

Setting the Benchmark for Confidence and Ease of Use in FT-IR Microscopy

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Introduction

Infrared microscopy combines the power of FT-IR spectroscopy with the magnification of microscopy to enable the identification of small particles. While powerful, the technique requires not only the interpretation of infrared spectra but also adds several steps beyond those required for a typical FT-IR measurement. These include: sample preparation, sample placement and viewing, experimental setup, use of liquid nitrogen, and finally the extraction of chemical and physical information. In the last decade, chemical imaging significantly improved the analytical power and speed of infrared microscopy, and has emerged as the state of the art technology. However, imaging did not simplify the fundamental workflow of infrared microscopy.

The Thermo Scientific Nicolet™ iN™10 FT-IR microscope is equipped with tools to accomplish the infrared micro-analysis workflow seamlessly and provide answers with the degree of confidence required by modern laboratories.

Verifying Instrument Performance

FT-IR spectrometers typically offer software and hardware tools tailored to check the performance status of the instrument. By contrast, infrared microscopes are considered “accessories” to the spectrometer and therefore are not independently monitored.

Quality-driven industrial laboratories, forensic science departments, and pharmaceutical environments require confidence in the results they produce from all instrumentation and must comply with international regulation standards. The Nicolet iN10 FT-IR microscope offers a comprehensive package to answer these needs. It includes FT-IR performance verification that complies with the industry-standard ASTM E-1421 and European Pharmacopoeia methods, and NIST traceable standards conveniently mounted in a practical plate to fit on the microscope stage. The instrument performance qualification can be fully automated (with the exception of the micro-ATR slide insertion, which is manual) for consistency and confidence in the system experimental conditions.



Figure 1: Performance verification plate for microscopes. The plate includes NIST traceable polystyrene standards and predefined locations for background collections offering a comprehensive performance verification test in any sampling mode, including transmission, reflection and micro-ATR. Performance verification is fully automated for consistency of experimental conditions.

Loading, Locating, and Viewing the Sample

Most users evaluate FT-IR microscopes by performance, analytical flexibility, and availability of automated features, which are perceived as simplicity of use. While these are excellent criteria to select a microscope, several aspects of the complete infrared microscopy analysis workflow are overlooked. Loading and locating the sample on the microscope stage is one of the first bottlenecks that requires microscopy skills and time. This process involves several steps, including moving the stage to a comfortable location for sample loading, positioning the sample under the objective field of view, focusing, and illuminating the sample for optimal visual observation.

OMNIC™ Picta™, the software user interface of the Nicolet iN10 FT-IR microscope, has been specifically designed to make these operations fast, simple and reliable by combining software automation and intuitive sampling tools.

Loading the Sample

The eject function, similar to the one available in any computer optical drive, positions the stage for quick sample loading with a single mouse click.



Key Words

- Confidence in Results
- Experiment Setting
- Location
- Sample Preparation
- System Performance Validation
- System Setup

Locating the Sample

A graphical representation of the most common infrared microscope slides allows the user to simply click in the area of the slide where the sample was placed. The stage then drives quickly to the region selected. This capability, combined with automatic focus and image optimization, allows the user to quickly set the sample in position for infrared analysis.

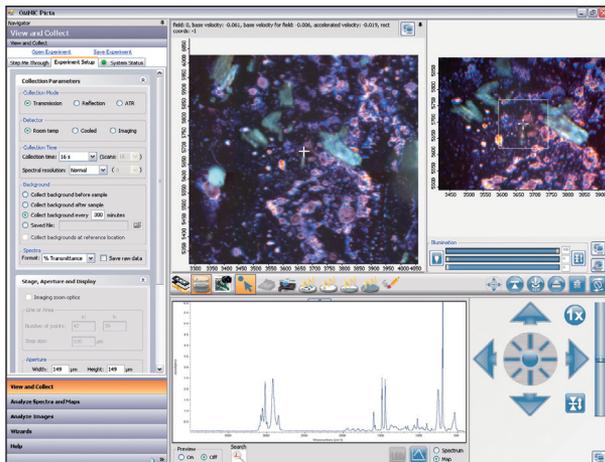


Figure 2: OMNIC Picta user interface main view. Instrument parameters are all grouped on the left panel, with icons representing several typical operations needed in infrared microscopy, allowing anyone – regardless of microscopy skills – to acquire superior results and provide reliable answers.

Viewing the Sample

Viewing of the sample is a significant source of frustration in the operation of an infrared microscope. Sample viewing on the Nicolet iN10 – obtained through color CCD digital video imaging – provides a more comfortable experience with an “export” option to send the image to a second monitor connected to the personal computer. Another common problem is the joystick. While some users like the direct control of their sample location, preferring the manual stage, others prefer a joystick, which is unanimously considered a cumbersome object on their lab bench. The Nicolet iN10 FT-IR microscope satisfies both preferences. It may be equipped with either a manual or motorized stage. The latter can be controlled by either a physical joystick or a virtual joystick implemented in OMNIC Picta; the closest experience to the latest web-surfing technologies, providing optimal convenience and ergonomics.

