

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

Edwin van der Pol<sup>1,2</sup>

Guus Sturk<sup>1</sup>, Ton van Leeuwen<sup>2,3</sup>,  
Rienk Nieuwland<sup>1</sup>

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*<sup>1</sup>Laboratory Experimental Clinical Chemistry; <sup>2</sup>Biomedical engineering and Physics, Academic Medical Center, Amsterdam, The Netherlands*

*<sup>3</sup>Biomedical Photonic Imaging, University of Twente, Enschede, The Netherlands*



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# Disclosures for Edwin van der Pol

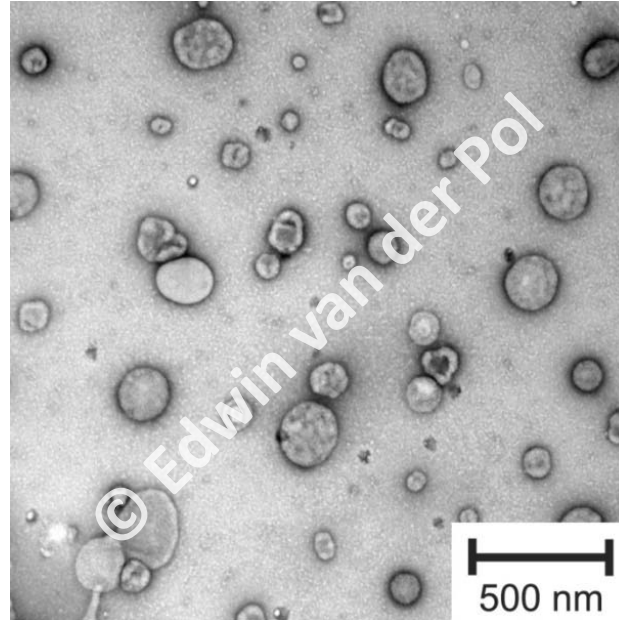
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Presentation includes discussion of the following off-label use of a drug or medical device:

<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

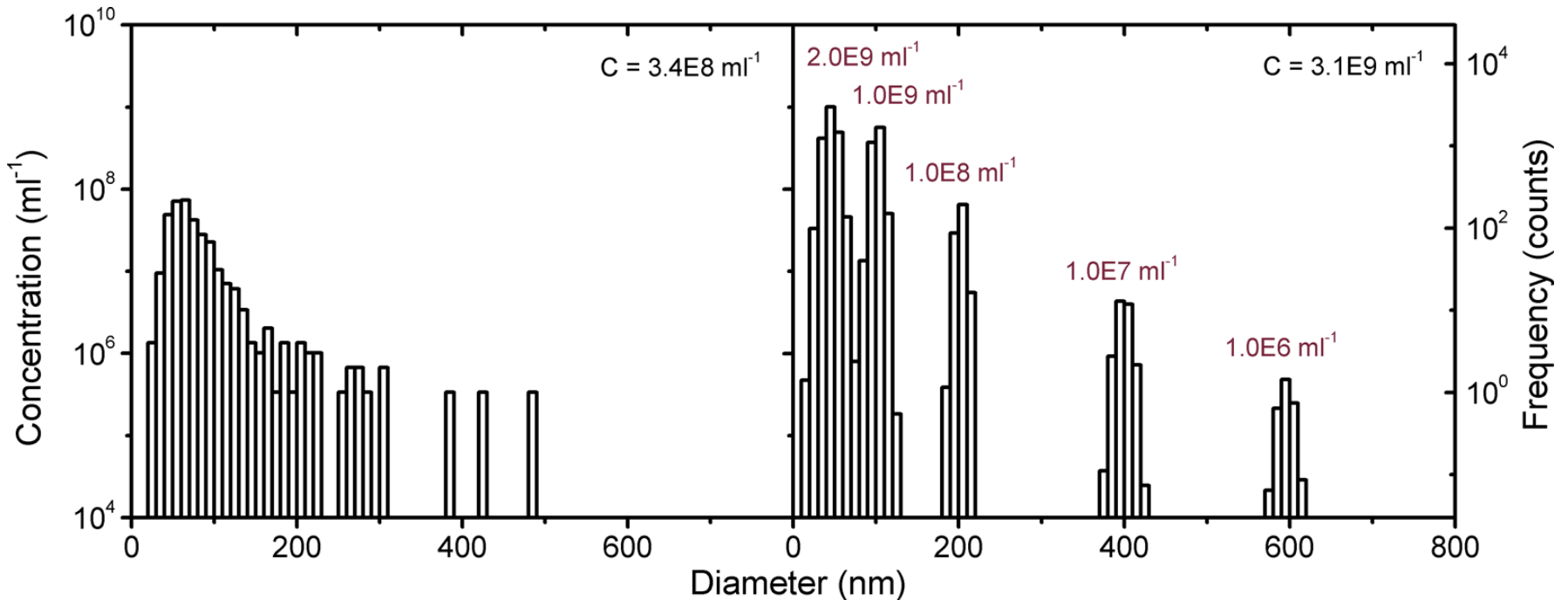
# Methods

- standard population<sup>1</sup> 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

