

Raman and CARS microspectroscopy of neutrophils

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Aim of the project

- Generally, get some experience in advanced methods of nonlinear optical imaging based on Raman and CARS microspectroscopy realizing with high contrast and spatial resolution
- Be acquainted with the whole circle of the measurement procedure at the “CARS” Raman microspectrometer
- Attempt in revealing some spectroscopic markers and chemically selective Raman/CARS images for neutrophils

Raman spectroscopy

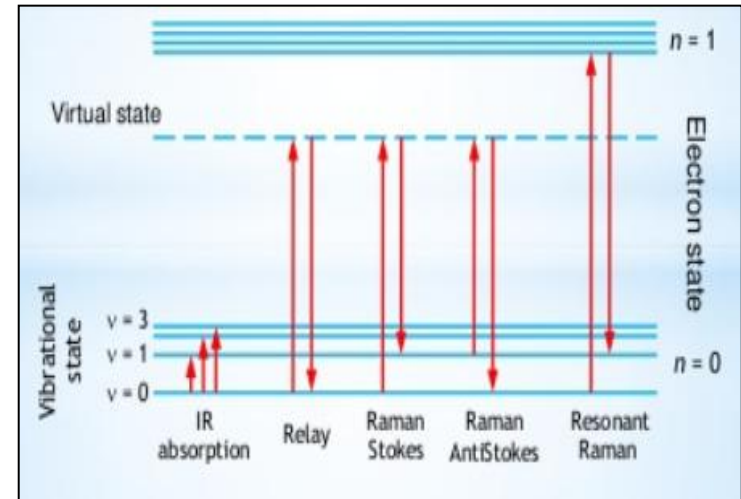
- Molecular vibrational spectroscopy
- Analytical technique to provide chemical and structural information about samples
- „Fingerprint spectroscopy“

- Advantages over the IR:

no suffer of aqueous systems, non-destructive, simple sampling

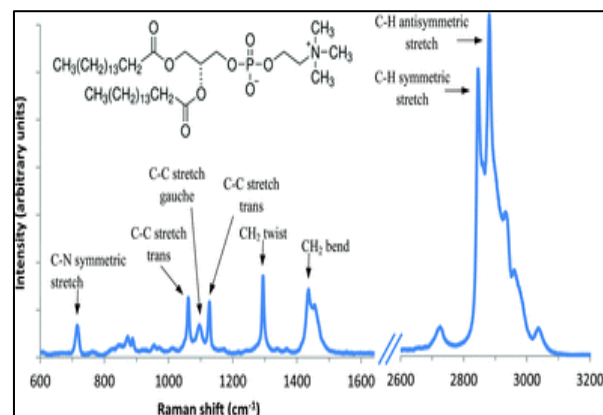
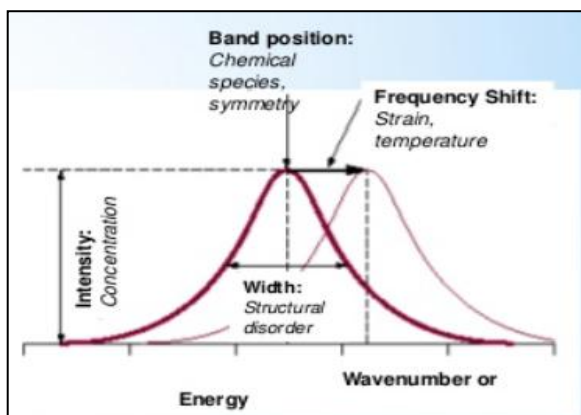
- Raman microscopy (imaging):

a good alternative to fluorescence microscopy with advantages of no photobleaching of the fluorophore and stable work with live cells



What can Raman spectra tell us?

