Introduction to Infrared and Raman spectroscopy

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Optical microscopy course 2012
31.10.2012

Types of Spectroscopy
= study of interaction between radiation and matter

- X-ray photoelectron spectroscopy (XPS)
- Infrared absorption spectroscopy (IRS)
- Ultraviolet and visible absorption spectroscopy (UV-VIS)
- Nuclear magnetic resonance spectroscopy (NMR)
- Raman spectroscopy
In the year 1800 the astronomer Friedrich Wilhelm Herschel analyzed the spectrum of sunlight. Herschel created the spectrum by directing sunlight through a glass prism so that the light was divided into its different colors. He measured the heating ability of each color using thermometers with blackened bulbs. When he measured the temperature just beyond the red part of the spectrum he noticed some kind of invisible radiation. Herschel concluded that there must be a different kind of light beyond the red portion of the spectrum, which is not visible to the human eye. This kind of light became known as "infrared" (below red) light.

Herschel then placed a water-filled container between the prism and thermometer and observed that the temperature measured was lower than the one measured without the water. Consequently, the water must partially absorb the radiation. This was the beginning of infrared spectroscopy.

Infrared spectroscopy measures the infrared light that is absorbed by a substance. This absorption depends on the wavelength of the light.
The electromagnetic spectrum

Interaction of radiation and matter

If matter is exposed to electromagnetic radiation, e.g. infrared light, the light can be absorbed, transmitted, reflected, scattered or undergo photoluminescence.
**ORIGIN OF THE SPECTRAL REGIONS**

<table>
<thead>
<tr>
<th>Type of radiation</th>
<th>Wavelength range</th>
<th>Type of transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma rays</td>
<td>$10^{-12} - 10^{-10}$ m</td>
<td>nuclear</td>
</tr>
<tr>
<td>X-rays</td>
<td>1 nm - 1 pm</td>
<td>inner electrons</td>
</tr>
<tr>
<td>Ultraviolet light</td>
<td>400 - 1 nm</td>
<td>outer electrons</td>
</tr>
<tr>
<td>Visible light</td>
<td>700 - 400 nm</td>
<td>outer electrons</td>
</tr>
<tr>
<td>Infrared light</td>
<td>2.5 μm - 700 nm</td>
<td>vibrations, rotations</td>
</tr>
<tr>
<td>Microwaves</td>
<td>1 mm - 2.5 μm</td>
<td></td>
</tr>
<tr>
<td>Radiowaves</td>
<td>$10^8 - 1$ m</td>
<td>spin flips</td>
</tr>
</tbody>
</table>

**Interaction of radiation and matter**

**Vibration theory**

- IR spectroscopy is based on the absorption of infrared light.

- Absorption excites molecular vibrations and rotations, which have frequencies within the infrared range.

- Simple model of an **harmonic oscillator** describes IR absorption.

*Mechanical model of a vibrating diatomic molecule*
According to Hooke’s Law, for small deflections the restoring force is proportional to the deflection:

\[ F = -k \cdot \Delta r \]

In a molecule the force constant \( k \) is a measure of the bond strength between the atoms.

For a harmonic oscillator it is possible to calculate the vibrational frequency, \( \nu \), of a diatomic molecule as follows:

\[ \nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \]

\[ \mu = \frac{m_1 \cdot m_2}{m_1 + m_2} \]

being the reduced mass.

Typically in infrared spectroscopy, wavenumbers are used:

\[ \nu = \frac{v}{c} = \frac{1}{2} \text{ (cm}^{-1}\text{)} \]

Vibration theory
On the basis of the equation above it is possible to state the following:

1) The higher the force constant \( k \), i.e. the bond strength, the higher the vibrational frequency, \( \nu \), (in wavenumbers).

3 absorption peaks for different force constants.
Note: in infrared spectroscopy highest wavenumber to the left.
Vibration theory

2) The larger the vibrating atomic mass, the lower the vibrational frequency, $\tilde{v}$.

![Graph showing absorption peaks for different atomic masses.](image)

3 absorption peaks for different atomic masses. Note: infrared spectroscopy highest wavenumber to the left.

