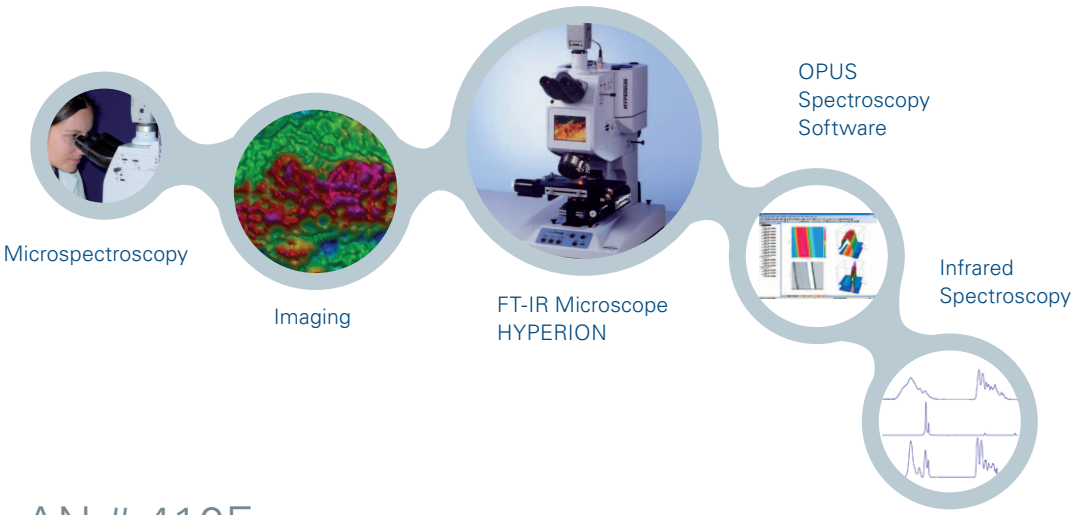

Application Note AN # 410E

FT-IR Microscopy - a new and powerful dimension of imaging

FT-IR microscopy is a well-established method for the chemical identification of particles or contaminations and for visualizing the distribution of certain substances in complex compounds. Due to the usage of modern focal plane array detectors, this method has advanced to a new imaging technique during the last years. It allows for the measurement of even large sample areas with a very high lateral resolution within a few minutes. After the measurement, the results are evaluated on the basis of easily comprehensible images.

For more than two decades, even very small samples have been analyzed using the Fourier transform infrared (FT-IR) spectroscopy. For this purpose, microscopes with mirror optics have been developed which allow not only a for visual viewing but also for infrared spectroscopic analysis of a sample. Recently, FT-IR microscopes equipped with focal plane array (FPA) detectors consisting of a matrix of 16 x 16 up to 128 x 128 detector elements are used. This new array detector allows to acquire up to 16,000 pixels/spectra simultaneously and has therefore enabled the FT-IR microscopy to become also an imaging technique.

Applications

The FT-IR microscopy can be used for a wide range of applications. Typical applications are the chemical identification of particles and smallest contaminations, the examination of the homogeneity of coatings and the analysis of the distribution of a multitude of different components in a complex mixture. An important advantage of this technique is that nearly all kinds of samples can be analyzed using it. Moreover, it is a non-invasive method which does not require staining or labeling of the sample under examination. The molecules are identified on the basis of their characteristic vibrations to which they are excited by the impinging IR beam. In this way, FT-IR imaging delivers information about the molecular composition of the analyzed sample area. That is why this image technique is also called 'chemical imaging'.

Spatial resolution

For FT-IR microscopy like for other types of optical microscopy, the lateral resolution is limited by the light diffraction. The smallest distance (δ) at which two points of a sample can still be separated is described by the following formula:

$$\delta = 0{,}61\ \lambda / NA,$$

with NA being the numerical aperture of the objective and λ being the wavelength of the light.

Because the numerical aperture of the mirror objectives in FT-IR microscopes is about 0.6, the above formula can be reduced as follows:

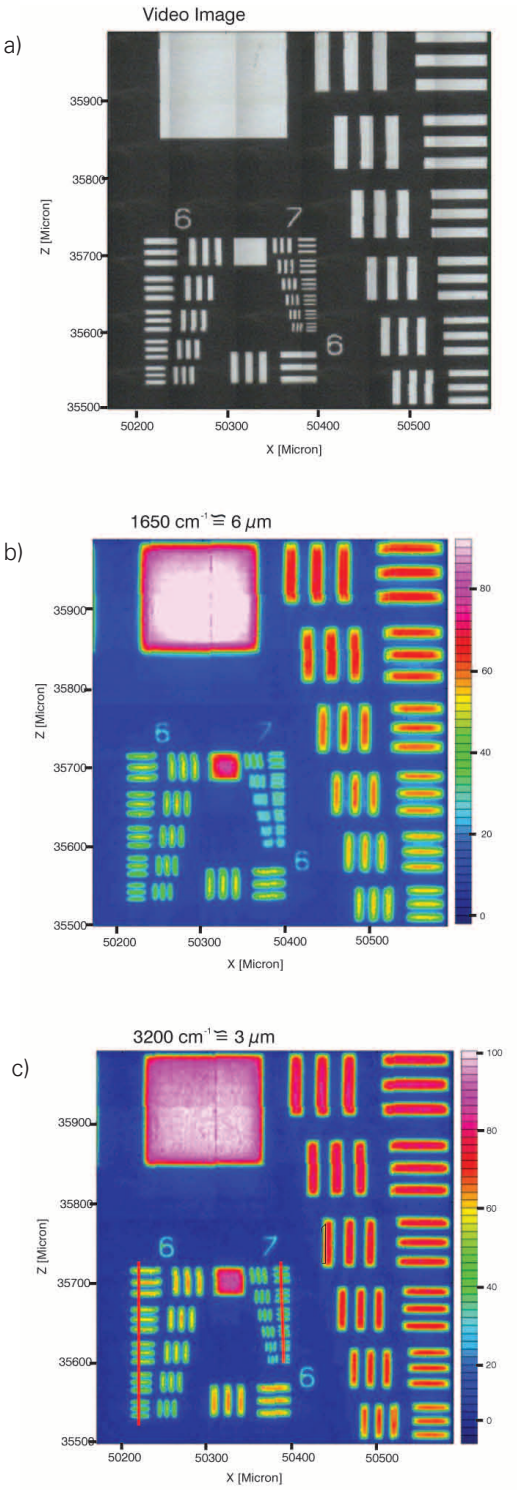

Figure 1: Pattern of metal strips on glass. The upper a) image is a video image of the sample. The video image covers a sample area of about 400 x 500µm. This sample area has been measured in the reflectance mode with a pixel resolution of 1,1 µm using the FT-IR imaging system BRUKER HYPERION 3000 equipped with a FPA detector (64 x 64 detector elements) and a 36x objective (NA=0.5). In b the IR image at 1650cm^{-1} (≙ 6 µm wavelength) is shown, in c the IR image at 3200cm^{-1} (≙ 3 µm wavelength), respectively.

Distance δ = Wavelength λ.

Figure 1 shows the visible image of a sample which consists of a regular pattern of metal strips on glass. The strips are arranged in groups of parallel oriented strips of different size and separated by different wide gaps. The sample area of about 400 x 500µm (shown in figure 1) has been measured in reflection mode with a pixel resolution (size of the sampling area which is imaged on one detector pixel) of

1.1 µm using the FT-IR imaging technique. The intensity of the spectra at the wavelengths of 1650cm^{-1} (6 µm; fig 1b) and 3200cm^{-1} (3 µm; fig 1c) are displayed in false color. The two false color images reveal that the rectangular form of the metal strips is rendered sharper and that the groups of the smaller strips are resolved better at a wavelength of 3 µm than at a wavelength of 6 µm. This result shows that the lateral resolution of a well designed FT-IR imaging system is only limited by the light diffraction. This fact becomes more obvious when you have a closer look at the cut along the red lines in group 6 and 7 marked in fig 1c.

The strips in group 6 separated by 14 to 8 µm are well-resolved at either wavelengths, 3 µm and 6 µm as shown in fig 2 top. If, however, the strip pattern and the gaps between the individual strips become smaller, as in group 7, the lateral resolution achieved at a wavelength of 3 µm is significantly better than at a wavelength of 6 µm. According to the theoretical expectations, it was found that structures

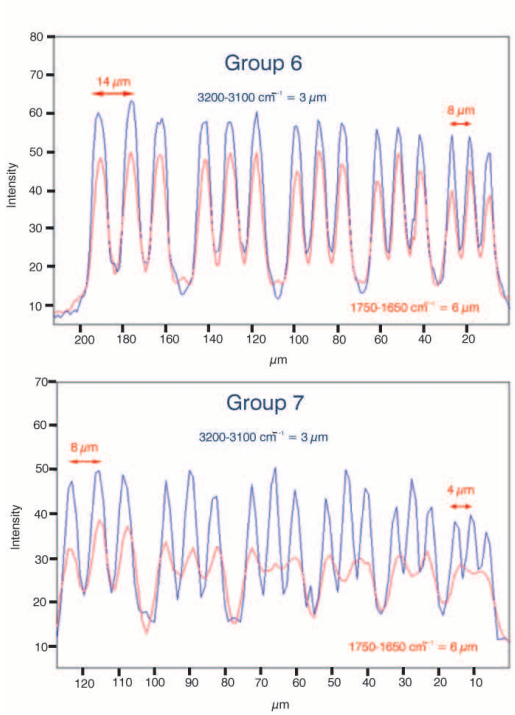

Figure 2: Resolution profile. This figure shows a cut through group 6 and 7 according to the red lines drawn in figure 1c. The blue trace corresponds to the profile at 3200 cm^{-1} and the red trace corresponds to the profile at 1650 cm^{-1}.

can be resolved up to 6 µm at a wavelength of 6 µm. And in case of a wavelength of 3µm, the resolution limit was shown to be about 3µm.

