

Novel methods to detect microparticles *and improved analysis with flow cytometry*

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Disclosures for Edwin van der Pol

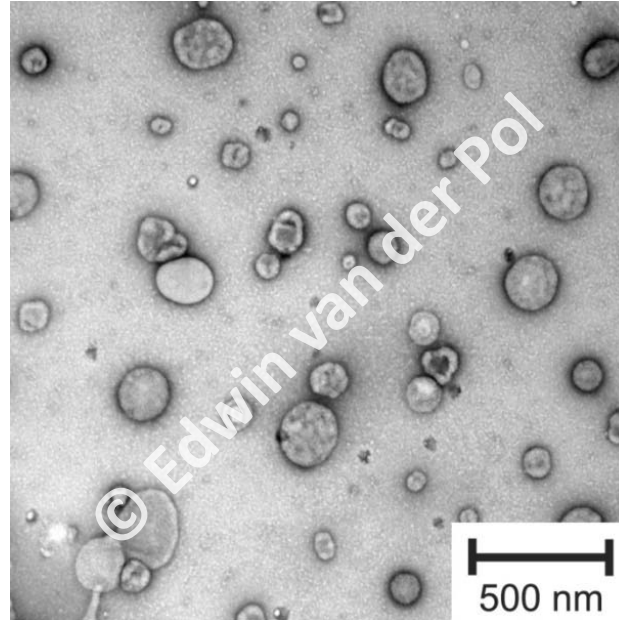
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Presentation includes discussion of the following off-label use of a drug or medical device:

<N/A>

Introduction



- body fluids contain cell-derived vesicles
- clinically relevant information
- problem: vesicle detection

Objective

- explore the ability of novel and conventional methods to detect the *size* and *concentration* of vesicles in suspension

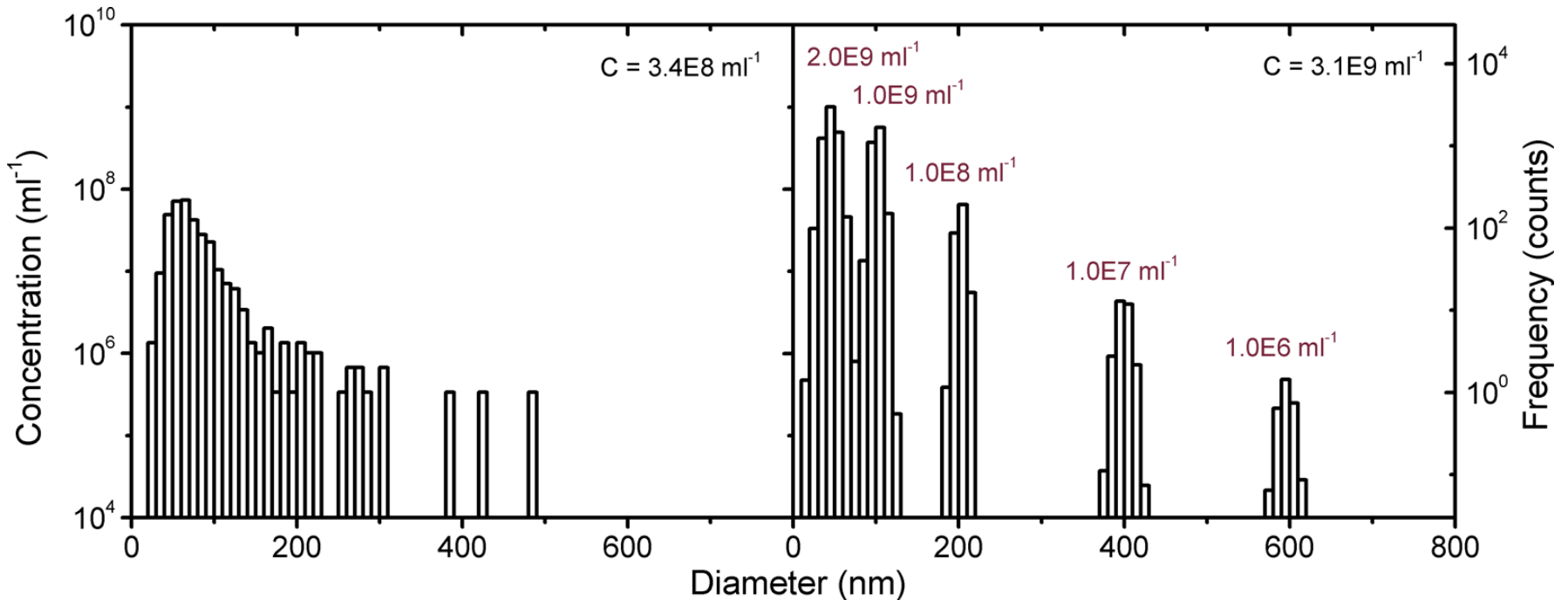
Methods

- standard population¹ of
 - vesicles prepared from human cell-free urine ($n=5$)
 - mixture of polystyrene beads
- analyzed by
 - Transmission Electron Microscopy
 - Nanoparticle Tracking Analysis
 - Resistive Pulse Sensing
 - Flow cytometry

