Optical detection and characterization of extracellular vesicles

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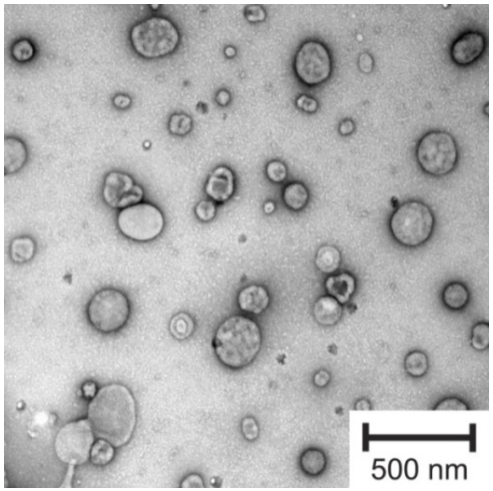
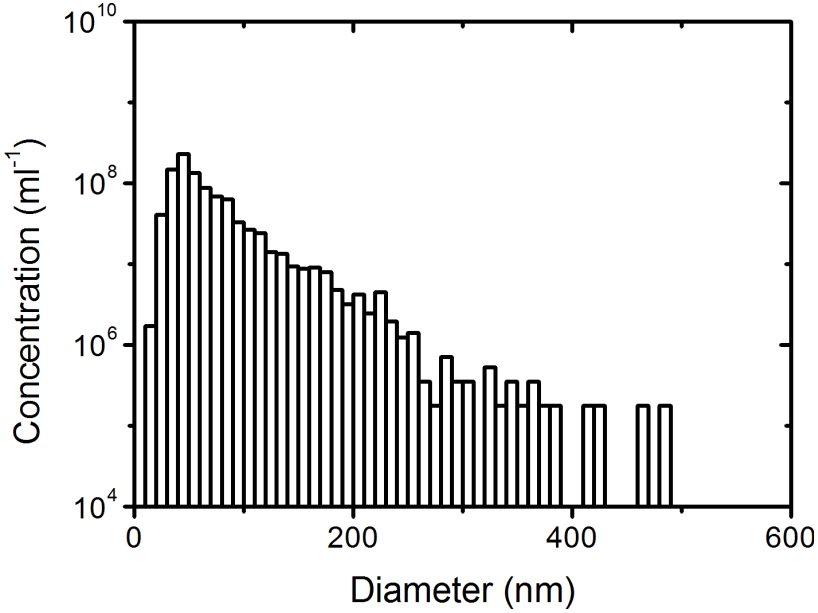
Introduction





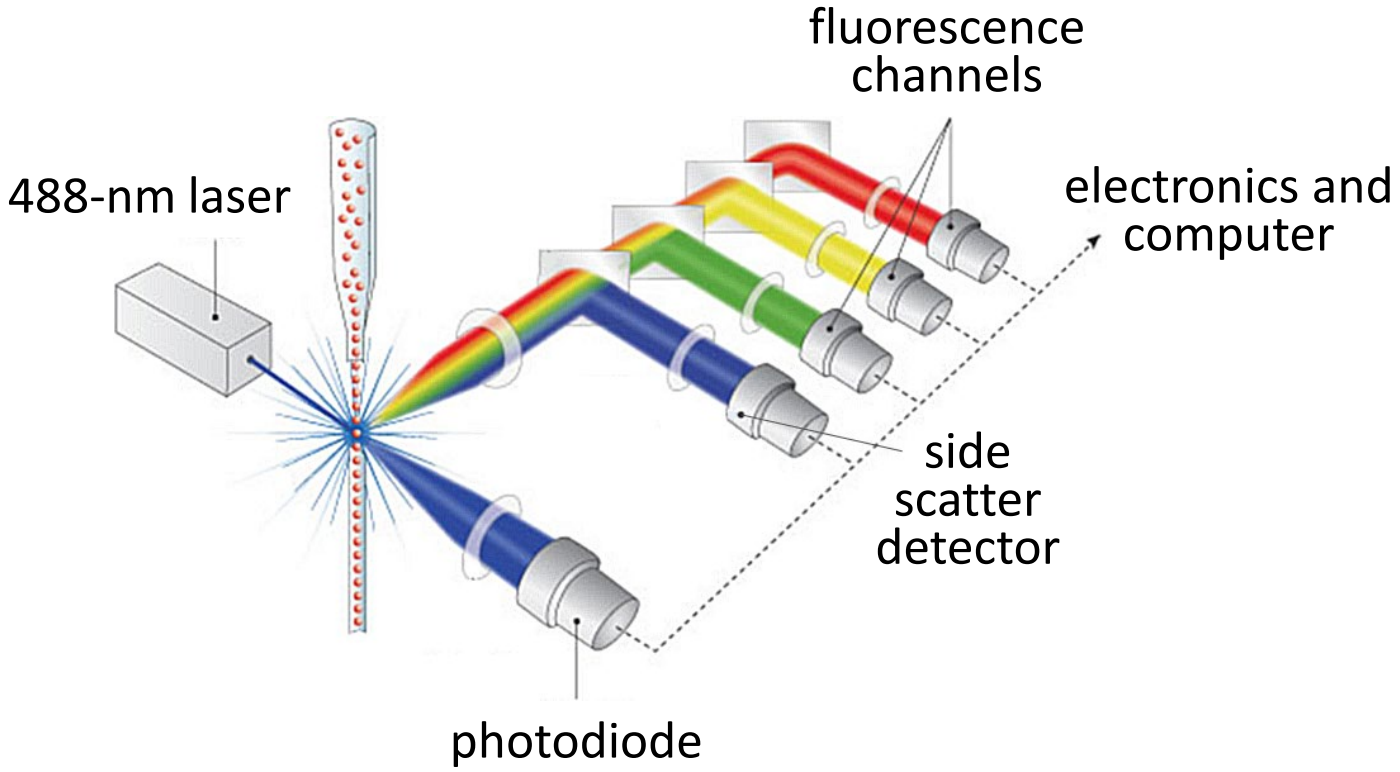
- cells release vesicles:
  spherical particles with phospholipid bilayer
- clinically relevant
- detection and isolation cumbersome

Objective (part 1)

