Single versus coincidence detection of cell-derived vesicles by flow cytometry

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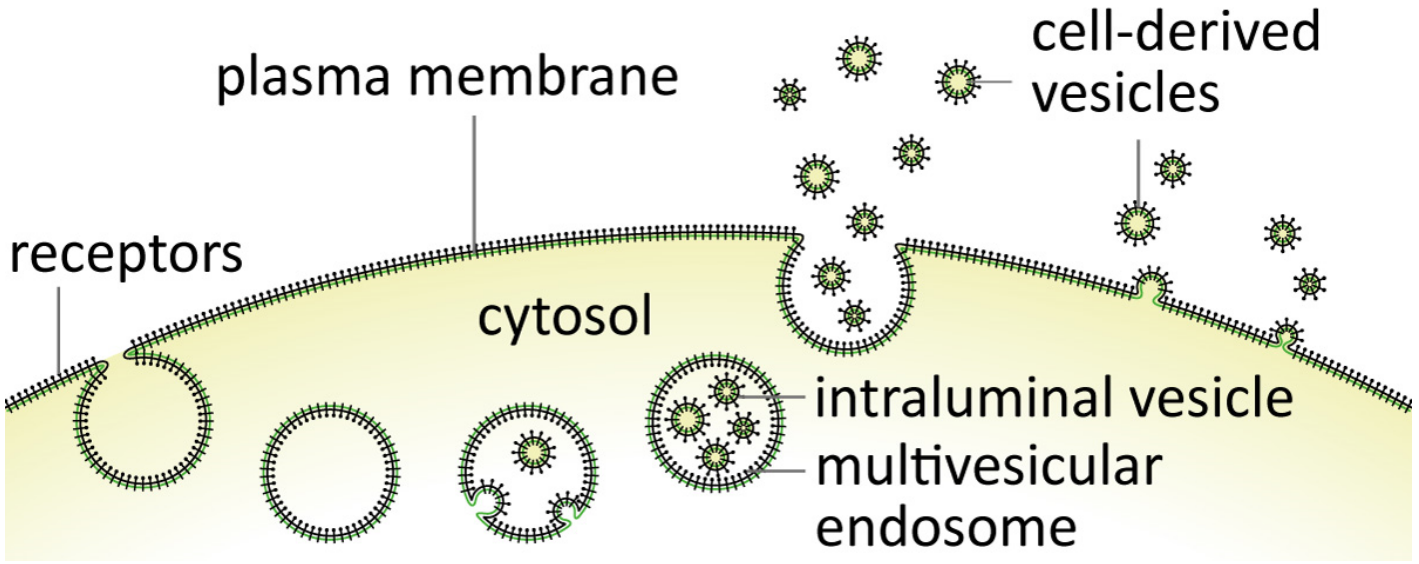
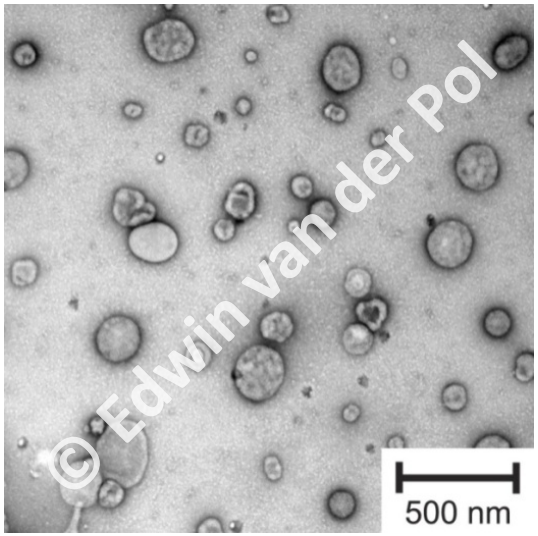
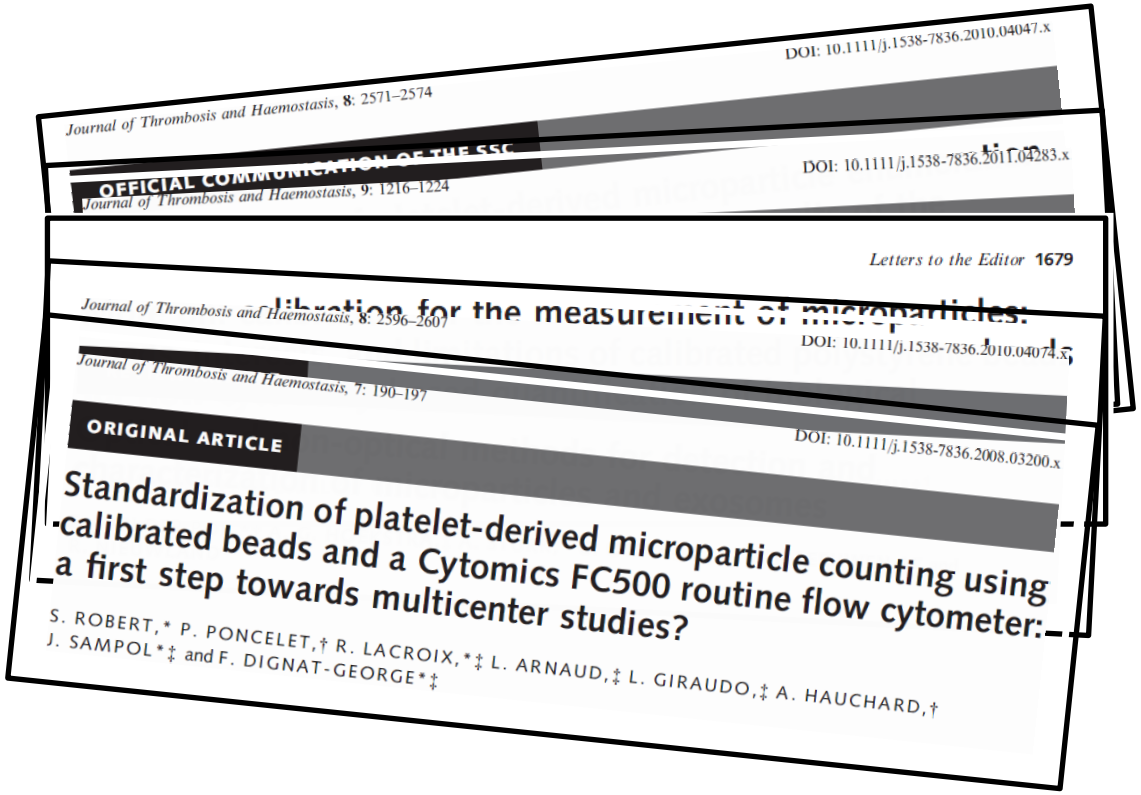
Introduction to cell-derived vesicles



- cells release vesicles:
  spherical particles with phospholipid bilayer
- specialized functions
- clinically relevant