Transmission Electron Microscopy

Philips CM-10

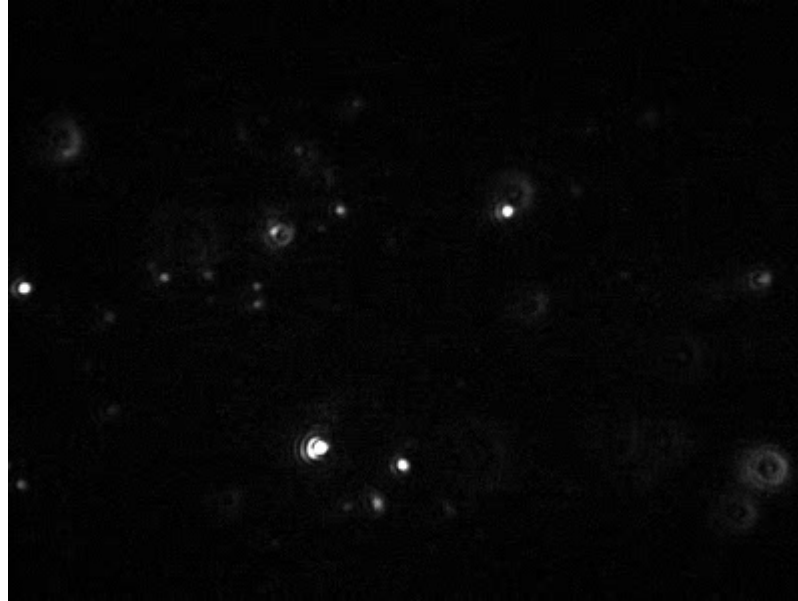
According to manufacturer



Vesicles

Beads

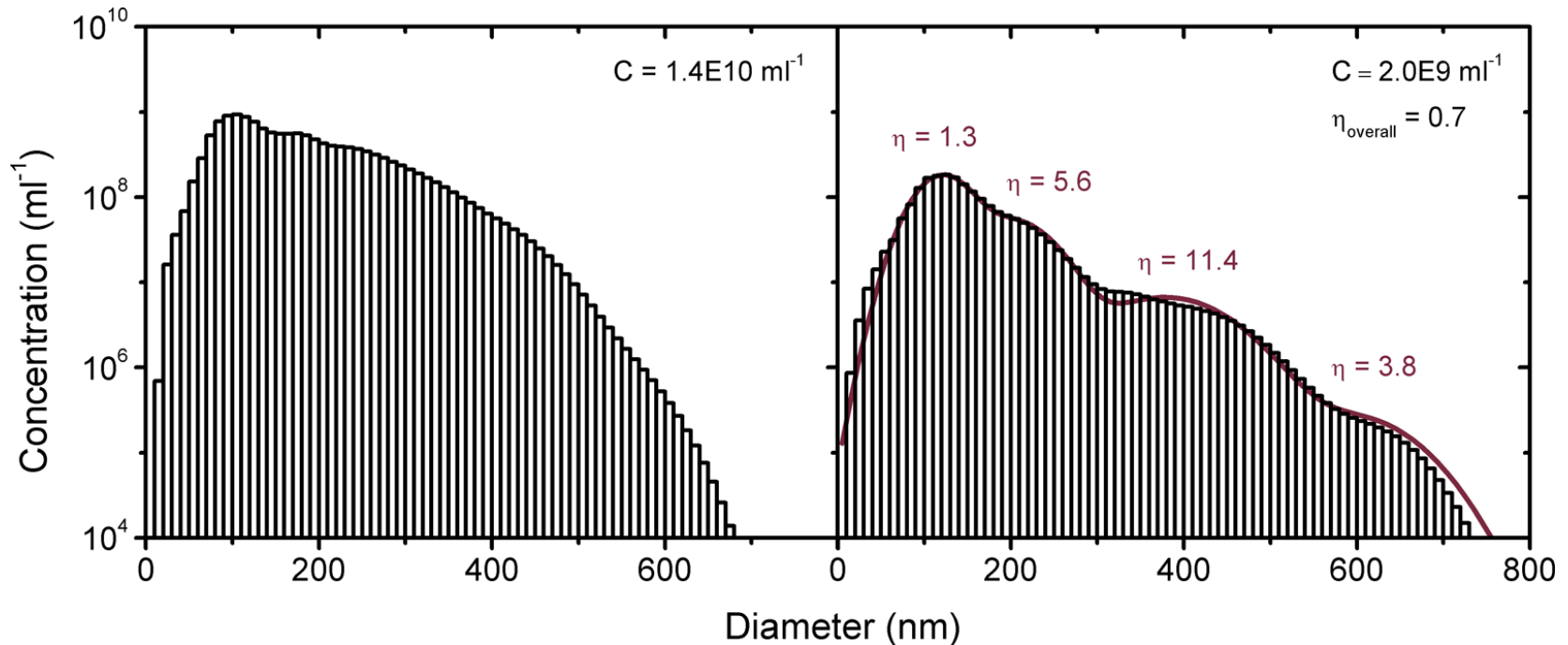
Nanoparticle Tracking Analysis



- determines *size* and *concentration* of vesicles in suspension
- recently successfully extended with *fluorescence detection*²

