Label-free identification of single cell-derived vesicles by Raman microspectroscopy

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Auguste Sturk[2], Ton van Leeuwen[1],
and Rienk Nieuwland[2]
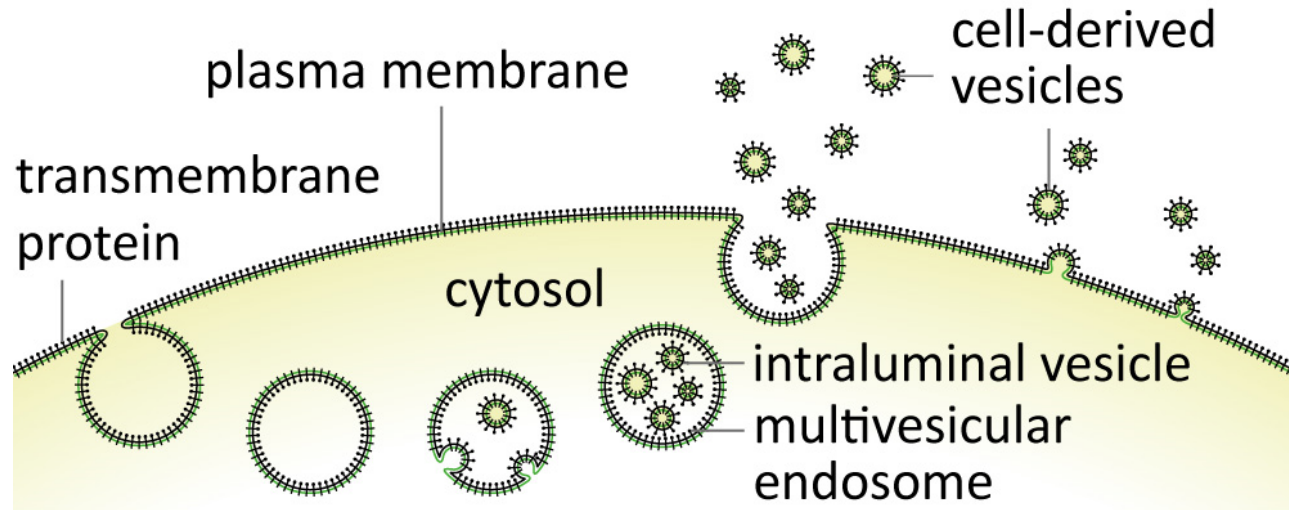
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Introduction – motive

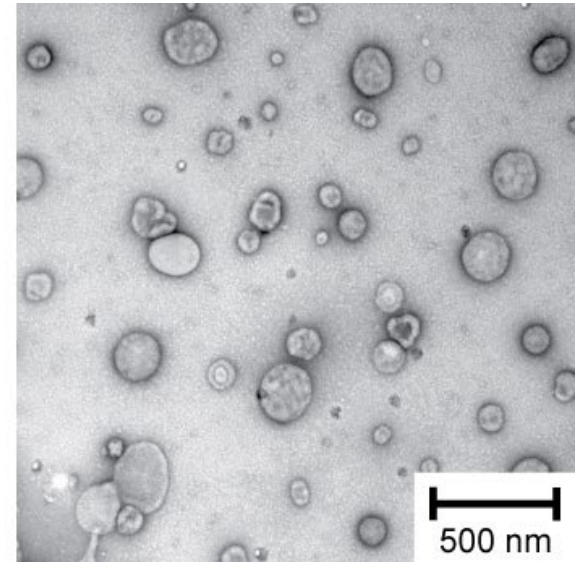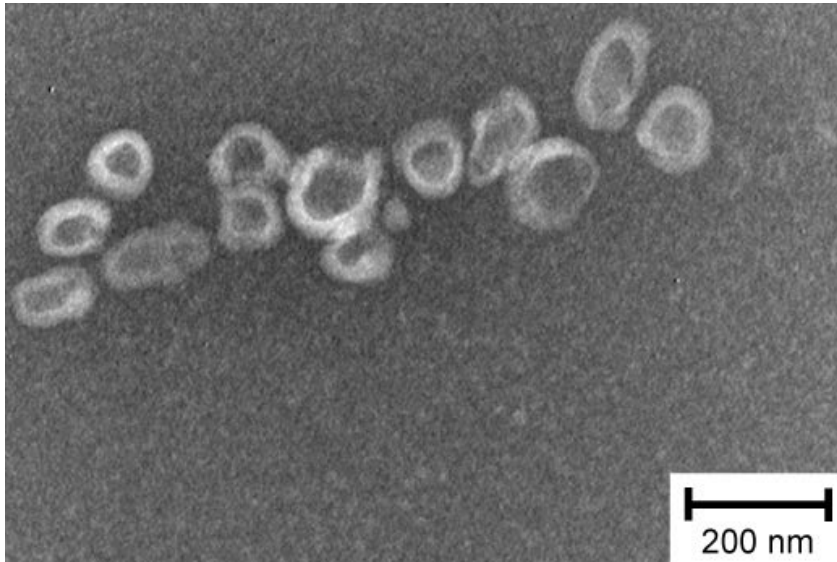figure adapted from Coumans et al., Clin Cancer Res (2012)
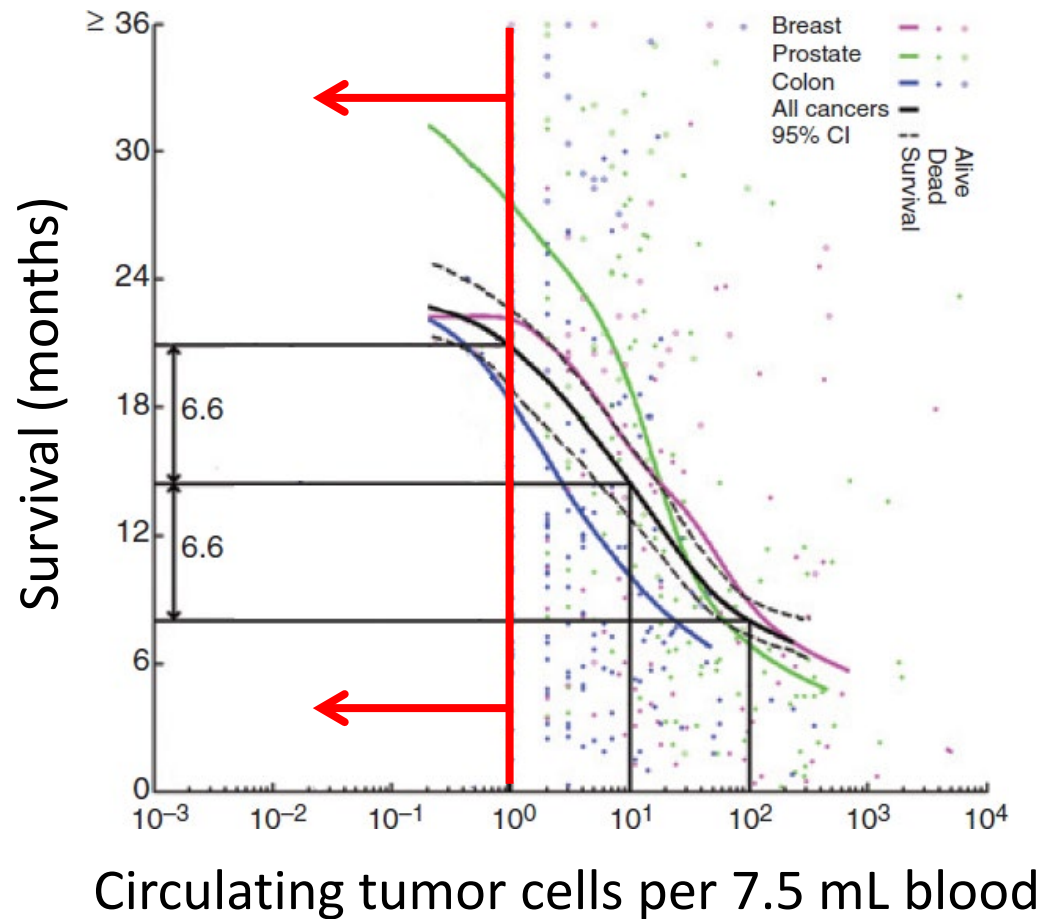
Introduction – cell-derived vesicles

