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

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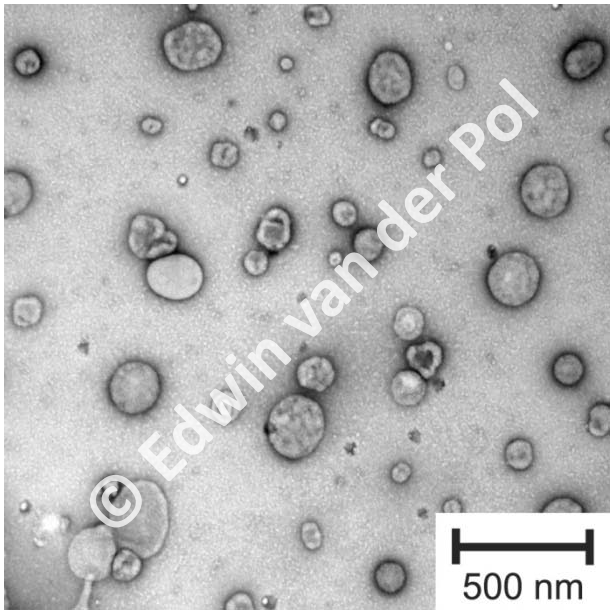
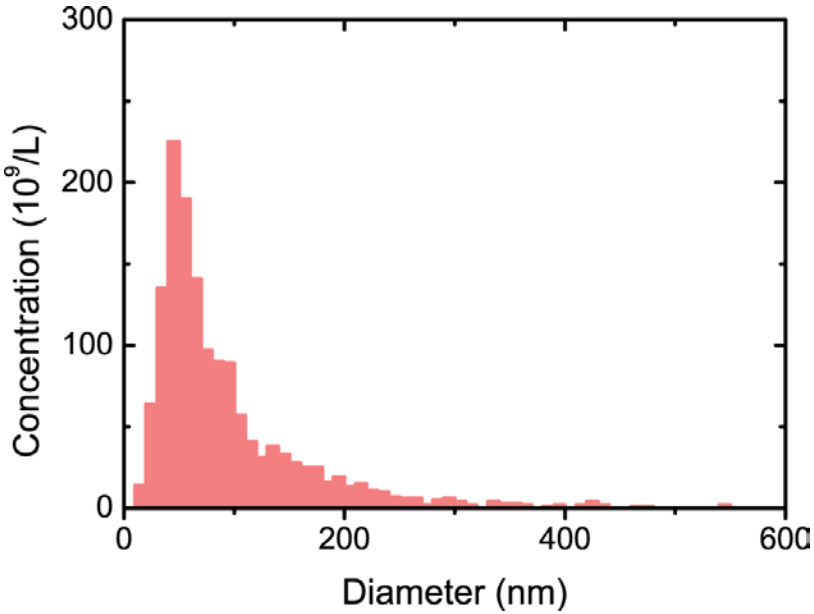
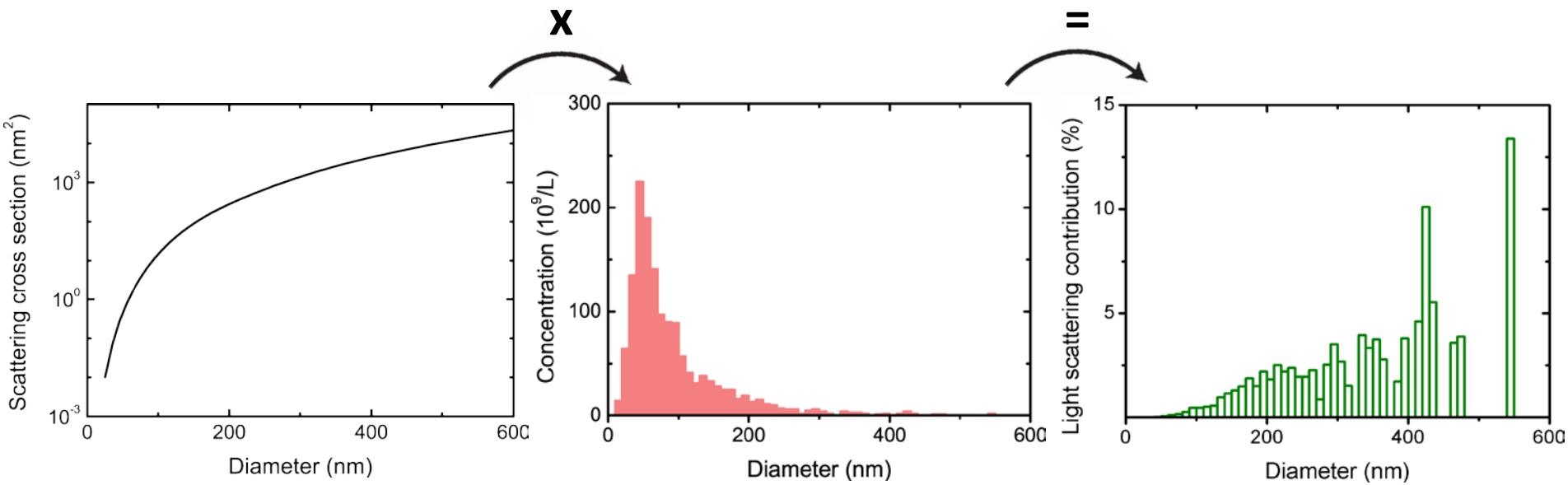
Introduction

