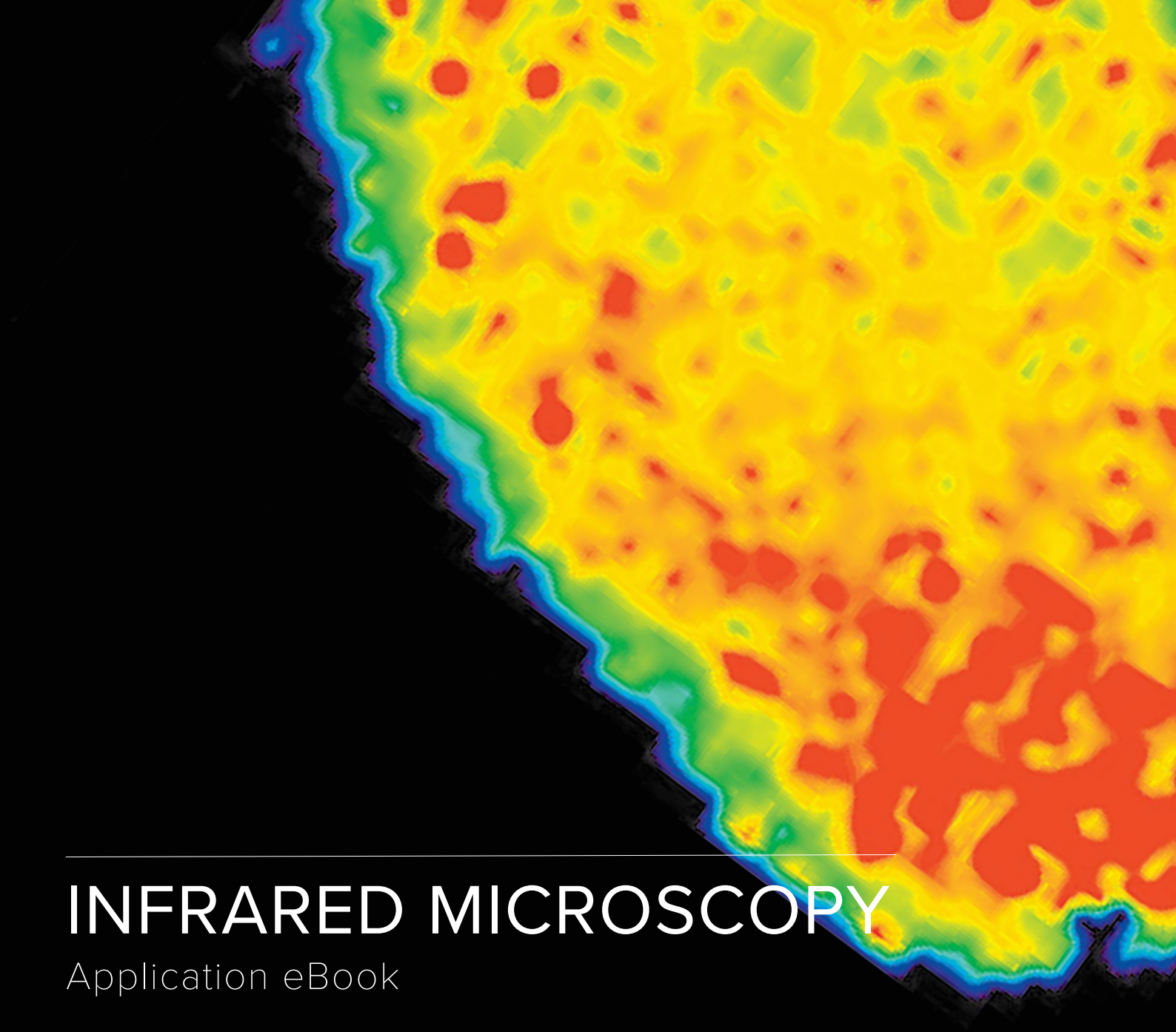


INFRARED MICROSCOPY

Application eBook

JASCO

Introduction to Infrared (IR) Microscopy-spectroscopy

Material Science

Life Science

Environmental Science

Introduction to Infrared (IR) Micro-spectroscopy

FTIR micro-spectroscopy is an analytical technique for imaging materials using functional group(s) for sample identification, surface analysis, multilayer film characterization, and particles characterization. Requiring minimal sample size and preparation, measurements can be made without lengthy procedures to manipulate samples or the use of solvents that would create additional laboratory waste. The use of an IR microscope allows the evaluation of small sample areas to provide useful information for quality control in manufacturing, product development, and failure analysis. The non-destructive nature of IR micro-spectroscopy means that fragile samples, such as biomedical and medical samples, can be readily analyzed. Samples can also be subjected to further analysis by complementary spectroscopic and non-spectroscopic measurements including Raman, UV-Visible and fluorescence. Upon collection of a spectrum, an ever-growing range of spectral libraries can be used to assist in sample identification. Alternatively, through functional group analysis using characteristic absorption bands in the spectra, structural elucidation can be achieved to identify unknown molecular structures for newly created materials, foreign materials in a sample (i.e. forensic studies), or a mixture of compounds (which can also be identified from spectral library searches). There are several measurement methods that can be employed in IR micro-spectroscopy, including transmission, reflection, and Attenuated Total Reflectance (ATR).

Transmission FTIR Microscopy

Transmission microscopy is one of the most common methods for analyzing solids, liquids, and gases, and it is the method of choice for analyzing films, laminates, and fibers. For successful transmission measurements, light is focused through the sample using a size-adjustable aperture and then collimated by a condenser onto a detector. Samples must be relatively thin (less than 50 µm) to allow the light to adequately pass through the sample. Transmission measurements provide the highest sensitivity and best detection of all IR sampling techniques.

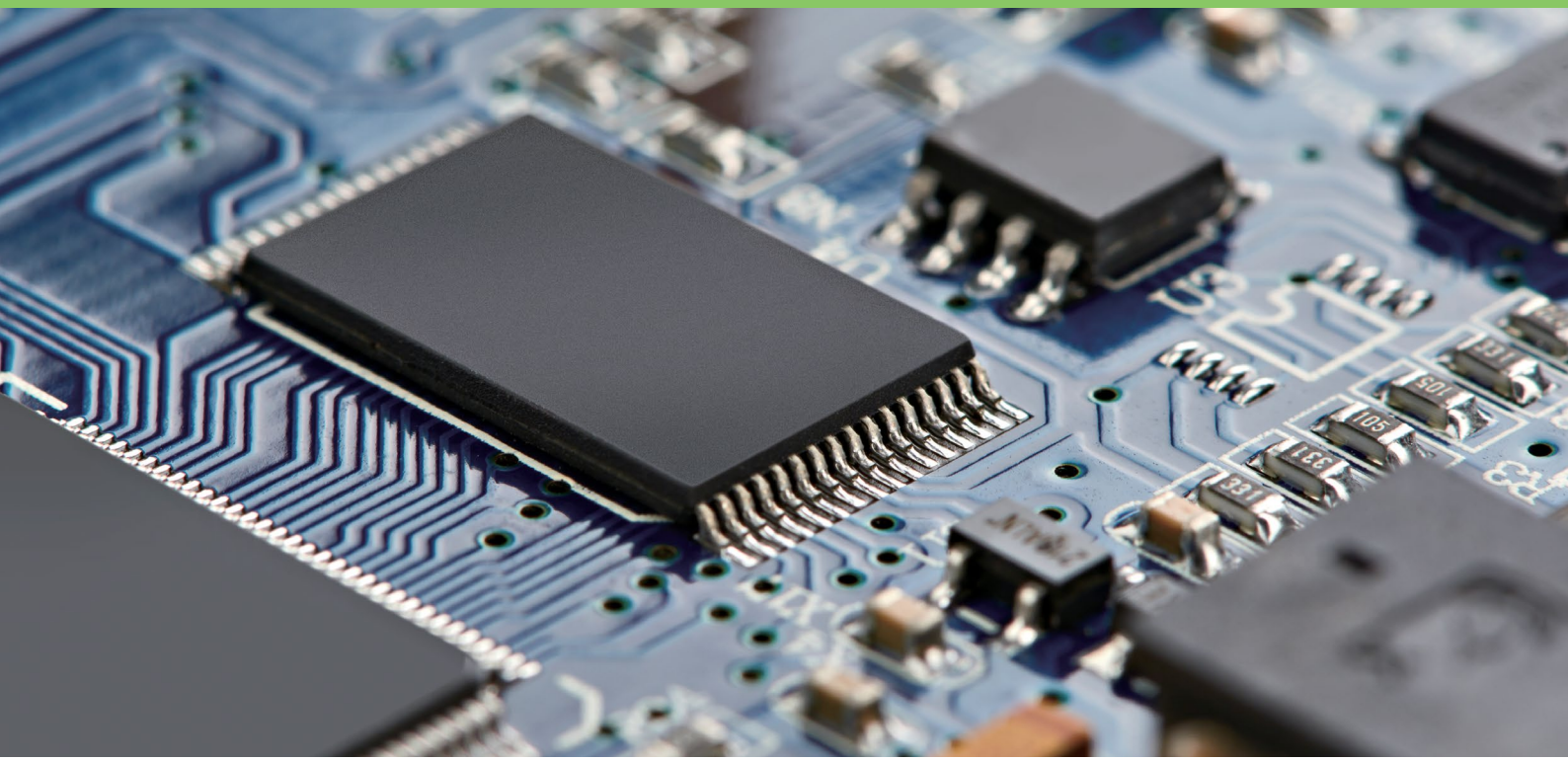
Reflection Absorption (including Grazing Angle) FTIR Microscopy

Reflection measurements work best for reflective materials, but it is not effective for dark materials or non-reflective substrates. Reflection measurements are performed with a single Cassegrain objective, which uses a primary and secondary mirror to direct the IR beam onto the sample and return the reflected light to the detector, typically with angles from 25 – 35° depending on the magnification of the Cassegrain objective.

Grazing angle reflection is often used to examine the thinnest of surface coatings by 'grazing' the sample at a very shallow incident angle, which results in longer sample path lengths providing greater sensitivity. Coatings on 'shiny', highly reflective substrates are excellent candidates for infrared reflection absorption studies. As the surface coating layer to be measured becomes thinner, incidence and collection angles can be adjusted from 45 – 75° until the 'grazing' angle is reached, generally considered to be maximal at 85°. The term reflection absorption' describes the process of the incident beam as it passes through the coating, reflects from the substrate, and passes back through the coating again before reaching the detector. As the incident and collection angles approach the grazing angle, the incident beam strikes the coating at shallower angles and the path length becomes maximal, enhancing the absorption intensity. Infrared Reflection Absorption Spectroscopy (IRRAS) is often used to study monolayer coatings on metals and other substrates.

Attenuated Total Reflectance (ATR) FTIR Microscopy

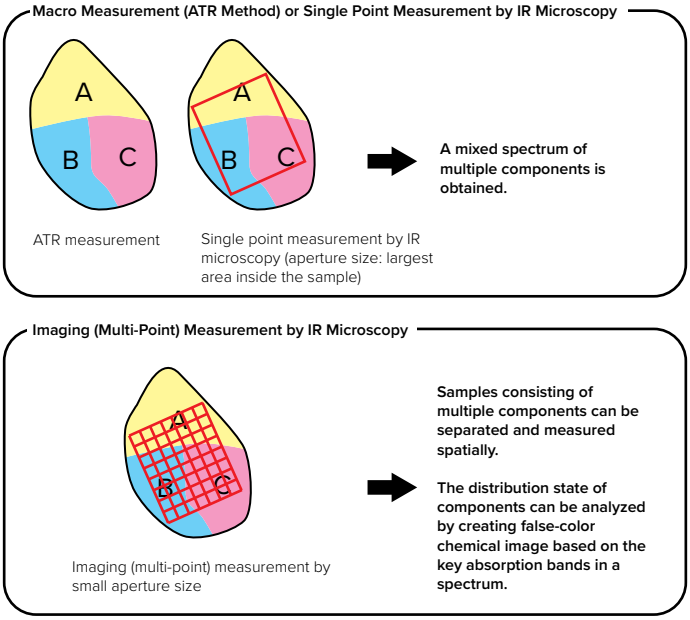
An ATR sampling accessory consists of a series of focusing mirrors that direct the spectrometer's IR beam into a crystal prism (typically at a 45° angle, but for optimal measurement, the angle can be different for some ATR accessories including ATR microscope objectives). Even though total internal reflection occurs within the prism, an evanescent field is propagated from the crystal surface into the sample that is in intimate contact with the prism; for solid materials this requires a device to compress the material against the prism. Following molecular interaction with the light, the waveform is passed onto the detector. The prisms used in ATR accessories are selected according to specific application requirements and can be manufactured from IR transmissive crystal materials, including diamond, zinc selenide (ZnSe), zinc sulfide (ZnS), and germanium (Ge). The penetration depth (and hence effective path length) of light interaction with the sample is dependent on the refractive index of both the sample and crystal prism, as well as the wavelength of measurement. In the mid-IR and fingerprint region, the penetration depth is typically in the region of several microns but is smaller as the wavelengths approach the near-IR. Spectra measured using ATR can be



Analysis of Foreign Materials

In the manufacturing process of industrial products, the inclusion of foreign materials can cause defective products. FTIR spectroscopy is an effective tool for the analysis and identification of causes of failure. If a foreign material consists of a single component, it can be relatively easy to identify the component using macro-ATR; however, since foreign materials often contain several components, they are harder to analyze and identify using macro-ATR, which in a spatially wide area cannot discriminate a mixture of components. In such cases, imaging measurements using IR microscopy is more suitable for analysis of foreign materials. During imaging, the measurement area is finely divided into a grid pattern and a spectrum at each point is measured.

This facilitates the identification of each spatially separated component. In addition, by creating false-color chemical images, the spatial distribution of multiple components can be imaged, which cannot be seen directly from the observed images. This section includes examples of foreign material analysis using IR Microscopy.



Microscopic Transmission Method

Analysis of Foreign Materials in Silicon Wafers

Foreign materials in silicon wafers (Fig. 1) were measured using high-speed FTIR imaging, and comparison with a spectral database was performed for each measurement position. As a result, it was found that the foreign materials that could not be identified by visual observation were composed of three components: protein, calcium carbonate, and cellulose (Fig. 2). A false-color chemical image was created based on the peak height of the dominant absorption band for each of the components, which allowed us to spatially separate and display the complex distribution of foreign material in a silicon wafer consisting of protein, calcium carbonate, and cellulose and their relative concentrations (Fig. 3).

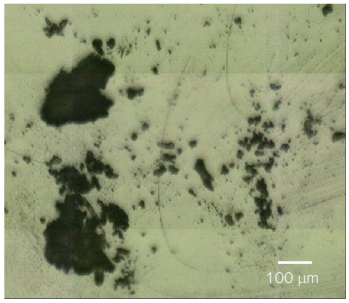


Fig. 1 Observation view

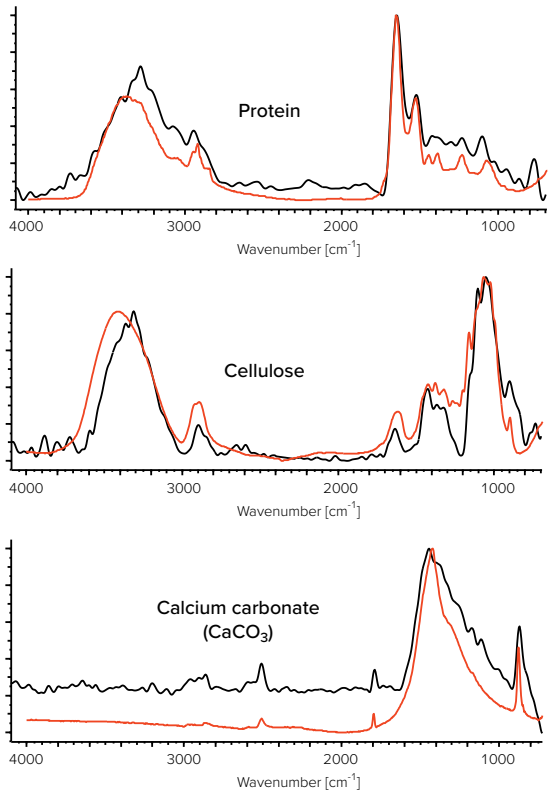


Fig. 2 Database search results
(black: measurement result, red: database search result)

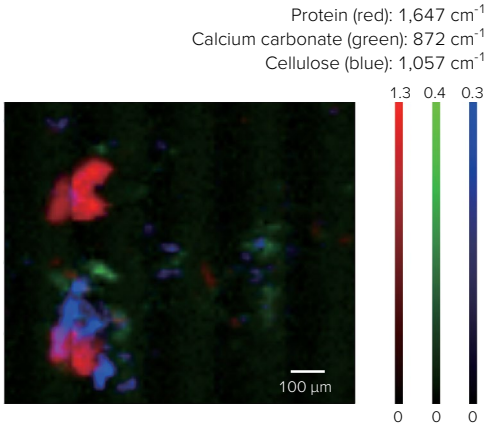


Fig. 3 Imaging result (peak height)

Microscopic Transmission Method

Analysis of Foreign Materials on Rubber Surfaces

White foreign materials that were found adhered to a sample of rubber packaging were removed and compressed between two KBr plates for imaging measurement (KBr plate method). After sample preparation, the imaging measurement was performed using FTIR microscopy.

Four unique spectra were found at various sample positions in the mapping measurement. (Figs. 4 – 7). Each spectrum can be interpreted as containing the following components:

Spectrum #1 (Fig. 4): This result suggests that the spectrum is a mixture of silicone and protein because there are peaks attributed to the Si-CH_3 stretching (near 1,260 cm^{-1}), to the Si-O stretching (near 1,000 to 1,100 cm^{-1}), to the Si-C stretching (near 800 cm^{-1}) and to the amide I and amide II bands (near 1,650 cm^{-1} and 1,550 cm^{-1}, respectively).

Spectrum #2 (Fig. 5): This result suggests that the spectrum is a protein because there are peaks attributed to the amide I and amide II bands and the N-H stretching (near 3,300 cm^{-1}).

Spectrum #3 (Fig. 6): This result suggests that the spectrum is a zeolite (aluminosilicate, a type of silicate) or talc (magnesium hydroxide and silicate) from the strong peak around 1,020 cm^{-1}.

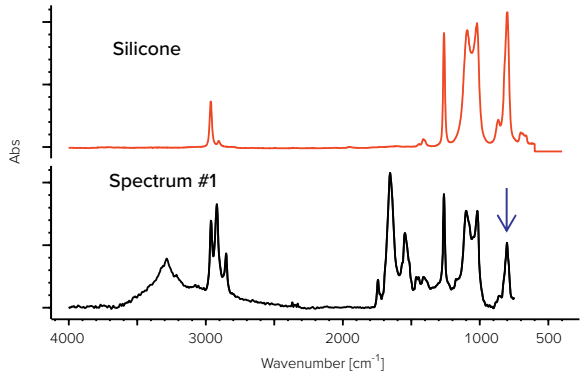


Fig. 4 Typical spectrum of mixed foreign material
and database search results (Spectrum #1)

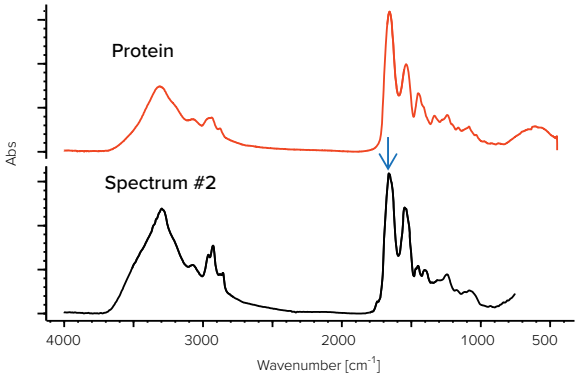


Fig. 5 Typical spectrum of mixed foreign material
and database search results (Spectrum #2)

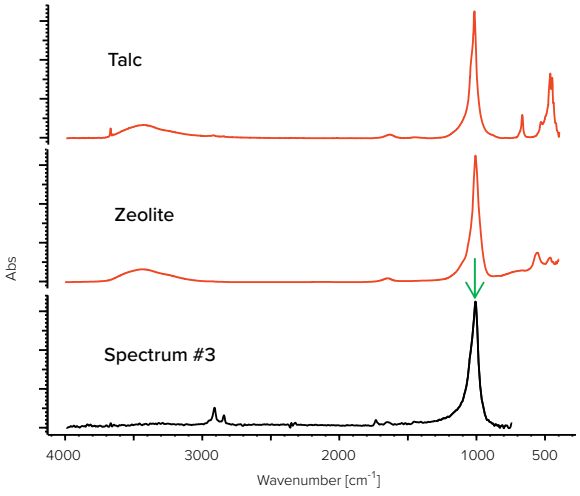


Fig. 6 Typical spectrum of mixed foreign material
and database search results (Spectrum #3)

Spectrum #4 (Fig. 7): This result suggests that the spectrum is an oil because there is a peak attributed to a C-H stretching (near 2,940 cm^{-1}) and a peak attributed to a C=O stretching (near 1,750 cm^{-1}) with a close match to the spectral database oil spectrum.

