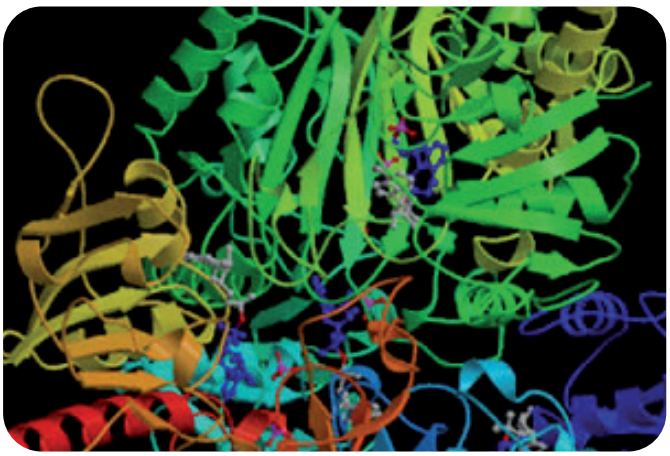


Application Note AN B407

Biomolecules, Cells and Tissue Studied by IR-Spectroscopy

For more than 30 years, molecules have been analyzed in the solid, liquid and gaseous state on the basis of their specific vibrations. So, the (FT-) IR spectroscopy has become an indispensable tool in the chemical and pharmaceutical industry for characterizing materials and identifying substances. In the last years, however, a completely new application field in the area of Life Science has opened up to this non-invasive analysis technique. Pharmaceutical and biotechnological companies use this technique to analyze both proteins and cellular systems in order to develop new drugs and products.

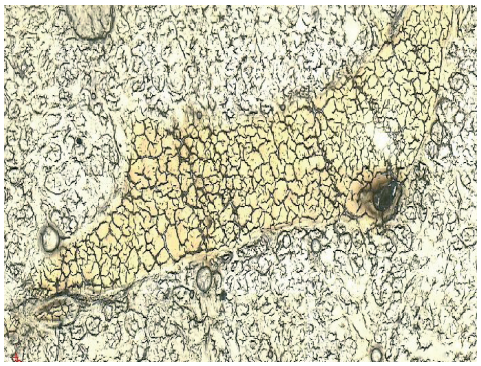
Infrared light excites molecules to vibrate. The molecules absorb only light of certains wavelengths that correspond to their specific vibration frequencies. Therefore, the position of the corresponding band in the absorption spectrum is characteristic for a certain vibration and, according to the Lambert-Beer law, the band intensity is directly proportional to the concentration of the vibrating molecule. Today's Fourier-transform infrared (FT-IR) spectrometer cover the whole (mid) infrared region and allow the acquisition of high-quality spectra within seconds.

Insight into protein conformation

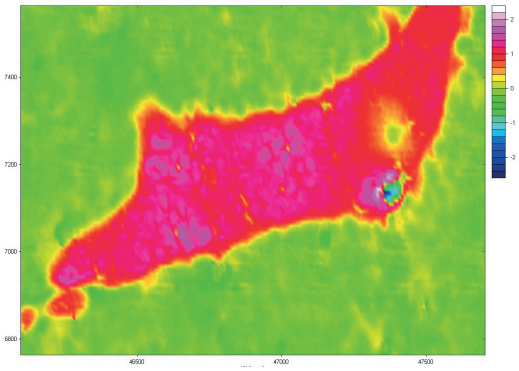
Proteins become more and more important as active agents in medical drugs. Especially therapeutic antibodies are a promising approach for the treatment of diseases that have been incurable so far. Under what conditions remains the protein stable? How long lasts this stability? These and other questions have to be answered in the course of the formulation optimization. The classical method to detect the aggregates of the protein is the size-exclusion chromatography. The denaturation process observed with this method mostly starts mechanistically at an earlier stage, namely with conformational changes of the protein and often under the formation of a typical ß-sheet structure. Using the FT-IR spectroscopy, conformational changes can be detected with a very high sensitivity. So, this technique allows to recognize instable formulations already at an very early stage. Furthermore, the aggregation process can be monitored directly using the FT-IR technique. To ensure a stable formulation, the proteins are often kept in complex buffers with a large number of additives like sugars, polyalcohols or amino acids and they are stored either in dissolved state (liquid formulation) or in powder-form (solid formulation) after the lyophilization. The FT-IR spectroscopy now gives the opportunity to measure the structural changes of proteins both in liquid and solid formulations easily and quickly regardless of the buffers or additives.

