ICAR-National Agricultural Higher Education Project

Fourier Transform infrared (FTIR) spectrometer

A Laboratory Handbook

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Introduction

In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis.

FTIR stands for Fourier transform infrared. Fourier-transform infrared spectroscopy, is concerned with the vibration of molecules. Each functional group has its own discrete vibrational energy which can be used to identify a molecule through the combination of all of the functional groups. This makes FTIR microscopy ideal for sample ID, multilayer film characterization, and particle analysis. the preferred method of infrared spectroscopy.

In the 1970s, the first commercial Fourier Transform infrared (FTIR) spectrometer appeared. In FTIR spectrometers, spectra are generated in the time domain by following the position of a moving mirror and the occurrence of constructive and destructive interference. A Fast Fourier Transform (FFT) then converts the signal from the time into the frequency domain. Since the FFT calculation takes time to compute, the development of commercial FTIR spectroscopy closely followed the trend of the increased power and miniaturization of computers. By the 1980s, dispersive infrared spectrometers were phased out for the more desirable interferometer type spectrometers as computers became more widespread.

FTIR spectrometers have three main advantages over dispersive spectrometers:

- The full spectrum reaches the detector simultaneously (instead of a small band of radiation as in grating-based spectrometers), the spectrum takes much less time to collect.
- Since there are fewer optics and no slits, the total power at each data point is much greater.
- The resolution is much greater than with a grating, enabling spectral subtraction, concentration curves, and library searches among other functions.

Other reasons why Fourier transform infrared spectroscopy is preferred over dispersive or filter methods of infrared spectral analysis are as follows:

- It is a non-destructive technique
- It provides a precise measurement method which requires no external calibration
- It can increase speed, collecting a scan every second
- It can increase sensitivity – one second scans can be co-added together to ratio out random noise
- It has greater optical throughput
- It is mechanically simple with only one moving part

FTIR Spectroscopy Principles

The electromagnetic spectrum consists of different regions corresponding to different energy (E), frequency (v), and wavelength (λ) ranges as seen in Figure 1. The unit for near-, mid-, and far-infrared, the wavenumber (cm-1), is derived from the inverse relationship between wavelength and frequency.
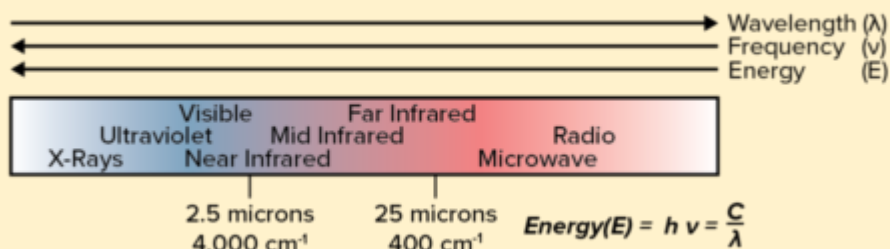

Figure 1. Electromagnetic Spectrum

FTIR spectroscopy takes advantage of how IR light changes the dipole moments in molecules (Figure 2) that correspond to a specific vibrational energy.

Figure 2. Interaction between infrared light and matter

Vibrational energy corresponds to two variables: reduced mass (μ) and bond spring constant (k) (Equation 1). For k constant, we can look at C-C, C=C, and C≡C showing an increase of 800 cm-1 across the series (Table 1). Substituting atoms in a C-C bond with nitrogen and oxygen causes a shift of 100 cm-1 (Table 2). By looking at the two series, it can be seen that bond strength alters the wavenumbers more than mass.

