Size and refractive index determination of submicrometer particles by flow cytometry

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Frank Coumans

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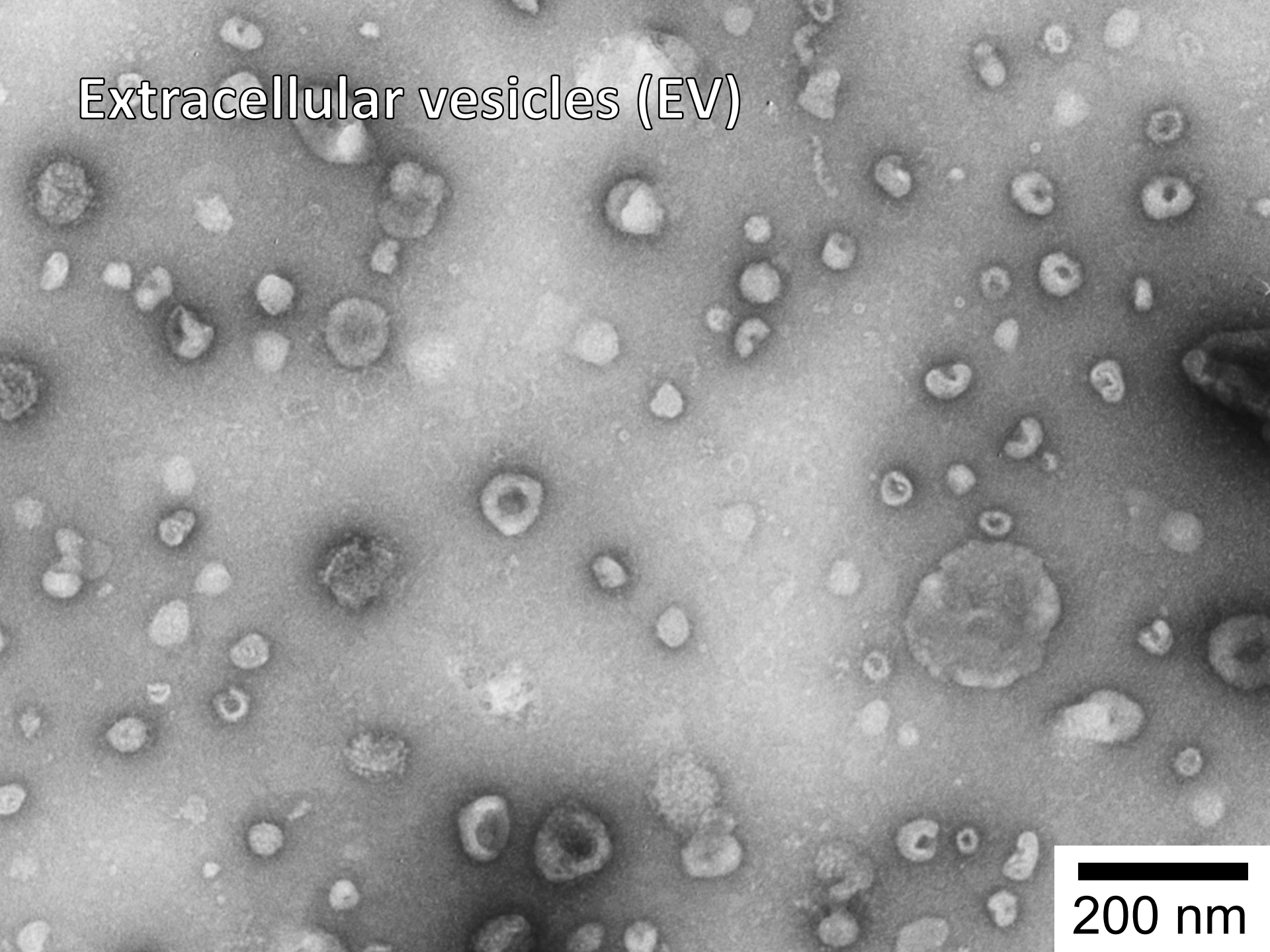
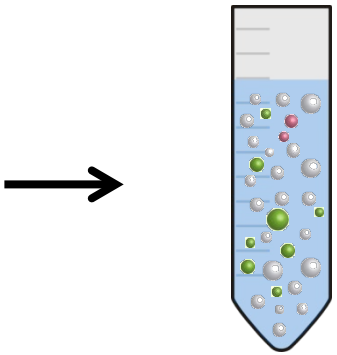
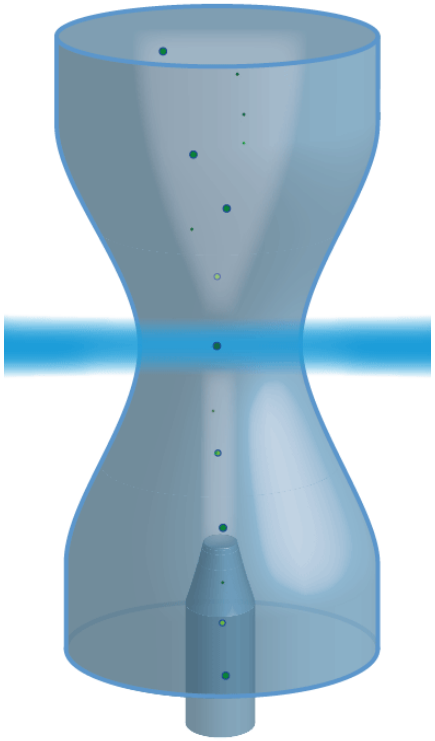

Extracellular vesicles (EV)
200 nm

EV-based “liquid biopsy”

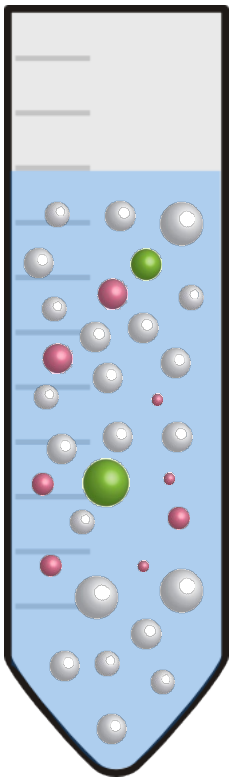




Hematology parameter	Concentration (vesicles mL^{-1})
Platelet vesicle count	$2.3 - 6.2 \cdot 10^9$
Erythrocyte vesicle count	$7.0 - 8.6 \cdot 10^{10}$
Reticulocyte vesicle count	$3.9 - 15.6 \cdot 10^8$
Leukocyte vesicle count	$6.2 - 16.4 \cdot 10^7$
Total vesicle count	$7.3 - 9.4 \cdot 10^{10}$

- nanoparticle flow cytometry can determine the concentration of an EV subpopulation

Problem 1: EV or similar-sized particle?