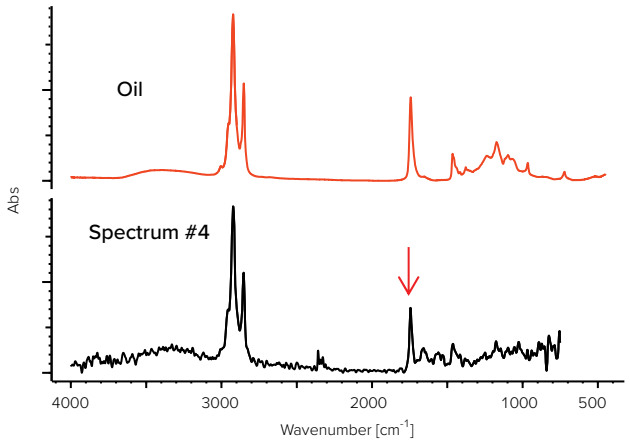


Fig. 7 Typical spectrum of mixed foreign material and database search results (Spectrum #4)

Next, a false-color chemical image was created using the peak heights of the key absorption bands of each component (indicated by the arrows in the spectra), which was then compared with the optical image (Figs. 8 and 9). The false-color chemical image shows that most of the foreign material is oil and protein, with trace amounts of silicone and zeolite or talc.

Imaging measurements enable the identification of each component in the mixed foreign material and the spatial distribution throughout the sample. The identification of the components in the foreign material is expected to lead to a review of the manufacturing process to avoid introduction of foreign materials in the end-product.

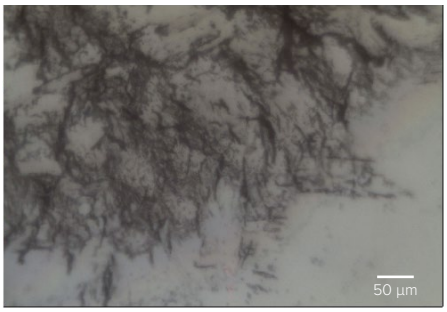


Fig. 8 Observation view

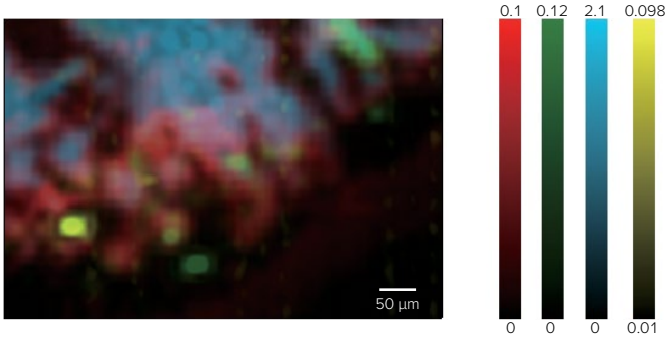


Fig. 9 False-color chemical image showing the spatial distribution of each component (red: oil, green: zeolite or talc, blue: protein, yellow: silicone)

Microscopic Reflection Absorption Spectroscopy (RAS) Method

Analysis of Foreign Materials on Solders

The Reflection Absorption Spectroscopy (RAS) method can be used when an object to be measured is adhered on a highly reflective surface, such as a metal plate. In this method, the absorption spectrum of the sample is acquired and evaluated by reflecting IR light from the reflective surface.

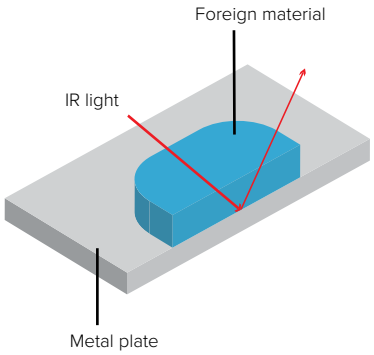


Fig. 10 Overview of reflection absorption method

Here we present the evaluation of an adhered foreign material on solder using the RAS method. The results indicated that it was rosin (a natural resin derived from pine resin).

The RAS method has the benefit of being a non-contact, non-destructive measurement technique. For optimal sample measurement, some sample preparation may be needed to avoid saturation of the signal or baseline curvature in the spectrum. Thick samples may need to be sliced to avoid signal saturation. If the sample or underlying surface is rough, the spectral baseline may exhibit curvature due to light scattering, so sample preparation is advised to improve the surface condition.

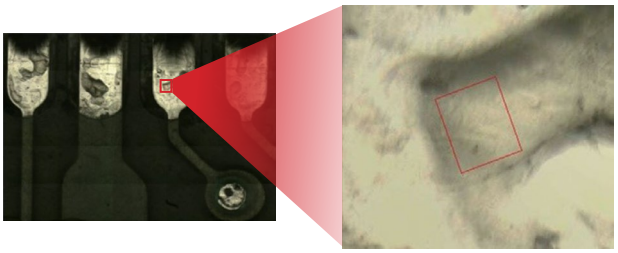


Fig. 11 Observation view (measurement size: 100 µm × 80 µm)

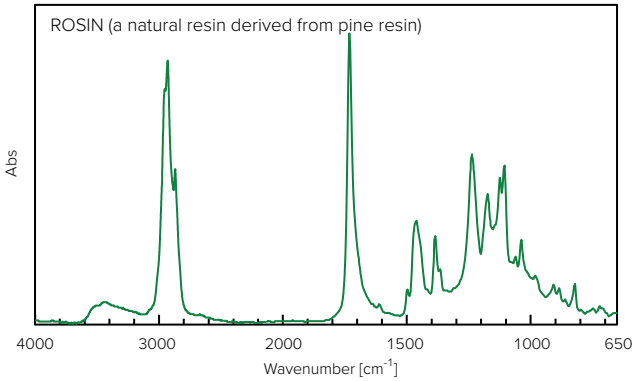


Fig. 12 Microscopic reflection absorption spectrum of foreign material on solder

Tips

Simple Sample Preparation using a Diamond Cell

For transmission IR spectroscopy, the sample must be thin enough to allow the transmission of light. Common sample preparation methods for overly thick samples include thinning the sample by applying pressure between KBr plates or sectioning with a microtome or slicer, which are both effective methods to measure transmission IR spectra. To simplify such sample preparation, JASCO offers the DC-500 diamond cell, which enables the user to perform IR transmission measurements without using a press or a slicer. The sample is placed between two diamond plates and pressure is applied carefully by turning two knobs, enabling sample thinning for the transmission measurement. Another feature of the diamond cell is the surface smoothness of the diamond plates used, which makes it easy to check for foreign materials during microscopic observation.

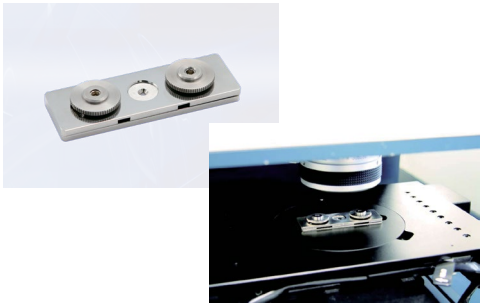
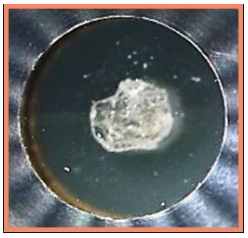


Fig. 13 Diamond cell

As an example, a transmission FTIR spectrum of a rubber sample was measured using a diamond cell (Fig. 14). Sample freezing may be required when preparing a rubber sample for sectioning with a microtome. However, the diamond cell can adjust the thickness of the rubber sample easily without freezing. From the observation view, the sample could be thinned by applying pressure to the diamond cell. In the IR spectrum, the peak position around 2,900 cm^{-1} is saturated and cannot be resolved before pressure is applied, but applying pressure and thinning the sample allows the spectrum to be completely resolved.

Before applying pressure



After applying pressure

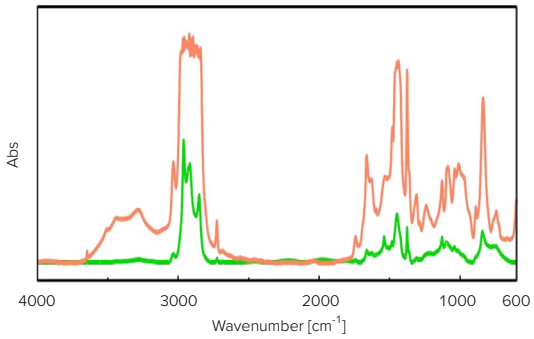


Fig. 14 IR spectra of rubber samples measured using a diamond cell (red: before applying pressure, green: after applying pressure)

The diamond cell can be easily adjusted to create a sample thickness suitable for a wide variety of IR transmission methods. When used with an IQ Frame, it can also be used for correlated analysis using IR and Raman spectroscopy.

Tips

KnowItAll

Wiley's KnowItAll, which is a highly regarded spectral library and search software, is used as standard for spectral identification. The search software starts from one click in the IR Microscope Imaging Analysis software (Fig. 15).

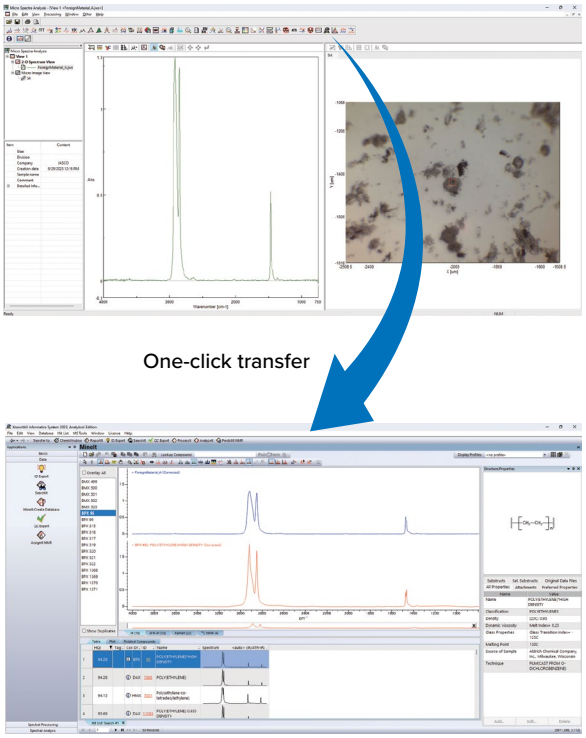


Fig. 15 KnowItAll

KnowItAll includes various functions, such as spectral/peak/structure search, mixture analysis, spectral interpretation, support, and more. It includes 12,600 Wiley original IR spectra and 625 JASCO original IR spectra as the standard reference library. Wiley's 339,000 IR spectra library is also available as an option.

Mixture Analysis (ID Expert)

For unknown samples containing multiple components, up to five components can be identified in a single spectrum using the IDExpert.

Spectral Interpretation Support (AnalyzeIt)

AnalyzeIt allows users to identify specific functional groups that match the absorption peaks in a sample spectrum.

User Database Builder (MineIt)

MineIt allows users to create their own database, including the sample spectra, structural formula, and physical properties. It enables the user to manage and share the measurement data that is specific to the user's needs.

Multi-Technique Simultaneous Spectral Searching (SearchIt)

SearchIt is a "simultaneous" multi-technique spectral search, which allows users to search IR and Raman spectra simultaneously to find the most relevant matches in each database linked to each other by chemical structure.

Microscopic ATR Method

Analysis of Foreign Materials on Resin Products

Attenuated Total Reflectance (ATR) is an effective surface measurement technique that can be used when a sample is too thick for the RAS method, which leads to signal saturation, or when the substrate is non-reflective. It is an ideal technique when only information about foreign materials adhering to a substrate's surface is needed..

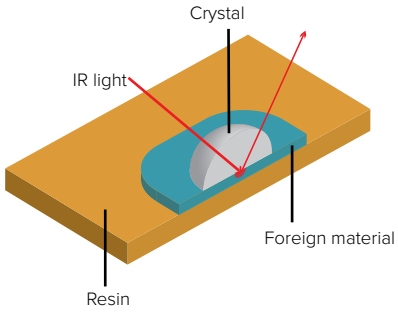


Fig. 16 Overview of the ATR method

As an example, a foreign substance adhered to a resin (Fig. 17) was evaluated using an ATR fitted with a Zinc Selenide (ZnSe) crystal, which has a typical penetration depth of approximately 1 – 2 µm. The results show that the adhered particles were azo dyes with characteristic peaks of azo compounds in the 1,300 – 1,500 cm^{-1} region (Fig. 18). If the thickness of the adherent is quite thin (1 µm or less), and information only about the adherent is required without interference from the substrate, a Germanium (Ge) crystal, which has a higher refractive index that results in a smaller penetration depth, may be a better alternative. If the surface of the sample matrix obstructs the measurement point, it may interfere with the conventional ATR measurement. In this case, it may be necessary to use a projection-type ATR accessory, such as the ATR-5000-G45, which has the benefit of extending the prism of the ATR with a narrower projection.

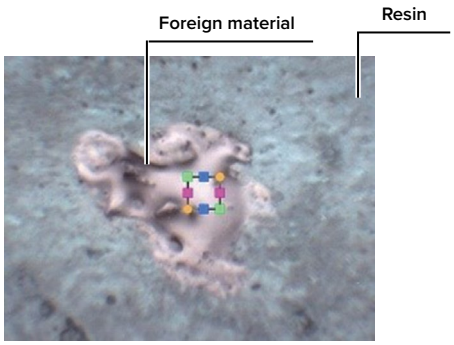


Fig. 17 Observation view

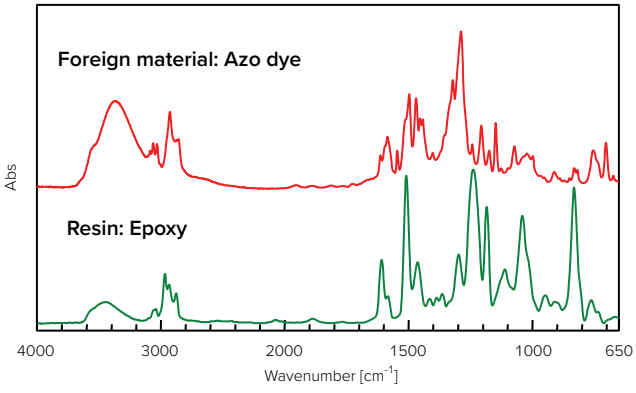


Fig. 18 Spectra of the resin (green: epoxy) and the foreign material (red: azo dye)

ATR Imaging Measurement of Particles Suspended in Machine Oil

When using the ATR method, it can be difficult to confirm that the crystal is in intimate contact with the sample, especially for fluidic samples that may move as the crystal encounters the sample. This problem also makes FTIR mapping measurements of a fluidic sample with an auto-stage extremely difficult because the ATR crystal contact point must be moved for each spectrum to be measured. By using IQ Mapping with a ClearView ATR objective, it is possible to visually confirm that the crystal is in contact with the correct sample measurement area. Since the crystal does not move during IQ Mapping measurements, accurate sample contact is maintained without disturbing the position of the sample.

ClearView ATR objectives allow both observation of the sample and ATR spectral measurement using a single objective, by switching the crystal position between Survey mode (up) and ATR mode (down). The ClearView ATR objective is first used in Survey mode to observe and identify the desired sample measurement area. Once the crystal is in contact with the sample, the accurate selection of the sample measurement area is confirmed. This single-point contact between the sample and the crystal also prevents contamination during mapping measurements. To demonstrate the effectiveness of this ATR imaging technique, a mapping measurement of particles suspended in machine oil was performed, and a false-color chemical image was created using the 1,720 cm^{-1} peak height, which is a dominant absorption peak in the polymethyl methacrylate (PMMA) spectrum. (Figs. 19–21). From the observed image and the false-color chemical image, it was confirmed that the distribution and chemical composition of the particles could be well mapped and imaged, and from the spectrum the identity of the suspended particles was confirmed as PMMA.

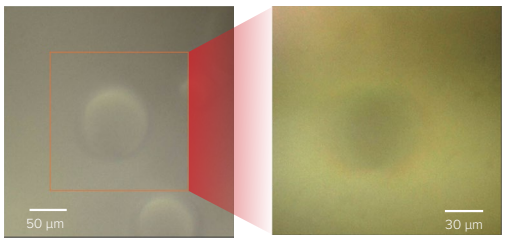


Fig. 19 Observation view
(left: sample before crystal contact, right: sample after crystal contact)

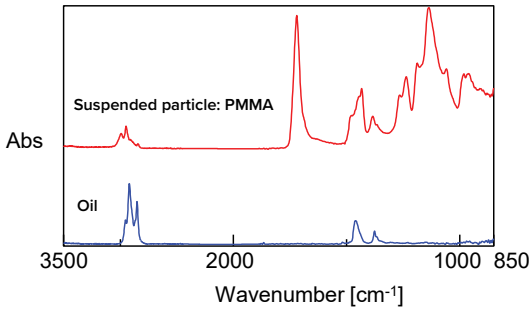


Fig. 20 Spectra of the machine oil (blue) and the suspended particle (red: PMMA)

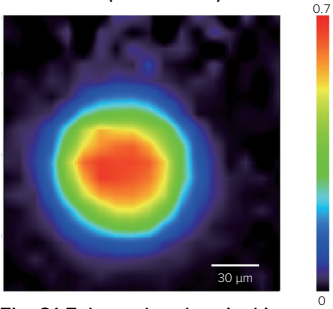


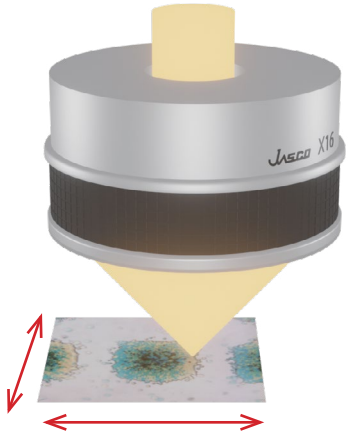
Fig. 21 False-color chemical image (peak height: 1,720 cm^{-1})

Tips

IQ Mapping

In a typical sample mapping measurement using IR microscopy, it is necessary to move the sample stage from one measurement point to the next. By scanning the IR light across the sample (IQ Mapping), JASCO's IR microscopes can perform mapping measurements without the need to move the sample stage, making it possible to perform a mapping measurement even when using a manual sample stage. This unique feature is especially beneficial for ATR measurements, as the sample area in contact with the ATR crystal can be mapped without repositioning the objective prism, which can disturb the sample.

Conventional mapping method (stage scanning)



IQ Mapping method (IR light scanning)

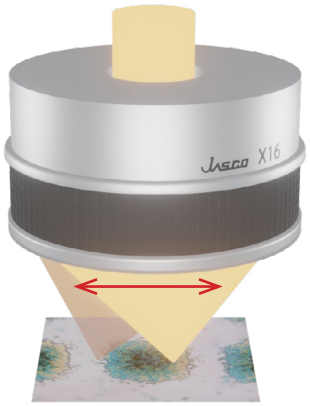


Fig. 22 IQ Mapping allows the sample measurement area to be moved while using a stationary Cassegrain objective to build up the sample image.

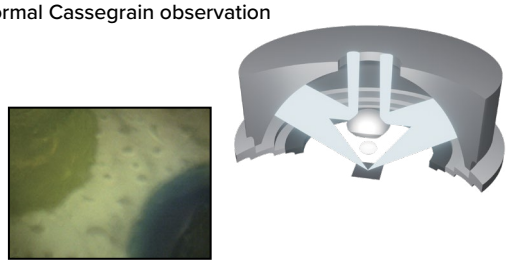
Tips

ClearView ATR Objectives

In conventional microscopic ATR measurement, the sample is observed with a standard reflection Cassegrain objective to determine the measurement point(s) in advance, and then the Cassegrain objective is switched to an ATR objective for measurement.

However, sample observation cannot be performed after switching to the ATR objective, and it is not certain that the target measurement area of the sample is in contact with the center of crystal. Reviewing the measurement results is the only way to confirm whether the target sample area has been correctly measured, which can be a waste of time, especially when the sample has been disturbed.

Normal Cassegrain observation



ATR Contact observation

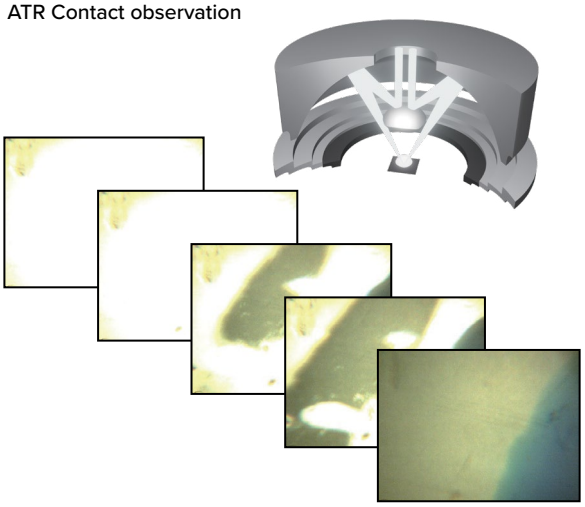


Fig. 23 Optical system of of a ClearView ATR objective

JASCO's ClearView ATR objectives can be used to observe the sample surface while the crystal is in direct contact with the sample, in addition to the usual sample observation, allowing the target sample area to be measured with absolute certainty.