- determine
  - size
  - concentration
  - composition (proteins, lipids, RNA, DNA)

  of *single* vesicles in suspension at high throughput

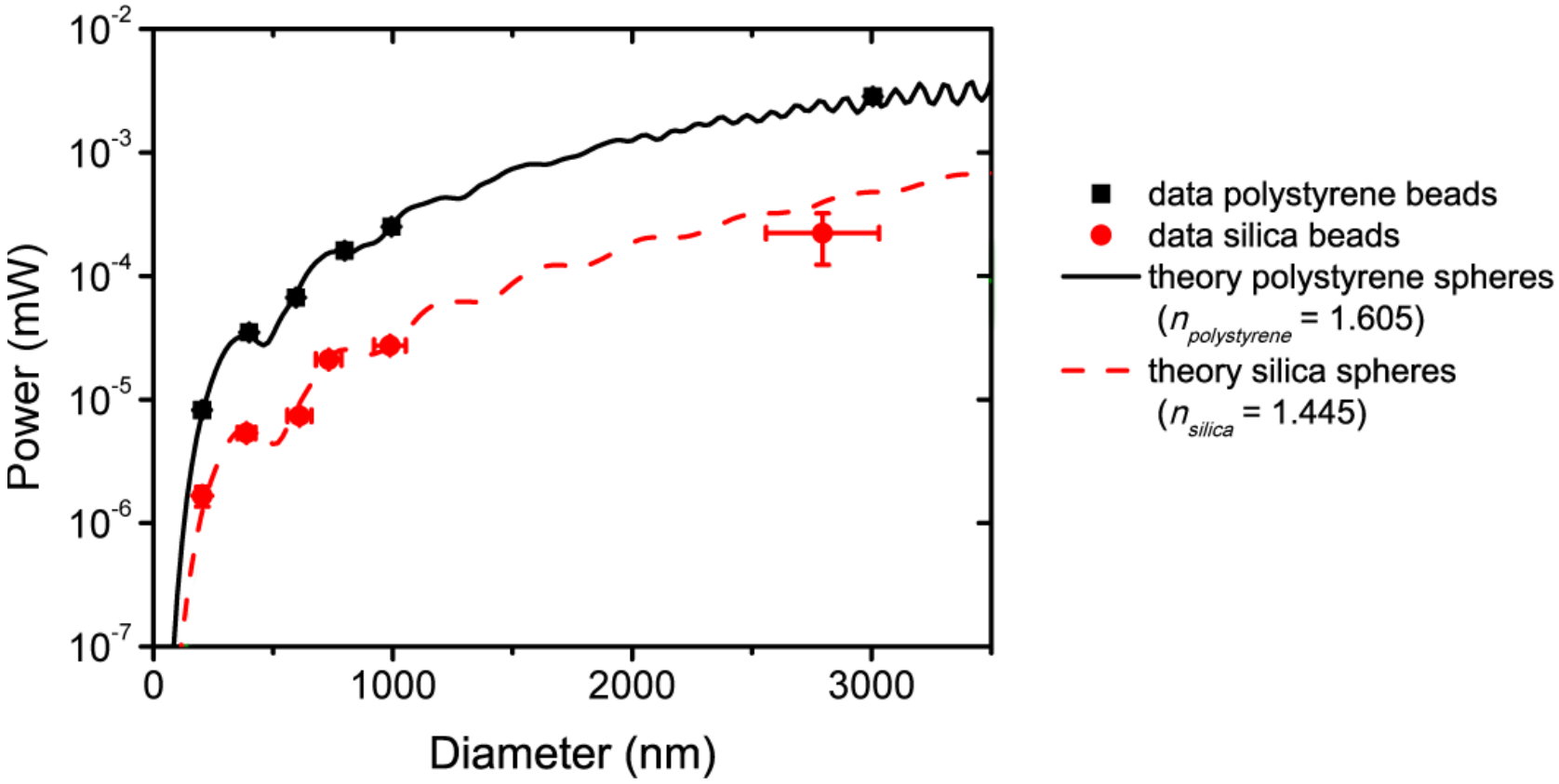
Conventional methods



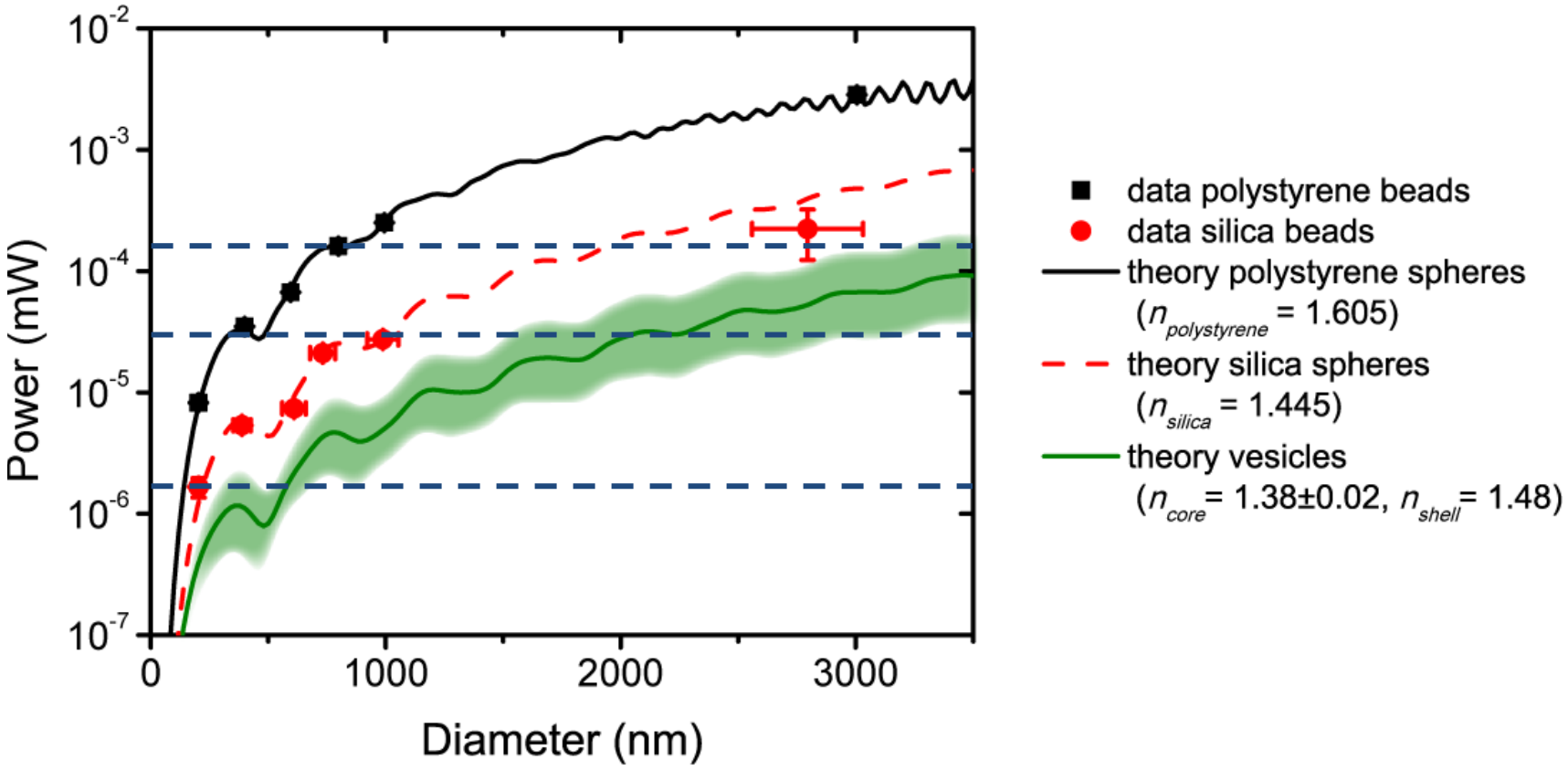
- flow cytometry
- transmission electron microscopy

