

FTIR MICROSCOPY

JASCO X16

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FT/IR-6700 with IRT-5200

A Brief History of IR Microscopy

In recent years there has been an explosive growth in the applications of FTIR Imaging Microscopy and the data resulting from these imaging systems. From materials research to the imaging of bacteria, tissues, cancer cells and disease as well as uses as a quality control tool for polymers and pharmaceuticals. As new demands emerge for the analysis of increasingly complex samples, FTIR imaging systems must continuously evolve to offer the most comprehensive solutions.

Selecting an IR microscope can be a challenging process, there are many types of systems with different features and functionality; this guide aims to demystify some of the technology that is used.

FTIR imaging microscope systems can provide useful information from a simple spectrum of a very small contaminant in a larger matrix or detailed information about the distribution of the chemical constituents together with spatial information, i.e., the variation and distribution of layers in a polymer laminate. In the past, FTIR imaging microscopes and imaging systems have been the realm of research laboratories or users with extensive experience in infrared spectroscopy. An FTIR imaging microscope with a linear array (LA) detector can provide a rapid, powerful tool for the examination of a wide array of samples. Combining this flexible imaging system with advanced data processing software can easily provide fast, visually relevant data.

This ebook is intended to be a brief overview of some of the key considerations when selecting an IR microscope for your laboratory.

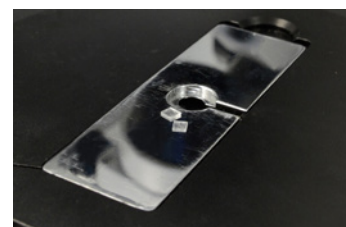
Sample Preparation and Measurement

Sample preparation is an extremely important factor when selecting and using an IR microscope; understanding the nature of the sample and the best method for measurement is necessary to obtain good data. Sample preparation is normally required to get a good IR spectrum, the same rules apply as when using a standard FTIR spectrometer. Is the sample a powder, film, gel, liquid or solid? Is it too thick? Is it reflective? The nature of the sample and the spatial resolution required will dictate whether transmission, reflection, ATR, or Grazing angle reflectance can be used. The sample may also require a different spectral range than the mid IR. Sensitivity can be an issue especially at higher spatial resolution with light from a very small area reaching the detector – in this case the choice of detector is important to get the fastest measurement in the shortest time, especially when mapping a large sample area for chemical composition.

Transmission Sampling

Transmission analysis of samples is probably the most common and most universal method of analyzing solids, liquids and gases. Transmission measurements provide the highest sensitivity and best detection of all infrared sampling techniques. In some cases sample handling for microscopy can be made easier by compressing the sample, such as a small fragment or filament between two KBr plates to form a pellet.

KBr (potassium bromide) pellets are the most recognized method for obtaining infrared spectra of powder or ground solid samples. Other ionic salts may be used for the pellet matrix such as NaCl, KCl or CsI, but KBr is preferred. These materials are very moisture-sensitive and must be handled carefully as they will quickly absorb atmospheric water vapor.



Transmission Sample

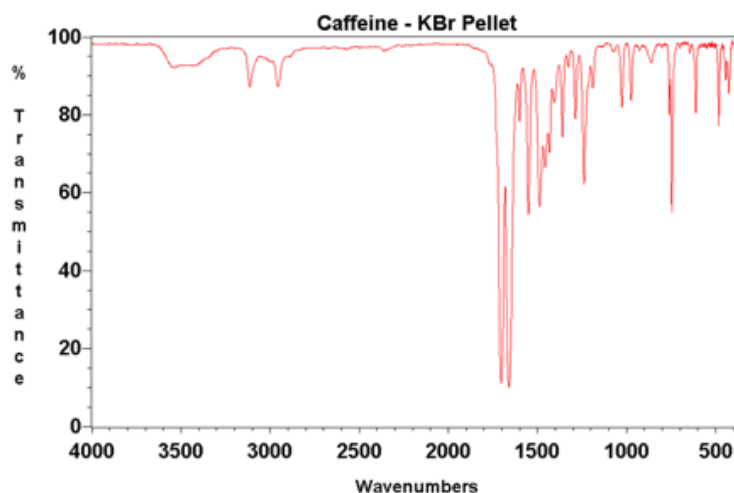
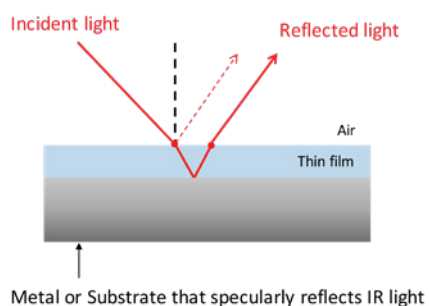


Fig. 1 Transmission spectrum of caffeine in KBr pellet

Specular, Reflection-Absorption and Grazing Angle Spectroscopy

Reflectance measurement is generally applied to samples with flat, shiny (specular) surfaces. A cassegrain reflectance objective directs the incident beam onto the samples flat surface and collects the reflected energy at the same angle.

The incident and collection angles are typically fixed at 30-35 degrees (where 90 degrees is parallel to the sample surface).



Reflection is typically used to measure thin films, such as oil on a specular substrate (e.g. metal). In this case, only the sample absorption spectrum is obtained, therefore a Kramers-Kronig transform is not required.

Specular reflectance measurement is generally used for samples that show reasonable reflectance such as polymer sheets, semi conductors, painted or coated metal, glass or paper, etc. Any flat, coated surface may be a subject of specular reflectance analysis.

Measurement of polymers and other samples often result in spectra with derivative bands and other distortions. The Kramers-Kronig transform removes the 'imaginary' specular component and calculates the 'real' absorption spectrum.