Even if the target object shifts and becomes off center during contact of the crystal and sample, IQ Mapping allows the aperture controlling the IR light path to be moved to the target object for measurement. With IQ Mapping, the measurement position can be adjusted to any desired location by moving the aperture around the entire field of view of the objective.

Combined IR and Raman Analysis

Cross-Section Analysis of a Multilayer Film

Cross-sections of a multilayer laminate film were prepared; measurement areas were identified, and the exact same area was mapped using both IR and Raman microscopes by utilizing the IQ Frame (Fig. 24). Principal component spectra were calculated by Multivariate Curve Resolution (MCR) from the spectral results (Figs. 25 and 26), and false-color chemical images were created from the principal component spectrum scores to illustrate the distribution of the identified components making up each layer (Figs. 27 and 28).

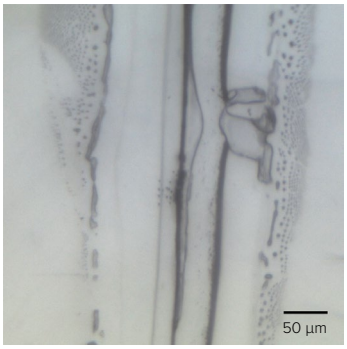


Fig. 24 Observation view

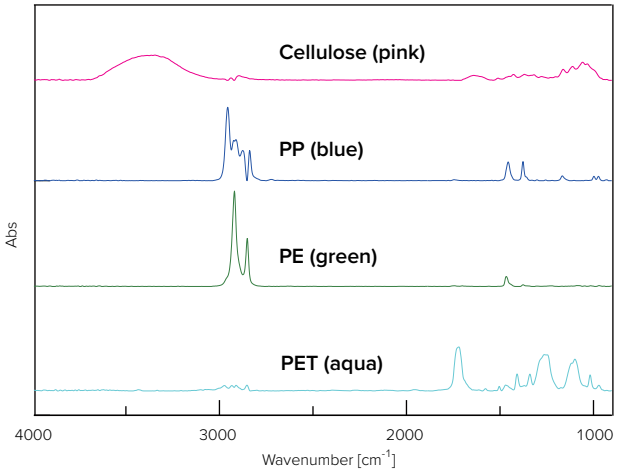


Fig. 25 Principal component spectra calculated by MCR (IR)

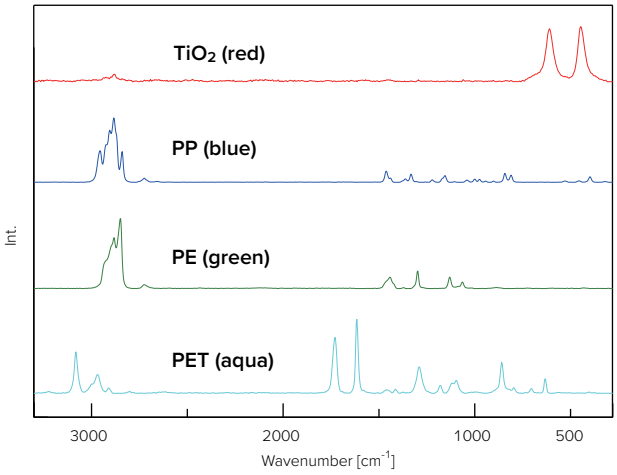


Fig. 26 Principal component spectra calculated by MCR (Raman)

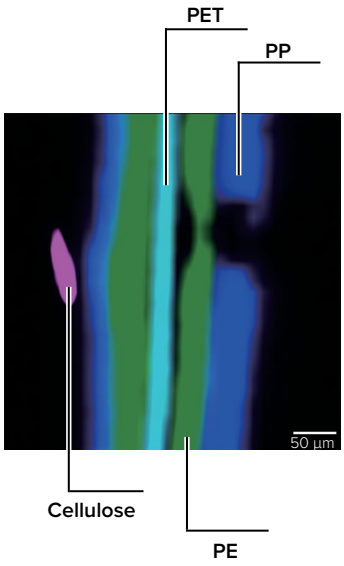


Fig. 27 False-color chemical image obtained using IR microscopy

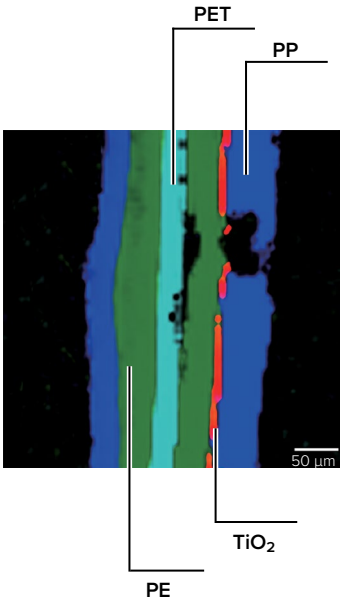


Fig. 28 False-color chemical image obtained using Raman microscopy

The distribution of polypropylene (PP), polyethylene (PE), and polyethylene terephthalate (PET) was visualized using both spectroscopic techniques. Cellulose was selectively identified using IR microscopy and titanium dioxide (TiO_2) was selectively identified using Raman microscopy as a very thin layer with a thickness of only a few µm. The fact that cellulose is not a constituent of the film sample measured in this study suggests that it is likely to be a contaminant. This example demonstrates that by using both Raman and FTIR, complementary information can be obtained for a more complete analysis.

Analysis of Paints and Coatings

Paints and coatings not only improve appearance and protect underlying substrates, but they can also provide functionality. In order to manufacture paints and coating films with better appearance, performance, and durability, it is necessary to analyze and understand the chemistry and functionality of these products at the molecular level. In addition, foreign material and impurity analysis is essential to investigate the product uniformity and identify defects to determine their cause and ensure the manufacture of defect-free products on the production line.

Since the main components of paints and coatings are organic compounds, including polymers, resins, pigments, and additives, FTIR is an effective analytical technique to evaluate their chemical composition, structure, and behavior. The use of an IR microscope also allows the evaluation of smaller sample areas, providing useful information for quality control in manufacturing, product development, and failure analysis.

Here we present an example of paint and coating film analysis using an IR microscope.

Microscopic ATR Method

ATR Imaging Comparison of Viscous Paint Samples

When performing ATR imaging, the measurement technique must be carefully selected to optimize the analysis. In conventional ATR imaging measurements, a motorized stage is used to repeatedly move the sample to measure a new area and to remake contact of the ATR crystal with the sample. Though this is generally an effective method for measuring wide areas of samples, when measuring tacky or viscous samples, the sample may adhere to the crystal after the first contact. This results in contamination of subsequent measurement points of contact, which makes it difficult to obtain accurate data due to the spectrum of the cross-contamination of the ATR crystal and sample surface.

ATR imaging with IQ Mapping requires only a single point of contact between the crystal and the sample since the IR light is scanned across the sample without having to move the motorized stage. This provides contamination-free measurements and minimizes the risk of the sample moving or becoming damaged during analysis.

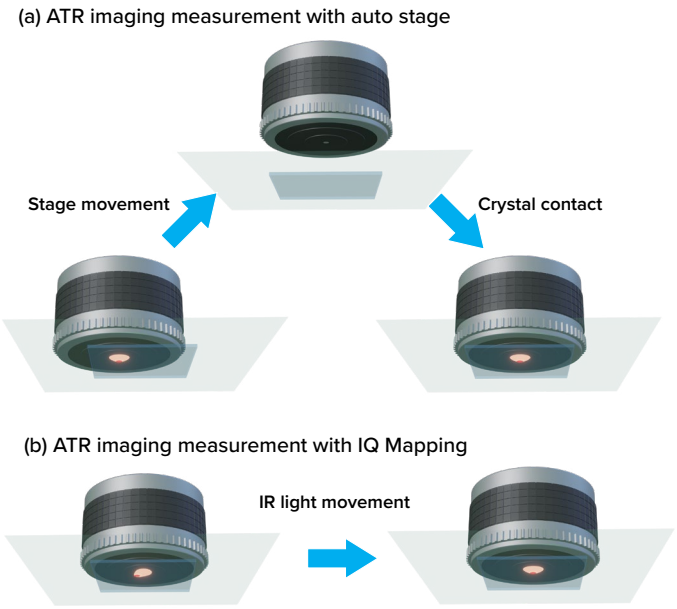


Fig. 1 Overview of ATR imaging measurement techniques

In this example, we measured a mixture of water-based and oil-based paints. First, an ATR imaging measurement was performed using an auto-stage (Figs. 2 – 4). Since the ATR crystal and the sample were in contact with each other frequently during the stage movement, contaminants adhered to the crystal and oil-based components could not be detected (Fig. 4). In addition, the shapeof the sample changed when the ATR crystal contacted the sample, resulting in a completely different shape between the observed image and the false-color chemical image (Figs. 2 and 3).

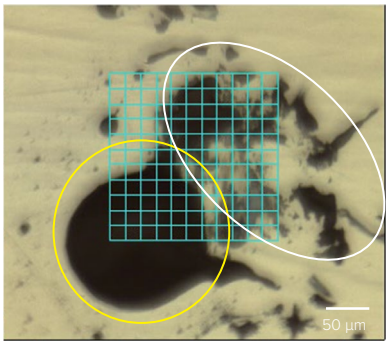


Fig. 2 Observation view
(yellow: water-based paint, white: oil-based paint,
aqua: measurement area)

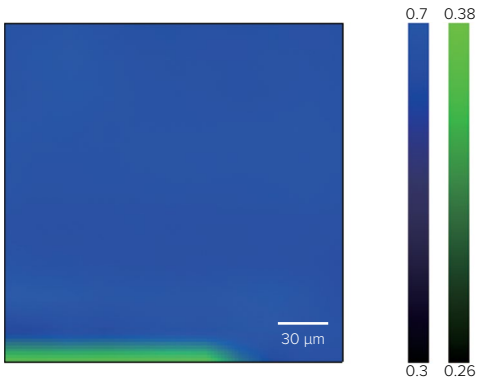


Fig. 3 False-color chemical image (green: water-based paint, blue: water-based paint + contamination)

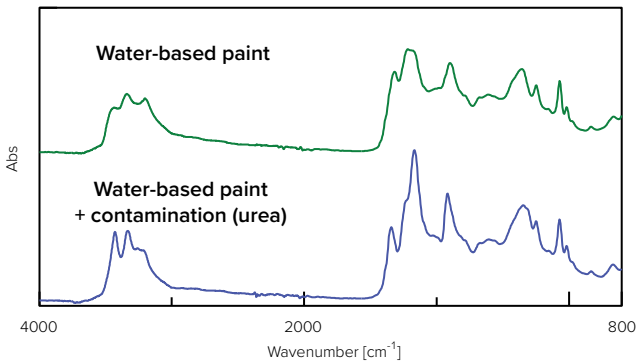


Fig. 4 Spectra of water-based paint (green) and water-based paint + contamination (urea) (blue)

Next, we show the results of an ATR imaging measurement using a Zinc Selenide (ZnSe) ATR crystal and IQ Mapping; changing the aperture measurement position without moving the sample stage. In this example, a sample comprised of a mixture of water-based and oil-based paints was prepared for measurement (Fig. 5).

Spectra with different components were detected depending on the measurement position, and each unique spectrum could be confirmed (Fig. 7). A false-color chemical image was created using the peak intensities of the main peaks at 1,666 cm^{-1} and 1,282 cm^{-1} (Fig. 6) for the water-based and oil-based paints, respectively. Selection of a suitable measurement technique allows the detection of different components at specific locations in a sample that appears to have only one component in the observed image, and to perform accurate sample identification at the microscopic level.

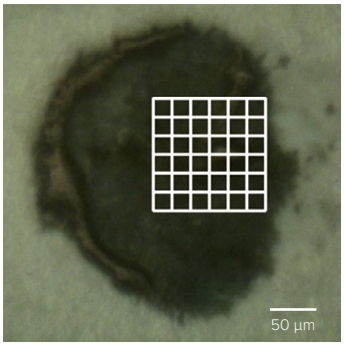


Fig. 5 Observation view
(white: measurement area)

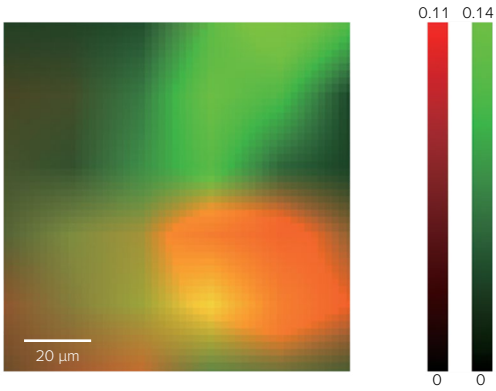


Fig. 6 False-color chemical image
(green: water-based paint, 1,666 cm^{-1} peak,
red: oil-based paint 1,282 cm^{-1} peak)

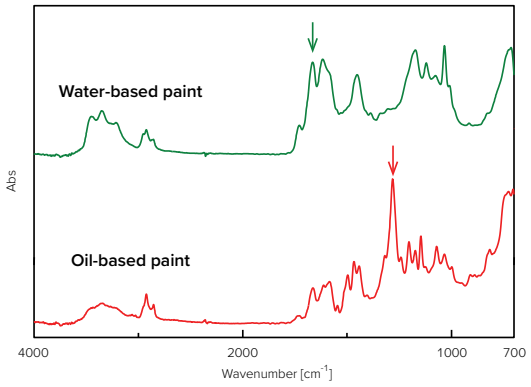


Fig. 7 Spectra of water-based paint (green) and oil-based paint (red)

IR Microscope Imaging Analysis Software Analysis Wizard

Imaging measurements using IR microscopy are widely used to visualize component distribution in a sample. However, when the area being measured is large, with a significant number of data points, there is a possibility of missing components that are localized in the sample. Data analysis skills and time are needed to analyze the large amounts of data generated from imaging measurements, which can be overcome by using model analysis software, such as Multivariate Curve Resolution (MCR). MCR is a function that leverages multivariate analysis to extract the principal component spectra from a collection of measured spectra to create relative concentration distributions. Therefore, MCR can be used to very quickly create false-color images of the each chemical distributed in a sample, together with an indication of their relative concentrations, without missing any of the individual components. In addition, by performing a database search on the obtained principal component spectra, the components can be readily identified. JASCO's IR microscope systems include Analysis Wizard, a group of software tools that make it easy to create false-color chemical images by guiding the user through the key parameters for sample analysis.

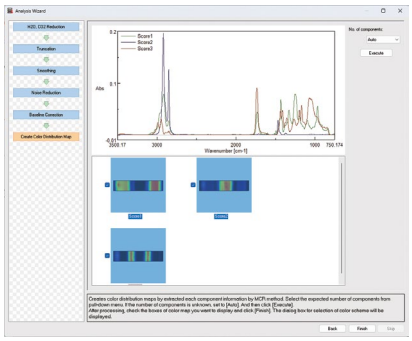
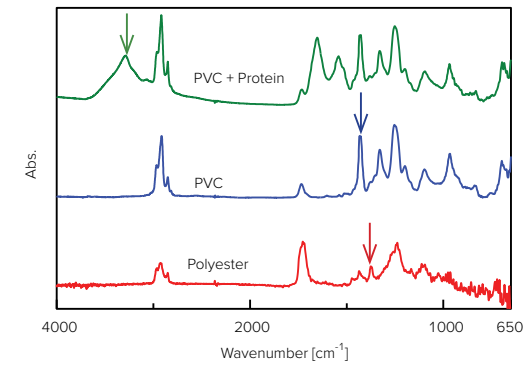
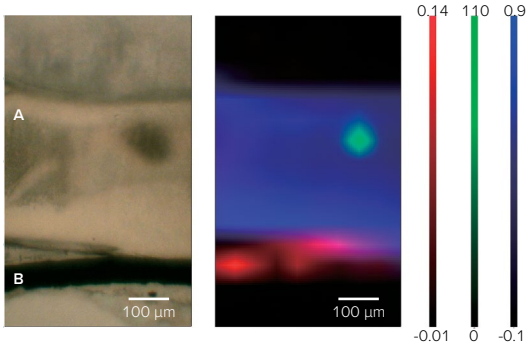


Fig. 8 Analysis wizard

Imaging analysis of a multilayer film was performed. Using the obtained spectra, a false-color chemical image was created from the intensity distribution of the key absorption bands (Fig. 9). From the obtained spectra, it was determined that there are three components in the multilayer film, and the distribution of these components was observed in the false-color chemical image. The results show that polyvinyl chloride (PVC) is distributed in layer A and polyester is distributed in layer B. In addition, the results show that the PVC layer contains protein as a foreign material. Next, using the same spectra, principal component spectra were modeled using MCR, and a false-color chemical image was created (Fig. 10). The model analysis was able to extract components that were not evident in the imaging analysis using key functional group bands and was able to determine the pure spectrum of the foreign material. It can be concluded that model analysis, such as MCR, can be used to obtain false-color chemical images without missing any components and allows the user to perform spectral database searches with high accuracy.



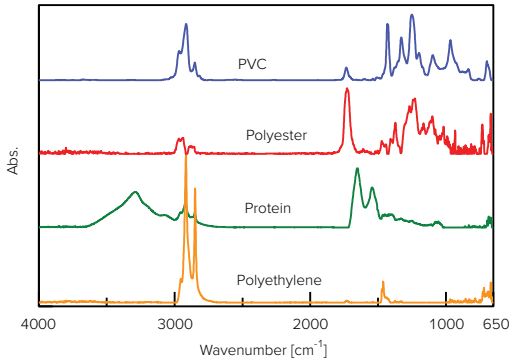
Obtained spectra



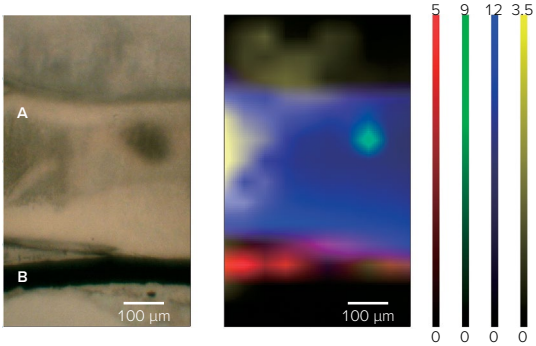
Observation view

False-color chemical image (red: polyester, green: protein, blue: PVC)

Fig. 9 Imaging analysis of multilayer film by key bands (False-color chemical image based on arrowed key bands)



Principal component spectra



Observation view

False-color chemical image (red: polyester, green: protein, blue: PVC, yellow: polyethylene)

Fig. 10 Imaging analysis of multilayer film by MCR

Combined IR and Raman Analysis

Analysis of Car Paint Chips

Analysis combining IR and Raman spectroscopy can provide more information about a sample and can increase confidence in the analytical results. When performing a combined analysis, correlated information from both methods is required, meaning the data must be accurately acquired from the same sample area. This can be accomplished by utilizing an IQ Frame. The IQ Frame enables accurate sample repositioning so that even when the sample is moved from one instrument to another, the exact measurement location(s) can be recorded and identified.

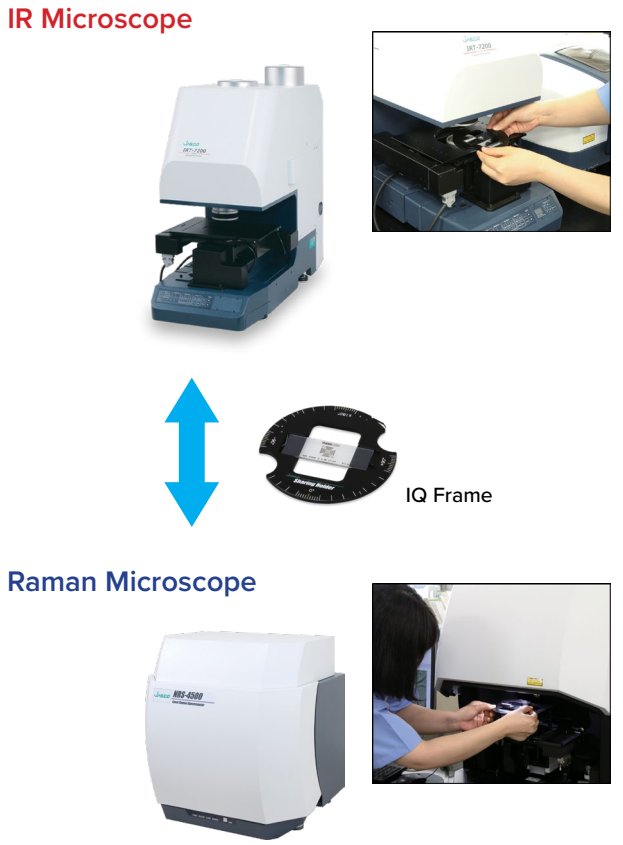


Fig. 11 IQ Frame operation between Raman and IR microscopes

In this application, analysis was performed on a paint chip obtained from a car body panel. A cross-section of the coating was made and sandwiched between two KBr plates (Fig. 12). First, IR microscopy imaging measurements were performed on a 200 µm square area. Then, principal component spectra were calculated by Multivariate Curve Resolution (MCR) from the measured spectra, and a false-color chemical image was created from the principal component spectra scores (Fig. 13). This imaging analysis revealed the distribution of four layers of mixed multiple organic components: acrylic + styrene, melamine + styrene, melamine + kaolin, and epoxy.