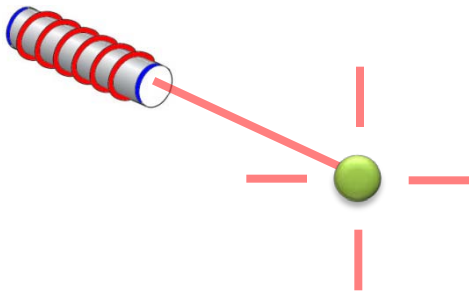


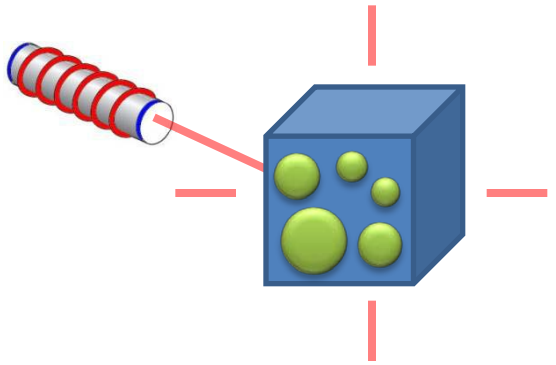


- body fluids contain cell-derived vesicles
- clinically relevant information
- problem: vesicle detection

Optical detection: light scattering





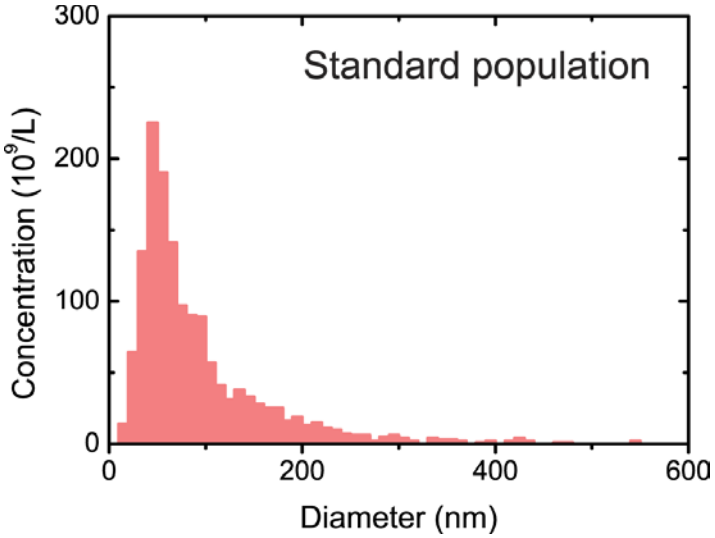


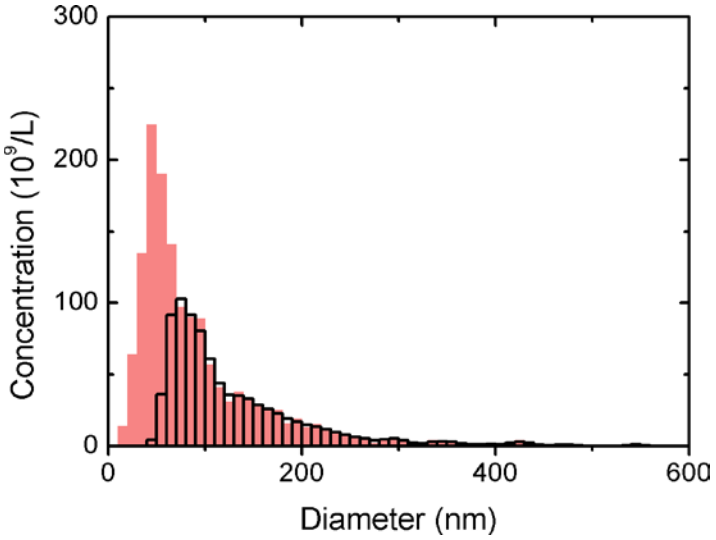
Outline

- 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

