Calibration of light scattering signals measured by flow cytometry

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Amsterdam UMC
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Conflict of interest

- Co-founder and shareholder of EXOMETRY

Quiz question

- Can NanoSight NS300 track extracellular vesicles with a diameter of 30 nm? Why (not)?

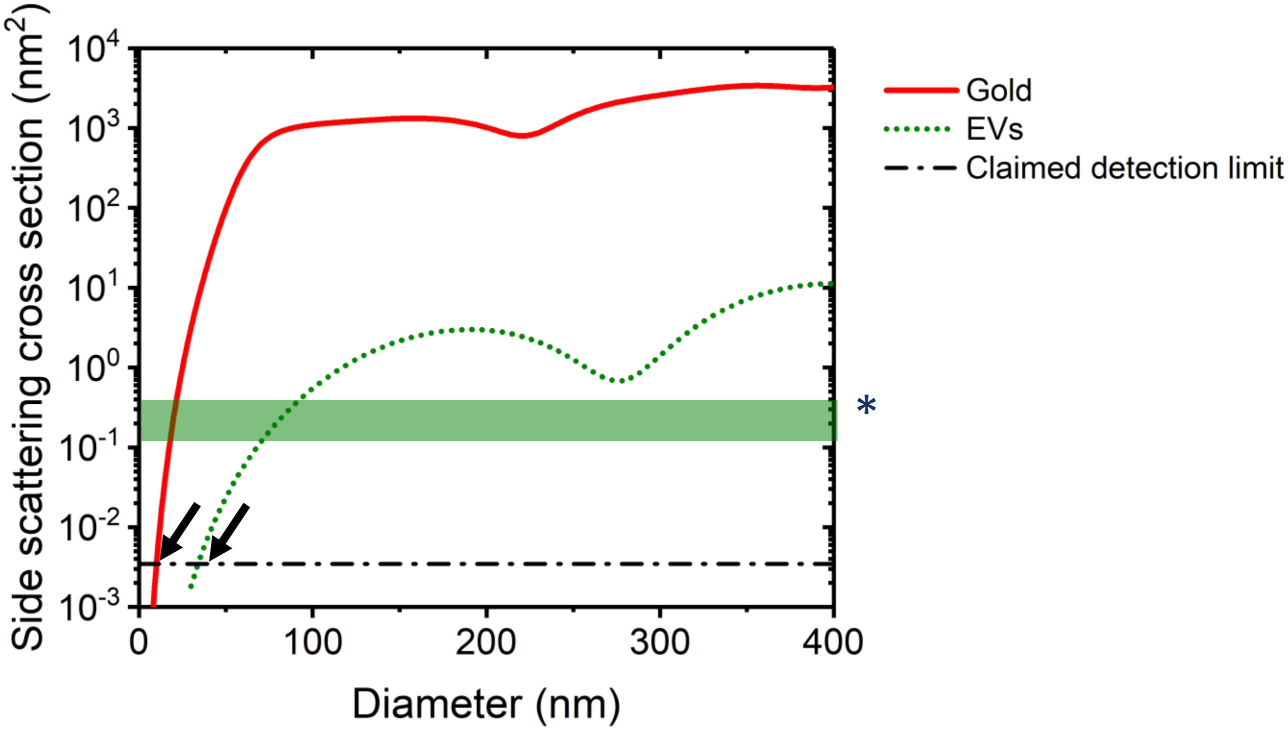
Quiz answer

The NS300 allows rapid, automated analysis of the size distribution and concentration of all types of nanoparticles from 0.01 - 1 µm* in diameter, depending on the instrument configuration and sample type.