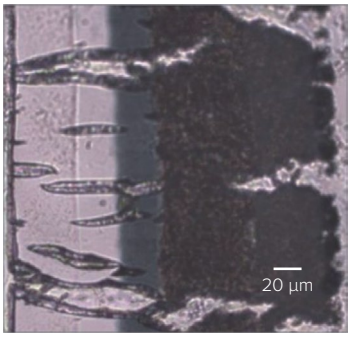
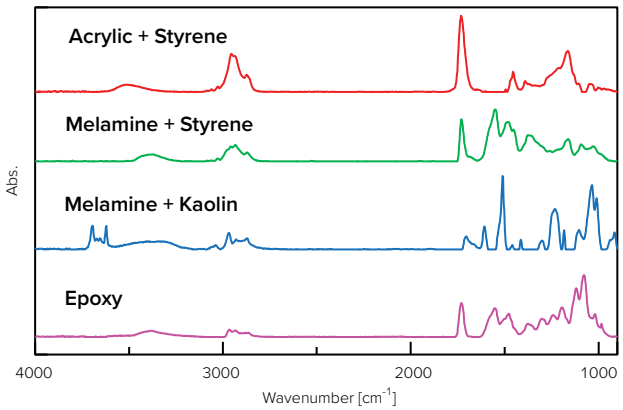
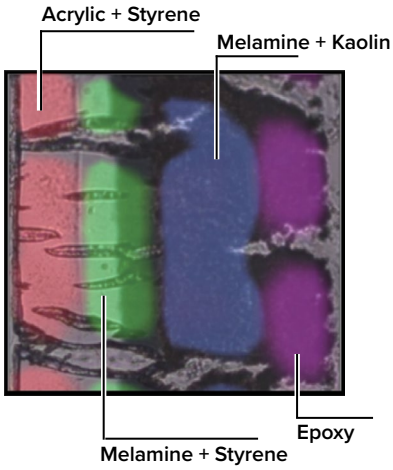


Fig. 12 Observation view

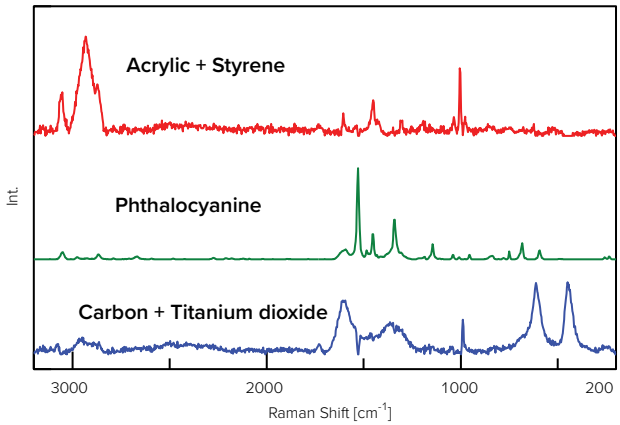


Principal component spectra

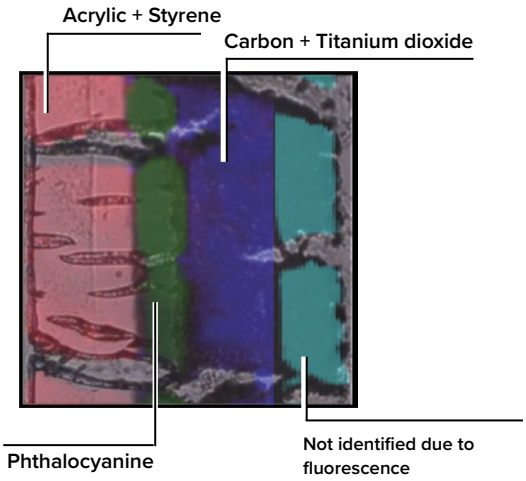


False-color chemical image

Fig. 13 Measurement results using IR microscopy



Principal component spectra



False-color chemical image

Fig. 14 Measurement results using Raman microscopy

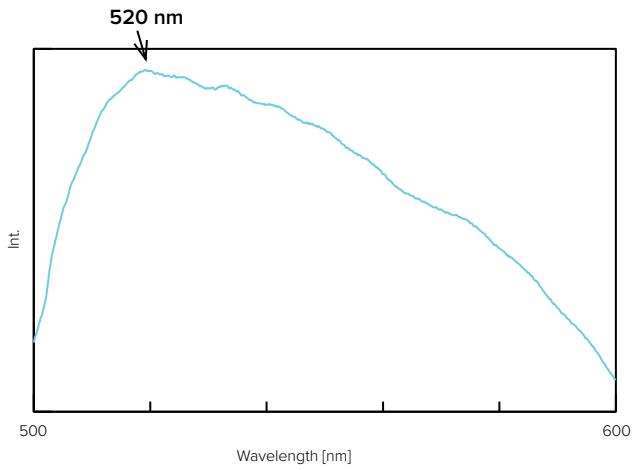


Fig. 15 Fluorescence spectrum of epoxy layer (405 nm excitation)

Next, Raman imaging measurements were performed on the same sample area. Principal component spectra were calculated by MCR using the acquired Raman spectra, and a false-color chemical image was created from the principal component spectra scores (Fig. 14). From the Raman imaging analysis, it was found that there were several layers containing phthalocyanine as pigment, carbon black, and titanium dioxide (TiO_2), as well as the layers containing acrylic and styrene, which were previously identified by IR microscopy. In the layer containing epoxy that was identified by IR microscopy, the Raman scattering peak could not be seen due to a significant amount of fluorescence caused by the presence of pigment components.

However, as a side benefit, fluorescence spectroscopy could be used to characterize and identify the chemical components in the pigments. Fluorescence measurement was performed using a shorter excitation wavelength of 405 nm (Fig. 15). The resulting emission spectrum indicates the presence of pigment components with a fluorescence maximum at 520 nm, which could be used to identify different pigment components. By combining the two techniques of IR and Raman microscopy (and additionally photoluminescence), a more detailed material analysis characterization is possible than by using either technique alone.

Tips

Combined Analysis using IQ Frame

Correlation molecular spectroscopy can provide more useful and detailed analysis of complex samples; however, when using microscopy, it can be difficult to locate the same sample area when transferring between systems for measurement. To overcome this barrier to correlated microspectroscopy, JASCO developed the unique IQ Frame, a sample measurement system that includes a calibrated sample holder together with advanced image matching technology (Fig. 16) that allows simple and accurate transfer between different microspectrometers for coordinated imaging and analysis using any combination of IR, Raman, and UV-Visible microscopy.

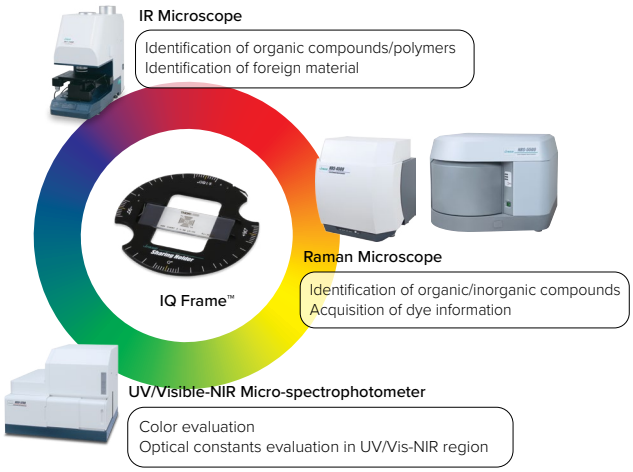


Fig. 16 Combined analysis using IQ Frame

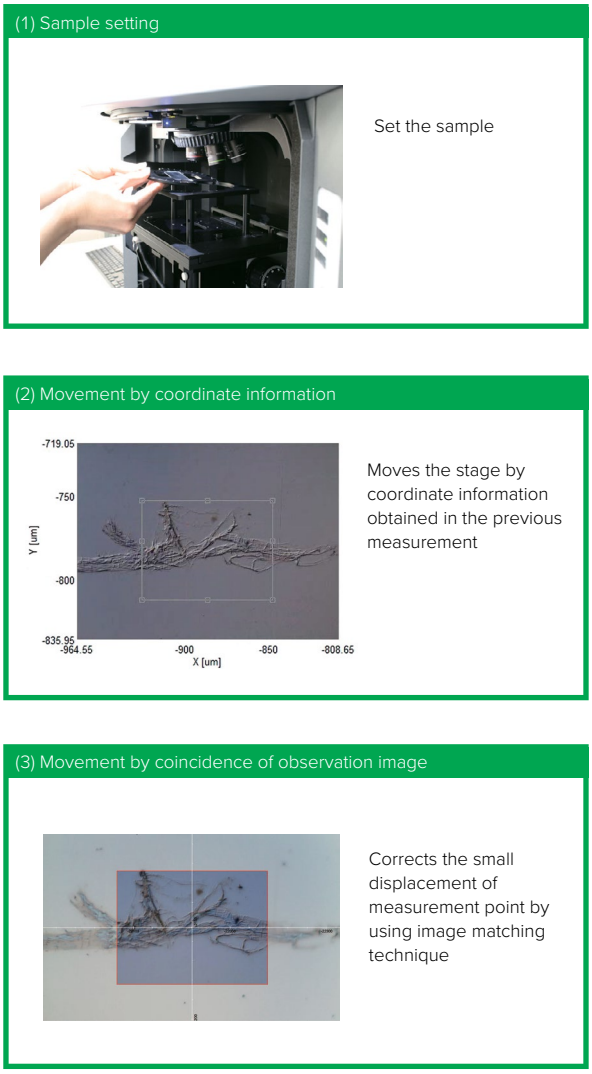


Fig. 17 Overview of image matching technique

Evaluation of Rubber Products

The chemical composition, physical structure, and quality of rubber are closely related to its performance and specifications. FTIR, which can be used to identify organic materials and analyze complex mixtures, is as an effective analytical technique for the research and development (R&D) and quality control of rubber products because it has the following key benefits:

- Little or no sample preparation is required
- The sample is not destroyed during measurement
- Analysis of a wide variety of materials is possible
- It can be measured relatively quickly

In addition to FTIR macro measurement, an IR microscope can provide the same information and analysis, but on much smaller sample areas, making it a useful technique for the identification, distribution and potential source of foreign materials in failure analysis. Furthermore, since chemical component distribution can be obtained, it can also be useful information for product R&D.
This section shows the evaluation of rubber products using IR microscopy.

Microscopic ATR Method

Analysis of a Rubber Sample with Surface Degradation

An IR microscope with an ATR objective was used to investigate the compositional changes in rubber upon degradation (Fig. 1). In order to analyze rubber materials that contain carbon black, a Germanium (Ge) crystal is used since it has a higher refractive index and smaller penetration depth. ATR measurements of the rubber samples before and after degradation were performed (Fig. 2), and then the difference spectrum between normal and degraded products was calculated (Fig. 3). It was concluded that zinc stearate was deficient in the degraded product.

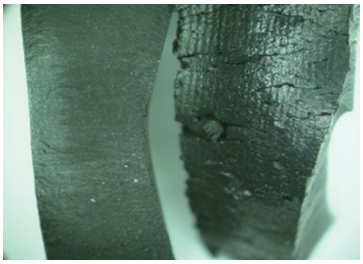


Fig. 1 Observation view
(left: normal product, right: degraded product)

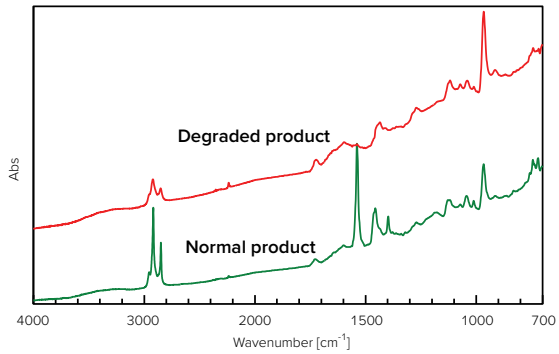


Fig. 2 Spectra of rubber samples before and after degradation
(red: degraded product, green: normal product)

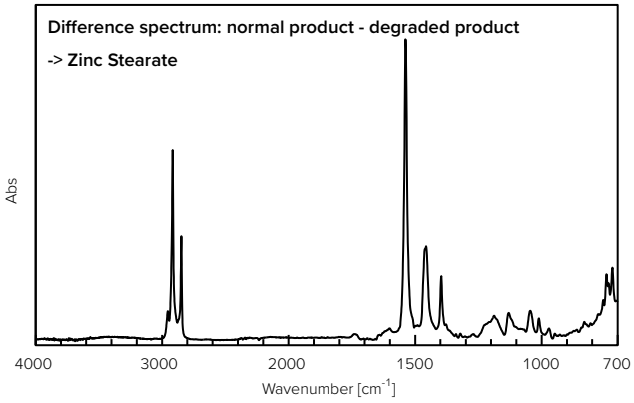


Fig. 3 Difference spectrum

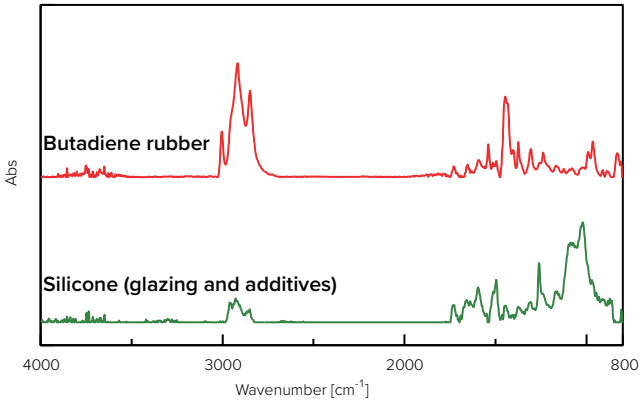


Fig. 5 Spectra of butadiene rubber (red) and silicone (glazing and additives) (green)

Microscopic ATR Method

Component Distribution Analysis of Rubber with a High Refractive Index

ATR imaging measurement was performed on a prepared cross-section of a tire spur (spike) (Fig. 4). In order to measure rubber containing carbon black, a Germanium (Ge) crystal is used since it has a higher refractive index and shallower penetration depth. In addition, a wide-area ATR objective that allows measurement across a wide single-contact point (mm scale) was used for this measurement. Principal component spectra were calculated by Multivariate Curve Resolution (MCR) from the measurement results (Fig. 5), and a false-color chemical image was created from the principal component spectra scores (Fig. 6). High-quality spectra of butadiene rubber (the main component of the tire) and silicone (an additive in the tire) were obtained, and the distribution of the components could be evaluated. The false-color chemical image also shows the mixture of butadiene rubber and silicone as the mixed color (yellow).

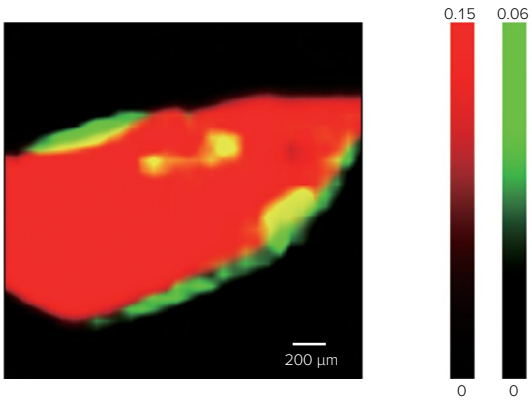


Fig. 6 False-color chemical image (red: butadiene rubber, green: silicone)

IQ Mapping for ATR

IQ Mapping, which provides wide-area imaging measurement for a single sample contact point, even for elastic samples, such as rubber, has enabled accurate analysis of the sample shape and chemical component distribution.

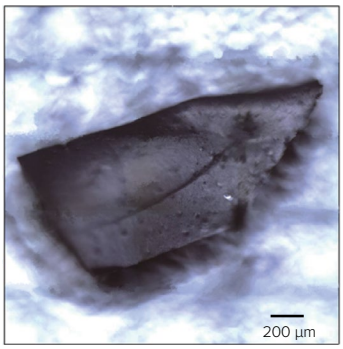


Fig. 4 Observation view of rubber tire fragment

Tips

Wide-Area ATR Objectives

ATR imaging measurements using IR microscopy have a wide range of applications, including the analysis of foreign materials and multilayer films, and the evaluation of additives, with the required spatial resolution and measurement area differing depending on the purpose of each analysis. The analysis of foreign materials in food and industrial products can visually be confirmed using ATR imaging; however, ATR measurement over a wide area is required since these foreign materials are often several hundred µm or larger in size. On the other hand, when evaluating the distribution of additives found in rubbers or resins, high spatial resolution in the micron order is required.

JASCO offers ATR objectives with different prism crystal materials, including Zinc Selenide (ZnSe), Zinc Sulfide (ZnS), diamond, and Germanium (Ge). The choice of Ge ATR objectives includes three different options for spatial resolution and sampling area with a single contact: ATR-5000-WG for a wide area at the mm scale, ATR-5000-MG for several hundred µm, and ATR-5000-G45 for high spatial resolution measurement to the diffraction limit. The features of each ATR objective are described below.

ATR Objective	ATR Imaging Area (single contact point)	Pixel Resolution (using a linear array detector)	Applications
ATR-5000-WG ("WG")	1,600 x 1,600 µm	50 x 50 µm	Screening analysis of large areas
ATR-5000-MG ("MG")	400 x 400 µm	12.5 x 12.5 µm	Analysis of distribution states on the order of tens to hundreds of µm
ATR-5000-G45 ("G45")	70 x 70 µm	2.2 x 2.2 µm	Analysis of micro-objects and micro-structures on the micron-order

To compare the analysis at different scales, a cross-section of a paper beverage container was prepared, and ATR imaging measurements using the above ATR objectives were performed for qualitative analysis of each layer. The entire area of the multilayer of approximately 800 µm could be imaged using the WG ATR objective, and the components of each layer were visualized (cellulose, polyethylene, and kaolin + calcium carbonate).

The MG ATR objective was used to measure the area around the central layer, and the polyethylene layer between the cellulose layers was detected, which could not be identified by the WG ATR objective due to its lower spatial resolution. The G45 ATR objective was used to measure the smaller area with high resolution, and was able to discriminate the layer as a single component (calcium carbonate).

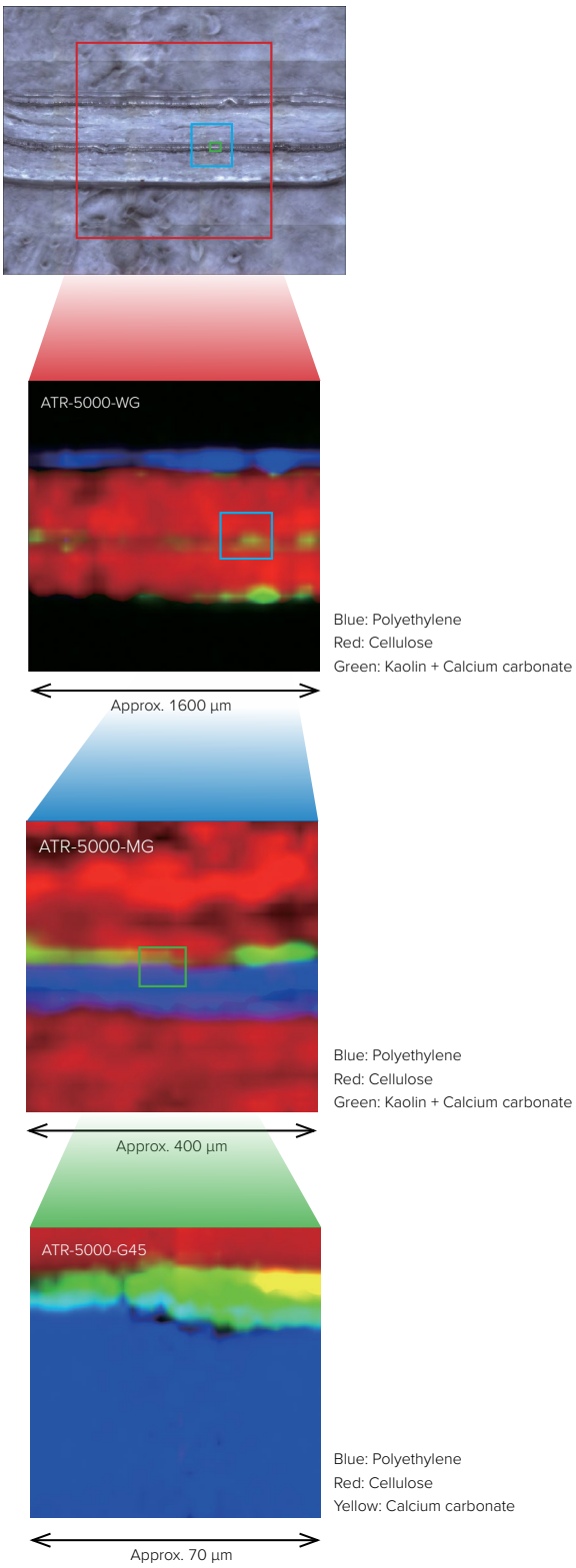


Fig. 7 Observation view and false-color chemical image (red: cellulose, blue: polyethylene, green: kaolin + calcium carbonate, yellow: calcium carbonate)

Evaluation of Some Physical Properties of Polymers

The physical properties of polymers are largely dependent on the chemical structure and orientation of their constituent molecules.

