

An overview of novel and conventional methods to detect microparticles and exosomes

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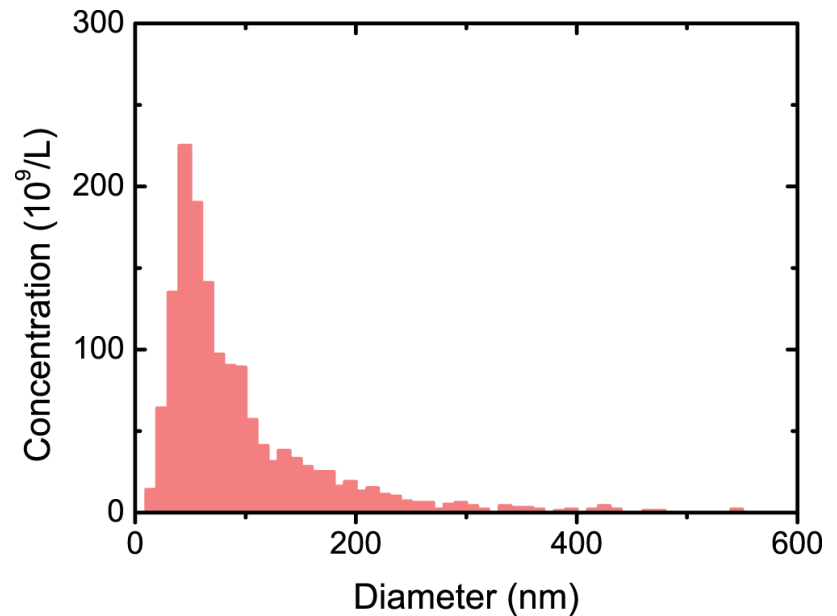
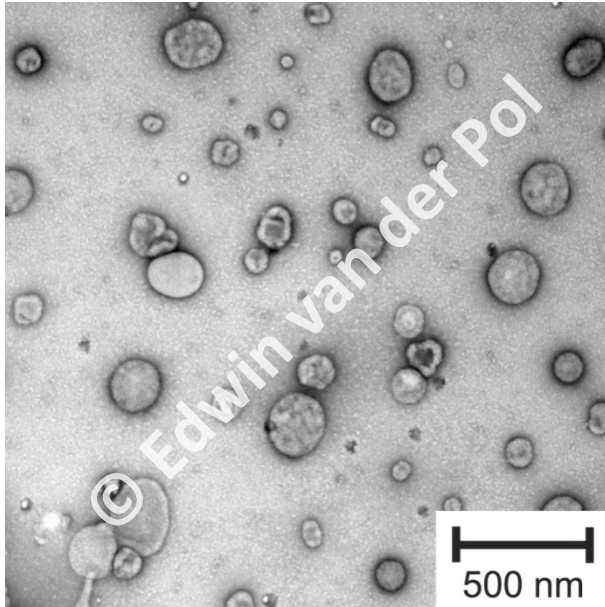
Academic Medical Center (AMC)

University of Amsterdam (UvA)

Laboratory Experimental Clinical Chemistry (Rienk Nieuwland)

