
TIMEGATED® RAMAN AS A TOOL
FOR EV CHARACTERIZATION
Seeing the unseen in
extracellular vesicles
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Timegated® Raman as a tool for extracellular vesicles characterization

Seeing the Unseen in EVs

Extracellular vesicles (EVs) are nanoscale particles released by cells and have gained significant attention in the field of biotechnology. They contain a diverse range of molecules and have various applications, including diagnostics and therapeutic delivery systems. EVs play a crucial role in cell communication and are involved in processes such as immune regulation and cancer metastasis. Monitoring the production, quality, and diagnostics of EVs is important, and Timegated® Raman spectroscopy offers benefits in this regard. EVs have potential in liquid biopsies for diagnostics, as their composition and quantity change in many diseases, making them useful as early diagnostic tools or prognostic indicators. Additionally, EVs hold therapeutic potential, similar to stem cells, and could be utilized in treating complex diseases.

Raman spectroscopy (RS) is a fast and non-destructive method able to reveal the biochemical composition of complex samples. RS is gaining interest in the extracellular vesicles (EV) field as a quality control tool. Here, we highlight the potential application of the time-gated (TG) Raman spectrometer in EV characterization.

Material and Methods

- Timegated® Raman spectrometer PicoRaman M3 with 532nm pulse laser
- Coupled with a MicroProbe and Olympus microscope
- EV samples:
  - Thrombocyte derived EVs, purified by UCx1 and UCx2
  - HEK293 cell derived EVs:
    - HEK293 cells infected with adeno-associated virus serotype 2/1 or 2/9
    - HEK293 expressing CD9-GFP

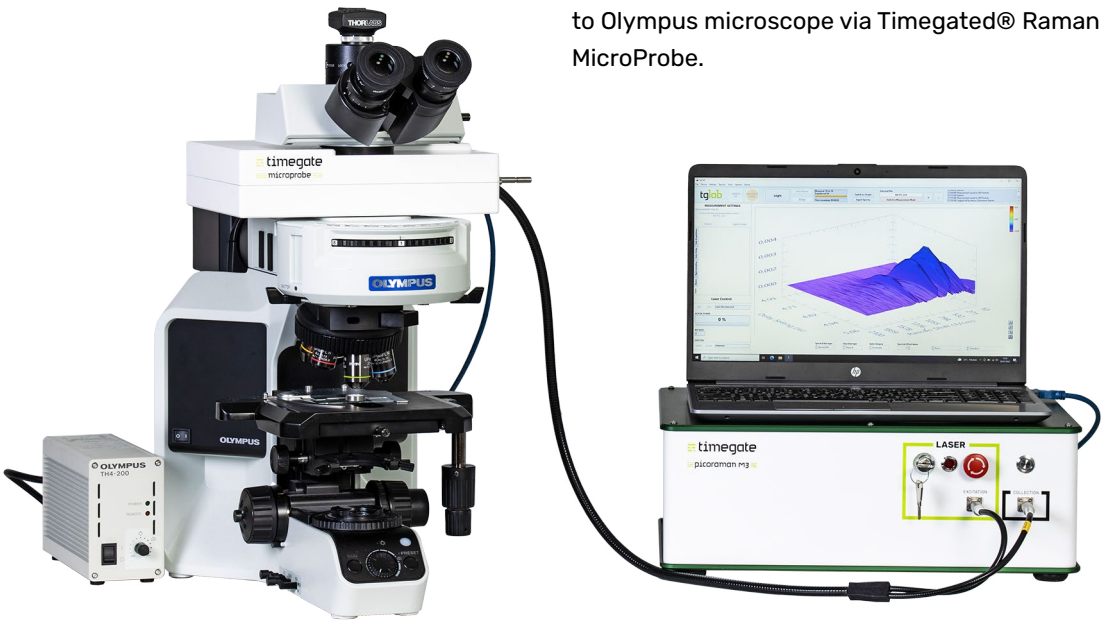

Figure 1 | Experimental setup: Timegated® Raman spectrometer PicoRaman M3 connected to Olympus microscope via Timegated® Raman MicroProbe.

Results

Raman spectra of platelet-derived EVs purified with different protocols show clear differences: the preparation that has been thought more thoroughly purification protocol displays a reduction in the intensity of peaks associated with proteins and amino acids shown in Figure 2. Regarding the EV, EV-GFP, EVs-AAV1 -AAV9, principal component analysis (PCA) displays major clusters highlighted in Figure 3, These findings indicate that TG Raman is a suitable tool for EV quality control since it discriminates EV preparation based on the degree of purity and distinguish between healthy EV preparation and preparation contaminated by AAV. In addition, TG Raman highlights the differences in the diverse AAV found in the EV preparations.