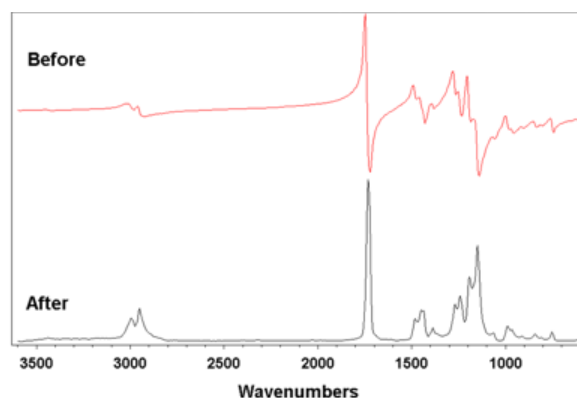


Fig. 2 Kramers-Kronig correction - Poly (methyl methacrylate)

Infrared Reflection Absorption Spectroscopy (IRRAS)

Coatings on 'shiny' substrates are excellent candidates for infrared reflection-absorption studies. As the coating gets thinner, incidence and collection angles can be varied from 45-75 degrees until the 'grazing' angle is reached, generally considered to be 85 degrees.

The term 'reflection-absorption' describes the progress of the incident beam as it passes through the coating, reflects from the substrate and passes through the coating again before reaching the detector.

As the incident and collection angles approach grazing angle, the incident beam strikes the coating at shallower angles and the pathlength through the sample gets longer, enhancing the absorption intensity.

Grazing angle reflection is used to examine the thinnest of surface coatings by 'grazing' the sample at a very shallow angle, the resulting longer sample pathlength providing greater sensitivity.

Infrared reflection-absorption spectroscopy (IRRAS) is often used to study monolayer coatings on metals and other substrates. Limited quantitation can be made when evaluating the composition and thickness of the coatings.

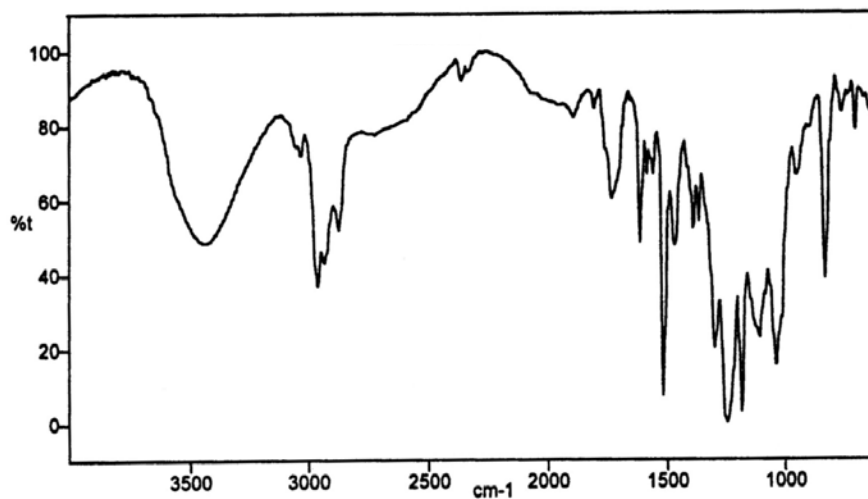


Fig. 3 Thin film coating on a metal can

Polarization of reflectance spectra can enhance the absorption but this is most significant at the grazing angle. The angle of polarization can have a dramatic effect if the monolayer is highly oriented.

Observing and Magnifying the Sample and How Cassegrain Objectives Work

Cassegrain objectives are reflective lenses that can be used for both visual observation and infrared measurement; they provide high magnification with minimal distortion or aberration. This optical system produces a focal plane with the measurement dimensions controlled by a variable slit mechanism.

For transmission and reflection measurement there are typically three levels of magnification - in the range 10x, 16x and 32x, either as a Cassegrain set (objective and condensing) for transmission or as a single Cassegrain objective solely for reflectance measurement.

In transmission measurement two Cassegrains are used together, one to condense, the other to 'de-magnify' the light after it passes through the sample. The focal planes of the Cassegrains are matched. Fibers, laminates, and thin films are often measured in transmittance mode. In reflectance mode, half the objective Cassegrain is used to bring the beam to the sample, the other half is used to direct the reflectance light to the detector. The spatial resolution is defined by the magnification and numerical aperture of the lens, this is typically lower in a cassegrain lens than a refractive lens, but cassegrains offer a much wider spectral range due to the fact there is no transmission through material to absorb the light.

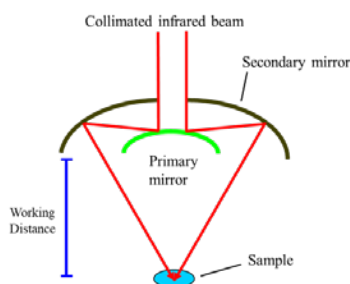
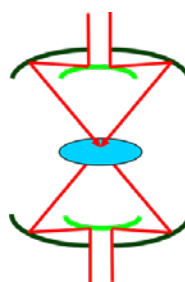
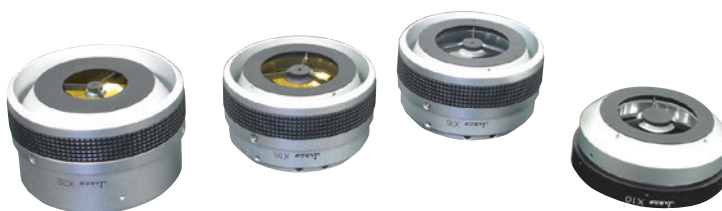


Fig. 4 Reflection Cassegrain



Transmission Cassegrain



Cassegrain Objectives

Refractive Objectives

As an alternative to Cassegrain objectives, high clarity refractive (light microscope) objectives with magnifications of 4x, 10x and 20x are often used for clearer visual observation and for use with polarization for higher contrast imaging.