Table 1   **Equation 1**   **Table 2**

C - C	1,400 cm⁻¹
C = C	1,600 cm⁻¹
C ≡ C	2,200 cm⁻¹

Increasing k

$$\tilde{\upsilon} = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}$$

C - C	1,400 cm⁻¹
C - N	1,350 cm⁻¹
C - O	1,300 cm⁻¹

Increasing μ

The FTIR Instrumentation

A FTIR spectrometer consists of four major parts: a light source, an interferometer, a sample chamber, and a detector (Figure 3).
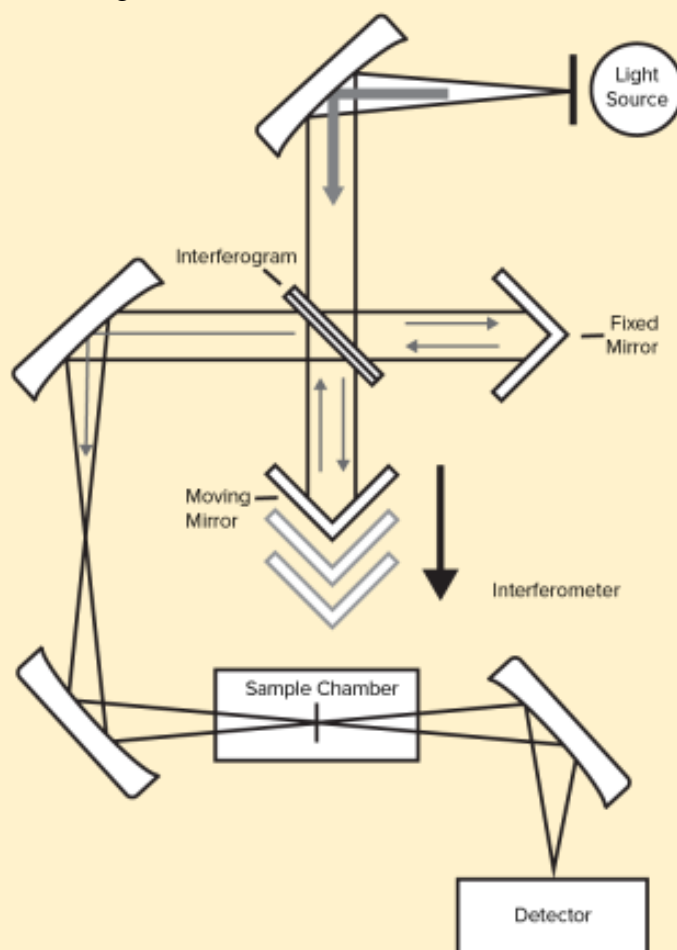

Figure 3. Digital Signal Acquisition by Interferometer

FTIR Instrumentation Process

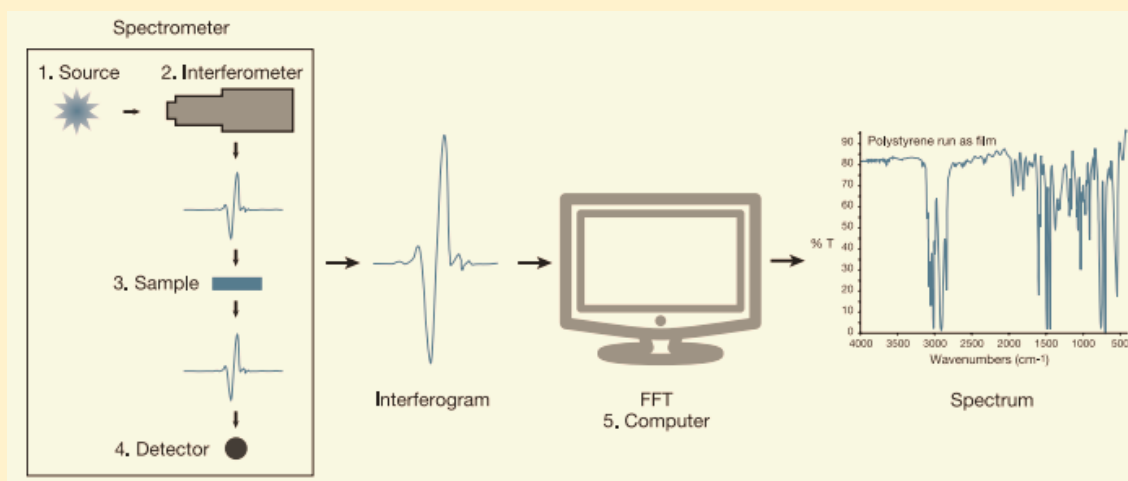The normal instrumental process depicted in Figure 4 is as follows:

Figure 4. FTIR instrumental process

1. **The Source:** Infrared energy is emitted from a glowing black-body source. This beam passes through an aperture which controls the amount of energy presented to the sample (and, ultimately, to the detector).

2. **The Interferometer:** The beam enters the interferometer where the "spectral encoding" takes place. The resulting interferogram signal then exits the interferometer. The interferometer uses a reference laser for precise wavelength calibration, mirror position control and data acquisition timing.

3. **The Sample:** The beam enters the sample compartment where it is transmitted through or reflected off of the surface of the sample, depending on the type of analysis being accomplished. This is where specific frequencies of energy, which are uniquely characteristic of the sample, are absorbed.