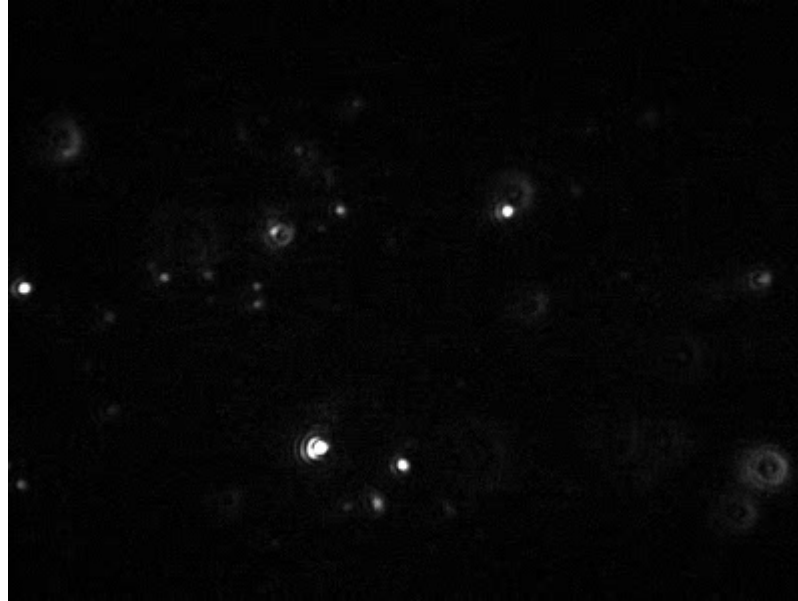
According to manufacturer



**Vesicles**

**Beads**

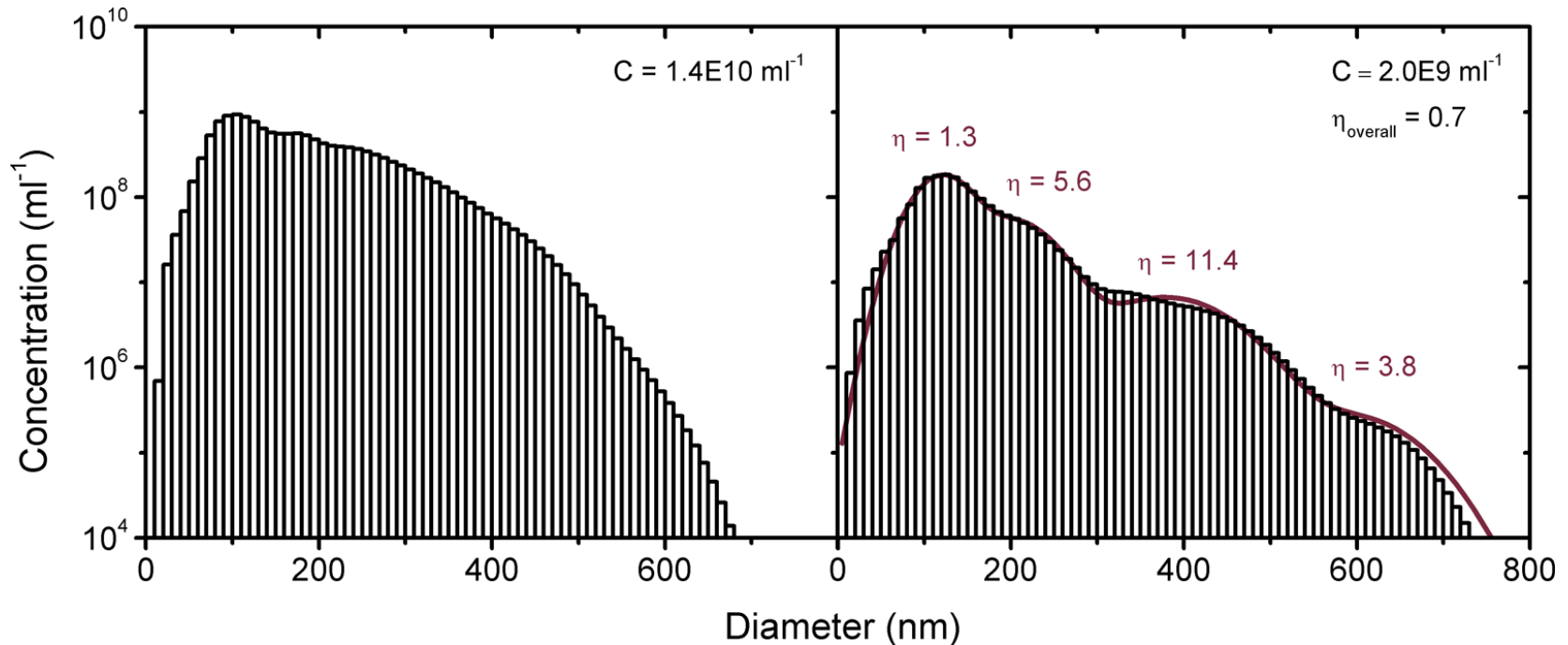
# Nanoparticle Tracking Analysis



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

# Nanoparticle Tracking Analysis

Nanosight NS500

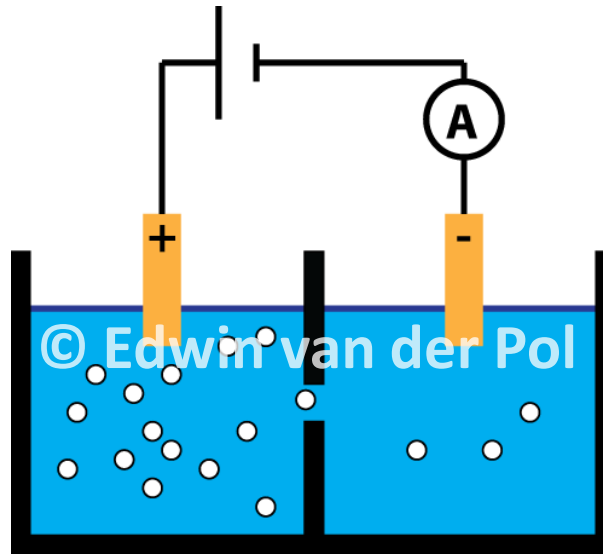


**Vesicles**

**Beads**



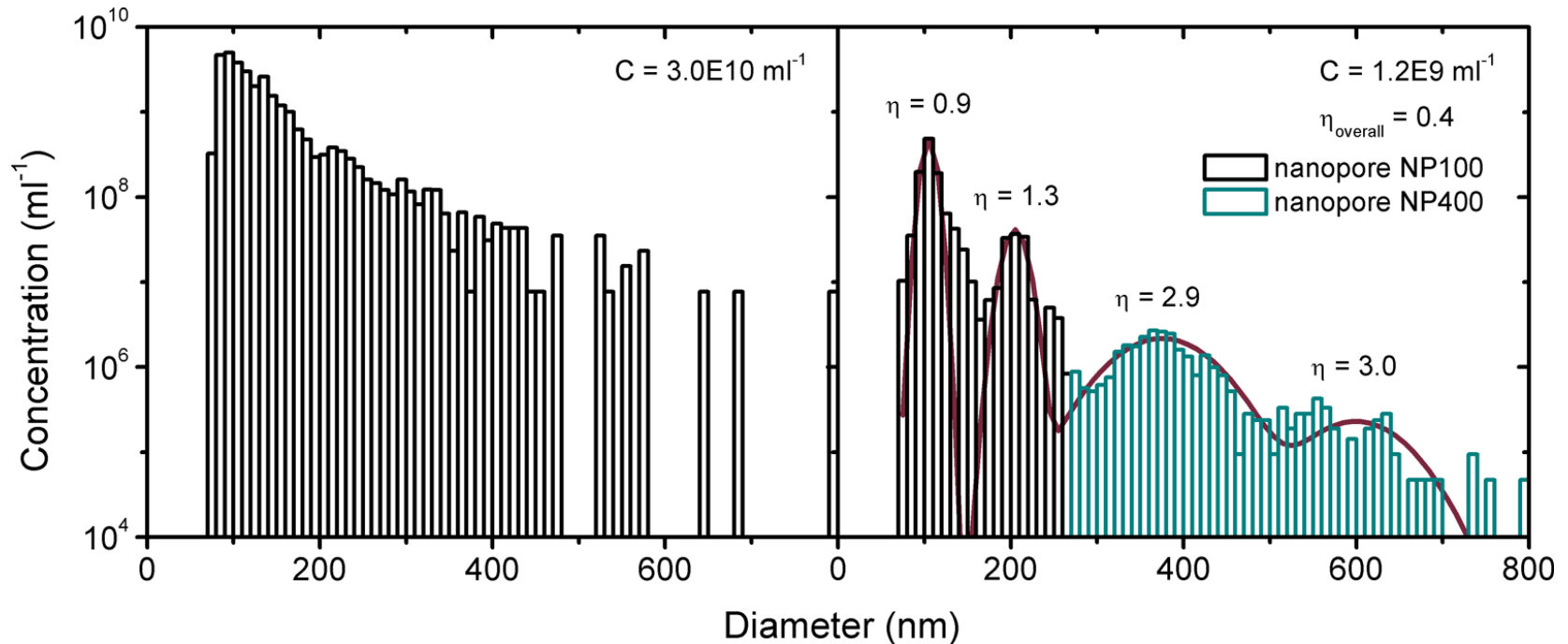
# Resistive Pulse Sensing



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

