Trapping of single extracellular vesicles in the evanescent field of an optical cavity

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  - Laboratory Experimental Clinical Chemistry
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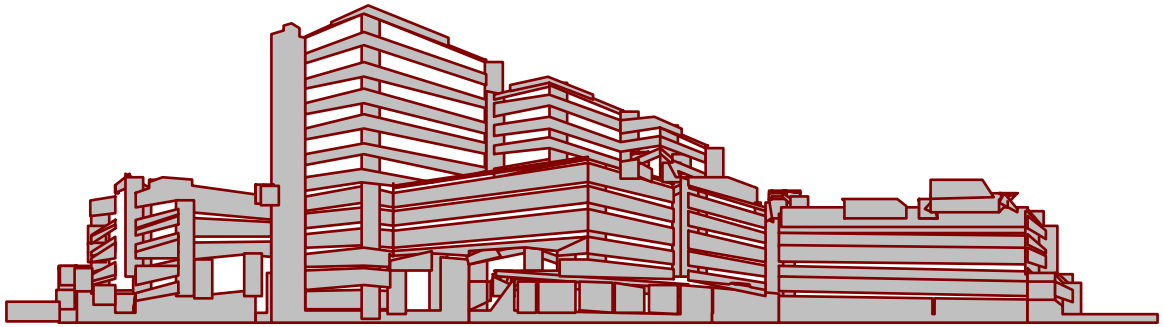
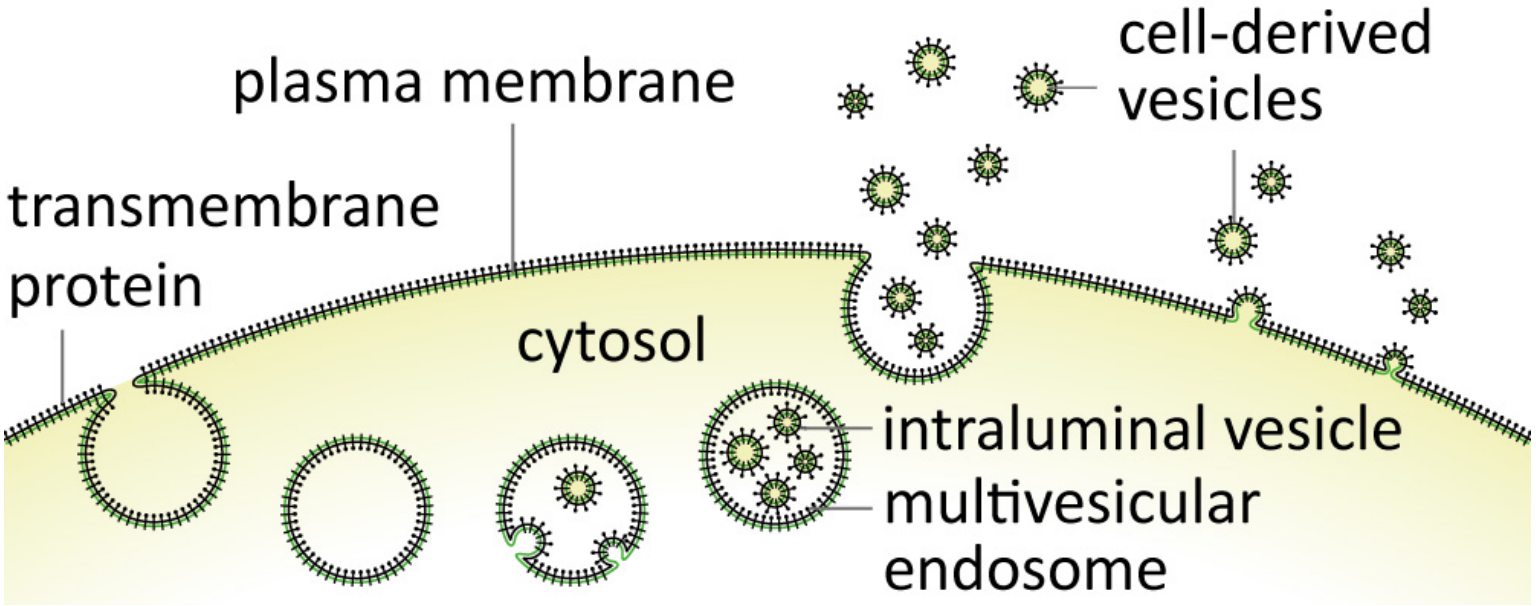
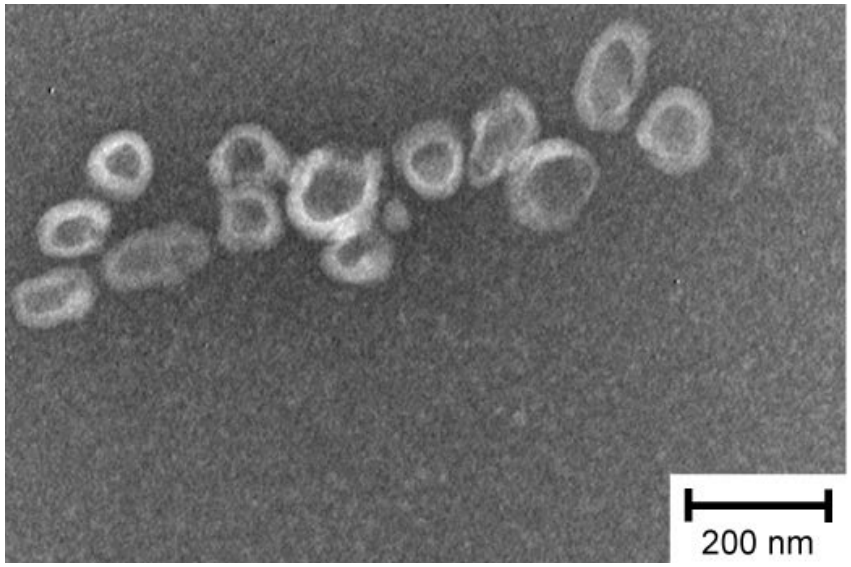


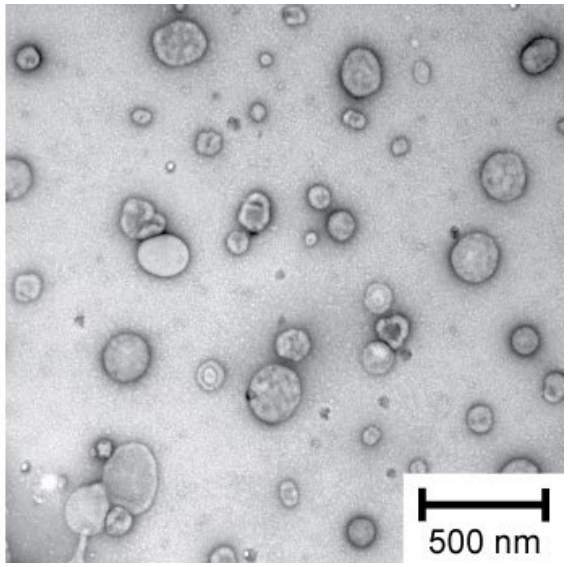
Introduction – extracellular vesicles



- cells release vesicles (e.g. exosomes): biological nanoparticles with receptors, DNA, RNA
- specialized functions
- clinically relevant