*sample and system configuration dependent

Source: malvernpanalytical.com

Quiz answer

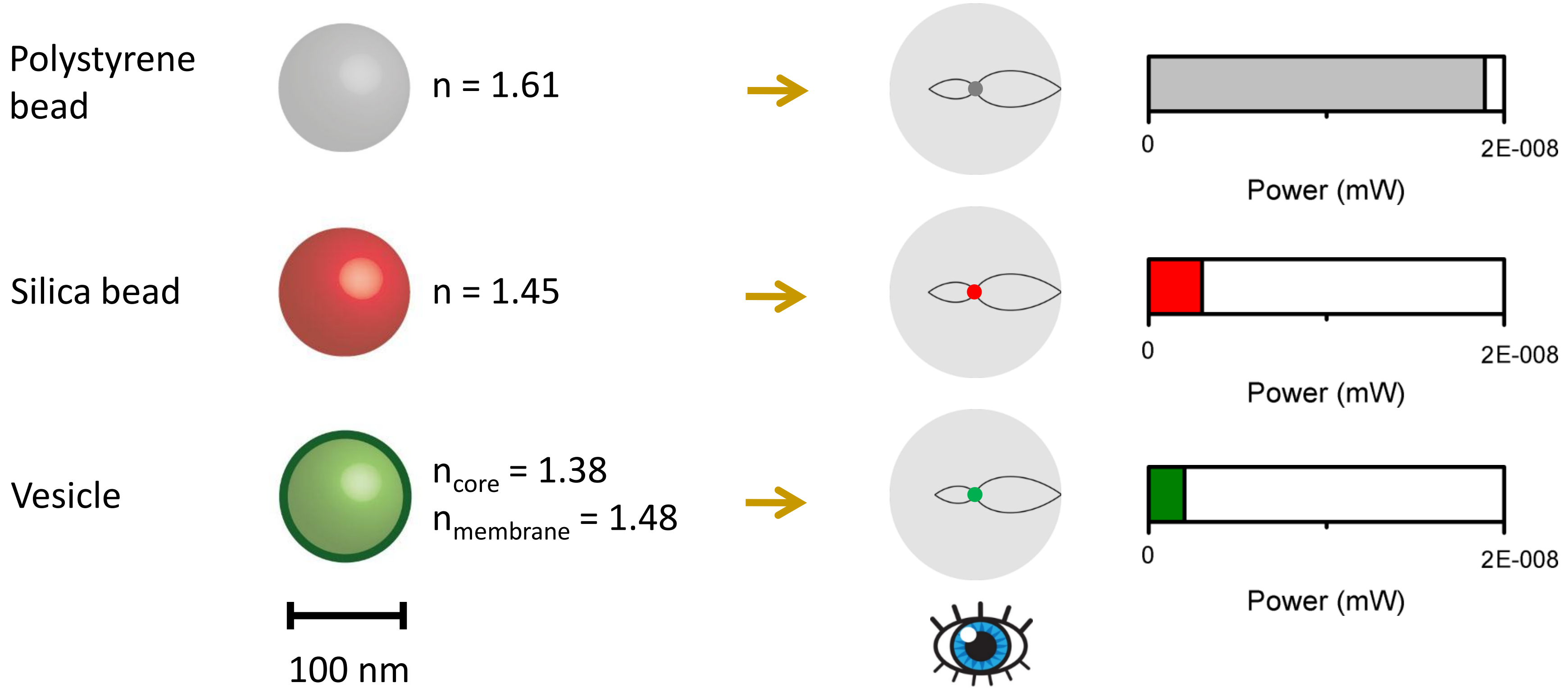


*van der Pol et al. *J Thromb Haemost* 2014

Outline

- ✔ Quiz answer
- Refractive index
- Calibration: from arbitrary units to standard units
- Accuracy and precision of “optical diameters”
- Relevance of calibration
- Summary

Light scattering and the refractive index



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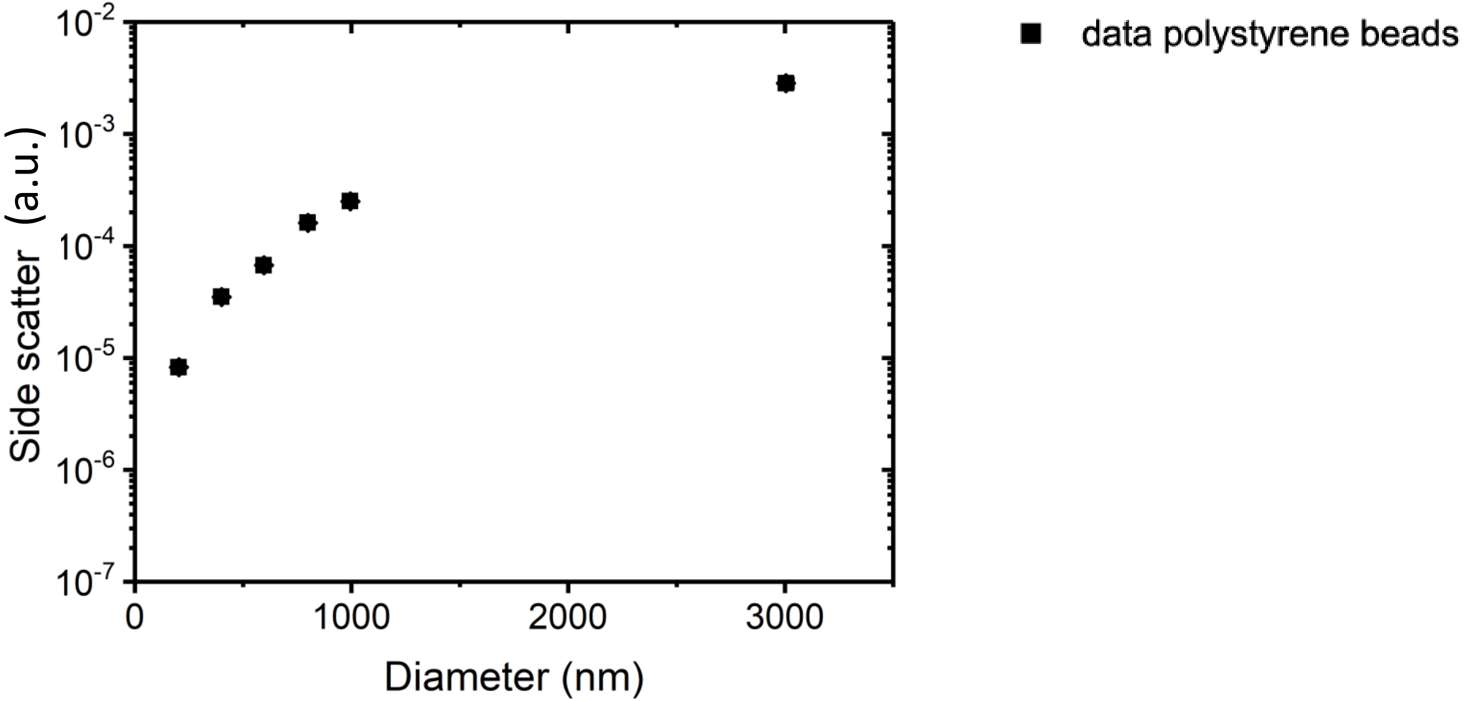
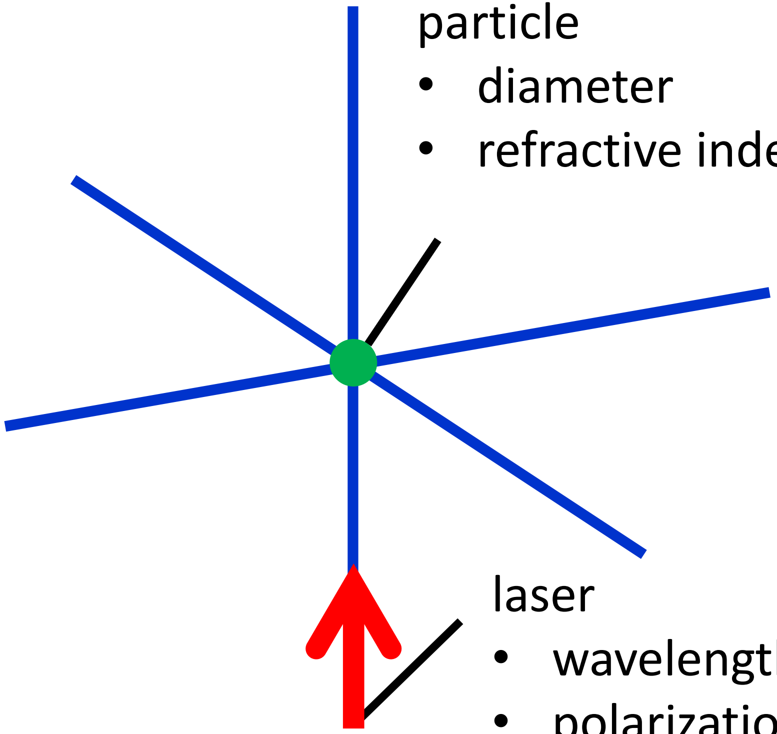
Relate scatter to diameter of beads



