application note

Lise-Meitner-Straße 6, D-89081 Ulm, Germany
Tel. +49 (0) 731 140 700, Fax. +49 (0) 731 140 70200
www.witec.de, info@witec.de

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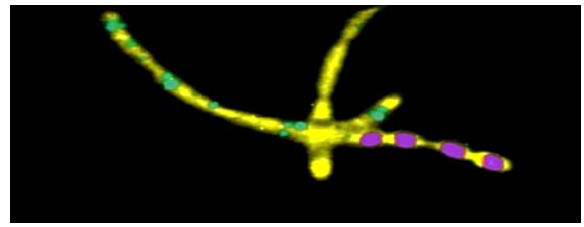
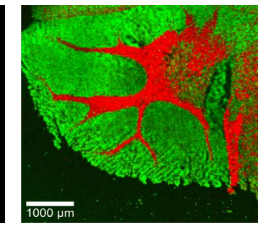
Confocal Raman Imaging in Life Science - Living Cells, Bacteria & Tissues

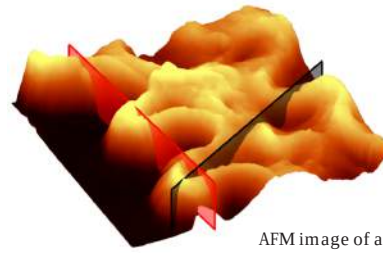
From measurements in liquids, to solid samples and soft tissues in life science research various samples are analysed on a regular basis. Through their convenient handling and versatile analysing possibilities all WITec microscope systems are particularly well-suited for life science applications: The flexible WITec imaging tools provide the opportunity to adjust the imaging technique to varying requirements and the powerful WITec software enables the comprehensive evaluation of the acquired data and the generation of depth profiles and 3D images.



Confocal Raman image of Bacillus Cereus.



Confocal Raman image of hamster brain tissue.



AFM image of a murine cardiac valve.

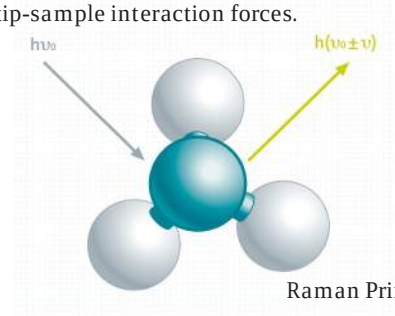
Working Principles

Confocal Raman Imaging

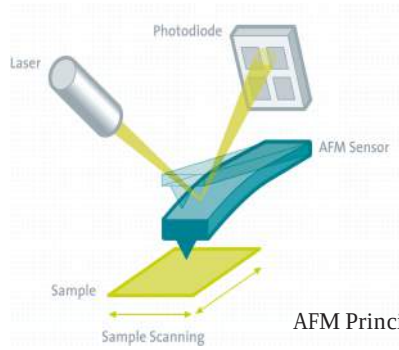
A Raman spectrum shows the energy shift of the excitation light (laser) as a result of inelastic scattering by the molecules in a sample. The excitation light excites or annihilates vibrations of the chemical bonds within the molecules which results in an energy shift of the photon scattered from this molecule. Different chemical species consist of different atoms and bonds, so each molecule can be easily identified by its unique Raman spectrum. As only molecular vibrations are excited (or annihilated), Raman spectroscopy is a nondestructive technique. In Raman imaging the Raman spectra are collected with a high-throughput confocal microscope/Raman spectrometercombination. A high-sensitivity CCD camera connected to a powerful computer and software system is used to detect the Raman signal. With specialized software tools the imaging capabilities can be expanded even further. For example, it is possible to generate images by integrating over selected spectral areas, determining the peak width, peak position or by even more sophisticated procedures such as the fitting of complete spectra or cluster analysis.

Atomic Force Microscopy (AFM)

In Atomic Force Microscopy the sample is scanned under the tip using a piezo-driven scanning-stage and the topography is displayed as an image. Atomic Force Microscopy provides spatial information parallel and perpendicular to the surface with resolution in the nm range. In addition to topographic high-resolution information, local material properties such as adhesion and stiffness can be investigated by analyzing the tip-sample interaction forces.



Raman Principle



AFM Principle

Raman-AFM Microscope alpha300 RA

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Lise-Meitner-Straße 6, D-89081 Ulm, Germany
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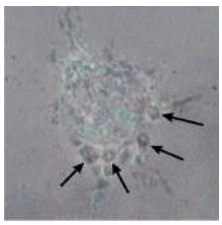
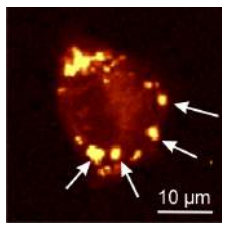
Living cells

To study living cells in their physiological surroundings without damaging them is a highly sought after capability in life science. The alpha300 combines such a nondestructive method with the cabability to identify chemical components inside a cell.

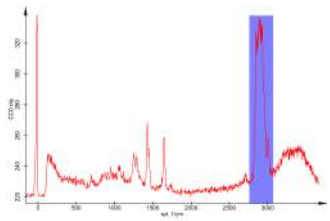
In this experiment, epithelial rat cells were investigated with the Spectral Imaging Mode of the alpha300 R. A spectrum at every pixel was taken (Scan Range: 40 x 40µm, 100 x 100 pixel, 10 000 spectra) using a 60 x Nikon (NA=1.0) water objective. The sample was excited with a 10 mW power 532 nm frequency doubled Nd: YAG laser.
Using the integrated video camera, a suitable cell was found (A). The Raman image in (B) shows the integral intensity of the CH-stretching band as indicated by the blue rectangle in (C) which shows one of the 10 000 spectra, acquired with an integration time of 100 ms. According to literature data, proteins and lipids can be identified by particular Raman bands.
In order to optimize the signal to noise ratio of the Raman images, a software tool performs a "fit procedure": From a small region of interest, a "basic spectrum" is generated by averaging all spectra in this particular region. This basic spectrum can be fitted to the measured spectra of the complete measurement, resulting in an optimized image.
The three spectra in (D) represent basic spectra of different regions of the cell with their corresponding images.
The blue spectrum corresponds to the mitochondriae. The green spectrum is the generated basic spectrum of the ER region (endoplasmatic reticulum). The basic spectrum of the nucleoli region is shown in red.
To distinguish the components of the cell, the three images generated by the fit procedure were illustrated with different colors and combined in one image (E). The blue features represent the mitochondriae and the red areas show the nucleoli inside the nucleolus. The endo-plasmatic reticulum and other different parts of the cell are clearly visible. Even the nuclear membrane is well-defined. Using this method, a "color-labelled" image can be created without dyeing the sample.



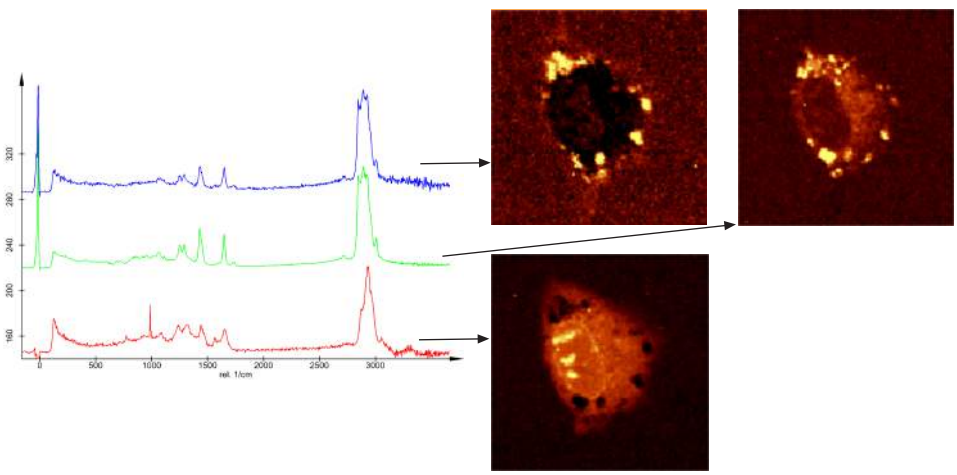
(A) Video Image



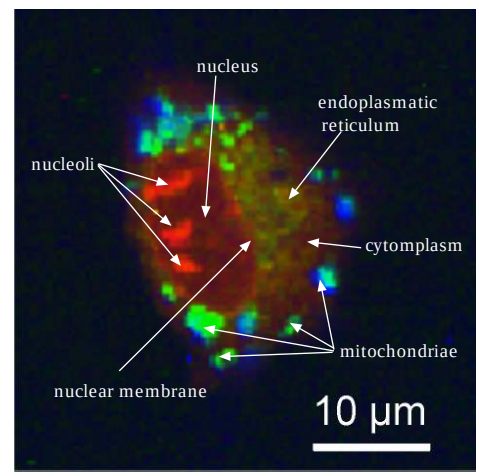
(B) Intensity in the CH-stretching band (2800/cm-3000/cm)



(C) One of the 10, 000 spectra



(D) Determination of basis spectra with corresponding images



(E) Color-coded image of the different regions of the cell.

