Dutch physics to elucidate detection of *bio* nanoparticles from body fluids

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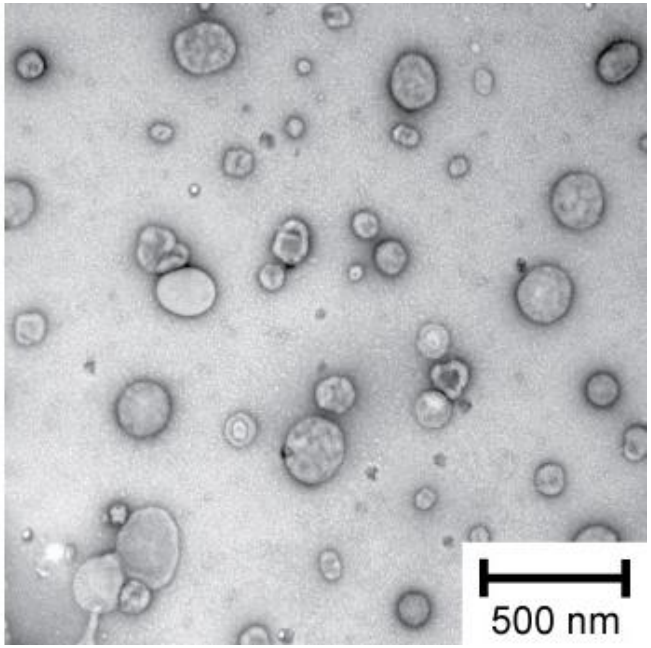
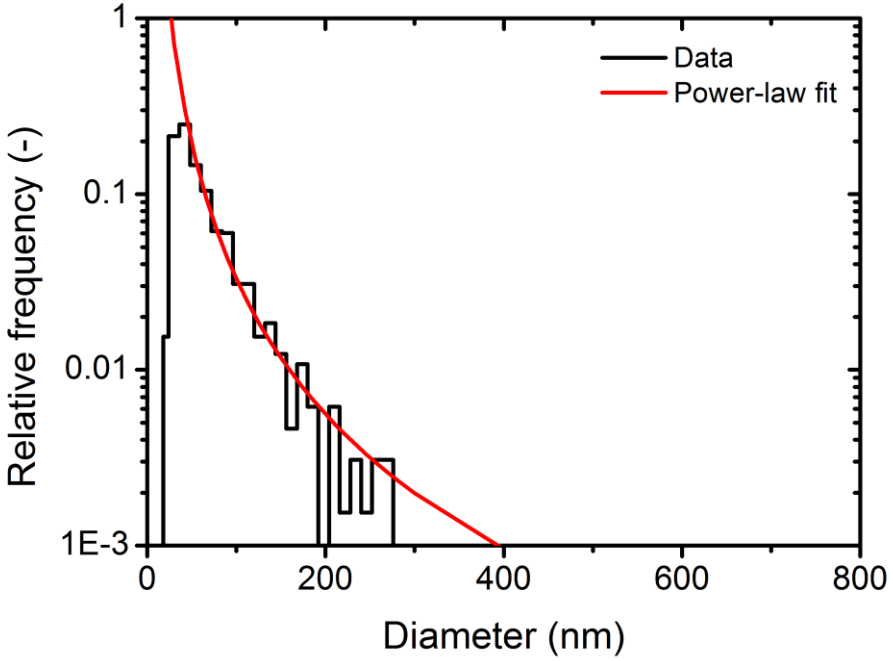
Extracellular vesicles

Introduction to extracellular vesicles



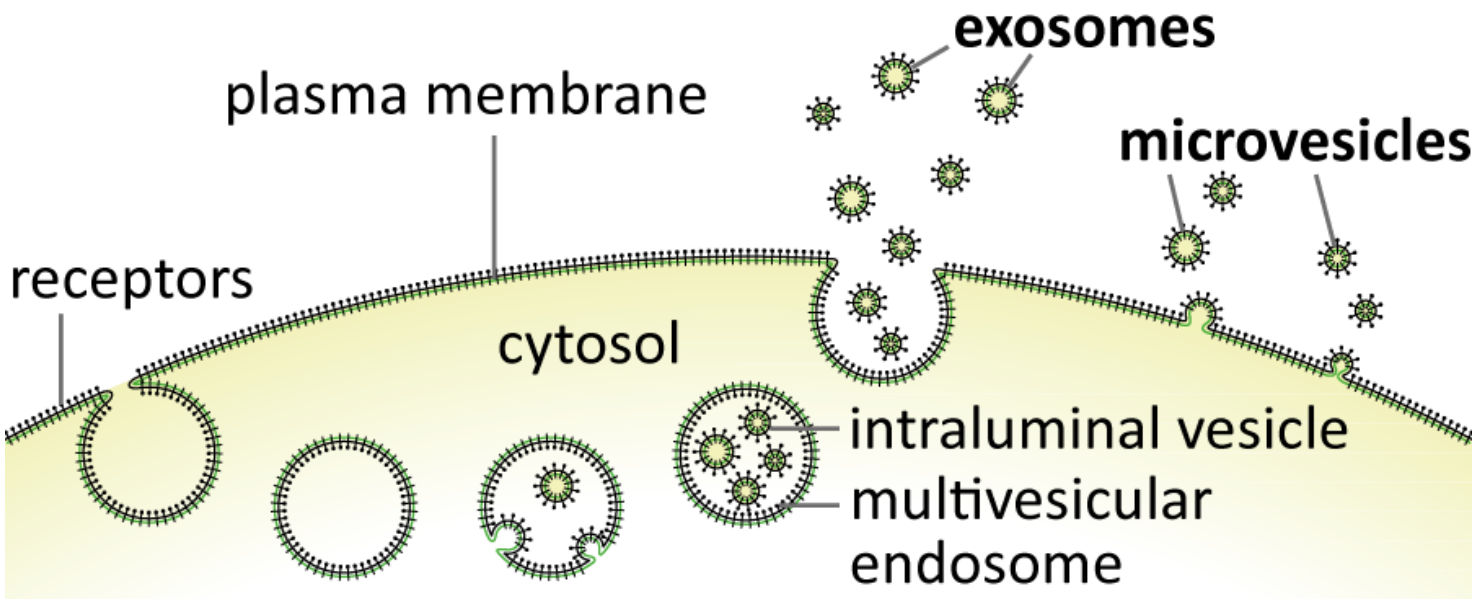


- extracellular vesicles are present in (body) fluids
  - e.g. blood, urine, saliva, sweat, ascites, cerebrospinal fluid
  - also cell culture media, ocean water*

size distribution by Vincent Fokkema (VSL)

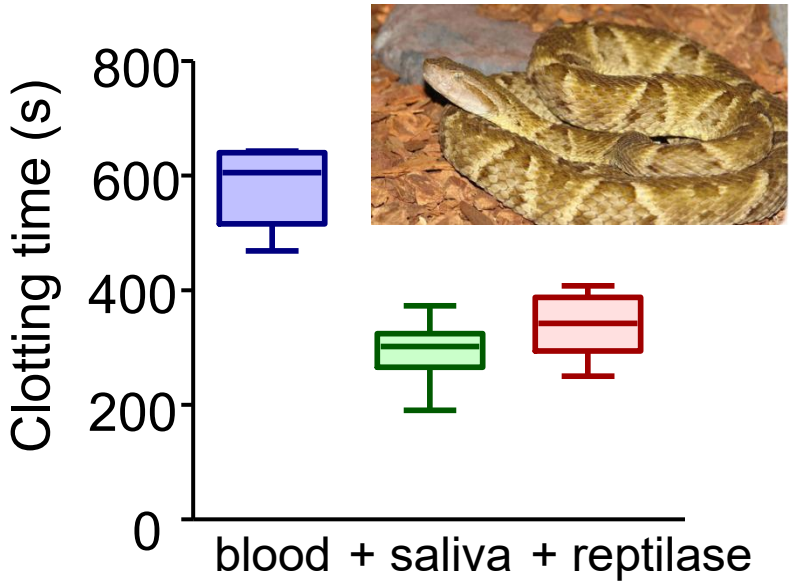
*Biller et al. *Science* (2014)