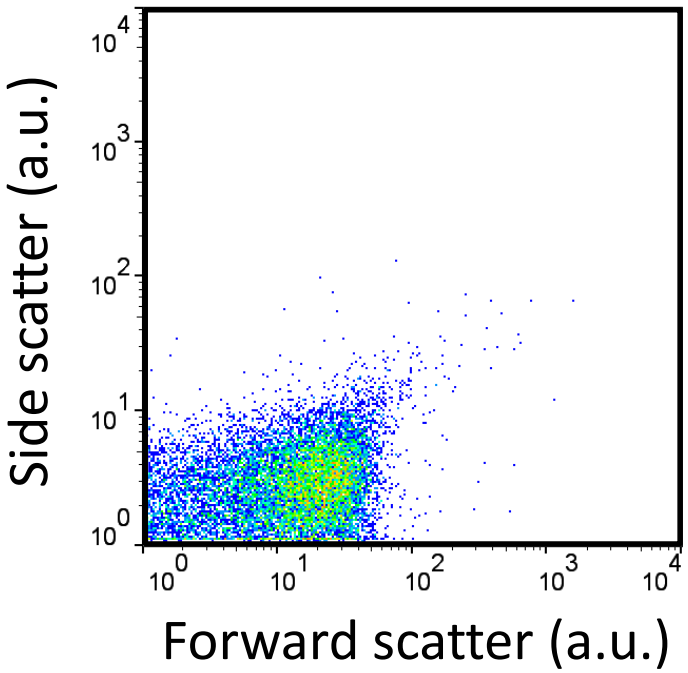


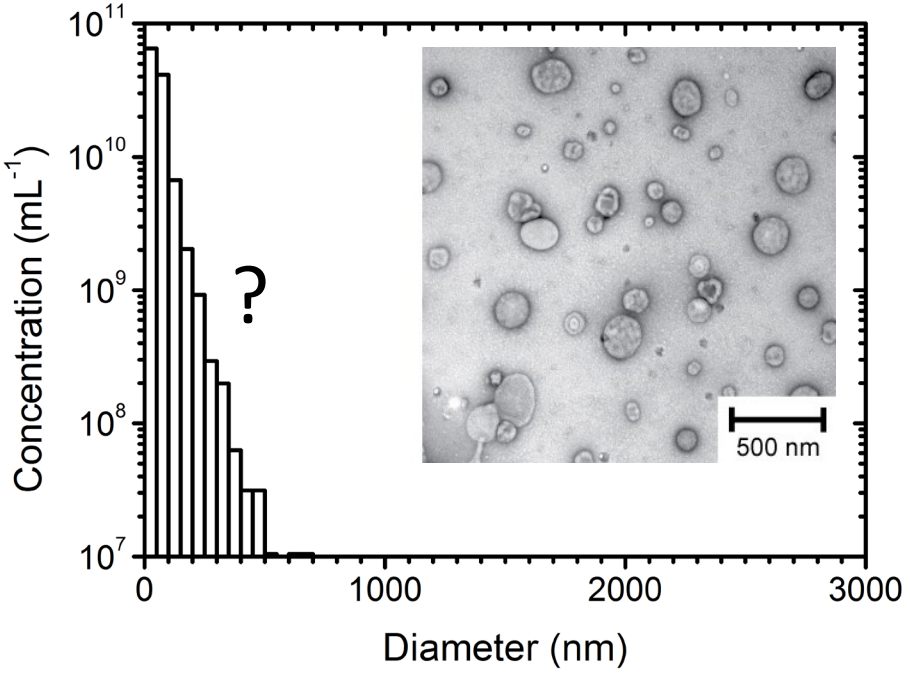
- extracellular vesicles ($n < 1.42$)*
- lipoproteins ($1.45 \leq n \leq 1.60$)
- protein aggregates ($1.53 \leq n \leq 1.60$)

* Konokhova *JBO* (2012), van der Pol *Nano Lett* (2014)

Problem 2: how to interpret data in a.u.?

same population of urine EV





BD FACSCalibur

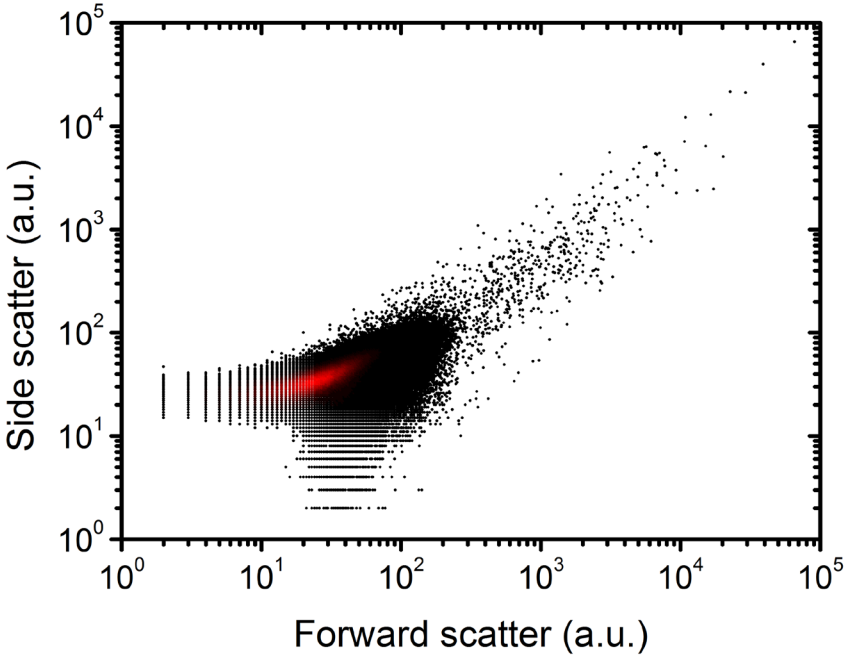
transmission electron microscopy

a.u.: arbitrary units

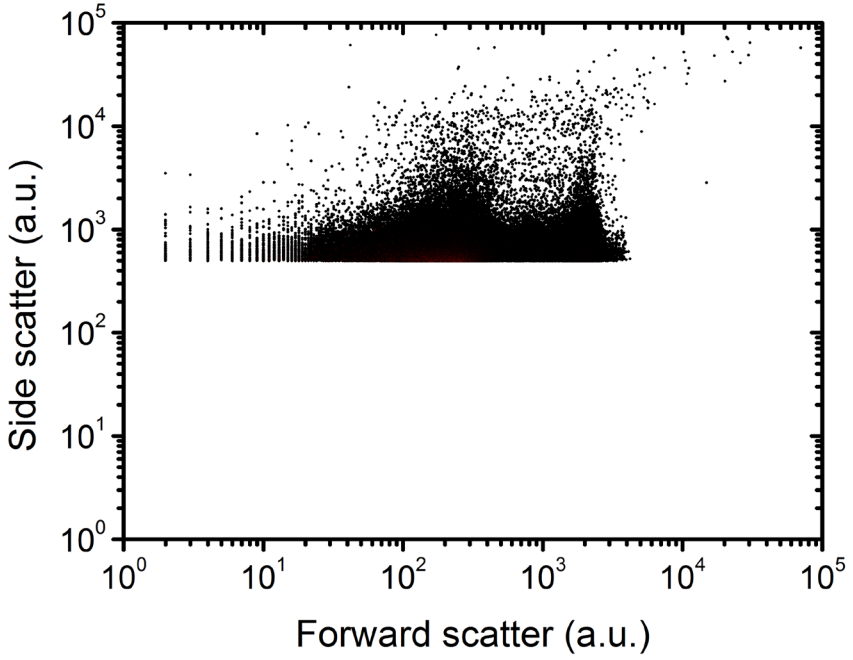
van der Pol et al. *J Thromb Haemost* (2012,2014)

Problem 3: how to compare data in a.u.?

same population of erythrocyte EV



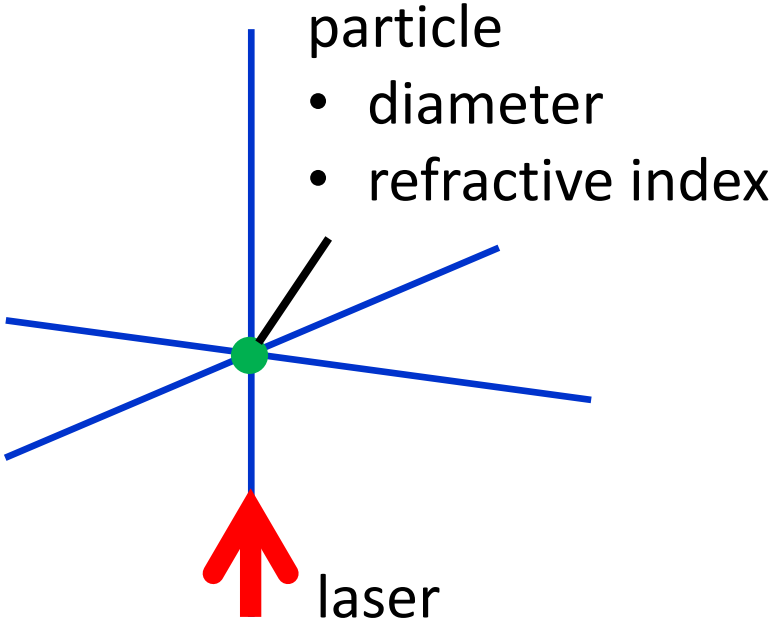
Apogee A50-micro



BD FACSCanto II

Goal

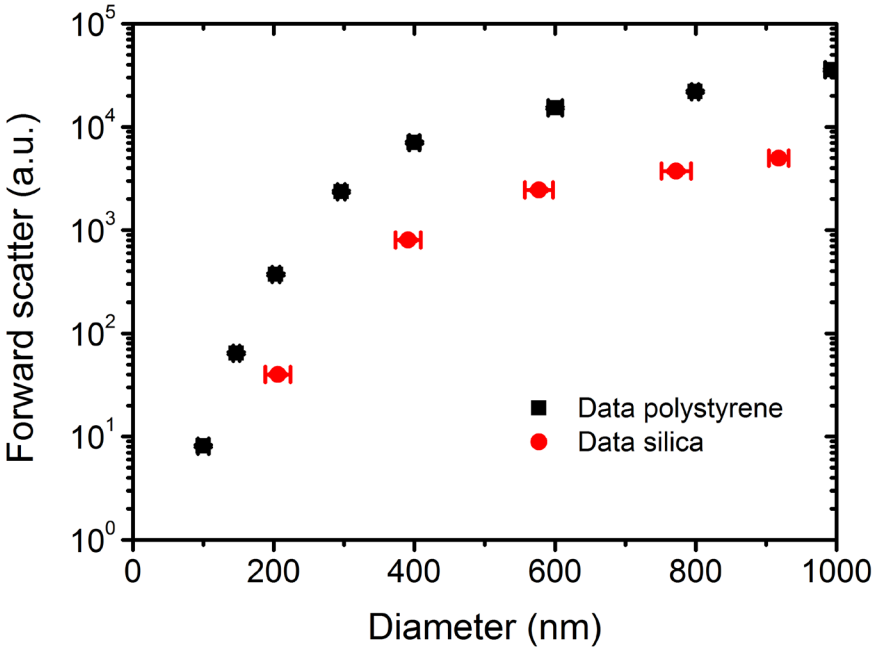
- obtain physical properties of particles from flow cytometry scatter signals