Samples courtesy of Dr. Angelika Rück, ILM, Ulm, Germany

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Tel. +49 (0) 731 140 700, Fax. +49 (0) 731 140 70200
www.witec.de, info@witec.de

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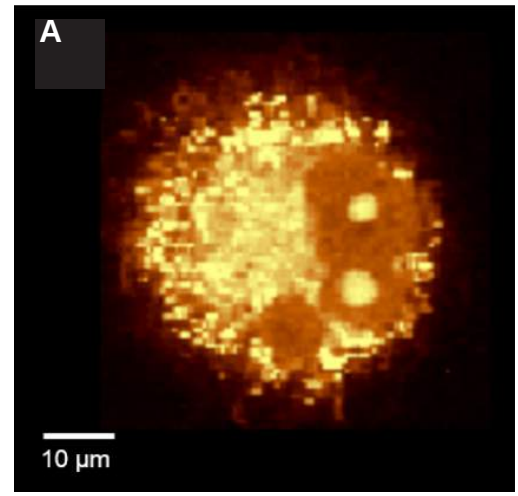
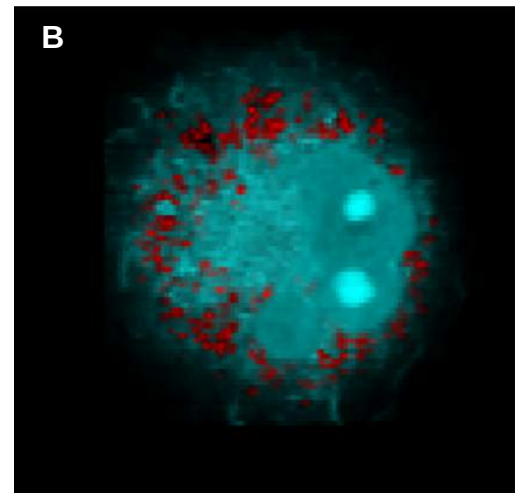
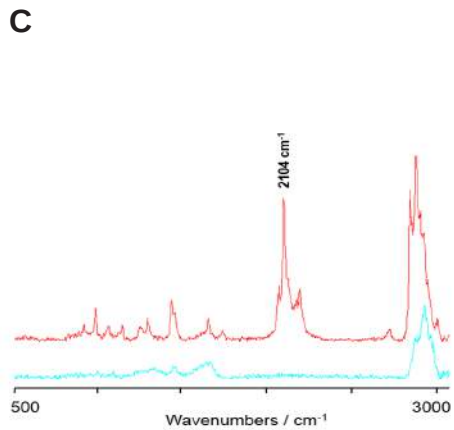
Investigating the intracellular components

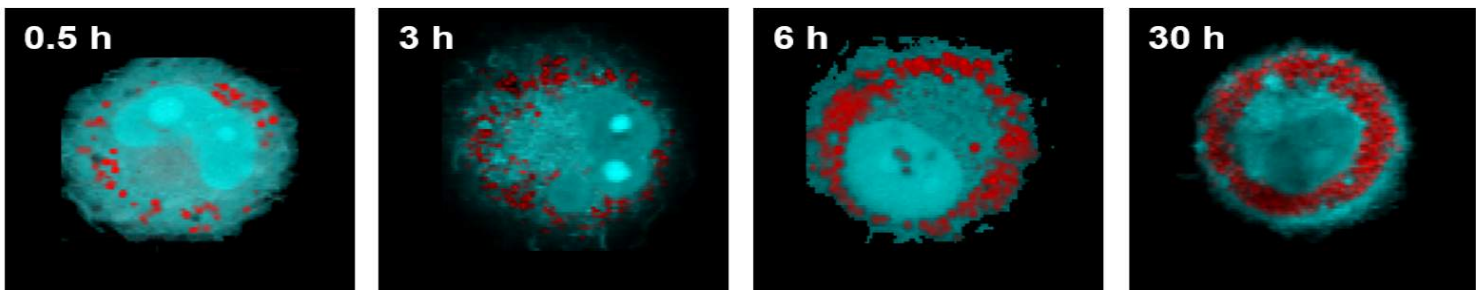
Confocal Raman microscopy was used to image intracellular components of macrophages. Macrophages take up low-density lipoproteins (LDL) for recycling and re-movement. If their export capabilities are overextended, macrophages store lipids and develop into foam cells. These foam cells contribute to cardiovascular diseases such as arteriosclerosis.
The subcellular lipid distribution of an in vitro THP-1 monocytes cell model was analyzed. The monocytes were differentiated into macrophages, incubated with deuterium labeled lipids and examined with the confocal Raman microscope alpha300 R with a 60x/NA=1.0 water immersion objective, an excitation wavelength of 488 nm, and 5mW laser power at the sample. For the lipid uptake quantification of each cell, the CD (2050-2275 cm^{-1}) to CH (2800 – 3020 cm^{-1}) ratio of the Raman scattering intensities were evaluated. Thereby the CD-stretching vibrations were used as marker for intracellular lipids, while the CH-stretching vibrations reflects the general density of the cells composition. A complete Raman spectrum was acquired at every image pixel. The WITec Project software was used for data evaluation and processing. For further information please refer to the figure legends.







(A) Raman image of a macrophage cell incubated with 400 µM of oleic acid for 3 hours, generated from the CH- stretching intensities. The image was recorded with 488 nm excitation using a 60x/NA=1.0 water immersion objective at a step size of 0.5 µm. (B) shows a Raman image reconstructed using a spectral unmixing algorithm, which decomposes the data set of the image into the most dissimilar spectral components. The associated spectral information is plotted in (C). Clearly visible are the Raman signals that originate from the CD stretching vibrations around 2104 cm^{-1}.



Raman images of macrophage cells incubated with 400 µM of oleic acid for different periods of time. After 30 hours macrophages store excessively lipids and develop into foam cells.

Images courtesy of Dr. Christian Matthäus, Leibniz-Institut für Photonische Technologien, Jena, Germany

Further reading:

C. Matthaeus, C. Krafft, B. Dietzek, B. R. Brehm, S. Lorkowski, J. Popp, Noninvasive imaging of intracellular lipid metabolism in macrophages by Raman microscopy in combination with stable isotopic labeling. Analytical chemistry 84, 8549-8556 (2012)10.1021/ac3012347).

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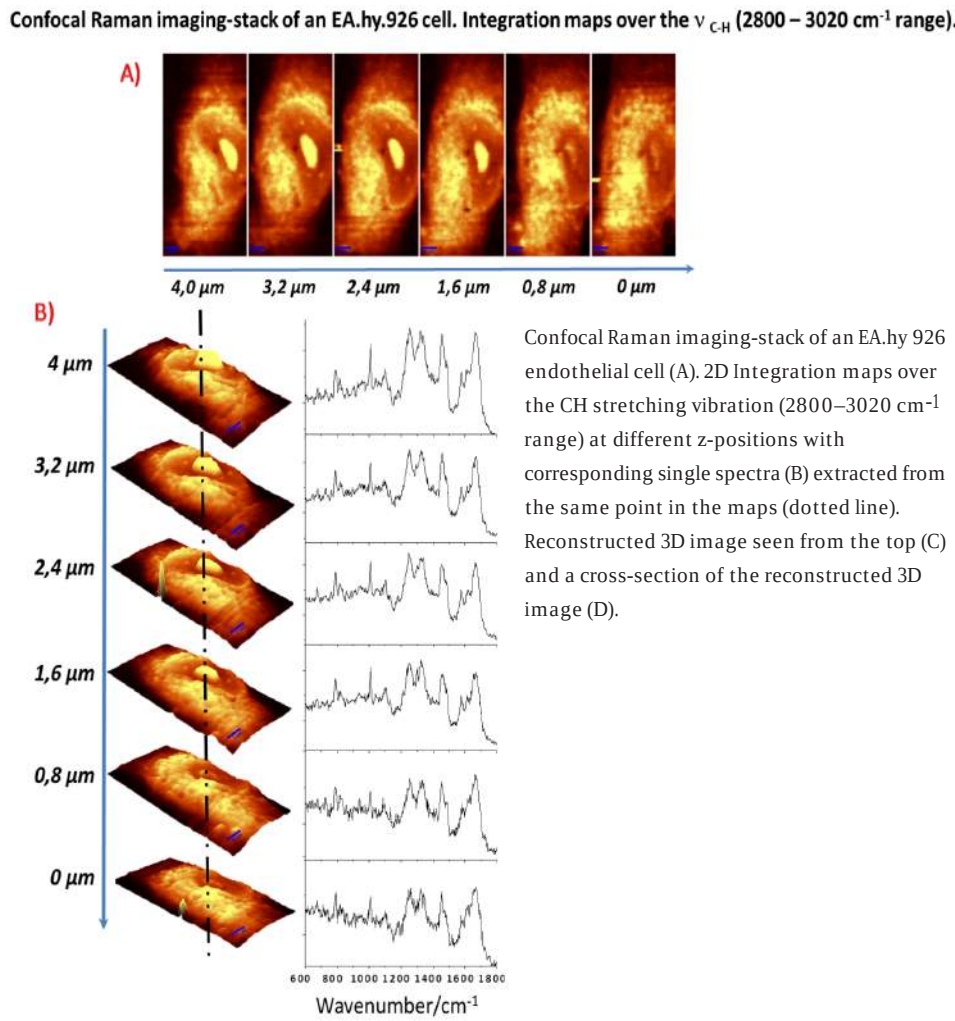
Lise-Meitner-Straße 6, D-89081 Ulm, Germany
Tel. +49 (0) 731 140 700, Fax. +49 (0) 731 140 70200
www.witec.de, info@witec.de

3D confocal Raman imaging of endothelial cells

In this study Raman imaging was used to three dimensionally study the heterogeneity of single endothelial cells and to define size, volume, shape and biochemical composition of the cellular organelles. The ability of confocal Raman imaging to construct 3D maps without disrupting the spatial integrity of the cell provides a unique insight into biochemical architecture and cellular processes of endothelium. 3D confocal Raman imaging can be obtained through subsequent measurement of several sample layers. And can be used e.g. for the early diagnosis of cancer by detecting subtle biochemical changes in cells and tissues associated with cancer development and progression or for morphological analysis of a tumor.
An alpha300 R confocal Raman microscope with a 60x water immersion objective for cells and an excitation wavelength of 532 nm was used for this study. The data acquisition was controlled by the WITec Project software package. All spectra were baseline corrected using a polynomial of degree 3 and the routine procedure for removal of cosmic rays was applied. The Image J processing program was applied to obtain 3D pictures of cells and tissues. For further information please refer to the figure legends.



