Introduction to Infrared Spectroscopy

i	Wherever you see this symbol, it is important to access the on-line course as there is interactive material that cannot be fully shown in this reference manual.

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1. Introduction to IR Spectroscopy

Spectroscopy can be defined as the interaction between matter and light. Infrared spectroscopy is a very powerful technique which uses electromagnetic radiation in the infrared region for the determination and identification of molecular structure as well as having various quantitative applications within analytical chemistry (Figure 1).

We do not aim to provide a mechano-quantic description of light and its interaction with atoms, as this is out of the scope of this module. However, it is important to note that atoms can absorb energy from electromagnetic radiation; this absorbed energy alters the state of the atoms within the molecule. These changes are usually manifest in alterations to the frequency and amplitude of molecular vibrations, which may be measured and plotted to produce an infrared spectrum.[1-4]

Infrared spectrometers use optical devices for dispersing and focusing electromagnetic radiation of IR frequency which is passed through the sample and any changes in absorbance measured against a reference beam.

There are three well defined IR regions (near, mid and far). The boundaries between them are not clearly defined and debate still persists, but broadly they are defined as:

- **Near infrared (12820-4000 cm^{-1})**: poor in specific absorptions, consists of overtones and combination bands resulting from vibrations in the mid-infrared region of the spectrum.
- **Mid-infrared (4000-400 cm^{-1})**: provides structural information for most organic molecules.
- **Far Infrared (400-33 cm^{-1}):** has been less investigated than the other two regions; however, it has been used with inorganic molecules.

The low energies, typically encountered within the infrared region, are not sufficient to cause electronic transitions; however, they are large enough to cause changes in the frequency and amplitude of molecular vibrations.
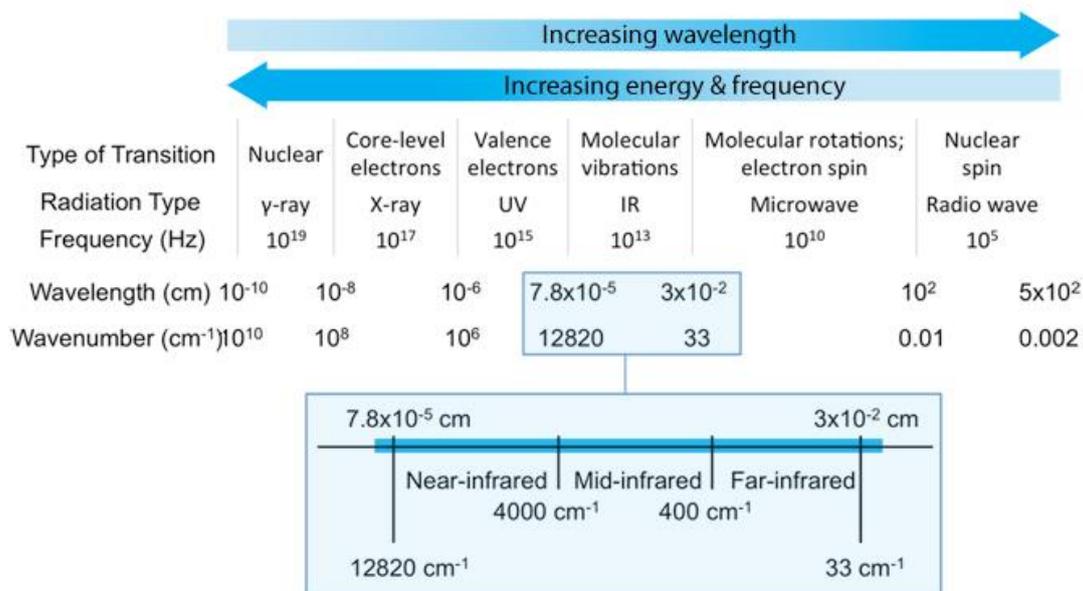
Figure 1: The electromagnetic spectrum and the infrared region.

2. Electromagnetic Spectrum

The electromagnetic spectrum is the range of all possible frequencies of electromagnetic radiation, each of which can be considered as a wave or particle travelling at the speed of light, often referred to as a photon. These waves differ from each other in length and frequency.

Frequency v - the number of wave cycles that pass through a point in one second. Measured in Hertz (Hz).

Wavelength λ - The length of one complete wave cycle (cm).

Frequency and wavelength are inversely related (Equation 1):

$$v = \frac{c}{\lambda} \quad (1)$$

Where:
c = speed of light 3 x 10^{10} cm/sec

The energy of a photon (E in Joules) is related to wavelength and frequency as follows (Equation 2):

$$E = hv = \frac{hc}{\lambda} \quad (2)$$

Where:
h = Planck's constant 6.6 x 10^{-34} Joules-sec

Energy is directly proportional to frequency; therefore, high energy radiation will have a high frequency.

Energy is inversely proportional to wavelength, hence, short wavelengths are high energy and vice versa (Figure 2).

Type of Transition	Nuclear	Core-level electrons	Valence electrons	Molecular vibrations	Molecular rotations; electron spin		Nuclear spin	
Radiation Type	γ-ray	X-ray	UV	IR	Microwave		Radio wave	
Frequency (Hz)	10^{19}	10^{17}	10^{15}	10^{13}	10^{10}		10^{5}	
Wavelength (cm)	10^{-10}	10^{-8}	10^{-6}	7.8×10^{-5}	3×10^{-2}		10^{2}	5×10^{2}
Wavenumber (cm^{-1})	10^{10}	10^{8}	10^{6}	12820	33		0.01	0.002

Increasing wavelength →

← Increasing energy & frequency
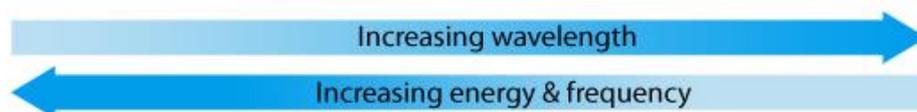
Figure 2: Electromagnetic spectrum.

3. Electromagnetic Radiation and Spectroscopy

The frequency and wavelength of electromagnetic radiation varies over many orders of magnitude. The electromagnetic spectrum is divided according to the type of atomic or molecular transition that gives rise to the absorption or emission of photons; UV, IR, microwave, radio wave etc. (Table 1).

Absorption spectroscopy relies on the absorption of energy from a photon which subsequently promotes the analyte from a lower-energy state to a higher-energy, or excited, state. As the energy of the photon changes the type of transition that the analyte undergoes will change. For example in IR spectroscopy, the absorption of relatively low IR radiation results in the vibration of chemical bonds within the analyte; a process which requires a fairly low energy input. Whereas, higher energy photons, such as those found in the UV-visible region of the electromagnetic spectrum, will promote valence electrons to move from their ground state to excited state energy levels within the atoms of an analyte; a process that requires a much greater energy input.

Type of Energy Transfer	Region of the Electromagnetic Spectrum	Spectroscopic Technique
Absorption	γ-ray	Mossbauer
	X-ray	X-ray absorption
	UV-Vis	UV-Vis
		Atomic absorption
	Infrared	Infrared (IR)
		Raman
	Microwave	Microwave
		Electron spin resonance (EPR)
	Radio waves	Nuclear magnetic resonance (NMR)
Emission (thermal excitation)	UV-Vis	Atomic emission
Photoluminescence	X-ray	X-ray fluorescence
	UV-Vis	Fluorescence
		Phosphorescence
		Atomic fluorescence

(left axis: Increasing energy & frequency)

Table 1: Electromagnetic spectrum region, type of energy transfer, and the associated spectroscopic technique.

Frequency vs. Wavelength

Low Frequency = Long Wave Length

Low Energy   High Energy

(NOTE: Frequency refers to the number of crests of waves
of same wavelength that pass by a point in one second.)
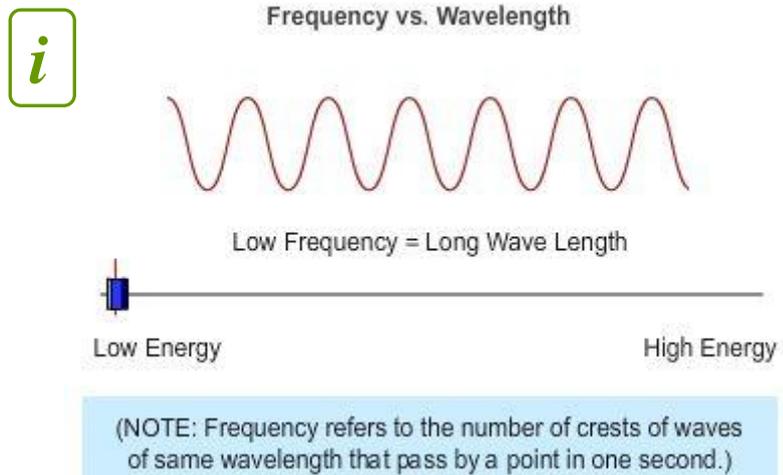
Figure 3: Relationship between frequency and wavelength.

4. Infrared Regions

Infrared spectroscopy can be rationalized as the spectroscopy that deals with electromagnetic radiation of infrared frequency. As previously explained, there are three well defined infrared regions; each of them has the potential to provide different information: (Figure 4)

- **Far-Infrared (400-33 cm^{-1}):** vibrations of molecules containing heavy atoms, molecular skeleton vibrations and crystal lattice vibrations
- **Mid-Infrared (4000-400 cm^{-1}):** useful for organic analysis
- **Near Infrared (12820-4000 cm^{-1}):** overtones; very useful for quantitative analysis

Infrared spectroscopy is one of the most useful and widely used methods to perform structural analysis.