VIBRATION OF A POLYATOMIC MOLECULE

- In diatomic molecules, the vibration occurs only along the chemical bond connecting the nuclei.
- In polyatomic molecules, all the nuclei perform their own harmonic oscillations.
  - These complicated vibrations can be expressed as a superposition of a number of independent "normal vibrations".
  - An $N$-atom molecule has $3N$ degrees of freedom of motion.

![Diagram showing atomic motions in normal modes of vibrations in CO2.](image)

Figure 1-10 Atomic motions in normal modes of vibrations in CO$_2$. 
VIBRATION OF A POLYATOMIC MOLECULE: CH₂

Stretching vibrations:

<table>
<thead>
<tr>
<th>Symmetrical stretching</th>
<th>Antisymmetrical stretching</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="http://en.wikipedia.org/wiki/Infrared_spectroscopy" alt="Symmetrical stretching" /></td>
<td><img src="http://en.wikipedia.org/wiki/Infrared_spectroscopy" alt="Antisymmetrical stretching" /></td>
</tr>
</tbody>
</table>

Bending vibrations:

<table>
<thead>
<tr>
<th>Scissoring</th>
<th>Rocking</th>
<th>Wagging</th>
<th>Twisting</th>
</tr>
</thead>
</table>

Spectrum of a biological molecule

![Spectrum of a biological molecule](http://en.wikipedia.org/wiki/Infrared_spectroscopy)
VIBRATION OF A POLYATOMIC MOLECULE

- To determine whether the vibration is active in the IR or Raman spectra, the selection rules must be applied to each normal vibration.

- Vibrational transitions can be observed in either infrared (IR) or Raman spectra.

- A vibration is IR-active if the dipole moment is changed during the vibration.

- A vibration is Raman-active if the polarizability is changed during the vibration.

Some spectroscopy
**INFRARED SPECTRUM**

- **Example:** IR absorption spectrum of CO$_2$

**ORIGIN OF INFRARED SPECTRUM**

**Beer-Lambert Law**

- In IR spectroscopy, the absorption of infrared light is measured as a function of frequency.

- Beer-Lambert law relates the absorption of IR light to the properties of the material through which the light is traveling:

\[
I = I_0 e^{-\sigma l N}
\]

- Transmission through the sample:

\[
T = \frac{I}{I_0} = e^{-\sigma l N}
\]
ORIGIN OF INFRARED SPECTRUM

- It should be noted that transmission is not directly related to the concentration of molecules
  - In quantitative IR spectroscopy, absorbance ($A$) is typically used:
    $$A = -\ln \frac{I}{I_0} = \sigma N l$$
  - Absorbance is linearly related to concentration of the molecules!

EXAMPLE OF IR SPECTRAL ANALYSIS

- Quantitative analysis
  - Univariate analysis can be conducted e.g. by calculating the height or area of the specific absorption band
  - The absorption can be calibrated to yield absolute concentration values
    - Suitable especially for liquids
Some spectral analysis

Polyatomic Molecules
- The spectrum becomes more complex as the number of bonds increases

Polyatomic Molecules
- The spectrum becomes more complex as the number of bonds increases
Absorption Wavenumber [cm\(^{-1}\)]

**FT-IR Spectrum**

CH-stretch (lipids)

amide I

(proteins)

C-O- stretch (polysaccharides)

