Application Note AN M151

Fast Imaging of Large Biological Tissue Samples

Introduction

FTIR microscopy has developed into a powerful tool for the analysis of biological tissue. The obtained IR spectral images allow for the differentiation of components and the identification of malignant or diseased tissue. Besides the well-established high magnification objectives, Bruker offers a 3.5x IR objective for the HYPERION 3000 FTIR microscope to easily generate overview images of large biological tissue samples.

Samples of biological tissue are often large and show no distinct features in the visible image, which makes it impossible to identify relevant areas with the naked eye. Furthermore, high magnification leads to an even more tedious and time consuming search for the desired measurement area.

Figure 1: 3.5x IR objective with lens cap attached.

Keyword	Instrumentation and Software
Biological tissue	HYPERION 3000 IR-imaging software
Fast chemical imaging	3.5x IR objective
Disease pattern recognition	OPUS spectroscopic software
Transmission measurement	FPA detector
Microtome section analysis	Automated sample stage

To solve this issue and allow the user to focus on the investigative task, Bruker offers a high quality 3.5x IR objective that can be used directly for measurements in reflection mode or in combination with a 3.5x IR condenser, if transmission mode is required.

It is made from IR transparent materials that have been assembled for optimal IR imaging performance with the HYPERION 3000 microscope.

Therefore, this objective delivers excellent spectral quality over a wide spectral range with minimized chromatic aberration and excellent signal to noise ratio.

Example: Chemical Image of a Wheat Stem

An overview image of a wheat stem microtome cut placed on a CaF_2 plate is shown in figure 2. The red squares indicate the measurement area of the 5 x 6 FPA images that are combined into one large IR image by the spectroscopy software OPUS. Each square covers an area of 710 x 710 µm, resulting in a total area of 3.55 x 4.26 mm.

The presented measurement was performed with 4 cm^{-1} spectral resolution and ca. 40 seconds measurement time per frame resulting in a total analysis time of about 20 minutes.

Chemical images showing the distribution of the different biochemical components within the tissue sample are generated quickly by integration of the corresponding spectral bands (fig. 3). As an example the band at about 1740 cm^{-1} is representative for lipids whereas carbohydrates dominate the spectral range from 1169 – 1134 cm^{-1}.
A Winner-Takes-All (WTA) image can be seen in figure 3. The WTA model assigns the color of the dominant component to each individual image pixel thereby allowing the display of several components in one picture.

Carbohydrates are indicated in red, the resin matrix in white, lipids in blue and the seed coating in green. Corresponding example spectra are shown below in the respective colors.

The described measurement and evaluation clearly shows the usefulness of a low magnification objective for the rapid generation of overview images and the fast identification of relevant measurement areas for further in-depth analysis.

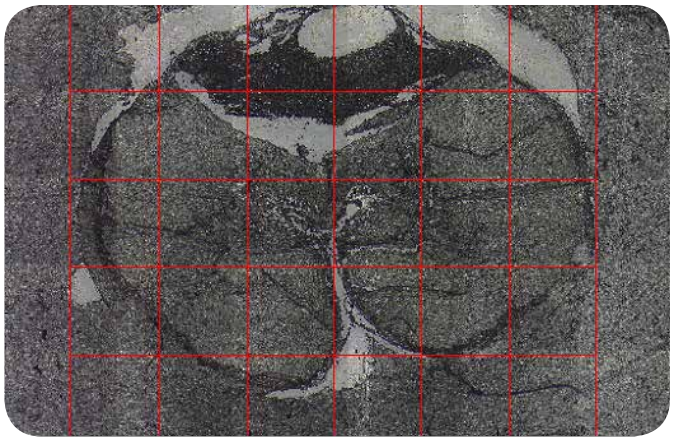

Figure 2: Visual overview image of a wheat stem microtome cross section placed on a CaF_2 plate.

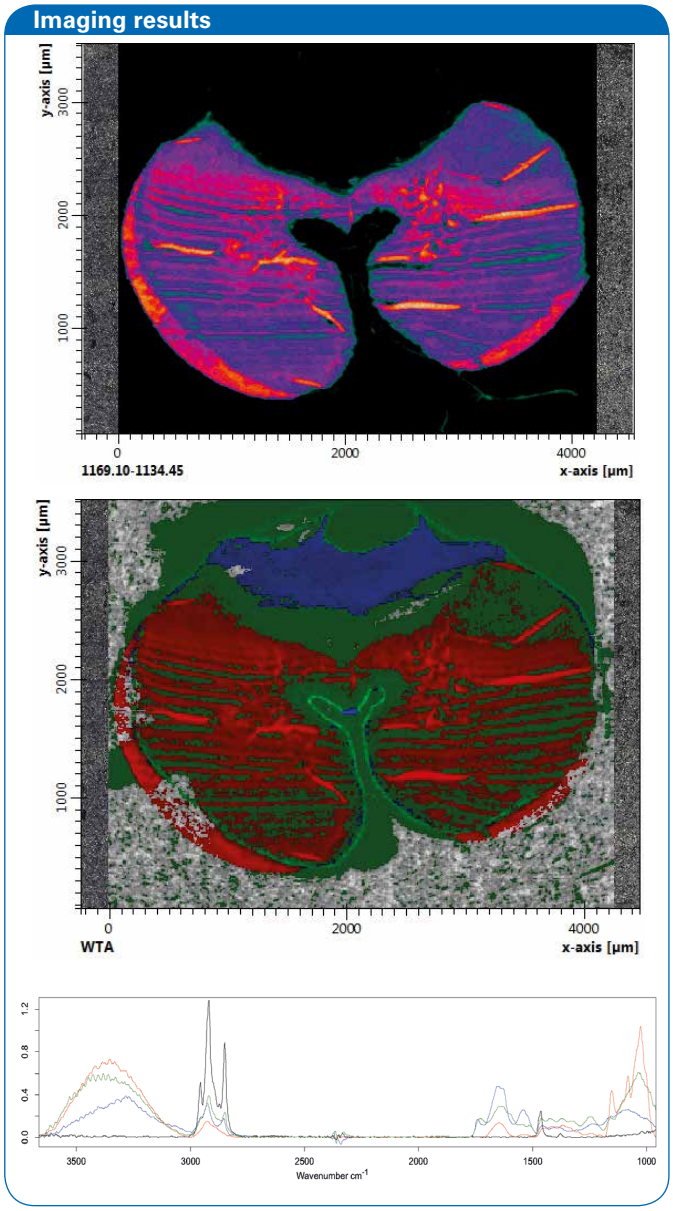

Figure 3: Top: IR chemical image showing the distribution of carbohydrates in a wheat stem cross section (strong color indicates a high concentration). Middle: Combined chemical image (The dominant component of the spectrum decides the color of the corresponding pixel) (red: carbohydrates, green: seed coating, white: resin matrix, blue: lipids) Bottom: Typical spectra from the IR image (colors correspond to the ones in the WTA image).

Bruker Optik GmbH
Ettlingen · Deutschland
Phone +49 (7243) 504-2000
Fax +49 (7243) 504-2050
info.bopt.de@bruker.com

Bruker Optics Inc.
Billerica, MA · USA
Phone +1 (978) 439-9899
Fax +1 (978) 663-9177
info.bopt.us@bruker.com

Bruker Shanghai Ltd.
Shanghai · China
Phone +86 21 51720-800
Fax +86 21 51720-899
info.bopt.cn@bruker.com

www.bruker.com/optics