Measuring extracellular vesicle concentrations: standardize the unknown

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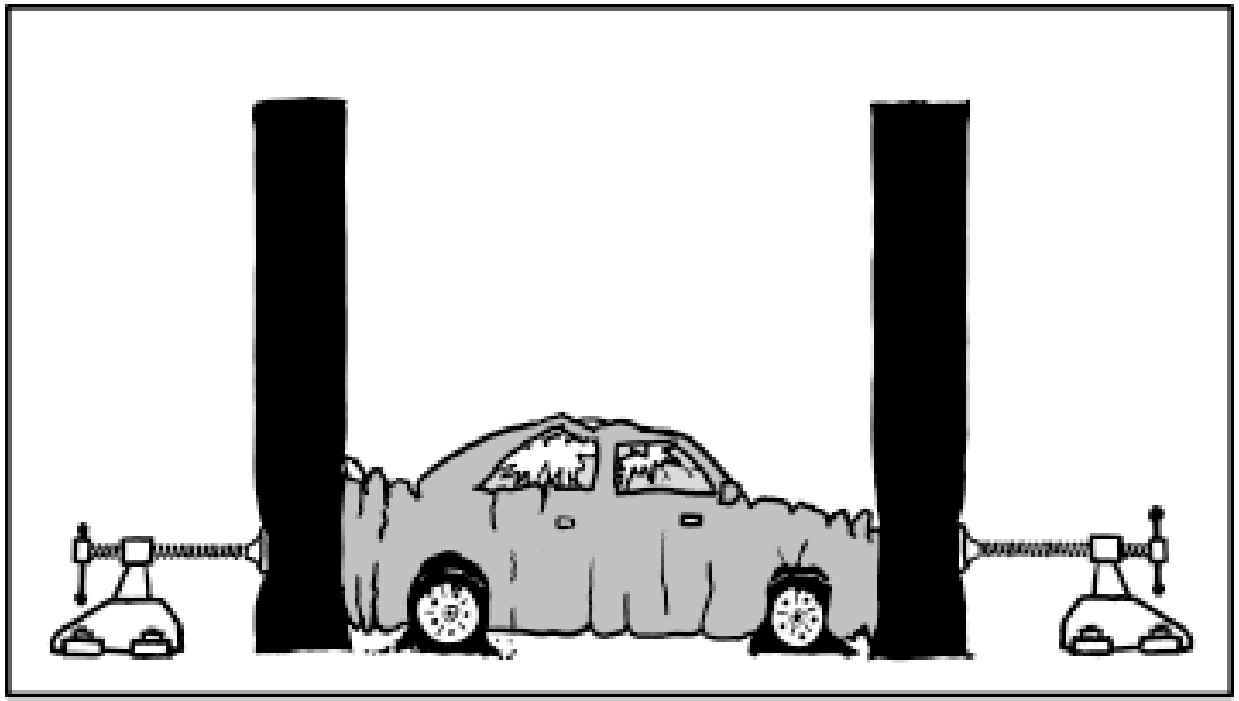
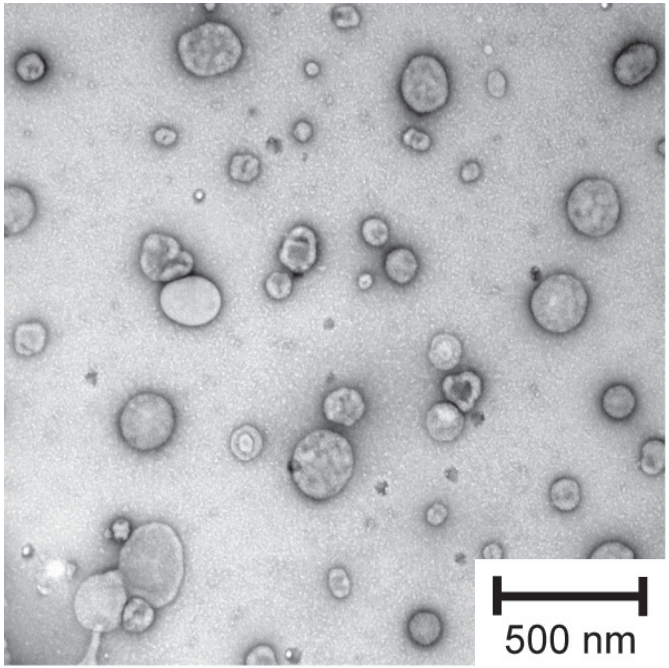
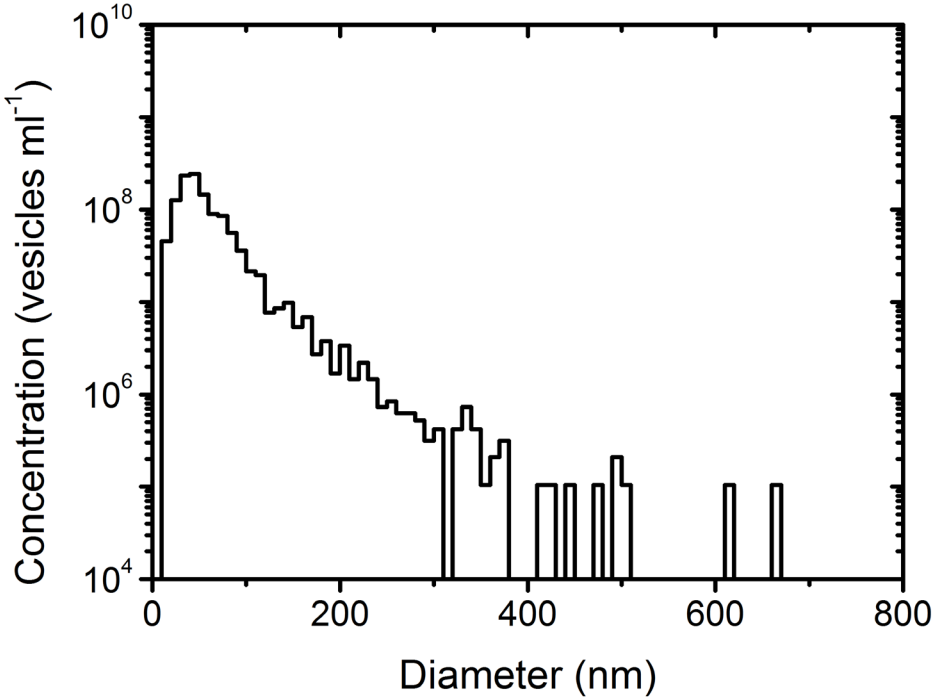


Image: xkcd.com

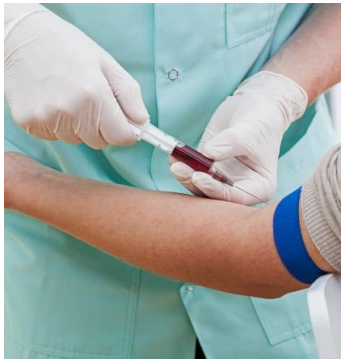
Extracellular vesicles





Why measure vesicle concentrations?