image adapted from www.semrock.com

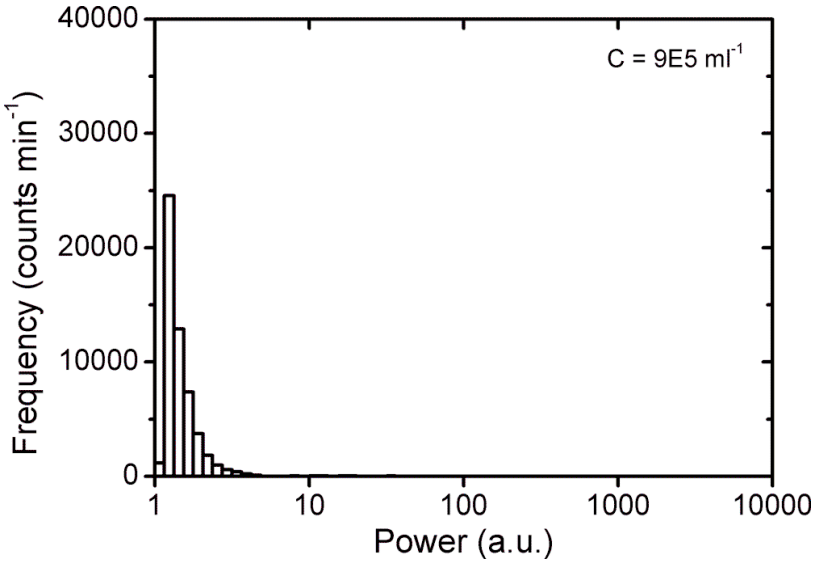
Results - flow cytometry



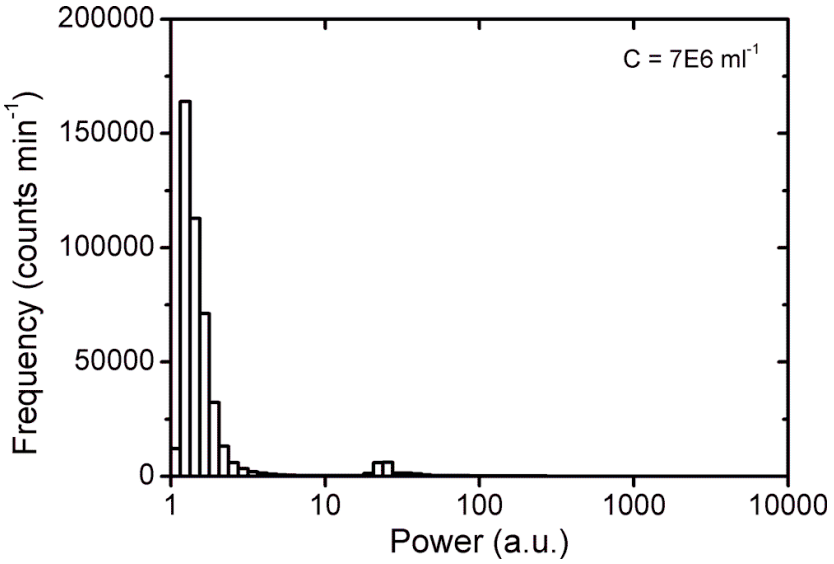
Results - flow cytometry



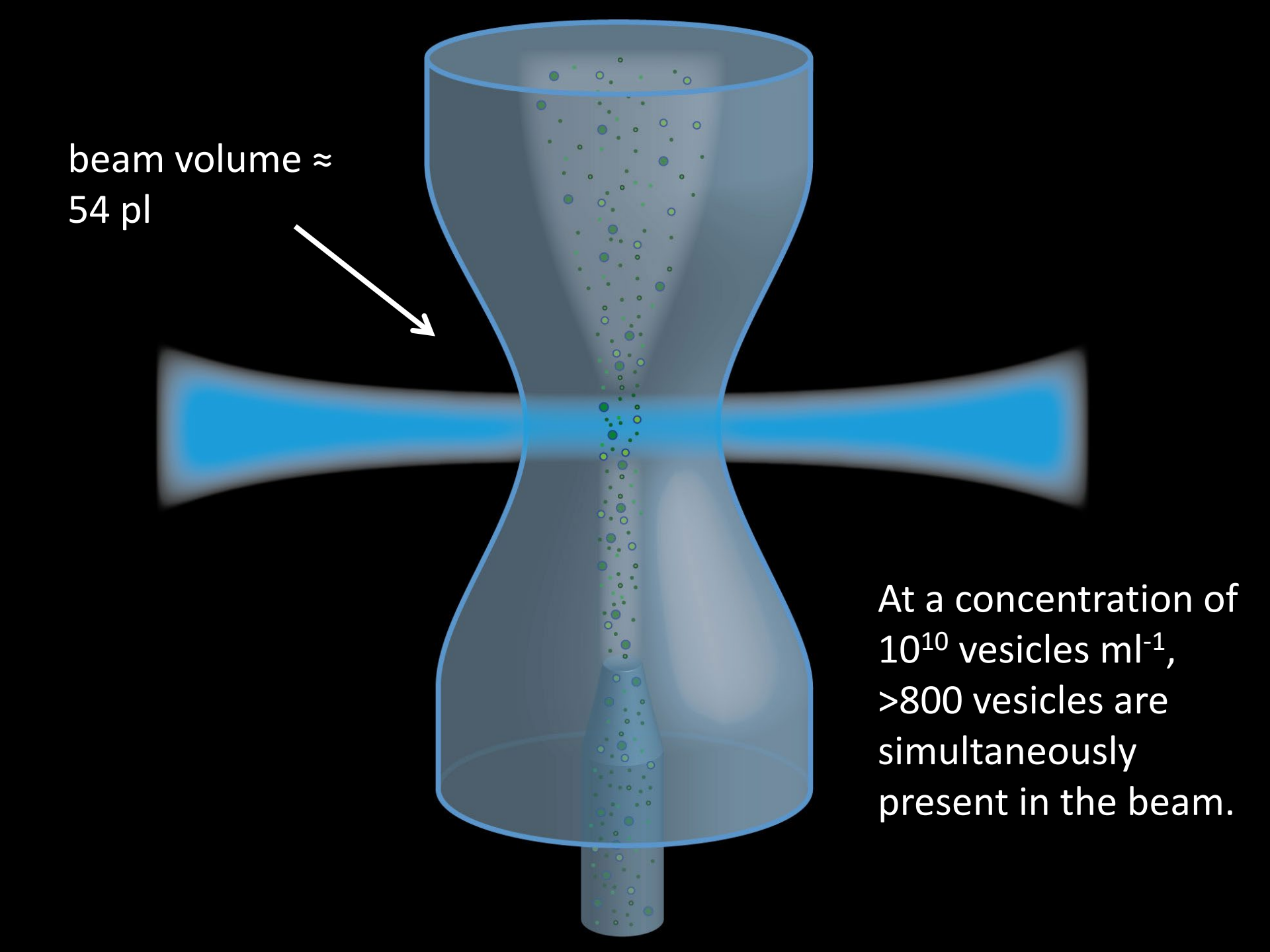
Results - *multiple* vesicles as single count



vesicles from human urine filtered with 220-nm filter



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