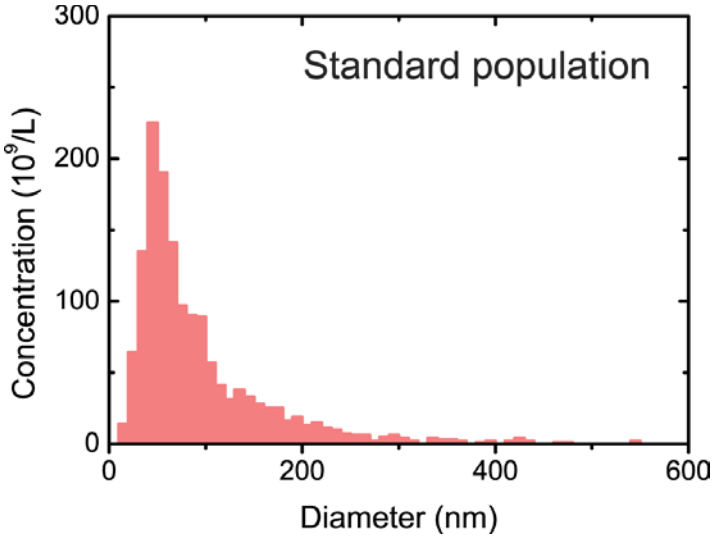
Example 1
Detection limit: **50 nm**
Size resolvability: **20 nm**

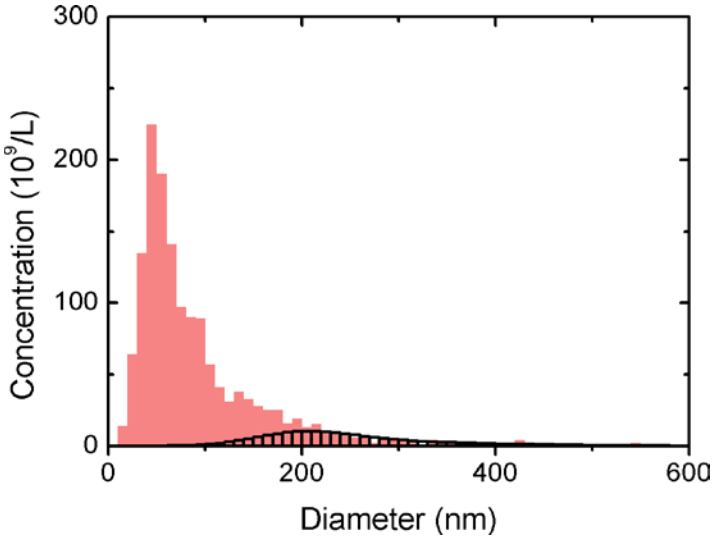




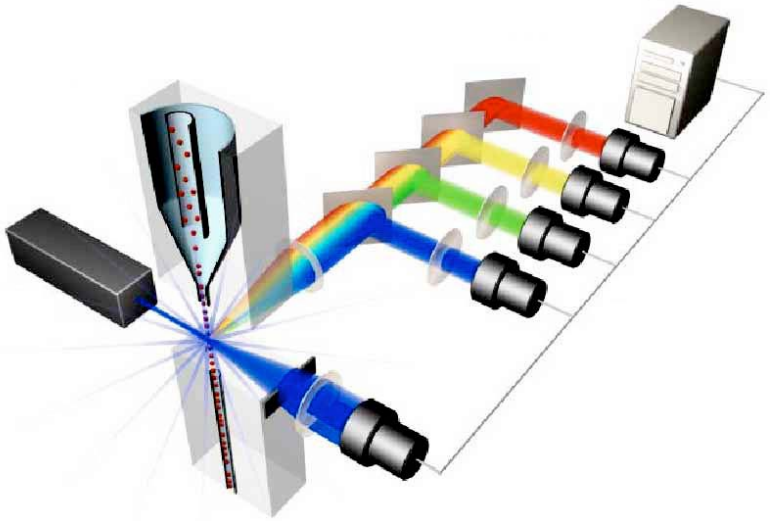
Approach: estimate capabilities of methods considering well-known limitations

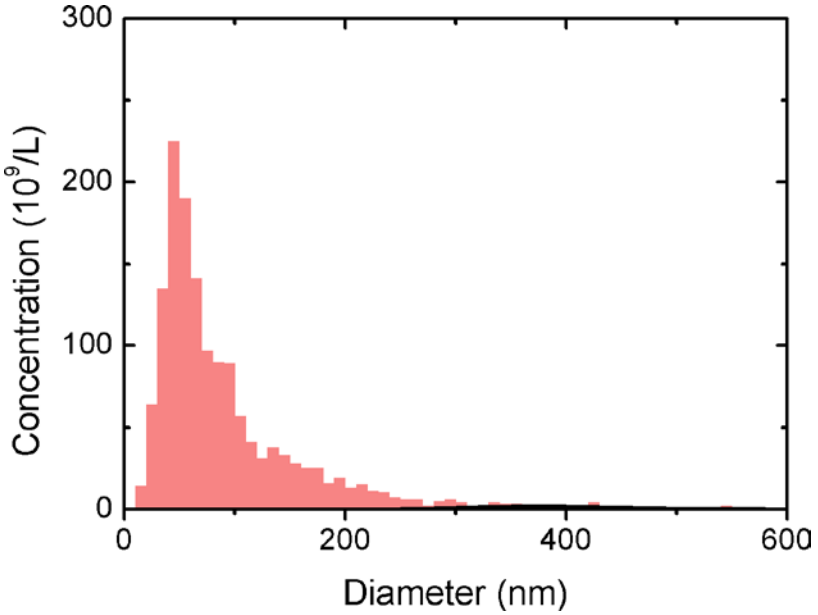
Example 2
Detection limit: **150 nm**
Size resolvability**: 100 nm**