- Blood plasma contains heterogeneous vesicle populations with clinical information*



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

*Gasecka et al. *Platelets* 2017

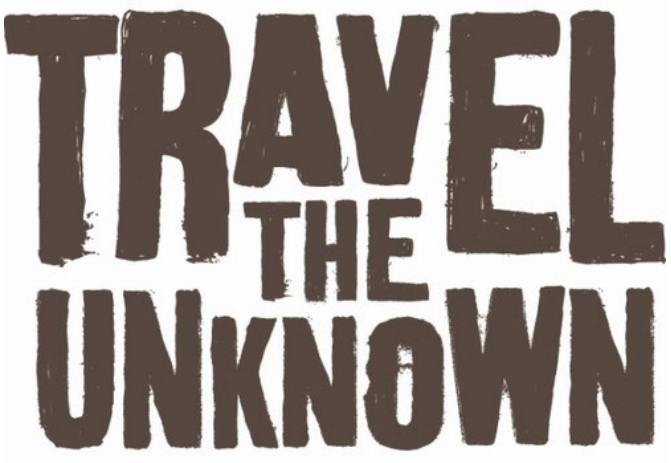
Problem definition

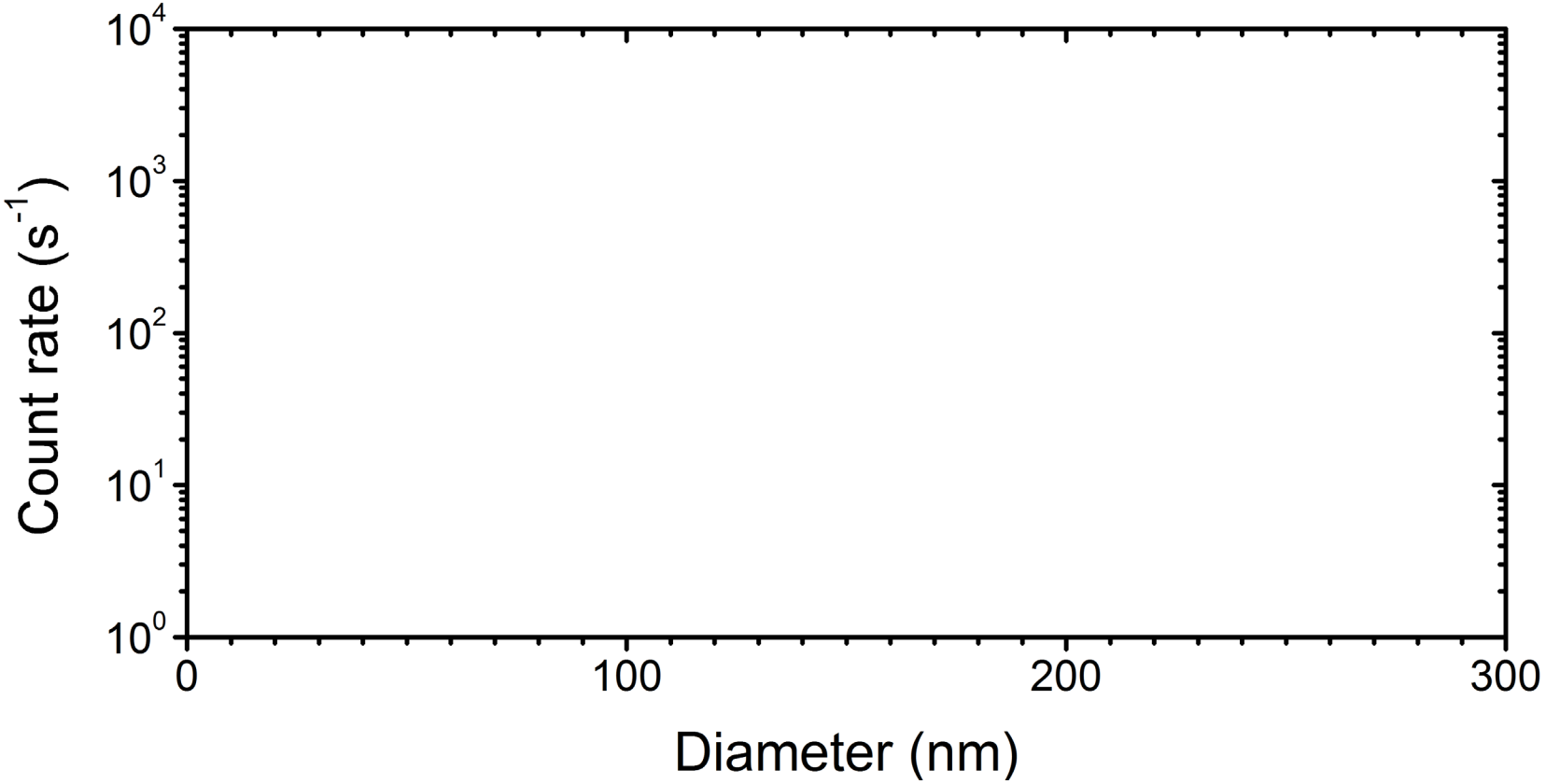
- Number concentration (vesicles / mL):
  number of vesicles per sample volume

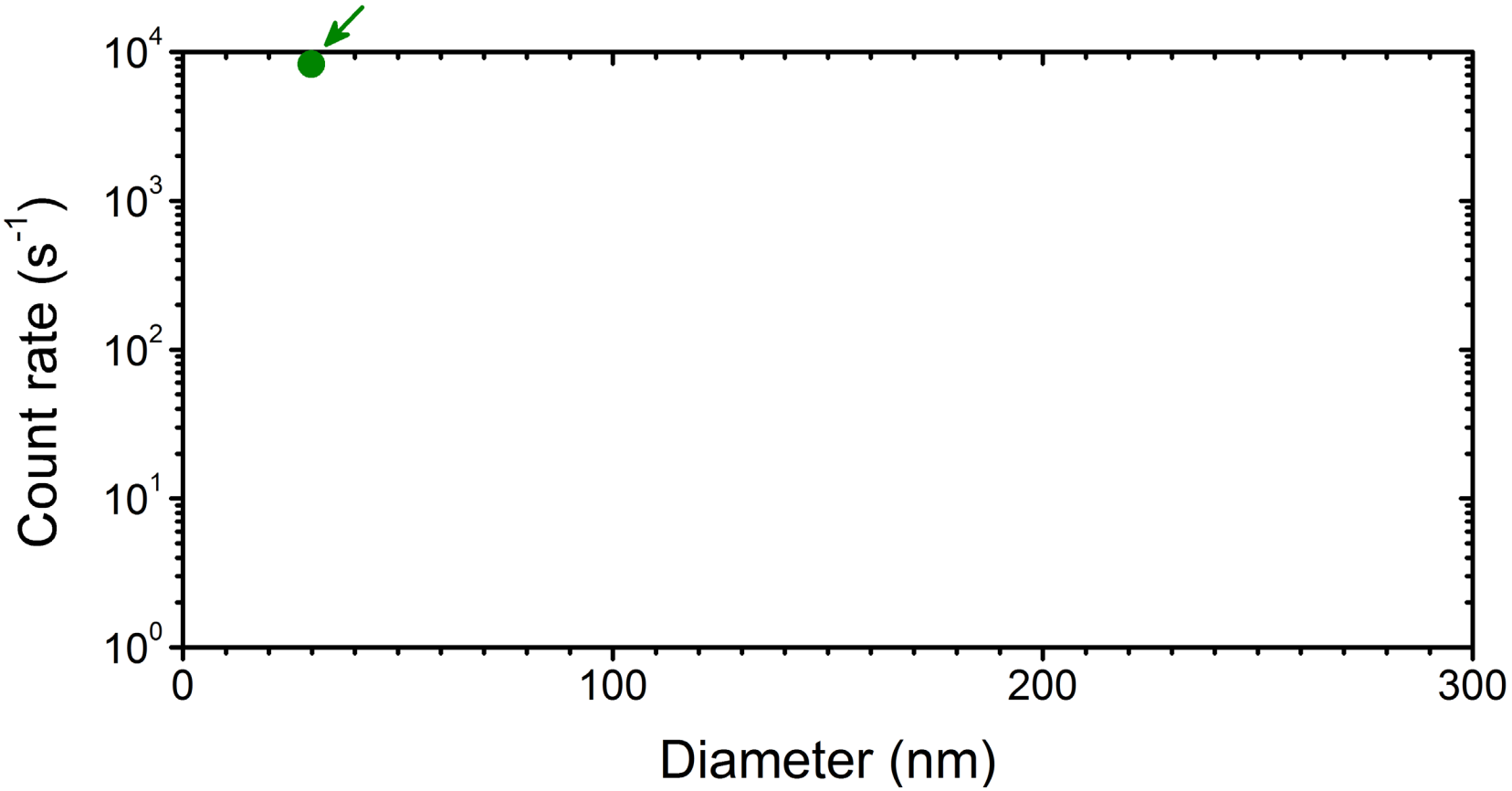
- Practical requirements:
  detect and characterize all vesicles
  in a known sample volume
  at a clinically relevant rate