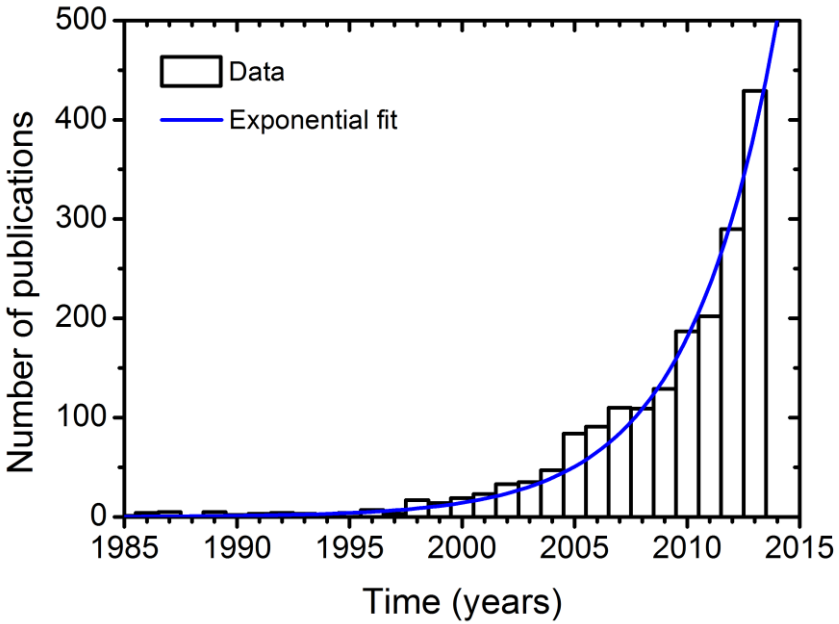
Introduction to extracellular vesicles



- cells release extracellular vesicles
- vesicles contain receptors, DNA, RNA
- specialized functions
  - e.g. communication, waste transport, coagulation

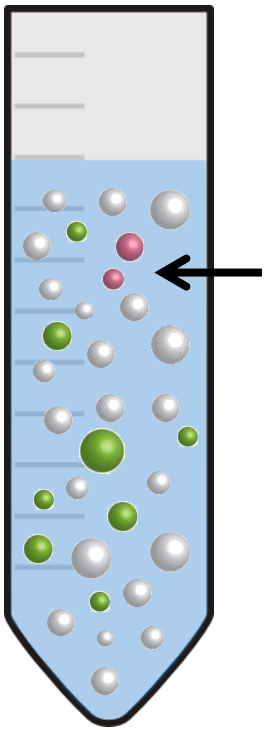
Function and clinical relevance of vesicles





- concentration, composition and function of extracellular vesicles correlate with disease

What do vesicle researchers need?



rare (tumor) vesicles

all vesicles →

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	$7.3 - 9.4 \cdot 10^{10}$

- tool to determine the concentration of a vesicle subpopulation

Vesicle concentration determination factsheet

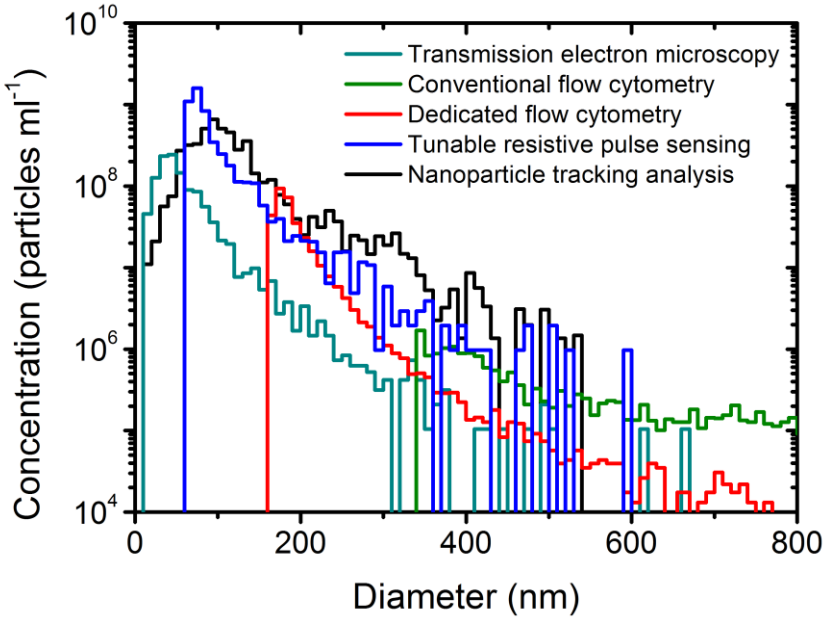
- reported concentration in plasma:
  $10^4 - 10^{12}$ vesicles mL^{-1}

- diameter <100 nm for >80% of vesicles
- vesicles differ
  - 25-fold in diameter
  - 20,000-fold in volume
  - 300,000-fold in concentration
  - 10,000,000-fold in scattered light
- vesicles originate from complex fluids

- instruments differ >1 order of magnitude in sensitivity

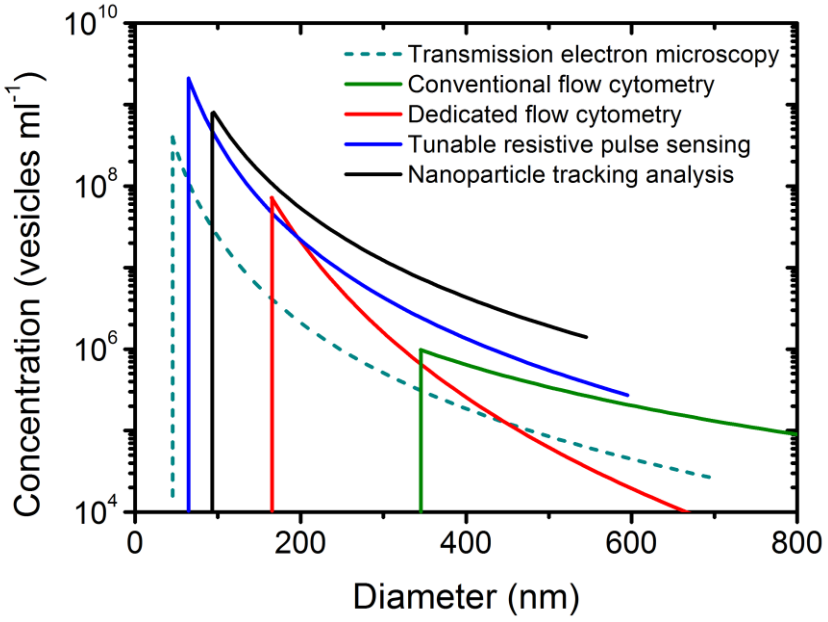
Size and concentration of vesicles

size distribution

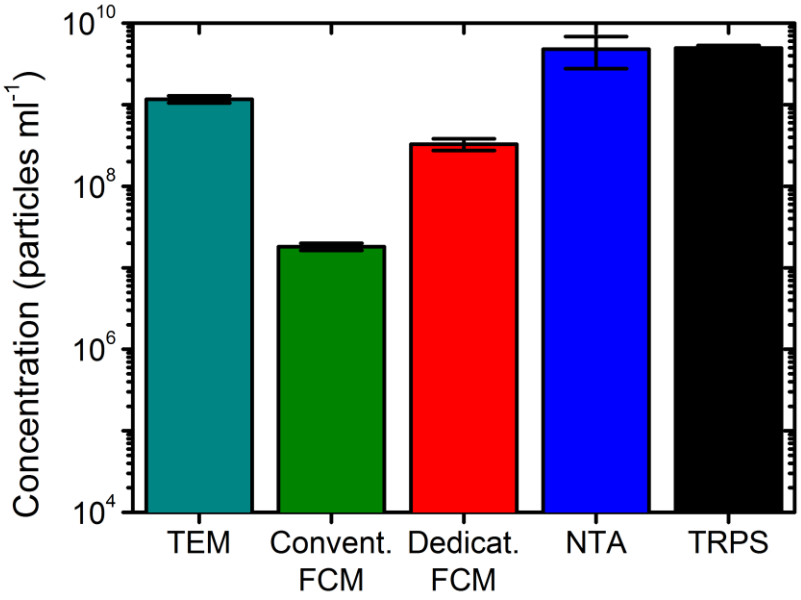


Size and concentration of vesicles

size distribution fitted



total concentration



- size distribution: power-law function
- smallest detectable size affects concentration

