An overview of Novel and Conventional Methods to Detect Microparticles and Exosomes

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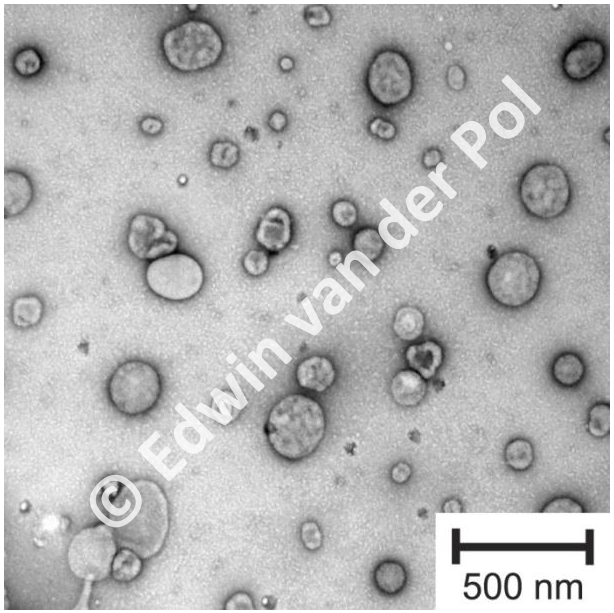
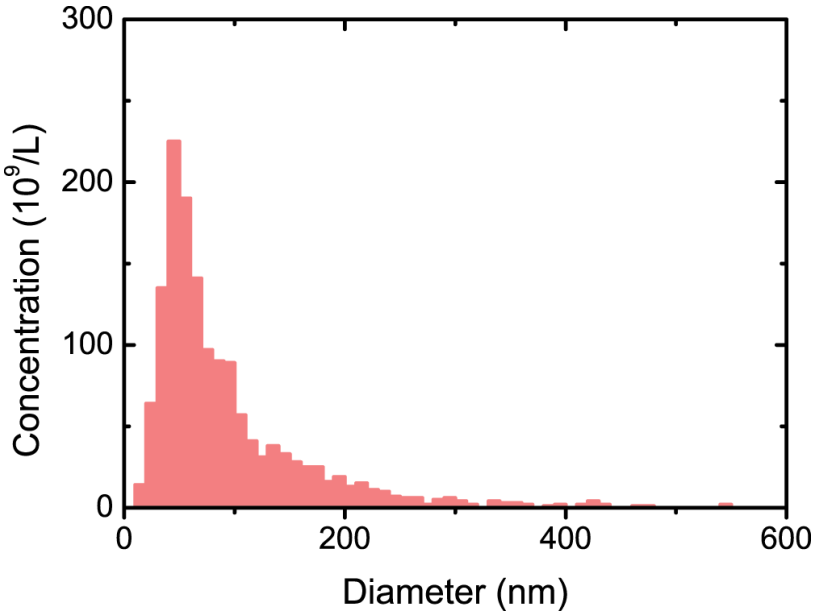
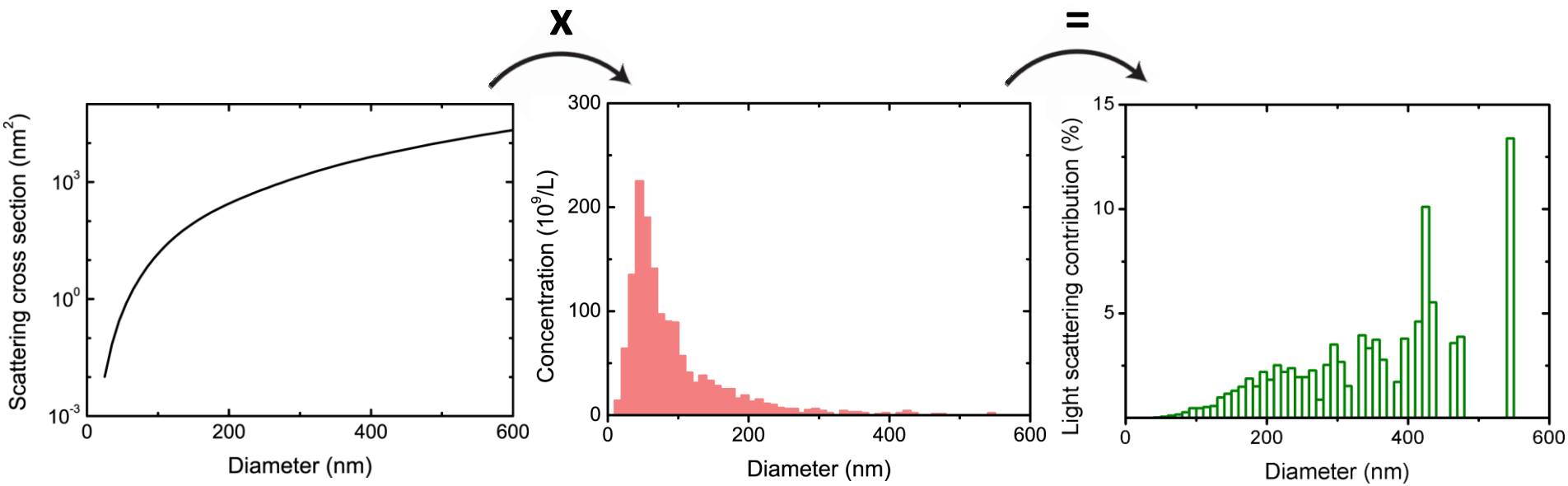
Introduction