Nanoparticle Tracking Analysis

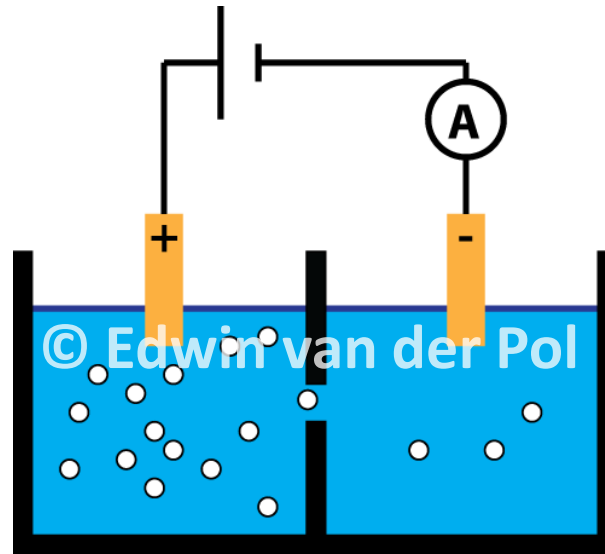
Nanosight NS500



Vesicles

Beads

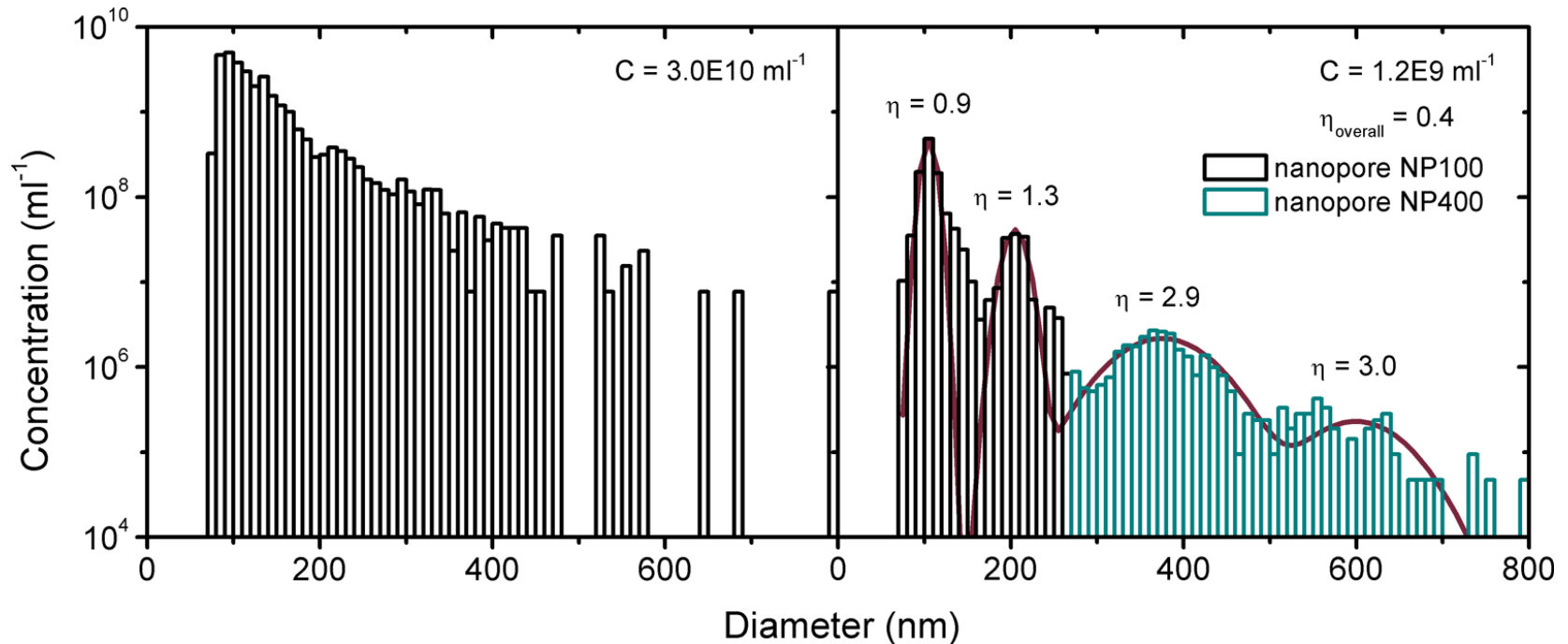
Resistive Pulse Sensing



- determines *size* and *concentration* of vesicles in suspension

Resistive Pulse Sensing

iZon qNano



Vesicles

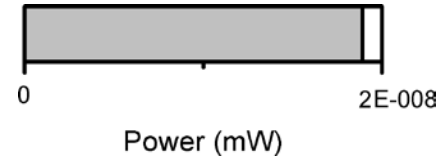
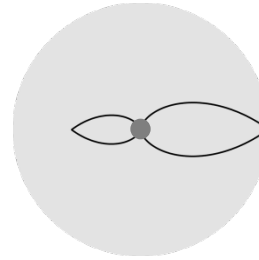
Beads

Flow cytometry and the refractive index

Polystyrene bead



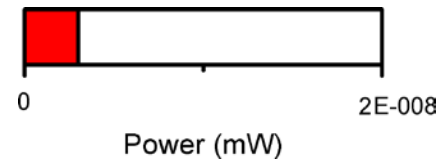
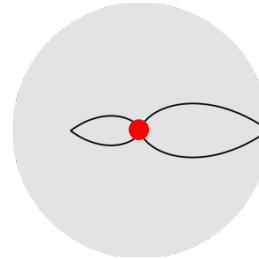
$n = 1.61$



Silica bead



$n = 1.45$

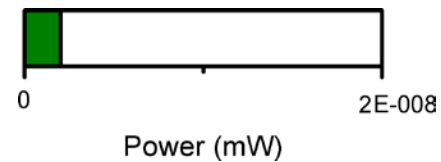
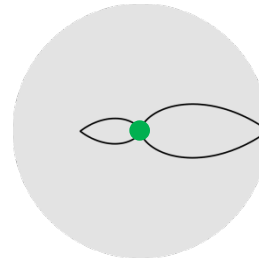


