Chemical spectroscopy of individual human milk extracellular vesicles

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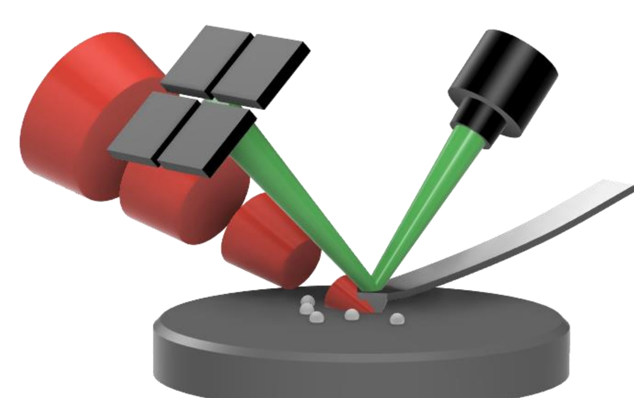
Introduction

01

Extracellular vesicles (EVs) are nanosized particles excreted by cells, which are associated with various physiological and pathological functions. They play a key role in intercell communication and are used as transport vehicles for various cell components [1].
State of the art analysis methods are not able to provide label free chemical information at the single vesicle level [2], hence, new analysis techniques are required to study the chemical difference within EV (sub-)population. We introduce a protocol to profile structure and composition of individual EVs with the help of photothermal scanning probe infrared spectroscopy (AFM-IR).

Tapping Mode AFM-IR

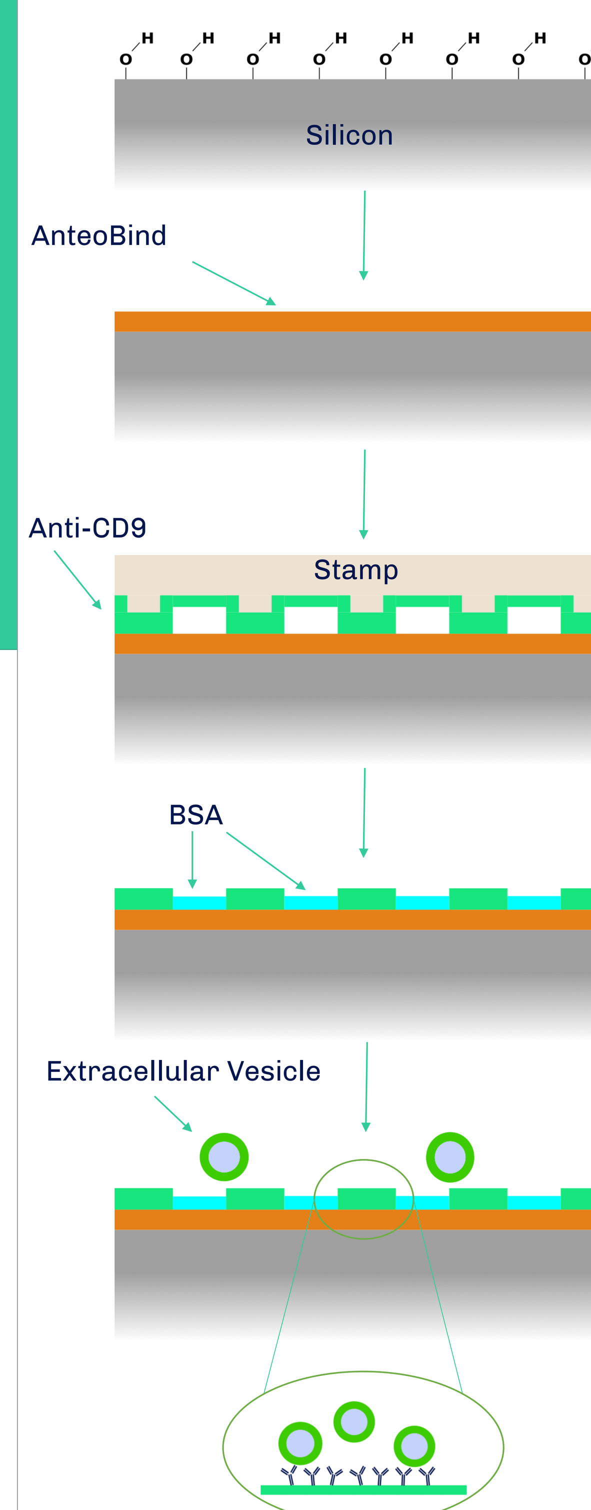
02



AFM-IR measurements were performed using a Bruker nano-IR 3s coupled to a MIRcat-QT external cavity cascade laser array (EC-QCL) from Daylight Solutions. The measured spectra cover a range from 910cm^{-1} to 1950cm^{-1} and were obtained using tapping mode. Cantilevers used were gold-coated and had a first free resonance at 300 kHz ± 100 kHz and a spring constant between 20 Nm^{-1} and 75 Nm^{-1}

Sample Preparation

03



The sample preparation consisted of the following steps:

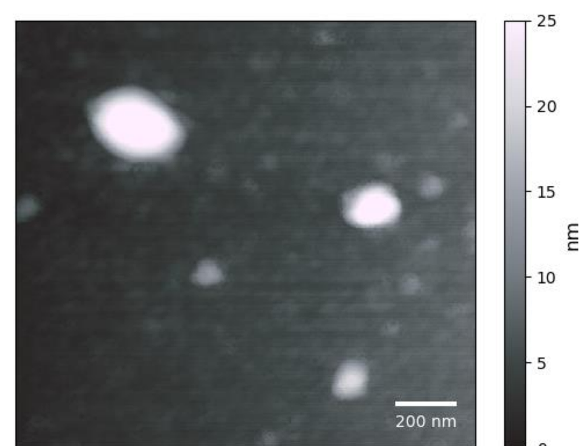
- Coating Si with 100 µL AnteoBind© Biosensor
- Stamping 20 µg/mL anti-CD9 antibodies
- Backfill pattern with 100 µL bovine serum albumin (BSA) in phosphate buffered saline (PBS)
- Incubate with 50 – 100 µL 1:10 diluted EV sample

Hyperspectral Imaging

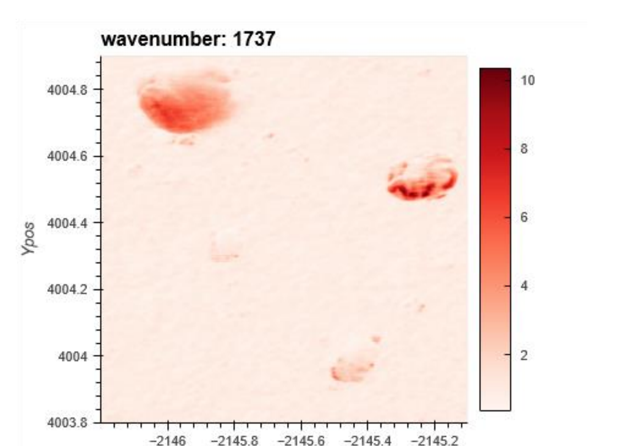
05

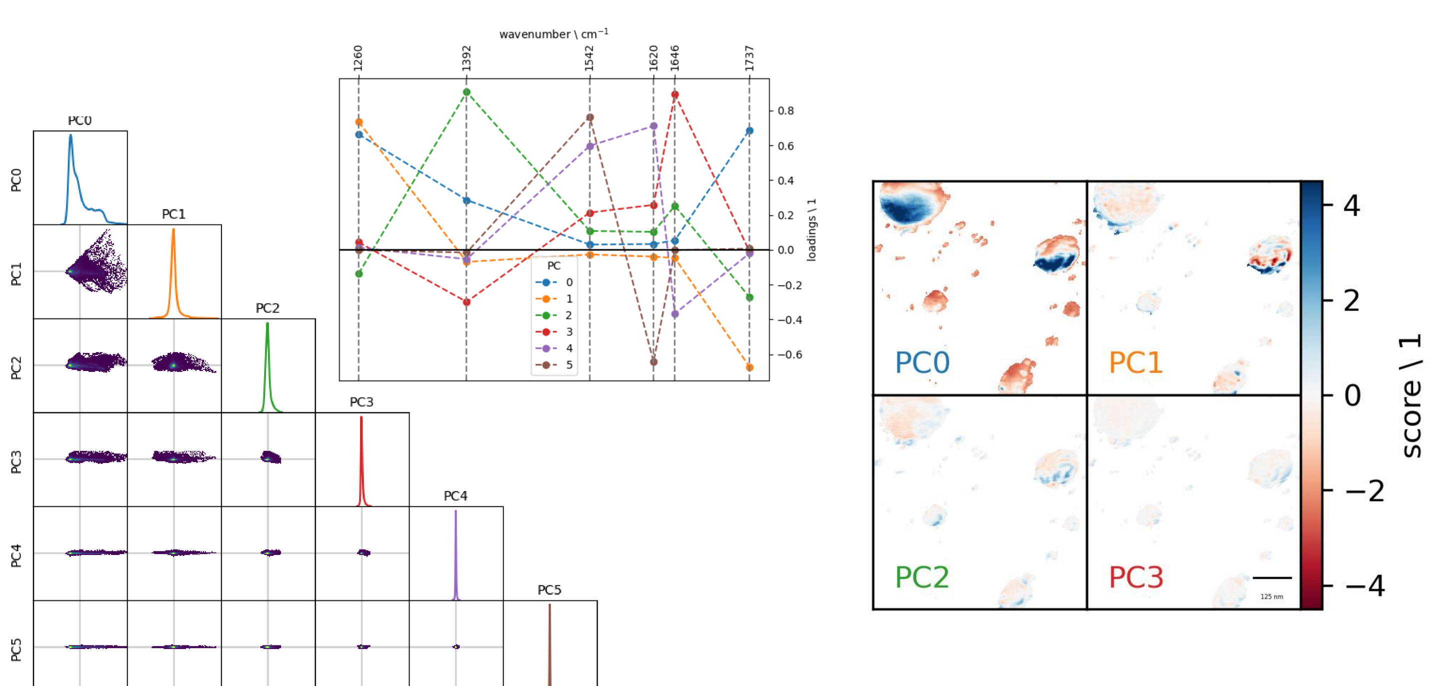
Hyperspectral images were assembled from multiple single wavelength AFM-IR images

Topography image



AFM-IR image (1737 cm^{-1})





To find trends in single pixel data, chemometrics are required. Principal component analysis (PCA) reveals trends in the data set.

