

P20 – TERS for Labelfree Cell Diagnostic

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In order to investigate cell-cell interactions or interactions between drugs and cell surfaces, information on the cell membrane composition is essential. It is known that cell surface glycoproteins are acting as cell specific identifiers for cell-cell interactions. Those macromolecules are often important integral membrane proteins, where they play a role as a receptor for active ingredients and second messengers.

A common method to identify membrane proteins is antibody labeling. Depending on the nature of the markers it is possible to use fluorescence or Raman spectroscopy as an analytical method. Especially silver and gold-labeled antibodies turned out to be very interesting as they can be used to increase the sensitivity of Raman labels via a plasmon enhancement¹. However, the lateral resolution capability with respect to the location of specific protein arrangements of this method is limited. A limitation of labeling with antibodies is the selectivity of the marker. Different and specific markers must be chosen for each protein of interest.

To provide spectroscopic information with high spatial resolution tip-enhanced Raman scattering (TERS) was chosen². The combination of an atomic force microscope (AFM) with a Raman microscope simultaneously provides information on the topography and the molecular structure of a sample with high sensitivity.

We present TERS measurements on colon cancer cells (cell line HT29, fixed with 2% formaldehyd) and demonstrate the distinction of different membrane proteins. In particular, an area of 90x90 nm was analyzed. Within this area spectra were recorded on a square grid with a spacing of 10 nm. The TER spectra were processed using multivariate data analysis like principle component analysis and cluster analysis.

Based on the clustering, a band assignment of the mean spectrum of each cluster was done. As expected all the TERS bands can be attributed to proteins or lipids, the known components of the cell membrane. By correlating the band assignment and the cluster analysis the location of distinct cell membrane components can be shown.

We demonstrate that he combination of high lateral resolution and specificity of TERS potentially allows a direct characterization of single membrane proteins.

References

- [1] J.Kneipp, H. Kneipp, K. Kneipp, Chem. Soc. Rev. 27 (2008), 1052–1060.
- [2] E. Bailo, V. Deckert, Chem. Soc. Rev. 37 (2008), 921-930.



TERS mapping for label-free cell diagnostic



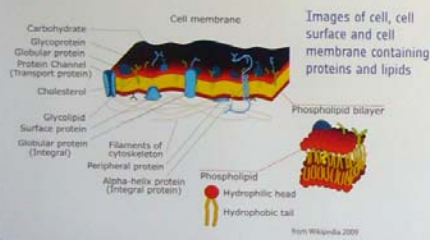
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Introduction

- Characterization of cell surfaces is essential for understanding cell-cell and cell-drug interaction
- Aim: Membrane protein detection, localization and differentiation of colon cancer cells (HT29) *in vivo* using a Tip-enhanced Raman spectroscopy (TERS) back reflection setup
- First step: → membrane protein detection on fixed HT29 cell surfaces
- Next step: → Protein detection *in vivo*

Cell surface overview

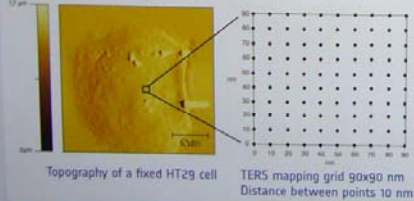


TERS set-up

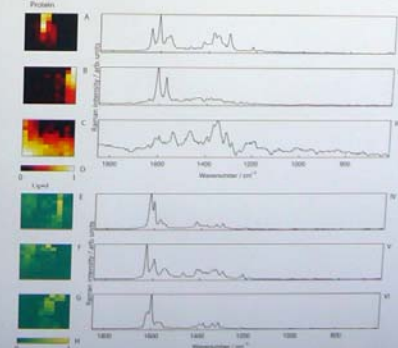
- JPK NanoWizard I
- Horiba JobinYvon LabRam
- Coherent Laser
 - @530 nm
 - power 970 μW
 - 5s acquisition time



Atomic force microscopy (AFM)



Multispectral data unmixing of TERS spectra with N-FINDR



Results

- TERS mapping on fixed HT29 cells has been achieved for the first time
- TERS provides:
 - estimated signal enhancement factor of at least 3.2×10^6
 - 18 fold shorter acquisition time compared to common Raman
 - better signal-to-noise ratio compared to common Raman
- Localization and distinction of protein and lipid sites achieved
- Structural protein characteristics (e.g. α -helix or β -sheet) and conformational changes detectable

Outlook

- TERS mapping on cells *in vivo*
- Differentiation of several membrane proteins and lipids
- Cell membrane protein diagnostic in real time during cell-drug interaction

- N-FINDR[®] analysis
 - unmixing spectra based on a set of component spectra (endmembers)
 - finds the most pure spectra [I-VI]
 - shows distribution of the spectral constituents in each endmember [A-H]
- Determination of protein sites
 - complete spectrum assigned for determination
 - still no single protein spectrum

Protein		Lipid	
Wavenumber / cm ⁻¹	Assignment	Wavenumber / cm ⁻¹	Assignment
1641-1631	Amide I	1731-1725	C=O
1574-1481	Amide II	1652-1599	C=C
1350-1232	Amide III	1337-1302	CH
1004	Phe	1275	PO

© 2008, Michael E. York. Multispectral Spectral End-member Determination by Nonnegative Factorization. Proceedings of the 16th International Conference on Artificial Intelligence Systems, Toronto, May 21, pp. 207-216. Springer, NY, 2008.

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 The research project "Merkelzell Diagnostik mit Nanosensoren/AFM" (0310022/B and 0310022/C) is financially supported by the Federal Ministry of Education and Research (BMBWF) Germany.

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