Vesicle



$n_{\text{inside}} = 1.38 \pm 0.02$

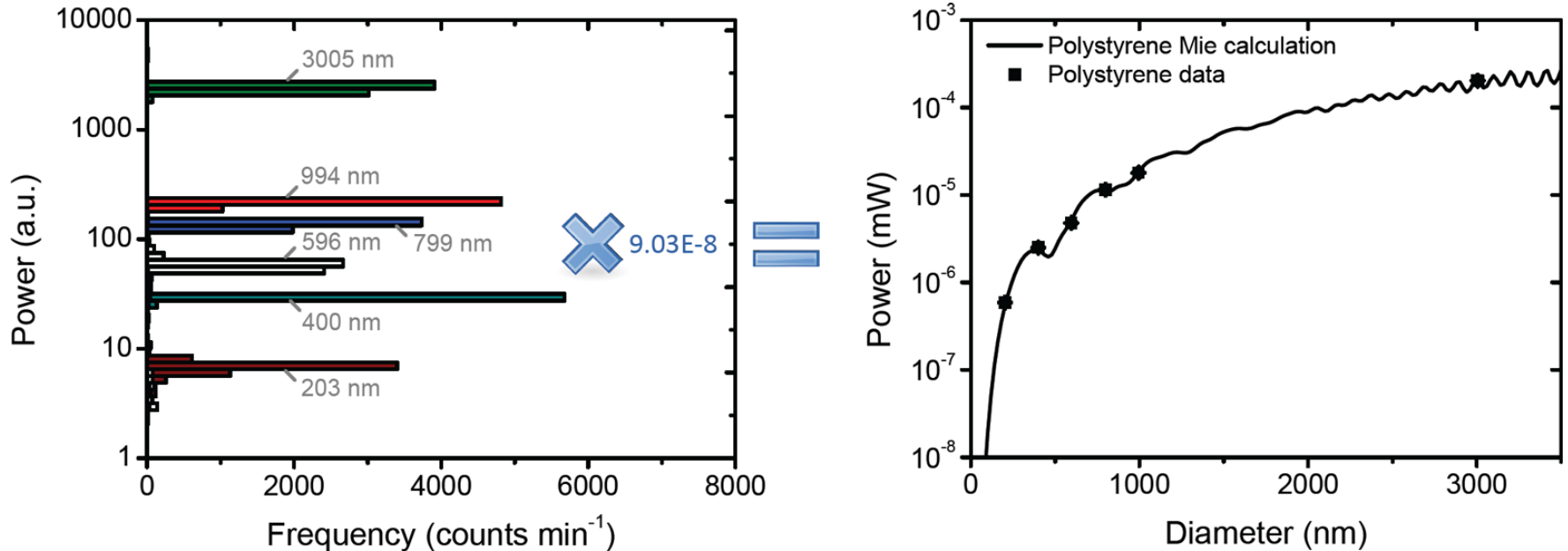
$n_{\text{membrane}} = 1.48$



100 nm

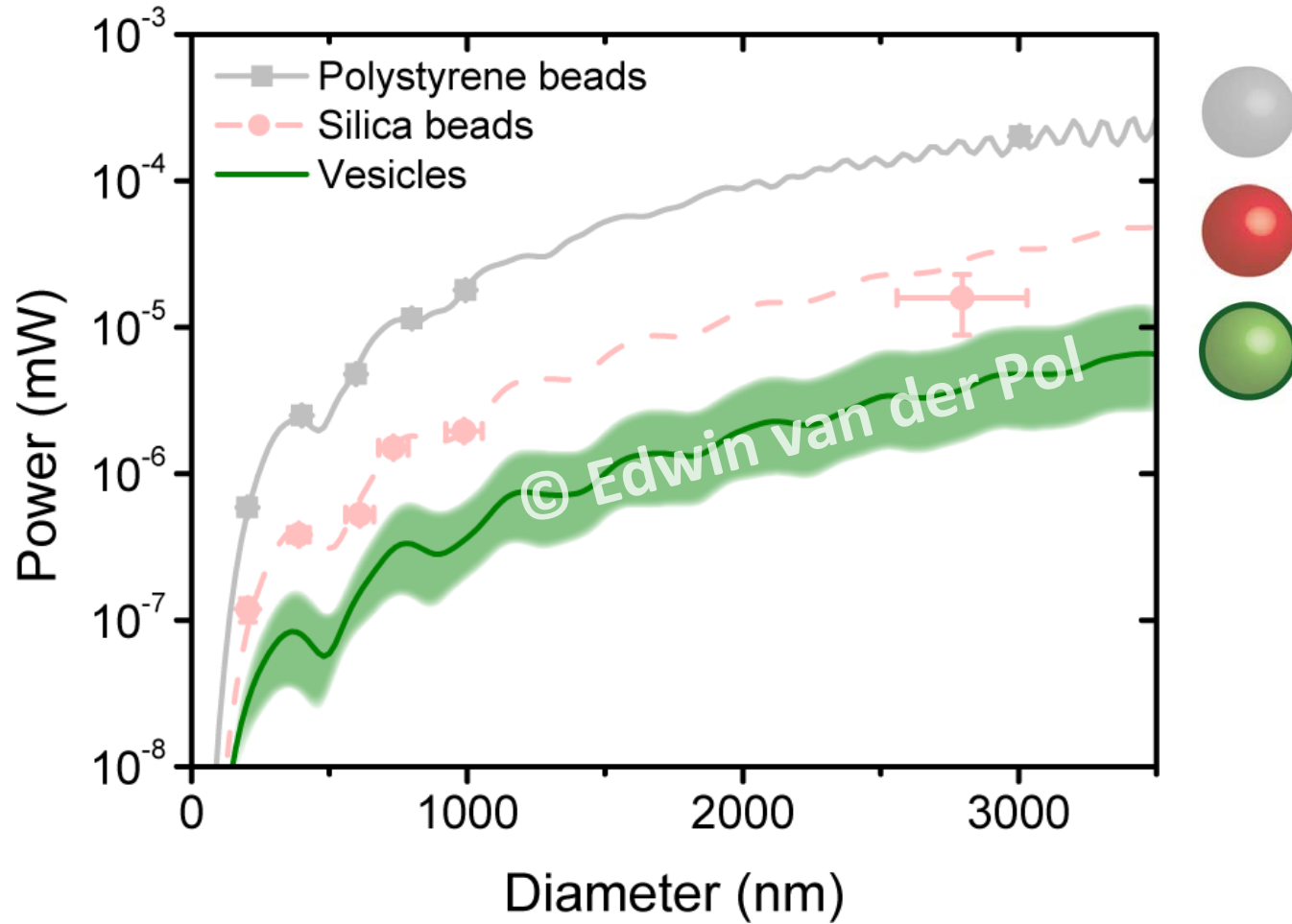


Flow cytometer calibration