4. **The Detector:** The beam finally passes to the detector for final measurement. The detectors used are specially designed to measure the special interferogram signal.

5. **The Computer:** The measured signal is digitized and sent to the computer where the Fourier transformation takes place. The final infrared spectrum is then presented to the user for interpretation and any further manipulation.

Because there needs to be a relative scale for the absorption intensity, a background spectrum must also be measured. This is normally a measurement with no sample in the beam. This can be compared to the measurement with the sample in the beam to determine the "percent transmittance." This technique results in a spectrum which has all of the instrumental characteristics removed. Thus, all spectral features which are present are strictly due to the sample. A single background measurement can be used for many sample measurements because this spectrum is characteristic of the instrument itself.

Working of Interferometer

Fourier transform infrared (FTIR) spectrometry was developed in order to overcome the limitations encountered with dispersive instruments. The main difficulty was the slow scanning process. A method for measuring all of the infrared frequencies simultaneously, rather than individually, was needed. A solution was developed which employed a very simple optical device called an interferometer.

Monochromatic light is a superposition of waves of the same frequency. The light intensity is a maximum when the individual waves have the same phase and constructively interfere with each other (Figure 5a). The light intensity decreases as the phase difference becomes larger and reaches a minimum when the individual waves are out of phase and destructively interfere with each other (Figure 5b). In a FTIR spectrometer, this phase difference is varied using a dual light path and two mirrors: one mirror that is fixed in place and another mirror that moves at a constant speed.
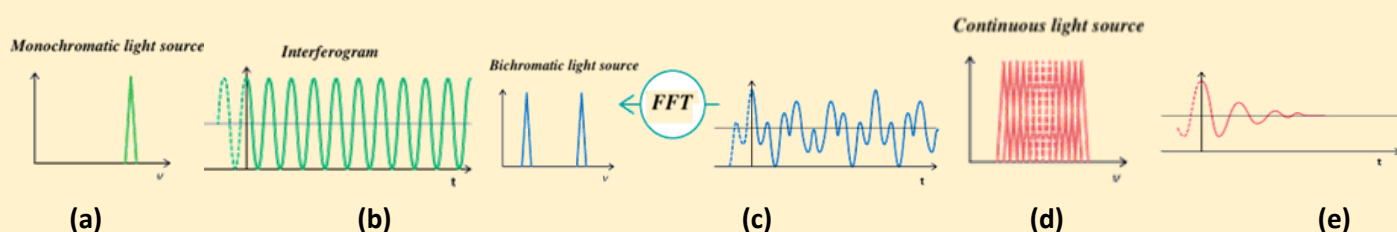

Figure 5a-e Working of Interferometer

For a monochromatic light source, a plot of the detected light intensity against time (or position of the moving mirror) appears as a sinusoidal wave (Figure 5c). The time domain signal of this waveform is converted to the frequency domain via a Fast Fourier Transform (FFT), producing a single peak at the frequency of the monochromatic light. For a bichromatic light source, containing two waves of different frequencies, a more complicated waveform is produced. Taking a Fourier transform of this waveform produces two peaks corresponding to the two different frequencies of light (Figure 5d). For a continuous light

source, its spectrum corresponds to a superposition of waves of different frequencies, all in phase with each other. Before the mirror is moved (when the time t = 0), there is no phase difference between the optical paths, and the light intensity at all wavenumbers is highest. As the mirror moves (when the time t > 0), destructive interference occurs, and the signal strength gradually converges to zero. The resulting plot of signal intensity against time is referred to as an interferogram. By taking a Fourier transform of the interferogram, the original frequency spectrum can be calculated (Figure 5e).