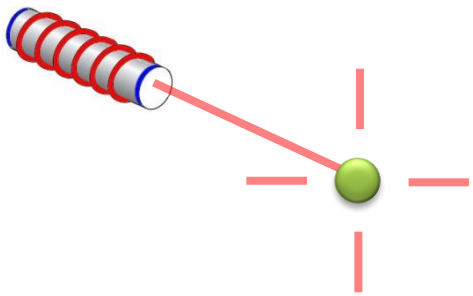


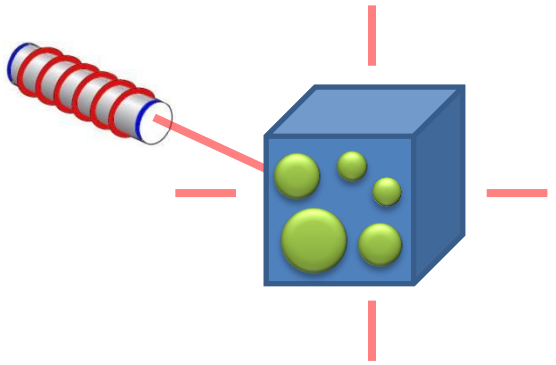


- body fluids contain cell-derived vesicles
- clinically relevant information
- problem: vesicle detection

Optical detection: light scattering







Outline

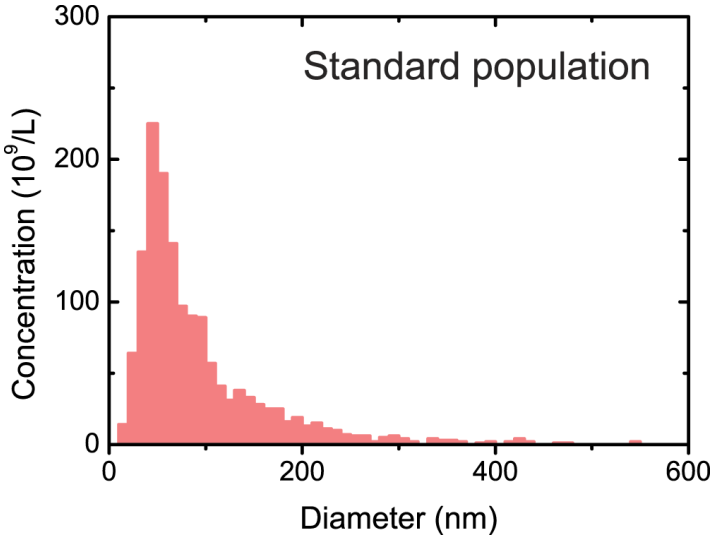
exploration of detection methods
- Flow cytometry (FACS)
- Dynamic Light Scattering (DLS)
- Nanoparticle Tracking Analysis (NTA)
- Atomic Force Microscopy (AFM)
- Impedance-based flow cytometry

