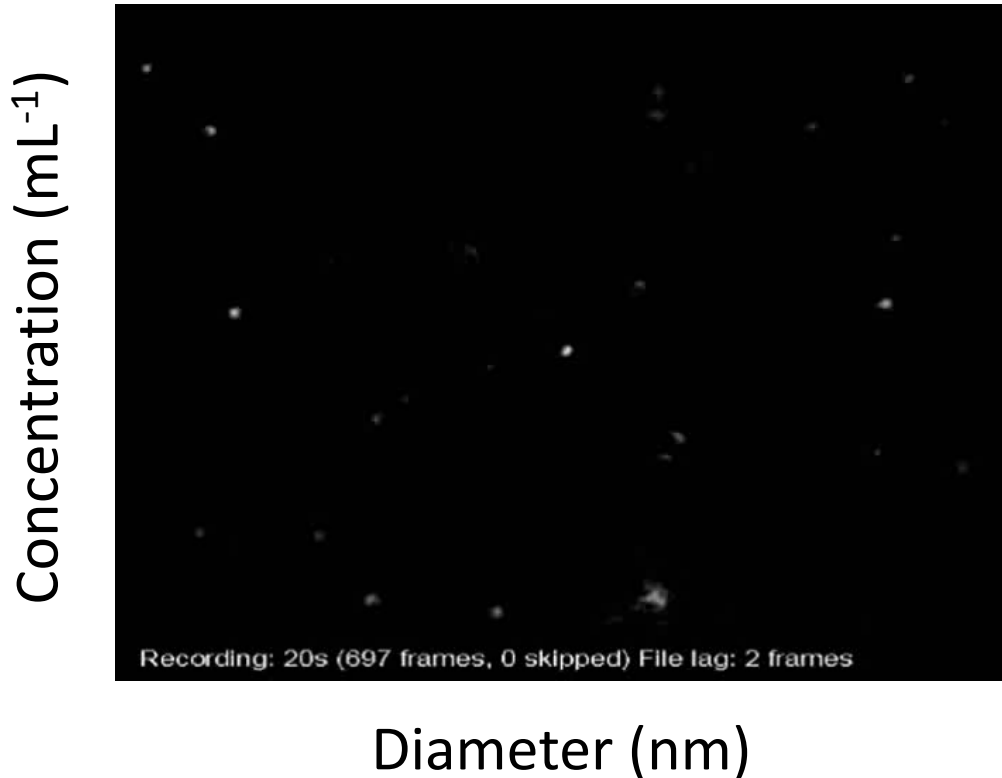
- the refractive index
 - is defined as $n = c_{vacuum} / v_{medium}$
 - affects refraction and reflection

Motives of studying the refractive index



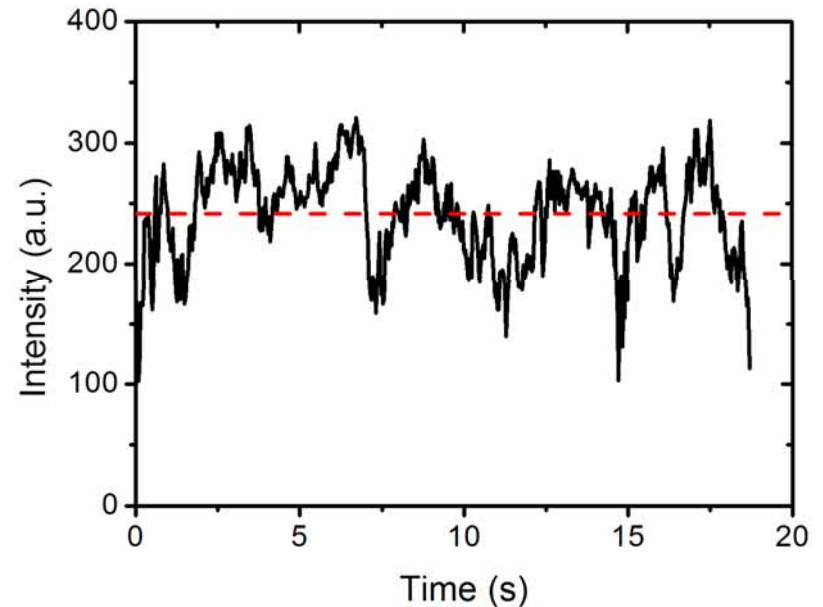
- new label-free parameter
 - cellular origin
 - distinguish vesicles from contamination
- relate light scattering to vesicle diameter
- detection range