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

Conclusion (part 1)

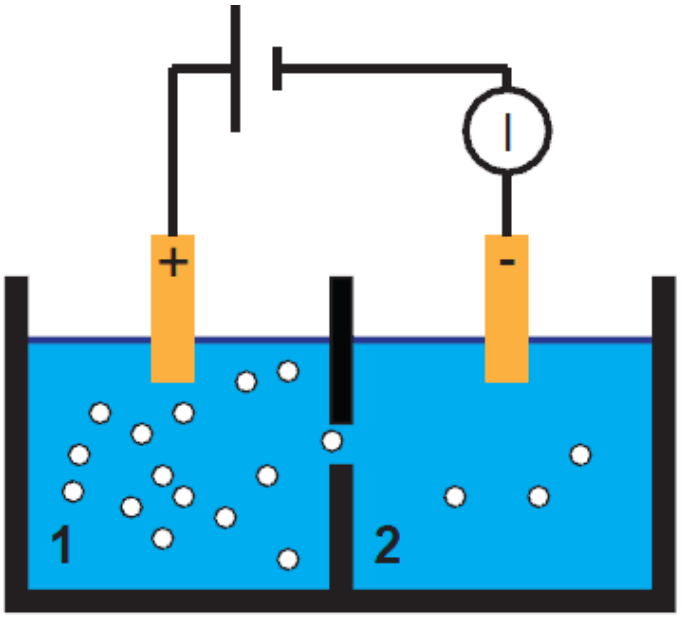
- vesicle detection by flow cytometry
  - scattering power related to bead diameter
  - detection limit is 300 – 700 nm for *single* vesicles
  - *multiple* vesicles are simultaneously illuminated

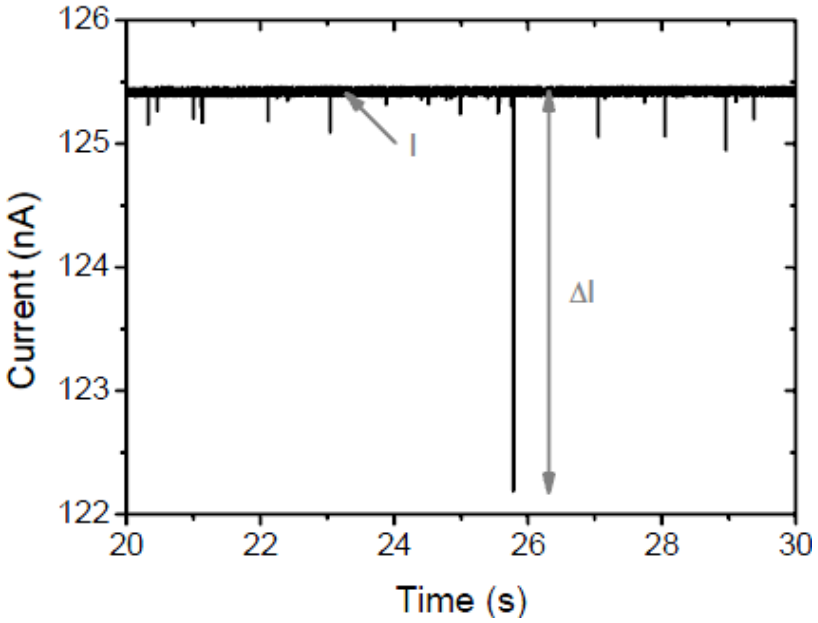
Objective (part 2)

