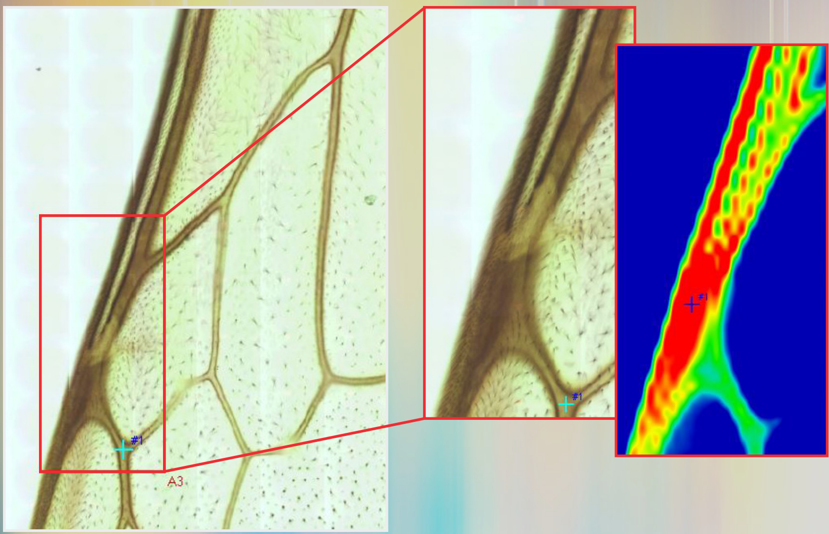
Technical note



NIR Microscopy Provides Advantages Beyond Traditional Mid-IR Analysis

Introduction

Fourier transform infrared (FTIR) micro-spectroscopy combines infrared (IR) spectroscopy with visualization. Since IR spectroscopy exhibits high specificity to molecular structure and chemical composition, FTIR micro-spectroscopy is particularly useful for analyzing the spatial distribution of chemical species. FTIR micro-spectroscopy typically involves mid-infrared (mid-IR) spectra (4,000-400 cm^{-1} wavenumber range, or ~2,500-25,000 nm in wavelength). Absorptions in the mid-IR correspond to fundamental vibrations of the chemical bonds associated with the atoms of the molecules. The characteristic frequencies of mid-IR absorptions from different vibrational bands allow for easy band assignment and interpretation, but the mid-IR often requires thin specimens and more involved sample preparation. Conversely, near infrared (NIR) absorptions arise from overtones and combinations of fundamental vibrations, in the range of 12,500 to 4,000 cm^{-1} wavenumbers or wavelengths from 800 to 2,500 nm. Overtones and combination bands in NIR intrinsically have lower molar absorptivities and this leads to increased penetration depth into the samples. Therefore, NIR spectra are more readily obtained with minimal sample preparation. Furthermore, the NIR radiation passes through many materials, such as glass or clear plastics, allowing spectra to be collected in containers like blister packs.

The Thermo Scientific™ Nicolet™ RaptIR+™ FTIR Microscope has been designed to take advantage of these attributes of NIR radiation. The RaptIR+ provides the option to easily swap detectors from a mid-IR MCT detector to an InGaAs NIR detector. A single button in the software runs an automated routine to optimize the detector. When coupled with the Thermo Scientific™ Nicolet™ iS50 FTIR Spectrometer, the RaptIR+ FTIR microscope allows easy switching of the excitation source to a white light source, and the beamsplitter to a CaF_2 beamsplitter. Thanks to these features, a mid-IR microscope can be converted into an NIR microscope in a matter of minutes.

In this technical note, several example applications of the Thermo Scientific Nicolet RaptIR+ FTIR Microscope with its user-swappable InGaAs detectors are presented to illustrate the advantages of NIR microscopy.

Nicolet RaptIR+ FTIR Microscope.

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Results

Figure 1 shows the visual images (left) and representative NIR spectra (right) of a caffeine tablet in blister packaging along with NIR correlation images. The spectra are identified as caffeine (a) and corn starch (b), respectively. Corn starch is the primary excipient in this tablet. The correlation images (inserts) built on the NIR spectra show the spatial distribution of the two components. In this example, the tablet was analyzed as received with no sample preparation. Because the outer plastic packaging is translucent it allows for direct focusing onto the tablet surface. The packaging is thin enough that the NIR absorbances of the plastic are small and do not interfere with the analysis of the tablet.