Introduction – extracellular vesicles

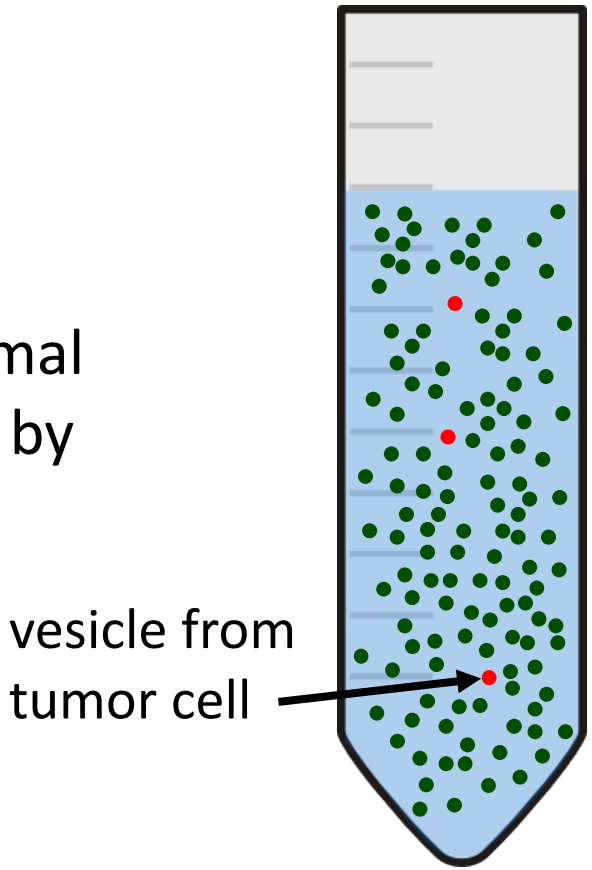




- vesicle detection is cumbersome
  - small size (< 500 nm)
  - low refractive index (~1.4)
  - fluorescent antibody labeling involves practical problems

Motive and goal

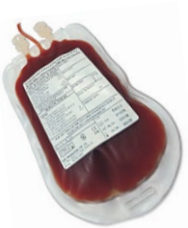
- Clinical motive
  - count tumor vesicles in blood for therapy monitoring
- Goal
  - distinguish tumor vesicles from normal vesicles in solution *without labeling* by Raman microspectroscopy



Sample – vesicle isolation

erythrocyte vesicles

- centrifuge (3·20 min, 1560·*g*) *

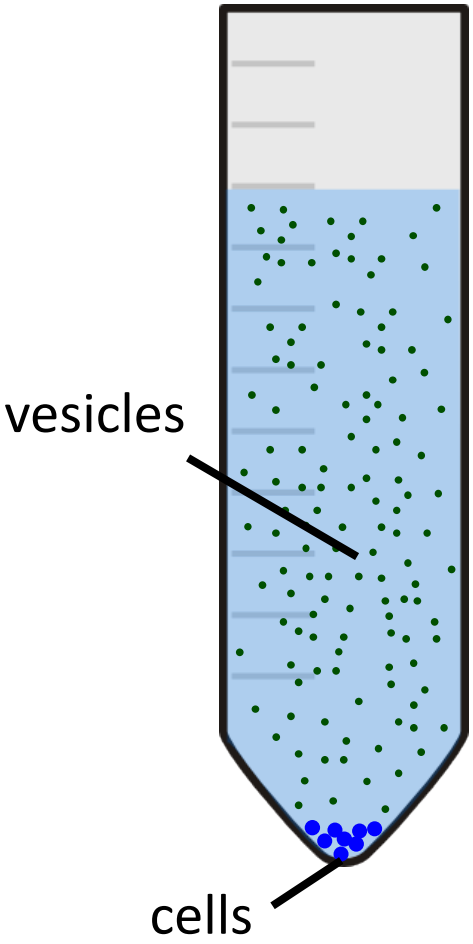


platelet vesicles

- centrifuge (3·20 min, 800·*g*)

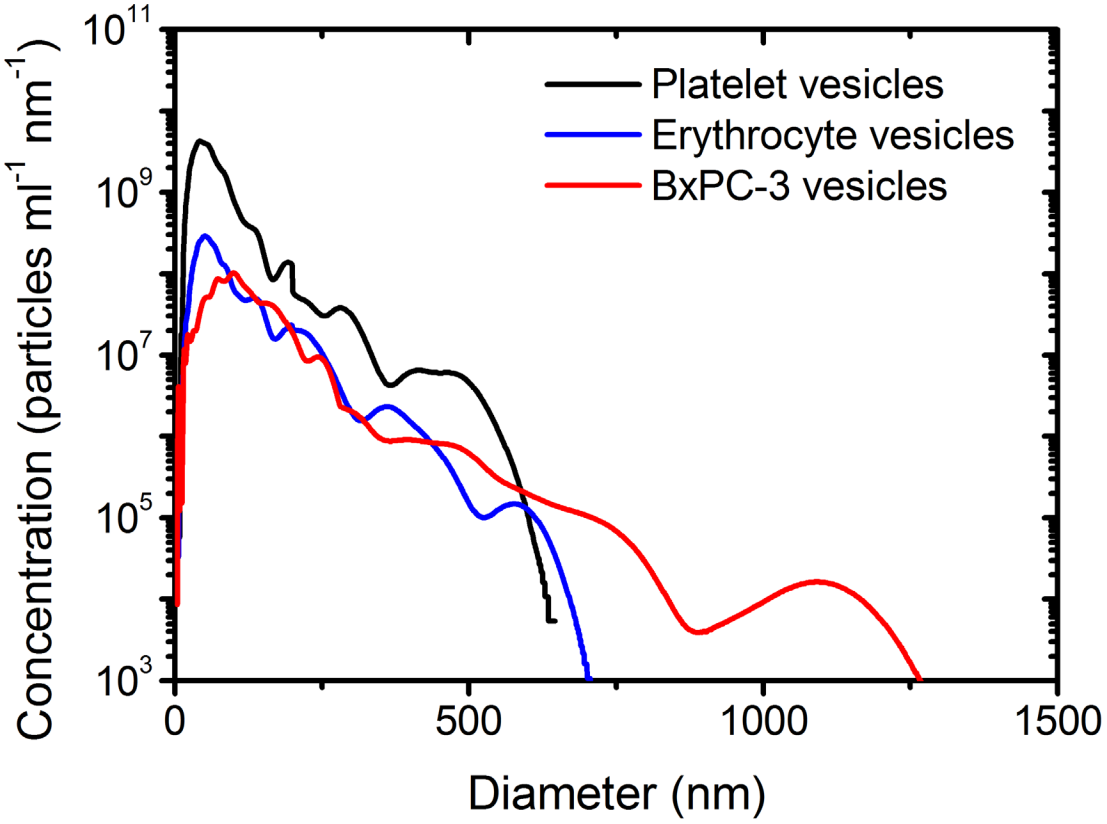
tumor vesicles from a human pancreatic adenocarcinoma (BxPC-3) cell line