Outline measuring vesicle concentrations

- Speed versus size
- Sample volume determination
- Detection limits
- Standardize the unknown




Count rate (s^{-1})
10^4
10^3
10^2
10^1
10^0
0
100
200
300
Diameter (nm)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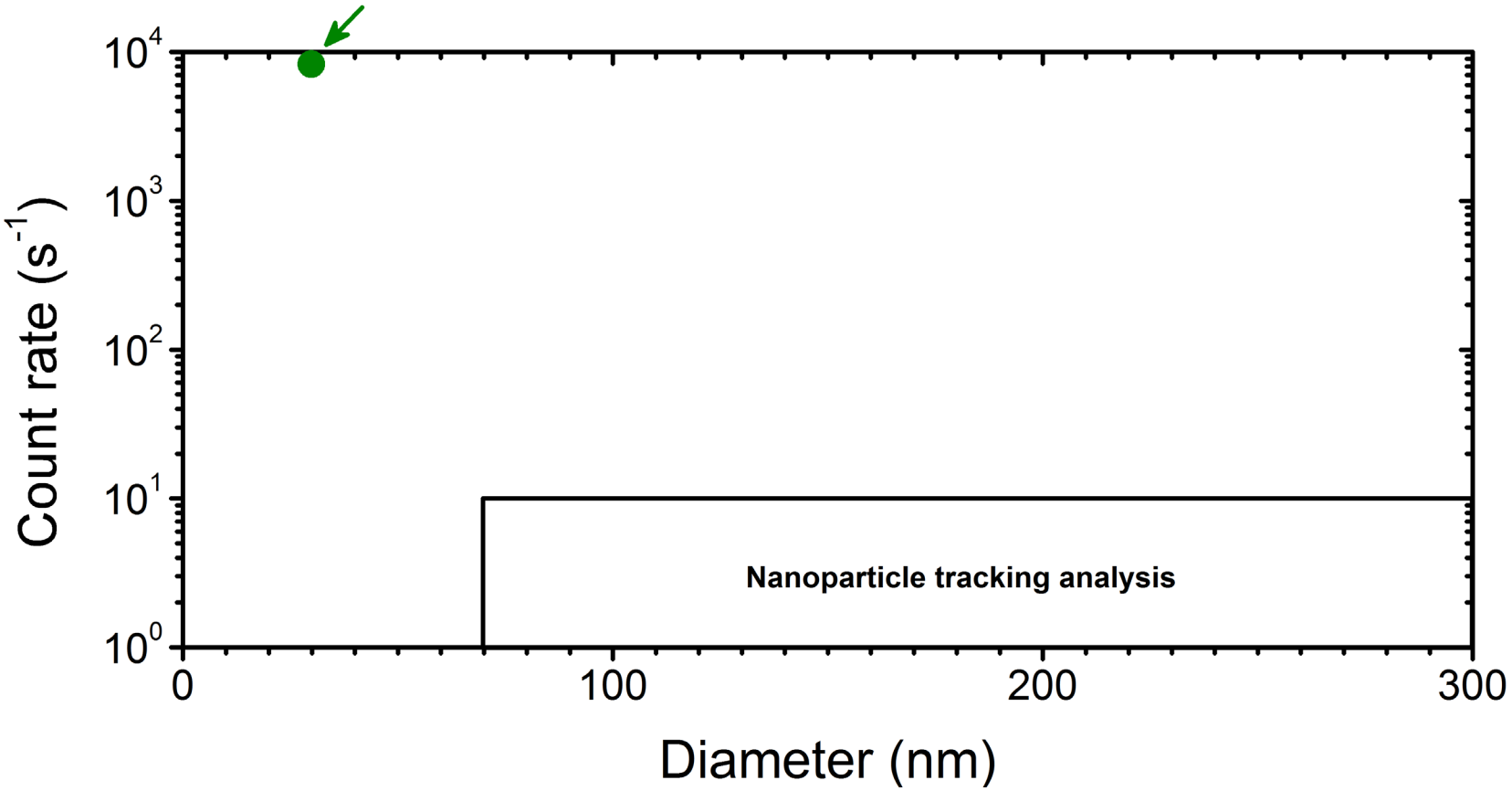
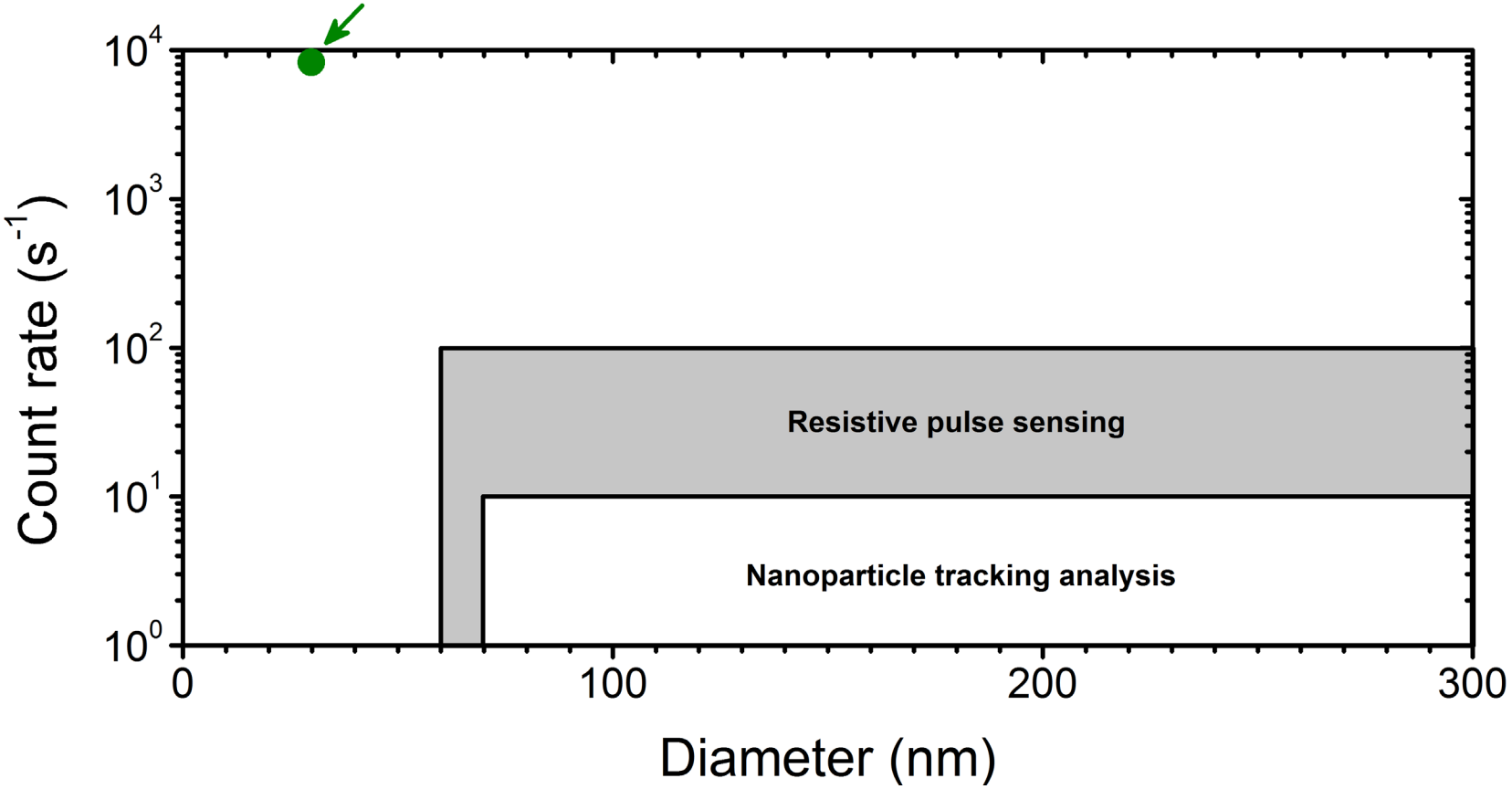
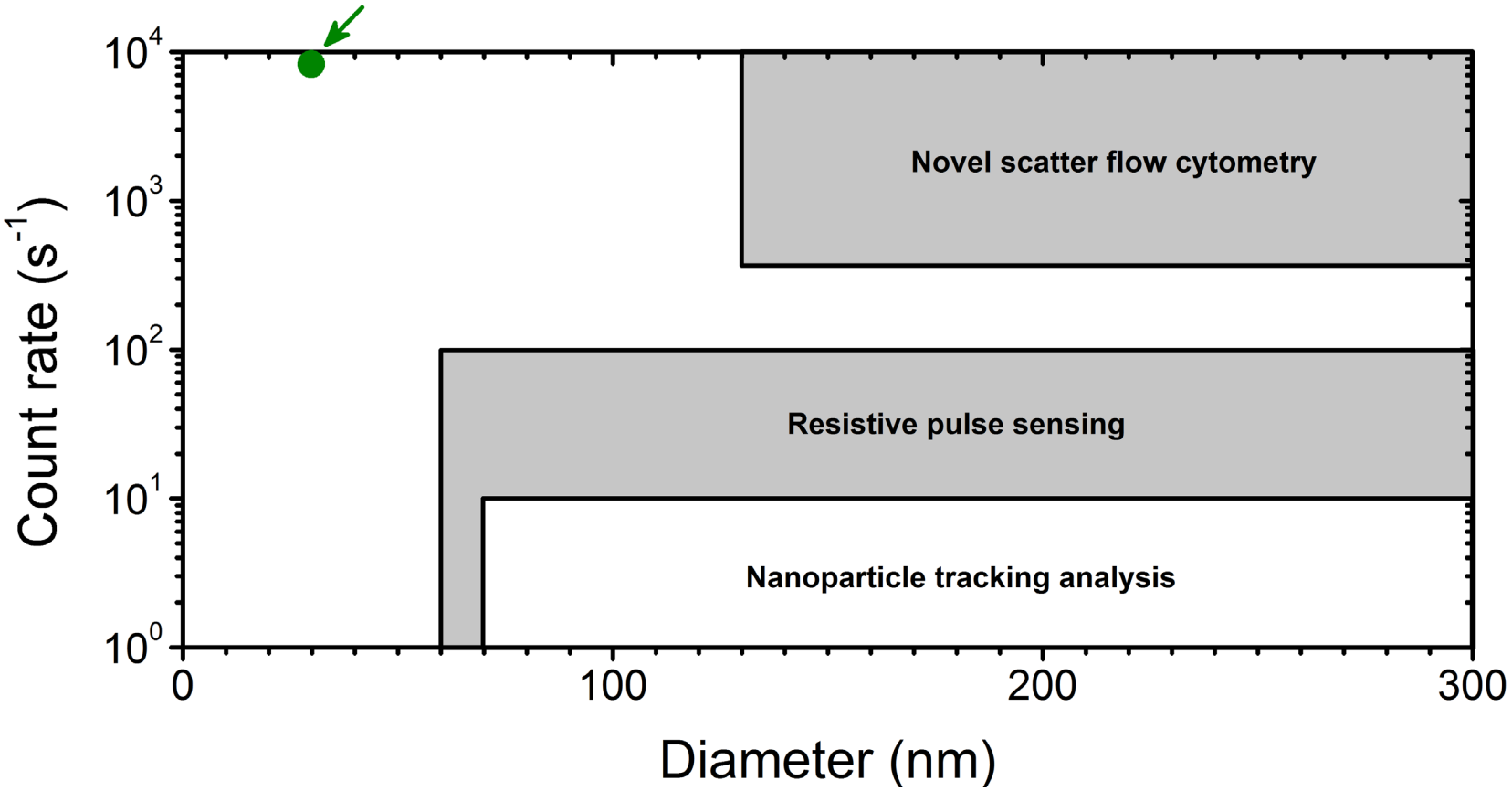