Mie theory



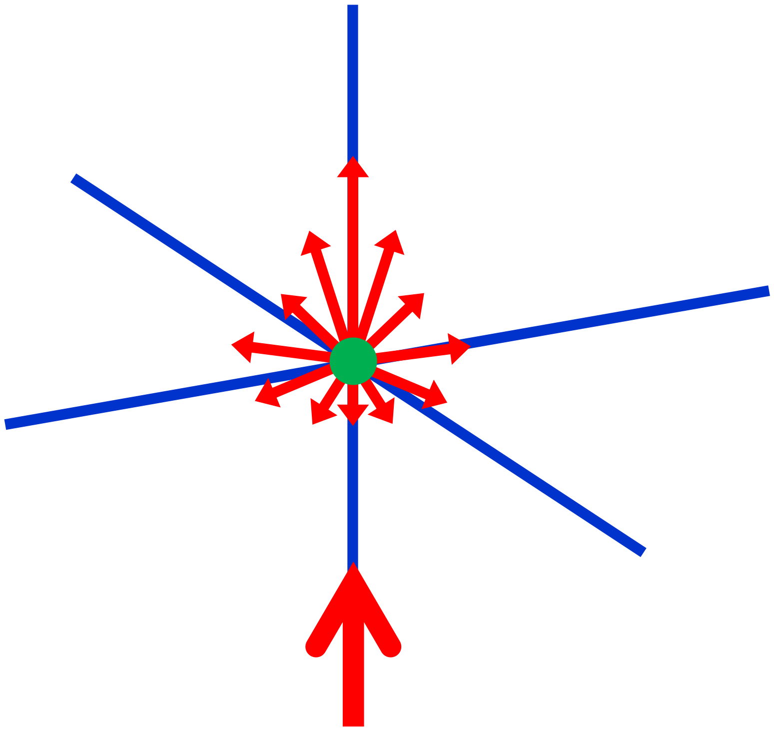
particle

- diameter
- refractive index

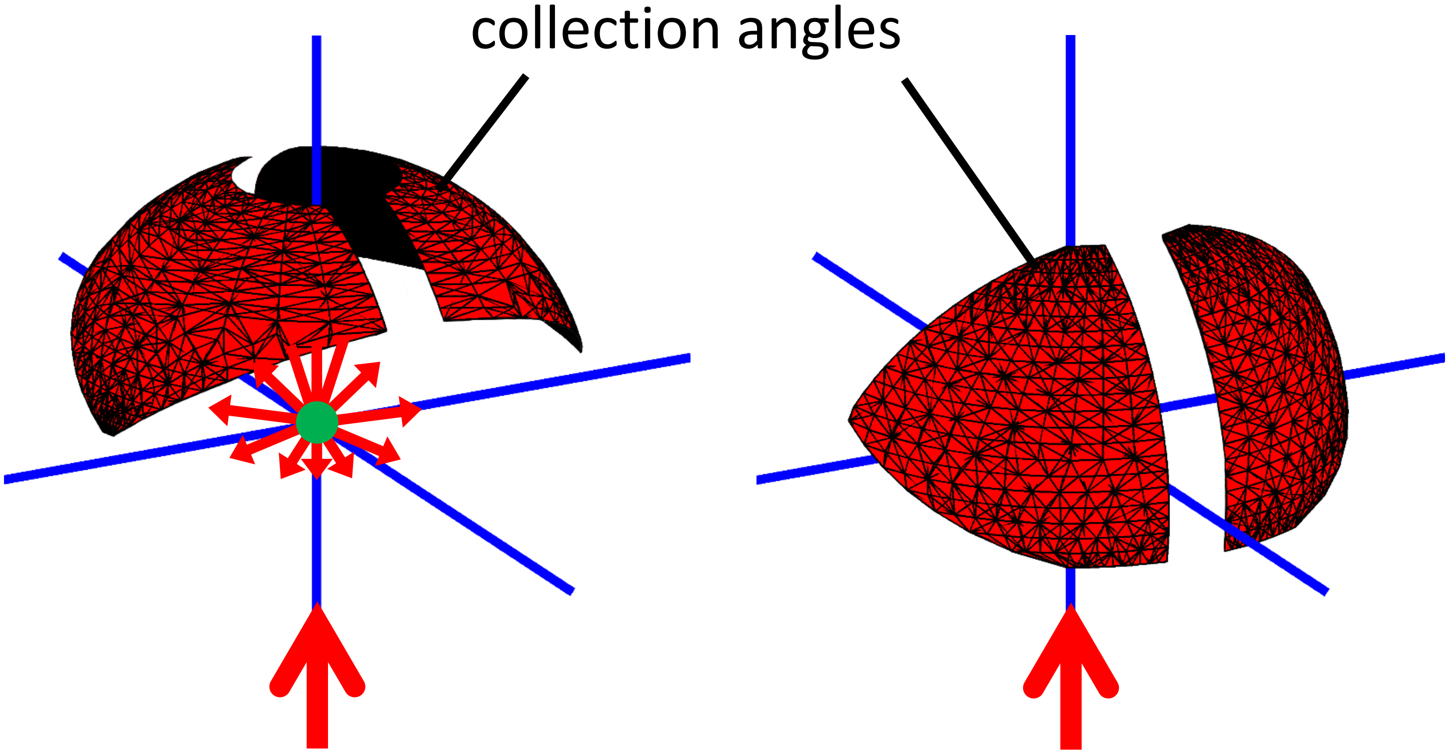