- (tumor) cells release vesicles:
  spherical particles with phospholipid bilayer
- specialized functions

Introduction – cell-derived vesicles

- detection and isolation cumbersome
  - small size (< 500 nm)
  - low refractive index (~1.4)
  - fluorescent antibody labeling involves practical problems
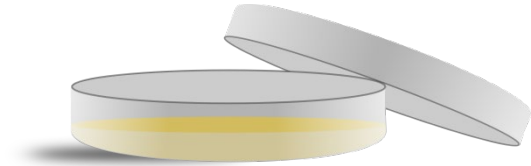
# Goal

erythrocyte vesicles

platelet vesicles

Human pancreatic adenocarcinoma (BxPC-3) vesicles

- distinguish tumor (BxPC-3) vesicles from erythrocyte and platelet vesicles directly in solution *without labeling* by Raman microspectroscopy

Method 1 – Single particle tracking

microscope
objective
NA = 0.4

vesicles in
solution

laser beam

metalized
surface

glass
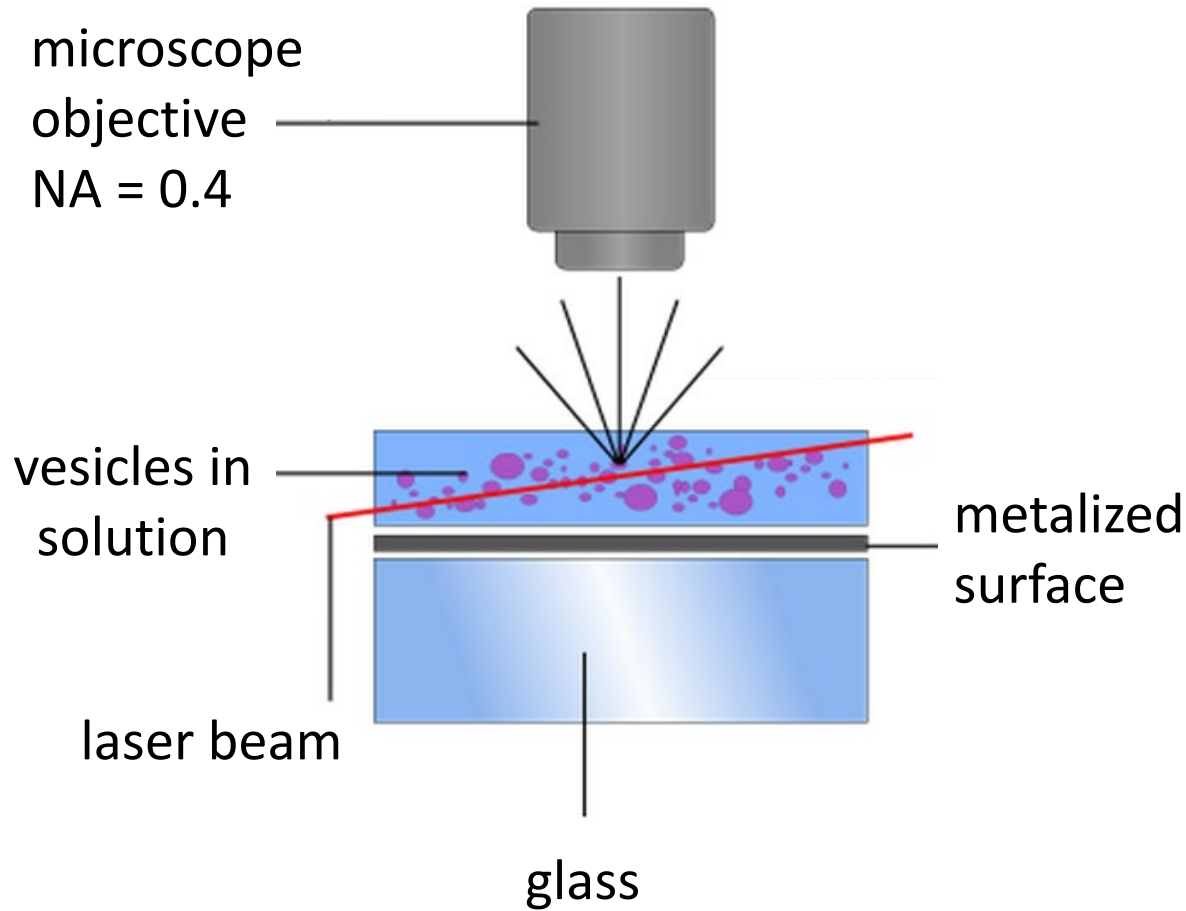
$$\langle x^2 \rangle = \frac{2k_B T \tau}{3\pi\eta d}$$