Given that the molecule under investigation is infrared active, (i.e. it absorbs Infrared radiation), then different types of structural information can be obtained.

Information achievable with Infrared spectroscopy includes:

1. The type of atoms within the molecule.

$$C-H \quad C-F$$

2. The type of bonds between atoms.

$$C-NH_2 \quad C\equiv N$$
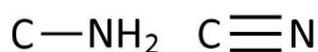
3. The molecular structure. More often than not, infrared spectroscopy is insufficient to determine the complete structure and additional techniques (such as NMR, mass spectroscopy, etc.) are used to solve the puzzle.
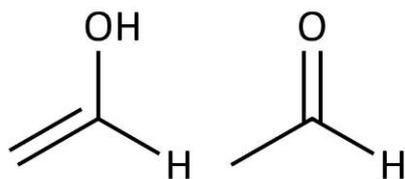
Both structures have the molecular formula C_2H_4O

4. From a quantitative point of view, infrared spectroscopy has a very well gained reputation for its power, flexibility, and reliability.
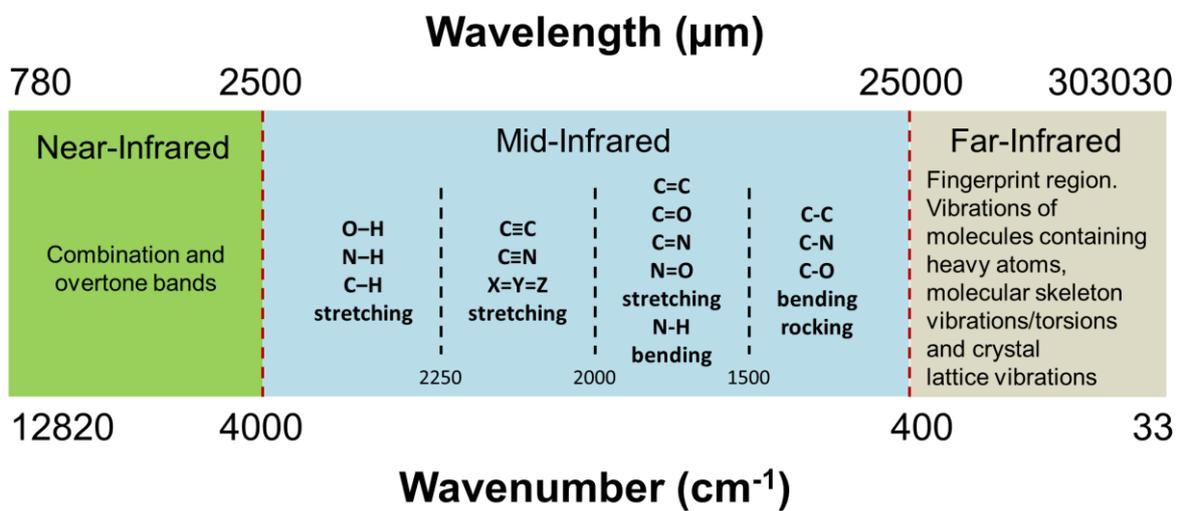
Figure 4: Infrared spectroscopy regions (oversimplified).

5. Molecular Vibrations

The absorption of light will increase both amplitude and frequency of molecular vibrations.[4-5] When the radiant energy matches the energy of a specific molecular vibration, absorption occurs. Molecules with a permanent dipole moment, such as water, HCl, and NO, are infrared active.

The HCl molecule possesses a permanent dipole moment, so it is infrared active.

$$\delta+ \quad \delta-$$
$$H—Cl$$
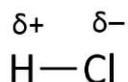
The O_2 molecule does not possess a permanent dipole moment, so it is not infrared active.

$$O=O$$
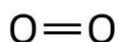
In the case of alkenes (C=C) and alkynes (C≡C) if the bond is symmetrically substituted no band will be seen in the IR spectrum, however, if the bond is asymmetrically substituted a stretching frequency corresponding to the alkene or alkyne bond will be present (Table 2).

Oscillator	Wavenumber (cm^{-1})
C-H	3320-2700
-C=C-	1690-1590
C=O	1870-1590
C-O	1300-1050
C≡C	2250-2150
C-Cl	800-600

Table 2: Wavenumbers for selected diatomic oscillators.

In order to understand molecular vibrations, a bond can be treated as a simple harmonic oscillator composed of two masses (atoms) joined by a spring. Figure 6 depicts a diatomic molecule with two generic atoms (of masses m_1 and m_2) connected by a spring.
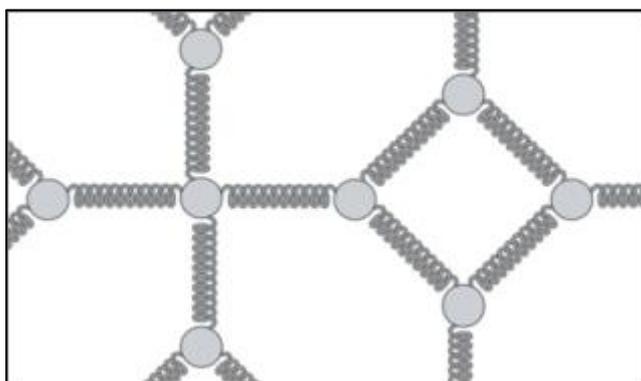
Figure 5: Representation of a polyatomic molecule.
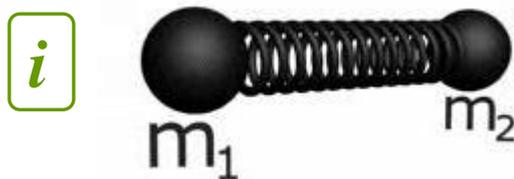
Figure 6: Representation of a diatomic molecule. If masses m1 and m2 are equal, no change in the dipole moment will occur as the molecule vibrates.

The classical vibrational frequency for a diatomic molecule (with force constant k and masses m_1 and m_2) has been derived from Hooke's Law (Equation 3):

$$v = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} = \frac{1}{2\pi c}\sqrt{\frac{k(m_1 + m_2)}{m_1 m_2}} \quad (3)$$

Where:

μ = reduced mass = $\frac{m_1 m_2}{m_1 + m_2}$

In terms of the wavenumber ($\bar{v}$) (Equation 4):

$$\bar{v} = \frac{v}{c} = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} = \frac{1}{2\pi c}\sqrt{\frac{k(m_1 + m_2)}{m_1 m_2}} \quad (4)$$

Where:

c = speed of light = 3 x 10^{10} cm/sec

6. Calculation of Molecular Vibrations

The stretching frequency $\bar{v}$ (cm^{-1}) of C-H can be calculated as follows:

$k = 5 \times 10^5$ dyne/cm
$m_1 = C = 12/6.02 \times 10^{23} = 1.99 \times 10^{-23}$ g
$m_2 = H = 1/6.02 \times 10^{23} = 0.167 \times 10^{-23}$ g
$c = 3 \times 10^{10}$ cm/sec

$$\bar{v} = \frac{1}{2\pi c} \sqrt{\frac{k(m_1 + m_2)}{m_1 m_2}}$$

$$\bar{v} = \frac{1}{2 \, x \, 3.14 \, x \, 3x10^{10}} \sqrt{\frac{5x10^5(1.99x10^{-23} + 0.167x10^{-23})}{1.99x10^{-23} \, x \, 0.167x10^{-23}}}$$

$$\bar{v} = 3023 \, cm^{-1}$$

The observed values for C–H bonds are in the region 3320-2700 cm^{-1}, which differ from the calculated values as the calculation does not take into account the environment of the C–H group within the molecule.

Force constants (k):

Single bond   = 5×10^5 dyne/cm

Double bond = 10×10^5 dyne/cm

Triple bond   = 15×10^5 dyne/cm

Remember higher wavenumber = higher frequency

The vibrational frequency of a bond will increase as 1) the strength of the bond increases and/or 2) the reduced mass (μ) decreases.

For example compare the calculated frequencies for the following bonds:

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powered by **crawford scientific**

HPLC GC MS SPE

1) Increase in bond strength from single to double to triple bond (Figure 7).

C–N bond

k = 5x10^5 dyne/cm

m$_1$ = C = 12/6.02x10^{23} = 1.99x10^{-23} g

m$_2$ = N = 14/6.02x10^{23} = 2.33x10^{-23} g

c = 3x10^{10} cm/sec

$$\bar{v} = \frac{1}{2\pi c}\sqrt{\frac{k(m_1 + m_2)}{m_1 m_2}}$$

$$\bar{v} = \frac{1}{2\ x\ 3.14\ x\ 3x10^{10}}\sqrt{\frac{5x10^5(1.99x10^{-23} + 2.33x10^{-23})}{1.99x10^{-23}\ x\ 2.33x10^{-23}}}$$

$$\bar{v} = 1145\ cm^{-1}$$

C=N bond

k = 10x10^5 dyne/cm

m$_1$ = C = 12/6.02x10^{23} = 1.99x10^{-23} g

m$_2$ = N = 14/6.02x10^{23} = 2.33x10^{-23} g

c = 3x10^{10} cm/sec

$$\bar{v} = \frac{1}{2\pi c}\sqrt{\frac{k(m_1 + m_2)}{m_1 m_2}}$$

$$\bar{v} = \frac{1}{2\ x\ 3.14\ x\ 3x10^{10}}\sqrt{\frac{10x10^5(1.99x10^{-23} + 2.33x10^{-23})}{1.99x10^{-23}\ x\ 2.33x10^{-23}}}$$

$$\bar{v} = 1619\ cm^{-1}$$

C≡N bond

k = 15x10^5 dyne/cm

m$_1$ = C = 12/6.02x10^{23} = 1.99x10^{-23} g

m$_2$ = N = 14/6.02x10^{23} = 2.33x10^{-23} g

c = 3x10^{10} cm/sec

$$\bar{v} = \frac{1}{2\pi c}\sqrt{\frac{k(m_1 + m_2)}{m_1 m_2}}$$

$$\bar{v} = \frac{1}{2\ x\ 3.14\ x\ 3x10^{10}}\sqrt{\frac{15x10^5(1.99x10^{-23} + 2.33x10^{-23})}{1.99x10^{-23}\ x\ 2.33x10^{-23}}}$$

$$\bar{v} = 1983\ cm^{-1}$$

Figure 7: Comparison of wavelength, stretching frequency, and wavenumber of bonds with different strengths (i.e. single, double, and triple bonds).