Confocal Raman imaging-stack of an EA.hy 926 endothelial cell (A). 2D Integration maps over the CH stretching vibration (2800–3020 cm^{-1} range) at different z-positions with corresponding single spectra (B) extracted from the same point in the maps (dotted line). Reconstructed 3D image seen from the top (C) and a cross-section of the reconstructed 3D image (D).

Images courtesy of Prof. Malgorzata Baranska, Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Krakow, Poland

Further reading:

K. Majzner, A. Kaczor, N. Kachamakova-Trojanowska, A. Fedorowicz, S. Chlopicki, M. Baranska, 3D confocal Raman imaging of endothelial cells and vascular wall: perspectives in analytical spectroscopy of biomedical research. Analyst 138, 603-610 (2012); (10.1039/c2an36222h).

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Lise-Meitner-Straße 6, D-89081 Ulm, Germany
Tel. +49 (0) 731 140 700, Fax. +49 (0) 731 140 70200
www.witec.de, info@witec.de

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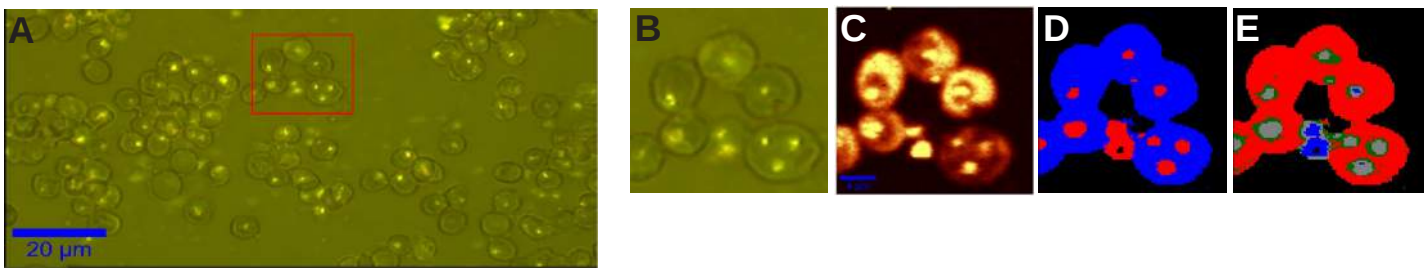
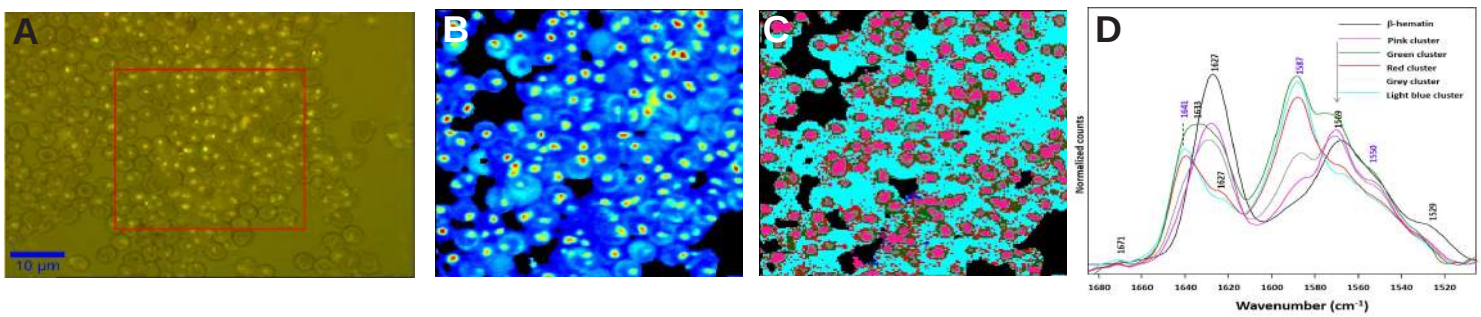
Raman microscopy in malaria diagnosis

Raman Microscopy can be applied as rapid and accurate diagnostic tool for malaria. Malaria is caused by the parasite Plasmodium that infects human blood cells. In the blood cells it catabolizes large amounts of haemoglobin into an insoluble bio-mineral known as haemozoin (malaria pigment). Besides serological antigen detection, this pigment is widely used for malaria diagnosis. A common microscopic method to detect haemozoin in malaria diagnosis is the dark-field microscopy. Through dark-field microscopy haemozoin is presented as bright intracellular spots. The combination of dark-field and Raman microscopy enables the comprehensive characterization of these spots and provides information about the stage of the disease.

In the following study Raman spectroscopy was applied to detect and image small inclusions of haemozoin in Plasmodium falciparum infected red blood cells. Therefore the confocal Raman microscope alpha300R with a 532 nm Nd:YAG laser and a 60x/NA=1.0 water immersion objective was used. The presence or absence of haemozoin was detected by the strong 1569cm^{-1} Raman band, which was used as marker band for haemozoin. After data acquisition the spectral data was further processed with the WITec software. A cluster analysis was applied in order to facilitate the identification of haemozoin within the cells and to enable the classification of the different stages of disease. A cluster analysis is suitable for the automatic identification of similar spectra and the classification of multi-spectrum data into a user-defined number of clusters. Thus color-coded, user-selected clusters images can be generated. The Raman results were compared with the dark-field micrographs in order to verify the analyzing method. For more details please refer to the figure legends.



Verification of the method by comparison of dark-field and Raman microscopy (A) Visible micrograph showing the partial dark-field effect lighting up haemozoin deposits in several parasite infected cells. (B) Zoom-in of the red square in (A): The infected erythrocyte towards the bottom right corner is triply infected. (C) Chemical Raman map generated by integrating the region between 1700 and 1500 cm^{-1}. The lighter color shows regions of stronger counts. (D and E) Unsupervised Hierarchical Cluster Analysis (UHCA) map generated for the 1700–1500 cm^{-1} range for 2 clusters (D) and for 5 clusters (E). The haemozoin particles can be clearly indentified by Raman cluster analysis.



Identification of haemozoin in different stages of disease (A) Visible partial dark-field micrograph of a thick film of malaria infected cells showing the malaria pigment haemazoin as light intracellular dots (B) Chemical map of the 1569 cm^{-1} Raman band of approximately the area bounded by the red square in (A). The yellow and red colors show the haemozoin deposits in the blood cells. (C) Cluster analysis performed using the range between 1700 cm^{-1} and 1300 cm^{-1} showing 5 clusters. The pink cluster correlates to the haemozoin deposits within late-stage of infected blood cells. The green and grey clusters are a mixture of haemoglobin and haemozoin. The light blue cluster correlates well with haemoglobin within the cell while the red-sub-micron sized dots (300 nm) appear to be inclusions of haemozoin observed in cells of different infection stages. (D) Mean extracted spectra from 5 cluster showing the region between 1600 and 1500 cm^{-1}. The colors correspond to (C) Note the differences in the red and light blue spectra. The strong shoulder on the pink spectrum centered at 1569 cm^{-1} indicates that the sub-micron-dots observed in (C) and are from inclusions of haemozoin.

Images courtesy of Prof. Bayden Wood, Centre for Biospectroscopy, School of Chemistry, Monash University, Clayton, 3800, Victoria, Australia

B. R. Wood, A. Hermelink, P. Lasch, K. R. Bambery, G. T. Webster, M. A. Khiavi, B. M. Cooke, S. Deed, D. Naumann, D. McNaughton, Resonance Raman microscopy in combination with partial dark-field microscopy lights up a new path in malaria diagnostics. The Analyst 134, 1119-1125 (2009)10.1039/b822603b).

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Lise-Meitner-Straße 6, D-89081 Ulm, Germany
Tel. +49 (0) 731 140 700, Fax. +49 (0) 731 140 70200
www.witec.de, info@witec.de

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Bacteria

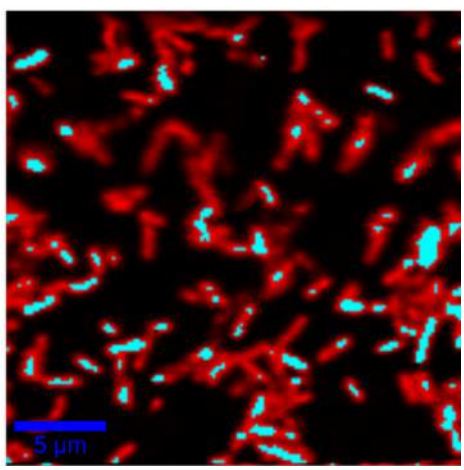
It has been reported that Raman Microscopy can be used to classify bacteria by their individual Raman spectra down to the sub-strain level. A high-resolution Raman imaging system also allows a single bacterium to be evaluated for metabolic products or drug detection inside the bacterium or to distinguish the intra- and inter-cellular heterogeneity. In the following study, Legionella Bozemanii and Bacillus Cereus are investigated with an alpha300 R Confocal Raman Imaging system at the single cell level.

