

focus on Spectroscopy

Overview and Comparison of Portable Spectroscopy Techniques: FTIR, NIR and Raman

By Dr Heather Murray, Analytik

Walk through the majority of R&D departments and quality laboratories and you will see FTIR, near-infrared (NIR) and Raman spectrometers aplenty. These now ubiquitous instruments are used to support the manufacture and development of a vast range of products of all shapes and sizes and material types from pharmaceuticals to pasta, paint, petroleum and polymer films. A single spectrometer can be used to verify the identity of bulk materials, check for contamination, ensure intermediates are processing as expected, and confirm final product to be within specification. Developments in sampling accessories and advancements in software (simplified user interfaces, library searching capabilities as well as processing algorithms) have greatly increased ease of use; these instruments are now well suited to the non-specialist user. However, these systems, in the main, are still kept in the laboratory and samples are brought to the instrument for often quite simple analysis.

Vibrational spectroscopy techniques are extremely well suited to be used as portable or handheld. Their simplicity, speed, selectivity, and ability to operate without sample preparation make them ideal to be used outside the lab in more difficult environments. Indeed, for specialised on-line applications FTIR, NIR and Raman have all found a niche in process monitoring in challenging environments. But it is by bringing the analyser to the sample and making the measurement when and where required that allows the full benefits of vibrational spectroscopy to be realised. This paper compares the three techniques, discusses their similarities and differences, but perhaps more importantly indicates where they are best deployed in order to maximise their effectiveness away from the laboratory.

The Requirements for Portable Spectroscopy

In comparing field based instruments to lab instruments emphasis is rightly put on the need for robustness for field work. Instruments may have to tolerate harsh conditions and yet maintain reliability and accuracy. They must be easy to operate, battery powered, lightweight with an ergonomic design and intuitive user interface. Sample accessories must be robust, with limited or no sample preparation required. Finally, if they are to be used successfully they must be capable of fast analysis, and give an unambiguous result such as pass/fail.



Figure 1: FTIR, NIR, and Raman portable spectrometers supplied by Analytik

FTIR Spectroscopy

Instrument design

IR spectroscopy is the measurement of the absorption of light across the range 4000 – 400 cm^{-1} . This can be done in two ways, firstly, by breaking down light and shining each wavelength at a sample, measuring how much of the light is absorbed, and repeating for each different wavelength, this is known as the 'dispersive' method. Secondly, there is the Fourier Transform method, which uses a broadband source rather than monochromatic, this source is manipulated by an interferometer to create an energy interference pattern which is then absorbed/transmitted by the sample. The detected radiation is then Fourier transformed which when automatically ratioed against a background with no sample gives a spectrum across the entire wavelength range. Because of faster data collection, greater sensitivity and precision virtually all IR spectrometers today are FTIR. As a consequence the technique of Mid-IR spectroscopy is now nearly always referred to as FTIR.

Background

FTIR is the most commonly used vibrational technique for material identification and authentication. When light is passed through a sample some of the wavelengths of light are absorbed whereas others are transmitted. At its simplest this can be described as the light absorption causing a change in the distribution of charge (dipole) across the molecule. This is the case for most covalent chemical bonds and therefore most organics are known to be 'IR active'. A wide range of inorganics are also IR active such as nitrates, phosphates, chlorates and sulphates.

In FTIR, light absorption by the sample is measured across a range from 400 – 4000 cm^{-1} or (25000 – 2500 nm) corresponding to fundamental molecular vibrational modes. A plot of absorbance, % transmittance or reflectance against wavenumber is termed an 'IR spectrum'. Absorptions corresponding to the vibrational frequencies of different bonds within the molecule can be used to identify a particular functional group e.g. -OH, -NH₂, -CH, -CH₂, -CH₃, -SH, -CN, -CO, -CS and versions of C-C as the positions of these bonds in the IR range are well known. However, the FTIR spectrum of a material can be complex as there may be many absorptions associated with the molecule which may interact, producing a characteristic pattern for the complete molecule. Identification of a material is then possible without having to characterise each absorption by matching its FTIR spectrum against others providing they were all obtained under the same conditions, especially interesting is the fingerprint region (below 1400 cm^{-1}), which contains the greatest number of unassigned vibrations.