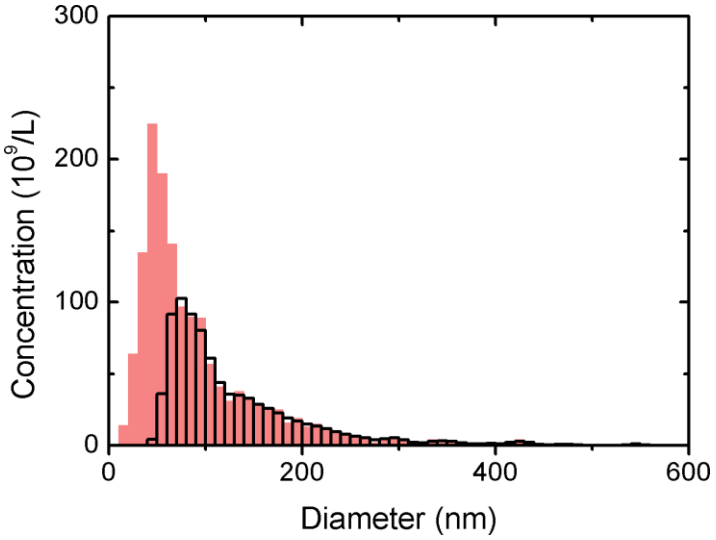
future developments

conclusions

Approach: estimate capabilities of methods considering well-known limitations

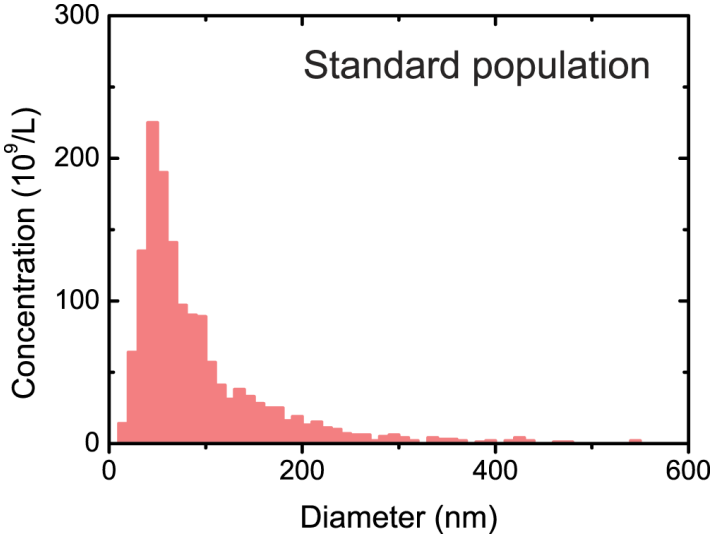
Example 1
Detection limit: **50 nm**
Size resolvability: **20 nm**

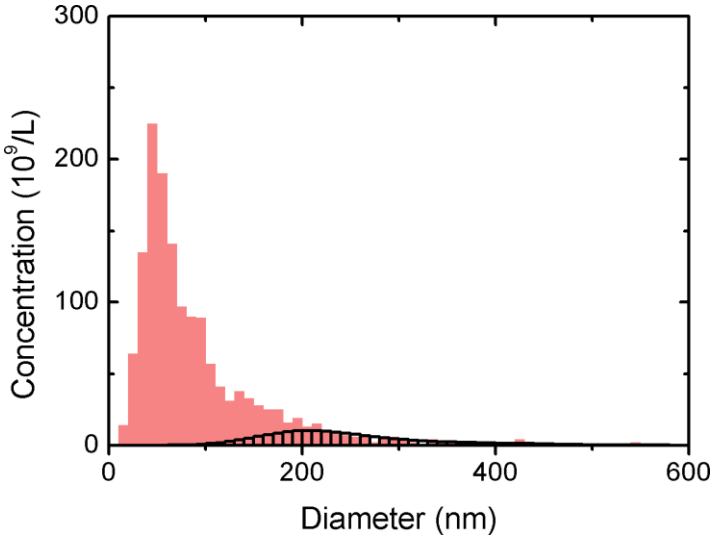




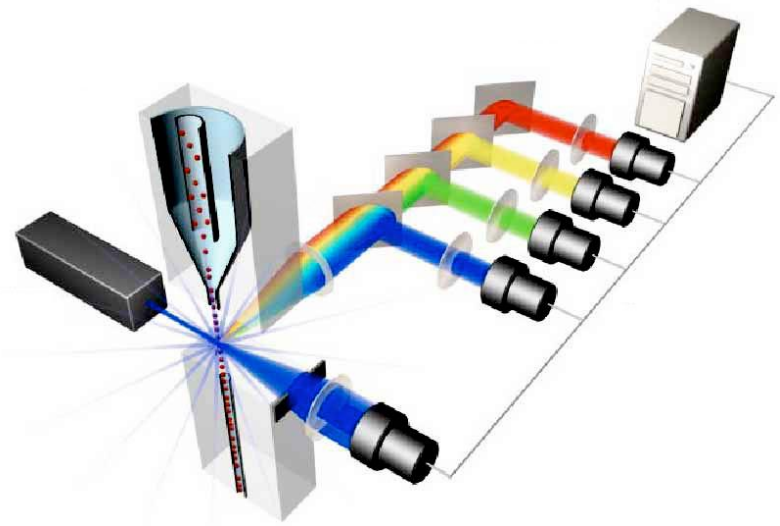
Approach: estimate capabilities of methods considering well-known limitations