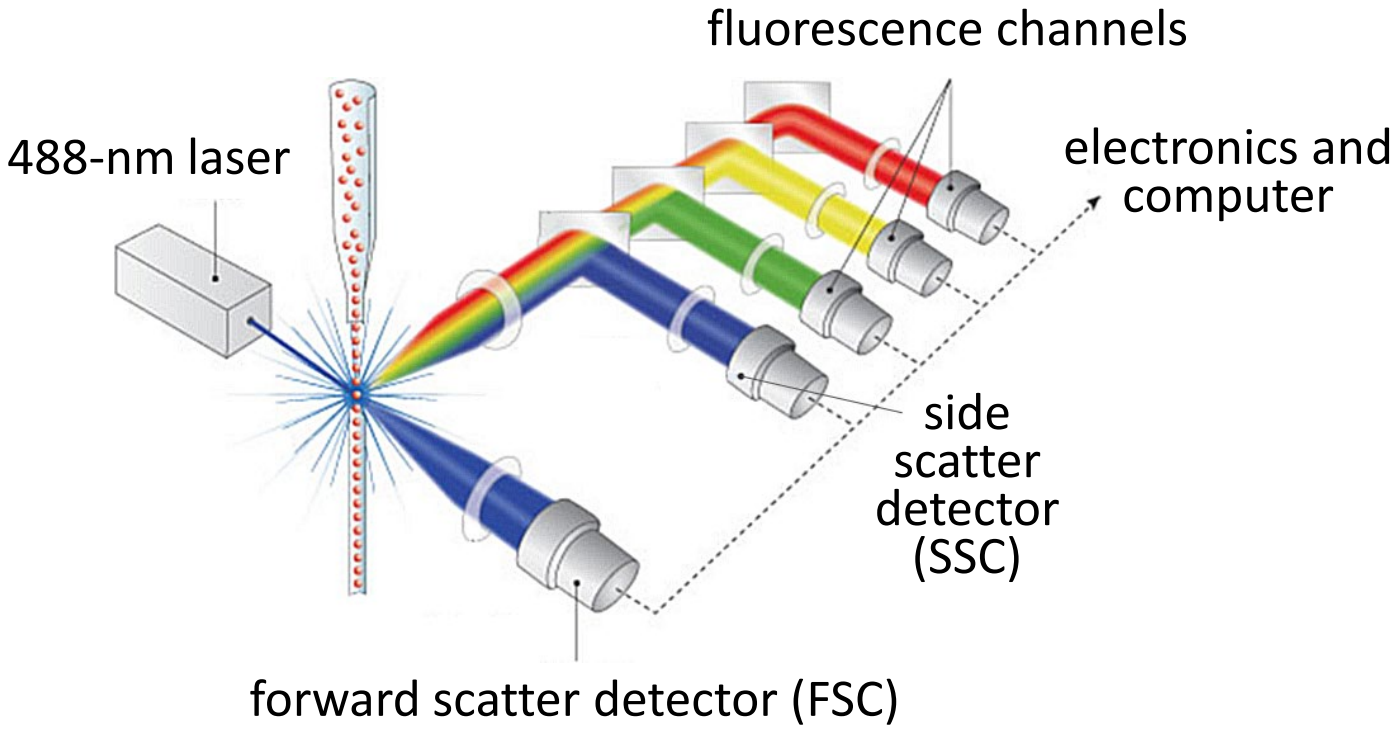
Introduction to cell-derived vesicles





- vesicles are studied mostly by flow cytometry
- mechanism causing detection incompletely understood

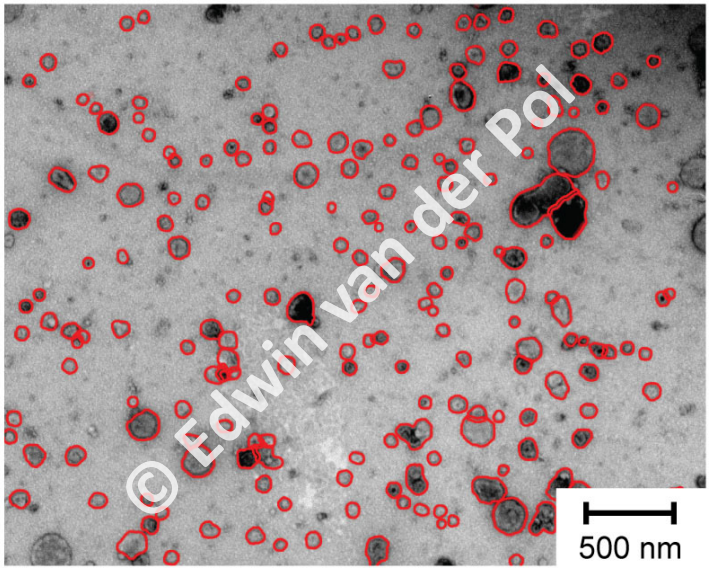
Introduction to flow cytometry

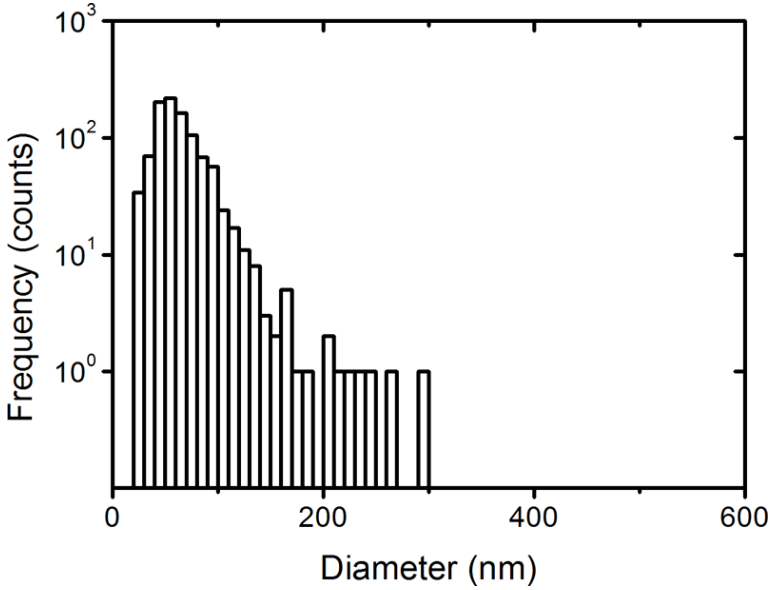


- smallest detectable polystyrene bead is 200 nm
  n = 1.61

image adapted from www.semrock.com

Problem



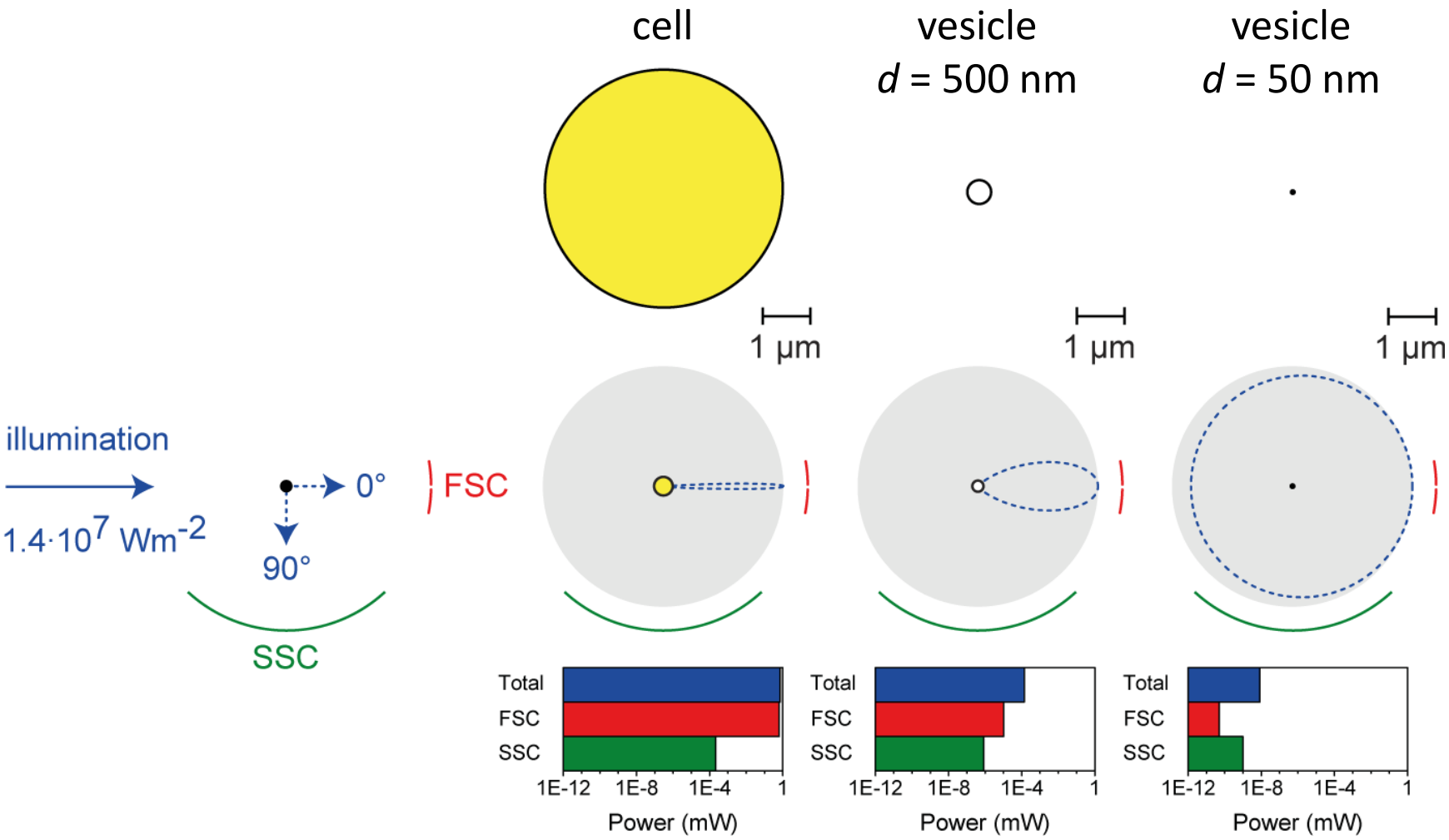


- diameter of vesicles is <300 nm, *n* = ~1.4
- against expectations, vesicles are detected by flow cytometry