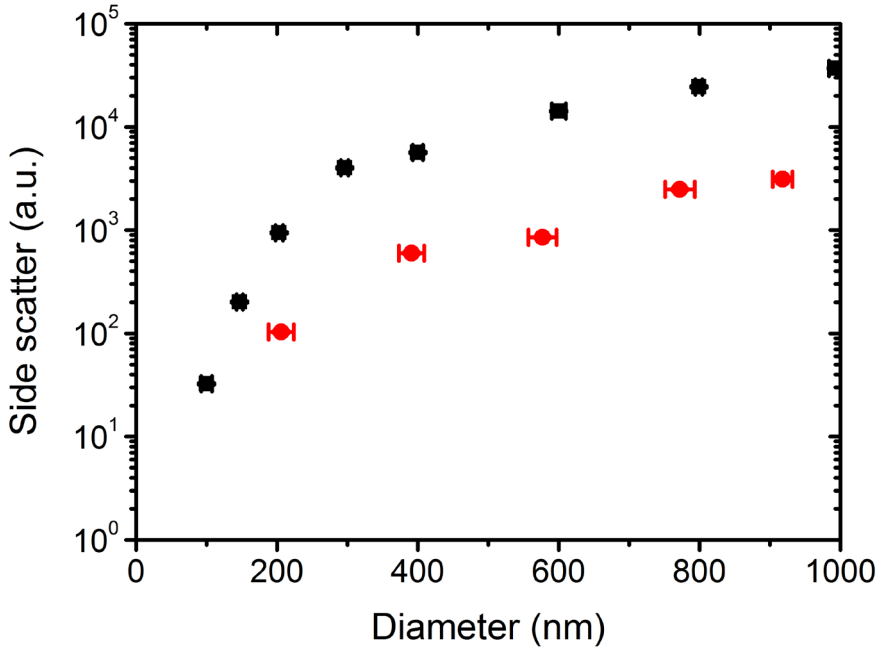


Approach

- calibrate instrument (Apogee A50-micro)
  - calibrate FSC and SSC
  - derive size from Flow Scatter Ratio (Flow-SR = SSC/FSC)
  - derive refractive index from size and FSC
- validate Flow-SR
  - beads mixture
  - oil emulsion
- apply Flow-SR
  - EV and lipoprotein particles from blood

Calibrate forward scatter and side scatter





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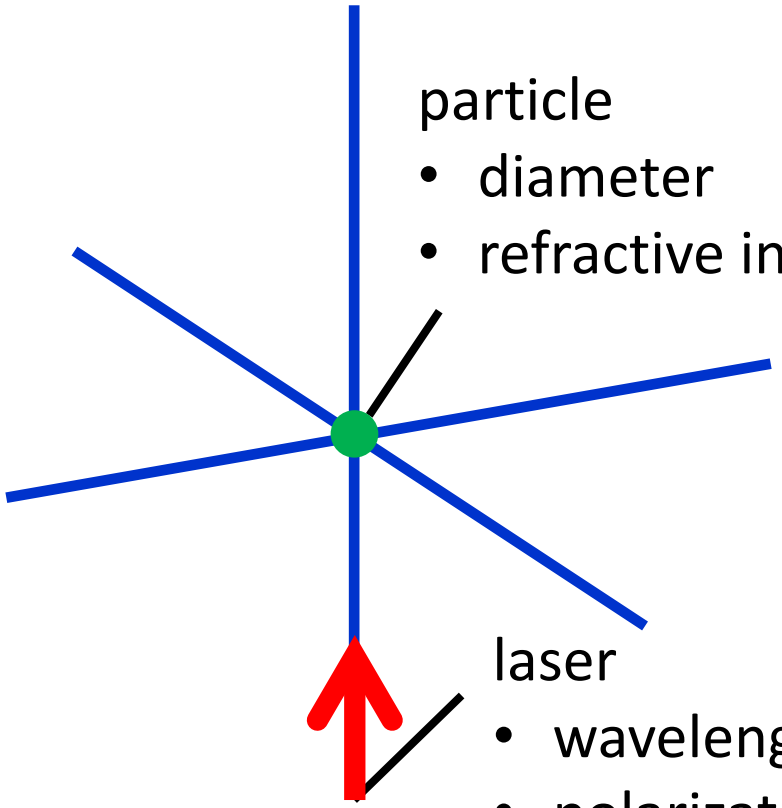
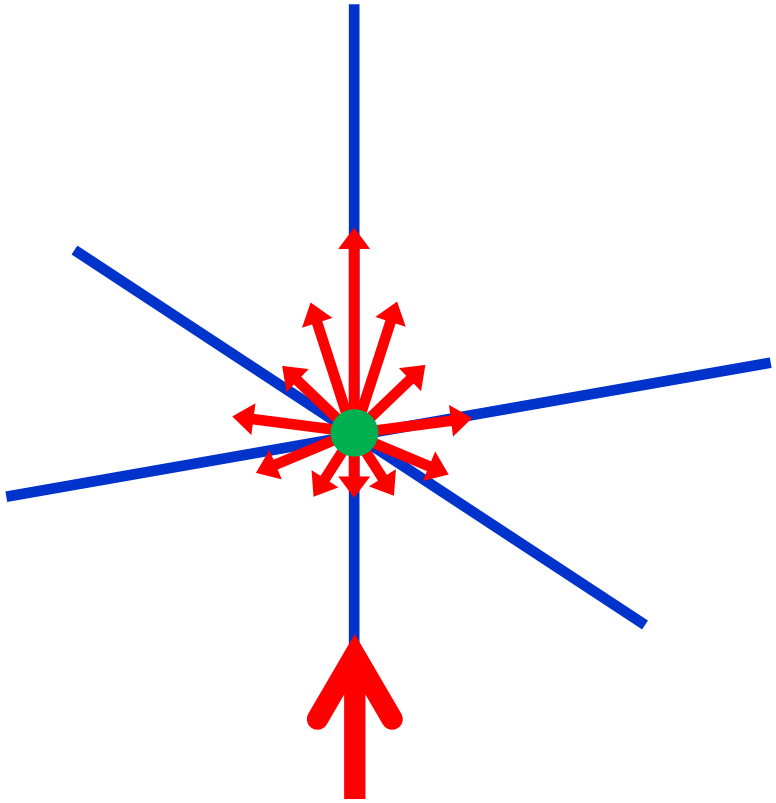


