# 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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| | |
|---|---|
| **Research Support/P.I.** | **No relevant conflicts of interest to declare** |
| **Employee** | **No relevant conflicts of interest to declare** |
| **Consultant** | **No relevant conflicts of interest to declare** |
| **Major Stockholder** | **No relevant conflicts of interest to declare** |
| **Speakers Bureau** | **No relevant conflicts of interest to declare** |
| **Honoraria** | **No relevant conflicts of interest to declare** |
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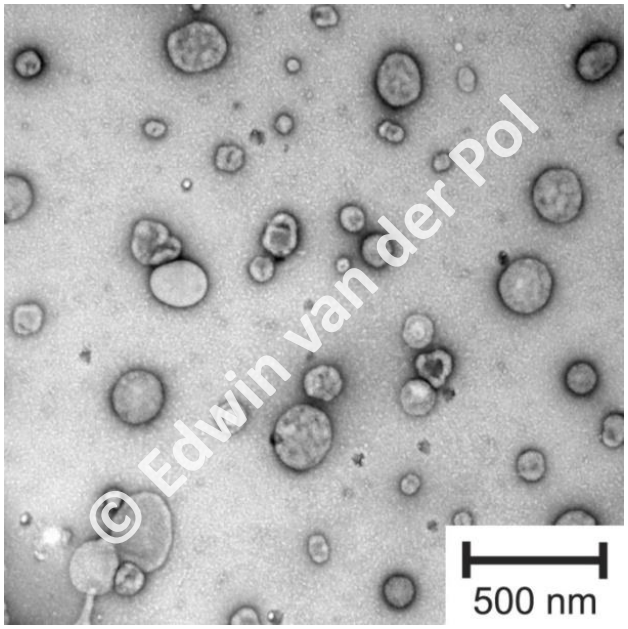
Presentation includes discussion of the following off-label use of a drug or medical device:
<N/A>

# 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

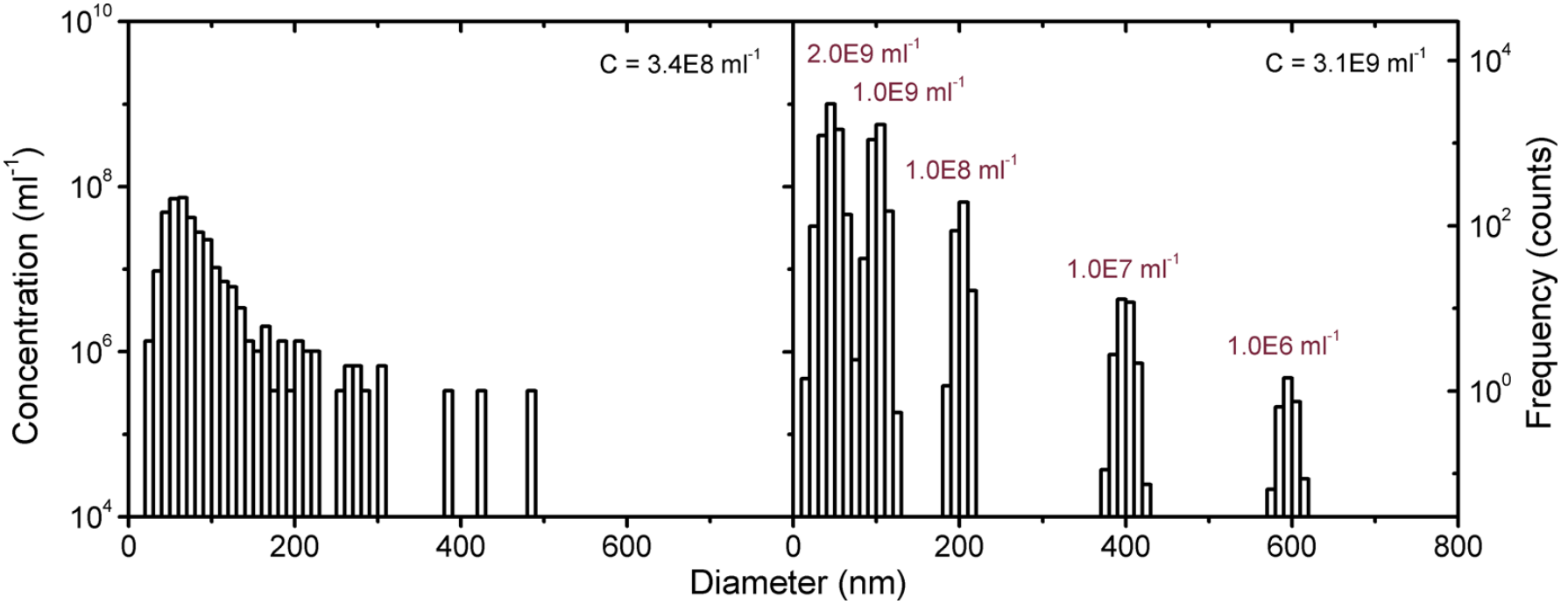
- standard population[1] of
  - vesicles prepared from human cell-free urine (*n=5*)
  - mixture of polystyrene beads
- analyzed by
  - Transmission Electron Microscopy
  - Nanoparticle Tracking Analysis
  - Resistive Pulse Sensing
  - Flow cytometry