x: displacement
k_B: Boltzmann constant
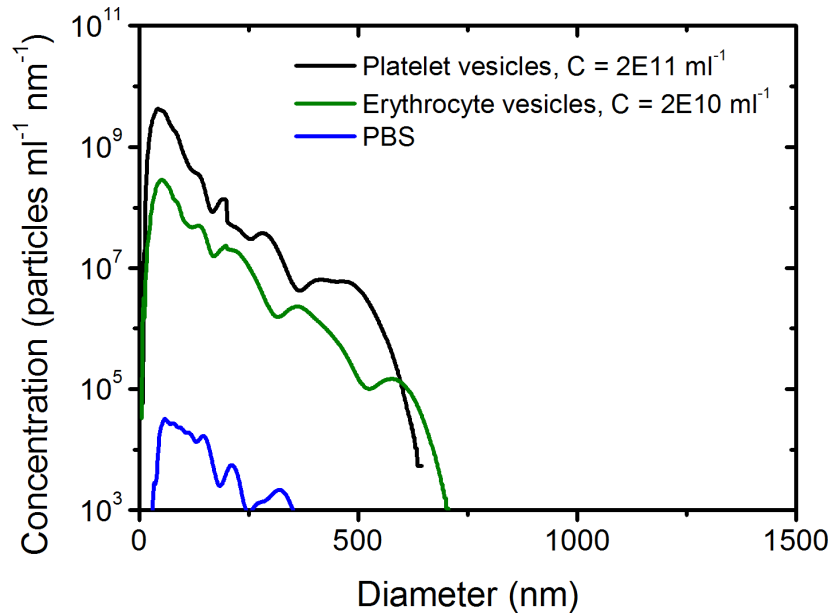T: temperature
t: observation time
η: viscosity
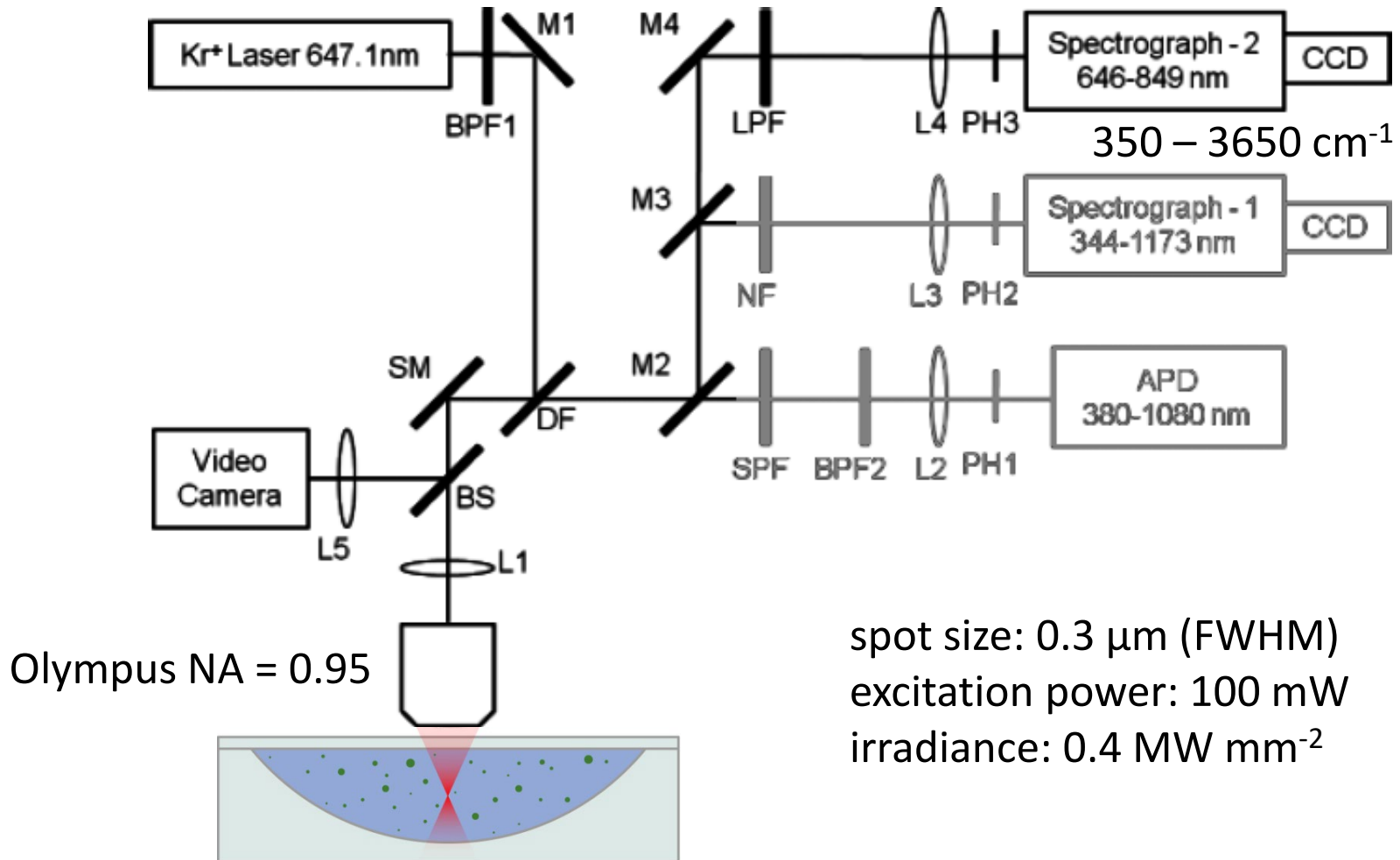d: diameter

Method 1 – Single particle tracking

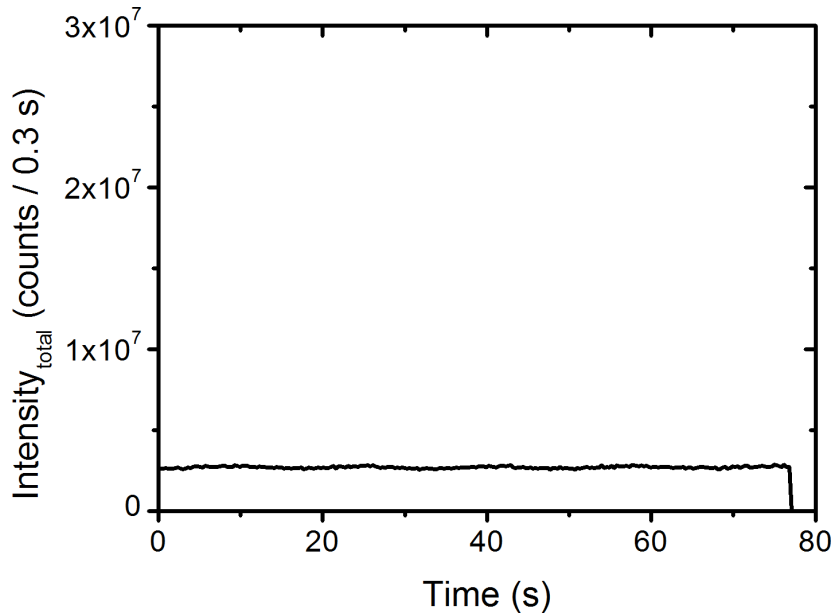- single particle tracking applied to determine size and concentration of vesicles
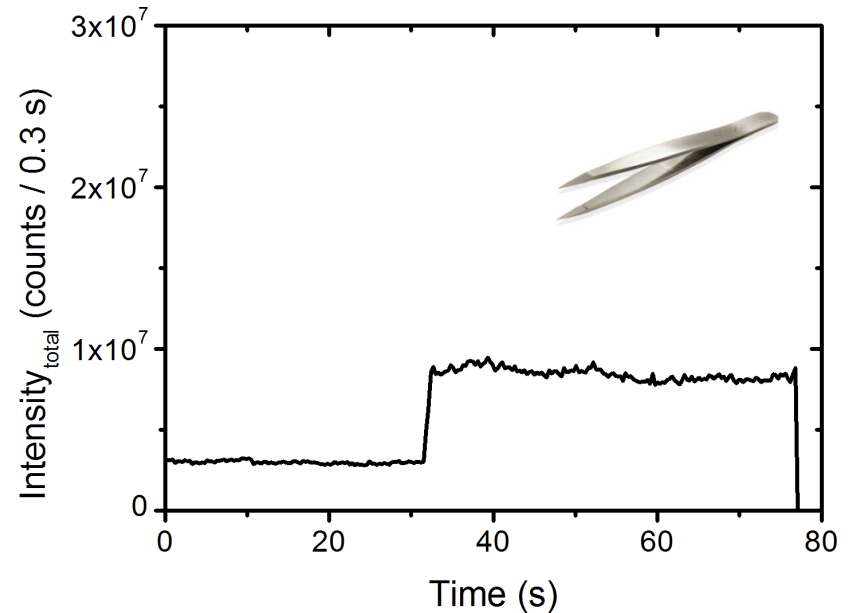
Results – Single particle tracking
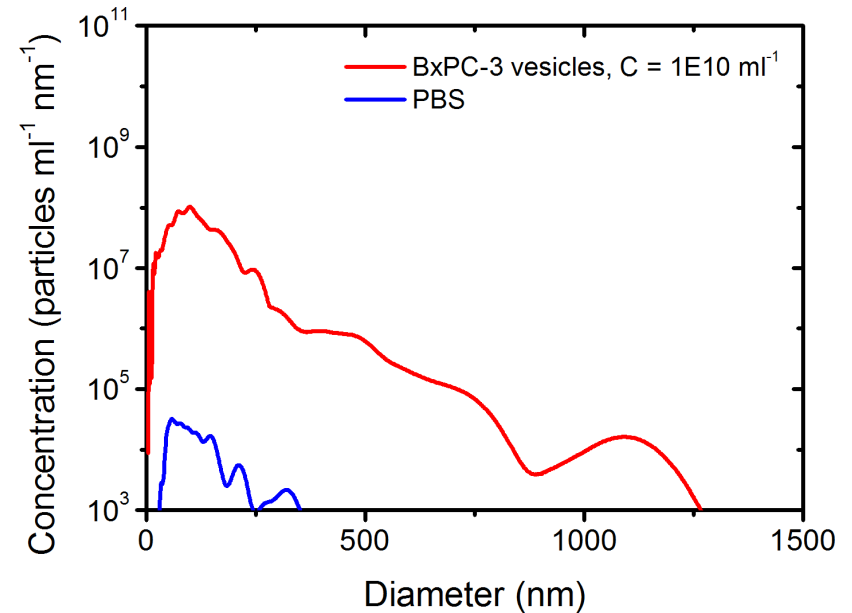
Method 2 - Raman microspectroscopy