ATR Objectives

After transmission and reflection measurement, Attenuated Total Reflectance (ATR) is one of the most widely used measurement techniques in FTIR spectroscopy. ATR is useful for samples that are non-reflective or non-transmissive, and can effectively control the path length for measurement.



Refractive Objectives

Snell's law predicts that at a critical angle, light energy penetrating a crystalline media with a refractive index greater than air will reflect radiation along the crystal. As the light beam strikes the interior surface of the crystal, an 'evanescent' wave penetrates a defined depth into a sample in 'intimate contact' with the crystal surface. The energy that penetrates the sample is absorbed corresponding to the vibrational frequencies for that sample. The ATR mechanism only allows for a finite depth of penetration into a sample in contact with an ATR element. For the majority of the ATR crystals, the depth of penetration is limited to 1-8 microns dependent upon wavelength. There are a number of different crystals used for ATR accessories, including diamond, germanium (Ge), zinc selenide (ZnSe); zinc sulfide (ZnS).

The relative peak intensities of mid IR spectra are a little different when compared to transmission spectra because as the wavelength gets longer, the depth of penetration into the sample is greater. Absorption wavelength values of mid IR spectra are directly comparable to transmission data, but the changes in relative spectral intensity can be misleading.

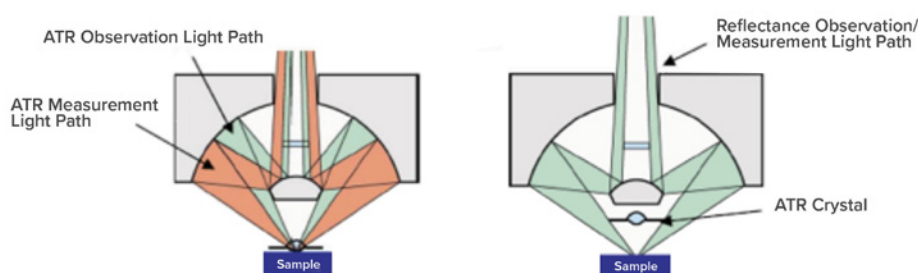
Spatial resolution can also be extremely important in FTIR microscopy, an ATR objective offers a smaller measurement area than can be achieved even with a x32 transmission Cassegrain. The IRT Series of microscopes has a wide range of ATR objectives including diamond, germanium and zinc selenide, and Clear View for observation directly through the ATR.



ATR Objectives

Clear View ATR

The unique design of the Clear View ATR objectives provides both observation of the sample and ATR spectral measurement using the same Cassegrain; by simply changing the crystal position (up and down).



ATR Observation / Measurement Mode

- Observe / measure the sample using ATR objective lens only
- Observe the sample even when the ATR crystal is in contact with the sample
- Perform ATR measurements while observing the sample in real-time

In addition, two special objectives, ATR-5000-SD and ATR-5000-SS offer simultaneous sample observation during data acquisition, something that is not possible with conventional ATR objectives. This innovative feature allows the selection of a specific area of the sample while observing the entire area of the sample that is in contact with the crystal element.