Digital Signal Acquisition by Interferometer

FTIR detectors observe interferograms as analog signals, but in order to perform a Fourier transform, the analog signals first need to be converted to digital signals. In a FTIR spectrometer, monochromatic light from a timing laser is used as an internal reference to track the position of the moving mirror using the precisely known wavelength of the laser. The intensity of the output interference signal is measured as a function of time. Each data point (or digital signal) in the infrared interferogram is taken when the timing laser inte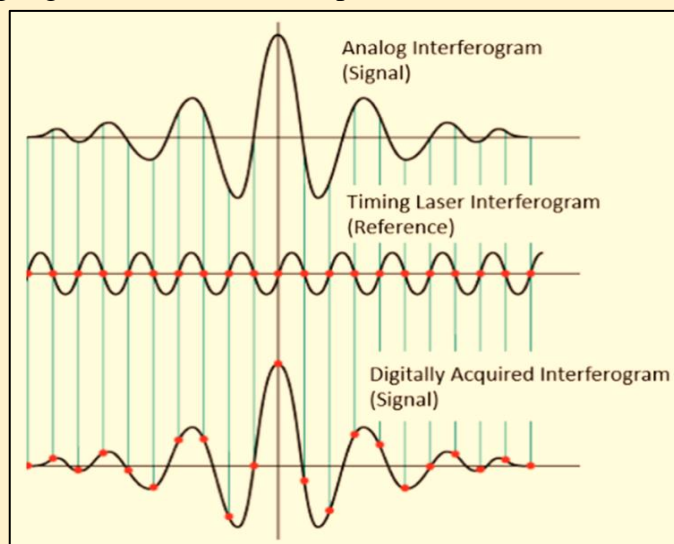rferogram has neither constructive nor destructive interfere allowing for an interferogram to be obtained at equal intervals (Figure 6). Helium-Neon (HeNe) lasers are the industry standard due to their excellent wavelength stability of 632.8 nm compared to solid-state or diode lasers. This laser stability allows for spectral additions, library searches, and other functions that require high wavenumber accuracy.

Figure 6. Digital Signal Acquisition by Interferometer

Attenuated Total Reflectance (ATR) FTIR

The use of Attenuated Total Reflectance (ATR) in FTIR spectroscopy has become the primary sampling method for FTIR spectroscopy. The major advantage is the lack of sample preparation for liquid and solid samples. When light reflects off of certain materials (diamond, ZnSe, etc.) at a critical angle, the light undergoes total internal reflectance with the propagation of an evanescent field from the surface of the crystal that interacts with the material (sample) in contact, Figure 7.
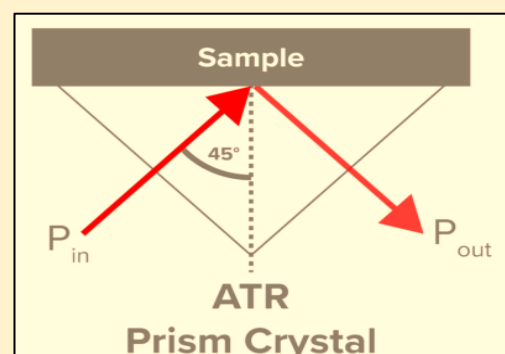

Figure 7. ATR Prism Crystal: Working

The penetration depth is dependent on the refractive index of both the sample (generally ~1.5) and the crystal itself. Since the refractive index is defendant on wavelength, spectra taken with ATR have slightly different intensity ratios across the spectrum and may need to be corrected to compare to transmission spectra. The three most common crystals are diamond, zinc selenide, and germanium, each having advantages and disadvantages (Table 3). Diamond crystals are rugged, have a penetration depth of 1.5 microns, low wavenumber cutoff (200 cm-1) but have poor throughput in the 2200 cm-1 region. ZnSe has exceptional throughput but a high cutoff (650 cm-1). Germanium has a very low penetration depth (0.8 microns) and is useful for highly absorbing substances

Table 3: Features of each prism

Prism	Feature of Prism	Suitable Sample	Non-Suitable Sample	Remark
ZnSe	Good throughput	General organic substance	Hard powder, acid alkaline, high RI sample	In case of hard powder or hubbly sample, diamond is recommended
Diamond	Intensity, durability, measurement	Hard powder, General organic substance	High RI sample	Poor S/N ratio in the region around 2000 cm-1, due to internal absorption
Germanium	Analysis for high RI sample	Sample including carbon	Hard powder, acid alkaline	Weak absorption due to small depth of penetration

Identification of Molecules using FTIR Spectroscopy

Since every functional group is composed of different atoms and bond strengths, vibrations are unique to functional groups, and classes of functional groups Figure 5 (e.g. O-H and C-H stretches appear around 3200 cm^{-1} and 2900 cm^{-1}, respectively).