Low-cost materials such as glass and quartz are NIR transparent, eliminating the need for specialized window materials such as KBr and BaF_2 that would be required for mid-IR measurements. Samples typically need to be prepared in a specific way for mid-IR analysis, but NIR is more forgiving. Figure 2 shows an example of a honeybee wing that was prepared for visual microscopy. The wing is mounted between a glass microscope slide and a glass coverslip. The adhesive used prevents the mounting from being disassembled for IR microscopy analysis. However, NIR can be used to collect spectral data though the glass. The mounting materials contribute to the spectral features, but those contributions can be subtracted to give a spectrum that resembles the spectrum of chitin, a major component that makes up the bee wings.

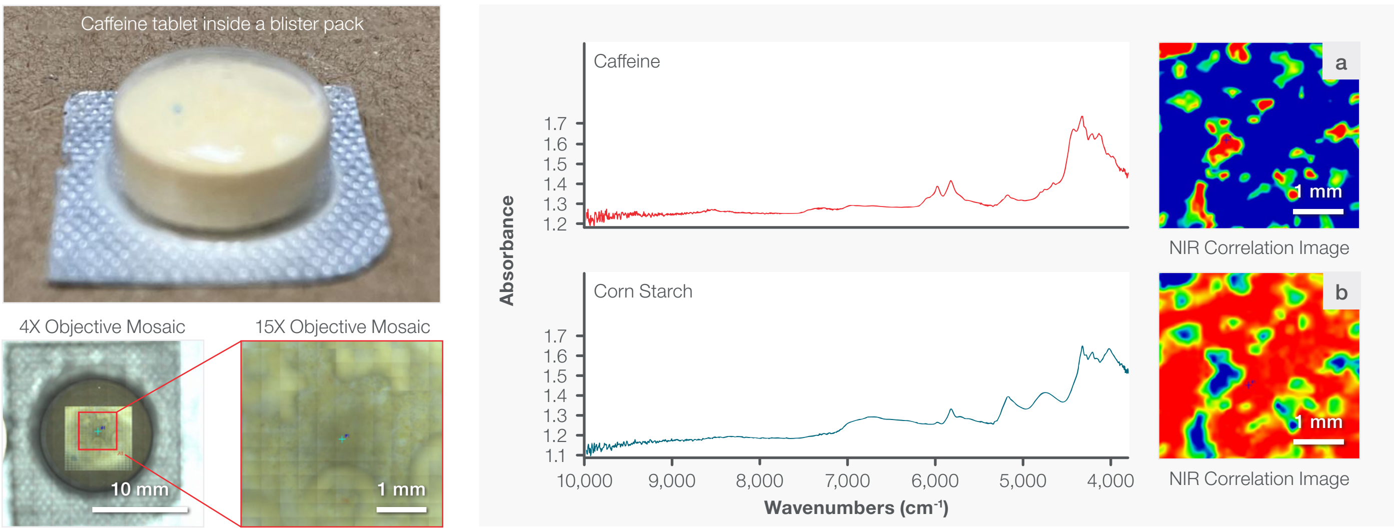

Figure 1. Left: Visual images of a caffeine tablet inside a blister pack. Right: Representative NIR spectra (through the blister pack) and correlation images showing the spatial distribution of (a) caffeine and (b) corn starch. The red areas on the images indicate high correlation with the spectra of the material specified and blue areas indicate an extremely low contribution.