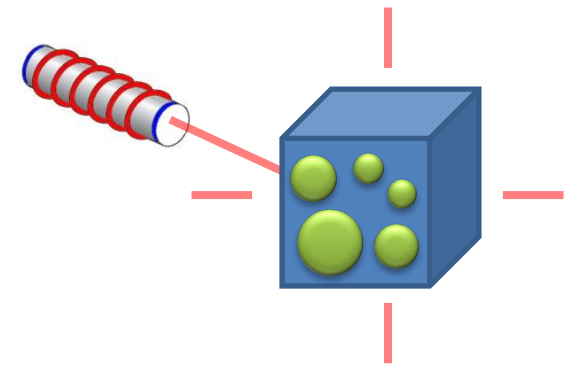
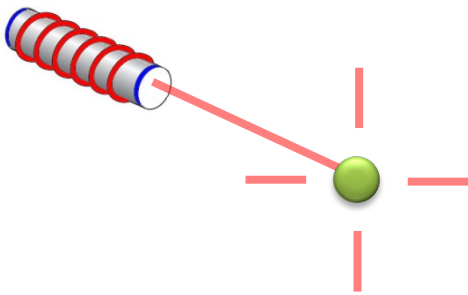
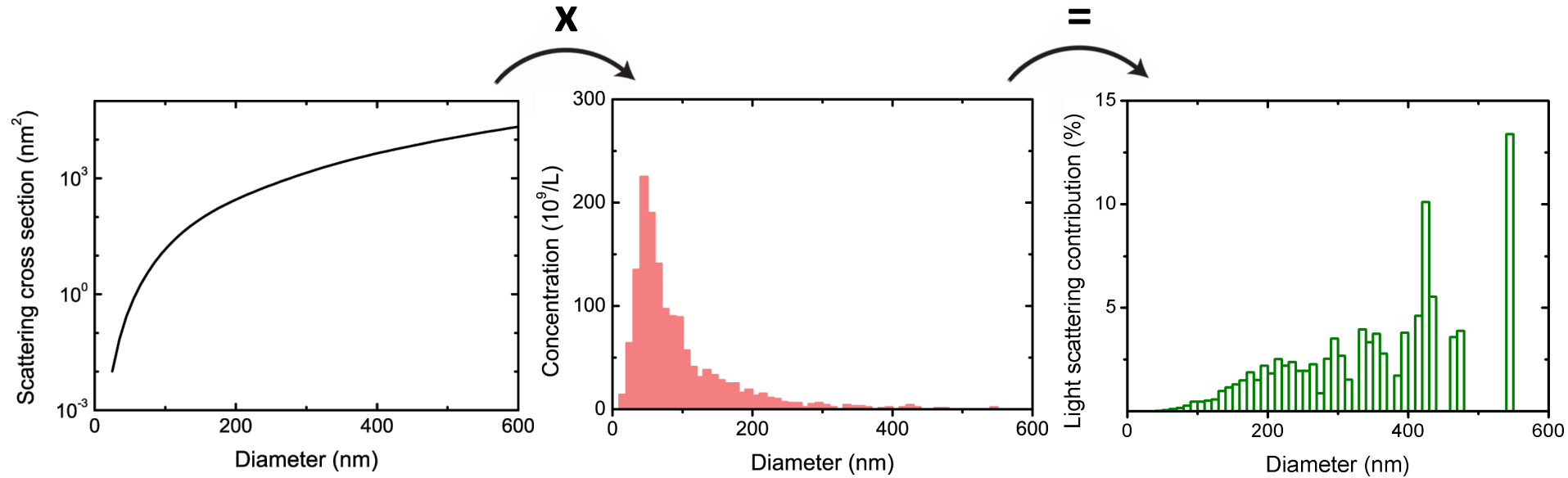
Biomedical Engineering & Physics (Ton van Leeuwen)

Introduction



- body fluids contain cell-derived vesicles
- clinically relevant information
- problem: vesicle detection

Optical detection: light scattering



Outline

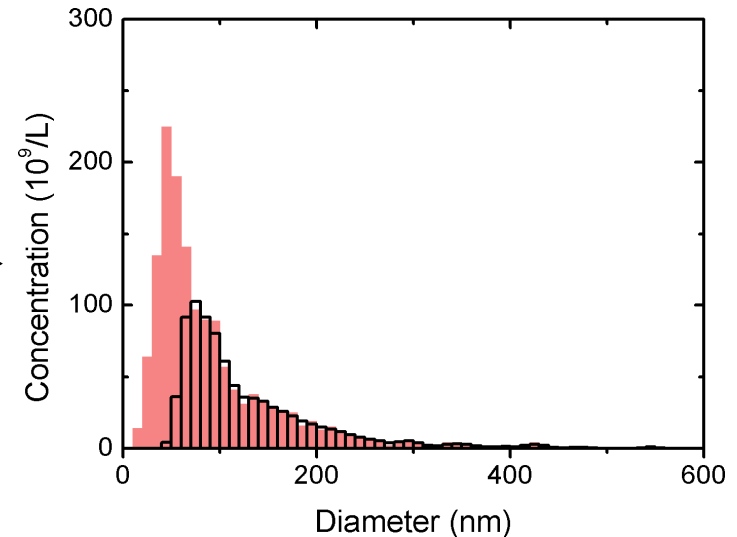
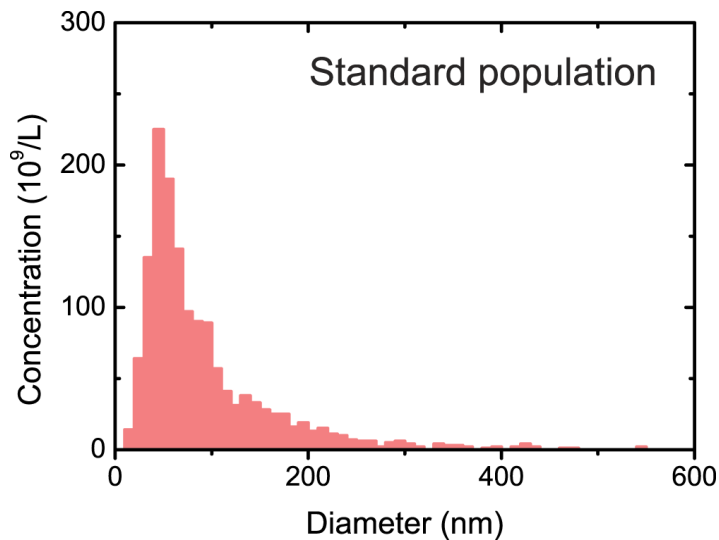
- exploration of detection methods
 - Flow cytometry (FACS)
 - Dynamic Light Scattering (DLS)
 - Nanoparticle Tracking Analysis (NTA)
 - Atomic Force Microscopy (AFM)
 - Impedance-based flow cytometry
- future developments
- conclusions