Flow cytometry (FACS)

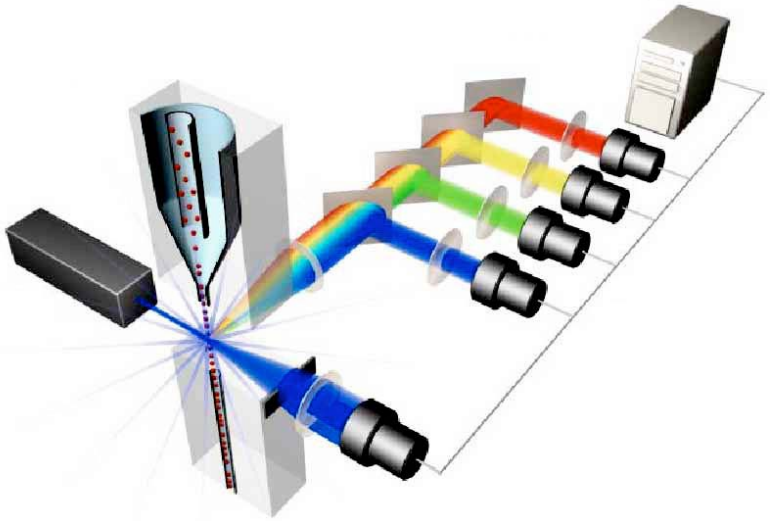


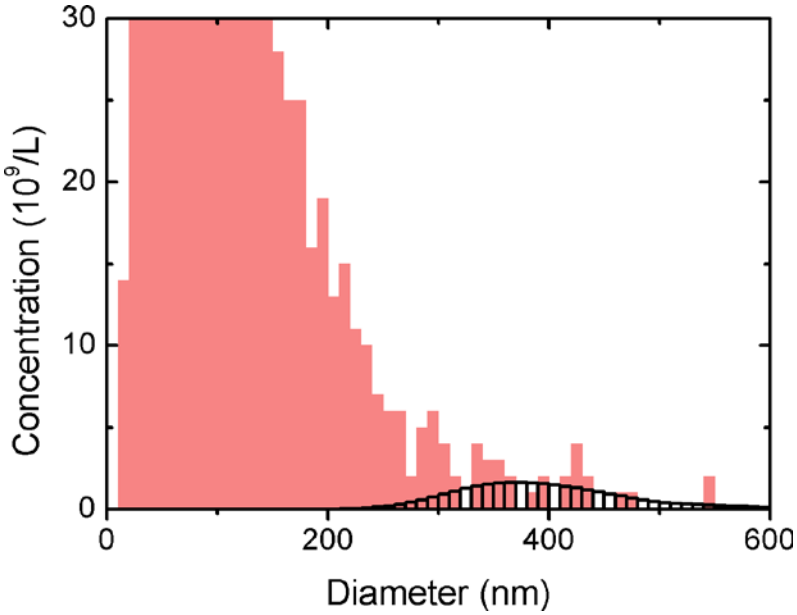


- developed for cell detection (>1 µm)
- smallest detectable polystyrene bead (n=1.6): ~300 nm[1,2]
- detection efficiency of vesicles (n≈1.4) by FACS: < 2%

1. Robert S. et al. J. Thromb. Haemost. 2009; **7**: 190-7
2. Perez-Pujol. et al. S. Cytom. Part. A. 2007; **71**: 38-45

Flow cytometry (FACS)