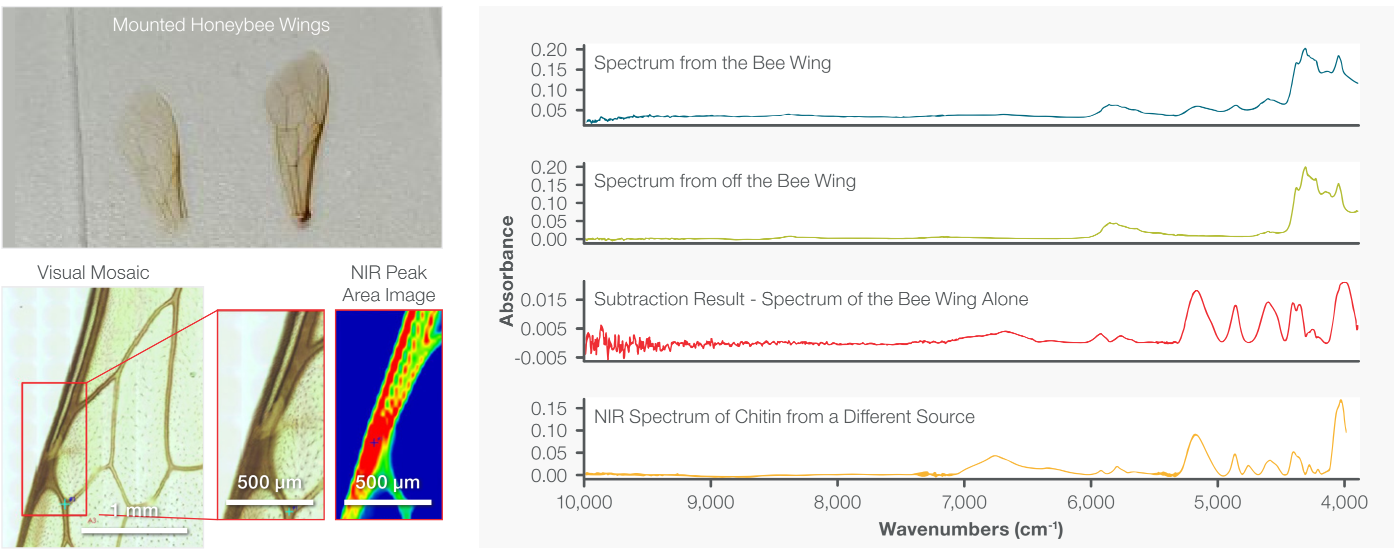

Figure 2. Visual Images of the mounted honeybee wings. The wings are mounted on a glass microscope slide with a glass cover slip secured on top of the wings with an adhesive. A NIR image of the wing is shown as well as a spectrum of the wing material. The contributions of the mounting material were subtracted off to give a spectrum of the honeybee wing which can be compared to a NIR spectrum of chitin from a different source.

FTIR spectroscopy and micro-spectroscopy have been extensively used in microplastic (MP) research to characterize polymer types and to trace their fate and transport in different environmental matrices [https://www.tandfonline.com/doi/full/10.1080/10643389.2020.1807450]. Large MPs (>200–300 µm) are often identified by Attenuated Total Reflection (ATR)-FTIR, whereas small MPs (down to 20 µm) are characterized by FTIR microscopy, most commonly mid-IR microscopy. However, particles with size range of 100-500 µm can be too small to manipulate for ATR measurements, but too big/thick for mid-IR transmission microscopy measurements. For example, Figure 3 shows the visual image, chemical image, and FTIR spectrum of a polyester particle (~400 µm). A substantial amount of the mid-IR spectral details is lost due to the over-absorbing peaks. Figure 3 also demonstrates another common challenge associated with MP analysis by FTIR microscopy: The filter material, Al_2O_3 in this case, has strong absorption in the fingerprint region, blocking spectral features from polyester, and causing further information loss. The choice of mid-IR transparent filter materials remains limited.