$350 - 3650$ cm^{-1}

Olympus NA = 0.95

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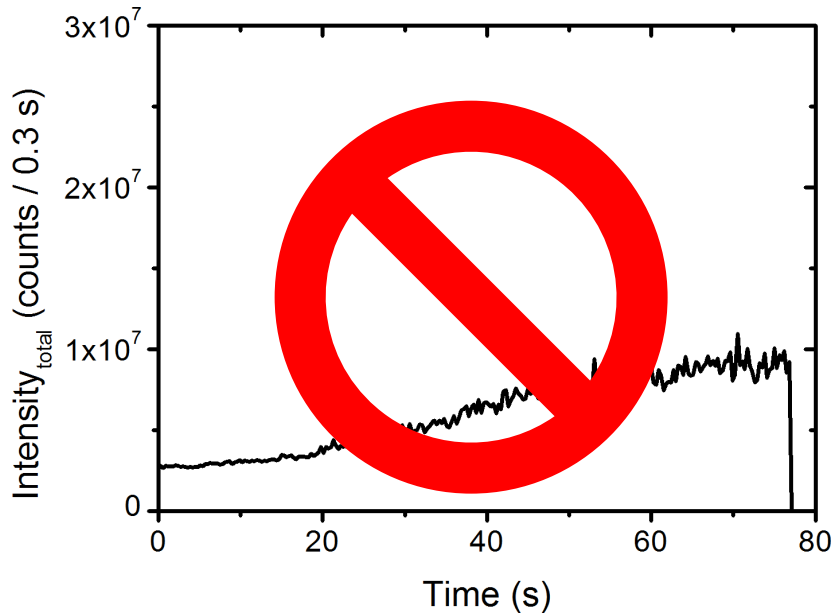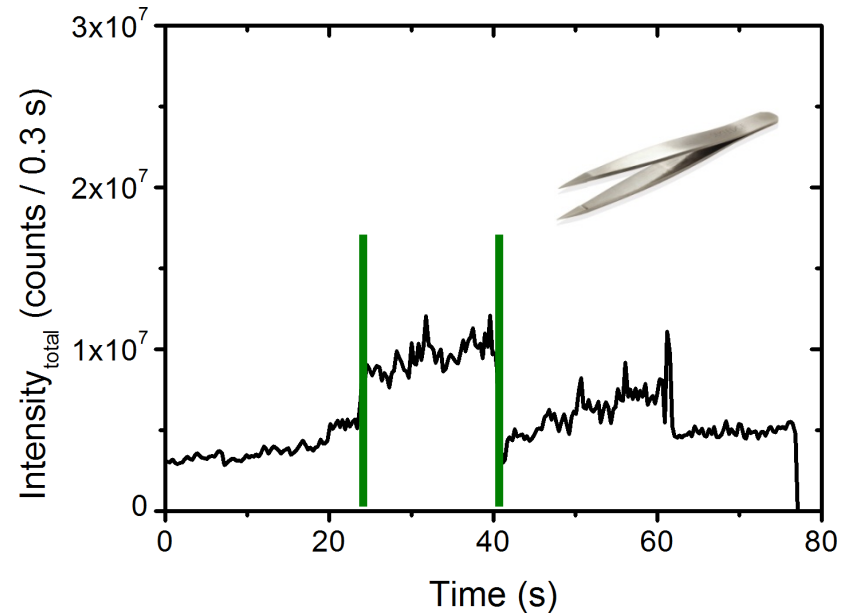
Methods - Raman microspectroscopy
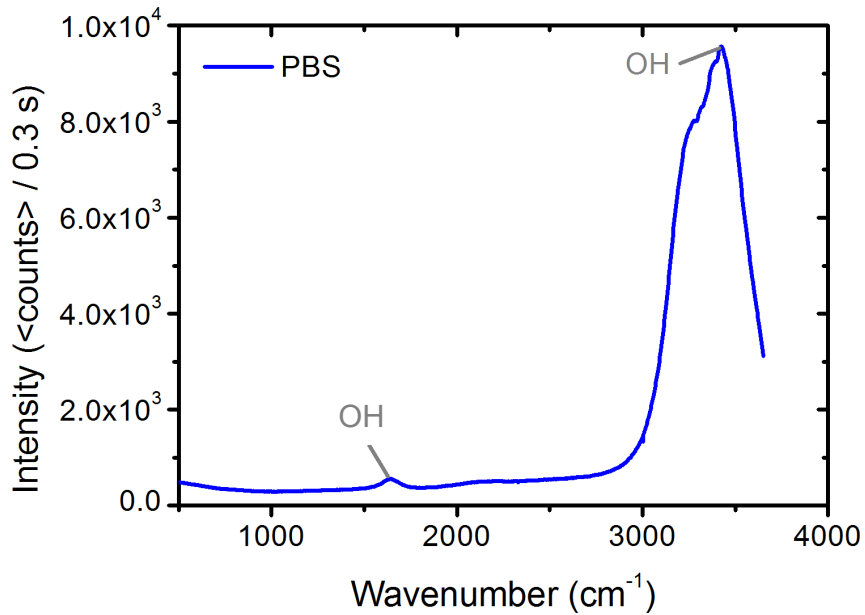
PBS