Approach: estimate capabilities of methods considering well-known limitations

Example 1

Detection limit: **50 nm**

Size resolvability: **20 nm**

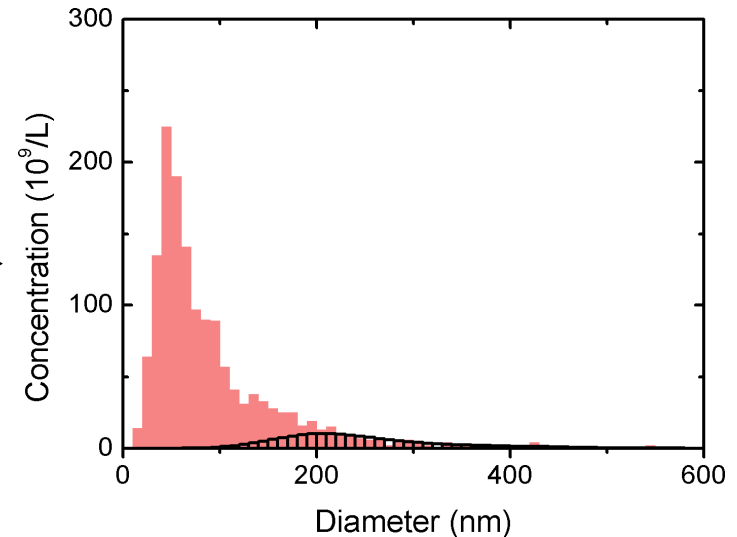
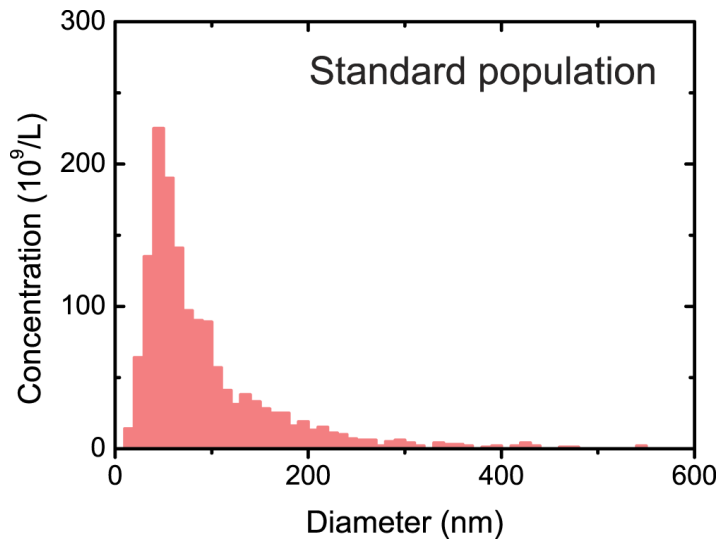


Approach: estimate capabilities of methods considering well-known limitations

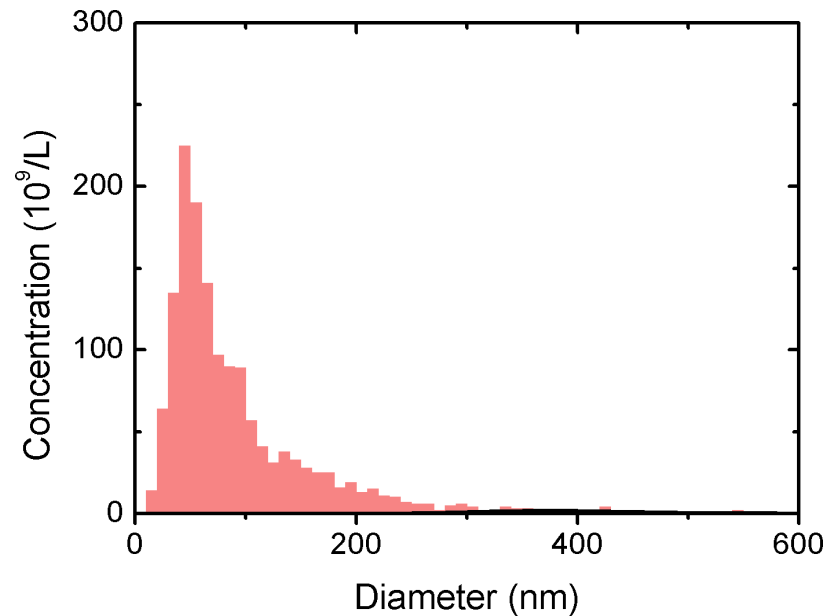
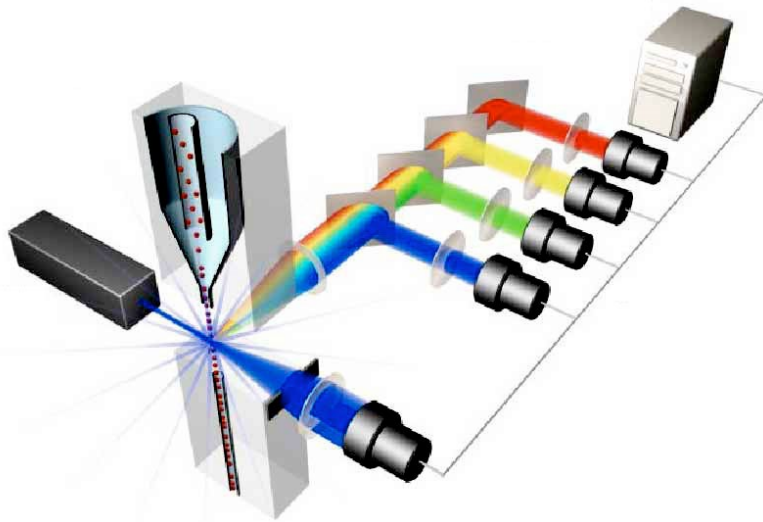
Example 2