- determine
  - size
  - concentration
  - composition (proteins, lipids, RNA, DNA)

  of *single* vesicles in suspension at high throughput

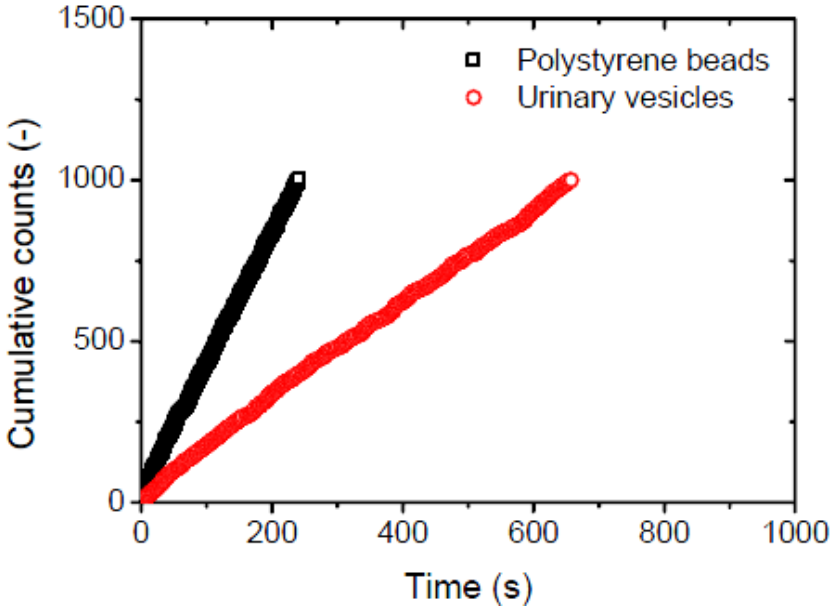
Method: resistive pulse sensing

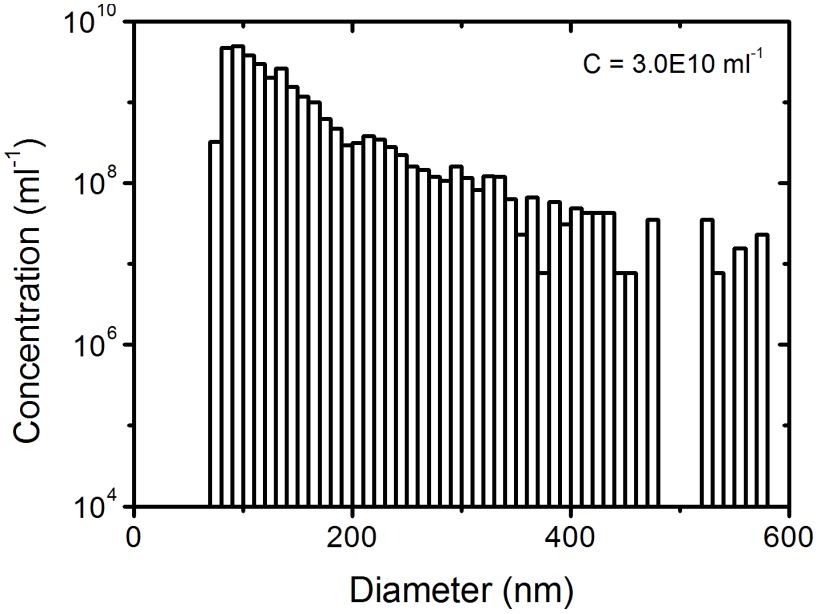




- diffusion
- electrophoresis
- osmosis
- pressure

Results: resistive pulse sensing





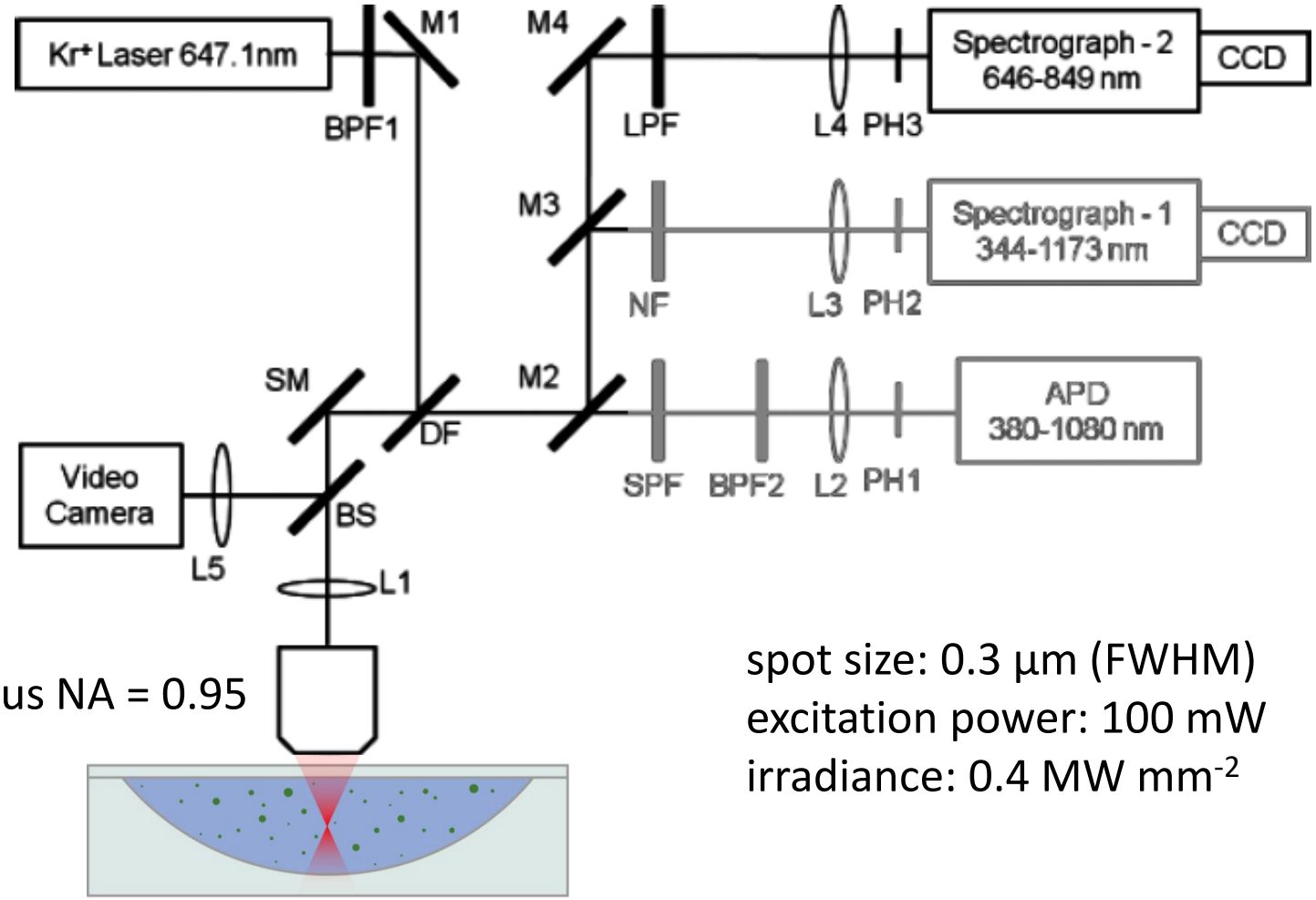
- size and concentration obtained
- detection limit: ~80 nm