Goals

- optimize detection settings
- measure light scattering power of beads
- describe measurements by Mie theory
- determine size of smallest detectable *single* vesicle
- investigate role of *multiple* particles in detection volume by titration

Methods – optimize settings flow cytometer



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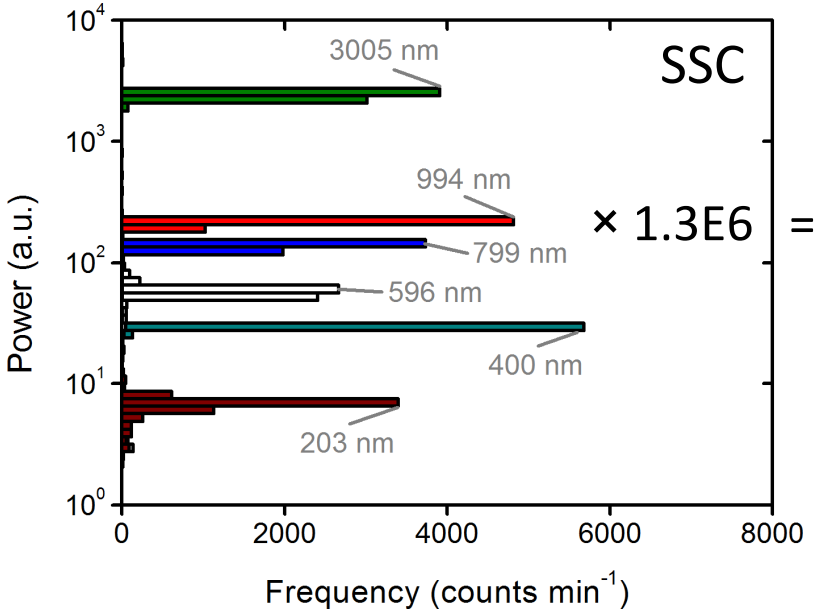
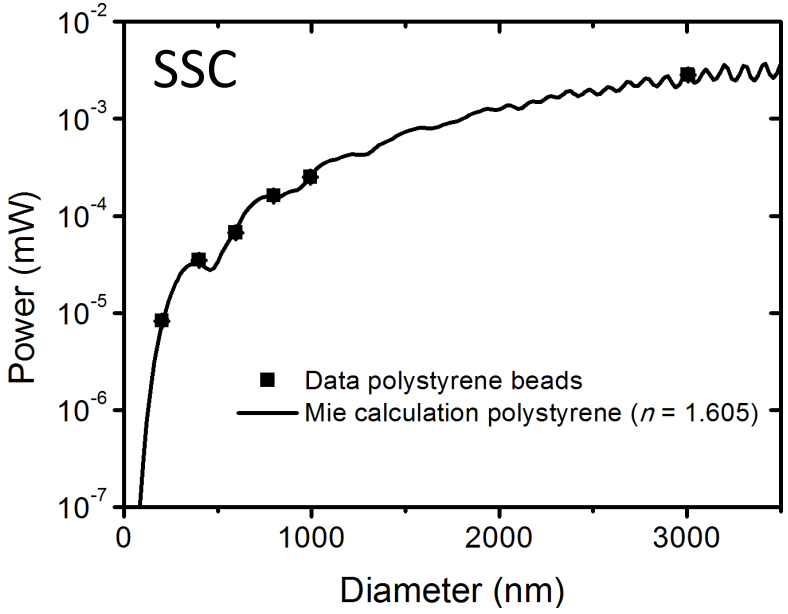
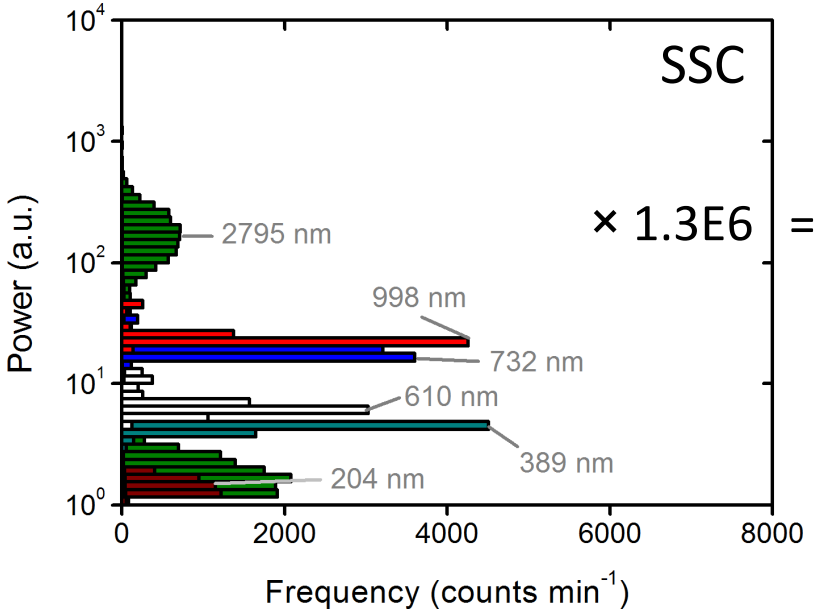
Results – scattering power of polystyrene beads