- Vibrational frequencies are characteristic to chemical bonds or group of bonds in a specific molecule
- Shifts of vibrational frequencies are sensitive to local environment of a molecule
- Peak intensity is proportional to the concentration of a substance

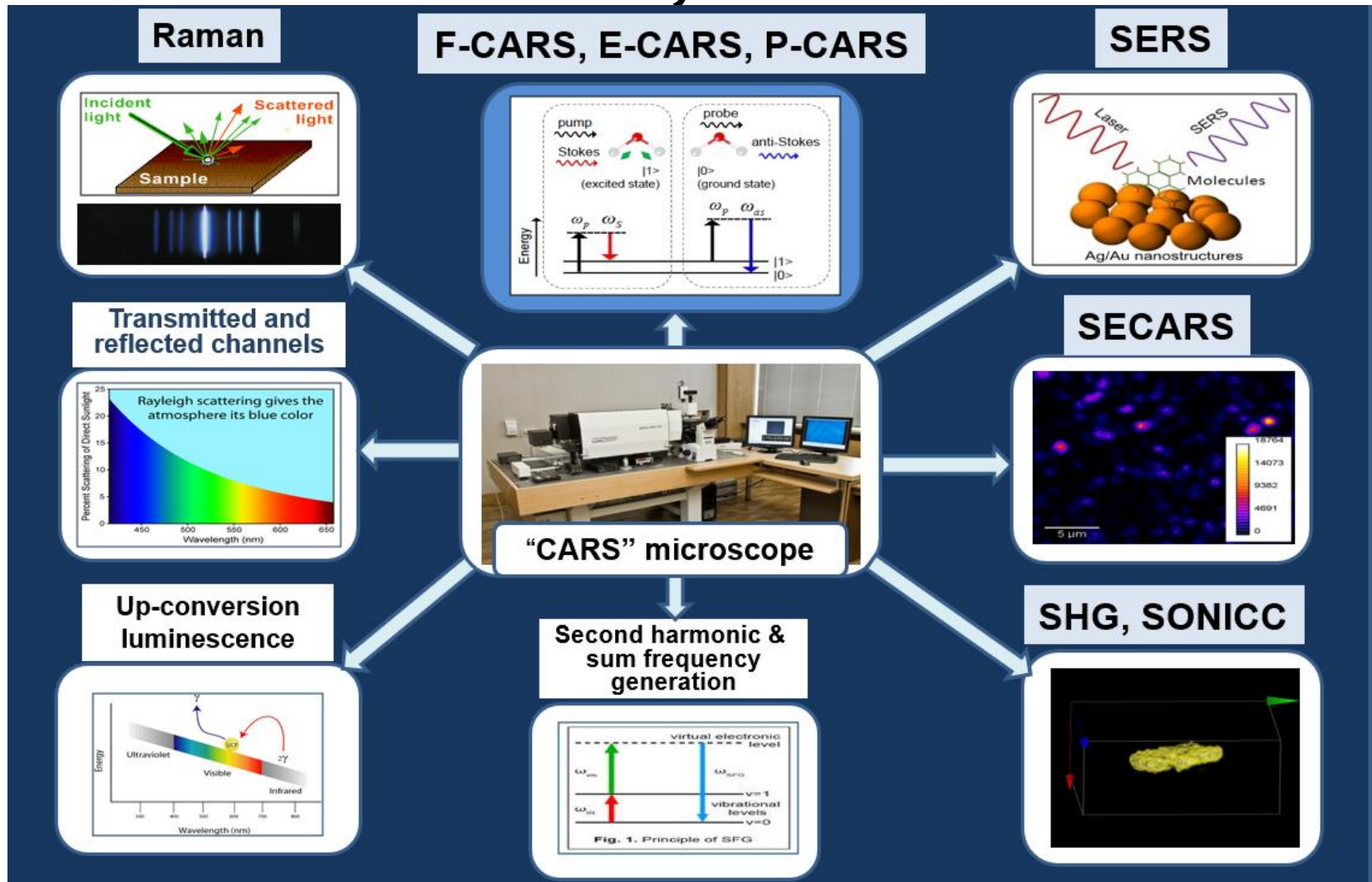


Applications of Raman spectroscopy

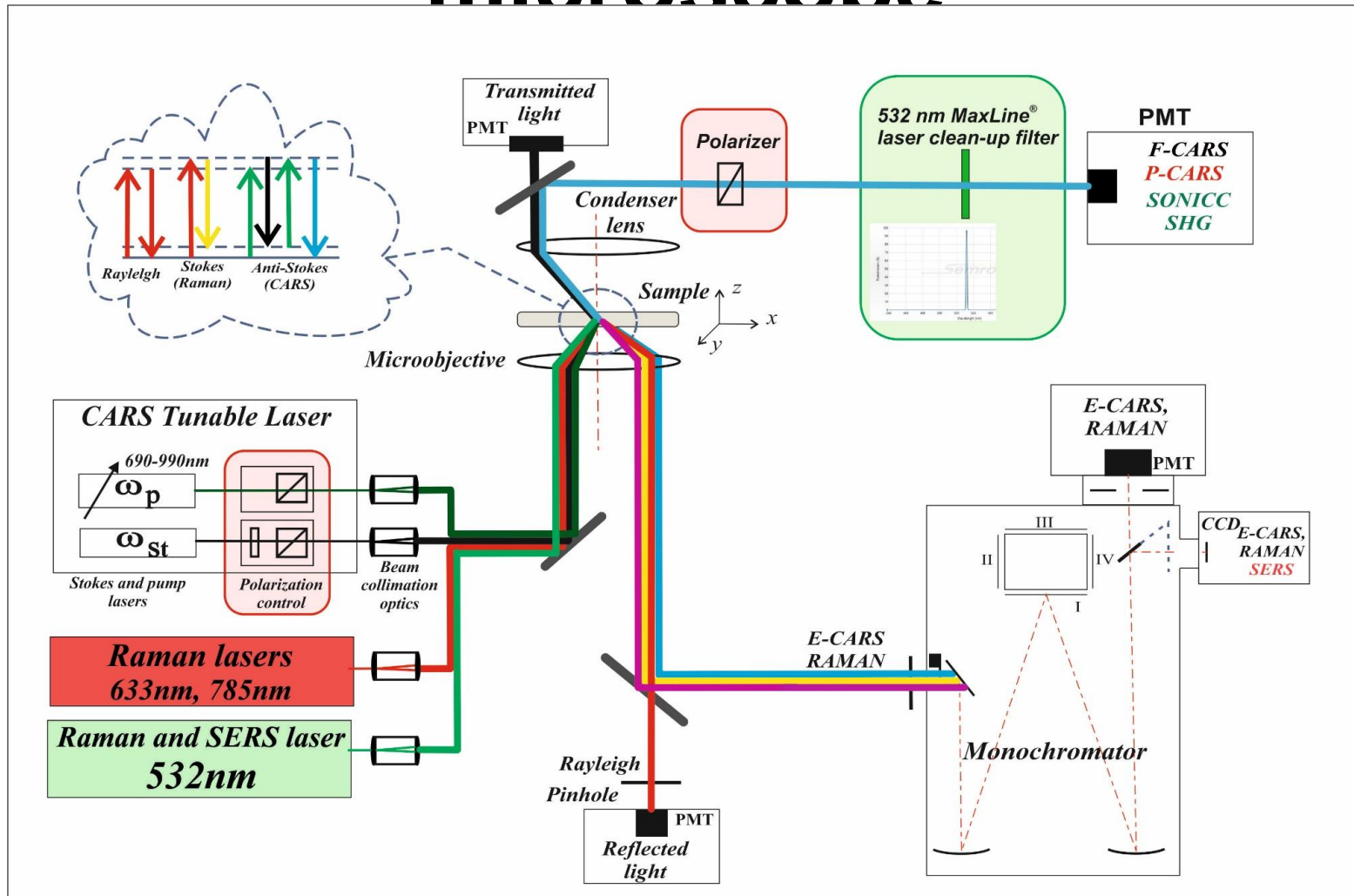


- Basically, any application where non-destructive, microscopic, chemical analysis and imaging is required