amide II

as a snapshot of the complex composition of biomolecules in the cells

**IR absorption of different organic molecular classes**

<table>
<thead>
<tr>
<th>Class</th>
<th>2,900</th>
<th>2,800</th>
<th>1,750</th>
<th>1,700</th>
<th>1,650</th>
<th>1,600</th>
<th>1,550</th>
<th>1,500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol and Phenol</td>
<td>3,500</td>
<td>2,900</td>
<td></td>
<td></td>
<td>1,380</td>
<td>1,320</td>
<td>1,280</td>
<td>1,240</td>
</tr>
<tr>
<td>Ether</td>
<td></td>
<td></td>
<td></td>
<td>1,270</td>
<td>1,230</td>
<td>1,190</td>
<td>1,150</td>
<td>1,110</td>
</tr>
<tr>
<td>Amine</td>
<td>3,500</td>
<td>2,900</td>
<td></td>
<td></td>
<td>1,600</td>
<td>1,560</td>
<td>1,520</td>
<td>1,480</td>
</tr>
<tr>
<td>Nitro compound</td>
<td></td>
<td></td>
<td></td>
<td>1,380</td>
<td>1,340</td>
<td>1,300</td>
<td>1,260</td>
<td>1,220</td>
</tr>
<tr>
<td>Ketone</td>
<td></td>
<td></td>
<td></td>
<td>1,700</td>
<td>1,660</td>
<td>1,620</td>
<td>1,580</td>
<td>1,540</td>
</tr>
<tr>
<td>Ketone</td>
<td></td>
<td></td>
<td></td>
<td>1,700</td>
<td>1,660</td>
<td>1,620</td>
<td>1,580</td>
<td>1,540</td>
</tr>
<tr>
<td>Carboxylic Acid</td>
<td>3,500</td>
<td>2,900</td>
<td></td>
<td>1,500</td>
<td>1,460</td>
<td>1,420</td>
<td>1,380</td>
<td>1,340</td>
</tr>
<tr>
<td>Carboxylate</td>
<td></td>
<td></td>
<td>1,700</td>
<td>1,660</td>
<td>1,620</td>
<td>1,580</td>
<td>1,540</td>
<td>1,500</td>
</tr>
<tr>
<td>Amide</td>
<td>3,300</td>
<td>2,100</td>
<td>1,870</td>
<td>1,830</td>
<td>1,500</td>
<td>1,460</td>
<td>1,420</td>
<td>1,380</td>
</tr>
</tbody>
</table>
The mid-infrared spectrum (4000-400 cm\(^{-1}\)) can be approximately divided into four regions:

1. **X-H stretching region (4000-2500 cm\(^{-1}\))**
   - O-H stretching $\rightarrow$ 3700-3600 cm\(^{-1}\)
   - N-H stretching $\rightarrow$ 3400-3300 cm\(^{-1}\)
   - C-H stretching $\rightarrow$ 3100-2850 cm\(^{-1}\)

2. **Triple-bond region (2500-2000 cm\(^{-1}\))**
   - C≡C bonds $\rightarrow$ 2300-2050 cm\(^{-1}\)
   - C≡N bonds $\rightarrow$ 2300-2200 cm\(^{-1}\)

3. **Double-bond region (2000-1500 cm\(^{-1}\))**
   - C=O bond $\rightarrow$ 1830– 1650 cm\(^{-1}\)
   - C=C stretching $\rightarrow$ $\approx$1650 cm\(^{-1}\)

4. **Fingerprint region (1500-600 cm\(^{-1}\))**
   - In this range, a spectrum of a molecule may have a hundred or more absorption bands present, but there is no need to assign the vast majority
   - The spectrum can be regarded as a "fingerprint" of the molecule
Fourier-Transform Infrared (FTIR) spectroscopy

FTIR SPECTROSCOPY IN SIB-labs KUOPIO

- Thermo Nicolet Nexus 8700:
  - Spectral range: 11000–375 cm\(^{-1}\)
  - Spectral Resolution: \(\approx 0.09\) cm\(^{-1}\)
  - Two detectors: TE Cooled DTGS and MCT-A
  - Beamsplitter: XT-KBr
  - Can be used to study both solid or liquid samples
  - Easy to use
In order to obtain IR light absorption at many discrete wavelengths, broadband light source is used containing full spectrum of wavelengths to be measured
- Possible to measure simultaneously absorption at many wavelengths

Nowadays, Fourier-transform infrared (FTIR) spectrometers are predominantly used and have improved the acquisition of infrared spectra dramatically
- Based on the interference of IR light between two beams → Interferogram
- Interferogram is a signal produced as a function of the change of pathlength between the two beams
- The two domains of distance and frequency are interconvertible by the Fourier-transform

Principle of FTIR device:

System consists following parts:
- HeNe-laser → internal calibration
- A glowing black-body IR-source
- Fixed mirror
- Moving mirror
- MCT detector
- Computer for FFT and data analysis

System consists only one moving part – a moving mirror!
The moving mirror produces an optical path difference between the two arms of the interferometer.