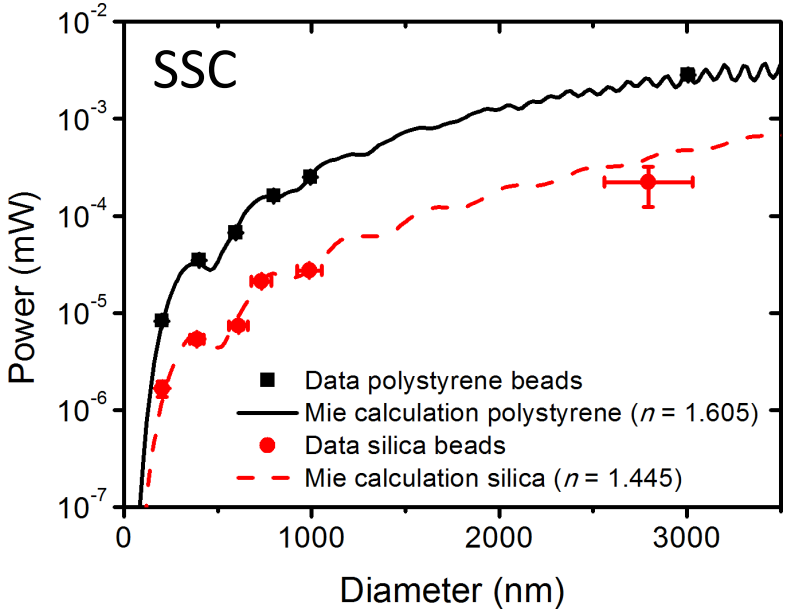


× 1.3E6 =



Results – scattering power of silica beads





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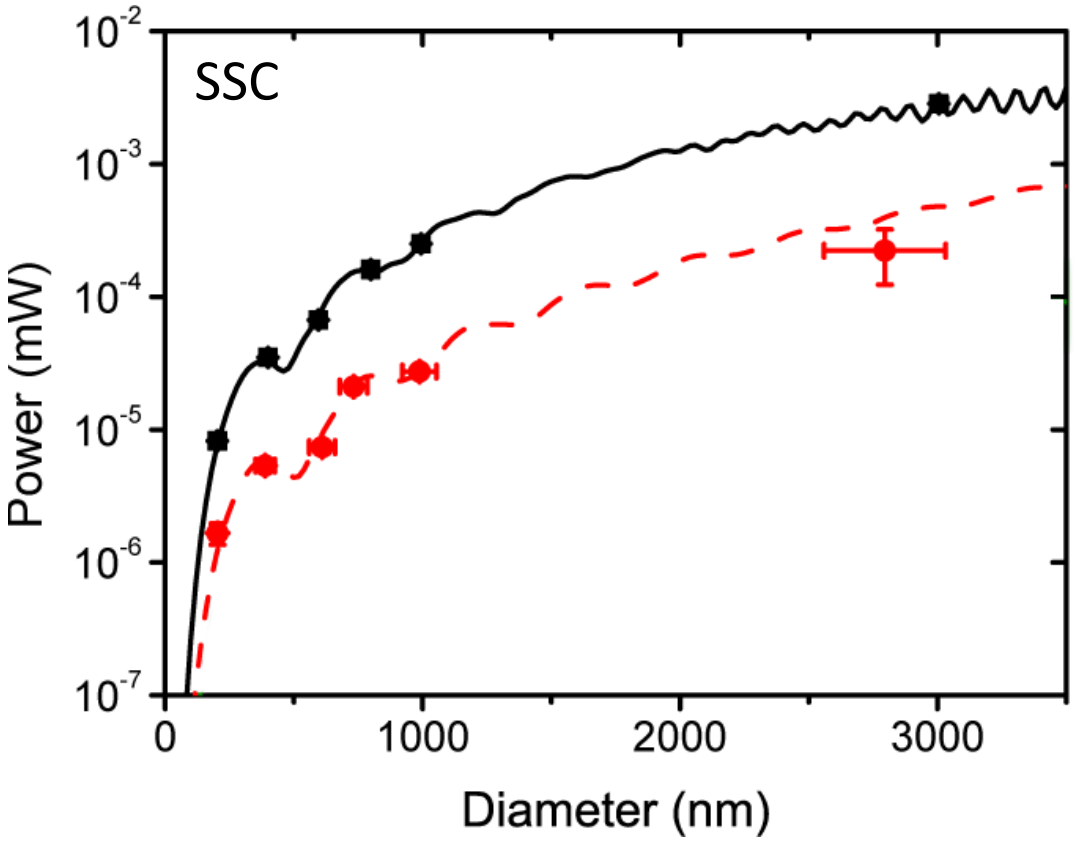
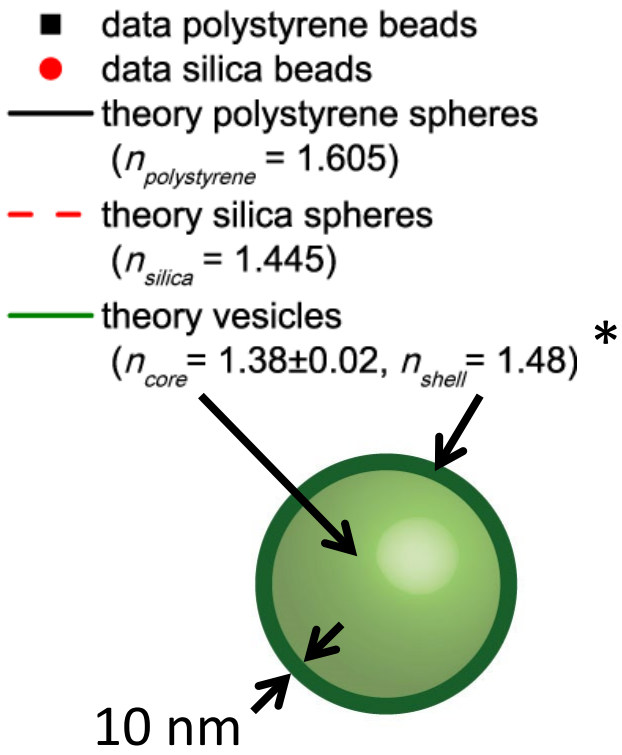
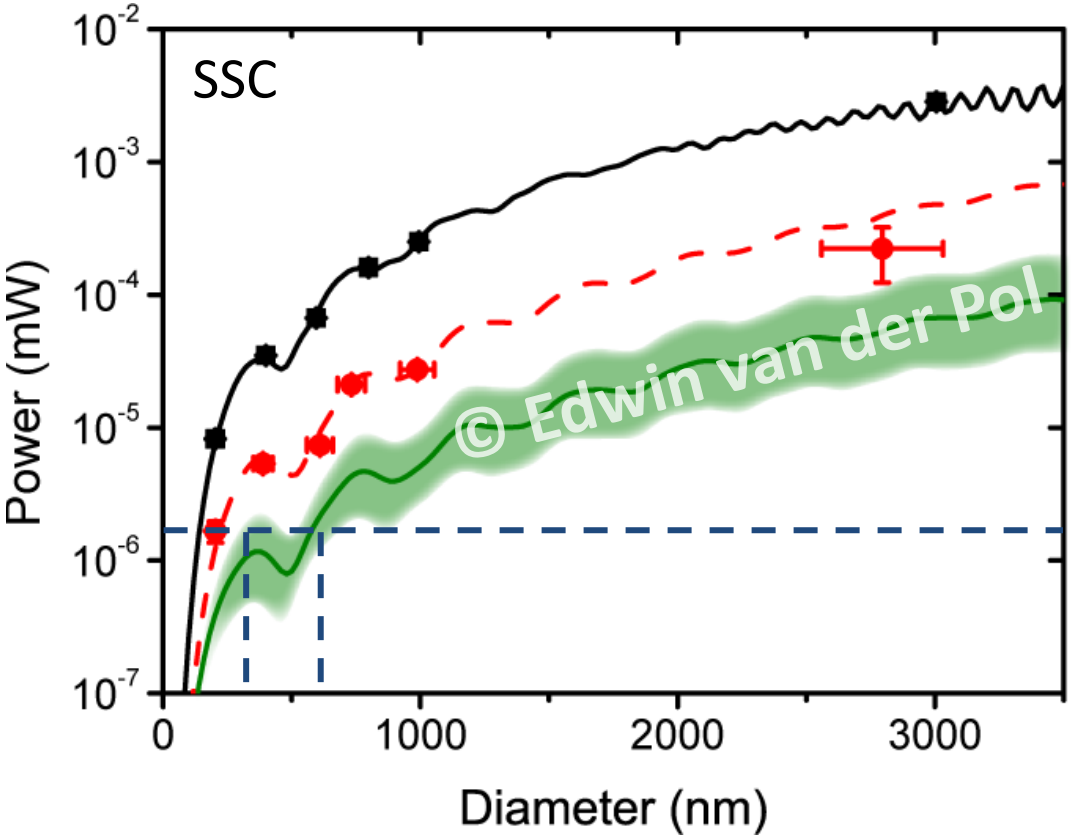
Results – scattering power vs. diameter

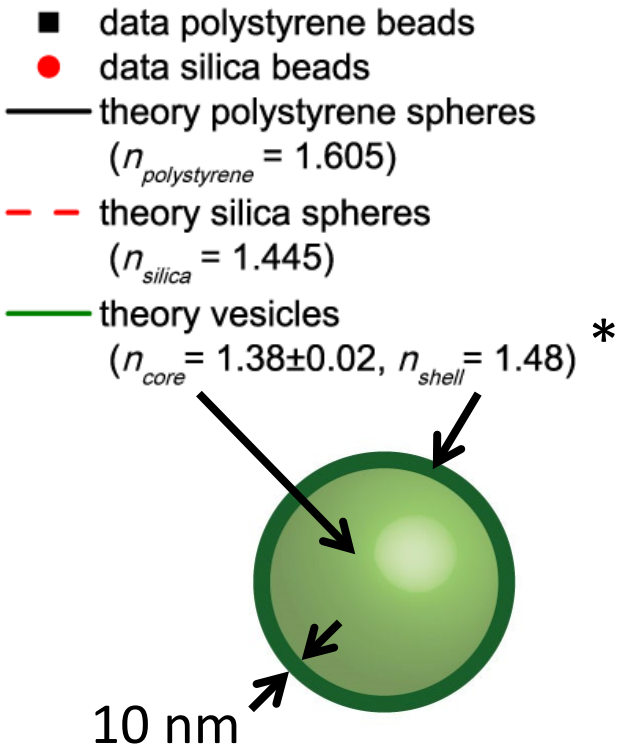




* van Manen et al., Biophys J (2008)