Detection limit: **150 nm**

Size resolvability: **100 nm**

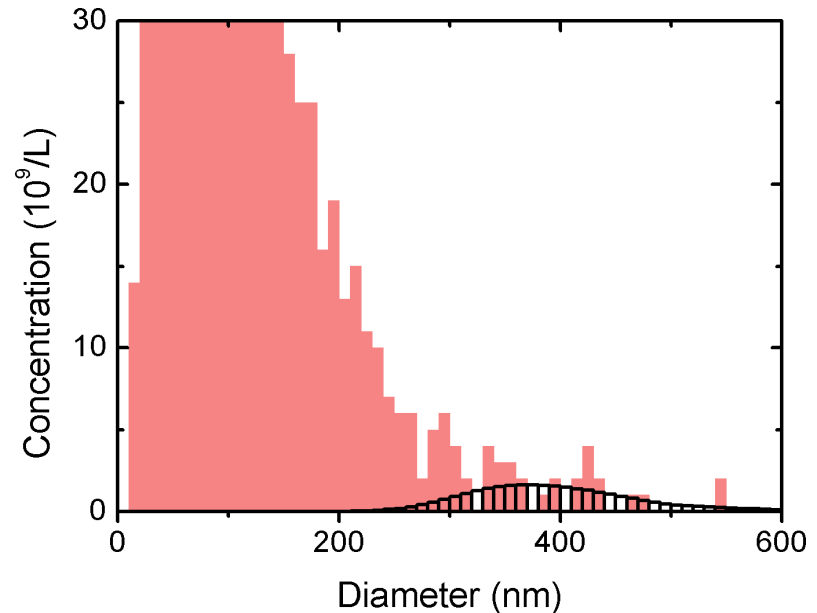
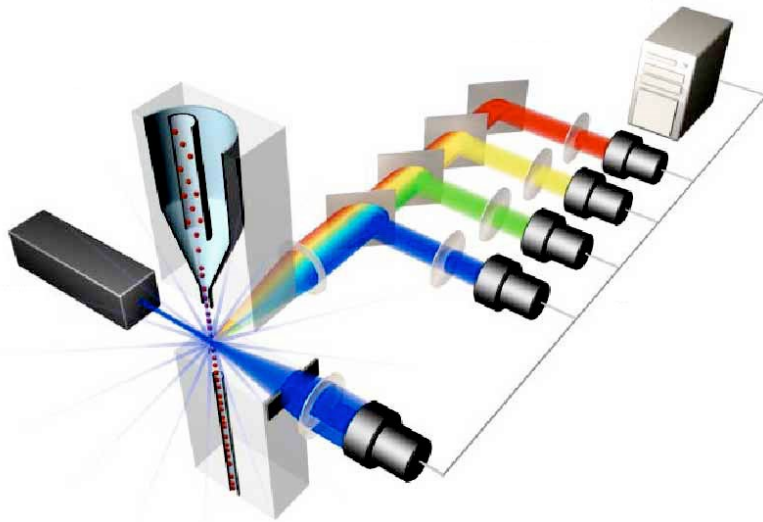


Flow cytometry (FACS)



- developed for cell detection ($>1 \mu\text{m}$)
- smallest detectable polystyrene bead ($n=1.6$): $\sim 300 \text{ nm}^{1,2}$
- detection efficiency of vesicles ($n \approx 1.4$) by FACS: $< 2\%$

Flow cytometry (FACS)