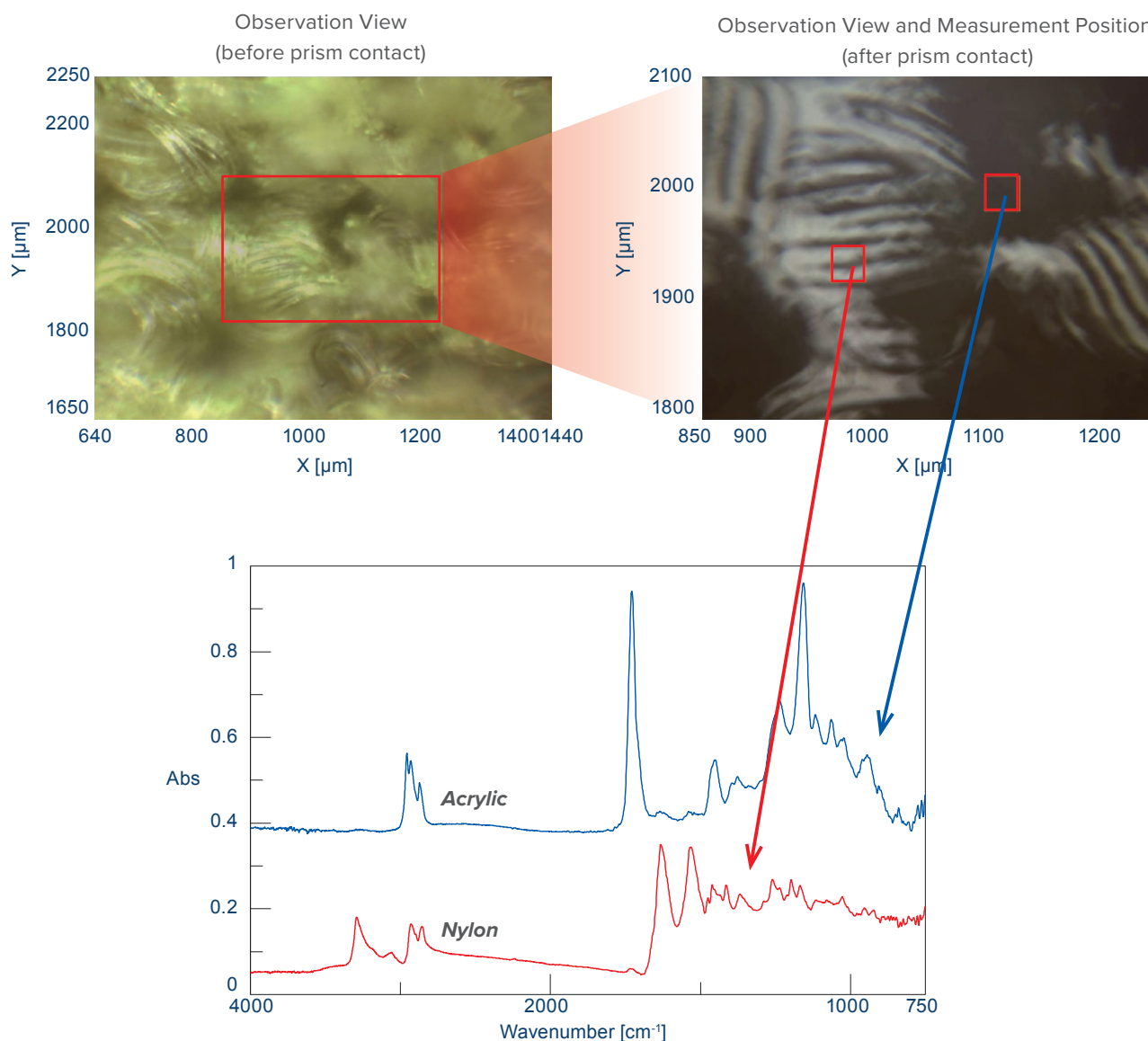


Fig. 5 Measurement of fibers by ATR

Care and Cleaning of ATR crystals

ATR crystals can be cleaned with the appropriate solvent that will remove the sample without damaging the surface. Dilute acid or alkaline solutions should be used sparingly and the ATR crystal immediately rinsed after use. Take note of the PH ranges for specific crystal materials. Cleaning solvents may attack the bonding material used in some ATR crystal assemblies. Strong acids or bases should be removed from the crystal as soon as possible after data collection. ATR crystals are chosen for the effective spectral range and the relative depth of penetration into the sample. Ideal for aqueous solutions, some ATR crystal materials are also available as liquid transmission cell windows.

Preparation of Thin Sections of Samples – Slice Master

IR transmission spectroscopy and particularly microscopy of solid samples requires relatively short path lengths, typically in order of a few to 10s of microns. A common technique for sectioning biological materials and polymer films is the use of a microtome. This is an expensive instrument and requires time consuming sample preparation, such as embedding. The Slice Master is a convenient alternative to the microtome and is a compact slicer that can create thin sections quickly and easily. It is a powerful tool for multi-layer film analysis and/or cross-sectional analysis. Four models are available, and can be selected according to the requirements of sample preparation.

Vertical Slicer

Easily cuts samples vertically for cross-section observation.

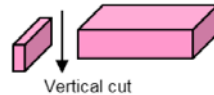
Cross sectioning

Capable of **vertically cutting** thin film and card materials with a specified thickness.

Creation of segments/slices

Includes a removable sample holder.

Thin sections are made by removing the sample holder, placing the sample in the holder, mounting it on the unit, and then cutting.



Angled Slicer

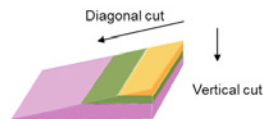
Observe wide cross- sections of samples with thin layers such as coatings. (Approximately 4 times as wide as a vertical cross-section area).

Cross sectioning

Capable of cutting at a 15° angle for thin film and card materials up to a certain thickness.

Creation of segments/slices

Includes a removable sample holder.



Multi-Angle Slicer

Enables the cutting of samples at a 15° angle while observing them under a microscope. Cutting at a 15° angle produces four times as much cross section surface area as cutting vertically. Variable cutting angle for cross-section observation of small samples.

Cross sectioning

Capable of cutting at a 45°-90° angle for thin film and card materials up to a certain thickness.

Creation of segments/slices

Includes a removable sample holder.



Tablet Slicer

For clean cutting fragile samples (such as tablets, cereal grains etc.). Variable angle cutting. Able to cut from 3 to 10 mm.



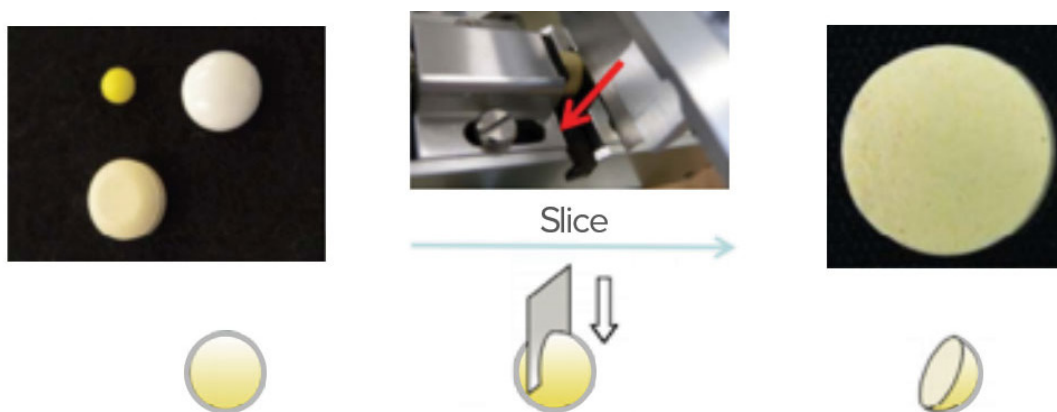


Fig. 6 Cutting images of the pellet sample and globular sample. Blade is super hard type.