Results – scattering power vs. diameter





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Goals

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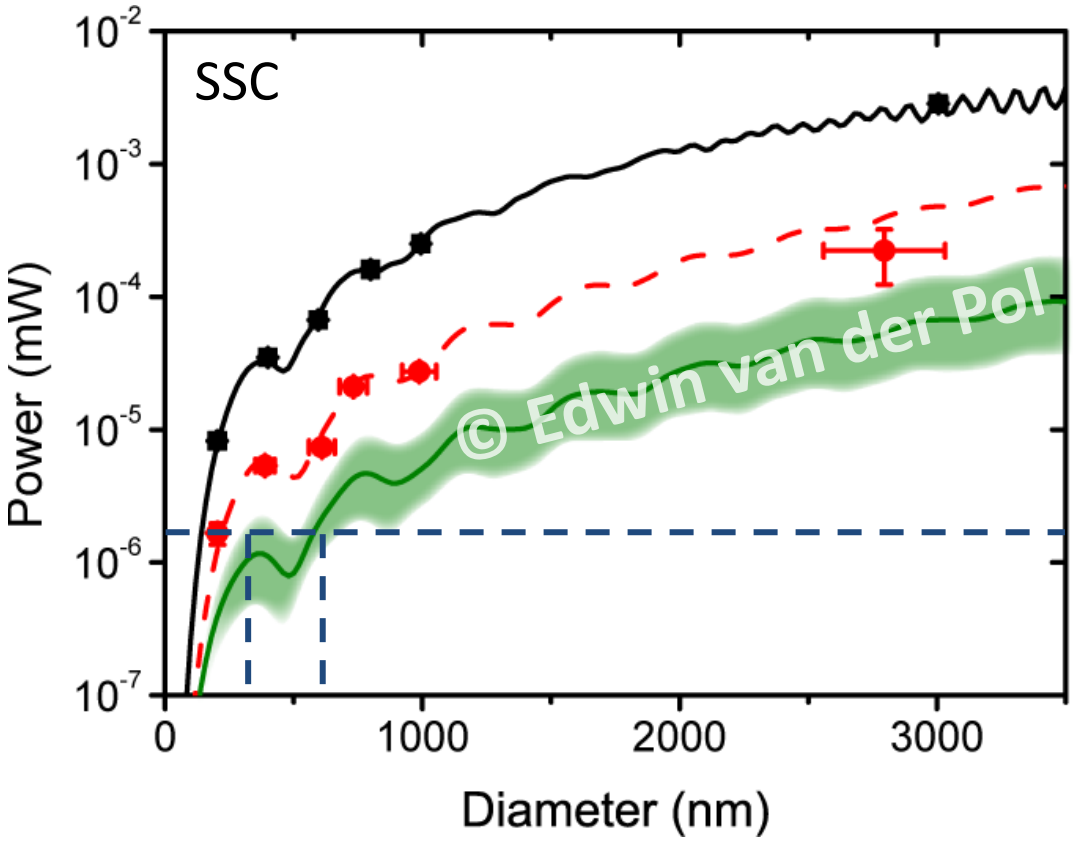

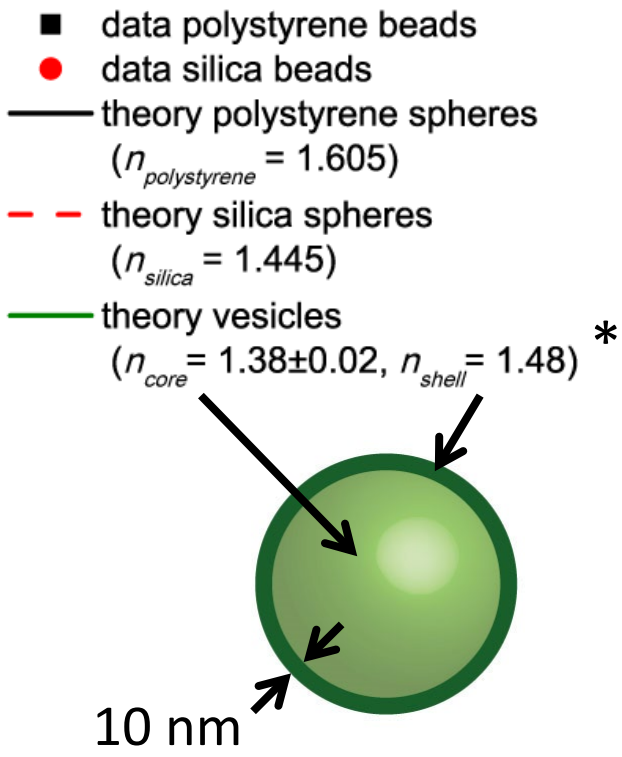

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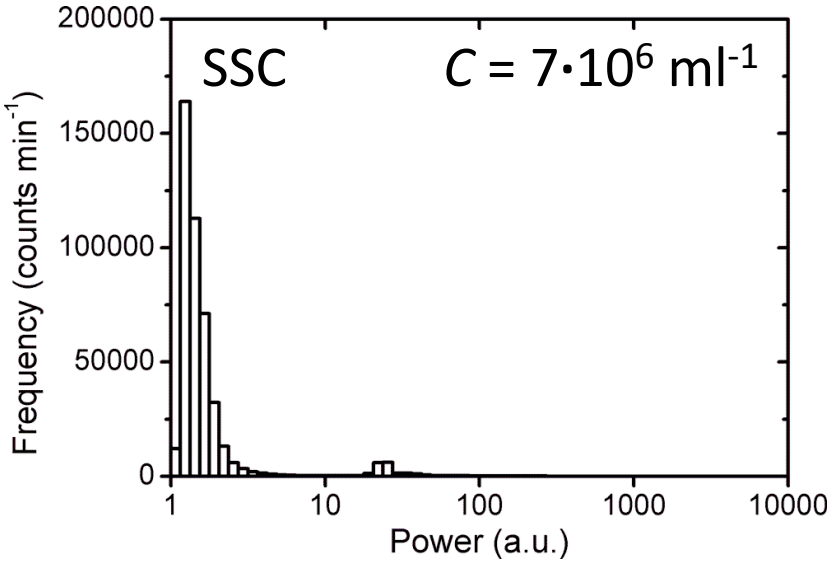
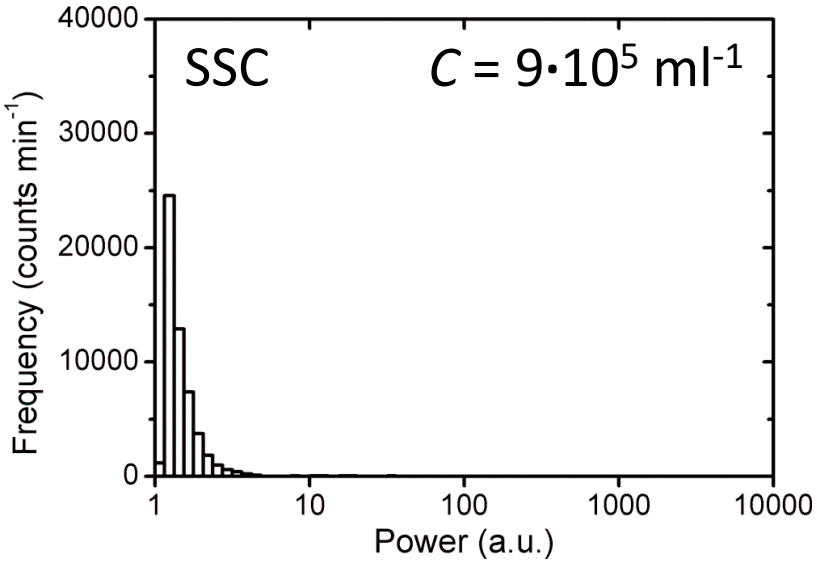
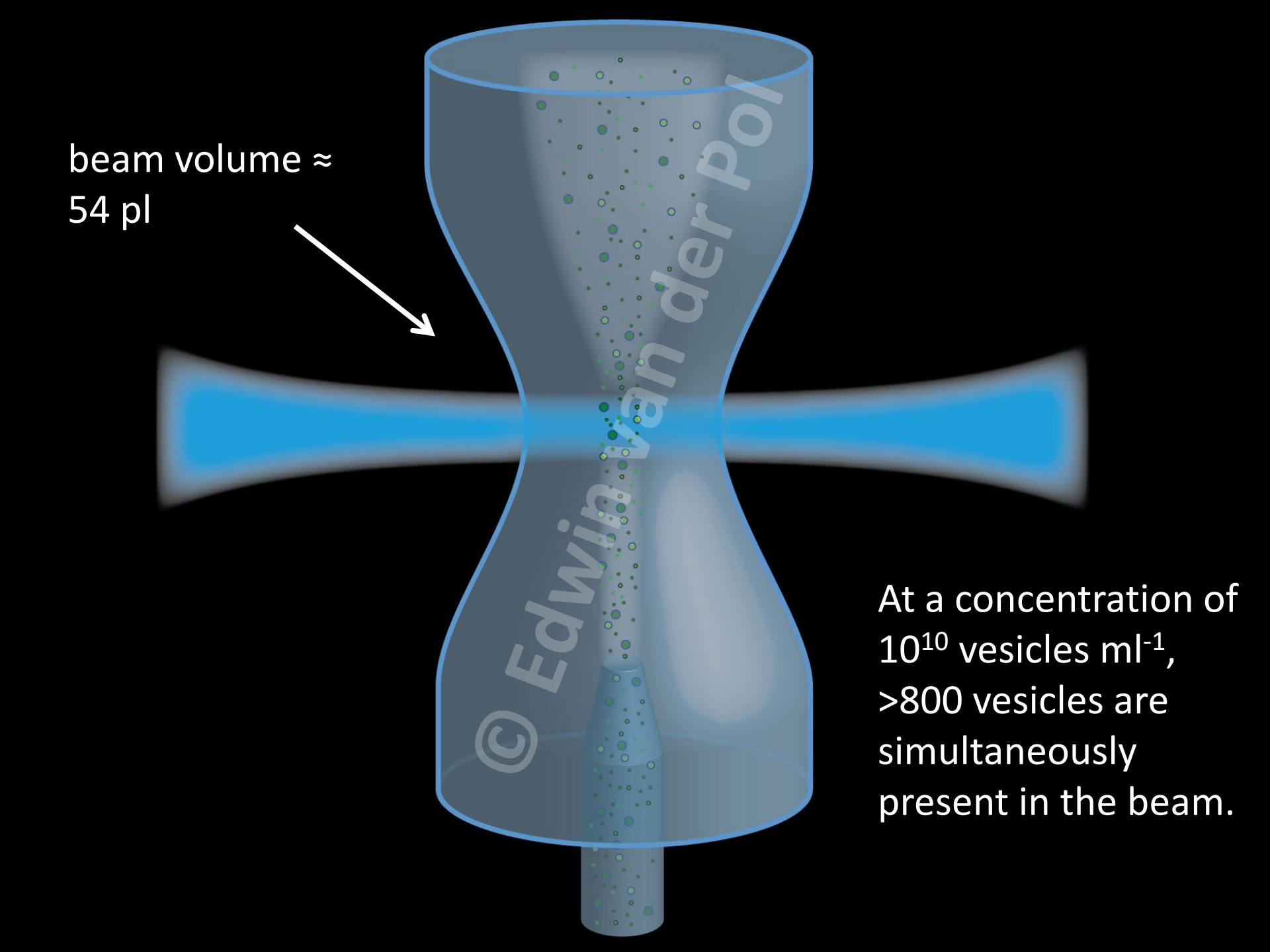
Results – *multiple* vesicles as single count