particle
- diameter
- refractive index

laser
- wavelength
- polarization
- power

Calibrate forward scatter and side scatter



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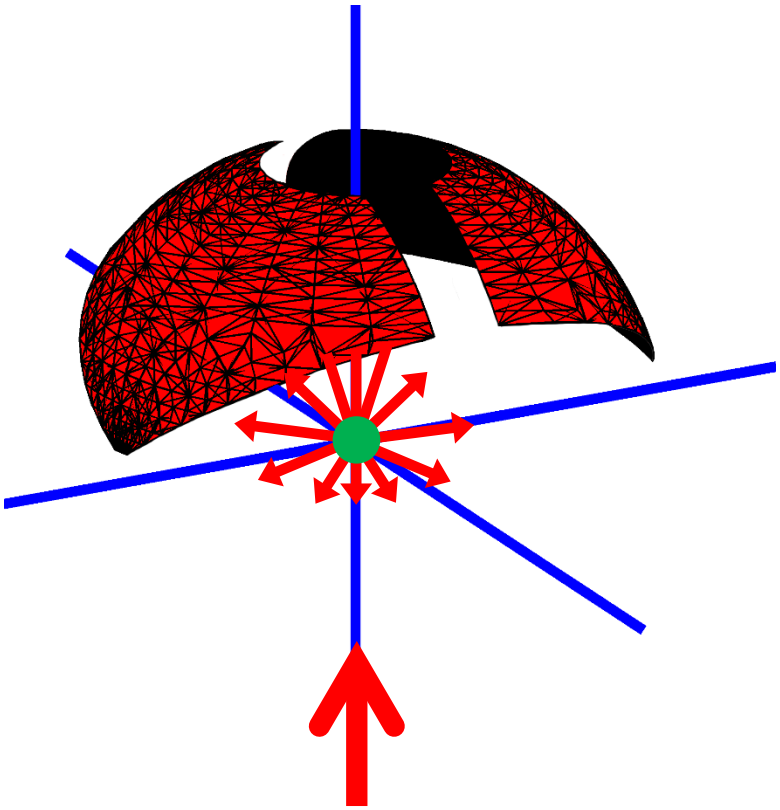
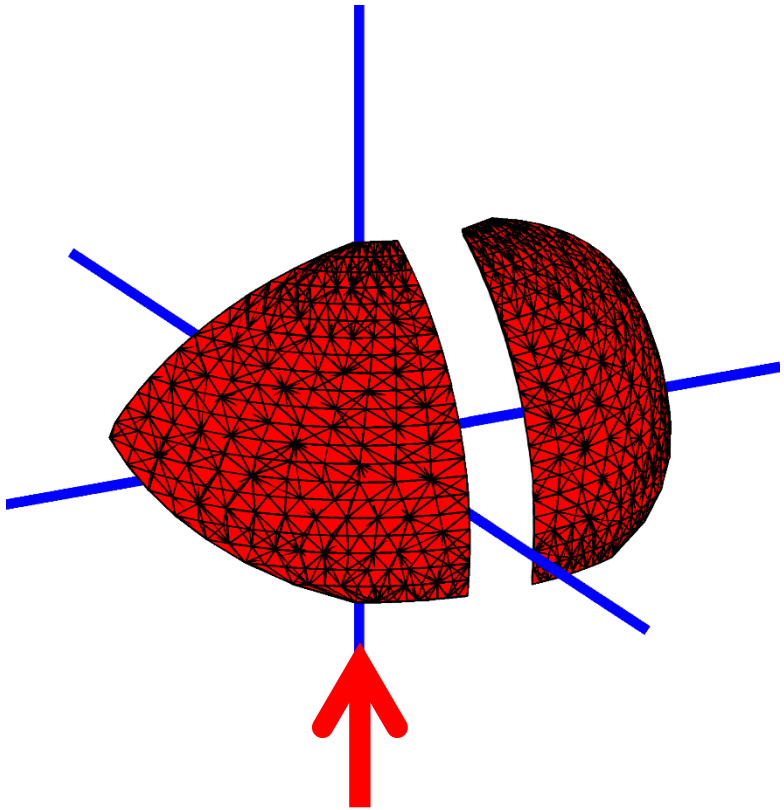
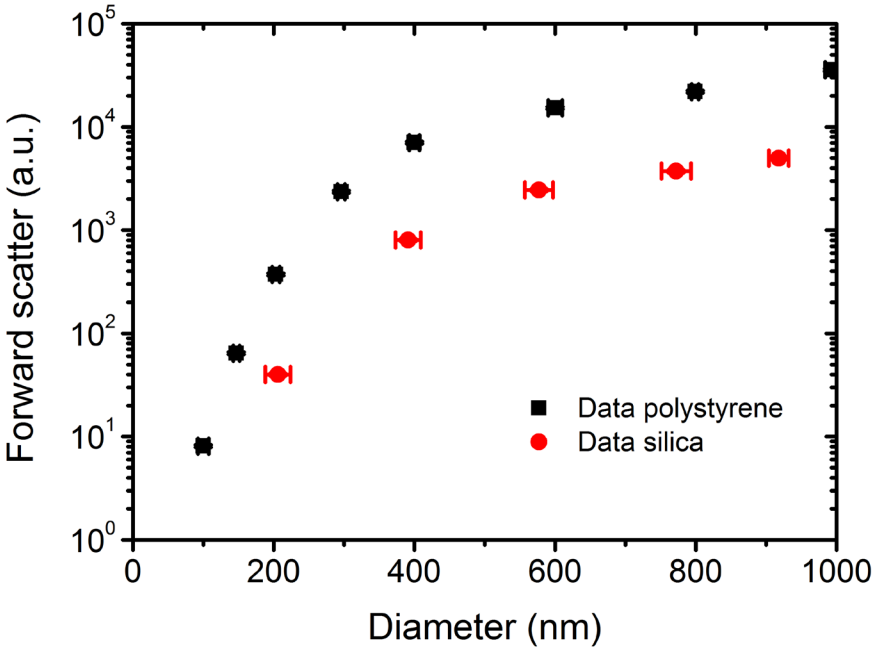


