Detection of extracellular vesicles: size does matter

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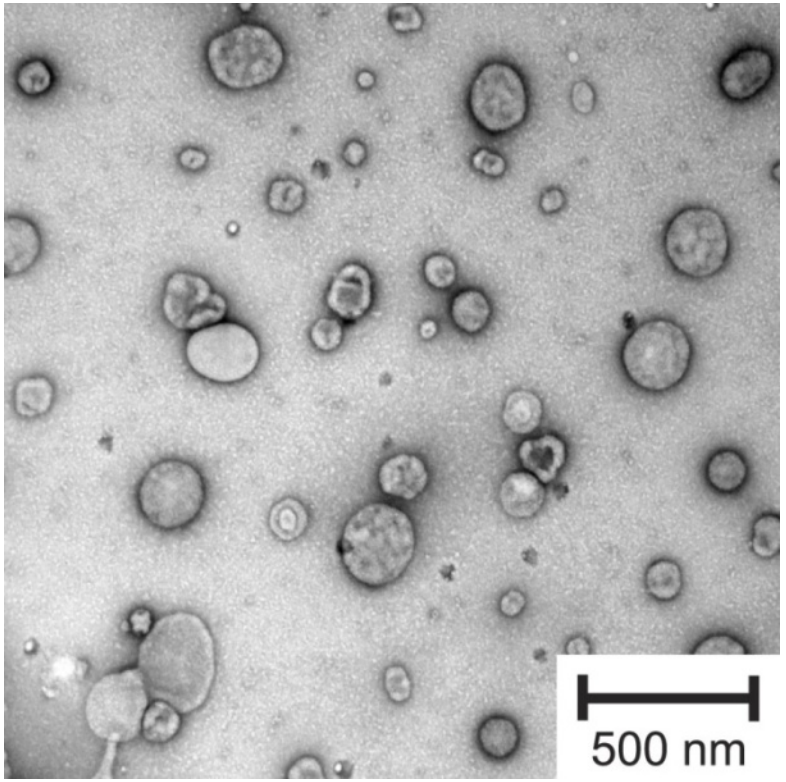
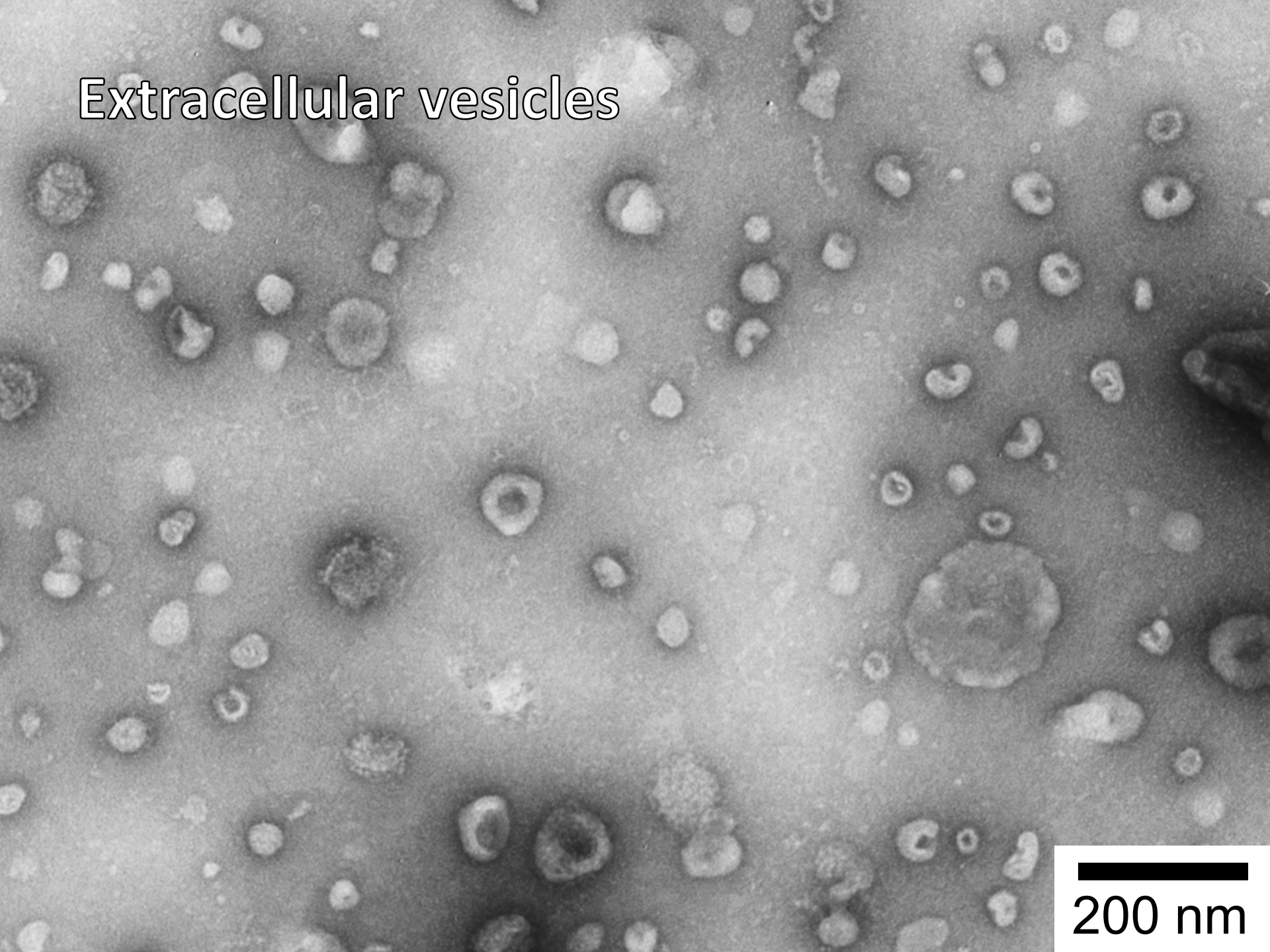
Amsterdam UMC
University Medical Centers

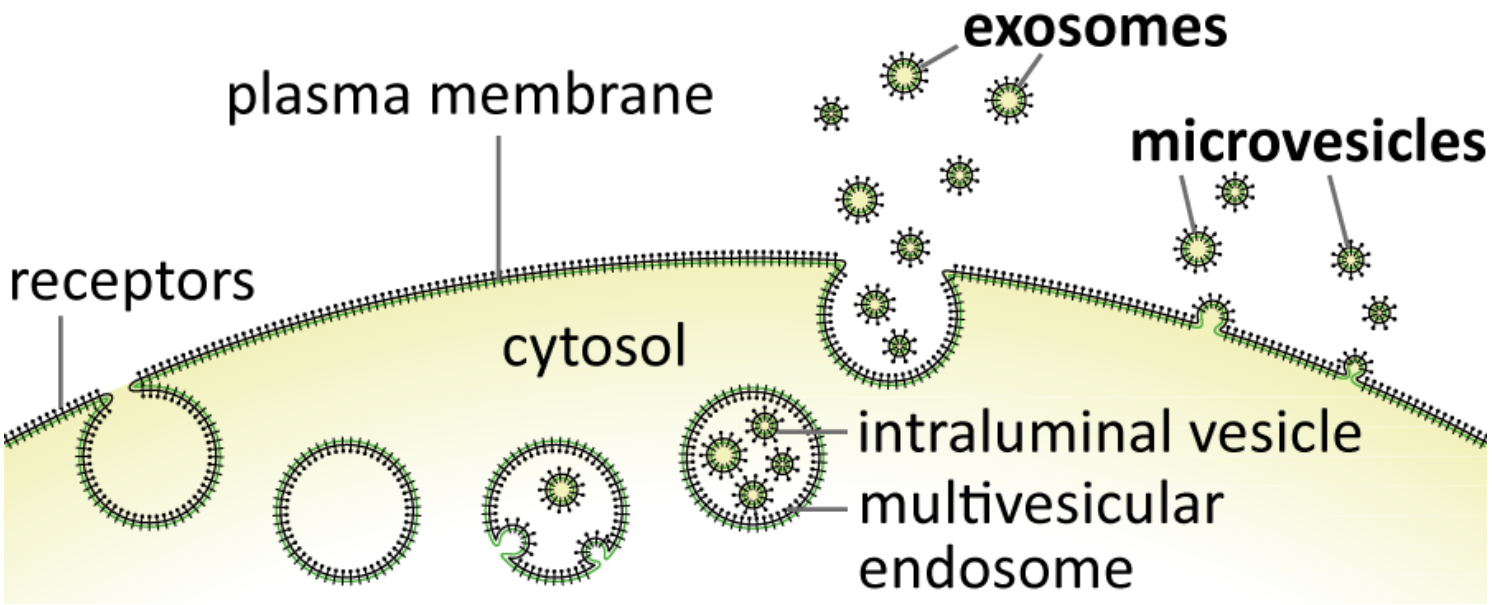
Outline

- PhD research
  - Extracellular vesicles
  - Size does matter
  - Flow cytometry
- Outlook
  - Standardisation
  - Clinical studies
  - Small *and* fast




Extracellular vesicles
200 nm

Extracellular vesicles

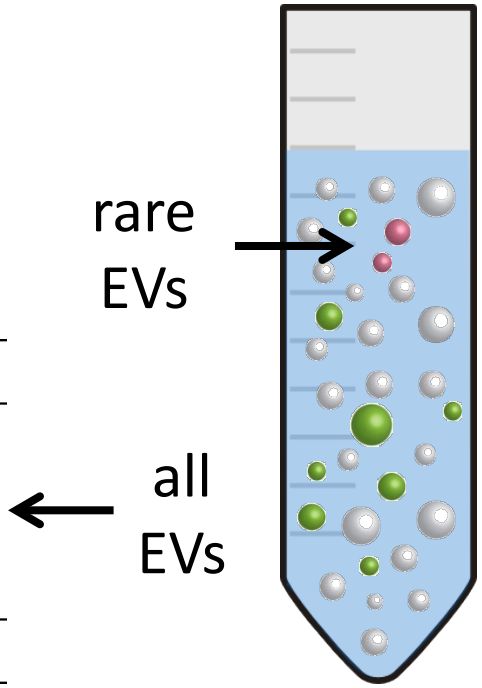


- Cells release EVs:
  biological nanoparticles with receptors, DNA, RNA
- Specialized functions
- Clinically relevant

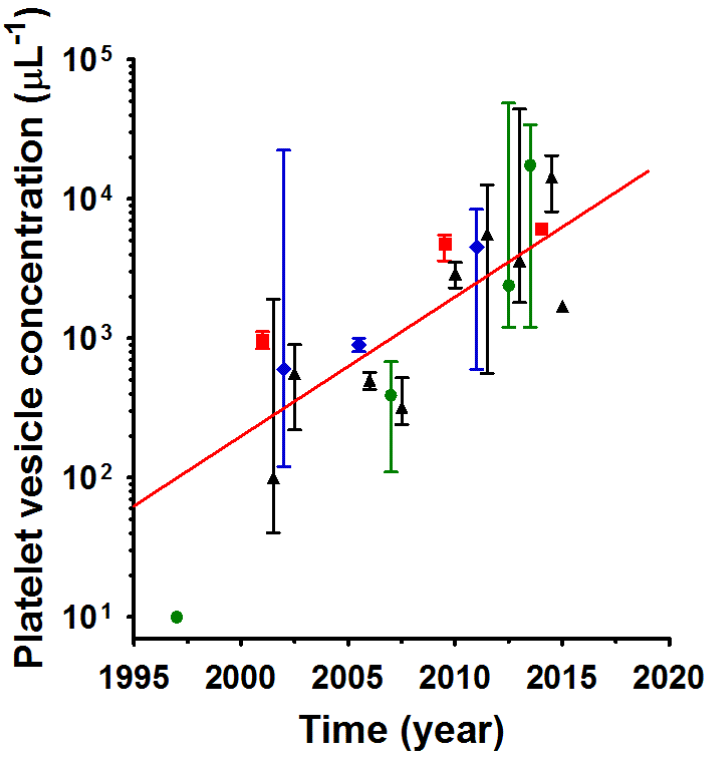
van der Pol et al. *Pharmacol Rev* 2012

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



Problem: counting vesicles is difficult



"Gąsecka's law"

- Reported concentrations of blood vesicles differ >10^6-fold
- Clinical data cannot be compared