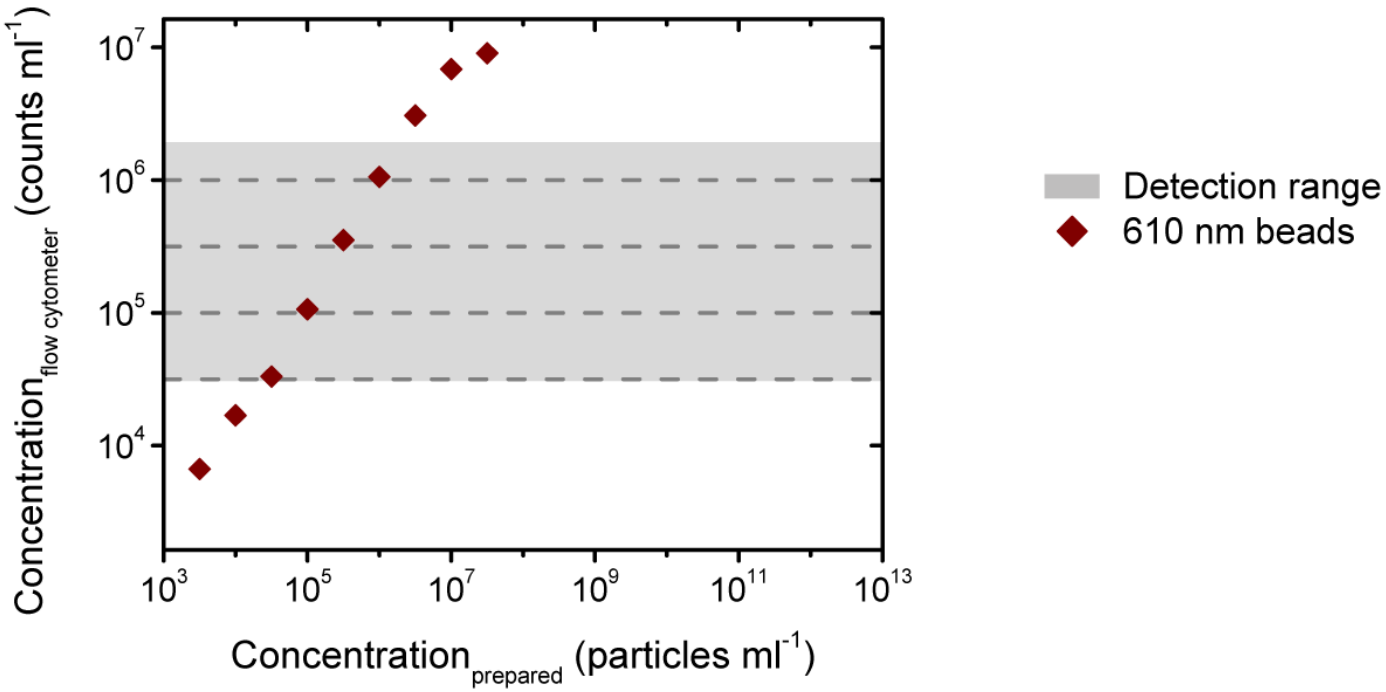
89-nm silica beads at concentration 10^{10} beads ml^{-1}