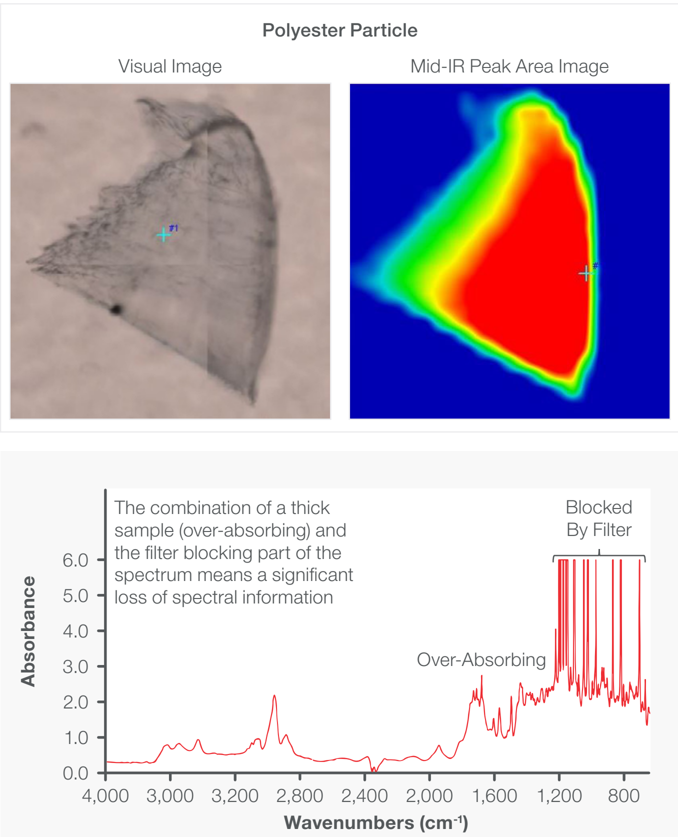

Figure 3. Mid-IR analysis of the polyester particle. Significant loss of spectral details of polyester due to over-absorbing peaks and the interference of the Al_2O_3 filter.

Figure 4 shows the NIR microscopy analysis of 5 different MP particles, with size ranging from 100-500 µm. All NIR spectra (bottom) exhibit well-defined spectral features despite the size of the particles, owing to the relatively weak absorptions intrinsic to NIR. The spectra are also free of interference from the filter material, Al_2O_3. When appropriate NIR libraries are available, NIR microscopy could be a viable alternative to mid-IR for large microplastic particle analysis.

Reflectance measurements account for a sizeable portion of the FTIR microscopy experiments because transmission measurements require thin specimens or small amounts of samples dispersed in non-absorbing matrices. Reflectance is the sum of specular reflection, where light is reflected from the surface, and diffuse reflection, where light is scattered after penetrating into the sample. The relative amounts of specular and diffuse reflection depend on the absorptivity and particle sizes of the sample, as well as wavelength. Small particle size and weak absorption promote diffuse reflection.

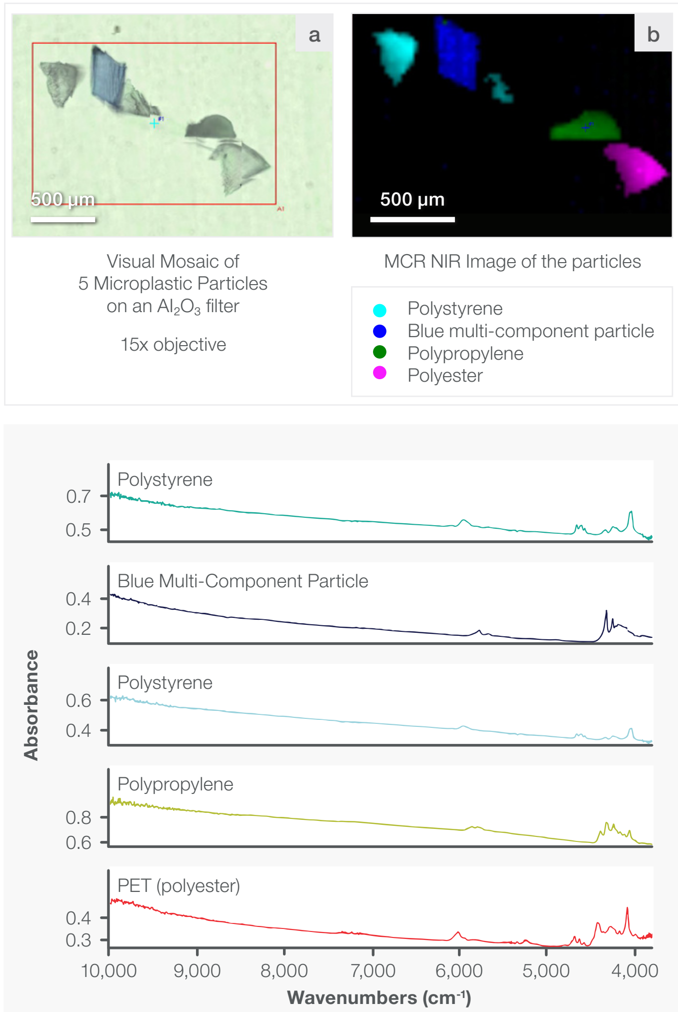

Figure 4. Top: (a) Visual mosaic image of the 5 microplastic particles. (b) MCR (multivariate curve resolution) NIR image of the 5 microplastic particles. The colors indicate the different types of polymers. Bottom: Representative NIR spectra from the 5 microplastic particles.