laser

- wavelength
- polarization
- power

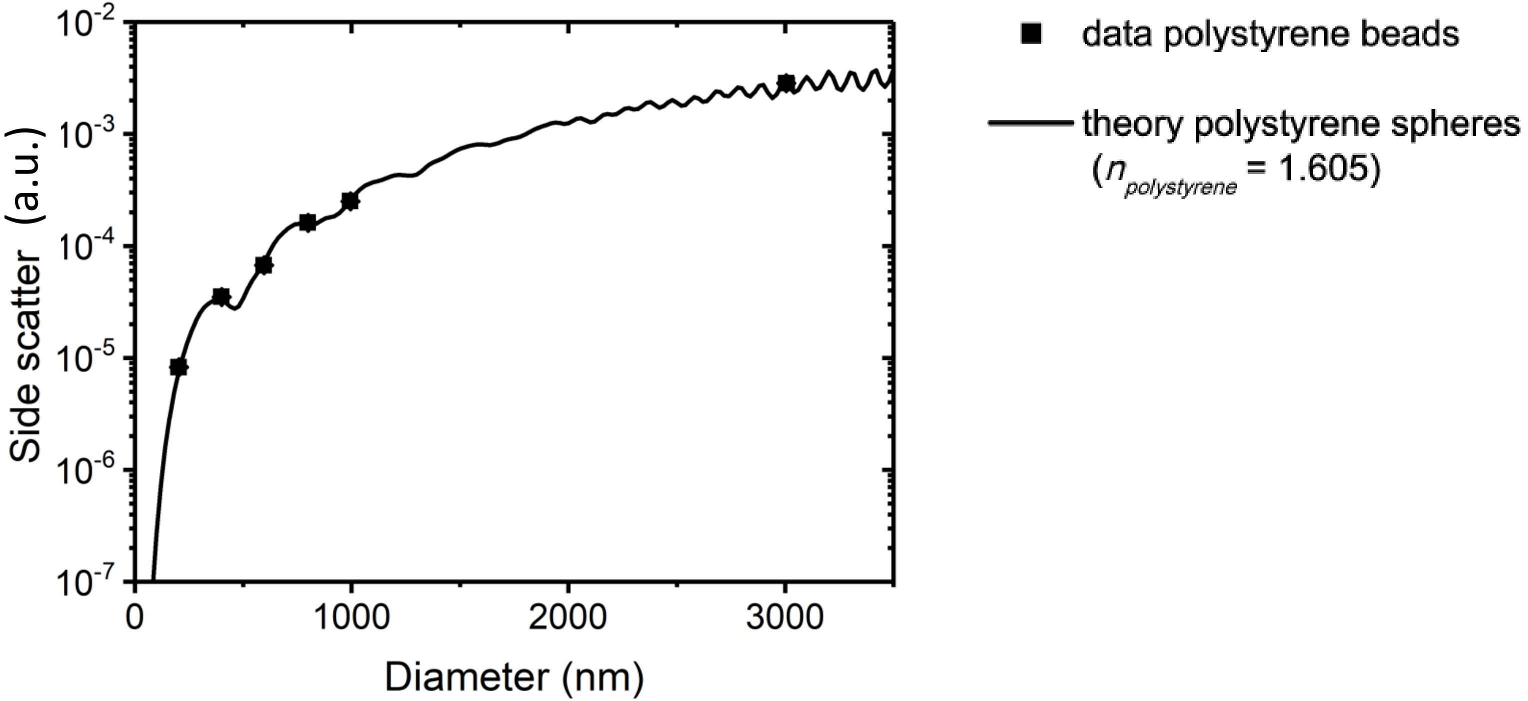
Mie theory



Mie theory

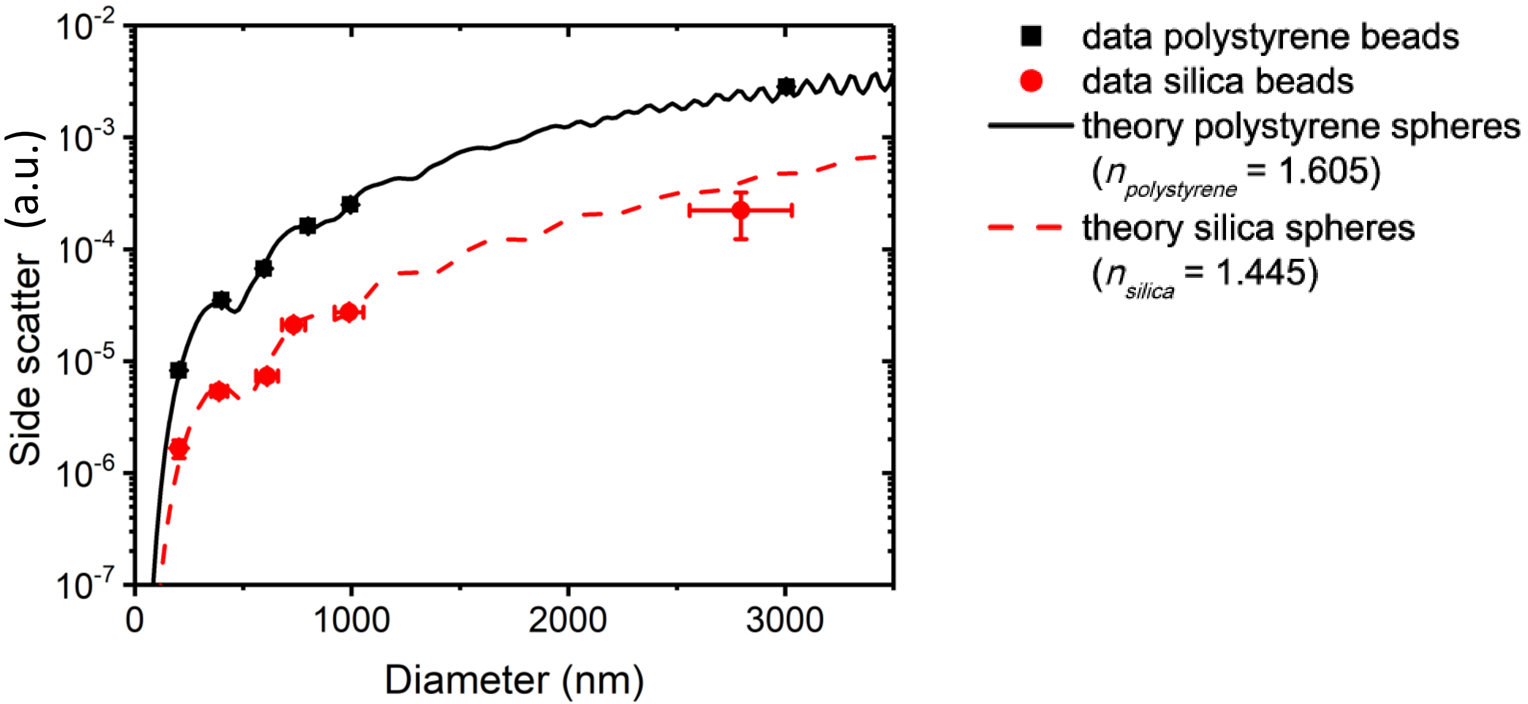


