Non-supervised algorithms for Raman spectral decomposition in the in-vitro study of oxide nanoparticles effects on human cells

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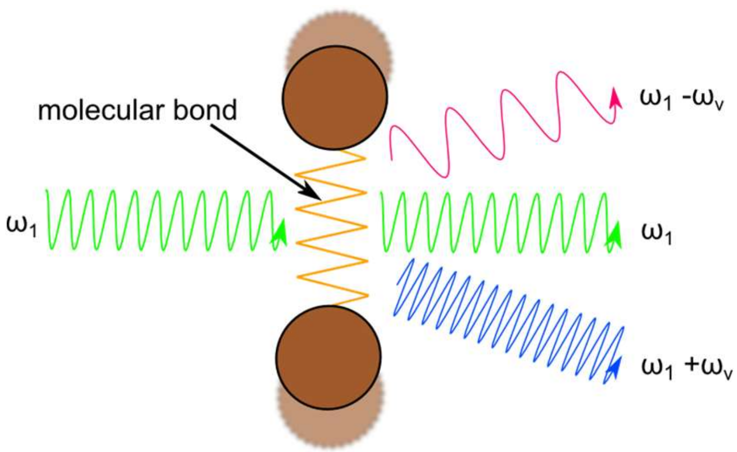
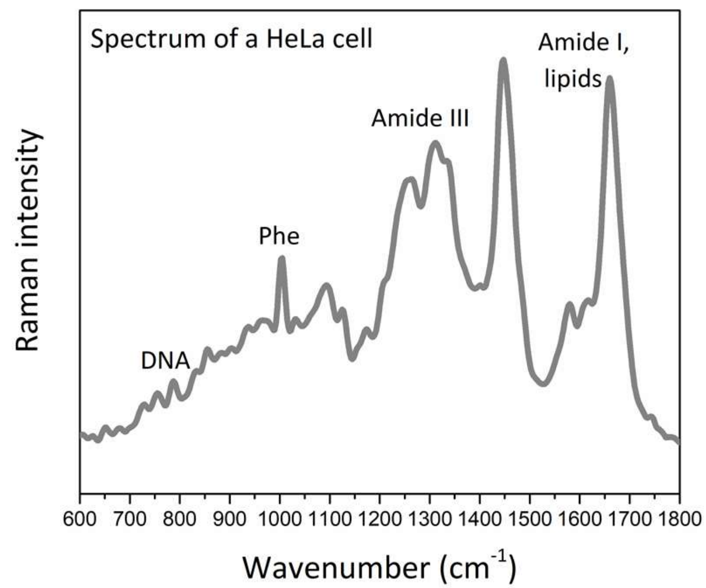
Raman spectroscopy

- Raman spectroscopy is a inelastic light scattering technique that can be used to probe molecular vibrations, electronic excitations, magnons etc.
- Molecular vibrations have specific energies (wavenumbers) that sometimes enable identification of molecules within a material