Tumor (BxPC-3) vesicle

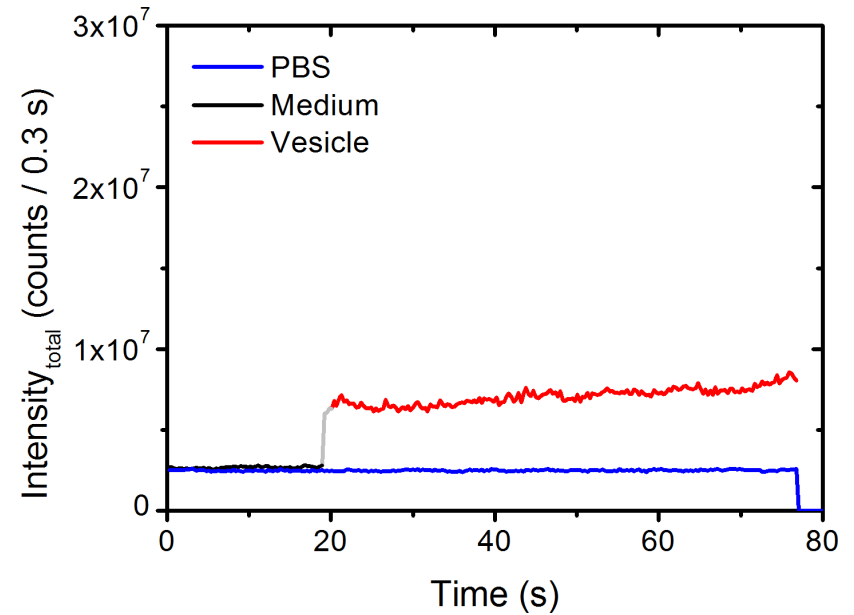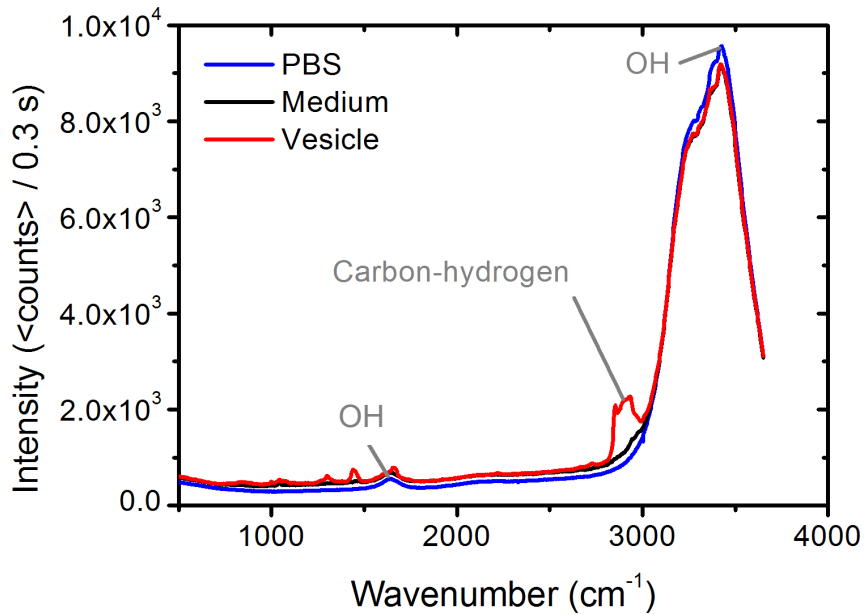# Methods - Raman microspectroscopy
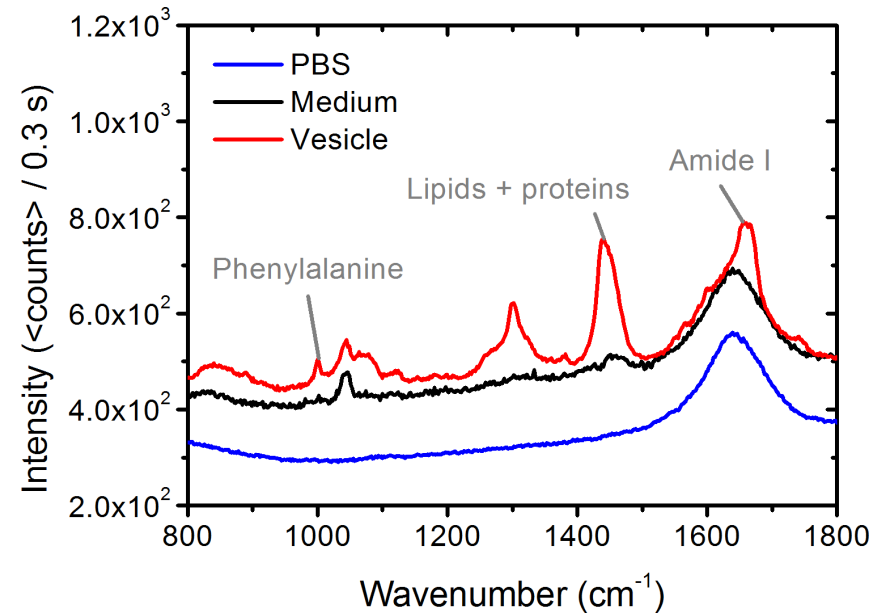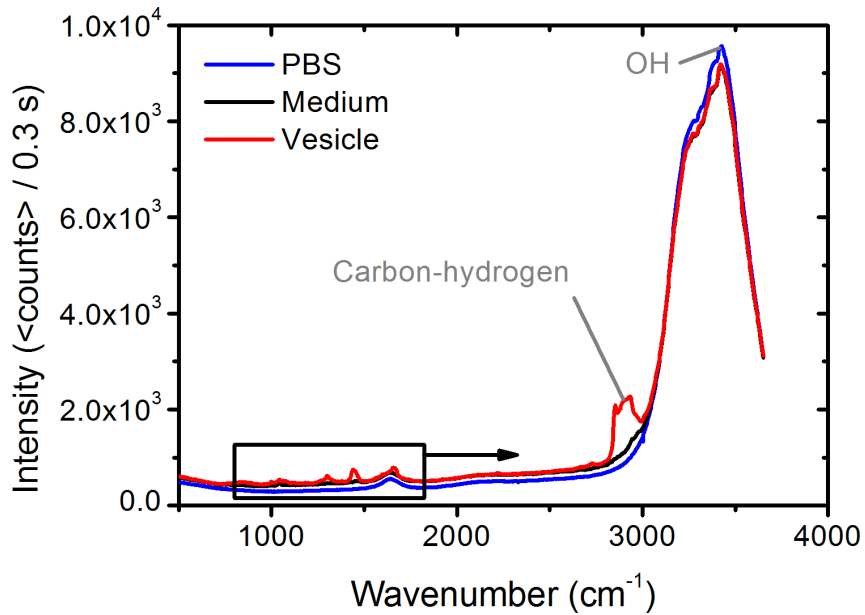