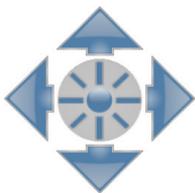


Figure 3: OMNIC Picta users interface includes a virtual joystick. The control in the center can be dragged by mouse, driving the stage to the desired location while the arrows provide controlled steps, Mosaic image zoom is controlled by the mouse-wheel.

Setting the Experimental Conditions

While an infrared spectrometer delivers fast and reliable results through standardized operation procedures, an infrared microscope requires more instrumental parameters that typically do not fit a SOP. In routine FT-IR analysis, sample size and infrared beam focus are generally not a concern since modern sampling accessories are pre-aligned and largely insensitive to sample volume or thickness (especially single reflection ATR).

In contrast, sample size and beam focus are fundamental variables in infrared microscopy and are specific to each sample measured. Specifically, infrared microscopy requires the adjustment of apertures to match the beam size to the sample dimensions. Further optimization may be required depending on the selected sample mode and background requirements. Understanding and becoming familiar with these experimental requirements is crucial for successful infrared microscopy analysis. However, today's laboratories are challenged with multi-tasking responsibilities and must quickly provide unambiguous answers at the lowest cost per analysis. Therefore, their “learning time” is limited.

The Nicolet iN10 FT-IR microscope has been designed starting from the premise that everything that is not strictly related to the analyst's goal of obtaining the answer must be controlled behind the scenes. OMNIC Picta reduces the gap between the simplicity of infrared spectroscopy and the complexity of infrared microscopy, as never before.

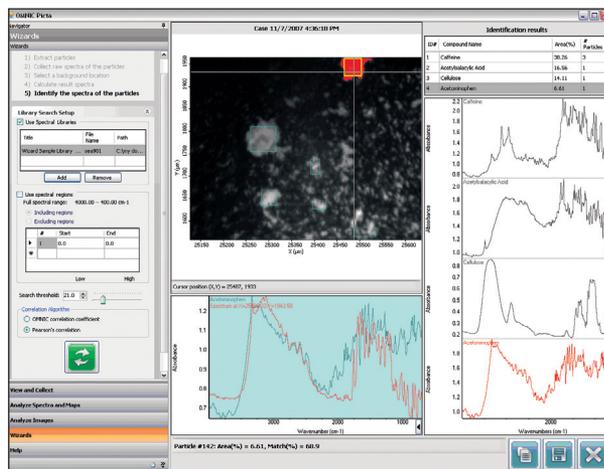
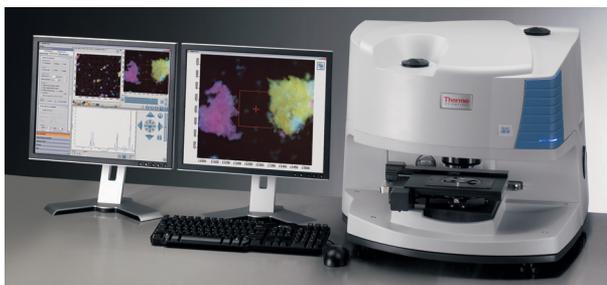


Figure 4: Particle analysis wizard of OMNIC Specta™ allows any user to collect, identify and measure samples in just a few mouse clicks. OMNIC Picta includes other wizards specifically designed for cross-section analysis and automatically extracts different components from area maps.

OMNIC Picta performs many smart, yet simple, operations automatically. The optics are parked when not in use. The selection of background location and collection parameters are set depending on the sample analysis mode. Optical sensors help protect the system from damage that may otherwise occur through incorrect operation. Icons and controls that do not apply to the user-selected sampling mode are hidden, to eliminate confusion and potential mistakes.

OMNIC Picta includes wizards to guide the user efficiently through the measurement of several common sample types. These include particles, fibers, randomly distributed mixtures and laminates. As an example, the particle analysis wizard locates sample features from the video image, measures the size of each feature and uses the dimensions to adjust the aperture for each one. After collecting a spectrum for each particle, the wizard automatically moves to an appropriate background location and collects as many background spectra as needed. Once the acquisition is complete, the wizard displays the spectrum of each feature and its relative size. If necessary, an identification routine will automatically search the spectrum of each feature against user-selected libraries, group similar features by material, class distribution and relative percentages. OMNIC Picta provides useful information simply and efficiently.



The Nicolet iN10 integrated FT-IR microscope is a completely independent infrared microanalysis system, which does not require an external spectrometer.

Answers with Confidence

Confidence in the results is essential to formulate accurate conclusions while providing justifiable answers. In fact, a very common use of infrared microscopy is failure analysis for industrial environments, or trace evidence analysis in forensic science labs. In both cases, laboratory scientists have to take direct responsibility for providing answers that significantly resolve quality problems or criminal investigations.

The identification of pure compounds is typically a simple task obtained through database comparison and correlation against thousands of spectra. Library searching is universally considered a very efficient and reliable tool for the identification of unknown materials. However, identification gets complicated when the sample is composed of multiple materials. In this case, the analyst has to select different regions of the sample in an attempt to obtain individual spectra of pure materials – sometimes with no specific knowledge – and search the unknown over multiple iterations.