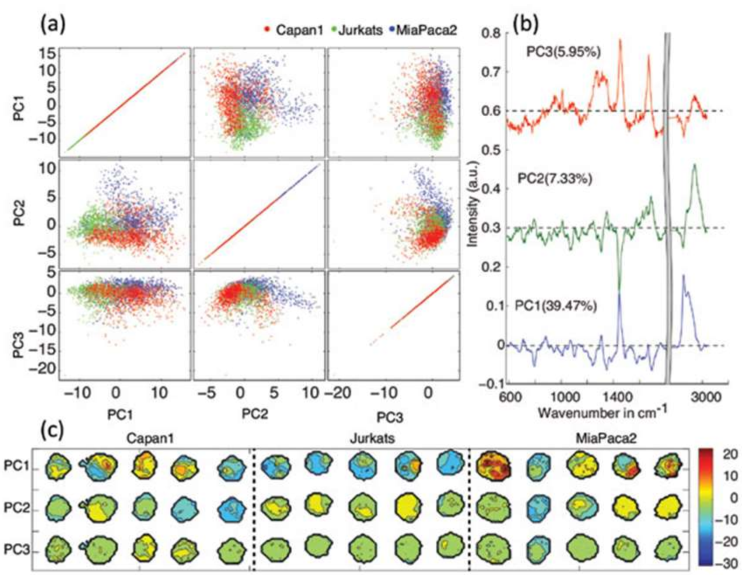


Raman spectroscopy modalities

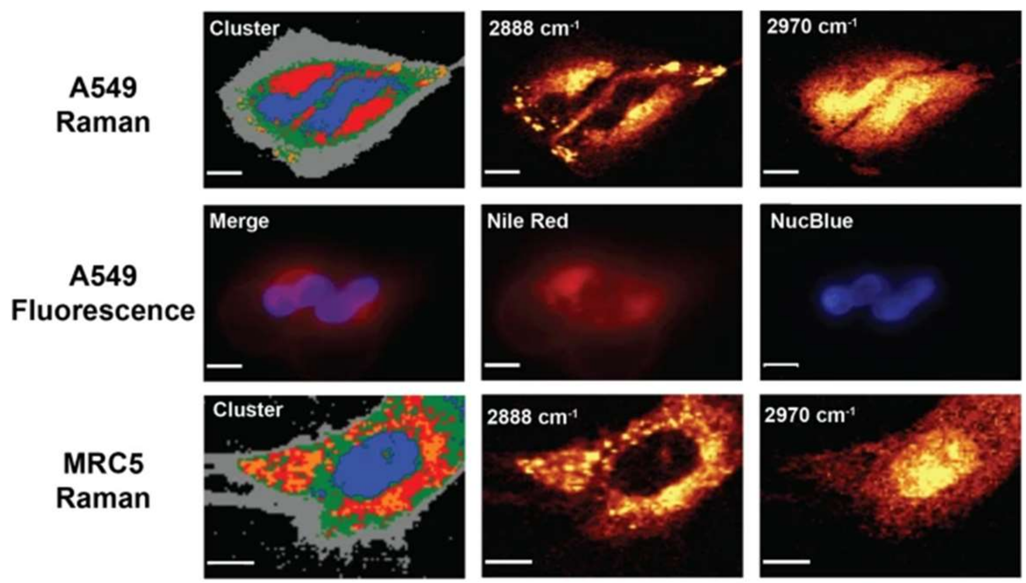
- Spontaneous, CARS, SRS, enhanced-SERS
- Common applications:

✓ Separating healthy and cancer cells/tissues based on vibrational spectra

✓ Raman imaging – obtaining Raman maps based on the vibrational wavenumbers belonging to specific biomolecules



Popp et al, *Spectrosc. Suppl.* 34 (2019)



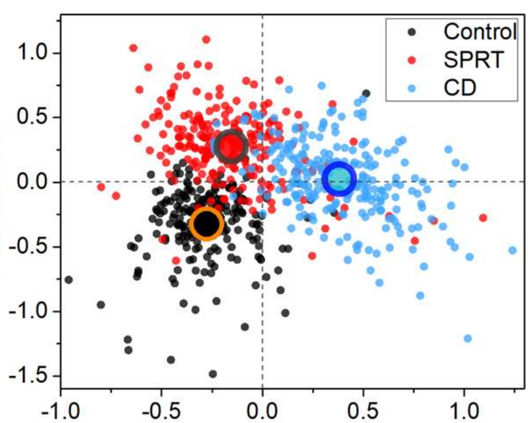
Surmacki et al, *Sci. Rep.* 8 (2018)

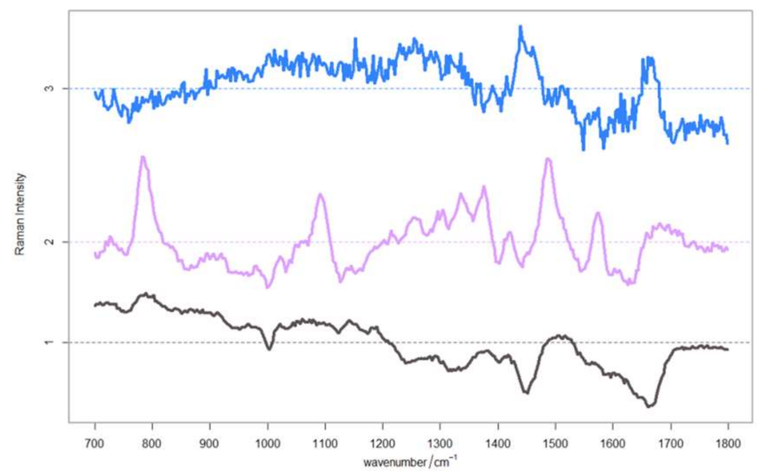
Non-supervised algorithms

- Non-supervised multivariate methods:
  - Exploratory data analysis
  - Data dimension reduction
  - Clustering according to the components that present spectral variance
- Principal component analysis (PCA):

  Loadings (basis vectors) are orthogonal and represent the directions of maximum variance between the spectra in the set

  Scores – projections of original dataset onto loading vectors





Non-supervised algorithms

- PCA:

  orthogonality of loading vectors, unique solution of equation, low sparseness, troublesome interpretability

- nnPCA - PCA with constraint of non-negative loading vectors:

  non-orthogonality of loading vectors, higher sparseness, non-negative values, good interpretability

- NMF - non-negative matrix factorization:

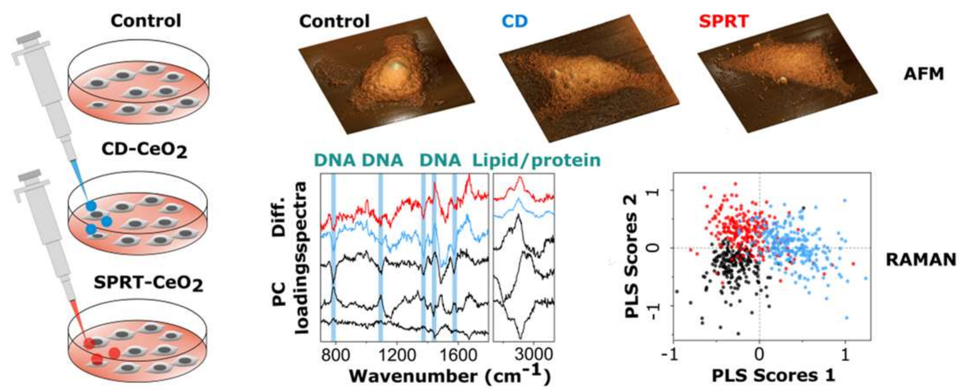
  non-orthogonality of loading vectors, non-unique solution of equation, non-negative values, good interpretability