Relate scatter to diameter of beads



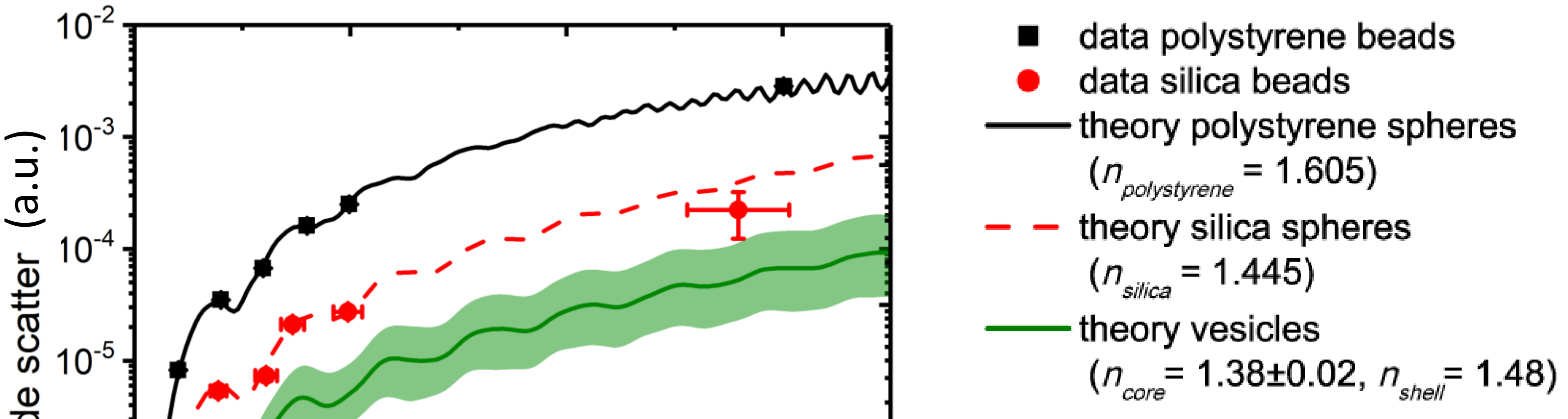
Model: de Rond et al. *Curr Protoc Cytom* 2018