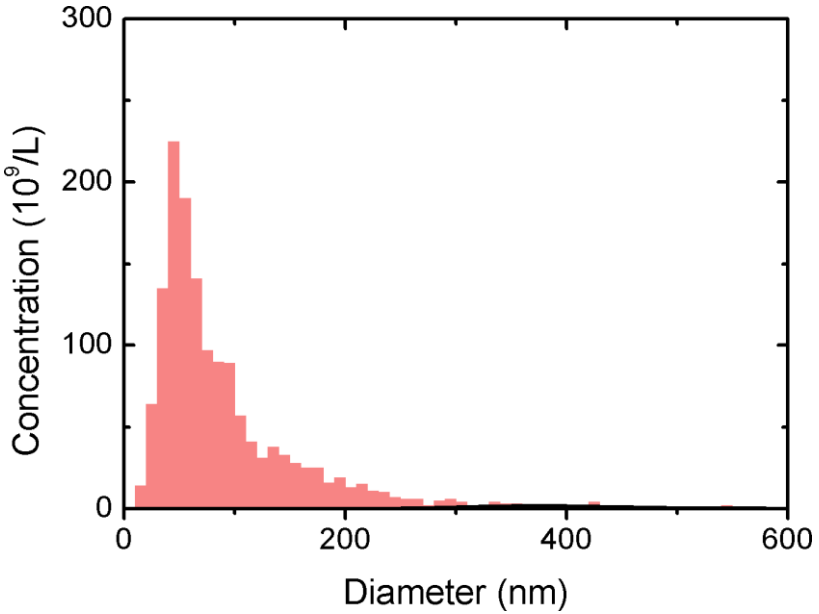
Example 2
Detection limit: **150 nm**
Size resolvability**: 100 nm**





Flow cytometry (FACS)





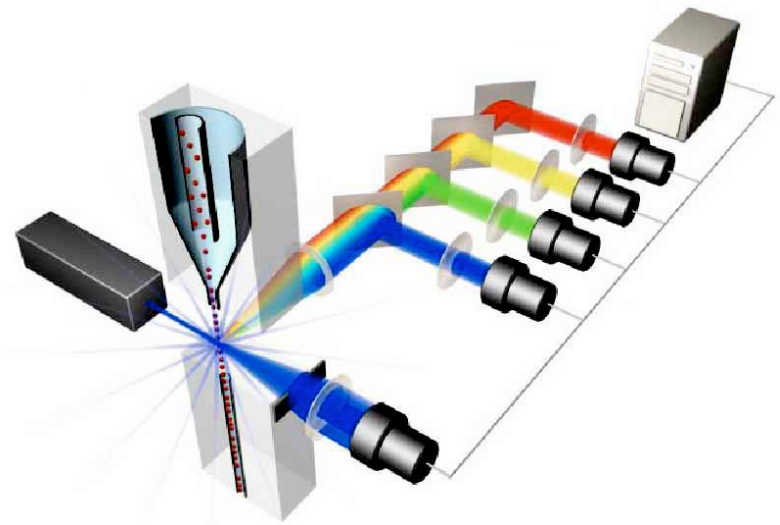
developed for cell detection (>1 µm)

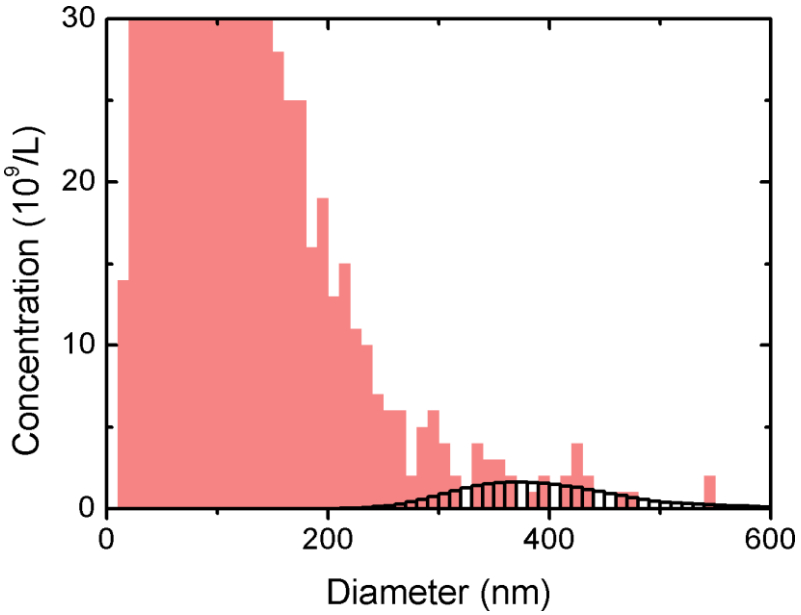
smallest detectable polystyrene bead (n=1.6): ~300 nm[1,2]

detection efficiency of vesicles (n≈1.4) by FACS: < 2%

1. Robert et al. *JTH* 2009
2. Perez-Pujol et al. *Cytom. Part. A* 2007

Flow cytometry (FACS)