Diamond Compression Cell

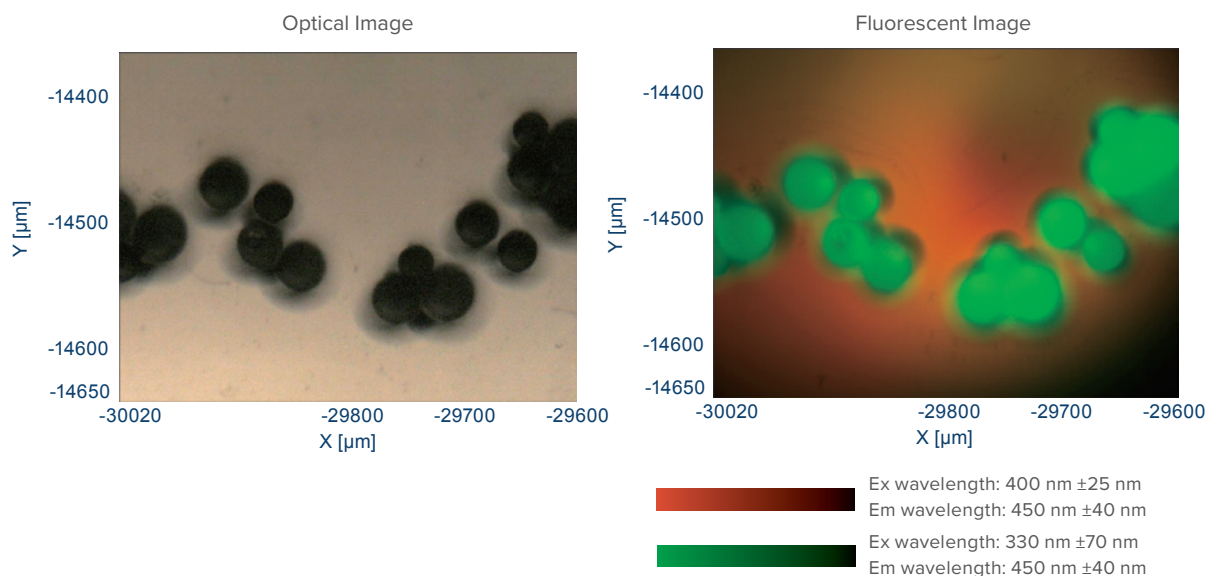
For transmittance measurement with IR microscopy where the sample is too thick to obtain good transmittance spectra, the sample can be compressed using a compression cell with diamond windows, which exerts high pressure on the sample. This accessory is used with a variety of sample types, such as monofilaments, polymer materials, rubber products, hair, biological tissue etc.



Sample Observation

Fluorescence Observation

Fluorescence observation can be used to identify fluorescent samples which cannot be seen with visible light. Two options are available with different wavelength ranges. The wavelength range is selected with a filter.



Differential Interference Contrast Observation

For observing a clear sample or a sample with low contrast, the DIC-5000 can accentuate the contrast for improved sample observation. The DIC-5000 is typically used with a 10x refractive objective lens.



Optical Image



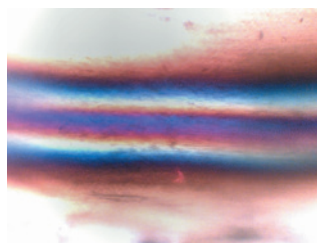
Differential Interference
Contrast Image

Visible Polarization Contrast Observation

There are some sample types that may be difficult to observe using visible light, these include monocrystals, minerals, and contaminants in polymer films etc. The use of polarized light dramatically improves the visualization of these samples.



Optical Image

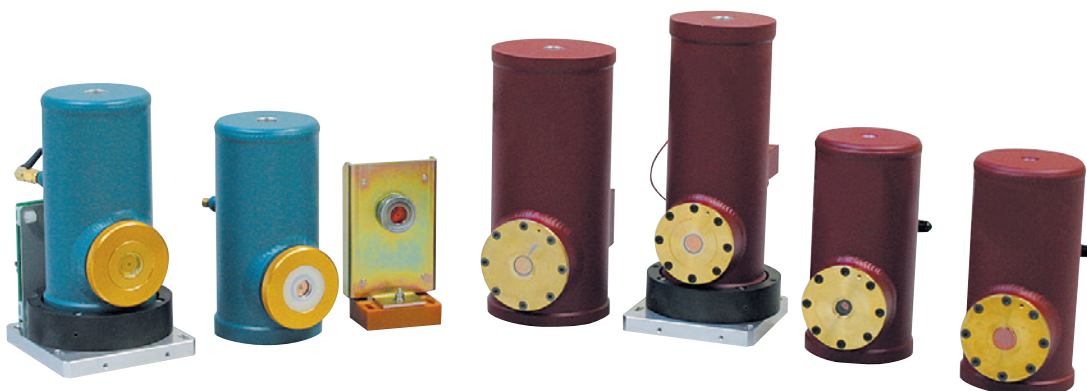


Polarization Image

Selecting a Detector for Sensitivity and Wavenumber Range

The many detectors that can be used in an FTIR microscope cover a wide wavelength range from the visible – (silicon photodiodes), NIR – (InSb or InGaAs), mid-IR – (TGS or MCT), far-IR – (Si bolometer).

The IRT Series has a choice of standard detectors with the option of a second detector. The simplest detector, a Peltier cooled DLaTGS detector is used in the mid IR region with good sensitivity, however with much greater sensitivity a LN₂ cooled mid-band MCT detector is better for measuring smaller microscopic areas. The optional second detector can be installed with a choice of either a fixed detector or a cassette system for interchangeable detectors, both can be selected from a wide range of options. To increase speed, especially for imaging or dynamic measurement a 16 element linear array detector (MCT or InSb) dramatically improves throughput.



*Detectors
High S/N ratio (MCT & TGS)*

Linear Array Detector

The 16 element linear array detector allows 16 spectra to be acquired simultaneously, for greater throughput. Although the measurement is slightly slower than a focal plane array detector (the fast processing speed makes up for this and the linear array with higher sensitivity also offers greater wavenumber range into the far IR).