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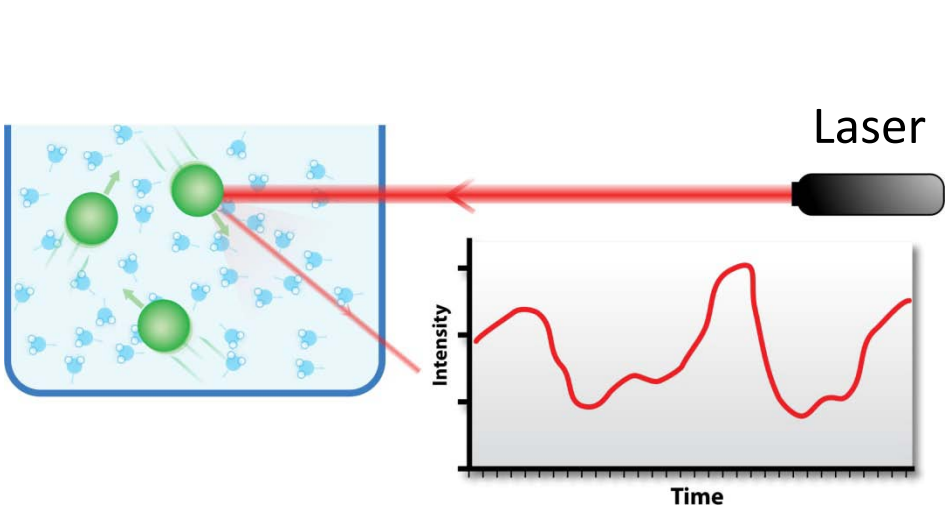
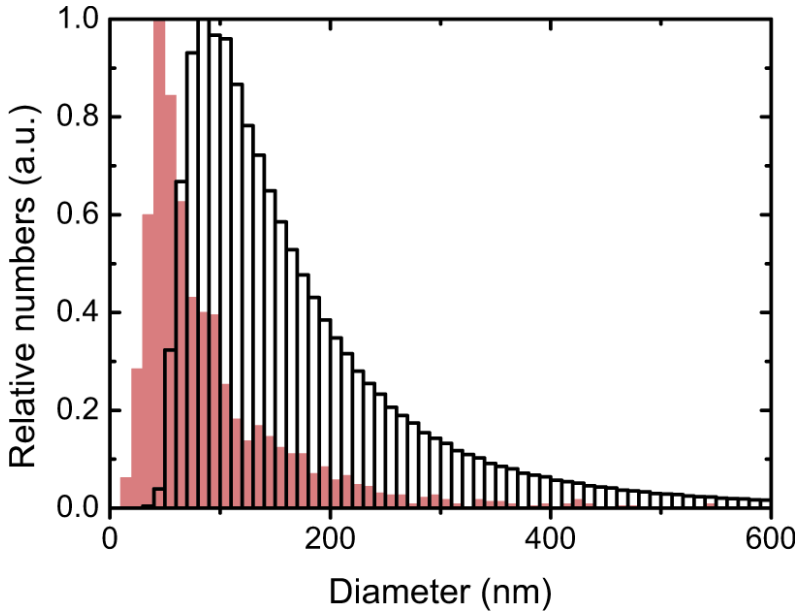
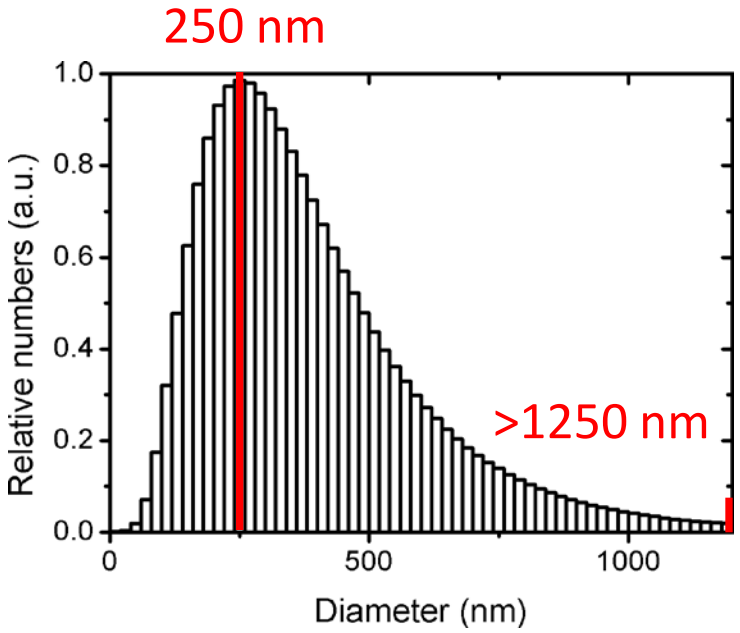
Dynamic Light Scattering (DLS)