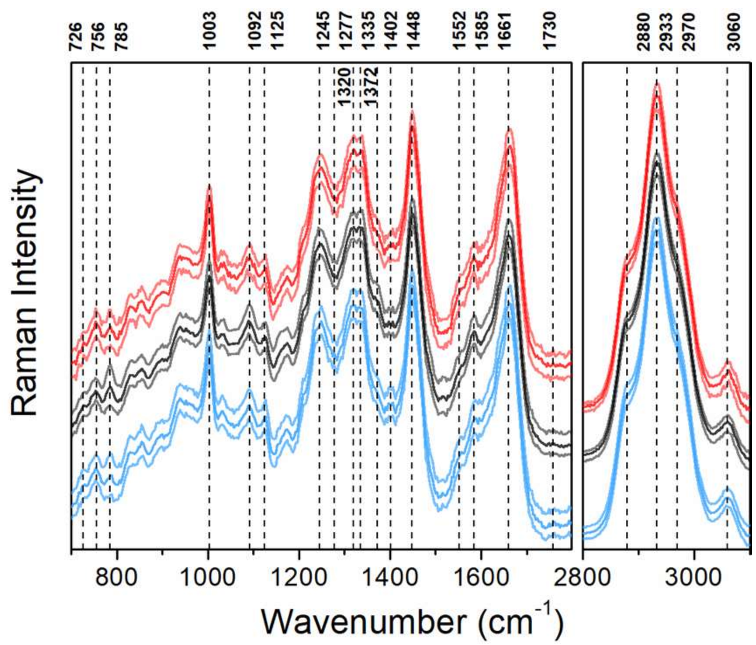
  $X_{nxp} = W_{nxk} * H_{kxp} + R$

k – reduced dimension, n – number of samples, p-number of features

Raman spectra of CeO_2 NP_s-treated cells

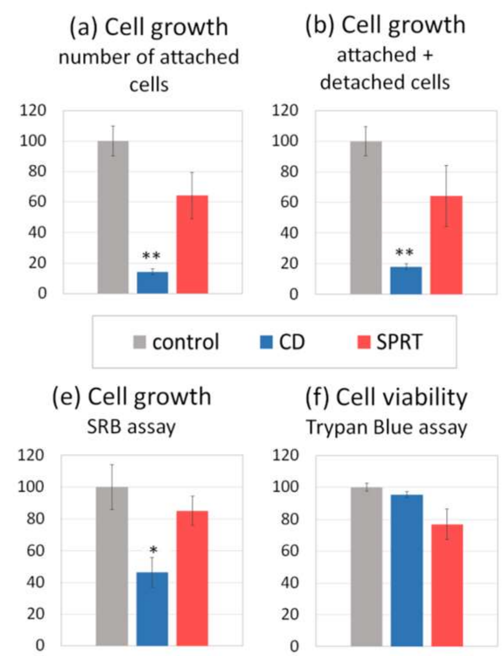
λ= 532 nm



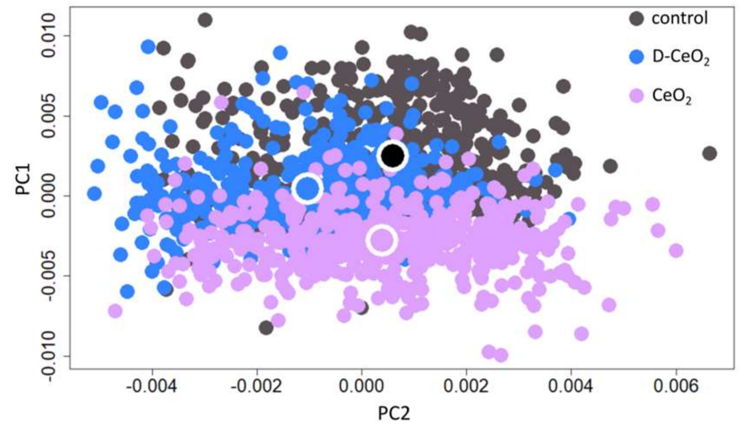


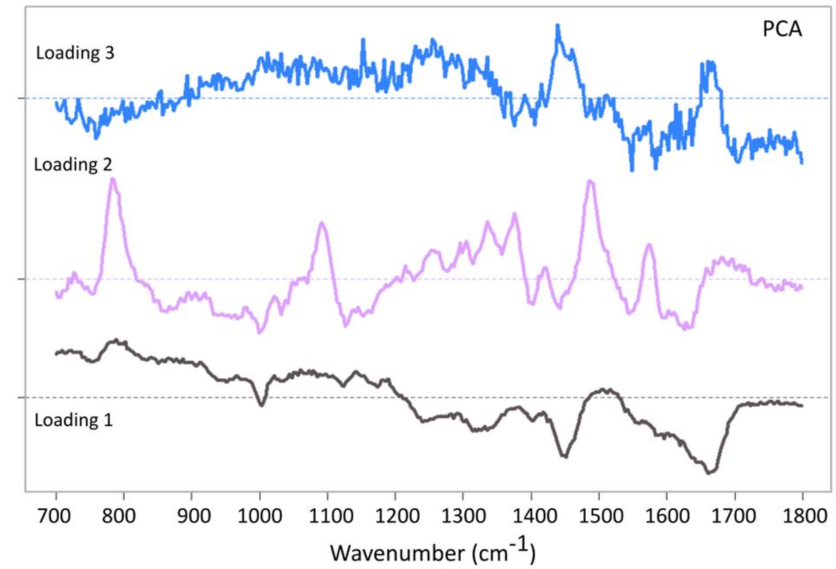
CeO_2 (SPRT)
Control
D-CeO_2 (CD)

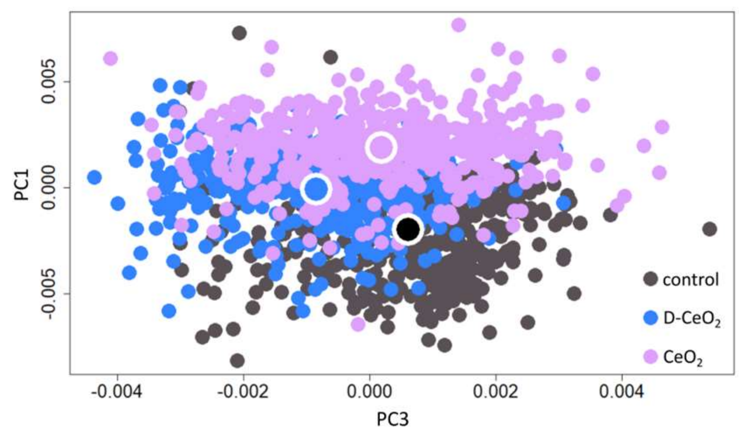
Mean spectra of HeLa cells
+/- standard deviation