- centrifuge (10 min, 180·*g*)



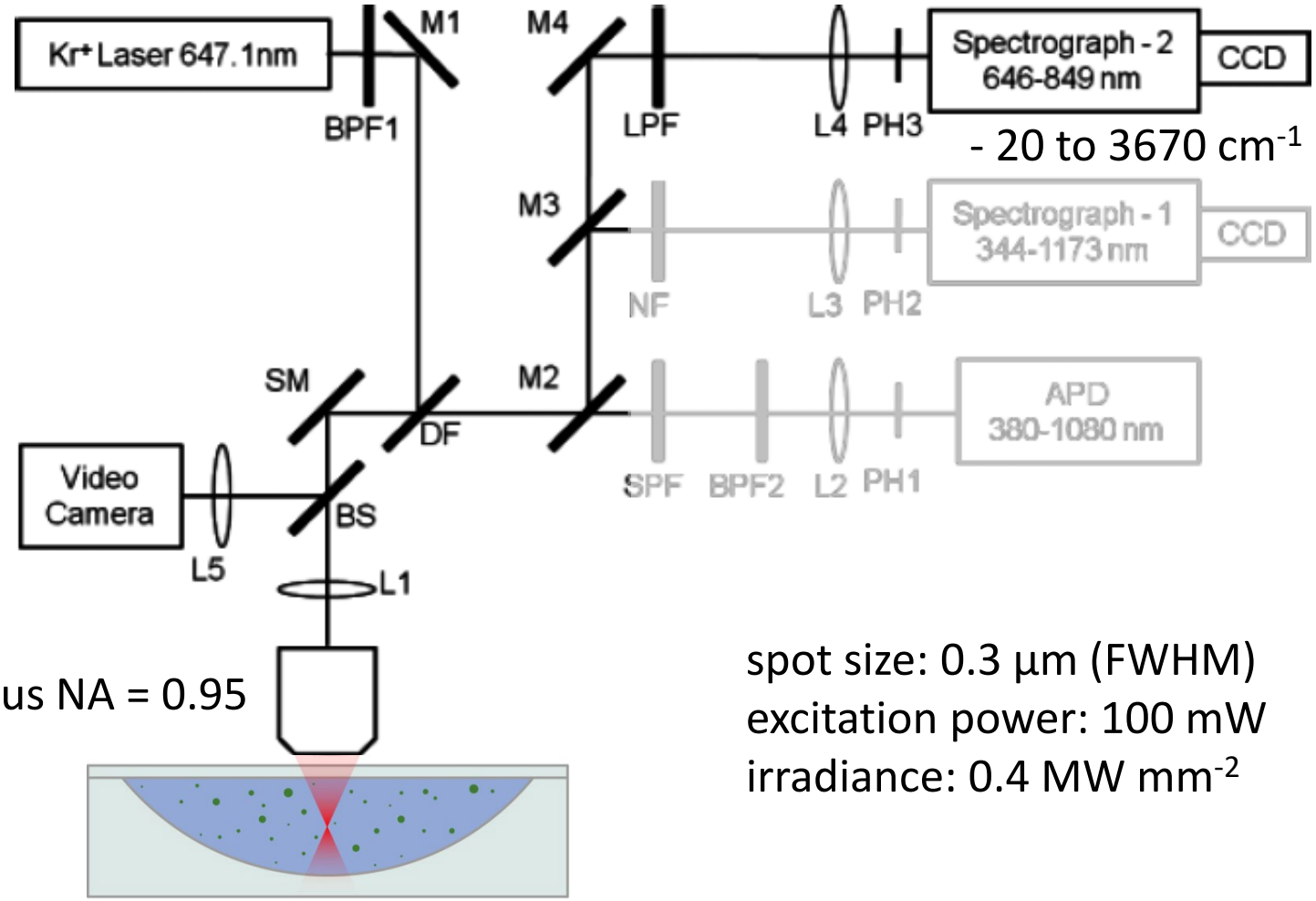
* more information on vesicle isolation: metves.eu

Sample – Vesicle size distribution



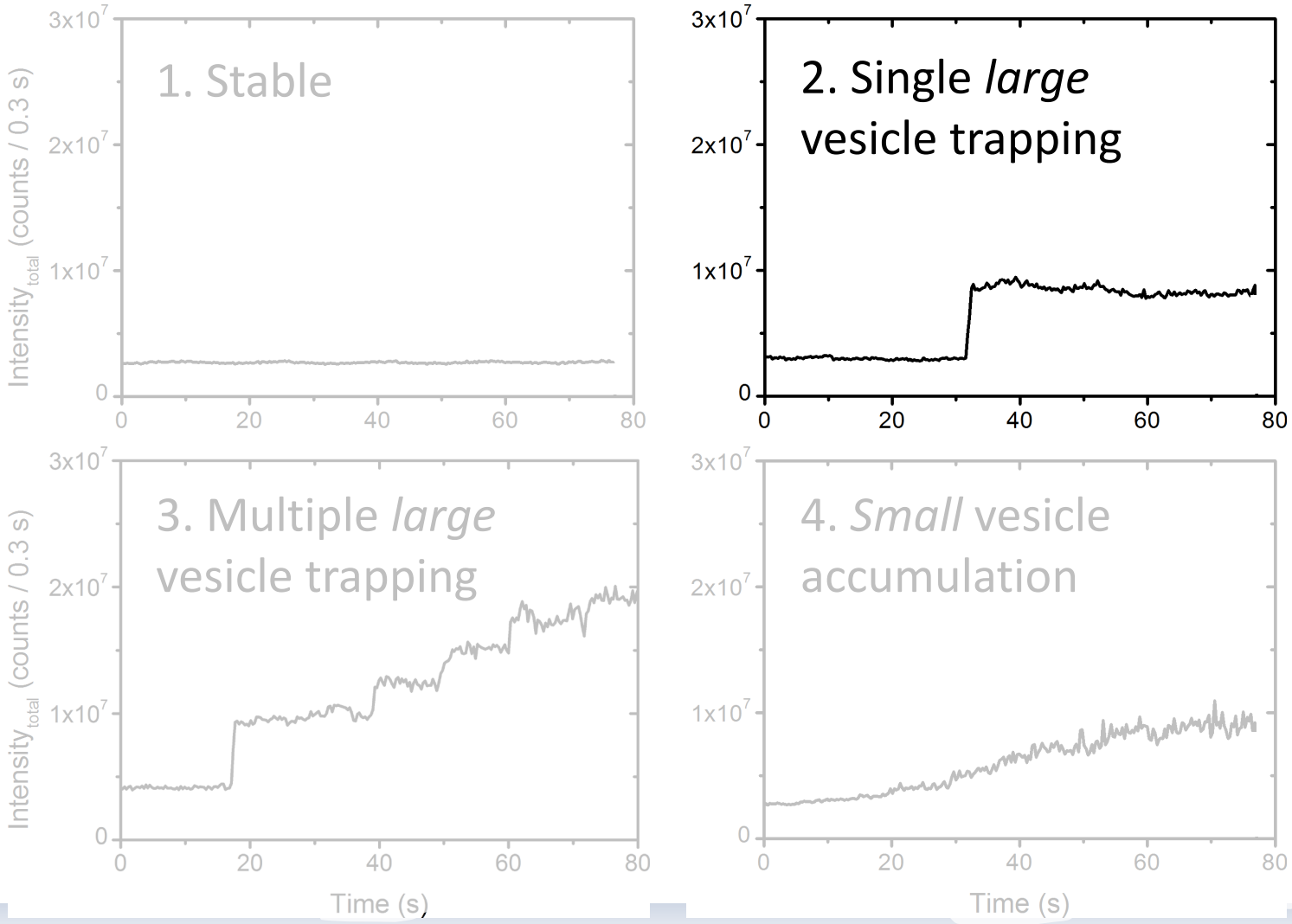
- data obtained with nanoparticle tracking analysis