2) Decrease in reduced mass (μ) (Figure 8).

C–S bond

k = 5x10^5 dyne/cm

m$_1$ = C = 12/6.02x10^{23} = 1.99x10^{-23} g

m$_2$ = S = 32/6.02x10^{23} = 5.32x10^{-23} g

c = 3x10^{10} cm/sec

$$\bar{v} = \frac{1}{2\pi c}\sqrt{\frac{k(m_1 + m_2)}{m_1 m_2}}$$

$$\bar{v} = \frac{1}{2\ x\ 3.14\ x\ 3x10^{10}}\sqrt{\frac{5x10^5(1.99x10^{-23} + 5.32x10^{-23})}{1.99x10^{-23}\ x\ 5.32x10^{-23}}}$$

$$\bar{v} = 985\ cm^{-1}$$

C–H bond

k = 5x10^5 dyne/cm

m$_1$ = C = 12/6.02x10^{23} = 1.99x10^{-23} g

m$_2$ = H = 1/6.02x10^{23} = 0.167x10^{-23} g

c = 3x10^{10} cm/sec

$$\bar{v} = \frac{1}{2\pi c}\sqrt{\frac{k(m_1 + m_2)}{m_1 m_2}}$$

$$\bar{v} = \frac{1}{2\ x\ 3.14\ x\ 3x10^{10}}\sqrt{\frac{5x10^5(1.99x10^{-23} + 0.167x10^{-23})}{1.99x10^{-23}\ x\ 0.167x10^{-23}}}$$

$$\bar{v} = 3023\ cm^{-1}$$

Figure 8: Comparison of wavelength, stretching frequency, and wavenumber of bonds with different reduced masses (μ).

7. Infrared Active Modes

A molecule that is infrared active must undergo a change in its dipole moment when vibrating. The simplest modes of vibration that are infrared active are stretching and bending modes (Figure 9).[3-5]
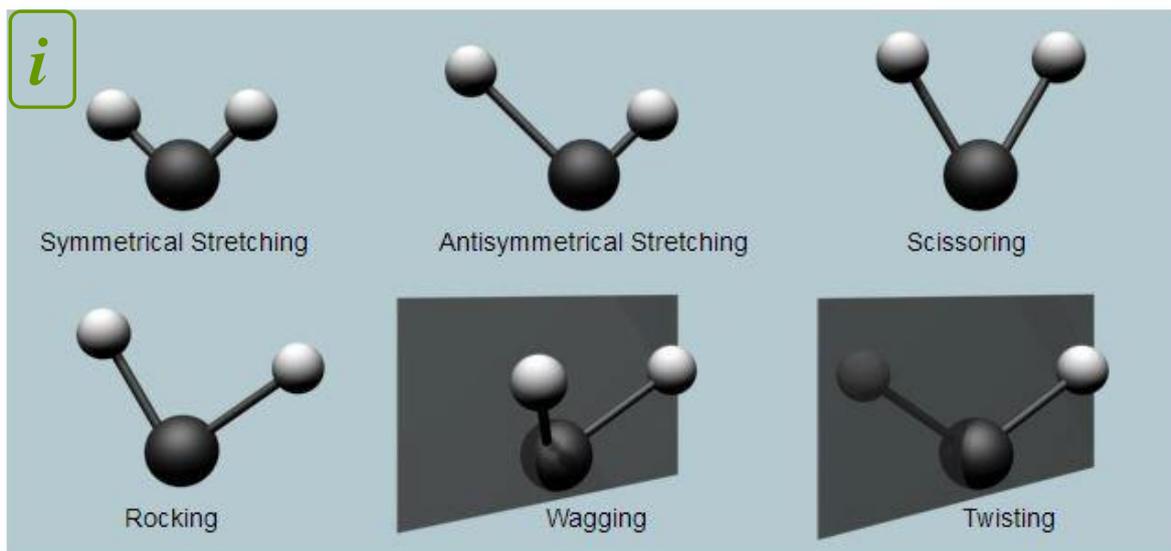
Figure 9: Modes of vibration (oversimplified).

For simplicity, we are going to illustrate the vibration modes in a linear molecule (acetylene in this case).
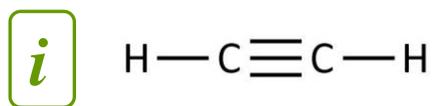
The symmetric C≡C stretching will not alter the acetylene's dipole moment and **it is not** infrared active.
However, substitution of either of the H atoms in acetylene produces an asymmetric alkyne bond which will be IR active.

Homonuclear diatomic molecules such as Cl_2, H_2, N_2, etc. will exhibit no infrared active modes as no change in their dipole moment is experienced during vibration.

8. Absorption Considerations

The amount of infrared radiation absorbed by the sample, at any given wavelength, can be measured in different ways. The use of 'transmittance' is common place in infrared spectroscopy. [2-4, 5-7]

Figure 10 illustrates a beam of infrared radiation of power I_0, directed at a sample solution.
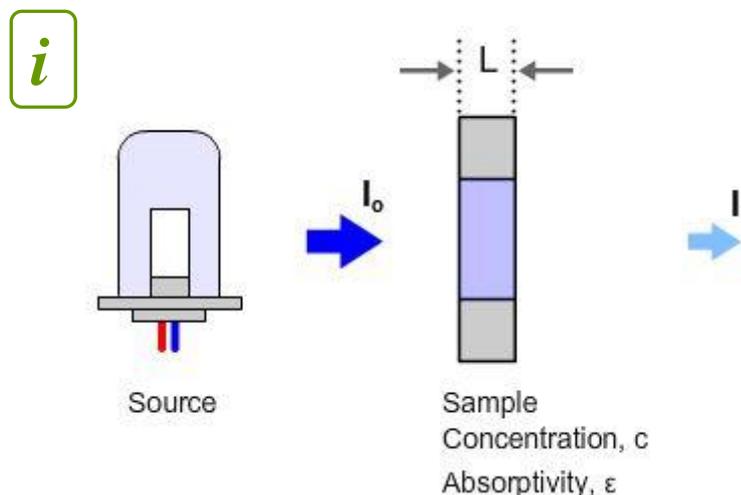
Figure 10: Absorption of infrared radiation of radiant power I_0. The radiation leaving the sample has radiant power I.

The transmittance can be calculated as follows (Equation 5):

$$\%T = \frac{I}{I_0} \; x \; 100 \;\; (5)$$

Where:
I_0 = intensity of the incident radiation (light)
I = intensity of the radiation (light) leaving the sample

Absorbance is given by Equation 6:

$$A = log\left(\frac{I_0}{I}\right) \;\; (6)$$

From the previous expression it follows that (Equation 7):

$$A = 2 - log(\%T) \;\; (7)$$

Absorbance is related to concentration *via* the Beer-Lambert law (Equation 8):

$$A = \varepsilon l c \quad (8)$$

Where:
ε = molar absorptivity (Lmol^{-1}cm^{-1})
l = path length (cm)
c = concentration (molL^{-1})

ε is sometimes referred to as the extinction coefficient.

Infrared (IR) radiation of all wavelengths is transmitted from the source. Some of the wavelengths of IR radiation will be absorbed by the sample and some of them will pass through (they are transmitted). The IR radiation which is transmitted is measured by the detector resulting in a unique IR spectrum for the sample of interest. This spectrum represents the IR absorption and transmission of that molecule. No two unique molecules will produce the same IR spectrum, resulting in IR spectroscopy being a very useful tool for molecular characterization and quantification.
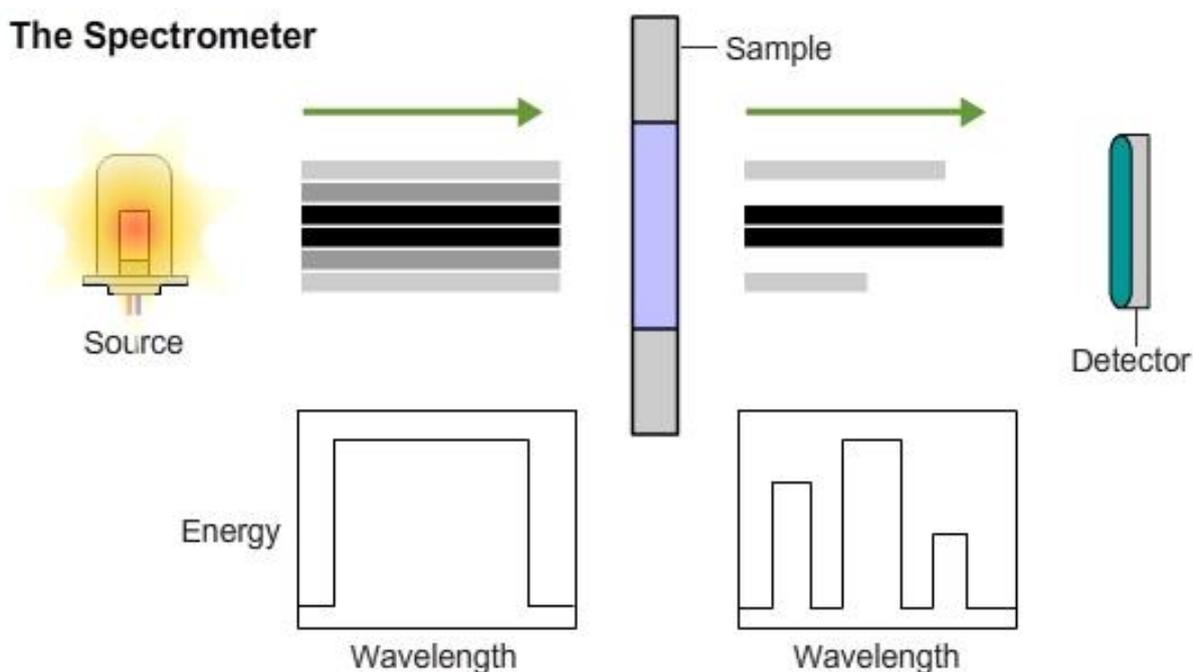
Figure 11: Sample absorption of IR radiation.