# Resistive Pulse Sensing

iZon qNano



**Vesicles**

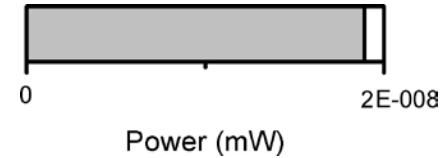
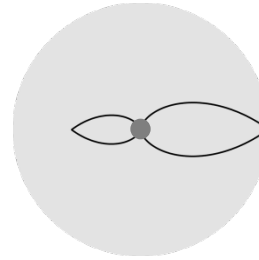
**Beads**

# Flow cytometry and the refractive index

Polystyrene bead



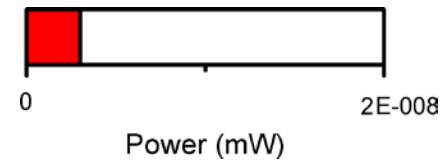
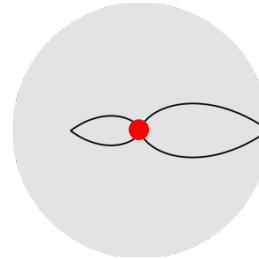
$n = 1.61$



Silica bead



$n = 1.45$

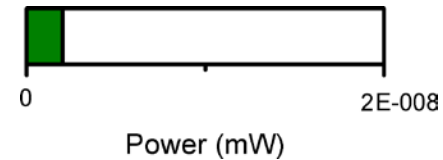
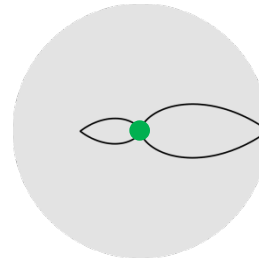