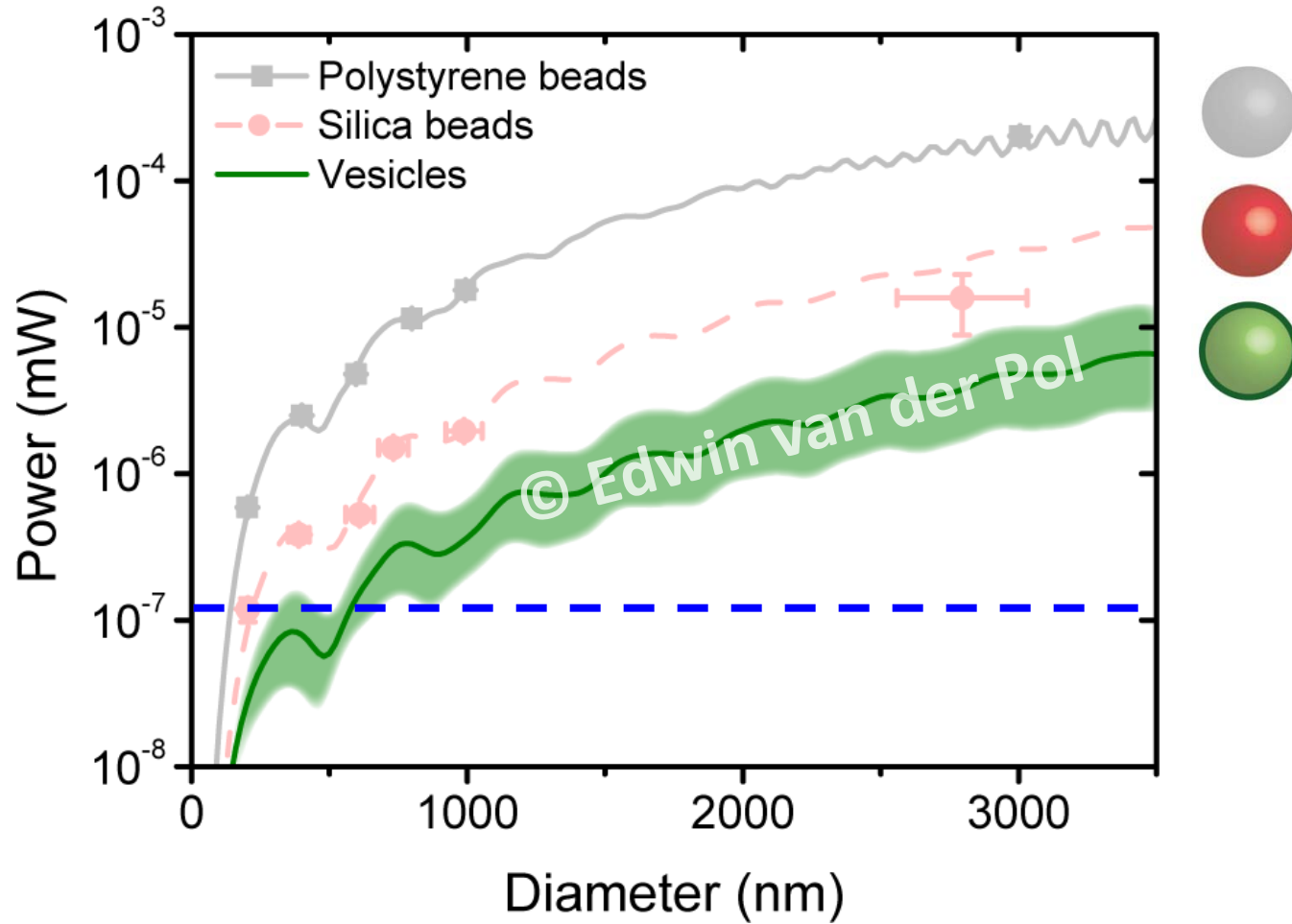


- absolute scattering power (mW) is calculated by Mie theory to relate detected scattering power (a.u.) to the diameter of beads