Legionella Bozemanii

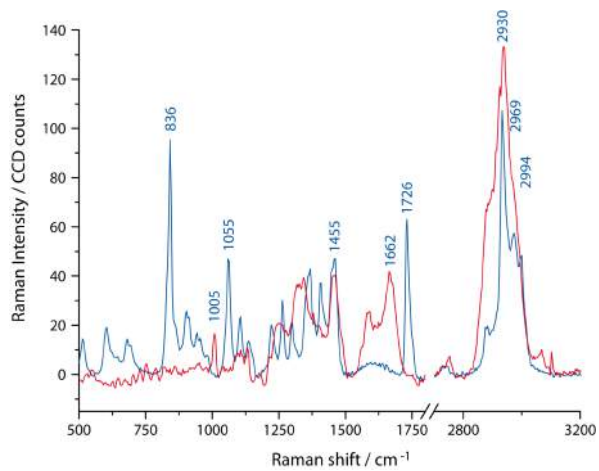
Legionella strains can produce Poly-ß-hydroxybutyric acid (PHB) in response to physiological stress, which serves as an energy storage molecule that can be detected with Raman spectroscopy. In this experiment a Raman image was acquired with a scan range of 25x25 µm. (A) shows the resulting color-coded Raman image. The blue area corresponds to the integral intensity of the C=O ester stretching band at 1726/cm. The red area depicts the cell body imaged using the protein amide I band at 1662/cm. The corresponding Raman spectra are shown in (B). The Raman image clearly reveals that the bacteria cells can contain different levels of PHB. Cells that contained little or no PHB could be discriminated from cells with a very high concentration.

Bacillus Cereus

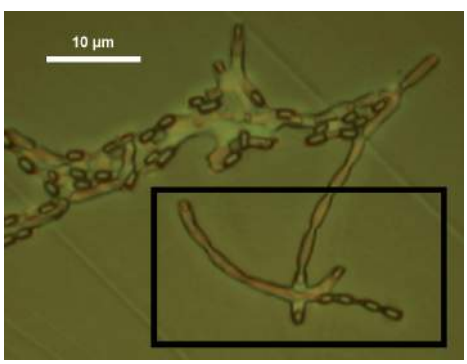
In a second experiment Raman Imaging was performed on Bacillus Cereus. This strain is also able to produce PHB as a storage material, accumulating it as intracellular granules. (C) shows the video image indicating the region where the Raman image was acquired, which is shown in (D). The Bacullus strain used here is also able to form three different cellular components which can be observed in the Raman image: Vegetative cells without PHP (yellow) and vegetative cells incorporating traces of PHB (green). Additionally, Bacillus Cereus can form spores after entering the stationary phase of growth and accumulation of PHB. In the Raman image four spores can be seen at the right edge of the chain (magenta). The fact that all three components can be detected together highlights the diagnostic power of Confocal Raman Imaging for identifying phenotypic heterogeneity at a single cell level.



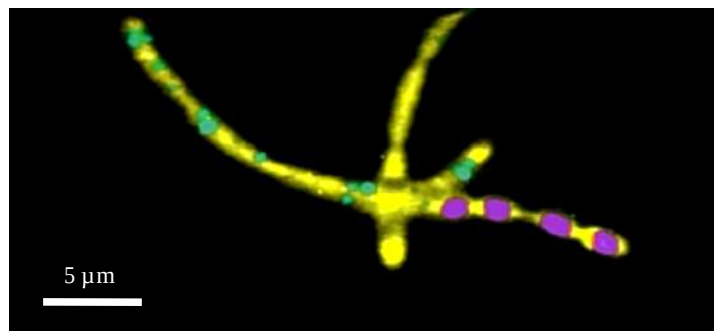
(A) Raman Image of Legionella Bozemanii



(B) Corresponding Raman spectra: Red: Vegetative cells (red) and PHB (blue).



(C) Vido image of Bacillus Cereus



(D) Raman Image of Bacillus Cereus as indicated in (C). Vegetative cells (yellow), PHB (green), spores (magenta).

Images courtesy of Dr. Antje Hermelink, Robert-Koch-Institute, Berlin, Germany; Further reading: Hermelink et. al., Analyst, 2009, 134, 1149-1153

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Lise-Meitner-Straße 6, D-89081 Ulm, Germany
Tel. +49 (0) 731 140 700, Fax. +49 (0) 731 140 70200
www.witec.de, info@witec.de

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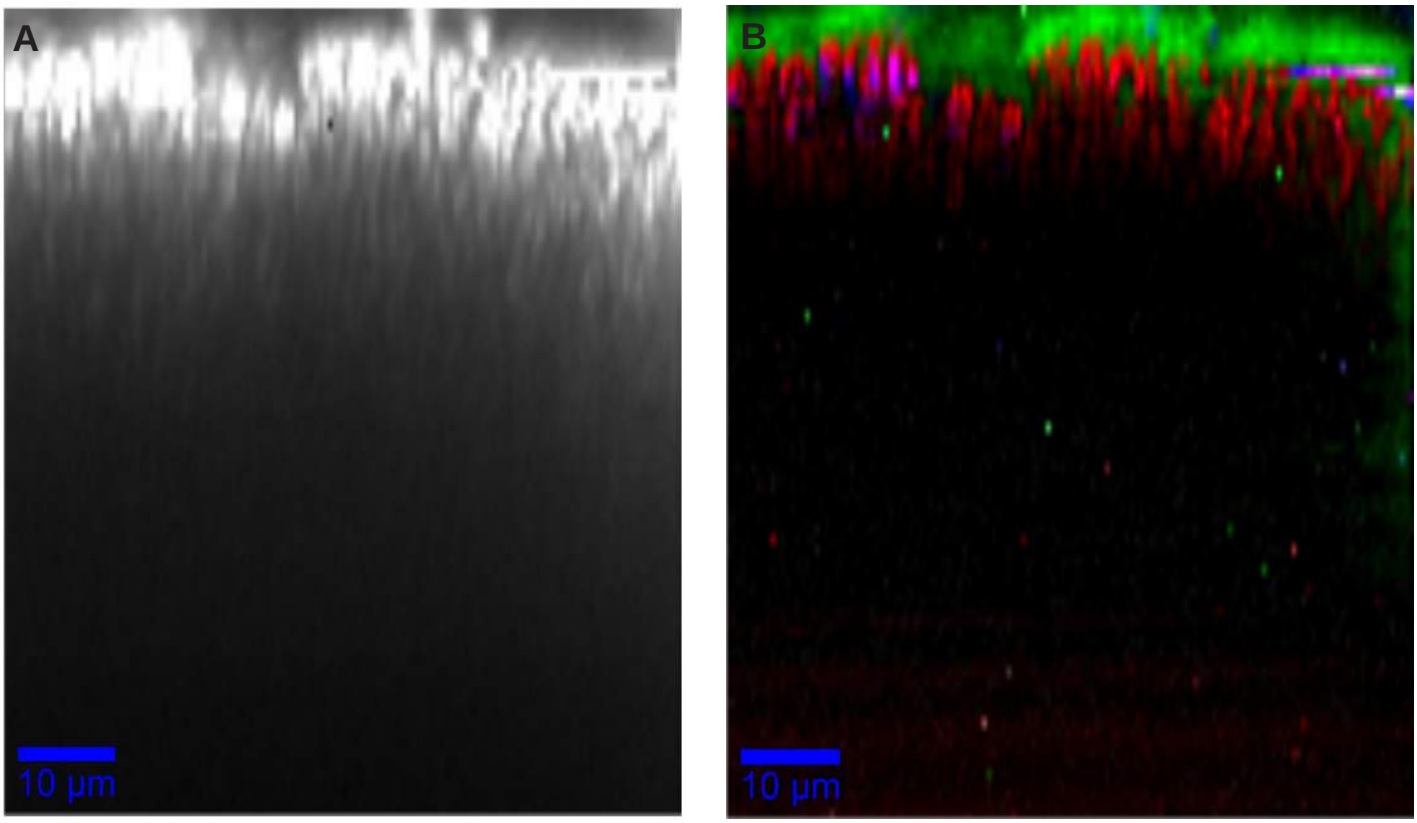
Imaging of bacterial biofilms

In this confocal Raman study complex microbial aggregates formed of dense, highly hydrated, highly structured clusters of bacterial cells are analyzed and imaged. With confocal Raman microscopy it was possible to achieve an in-depth analysis of the targeted microbial communities without additional, more invasive techniques or prior knowledge of the sample being required. A WITec confocal Raman microscope with a 60x/NA=1.0 water immersion objective, and 532 nm excitation wavelength was used to acquire x-z-Raman depth profiles. Different bacterial microcolonies could be distinguished and visualized. The spatial resolution was 350 nm laterally (x-direction) and 2 µm in z-direction. For further information please refer to the figure legend.



Spectral imaging based on (resonance) Raman imaging focused on the heme moiety in cytochrome-c. Set of two spectral images cutting through the outer layer of a nitrifying biofilm granule, revealing a dense multilayer of different bacterial microcolonies: two types of Nitrosomonas (green and red) and one unidentified species (blue). (A) full spectral intensity as recorded (B) Raman analysis of (A). Image dimensions: 140 x 80 spectra covering 70 x 80 µm^2 (x/z depth image).

