Detection of microparticles by flow cytometry

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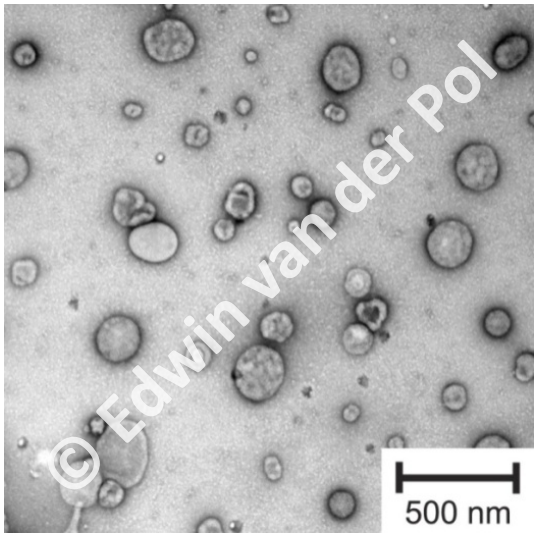
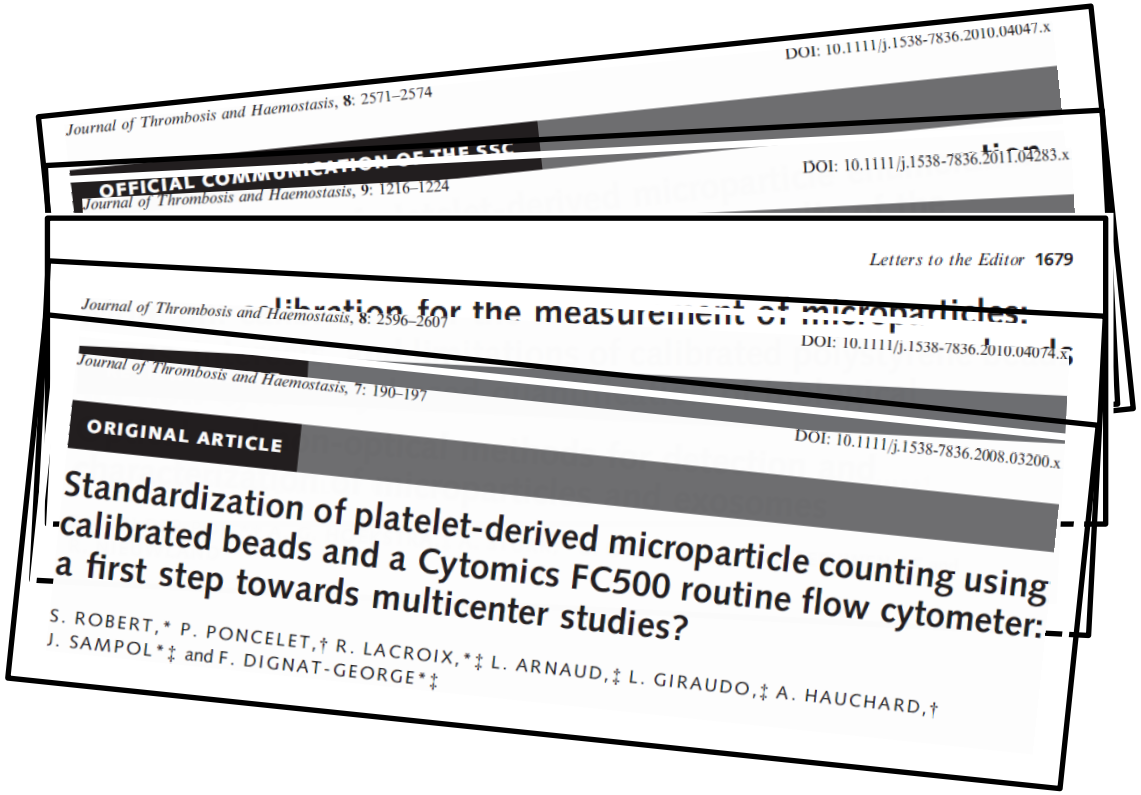
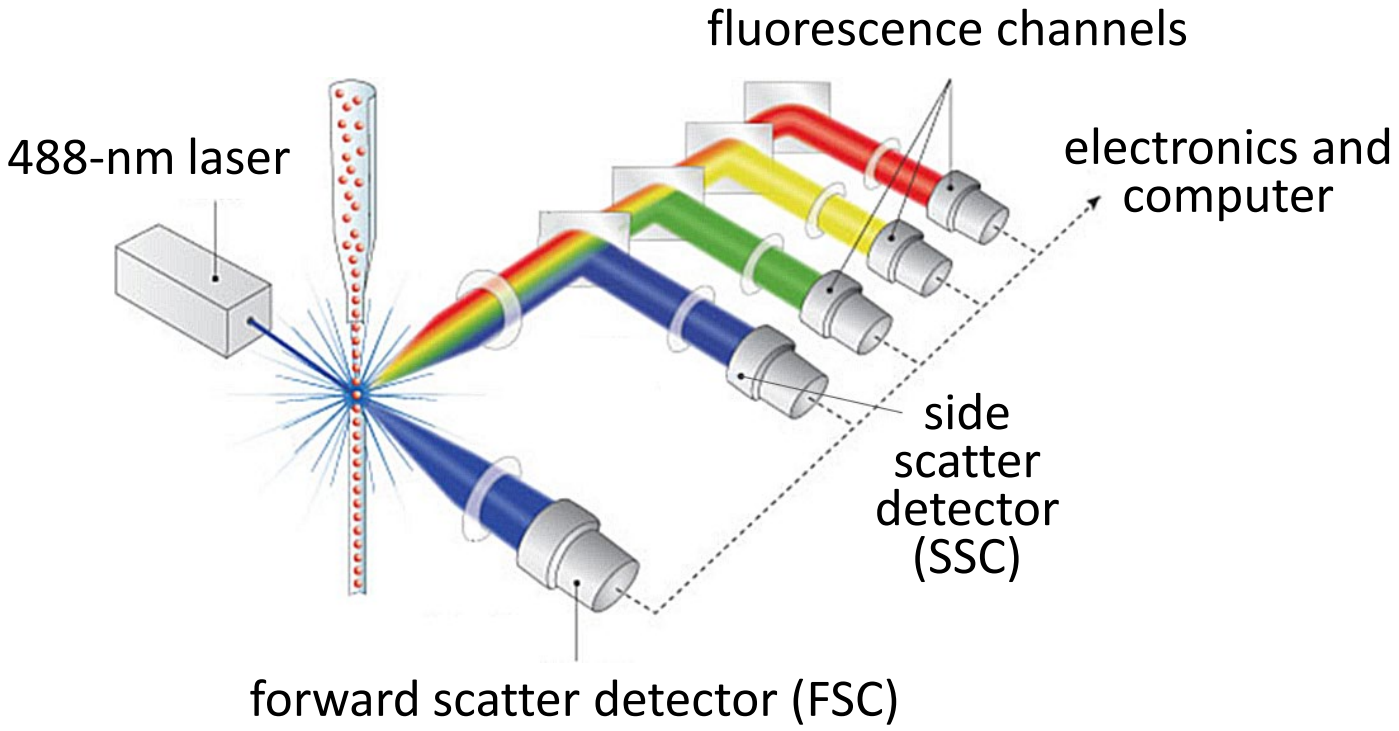
Microparticles and flow cytometry





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

Introduction to flow cytometry

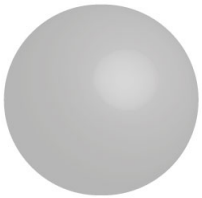


- smallest detectable polystyrene bead is 200 nm
  n = 1.61

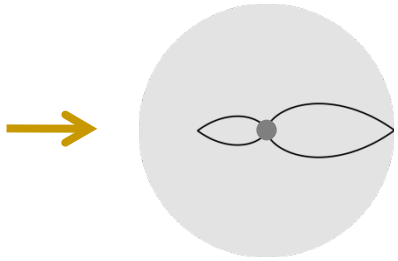
image adapted from www.semrock.com

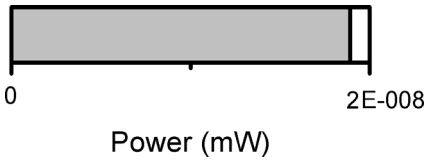
Light scattering and the refractive index