The molecular orientation of polymers is closely related to the functional properties of the material. For example, in the manufacturing of polypropylene (PP) films, different orientation states are used depending on the required application, such as strength, abrasion resistance, moisture resistance, transparency, etc. Some polymers can have the same molecular structure but different crystal structures, which these give rise to differences in physical properties, such as hardness, melting point, transparency, and strength, and can be exploited for different applications. Therefore, it is very important to elucidate and understand the orientation and crystal structure of polymeric materials.

IR spectroscopy is extremely effective for evaluating the properties described above. Polarization measurement, where a polarizer is inserted into the optical path, can be used to evaluate the orientation state of a sample. Characteristic peaks of crystals are found in the low wavenumber region of an IR spectrum, and these specific peaks can be used to determine the state of the crystal structure. Furthermore, IR imaging can be used to visualize the distribution of orientation state and crystal structure in a sample.

This section shows an example of using IR microscopy to evaluate the distribution of the orientation and crystal structure of polymers.

Microscopic Transmission Method

Orientation Distribution Analysis of Polypropylene

Polarization imaging measurements are useful in evaluating the distribution of molecular orientation in a sample. The example described here demonstrates the use of IR polarization imaging to evaluate the molecular orientation distribution of a stretched polypropylene material.

A comparison of spectra acquired using different angles of polarization (perpendicular and parallel directions) revealed a difference in peak height at 1,304 cm^{-1}, (CH_2 wagging and twisting) (Fig. 1), and at 809 cm^{-1} (C-C stretching band of the polymer chain backbone).

Exploiting this variation, a false-color chemical image was created from the peak height ratio of 809 cm^{-1}/1,304 cm^{-1} (Fig. 2). There is a correlation between molecular orientation and peak height ratio, indicating that the polypropylene molectular chain is highly oriented when the peak height ratio is large. It was confirmed that areas with high and low orientation are distributed periodically throughout the polypropylene film.

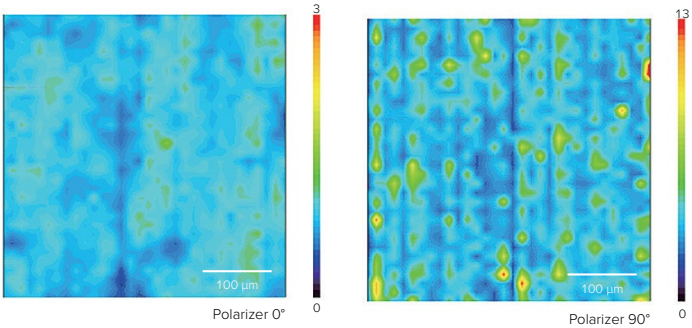


Fig. 1 Polarization false-color chemical images (Intensity ratio between 809 cm^{-1} and 1,304 cm^{-1})

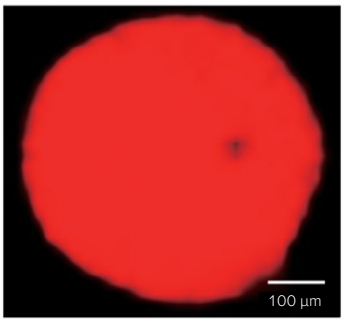
Microscopic Transmission Method

Evaluation of Polymer Crystallinity by IR Mapping

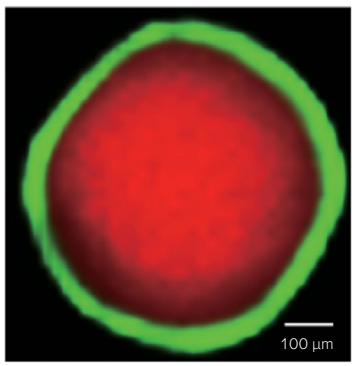
Some polymers used in plastics and fibers have the same molecular structure but different crystal structures. The crystal structure impacts the chemical, physical, thermal, and mechanical properties, such as hardness, transparency, and melting point. Therefore, it is very important to evaluate the crystal structure of polymer materials to assess their use for specific applications.

Here, we present the results of the investigation into the internal structure of two different polymeric products (fishing lines). The two fishing lines have different functions, characteristics, and costs, but each is manufactured from polyvinylidene fluoride (PVDF), which is known to possess many crystal structures (polymorphs). A thin cross-section of each fishing line was prepared, sandwiched between two KBr plates, and FTIR imaging measurement was performed using the microscopic transmission method on an IRT-5200 FTIR microscope. Since α- and β-type crystal structures have characteristic peaks around 763 cm^{-1} and 840 cm^{-1} respectively, a false-color chemical image was created using the peak height of each characteristic polymorph peak (Fig. 2). Fishing line #1 was found to be composed entirely of the β-polymorph. In contrast, it was confirmed that fishing line #2 has mostly β-crystal structure at its center with an external coating of approximately 50 µm, comprised of a mixture of α- and β-type polymorphs.

In conclusion, the cross-sectional FTIR imaging of the fishing lines demonstrated that fishing line #2 has the greater strength due to its different outer layer of PVDF, comprised of α+β crystal polymorph structures.



Fishing line #1



Fishing line #2

Fig. 2 False-color chemical images (red: β type (840 cm^{-1}), green: α+β type (840 cm^{-1} and 763 cm^{-1}))

Tips

Advantages of a Grazing Angle Reflection Objective

Grazing angle reflection objectives are used for high sensitivity Reflection Absorption Spectroscopy (RAS) measurements. By using a grazing angle reflection objective (Fig. 3), very high sensitivity measurements of thin films on reflective metal surfaces can be measured, which is useful for the analysis of Langmuir-Blodgett (LB) films, oxide films on metal surfaces, and processed films produced by various methods. Recently, grazing angle objectives have been used for the in-situ measurement of the vapor deposition processes onto silicon substrates in chemical vapor deposition (CVD) reaction chambers. RAS can analyze films as thin as several 10 Å if the sample has a high absorption coefficient. The grazing angle reflection objective can also perform high sensitivity measurements of small areas.

Fig. 3 Grazing-angle reflection objective

A polymethyl methacrylate (PMMA) film of approximately 50 nm in thickness was made by evaporating a PMMA solution onto a gold mirror. The film was measured on the gold mirror using a grazing angle reflection objective by the conventional RAS method. While only a small amount of absorption was observed using the traditional RAS method, a grazing angle reflection objective could perform the measurement with an order of magnitude greater sensitivity, as seen in the spectra (Fig. 4) below.

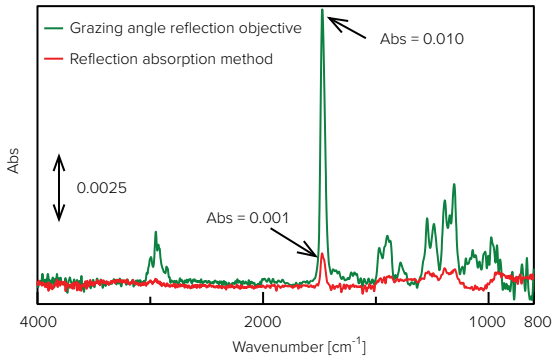


Fig. 4 IR spectra of PMMA film on gold mirror

Tips

Vacuum Option

In general, since an FTIR spectrometer only has a single beam, it cannot ratio the reference light (background) and measurement light (sample) simultaneously, which creates a time lag between the background and sample measurements. FTIR spectrometers are very sensitive to the strong vibrational dipoles of atmospheric water (H_2O) vapor and carbon dioxide (CO_2). Even small changes in concentration of H_2O vapor or CO_2 (both present in the operator's breath) in the atmosphere results in noise that can be readily detected in the IR spectrum, adversely affecting spectral analysis. In measurements that are likely to be sensitive to this type of interference, it is common to purge the optical path with an IR-inert gas, such as nitrogen (N_2) or dry air, or to remove atmospheric noise through data processing. The most effective solution to remove atmospheric effects is to completely evacuate the IR optical path. Performing measurements under vacuum enables the collection of high-quality spectra with high accuracy and speed without atmospheric influence.

To illustrate the effectiveness of a full-vacuum FTIR, a spectrum of a silicon wafer was measured using the transmission method under both atmospheric and full-vacuum conditions (Fig. 5). When measuring a sample with a high refractive index and a highly reflective surface, such as a silicon wafer, IR light may be reflected from the surface (back reflection) and returned to the interferometer. When this light passes through the sample chamber for the second time and enters the detector, the effect of atmospheric noise may increase due to the time between the measurement light and the reference light measured without the sample present. When performing qualitative analysis of a sample, the sample can be tilted to prevent the light from returning to the interferometer, but this is not suitable for quantitative analysis or optical characterization. By using a full-vacuum system, high-quality IR spectra without atmospheric noise can be obtained, even without tilting the sample.

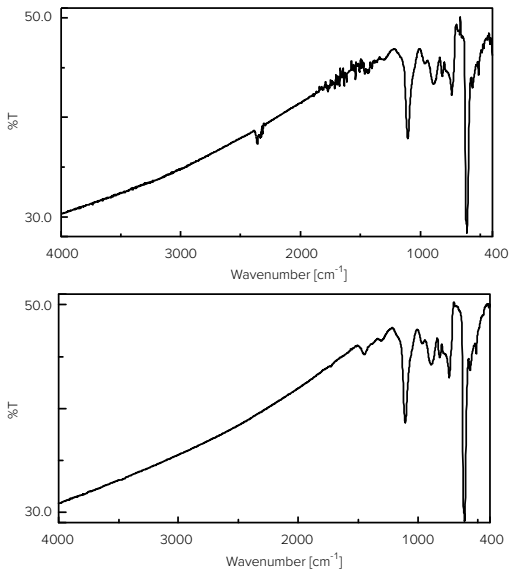


Fig. 5 Measurement example of epitaxial film on silicon wafer

In addition, when measuring small peaks, it may be necessary to eliminate environmental interference due to the IR absorption of H_2O vapor and CO_2 that appear in the spectrum. Measurement under vacuum conditions is very effective at eliminating atmospheric noise and can provide a clear spectrum free of peaks caused by H_2O vapor and CO_2. In the following example, a grazing angle reflection measurement of a tristearin film formed on a gold mirror was performed under atmospheric and full-vacuum conditions (Fig. 6). The spectrum collected under vacuum is cleaner, without interfering peaks from atmospheric noise.

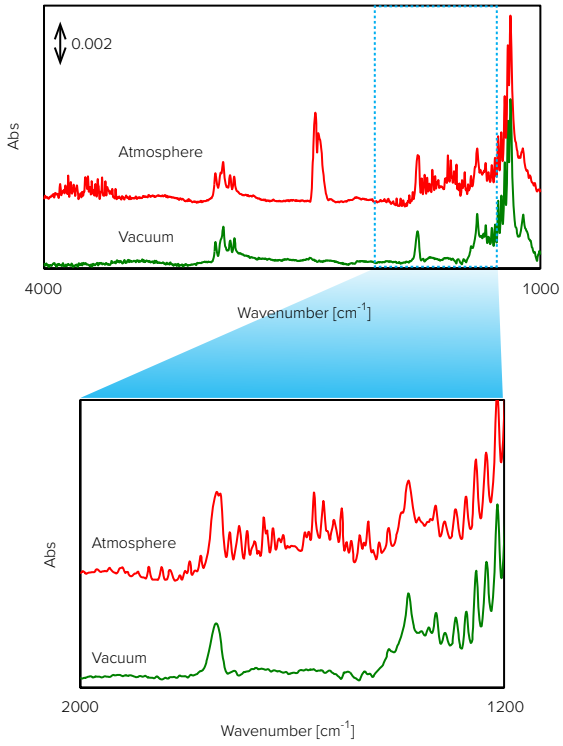
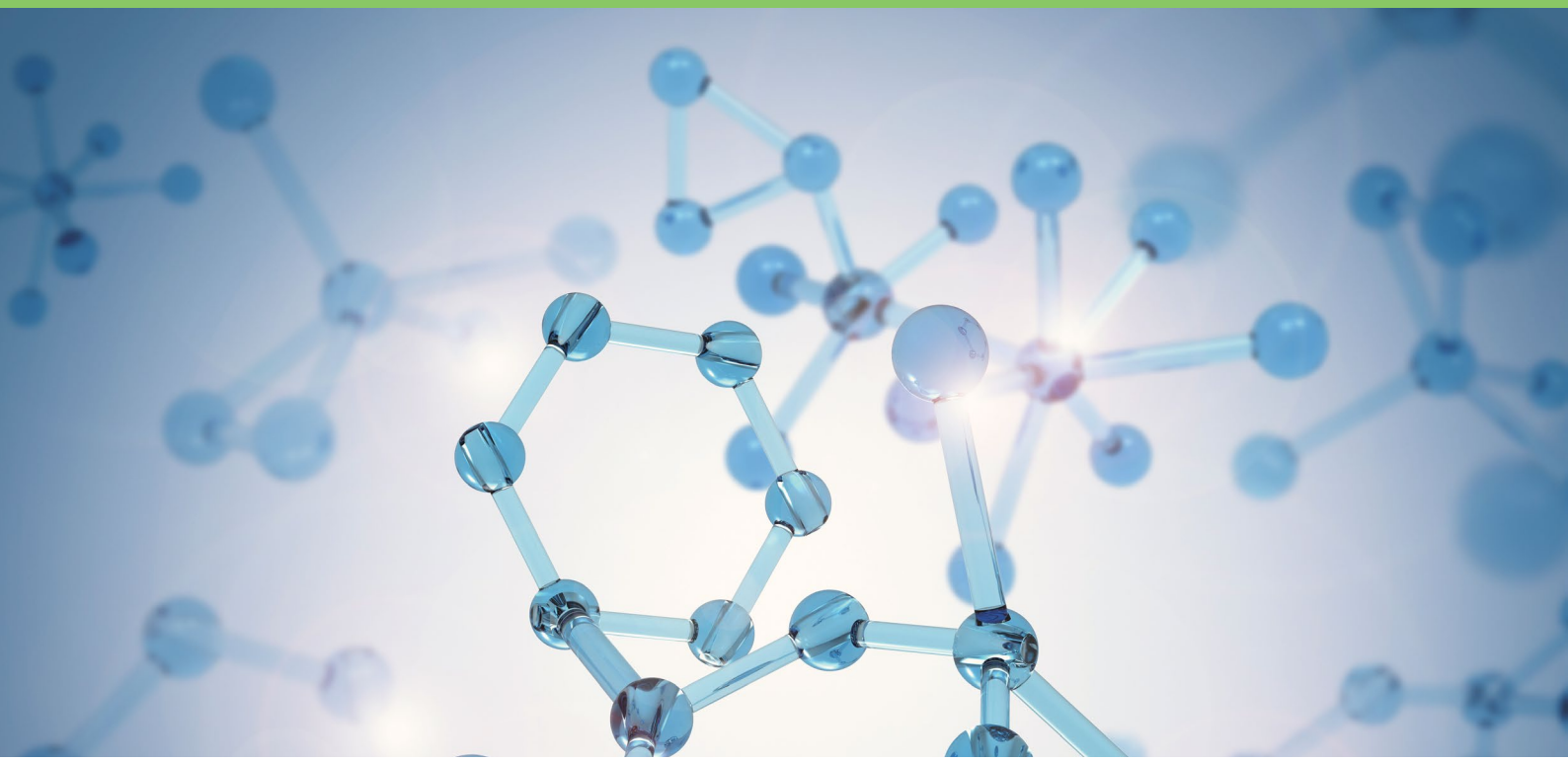


Fig. 6 IR spectra of tristearin film on gold mirror (Grazing angle reflection measurement)

Full vacuum IR microscope system

The vacuum option is available for the FT/IR-6X and the FT/IR-8X systems such as seen above.



Dynamic Imaging

FTIR spectroscopy has three methods for time course measurement: interval measurement, rapid scan, and step scan with time resolutions of 1 sec, 50 msec, and 5 msec (option: 10 nsec), respectively.

Here, we will review the basic features and scanning method of the moving mirror in the interferometer for each time course measurement. “Interval measurement” is performed with regular scanning while rapid scan measurement uses a faster scan rate of the moving mirror. In step scan measurement, the moving mirror stops at each data-sampling point and the sample is perturbed at each of the points.

One of the main features of a Fourier-Transform Infrared (FTIR) spectrometer is the ability to simultaneously measure multiple wavelengths. FTIR spectroscopy can acquire an interference pattern of waves generated by light with multiple wavelengths in the time domain, which is created by scanning a moving mirror in the interferometer along its optical axis to obtain an intensity distribution (spectrum) for each wavelength. This distribution is then converted from the time domain into the frequency domain (cm^{-1}) using a Fast Fourier Transform (FFT). Since an FTIR spectrometer can acquire an IR spectrum faster than a dispersive IR spectrometer, FTIR spectroscopy is a powerful analytical technique for time-dependent measurements, which can be used to monitor the structural change in a sample over time.

The IRT-7000, a linear array imaging microscope, has three time course measurement methods: interval measurement, rapid scan measurement, and step scan measurement. Each measurement method uses a different scanning method for the moving mirror in the FTIR interferometer as noted above.

Interval Measurement

Interval measurement is performed using normal moving mirror scanning speeds, offering a typical minimum time resolution of around 1 sec.

Applications: Monitoring of structural changes in a sample or changes in a gas concentration, etc. Figure 1 shows spectra of room air over a period of 12 hours. The temporal changes in the environmental CO_2 concentration can be monitored by plotting the intensity of the 2,360 cm^{-1} peak against time.

Rapid Scan Measurement is performed by high-speed scanning of the moving mirror, which can increase the minimum time interval of the IR microscope to 50 msec (with a spectral resolution of 16 cm^{-1}).

Rapid scan mode can be used for studying kinetics, monitoring dynamic processes, such as photo-polymerization reactions, orientation relaxation of polymer films, and depth profiling. Figure 2 shows the rapid scan spectra over the course of 50 seconds of a UV curing reaction where the C=C double bond is seen to decrease as the polymerization proceeds to form C-C single bonds (not shown in figure).

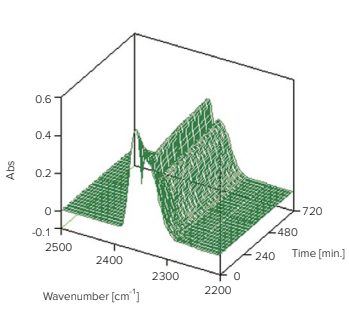


Fig. 1 CO_2 concentration monitoring (Interval measurement data)

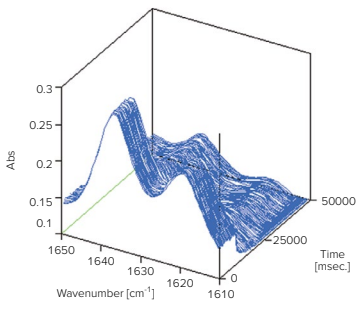


Fig. 2 Curing process of UV curable resin (Rapid scan measurement data)

Step Scan Measurement can only be applied to measure the dynamics of samples that have a reversible/repeatable response. Typical examples include studying the effects of applying stress to samples, changes in applied electric field, or changes in temperature. The minimum time resolution in step scan mode is 5 µsec (optionally 10 nsec).

To make a complete spectral scan, the moving mirror is stopped and dithered at each frequency sampling point while reversible perturbations are made to the sample.

Applications: Monitoring the orientation relaxation process of liquid crystals, ferroelectric transitions of polymers, light-induced protein reactions, and other systems with reactions in the microsecond time scale.

The FTIR spectra measured during the relaxation process of the orientation of a liquid crystal in response to an applied electric field are shown in Fig. 3. The peak intensity transition at 2,925 cm^{-1} (bottom of Fig. 3) is attributed to C-H stretching. The peak intensity fluctuated in response to the applied voltage and decreased in two phases - rapidly and then moderately. It is known that the liquid crystal in the area near the electrode has a faster orientation relaxation than the bulk crystal and this difference can be readily observed using an FTIR spectrometer with step scan time course measurement.