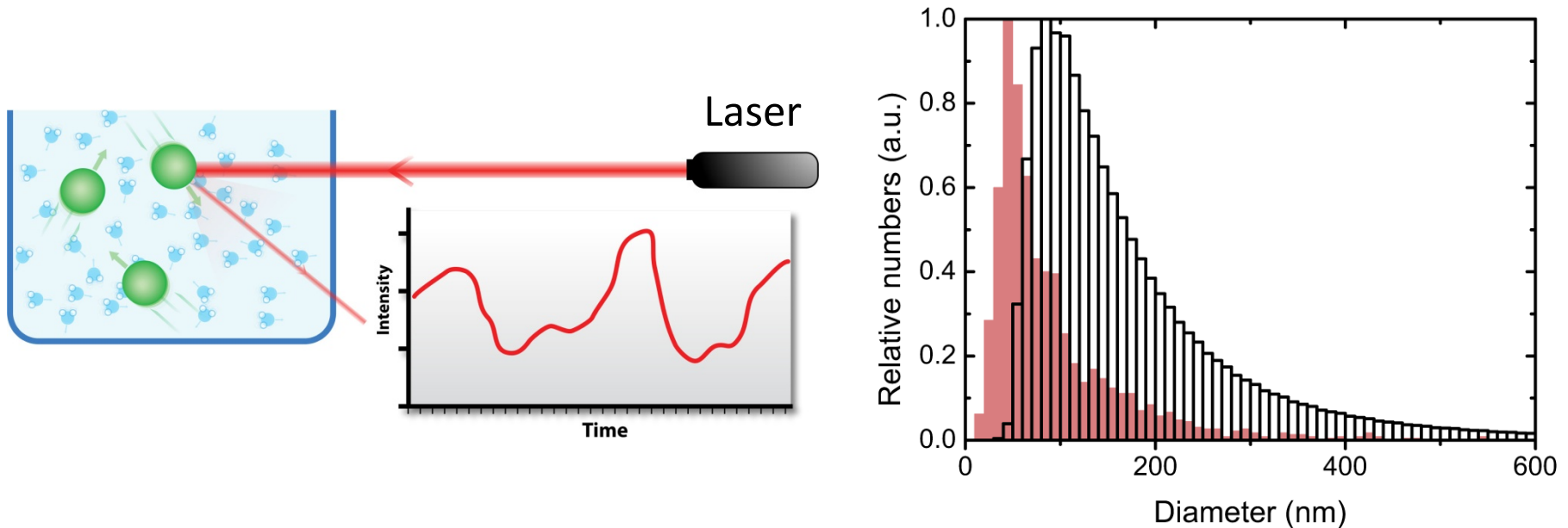


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1. Robert et al. *JTH* 2009

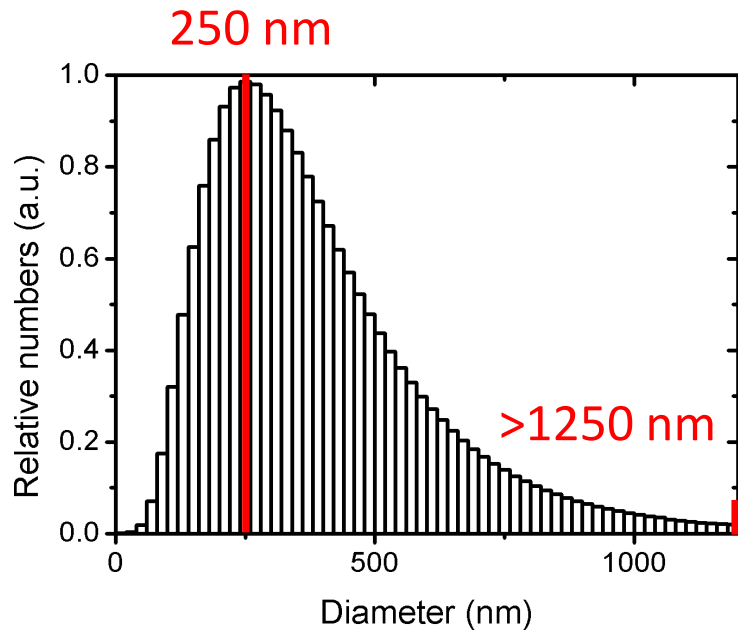
2. Perez-Pujol et al. *Cytom. Part. A* 2007

Dynamic Light Scattering (DLS)

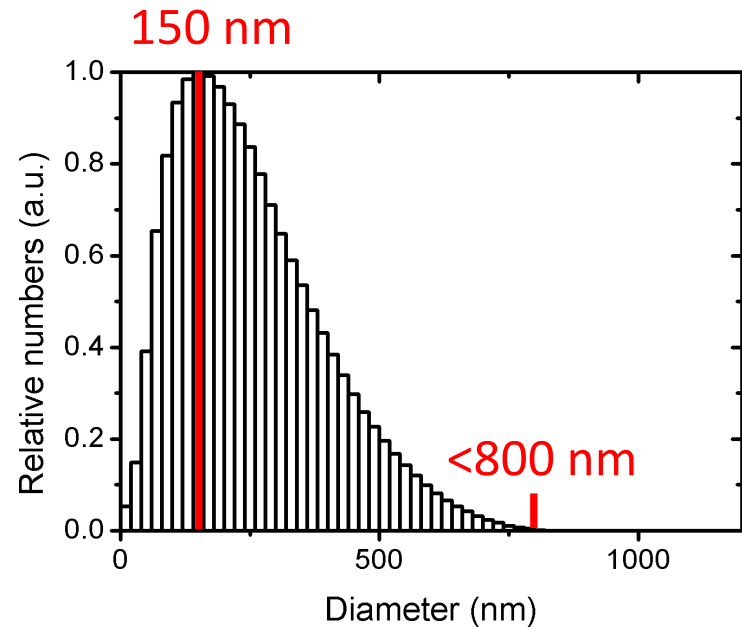


- Brownian motion depends on vesicle diameter
- determines mean *size* of vesicles in fluids
- difficulty with polydisperse samples
- result strongly depends on mathematical algorithm