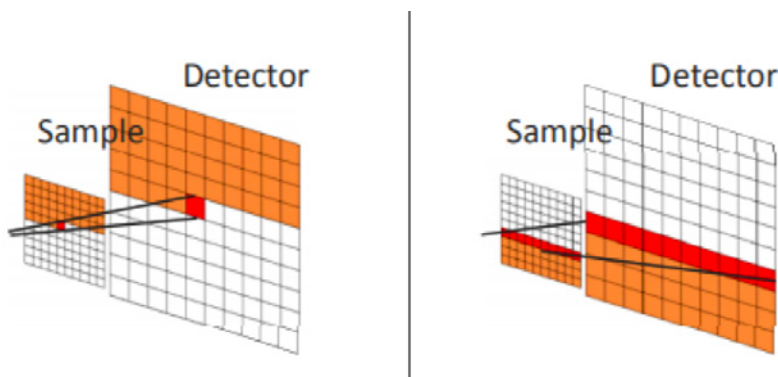


Fig. 7 Comparison of single point detector with Linear Array Detector

Mapping & Imaging Measurement

IR mapping and imaging measurement are widely used to visualize the chemical distribution and physical structure in a wide range of sample matrices. Samples that are often analyzed using this technique include polymer films and laminates, semi-conductors, contamination from foreign bodies and forensics; more recently biological tissues have been imaged, especially for the distribution of molecules such as proteins and lipids for diagnostic purposes.

To develop a large area, mapping and imaging can be time consuming as it may require the acquisition of many data points to build up a complete surface image. Typically an automated stage is required to move the sample in the view of the objective cassegrain, as well as imaging software capable of easily creating a measurement grid, spectral acquisition and extraction of the key vibrational bands with which to build the image.

Modern FTIR microscopes include a large amount of automation to make the measurement process less cumbersome for the user. The IRT-5000 and 7000 series have many automated features which can speed up the process and develop results and images in real time.

IQ Mapping is a unique feature that allows the measurement area to be moved in a stationary cassegrain to build up an image map without moving the stage. IQ Mapping can be used with transmission, reflection and ATR measurements. It is exceptionally useful for ATR measurement as an area in contact with the crystal can be mapped without lifting and repositioning the objective prism, which normally causes damage to the surface during the process. IQ Mapping also provides imaging for soft samples, like gels or even viscous liquids. The ATR prism will agitate the sample if it is lifted and repositioned, but using The Clear View SS type ATR objective allows the sample to be viewed and measured without disturbing the sample once it is in position.

The use of 'multi detector' units can significantly increase the speed of acquisition. Focal plane array and linear array detectors can image an entire section much faster than a single point detector.

Below is an example of impurity analysis from measurements using IQ Mapping with the IRT-5000 FTIR Microscope (Fig. 8).

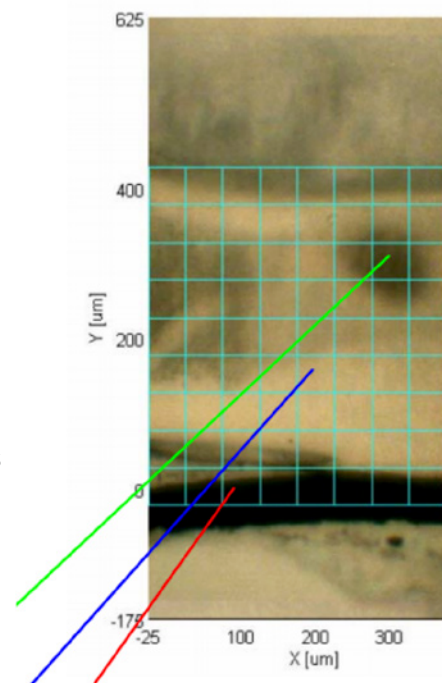
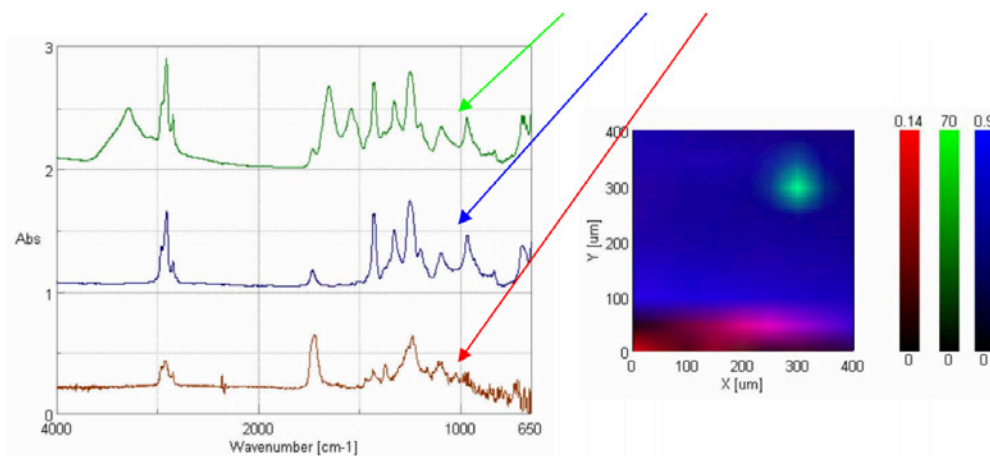


Fig. 8 Observation image (yellow-green grid shows measurement points)



IQ Mapping with ATR allows measurement without any contamination, as the number of contacts between sample and prism is reduced to only one. The sample shown in Fig. 9 was measured by using a ZnSe ATR prism.

The result of the mapping measurement found spectra of several different components, depending on the measurement positions (Fig. 10).

The sample measured was a mixture of water-based and oil-based marker inks, with specific peaks for the different chemical structures identified in the spectra below. A color distribution map using the specific peaks for components at 1666 cm^{-1} and 1282 cm^{-1} is shown below. As demonstrated here, mapping measurement can detect different components and their distribution in the sample which appear to be a single component in the visible image.