For path differences of $\delta = (n + 1/2)\lambda$, the two beams interfere destructively in the case of the transmitted beam and constructively in the case of the reflected beam.

**Broadband IR source**

- Frequency distribution of a black body source
- Optical retardation
- Resulting detector signal
INFRARED SPECTROSCOPY IN PRACTICE

- The actual IR absorption spectrum is obtained with Fourier Transform:

The essential equations for a Fourier-transformation relating the intensity falling on the detector, \( I(\bar{\nu}) \), to the spectral power density at a particular wavenumber, \( \bar{\nu} \), given by \( B(\bar{\nu}) \), are as follows:

\[
I(\bar{\nu}) = \int_{-\infty}^{+\infty} B(\bar{\nu}) \cos(2\pi \bar{\nu}) d\bar{\nu}
\]  

(2.1)

which is one half of a cosine Fourier-transform pair, with the other being:

\[
B(\bar{\nu}) = \frac{1}{2\pi} \int_{-\infty}^{+\infty} I(\bar{\nu}) \cos(2\pi \bar{\nu}) d\bar{\nu}
\]

(2.2)

- **Two interferograms are produced:**
  1) With the sample in the light beam
  2) Without a sample in the light beam (Reference)

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**Transmission spectrum**

- An interferogram measured without any sample in the optical path is Fourier transformed. This results in reference spectrum.
Transmission spectrum

- An interferogram measured without any sample in the optical path is Fourier transformed. This results in reference spectrum.

- A second interferogram, measured with the sample in the optical path, is Fourier transformed. This results in sample spectrum. Spectrum looks similar to the reference spectrum, but shows less intensity at those wavenumbers where the sample absorbs radiation.

- The final transmission spectrum is obtained by dividing the sample spectrum by the reference spectrum:
In FTIR microspectroscopy, an IR spectrometer is combined with a microscope facility in order to study very small samples (5-10 μm). FTIR microspectroscopy can be used to produce 2D or 3D “chemical image” of a sample. Each pixel (or spatial location) is represented by an infrared spectrum. Thousands of interferograms can be collected simultaneously and then transformed into infrared spectra. Pixel size < 10 μm.

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**Advanced techniques: FTIR MICROSCOPY**

- FTIR MICROSCOPY IN SIB-labs KUOPIO
  - PerkinElmer Spectrum Spotlight 300:
    - Microscope and FTIR imaging unit in the same package
    - Modern FTIR imaging system
    - Produces images of sample chemical composition
    - Pixel size of 6.25 μm and 25 μm
    - Two detectors: single element and 16 element linear detector
    - Spectral range: 7800-700 cm⁻¹ (imaging) and 7800-600 cm⁻¹ (microscope)
    - Works in transmission and reflection (ATR) modes
Example

- Mineral and collagen distribution in bone

Raman spectroscopy
Mr Raman

Venkata Raman
The Nobel Prize in Physics 1930
Chandrasekhara Venkata Raman, born on November 7th, 1888.
In 1922 he published his work on the "Molecular Diffraction of Light", the first of a series of investigations with his collaborators which ultimately led to his discovery, on the 28th of February 1928, of the radiation effect which bears his name ("A new radiation", Indian J. Phys., 2 (1928) 387), and which gained him the 1930 Nobel Prize in Physics.