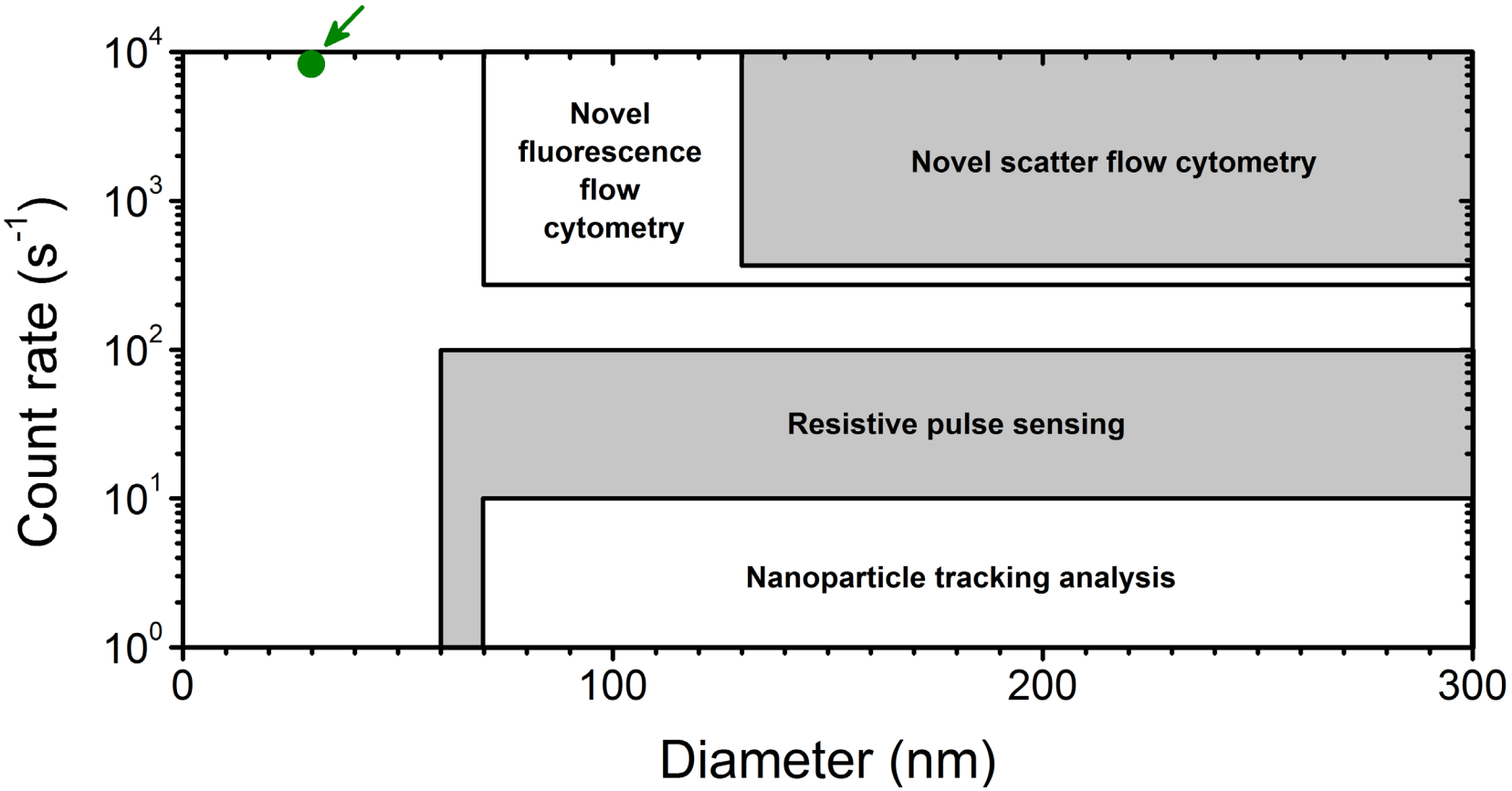
Coumans et al. *Circ.Res.* 2017

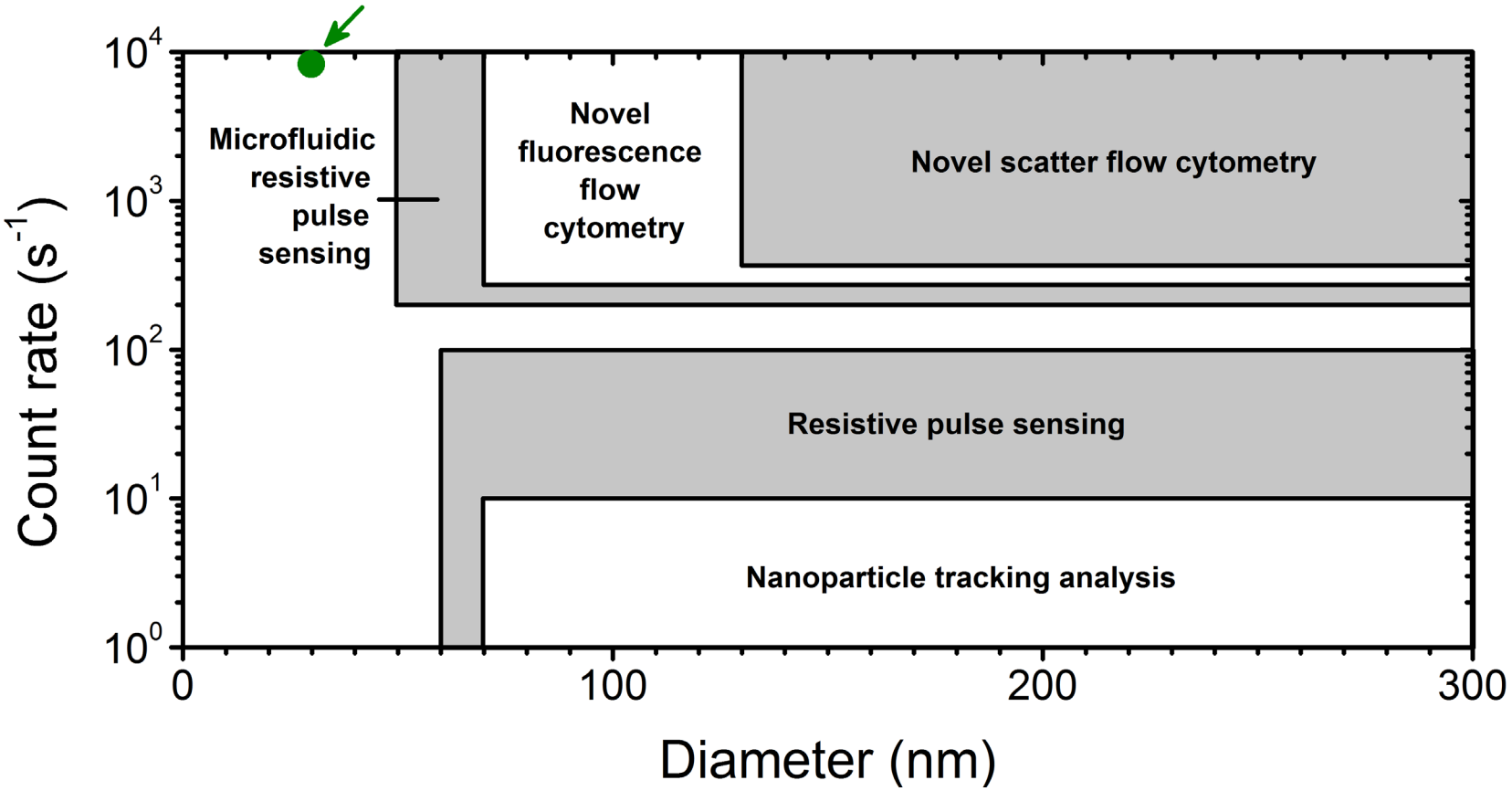


Coumans et al. *Circ.Res.* 2017

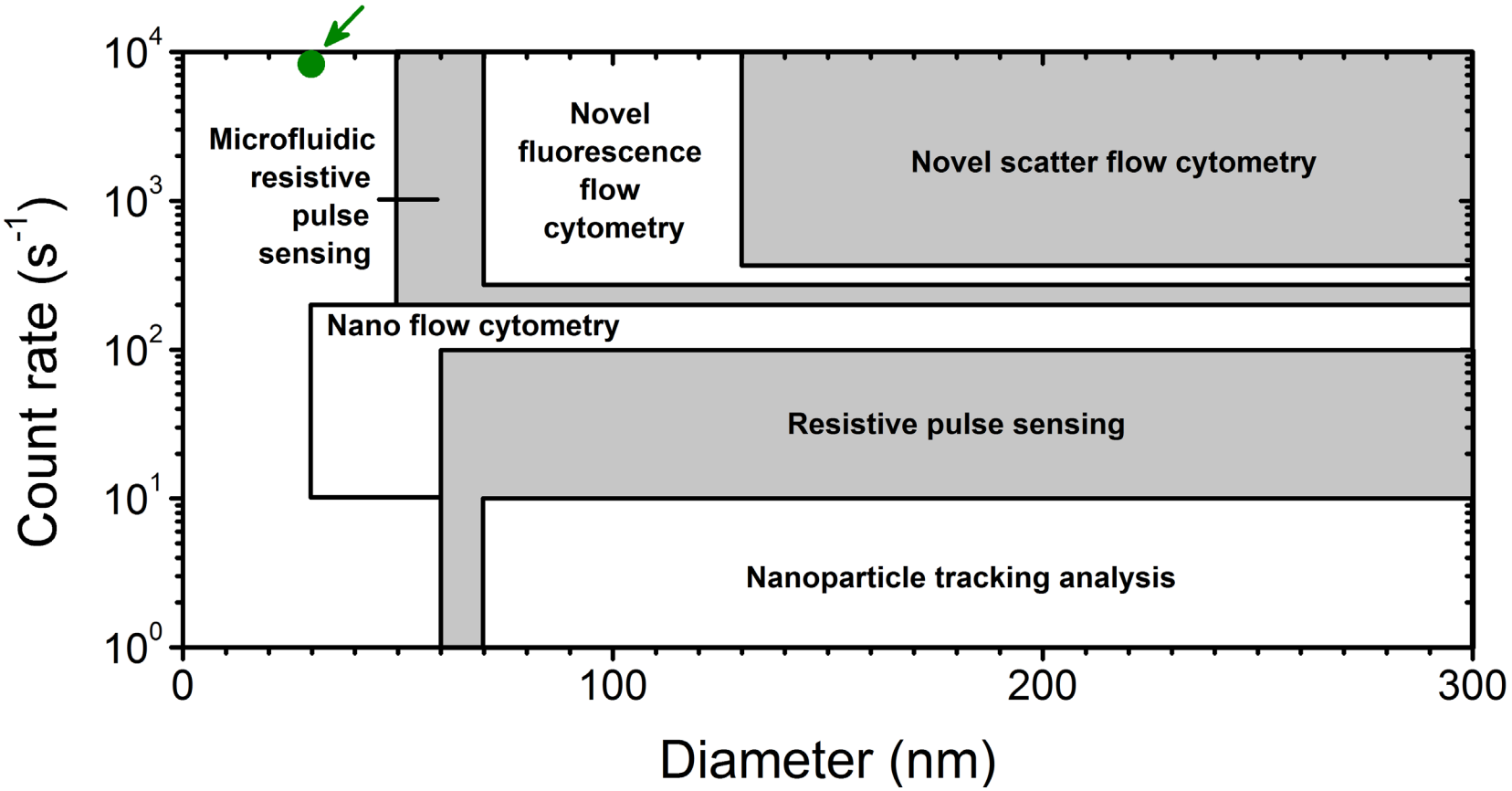


Novel scatter flow cytometry: van der Pol et al. *JTH* 2014


Count rate (s^{-1})
10^4
10^3
10^2
10^1
10^0
0
100
200
300
Diameter (nm)
Novel fluorescence flow cytometry
Novel scatter flow cytometry
Resistive pulse sensing
Nanoparticle tracking analysis