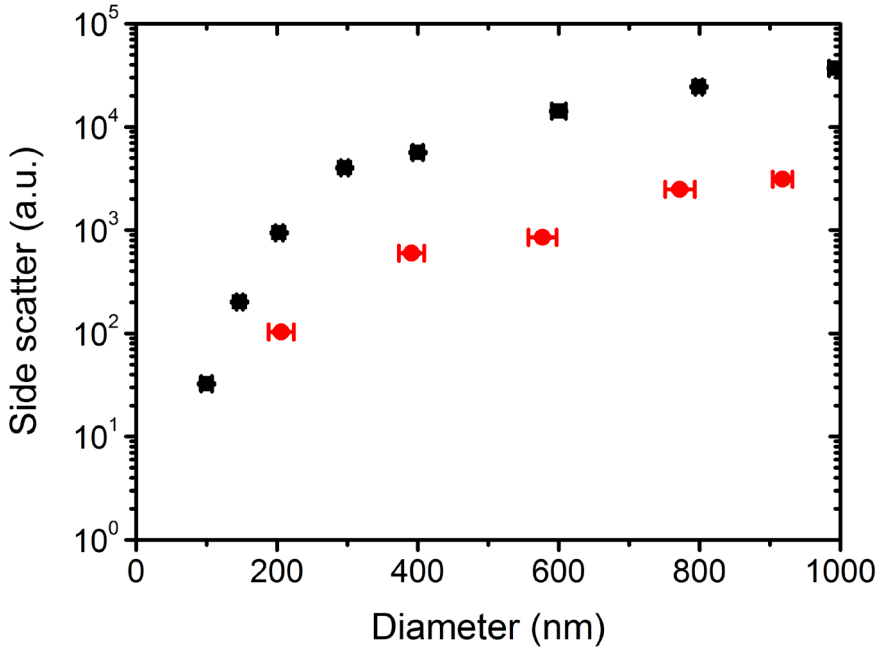
forward scatter



side scatter

Calibrate forward scatter and side scatter





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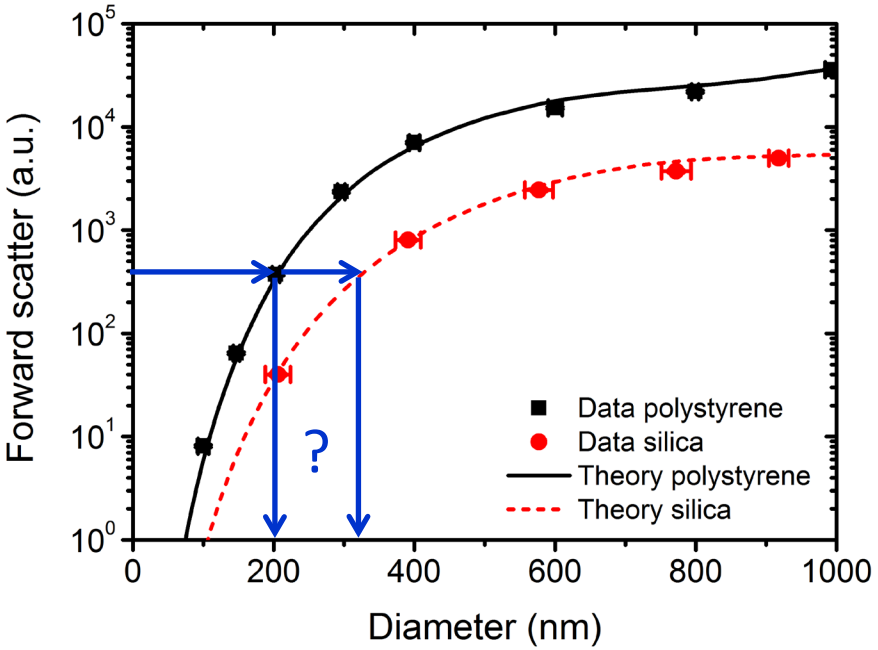
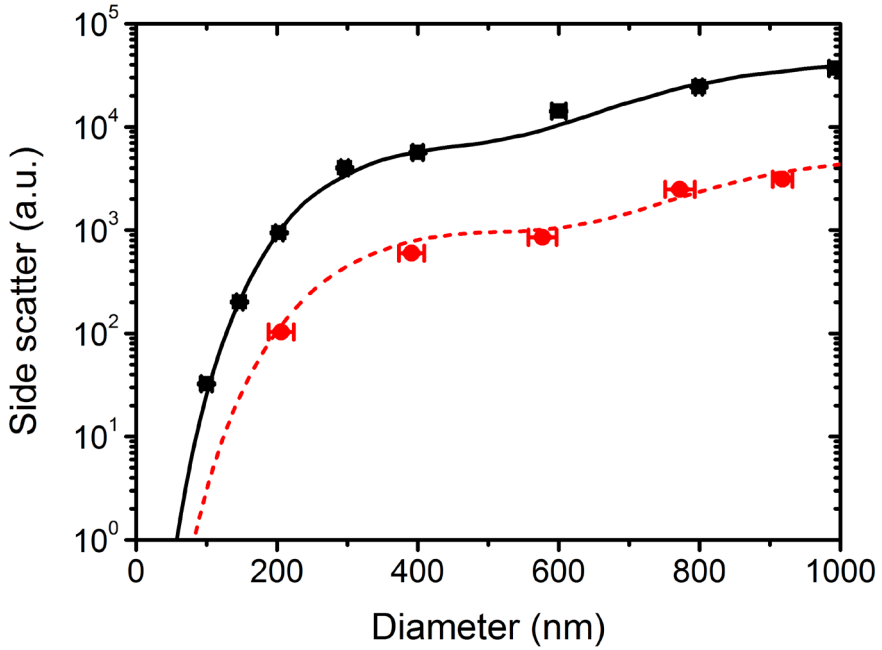
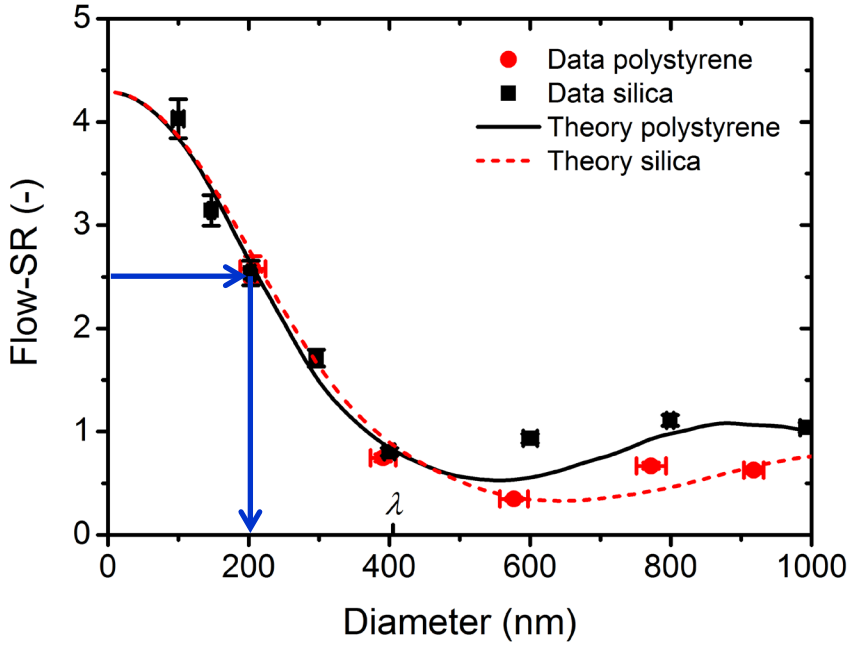




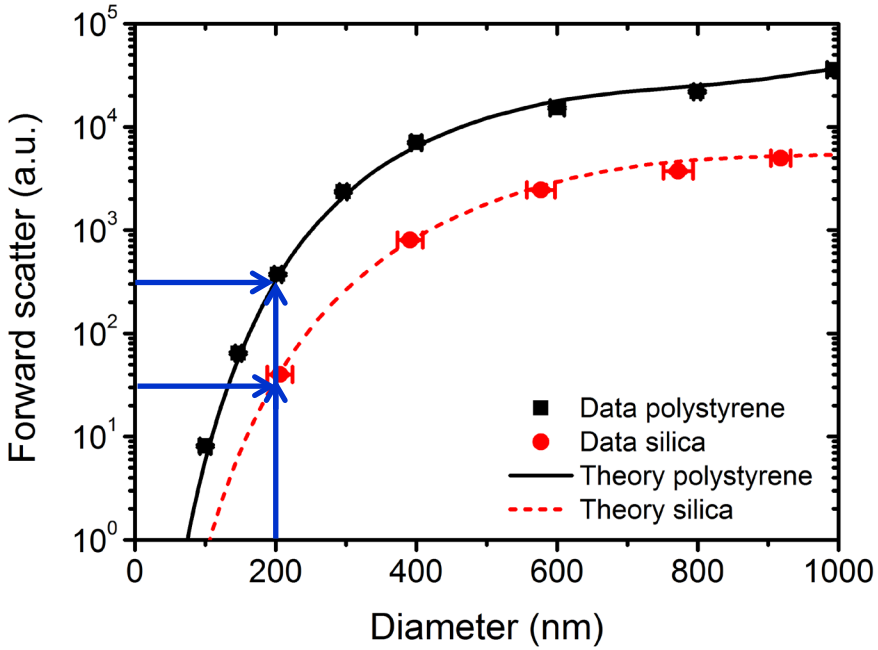
$$\text{Flow-SR} = \frac{\text{side scatter}}{\text{forward scatter}}$$

Derive size from Flow-SR



$$\text{Flow-SR} = \frac{\text{side scatter}}{\text{forward scatter}}$$

Derive refractive index from size and FSC



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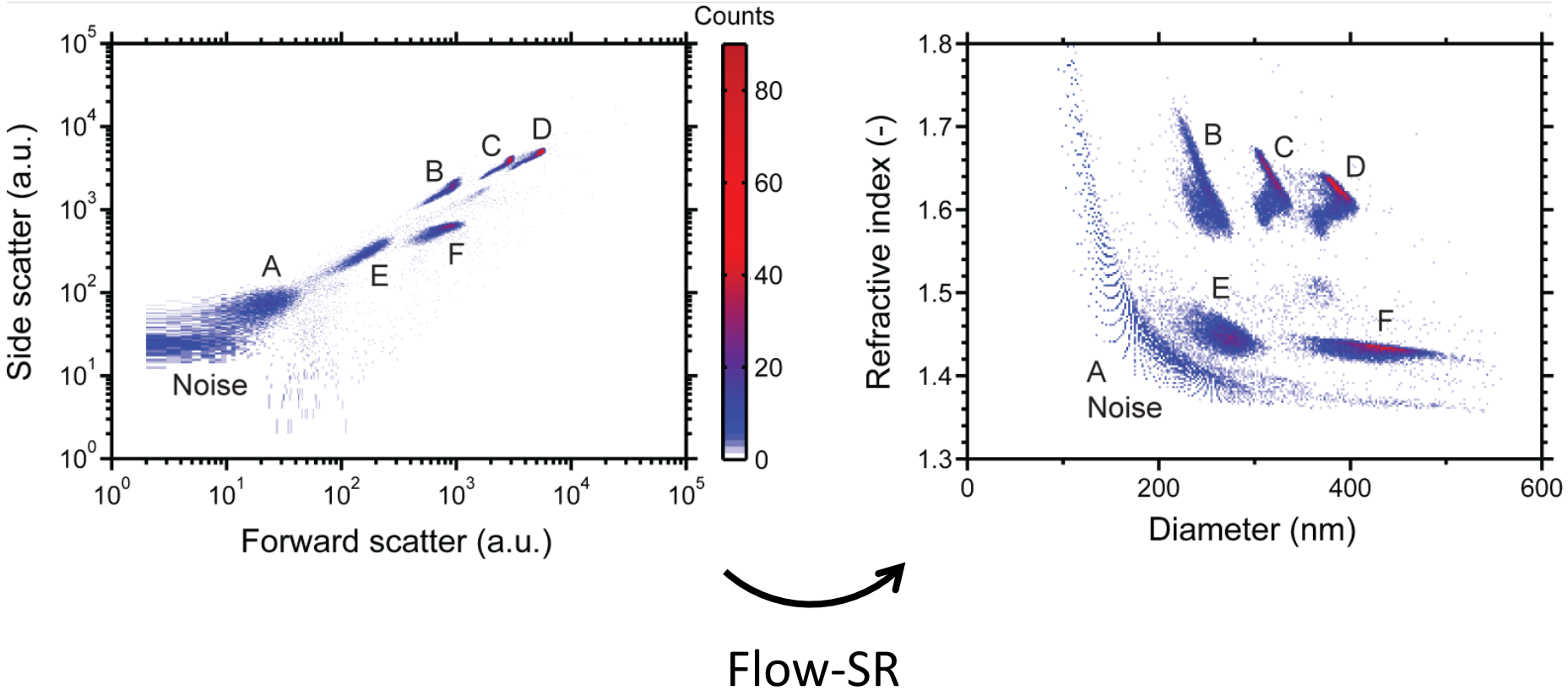
Validate Flow-SR with a beads mixture