1. van der Pol et al. *JTH* 2010

# Transmission Electron Microscopy

Philips CM-10

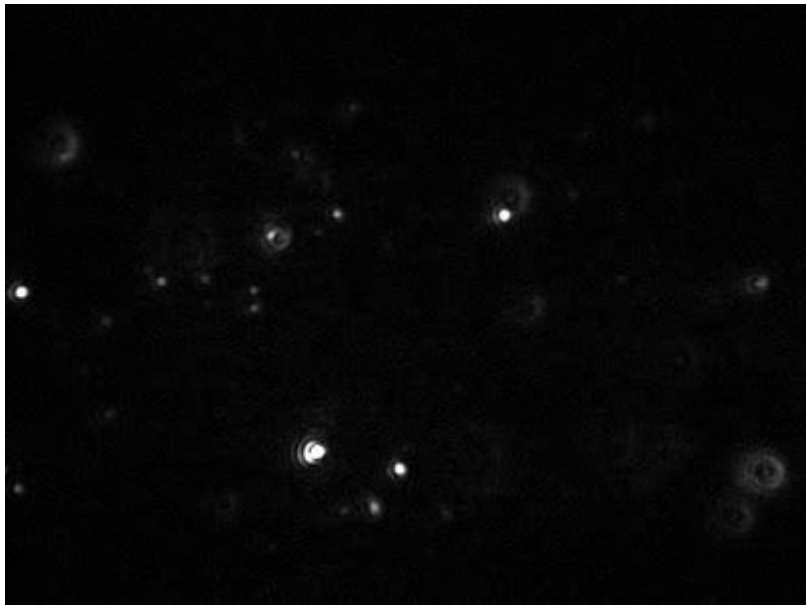
According to manufacturer




**Vesicles**

**Beads**

# Nanoparticle Tracking Analysis

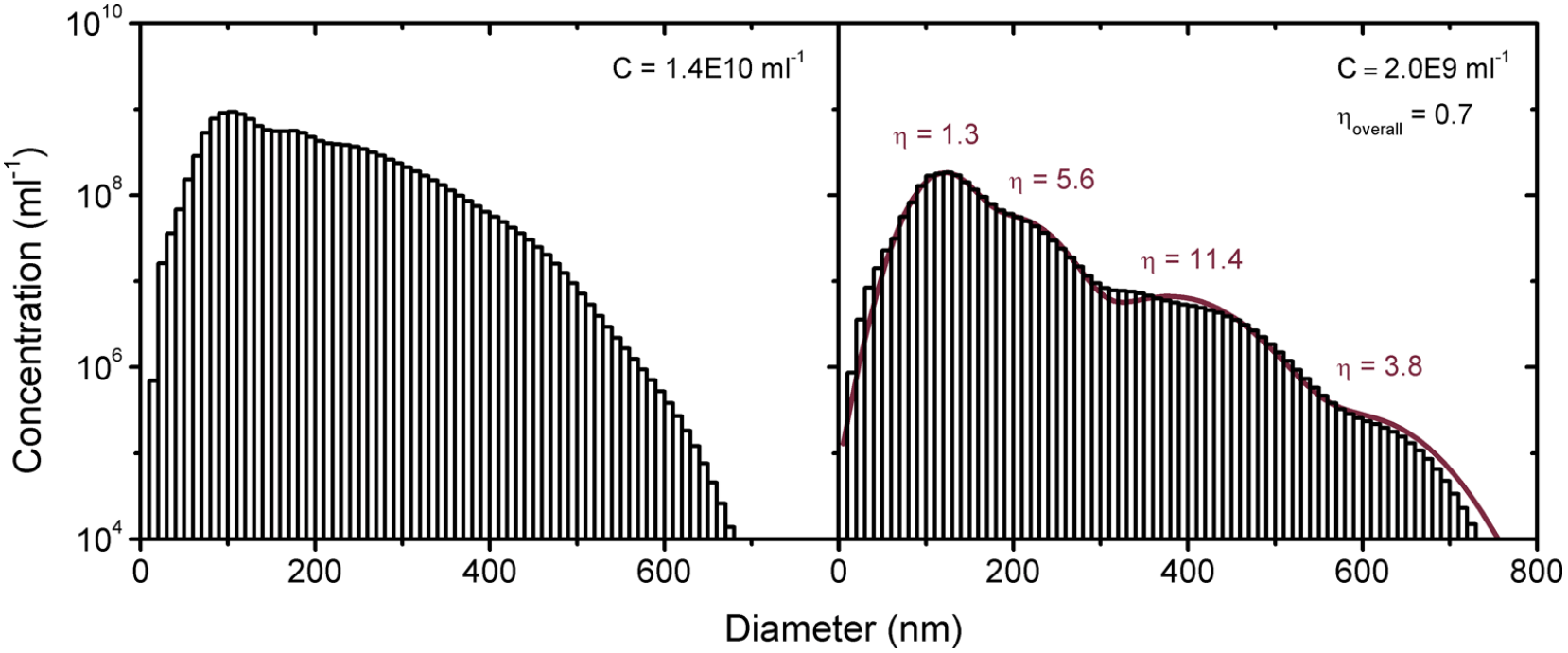


- determines *size* and *concentration* of vesicles in suspension
- recently successfully extended with *fluorescence detection*[2]