9. The IR Spectrum

Historically, infrared spectra have been represented as percent of transmittance versus either the wavenumber or the wavelength. The use of wavenumbers, is standard, with the use of wavelength (expressed in nm or μm) having fallen out of favor.[4-6, 8-10]

In terms of wavenumbers the infrared region spans from 33 to 12820 cm^{-1}. However, most infrared analyses are carried out in the mid-infrared region (400 to 4,000 cm^{-1}).

By convention, the wavenumbers are plotted in decreasing order from left to right. A typical IR spectrum is illustrated in Figure 12.
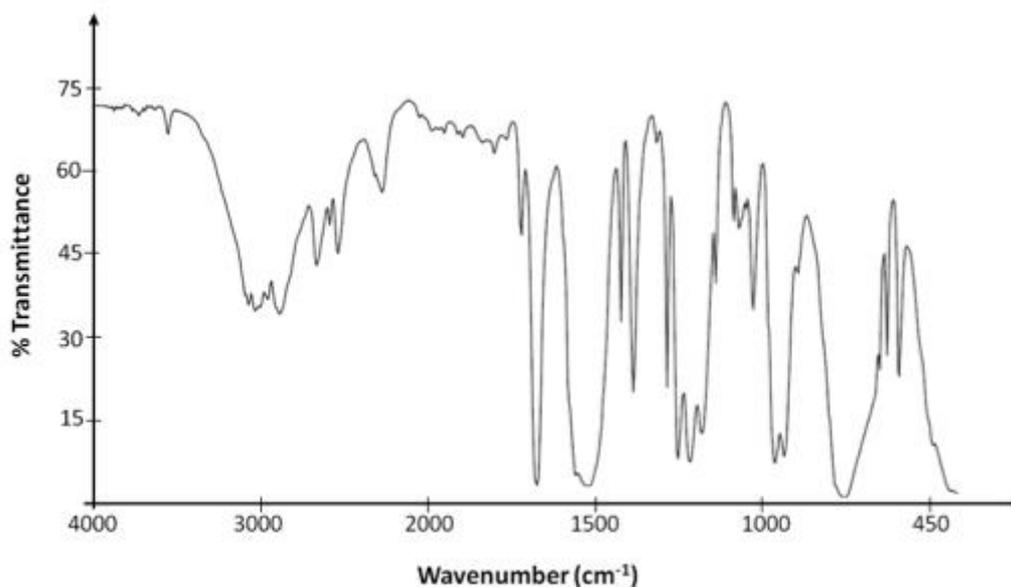
Figure 12: Typical IR spectrum.

The wavenumber $\bar{\upsilon}$ can be found by using the following expression (Equation 9):

$$\bar{\upsilon} = \frac{1}{\lambda} \quad (9)$$

The wavenumber is usually expressed as cm^{-1}.

Example: Calculate the wavenumber (in cm^{-1}) corresponding to infrared radiation of wavelength 2,500 nm.

$$\bar{\upsilon} = \frac{1}{\lambda} = \frac{1}{2500} \; x \; \frac{1}{10^{-7}} = 4000 \; cm^{-1}$$

Note: 1 nm = 1 x 10^{-7} cm

10. Infrared Absorption Signals

Selected IR frequencies.[11]
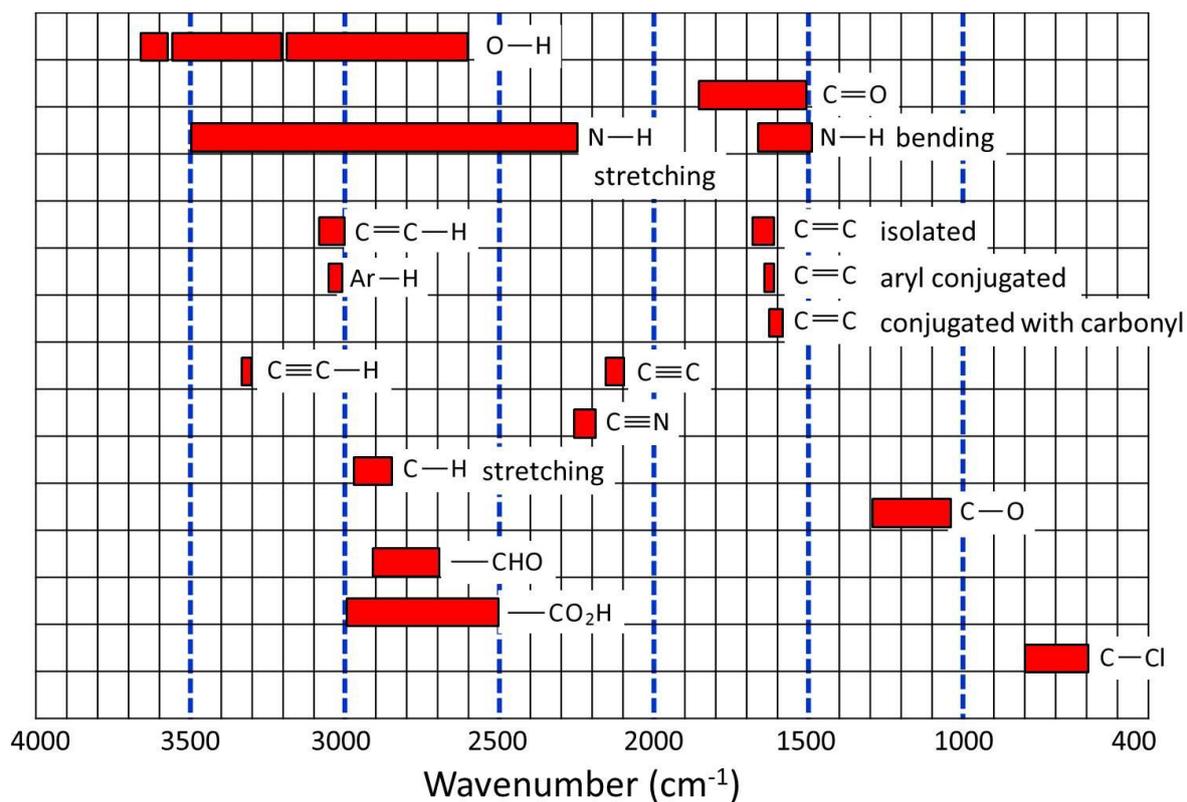
The vibrational frequencies of certain functional groups will correspond to the absorption of certain wavelengths of IR energy. Absorption of these wavelengths by a sample can therefore act as a diagnostic or fingerprint, to indicate the possible presence of these groups within the analyte molecule. Some typical frequencies are shown in the table above.

11. Dispersive IR Instruments

Most IR spectrometers can be categorized into two classes: dispersive and Fourier Transform instruments.[2-4]

The basic design of a dispersive single beam instrument includes a source of infrared radiation, a monochromator, and the detector (Figure 13).

After interacting with the sample (or the blank), infrared radiation is dispersed by a monochromator into its individual frequency components and information on which frequencies were absorbed can be obtained using a photodiode array detector.
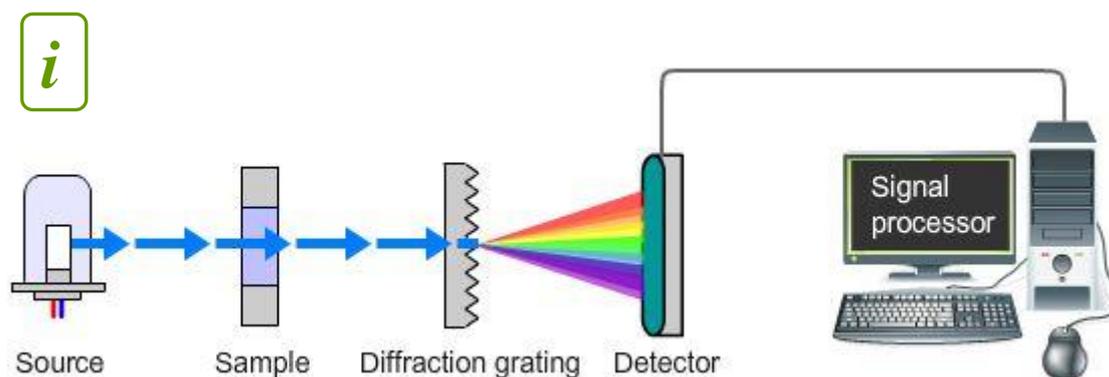
Figure 13: Basic concept of a single beam IR instrument.