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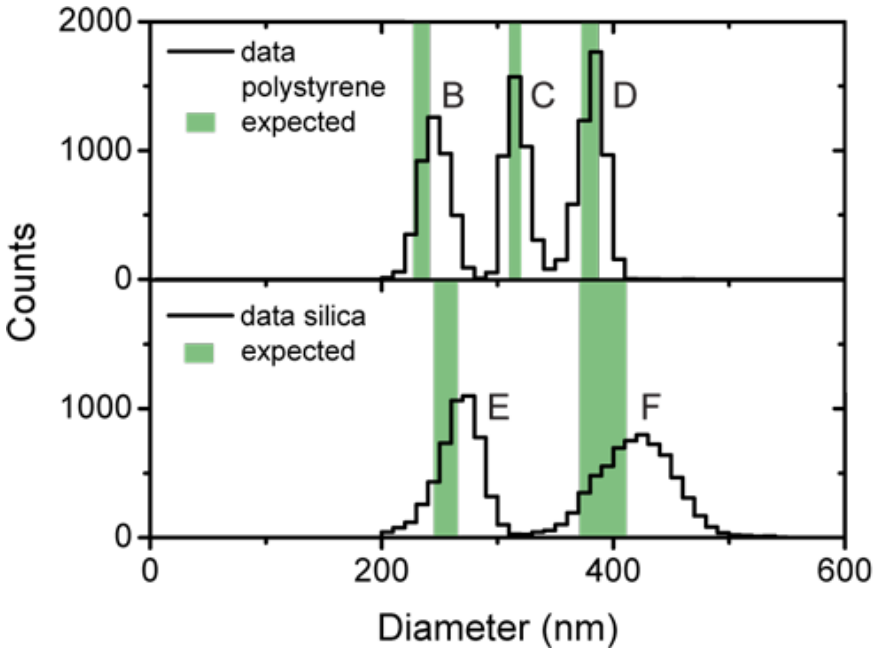
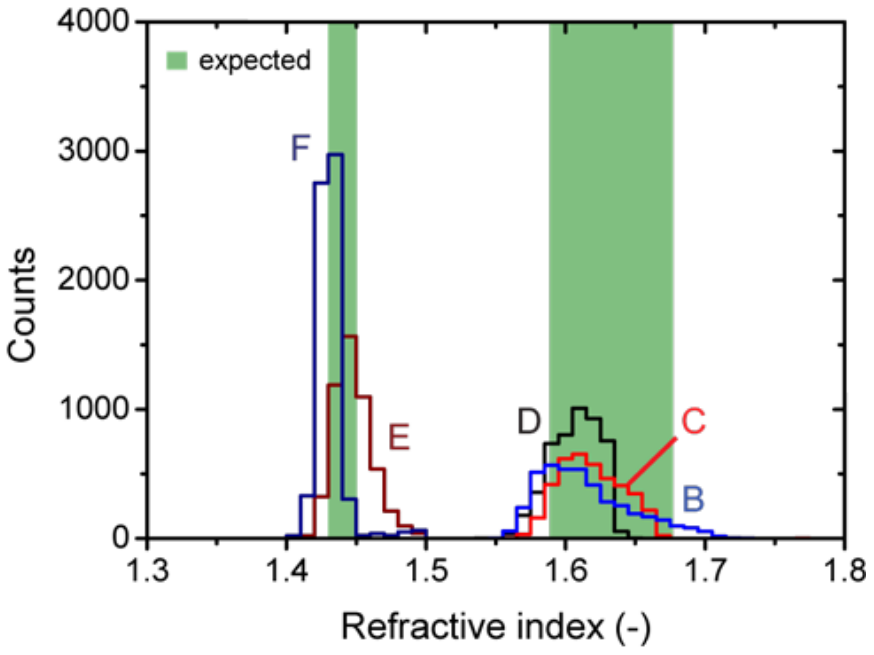