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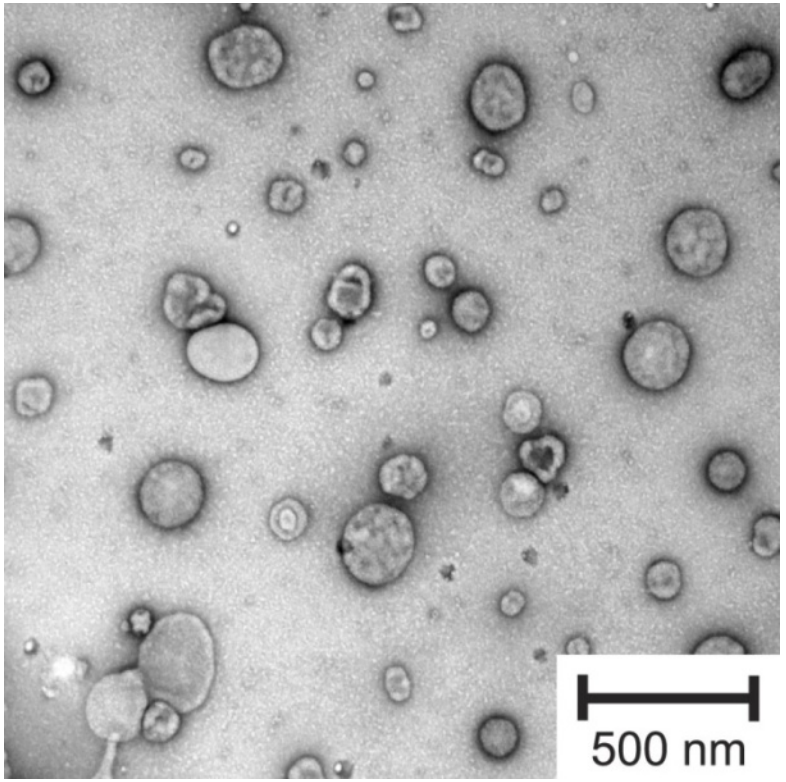
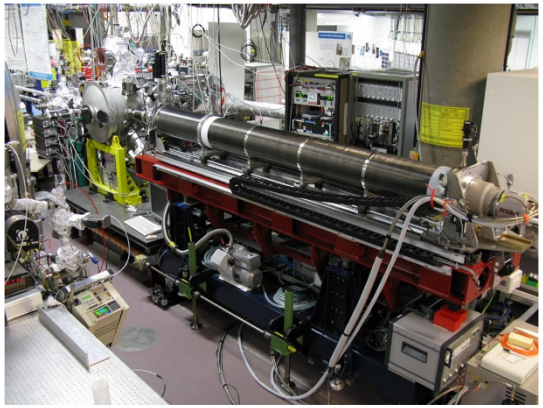
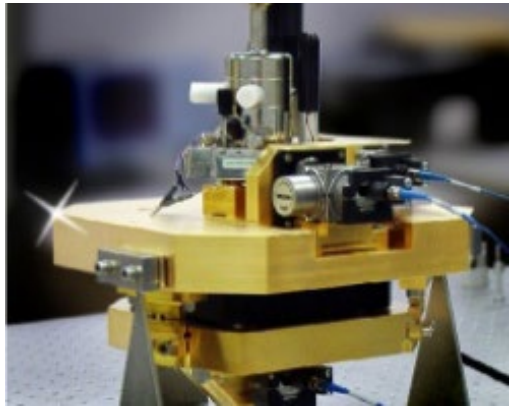


Size distribution of extracellular vesicles?

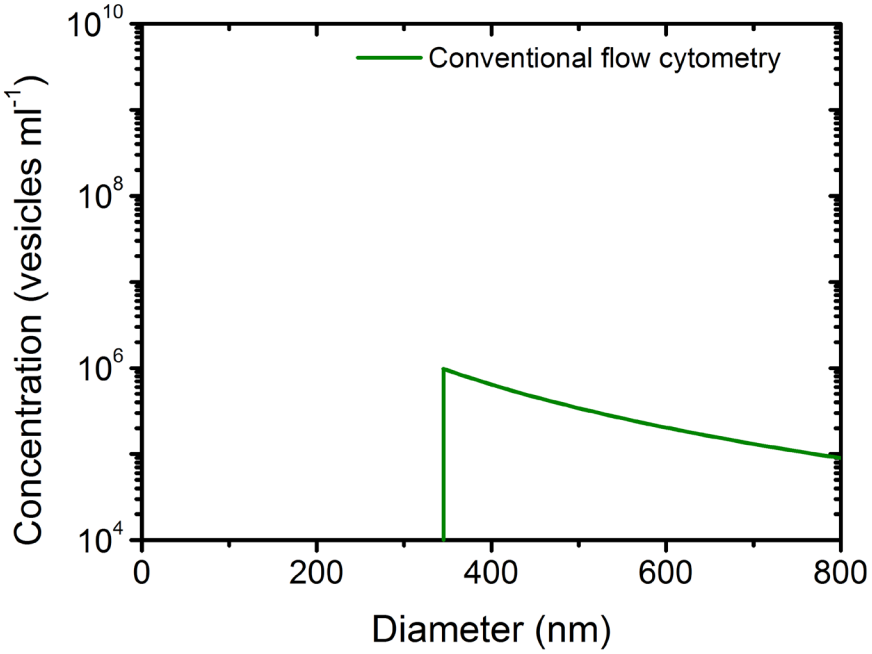
Extracellular vesicles

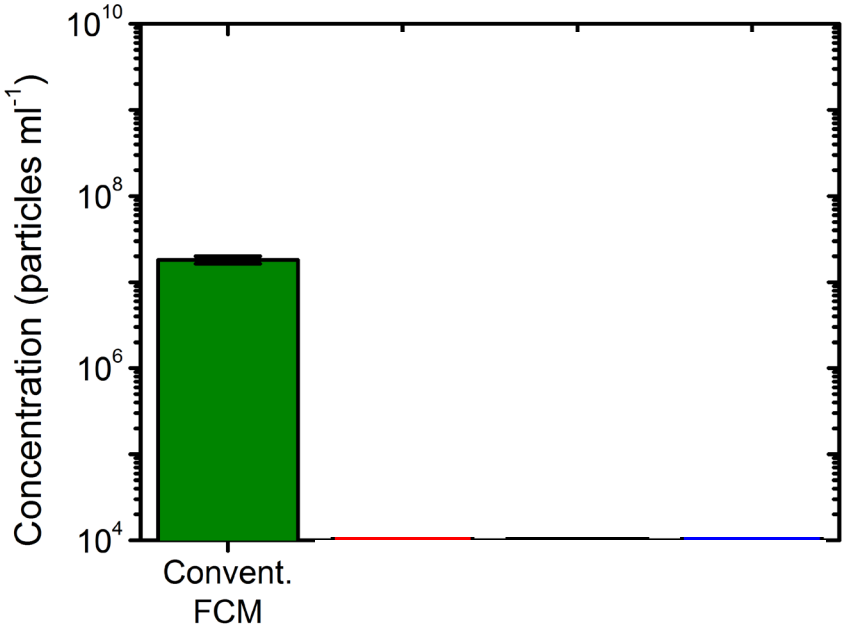
Reference particles





Size distribution of extracellular vesicles





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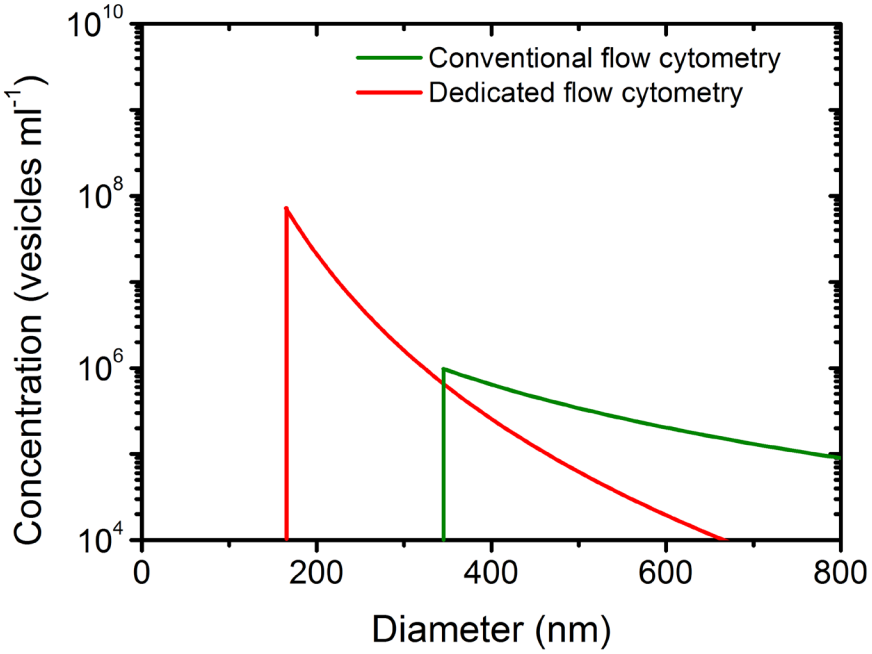

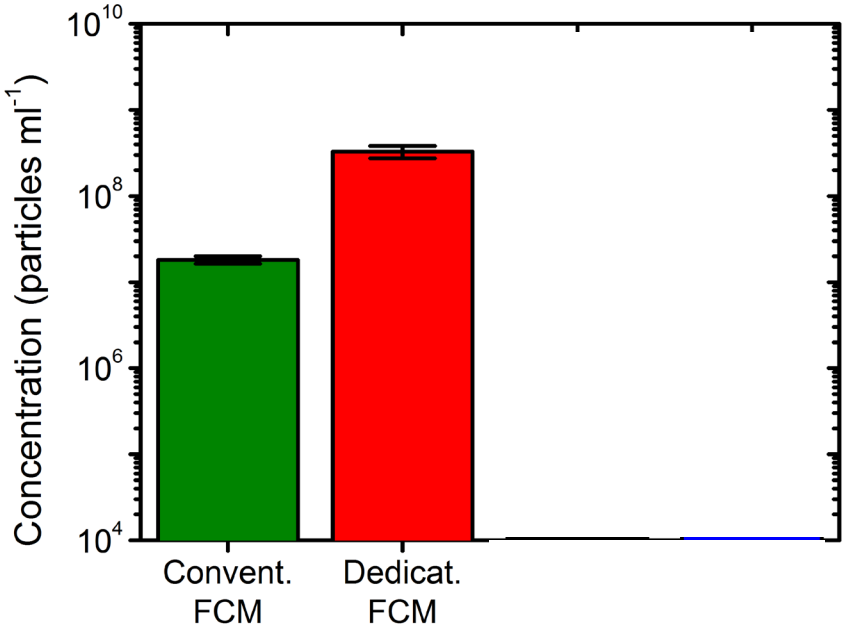

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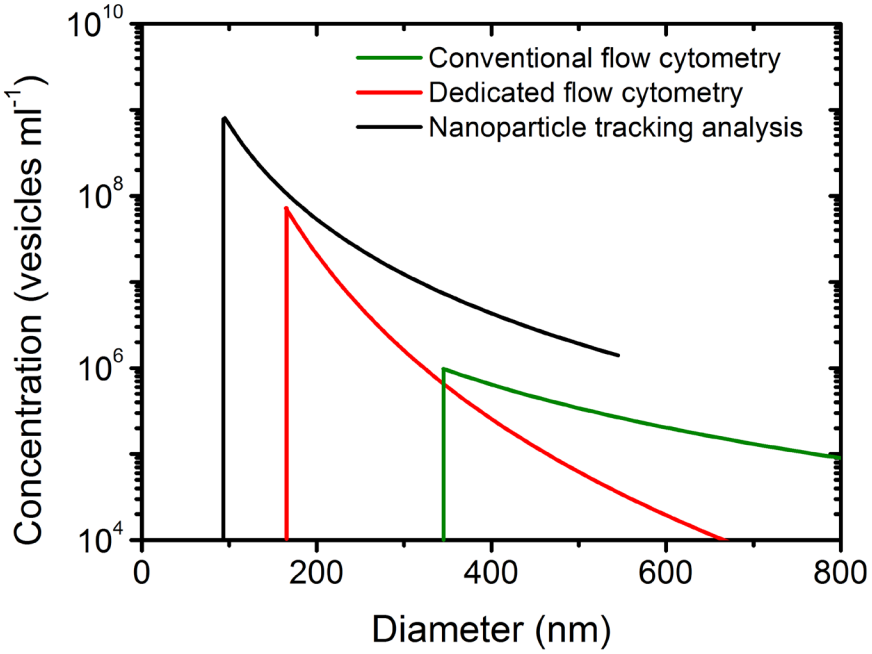