Vesicle



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

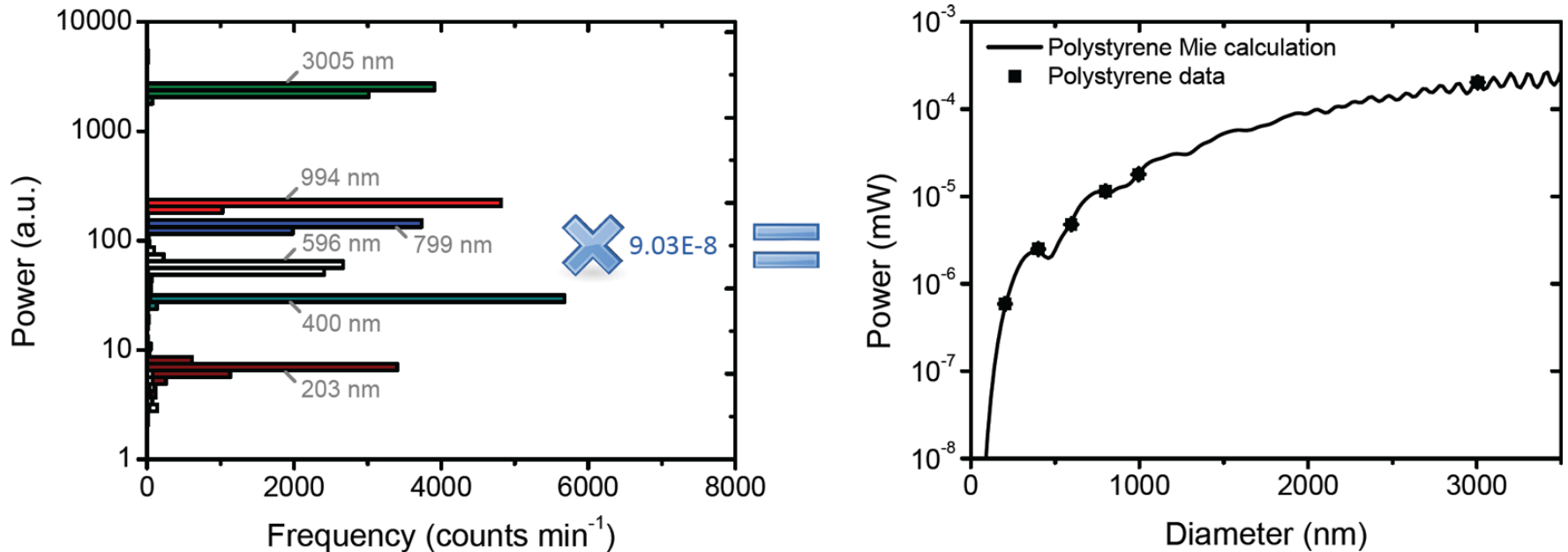
$n_{\text{membrane}} = 1.48$



100 nm

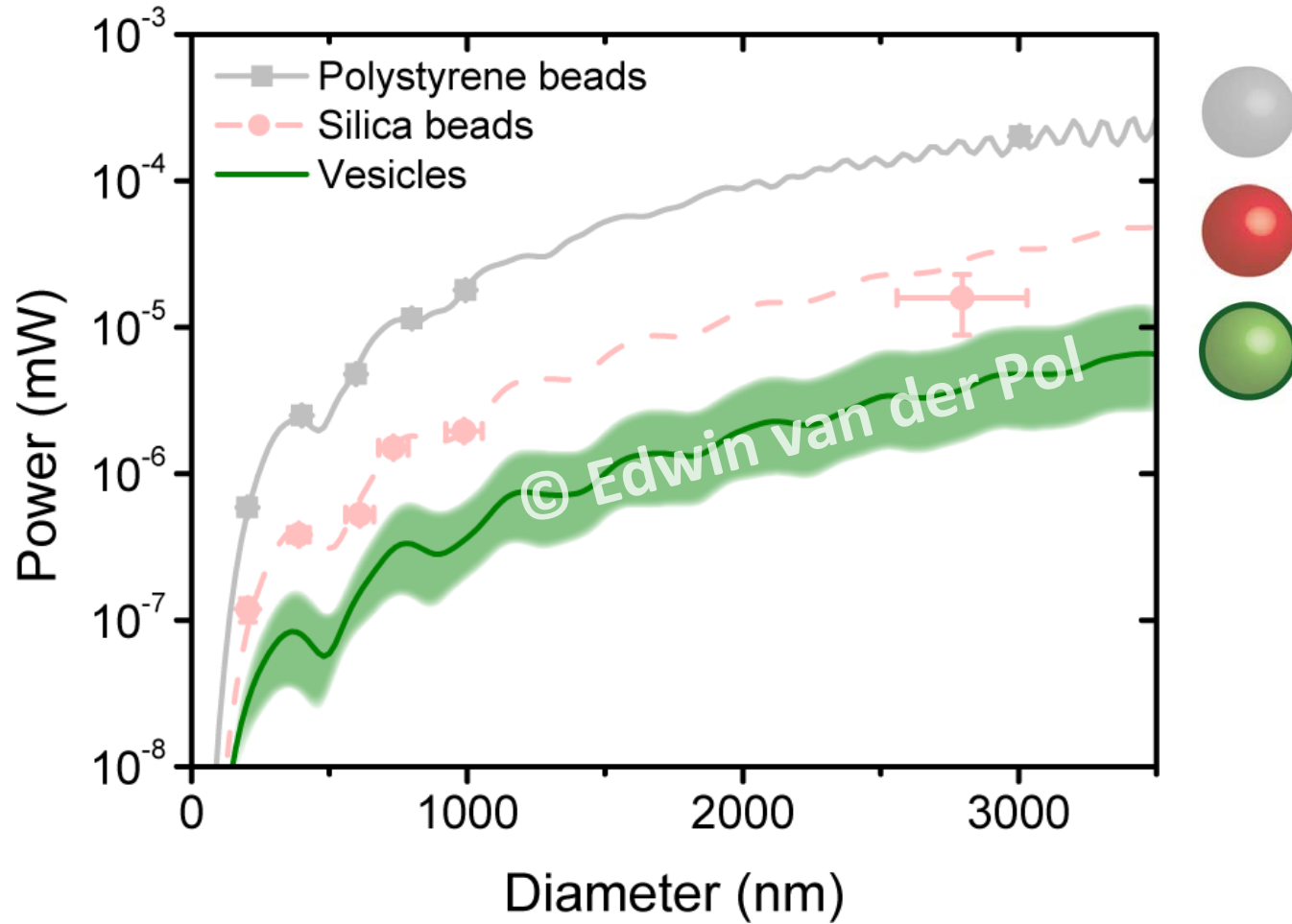