Objective

- determine
  - size ✓
  - concentration ✓
  - composition (proteins, lipids, RNA, DNA)

  of *single* vesicles in suspension at high throughput

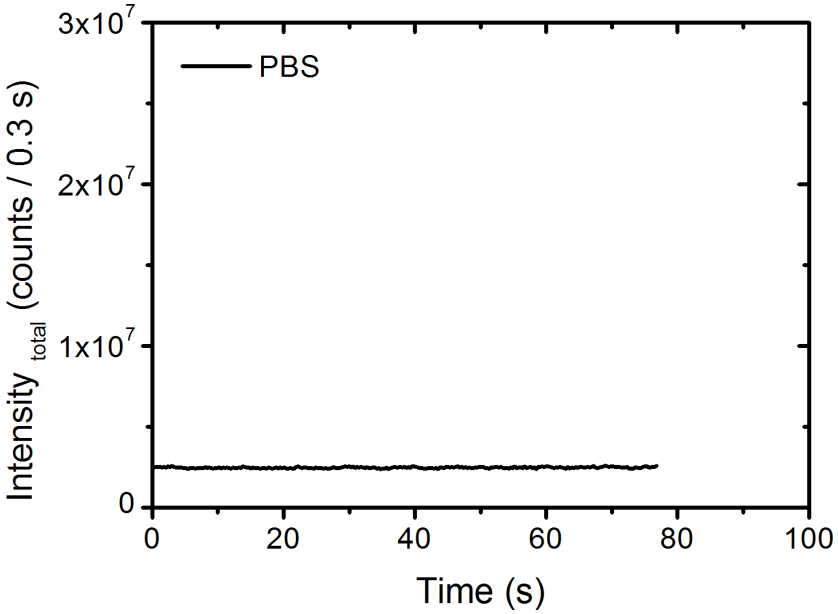
Method: Raman microspectroscopy

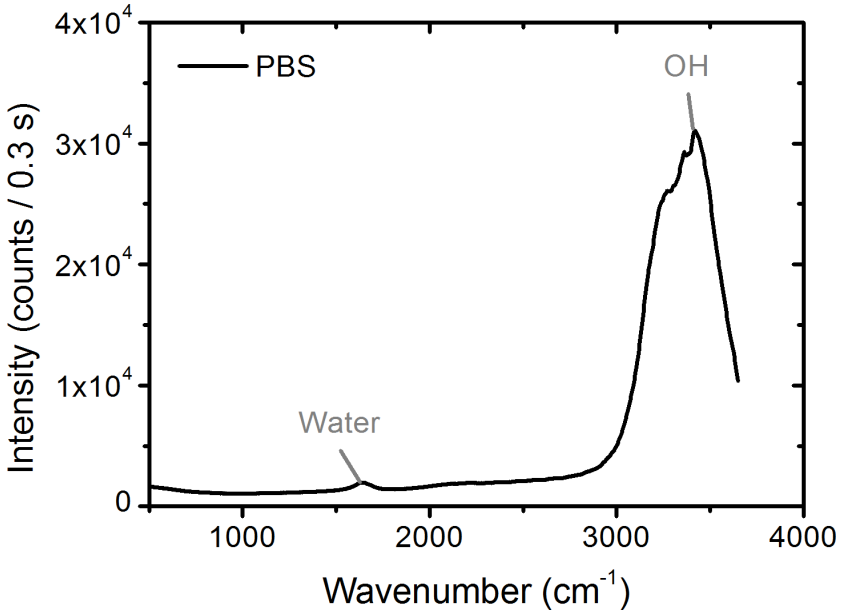


Olympus NA = 0.95

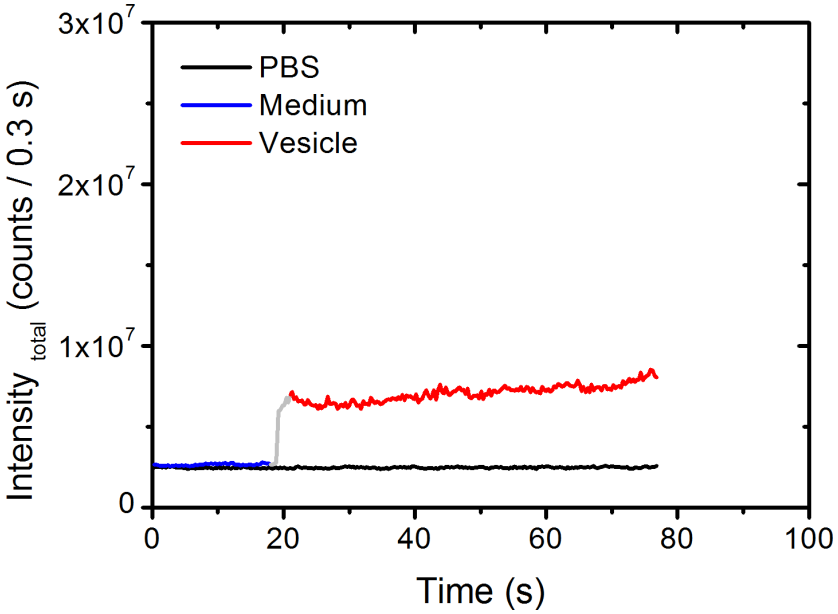
spot size: 0.3 µm (FWHM)
excitation power: 100 mW
irradiance: 0.4 MW mm^{-2}