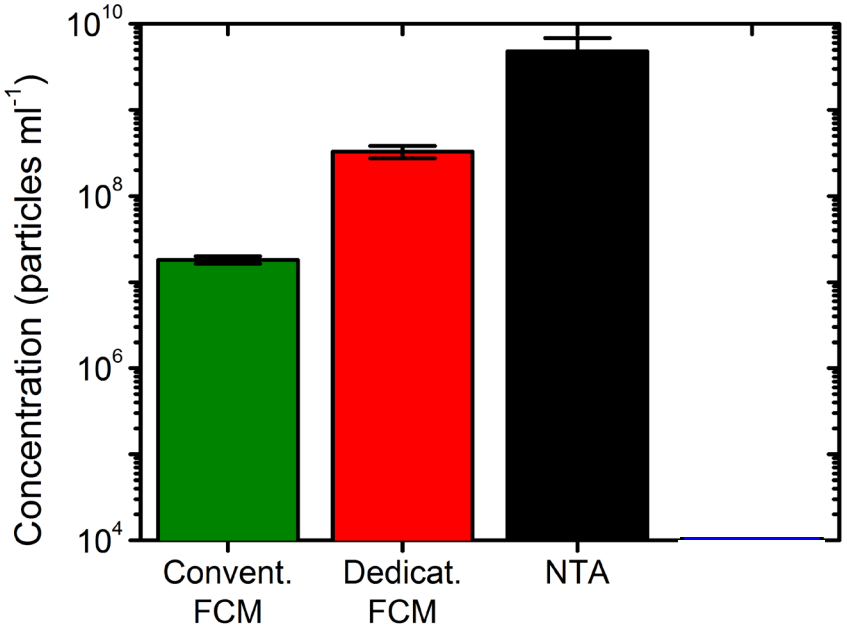

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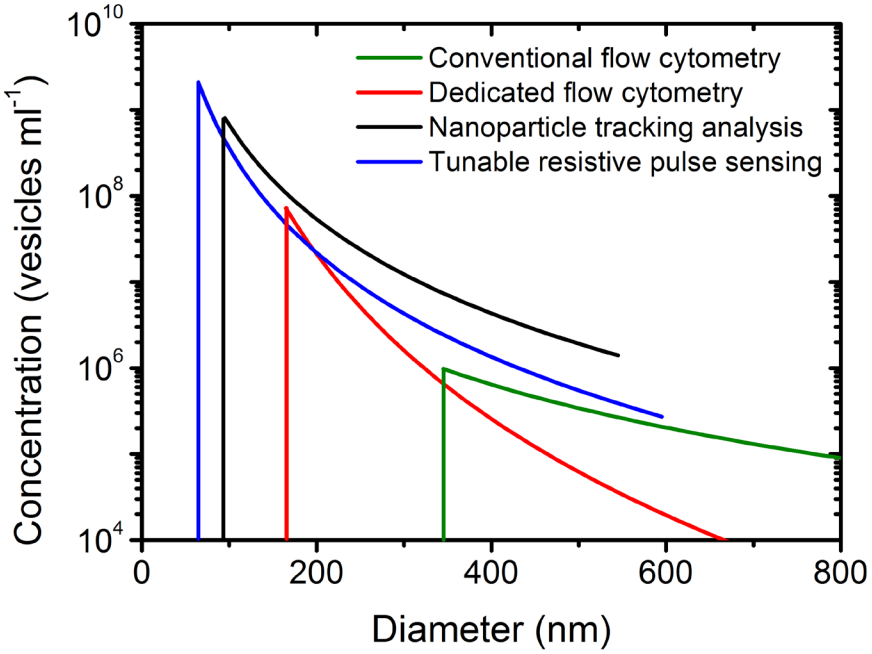

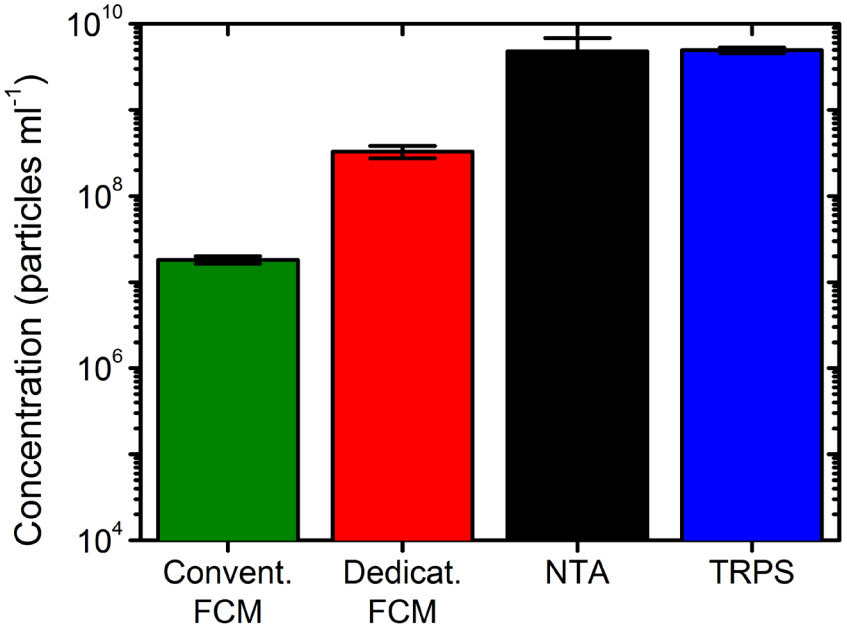

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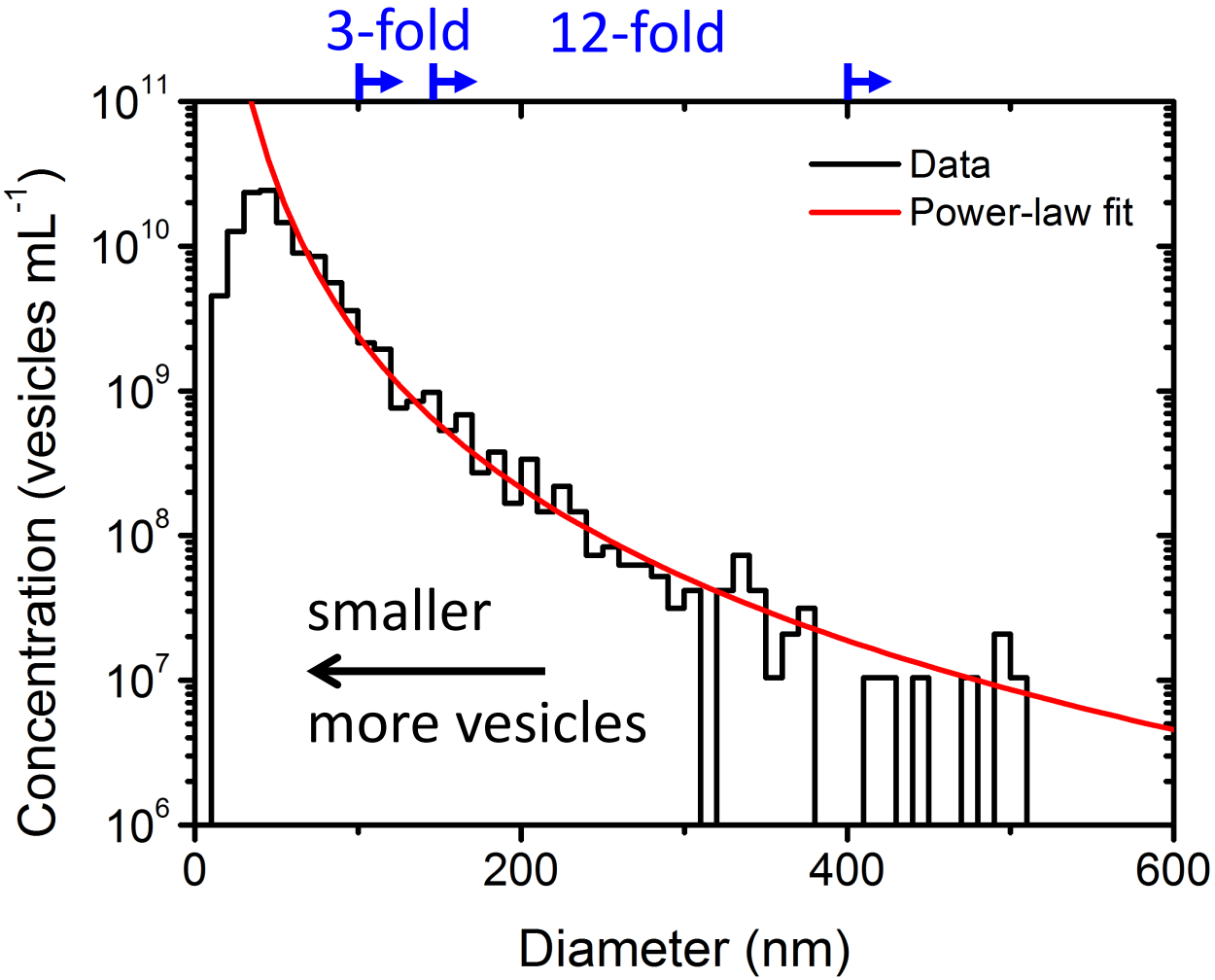

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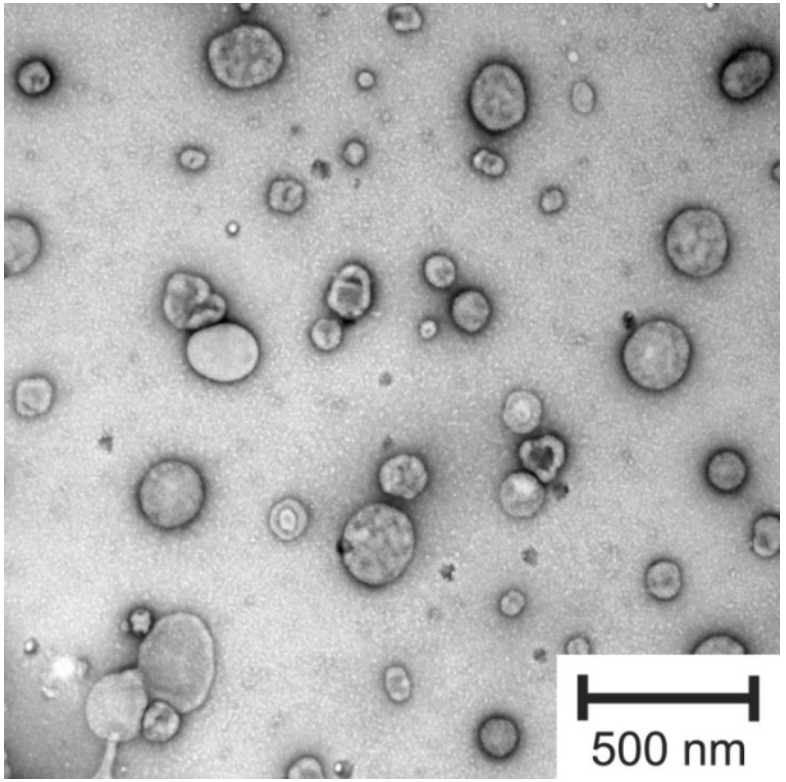
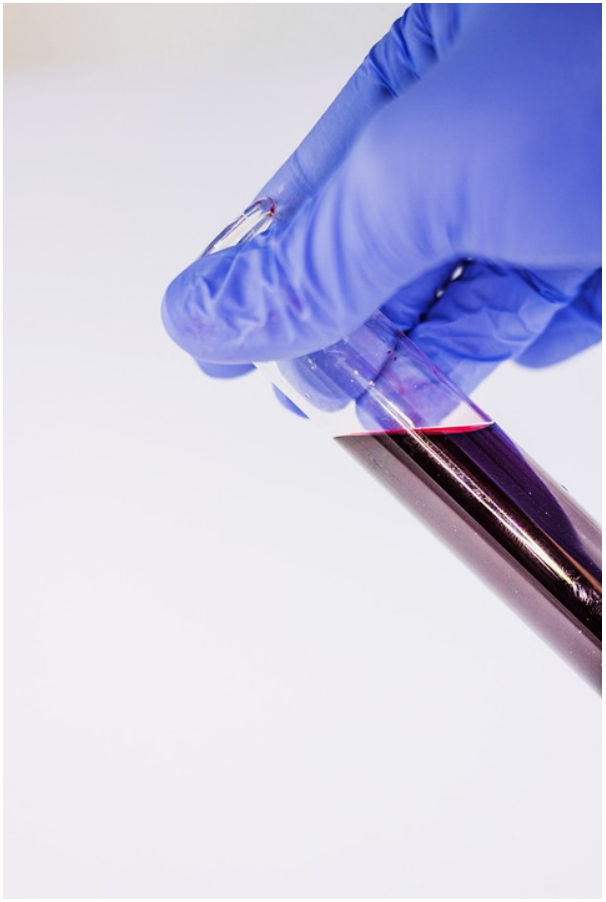