Interpretation

Since FTIR has such high molecular selectivity it is relatively simple to interpret making it especially useful in identifying unknown samples. There are a number of automatic search methods for FTIR spectra including simple peak picking, Euclidean distance or first derivative algorithms. Compared to NIR and Raman there are many more reference texts, correlation charts and electronic libraries containing FTIR spectra. By comparing the peak position, intensity, integrated area and shape of peaks of an unknown to all others, a list of the closest matches can be created. Spectral subtraction of major components to allow minor components to be identified by library searching is also a useful technique to identify contaminants for example.

FTIR is also used extensively for quantitative analysis from a few ppm to the % level. Simple methods following the Beer-Lambert Law can be constructed as absorbance is linearly proportional to sample concentration as shown below:

$$A = a \cdot b \cdot c \text{ Where: } A = \text{absorbance, } a = \text{absorptivity, } b = \text{pathlength, } c = \text{concentration}$$

Sampling

There has been a great deal of progress over the past decade or so in the development of sampling accessories for FTIR. IR absorptions are strong; and historically samples were diluted before measurement by preparing a KBr pellet or Nujol mull. The late 1990s saw the introduction of easy to use sampling accessories based on reflectance. The advent of these Attenuated Total Reflectance (ATR) and Diffuse Reflectance (DRIFT) accessories removed the requirement for time consuming sample preparation reducing the time required to analyse a

single sample to just a few seconds. Recently transmission accessories with fixed and variable pathlengths for quantitative work on liquids have been developed. Further useful, but somewhat niche sampling accessories have also been developed based on specular reflection and grazing angle techniques (all are described below) resulting in an incredibly versatile instrument.

Table 1: A selection of FTIR sample interfaces for the portable FTIR range from Agilent

Sampling interface/ accessory	Description
Fixed path %T	For rapid quantitation of minor components in a liquid such as viscous oils, fuels and lubricants. A 100 micron pathlength transmission accessory does not require sample preparation and is easy to clean between samples.
Variable path %T	Variable selectable pathlength transmission accessory for minor/major bands in liquids.
Diffuse/DRIFT	For solid samples including powders with low reflectance including artwork, soils, rocks and minerals, composites, rough plastics, fabrics and corrosion on metal surfaces. Diffuse reflectance can be used both as a contact and non-contact measurement with a penetration depth between 200 and 500 micron.
ATR	For the analysis of solids, liquids, pastes and gels which are highly absorbing or non-reflective. The most common ATR interface is a diamond window, impervious to corrosion and scratching. Samples require good contact with the diamond window. Using the diamond ATR only the top 2-3 microns are analysed. For very highly absorbing samples such as carbon filled elastomers and rubbers an ATR fitted with a Germanium crystal is required. Only the top 0.5 to 2 micron is sampled. Further ATR crystal materials are available.
Specular	For the analysis of films and coatings on reflective metal surfaces such as aluminum or steel. The angle of incidence is 45 degrees. The infrared energy passes through the film, reflects off the metal substrate and passes back through the film. In addition, it can be used for the analysis of smooth, opaque samples where infrared light reflects off the surface.
Grazing angle	For the analysis of very thin (sub-micron) films on highly reflective metal surfaces such as the detection of trace contamination for cleaning validation studies. Similar in concept to the specular reflectance interface, differing in the angle of incidence (82 degrees versus 45). The increased angle of incidence causes more interaction of the infrared energy within the thin sample and has the secondary benefit of increasing the pathlength of the sample and has increased sensitivity versus specular.

Traditionally FTIRs were large and generally required to be operated through a PC, making them impractical for many applications other than in the laboratory.