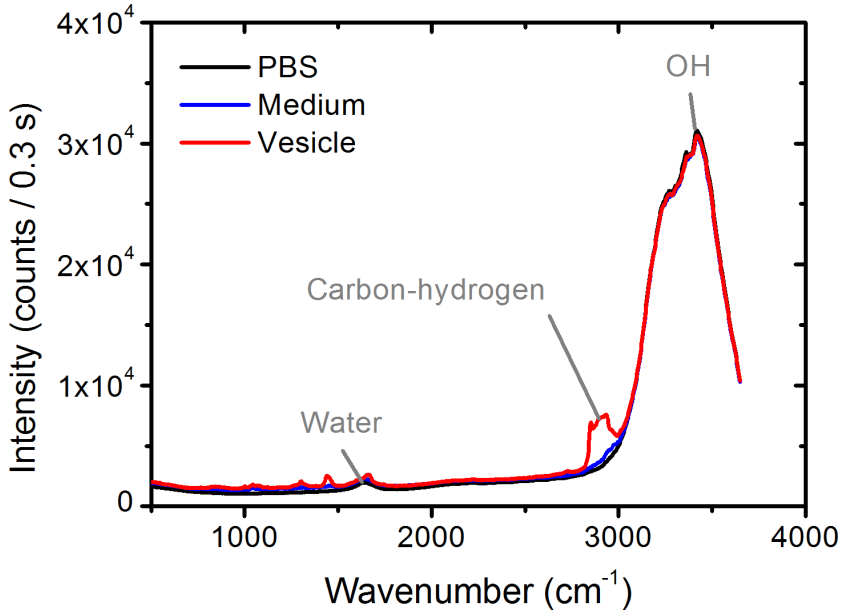
Results: Raman spectrum of PBS



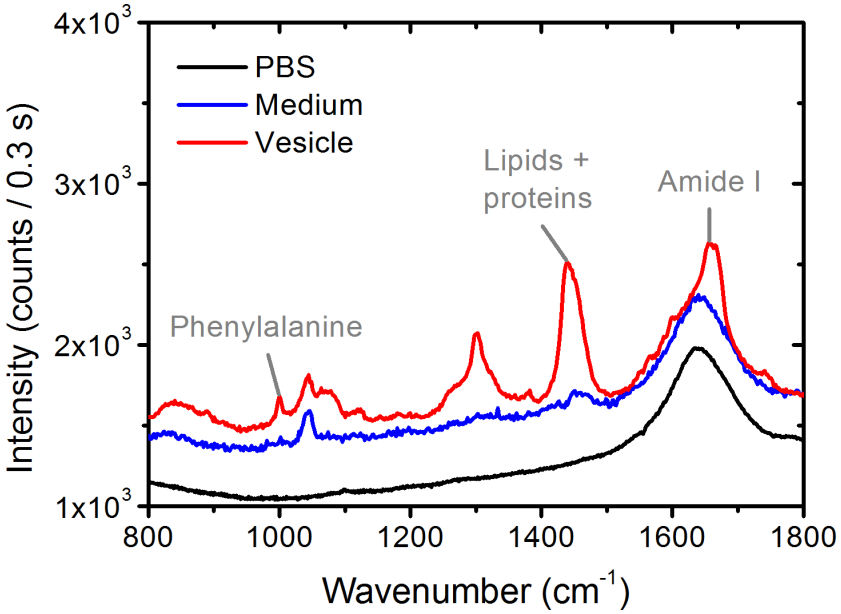


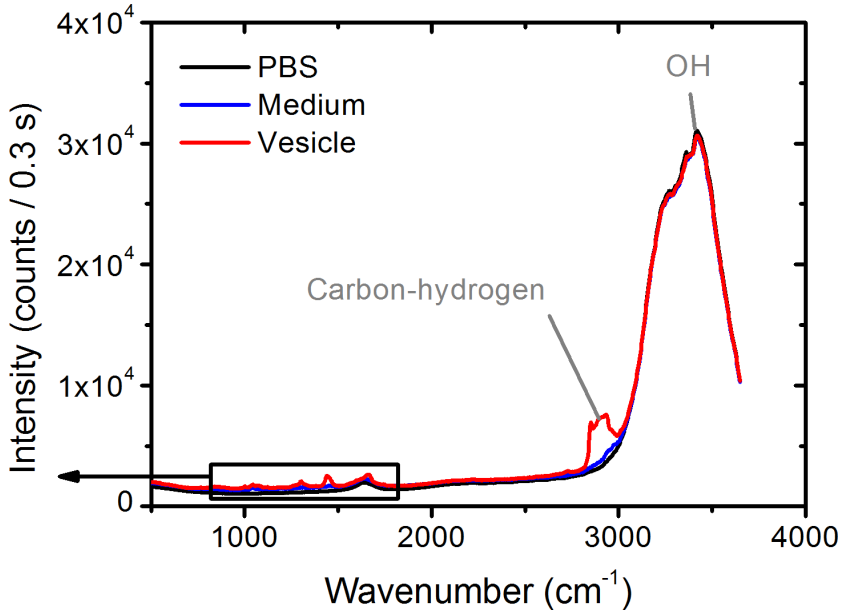
Results: Raman spectrum of tumor cell vesicle