Vesicle detection by flow cytometry

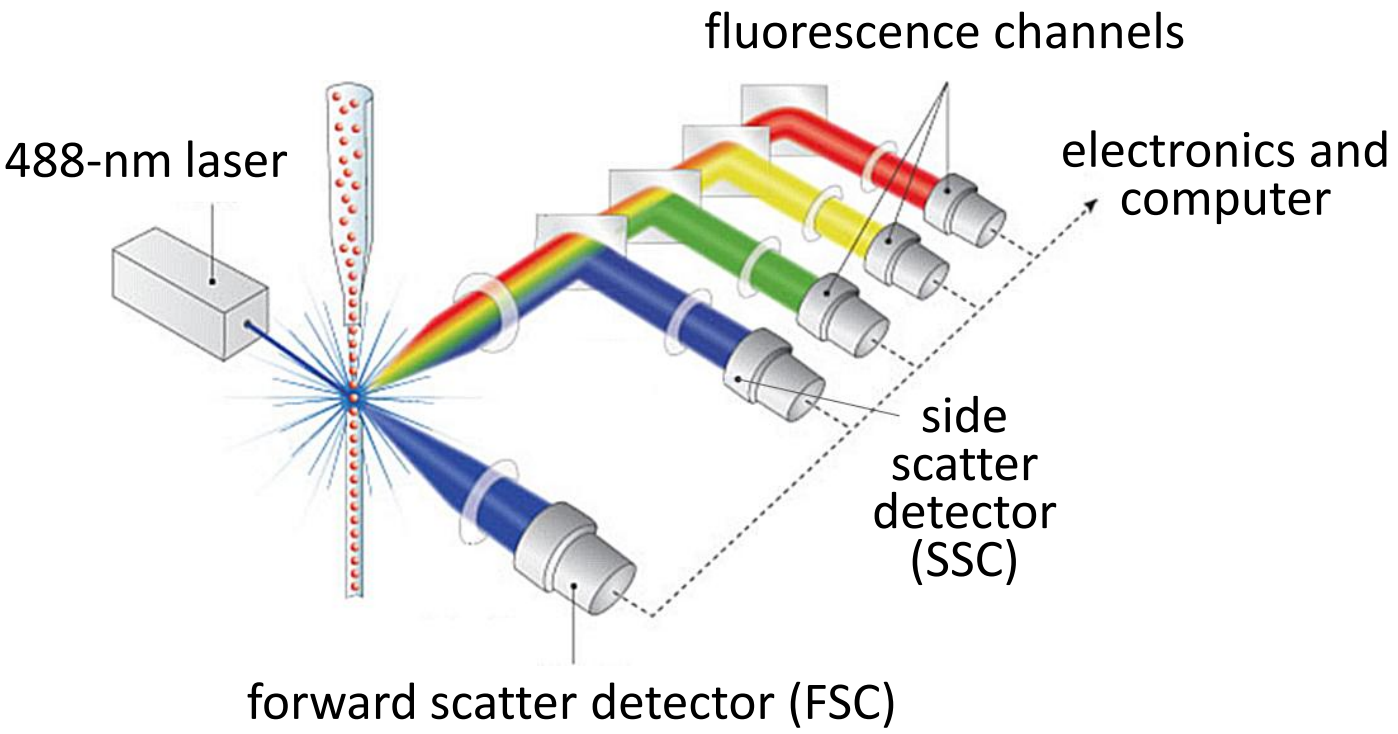
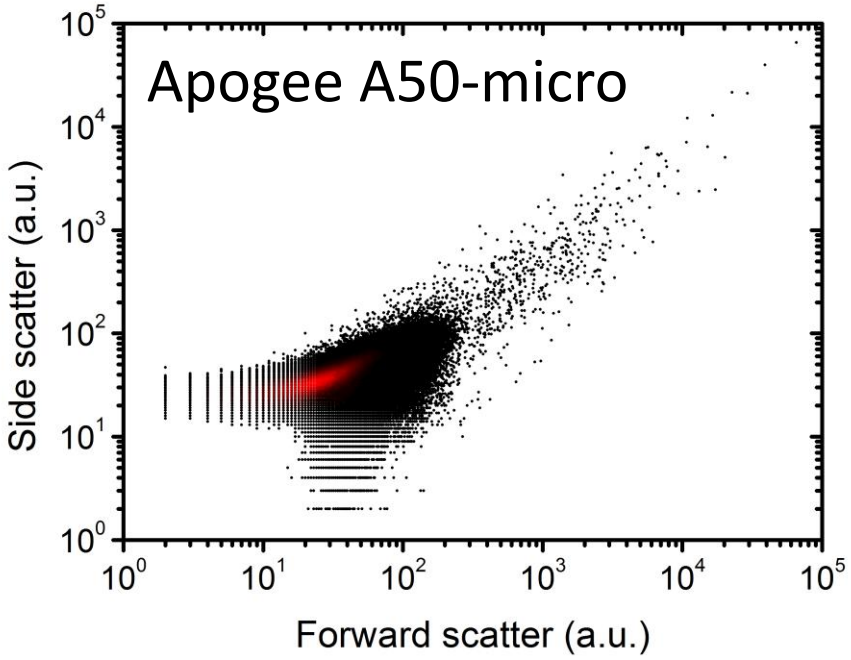
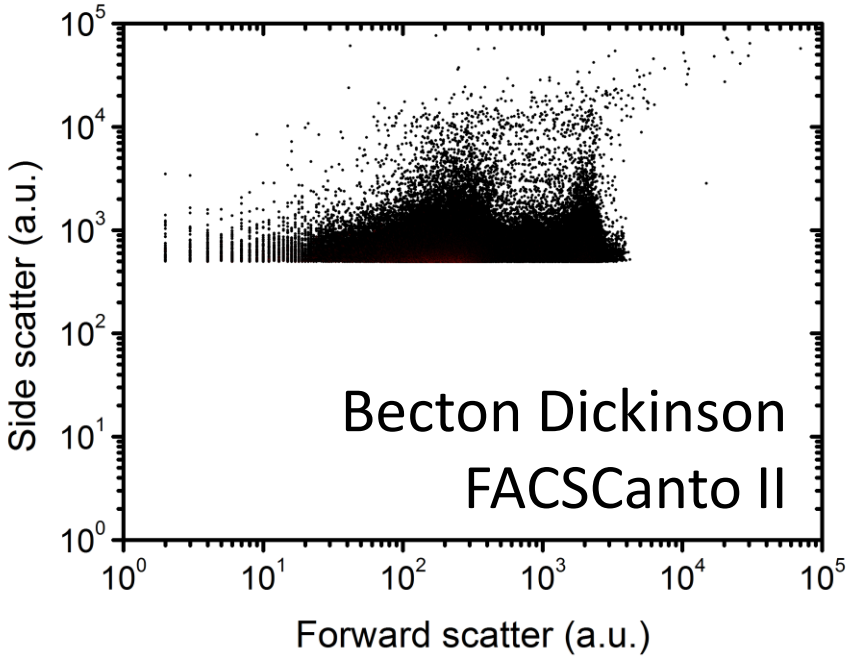


image adapted from semrock.com

Problem: arbitrary units hamper data interpretation and comparison

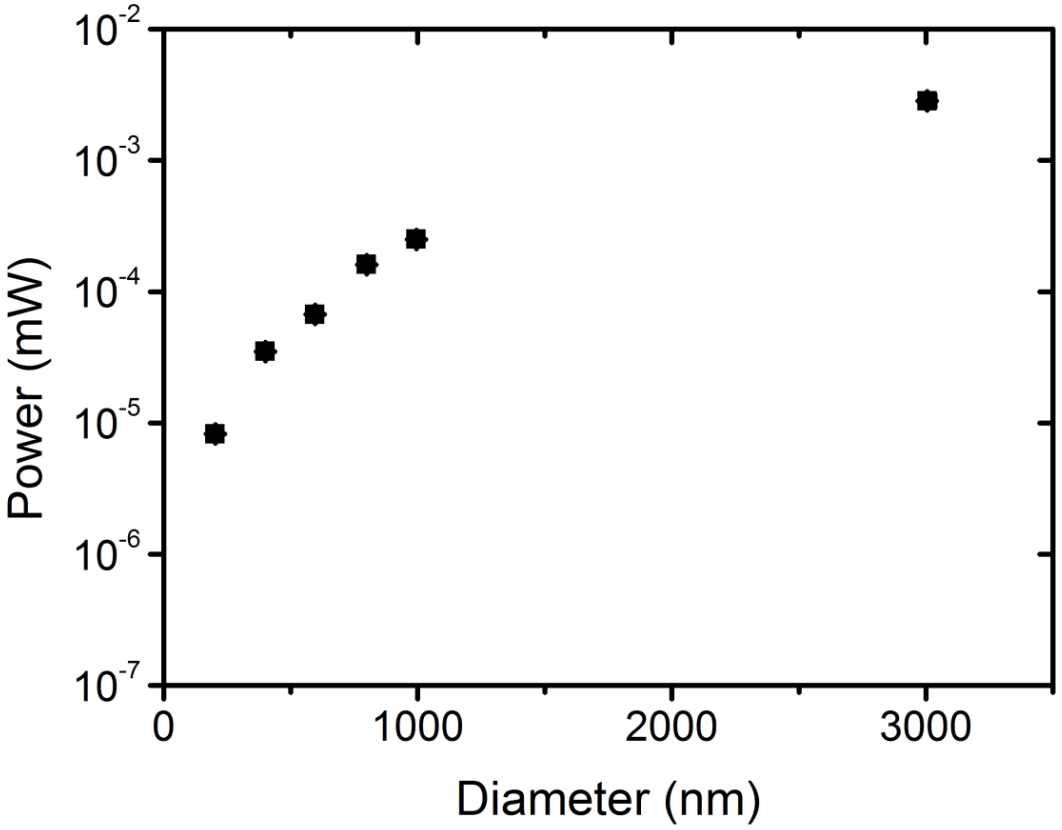
same population of vesicles from erythrocytes