2. Dragovic et al. *Nanomedicine* 2011

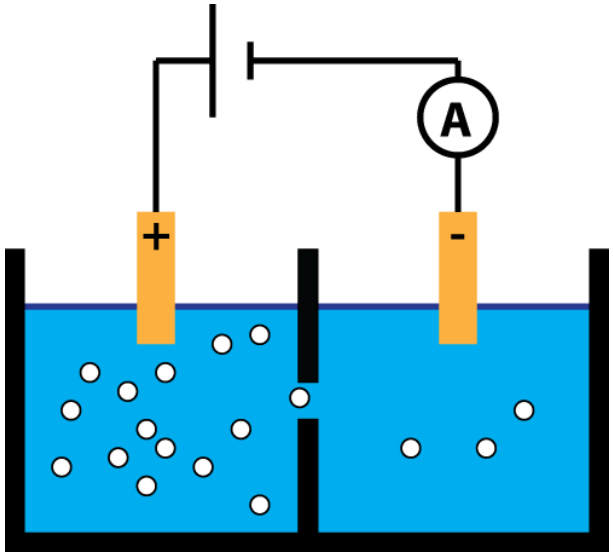
# Nanoparticle Tracking Analysis

Nanosight NS500




**Vesicles** **Beads**

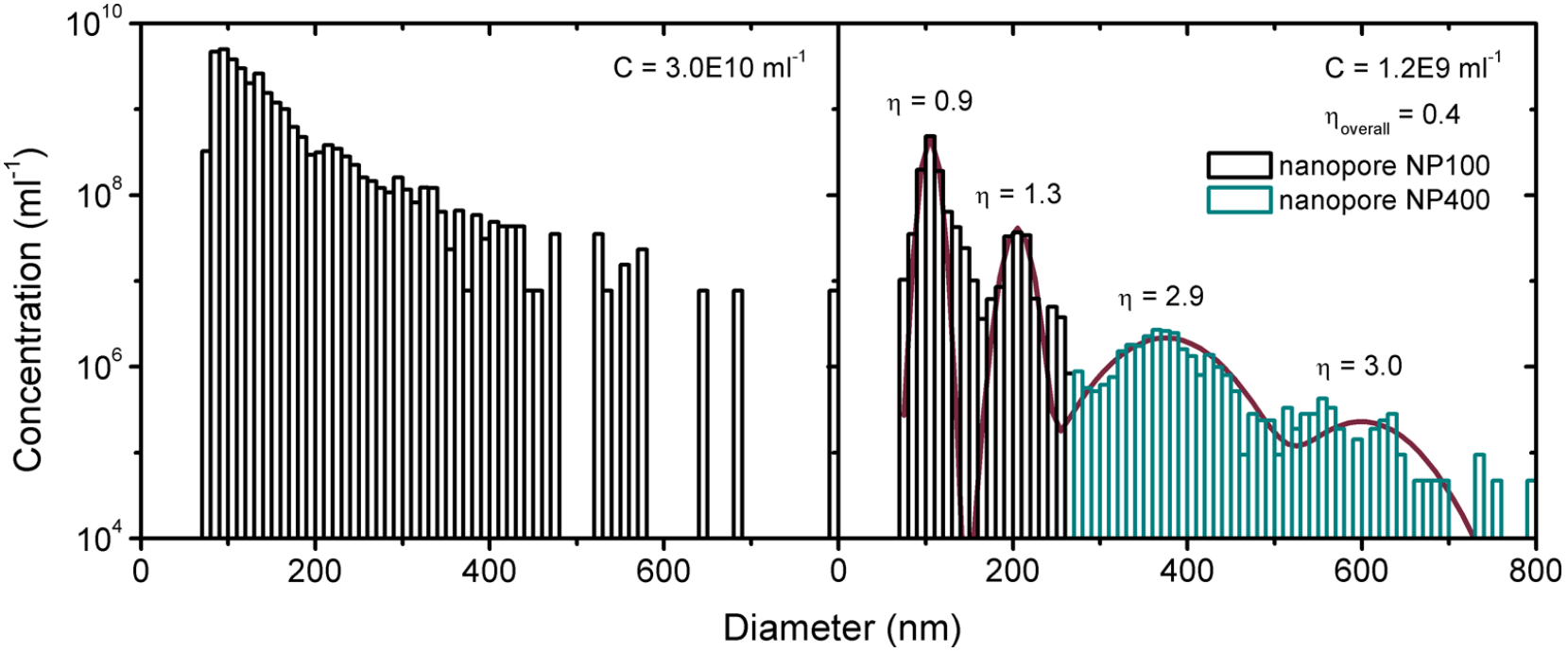
# Resistive Pulse Sensing




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

# Resistive Pulse Sensing

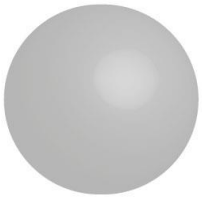
iZon qNano



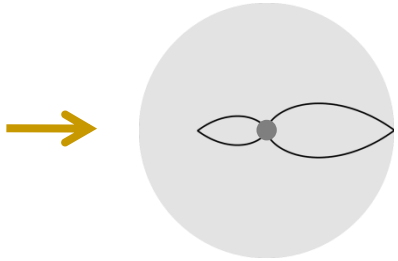

**Vesicles** **Beads**

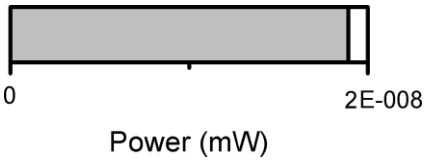
# Flow cytometry and the refractive index