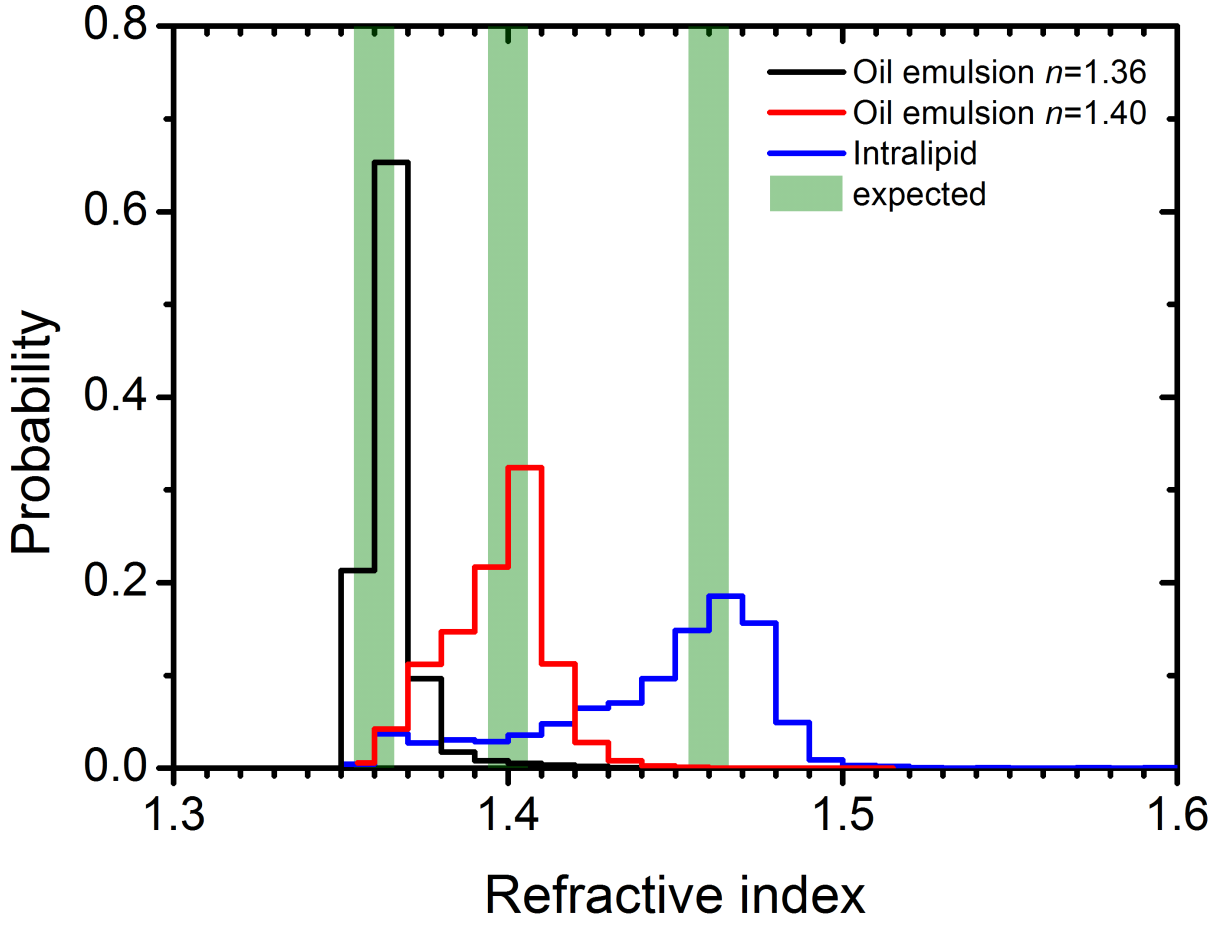


measurement error < 8%

CV < 8%

CV < 2%

Validate Flow-SR with oil emulsions



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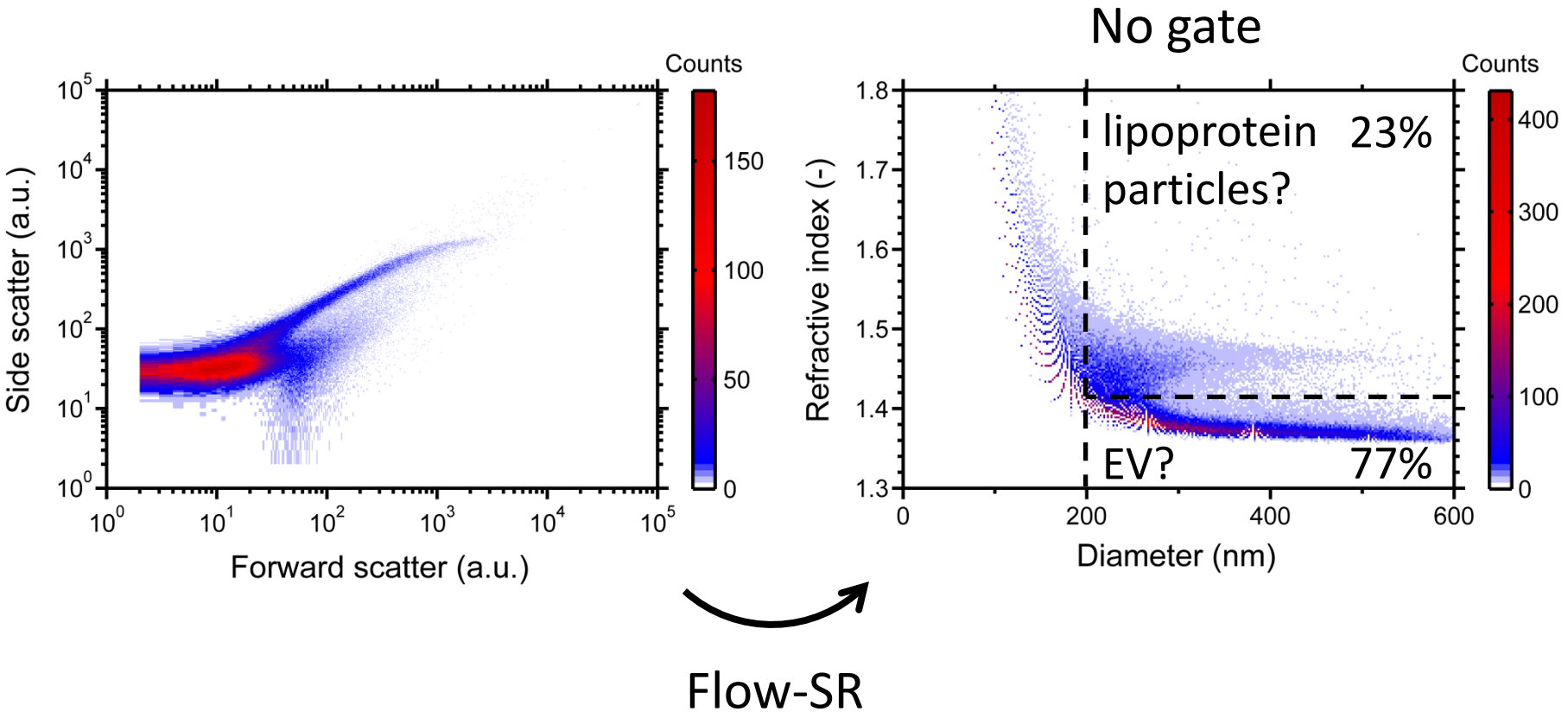
Supernatant of outdated platelet concentrate



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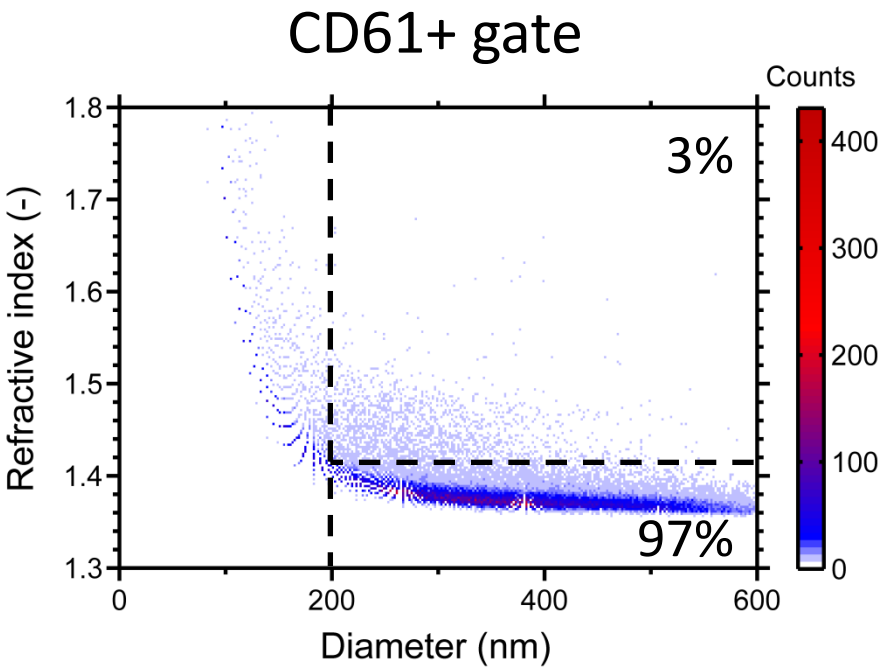
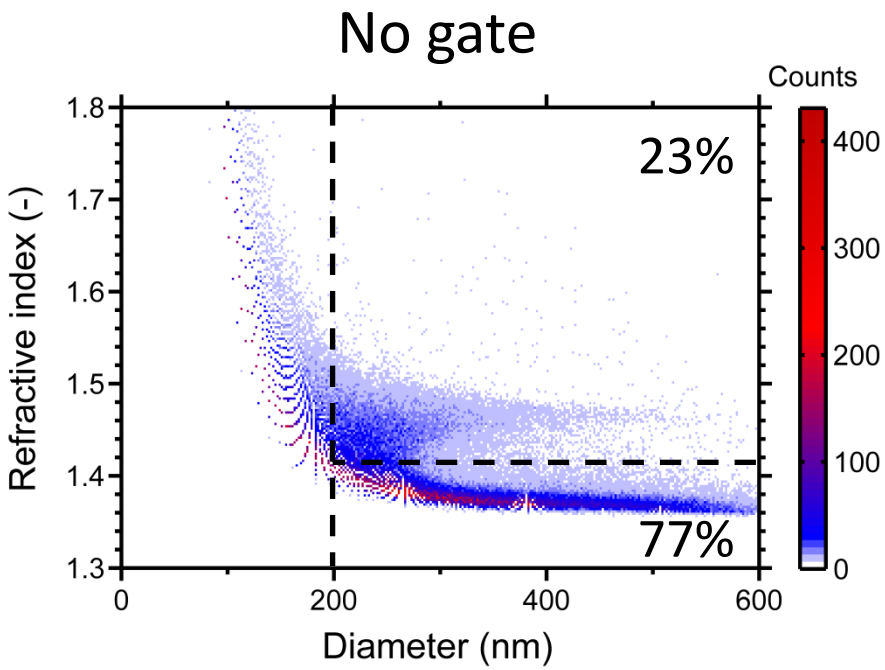
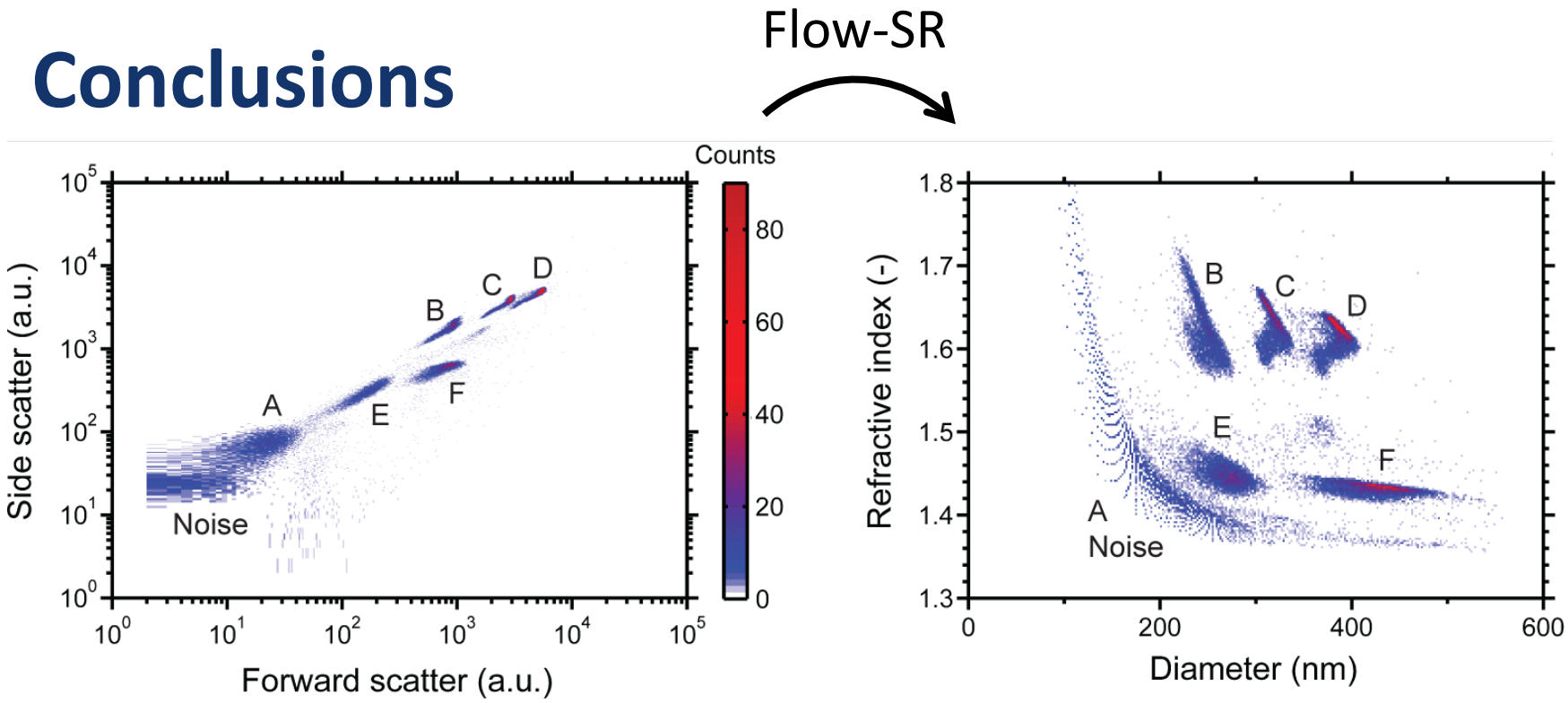