Analysis of cells

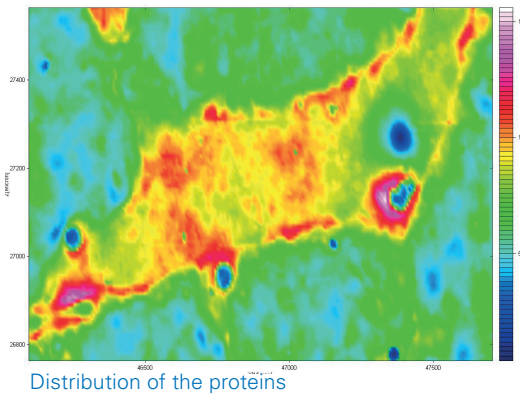
Already in the 1980s, Dieter Naumann and Harald Labischinski from the Robert-Koch Institut in Berlin have developed



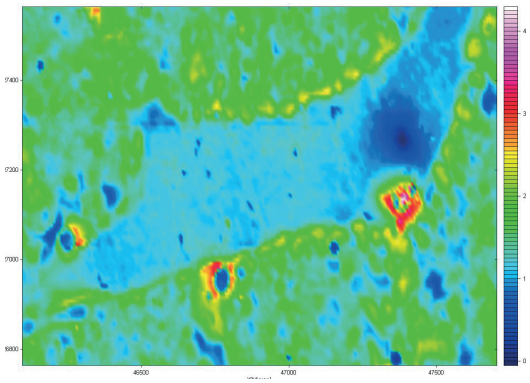
Visible Video Image



Distribution of the active agent



Distribution of the proteins



Distribution of the lipids

Figure 1: Tissue section (1.6 x 0.8 mm) of a mouse kidney (thickness: 10 µm). In the visible video image, the active agent is discernible by the yellowish staining. The whole sample area has been measured using the FT-IR imaging technique. For each image point (4 x 4 µm) a full IR spectrum was received. The intensity of the individual absorption bands is proportional to the concentration of corresponding absorbing molecule class. The distribution of the individual molecule classes has been visualized by a false color image reflecting the signal intensities of certain absorption bands. The figures show the distribution of the active agent, the proteins and the lipids. It is clearly visible that the active agent is mainly located in the protein-rich region, whereas, the lipids are concentrated in distinctly separated areas. The measurement has been performed using a Bruker Hyperion 3000 microscope.

a method for identifying microorganisms that bases on the FT-IR spectroscopy. The most amazing aspect of this method is that many microorganisms can be identified even to strain level! Due to this fact the IR-method allows to gather information about the origin of a certain strain which is imortant for tracking contamination routes e.g. in pharma or food products.

Imaging of tissues

Only for a few years, modern FT-IR microscopes have been equipped with multielement detectors. This technique enables the analysis of tissue sections with a spatial resolution of up to 0.5 µm within a few minutes. During the measurement, a complete infrared spectrum is acquired at each image point. The result is an image showing the distribution of the main biochemical components (especially lipids, proteins and polysaccharides) in the analyzed tissue area (see figure 1). This measurement technique requires neither chemical labeling of the components nor staining of the sample. To visualize also the distribution of the individual proteins or active agents in the same sample, today's imaging infrared microscopes are equipped with fluorescence channels. Typically, unstained cryomicrotome sections with a thickness of 6 to 10 µm are analyzed. The analytical questions that can be answered with this technique range from characterizing cancer tissues and confirming the presence of implant material to detecting amyloid structures.

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