Images courtesy of Dr. Ann-Kathrin Kniggendorf, Hannoversches Zentrum für Optische Technologien, Gottfried Wilhelm Leibniz Universität Hannover, Germany

Further reading:

A.-K. Kniggendorf, M. Meinhardt-Wollweber, Of microparticles and bacteria identification--(resonance) Raman micro-spectroscopy as a tool for biofilm analysis. Water research 45, 4571-4582 (2011)10.1016/j.watres.2011.06.007).

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Lise-Meitner-Straße 6, D-89081 Ulm, Germany
Tel. +49 (0) 731 140 700, Fax. +49 (0) 731 140 70200
www.witec.de, info@witec.de

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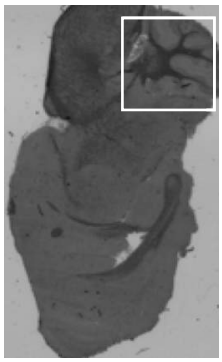
Tissues

Programmable Large Area Scan - Hamster brain cross section

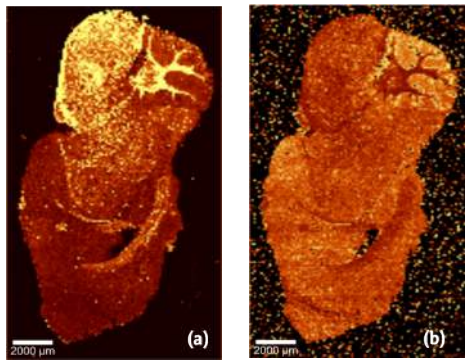
For Raman large-area scanning on biological tissues one quite often faces a variety of obstacles. For example, fluorescence might influence the quality of the Raman spectra or the sample might not be entirely flat over the complete scan range. In order to reduce the fluorescence, a bleaching sequence before acquiring the Raman spectra sometimes eliminates the fluorescence signal. To correct for insufficient sample flatness or tilt, one approach can be to measure the sample height at selected spots. These values can then be used to adjust the focus accordingly over the complete scan range. With the alpha500, bleaching and tilt compensation can be achieved by performing a programmable large-area scan.
In the following study a hamster brain cross section is moved under the laser beam and the focus position is set using the values from the predefined tilt compensation. At each image pixel a pre-bleaching sequence of 2 s is executed before the Raman spectrum acquisition which took 1 s.

In (A) a video image of the complete brain cross section can be seen. (B) shows the corresponding Raman image after evaluating the integral intensity of the CH-stretching band (a) and its center of mass (b), respectively. A second large-area scan was performed at the area indicated in (A). The results are depicted in (C) - (E) showing different images of the gray and white brain matter of that region with the corresponding spectra. This can be achieved by evaluating the Raman imaging data in more detail.

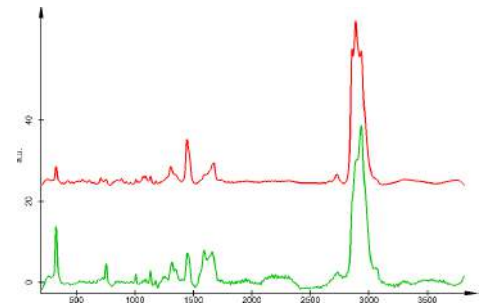
Following the large-area scans a high resolution zoom-in image was acquired at the position indicated in (C), revealing differences in the chemical properties on the sub-micron scale. (F a+b)



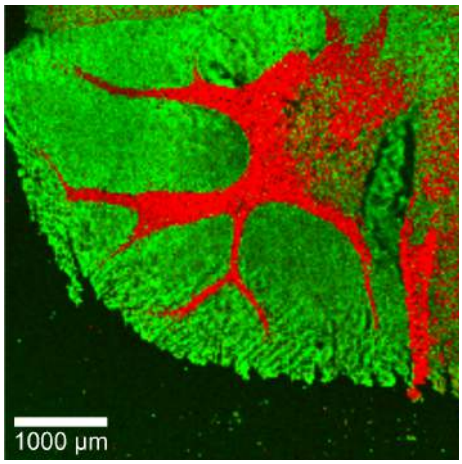
(A) Video Image



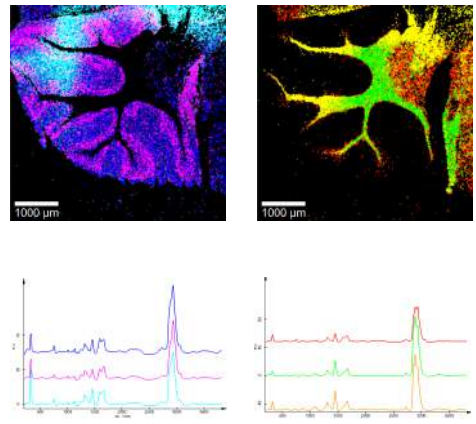
(B) Raman Images of the complete brain cross section; (a) Intensity of the CH-stretching band (2800/cm-3000/cm) (b) Center of Mass of the CH-stretching band



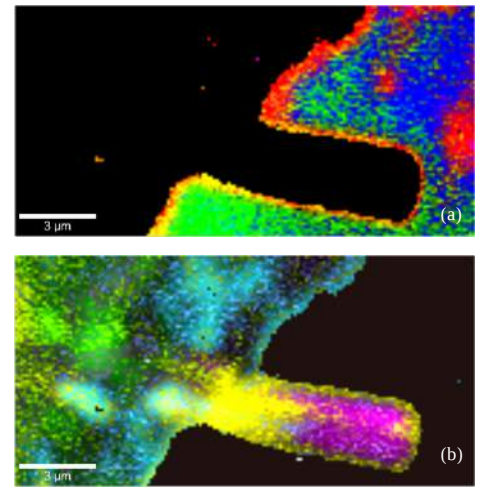
(D) Raman spectra of the gray (green) and white (red) brain matter used for the image generation as shown in (C).



(C) Raman image of the gray (green) and white (red) brain matter as indicated in (A).



(E) Detailed evaluation of the gray (left) and white (right) brain matter using further characteristic spectral features of the individual material.



(F) Zoom-in image at the border position between gray and white brain matter as indicated in (C). It shows the sub-micron differentiation of chemical properties of the brain material.

Samples courtesy of Dr. Peter Lasch, Robert-Koch-Institute, Berlin, Germany

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Lise-Meitner-Straße 6, D-89081 Ulm, Germany
Tel. +49 (0) 731 140 700, Fax. +49 (0) 731 140 70200
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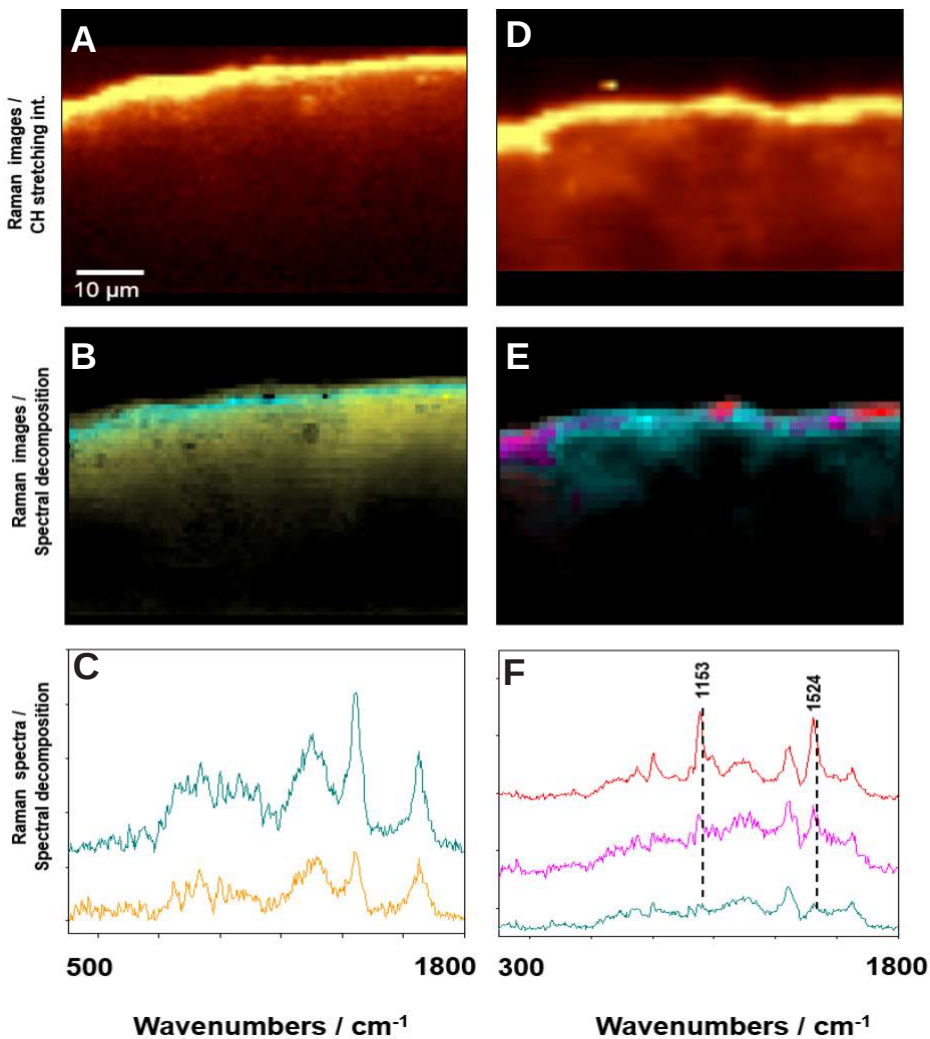
Depth profiles of human skin

In the following study the transdermal delivery of pharmaceutical agents was investigated with Raman depth profiles of human skin tissue. Skin penetration experiments were performed by applying beta-carotene to the skin biopsies. After incubation x-z-Raman profiles of the intact full thickness skin biopsies were generated and spectral images were acquired using an alpha300 R+ microscope with a 50x 0.9NA objective and 785 nm excitation wavelength. Depth profiles were collected employing the x-z-scan modus starting at the bottom end of the image. The spectral information in the images highlight the different components of the tissue and the presence of the agents. Presented are Raman images of untreated skin and diffusion patterns for beta-carotene. For further information please refer to the figure legend.