Figure 5 shows the visual images of a pharmaceutical tablet using 4x (a) and 15x (b) objectives, respectively. The tablet contains three active components: aspirin, acetaminophen, and caffeine; and the excipient, cellulose. Figures 6 and 7 show the correlation images (top) and spectra (bottom) of the tablet using mid-IR and NIR microscopy, respectively. With respect to the correlation images, mid-IR and NIR microscopy produced comparable results, but the mid-IR correlation images appear to be slightly grainier, particularly evident in the cases of high-concentration components acetaminophen and aspirin.

The appearance of reflection spectra depends on the relative contributions of specular reflection and diffuse reflection. The amount of specular reflection is determined by the refractive index. The refractive index has a minimum on the high wavenumber side of an absorption band and a maximum on the low wavenumber side. Consequently, the specular reflection spectrum in the mid-IR resembles a first derivative. Since absorption in NIR is weaker than in mid-IR, there are small variances in refractive index; hence, negligible specular reflection in NIR. Diffuse reflection arises from scattered/reflected light at different angles as the incident light penetrates into the sample. As the incident light traverses a path inside the sample some of it is absorbed; this is why the diffuse reflection spectra resemble transmission spectra. In mid-IR diffuse reflection, strong bands can become totally absorbing. In NIR, because the absorbance is weaker, scattering occurs more efficiently.

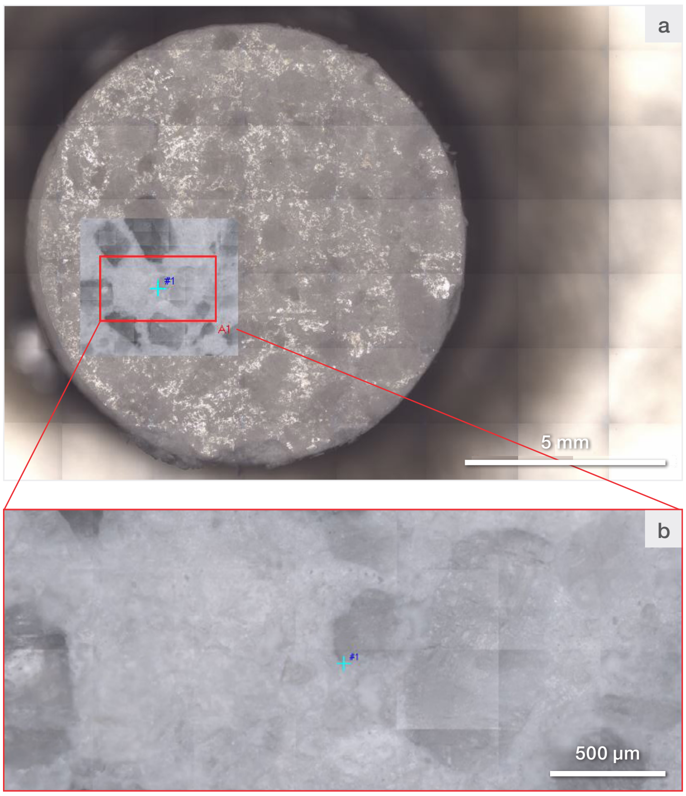

Figure 5. Visual Mosaic Images of a Migraine Relief Tablet.
(a) Mosaic of the whole tablet using the 4x objective.
(b) Mosaic image of the area of interest using the 15x objective.