DLS applied to vesicles



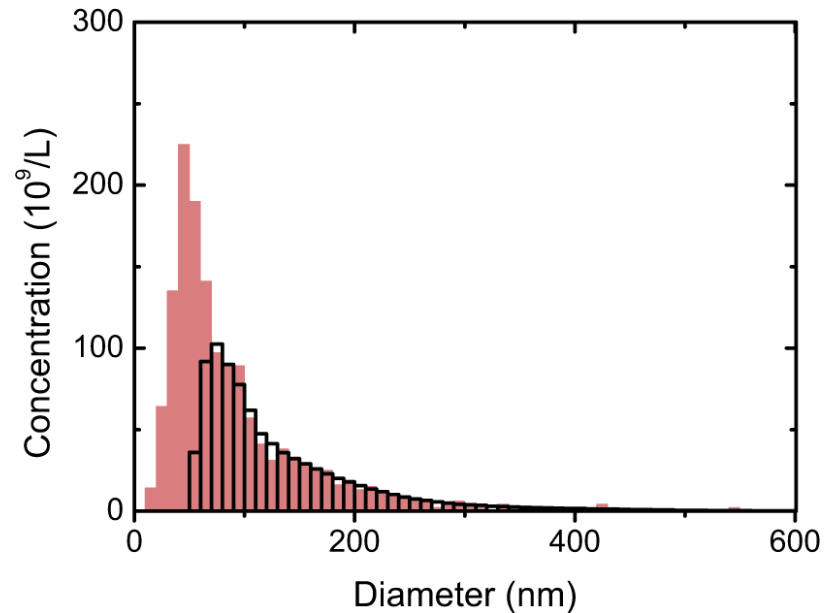
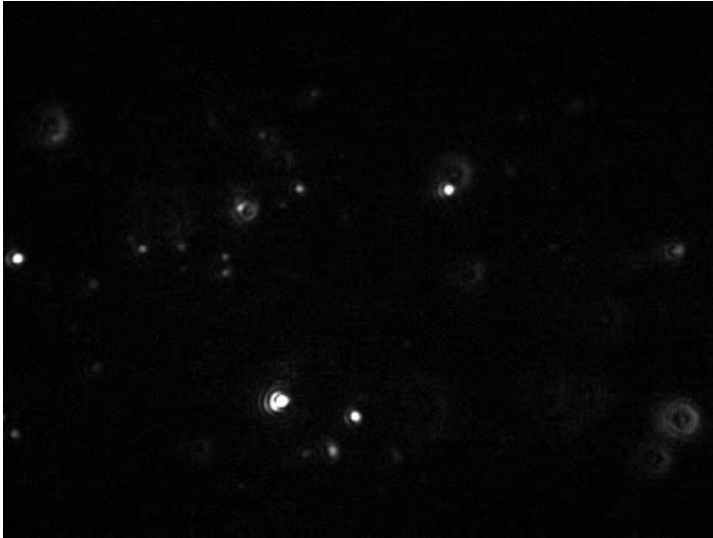
N5 Submicron Particle Size
Analyser (Beckman Coulter)³