Nanoparticle tracking analysis (NTA)



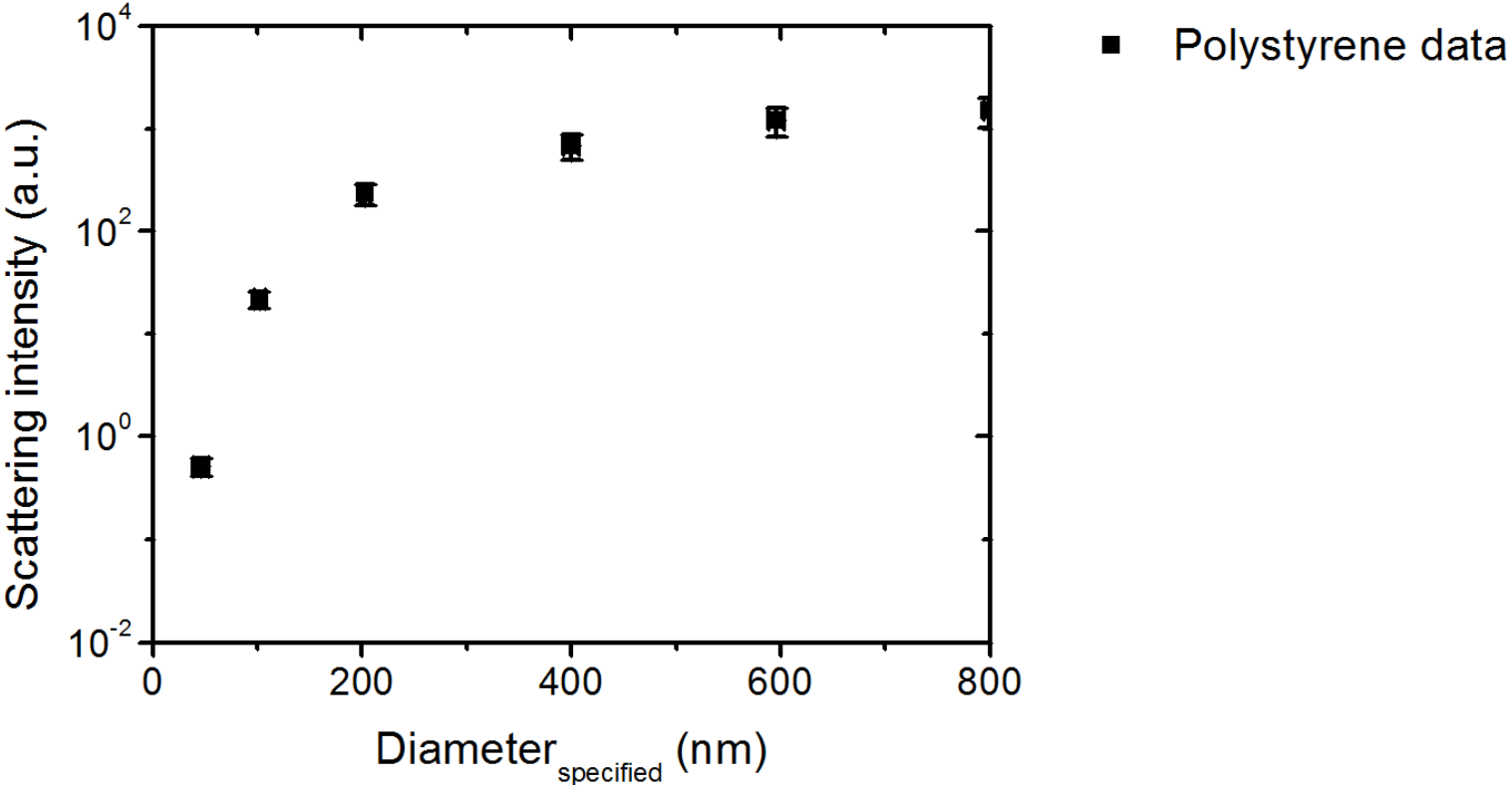
- determine size and concentration of vesicles
- additional parameters: light scattering or fluorescence