Polystyrene bead



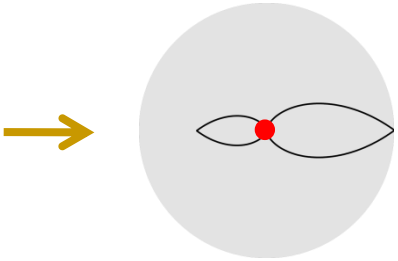
n = 1.61

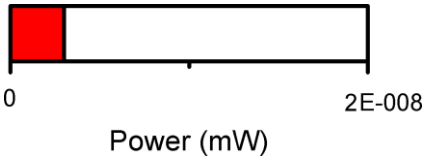





Silica bead

n = 1.45

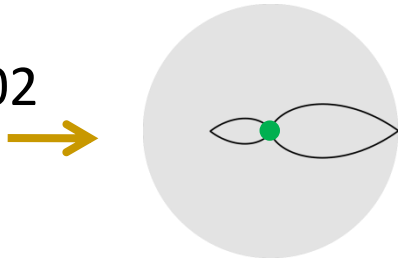


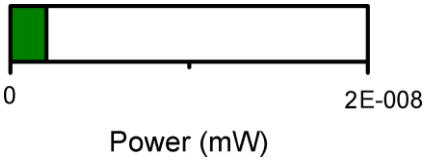