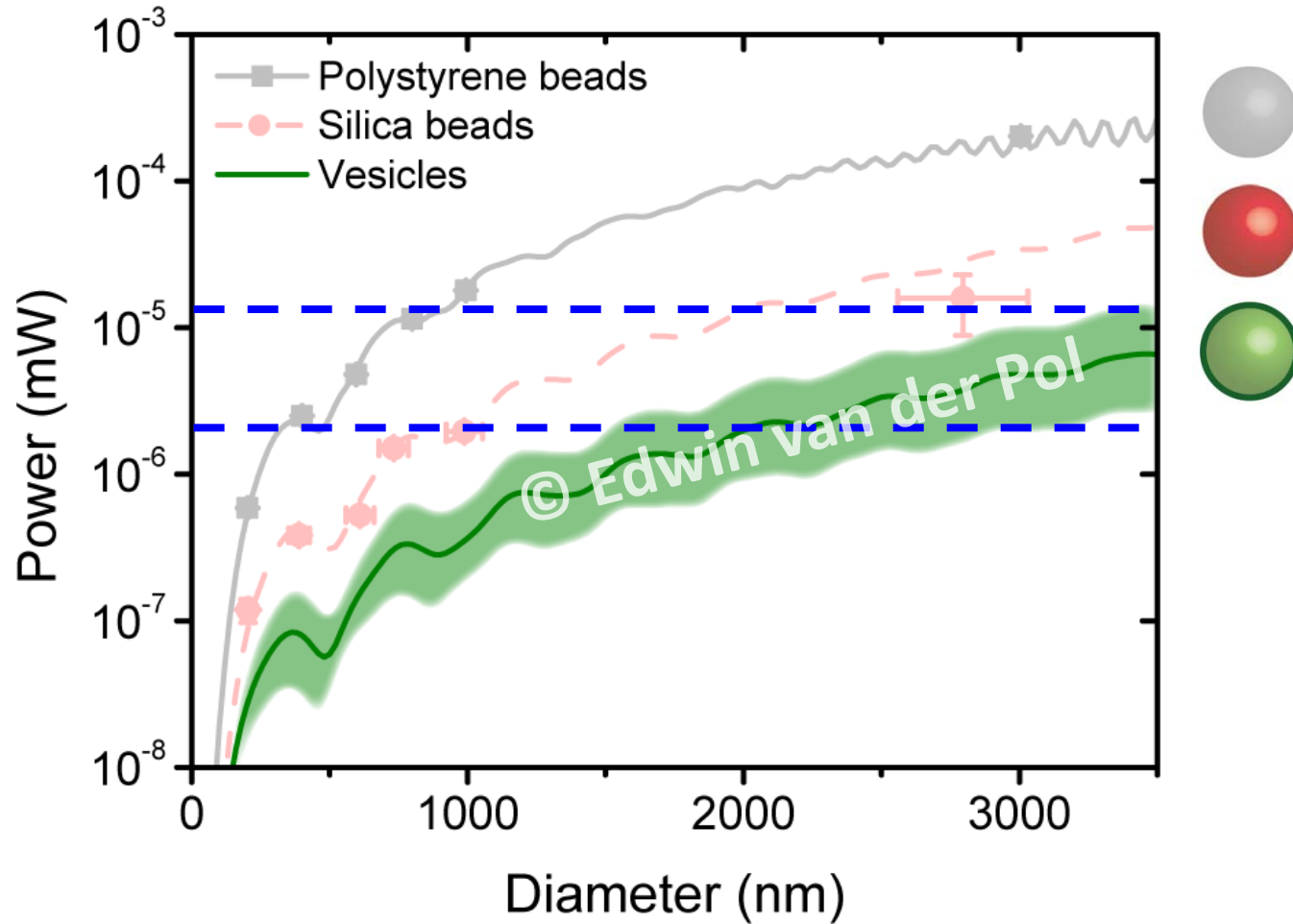
Flow cytometry calibration



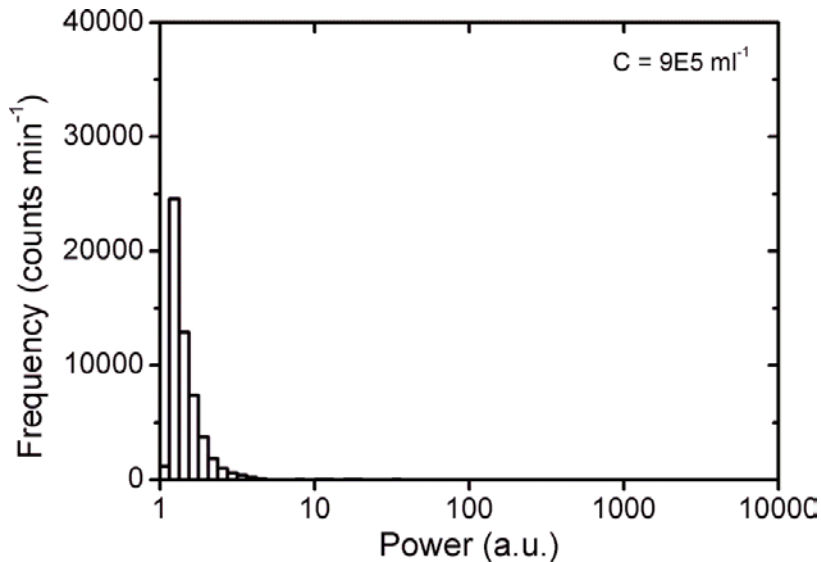
Flow cytometry detection limit



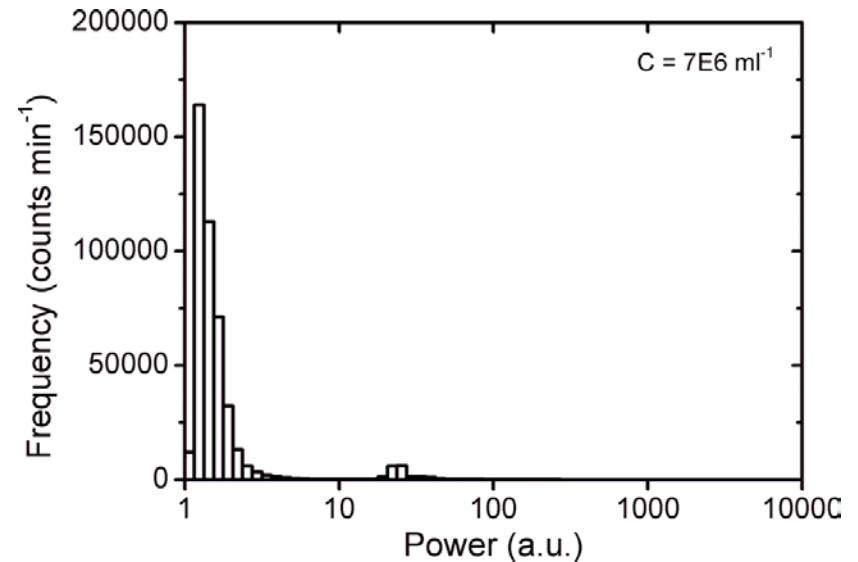
Flow cytometry detection limit



Flow cytometry detects *multiple vesicles as single count*