Method – measure light scattering by NTA

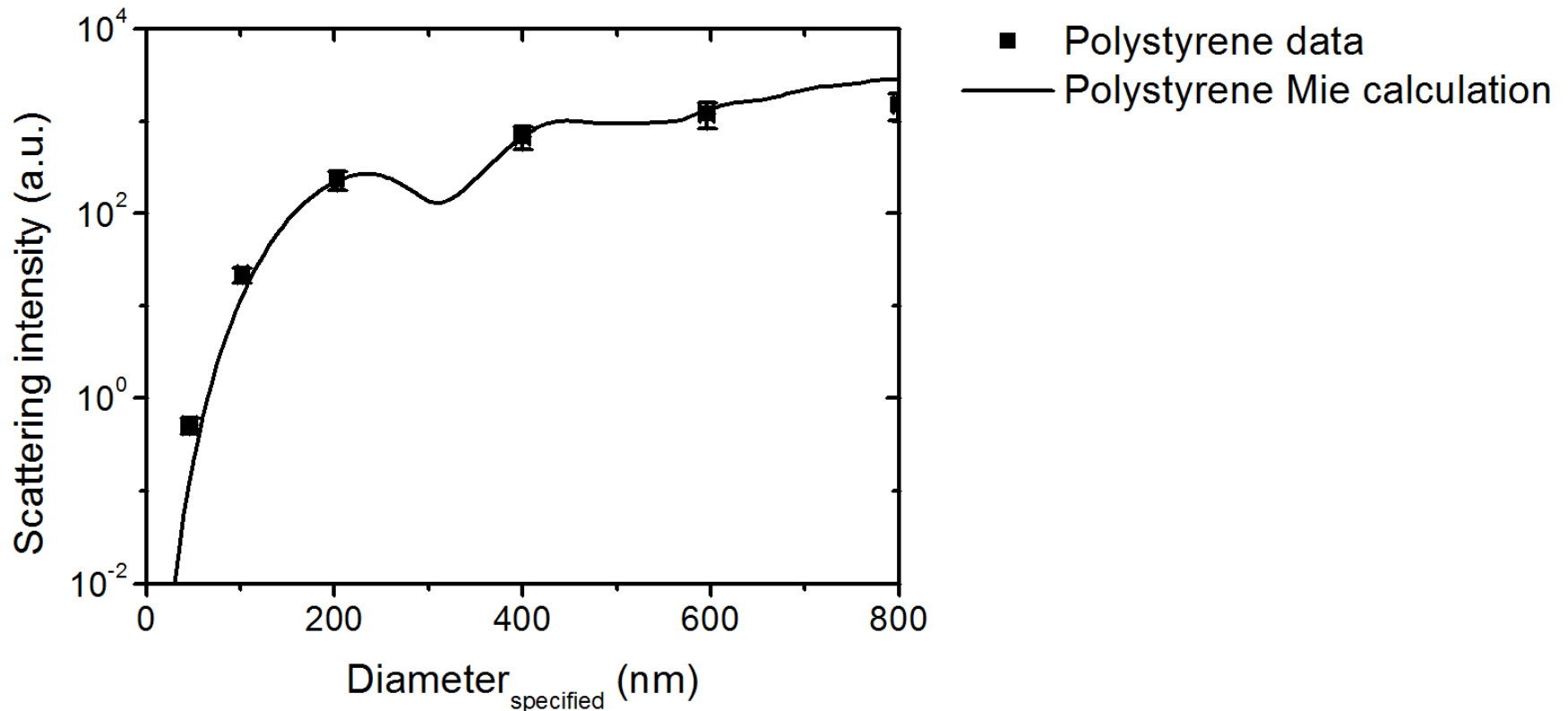


- no pixel saturation
- video processing by NanoSight NTA 2.3
 - intensity corrected for camera shutter time and gain