Polystyrene bead



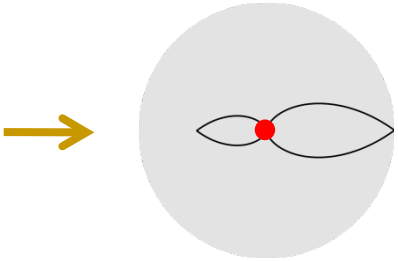
n = 1.61

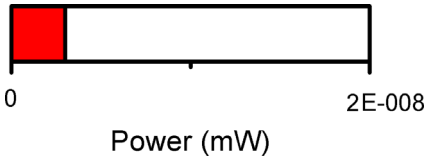




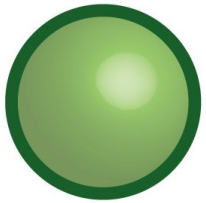
Silica bead

n = 1.45



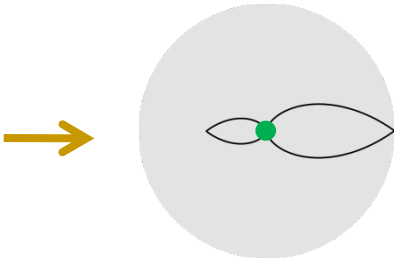


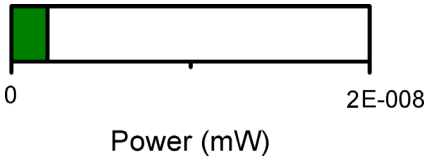
Vesicle



n_{core} = 1.38

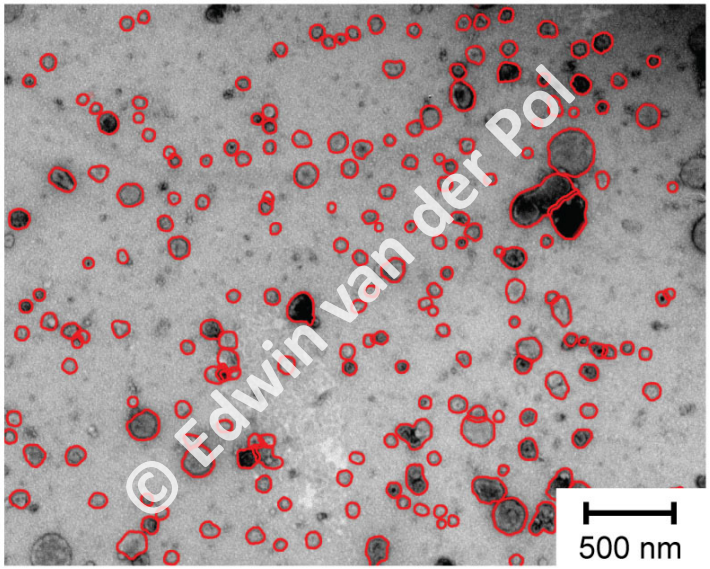
$n_{membrane}$ = 1.48

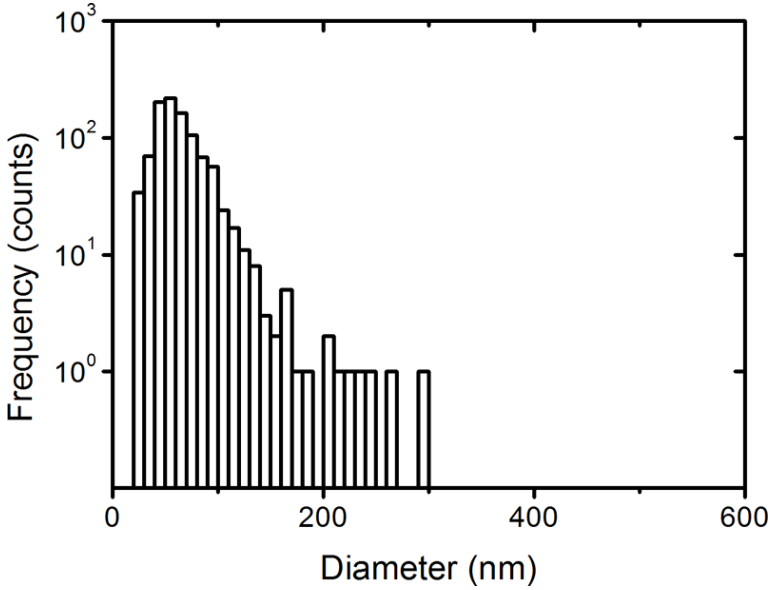




100 nm

Problem



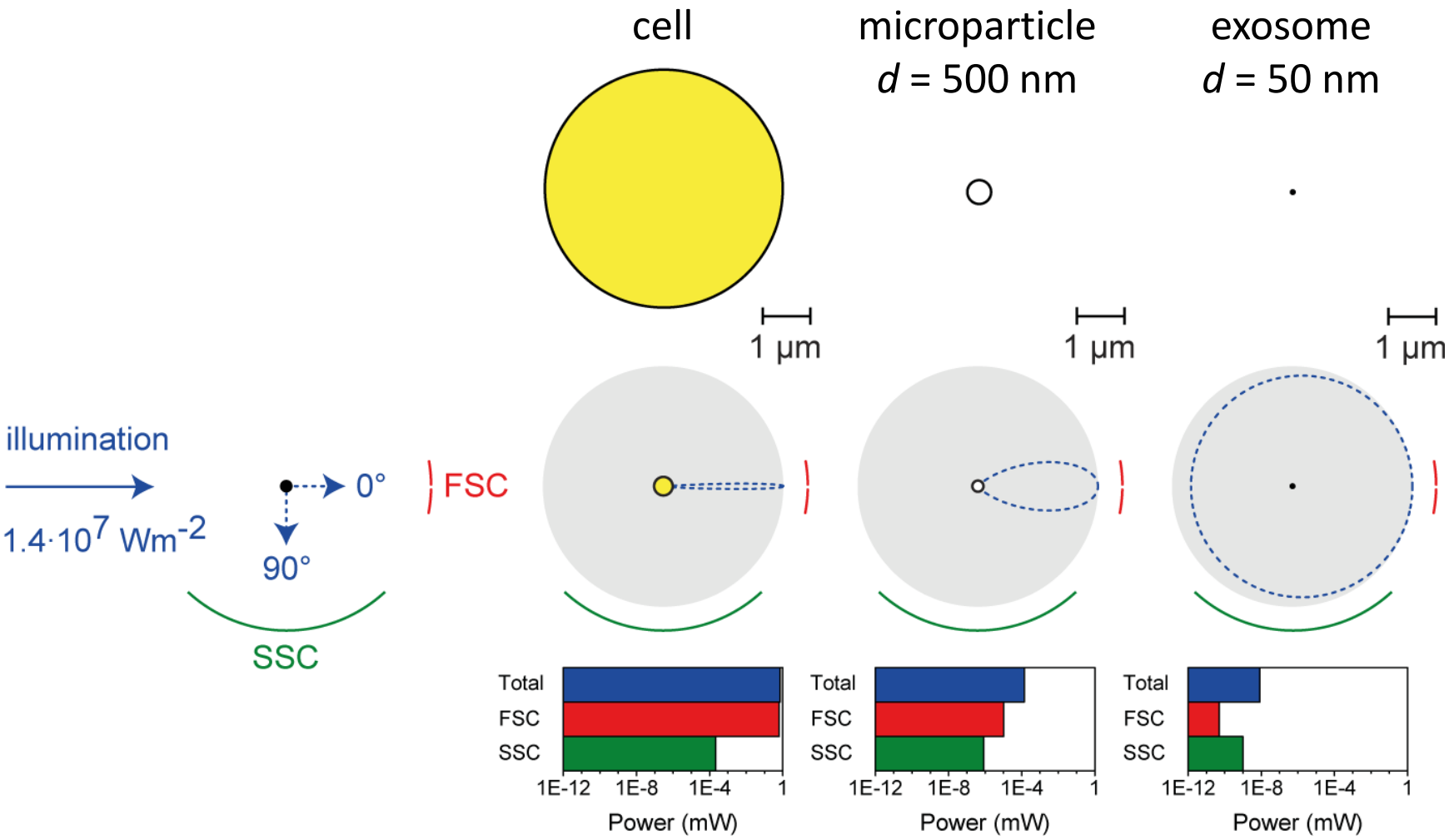


- diameter of vesicles is <300 nm
- against expectations, vesicles are detected by flow cytometry

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 dilution series
- prospects