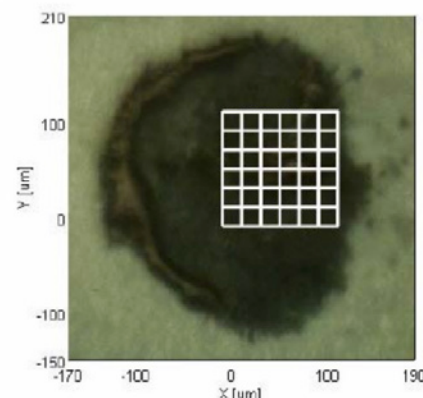


Fig. 9 Sample observation image (white grid shows measurement points)

Combining data from IR and Raman Measurements - IQ Frame

The combination of data from FTIR and Raman microscopy can provide richer information about structure and chemical composition. IQ Frame can be used with both IRT and NRS series of IR and Raman microscopes. The exact sampling positions can be shared between the two instruments (IR microscope and Raman spectrometer). IQ Frame consists of shared holder and a dedicated program, which enables automated searching and identification of the sampling position using an image matching function. This is useful for evaluating complex materials (including organic compounds and inorganic compounds).

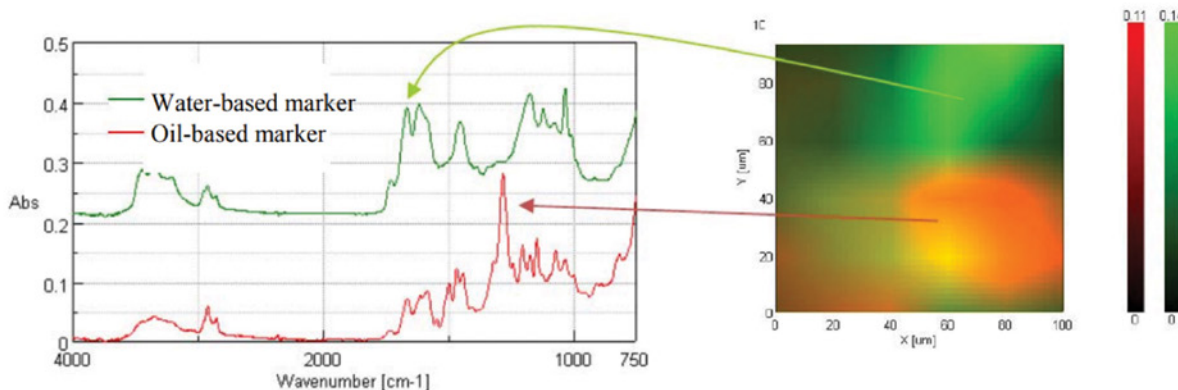


Fig. 10 Measured spectra of impurity

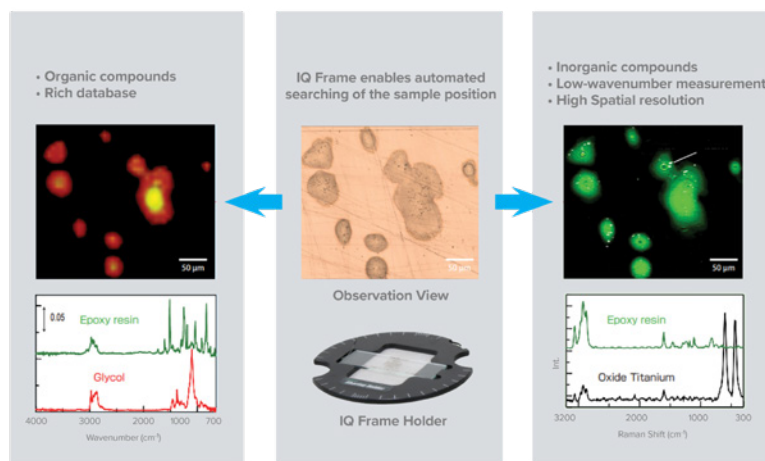


Fig. 11 Transfer sample between IR and Raman microscopes

Chemometrics

Chemometrics is a statistical method that can be used to analyze spectral data by comparing unknown variables to a set of known or referenced data. Quantitative and qualitative analysis can be performed using five multivariate algorithms, including Classical Least Square (CLS), Principal Components Regression (PCR), Partial Least Square (PLS), Principal Components Analysis (PCA) and Multivariate Curve Resolution (MCR). In FTIR data analysis, chemometrics is used to determine the amount of components in a mixture.

Classical Least Square (CLS) is a prediction model that enables the determination of concentration of unknown components based on the concentration of known multicomponent systems, assuming that the system can be represented by a linear matrix. The matrix sequence for CLS is based on the Beer Lambert law,

$$\text{Abs} = C \times k \text{ (C: concentration, k: absorptivity coefficient) + errors.}$$

A calibration model is created to calculate the correlation coefficient for each component's horizontal axis from the standard spectrum for which the multiple component concentrations are known. Calculations based on this method are very fast, but not recommended for mixtures of constituents that interact.

Inverse Least Square (ILS) uses a variation of the CLS method, where each concentration is a function of the absorbance at a series of given wavelengths. While this calculation is fast and allows for the quantification of more complex mixtures, wavelength selection can be difficult and time consuming since multiple wavelengths must be used to accurately describe each known component in the mixture. Several samples are needed for a more accurate calibration.

Principal Component Regression (PCR) is a method that combines Principal Component Analysis with Inverse Least Squares regression to create a quantitative model for complex samples. Instead of being based on Beer's law with the aim to calculate the absorptivity coefficients for the constituents of interest from a regression of the concentration, PCR regresses concentrations on PCA scores.

Principal Components Analysis (PCA) is a method used to reduce data to set variables, principal components, and scores and loadings based on data distribution. Each principal component calculated contains information on the variation of the data; scores are used to classify the data, and loading provides an indication of the importance of components, allowing the clustering of the scores.