Raman depth profile images of human skin. Images where collected using a 785 nm excitation at a step size of 1 µm. (A) and (B) show Raman images of normal untreated skin(B) was preprocessed using a spectral unmixing algorithm. The associated spectra plotted in (C) exhibit the typical protein characteristic peaks at 1650 – 1800 cm^{-1}. The Raman images (D) and (E) show depth profile images of skin treated with a beta-carotene formulation. The distribution of beta-carotene is plotted in red and magenta. The associated spectra plotted in (F) show the characteristic Resonance Raman bands of beta-carotene at 1524 and 1153 cm^{-1}.

Images courtesy of Dr. Christian Matthäus, Leibniz-Institut für Photonische Technologien, Jena, Germany

Further reading:

M. Ashtikar, C. Matthaus, M. Schmitt, C. Krafft, A. Fahr, J. Popp, Non-invasive depth profile imaging of the stratum corneum using confocal Raman microscopy: First insights into the method. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 50, 601-608 (2013); (10.1016/j.ejps.2013.05.030).

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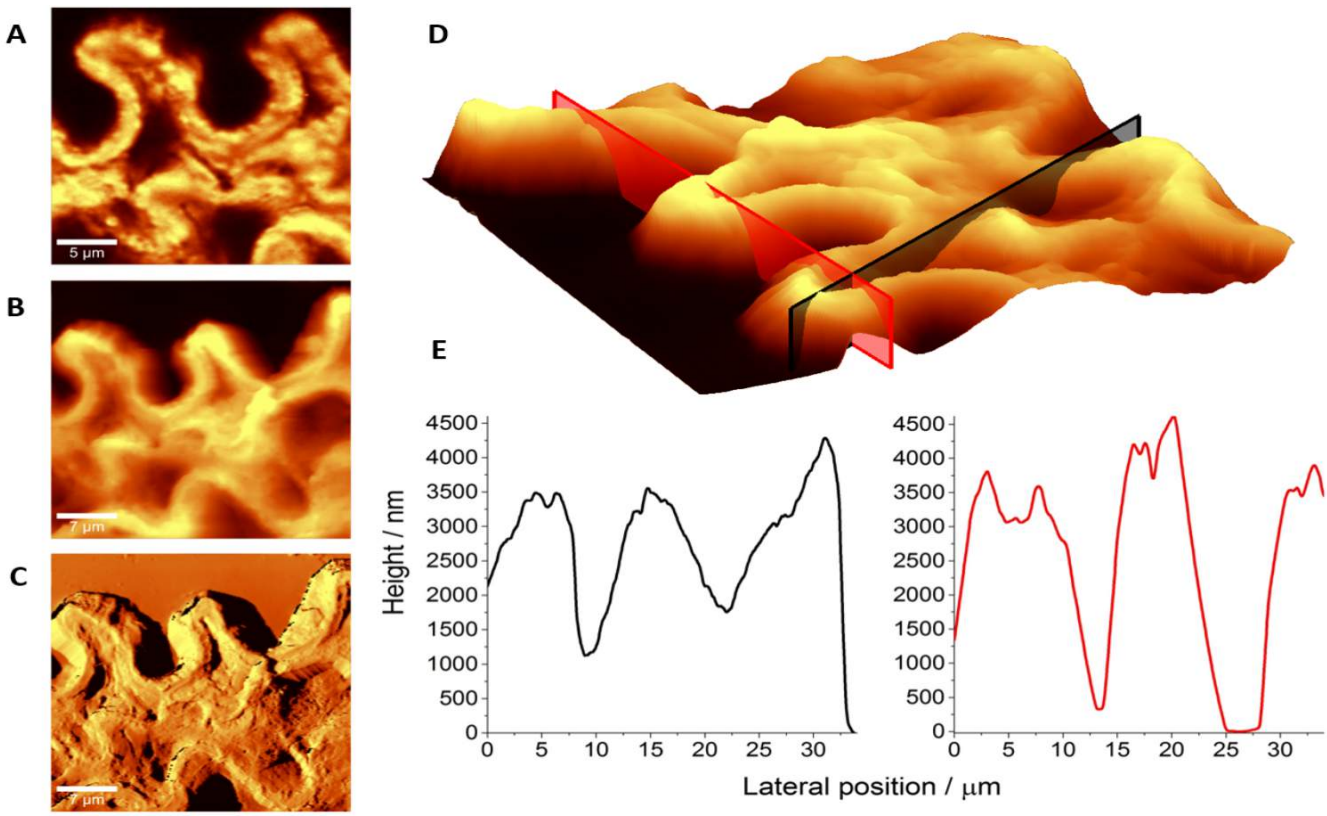
Lise-Meitner-Straße 6, D-89081 Ulm, Germany
Tel. +49 (0) 731 140 700, Fax. +49 (0) 731 140 70200
www.witec.de, info@witec.de

Imaging of vessels with combined Raman-AFM

Vessels consist of three main parts including tunica intima, media, and adventitia. Changes within the vessel tissue structure and biochemical composition can indicate pathological alterations of the tissue, especially in endothelial cells. Those changes can be symptoms of vessel diseases e.g. diabetes, hypertension and atherosclerosis. Immunohistochemical (IHC) staining is a well-established method in pathology to analyze the cellular compartments of a sample. While for IHC staining the sample is treated with fluorescent dyes, Raman spectroscopy distinguishes specific tissue and cell constituents by integration of the appropriate Raman band. In many cases the information obtained with these two methods is the same.

In the following study, an alpha300 RA fully integrated Raman-AFM microscope was used to investigate murine vessels. The Raman-AFM cross-section analyses of the vascular wall status are useful to monitor ex vivo disease progression and effects of treatment in experimental diabetes, atherosclerosis or hypertension. While Raman spectroscopy provides specific biochemical information about the sample composition, AFM (Atomic Force Microscopy) detects the topography, structure, and physical properties, e.g. stiffness, adhesion, of the sample's surface. Thus combined Raman-AFM analyses are useful for a comprehensive characterization of the vessel status and both pathological and physiological conditions can be investigated and distinguished. In the following figure an exemplary Raman-AFM analysis of a vessel wall cross section is shown. For further information please refer to the figure legend.



The images of the vessel wall cross section: Raman distribution image of organic vessel specimens (A). AFM AC (intermittent or tapping mode) images of the vessel topography shown in (B) and the phase image shown in (C). The phase image reveals e.g. the tissue stiffness. The 3D topography imaging (D) was used to position cross section (black and red lines). Through the cross section profiles (E) the thickness of the vessel walls can be determined. Data acquired with an alpha300 RA fully integrated microscope, 100x/NA=0.9 air objective, 532 nm excitation wavelength, 10 mW laser power at the sample. The integration time per Raman spectrum: 0.2 s. AFM topography image: AC (tapping) mode.

Images courtesy of Prof. Malgorzata Baranska, Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Krakow, Poland

Further reading:

M. Pilarczyk, A. Rygula, L. Matheuszuk, S. Chlopicki, M. Baranska, A. Kaczor, Multi-methodological insight into the vessel wall cross-section: Raman and AFM imaging combined with immunohistochemical staining. Biomedical Spectroscopy and Imaging, (2013)10.3233/bsi-130048).

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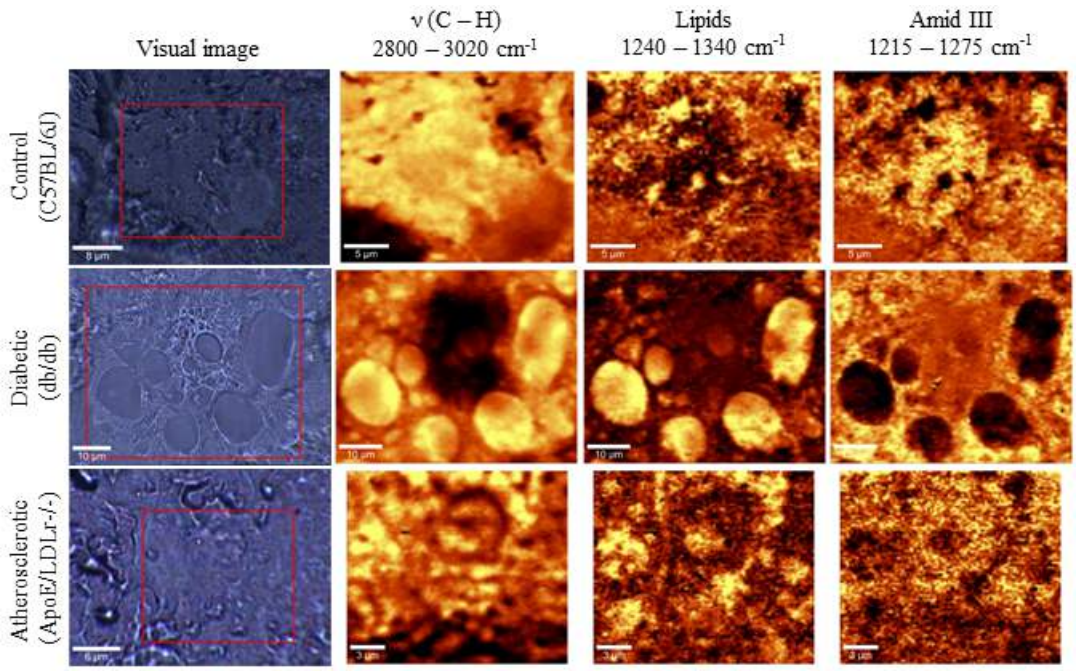
Lise-Meitner-Straße 6, D-89081 Ulm, Germany
Tel. +49 (0) 731 140 700, Fax. +49 (0) 731 140 70200
www.witec.de, info@witec.de