Problem: count the black grains



Solution: flow cytometry

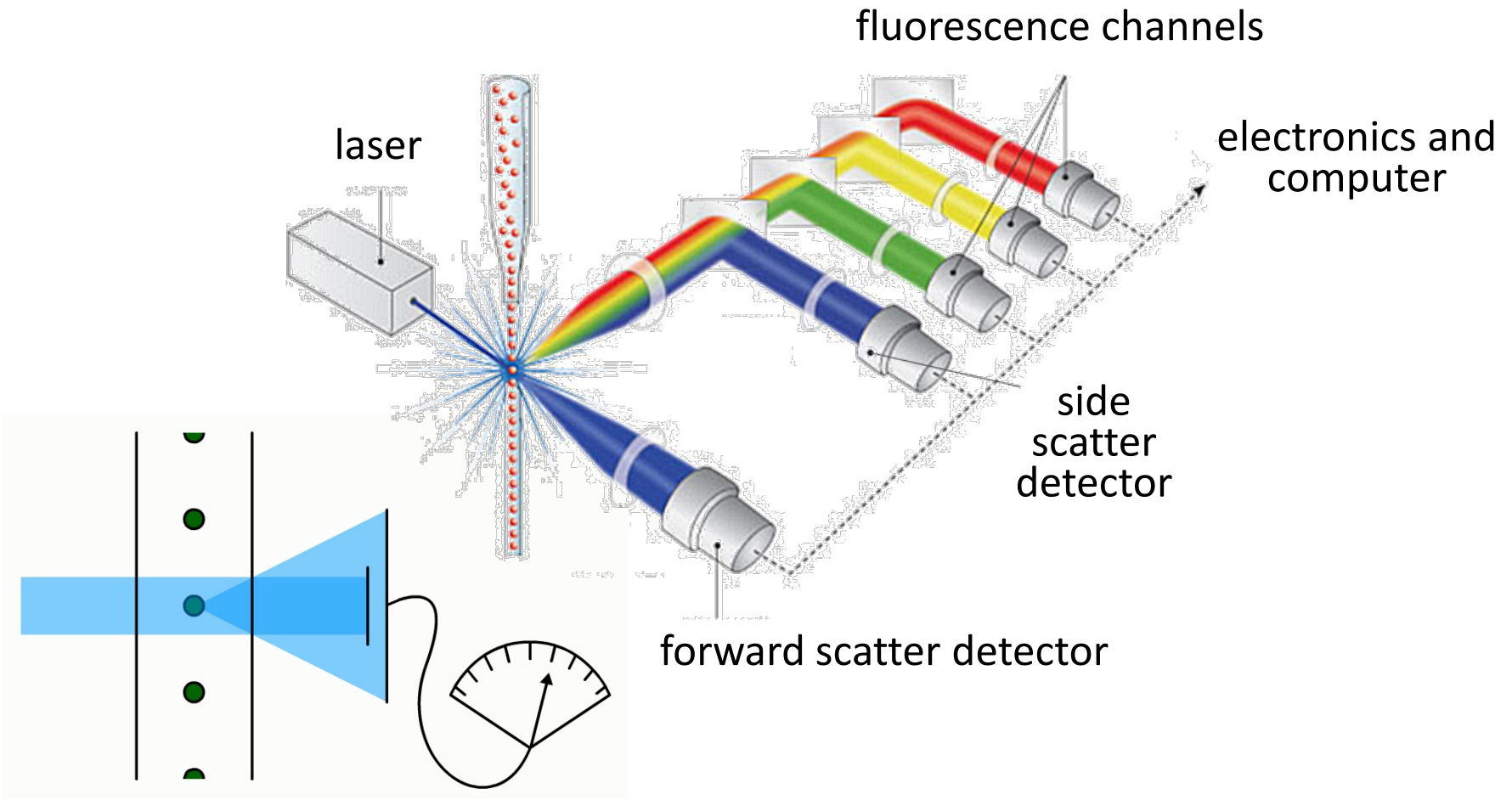
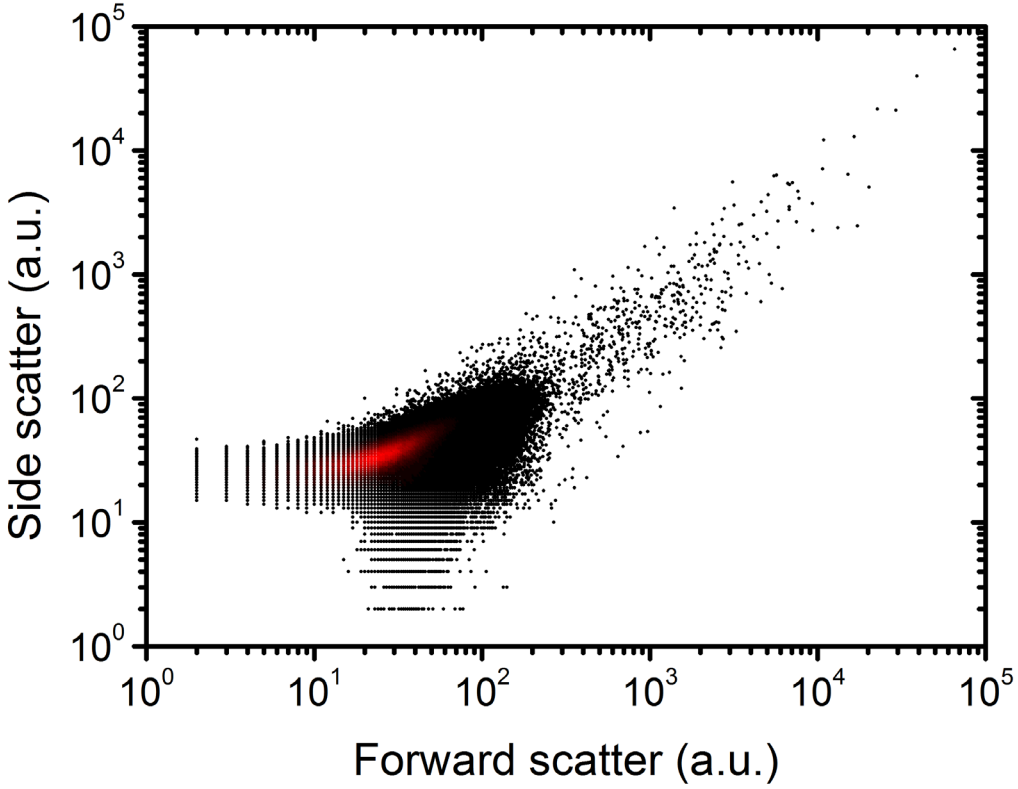


image: semrock.com

Problem: which size of vesicles do I measure?

Flow cytometry:



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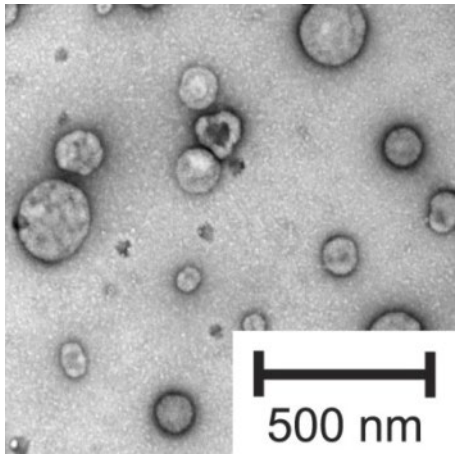
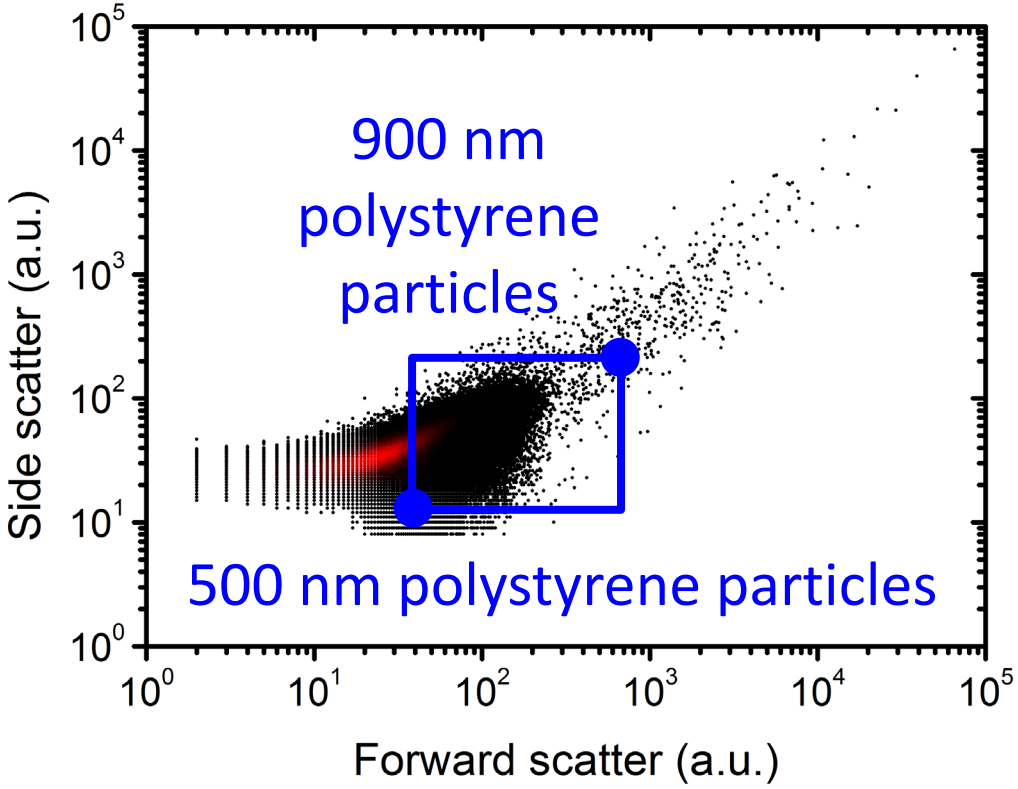
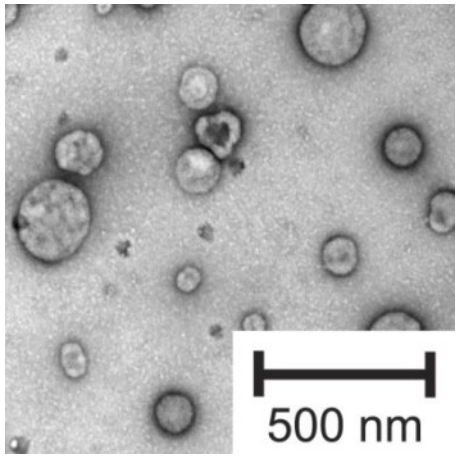