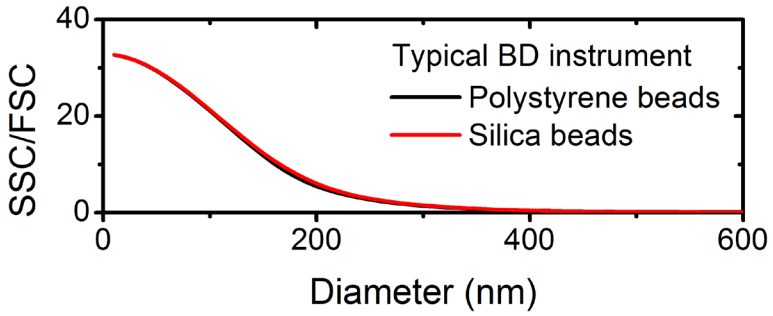
centrifuged 3-fold, 1550 × *g*, 20 min

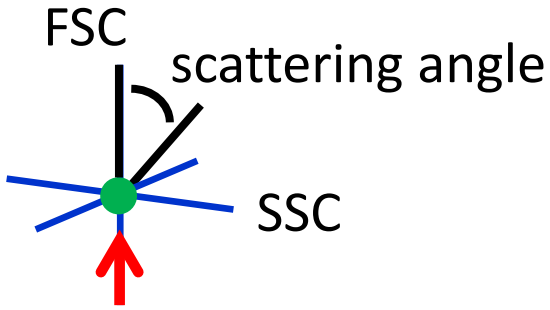
Conclusions



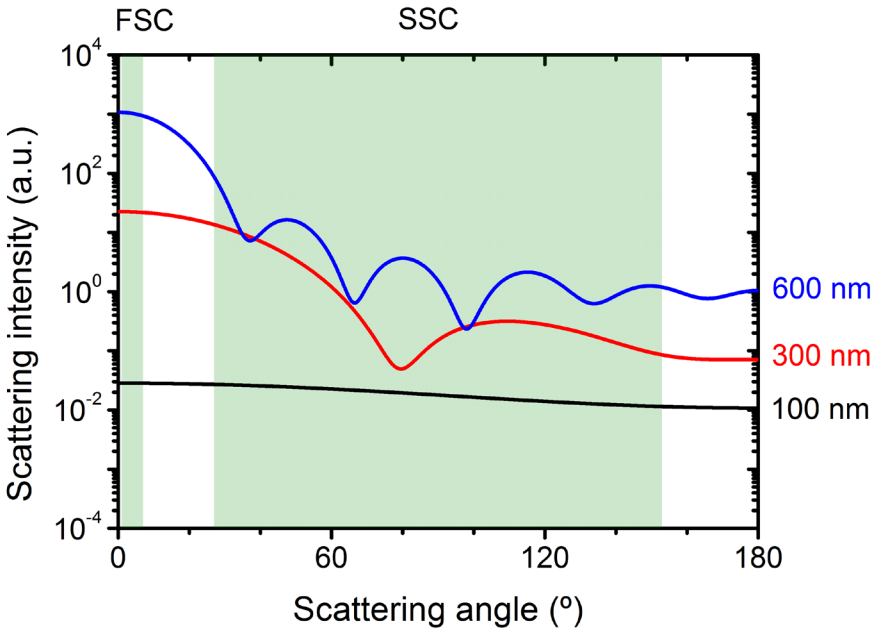
- Flow-SR enables size and refractive index determination of nanoparticles by flow cytometry
  - data interpretation and comparison
  - label-free identification

Discussion – Flow-SR principle and applicability

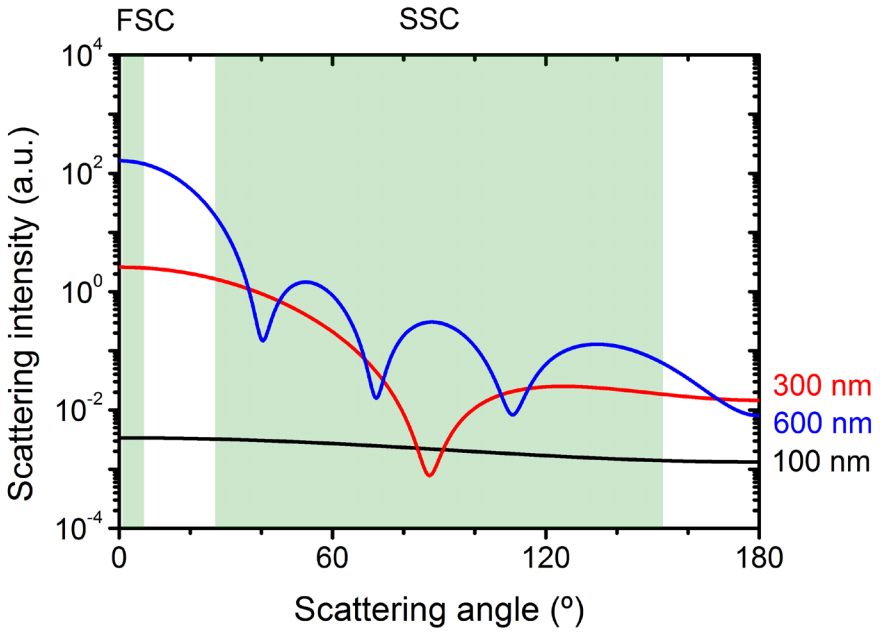




Polystyrene beads



Silica beads



Acknowledgements

- Vesicle Observation Center, Academic Medical Center, University of Amsterdam
  - Ton van Leeuwen
  - Rienk Nieuwland
  - Frank Coumans
  - Leonie de Rond

Vesicle Observation Center

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