Multimodal optical platform at FLNP, JINR

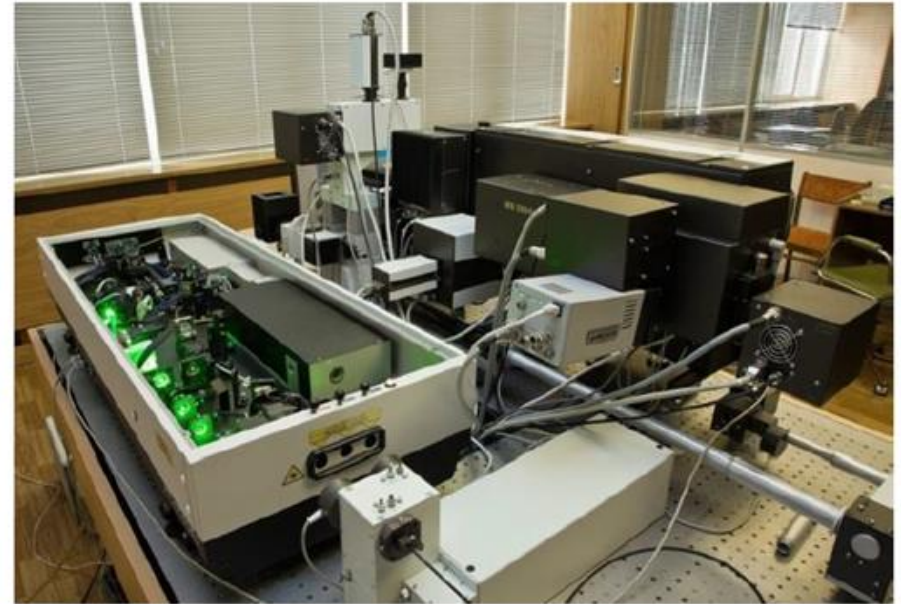
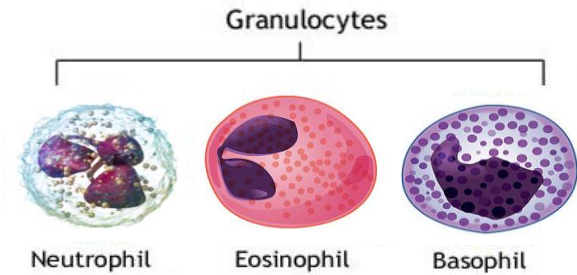