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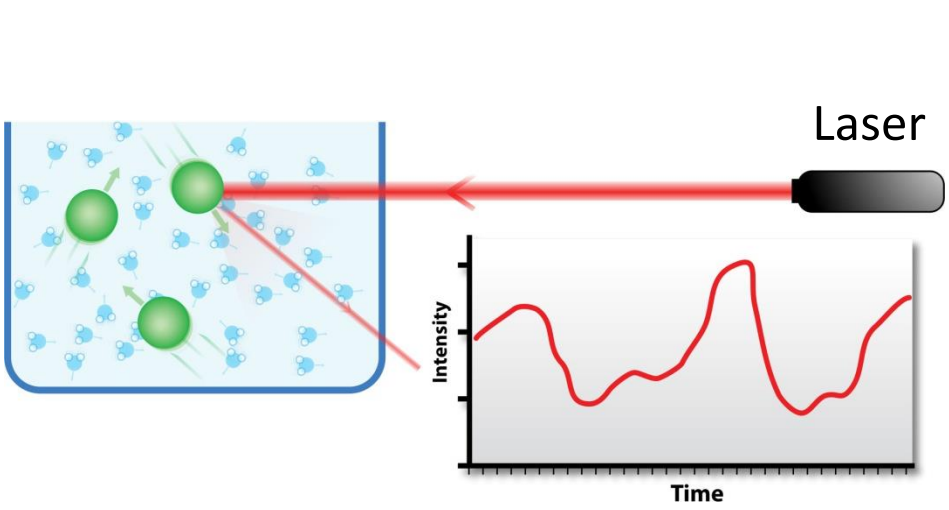
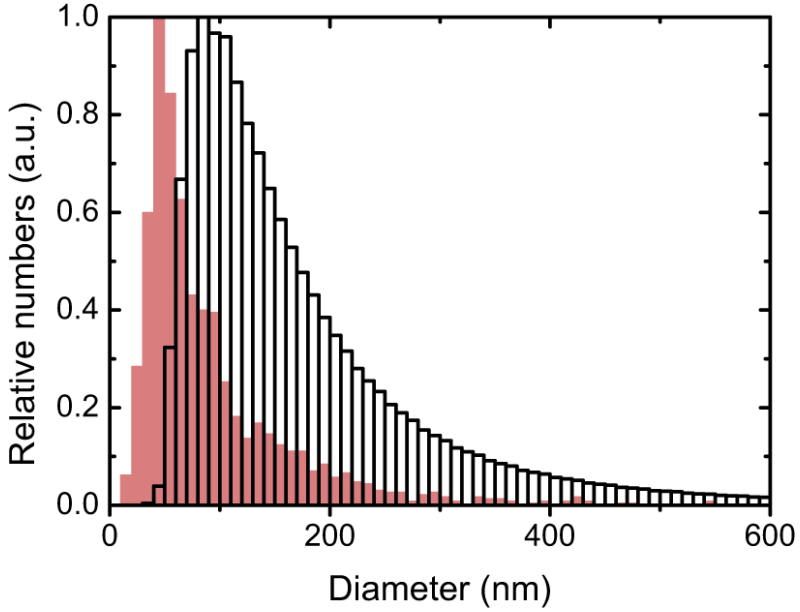
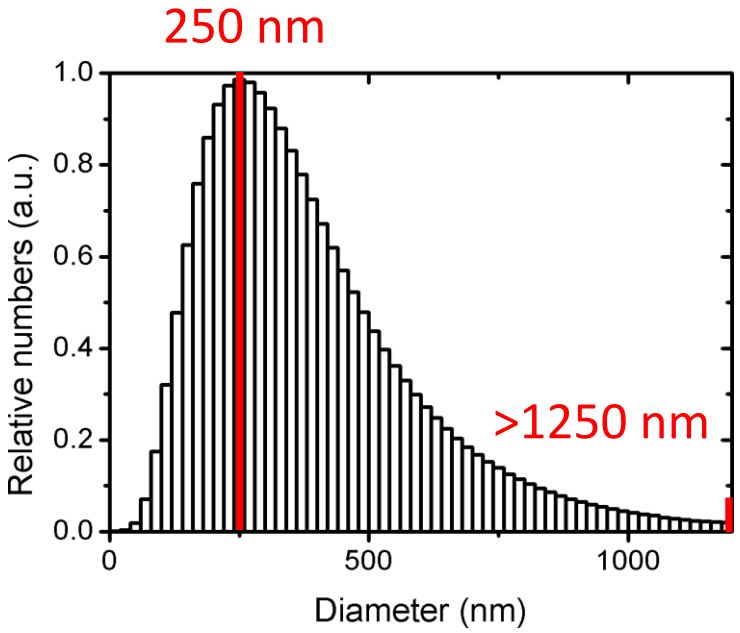
Dynamic Light Scattering (DLS)