Sources and detectors for infrared radiation have limited stability; with light intensity and detector sensitivity changing over time, or with fluctuations in temperature etc. The blank (reference or background) and sample measurements should be made one after the other to ensure they are made under the same analytical conditions. This limitation is minimized by the use of double beam instruments which are capable of measuring the sample and reference simultaneously.

Double beam instruments use 'choppers' to control the path of the radiation, alternating between the sample and the reference (Figure 14). These instruments use the known speed of rotation of the beam chopper to compare and resolve the information reaching the detector.

The use of an opaque surface provides the means for adjusting the 0% transmittance response of the detector.
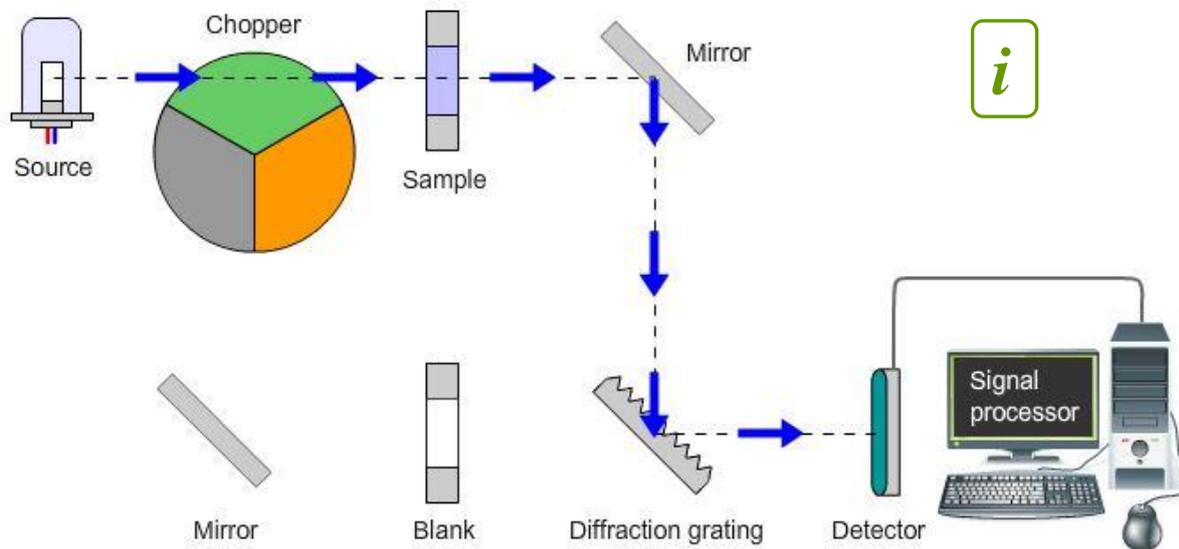
Figure 14: The double beam IR instrument.

Finally, it is easier to correct for absorption of infrared radiation by carbon dioxide and water (present within the instrument background) with double beam instruments than with their single beam counterparts.

12. FTIR Instruments

FTIR stands for Fourier Transform Infrared. FTIR spectrometers consist of an IR source, interferometer, sample cell or chamber, detector and a laser.[7,9,11] A schematic of an FTIR instrument is shown below (Figure 15).

IR source

IR radiation is emitted from a glowing black body source. IR radiation passes through an aperture which controls the amount of radiation that reaches the sample, and therefore, the detector.

Common IR sources are:

1. Silicon carbide rods which are resistively heated and commonly known as a Globar. An electric current is passed through the rod which becomes very hot (1300 K) and emits large amounts of IR radiation. Previously, cooling with water was required to avoid damaging electrical components; however, advances in metal alloys have led to the production of Globars that do not require cooling by water.
2. Nichrome and Kanthanl wire coils were once popular IR sources and did not require cooling as they ran at lower temperatures than Globars, however, this also resulted in lower amounts of IR radiation being emitted.
3. Nernst Glowers are manufactured from a mixture of refractory oxides and are capable of reaching hotter temperatures than a Globar; however, they are not capable of producing IR radiation above 2000 cm^{-1}.

Interferometer

The first interferometer was invented by Albert Abraham Michelson, who received a Nobel Prize for his work in 1907. Without this essential piece of optical equipment the modern day FTIR system would not exist. The interferometer consists of a beam splitter, a fixed mirror, and a moving mirror.

Beam Splitter

The beam splitter is made of a special material which transmits half of the incident radiation and reflects the other half. IR radiation from the source strikes the beam splitter and is separated into two beams. One beam is transmitted through the beam splitter to the fixed mirror while the other beam is reflected from the beam splitter to the moving mirror. Both mirrors reflect the radiation back to the beam splitter where the two beams interfere to produce an interferogram.

Moving Mirror

The moving mirror is a flat highly reflective surface mounted on air bearings that allow for high speed movement of the mirror (movements are made once every millisecond). The moving mirror only moves a few millimeters away from the beam splitter.

Fixed Mirror

The fixed mirror is a flat highly reflective surface.
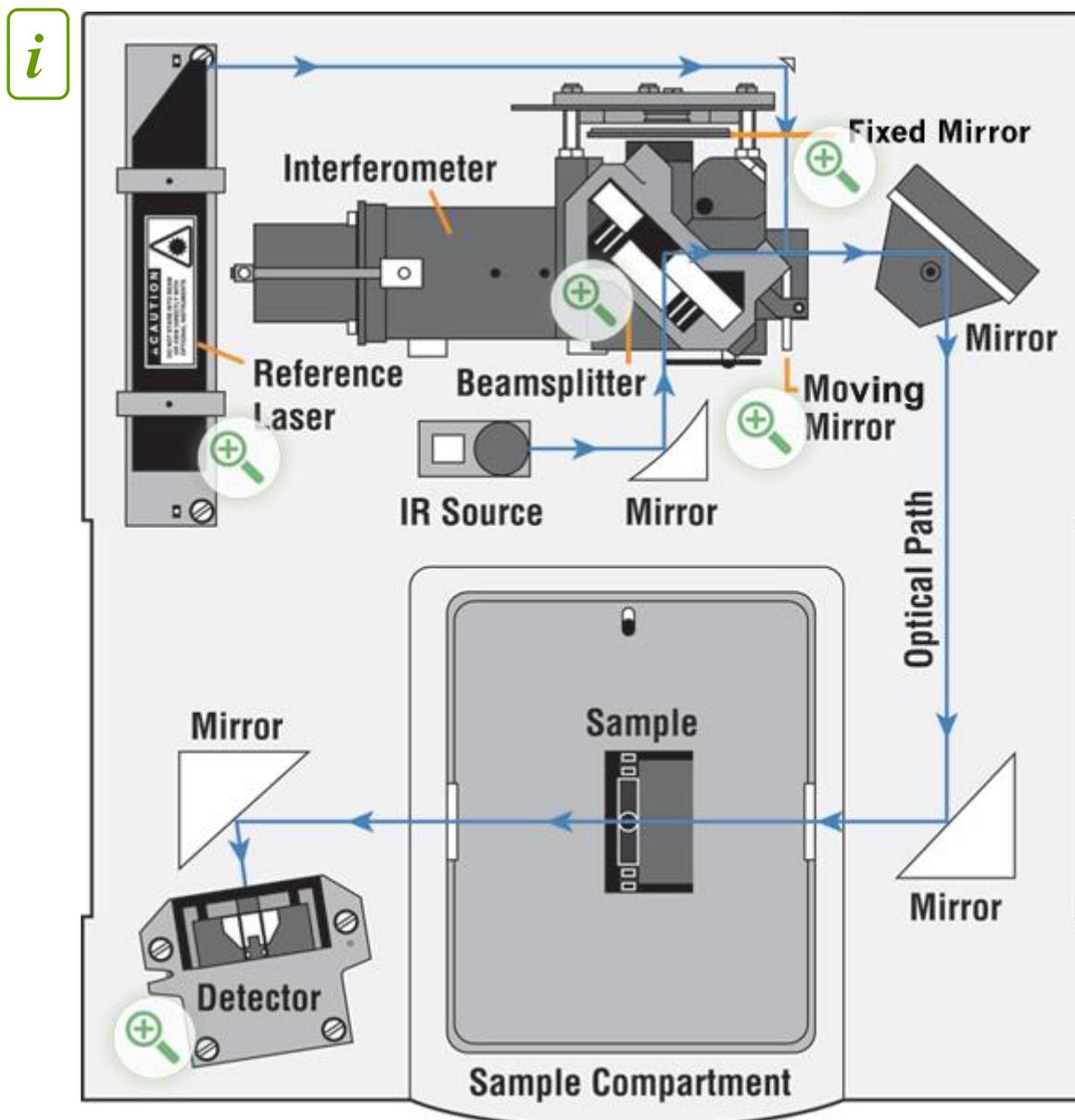
Figure 15: Operational Schematic of a Thermo Nicolet FTIR Instrument. Image reproduced with permission from Thermo Fisher Scientific (Madison, WI, USA).

Laser

Many instruments employ a Helium-Neon laser as an internal wavelength calibration standard. It is imperative that the position of the moving mirror is known at any given moment. The moving mirror moves back and forth at a precise constant velocity that is timed using a very accurate laser wavelength.

The intensity of the laser beam is measured at two points in the interferometer. As the mirror moves the intensity at these two points will rise and fall due to the enhancement and cancellation of the HeNe beam paths, producing a sine wave of intensity vs. mirror position. The number of "fringes" in the sine wave allows the instrument to know exactly how far the mirror has moved, and the relative phase of the sine wave tells the instrument in which direction the mirror is moving.