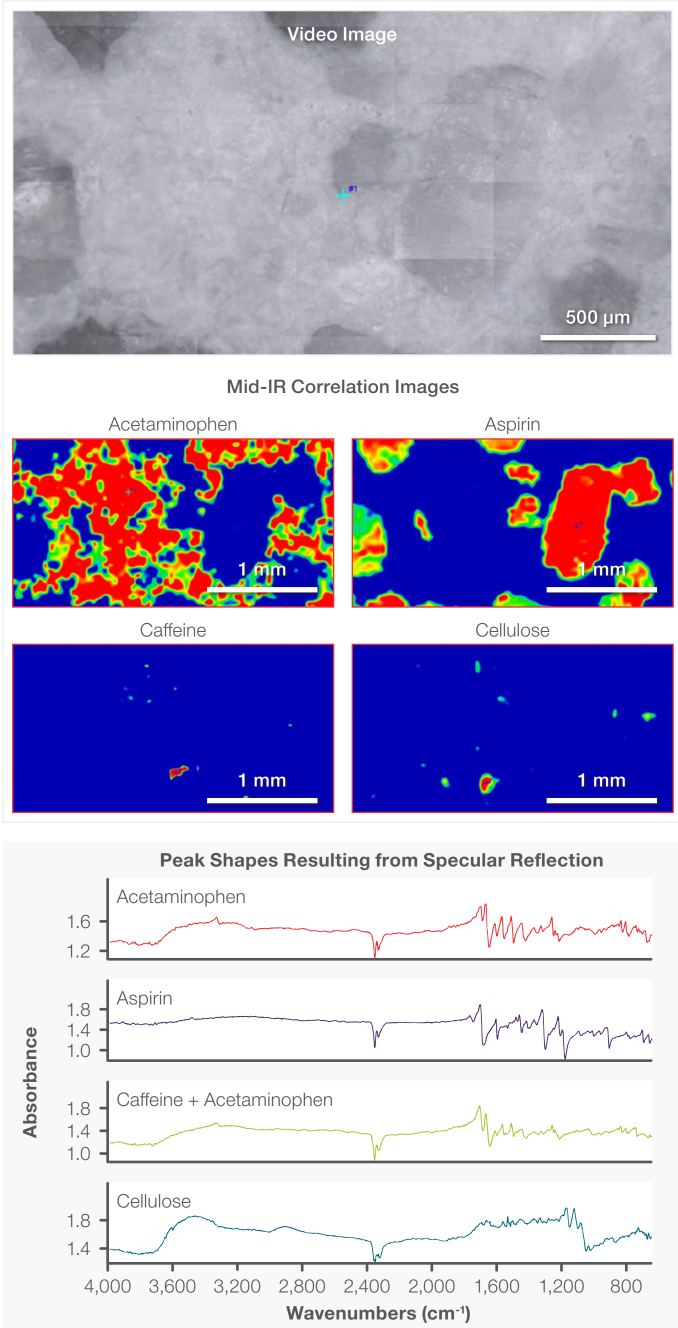

Figure 6. Top: Mid-IR correlation images of a pharmaceutical tablet. The images show the spatial distribution of the tablet components based on the Mid-IR spectra. The red areas indicate high correlation with the spectra of the material specified and blue areas indicate an extremely low contribution. Bottom: Representative spectra from the Mid-IR analysis of the tablet area.