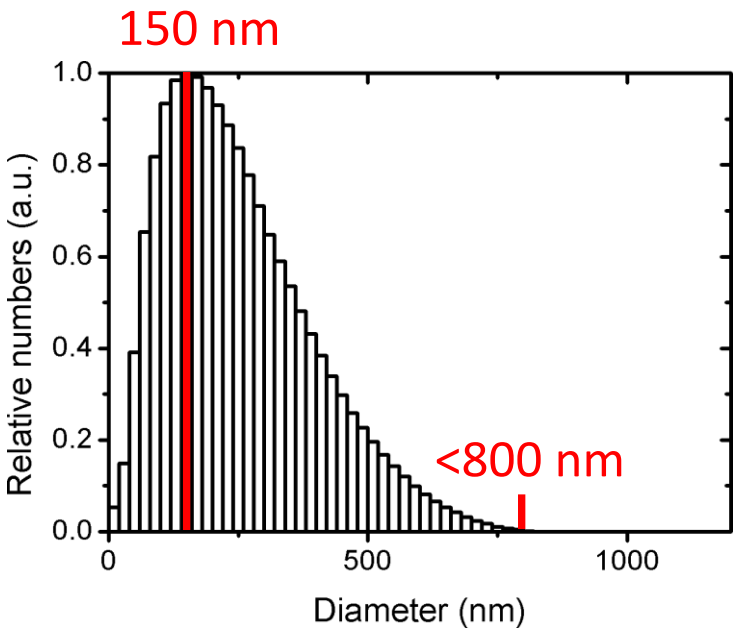


Brownian motion depends on vesicle diameter
determines mean *size* of vesicles in fluids
difficulty with polydisperse samples
result strongly depends on mathematical algorithm

DLS applied to vesicles



N5 Submicron Particle Size Analyser (Beckman Coulter)[3]

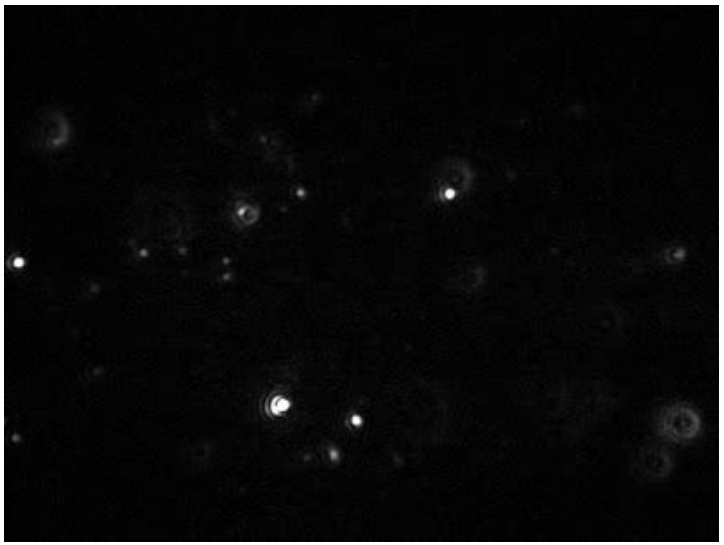


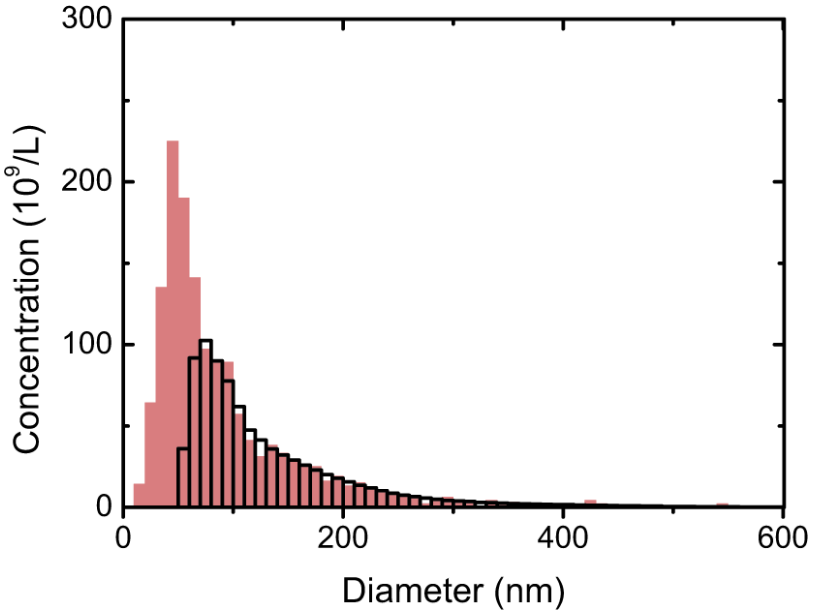
Zetasizer Nano S (Malvern Instruments Ltd)[3]

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

3. Lawrie et al. *Vox Sang* 2009

Nanoparticle Tracking Analysis (NTA)