Vesicle

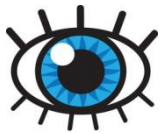
$n_{inside}$ = 1.38 ± 0.02

$n_{membrane}$ = 1.48

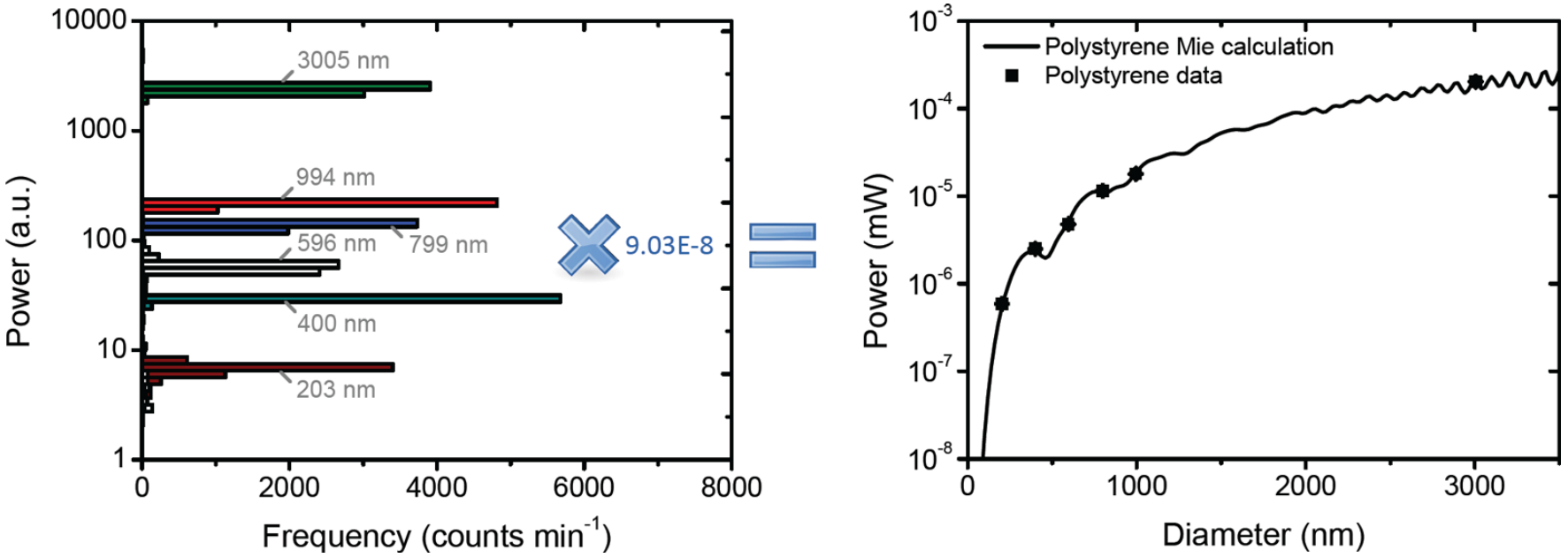





100 nm

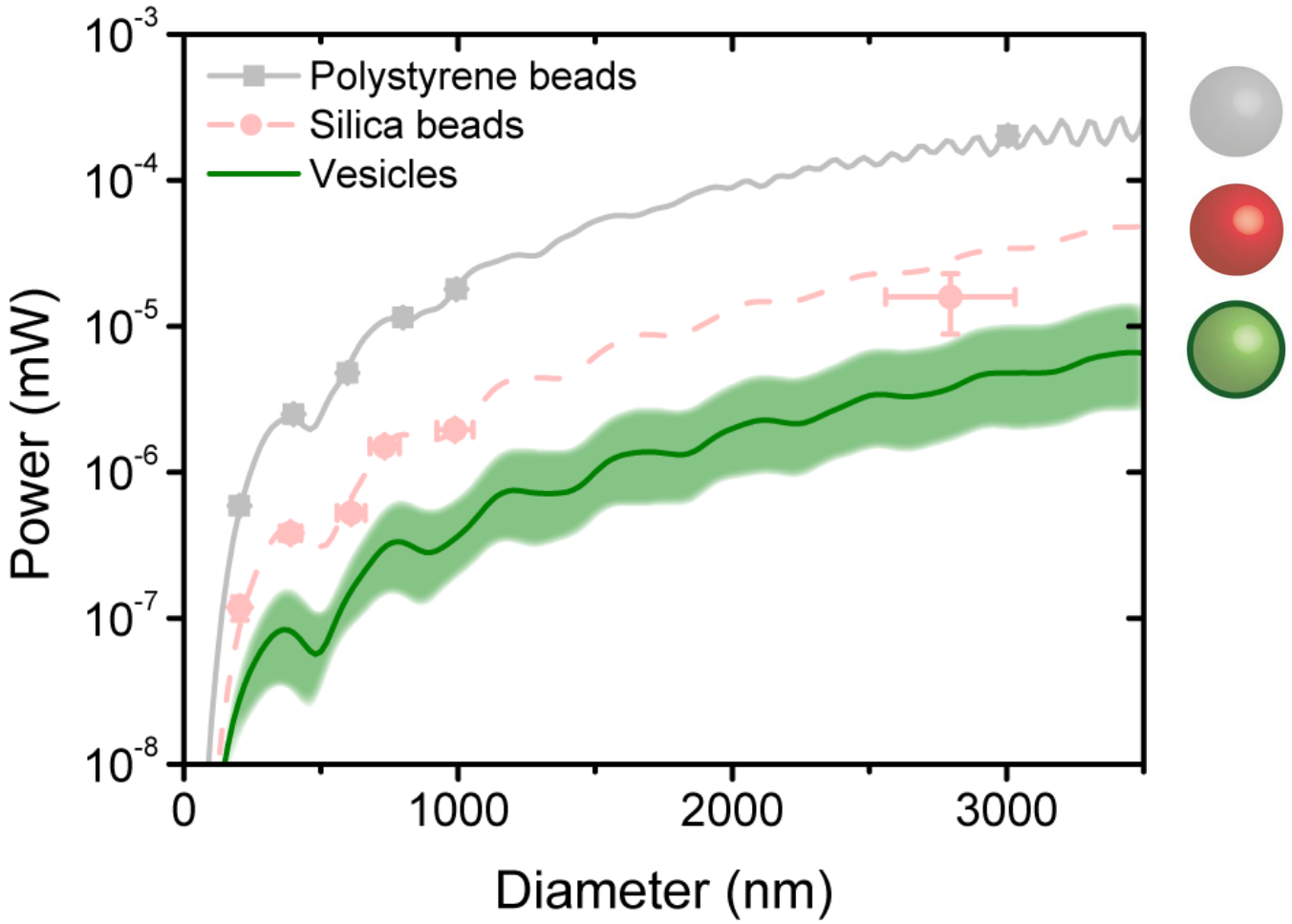


