

Label-free distinction between platelet and erythrocyte microparticles by Raman microspectroscopy

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Disclosures for Edwin van der Pol

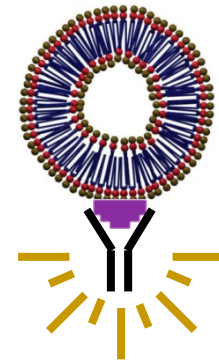
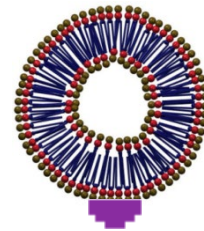
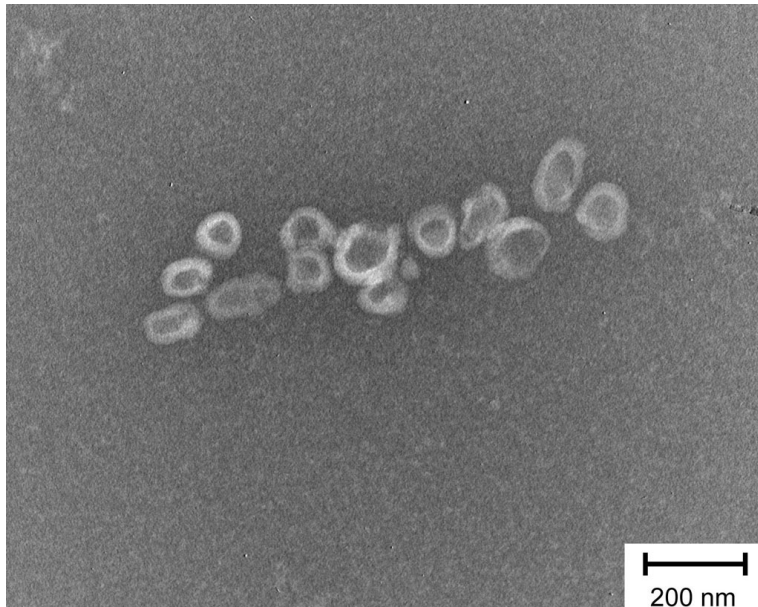
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Scientific Advisory Board	No relevant conflicts of interest to declare

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

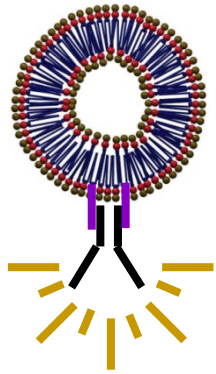
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Introduction

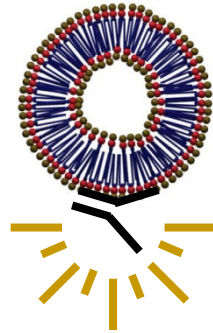


- body fluids contain cell-derived vesicles
- clinically relevant information
- fluorescent antibody labeling