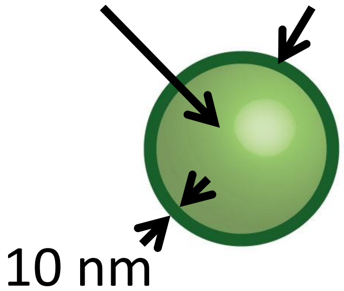
Relate scatter to diameter of beads



Model: de Rond et al. *Curr Protoc Cytom* 2018

Relate scatter to diameter of EVs





Turn your flow cytometer into a particle sizer!

Deriving Extracellular Vesicle Size From Scatter Intensities Measured by Flow Cytometry

Leonie de Rond,[1,2,3] Frank A. W. Coumans,[1,2,3] Rienk Nieuwland,[2,3] Ton G. van Leeuwen,[1,3] and Edwin van der Pol[1,2,3,4]

Current Protocols in Cytometry, e43 (2018)

- Program yourself
- Use your own beads + FCM_{PASS} (by Josh Welsh)
- Fully automated (Rosetta Calibration, COI)

Flow cytometry derives “optical diameters”

Measures	Assumes	Derives
Fluorescence	Size to fluorescence relation of targeted particles is similar to reference liposomes	Optical diameter[1]
Light scattering	Spherical / concentric particles Particle refractive index Known optical configuration	Optical diameter[2]

1. Stoner *Cytom. Part A* 2016
2. de Rond et al. *Curr. Protoc. Cytom.* 2018

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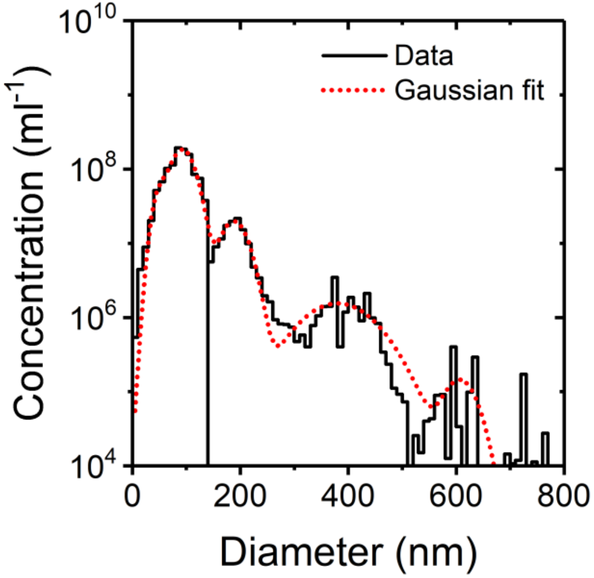
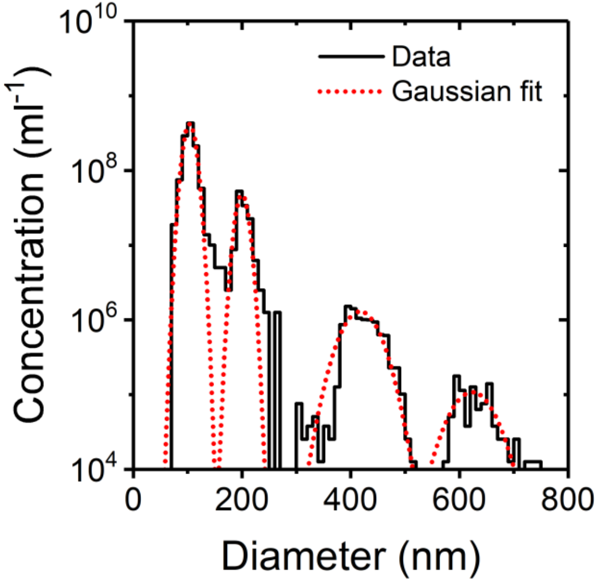
Sizing accuracy and precision using bead mix

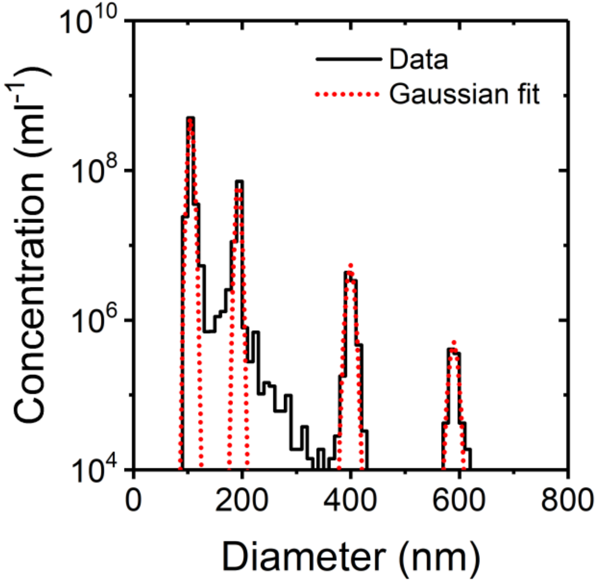
NTA
(Nanosight NS500)