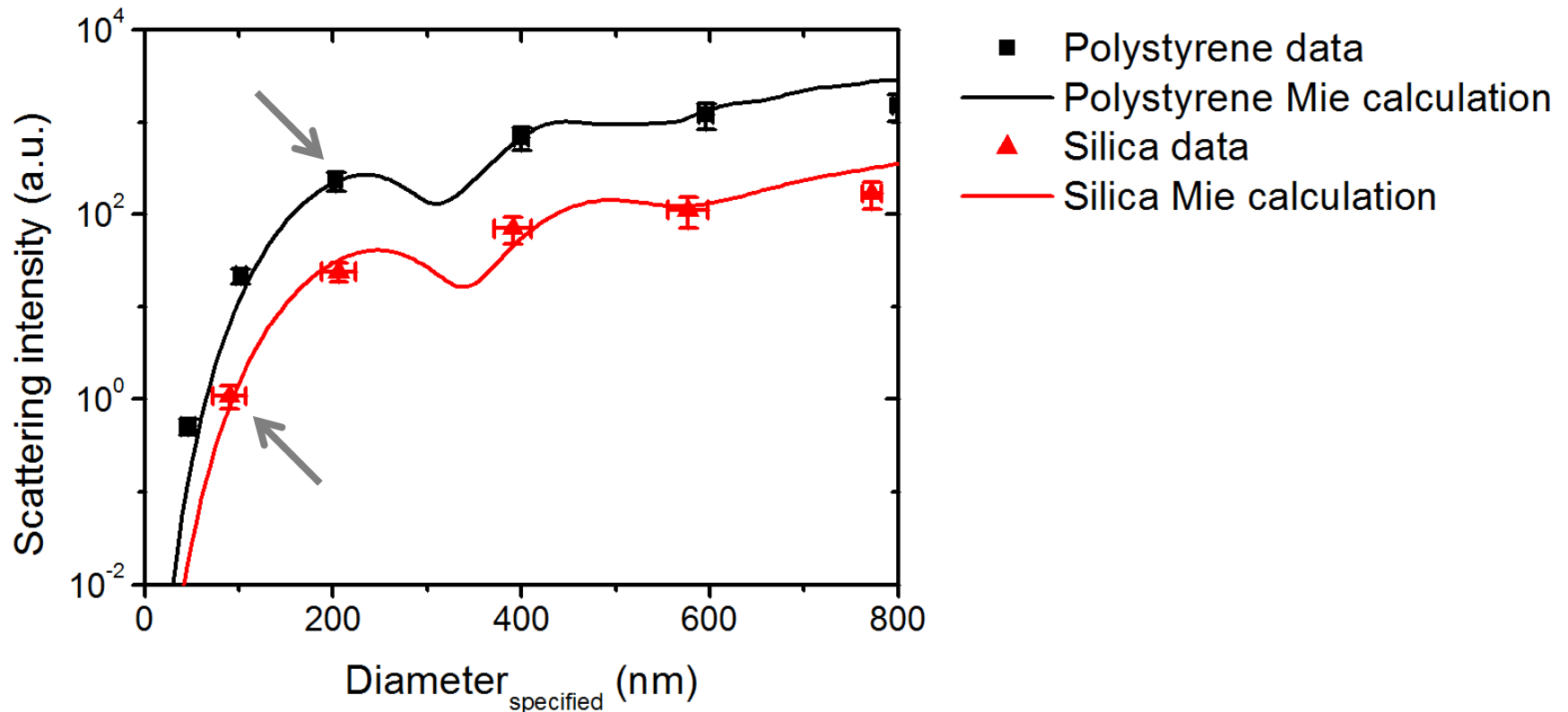
Scattering power versus diameter of beads



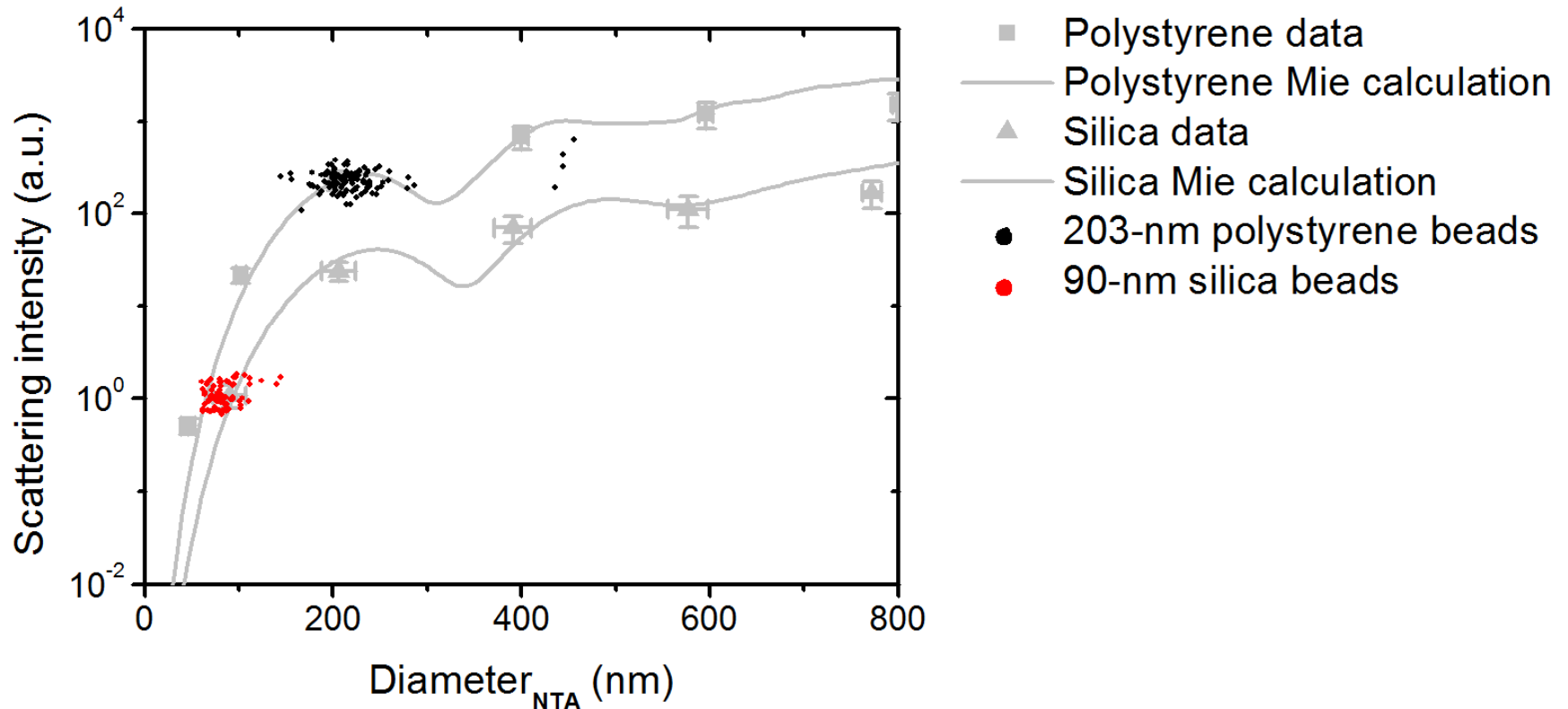
Scattering power versus diameter of beads