Methods – optimize flow cytometer settings



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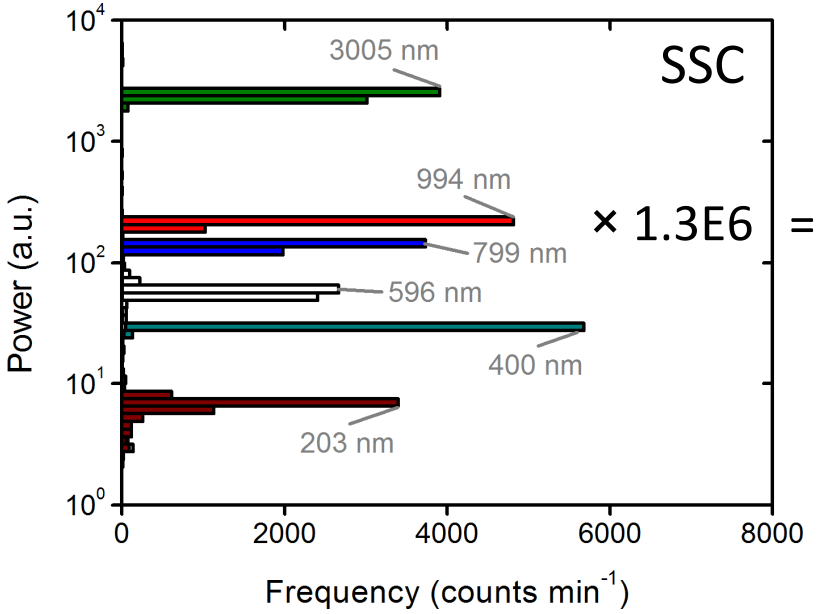
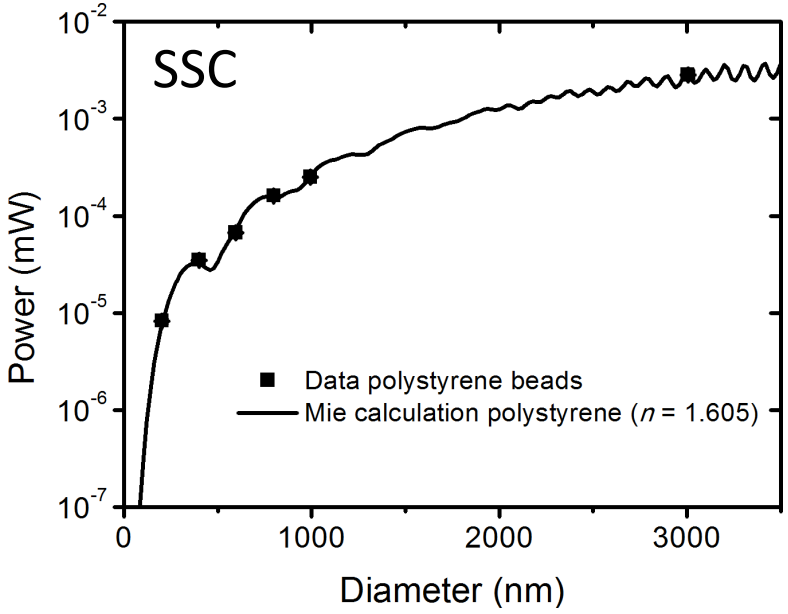
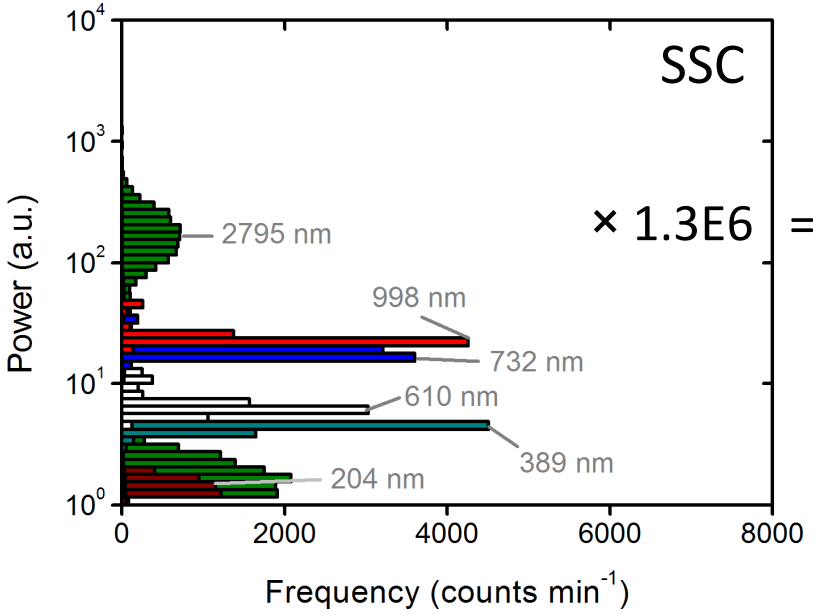
Results – scattering power of polystyrene beads