Score plots applied onto the pixels. Every PC shows up at different pixels, thus underlining the difference in the vesicles.

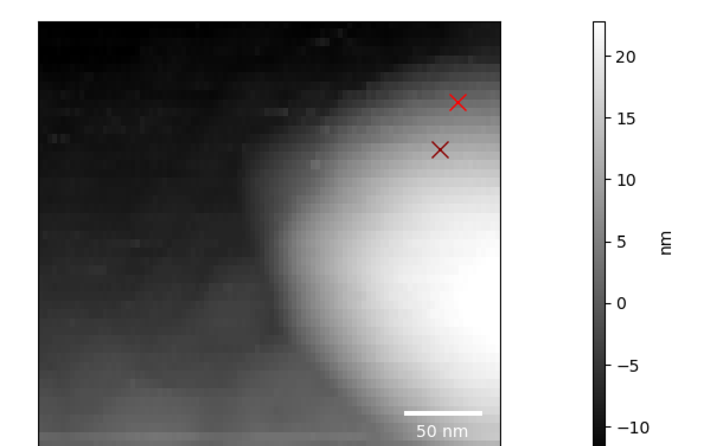
Single Point Spectroscopy

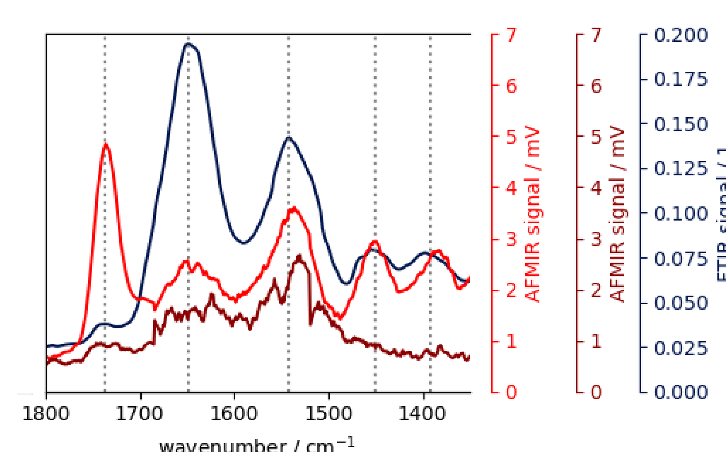
04

AFM-IR spectra compare well to bulk FTIR spectra of EVs [3].

Relevant bands:

Wavenumber in cm^{-1}	Spectral assignment
1392 cm^{-1}	COO$^-$ symmetric stretch
1451 cm^{-1}	CH_2 bending of lipidic acyl chains
1542 cm^{-1}	Amide II
1648 cm^{-1}	Amide I
1737 cm^{-1}	saturated ester C=O stretch





Conclusion

06

We had set out to find a method to obtain label-free chemical information of single extracellular vesicles. We developed an immbolization protocol to selectively deposit EVs on an AFM-IR compatible substrate via an anti-CD9 antibody.
Nanoscale spatial resolution AFM-IR spectra of EVs compare well to FTIR bulk reference spectra. For high throughput measurements of many EVs, hyperspectral images were assembled from many tapping mode AFM-IR images.

Image segmentation was employed to find all pixels belonging to the same vesicles. This enabled us to study composition of EVs within different (sub-)populations of vesicles.

In short:
✓ Specific immobilization of EVs developed
✓ Label-free determination of chemical composition of single vesicles possible

[1] Kanchan Vaswani, Murray D. Mitchell, Olivia J. Holland, Yong Qin Koh, Rebecca J. Hill, Tracy Harb, Peter S. W. Davies, and Hassendrini Peiris. A Method for the Isolation of Exosomes from Human and Bovine Milk. Journal of Nutrition and Metabolism, 2019:1–6, December 2019.
[2] Théry, Clotilde, et al. "Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines." Journal of extracellular vesicles 7.1 (2018).
[3] Victoria Ramos-Garcia, Isabel Ten-Doménech, Alba Moreno-Giménez, Maria Gor-maz, Anna Parra-Llorca, Alex P. Shephard, Pilar Sepúlveda, David Pérez-Guaita,Máximo Vento, Bernhard Lendl, Guillermo Quintás, and Julia Kuligowski. ATR- FTIR spectroscopy for the routine quality control of exosome isolations. Chemomet-rics and Intelligent Laboratory Systems, 217:104401, October 2021.

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TUMOR LN-oC

The authors acknowledge financial support through the COMET Centre CHASE, funded within the COMET – Competence Centers for Excellent Technologies programme by the BMK, the BMDW and the Federal Provinces of Upper Austria and Vienna. The COMET programme is