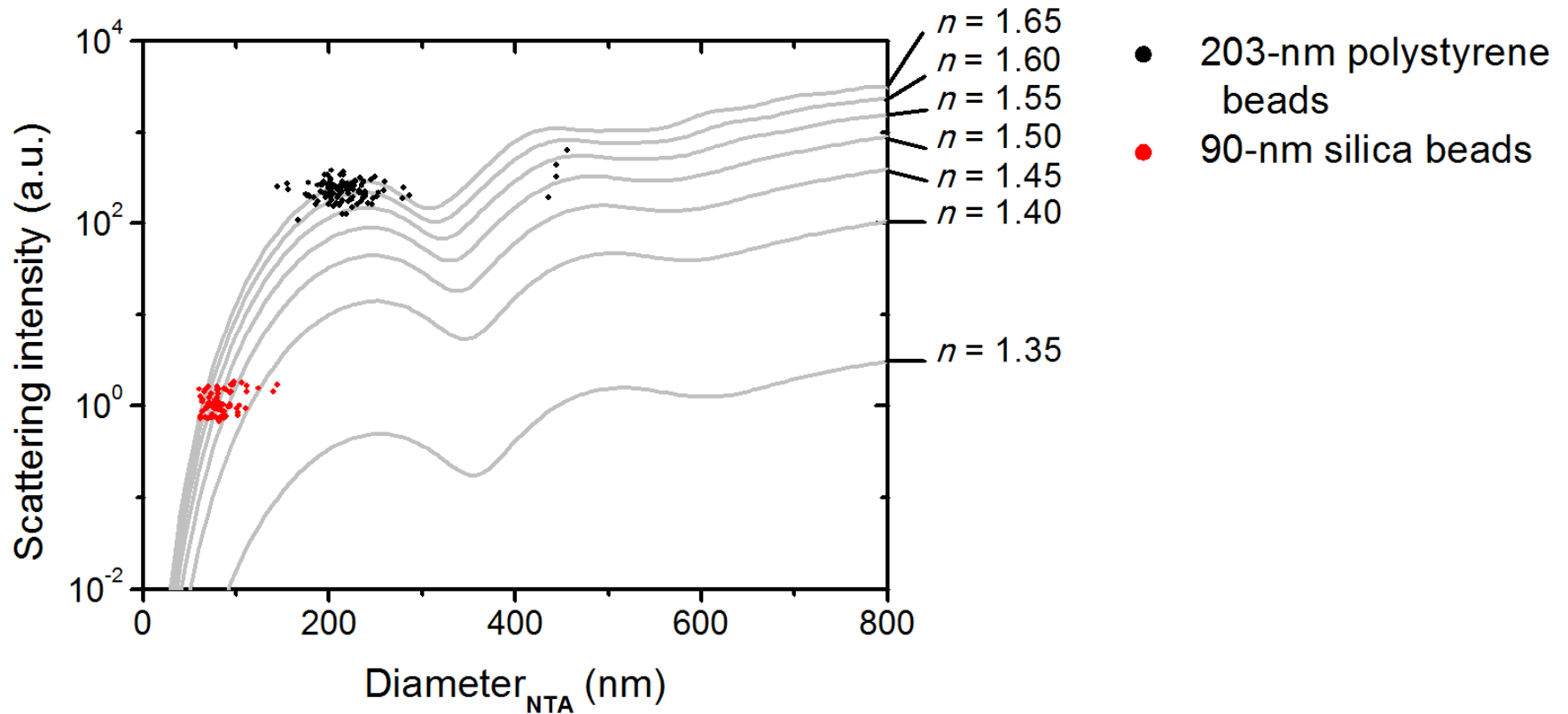
Scattering power versus diameter of beads