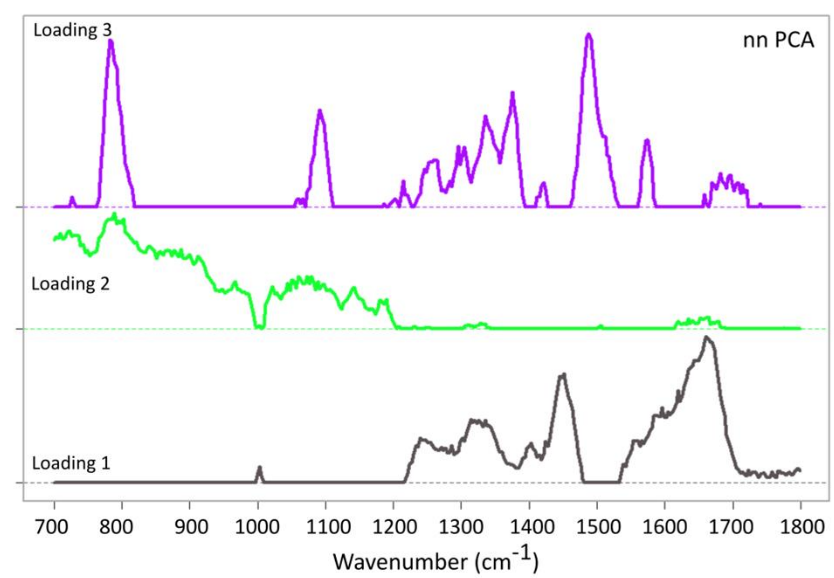


Raman spectra of CeO_2-treated cells



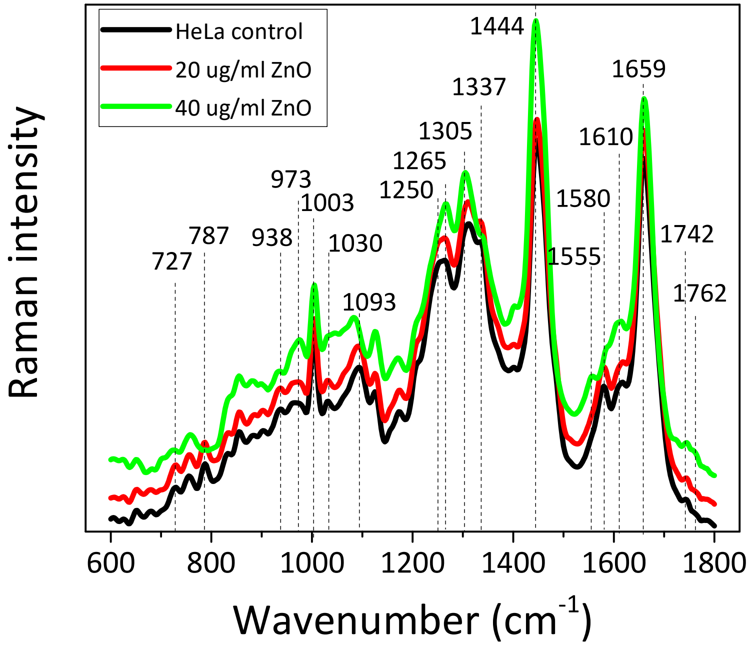






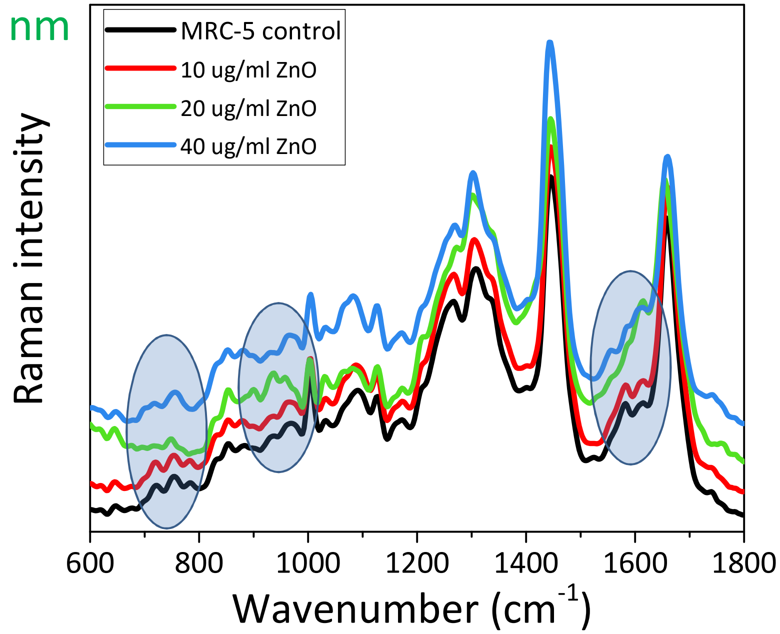
Raman spectra of ZnO NP_S-treated cells

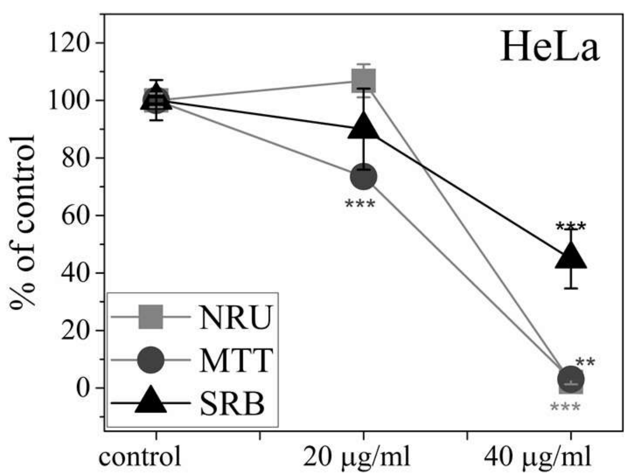
Mean Raman spectra of HeLa cells

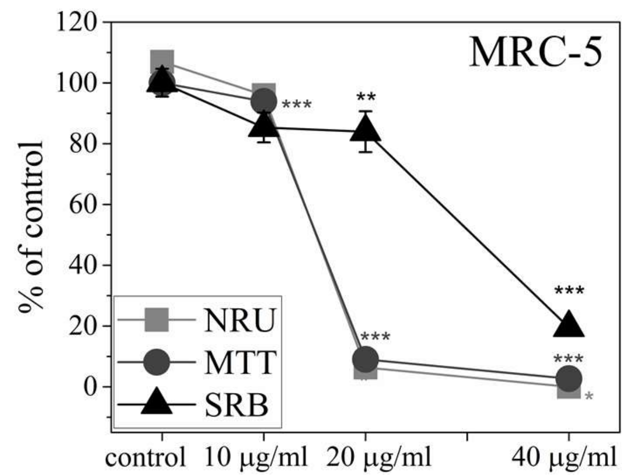


λ= 532 nm