Flow cytometry calibration before 2012

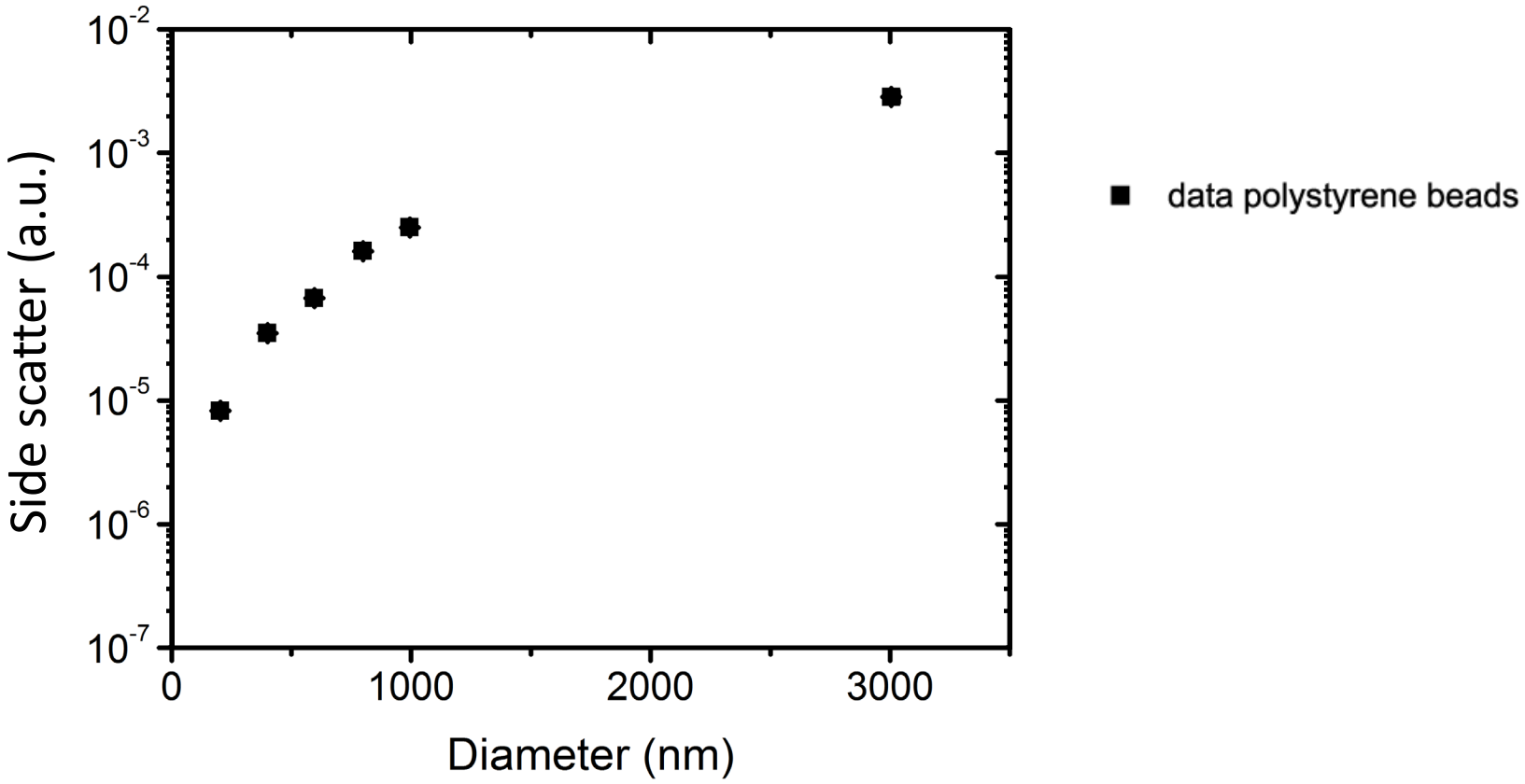
Flow cytometry:



?



Relate scatter to diameter of beads



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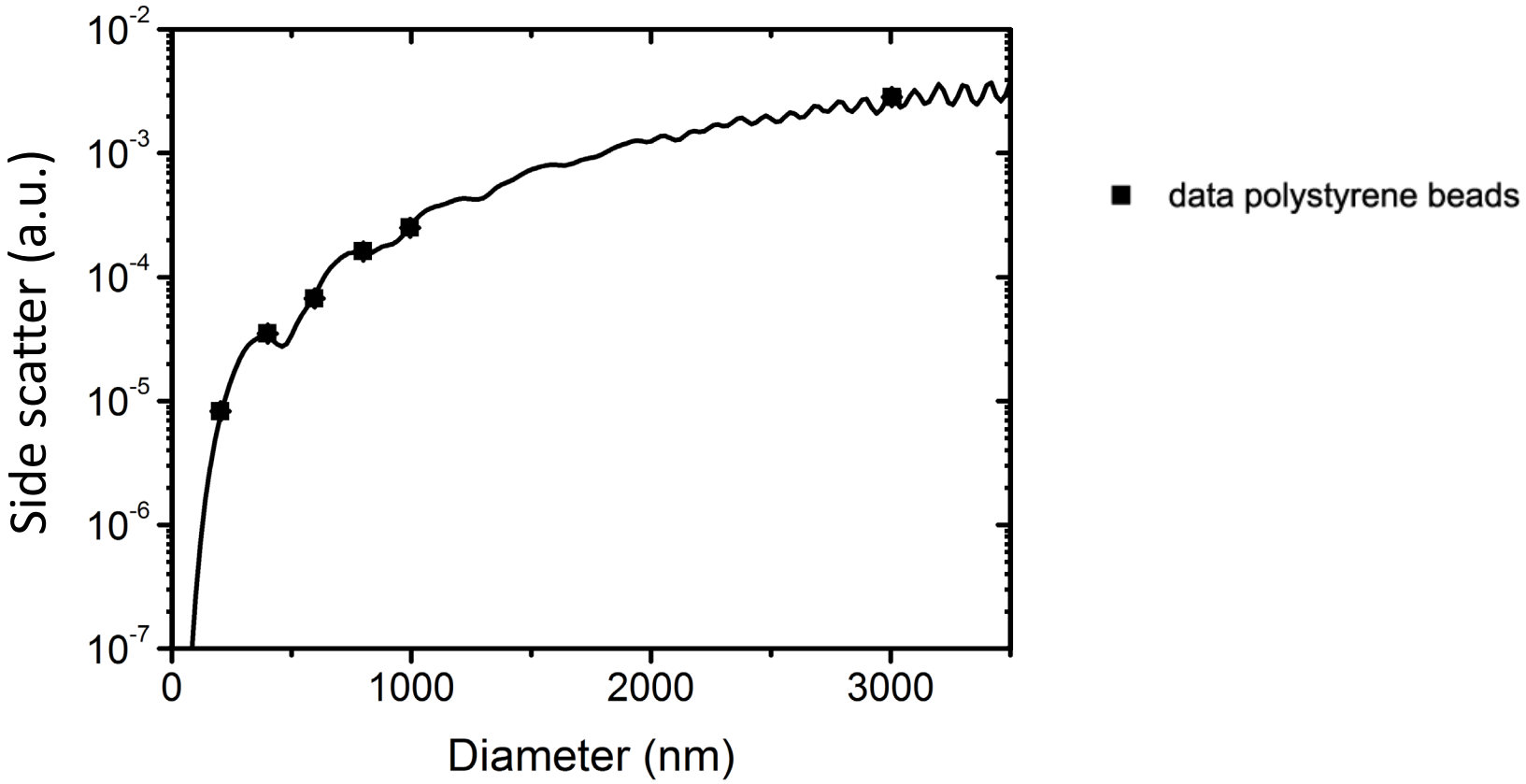

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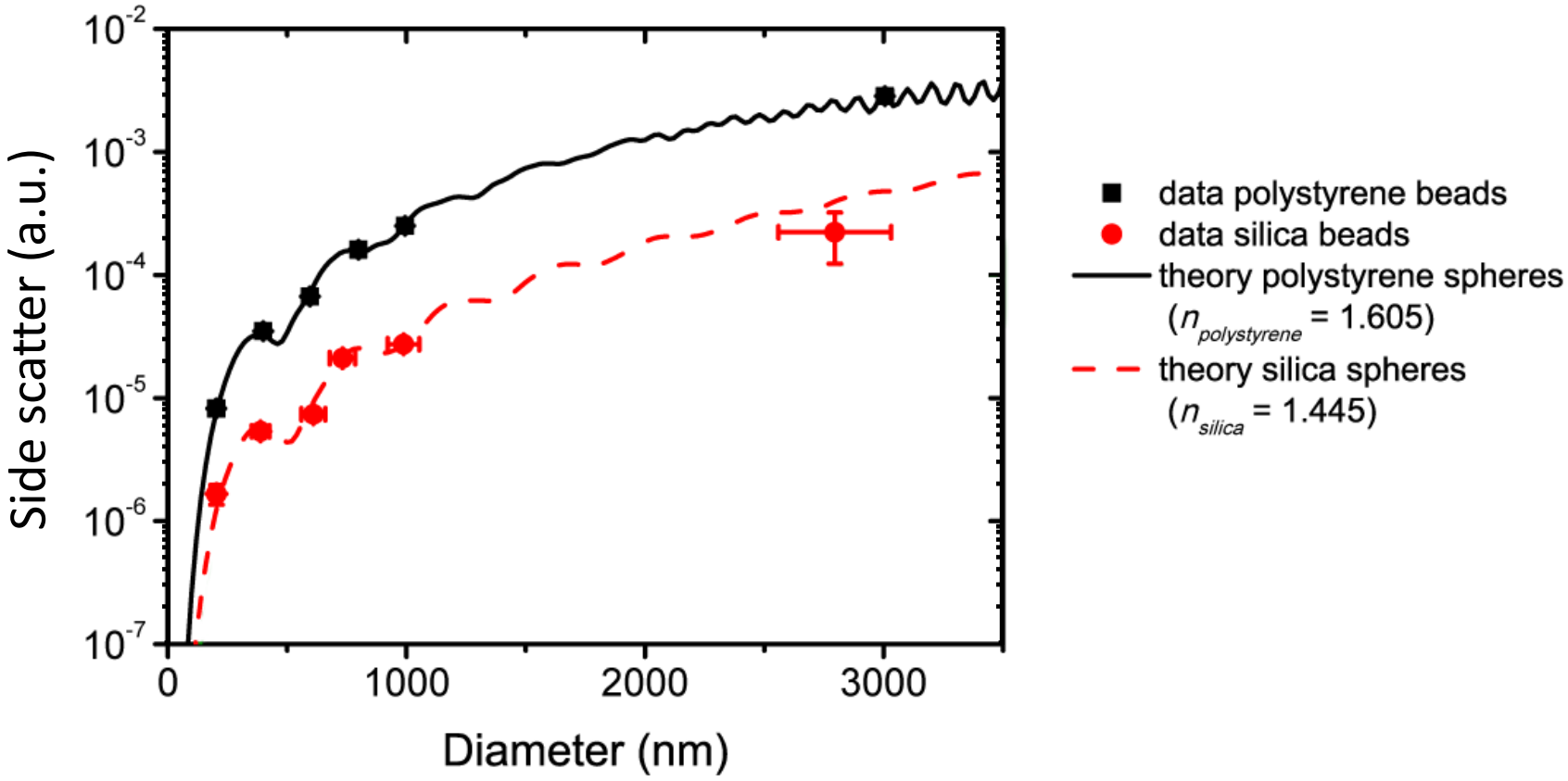
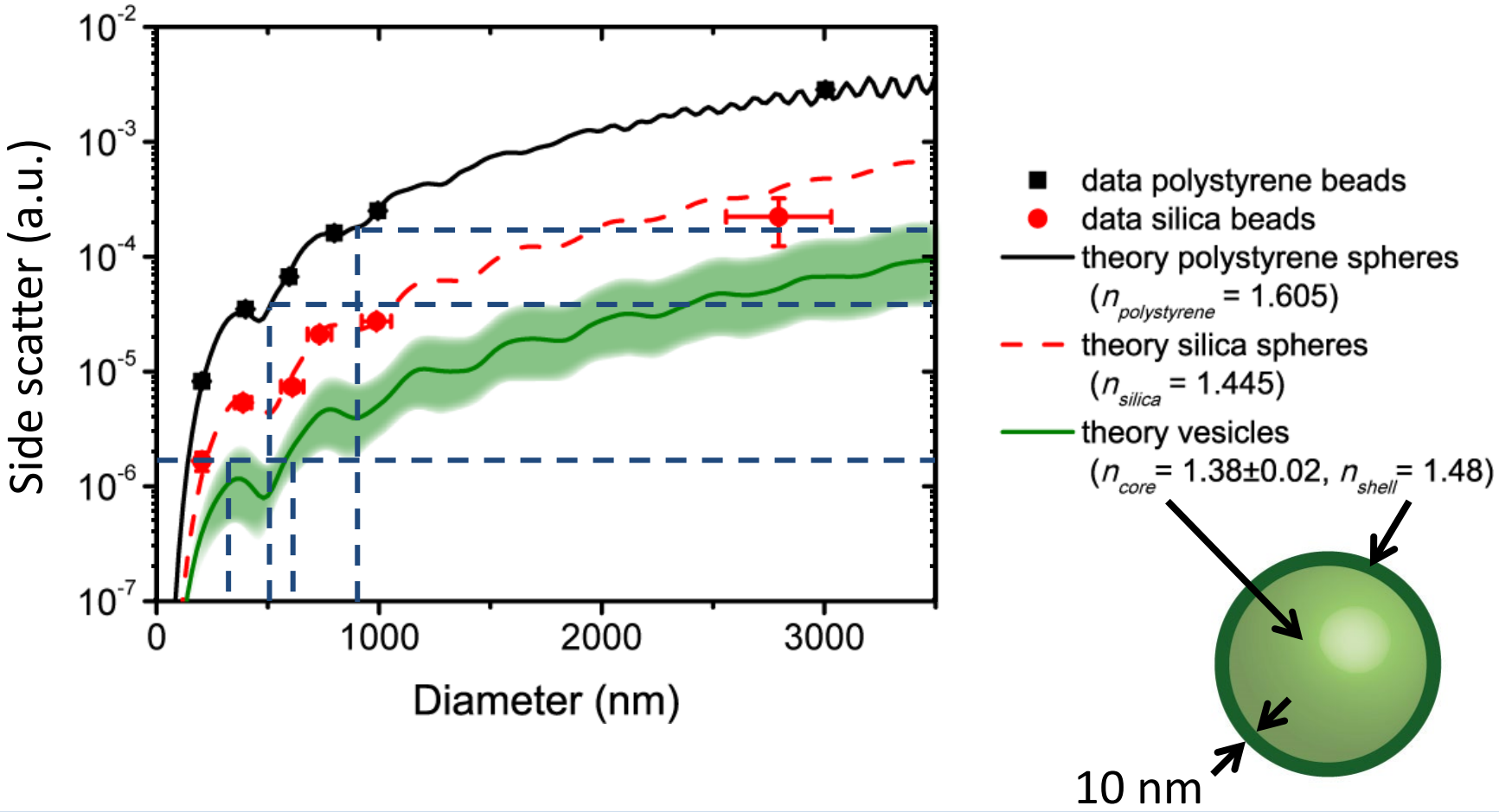
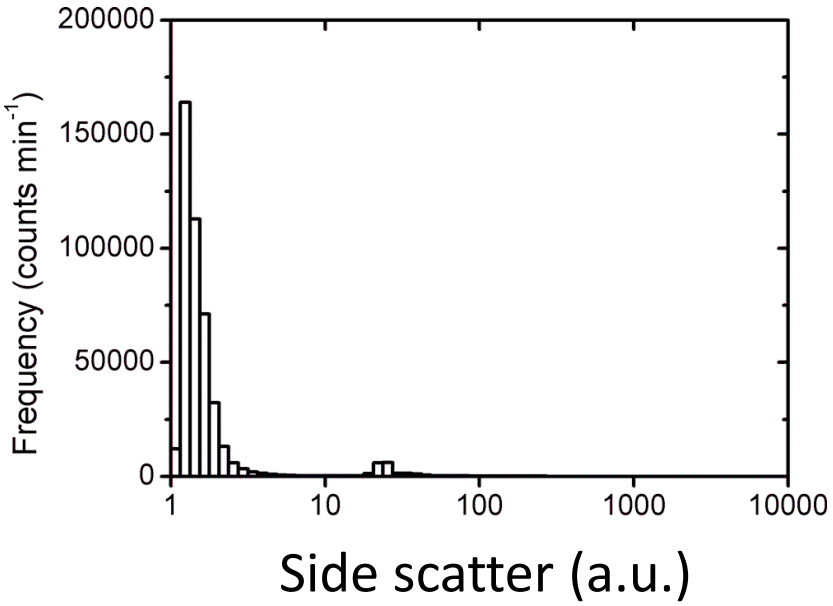