Methods – Raman microspectroscopy

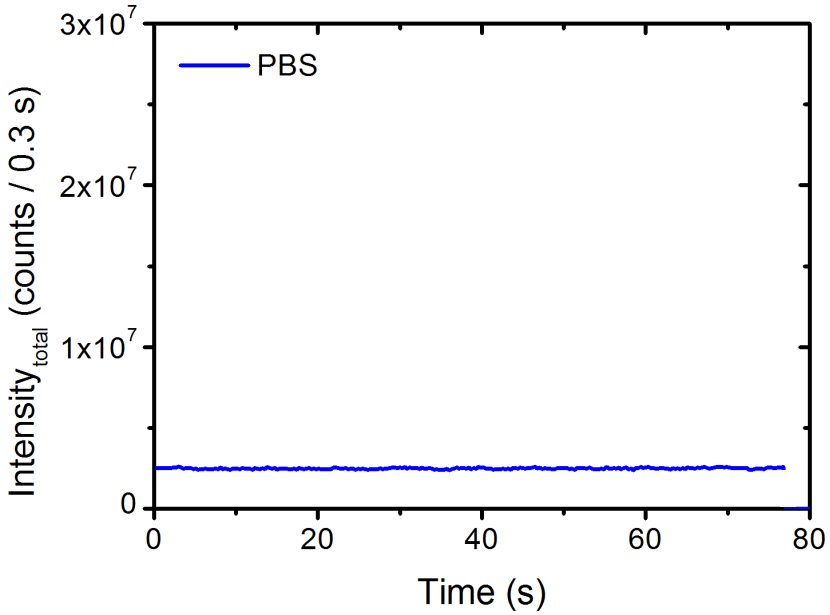


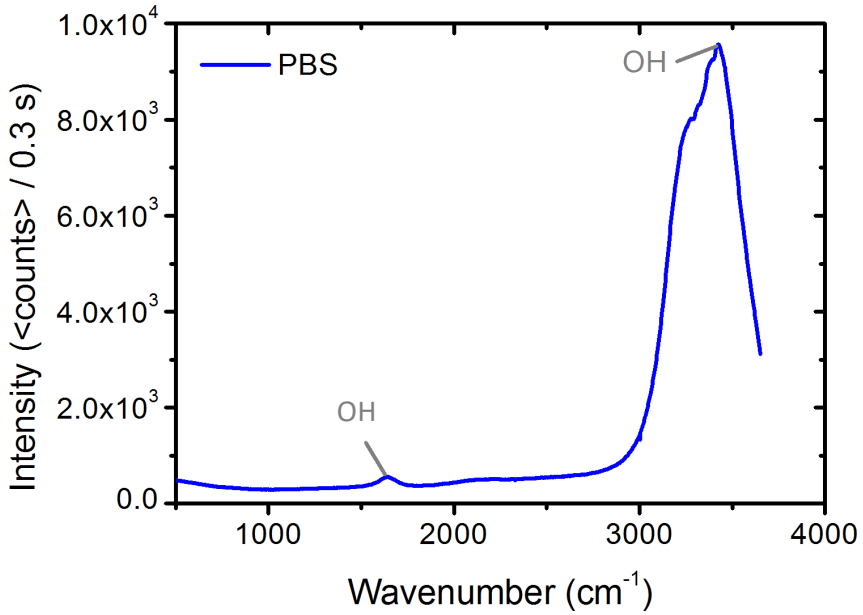
spot size: 0.3 µm (FWHM)
excitation power: 100 mW
irradiance: 0.4 MW mm^{-2}