# Flow cytometer calibration

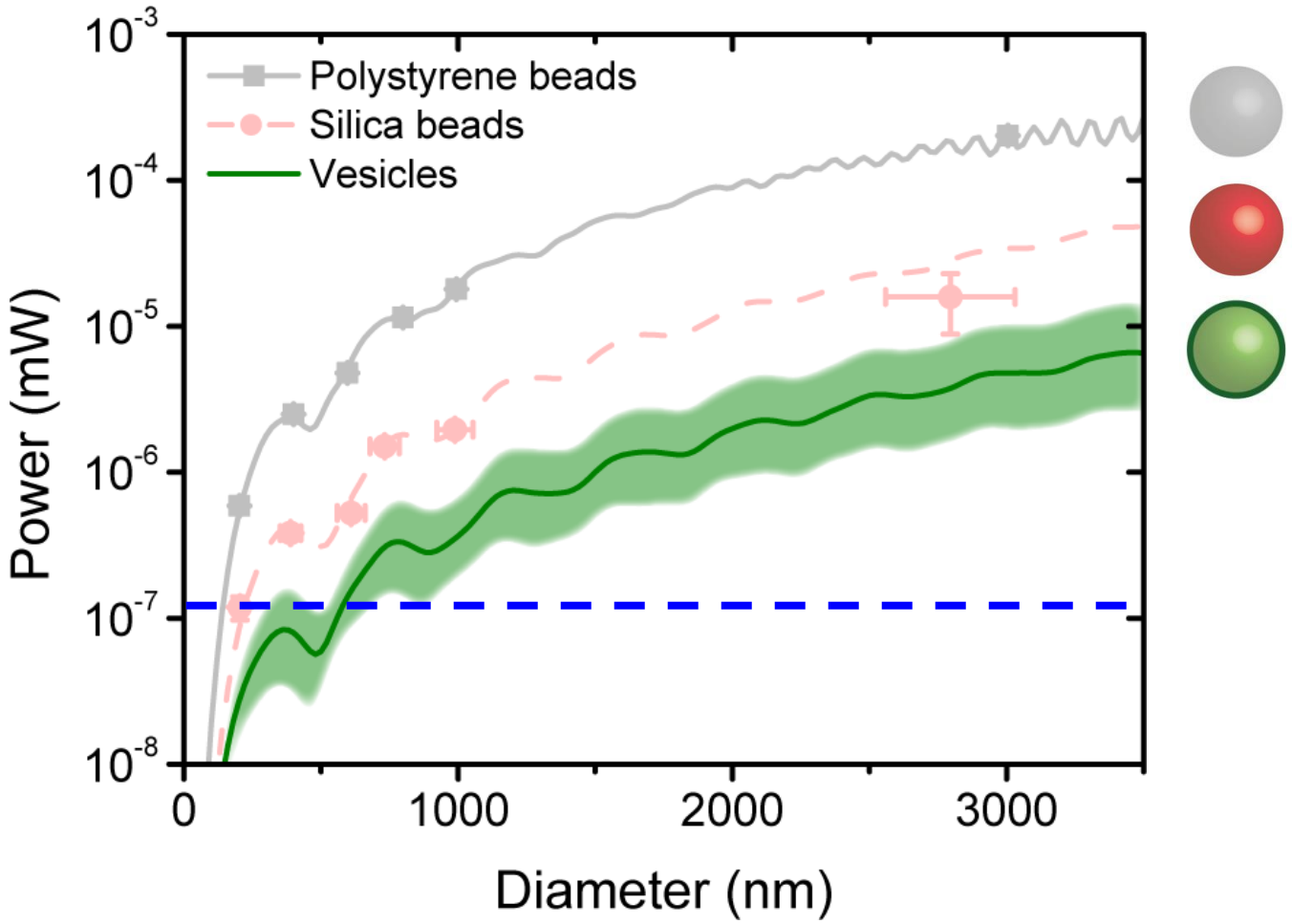



- absolute scattering power (mW) is calculated by Mie theory to relate detected scattering power (a.u.) to the diameter of beads