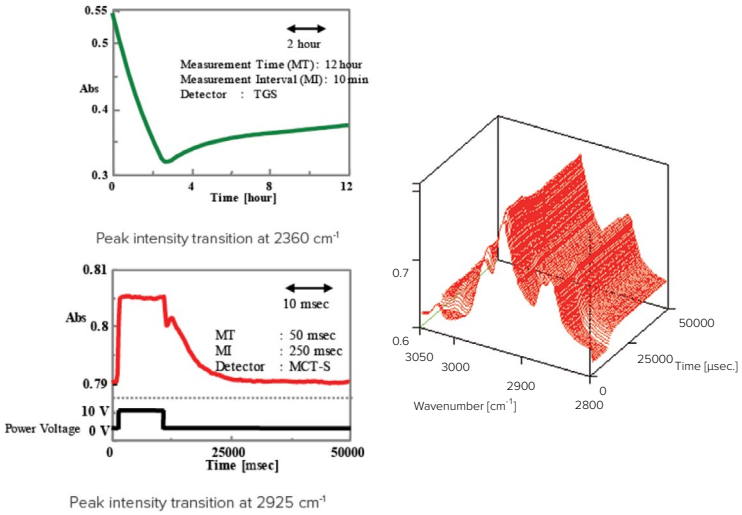


Fig. 3 Orientation relaxation process monitoring of liquid crystal (Step scan measurement data)

In recent years, there has also been an increasing need for time course mapping measurements to monitor the structural change in a micro-area plane over time.

This section shows examples of mapping measurements for monitoring the changes in molecular structure when a sample is irradiated with a UV light or subject to changes in the applied electric field.

Dynamic imaging measurements can be performed with an FT/ IR-8X spectrometer fitted with a step scan interferometer, in combination with an IRT-7200 linear array imaging microscope.

Time Course Imaging

Analysis of the Curing Process of UV Curable Resins

Time course mapping measurements were performed of an UV-cured resin applied on a metal plate. The measurement was started as the sample was irradiated with UV light to monitor the subsequent reaction. The spectra of the cured and uncured portions were also obtained (Fig. 4).

It can be observed that the peak attributed to the C-H out-of-plane bending vibration of the vinyl group (near 810 cm^{-1}) decreases

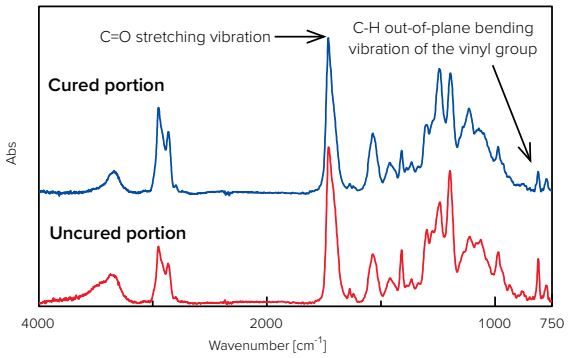


Fig. 4 IR spectra of cured and uncured portions (blue: cured portion, red: uncured portion)

over time, which is consistent with the advancement of the polymerization reaction as the C=C double bond is converted to a single bond with the curing of the resin. In contrast, the intensity of the C=O stretching vibration (around 1,730 cm^{-1}) is almost the same before and after curing, as this functional group does not undergo any reaction.

To illustrate the distribution of the curing over time across the sample, a stacked view was created from the ratio of the two peak heights (810 cm^{-1}/1,730 cm^{-1}) and using the peak at 1,730 cm^{-1} as an internal standard (Fig. 5). The z-axis indicates the time, and the change in color against the time at each location shows the differences caused by the progress in the chemical reaction distributed across the sample. The locality of UV light intensity and the degree of curing per unit area can be evaluated, where the lower intensity in the 810 cm^{-1} peak indicates a greater extent of reaction (Figs. 6 and 7).

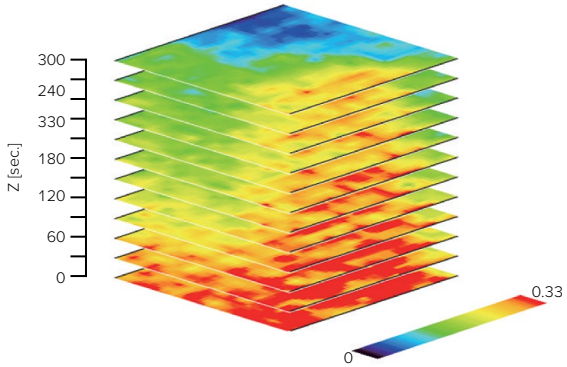


Fig. 5 Stacked false-color chemical images (peak height ratio 810 cm^{-1}/1,730 cm^{-1})

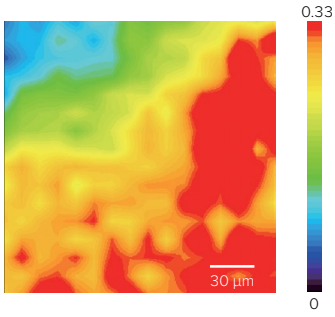


Fig. 6 False-color chemical image before UV irradiation (0 sec.) (peak height ratio 810 cm^{-1}/1,730 cm^{-1})

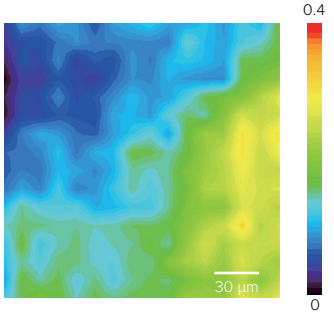


Fig. 7 False-color chemical image after UV irradiation (330 sec.)(peak height ratio 810 cm^{-1}/1,730 cm^{-1})

Heating and Cooling Stages for IR and Raman Microscopy

Two models of heating and cooling stages are available for sample measurements in different temperature ranges. These are operated with a dedicated program that controls the stage temperature, ramping of temperature, and automatically starts measurement when the sample reaches the measurement temperature at the set temperature ramping/falling rate.

Heating and cooling stage

By combining an IR microscope with a heating/cooling stage, thermophysical properties, observation images, and structural changes in molecules can be comprehensively evaluated, including crystallization, melting, and other phase transitions.

As an example of measurement, the structural change of benzoic acid was monitored when heated to 150 degrees Celsius. A 3D spectrum of benzoic acid as a function of temperature was obtained (Fig. 8). An observation image (Fig. 10) and the intensity of the peak attributed to the O-H bending vibration at 930 cm^{-1} (Fig. 9) was monitored at each temperature. From the results, it could be seen that the structure and state of benzoic acid changed at approximately 120 – 125 degrees Celsius. Since the melting point of benzoic acid is 122.4 degrees Celsius, it could be seen that this system accurately captured the intramolecular structural and state changes associated with the melting process.

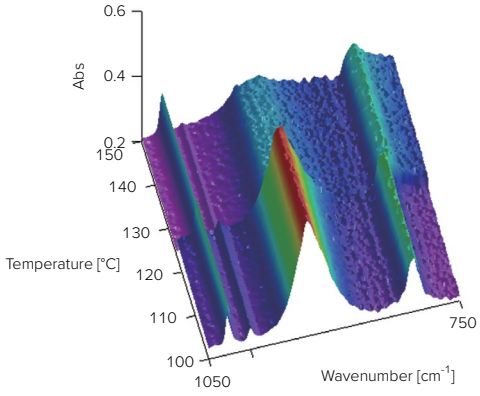


Fig. 8 3D spectrum of benzoic acid

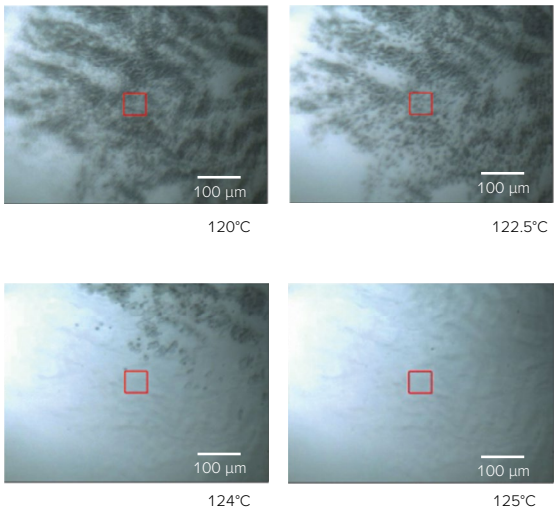


Fig. 9 Observation views of benzoic acid at each temperature (square: measurement area)

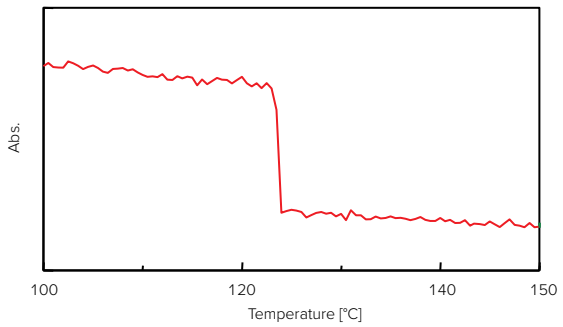


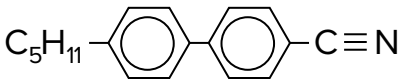
Fig. 10 Peak intensity change at 930 cm^{-1}

Compared to conventional macro FTIR spectroscopy, since the measurement area of an IR microscope is relatively small, it allows for accurate uniform heating/cooling) and yields high-precision analysis of the sample. This system can be used in a wide range of applications that include structural changes due to heating, such as the curing process of thermosetting resins, crystallization processes of drugs and polymers, protein denaturation, and polymorphic phase transitions.

Step Scan Imaging

Fast Time Course Analysis of Liquid Crystals with Applied Voltage

Step scan time course measurements were used to monitor the orientation relaxation process of liquid crystals when subjected toan electric field[1]. It is well known that the orientation of molecules in liquid crystals changes when a voltage is applied. Step scan FTIR measurement was used with an IR polarizer placed incident to the sample and parallel to the orientation direction of the liquid crystal to evaluate the changes in orientation. A typical nematic liquid crystal, 5CB (4-pentyl-4'-cyanobiphenyl), was used for the measurements (Fig. 11).



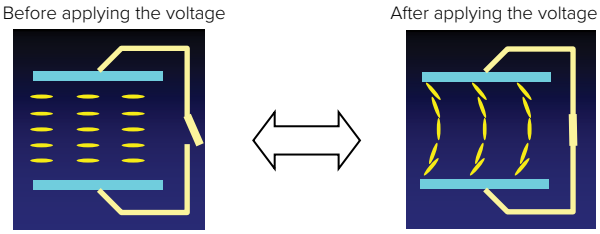


Fig. 11 Overview of sample (5CB) (yellow: sample)

The time resolution interval of the initial measurement was 250 µsec, with a total measurement time of 25 msec. A voltage of 5 V was applied to the liquid crystal for 10 msec, from 1 msec after the start of the measurement. Focusing on the intensity change in the peak attributed to the C-H anti-symmetric stretching vibration of the methylene group (at 2,925 cm^{-1}), the peak intensity changed in synchronization with the ON and OFF voltage, and the orientation of the molecules in the liquid crystal could be observed in the spectrum (Fig. 12).

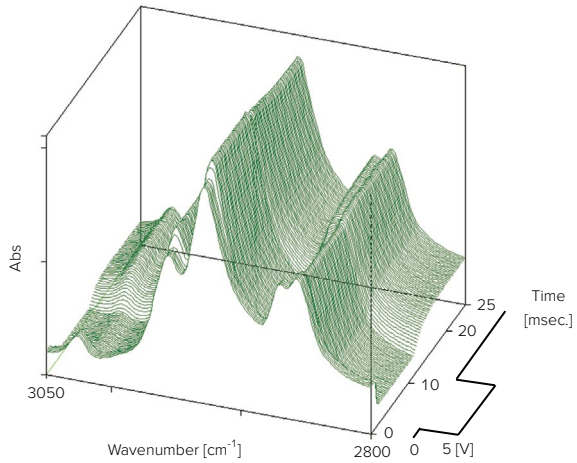


Fig. 12 Orientation relaxation process of liquid crystal (5CB)

Dynamic imaging measurement of the orientation relaxation process of liquid crystals was performed using an IR microscope (Fig. 13). The time resolution interval of this measurement was 1.0 msec, and the total measurement time was 120 msec. A voltage of 10 V was applied to the liquid crystal for 11 msec, from 19 msec after the start of the measurement. The images before and after the voltage was applied (19 msec and 21 msec, respectively) were entirely one color, indicating that the orientation of the liquid crystal molecules was uniform. After the applied voltage was stopped (32 msec, 40 msec, 60 msec, and 100 msec), the orientation of the liquid crystal molecules was heterogeneous. This indicates the localization of the reorientation process of liquid crystal molecules depends on its distribution through the structure; with faster reorientation near the point where the electric charge was applied, and slower reorientation in the bulk material.

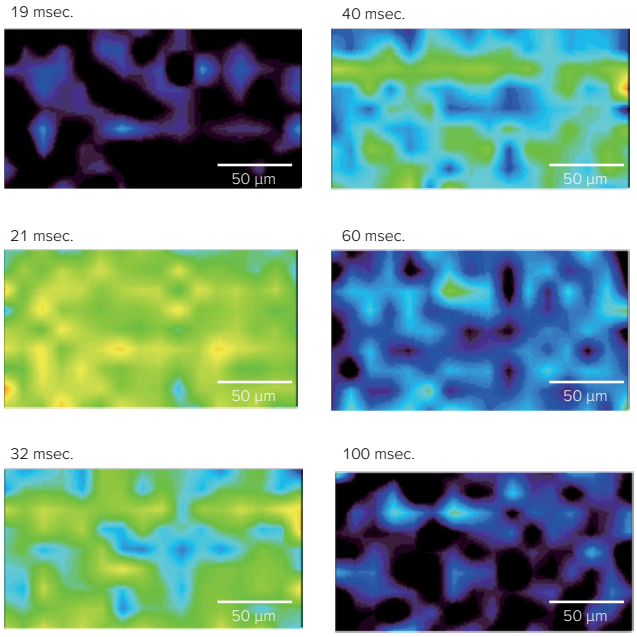


Fig. 13 Dynamic imaging results of the orientation relaxation process of liquid crystal (5CB)

As described above, step scan time course measurement can be used to monitor the orientation relaxation process of a liquid crystal when subjected to an applied electric field. Combining step scan FTIR measurement with an IR microscope enablesobservation of the localized changes in the in-plane distribution of the orientation relaxation process.

1) Sugiyama, H., Koshoubu, J., Kashiwabara, S., Nagoshi, T., Larsen R. A., Akao, K., Appl. Spectrosc., 62(1), 17-23 (2008).

Two-Dimensional (2D) Correlation

The two-dimensional (2D) correlation program (optional Spectra Manager software) can be used to calculate and display the correlation of peaks observed in the measured spectra acquired in three dimensions (3D), which can include time interval and temperature-dependent measurements. This enables correlation analysis of different functional group measurements to understand how the relationship between functional groups and the aggregation state of molecules change depending on time or temperature.

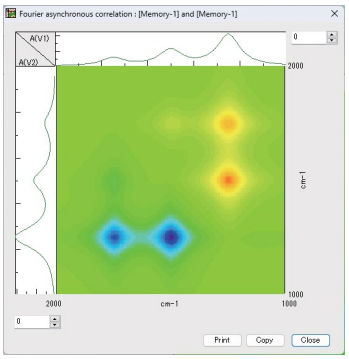


Fig. 14 Two-dimensional (2D) correlation program

In order to understand 2D correlation, simulated spectra (Fig. 15, Table 1) with varying peak intensities under the following conditions were synthesized and analyzed using the 2D correlation program. The correlation spectra of the composite waveforms as well as their interpretations are shown (Figs. 16 and 17).

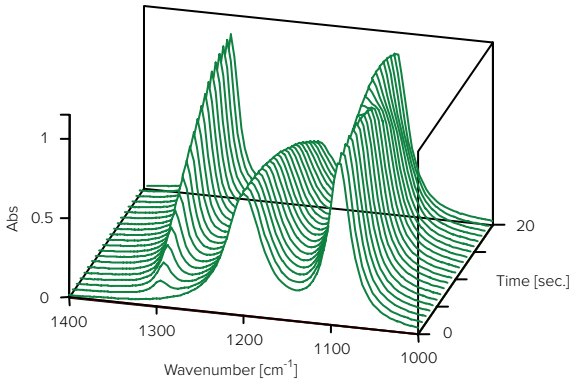


Fig. 15 Three-dimensional (3D) spectrum of composite waveform

Table 1 Summary of Simulation Spectra

Peak Position	1300 cm^{-1}	1200 cm^{-1}	Around 1100 cm^{-1}
Characteristics of peak	Increase	Slowly decrease	1090 cm^{-1}: Decrease 1110 cm^{-1}: Increase

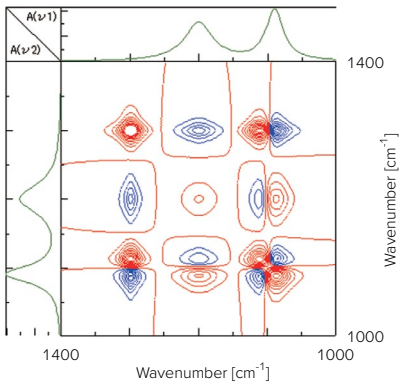


Fig. 16 Synchronous correlation spectra (red: positive value, blue: negative value)

**Synchronous correlation: This indicates the degree to which the behavior of the changes in peak intensity are similar. Positive values are indicated when the intensities of each peak are changing in the same direction while negative values are indicated when the intensities of each peak are changing in opposite directions.*

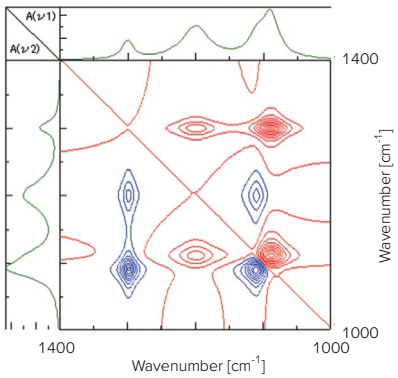


Fig. 17 Asynchronous correlation spectra (red: positive value, blue: negative value)

**Asynchronous correlation: This indicates the degree to which the behavior of the change in peak intensity is different. When the synchronous correlation is positive, negative values are indicated if the peak intensity change on the X-axis is slower than the peak intensity change on the Y-axis while positive values are indicated if the peak intensity change on the X-axis is faster than the peak intensity change on the Y-axis.*

2D correlation analysis can be applied to data from different molecular spectroscopy measurements; these 2D correlation results combining spectral analysis techniques (including near-IR, Raman, UV-Visible, or Circular Dichroism) with IR, and can provide additional information about peak assignment, lattice vibrations, and the relationship between intramolecular vibrations, color, or chiral information.

Component Distribution Analysis of Foods

IR microscopy is one of the most important analytical techniques in the field of food science; one example is the analysis of foreign materials to discover the nature and origins of contamination. Complaints about foreign materials discovered by consumers in food products are widespread, and product recalls are not only common but well publicized. Many such incidents are reported to include rubber or resin contamination caused by deterioration of components used in the production line equipment, and contamination caused by human error, etc. IR microscopy can be used to determine the identity of the foreign material contamination to help in pinpointing their source.

IR microscopy can also be used to investigate food quality. In food research and development (R&D), there is an interest in evaluating the relationship between the distribution of food components and its effect on flavor and taste. It is expected that IR microscopy, which can visualize the distribution of components, such as water and fats, will provide important information in food R&D.

This section shows examples of food component distribution analysis using an IR microscope.

Microscopic Transmission Method

Moisture Distribution Analysis of Foods

FTIR spectroscopy is useful for obtaining information about the nature of trace amounts of moisture in food products. Furthermore, FTIR mapping measurement of a cross-section of a food product can be used to visualize the moisture distribution in a food product.

Differences in moisture distribution were analyzed in noodle cross-sections of two types of fried noodles, cooked by amateurs and professionals, respectively.

In order to compare moisture distribution in the two different noodle preparations, the noodles were cut to a few mms thickness and sandwiched between CaF_2 windows in order to prevent drying, and fast imaging measurements were performed (Fig. 1).