# Flow cytometer calibration

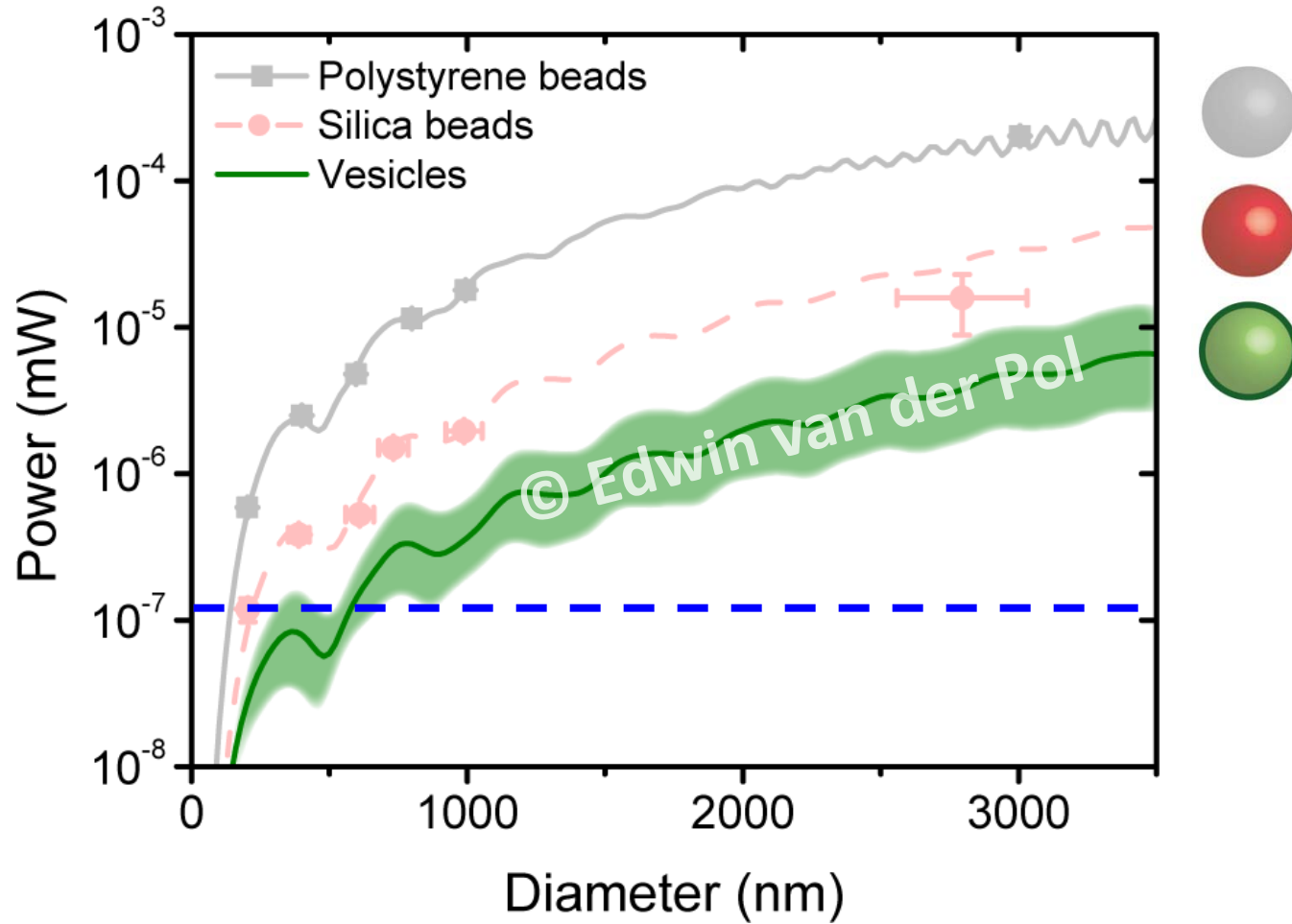


- absolute scattering power (mW) is calculated by Mie theory to relate detected scattering power (a.u.) to the diameter of beads