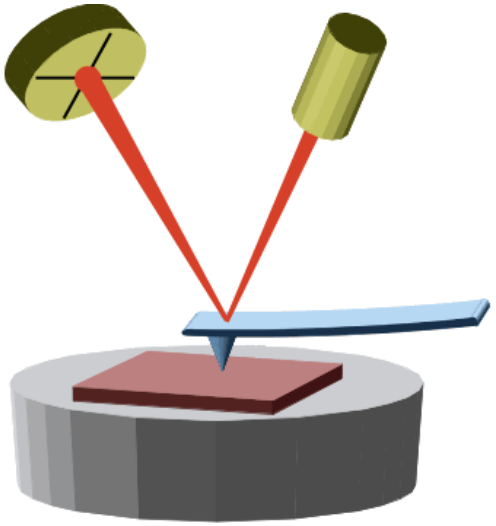


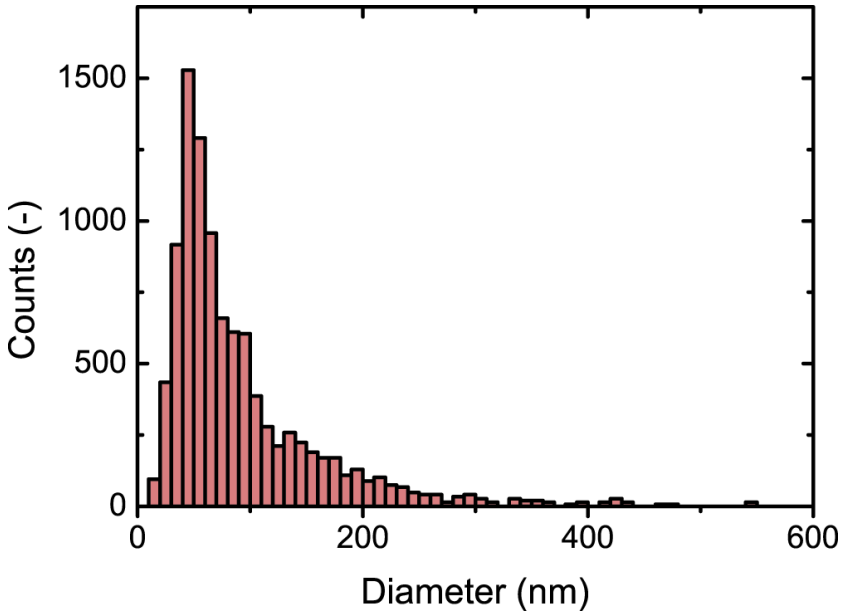


determines *size* and *concentration* of vesicles in fluids[4]
present detection limit:~50 nm for vesicles
can potentially be extended with fluorescence detection

4. Harrison et al. *JTH* (2009)

Non optical methods: Atomic Force Microscopy (AFM)

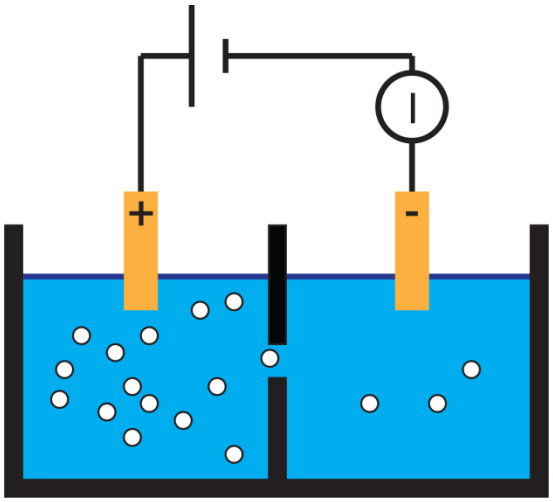


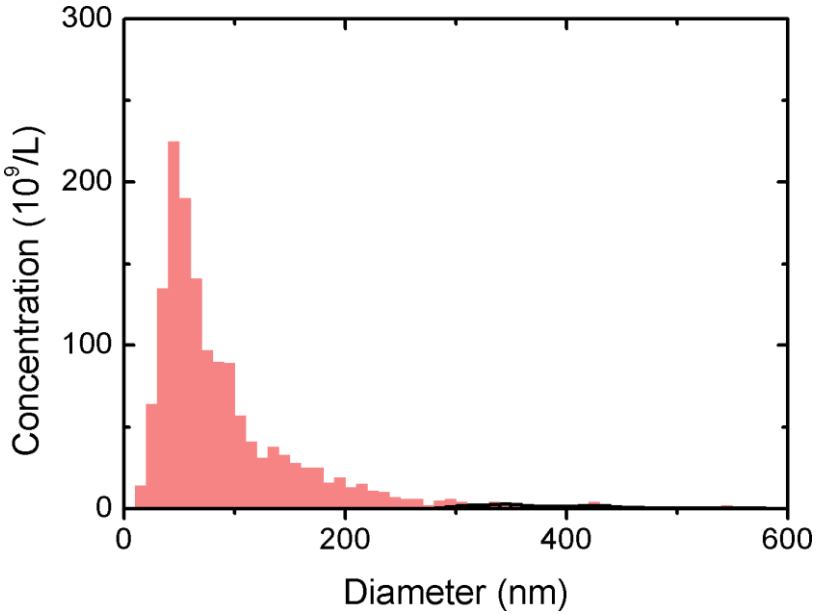


provides information on *size*, *concentration*, *biochemical composition*, and *cellular origin*[5]
binding efficiency and influence of binding on vesicle deformation unknown