Conclusions:

TG Raman can overcome the fluorescence interference given by GFP. This allows the acquisition or Raman spectra of GFP-EVs, which display no remarkable difference compared to the unmarked EVs.

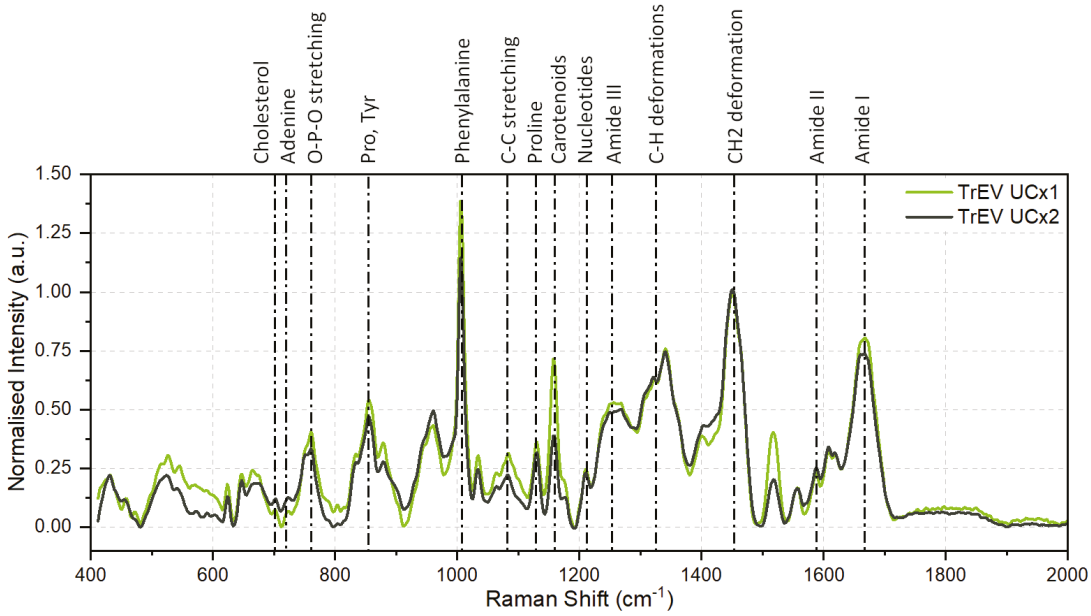

Figure 2 | Raman spectra of thrombocytes (TrEV) derived EVs purified by UCx1 (light green) and UCx2 (dark green). Tentative of peaks assignment is reported on top of the figure.

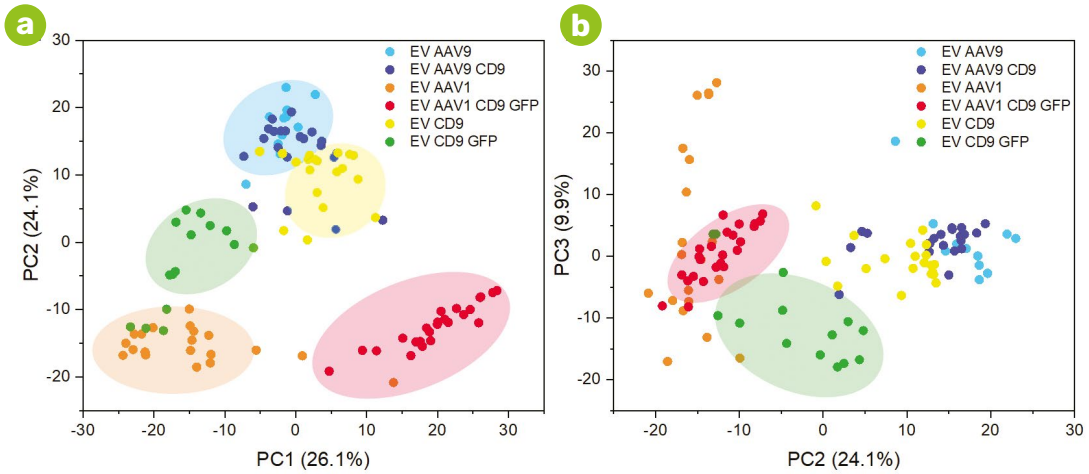

Figure 3 | Multivariate statistical analysis of Raman spectra. PCA analysis performed on spectra obtained on EV and EV-AAV. Samples marked with DC9 and/or GFP overexpress DC9 and/or express GFP.


Real-time process monitoring,
improved product quality,
and faster time to market
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