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


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

Results – counts from mixtures of beads



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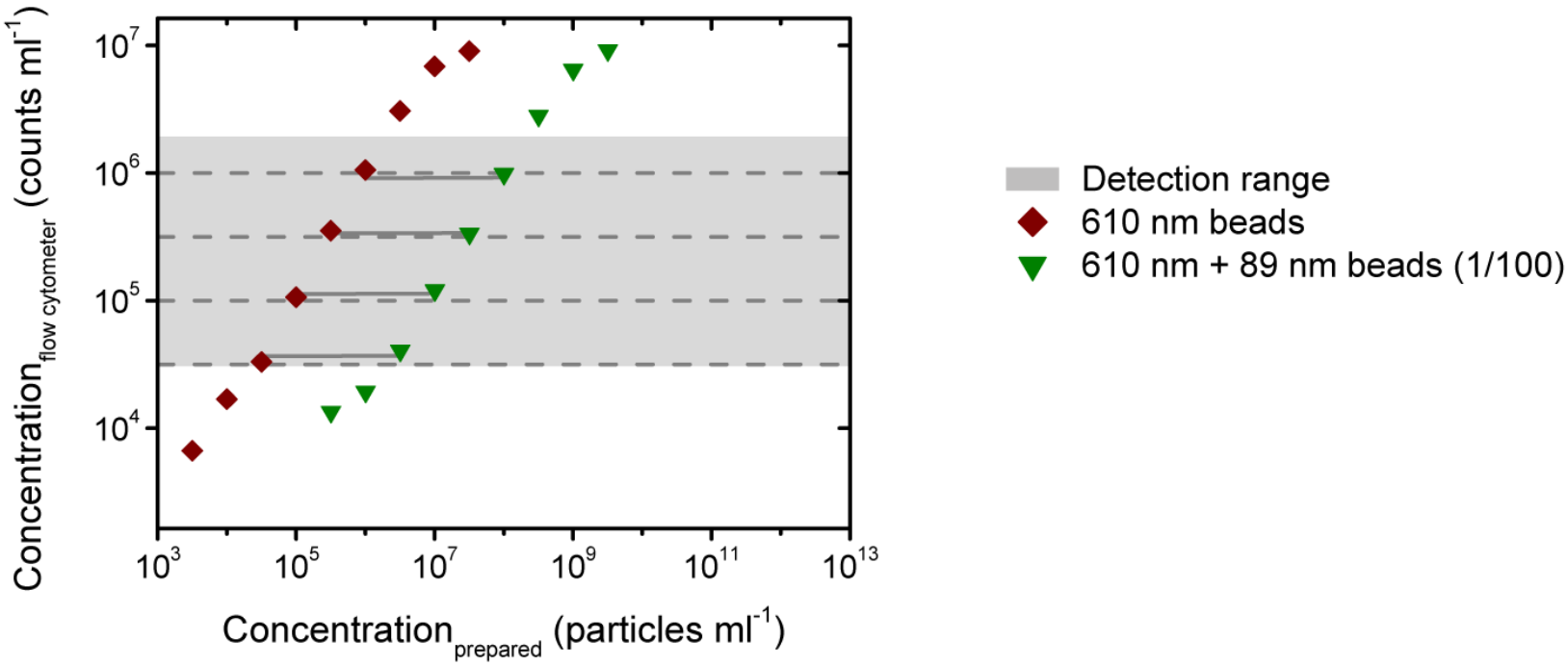
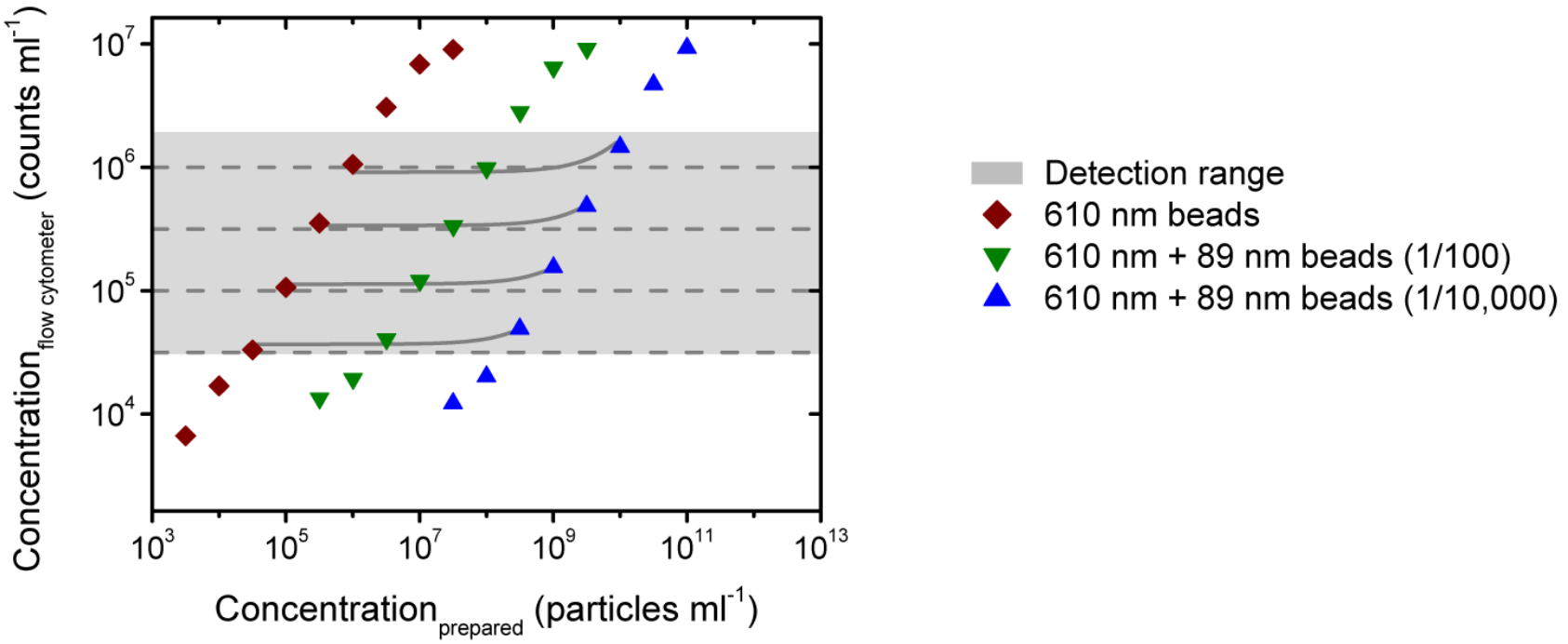


Results – counts from mixtures of beads



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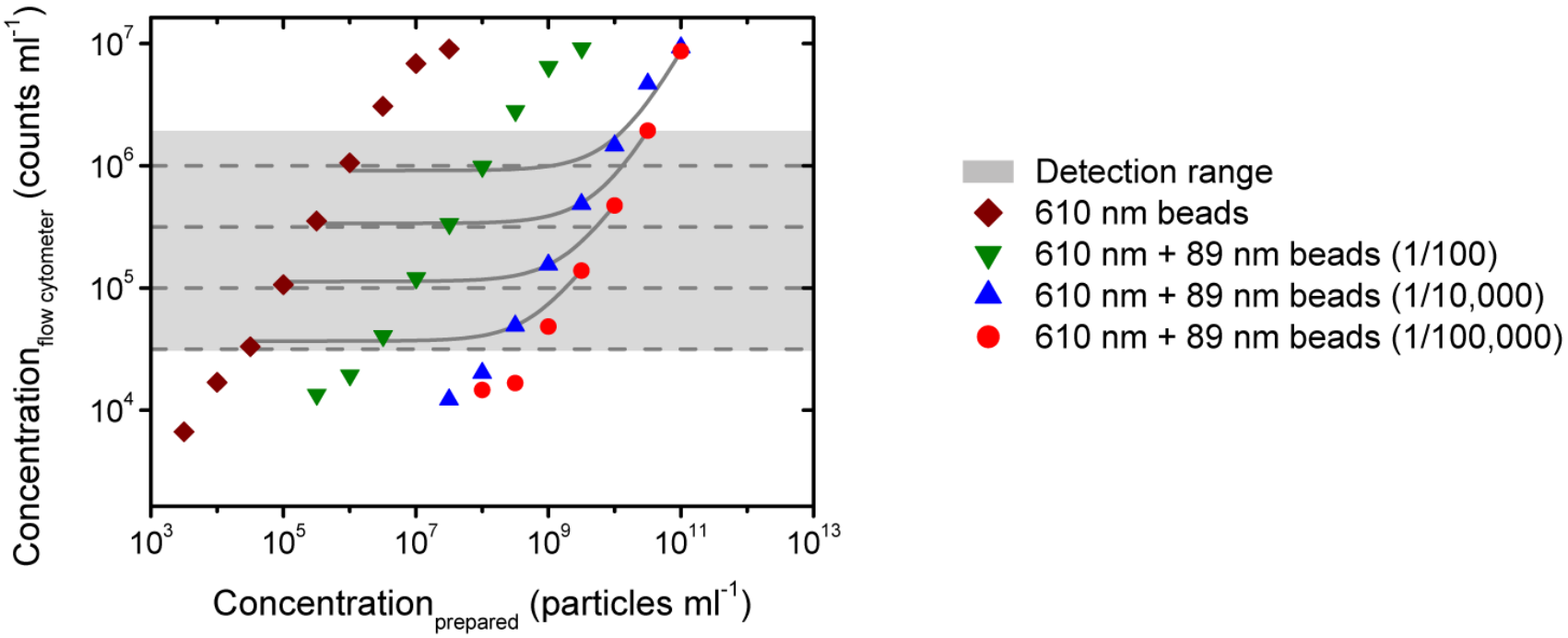