Mean spectra of MRC-5 cells





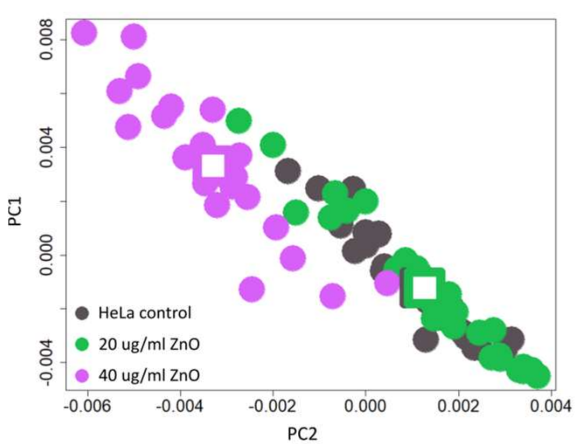


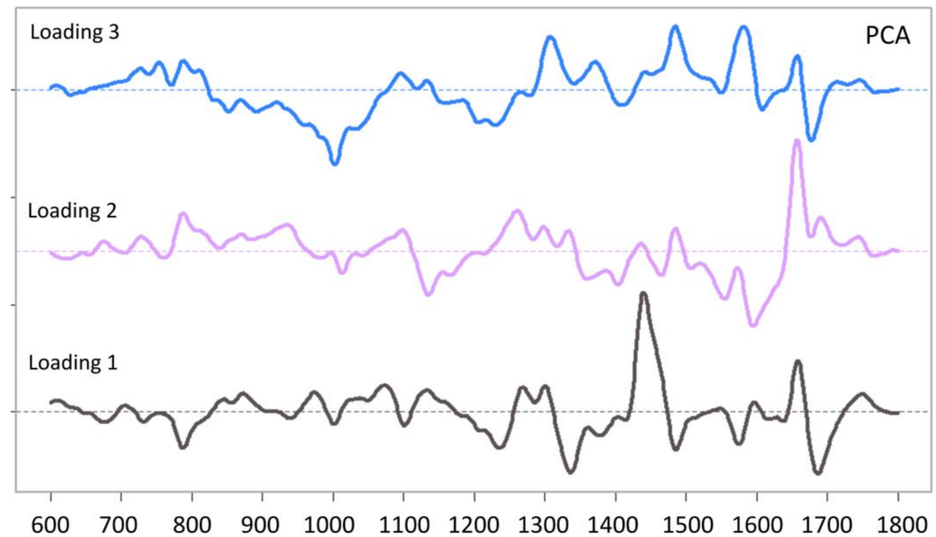
Raman spectra of ZnO NP_S-treated cells

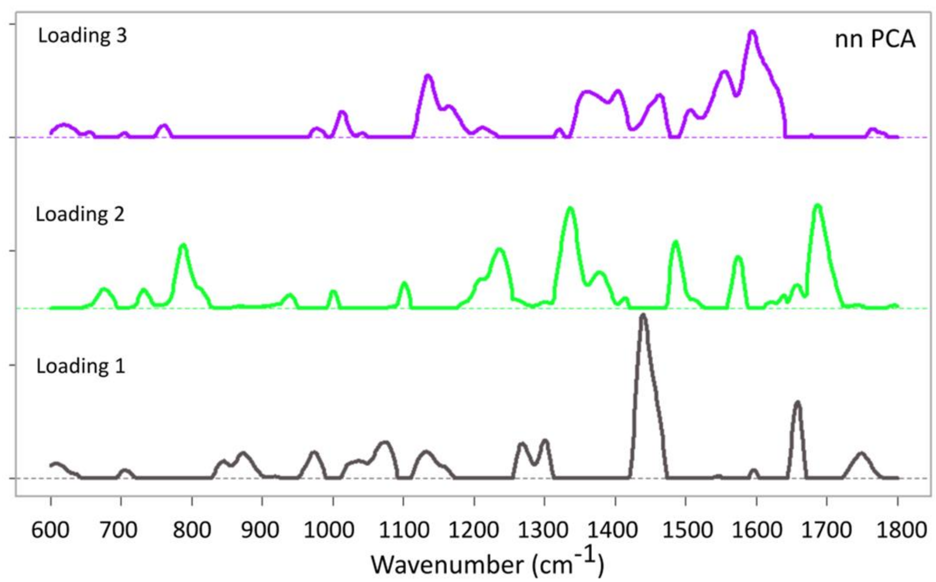
- HeLa cells
- nnPCA loading vectors resemble phosphatydilserine and DNA spectra
- With the increased ZnO concentration, cells show:

✓ Higher phosphatidylserine content

✓ Lower DNA content





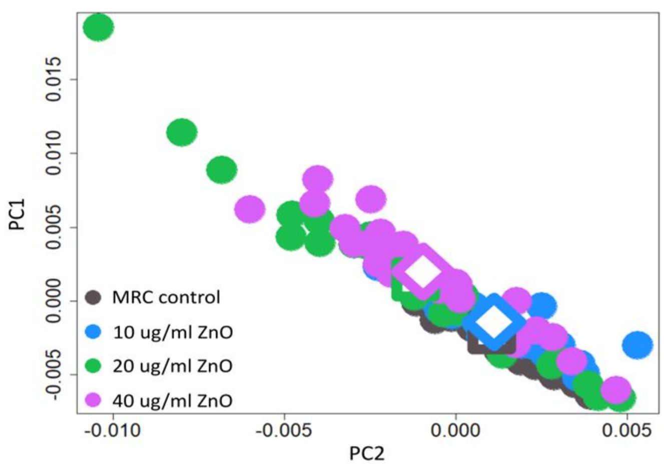


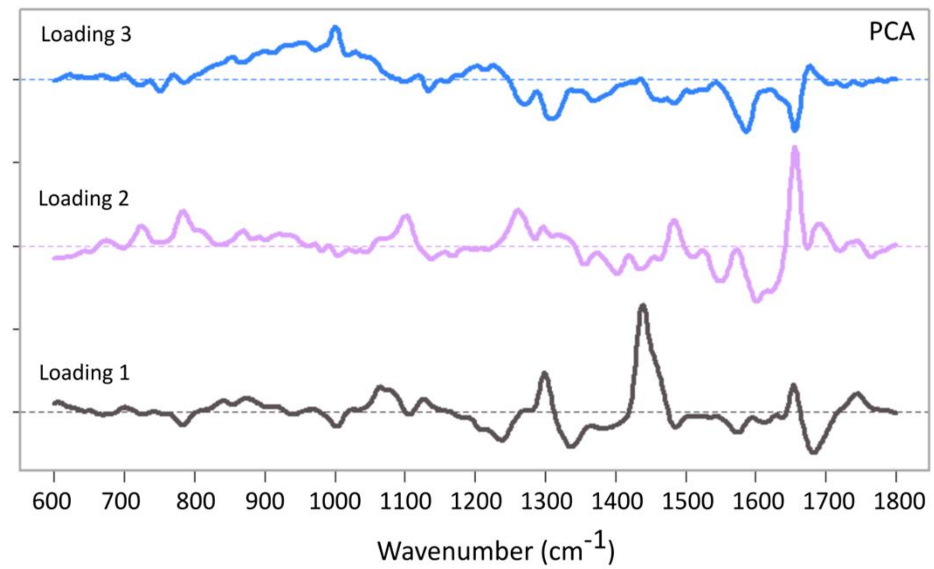
Raman spectra of ZnO NP_S-treated cells

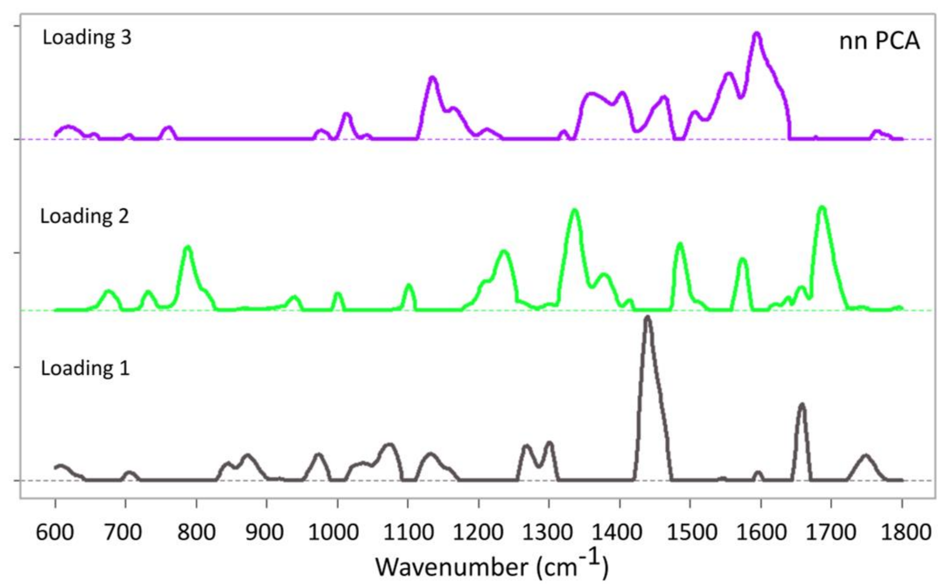
- MRC-5
- nnPCA loading vectors resemble unsaturated fatty acid and DNA spectra
- With the increased ZnO concentration, cells show:

✓ Higher unsaturated lipids content

✓ Lower DNA content

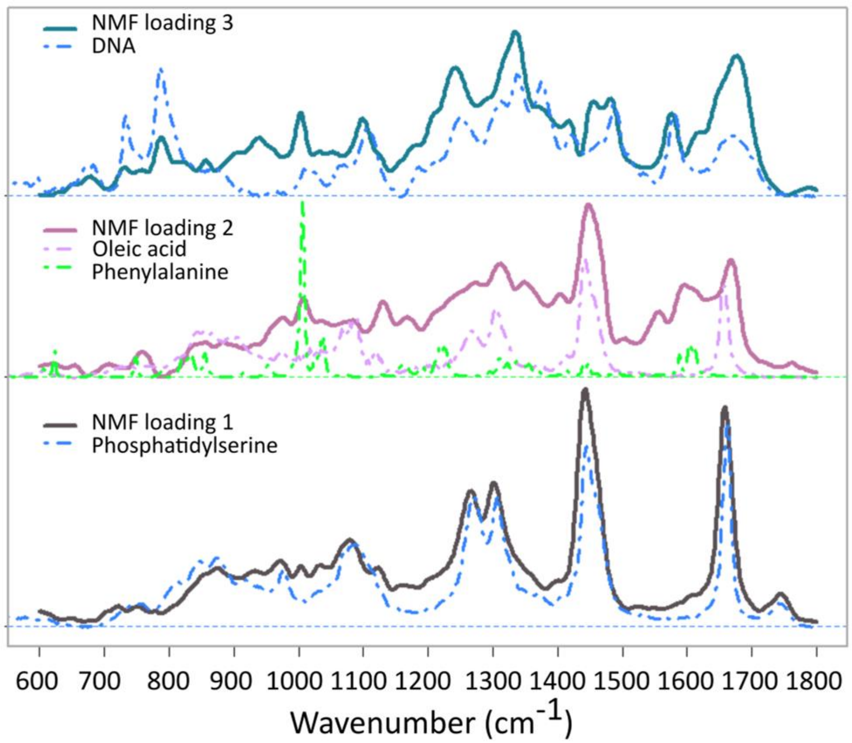


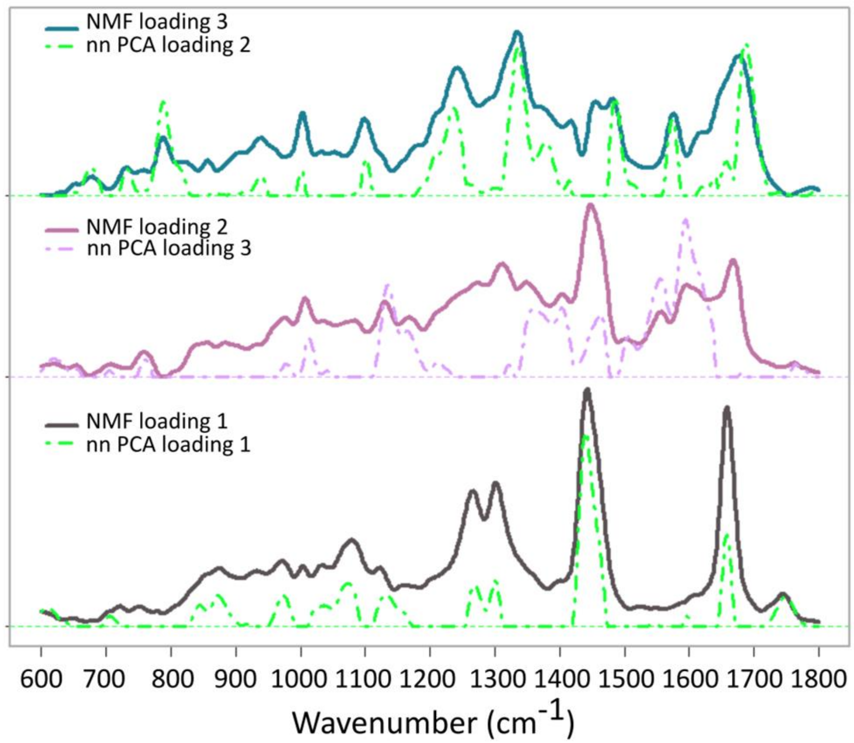




Raman spectra of ZnO NP_S-treated cells

- NMF: ns-NMF algorithm, dimension reduction to k=3
- 2 NMF loadings match the spectra of DNA and phosphathidylserine
- when number of components is higher than 3 variations of the same components reappear





Conclusions

- PCA, nn-PCA and NMF were used for spectral data analysis of cells treated with different concentrations of CeO_2 and ZnO NPs
- Better interpretability of the NPs effect on cells was demonstrated for nn-PCA, especially in the case of ZnO nanoparticles
- NMF provided similar conclusions as nn-PCA, but the choice of the number of latent components influences the correlation of loadings with the spectra of real biomolecules
- For NMF the best mathematical results are not best in terms of physical interpretation

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Thank you for the attention!