Detecting pathological changes in liver tissue by confocal Raman imaging

The liver is involved in most metabolic processes of the human body. Thus metabolic dysfunctions often affect the liver and cause altered tissue structures or composition. For instance a relation between diabetes mellitus type II and liver steatosis and cirrhosis has been described in literature. Furthermore liver dysfunctions may be associated with atherosclerosis. Raman spectroscopy has been widely applied to biological and biomedical samples as it has a number of useful advantages for studying such materials: Minimal or no sample preparation, non-destructively and the possibility to gain molecular information without the use of stains.

In the following study the biochemical alterations in diabetic and atherosclerotic tissue compared to normal liver tissue were investigated by confocal Raman imaging. Therefore liver tissues of atherosclerotic and diabetic mice models were used. The analyses were performed on a confocal Raman microscope alpha300 R equipped with a frequency-doubled Nd:YAG laser for 532 nm excitation and a 100x objective (NA=0.9). The Raman results were evaluated as follows: The Raman signal of the C-H stretching vibrations (2800 – 3020 cm^{-1}) originates from both lipids and proteins. To image saturated and unsaturated lipids the range between 1240 cm-1 and 1340 cm^{-1} was selected. The amide III region (1215 – 1275 cm^{-1}) was chosen to detect proteins. Thus protein- and lipid-rich regions within the liver tissue could be determined and distinguished. It could be shown that the lipid content in the diabetic mouse tissue was highest and relatively high in atherosclerotic mice. These data strengthen the assumption that liver steatosis and metabolic diseases such as diabetes or atherosclerosis are correlated.



A microphotograph of the cross-section of liver tissues taken from control (top), diabetic (middle) and atherosclerotic (bottom) mice. The red square indicates investigated areas (26.1 x 21 μm^2, 18.8 x 15.5 μm^2 and 19.2 x 16.7 μm^2 respectively). Raman maps of the lipid and protein distributions obtained by integration of the marker bands in the regions of: 3020–2800 cm^{-1} (lipids and proteins), 1240–1340 cm^{-1} (lipids) and 1215–1275 cm^{-1} (amide III, proteins). Scale bars as presented in each image.

Images courtesy of Prof. Malgorzata Baranska, Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Krakow, Poland

Further reading:

K. Kochan, K. M. Marzec, K. Chruszcz-Lipska, A. Jasztal, E. Maslak, H. Musiolik, S. Chlopicki, M. Baranska, Pathological changes in the biochemical profile of the liver in atherosclerosis and diabetes assessed by Raman spectroscopy. Analyst 138, 3885-3890 (2013); (10.1039/c3an00216k).

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Lise-Meitner-Straße 6, D-89081 Ulm, Germany
Tel. +49 (0) 731 140 700, Fax. +49 (0) 731 140 70200
www.witec.de, info@witec.de

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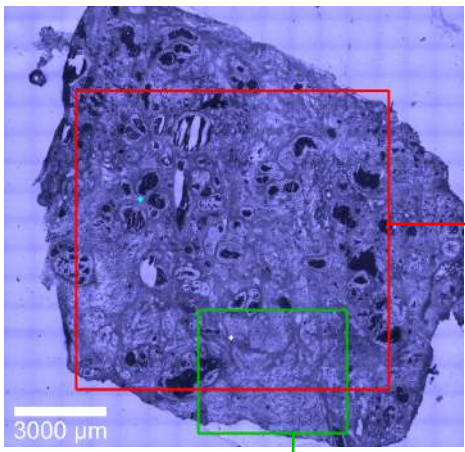
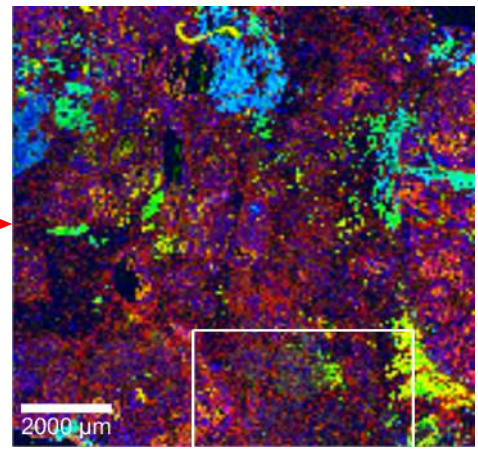
Histopathological human breast cancer tissue

For histopathological diseases or cancer detection, Raman imaging is considered to be a potential alternative for the currently used techniques, which require staining of the biopsy tissue before the manual microscopic investigation. As Raman imaging is a label-free characterization method for the sample's compounds, it is well-suited to generally accelerate the detection process or to establish automated and more reliable detection procedures. However, extended basic research must still be made in order to achieve clinical relevance for Raman imaging as a standard method for diagnosis.

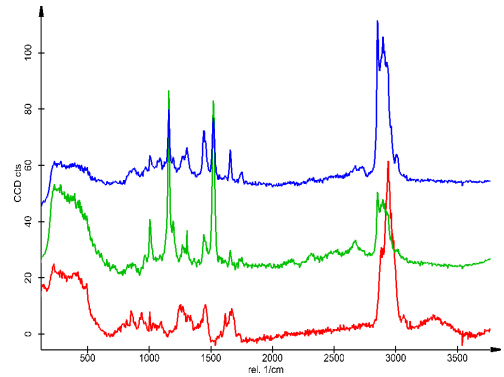
In the following study untreated histopathological breast cancer tissue was investigated with the WITec alpha500 Confocal Raman Microscope using its large-area scanning capabilities. (A) shows an overview video image of the sample. Based on this image, the scan ranges for the Raman imaging scans can be selected as indicated by the rectangles. The first large-area scan (red rectangle) was acquired in order to obtain an overview Raman image of the tissue sample followed by a second large area scan (green rectangle) at the area with potentially carcinogenic tissue. (B) - (D) show the resulting Raman Images and corresponding spectra. The imaging parameters for the first large-area scan are 10.2 mm x 9.7 mm, 150 x 150 pixel (= 22,500 Spectra). The scan range of the second scan was 4.85 mm x 4.00 mm and 150x150 pixels. Focusing on (D) reveals differences in the chemical composition of the tissue. Especially the tissue in the areal indicated with the orange area shows clear differences in the spectral characteristics (no red spectrum detectable). Using staining techniques (results not shown here) it could be shown that the carcinogenic tissue is located exactly at this area of the image. This results suggest, that a differentiation of healthy and carcinogenic tissue with Raman Imaging can be possible.



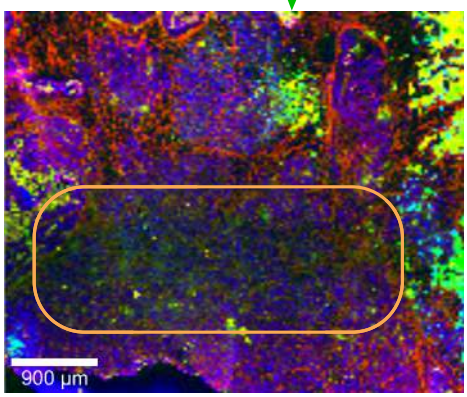
(A) Video Image of the unstained breast cancer tissue



(B) Large-area scan I (red rectrangle in A)



(C) Basis spectra used for image generation for the images in (B) + (D)



(D) Large-area scan II (green rectangle in A). The orange area indicates the location of the carcinogenic tissue.