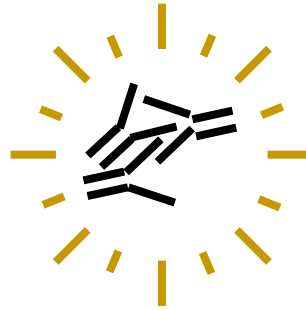
Problems fluorescent antibody labeling



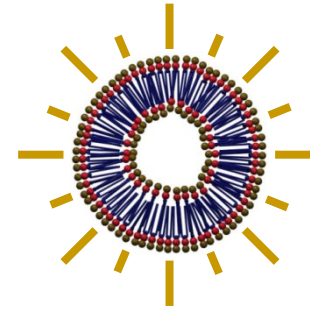
FC receptor
binding



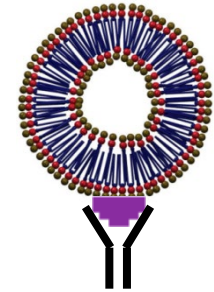
non-specific
binding



aggregates



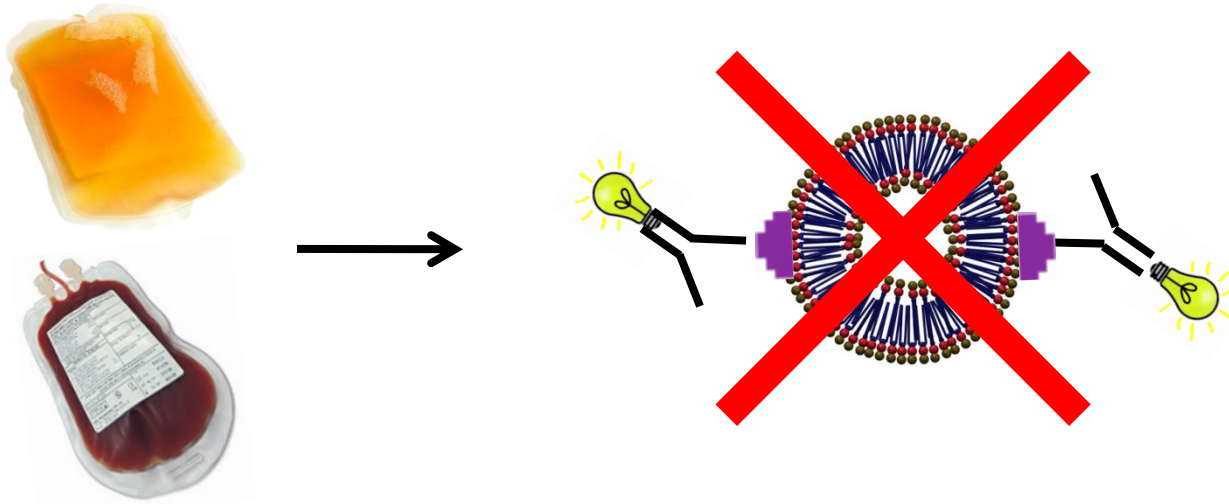
autofluorescence



photobleaching

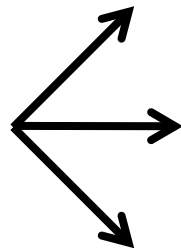
- practical problems¹
- laborious
 - controls, blanks
 - spectral overlap
- expensive

Goal



- distinguish *single* platelet from erythrocyte vesicles directly in solution without fluorescent antibody labeling