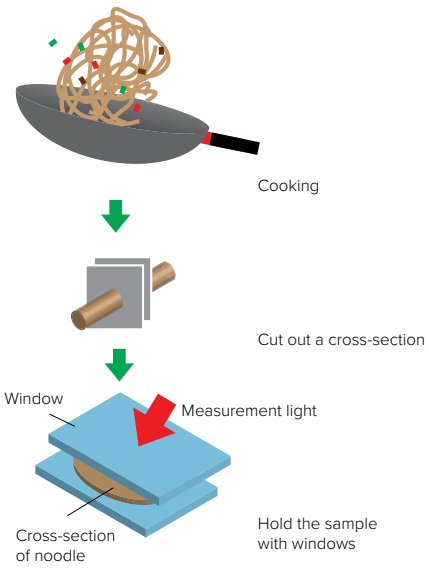


Fig. 1 Diagram of the measurement

The water content distribution in both noodle samples cooked by a professional and an amateur, respectively, was compared by calculating the peak area ratio of the water (1,882 – 2,321 cm^{-1}) and the starch (3,872 – 4,165 cm^{-1}), and the moisture distribution was analyzed (Fig. 2). The results show that the amateur-cooked noodles had the highest moisture content in the center, while the professionally cooked noodles had the highest moisture content in the middle layer (between the outermost shell and the center), rather than in the center. In parallel with the measurement, a sensory test was conducted with a group of 20 people, and almost all of them agreed that the professionally cooked noodles were more flavorful. It was confirmed that there is a relationship between the difference in "taste" and the moisture distribution in fried noodles.

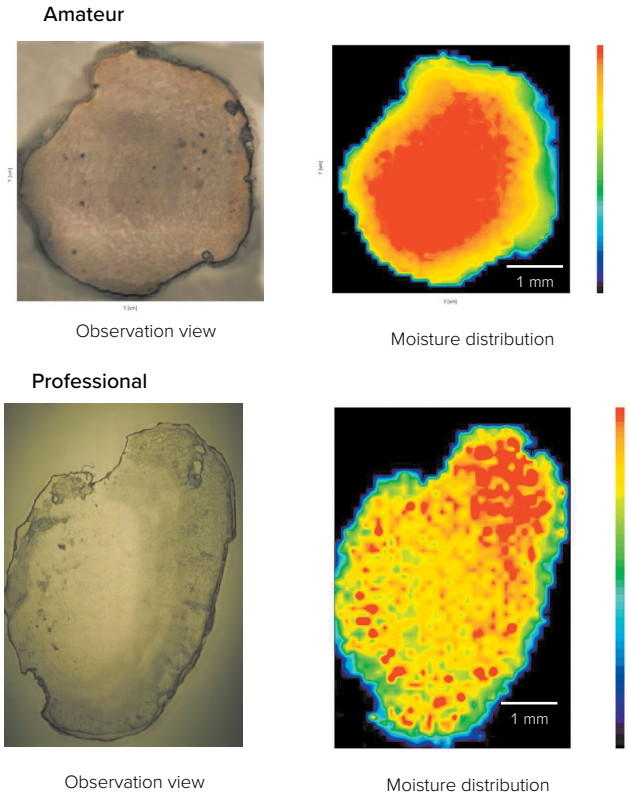


Fig. 2 Moisture distribution of fried noodles by different cooks

Microscopic ATR Method

Wide-Area ATR Measurement of Foods

FTIR imaging measurements can visualize the distribution of components on a sample surface. In order to evaluate the distribution of components on the surface of a cheese sample (Fig. 3), an ATR-5000-WG, which is capable of wide-area ATR imaging, was used.

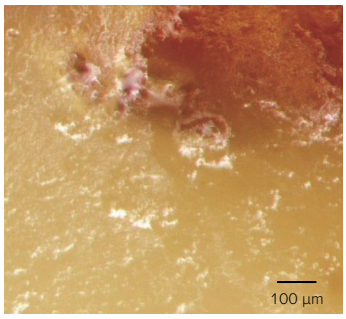


Fig. 3 Observation view

Principal component spectra were calculated by Multivariate Curve Resolution (MCR) analysis (Fig. 4), and a false-color chemical image showing the distribution of principal components was created using the peak areas of the key absorption bands (Fig. 5). As a result of the analysis, fats and proteins were detected as the main components, and their distribution in the surface was determined and visualized.

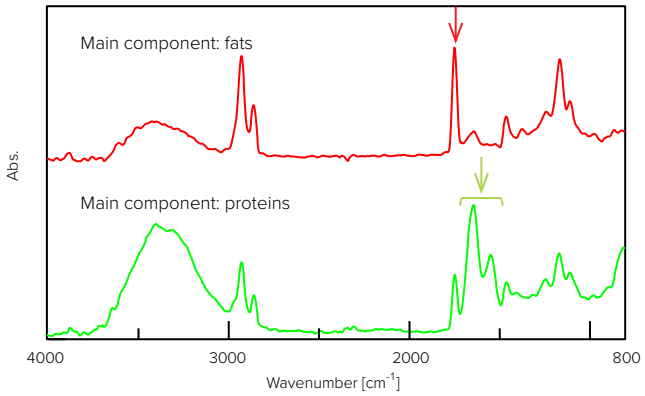


Fig. 4 Principal component spectra (red: fats, green: proteins)

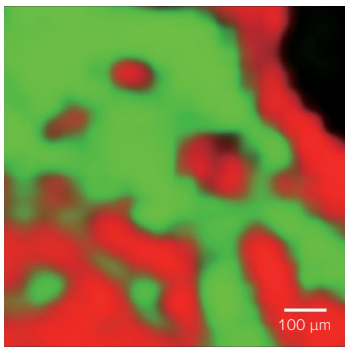


Fig. 5 False-color chemical image (red: fats, green: proteins)

Tips

Protein Secondary Structure Analysis

Many protein structures have been confirmed using x-ray crystallography, especially since the human genome has been sequenced and elucidated. Nuclear Magnetic Resonance (NMR) spectroscopy also provides excellent structural information about proteins and Circular Dichroism (CD) spectroscopy is an extremely useful technique for aqueous and solid phase samples. However, it is very difficult to analyze the secondary structure of a protein in a multi-component system with a structure like that of a biological tissue using the above methods while maintaining the structural integrity. In contrast, IR spectroscopy can be easily used even for non-crystalline samples, and thus the secondary structure of proteins in biological tissues can be analyzed in a more *in-vivo*-like state without purification.

JASCO's protein Secondary Structure Estimation (SSE) program can quickly perform protein secondary structure analysis using the amide I peak in an IR spectrum.

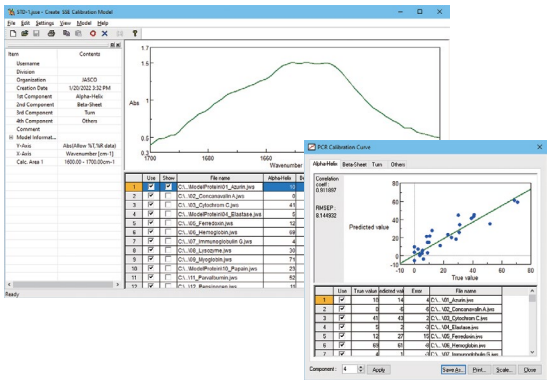


Fig. 6 SSE calibration modeling program

In conventional secondary structure analysis of proteins using IR spectroscopy, a heavy water solution (D_2O, deuterium) is often used to minimize the effect of the water (H_2O) peak, which coincides with the amide I peak. However, D_2O is more expensive and more difficult to handle than ordinary H_2O. To compensate for the effects of water in the amide 1 band, the SSE program in Spectra Manager automatically subtracts the H_2O peak from the spectrum so that protein secondary structure analysis can be performed using ordinary aqueous solutions.

Conventional secondary structure analysis has been performed from the amide I peak using band decomposition, which requires a certain amount of experience and can be user subjective. In the Spectra Manager SSE program, the protein secondary structure is estimated by Principal Component Regression (PCR) or Partial Least Squares (PLS) using a reference protein dataset with known secondary structure (confirmed by x-ray crystallography). The use of multivariate analysis minimizes the need for experience in data processing and removes the subjectivity involved with the band decomposition method initially described.

IR microscopy can also be used for *in-situ* surface analysis of samples with non-uniform structural distribution. With the Secondary Structure Imaging (SSI) program, spatial analysis can be performed non-destructively on biological samples. In the example described here, a hair sample was prepared using the KBr plate method, and an FTIR transmission measurement was performed (Fig. 7). The FTIR image confirmed that the percentage of -CH was higher in the center (medulla) and on the surface (cuticle) of the hair. Focusing on the secondary structure of the protein, it was confirmed that the percentage of α-helix was higher in the center (medulla) of the hair.

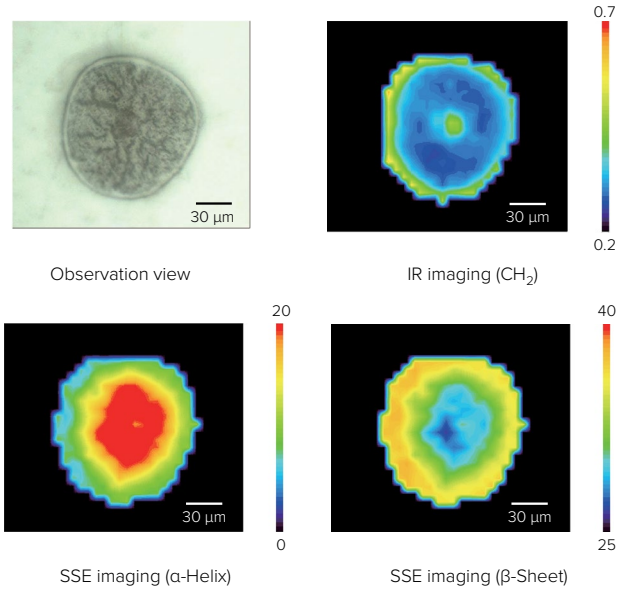


Fig. 7 Cross-sectional analysis of hair

This technology is now being used to analyze pathological tissue sections (e.g., cancer and atherosclerosis)[1] and food products (e.g., rice and wheat). Secondary structure analysis using FTIR microscopy is an effective, nondestructive analytical technique that can be applied to a variety of fields. FTIR microscopy with the IRT- 5000 Series FTIR microscope together with software tools, such as SSE and SSI, supports in-depth secondary structure analysis and distribution imaging.

1) Yamada, T., Miyoshi, N., Ogawa, T., Akao, K., Fukuda M., Ogasawara, T., Kitagawa Y., Sano K., Clinical Cancer Research. 8, 2010-2014 (2002).

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Applications of IR Microscopy in Pharmaceuticals and Medicine

Recently, IR microscopy has been attracting attention in the medical field for being able to discriminate between healthy and diseased tissues, for blood analysis, drug development, analysis, and identification of contaminants in medicines and medical devices.

In the field of pharmaceuticals, FTIR microscopy is widely used as an analytical method for drug development and quality control because it allows both qualitative and quantitative evaluation of active pharmaceutical ingredients (API) and additives in tablets. Furthermore, imaging measurements enable visualization of the distribution of API, fillers, and encapsulation, which is important information for tablet dissolution, drug uptake in patients, and quality assurance.

FTIR microscopy is also effective for measuring biological samples because it requires minimal sample preparation and can be performed with little or no damage to the sample. FTIR microscopy is used routinely in the medical field to obtain detailed information about the chemical composition of biological samples (proteins, lipids, carbohydrates, DNA, etc.), which is useful in research. Furthermore, imaging measurements can visualize the distribution of chemical components in biological samples to provide new insights into developing technologies.

In the following application, we present examples using IR microscopy for drug evaluation and the analysis of diseased tissue.

Microscopic Reflection Analysis

Near-IR Imaging of OTC Drugs (Sedatives)

Near-Infrared (NIR) spectroscopy is widely used for the non-destructive evaluation of foods and pharmaceuticals because this region of the spectrum readily transmits through glass containers and many background absorption peaks in the NIR region are weaker than those in mid-IR region, which is especially beneficial for reducing the interference of water. Recently, NIR has been introduced into the regulatory framework for Process Analytical Technology (PAT) proposed by the FDA and is mainly used to evaluate the mixing uniformity of samples in sealed vials and using in-line measurement to evaluate moisture content. NIR microscopy can also be used to determine and visualize the chemical content uniformity in pharmaceutical tablets and capsules.

There are a wide variety of measurement methods that can be used in NIR spectroscopy with instruments that include dispersive, filter, acousto-optic tunable filters (AOTF), and Fourier-transform (FT). FT-NIR is typically faster and has superior wavenumber range, wavenumber accuracy, and throughput compared to the other methods mentioned. Furthermore, NIR imaging provides nondestructive visualization of components on the surface of tablets and other materials, making it useful in drug design and quality control. NIR is also used to help solve problems in manufacturing processes and to better characterize final products.

This section describes the use of NIR imaging to evaluate the distribution of components in a tablet. An IR microscope with an FT-NIR spectrometer was used in reflection measurement mode for the imaging of a cross-section and the surface area of an OTC drug (sedative).

From the NIR mapping measurement of the tablet cross-section (Fig. 1), a false-color chemical image was created using peak heights specific to the unique functional groups for each component (Fig. 2). As a result, it was confirmed that the tablets consist of four layers of three components. Next, the tablet surface was analyzed, and the distribution of the components on the surface coating layer was expressed as a peak height ratio (4,380 cm^{-1}/4,680 cm^{-1}); typical of acetylsalicylic acid (Fig. 3). As a result, it was confirmed that the components in the surface coating layer were unevenly distributed.

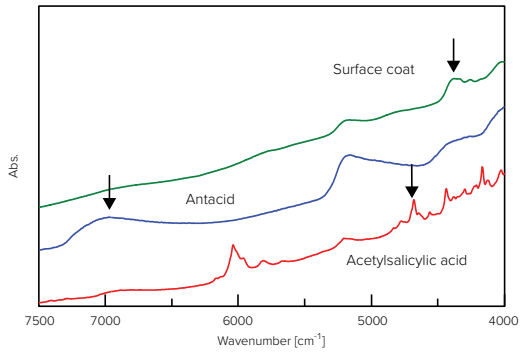


Fig. 1 Average spectra of each component (green: surface coat, blue: antacid, red: acetylsalicylic acid)

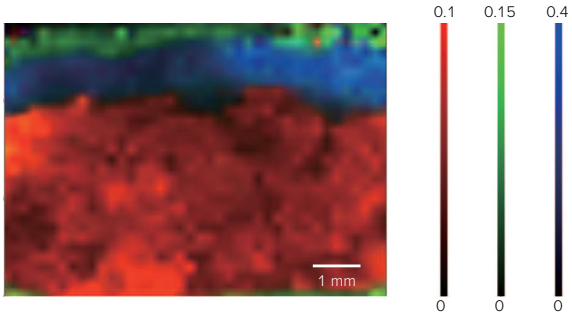


Fig. 2 NIR false-color chemical image of tablet cross-section (green: surface coat, blue: antacid, red: acetylsalicylic acid)

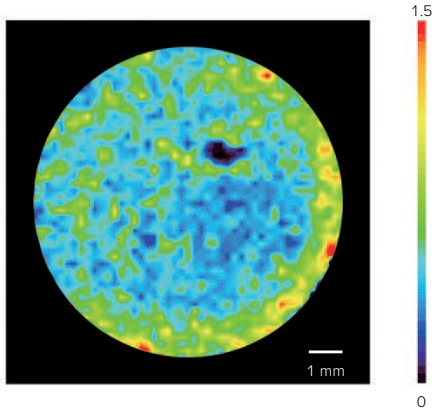


Fig. 3 NIR false-color chemical image of table surface coating layer (peak ratio 4,380 cm^{-1}/4,680 cm^{-1})

Microscopic Transmission Method

Measurement Example of Mouse Eyeball Cross-Sections

As an example of a biological sample, IR imaging measurements were performed on cross-sections of a mouse eyeball. The sections were mounted on BaF_2 slides (10 mm diameter, 1 mm thick) and measured in transmission mode (Fig. 4).

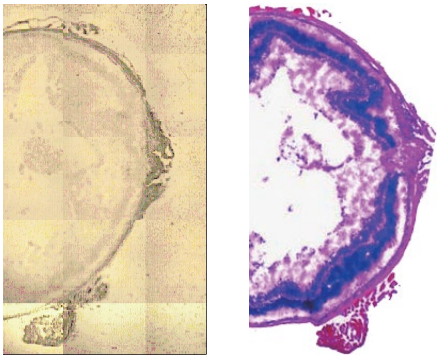


Fig. 4 Observation image (left) and H&E-stained image (right)

A false-color chemical image was created using the area ratio of the amide I peak (~1,640 cm^{-1}) and the C-H stretching vibration peak (~2,900 cm^{-1}), and false-color secondary structure distribution images of α-helix and β-sheets in the protein were also created (Fig. 5). As a result, the correspondence between the Hematoxylin and Eosin (H&E) stain image in each component tissue was confirmed. The two layers corresponding to the retina and optic disc have an abundance of α-helix structure, which indicates potential for a proliferative diseased state.

By further analysis of diseased samples, it is hoped that a causal relationship between disease states and protein structure can be revealed.

We would like to express our sincere gratitude to Dr. Miyoshi of Department of Tumor Pathology in University of Fukui, Japan, for providing the samples in this evaluation.

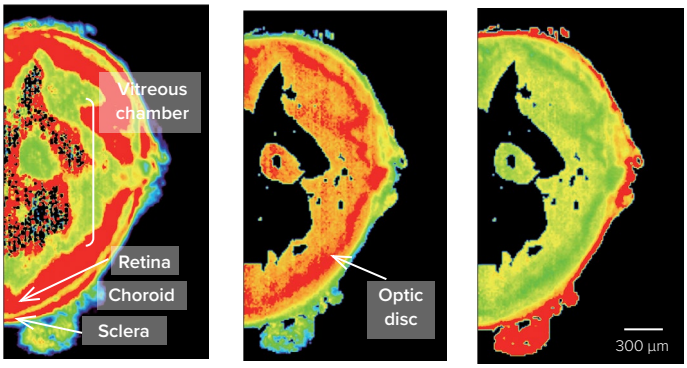


Fig. 5 False-color chemical images (left: peak area ratio, center: α-helix, right: β-sheet)

Analysis of Microplastics

There is a growing and alarming concern about the effects of microscopic plastics (microplastics) on the environment. Microplastics were first identified as contamination in the marine environment and food chain, but then developed to land-based ecosystems and the entire food chain, which has led to a long lasting and potentially devastating impact on the health of all animal life on the planet, including human health. The source and nature of microplastics is starting to be well understood, but a significant amount of research is still being done into the nature of microplastics, their distribution in the environment, and how to deal with them.

Here we present examples of microplastic evaluation using IR spectroscopy.

Microscopic Transmission Method

Imaging and Particle Size Analysis of Microplastic Particles

IR microscopy is one of the best methods to investigate and characterize types of environmental plastics. Microplastics can be measured without complicated sample preparation and chemical identification can be made with reference to spectral databases of known compounds. In addition, imaging measurements enable not only qualitative analysis, but also a more quantitative approach to chemical composition, as well as particle size and distribution analysis.

To confirm the validity of the evaluation of microplastics using an FTIR microscope, a suspension containing five different plastic polymers (PE: polyethylene, PP: polypropylene, PS: polystyrene, PET: polyethylene terephthalate, and PVC: polyvinyl chloride) was measured. The samples were collected on a polytetrafluoroethylene (PTFE) filter, and then mapping imaging measurements were made of the entire filter (Fig. 2).

Using the average spectrum of each component obtained from the imaging measurements (Fig. 1), a false-color chemical image was created using the peak heights indicated by the arrow in each spectrum (Fig. 3). By creating a false-color chemical image, it is possible to visualize the distribution of each of the five plastic polymers.

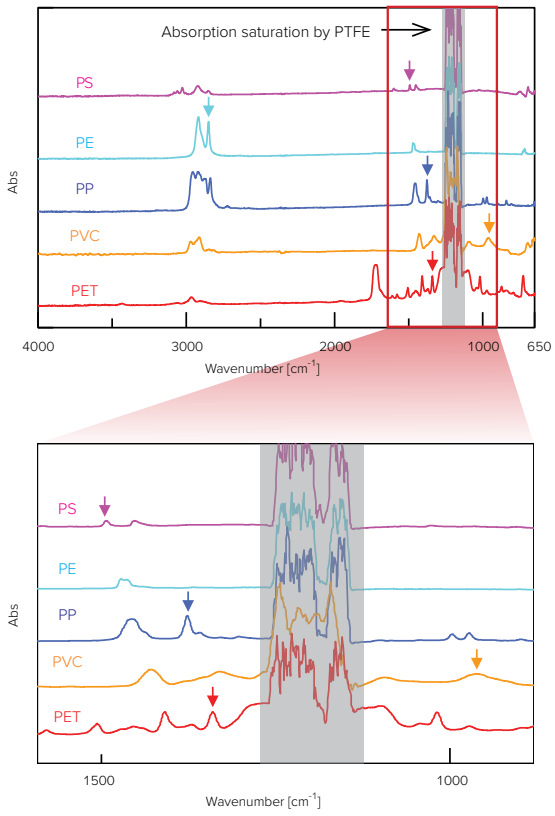


Fig. 1 Average spectrum of each component

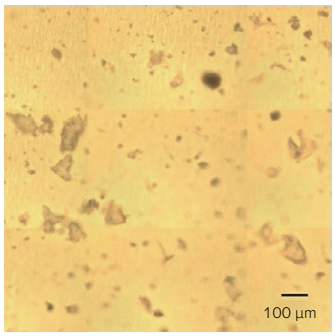


Fig. 2 Observation view

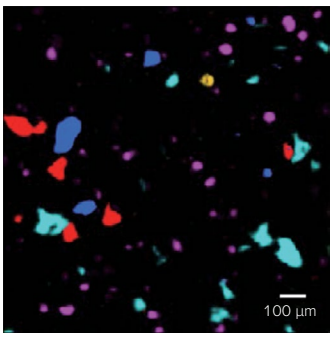


Fig. 3 False-color chemical image
(pink: PS, aqua: PE, blue: PP, yellow: PVC, red: PET)

Furthermore, as an example of particle size analysis, a stacked histogram of the relevant particle size dimensions and frequency for each component was created (Fig. 4). The JASCO Particle Analysis (JPA) program allows the analysis of particle size using both observation images and false-color chemical images.