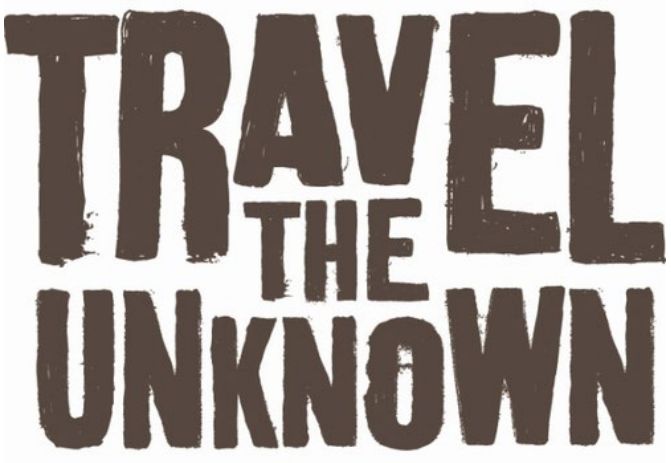
Microfluidic resistive pulse sensing: Fraikin *Nature Nanotech.* 2011



Nano flow cytometry: Zhu et al. *ACS Nano* 2014

Outline measuring vesicle concentrations

- ✔ Speed versus size
- Sample volume determination
- Detection limits
- Standardize the unknown



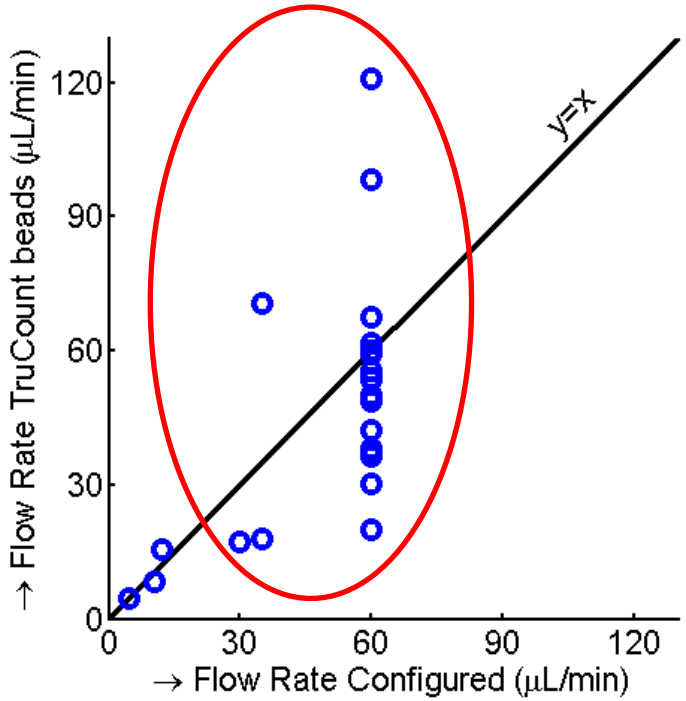
Sample volume determination

concentration [µL^{-1}] = particles / sample volume [µL]

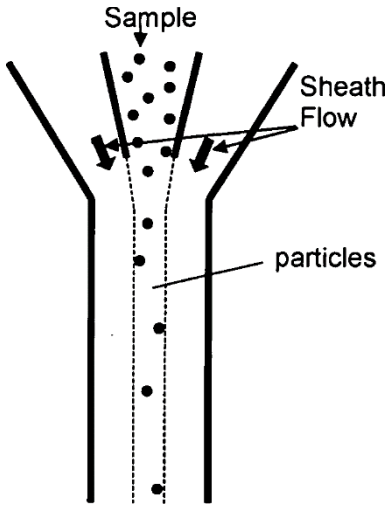
sample volume [µL] = flow rate [µL/min] · time [min]

- Flow rate
  - Specified
  - Calibrated
- Fixed volume

Calibrated versus specified flow rate

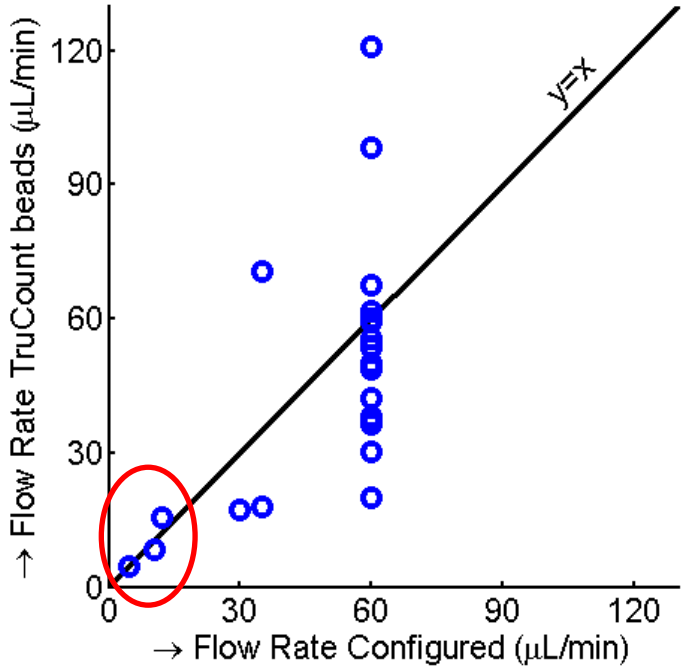


Most flow cytometers:

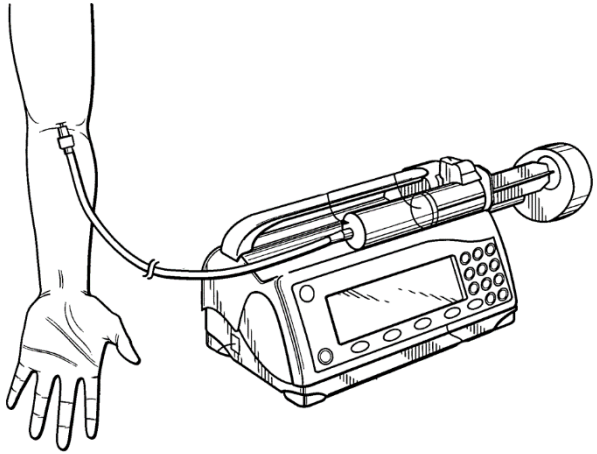


Differential pressure

Calibrated versus specified flow rate



Apogee A60-Micro:



Actuated syringe pump

Calibrated flow rate versus syringe pump

- Disadvantage syringe pump
  - Particles stick to tubing (>0.4%)
- Disadvantage calibration particles
  - Trucount beads
    - Error unknown
    - Too big (>3 µm) thus too bright
  - No sub-µm concentration reference particles

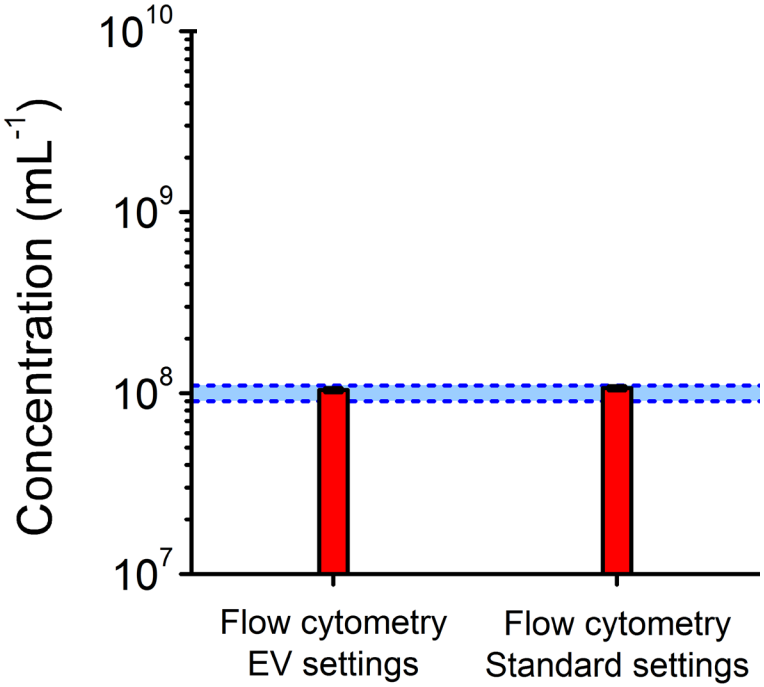
Sample volume determination

concentration [μL^{-1}] = particles / sample volume [µL]
sample volume [µL] = flow rate [µL/min] · time [min]