Methods

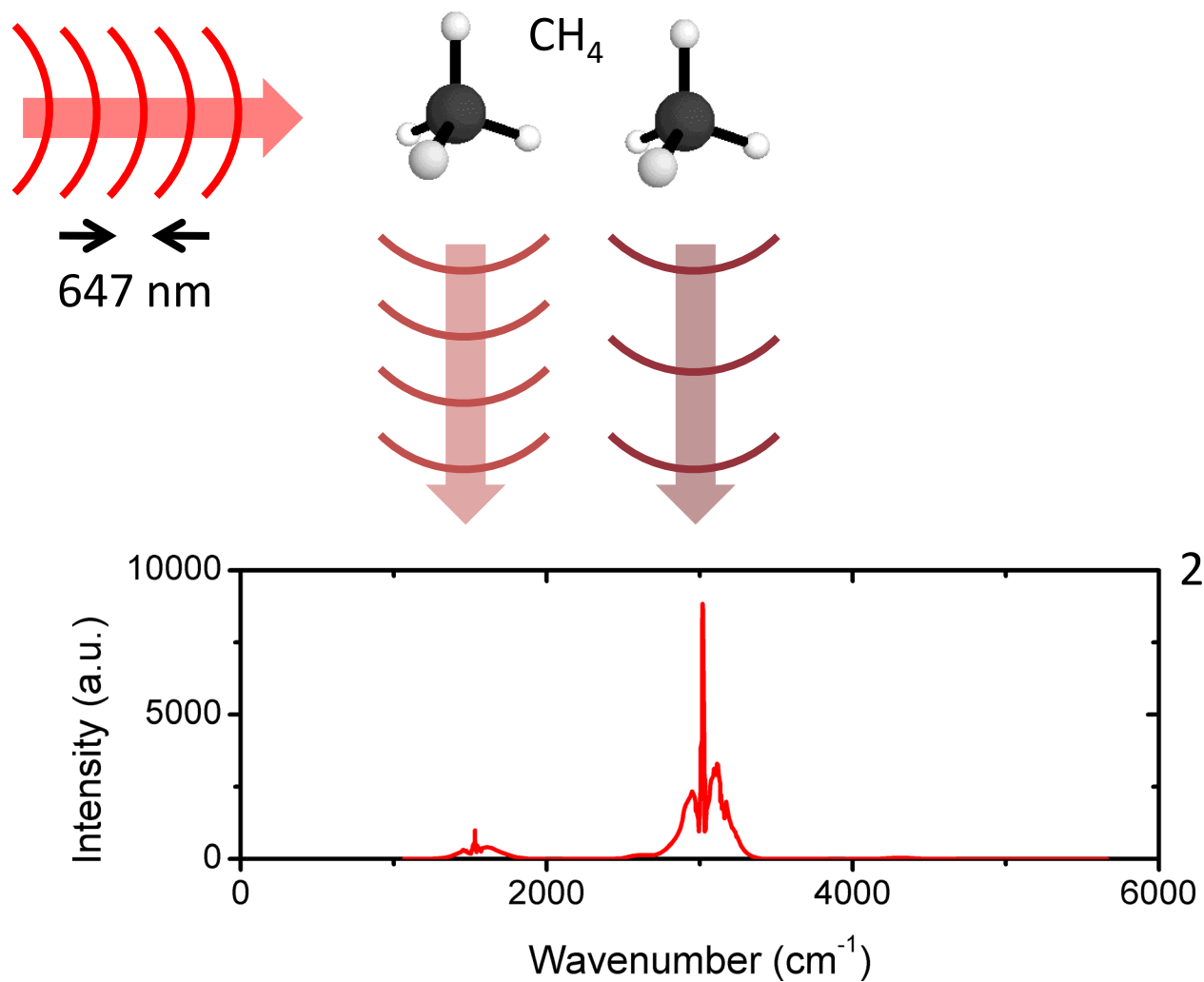


Flow cytometry

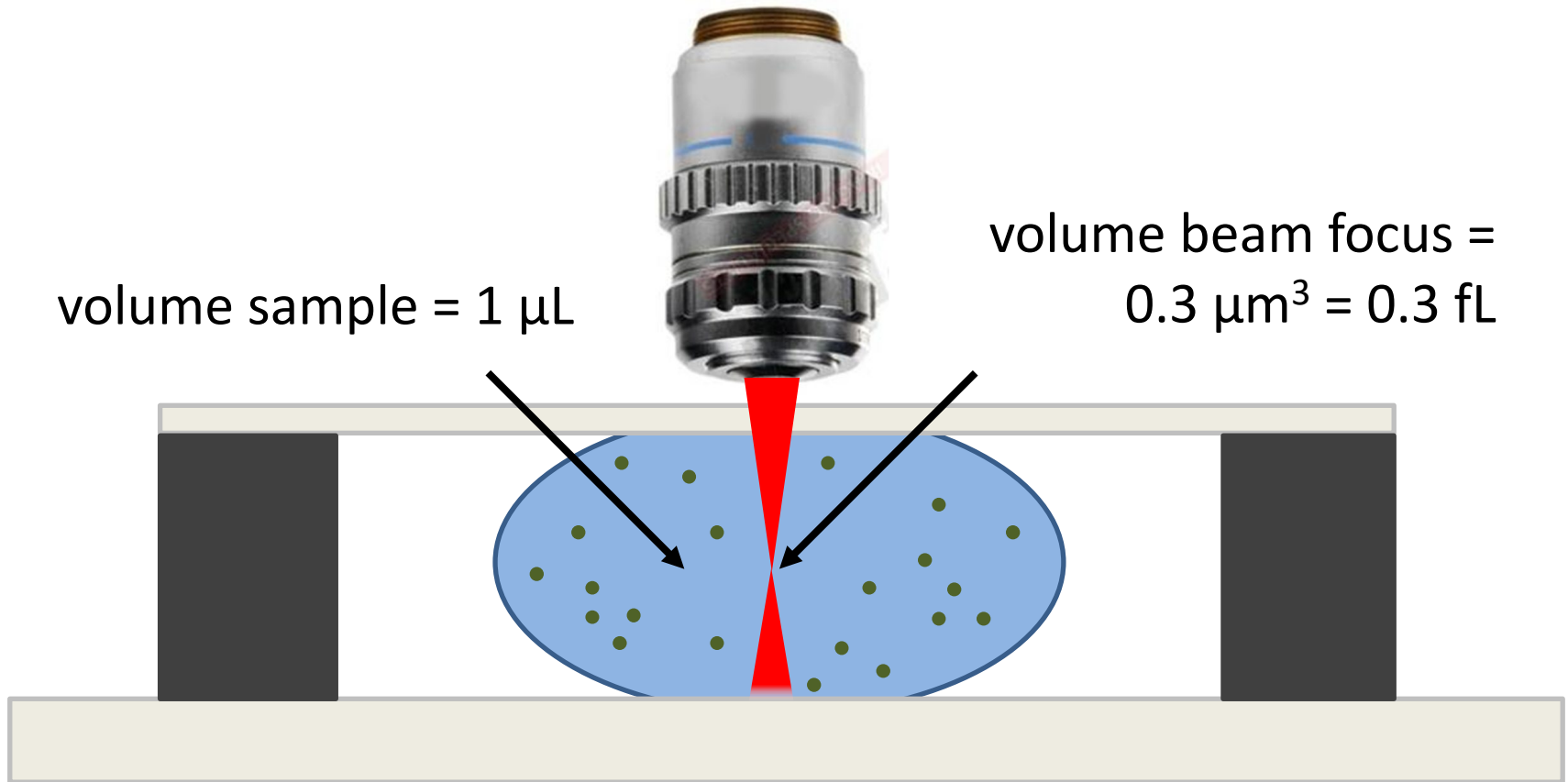
Transmission Electron
Microscopy

Raman
microspectroscopy

Raman spectroscopy



