Perfect Focus™: FT-IR Microscopy

APPLICATION BRIEF

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Locating and focusing on a sample under the microscope is the first step in any type of microscopic analysis. This operation is either carried out manually while the operator looks at the sample or automatically under software control. In the automatic mode, this focus operation typically uses the video camera on the microscope. In either case, if the sample is not focused, optimal results may not be obtained.

This situation is identical in micro FT-IR analysis. If the sample is not in the focus of the beam, the spectrum that is obtained is not optimal. An FT-IR microscope is a large complicated beam condenser that also allows you to view the sample. If the IR beam is not "condensed" (focused) at the sample, a good infrared spectrum may not be obtained. Obviously, this is more crucial as the sample size gets smaller.

With a conventional microscope, users focus on the plane of interest in a sample. If the sample is a thin layer, one typically focuses on the top. In a sample with depth, such as an occlusion in a plastic, the operator will focus into the sample to bring the contaminant into sharp contrast.

Algorithms to auto focus various devices such as cameras, inspection equipment and motorized microscopes, all use similar approaches of optimizing the visual signal from a sample. These approaches range from optimizing the brightness (simple) to using the power spectrum on visible images (more complicated). Although using the power spectrum of the pixels in an image is a better way to focus, it is not optimal for infrared spectroscopy.

Focus is defined as a point in an optical system where rays of light converge. The focus or focal point is then dependent on the distance between the viewing optic and the sample. If one uses visible radiation, wavelengths are 400 to 700 nm. In the mid-IR, the wavelengths are much longer: 2.5 to 20 microns (25,000 to 200,000 nm). Therefore the focus for visible light is not necessarily the focus for IR. If you want to obtain an infrared spectrum, you want the IR radiation converging at your very small sample. Therefore, focus in the visible is not as relevant as focus in the IR.

Focus in Infrared

PerfectFocus™ has been designed to provide a focus using the infrared radiation. Since part of microscope analysis is also to capture a visible image, we have provided the means to focus in both regions of the spectrum.

The coarse focus capability uses the full signal of the IR intensity from the source that has passed through the full optical path of the instrument to the sample. This signal is optimized for the sample much as an analyst might do when positioning a sample in a beam condenser. Figure 1 illustrates the total infrared signal through a microscope system including the sample.

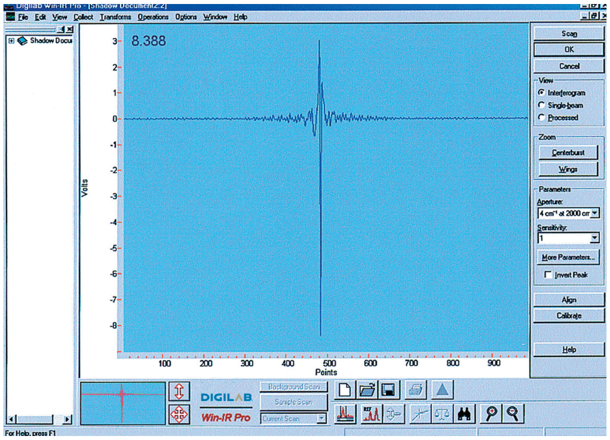
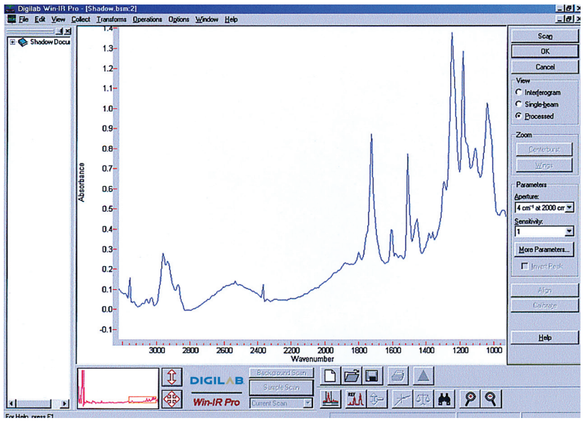


Figure 1. Total infrared signal (interferogram) through the UMA 600. This signal is used to focus the sample in the IR region of the electromagnetic spectrum.

Focus in Infrared (continued)

This signal is optimized for each sample. Using the monitor mode feature of the software, which displays the IR spectrum in real time, the analyst can then determine the quality of the spectrum (Figure 2).

Figure 2. Mid IR spectrum of the inside coating of a beverage can. This spectrum was collected in specular reflectance and results from one scan of the interferometer.



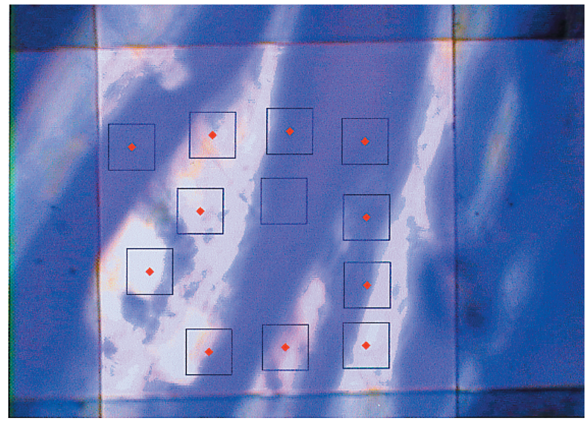
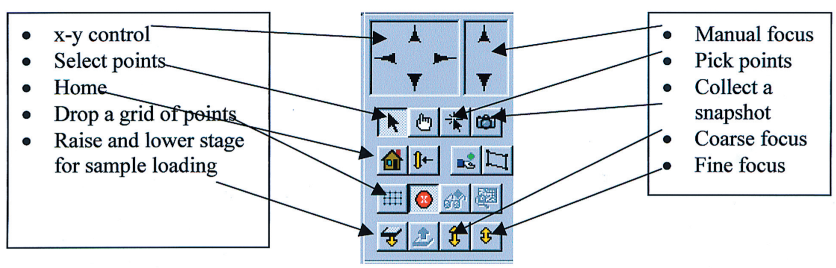


Figure 3. Sample of ink on a gold mirror. The red points indicate where the spectra will be collected. The dark edges show the patented Varian View in the visible while only collecting data from the area of interest.

The fine focus capability makes use of algorithms for visible focus, calculating the power spectrum, to bring the sample into sharp focus for visible image capture. This visible image capture is used to obtain a record of the sample analyzed and locations on the sample where spectra were collected (Figure 3).

An additional feature to simplify the analysis is the ability to load and unload the sample without loosing the focus. The load feature has simplified this. By simply pressing a key on the computer screen, the stage is lowered, allowing you to change the sample. With another key press, the stage is raised back into position for optimal analysis (Figure 4).

Figure 4. UMA 600 software control panel. Key features on this pad include:



Conclusion

Fine Focus in the visible range and PerfectFocus™ in the IR range. These two techniques provide the best technologies to focus your sample in the regions of the spectrum where you actually work. The visible is used for a record of the sample and the infrared for spectral data collection.

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