Erythrocyte vesicles

Erythrocyte vesicles
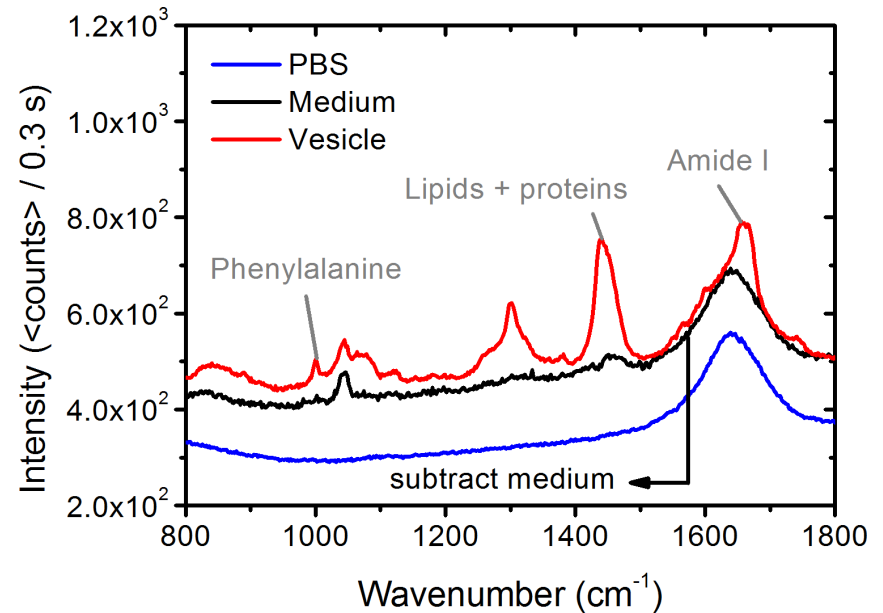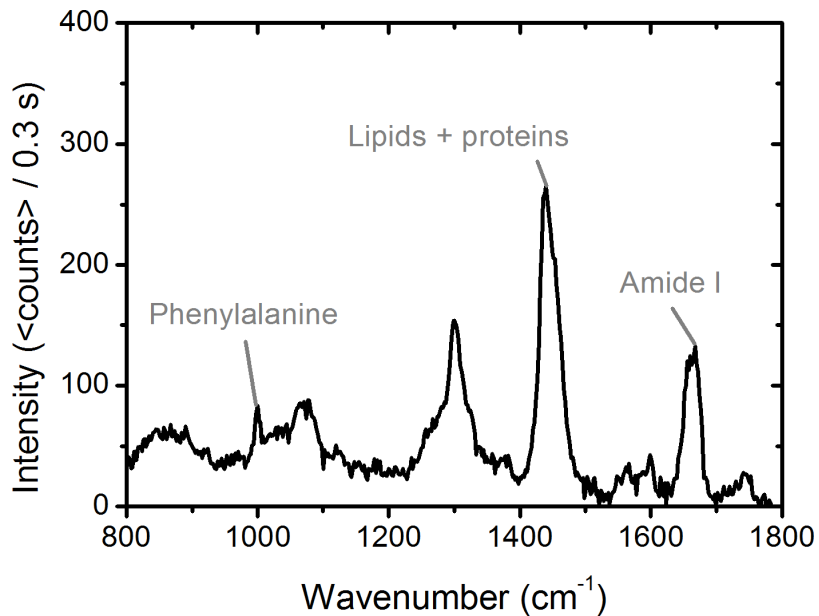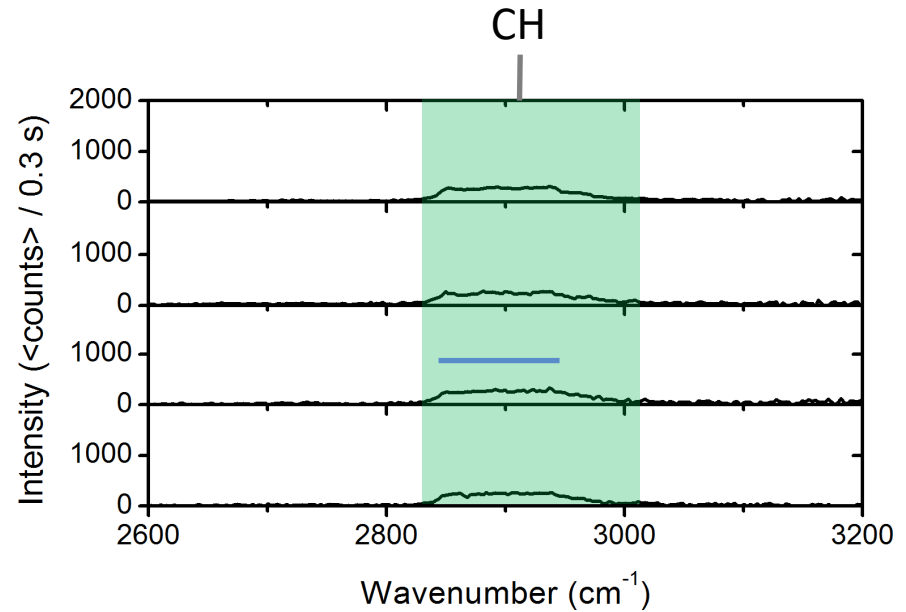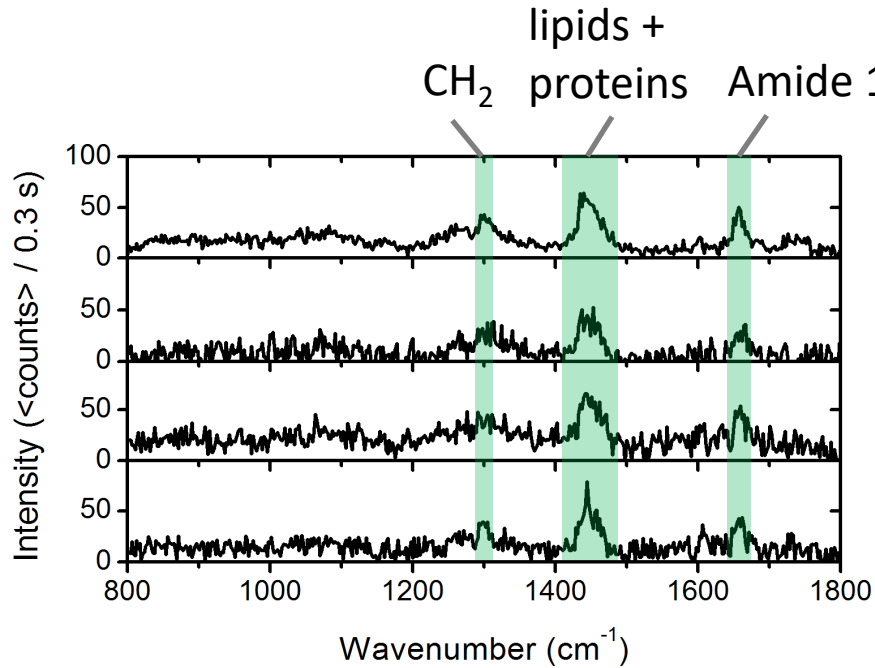
Methods - Raman spectrum of PBS
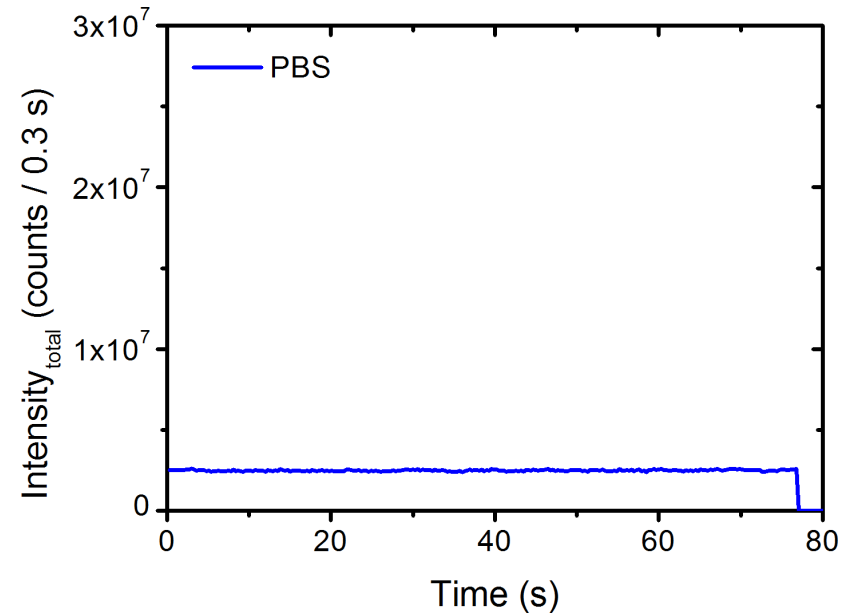
Methods - Raman spectrum of *single* tumor (BxPC-3) vesicle