Raman microspectroscopy³



3. Puppels G.J. et al. Nature 1990; **347**: 301-3

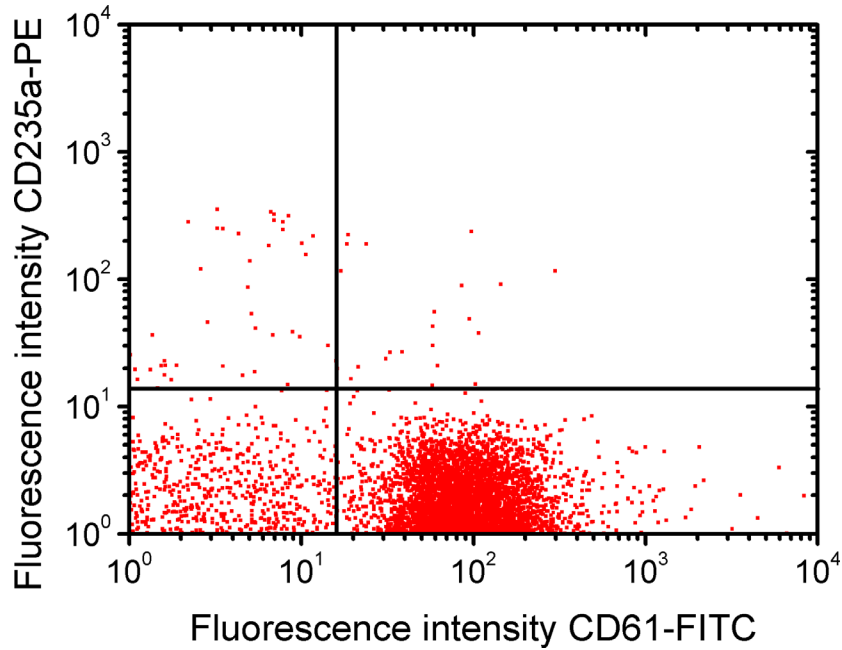
4. Uzunbajakava et al., Biophys. J. 2003; **84**: 3968-3981