RPS
(Izon qNano)

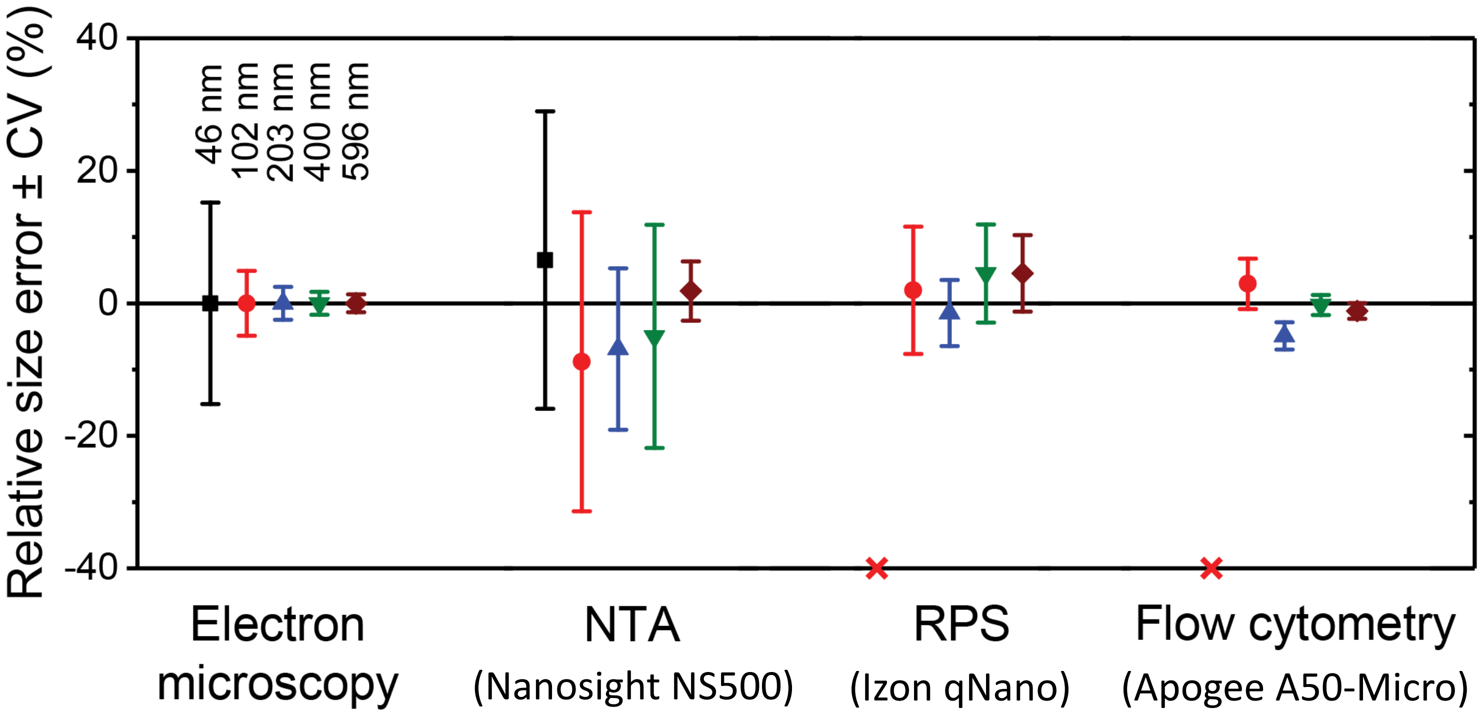


Flow cytometry
(Apogee A50-Micro)