Relate scatter to diameter of vesicles

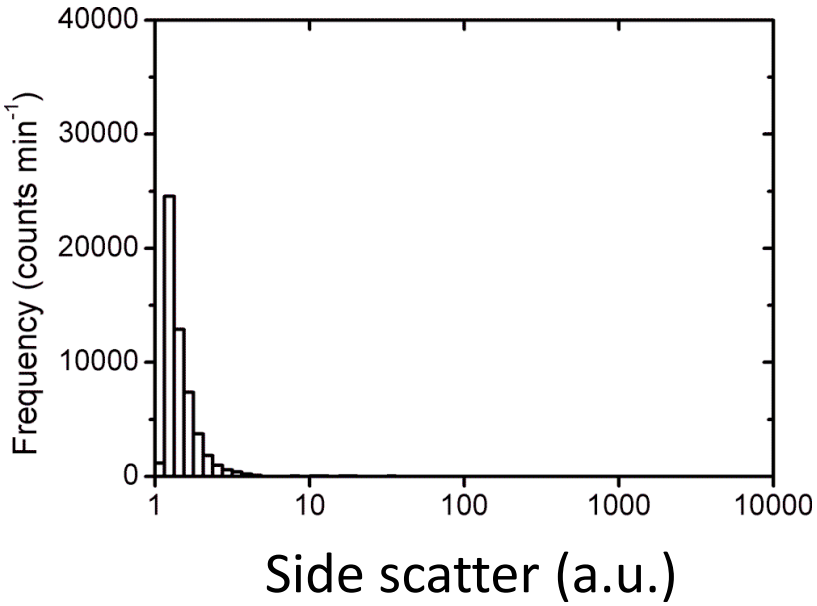


Particles that are too small to be detected generate a signal!

89 nm silica beads



urine EVs <220 nm



Flow cytometry

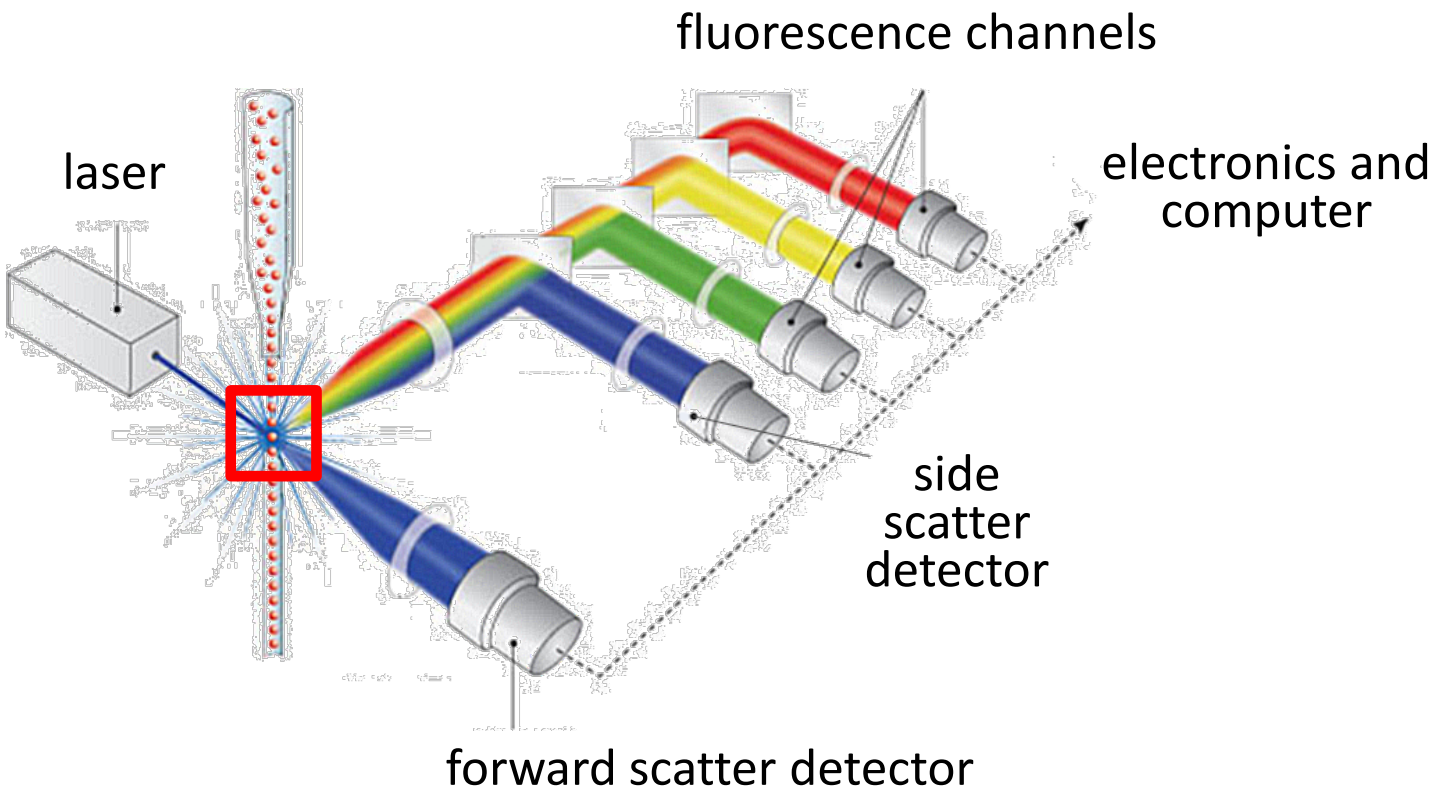
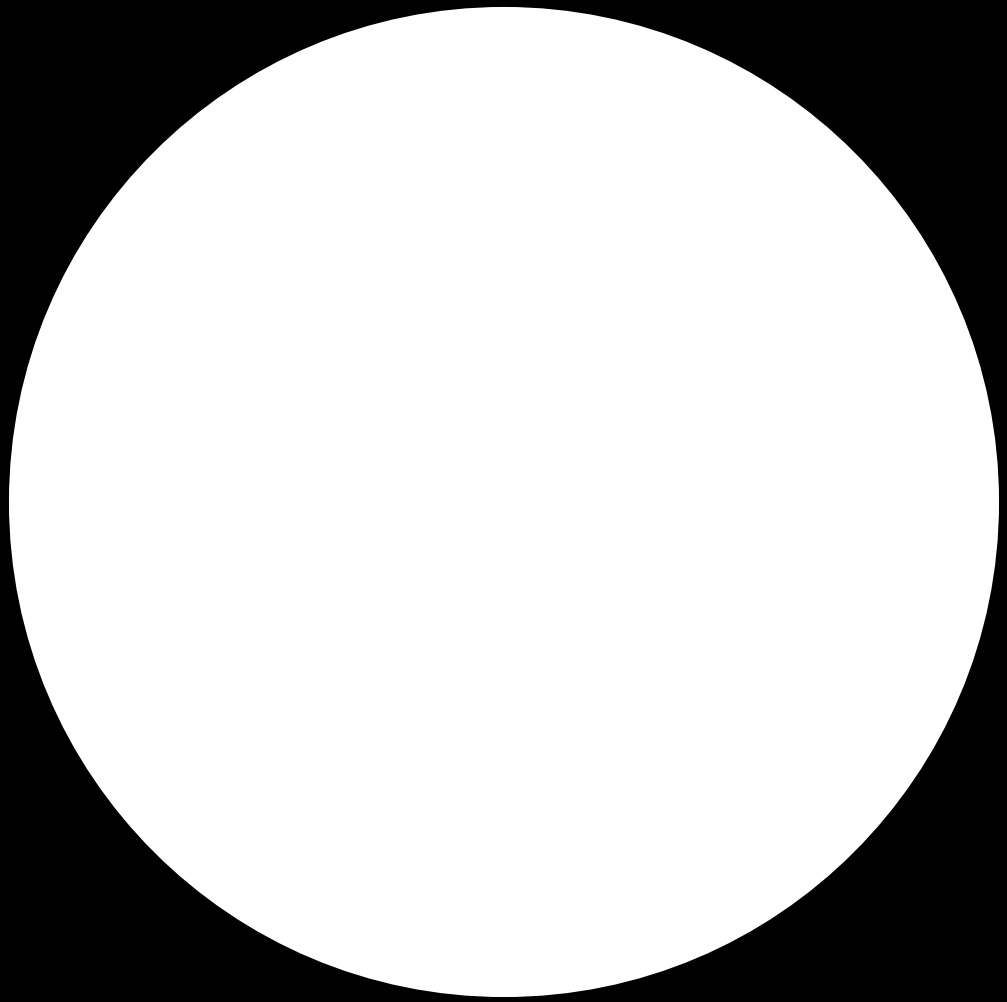
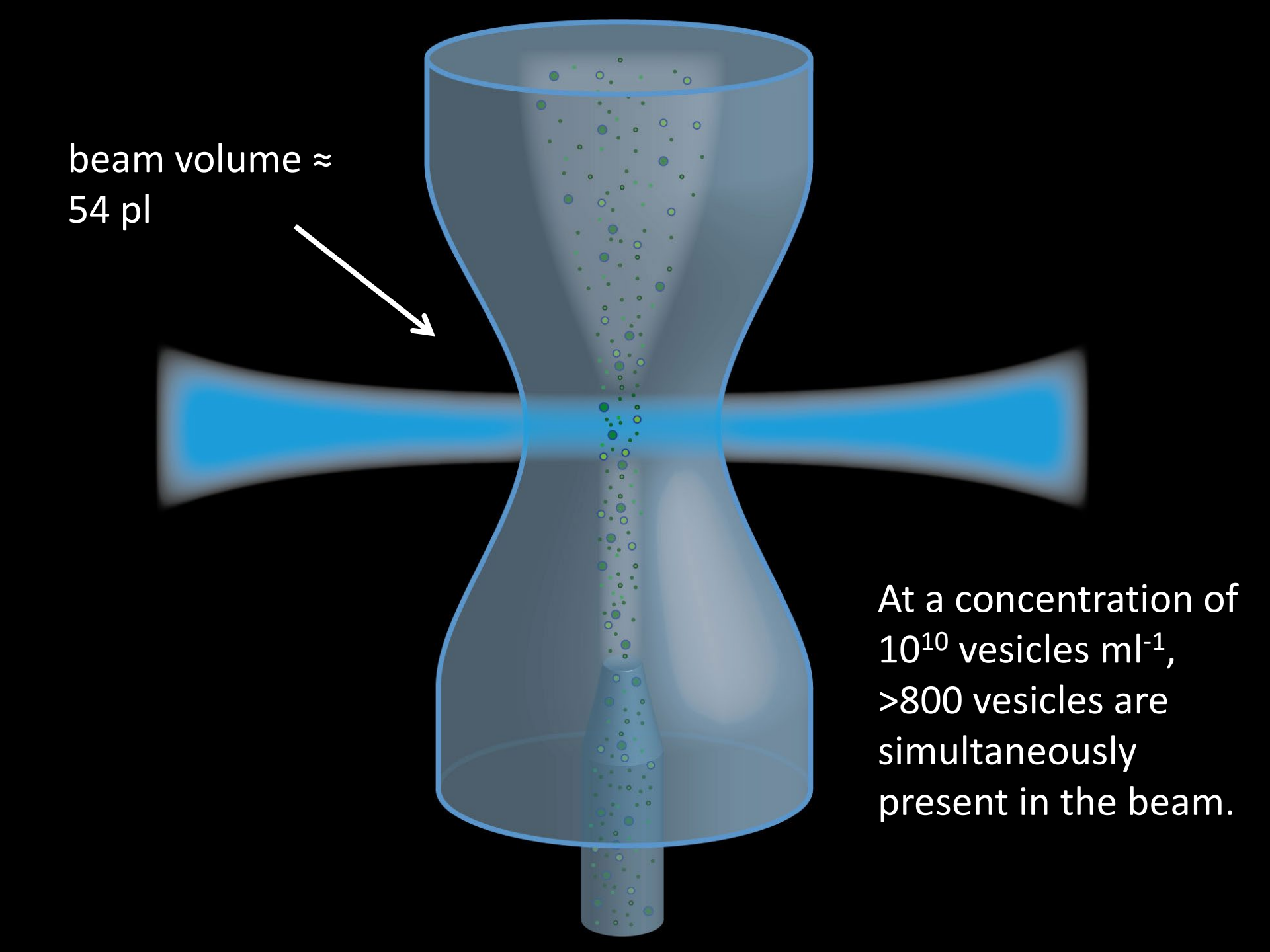
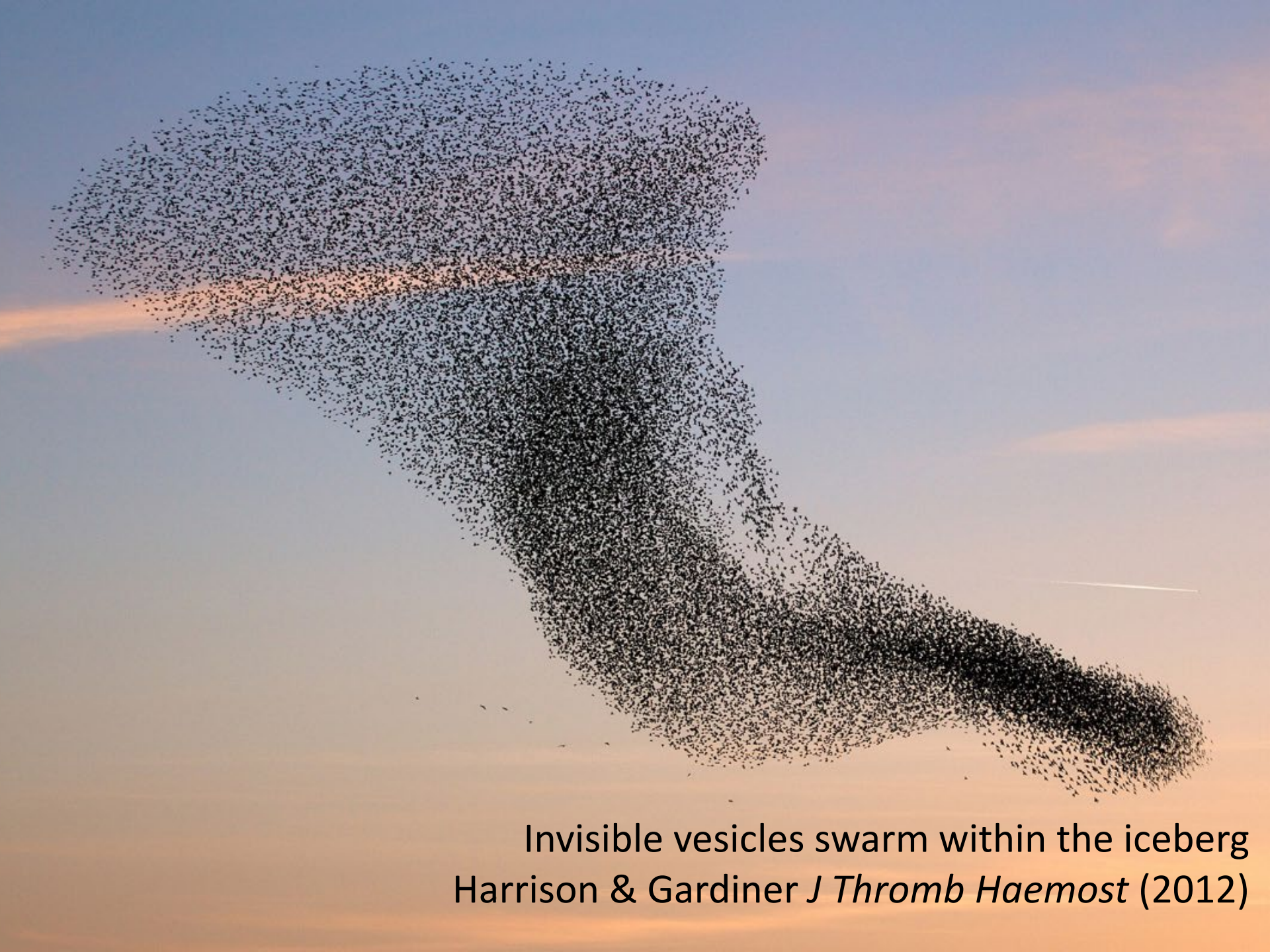