Impression of Raman microspectrometer

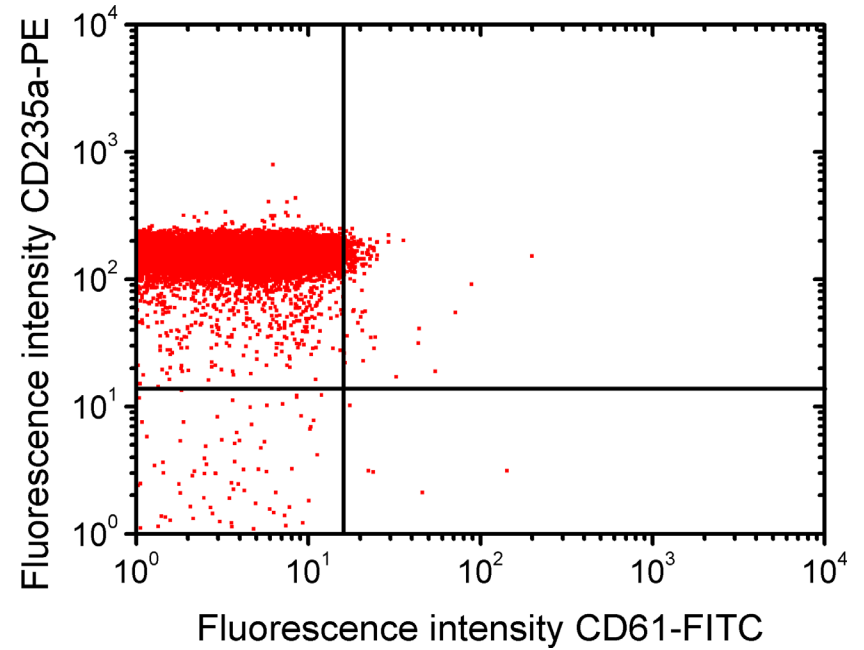


Image courtesy of Aufried Lenferink and Cees Otto, Medical Cell BioPhysics, University of Twente, Enschede, The Netherlands