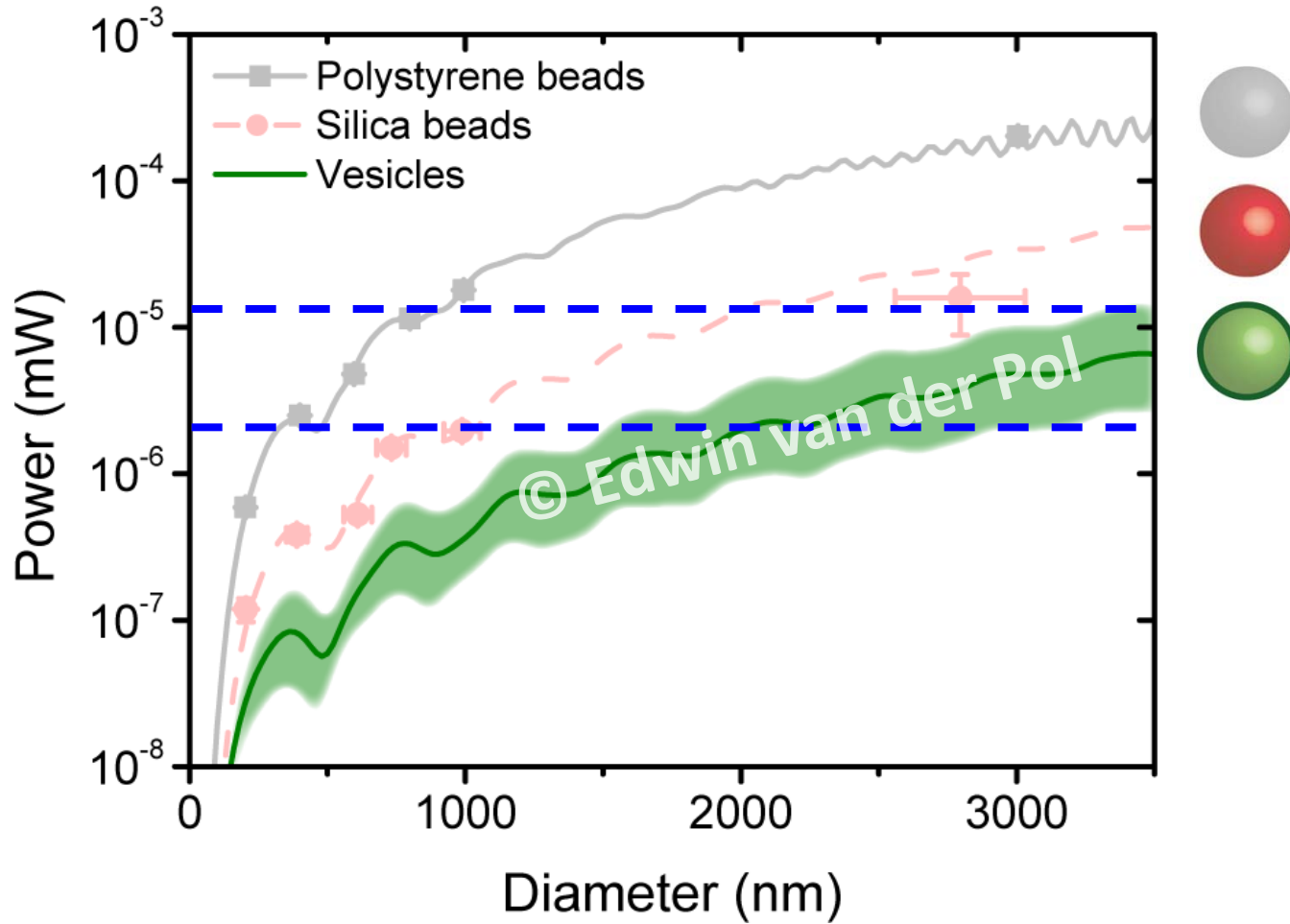
# Flow cytometry calibration



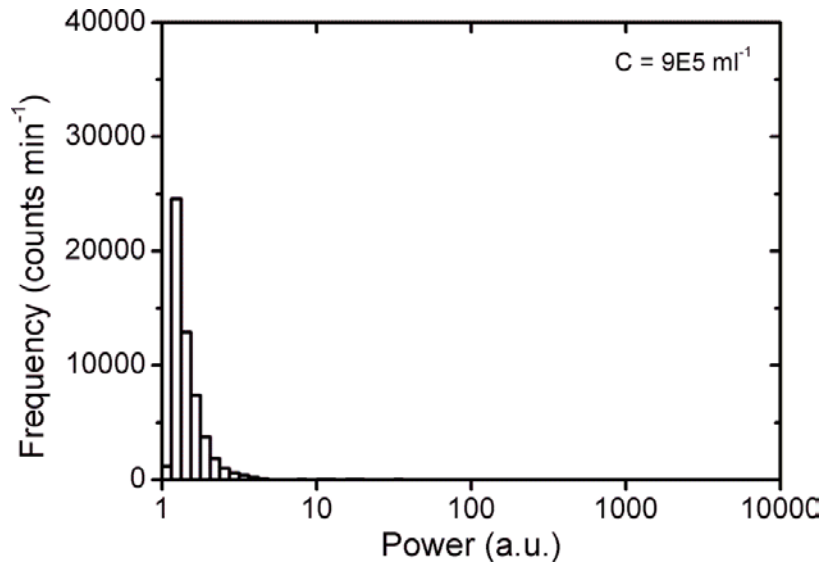
# Flow cytometry detection limit



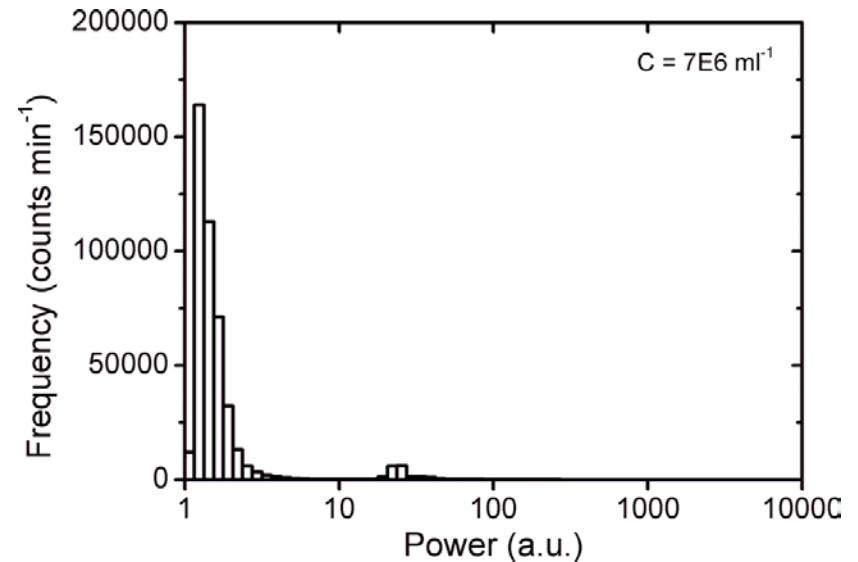