Optical diagram of the CARS microscope

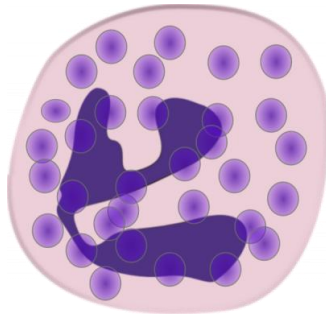


Experiment equipments

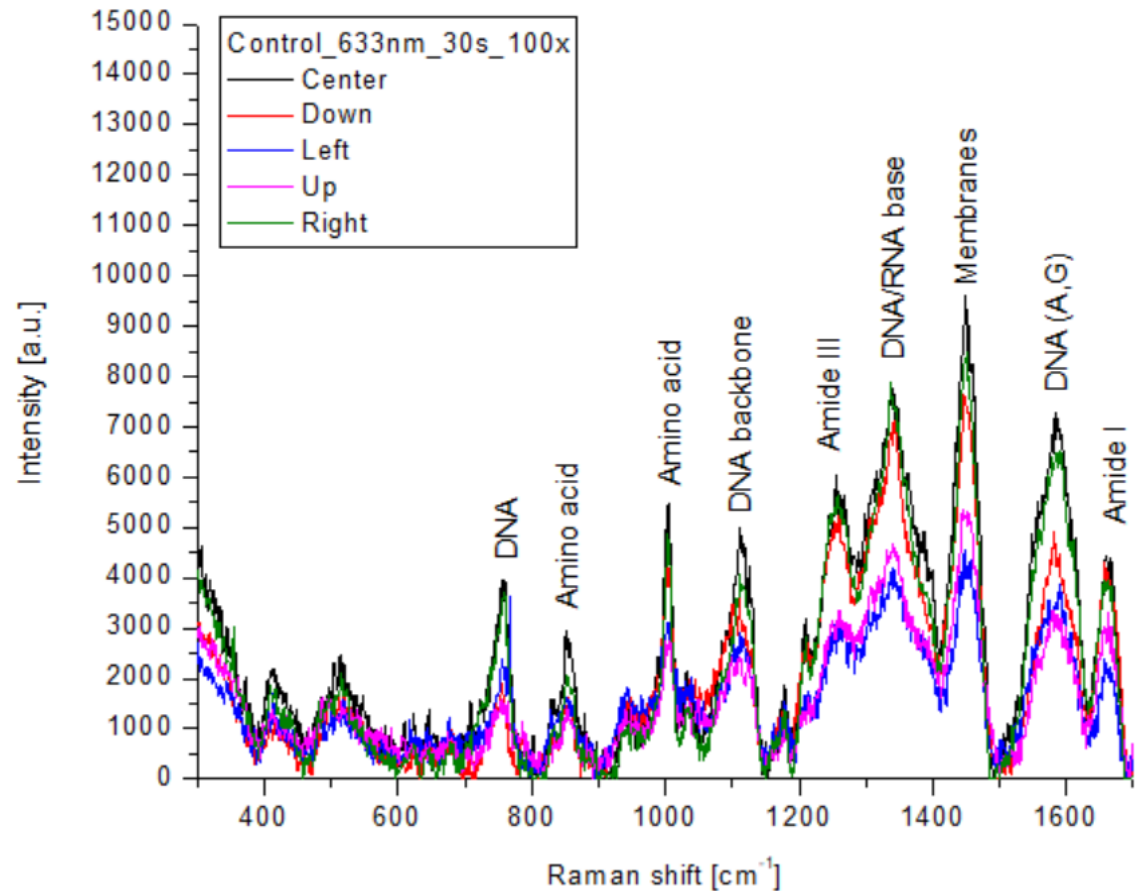
- CARS microscope
- Sample of neutrophils



Raman spectra of nonactivated neutrophils

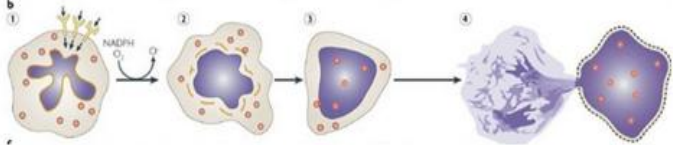
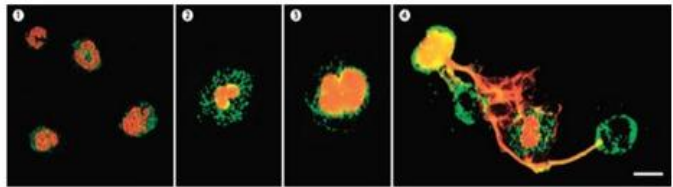