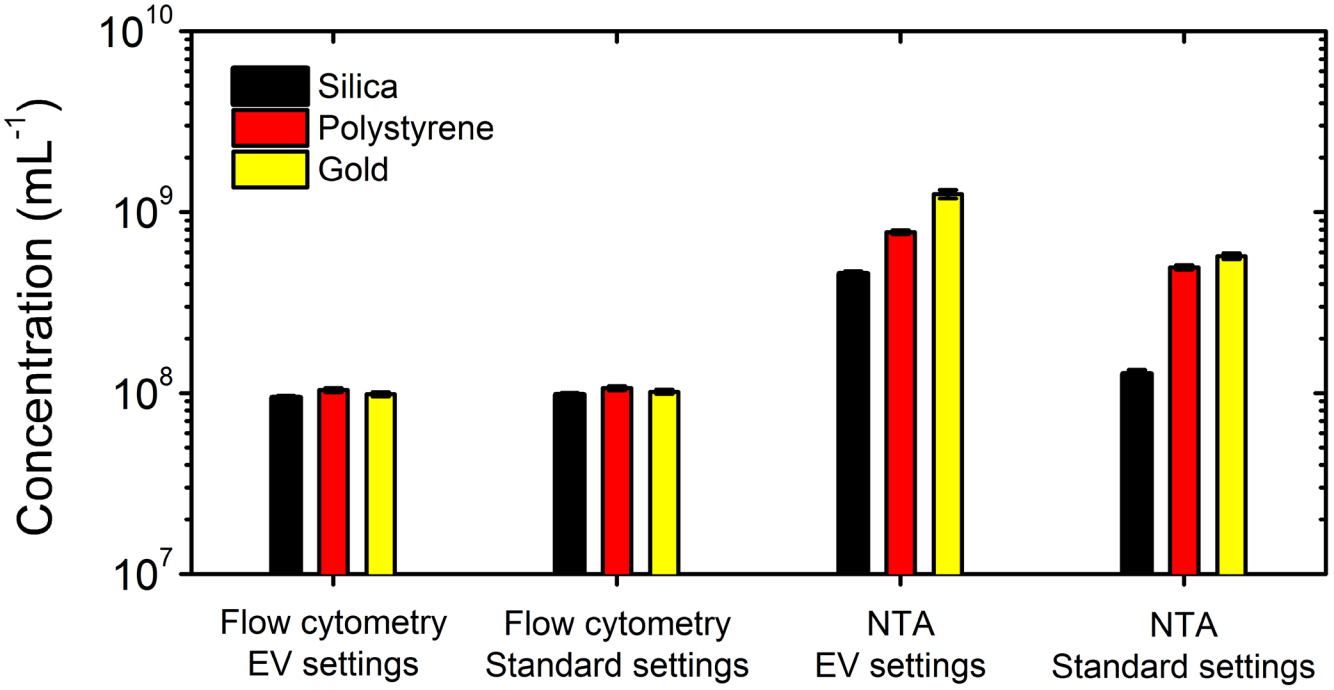
- ✔ Flow rate
  - ✔ Specified
  - ✔ Calibrated
- Fixed volume: nanoparticle tracking analysis (NTA), used by 58% of the research field*

*Gardiner et al. *JEV* 2016

Syringe flow rate versus fixed volume

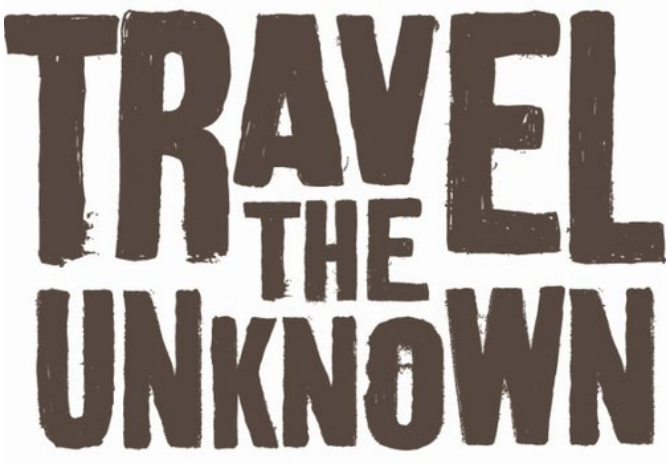


Syringe flow rate versus fixed volume



Outline measuring vesicle concentrations

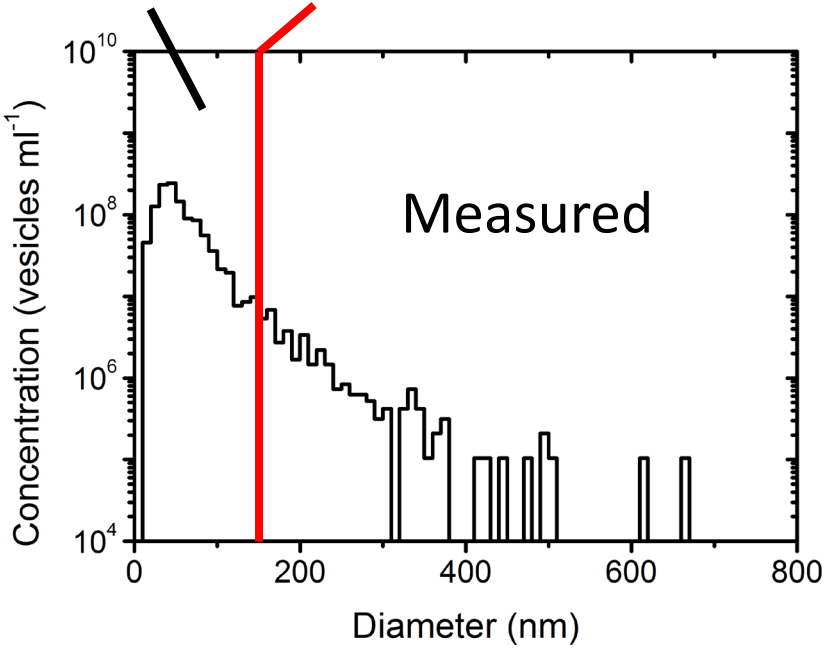
- ✔ Speed versus size
- ✔ Sample volume determination
- Detection limits
- Standardize the unknown

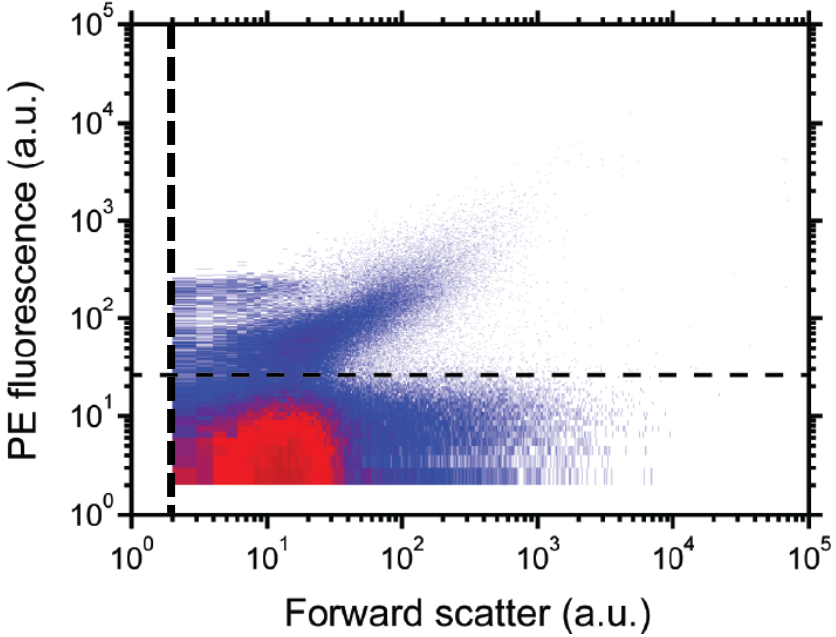


Detection limits and “the unknown”