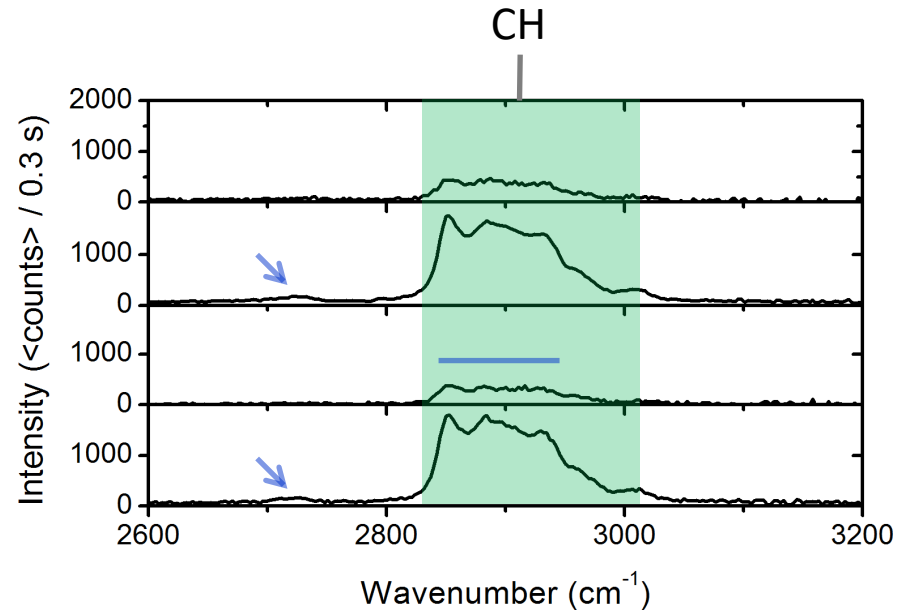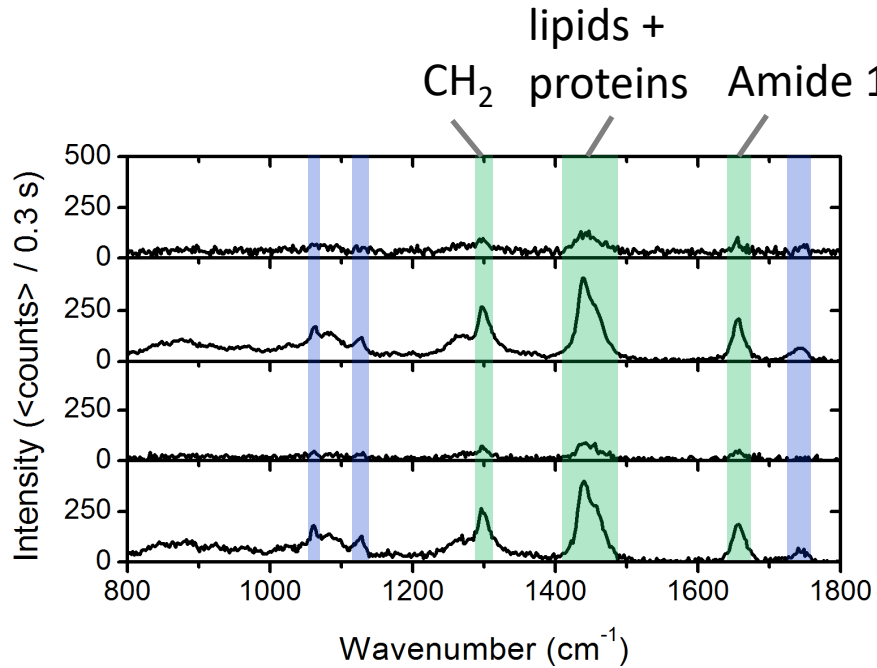# Methods - Raman spectrum of *single* tumor (BxPC-3) vesicle
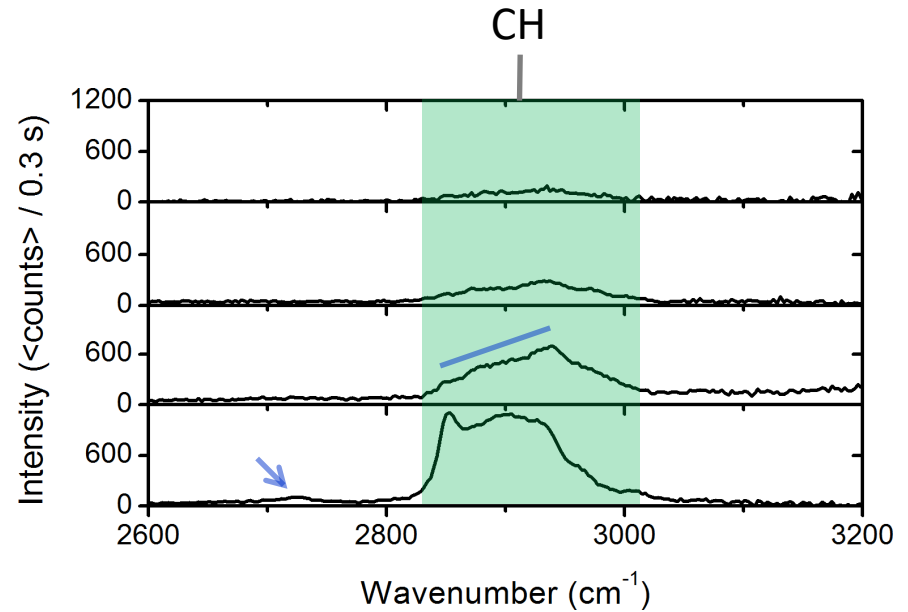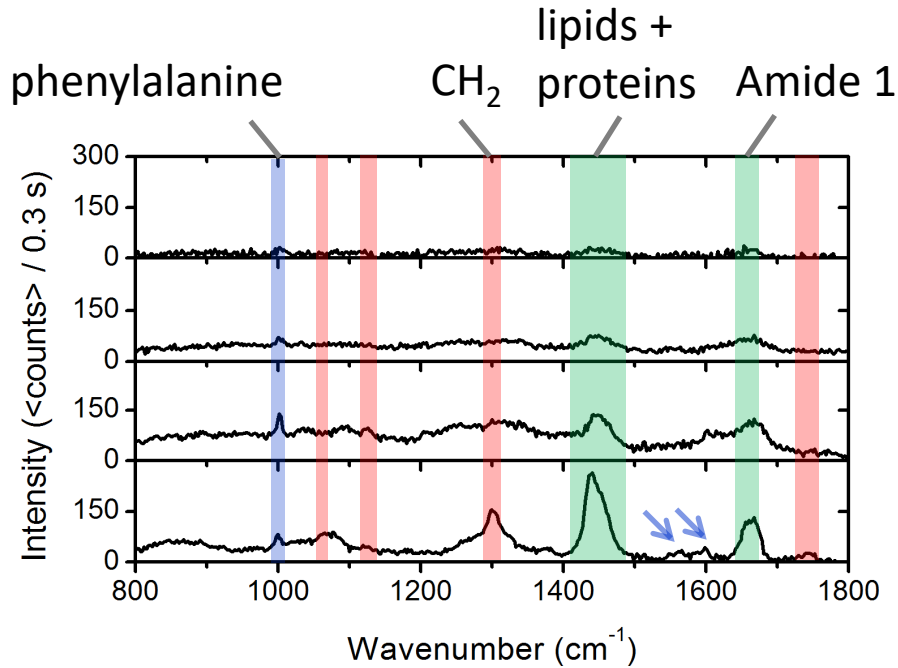
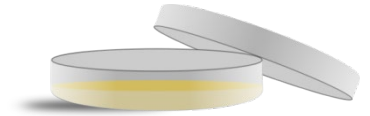# Methods - Raman spectrum of *single* tumor (BxPC-3) vesicle