Scattering power versus diameter of beads

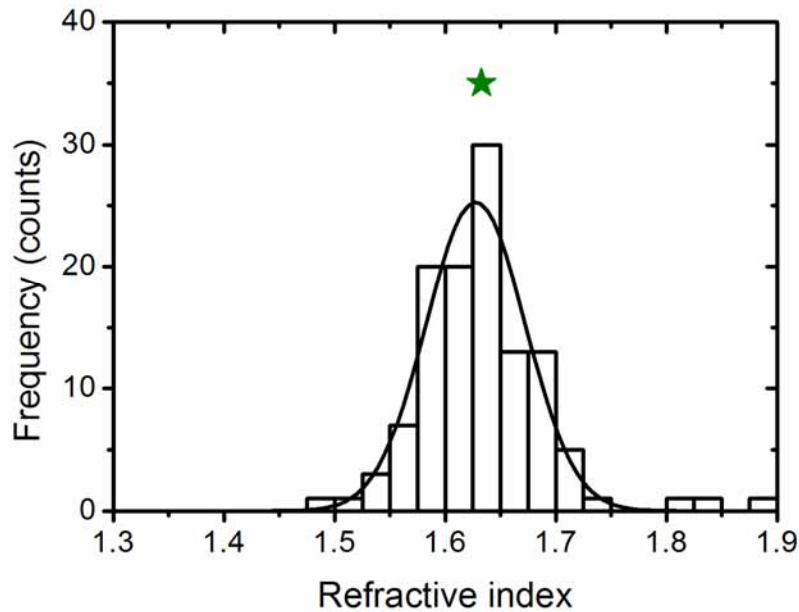


Scattering power versus diameter of beads



Validate method using beads

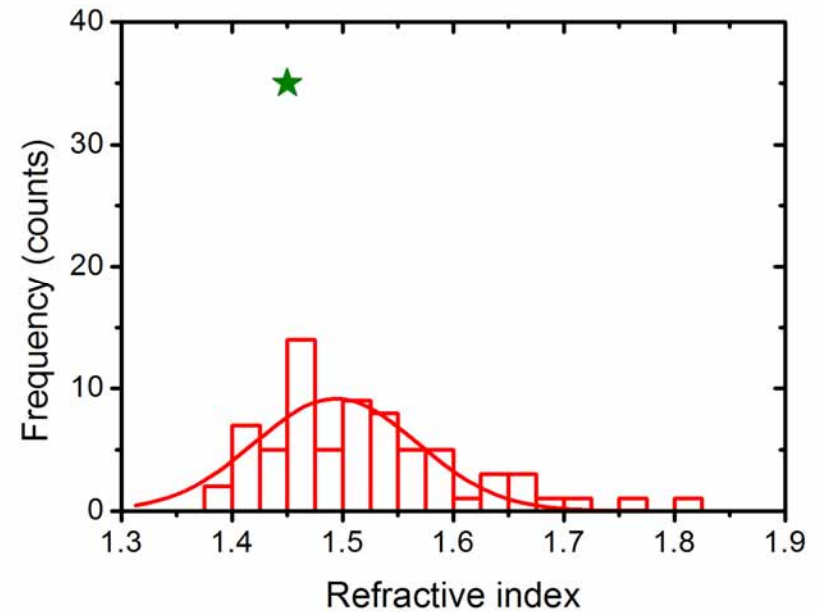
203-nm polystyrene beads