Zetasizer Nano S
(Malvern Instruments Ltd)³

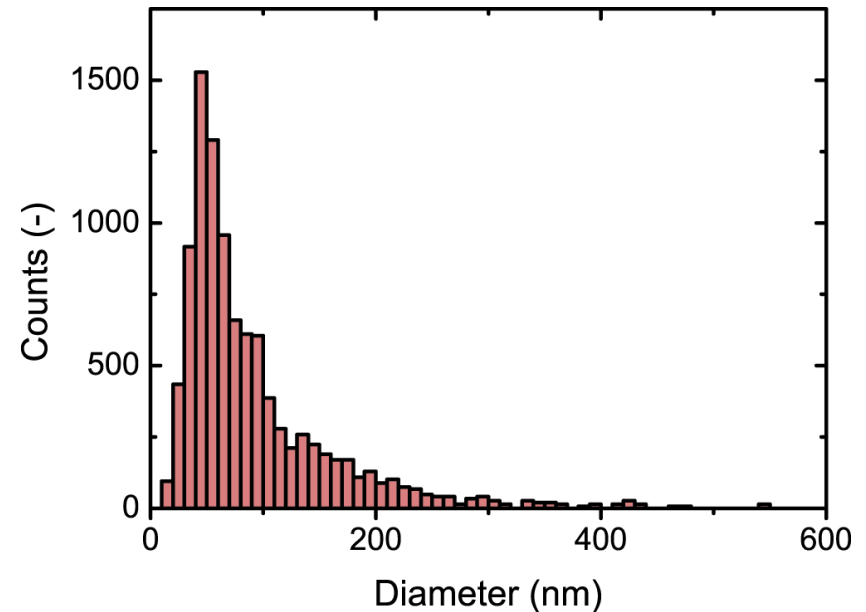
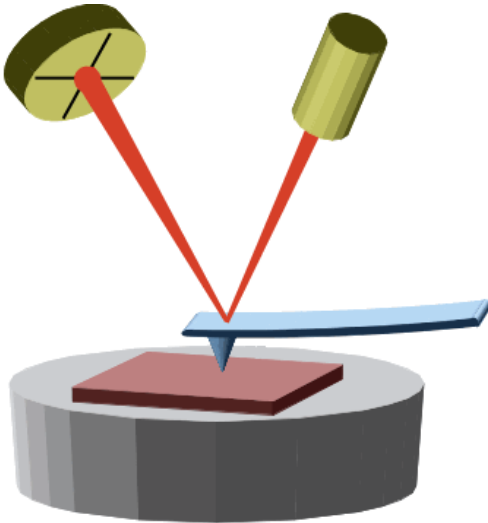
- results are system dependent
- no determination of absolute *size* and *concentration*

Nanoparticle Tracking Analysis (NTA)



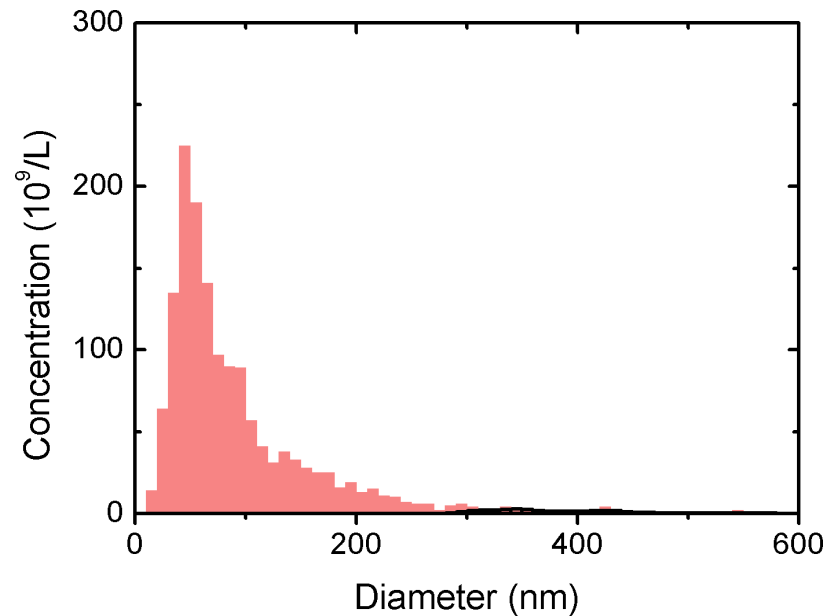
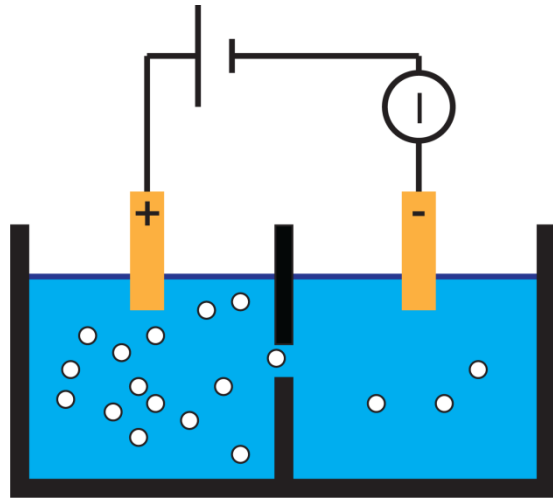
- determines *size* and *concentration* of vesicles in fluids⁴
- present detection limit: ~50 nm for vesicles
- can potentially be extended with fluorescence detection

Non optical methods: Atomic Force Microscopy (AFM)