Detector

There are two classes of infrared detectors; thermal and photonic detectors. Thermal detectors use the IR radiation as heat; whereas, quantum mechanical (photonic) detectors use the IR radiation as light which results in a more sensitive detector.

Thermal detectors: detect changes in temperature of an absorbing material (lithium tantalate ($LiTaO_3$), lead selenide (PbSe), germanium etc.). Many temperature dependent phenomena can be followed to measure the effects of the incident IR radiation. Bolometers and microbolometers use changes in resistance, while thermocouple and thermopiles use the thermoelectric effect. Golay cells monitor thermal expansion.

Photonic Detector: exhibit faster response times and higher sensitivity in comparison to their thermal counterparts, therefore, they are much more prolific in FTIR instruments. The materials used in these detectors are semiconductors with narrow band gaps. The incident IR radiation causes electronic excitations between the ground and first excited states, which in photoconductive detectors result in a change in resistivity which is monitored.

13. FTIR Operation

Prior to the development of FTIR spectrometry, the limitation within IR was the slow scanning process. FTIR allows for all the infrared frequencies to be scanned simultaneously, allowing for data to be collected in a matter of seconds rather than several minutes. This is achieved through the use of an optical device called an interferometer which produces a signal which is made up of all of the infrared frequencies.

Most interferometers consist of a beam splitter which splits the incident infrared beam into two separate optical beams. One beam is reflected from a fixed mirror, while the other beam is reflected from a mirror that is constantly moving in the instrument. The moving mirror typically moves by only a few millimeters from the beam splitter (Figure 16).

FTIR Operation

FTIR based on the Michelson interferometer. By changing the position of the moving mirror, a different optical path is established and different information is obtained.
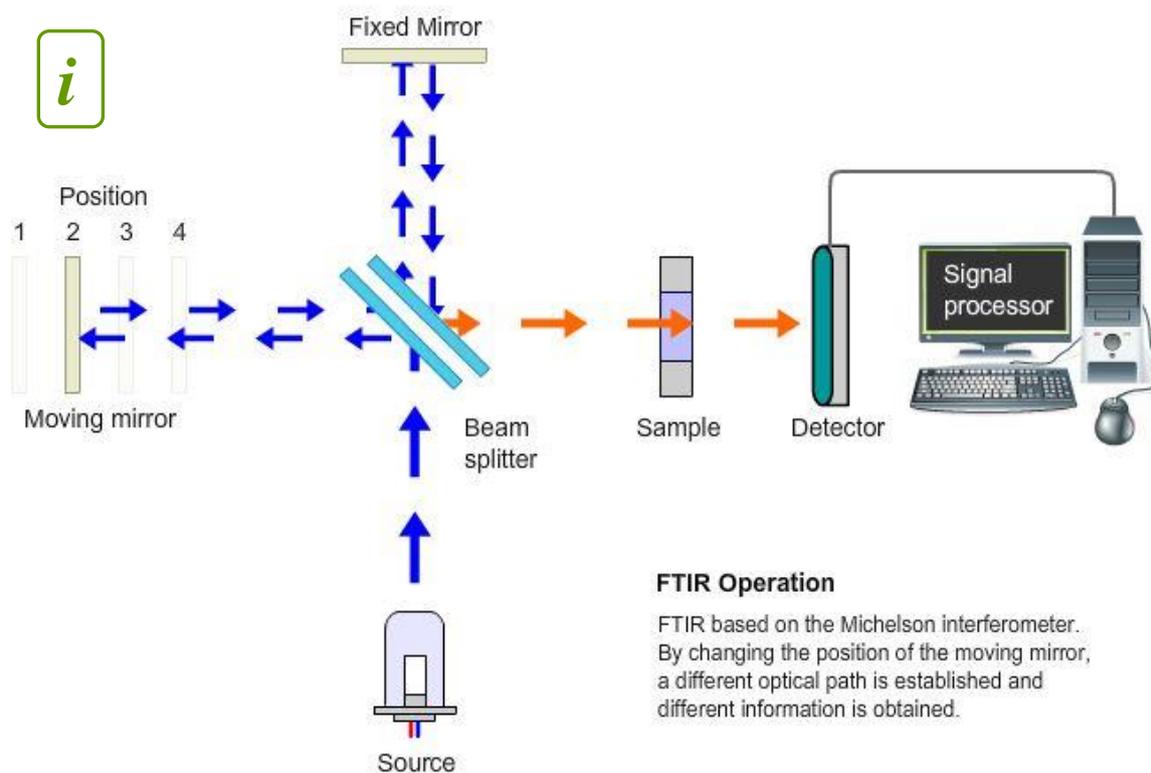
Figure 16: FTIR based on the Michelson interferometer. By changing the position of the moving mirror, a different optical path is established and different information is obtained.

The two beams are reflected from their respective mirrors and recombine at the beam splitter. The path length of the beam that is reflected from the fixed mirror remains constant, while the path length of the beam that is reflected from the moving mirror is constantly changing as the mirror moves. The signal that exits the interferometer is the result of these two beams interfering with each other, and is called an interferogram (Figure 17).
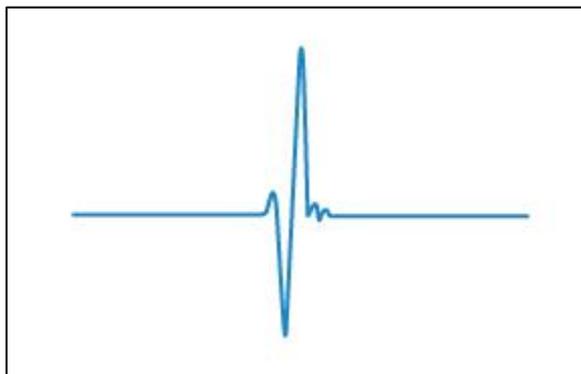
Figure 17: Interferogram.

The interferogram is unique in that every data point, which is a function of the moving mirror position, has information about every infrared frequency emitted from the source. This allows for all frequencies to be measured simultaneously.

The interferogram is converted to a more familiar IR spectrum (wavenumber vs. % transmittance) using the well-known mathematical technique called Fourier transformation. The transformation of the interferogram is carried out by the instrument software.

IR spectra are presented on a relative scale (%T), therefore, a background spectrum must be measured. A background spectrum is taken with no sample in the beam and is then subtracted from the sample spectrum to remove artifacts generated by the instrument or air (i.e. water, carbon dioxide, etc.).

FTIR Advantages

FTIR instruments have several advantages over dispersive IR instruments including:

Speed

All IR frequencies are measured simultaneously, resulting in measurements being taken in seconds rather than minutes. This is often referred to as the Felgett Advantage.

Sensitivity

The detectors utilized in FTIR instruments are highly sensitive which results in lower signal to noise ratios. This is known as the Jacquinot Advantage.

Simplicity

The only moving part in an FTIR instrument is the mirror in the interferometer; therefore, there is very little need for mechanical maintenance.

Internal calibration

The internal laser is used to self-calibrate the moving mirror in the FTIR instrument negating any need for timely or complicated external calibration. This is denoted as the Connes Advantage.

14. Sample Preparation

Proper sample preparation is required to obtain meaningful spectra with sharp peaks, which have good intensity and resolution. Ideally the largest peaks should be attributable to the compound being analyzed opposed to the background or sample matrix (water, CO_2, solvent etc.) and should ideally have an intensity of 2-5 %T for the strongest peaks in the spectrum (Figure 18). A transmission of 5 % is equivalent to an absorbance (A) = 1.3 (i.e. the amount of light that is absorbed by the sample), which is the upper detection limit for most detectors.

$$A = 2 - \log(\%T) \quad (7)$$

The equation above is worth remembering as it allows the absorbance of a sample to be calculated from the percentage transmittance data.

Peaks that are of higher intensity will be cut off and the sample will need to be prepared again.

Compounds can be analyzed in the vapor phase, as pure liquids, in solution, and as solids.
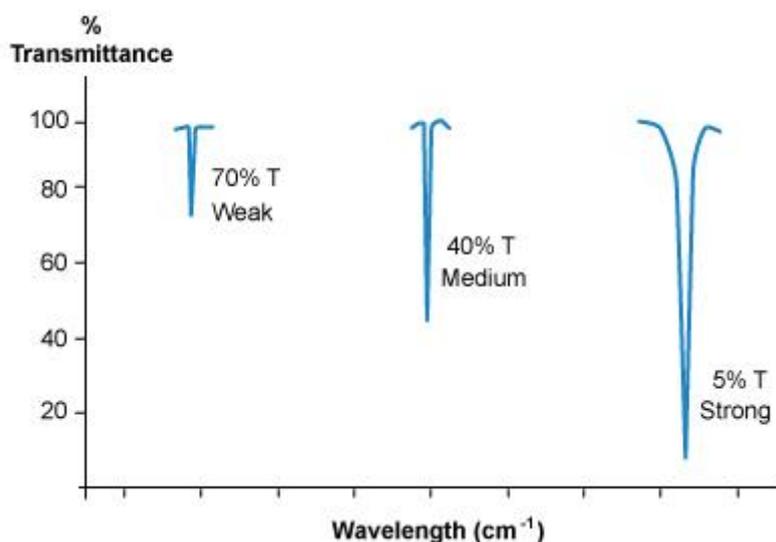
Figure 18: Classification of IR signals.

15.1. As a Liquid

A drop of the liquid is squeezed between two sodium chloride (NaCl) plates, which are transparent in the 4000-625 cm^{-1} region (Figure 19).

The plates are then placed in a holder and a spectrum is taken.