Results – Total intensity versus time

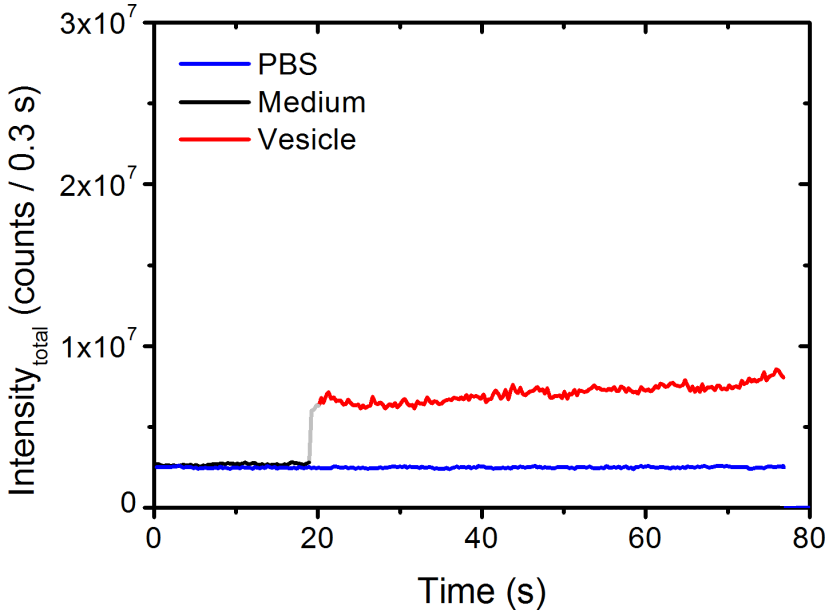


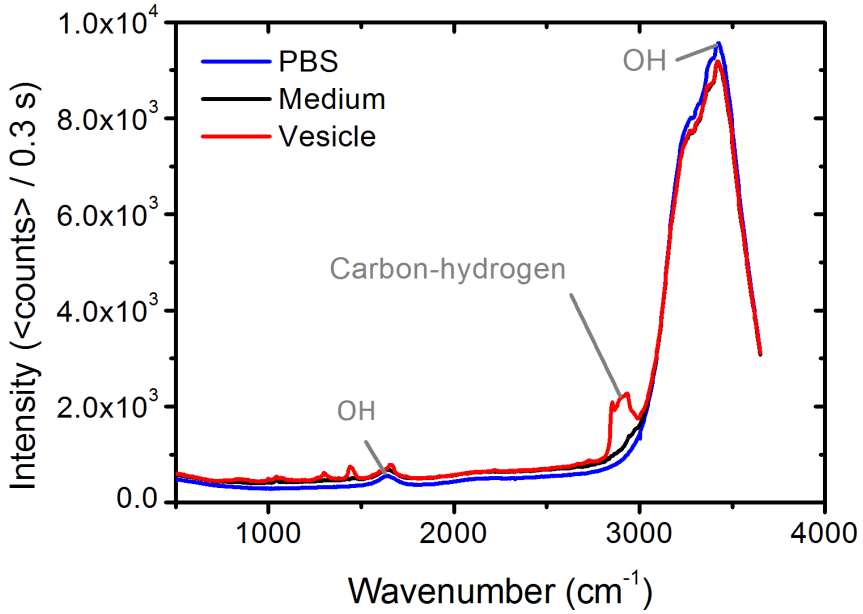
Results - Raman spectrum of PBS





Results - Raman spectrum of *single* tumor (BxPC-3) vesicle





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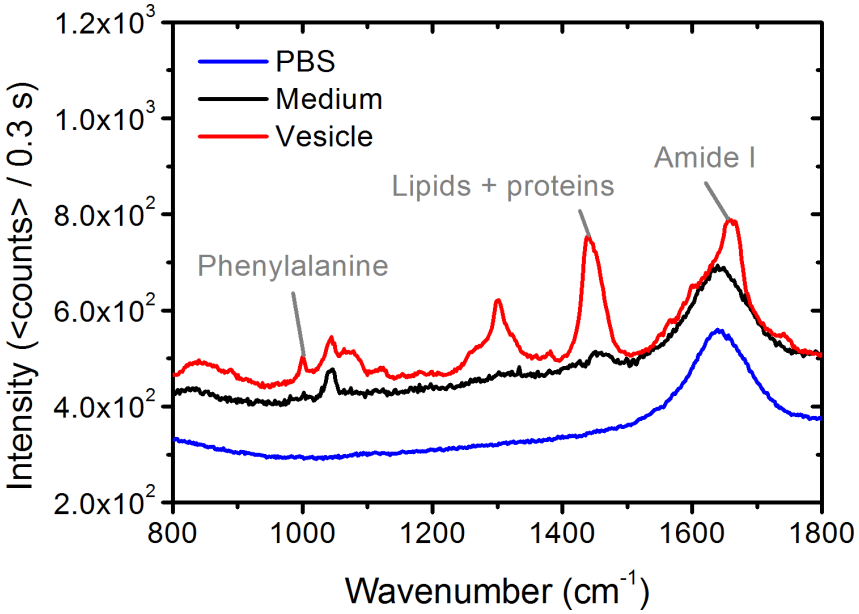