ORIGIN OF RAMAN SPECTRUM

- The origin of Raman spectra is markedly different from that of IR spectra

- In Raman spectroscopy, the sample is irradiated by intense laser beams in the UV-visible region ($\nu_0$)
  - Scattered light is observed

- Scattered light consists of two types:
  - Rayleigh scattering
  - Raman scattering
Rayleigh and Raman scattering:

**Rayleigh scattering:**
- Strong
- Same frequency as the incident light ($\nu_0$)
- Rayleigh is filtered out from the signal

**Raman scattering:**
- Very weak (~$10^{-5}$ of the incident light)
- Has frequencies $\nu_0 \pm \nu_m$
- $\nu_0 - \nu_m$ is called the Stokes line
- $\nu_0 + \nu_m$ is called the anti-Stokes line

In Raman spectroscopy, the vibrational frequency ($\nu_m$) is measured as a shift from the incident beam frequency ($\nu_0$)
Raman Spectroscopy

CHARACTERISTICS OF RAMAN SPECTROSCOPY

- Non-contact, non-destructive analysis
  - No sample preparation needed
  - Fast, sensitive, flexible...
- Scattering in general is dependent on the frequency of the excitation radiation to the fourth power
- Rayleigh scattering is about $10^5$ times stronger than Raman scattering
- Stokes and Anti-Stokes scattering are related to the population in the ground state and the first excited vibrational level
**RAMAN SPECTROSCOPY IN PRACTICE**

- Simple dispersive Raman spectrometer:

![Diagram of a simple dispersive Raman spectrometer](image)

**QUANTITATIVE RAMAN SPECTROSCOPY**

- In absorption spectroscopy, a background spectrum is without a sample and with the sample
  - The ratio of spectra is used to quantificate the amount of light transmitted through the sample

- Raman spectroscopy is based on scattering and a ratio method cannot be used to determine the amount of scattered light unless an internal standard is present in the sample
  - Quantification of Raman spectroscopy is much more complicated !!
  - Typically, ratios of bands inside the spectrum are calculated
Intensity of Raman Bands...

\[
I_{\text{Raman}} = KVCI_0
\]

Constant for each Raman band
Intensity of Raman band
Concentration
Volume
Intensity of Laser

Note: Lambert-Beer \( A = \sigma LN \)

**RAMAN SPECTROSCOPY IN SIB-labs KUOPIO**

- **Bruker Senterra 200LX:**
  - Dispersive Raman Microscope
  - 785 nm and 532 nm excitation lasers (easy switching)
  - Mounted to Olympus BX microscope allowing all optical microscopy capabilities
  - Wide spectral ranges:
    - 3300-80 cm\(^{-1}\) (785nm)
    - 4200-80 cm\(^{-1}\) (532nm)
  - Spectral resolution of 3 cm\(^{-1}\)
  - Spatial resolution \( \approx 1 \mu m \)
RAMAN SPECTROSCOPY IN SIB-labs KUOPIO

- Bruker Senterra 200LX:
  - Measurement options:
    - Point measurements or mapping
    - Confocal measurement
    - Measurement via Fiber optics

Raman spectroscopy vs. IR spectroscopy
Infrared & Raman Spectroscopy

**IR**

Absorption of IR light in a sample

**Raman**

Inelastic scattering of light at a molecule

**IR Raman**

RAMAN SPECTRUM vs. IR ABSORPTION SPECTRUM

barbital
**RAMAN SPECTROSCOPY vs. IR SPECTROSCOPY**

**Raman spectroscopy**
- Emission of scattered laser light
- **Senses polarizable vibrations**
- Little or no sample preparation is required
- Water and CO\textsubscript{2} are very weak scatterers
  - Aqueous samples can be directly measured
  - Purging is unnecessary
- Fiber optics can be used for remote analyses
- Quantitative analysis very challenging

**IR spectroscopy**
- IR light absorption
- **Senses dipole vibrations**
- Sample preparation required
- Short optical pathlength required
- Water and CO\textsubscript{2} strongly absorb IR light
  - Suitable for non-aqueous samples
  - Purging needed
- Allow quantitative analysis

**SUGGESTED READING:**

**Books:**

**Recommended web pages:**
- http://orgchem.colorado.edu/hndbksupport/irtutor/tutorial.html
- http://www.chemlin.net/chemistry/ir_spectroscopy.htm
- http://www.dolpoms.ac.uk/tipplib/raman/index.php
- http://webbook.nist.gov/