If the peaks in the spectrum are too intense the liquid can be wiped from one plate, then the spectrum taken again.

Figure 19: Sodium chloride (NaCl) plates in various conditions.

NaCl plates are very fragile and sensitive to water. Samples should never be dissolved in water and placed on a NaCl plate as it will fog up or dissolve. The plates should be held by the edges to avoid moisture from fingers damaging them. After a sample has been run, ethanol can be used to clean the plates. Moisture in the air can also damage NaCl plates; therefore, they should be stored in a desiccator. Cloudy or damaged plates (pitted, fingerprints etc.) will result in **poor spectra** with broad bands and spectra with less than optimum transmission (Figure 20). Cloudy plates can be restored by polishing.

Liquids can also be placed directly on an Attenuated Total Reflectance (ATR) plate which will be discussed later.
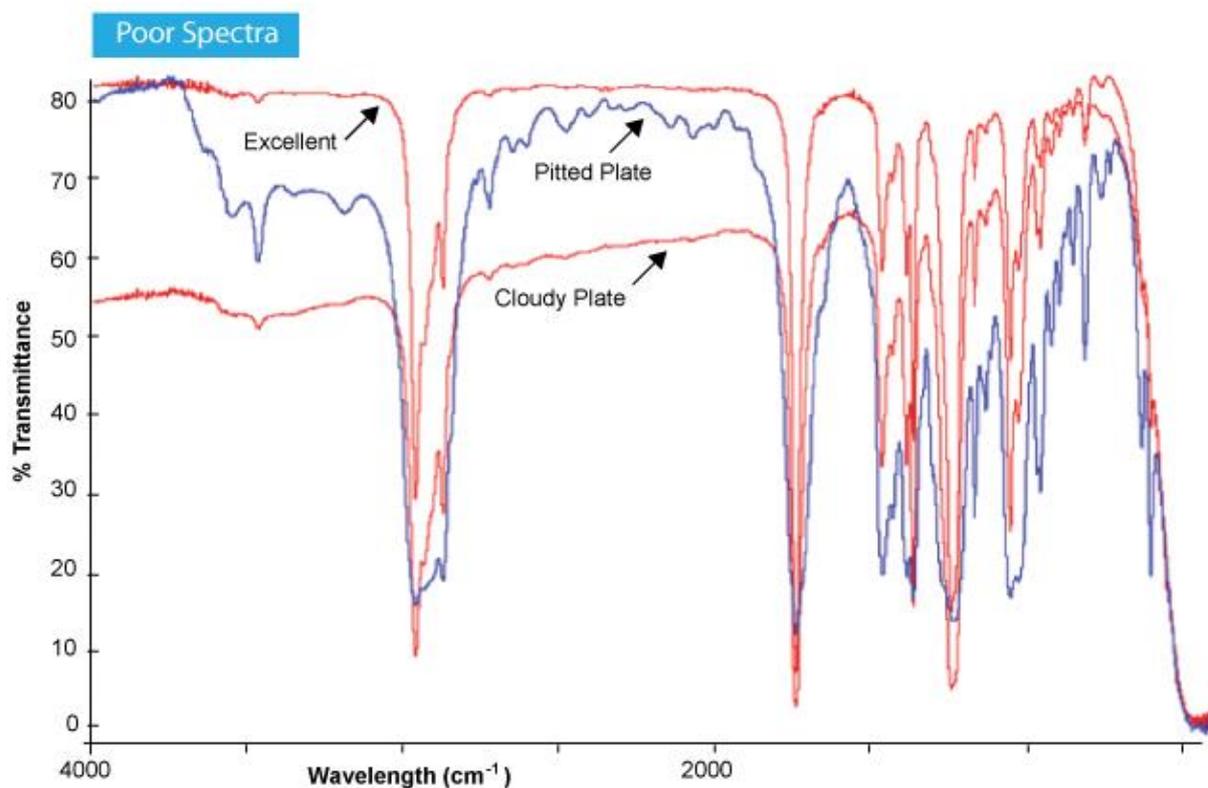
Figure 20: Representative spectra obtained with sodium chloride (NaCl) plates in various conditions.

15.2. As a Solution

Samples can be dissolved in an appropriate solvent to give a solution. The spectrum is then taken by placing a drop on a NaCl plate or by using a sodium chloride solution cell (Figure 21). Solvents should be free of water to avoid damaging the sodium chloride cell surfaces. A reference spectrum of the blank solvent should be obtained and subtracted from the sample spectrum.

When solvents absorb ~80% of the incident light, spectra cannot be obtained because insufficient light will be transmitted and detected. The regions in which common solvents absorb too strongly to give meaningful spectral information from a sample are shown in the table below.

If aqueous solvents must be used for solubility, special calcium fluoride cells can be used.

Figure 21: Sodium chloride solution cell. Image reproduced with permission from International Crystal Laboratories (Garfield, NJ, USA).

Transmission Characteristics of Common Solvents.

Transmission below 80%, with a 0.10 mm cell path are shown as darkened areas.
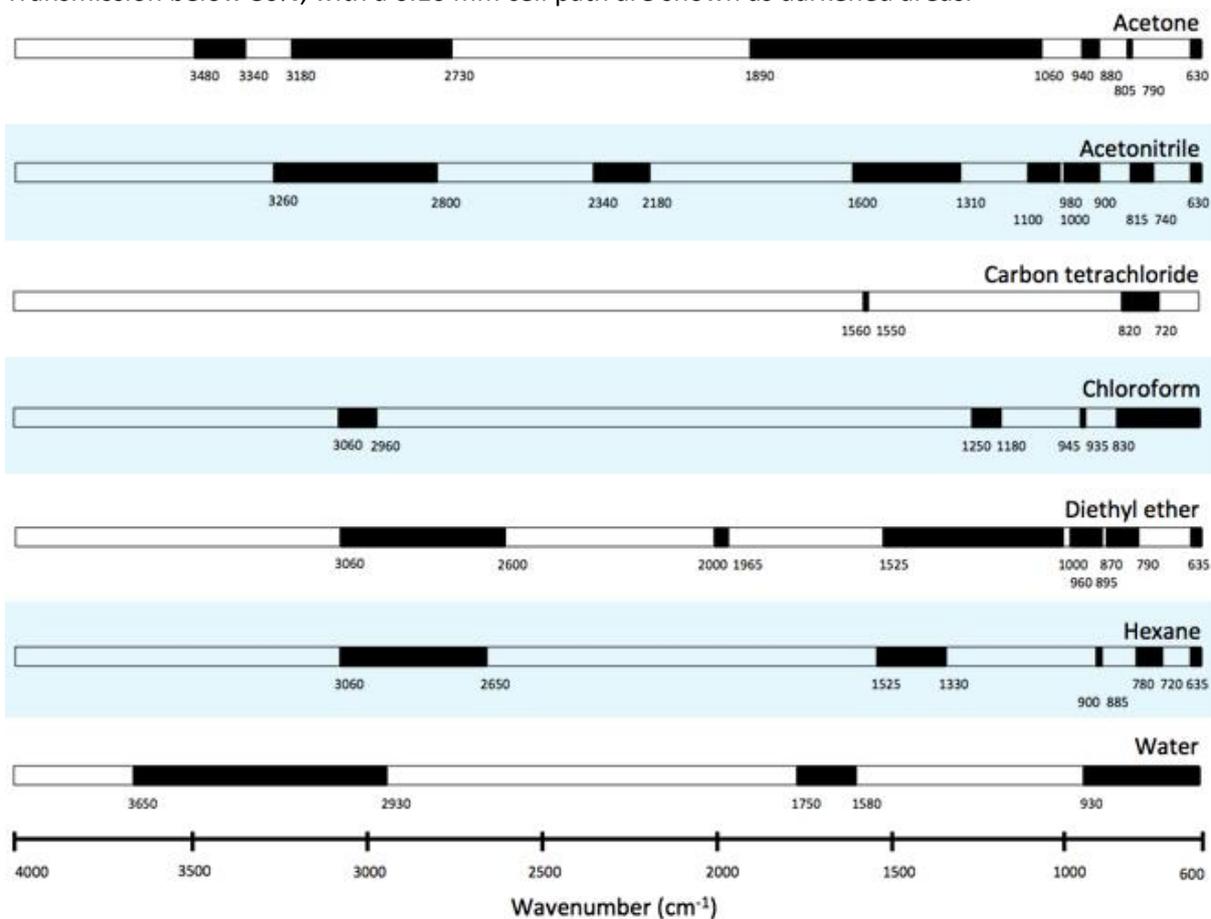
As a Nujol Mull

IR spectra of solid samples can be obtained using a Nujol mull. Nujol is a mineral oil which itself has an IR spectrum (Figure 22).
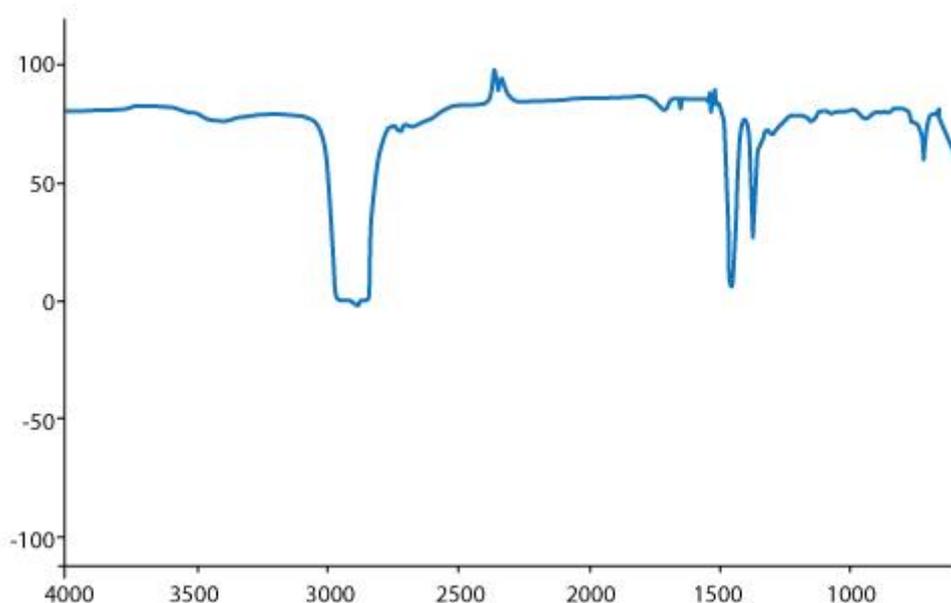
Figure 22: IR spectrum of pure Nujol.