Figure 2: ExoScan 4100 handheld FTIR

A handheld FTIR from Agilent, the ExoScan 4100 is shown left. With a PDA interface and simple push button operation the 4100 is rugged and easy to use. Moreover, the interferometer used in the 4100 forms the basis of the entire Agilent compact and portable FTIR range, with high sensitivity, full wavelength range and high resolution. The 4100 ExoScan is versatile - diffuse, grazing angle, specular and ATR sampling accessories are all easily interchangeable.

Application

At least one FTIR will be found in almost every quality laboratory, the output most often being used with a peak picking routine or a quantitative test to confirm the identity of materials or perform a contamination check. However, the cost of getting the sample to the lab can often be an unnecessary one as high performance portable FTIRs can now be used with confidence.

Use the 4100 ExoScan FTIR to determine if:

- a metal surface is properly cleaned in preparation for a manufacturing process such as painting or coating.
- a surface is properly prepared for a bonding process.
- the correct coating has been applied to a surface and that the thickness of that coating is accurate and precise.
- a surface has the expected homogeneity.

- anodisation and alodining processes have been correctly carried out.
- spots, streaks, stains, or blemishes on a surface are of concern.
- high value composite material has been damaged by heat, UV, or chemical exposure.
- polymers and composites are properly cured.
- incoming raw materials and outgoing finished products meet specifications.
- solids, liquids, gels and pastes meet specifications.

The strength of mid-IR absorptions (compared to NIR) is certainly an advantage when trying to identify a low level contaminant and troubleshooting production failures using FTIR is common as shown above. However, if a sample contains water its strong mid-IR absorption may swamp other more useful information and if natural products, which by their nature are heterogeneous are to be analysed then attention turns towards NIR as an alternative technique.

Near-Infrared (NIR) Spectroscopy

Background

Well used in food and agricultural analysis because of the extremely convenient and relatively large volume sampling, the applicability of Near-infrared spectroscopy (NIR) has improved greatly over the past decade. NIR characterises a material based on its absorption in the 4000 – 12500 cm⁻¹ (2500 – 800 nm) region, corresponding to vibrational overtones and combination modes; these modes are much weaker than the fundamental modes of mid-IR. The bands observed in NIR predominantly arise from stretching of O-H, C-H, and N-H bonds, and are greatly broadened relative to these same bands found in the mid-IR.

Due to broadening, and the commonality of these bands across organic molecules, the differences between NIR spectra of different compounds are often very subtle, resulting in a lower inherent molecular selectivity than mid-IR. Compared to mid-IR, NIR is a much less sensitive technique; it is therefore unsuited to the analysis of low-level components below a few percent. Because the sampling method is generally diffuse reflectance (as described below) the physical nature of the sample is extremely important in NIR, therefore the NIR spectrum comprises both chemical information (peak shape and position) and physical state (baseline slope). If information on the physical state of the sample is required (differentiating chemically identical samples differing only in particle size distribution for example) NIR is the preferred technique since the Raman spectrum is largely unaffected by particle size, and sample preparation in FT-IR (typically by ATR) destroys the original particle size distribution. Therefore some on-going maintenance of calibration models is often required when using NIR.

Interpretation

Qualitative analysis

Whereas the presence of a number of peaks in particular locations can be used to identify a sample by FTIR, since NIR bands are generally broad and ill-resolved peak picking algorithms are not used to identify a material. An alternative approach is to use discriminant analysis (pattern recognition) where a training set of known samples are analysed and classified into well defined groups. Later unknown samples can then be similarly classified. Powerful multivariate chemometric techniques such as Principal Components Analysis (PCA) are required for sample qualification with NIR data. There are many different methodologies available but they can be thought of as methods to compress the data in some way, separate different samples, and cluster similar samples together.

Quantitative analysis

As with qualitative analysis, if subtle differences in spectral features can be related to changes in spectral composition, NIR instruments can be calibrated to quantitate a number of constituents simultaneously. A training set of samples is required which should include the full expected measurement range and all possible process variations. Although this may be a labour intensive process initially, after calibration and independent validation, a great number of constituents can then be predicted in a single non-destructive NIR measurement.