Nonactivated neutrophil

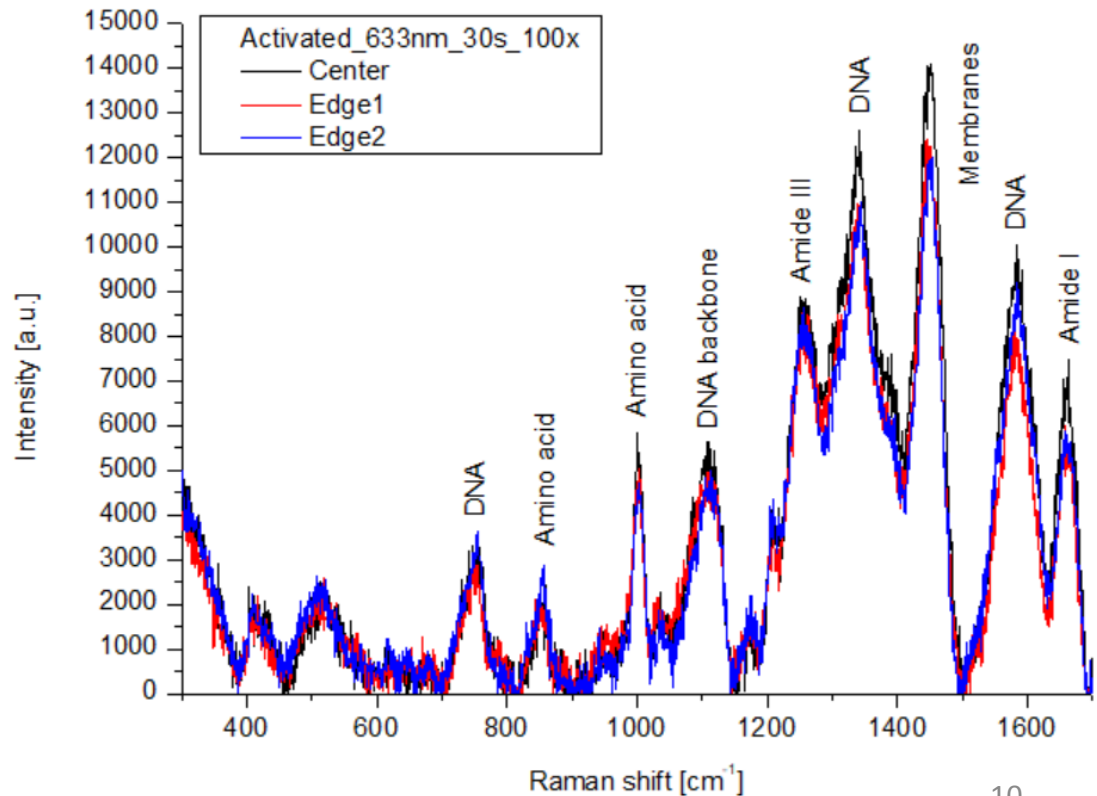
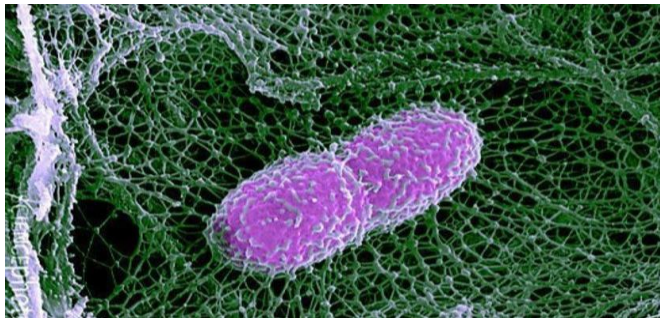