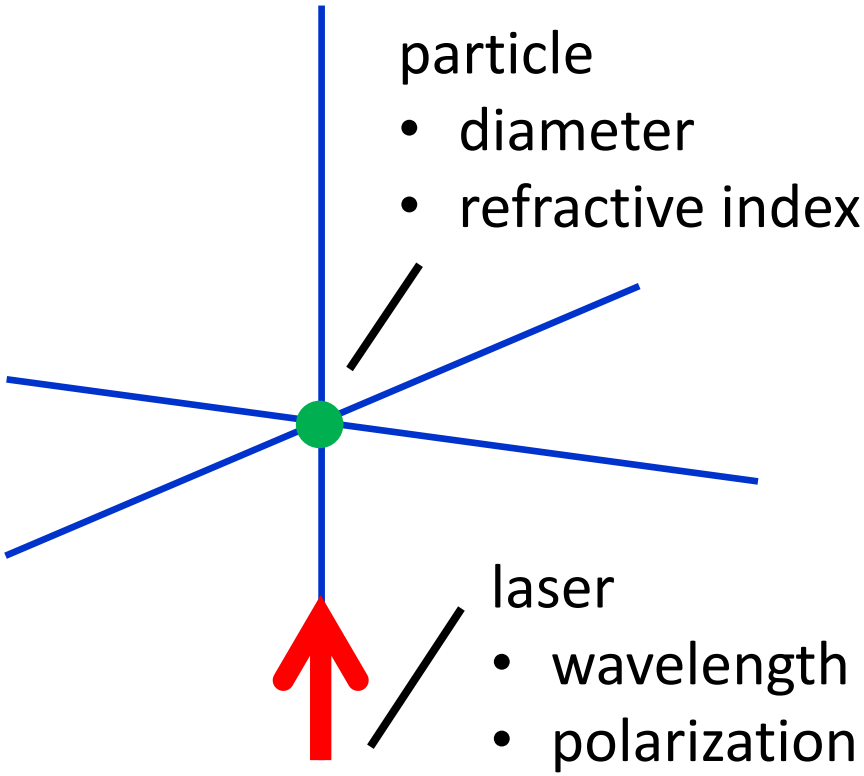
- goal: get insight in flow cytometry data

Relate scatter to diameter of beads



* Report A. Nicolet & F. Meli, www.metves.eu

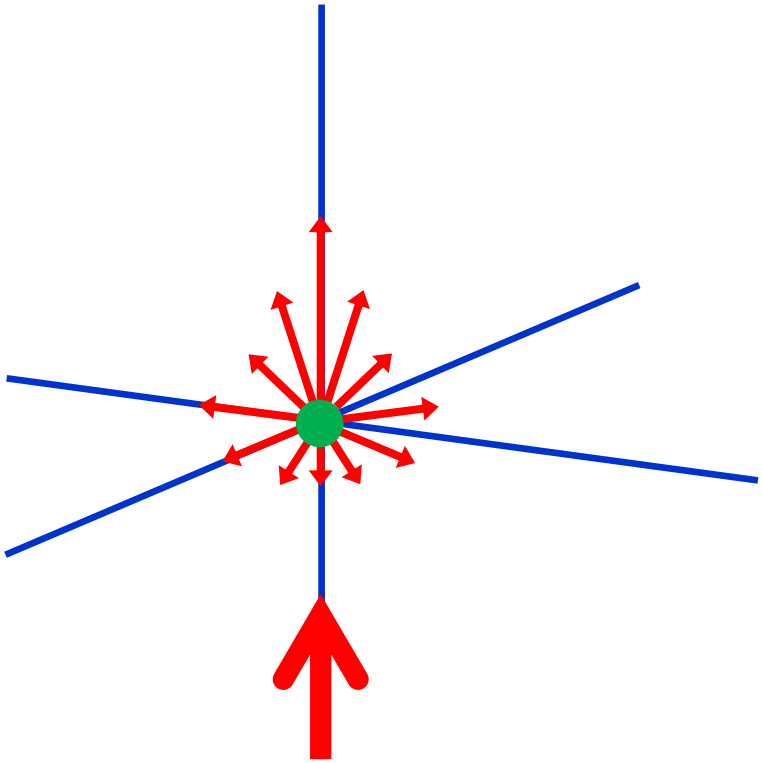
Relate scatter to diameter of beads with Mie theory



EXOMETRY

www.exometry.com

Relate scatter to diameter of beads with Mie theory



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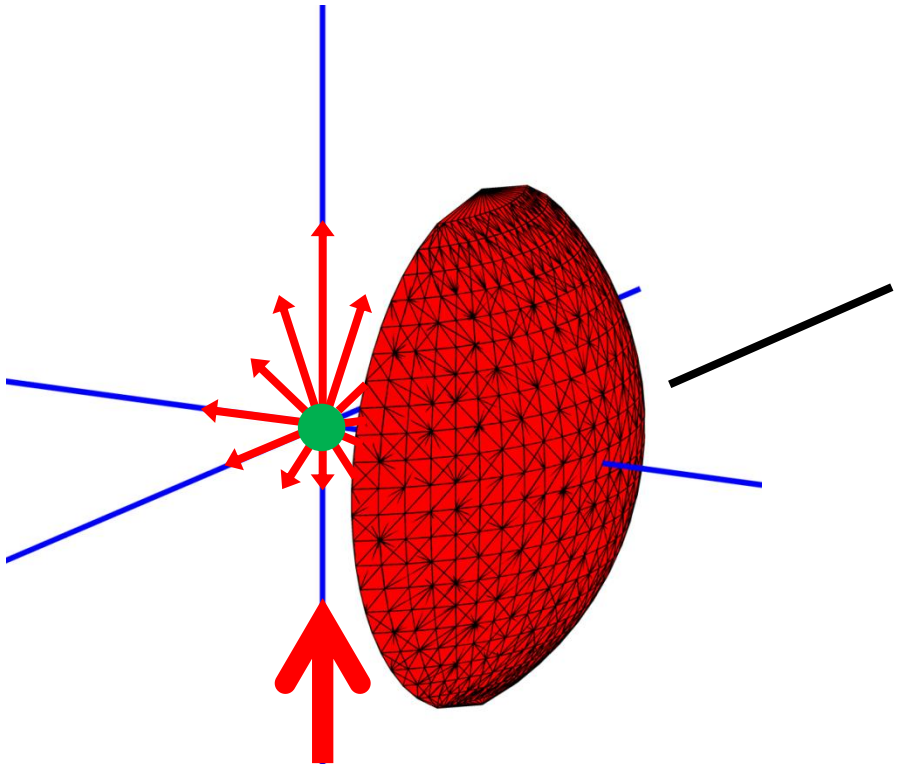
Relate scatter to diameter of beads with Mie theory

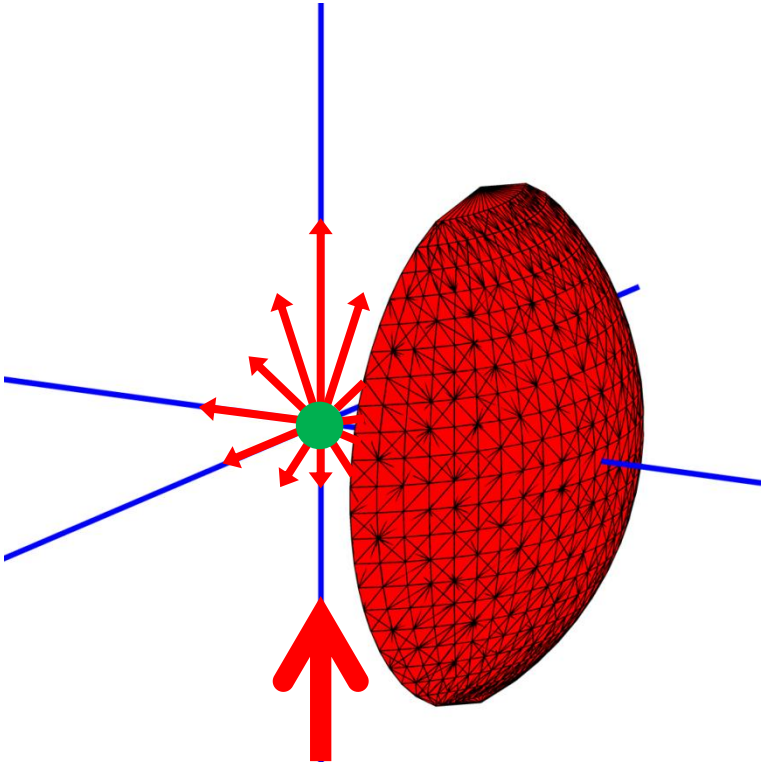


optical configuration

- collection angles
- collection efficiency
- obscuration bar
- diaphragm
- mirror