Sampling

The weakness of NIR bands is a great advantage in terms of sample preparation as for the majority of materials samples can be analysed 'as is' without any preparation. The most commonly used mode of sampling for solids in NIR is diffuse reflectance. Trigger operated fibre probes allow fast measurement often through original packaging materials. Transflectance (combined transmission and reflectance) and transmission are suitable configurations for liquids analysis.

Application

There has been much recent activity in the miniaturisation of NIR instrumentation with fast, no moving parts systems developed to be used for process control. A number of vendors supply portable systems used to measure moisture content and monitor blending processes, or identify an intermediate product on-line for example. A robust portable NIR spectrometer



from ASD Inc is shown (figure 3), this research grade instrument can be operated with a selection of fibre optic probes, both reflectance and transmission, and is ideal for field measurements since operation is truly independent using long life battery packs.

The GoLab mobile version of their LabSpec 4 series spectrometer is shown in Figure 3. The spectrometer can now be stored on a trolley for efficient transportation to the sample or can be easily hand carried (5.4kg).

Use the LabSpec 4 NIR to determine:

- Verification of identity of solids, liquids, powders, pellets and gels
- Intermediates processing as expected by
 - o Correct particle size
 - o Correct water content
- Finished products are in-spec by multicomponent quantitative analysis e.g. protein in grain
- ID verification through multi-layer polymer packaging
- Tablet composition including placebo identification

Figure 3: LabSpec 4 portable NIR spectrometer and GoLab trolley from ASD Inc.

The popularity of NIR in the food and feed industry coupled with the requirements of working with seasonal natural products has led to the construction of generic quantitative

calibrations which can be coupled with a number of NIR instruments. Although fully portable, the LabSpec 4 is a research grade instrument; subsequently these generic calibrations can be transferred to the LabSpec 4 NIR, allowing crop breeders, farmers, grain distributors, millers and bakers access to portable NIR without the requirement to build their own NIR calibrations.

NIR instrumentation is robust, analysis is fast, and sampling accessories are very easy to use. However, NIR spectral data is broad meaning subtle changes in NIR spectra are difficult to detect by human operators, and data treatment is often much more complex than that required for IR. If high molecular selectivity is required another technique which does not require any sample preparation is Raman spectroscopy.

Raman Spectroscopy

Background

Infrared and Raman activity is dependent on the symmetry of a molecule. Not all samples are infrared active, some are Raman active, and some are both. Whereas FTIR and NIR are closely related absorption spectroscopies, Raman is a scattering technique. Irradiation of a sample with an intense single wavelength light source results in some light scatter, most of which is at the same wavelength as the source and is called Rayleigh scatter, but a small amount will scatter with a different frequency, known as the Raman Effect. This process is not the same as the absorption process in FTIR and NIR, in the absorption case the IR energy absorbed matches the frequency of the vibration itself. Raman activity requires a change in the polarisability (or induction of a dipole) of a vibrational group within a molecule caused by the interaction of light which does not match the vibrational frequency.

A molecule showing IR activity (causing a change in the dipole) may or may not show the change in polarisability required to make it Raman active. Many absorptions which are weak in the mid-IR are strong in Raman, and in molecules with a centre of symmetry the techniques are mutually exclusive, FTIR and Raman are therefore said to be complementary. Symmetric vibrations give rise to intense Raman lines; non-symmetric ones are usually weak and sometimes unobservable.

Similarly to FTIR, it is a change in fundamental vibrational modes that cause a molecule to be Raman active resulting in exceptional molecular selectivity.

Interpretation

A major benefit of Raman over NIR can be its insensitivity to physical form of the sample. If Raman is being used for identification analysis this results in much simpler library and method development. Very often a single spectrum can be used to represent each compound type in an ID library, this should be contrasted with NIR spectral libraries which may require some careful sample selection to ensure all allowable physical differences in samples are represented in a model. If the physical form of samples in NIR is not considered at the design stage then over time NIR libraries tend to break down, and samples have to be added to the library later. This maintenance of calibrations can be a costly exercise. There are many commercial Raman libraries available and peak picking routines can be used in a similar way to the mid-IR.