Figure 5. Correlation chart with various functional group vibrations

Since the collection of vibrational energy bands for all of the functional groups a molecule is unique to every molecule, these peaks can be used for identification using library searches of comprehensive sample databases.
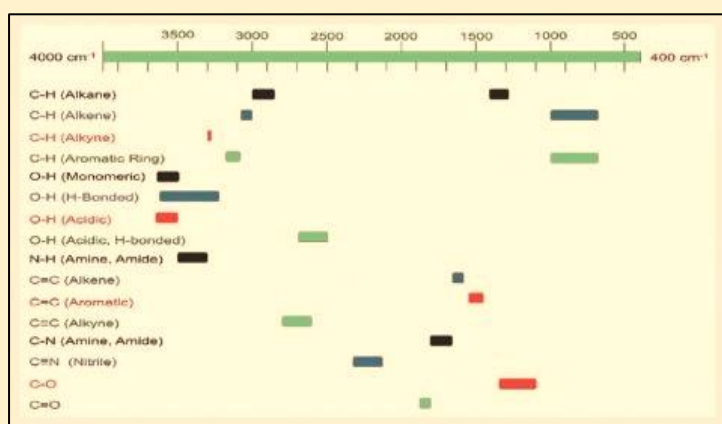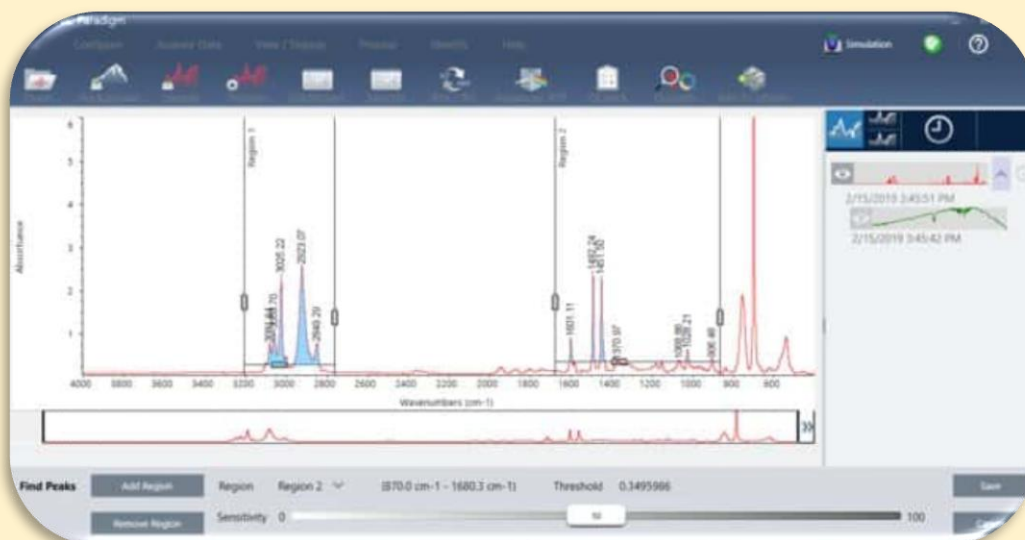
Glimpses of Thermo Scientific™ Nicolet Summit FTIR: OMNIC™ Paradigm Software installed in Microbiology Laboratory under NAHEP-CAAST Project

FT-IR Glossary

Absorption The transfer of energy from an infrared beam to the molecules present in the path of the radiation.

Absorbance Spectrum Absorbance is related to Transmittance as follows: $A = \log 1/T$ where A is absorbance and T is transmittance. Transmittance spectra are calculated by ratioing a sample single beam spectrum to a background spectrum. Absorbance is linearly proportional to concentration (see Beer's Law), and should be used for quantitative analysis.

Attenuated Total Reflection (ATR) Infrared radiation entering a prism made from a high-refractive index, infrared transmitting material can be totally internally reflected. This internal reflection creates an effect called the evanescent wave that extends beyond the surface of the crystal into the sample that is in contact with the crystal. In regions of the infrared spectrum where the sample absorbs energy, the evanescent wave will interact with the sample and a spectrum can be obtained. Attenuated Total Reflection produces a very short pathlength for the infrared light in the sample. This makes this technique ideal for highly absorbing materials such as aqueous solutions, rubbers, and polymers.

Background The characteristics of the environment of the spectrometer, including the detector and atmospheric conditions. A reference spectrum that accounts for the unique optics of a sampling accessory and an instrument. The background spectrum is the result of the output of the source, the response of the beamsplitter optics, sampling module or sample holder, and detector and any atmospheric absorptions inside the analyzer. Sample spectra are ratioed against a background spectrum so that the final spectrum is free of those features.