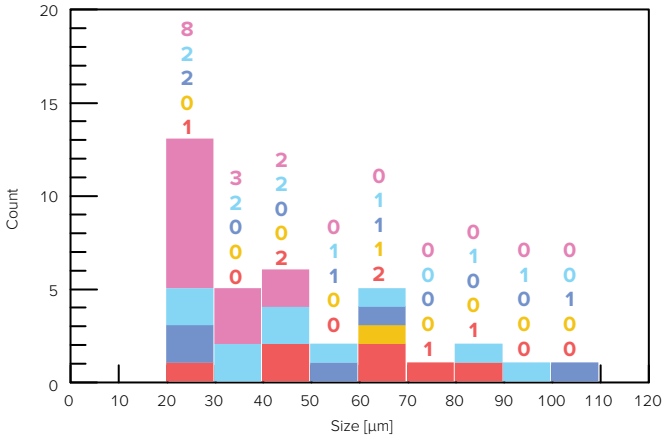


Fig. 4 Stacked histograms by component (X-axis: particle size)
(pink: PS, aqua: PE, blue: PP, yellow: PVC, red: PET)

IR microscopy allows the identification of the type of plastic present, and by creating a histogram from the results can illustrate trends in sample data.

We would like to express our sincere gratitude to TOSOH Analysis and Research Center Co.,Ltd., Japan, for providing this data.

Tips

JASCO Particle Analysis

The JASCO Particle Analysis (JPA) program is an add-in to the Spectra Manager Micro Imaging Analysis program, which allows the user to perform particle size analysis and statistical processing of sample data. The program automatically binarizes observed or false-color chemical images and performs particle size analysis using parameters such as sample size, area, perimeter, horizontal and vertical Feret diameter, aspect ratio, circularity, and color. In addition, statistical processing functions can be used to display histograms (stacked histograms for each component are also available), frequency distribution, correlation distribution, and component presence ratios.

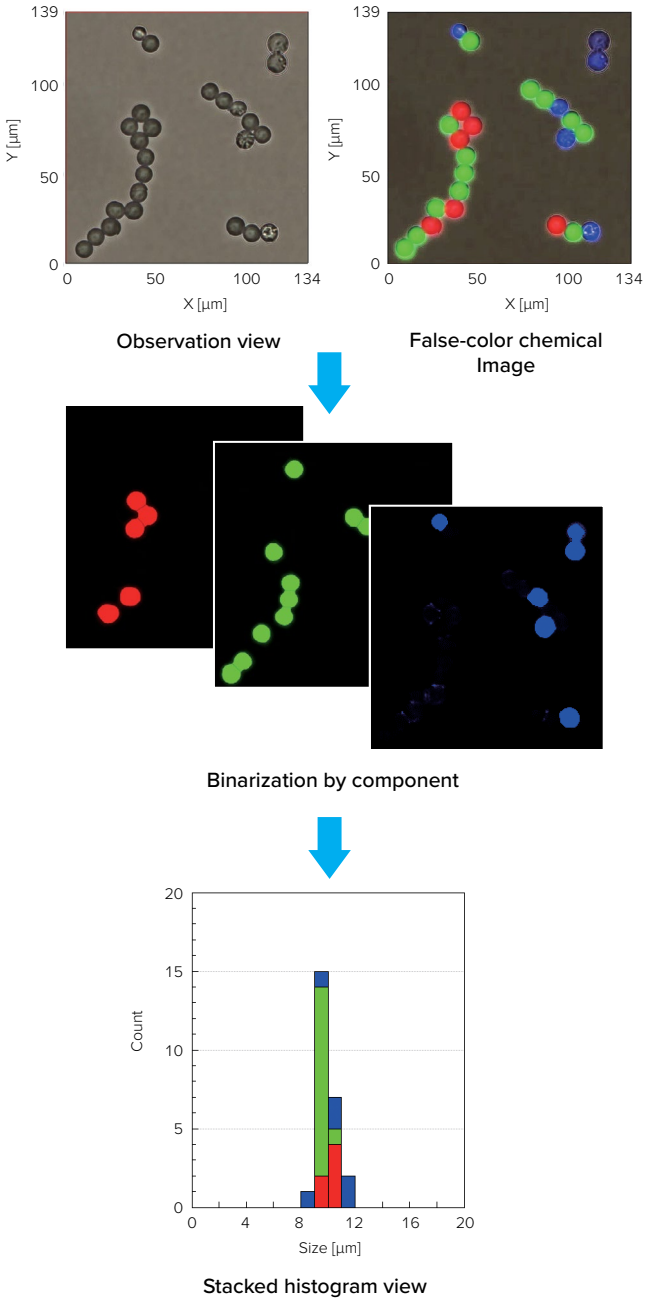


Fig. 5 Overview of JASCO Particle Analysis Program

Tips

IQ IR NAV Microscopy Imaging Analysis

JASCO's IQ IR NAV* makes it easy to analyze microscopic foreign materials and particulates. These functions are accessed from the Spectra Manager software through the Microscope Measurement program.

**IQ IR NAV can only be used with single-element detectors.*

Observation Optimization IQ IR NAV consists of the following two functions:

Focus NAV** executes auto-focus and auto-correction.

***Focus NAV is only available when using the auto XYZ stage.*

View NAV performs automatic brightness adjustment according to preset conditions with a single click.

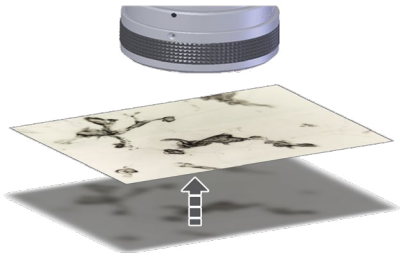


Fig. 6 Observation optimization

Search NAV Using Search NAV, measurement points can be automatically detected in real-time using an image recognition algorithm; the locations can then be registered and reviewed in a list of measurement points prior to acquiring data.

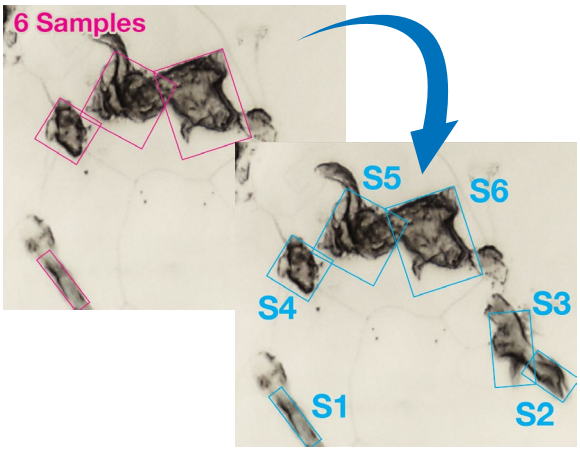


Fig. 7 Real-time sample recognition and registration of measurement points

The following additional functions can be used in combination with the optional add-in software Advanced Search NAV (ASN).

Refining of the Measurement Target The target measurement positions can be detected and filtered using parameters such as sample size, shape, circularity, contrast, and color.

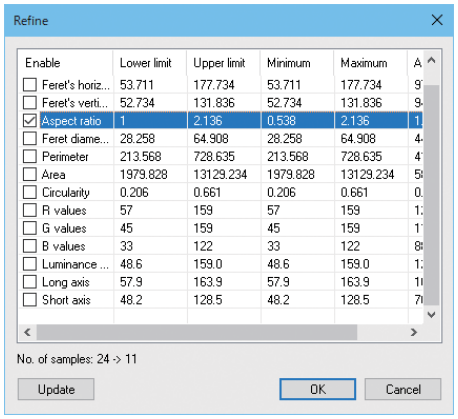


Fig. 8 Refining of the measurement target

Real-Time Analysis Result Display Using Advanced Spectra Search (ADSS), library search or spectral classification*** of the target measurement points can be performed automatically after sample measurement, and the results can be displayed as a compound name overlaid on top of the measurement position in the observation image.

*** See page 37 for more details..

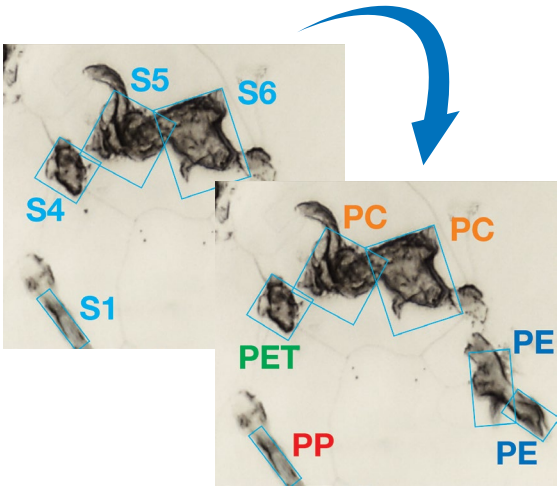


Fig. 9 Display of analysis results

Advanced Spectra Search (ADSS)

When performing qualitative analysis of an unknown sample from its FTIR spectrum, identification can be made using a library search against a database of known reference spectra. However, a library search will often output multiple spectra with similar features, and the final selection has to be made by the analyst. In order to assist the user in making the correct judgment, knowledge of spectral interpretation is beneficial, which can be a major obstacle for inexperienced analysts.

JASCO's Advanced Spectra Search (ADSS) program, which incorporates the “interpretation of a skilled analyst” into the software using machine learning, can overcome the above complications of spectral interpretation. The ADSS program has two functions: "Classification" and "Search".

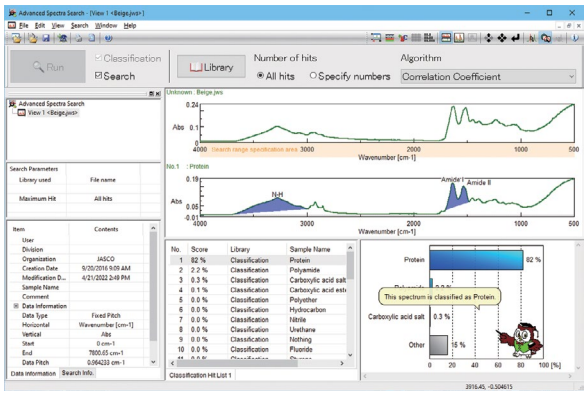


Fig. 10 Advanced Spectra Search program

"Classification" Function Based on the machine learning results of approximately 10,000 spectra, the classification function categorizes a spectrum of an unknown sample into one of 35 different types of chemical compounds (Table 1). Since this classification function has been trained mainly on spectra of foreign materials and polymers, it is particularly powerful for these analyses. Furthermore, since the training includes characteristics that depend on the difference in measurement methods and sample preparation, the acquired spectra can be used without any additional data processing. In addition to the fact that the type of substance can be estimated from the classified categories, key absorption bands unique to each compound and their potential functional groups are displayed in the spectrum, making it possible to visually confirm the validity of the results.

Table 1 Classification categories

Acetone	Acrylic resins	Alcohol
Carbonates	Carboxylic acids	Carboxylic acid esters
Carboxylic acid esters (oil)	Carboxylic acid salts	Cellulose and sugar
Epoxy resins	Fluorides	Hydrocarbons
Hydrocarbons (polyethylenes)	Hydrocarbons (polypropylenes)	Nitriles
Phenolic resins	Phosphates	Polyamides
Polycarbonates	Polyethers	Polyethers (polyacetal)
Polyesters	Polyimides	Polyvinyl acetates
Polyvinyl alcohol	Polyvinyl chlorides	Proteins
Silica	Silica (talc)	Silica (kaolin)
Silicone	Styrene	Sulfates
Urethanes	Water	

"Search" Function This function performs a library search. A correlation of similarity is made (scored) using a selected search algorithm, and the search results are reported in descending order of the score. A database library of more than 600 registered spectra, mainly of foreign materials and polymers, is available as standard, but it is also possible to build custom user-libraries.

In addition, using the dedicated microplastics library, "IR Plastics Library", with 153 spectra of 39 registered species, qualitative analysis specific to plastics and polymers can be performed.

Combined Microscopic Analysis

IR and Raman Study of Plastic Particles

A mixed sample of plastic particles was sprayed onto a substrate and the distribution of components (Fig. 12) was measured using both IR and Raman microscopy. Analysis using two complementary methods, in this case, IR and Raman spectroscopy, can provide more information about a sample with increased confidence in the results, compared to one method in isolation. When combining IR and Raman microscopy, information from the two methods can be obtained more accurately by utilizing the IQ Frame (Fig. 11, see page 16), which provides accurate and precise measurement positioning as the sample is transferred between the FTIR and Raman microscopes.

Fig. 11 IQ Frame operation between Raman and IR microscopes

The distribution of components in a sample of sprayed plastic particles (Fig. 12) was measured using both IR and Raman microscopy.

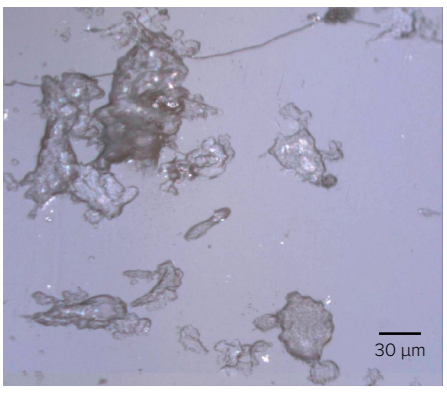
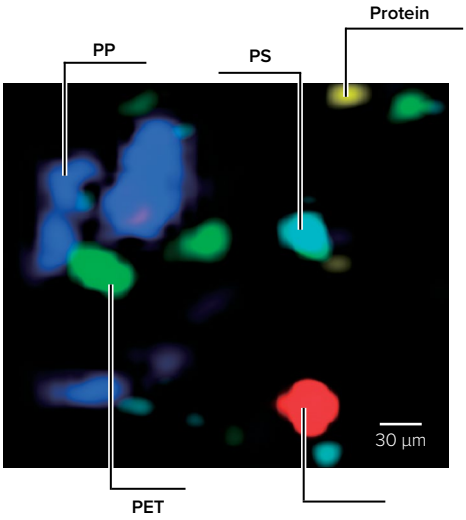


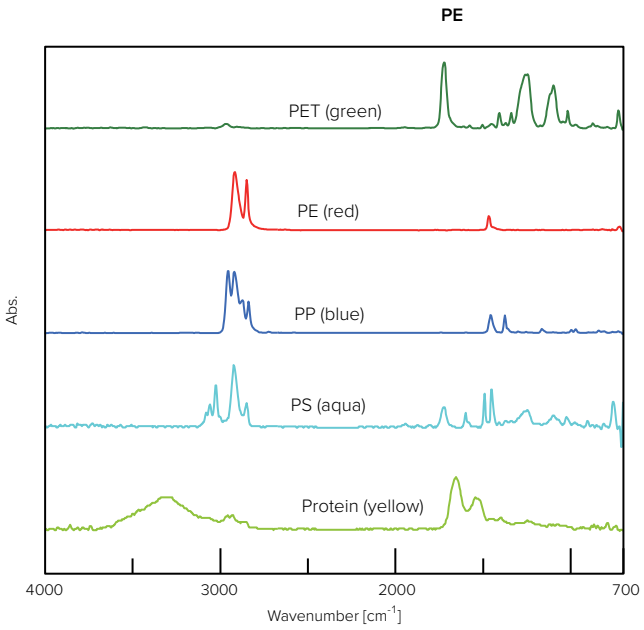
Fig. 12 Observation view

Measurement by IR Microscopy

Principal component spectra were calculated using multivariate analysis (MCR model analysis), and a false-color chemical image was created using the principal component spectrum scores (Fig. 13). FTIR analysis revealed the presence of proteins, in addition to four types of plastics: polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), and polystyrene (PS).



False-color chemical image (Score of Principal Component Spectra)

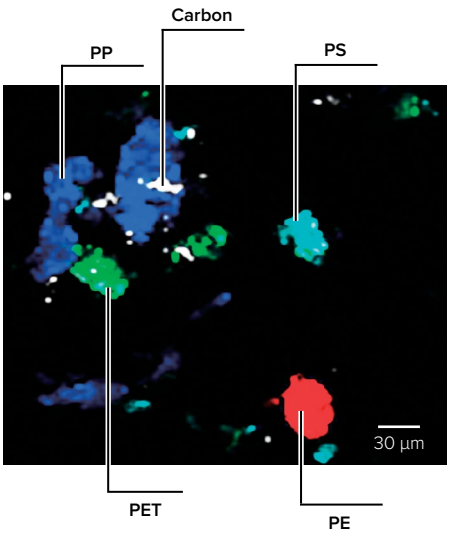


Principal component spectra

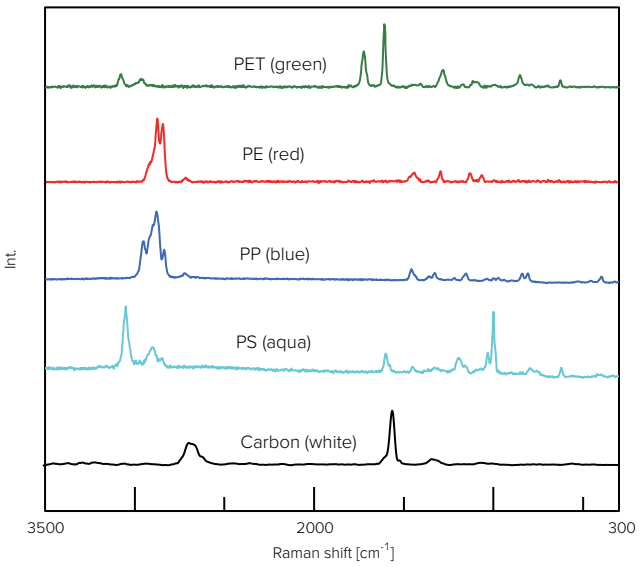
Fig. 13 Measurement results from IR microscopy

Measurement by Raman Microscopy

Raman imaging measurements were performed at exactly the same locations as were measured with the FTIR microscope. Each principal component spectrum was calculated using MCR model analysis, and a false-color chemical image was created using the peak heights of the key absorption bands (Fig. 14). The analysis revealed the presence of carbon, in addition to the same four types of plastics: PET, PE, PP, and PS.



False-color chemical image



Principal component spectra

Fig. 14 Measurement results from Raman microscopy

In addition to the four plastics, IR microscopy was able to detect protein, whereas Raman microscopy could not identify the protein but was able to measure carbon.

Using the false-color chemical images derived from the IR and Raman measurements, it was found that combining the results revealed the presence of a total of six components, while each of the methods alone could only detect five components.

In order to visualize the results of this analysis, the false-color chemical images from the Raman measurement were superimposed on the false-color chemical images from the FTIR measurement (Fig. 15). The combined false-color chemical image, included carbon, which was detected by Raman spectroscopy, in addition to the five components (PET, PE, PP, PS, and protein) that were detected by IR spectroscopy, demonstrates the advantage of combining the different attributes of IR and Raman spectroscopy, coupled with the benefits of IQ Mapping to be able to synchronize these measurements.

Thus, the use of IR and Raman spectroscopy together enables complementary analysis and increases the amount of information obtained from a single sample, thereby improving the scope, accuracy, and confidence in the analysis.

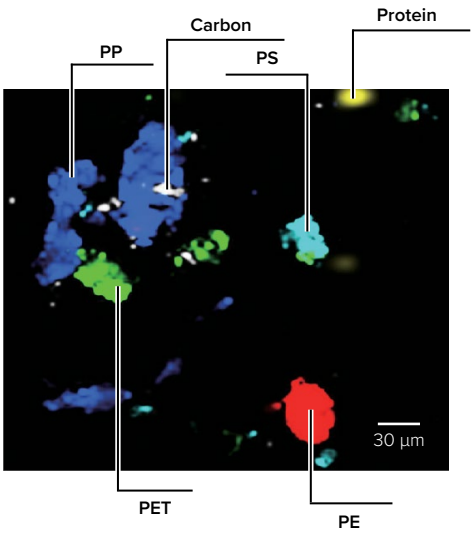


Fig. 15 Superimposition of false-color chemical images obtained from IR and Raman microscopes

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