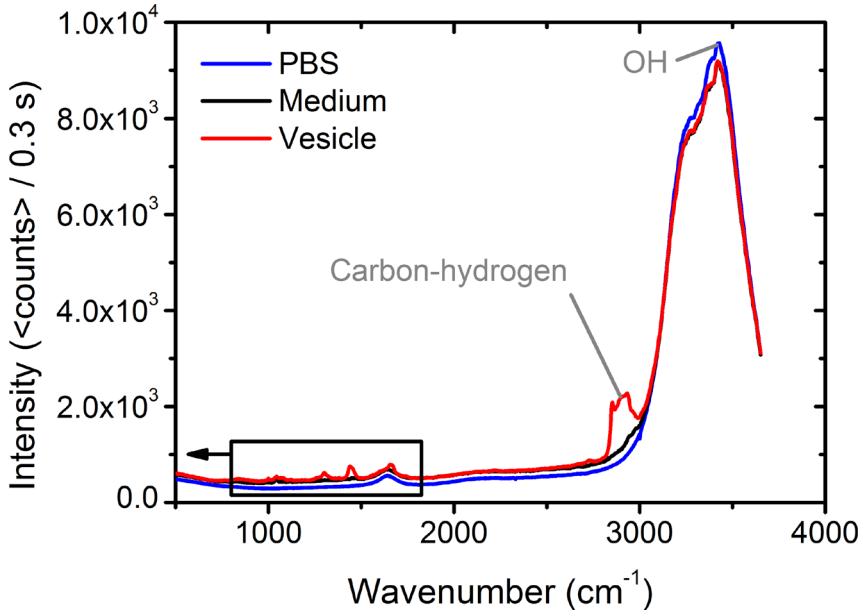

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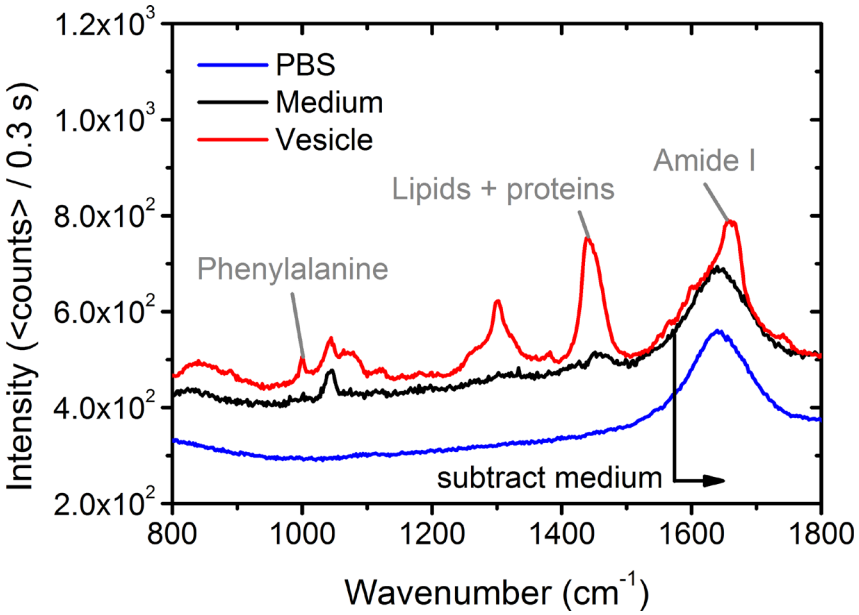
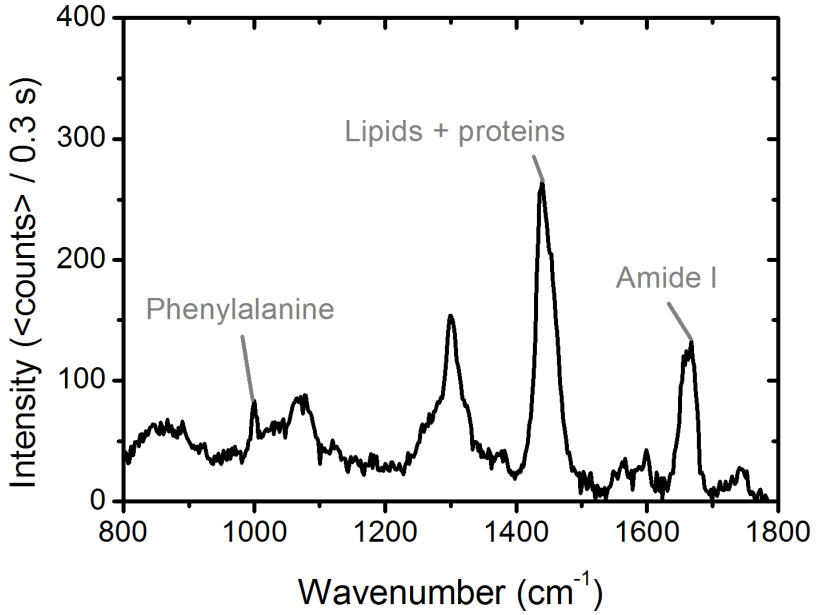
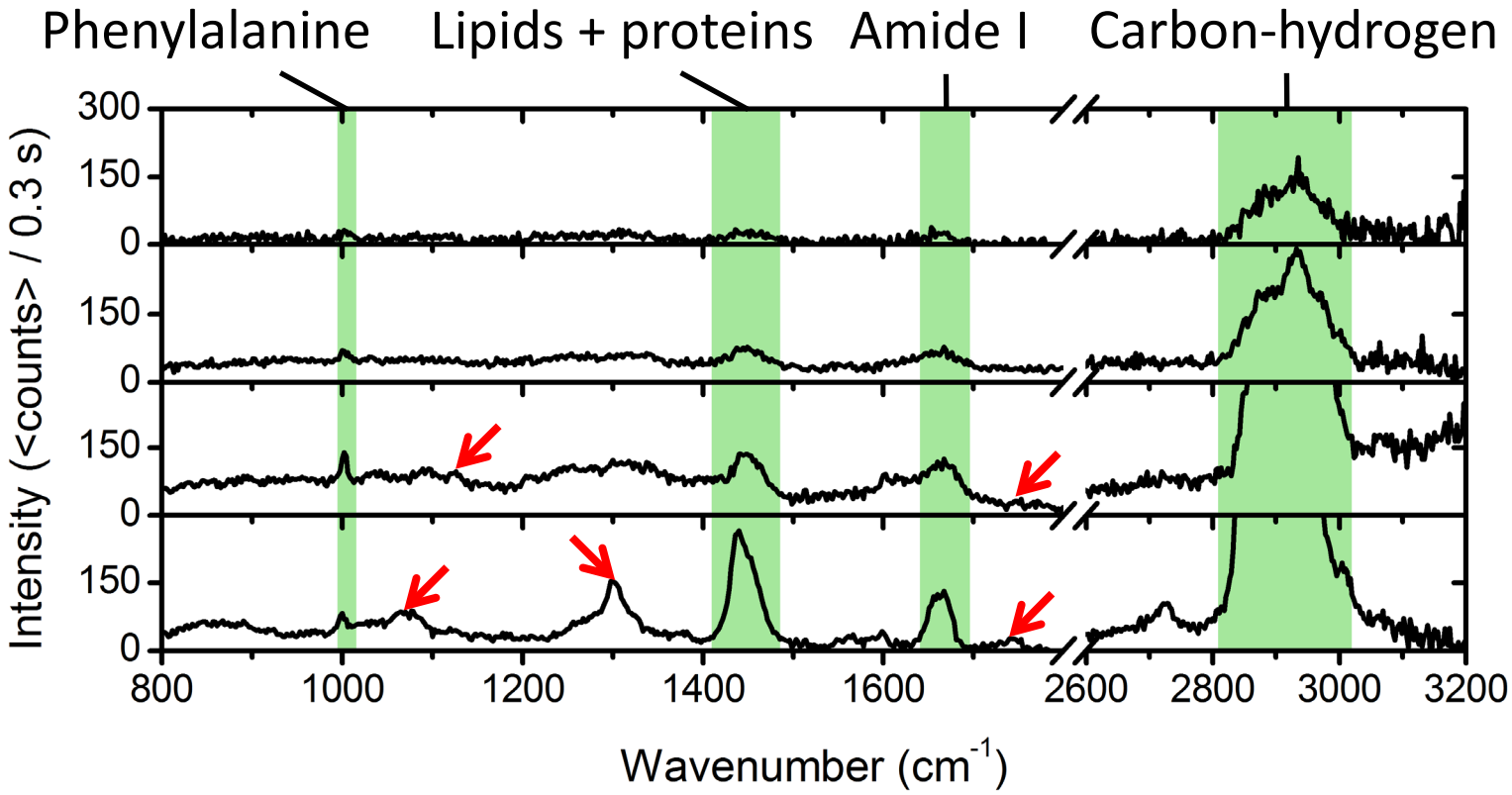