Accuracy: 1%

Coefficient of variation: 3%

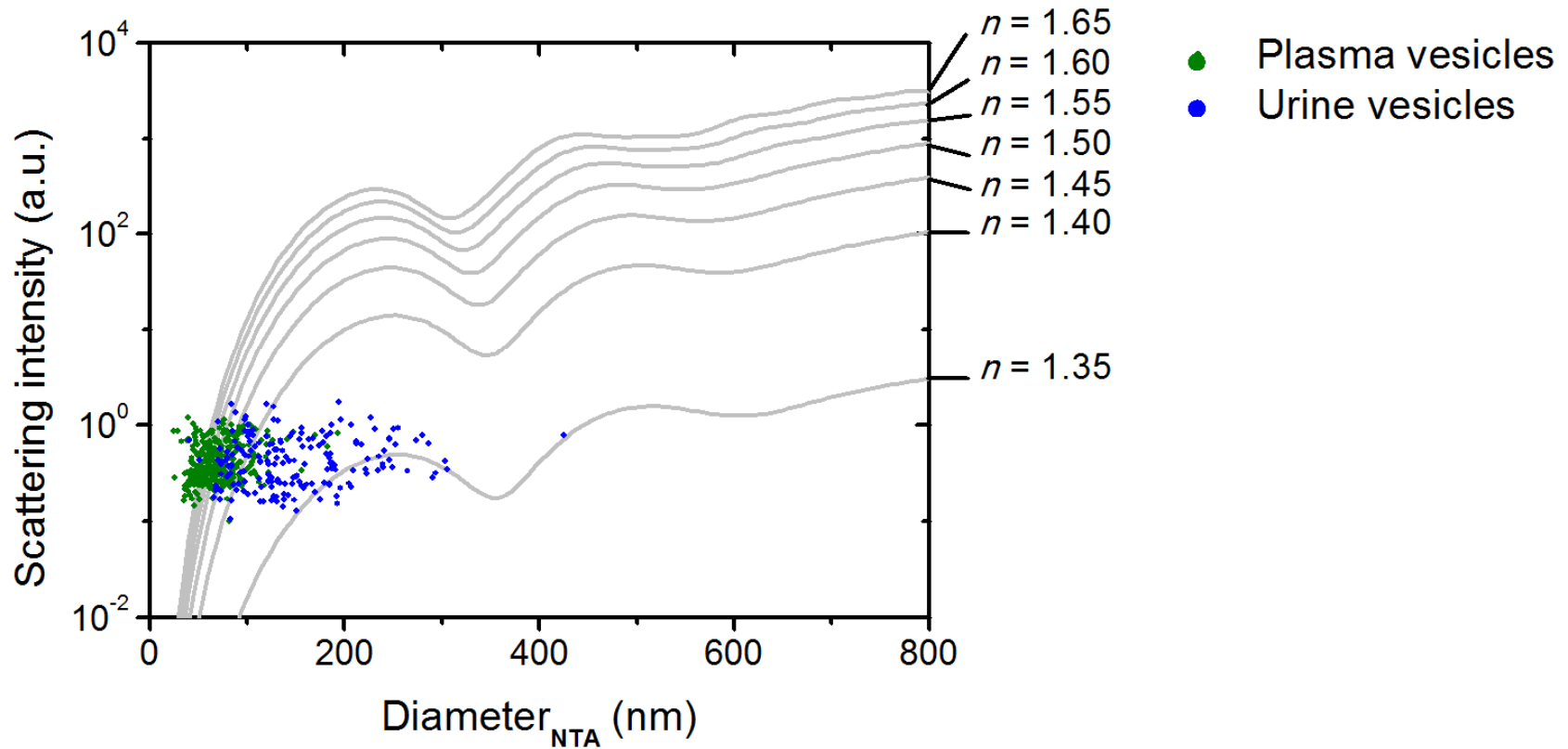
90-nm silica beads



Accuracy: 3%

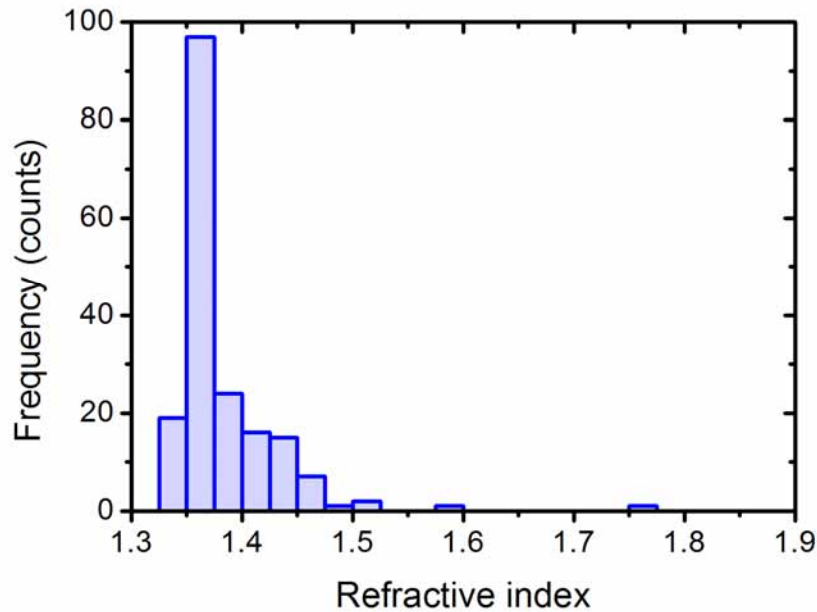
Coefficient of variation: 5%

Scattering power versus diameter of vesicles