This high spatial resolution has only been possible because the used pixel resolution (= edge length of each detector element) of 1,1 µm has been significantly higher than the structures to be resolved, i.e. all strips and gaps have been imaged on several pixels and not on only one single pixel (see figure 2 bottom).

IR analysis of a wheat stem

The examination of animal and vegetable tissues is also a typical application for FT-IR imaging. Figure 3 and 4 show the resulting data for a wheat stem tissue. The main biochemical components as proteins, carbohydrates and waxes/lipids could be identified by their characteristic absorption. The IR images in figure 4 reveal the distribution of these components within the tissue. This information can also be combined into a RGB image (figure 3) showing the distribution of proteins (green), carbohydrates (red) and waxes/lipids (blue) in parallel.

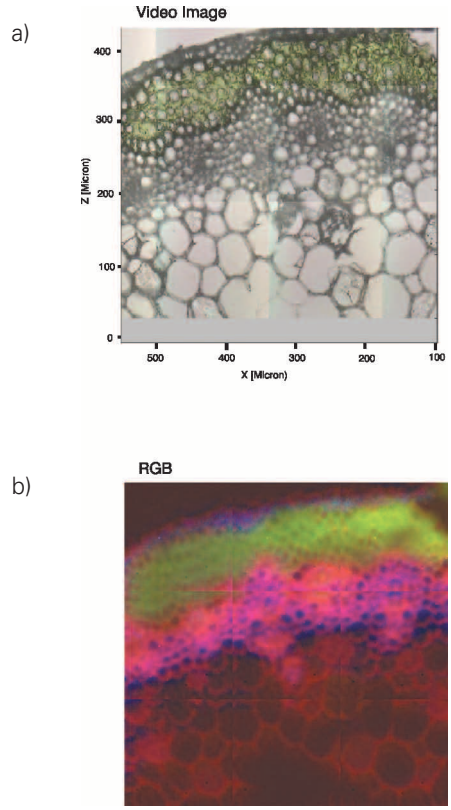

Figure 3: Wheat Stem. In a the video image of a wheat stem (microtome section; thickness of 10 µm) is shown . The video image covers a sample area of about 500 x 500 µm. The RGB image (b) shows the combined distribution of the proteins (green), carbohydrates (red) and the waxes/lipids (blue)

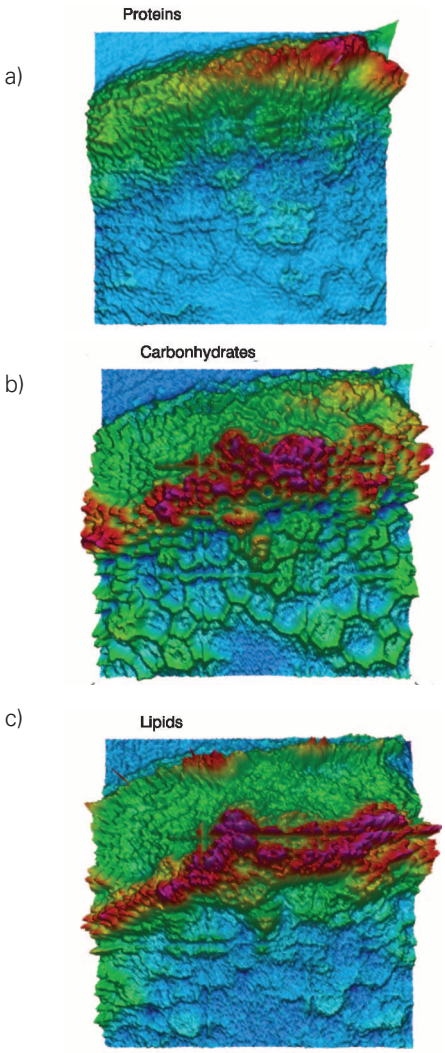

Figure 4: Wheat stem sample (see figure 3) area has been measured in transmittance with a pixel resolution of 2.7 µm using the FT-IR imaging system BRUKER HYPERION 3000 equipped with a FPA detector (64 x 64 detector elements) and a 15x objective (NA=0.4). The individual IR images show the distribution of proteins (1710 - 1480cm^{-1}, c), carbohydrates (1140 – 900 cm^{-1}, d) and waxes/lipids (1770 – 1700 cm^{-1}, e) within the tissue. The distribution of these components has been visualized by evaluating the intensity of the characteristic vibrations of each of these three biochemical components.

www.bruker.com/optics

Bruker Optics Inc.
Billerica, MA · USA
Phone +1 (978) 439-9899
Fax +1 (978) 667-5993
info@brukeroptics.com

Bruker Optik GmbH
Ettlingen · Germany
Phone +49 (7243) 504-2000
Fax +49 (7243) 504-2050
info@brukeroptics.de

Bruker Hong Kong Ltd.
Hong Kong
Phone +850 2796-6100
Fax +852 2796-6109
hk@brukeroptics.com.hk