# Flow cytometry detection limit



# Flow cytometry detects *multiple vesicles as single count*



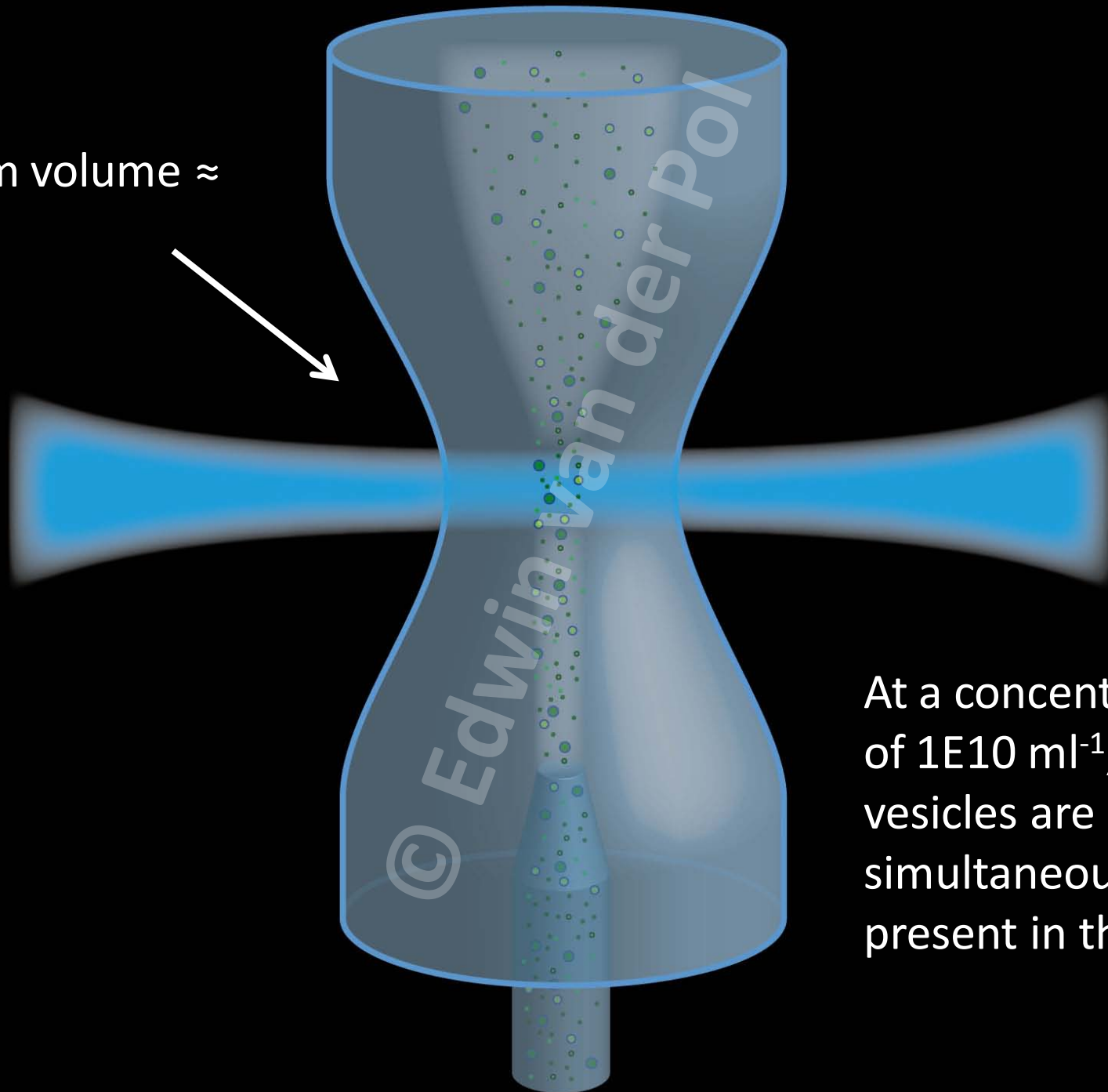
vesicles from human urine  
filtered with 220 nm filter



89 nm silica beads at  
concentration  $1E10 \text{ ml}^{-1}$



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)