Spectral processing and interpretation typically requires a significant amount of infrared spectroscopy skill, and even when they are available, it takes a considerable amount of time.

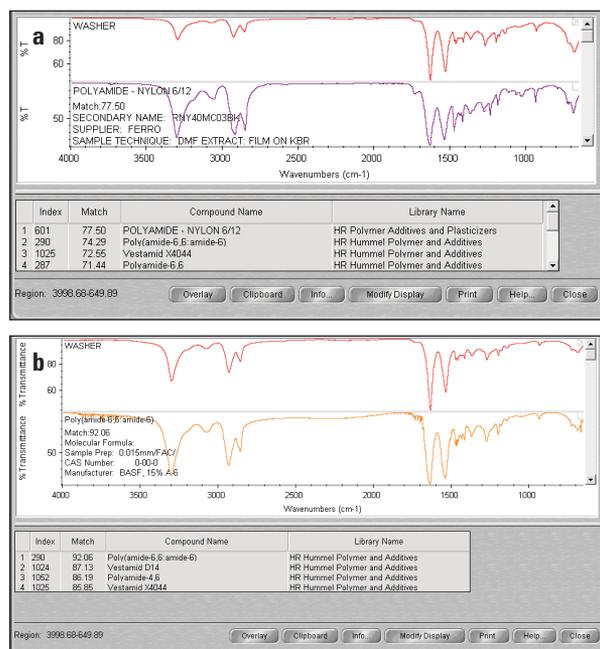


Figure 5: The original spectrum of a polymer material searched against transmission libraries can be identified with acceptable correlation match (top image). However, applying the OMNIC Picta unique ATR correction allows more accurate identification. In the bottom image, the same material after correction is identified as a different type of Nylon (6,6 instead of 6,12) and the correlation match improves from 77.5 to 92 (in a 0 - 100 scale). Detailed visual observation of the two results in the region between 1700 cm^{-1} and 900 cm^{-1} confirms that the second result is more accurate.

OMNIC Picta includes the spectral processing tools necessary to guarantee the highest confidence in search identification. The proprietary ATR correction function precisely calculates and removes optical effects introduced by the ATR crystal in the final spectrum, allowing identification against transmission libraries and superior correlation matches. This feature is extremely important since it does not require the user to create or purchase dedicated ATR libraries. Therefore, standard infrared transmission libraries can be utilized.

When analyzing mixtures, OMNIC Spectra (software designed specifically for spectral identification and interpretation) provides consistency in the search process and multi-component information, with no user intervention.

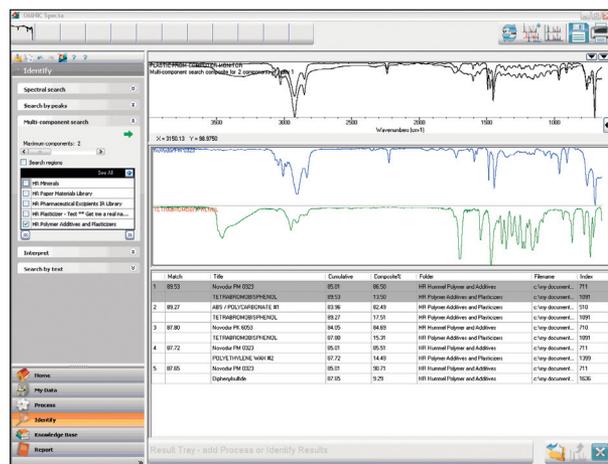


Figure 6: OMNIC Spectra multi-component identification of a polymer material fragment. The main compound is an acrylonitrile styrene butadiene techno-polymer (ABS) and several other peaks, which would not be identified by a simple search algorithm, match the spectrum of a bromide bis-phenol flame retardant.

Typically, the identification of mixtures by infrared spectroscopy is challenging. Even binary mixtures can be difficult to resolve. OMNIC Spectra is applicable for infrared microscopy results in cases where spatial isolation of particles is insufficient to obtain pure component spectra. OMNIC Spectra can identify up to four components in mixtures and provides semi-quantitative information by calculating the synthetic spectrum that best matches the unknown. The resulting co-addition of identified compounds and the cumulative match index build the level of confidence in the results.

Conclusions

The Nicolet iN10 FT-IR microscope sets a new benchmark for confidence in results and ease of use. It provides usable technology to address system performance verification, locating and focusing samples, error-free experiments, and interpreting results with extraordinary power.

Using the Nicolet iN10, microscopists and FT-IR users will benefit from the speed of analysis, exceptional ergonomics and confidence delivered by the instrument. Those who are new to the technique can now run their samples without becoming experts in microscopy.

The Nicolet iN10 is an integrated microscope, which means it does not require an external FT-IR spectrometer to operate. Thus, users of other brands of FT-IR spectrometers can take advantage of this new benchmark in infrared microscopy.

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