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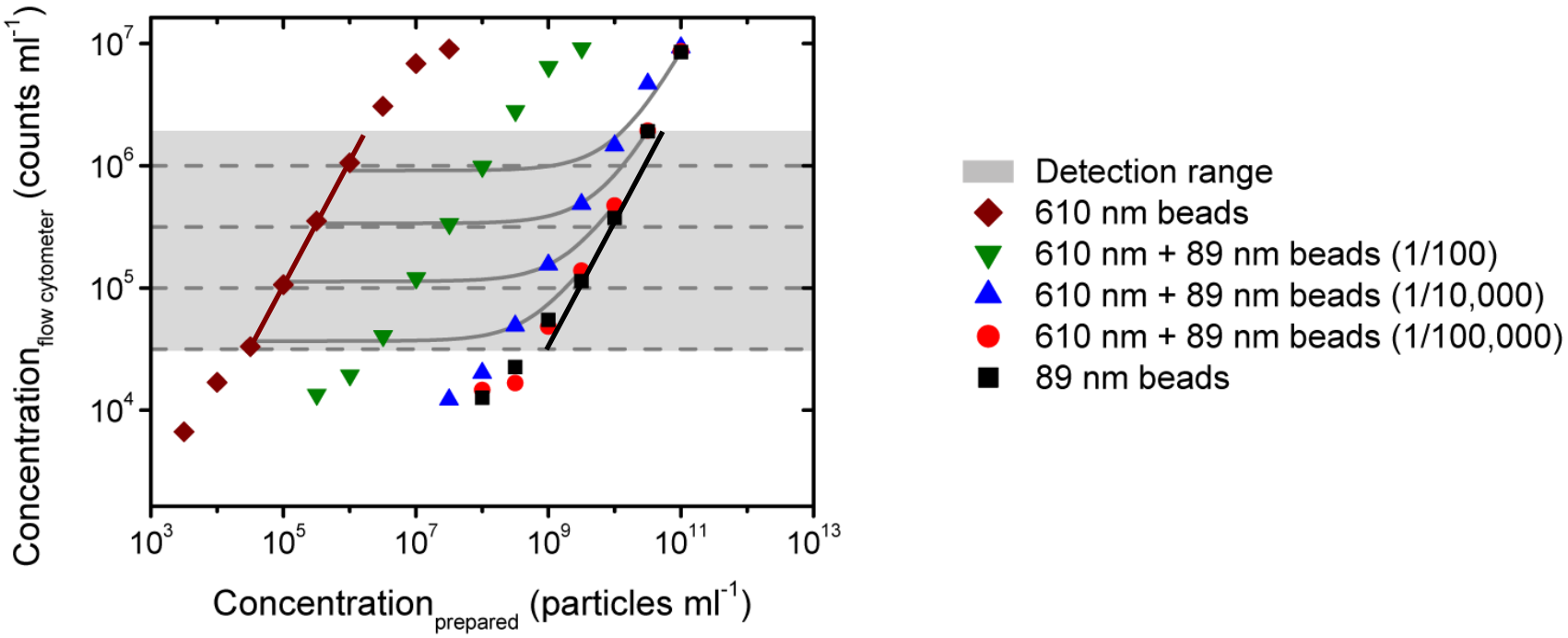
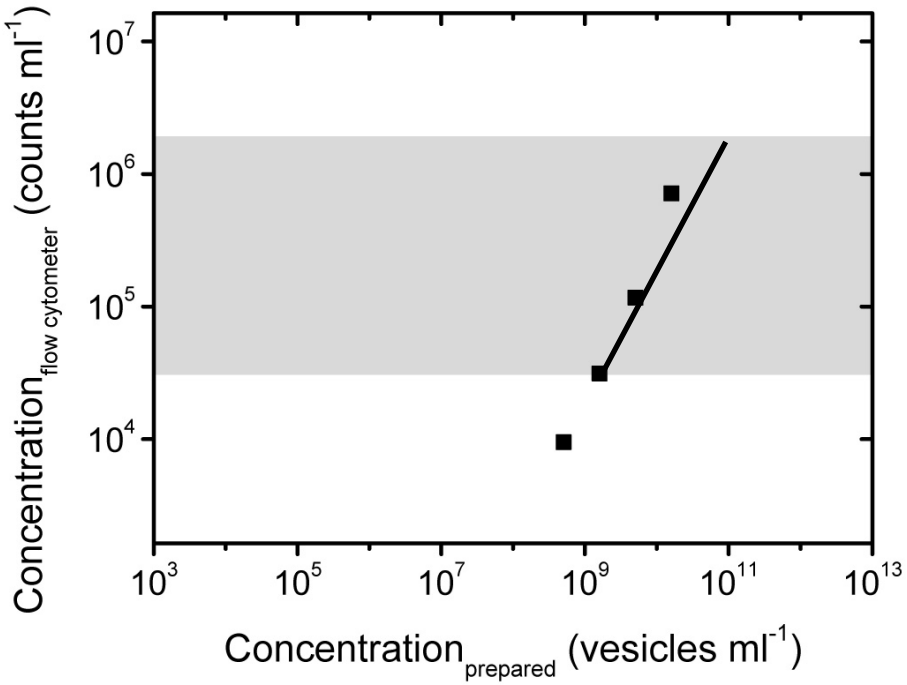


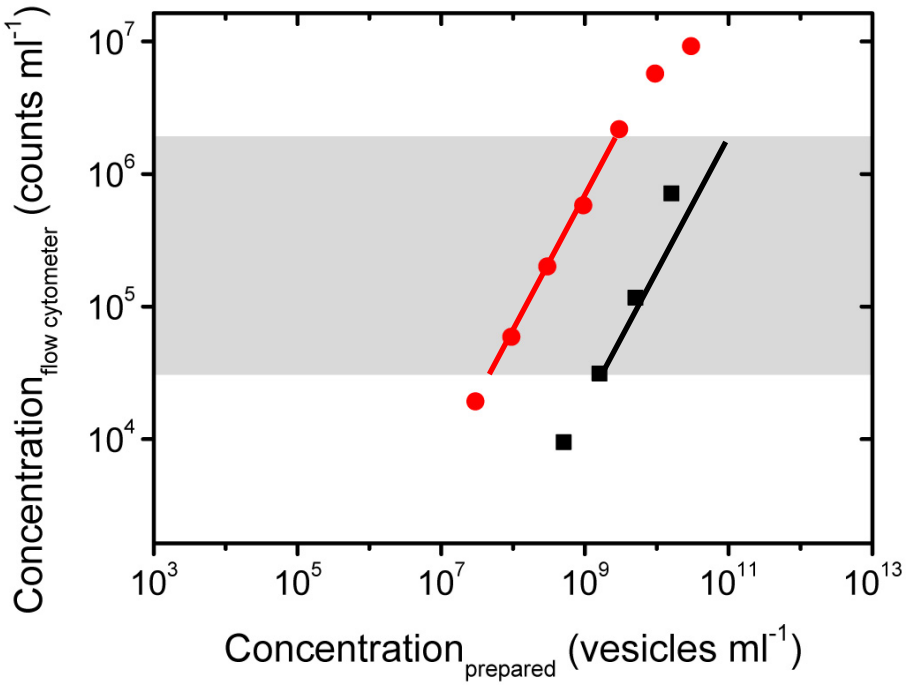
Results – counts from urinary vesicles



Detection range

Cell-free urine filtered with 220-nm filter

Results – counts from urinary vesicles

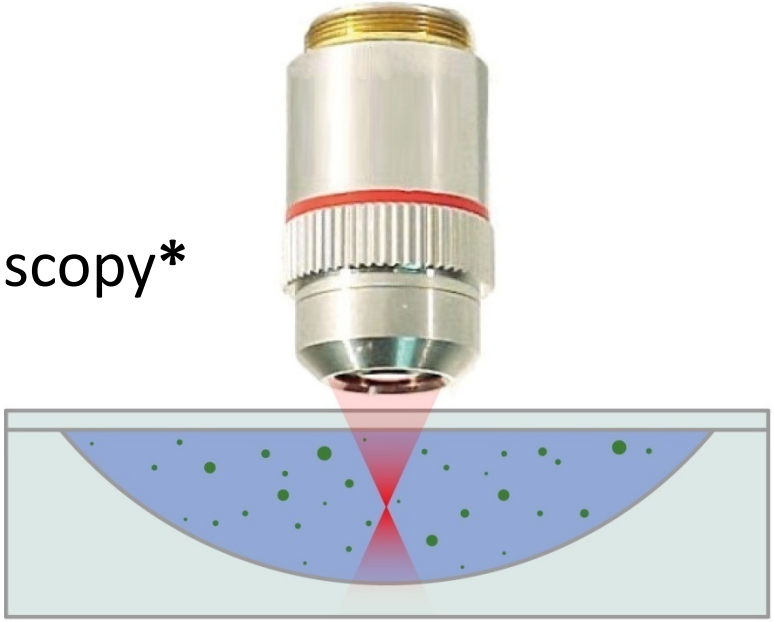


Conclusion

- vesicle detection by flow cytometry
  - scattering power related to diameter and refractive index for *single* beads and vesicles
  - single event signal attributed to scattering from *multiple* vesicles

Outlook vesicle detection

- increase sensitivity of flow “cytometry”
  - reduce detection volume
  - increase irradiance
  - maximize collection angle
  - shorter wavelength
- employ other techniques
  - Confocal Raman microspectroscopy*



* Proc SPIE 8591-11, Wednesday 1:50 pm room 309

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