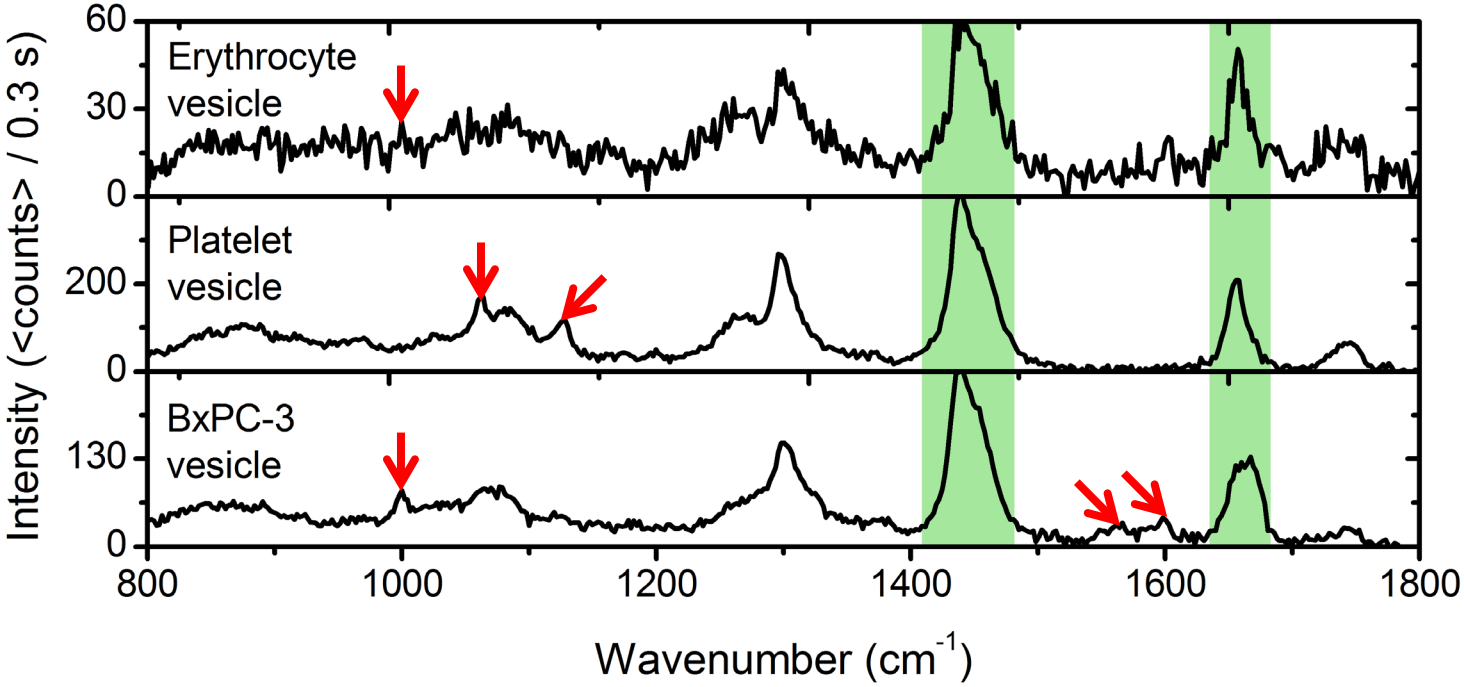


Results – Single BxPC-3 vesicle trapping



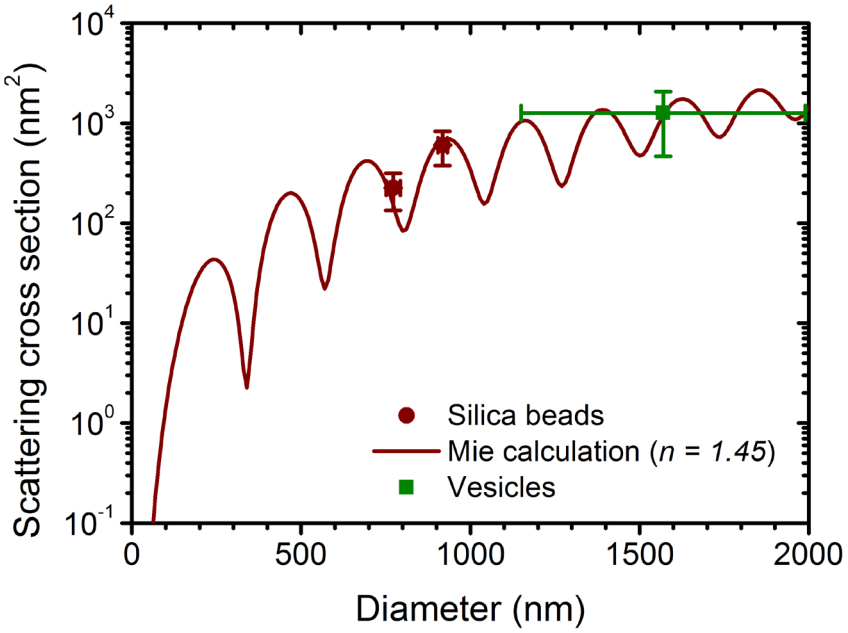
- BxPC-3 vesicles have different Raman spectra
- composition of vesicles from one cell type differs

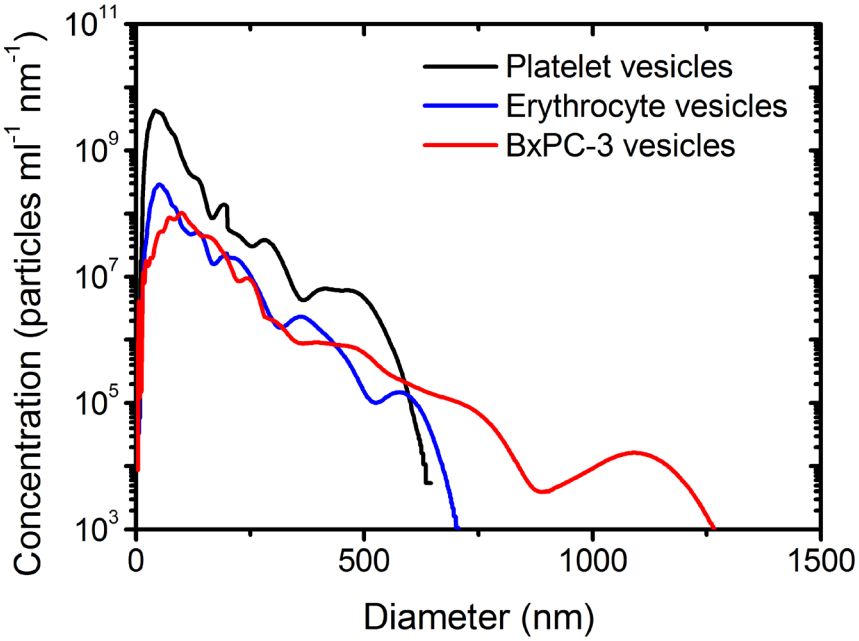
Results – Single vesicle trapping comparison



- Differences obtained! However,
  - only 12 single vesicles were trapped
  - low signal-to-noise ratio
  - vesicle size is unknown

Estimation of vesicle size by elastic scattering



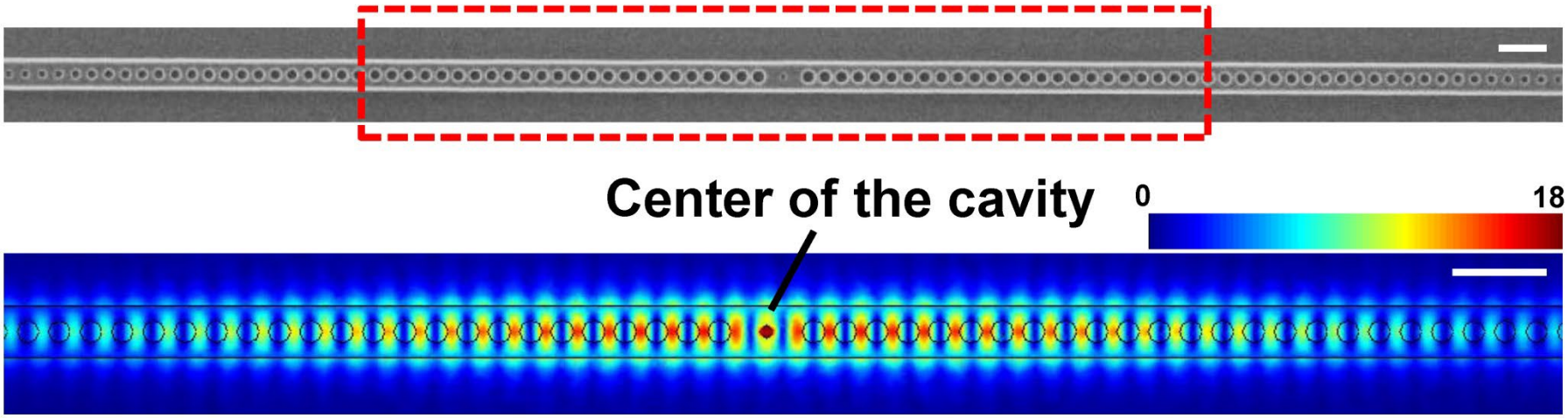


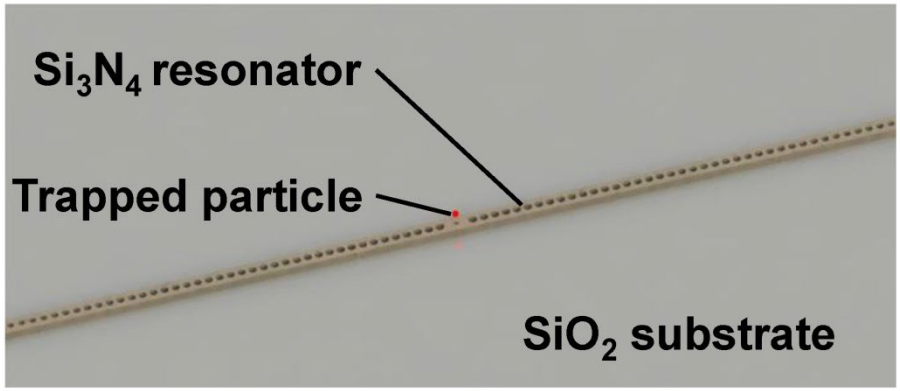
- vesicle diameter > 1 µm

Conclusion and discussion

- measured Raman spectrum of single vesicles
  - composition of vesicles from one cell type differs
  - more measurements on single vesicles required
- diameter of trapped vesicles > 1 µm
  - trapping of smaller vesicles required