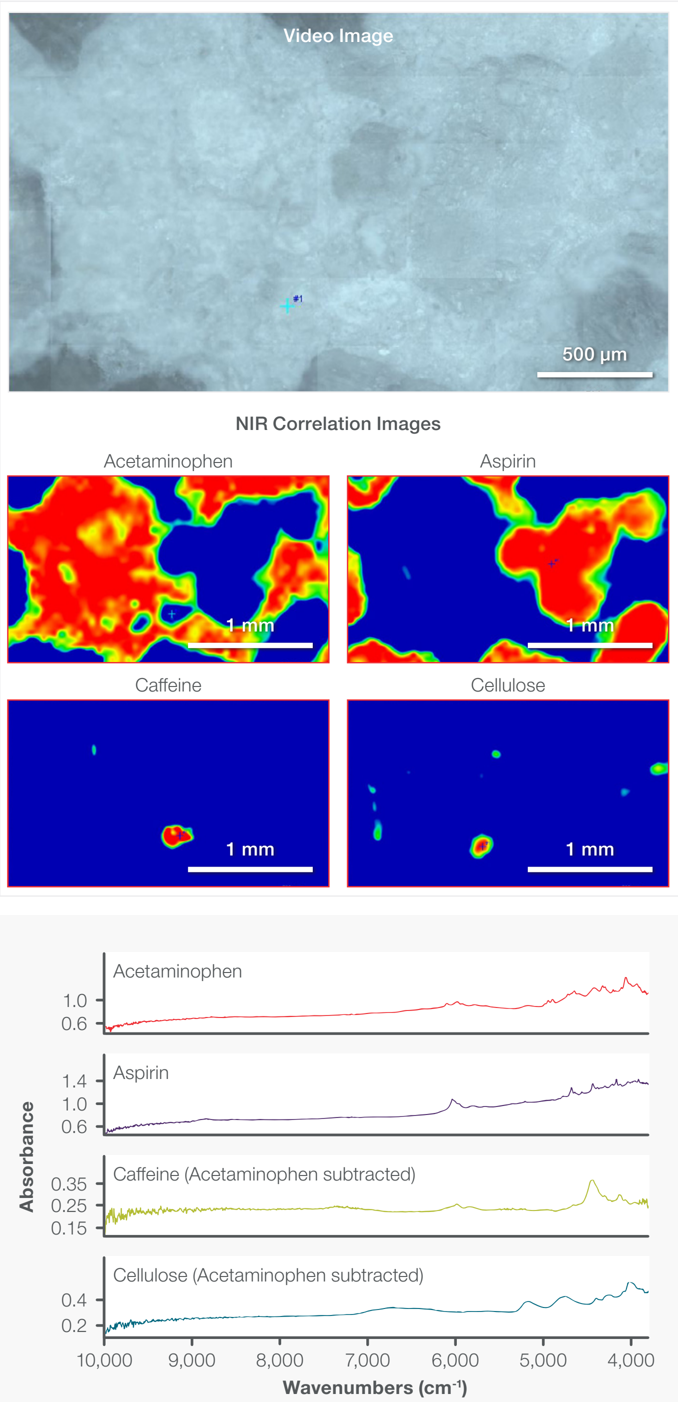

Figure 7. Top: NIR correlation images showing the spatial distribution of tablet components within the area of interest. The red areas indicate high correlation with the spectra of the material specified and blue areas indicate an extremely low contribution. Bottom: Representative NIR spectra from the various components.

Overall, while a reflection spectrum always has contributions from both specular and diffuse reflections, specular reflection dominates in mid-IR resulting in derivative-like peak shape (Figure 6), whereas diffuse reflection prevails in NIR giving rise to transmission-like peak shape (Figure 7). A mathematical operation called Kramers-Kronig (K-K) transformation is often applied to convert the specular reflection spectra to absorption type spectra. However, the conversion will distort any diffuse reflectance features. Note that specular reflection varies from particle to particle within the sample. This is why the correlation images in Figure 6 (top) are slightly grainier. When the components are known and a suitable library is available, as in the current case, NIR microscopy might be a preferred approach.

Summary

The Thermo Scientific Nicolet RaptIR+ FTIR Microscope unleashes additional analytical capabilities in the NIR region by offering user swappable InGaAs detectors. In this tech note, we have demonstrated the distinct advantages of NIR microscopy.

1. Glass is transparent in the NIR region, and this allows for the direct analysis of a bee wing sample prepared using glass materials that are not conducive to mid-IR analysis.
2. A tablet was successfully mapped by directly focusing on the tablet surface through a blister package. Negligible contribution from the packaging material was observed due to low NIR absorptivity. This illustrates a sampling advantage afforded by NIR over mid-IR analysis.
3. In the case of the analysis of microplastic particles between 100-500 µm where sample preparation is inconvenient, mid-IR transmission measurements cause over-absorbance. NIR microscopy offers a viable solution owing to the relatively weaker absorption in the NIR range.
4. Reflection measurements allow spectra to be obtained from a wide range of sample types. More efficient scattering (diffuse reflection) in the NIR results in transmission-like spectra that allow for direct library searching and improved correlation imaging.

Learn more at **thermofisher.com/RaptIR**