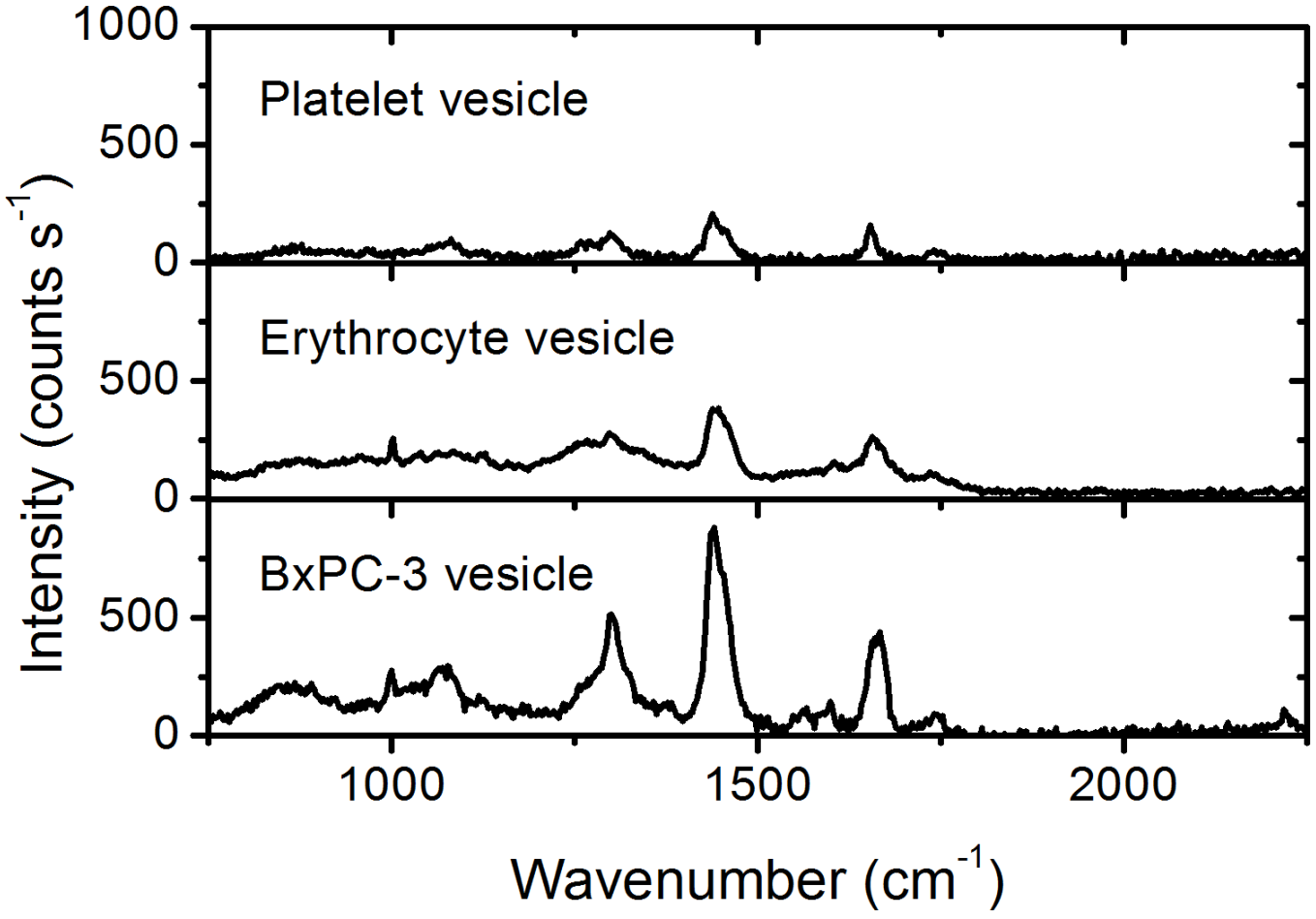


Results: Raman spectrum of tumor cell vesicle





Results: Label-free identification of vesicles

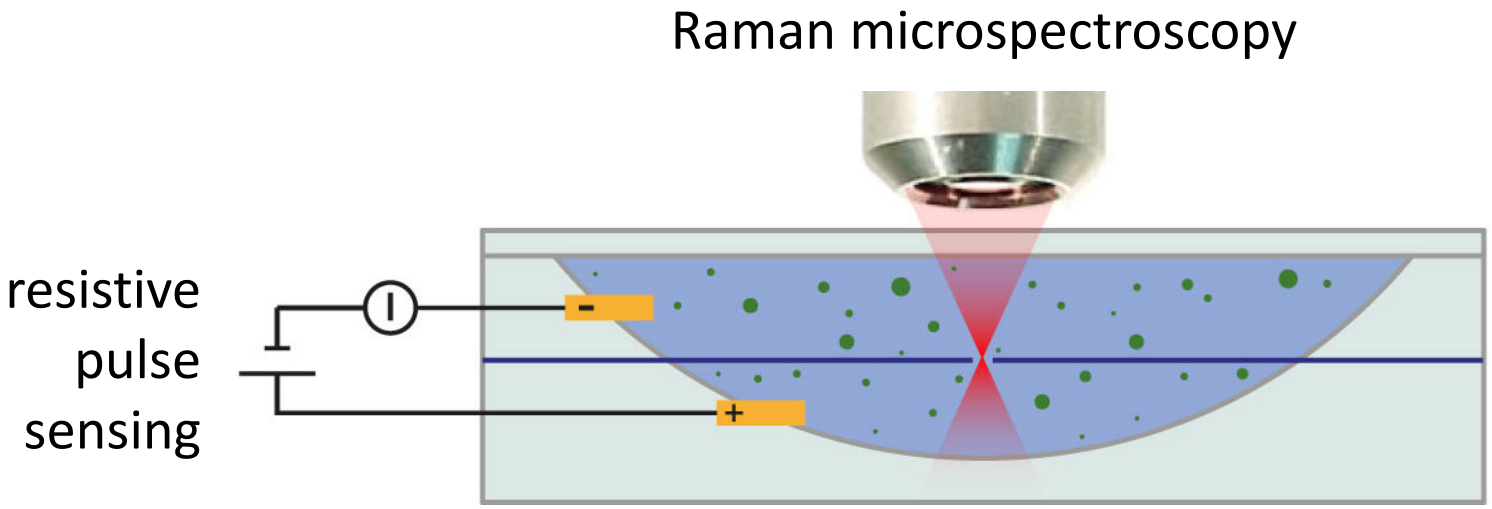


Conclusion

- determine
  - size ✓
  - concentration ✓
  - chemical composition ✓

  of *single* vesicles in suspension ~~at high throughput~~

Outlook



- simultaneously determine
  - size
  - concentration
  - chemical composition

  of *single* vesicles in suspension at medium throughput

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