Results flow cytometry

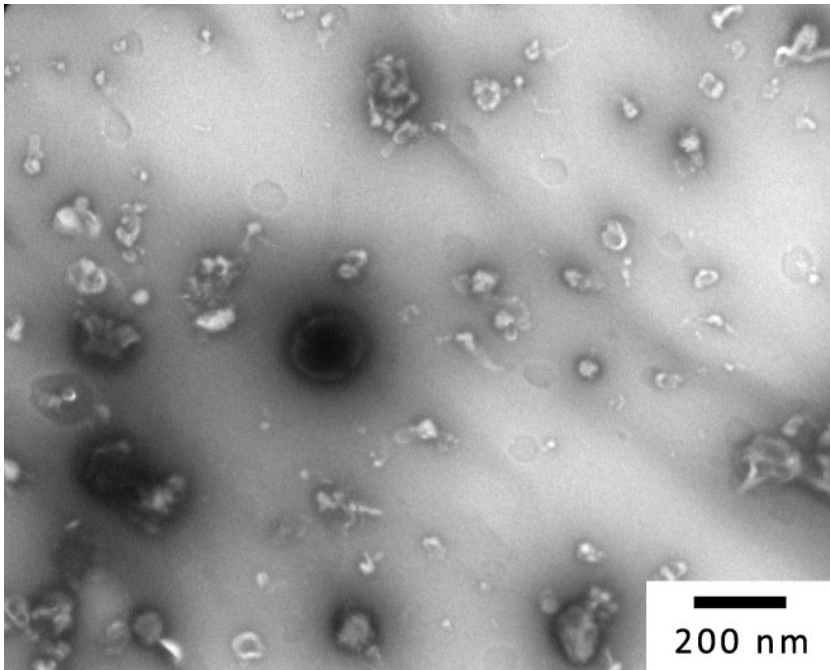


Platelet vesicles

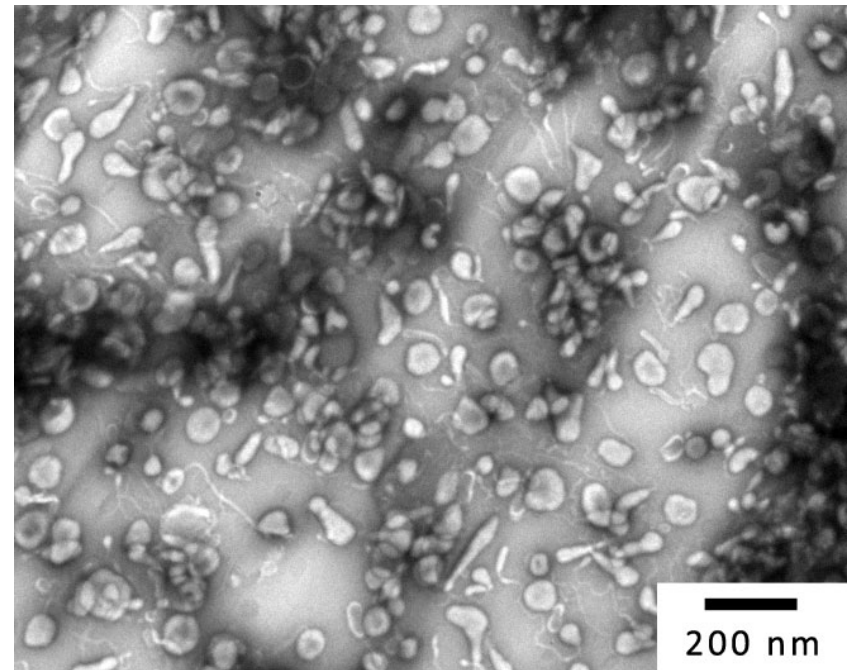


Erythrocyte vesicles

Results transmission electron microscopy

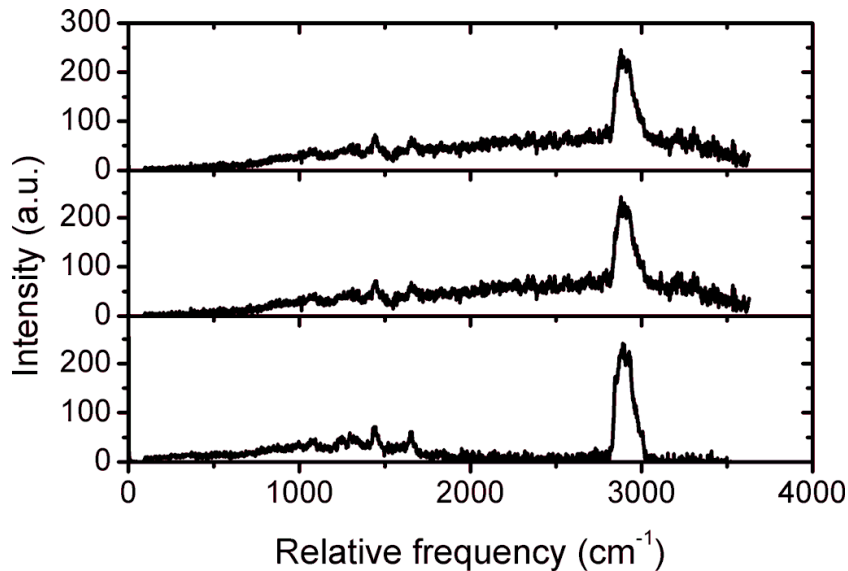


Platelet vesicles

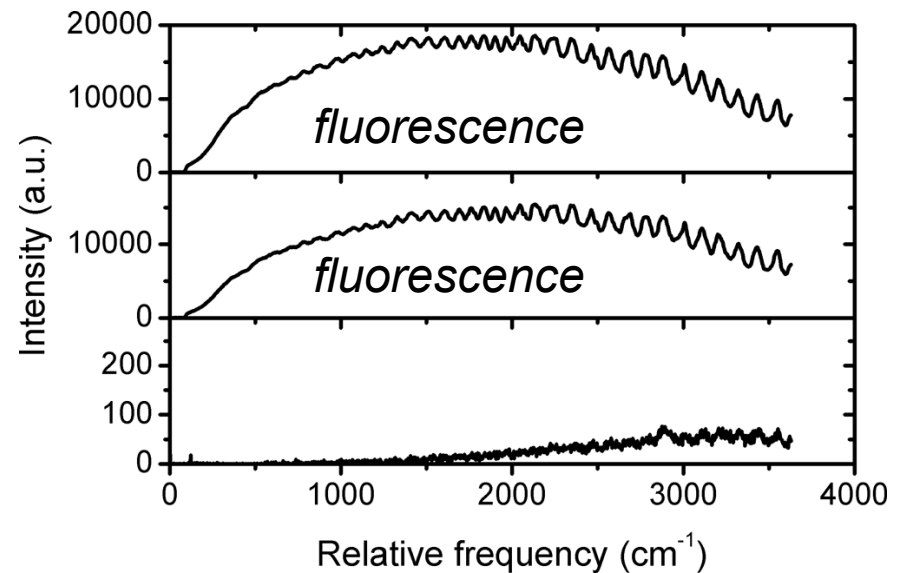