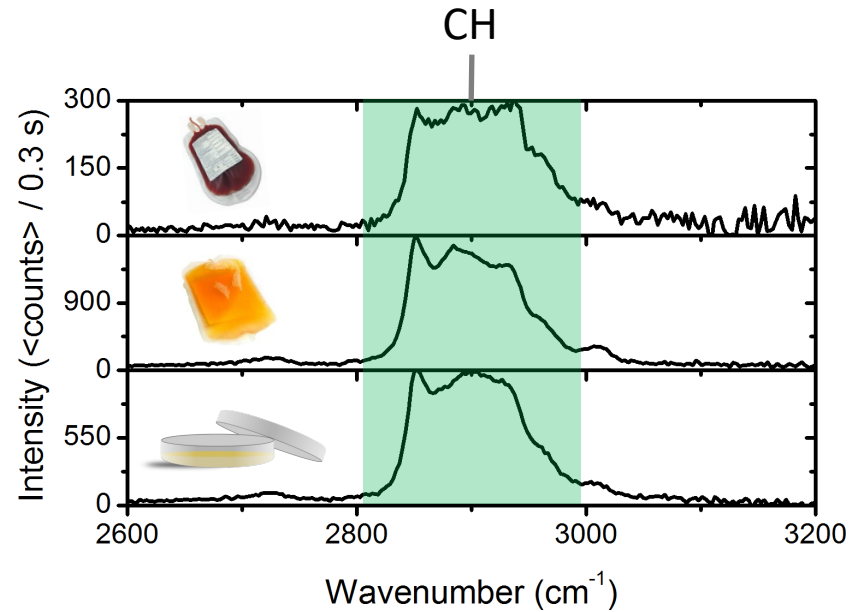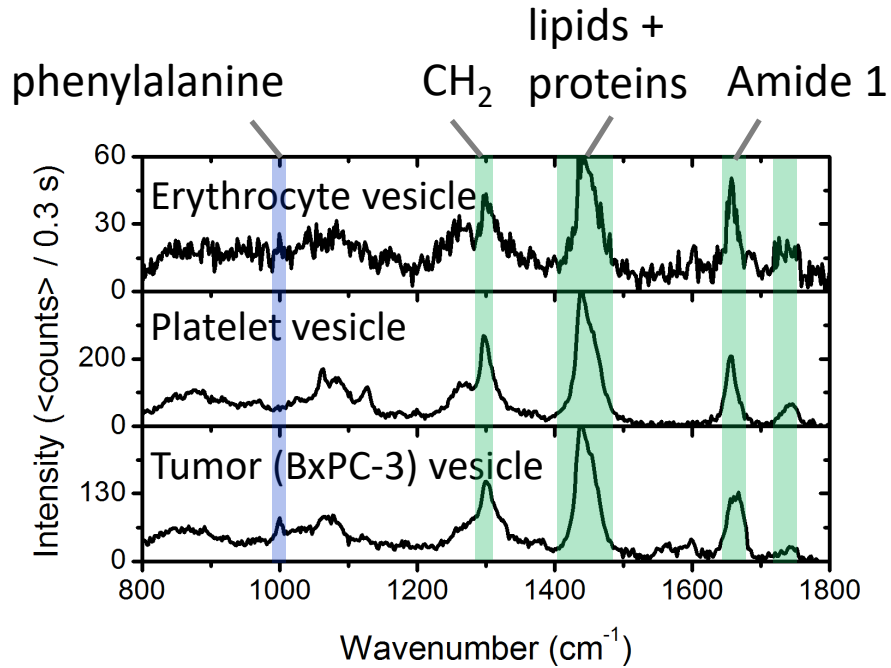# Results - Raman spectrum of *single* erythrocyte vesicles

Results - Raman spectrum of *single* platelet vesicles

Results - Raman spectrum of *single* BxPC-3 vesicles

Results - summary

Conclusion and discussion

- distinguished single tumor (BxPC-3) vesicles from erythrocyte and platelet vesicles directly in solution *without labeling*

- improvements
  - simultaneous sizing by single particle tracking
  - optimize isolation

- develop high throughput system
  - microfluidics
  - multiple probes
  - Coherent anti-Stokes Raman spectroscopy

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