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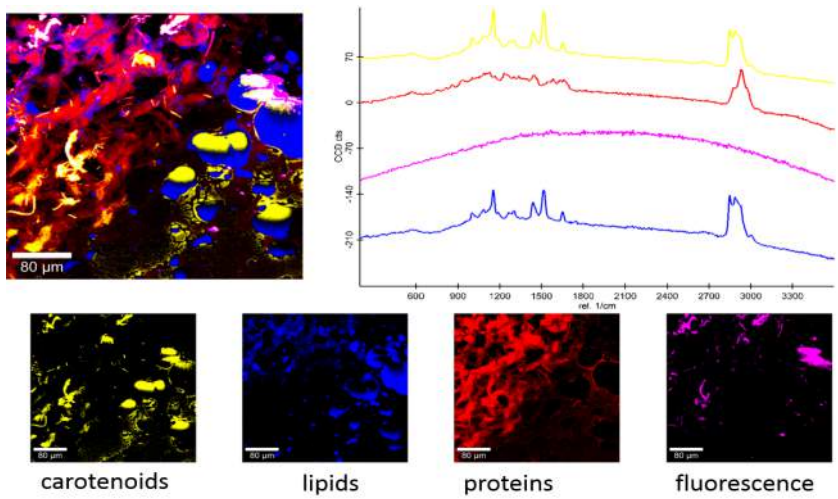
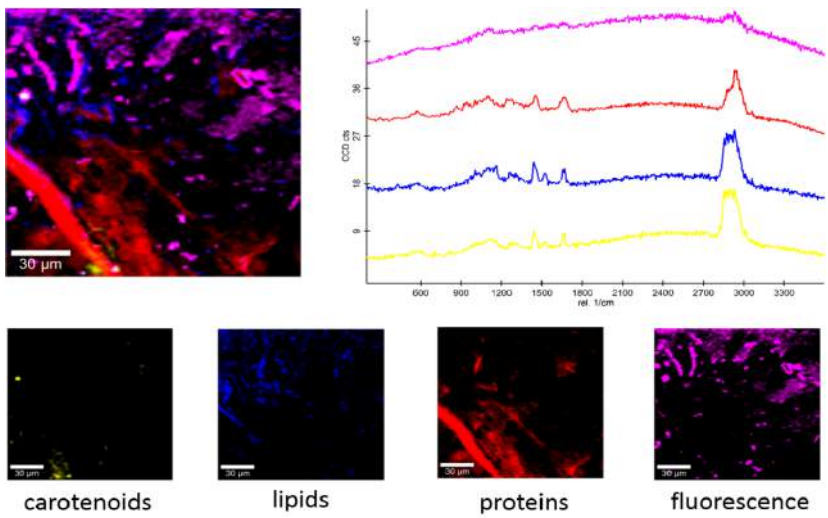
Comparison of noncancerous and cancerous tissue

Through confocal Raman imaging the general tissue composition as well as the appearance of tumor markers can be identified. Thus noncancerous and cancerous tissue can be distinguished and characterized without any external agent and stain or sample preparation prior to the experiments. In this study noncancerous and cancerous human breast tissue of the same patient was examined with an alpha300 R confocal Raman microscope, a 50x objective, and a 532nm excitation laser with 10 mW laser intensity at the sample. The WITec Project plus software was used for data acquisition and processing. The appearance of different tissue components was determined through their unique Raman spectra and the noncancerous and cancerous tissues could be distinguished by the altered appearance of carotenoids, lipids, and proteins. For further information please refer to the figure legends.



Breast tissue from the margin of the tumor mass: Raman image (350 x 350 mm,) integration time 0.03 sec, 1 accumulation; average spectra used for the basis analysis method, colors of the spectra correspond to the colors of the different areas in the Raman image; images for the filters for spectral regions: carotenoids (1490 – 1580 cm^{-1}), lipids (2850 – 2950 cm^{-1}), proteins (2900 – 3010 cm^{-1}), and fluorescence (2200 – 2300 cm^{-1}). Please note that carotenoids are clearly detectable in the tissue.



Breast tissue from the tumor mass (carcinoma ductale G3 infiltrans mammae): Raman image (150 x 150 mm,) integration time 0.03 sec, 1 accumulation; average spectra used for the basis analysis method, colors of the spectra correspond to the colors of the different areas in the Raman image; images for the filters for spectral regions: carotenoids (1490 – 1580 cm^{-1}), lipids (2850 – 2950 cm^{-1}), proteins (2900 – 3010 cm^{-1}), and fluorescence (2200 – 2300 cm^{-1}). Please note that the amount of detectable carotenoids is strongly reduced.

Images courtesy of Halina Abramczyk and Beata Brozek-Pluska, Lodz University of Technology, Institute of Applied Radiation Chemistry, Laboratory of Laser Molecular Spectroscopy, Wroblewskiego 15, 93-590 Lodz, Poland.

Further reading:

B. Brozek-Pluska, J. Musial, R. Kordek, E. Bailo, T. Dieing, H. Abramczyk, Raman spectroscopy and imaging: applications in human breast cancer diagnosis. The Analyst 137, 3773-3780

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Lise-Meitner-Straße 6, D-89081 Ulm, Germany
Tel. +49 (0) 731 140 700, Fax. +49 (0) 731 140 70200
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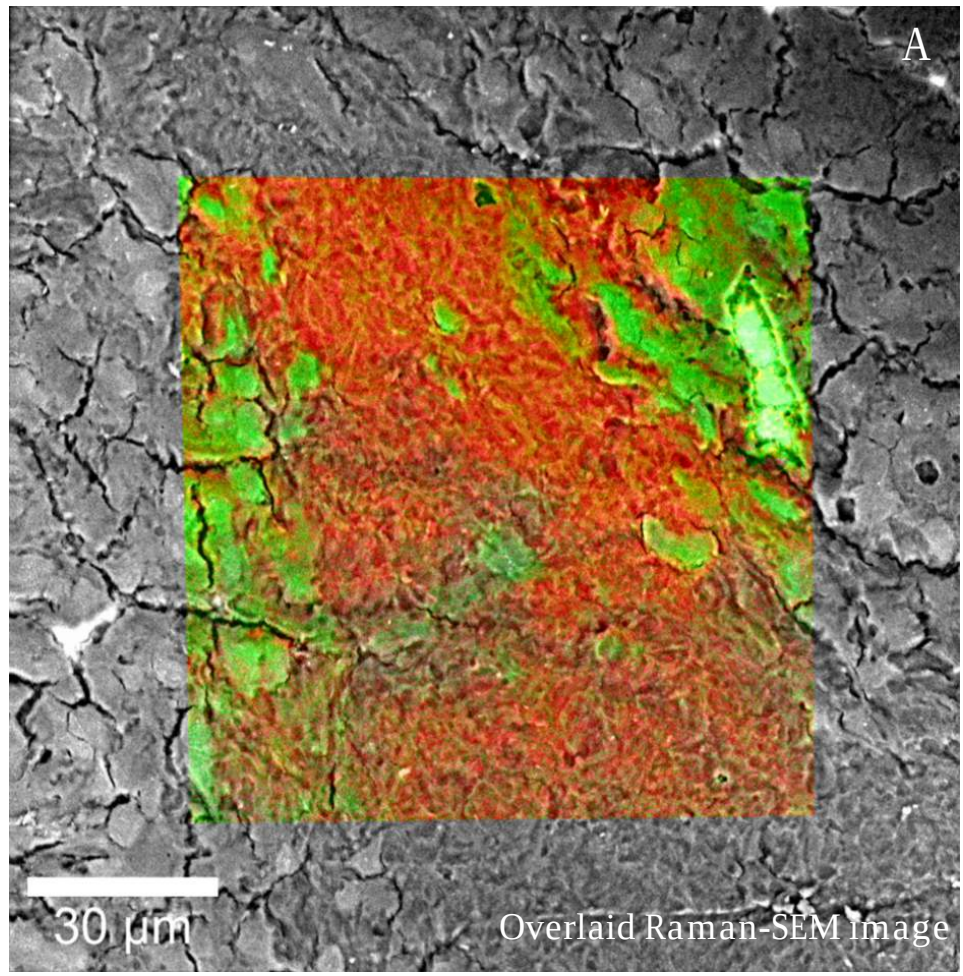
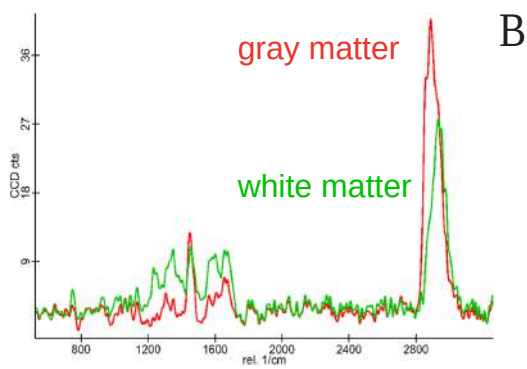
RISE Microscopy - Correlative Raman Imaging and Scanning Electron Microscopy (Raman-SEM)

RISE Microscopy is a novel correlative microscopy technique that combines SEM and confocal Raman Imaging. Through RISE Microscopy ultra-structural surface properties can be linked to molecular compound information. This unique combination opens up new dimensions for more comprehensive sample characterization.

Scanning Electron Microscopy (SEM)
Scanning Electron Microscopy (SEM) is a high-resolution imaging technique that can determine a sample's topography, morphology, surface structure and materials orientation. The images are acquired with nano- or micrometer resolutions and magnification ranges of 10 – 10.000x. Due to the large depth of field, it is also possible to generate a 3D appearance of the surface. SEM is a non-destructive method which allows combinations with other imaging techniques.

RISE: Combining SEM with confocal Raman imaging
Confocal Raman imaging facilitates the analysis and visualization of molecular compounds of a sample with a lateral resolution at the diffraction limit (~200 nm). The correlative Raman Imaging and Scanning Electron (RISE) Microscope combines the advantages of the SEM and the Raman imaging technique within one instrument. In this way ultra-structural surface information is linked to molecular compound information. To switch between the different RISE measuring techniques the sample is automatically transferred and re-positioned within the vacuum chamber of the microscope. The acquired results can be correlated and the images overlaid.





(A) Raman-SEM image overlay of a hamster brain tissue sample. In the color-coded Raman image the white brain matter is shown in Green and the gray brain matter in Red. Raman image: 100 µm x 100 µm, 300 x 300 pixels = 90,000 spectra, 50 ms integration time per spectrum. (B) The corresponding Raman spectra reveal the different spectral characteristics of the white and gray brain matter.