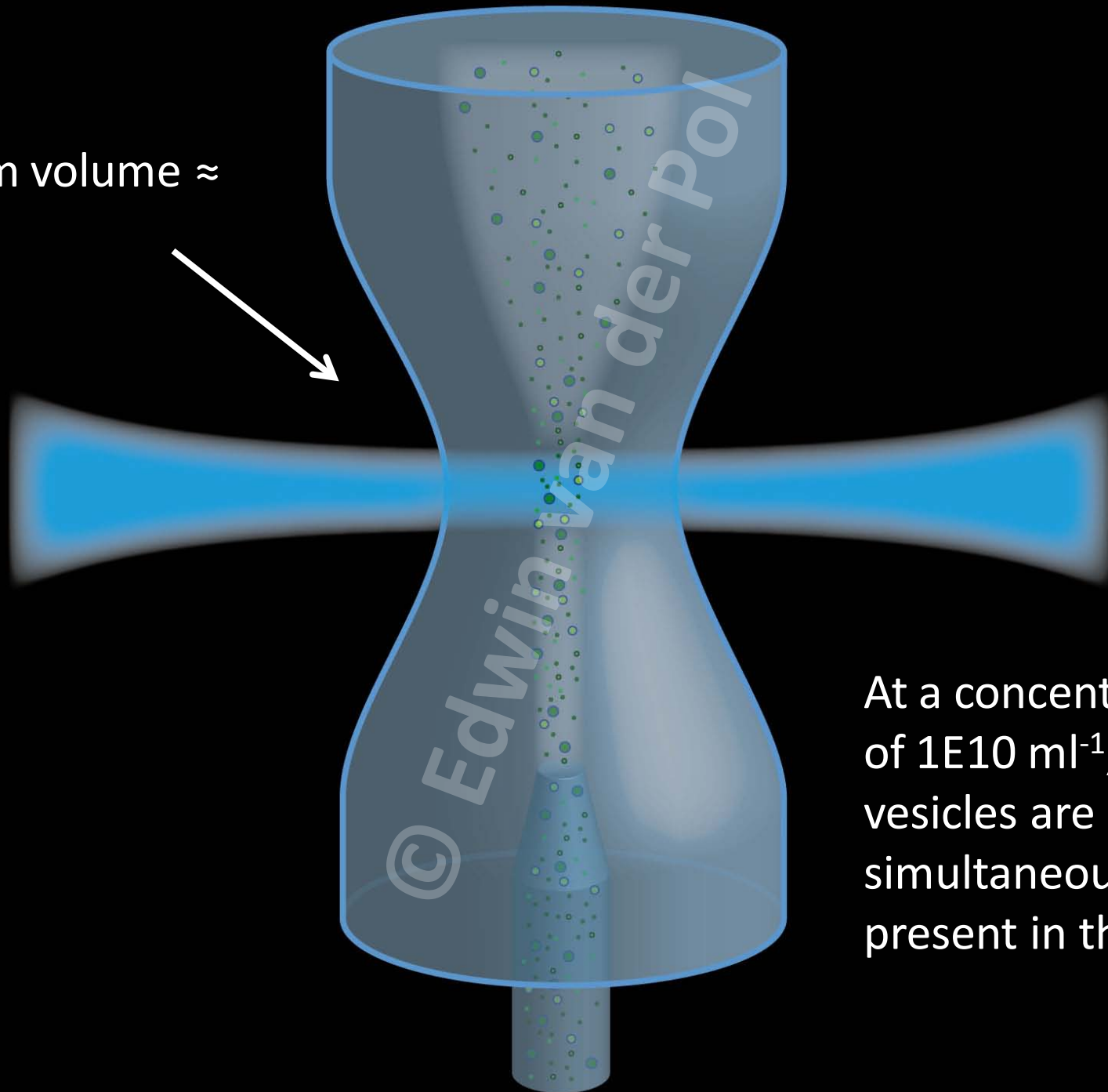


vesicles from human urine
filtered with 220 nm filter



89 nm silica beads at
concentration 1E10 ml⁻¹

Beam volume \approx
54 pl



At a concentration
of $1E10 \text{ ml}^{-1}$, 864
vesicles are
simultaneously
present in the beam

Conclusion

- Nanoparticle Tracking Analysis and Resistive Pulse Sensing are promising methods to determine size and concentration of *single* vesicles in suspension (P-MO-405)
- detection of vesicles by flow cytometry is attributed to scattering from *multiple* vesicles (P-MO-404)