5. Yuana et al. *Thromb. Haemost.* 2009

Impedance-based flow cytometry



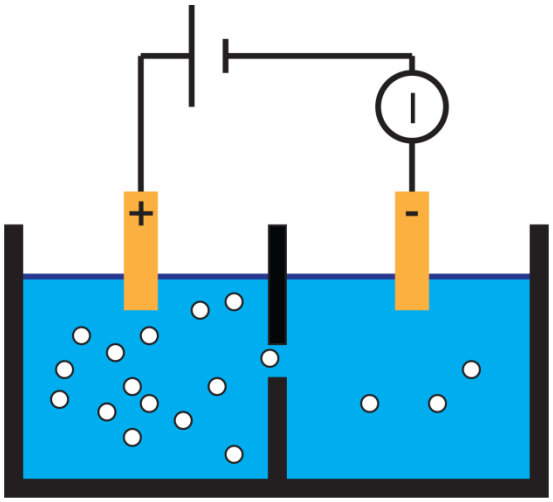


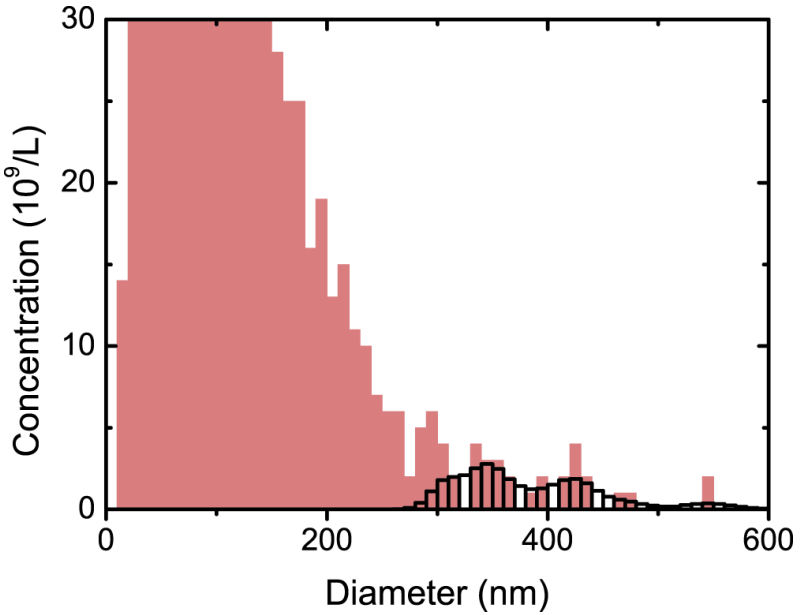
determines *size* and *concentration* of vesicles

present detection limit:~300 nm[6]

can be combined with flow cytometry

Impedance-based flow cytometry





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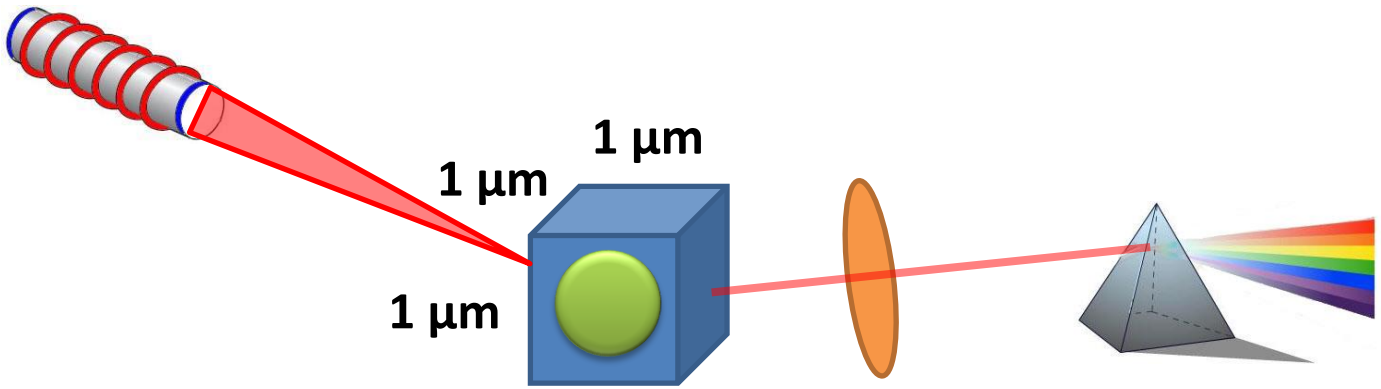
Overview

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 microspectroscopy[7]:
  - determine size, concentration, and chemical composition of vesicles in fluids *label-free*



7. Puppels et al. *Nature* 1990

Conclusions

vesicle detection remains challenging

applications of novel and conventional methods requires further investigation