image: semrock.com




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



Invisible vesicles swarm within the iceberg
Harrison & Gardiner *J Thromb Haemost* (2012)

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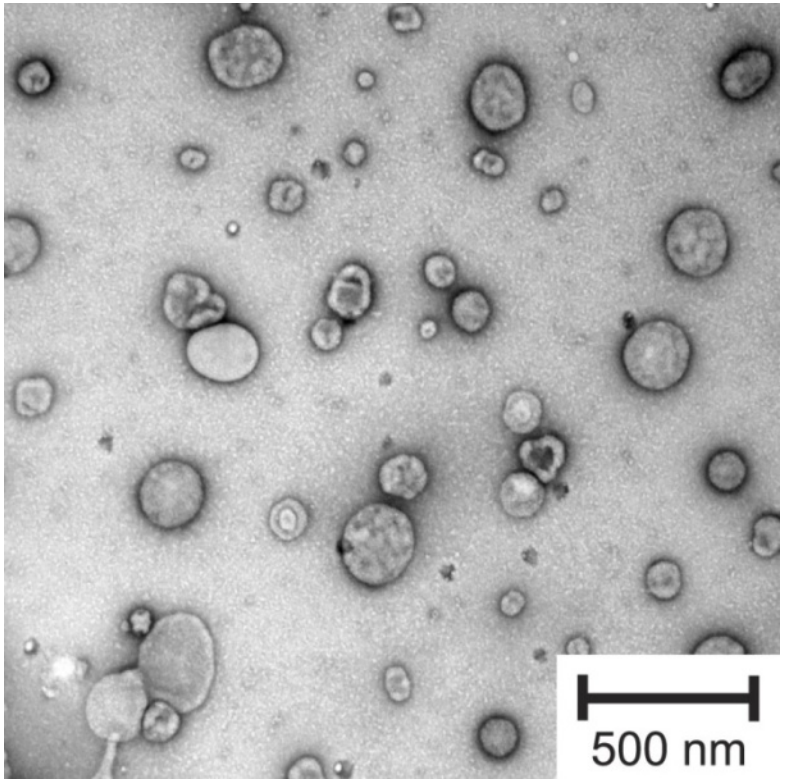
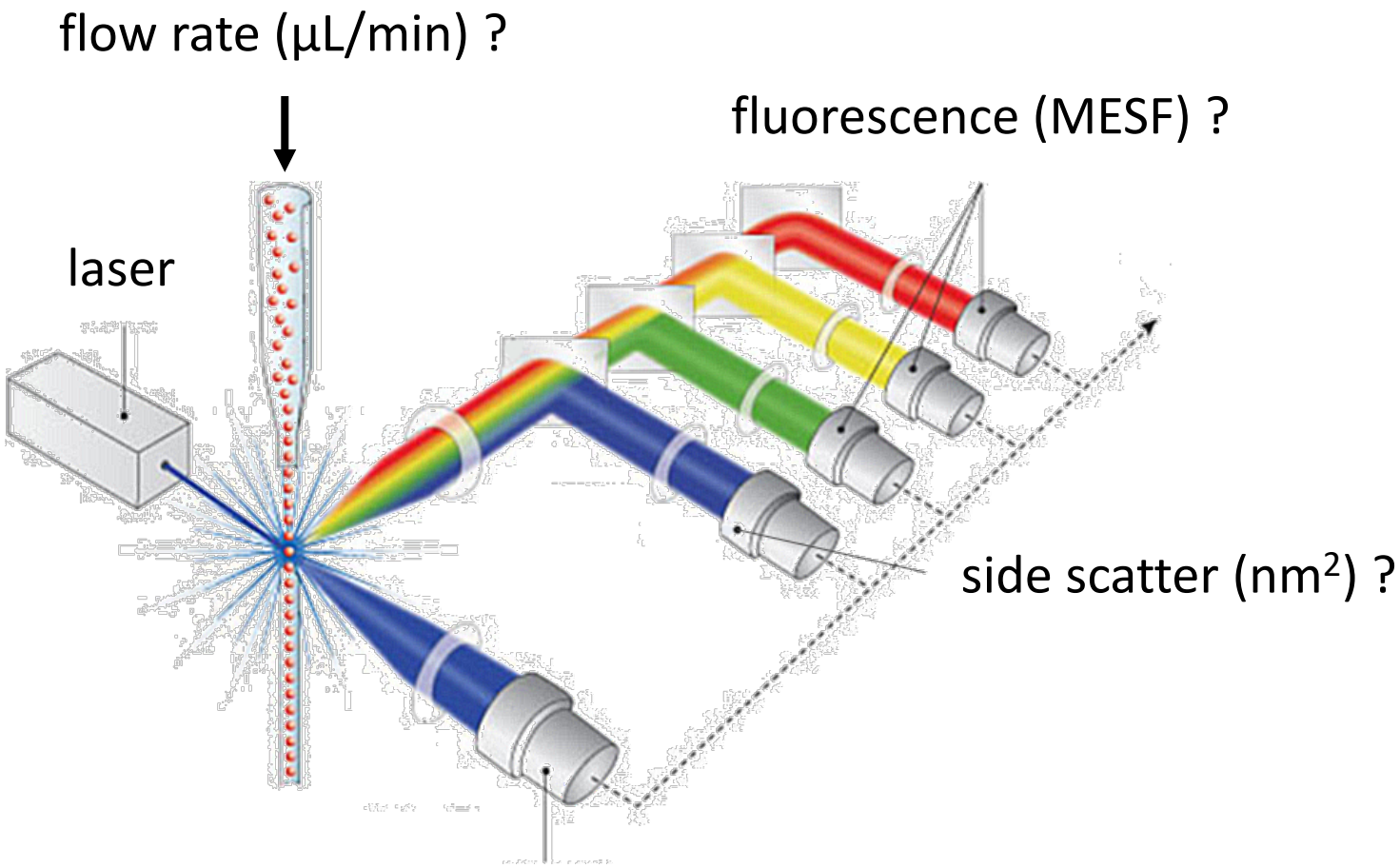


How to calibrate flow cytometers?