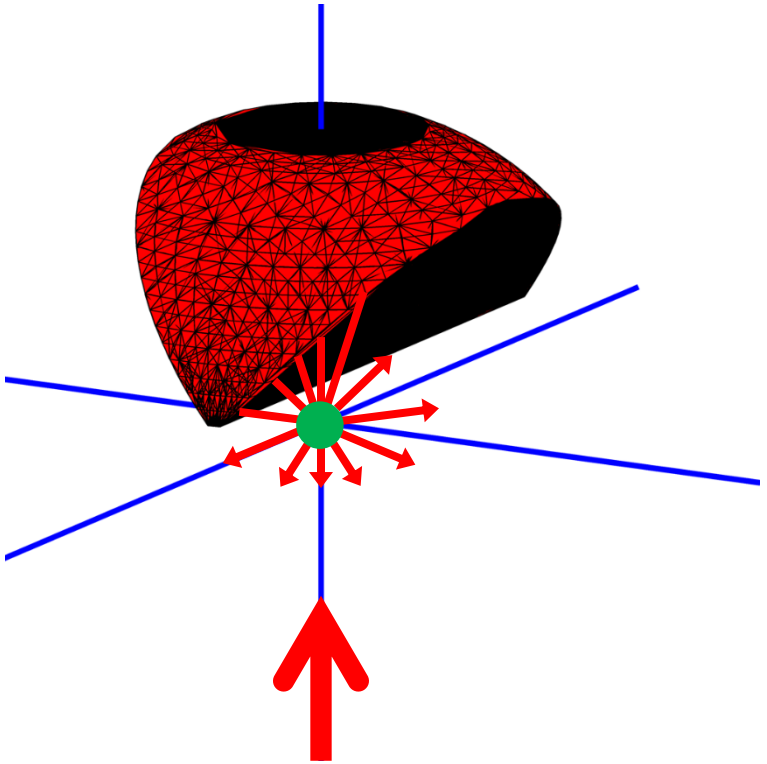
Relate scatter to diameter of particles with Mie theory