A potential pitfall of the Raman technique is an interference known as fluorescence where the molecule does absorb radiation and an electronic transition occurs. This is seen in the Raman spectrum as a baseline which may obscure the entire spectrum. Visible lasers used in many benchtop Raman instruments are of the correct excitation frequency to cause electronic transitions, changing the laser frequency to 785nm can reduce the number of samples showing fluorescence. Alternatively photobleaching is used to reduce fluorescence, although some samples will continue to fluoresce and will not produce acceptable spectra. Raman is obviously not an acceptable technique for these samples.

Sampling

Raman sampling methods are simple, the sample is contained in a glass vial or similar for a bench top system, very little sample is required and it can easily be used in a non-contact fashion through many common container materials such as plastics and glass (including amber coloured glass).

Although Raman signals are weak – a problem for Raman experiments in the past – the sensitivity of contemporary Raman instrumentation is such that acquisition times of one to tens of seconds are now the norm; comparable with FTIR and NIR.

Due in part to the use of relatively high power lasers, Raman was never as popular as FTIR outside research environments as the lasers required water cooling, specialist set up and maintenance, safety systems and large rooms to keep them in. The development of smaller FT-Raman systems did see a rapid rise in the use of Raman outside research. Recent developments in source and detector technology and the use of fibre optics have resulted in the availability of flexible yet robust systems capable of going on the production line, and small hand held devices used throughout the production process.

Application

The majority of Raman systems found in industry today are benchtop systems requiring a high level of skill in their operation. They are still more likely to be found in R&D, used for troubleshooting, rather than in routine QA. However, handheld Raman devices fill all the requirements as stated above for portable spectroscopy. The RAPID-ID and PHARMA-ID from DeltaNu are shown below.



Figure 4: RAPID-ID (left) and PHARMA-ID (right) handheld Raman spectrometers from DeltaNu

For the routine analysis of chemicals, polymers, medical devices, consumer products and packaging Raman spectroscopy is an ideal tool for use outside the lab. For the regulated industries a 21CFR part 11 compliant version, PHARMA-ID was developed for the following analyses:

- Incoming raw materials identification
- At-line troubleshooting
- Intermediate and final products inspection
- Counterfeit drug and packaging identification.

To the operator Raman spectra are sharp and easily understandable, method development is straightforward and analyses can be carried out in seconds with unambiguous yes/no or simple material ID as the result. Analysis of both solids and liquids is entirely non-destructive and subtle changes in physical characteristics in samples do not affect the result.

For a small percentage of samples the Raman spectrum will be swamped by fluorescence, if this effect cannot be removed or reduced by photobleaching or software baseline correction Raman is not a suitable technique and FTIR and NIR should be considered.

Technique summary			
	Handheld FTIR	Portable NIR	Handheld Raman
Typical Sample type	Solids, liquids, powders, gels, films. Samples with low water content Homogeneous samples	Solids, liquids, powders, gels, pellets, thick films. Higher water content OK Heterogeneous samples OK	Solids, liquids, powders, gels. Aqueous samples and glass containers OK Homogeneous samples
Applications	QA materials verification Trace contaminants Counterfeit detection Packaging analysis Quantitation Surface analysis	QA materials verification Moisture analysis Quantitation Tablet composition Counterfeit detection	QA materials verification Process troubleshooting Counterfeit detection (packaging and bulk product)
Deployed	Lab and mobile	Lab and mobile	Lab and mobile

Conclusion

The three common vibrational spectroscopic techniques, FTIR, NIR and Raman should no longer be confined to the laboratory. Portable instrumentation is robust, reliable and accurate, analysis is fast and unambiguous; and expert spectroscopy knowledge is not required for its operation.

Designed for where and when immediate analysis is needed - authentication of materials as they are received, troubleshooting production breakdowns, quantifying multi component mixtures - literally in the field, at final inspection and in the battle against counterfeiters, portable FTIR, NIR and Raman each have their own place.

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