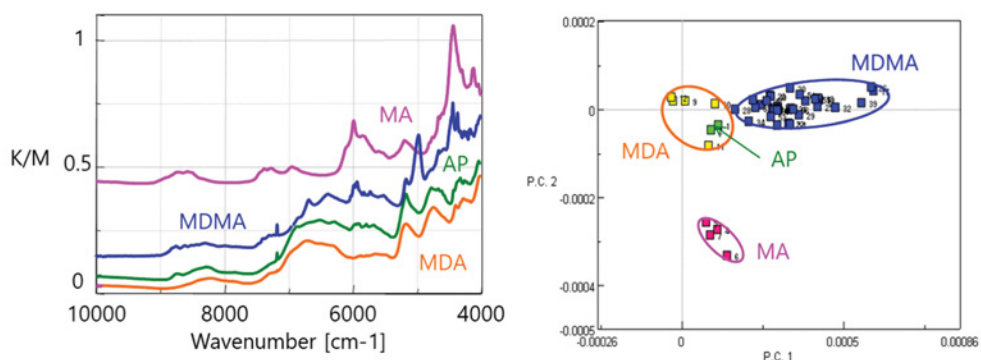


Fig. 12 Diffuse reflectance spectrum of drug tablets and PC1 and PC2 score plots

Partial Least Square (PLS) is a quantitative spectral decomposition technique that uses the concentration data during decomposition of the spectral matrix, directly correlating the concentration data to the spectral data. The variations in concentration are taken into account and related to the variations in the spectral data. This form of regression leads to components with larger concentrations to be weighted more heavily than components with smaller concentrations.

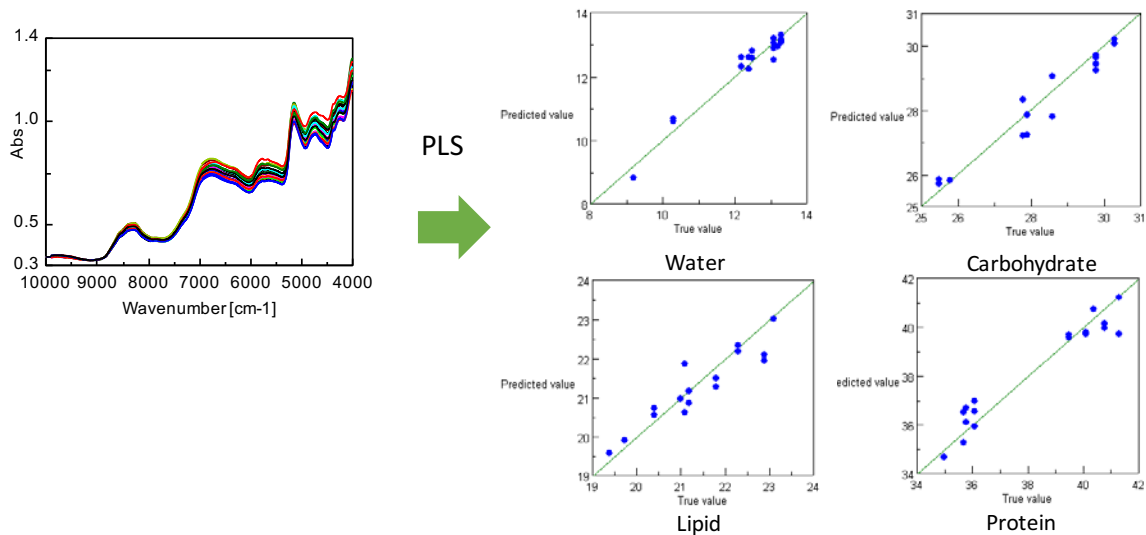
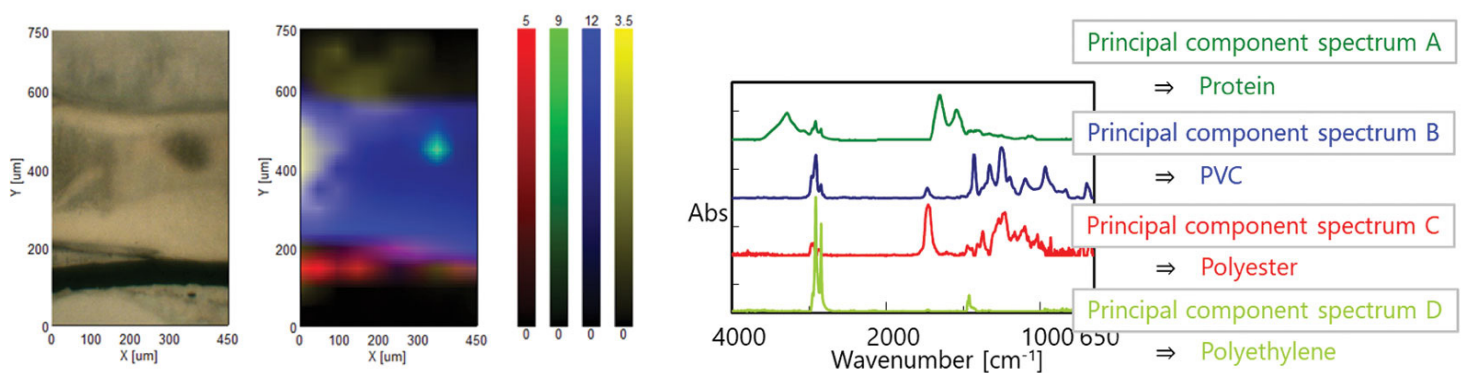


Fig. 13 PLS calibration of soy bean

Multivariate Curve Resolution (MCR)

In the case of multivariate curve resolution, the standard sample spectra are not known, only the matrix of spectra. Therefore, PCA is performed on multiple spectra and principal component spectra are then calculated. Different components can be found in an unknown sample. Below, a cross-section of a multilayer film was imaged using MCR with a FTIR microscope, the results demonstrated that the sample consists of four components.



Left: Optical Image and Right: Chemical Image (Green: protein, blue: PVC, Red: Polyester, Yellow: Polyethylene)

Principal component spectra (offset display)



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