Becton Dickinson FACSCalibur



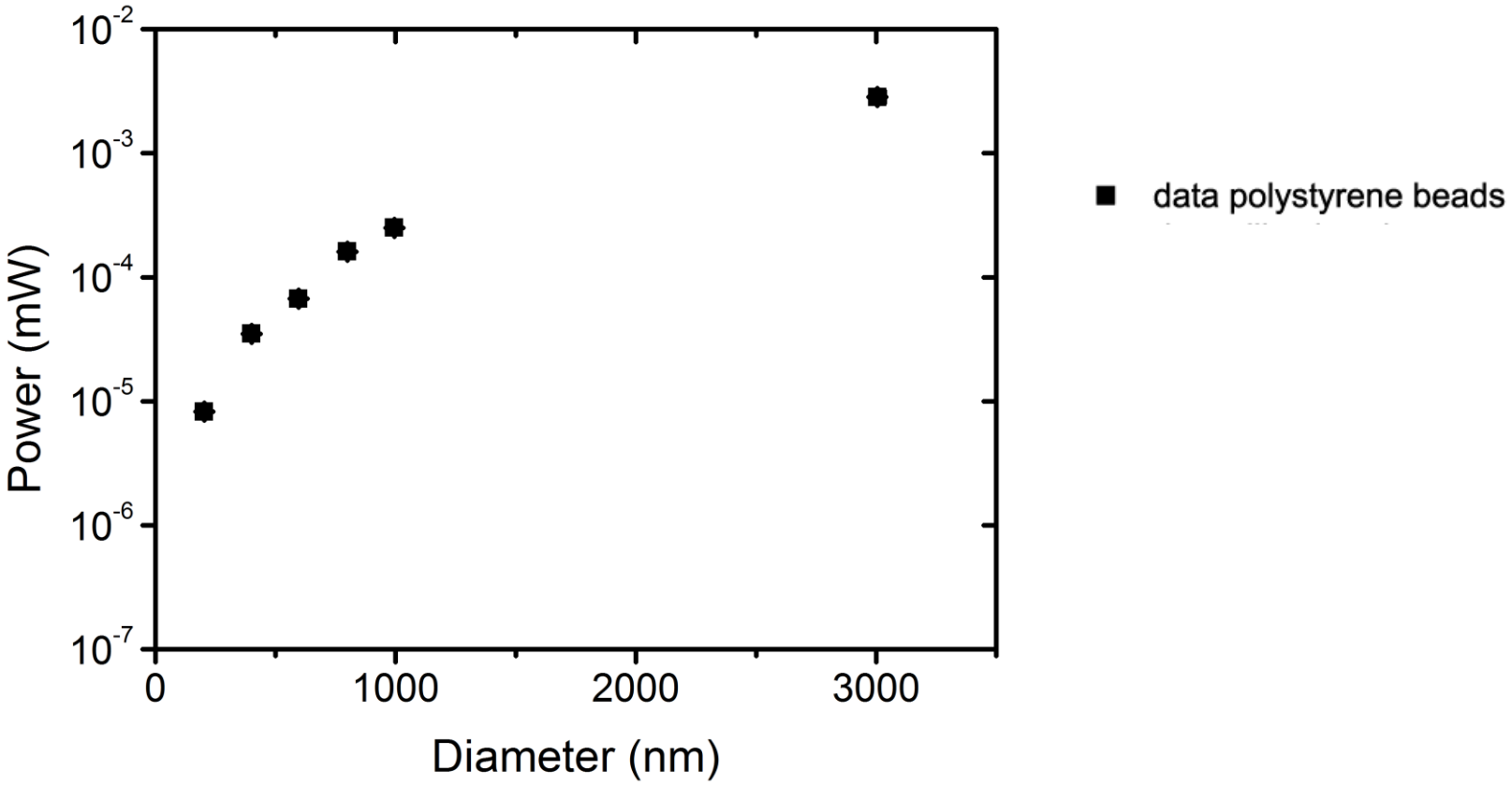
Side scatter

Apogee A50-micro

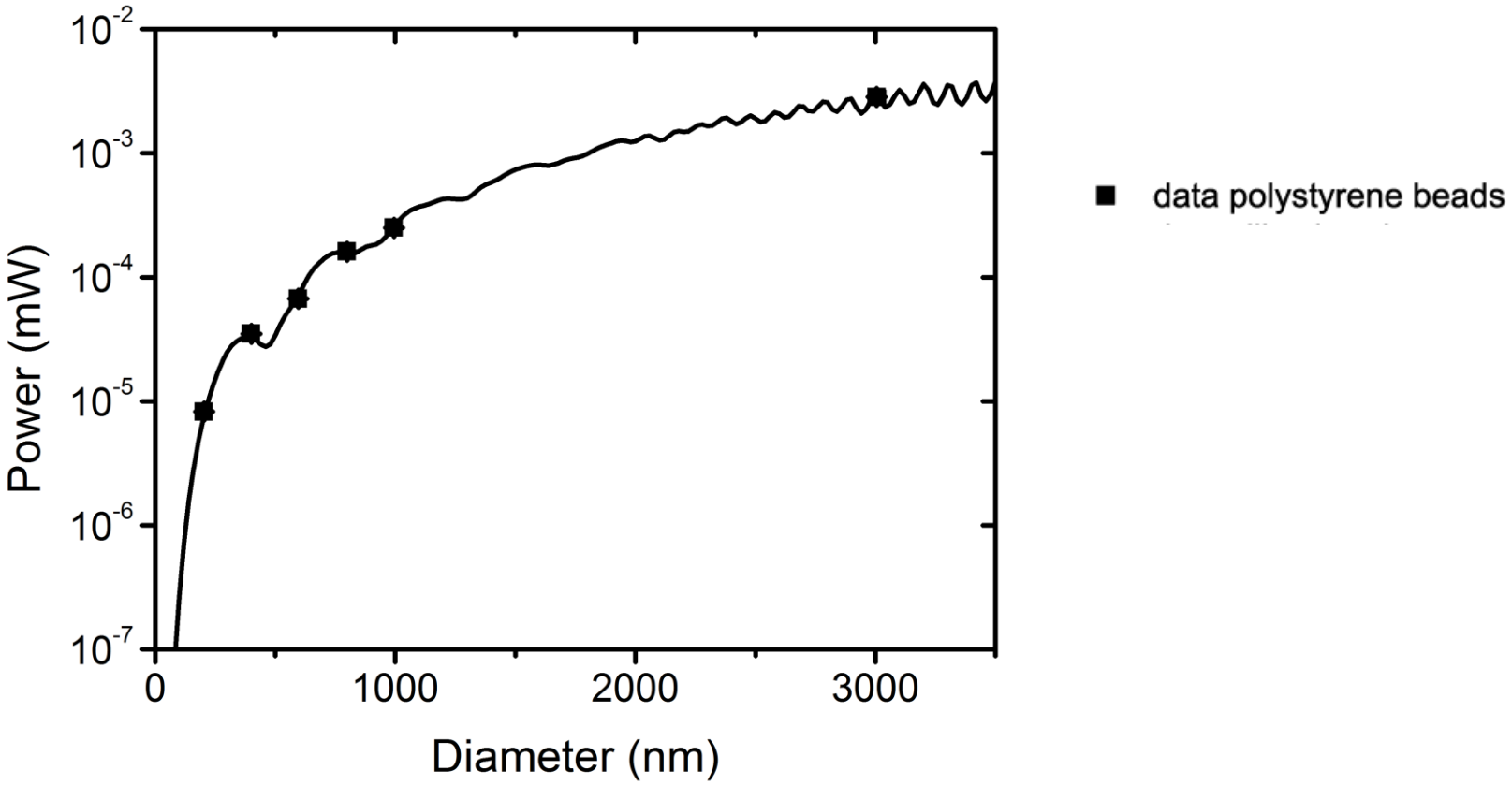


Forward scatter

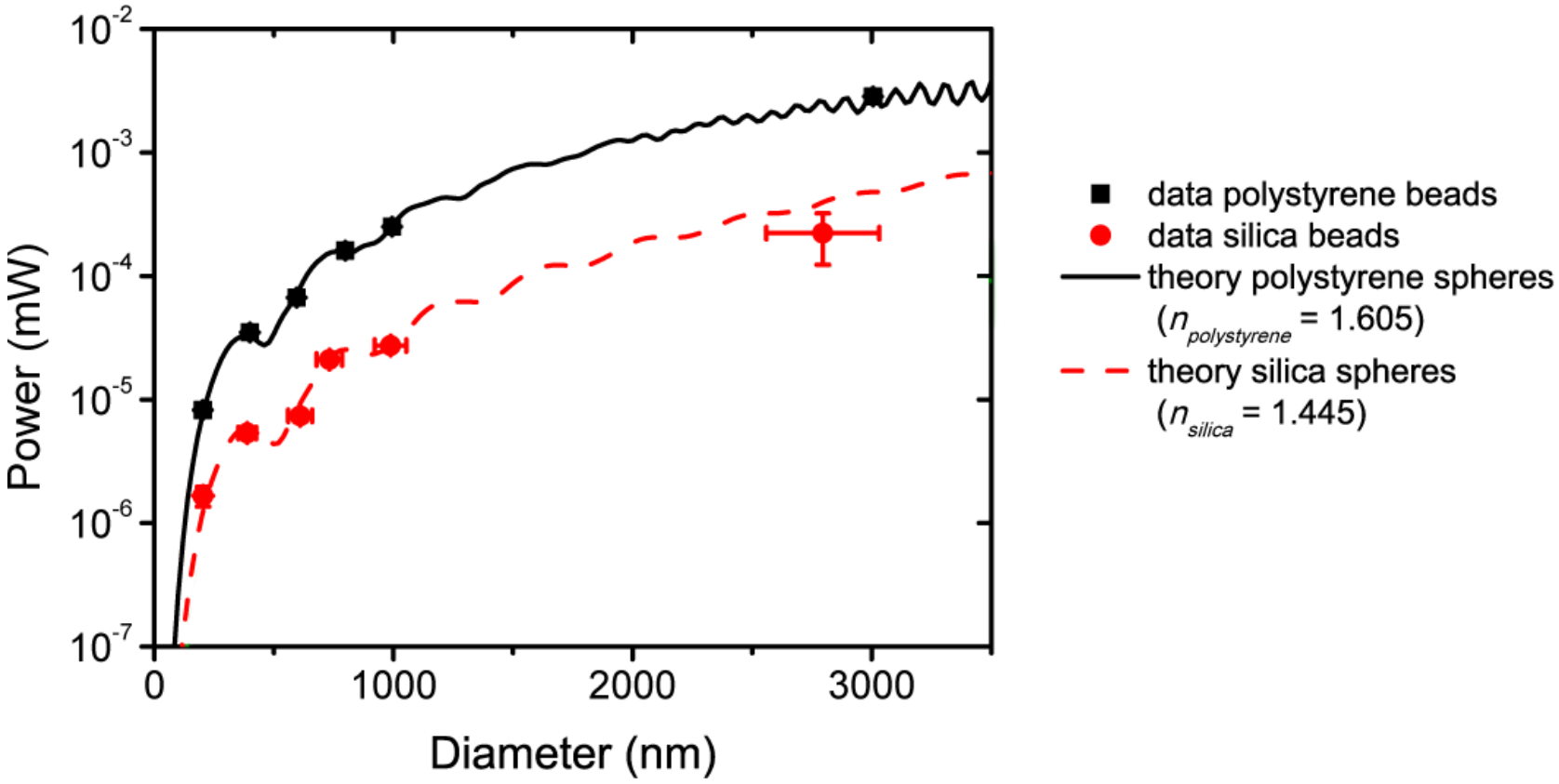
Relate scatter to diameter of beads



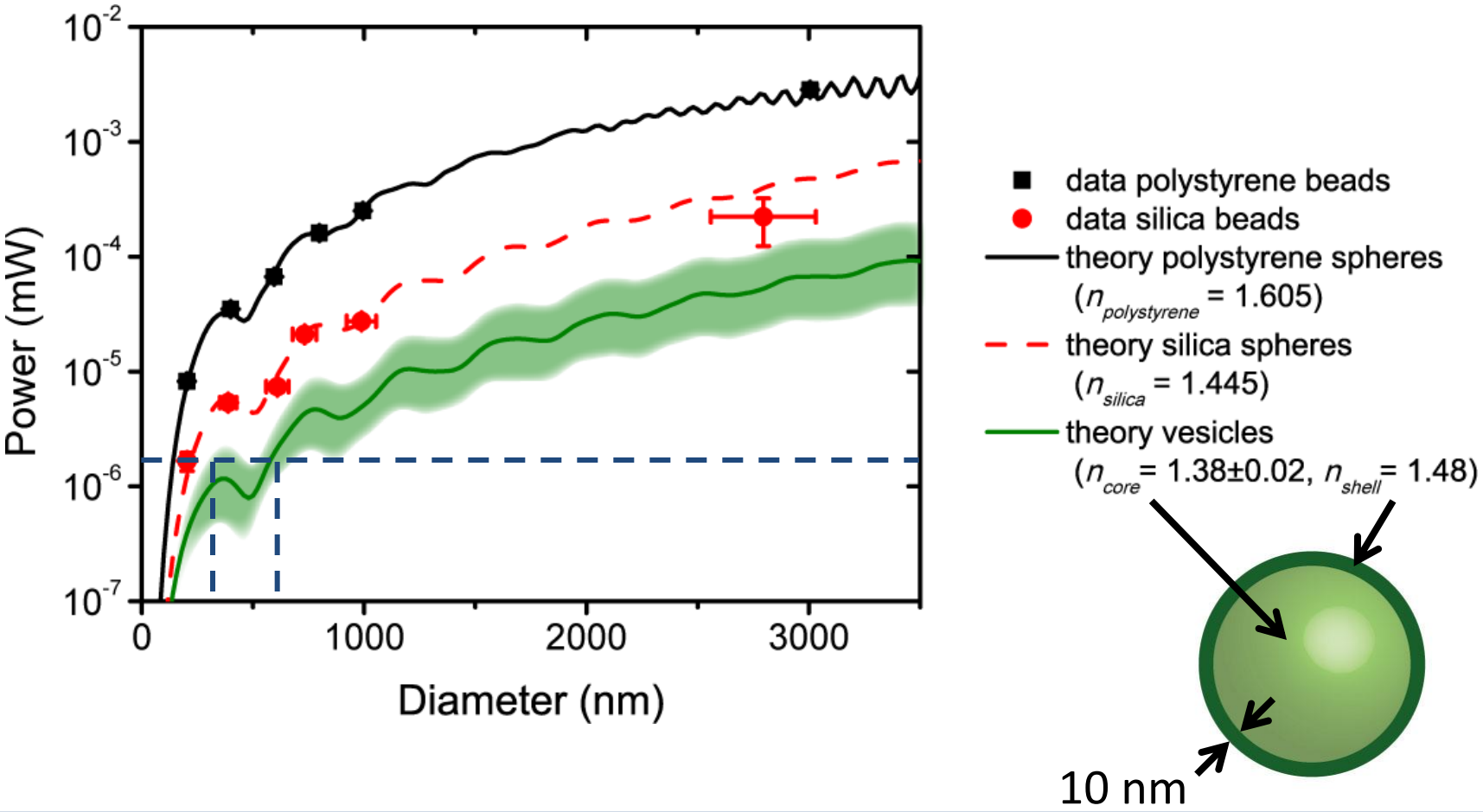
Relate scatter to diameter of beads



Relate scatter to diameter of beads

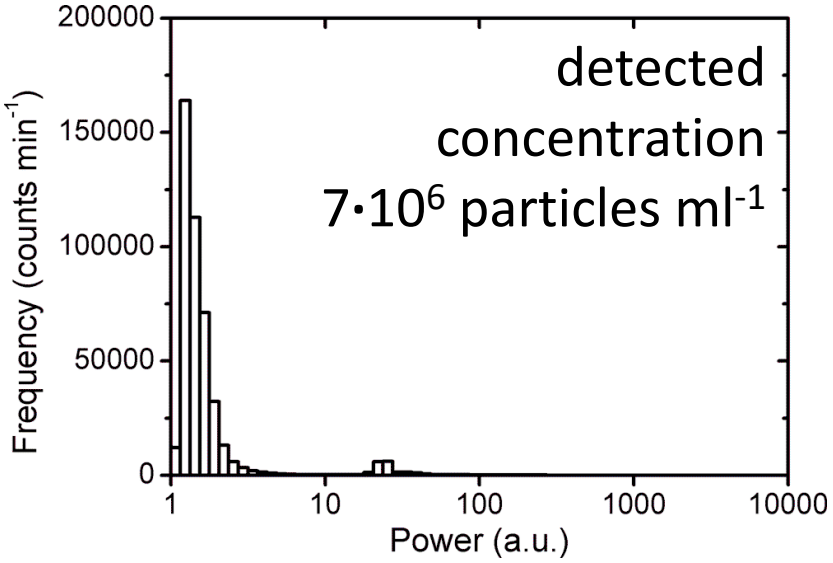


Relate scatter to diameter of vesicles

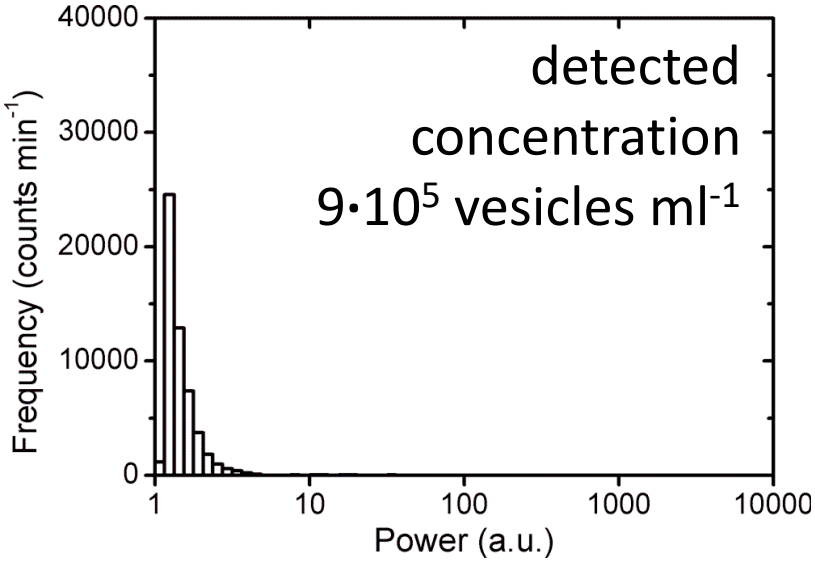