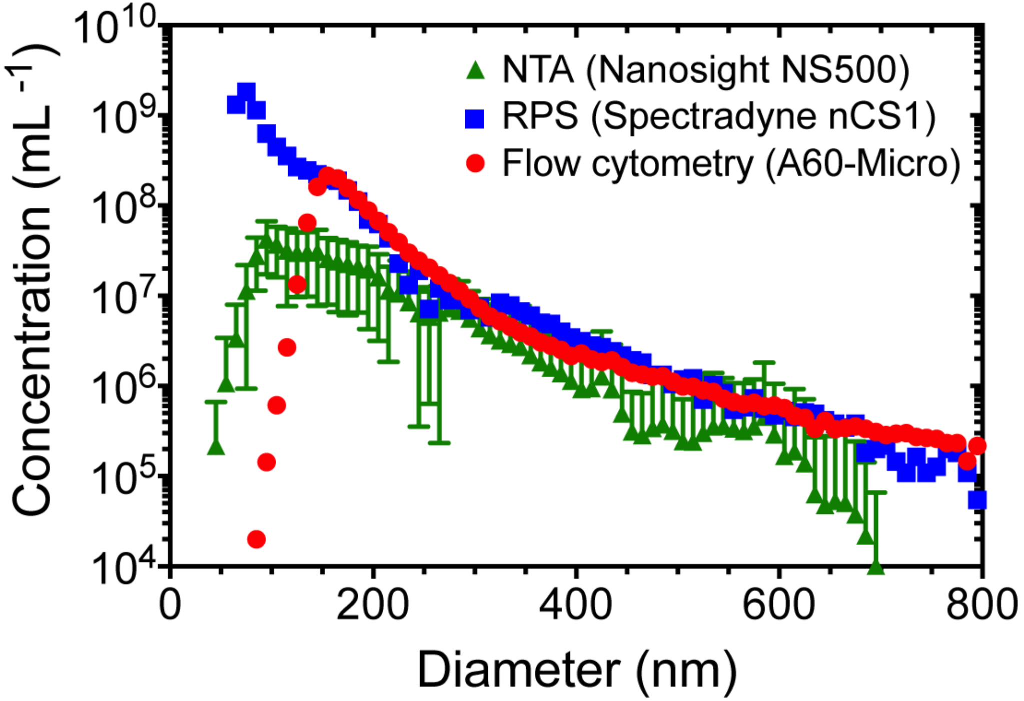


van der Pol et al. *J Thromb Haemost* 2014

Sizing accuracy and precision using bead mix



Extracellular vesicles in urine sample



Data and figure by Michael Cimorelli, manuscript in prep.

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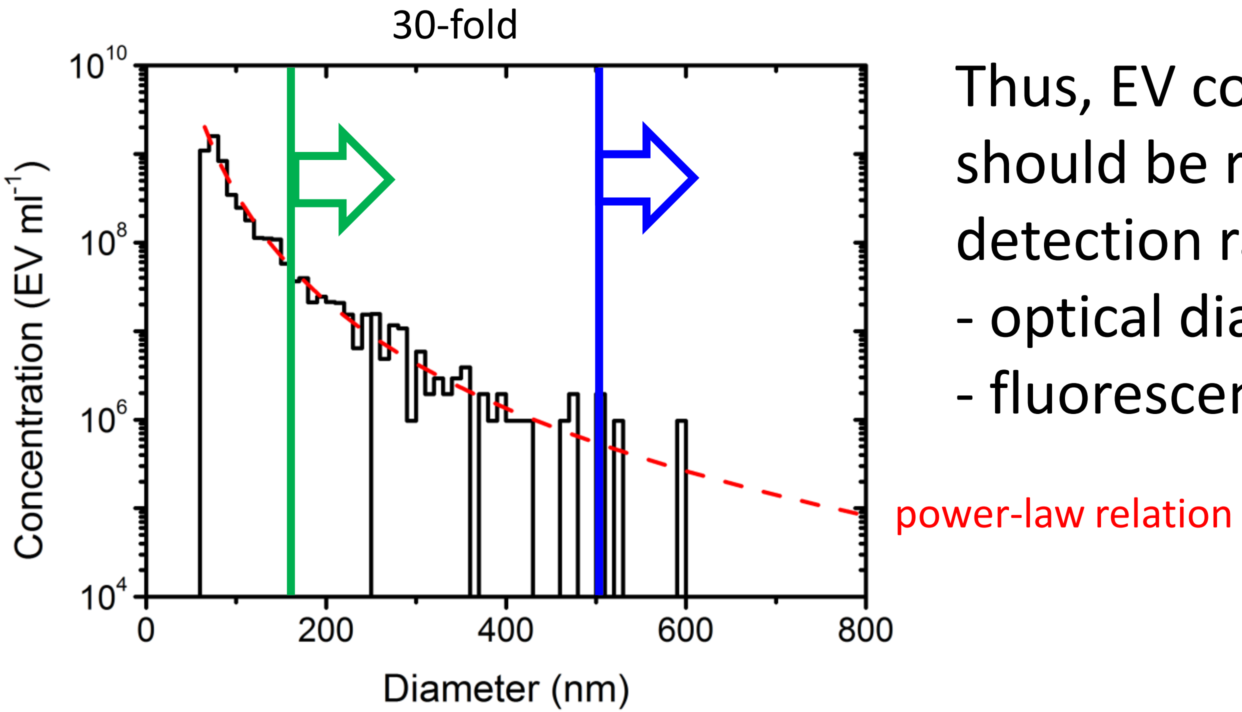
Optical diameter is relevant to standardization



Thus, EV concentrations should be reported with detection range, e.g.

- optical diameter range (nm)
- fluorescence range (MESF)

Summary

- Light scattering depends on
  - particle diameter, shape and *refractive index*
  - optical configuration of instrument
- Calibration: from arbitrary units to standard units
- Optical diameters are accurate and precise when assumptions are valid
- EV concentrations should be reported with detection range

Relevant literature

- A compendium of single extracellular vesicle flow cytometry.
  Joshua A. Welsh et al. *J Extracell Vesicles* **12**, 12299 (2023)

- Deriving extracellular vesicle size from scatter intensities measured by flow cytometry.
  Leonie de Rond et al. *Curr Protoc Cyt* **e43** (2018)

- MIFlowCyt-EV: a framework for standardized reporting of extracellular vesicle flow cytometry experiments.
  Joshua A. Welsh et al. *J Extracell Vesicles* **9**, 1713526 (2020)

Acknowledgements

- Amsterdam University Medical Centers
  - Michael Cimorelli
  - Rienk Nieuwland
  - Ton van Leeuwen
- Beckton & Dickinson
  - Joshua Welsh
- Scintillon Research Institute
  - John Nolan

- More information
  - edwinvanderpol.com
  - evflowcytometry.org
  - vesiclecenter.com
  - isev.org

Netherlands Organisation for Scientific Research

Amsterdam Vesicle Center

Amsterdam UMC University Medical Centers