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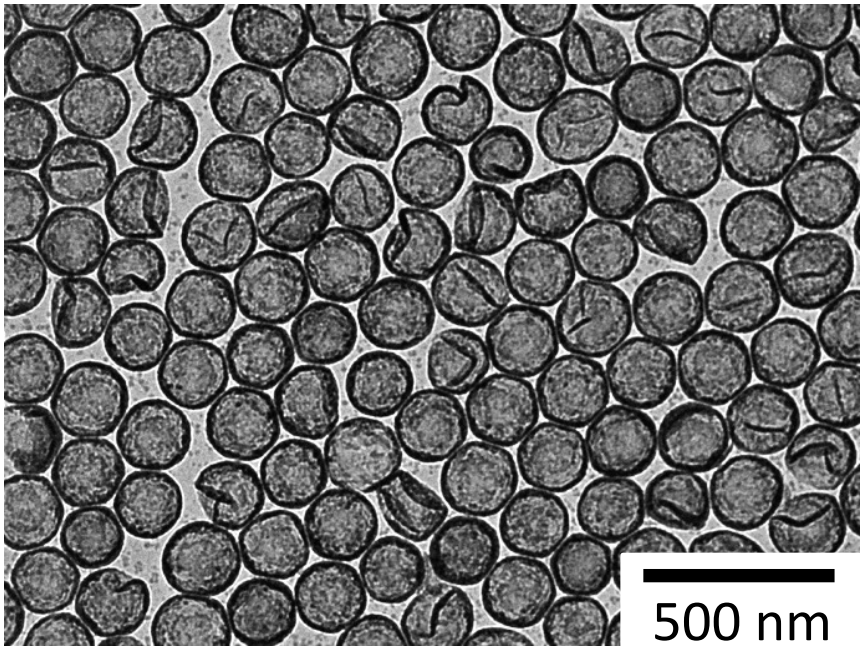
Solution



- One reference material to calibrate them all
- Properties similar to extracellular vesicles
  - scatter (size + refractive index)
  - fluorescence
  - concentration

Tolkien (1954)

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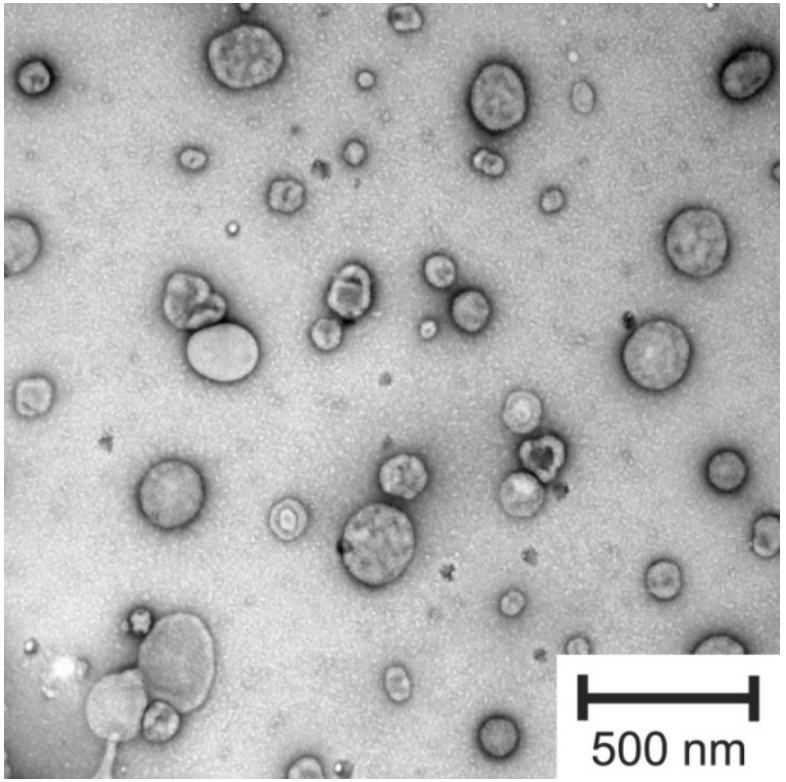

Clinical studies on extracellular vesicles

- Myocardial infarction
  - Therapy monitoring
- Stroke
  - Differentiate between ischemic stroke and hemorrhage stroke in acute phase
- Prostate cancer
  - Diagnoses

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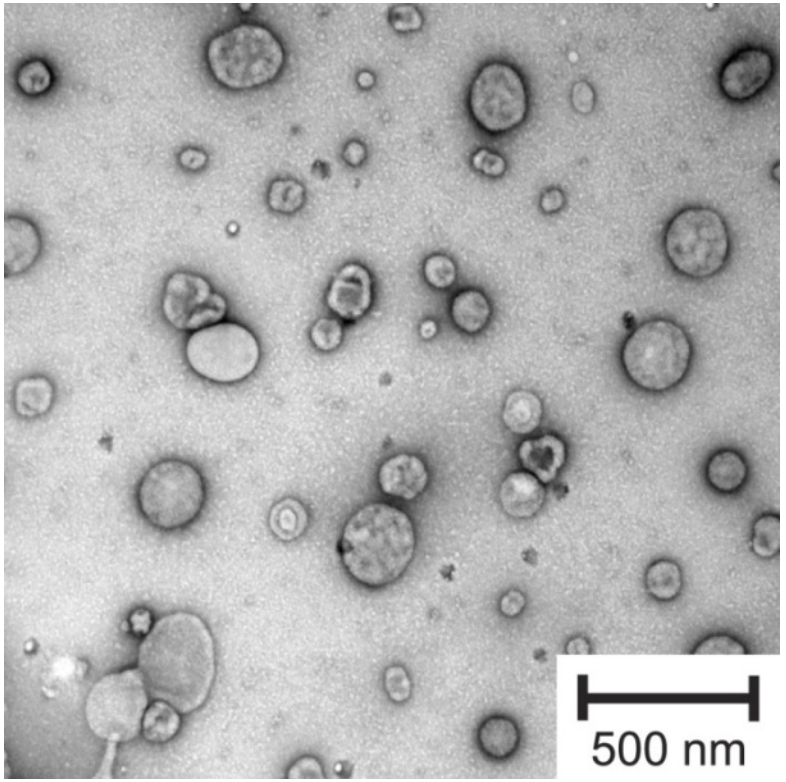
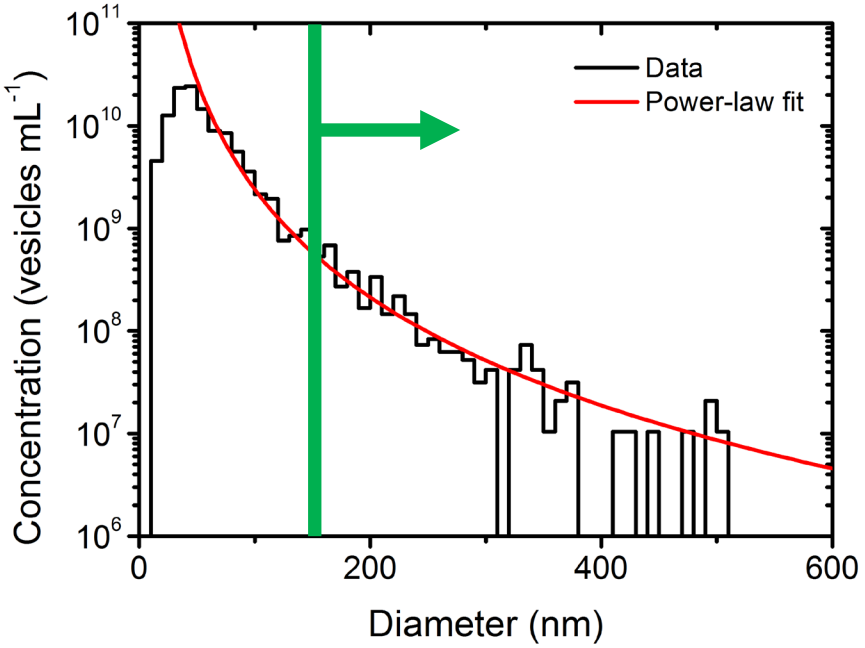


Problem: small *or* fast

vesicles diameter (nm)	Measurement time
>150	15 minutes
>30	24 hours



Solution: EV-Radar, small *and* fast

vesicles diameter (nm)	Measurement time
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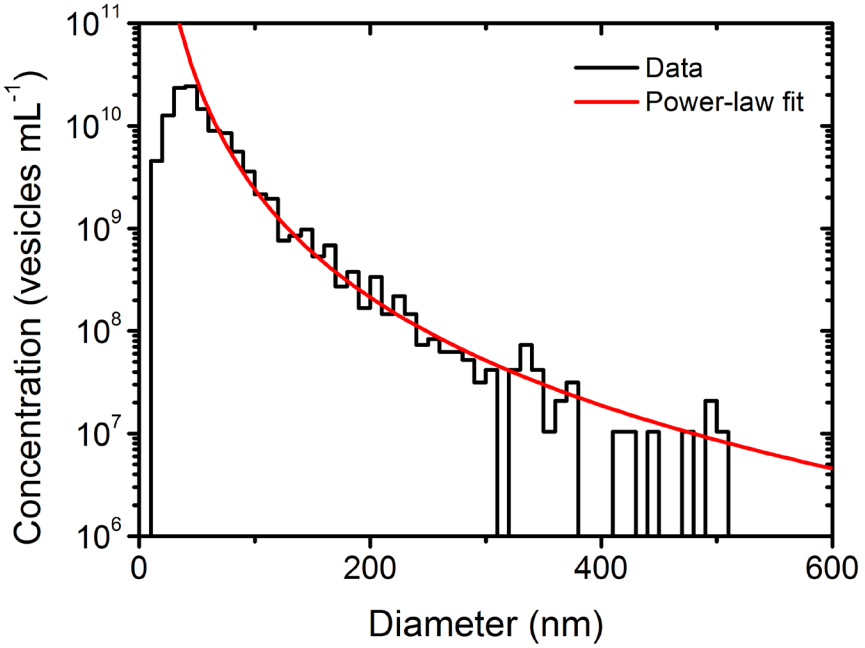
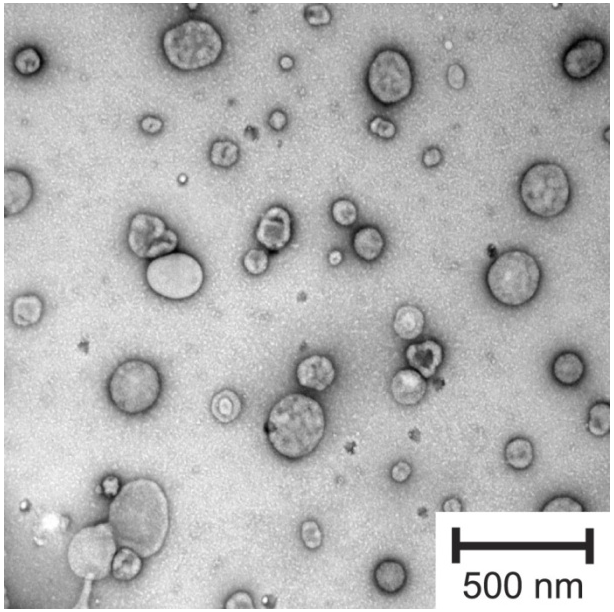


Conclusion



- Body fluids contain extracellular vesicles with clinical information
- The size of extracellular vesicle matters!


TIEN GULDEN
10

Acknowledgements

- My parents
- My wife

- Vesicle Observation Center
  Amsterdam University Medical Centers
  - Ton van Leeuwen
  - Rienk Nieuwland
  - Guus Sturk
  - Frank Coumans

- More info about extracellular vesicles:
  edwinvanderpol.com