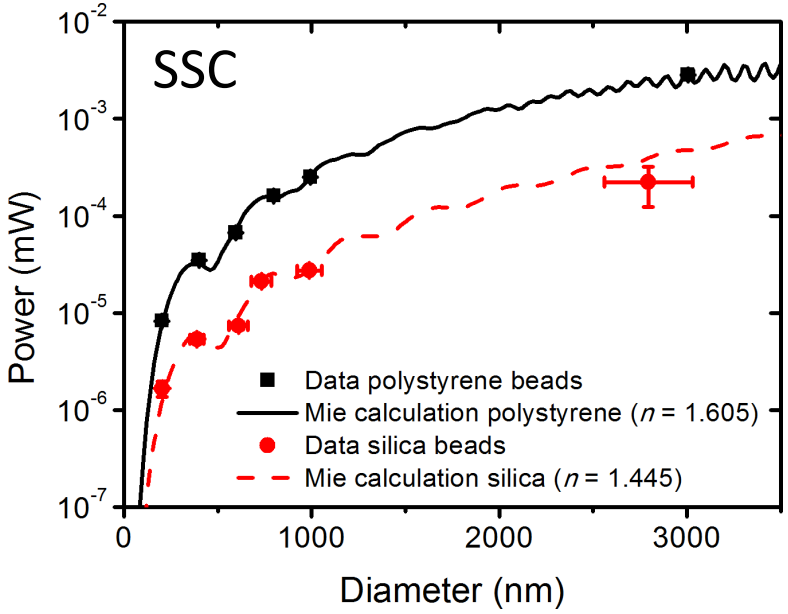


× 1.3E6 =



Results – scattering power of silica beads





Goals

- ✓ optimize detection settings
- ✓ measure light scattering power of beads
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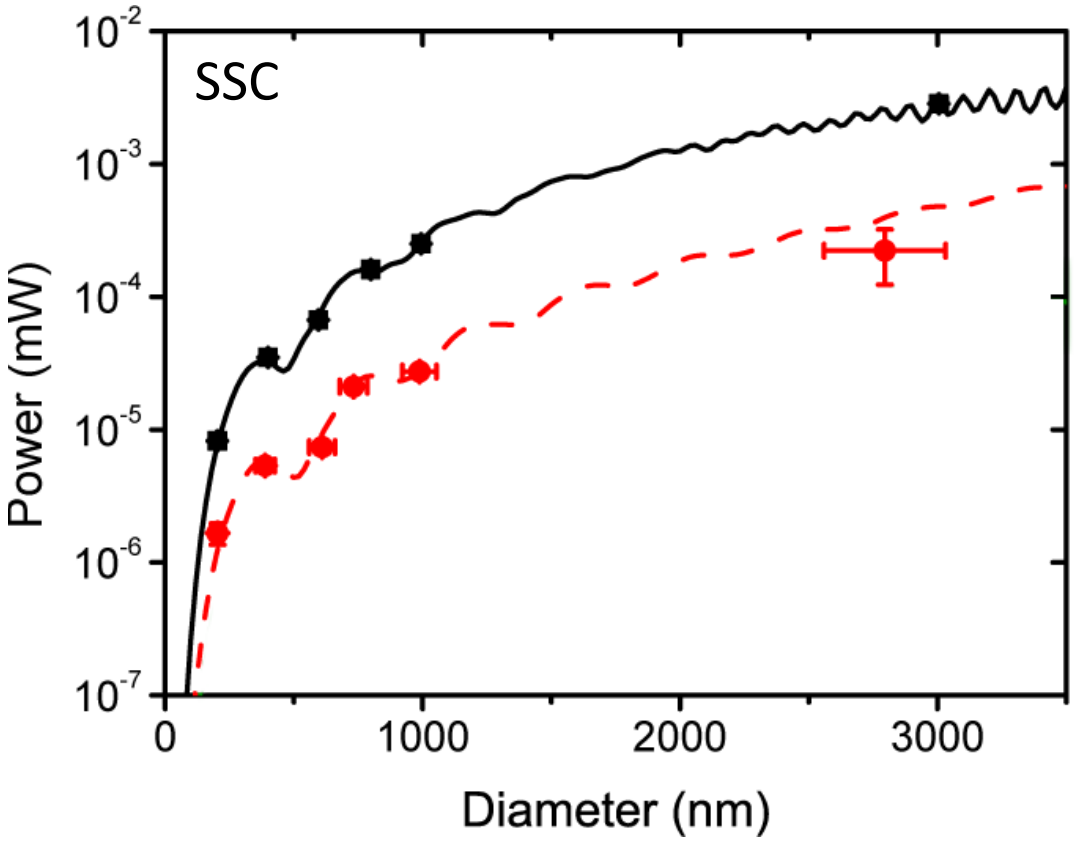
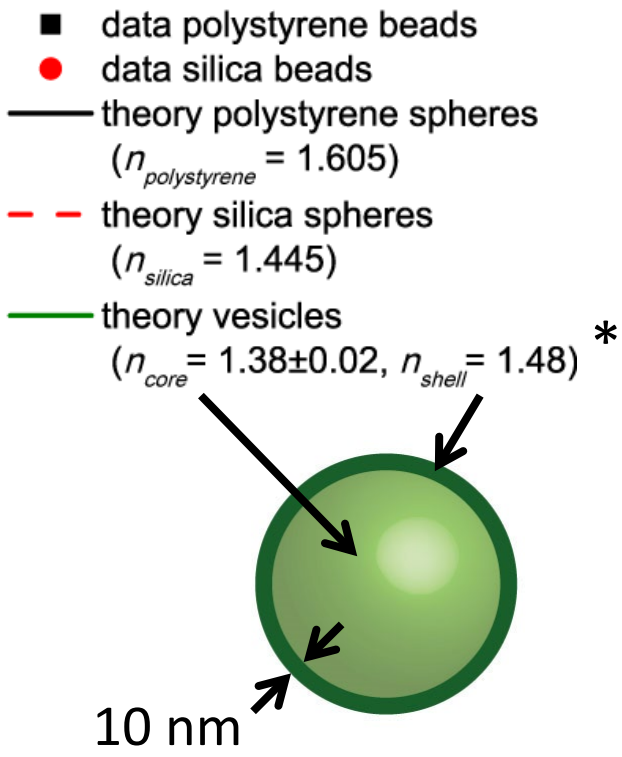
- prospects