Erythrocyte vesicles

Results Raman microspectroscopy

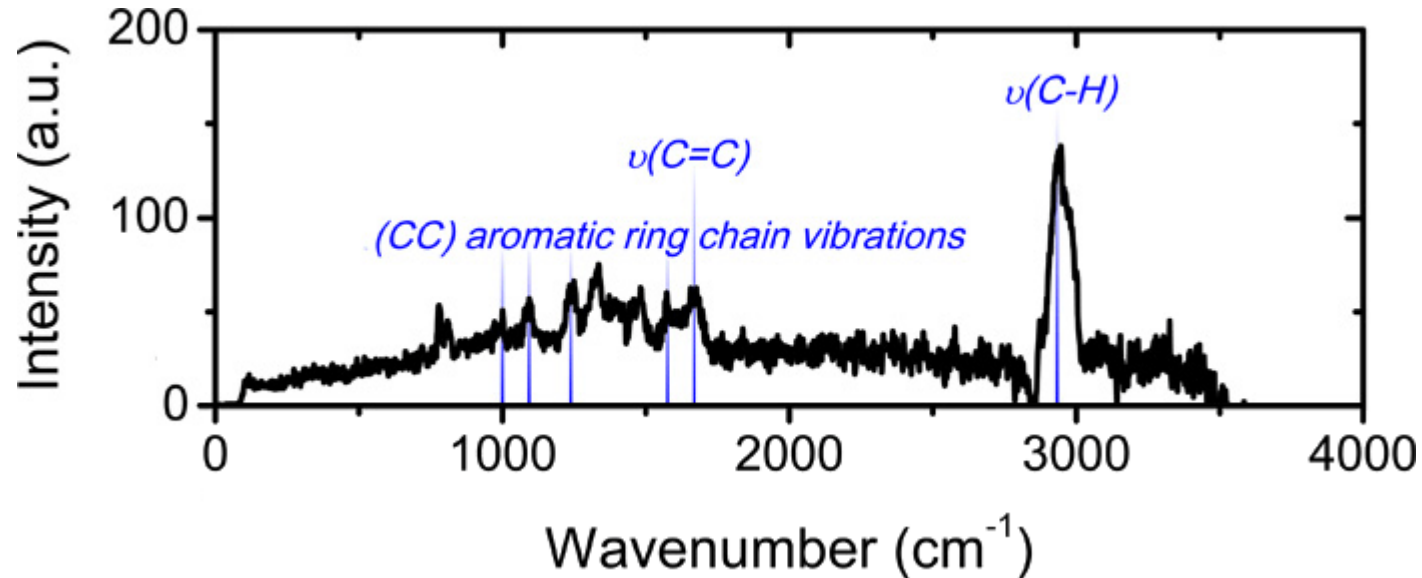


Platelet vesicles



Erythrocyte vesicles

Biochemical information from vesicle



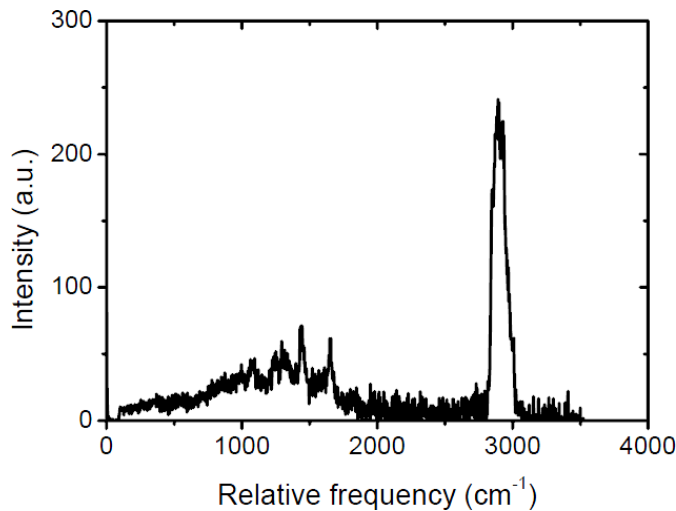
- biochemical information is obtained from peak locations

Future developments