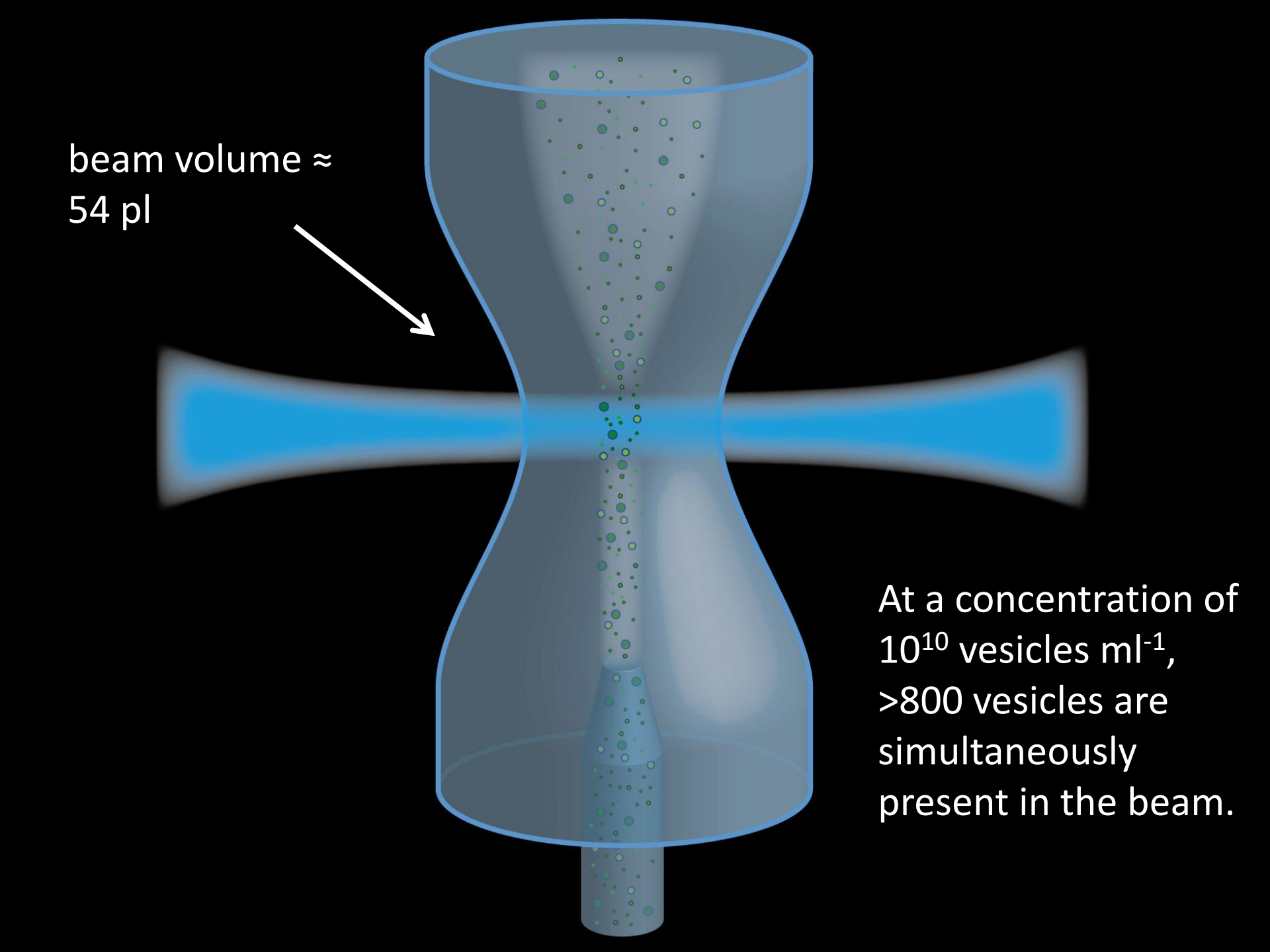
Particles that are too small to be detected generate a signal!

89 nm silica beads at concentration 10^{10} particles ml^{-1}



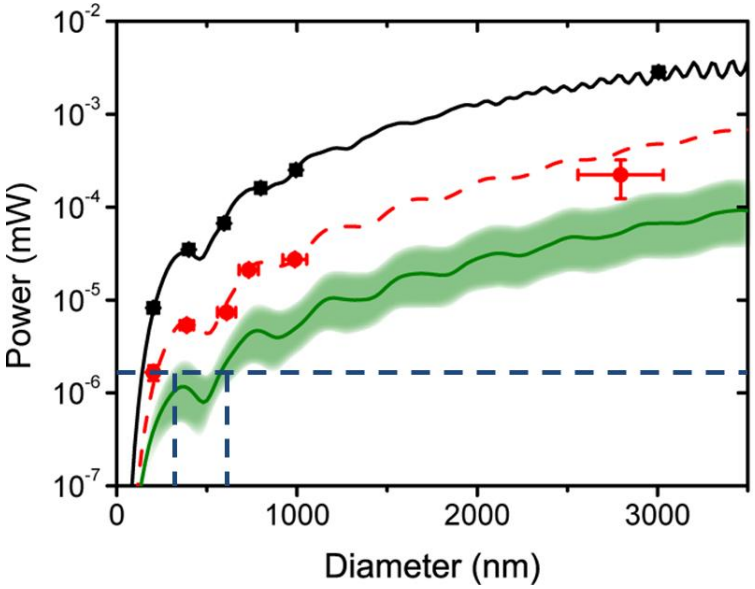
urine filtered with 220 nm filter concentration ≥ 10^{10} vesicles ml^{-1}

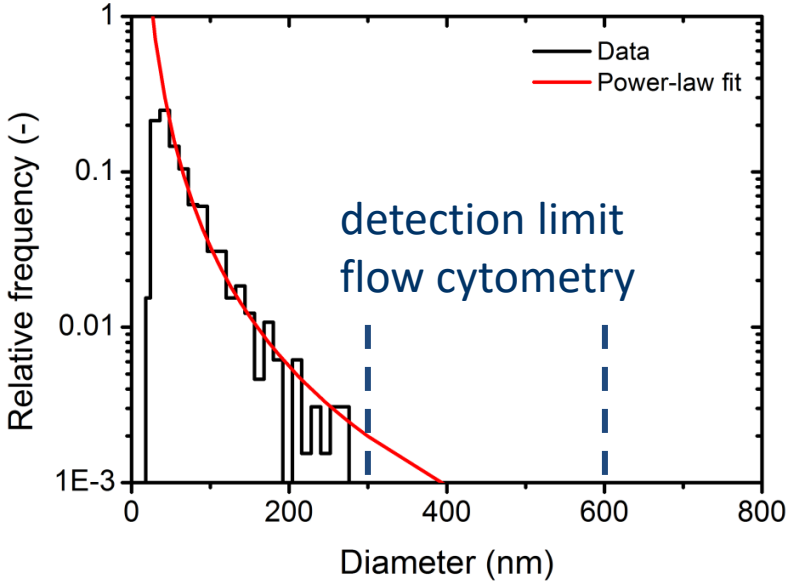



beam volume ≈
54 pl
At a concentration of
10^{10} vesicles ml^{-1},
>800 vesicles are
simultaneously
present in the beam.