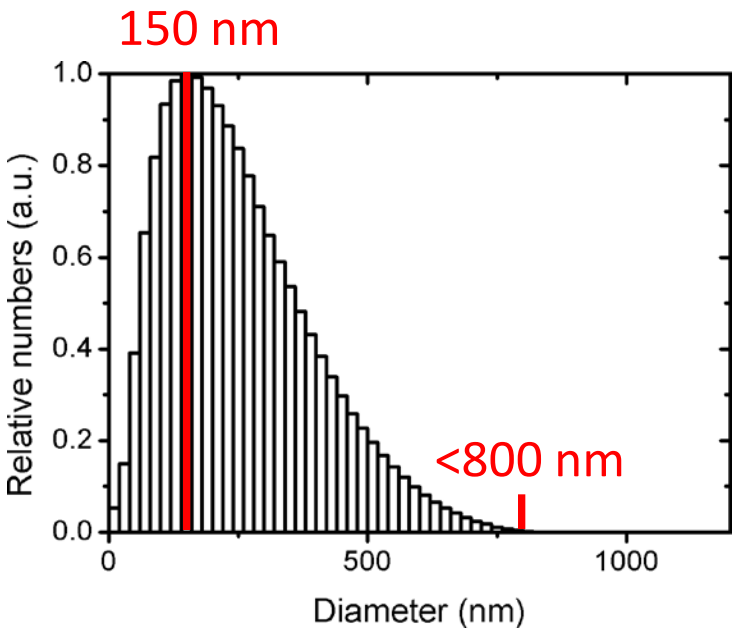


- Brownian motion depends on vesicle diameter
- determines mean *size* of vesicles in fluids
- difficulty with polydisperse samples
- result strongly depends on mathematical algorithm

DLS applied to vesicles



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

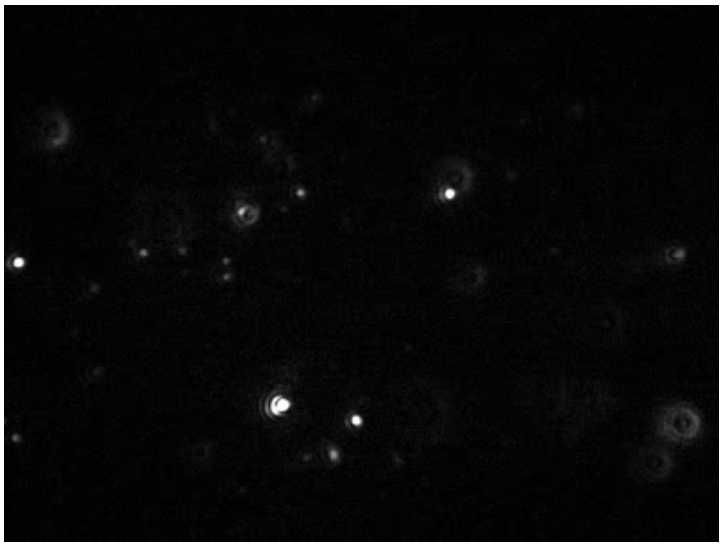


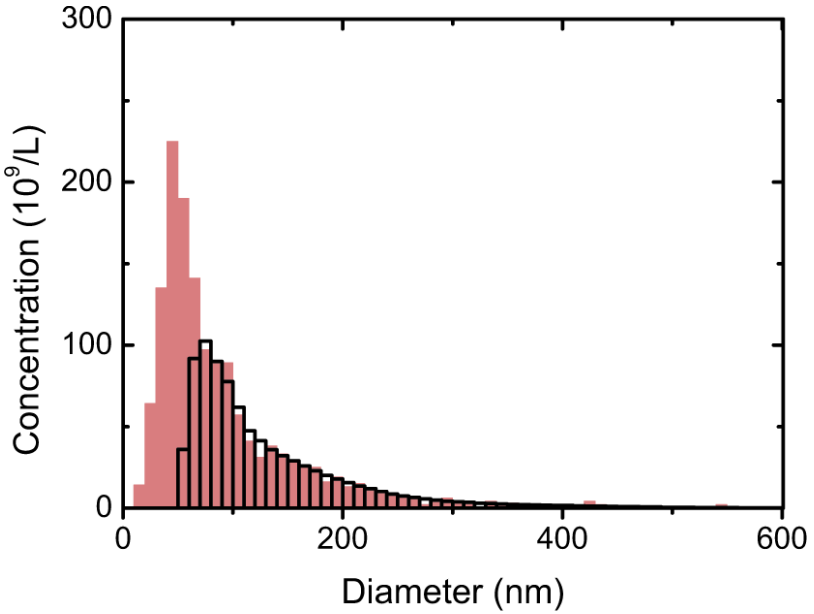
Zetasizer Nano S (Malvern Instruments Ltd)[3]

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

3. Lawrie A.S. et al. Vox Sang 2009; **96**: 206-12

Nanoparticle Tracking Analysis (NTA)