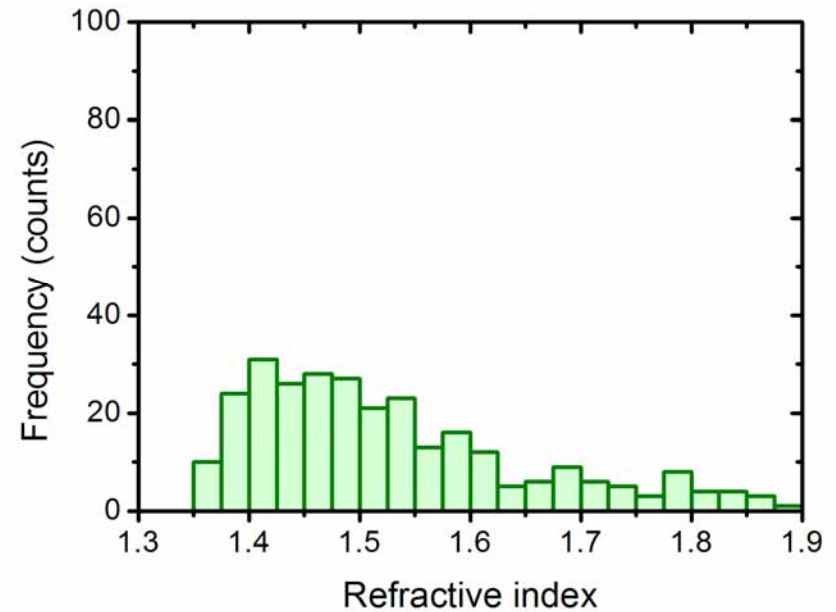
Refractive index distribution of vesicles by NTA

Urine vesicles



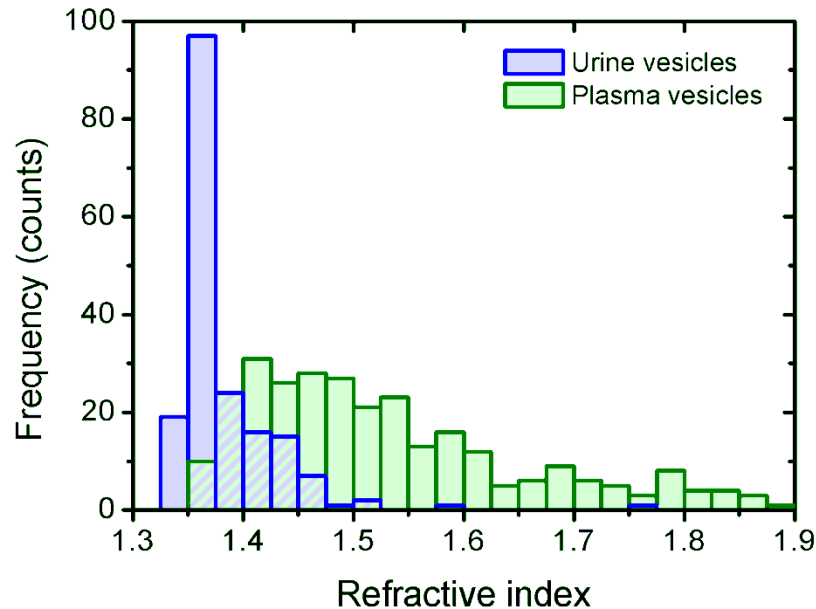
$n = 1.36$

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