Unknown

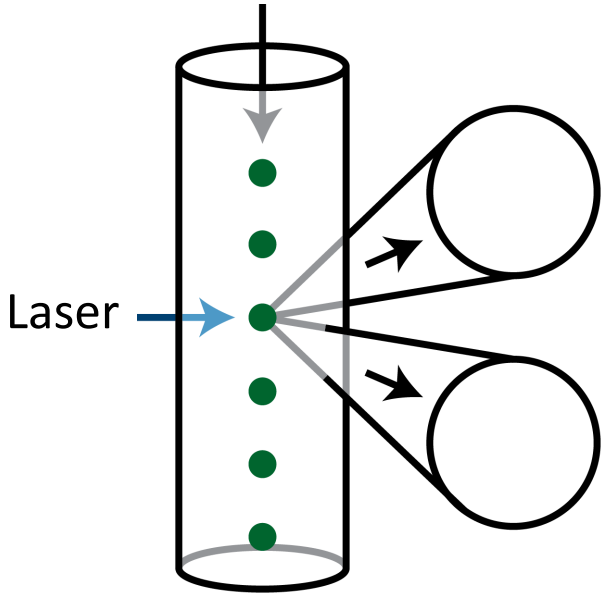
Detection limit itself is unknown and differs per detector!





Standardize “the unknown”

1. Calibrate flow rate (µL/min)

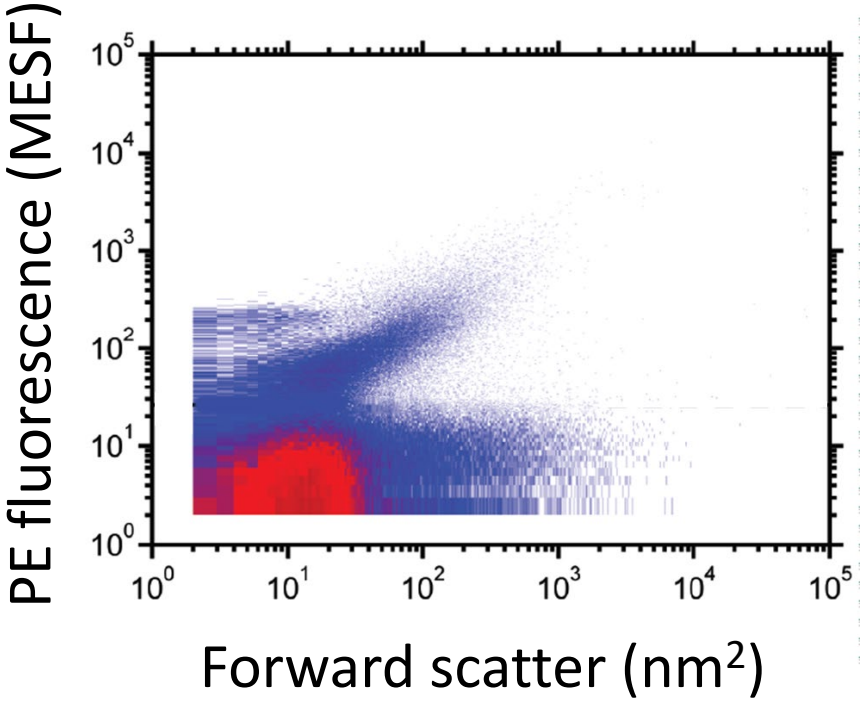


2. Calibrate scatter (nm^2)

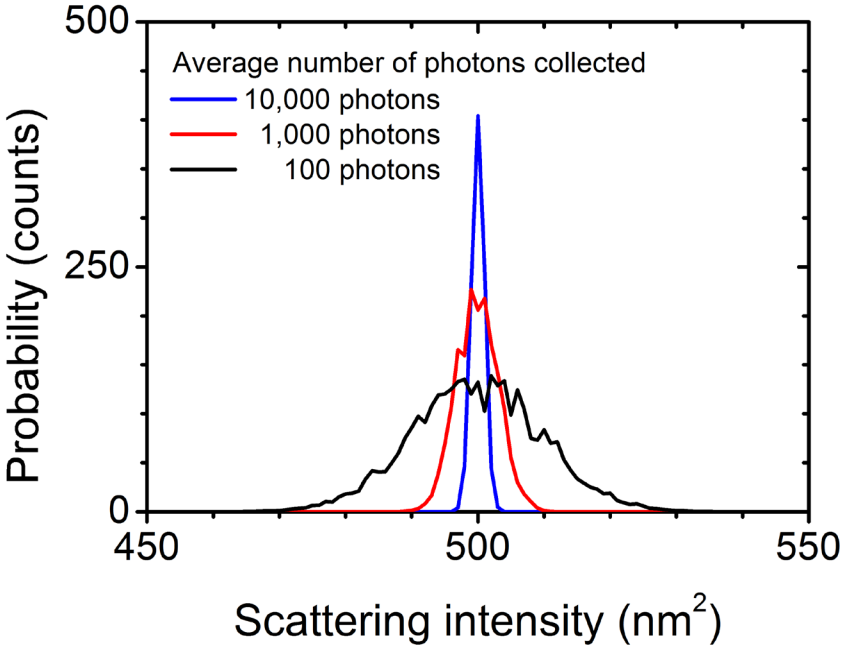
3. Calibrate fluorescence (MESF)

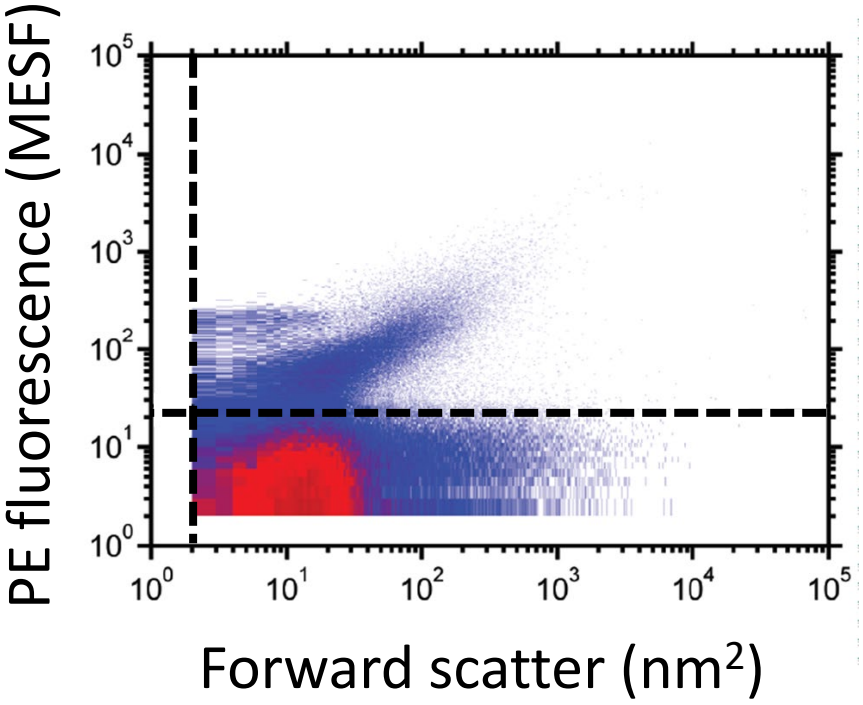
Data with comparable units

- Run beads of known
  - concentration (mL^{-1})
  - mean equivalent fluorescence intensity (MESF)
  - scattering intensity (nm^2) for the collection angles of the instrument



Detection limits with comparable units





Summary

- Vesicle concentrations are future clinical parameters
- NTA does not measure concentration (error >100%)
- To measure vesicle concentrations, calibrate
  - flow rate (µL/min)
  - fluorescence intensity (MESF)
  - scatter intensity (nm^2)

  and determine and report detection limits

Acknowledgements

- Academic Medical Center
  - Vesicle Observation Center
  - Biomedical Engineering & Physics
  - Laboratory Experimental Clinical Chemistry

- More information: edwinvanderpol.com