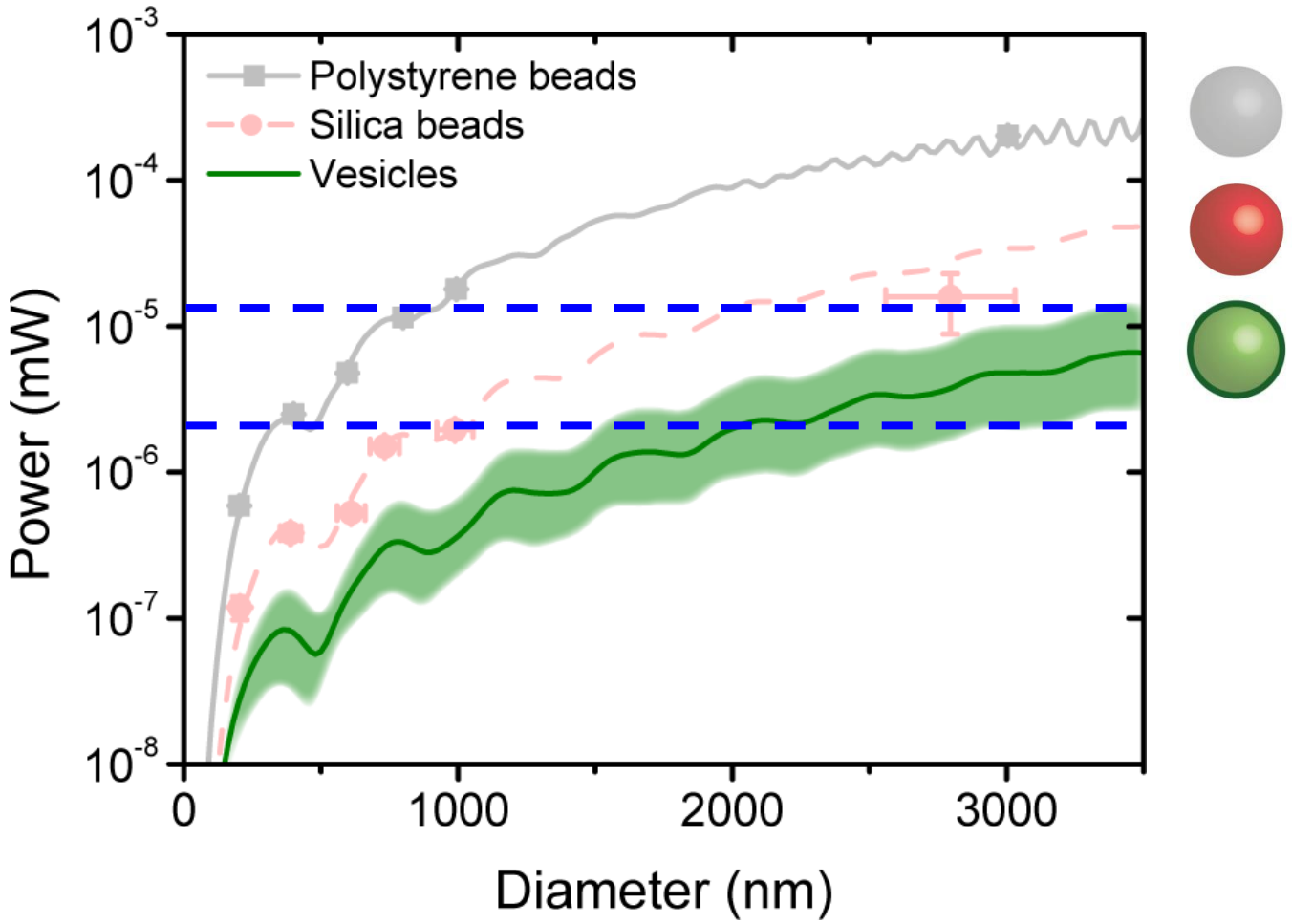
# Flow cytometry calibration



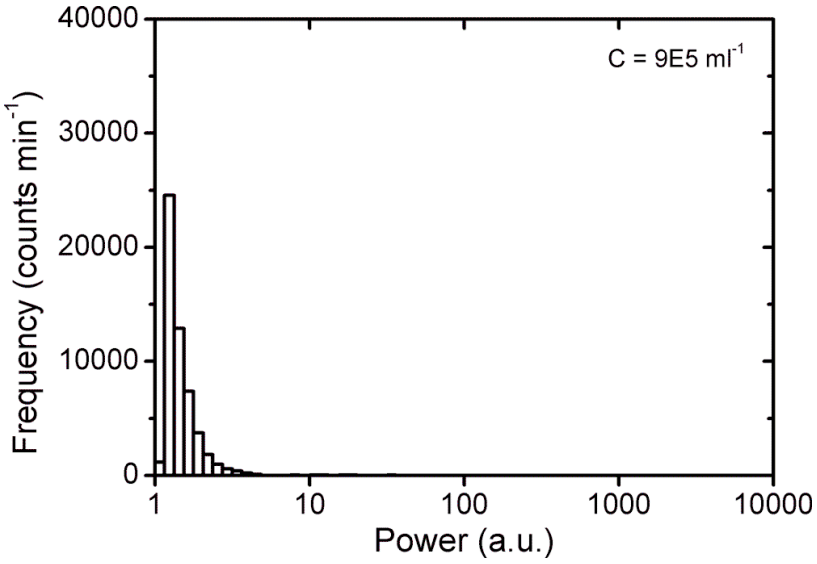
# Flow cytometry detection limit



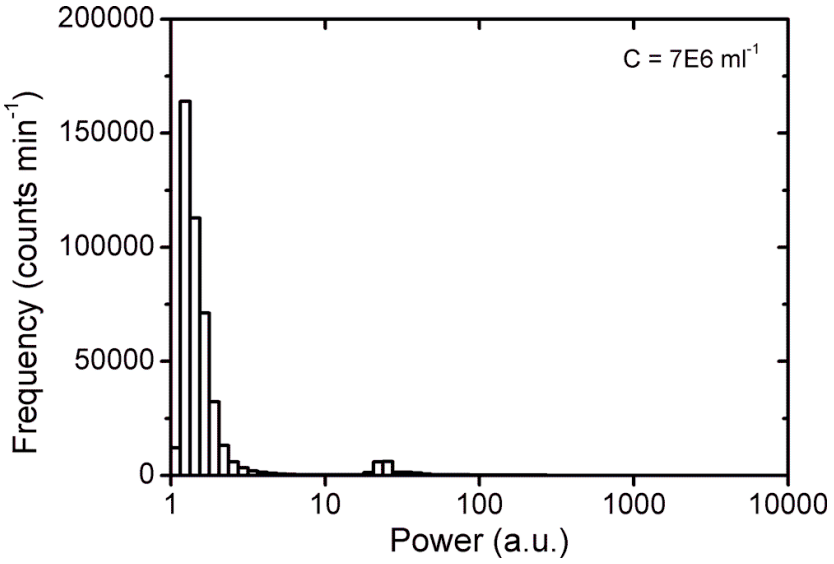
# Flow cytometry detection limit



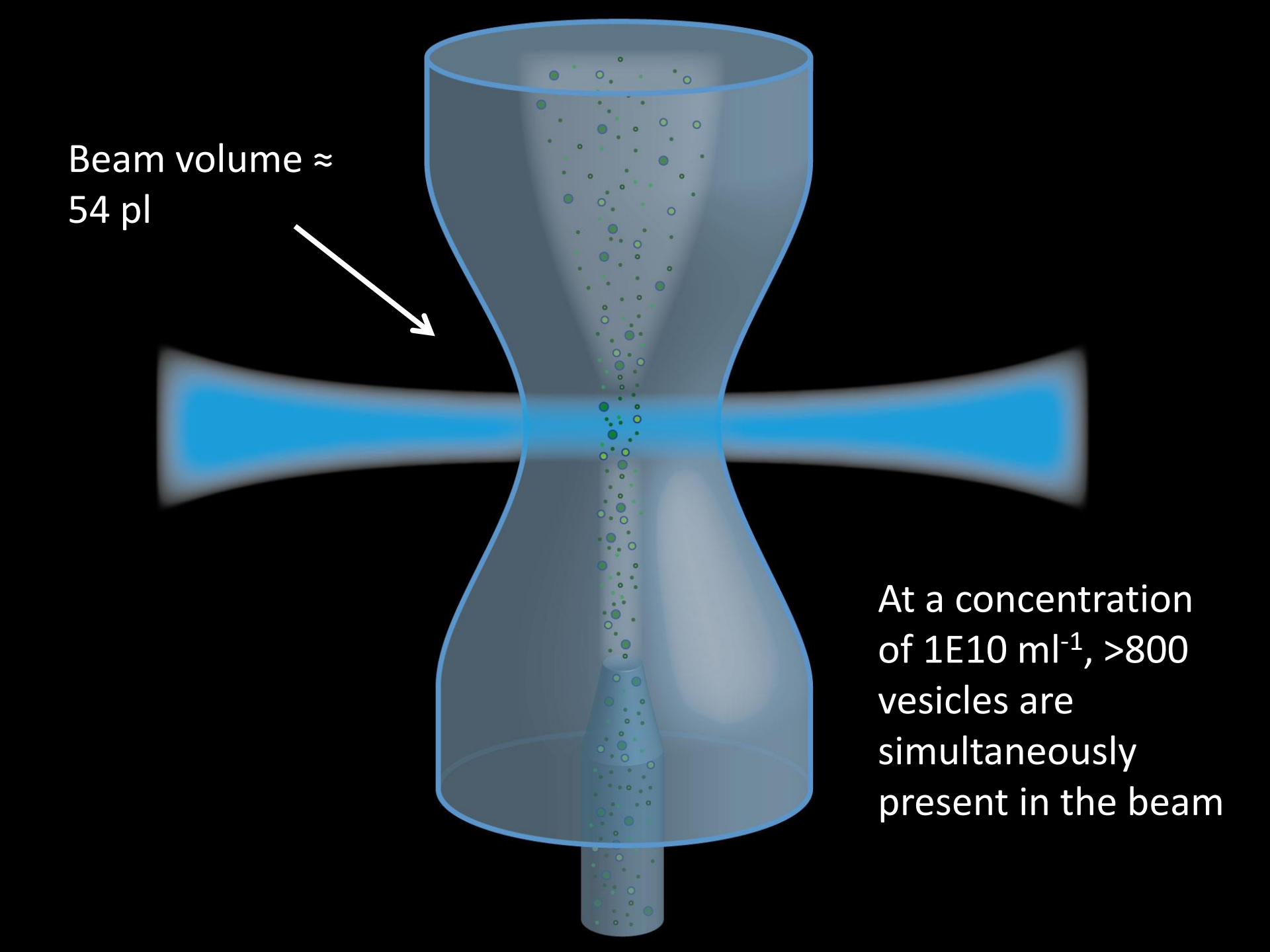
# Flow cytometry detects *multiple* vesicles as single count




vesicles from human urine filtered with 220 nm filter




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


Beam volume ≈
54 pl
At a concentration of 1E10 $ml^{-1}$, >800 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)