A small amount of sample is ground using a small agate mortar and pestle and a drop of Nujol (Figure 23). The mull is then pressed between two NaCl plates and the spectrum obtained. The mull should appear transparent and free of bubbles when properly prepared.   If the peaks in the spectrum are too strong one plate can be wiped clean and the spectrum re-run.

Figure 23: Agate mortar and pestle. Image reproduced with permission from Cole-Parmer (Hanwell, London, UK).

As a KBr Disc

A solid sample can be ground with 10-100 times its mass of pure potassium bromide (KBr). Solid samples should be finely ground before adding the KBr. This is then pressed into a disc using a special mold and a hydraulic press (Figure 24). The use of KBr eliminates any bands that may obscure analyte signals when using a Nujol mull. A band at 3450 cm^{-1} will often be present and is attributable to the OH group from traces of water. Water can be minimized by drying the KBr in an oven. Excessive grinding of the hygroscopic KBr can increase the water content.

Solid state spectra can differ greatly from solution state spectra due to intermolecular interactions between functional groups, i.e. hydrogen bonding. Conversely, solid state spectra will often exhibit a greater number of resolved bands which can aid in compound identification.

Material	Wavelength Range (μm)	Wavelength Range (cm^{-1})	Refractive Index at 2 μm
NaCl	0.25-17	40,000-590	1.52
KBr	0.25-25	40,000-400	1.53
KCl	0.30-20	33,000-500	1.5

Table 3: Material used for obtaining solid state IR spectra. Note these materials can also be used to produce plates and solution cells for obtaining spectra with liquids and mulls.

Reasons for Cloudy Discs

- KBr mixture not properly ground
- Sample was not dry
- Sample:KBr ratio too high
- Disc too thick
- Sample has a low melting point

Figure 24: KBr Press. Image reproduced with permission from Specac (Orpington, Kent, UK).

15. Attenuated Total Reflectance (ATR)

As has been discussed previously, IR spectra can be obtained from samples as liquids, solids, or mulls; however, the primary drawback is the sample preparation that is required to obtain good quality spectra. IR instruments which utilize an attenuated total reflectance (ATR) stage negate the necessity for complex and timely sample preparation resulting in good quality, reproducible spectra.

With traditional means of IR spectroscopy the IR radiation is passed through the sample and the resulting radiation which is transmitted is measured. Attenuated total reflectance measure the changes which occur in a totally internally reflected IR beam when it is in contact with a sample (Figure 25).
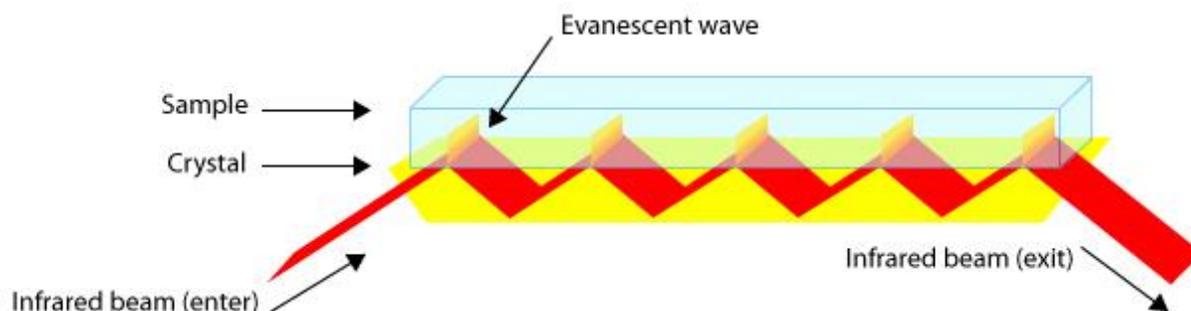
Figure 25: Evanescent wave.

The infrared beam enters the crystal which is made of an optically dense material (i.e. it has a high refractive index) at a particular angle of incidence, the IR beam is internally reflected (usually between five and ten times), this internal reflectance results in the production of an evanescent wave which can extend beyond the crystal surface and into the sample itself. The wave will usually penetrate into the sample with a depth of 0.5-2 μm. The depth to which the wave penetrates is dependent on the angle of the incident IR beam and the refractive index of the crystal material and sample itself.

When a samples absorbs the infrared radiation there is a change in the evanescent wave; in other words the wave is attenuated. The attenuated energy from each of the evanescent waves is then transferred back to the IR beam which exits the crystal and is measured by the detector to produce an IR spectrum.

Typical ATR Crystal Materials

In ATR instruments the crystal is an optically dense material which has a refractive index that is greater than the sample. Common ATR crystal materials are listed in Table 4. The most common are zinc selenide (ZnSe) and Germanium (Ge).

Zinc selenide is applicable to the analysis of liquids and non-abrasive pastes. It has a working pH range of 5 – 9. Germanium is more robust with a working pH range of 1 – 14 and can be used to analyze weak acids and alkalis. For a greater initial cost, instruments which utilize diamond as the ATR crystal material exhibit greater durability and robustness, with the crystal having to be replaced less in comparison to ZnSe and Ge.

Materials such as ZeSe and Ge can scratch easily; therefore, care must be taken when cleaning the

crystal surface. It is recommended that crystal surfaces are cleaned with lint free tissues soaked with solvents such as water, methanol or isopropanol.

Material	Wavelength Range (cm^{-1})	Refractive Index
ZnSe	20,000-500	2.43
ZnS	22,000-750	2.25
Ge	5,000-600	4.01
Si	10,000-100	3.42
Diamond	45,000-10	2.40

Table 4: Attenuated total reflectance (ATR) crystal materials.

ATR Instrument

In traditional ATR instruments the sample was clamped against the vertical face of the crystal. This design has now been replaced by horizontal ATR stages where the upper surface of the crystal is exposed (Figure 26). ATR accessory kits can be purchased which can be used to modify existing IR instruments.

Similarly to FT-IR instruments a background spectrum must be collected; this is taken from the clean ATR crystal. The background spectrum which is obtained can be a useful indication of the cleanliness of the ATR crystal; a line at 100% T should be obtained with no spectral features. In order for total internal reflectance to occur there must be good contact between the sample and crystal surface. Liquid samples can be placed directly onto the ATR crystal; the whole crystal must be covered. Similarly pastes or other viscous substances can be spread onto the crystal. In the case of solids, these are more readily analyzed on single reflection ATR instruments which are often made of diamond. High quality spectra can be obtained directly from powder samples placed on the ATR crystal. The amount of sample should entirely cover the crystal and does not need to be more than a few millimeters thick. In order to ensure that there is good contact with the crystal surface the instrument pressure arm is positioned over the sample and tightened; it may be necessary to apply greater pressure when analyzing high density polymers or coatings on metal surfaces, however, the user manual should always be consulted for optimum operating parameters.

The major advantages of ATR instruments are the lack of sample preparation, the ability to obtain high quality reproducible spectra, and due to their ease of use, the variation between users is minimized.
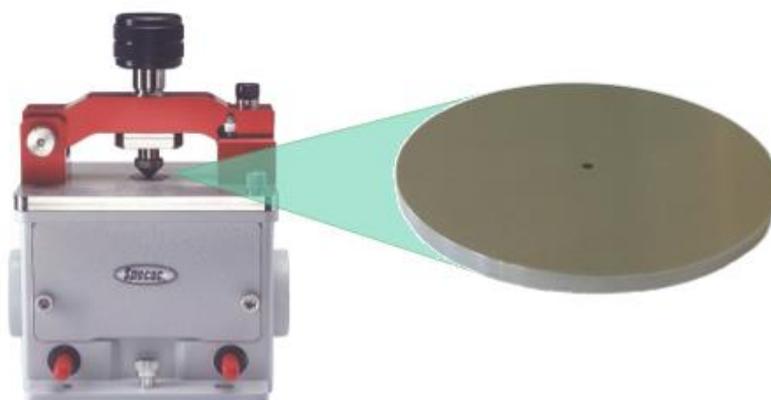
Figure 26: ATR Instrument. Image reproduced with permission from Specac (Orpington, Kent, UK).

16. Applications of IR Spectroscopy

IR spectroscopy has primarily been used for structural elucidation and identification of unknowns (by comparison with a spectrum of a standard).

Modern advances have seen the development of 2D IR techniques which have been applied to a myriad of different applications including isotope labelling studies of biological species,[12] the investigation of proteins, peptides, and hydrogen bond dynamics,[13] and also the study of nanocrystalline thin films.[14]

Other areas of note where IR spectroscopy is being utilized are in stem cell studies,[15] materials science,[16] catalysis,[17] and reaction kinetics.[18] Which demonstrates the applicability and flexibility of this analytical technique.

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