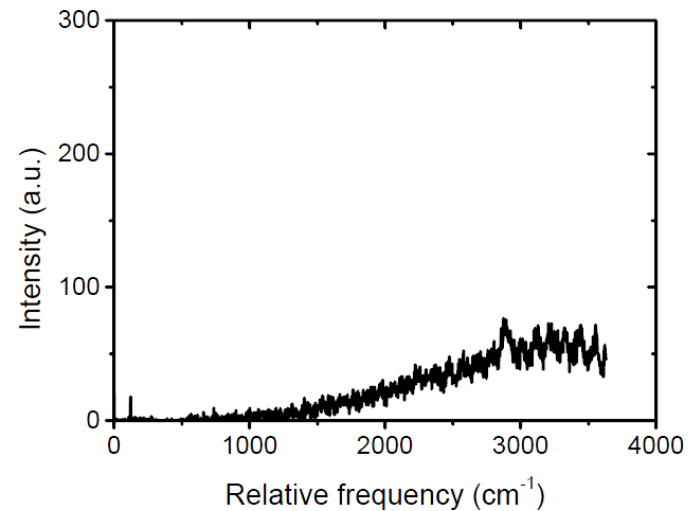
- develop high throughput system
- use longer wavelength to reduce autofluorescence
- trap and sort vesicles

Conclusion

- biochemical information is obtained from *single* vesicles in suspension without fluorescent antibody labeling



Platelet vesicles



Erythrocyte vesicles