Outlook: use NanoTweezer to trap vesicles

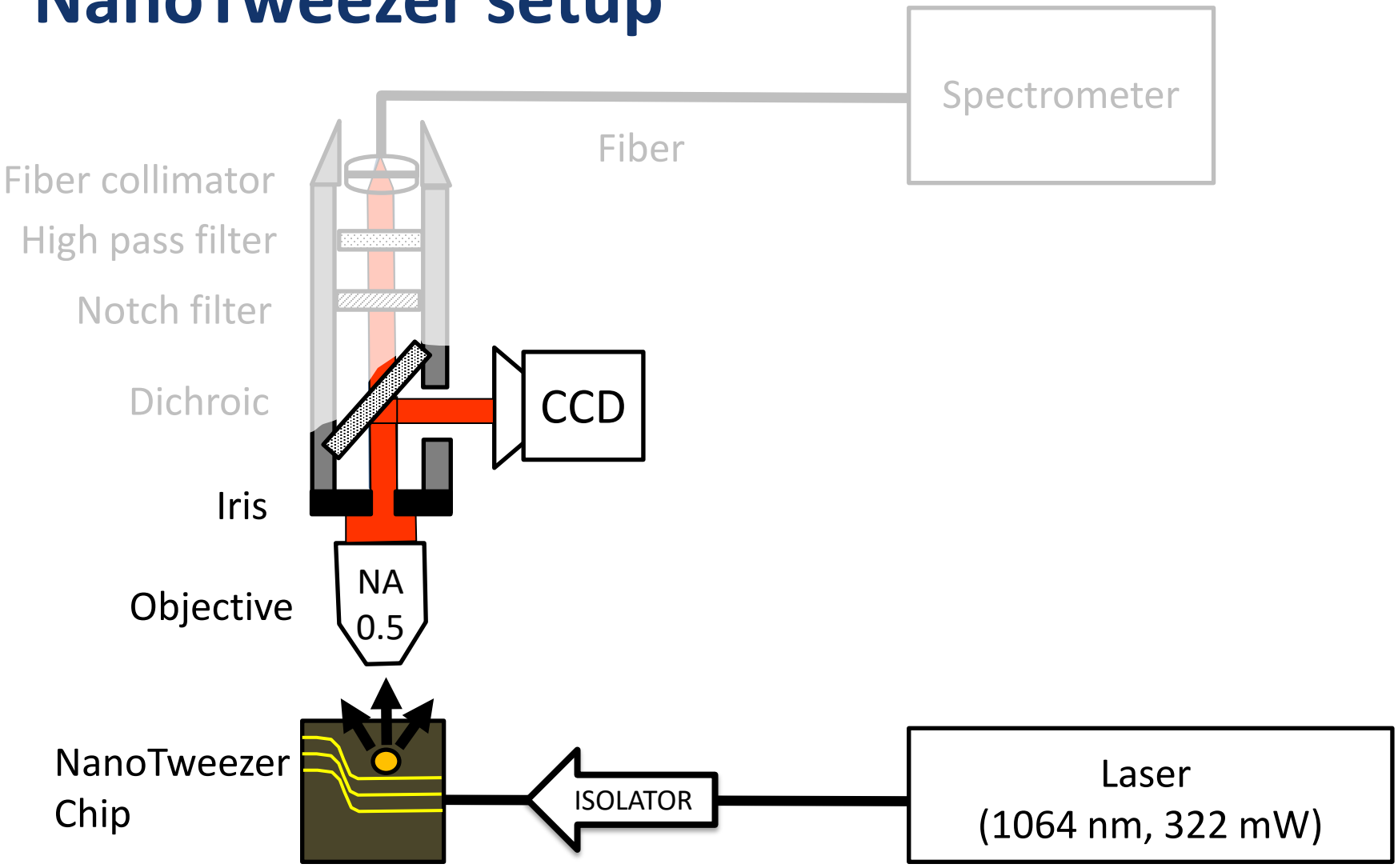




images adopted from Optofluidics (booth #4035) and Erickson et al., Lab Chip (2011)

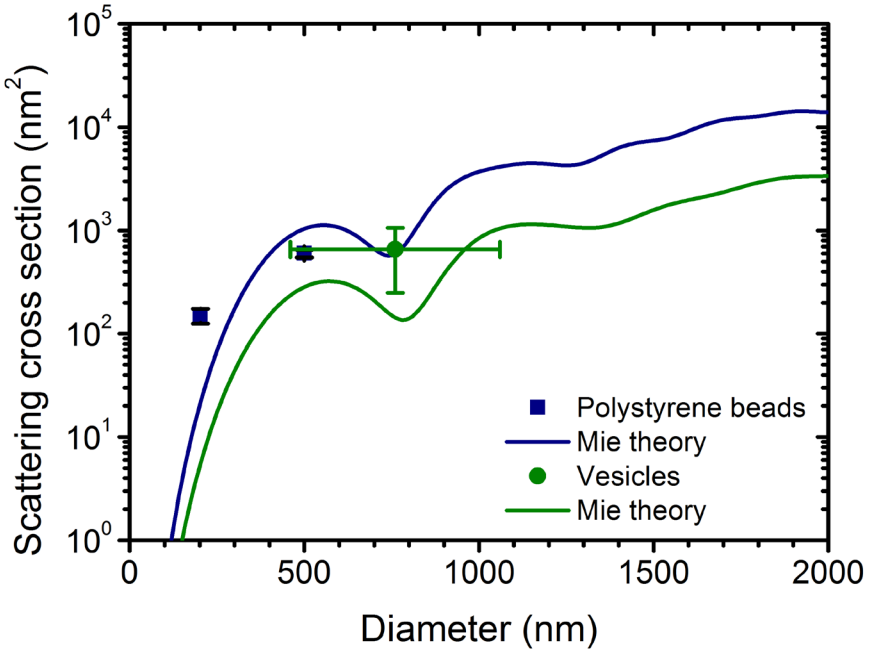
Optofluidics NanoTweezer

NanoTweezer setup



Trapping urinary vesicles with a NanoTweezer

Size estimation of vesicles trapped by the NanoTweezer



Outlook: Raman spectroscopy of single tumor vesicles with Nanotweezer