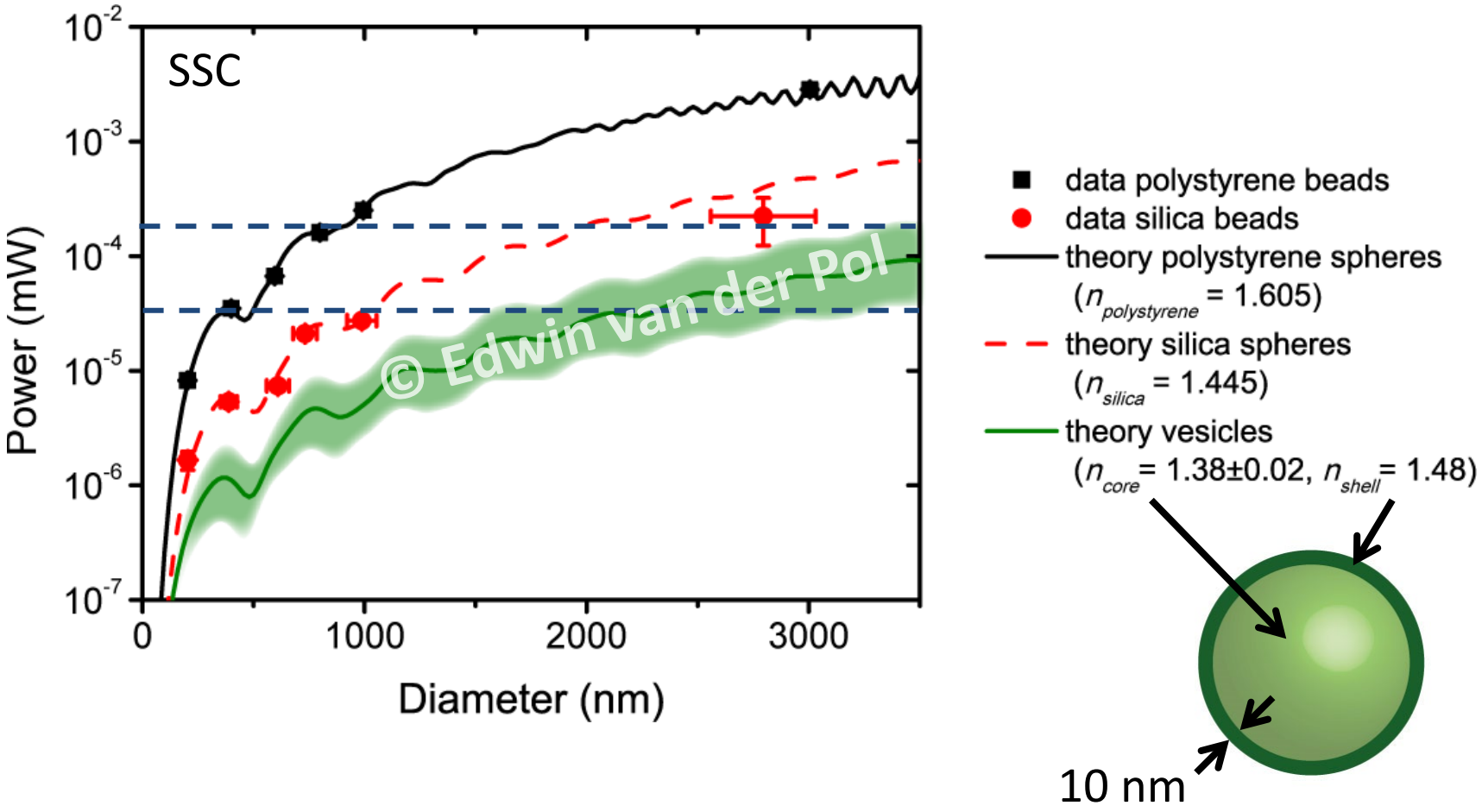
Results – scattering power vs. diameter



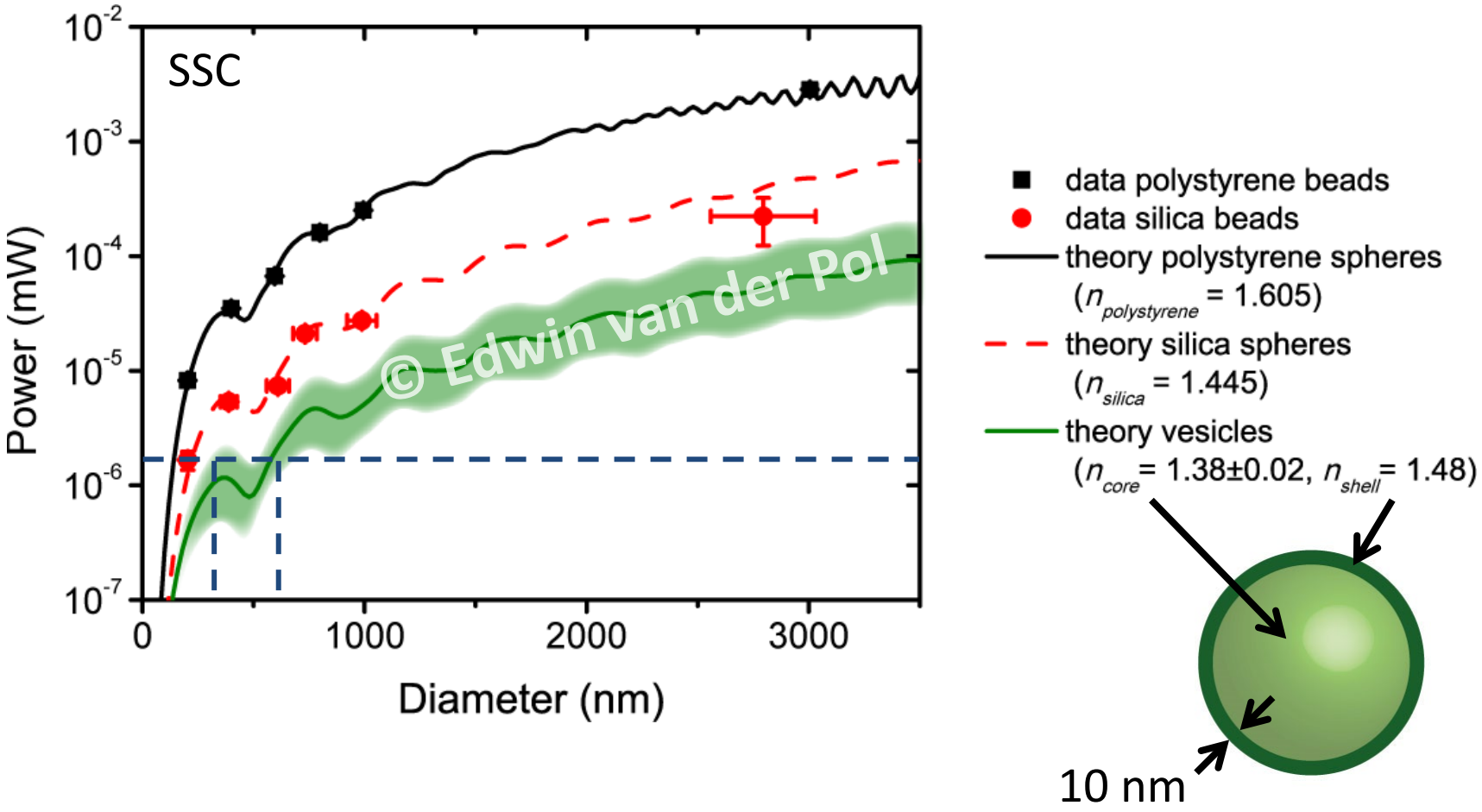


* van Manen et al., Biophys J (2008)
Konokhova et al., J Biomed Opt (2012)

Results – scattering power vs. diameter



Results – scattering power vs. diameter



Goals

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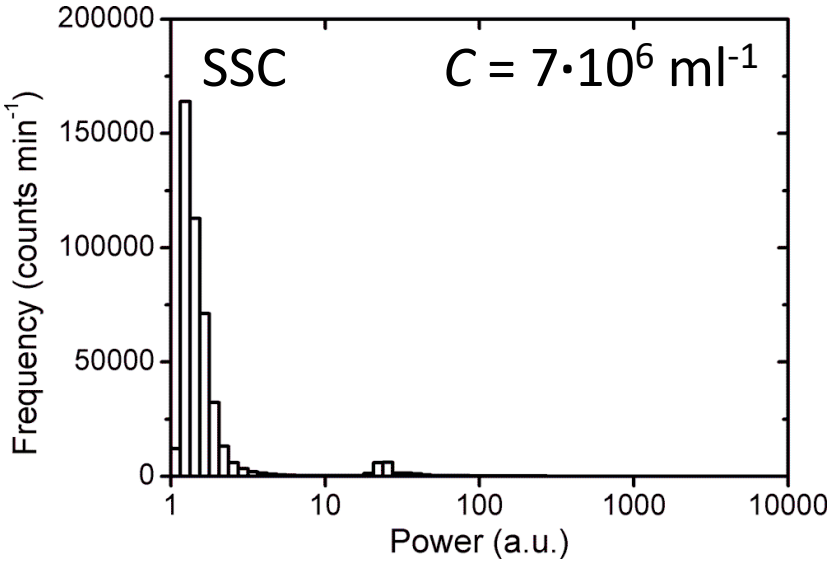
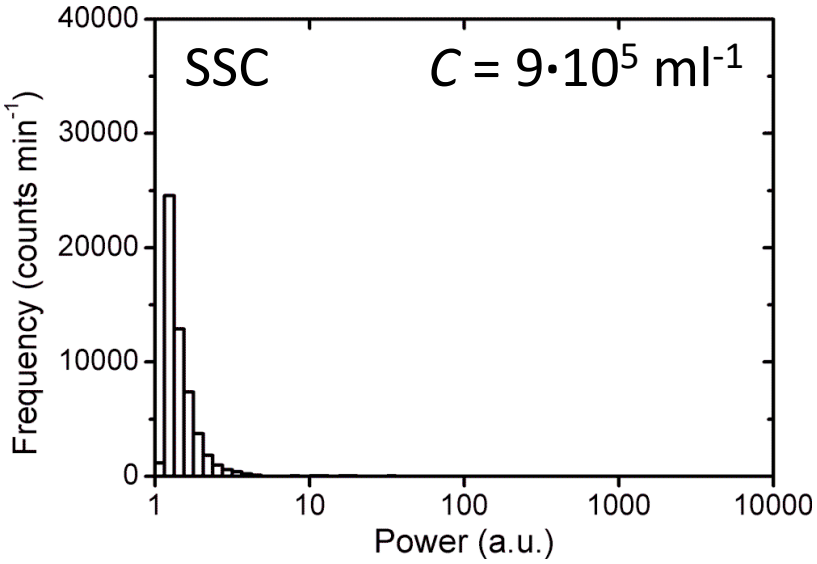
- prospects

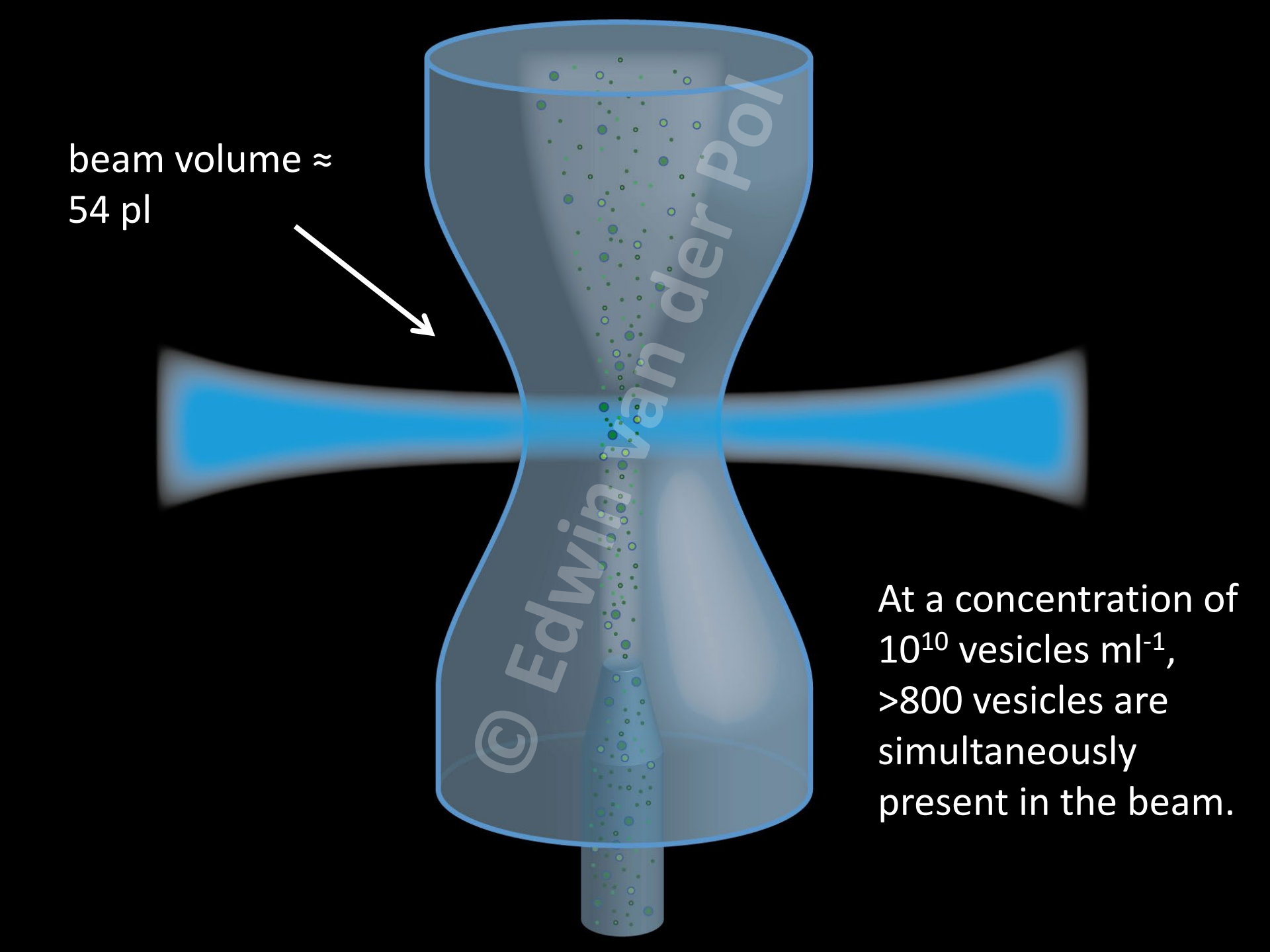
Results – *multiple* vesicles as single count