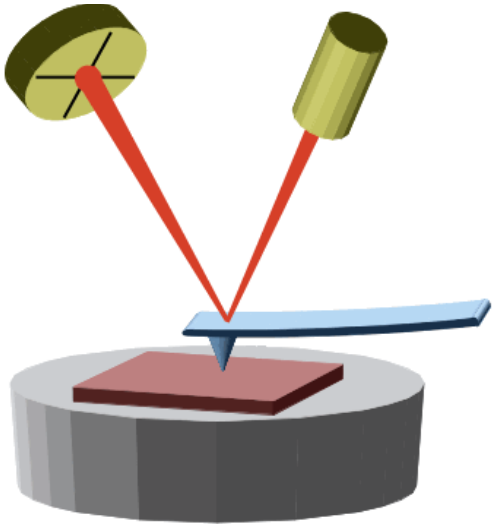


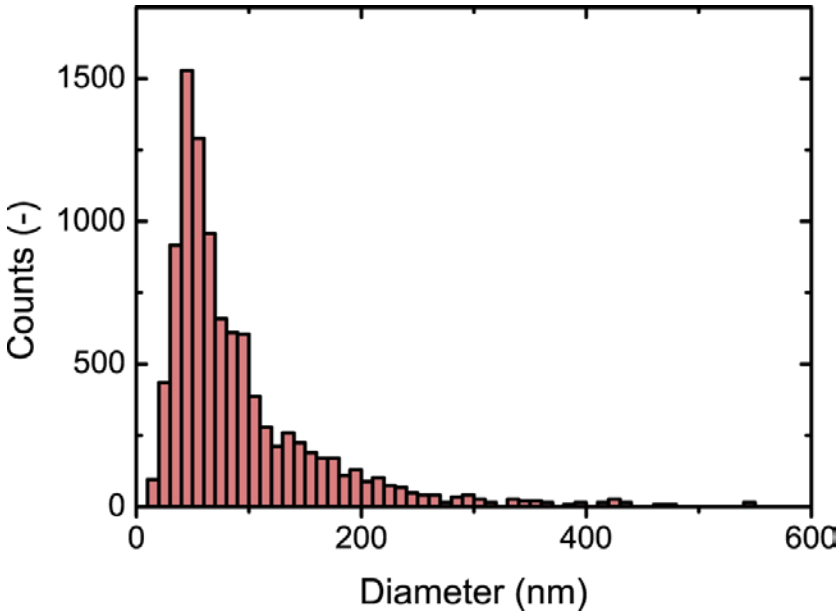


- determines *size* and *concentration* of vesicles in fluids[4]
- present detection limit:~50 nm for vesicles
- can potentially be extended with fluorescence detection

4. Harrison P. et al. Int. S. J. Thromb. Haemost. Boston, 2009, 185

Non optical methods: Atomic Force Microscopy (AFM)

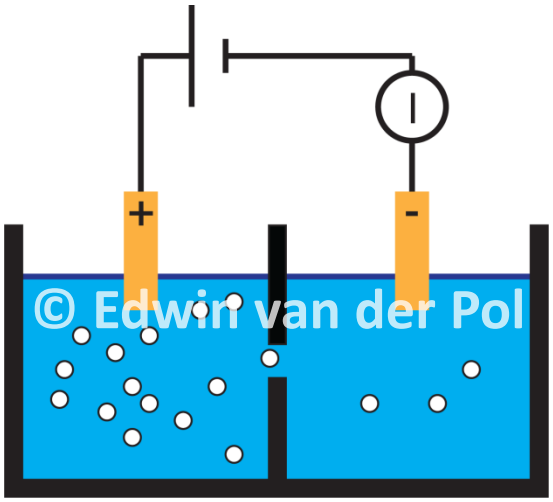


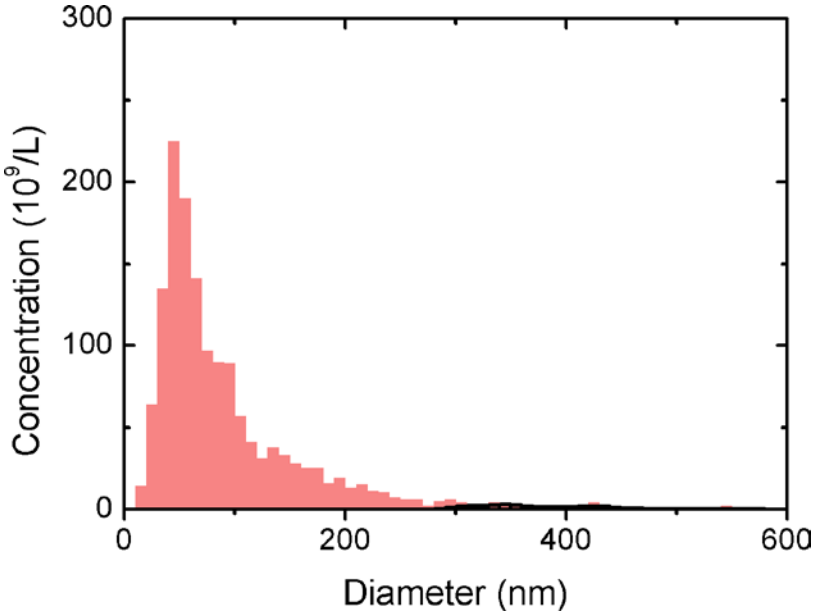


- provides information on *size*, *concentration*, *biochemical composition*, and *cellular origin*[5]
- binding efficiency and influence of binding on vesicle deformation unknown