Raman spectra of activated neutrophils

- 1,5 hour of activation

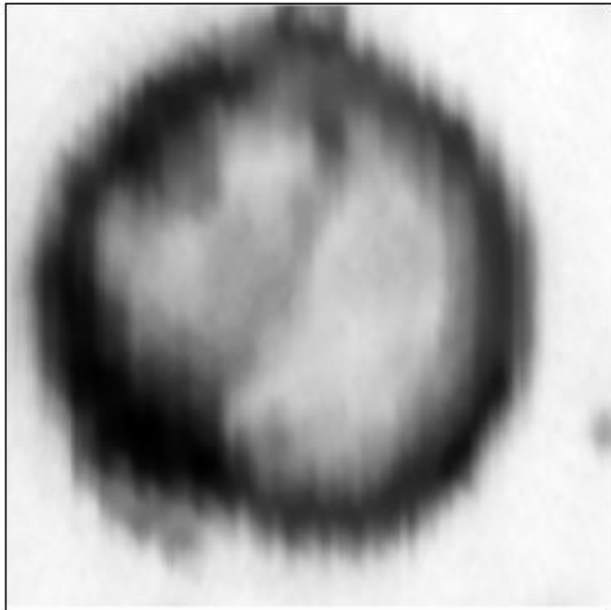


Activated neutrophil

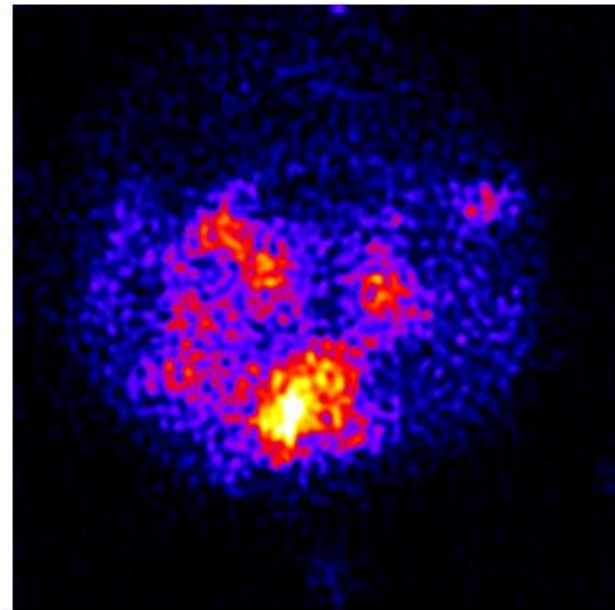


CARS images of nonactivated neutrophil

Micrograph image



CARS image
at Raman shift – 1580cm⁻¹

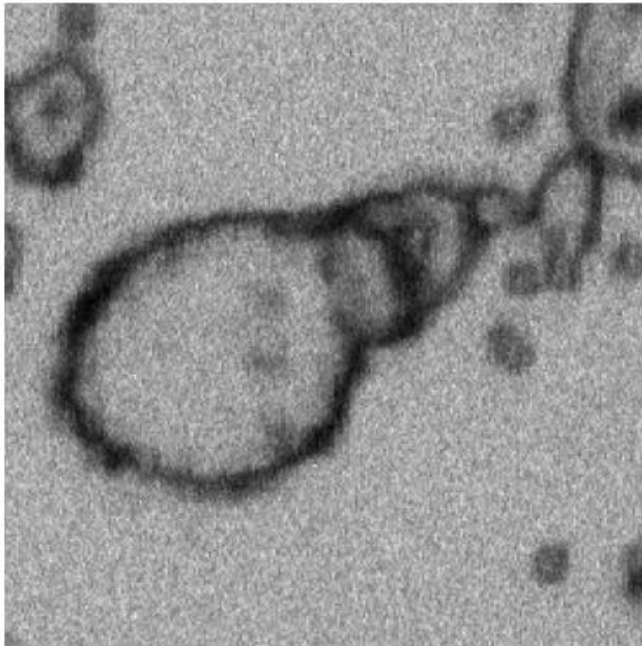


Scan area – 12x12 um

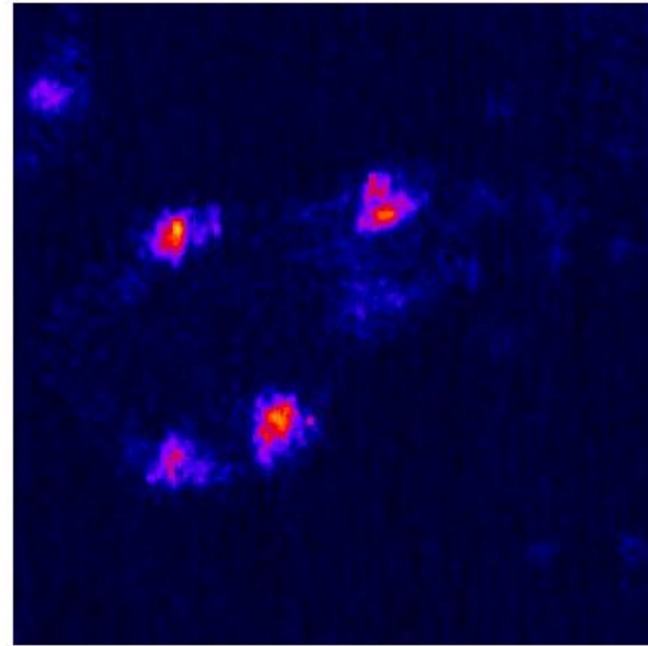
CARS images of activated neutrophil

- 1,5 hour of activation

Micrograph image



CARS image
at Raman shift – 1580cm⁻¹



Scan area – 24x24 um

**Thank you for your
attention**