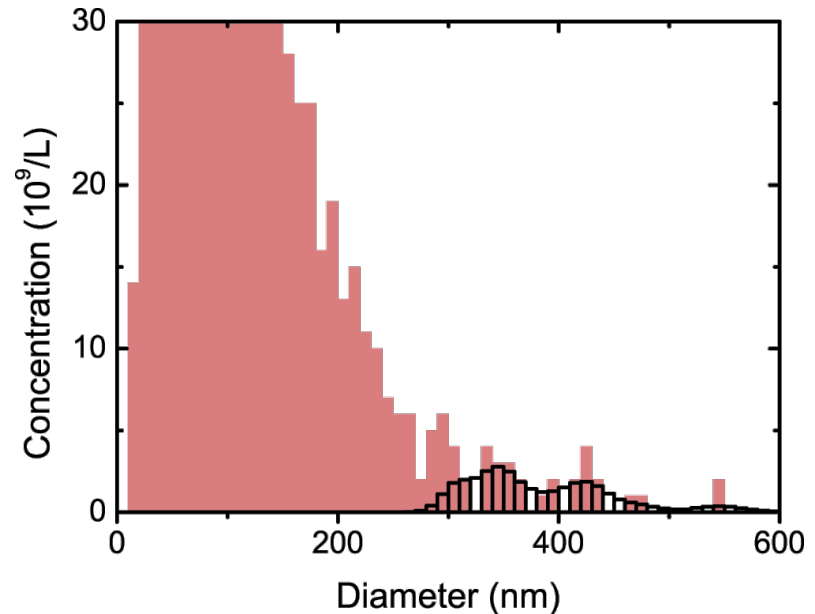
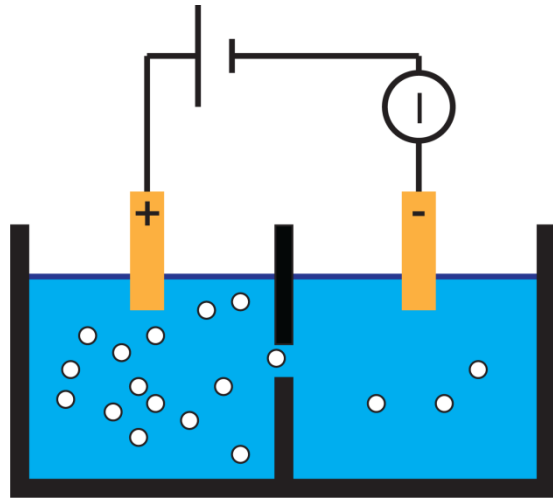
- provides information on *size, concentration, biochemical composition, and cellular origin*⁵
- binding efficiency and influence of binding on vesicle deformation unknown

Impedance-based flow cytometry



- determines *size* and *concentration* of vesicles
- present detection limit: $\sim 300 \text{ nm}^6$
- can be combined with flow cytometry

Impedance-based flow cytometry



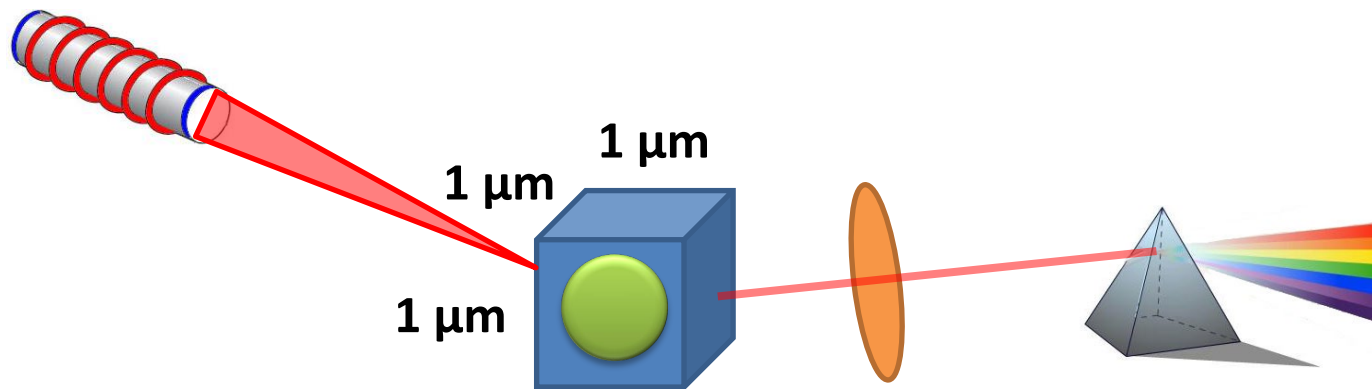
- determines *size* and *concentration* of vesicles
- present detection limit: ~300 nm⁶
- can be combined with flow cytometry

Overview

Method	Size	Concentration	Biochemical information	Measurement time
Transmission Electron Microscopy (TEM)	✓	✗	😐	hours
Flow cytometry (FACS)	✗	😐	✓	seconds
Dynamic Light Scattering (DLS)	😐	✗	✗	minutes
Nanoparticle Tracking Analysis (NTA)	😐	😐	to be investigated	minutes
Atomic Force Microscopy (AFM)	✓	😐	✓	hours
Impedance-based flow cytometry	✗	😐	✗	seconds

Future developments

- Raman microspectroscopy⁷:
 - determine size, concentration, and chemical composition of vesicles in fluids *label-free*



Conclusions

- vesicle detection remains challenging
- applications of novel and conventional methods requires further investigation