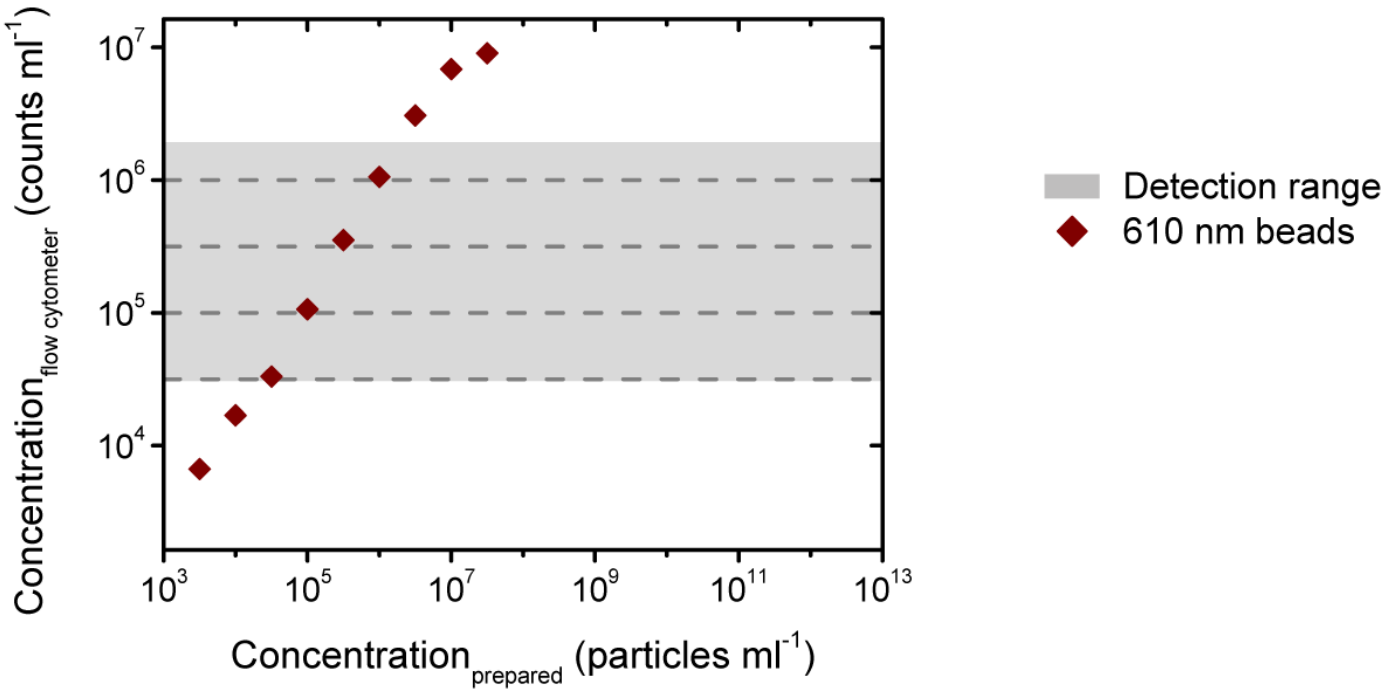
89-nm silica beads at concentration 10^{10} beads ml^{-1}



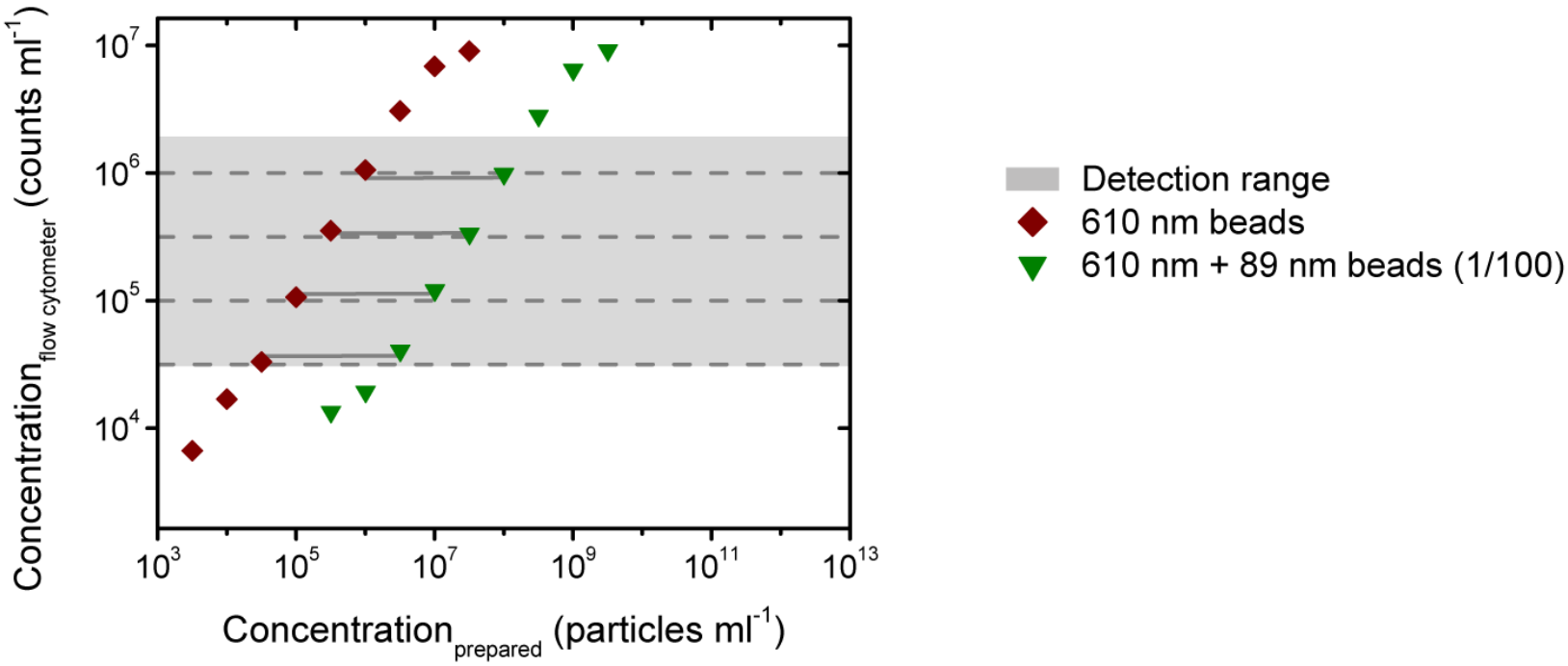
urine filtered with 220-nm filter concentration ≥ 10^{10} vesicles ml^{-1}


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.