Background Spectrum A single beam spectrum obtained without a sample in place. The background spectrum is the result of the output of the source; the response of the beamsplitter, optics, sampling accessory or holder, and detector; and any atmospheric absorptions inside the spectrometer.

Baseline The portion of spectrum or chromatogram that contains no bands or peaks. The baseline represents those regions where the sample absorbs little or no infrared energy.

Baseline Correction Removal of an offset, slope or curvature from the baseline of a spectrum.

Corrected Peak Area The area that is below a peak, above the baseline and between two vertical lines that define the left and right limits of the area.

Corrected Peak Height The difference in the Y axis unit value between the maximum of a peak and the baseline.

Far-IR (Far Infrared) The region of infrared radiation from 400 to 10 wavenumbers.

Fourier Transform (1) To convert an interferogram (data in the time domain) to a single-beam spectrum (data in the frequency domain) to reveal the response at all frequencies within the spectral range. (2) The mathematical operation used to convert an interferogram to a single-beam spectrum.

Frequency The number of light wave cycles that occur per unit time or space. Infrared frequency is typically expressed in wavenumbers (cm-1).

FT-IR (Fourier Transform Infrared) An infrared spectroscopic technique that uses an interferometer for data collection and a digital Fourier transformation to process the data.

FT-NIR (Fourier Transform Near Infrared) A near infrared spectroscopic technique that uses an interferometer for data collection and digital Fourier transformation to process the data.

FT-Raman (Fourier Transform Raman) Raman spectroscopy in which an interferometer is used as the dispersing element to achieve spectral resolution. Typically performed with a 1064 nm laser and either Ge or InGaAs detectors.

Interferogram A photographic or electronic recording of an optical interference pattern. The interferograms recorded by an infrared spectrometer are plots of infrared intensity versus optical path difference. Interferograms are Fourier transformed to obtain single beam spectra.

NIR (Near Infrared) A region of the electromagnetic spectrum extending from approximately 12000 cm-1 to 4000 cm-1.

Noise Random signals produced by a number of components in a spectrometer, including the detector and signal-processing electronics.

Quantitative Analysis The use of peak heights, band ratios, or peak areas to calculate the amount of a substance in an unknown sample.

Resolution A measure of how well closely spaced peaks in a spectrum are differentiated. The higher the resolution, the more peaks that can be revealed. Increasing the resolution requires that the distance traveled by the moving mirror be increased. The resolution is approximately

twice the data spacing value. May also refer to size of sample in a microscope; see spatial resolution and spectral resolution.

Sampling Spectrum The spectrum of a substance being analyzed.

Scan (1) To collect data with an interferometer.(2) One movement of the moving mirror from the point closest to the beamsplitter to the farthest point or vice versa.

Signal-to-Noise Ratio (SNR) The ratio of signal in a spectrum to the noise in a spectrum. Signal is often measured as the intensity of a peak, noise is measured as the random fluctuations of the baseline.

Spectral Resolution The ability of an instrument to distinguish between two spectral features that are close to each other in wavenumber. Resolution is measured in terms of the closest two features that can be distinguished. In FT-IR, high resolution is denoted by small numbers, e.g. 0.01 cm-1. Low resolution is denoted by large numbers, e.g. 64 cm-1.

Spectrum A graphical representation of the intensity of the infrared radiation reaching the detector at each frequency or wavelength measured. The intensity at a given frequency or wavelength is determined by the characteristics of the spectrometer and the sample, if one is present.

Wavenumber The number of waves per centimeter. Wavenumber is the inverse of wavelength. Often used as the X-axis unit of an FT-IR spectrum.

Suggested readings:
1. Berthomieu, C., & Hienerwadel, R. (2009). Fourier transform infrared (FTIR) spectroscopy. Photosynthesis research, 101, 157-170.
2. Talari, A. C. S., Martinez, M. A. G., Movasaghi, Z., Rehman, S., & Rehman, I. U. (2017). Advances in Fourier transform infrared (FTIR) spectroscopy of biological tissues. Applied Spectroscopy Reviews, 52(5), 456-506.
3. Fahelelbom, K. M., Saleh, A., Al-Tabakha, M. M., & Ashames, A. A. (2022). Recent applications of quantitative analytical FTIR spectroscopy in pharmaceutical, biomedical, and clinical fields: A brief review. Reviews in Analytical Chemistry, 41(1), 21-33.