5. Yuana Y. J. et al. Thromb. Haemost. 2009; **8**: 315-23

Impedance-based flow cytometry

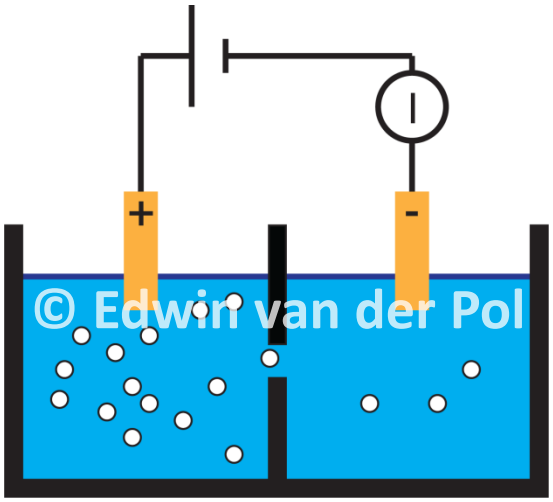


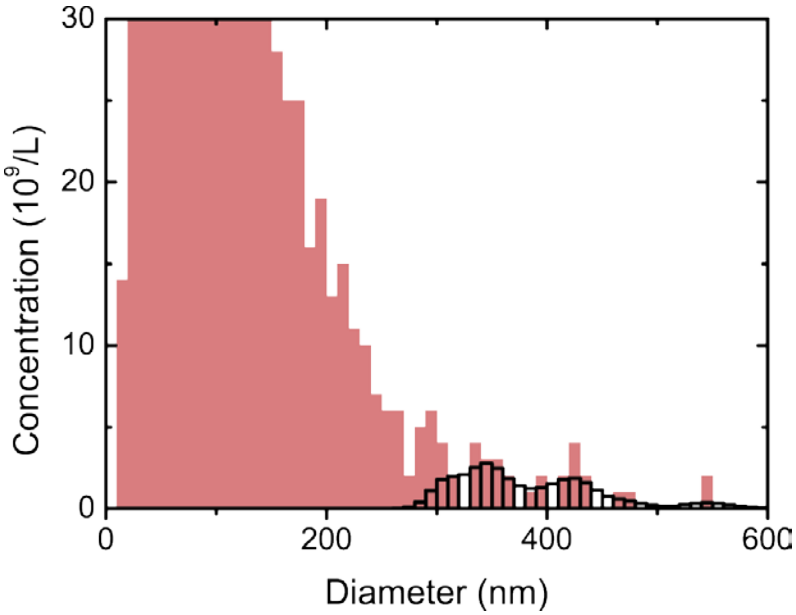


- determines *size* and *concentration* of vesicles
- present detection limit:~300 nm[6]
- can be combined with flow cytometry

6. Zwicker J.I. et al. Clin. Cancer Res. 2009; 15: 6830-40

Impedance-based flow cytometry





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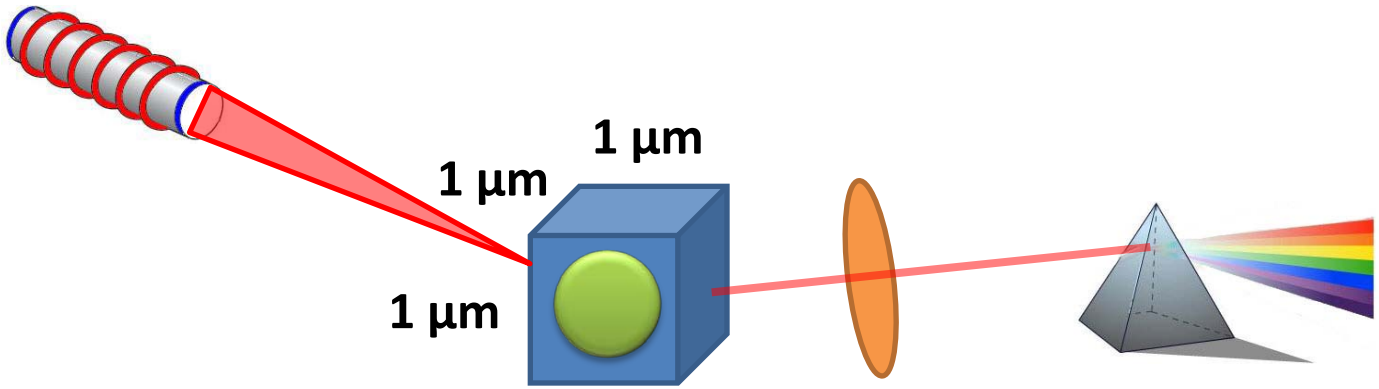
Overview

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 G.J. et al. Nature 1990; **347**: 301-3

Conclusions

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