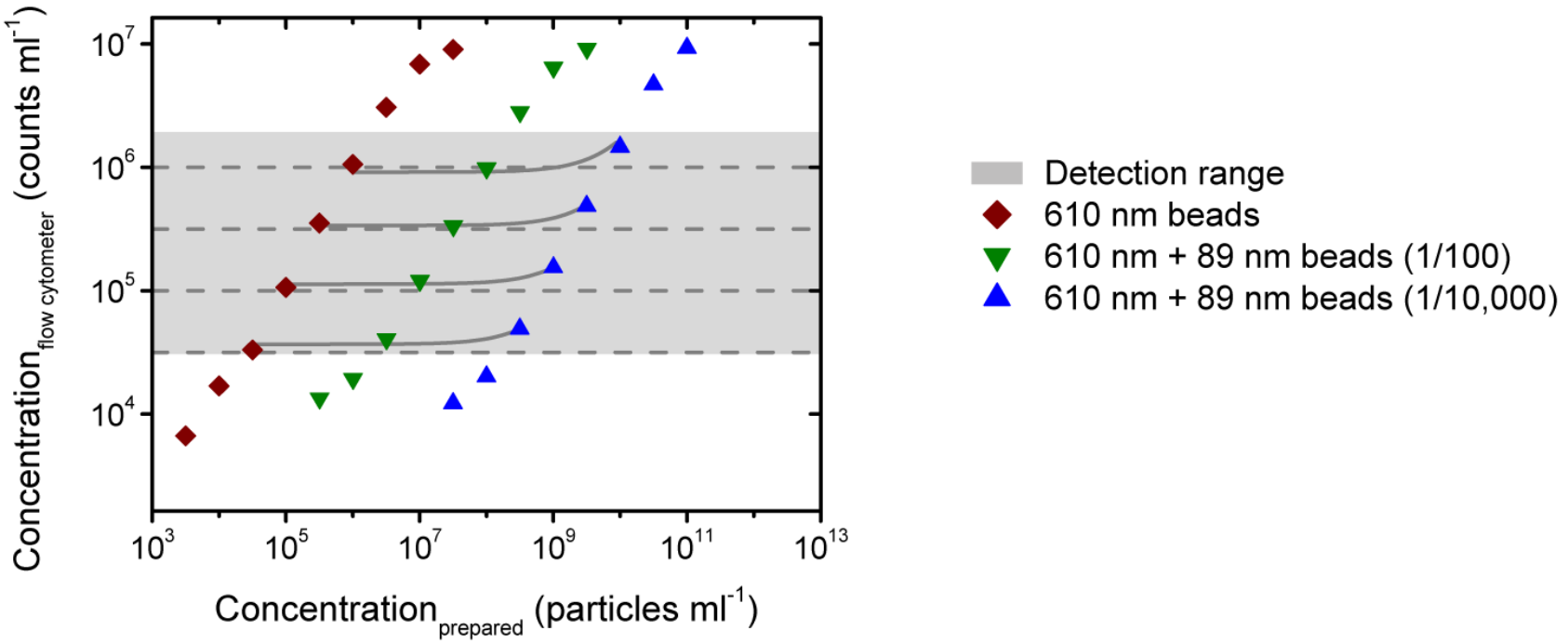
Results – counts from mixtures of beads



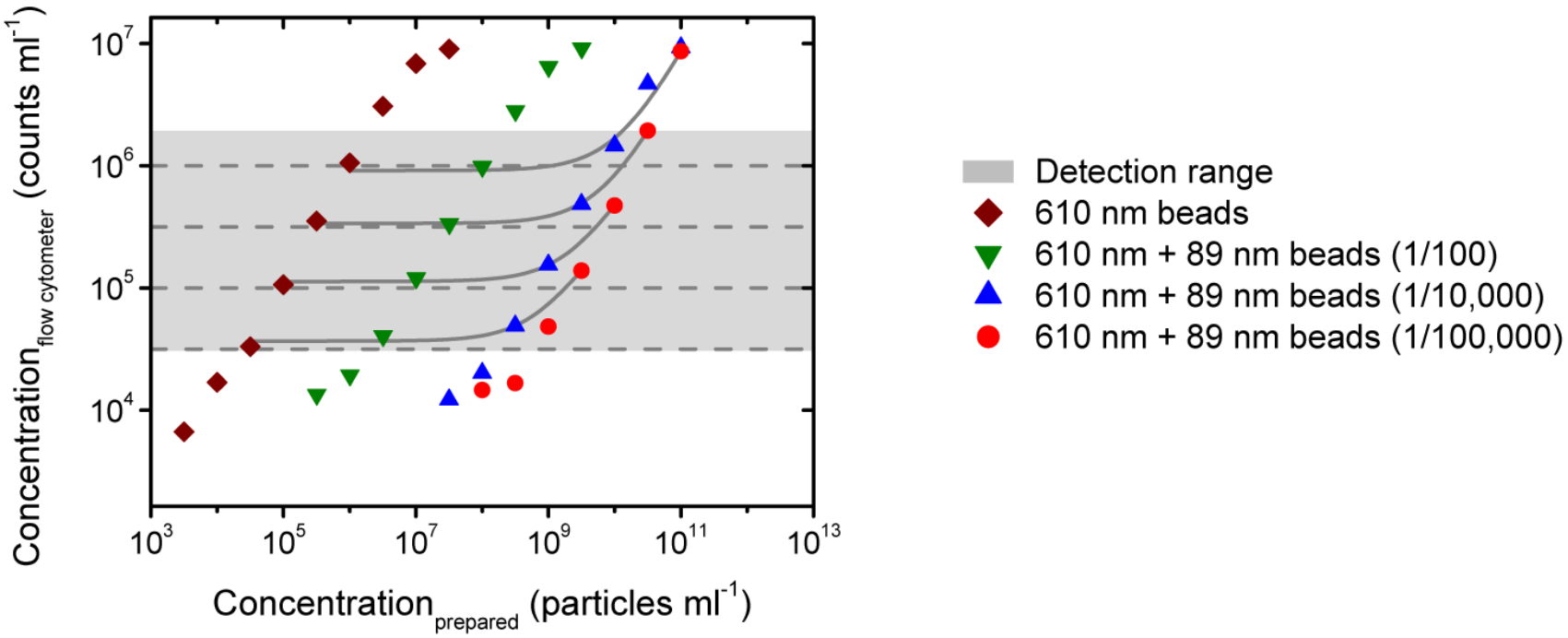
Results – counts from mixtures of beads



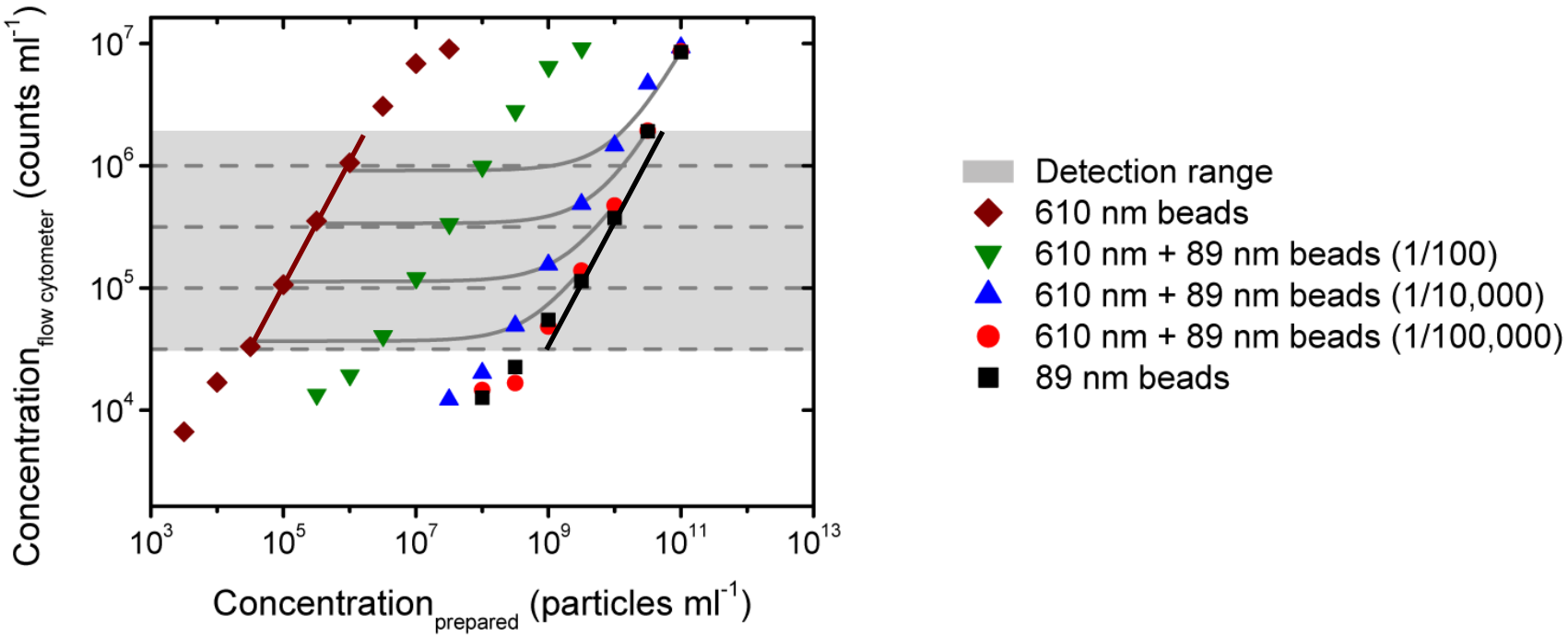
Results – counts from mixtures of beads



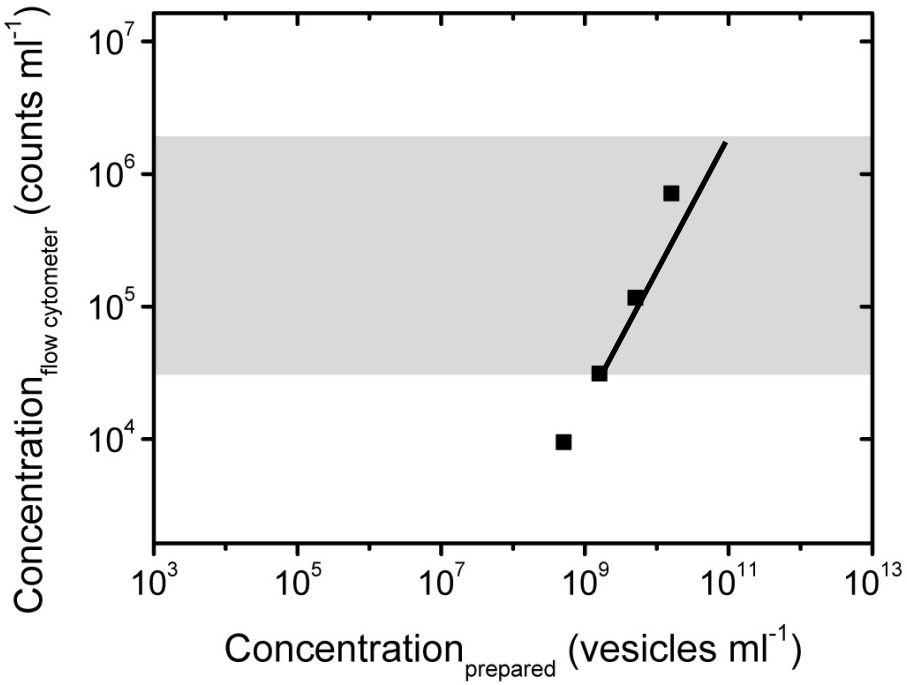
Results – counts from mixtures of beads



Results – counts from mixtures of beads



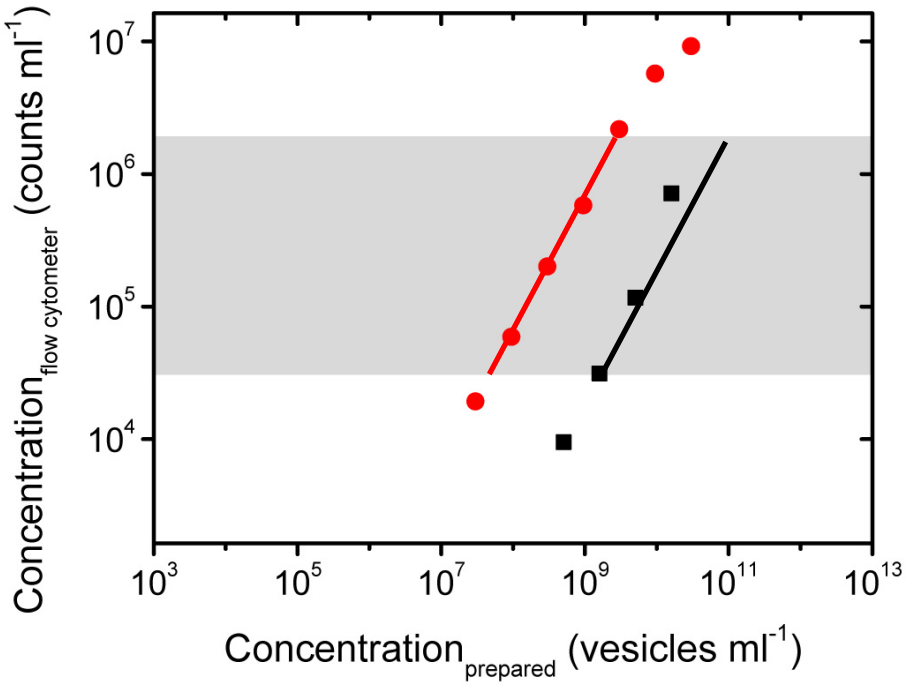
Results – counts from urinary vesicles



Detection range

Cell-free urine filtered with 220-nm filter

Results – counts from urinary vesicles



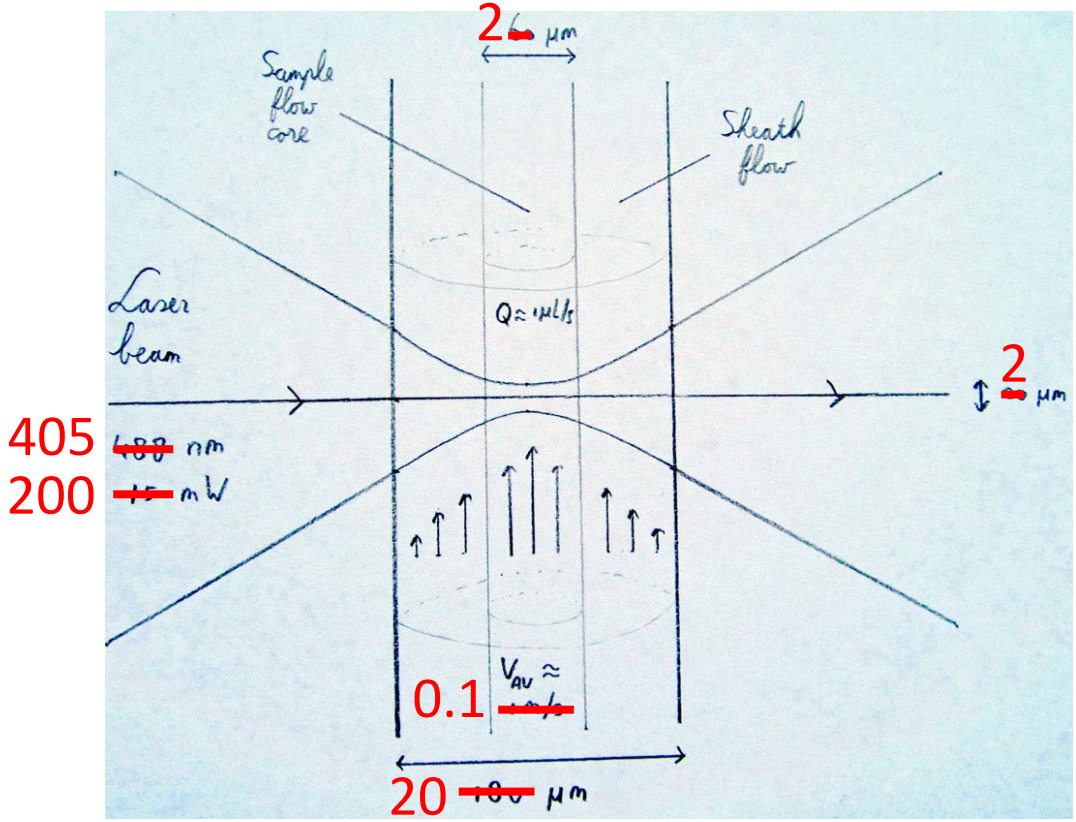
Conclusion

- 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

Prospects of vesicle detection by flow cytometry

- calibration should be based on experiments *and theory*
  - size distribution
  - refractive index
- flow cytometry is good
- increase sensitivity

Sensitivity should be increased



"A flow cytometer is unable to detect the smallest vesicle as long as you can detect cells with it."

Acknowledgements

- Anita Böing
- Anita Grootemaat
- Chi Hau
- Chris Gardiner
- Frank Coumans
- Guus Sturk
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- Marianne Schaap
- Martin van Gemert
- Paul Harrison
- Rienk Nieuwland
- Ton van Leeuwen

More on vesicle detection:
edwinvanderpol.com