Swarm detection

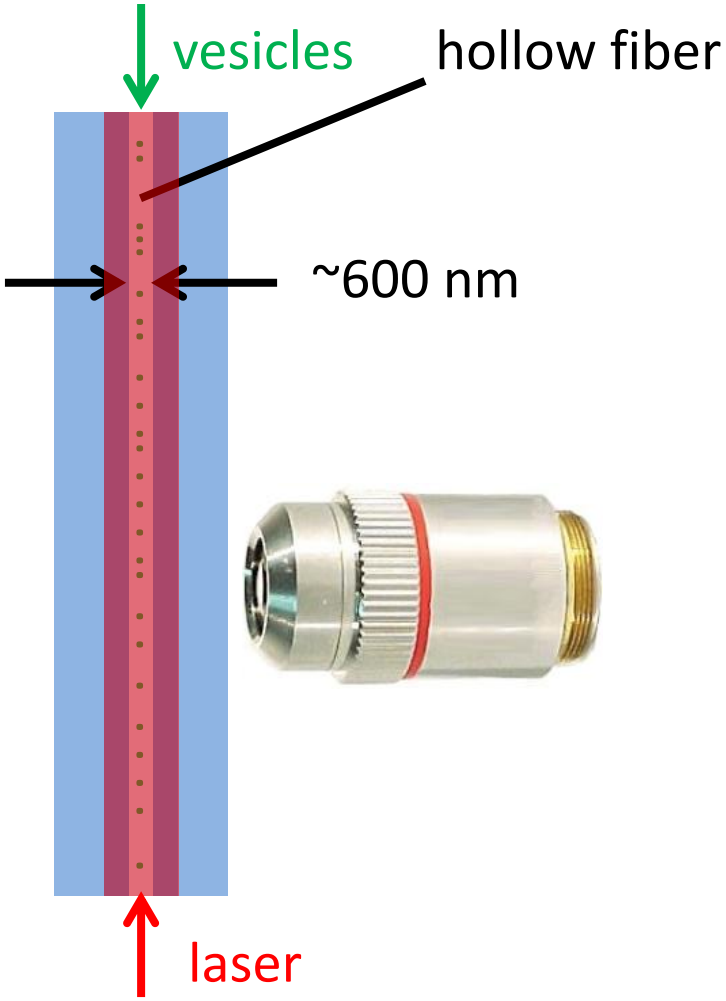
Summary vesicle detection by flow cytometry





- single event signal attributed to scattering from *multiple* vesicles
- flow cytometry detects <1% of all vesicles
- vesicle researchers need improved sensitivity!

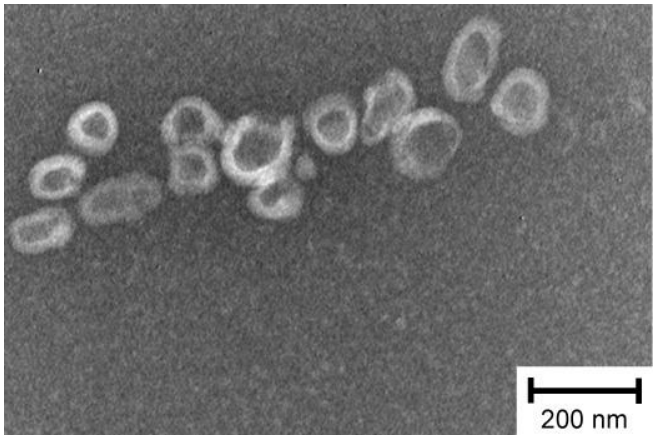
Nano-EPics

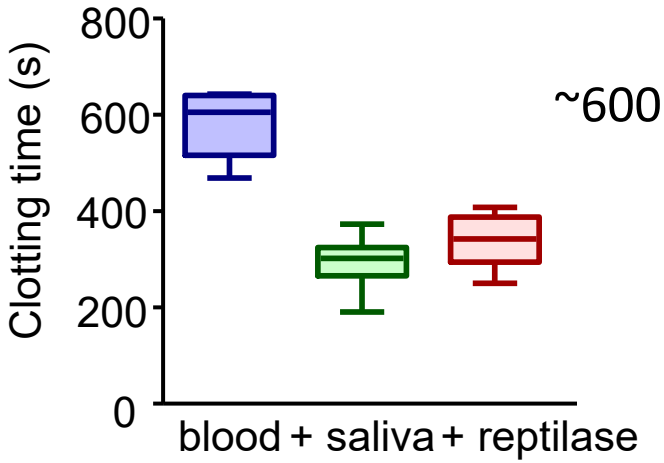


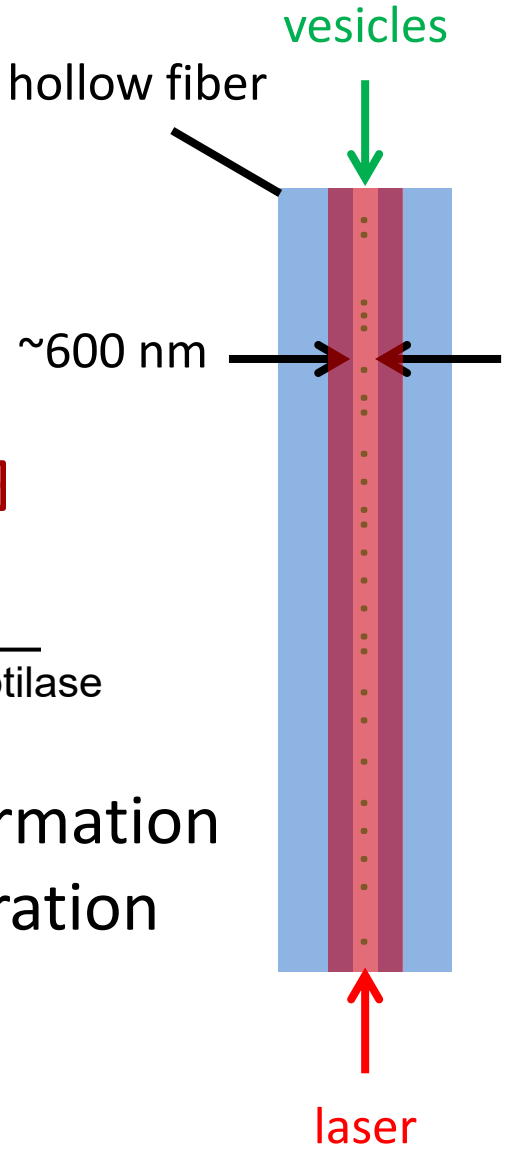
- contact: Sanli Faez
  s.faez@uu.nl
- fiber: Heraeus Quartz Glass

- scattering of single…
  - 35-70 nm urinary vesicles (this video)
  - 19 nm polystyrene beads (submitted paper)
  - contact: Sanli Faez (s.faez@uu.nl)

Summary



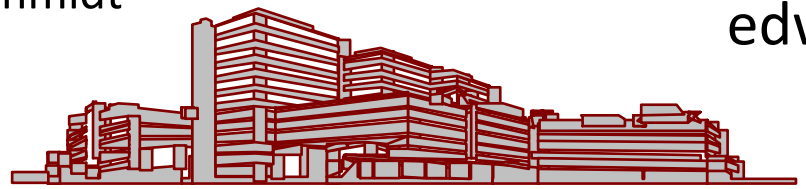




- extracellular vesicles contain clinical information
- tool is needed to determine the concentration of a vesicle subpopulation
- *you* may provide the solution

Acknowledgements

- Academic Medical Center
  - Biomedical Engineering & Physics
    - Ton van Leeuwen
    - Frank Coumans
  - Laboratory of Experimental Clinical Chemistry
    - Rienk Nieuwland
    - Guus Sturk
- University of Utrecht
    - Sanli Faez
- Heraeus Quartz Glass
    - Stefan Weidlich
    - Markus Schmidt



More information:
edwinvanderpol.com