FT-IR Spectroscopy

An introduction in measurement techniques and interpretation
History

• Albert Abraham Michelson (1852-1931)
  – Devised Michelson Interferometer with Edward Morley in 1880 (Michelson-Morley experiment)
  – Detects the motion of the earth through the ether
    • There was no!
    • Death knell for the ether theory
  – No detector was available
  – Nonexistence of Fourier Transform algorithms capable of being performed by human calculators
• Rubens and Wood presented the first real interferogram in 1911
History

• 1950-1960 Air Force Cambridge Research Laboratories at John Hopkins University tested high resolution spectrometers for astronomical applications

• “Cooley and Tukey” algorithm allowed computing of Fourier transforms in 1965
  – The critical factorization step used by Cooley and Tukey had already been recognized and described by Gauss as early as 1805!
What is FT-IR?

- Fourier Transform InfraRed – Spectroscopy
- Advancement of dispersive spectrometers
- Interferometer
  - Beamsplitter
  → Interferogram
- After recording Fourier Transformation
  - Mathematical technique
Interferometer

• Michelson Interferometer
  – Beamsplitter splits up light
    • Reflected on moveable and fixed mirror
    • Interference after recombination
    • By smoothly translating the movable mirror, the optical path difference between the beams reflecting off the two mirrors is varied continuously which leads to a change of wavelength → Interferogram
Interferogram

- Sum of cosine signals of all frequencies
- Translation of the symmetric interferogram into a spectrum by Fourier Transformation:

\[ S(\tilde{\nu}) = \int_{-\infty}^{\infty} I(x) \cos(2\pi \tilde{\nu} x) dx \]
Interferogram

- Experimental interferograms are asymmetric due to phase shifts
  - Complex FT required, including cosine and sine terms
Dispersive vs. FT-spectroscopy

• FT-devices have multiple advantages compared to dispersive devices:
  – **Multiplex-Advantage**: Capability of a complete wavelength scan at a time
    • Reduced background noise
    • Less scan time
  – **Throughput-Advantage**: Optical throughput is ten times higher
  – **Connes-Advantage**: Stable wavenumber because of internal reference (He-Ne-Laser)
Interpretation

- Typical absorbance positions:
  - “Lipids”
    - $=\text{CH}_2$: 3100-3000
    - $-\text{CH}_2-, -\text{CH}_3$: 3000-2850
  - Protein Amide I:
    - 1690-1600
  - Protein Amide II:
    - 1575-1480
  - Nucleic Acid:
    - $-\text{PO}_2$: 1225; 1084
Amide I & Amide II

- **Amide I:**
  - $-\text{C}=\text{O}$ \text{(stretch)}
    - prim. Amids
    - sec. Amids
    - tert. Amids

- **Amide II:**
  - $-\text{C}-\text{N}$ \text{(stretch)}; $-\text{C}-\text{N}-\text{H}$ \text{(deformation)}
    - prim. Amids
    - sec. Amids

- Secondary structure of proteins (α-Helix, β-sheet, random coil)
Amide I

- Here an example for varying secondary structure:
Sample Recording

• Solid samples are recorded with KBr pressed as a pellet
• Liquid samples are recorded with water as solvent
• Adjuvants have to be subtracted after recording to obtain the pure protein spectrum
• The spectrum is recorded with wavelength [cm⁻¹] on the abscissa and transmission [T%] or absorbance [A] on the ordinate
Interpretation
First steps

- Truncate the spectrum to the favored Amide region (Amide I: 1720-1580cm\(^{-1}\))
- Baseline correction
  - Fits a straight baseline to the non peak sections of a trace
Baseline correction
First steps

• Fourier Self-Deconvolution
  – High pass Fast Fourier Transform filter
  – Based on a method described by Griffiths and Pariente in 1986
  – Two filters
    • Exponential filter is used to sharpen spectral features
      – $\gamma$ equals the FWHH of the widest resolvable peak
    • Smoothing filter
      – FSD tends to increase the noise in the data
      – Bessel filter is applied
Fourier Self Deconvolution
Qualitative Interpretation

• The 2nd derivative
  – Convolution technique described 1964 by Savitzky and Golay
  – Reveals the secondary structure of the protein sample
Quantitative Interpretation

• Quantitative interpretation is a quite difficult technique
• Operator dependent method
• Starting with the deconvoluted trace a peak fitting is performed
  – Algorithm is described by Marquardt and known as the Levenberg-Marquardt method in 1963
• There are multiple solutions for one trace
  – Even though the algorithm appears to have found a minimum, there may be a better solution for a given number of peaks and line shapes
  – Operator has to give more “information”
Example
“Same, same but different”
And then?

• The peak report contains
  – Number of peaks
  – Peak positions
  – Area of the peak (AUC)

• The sum of the AUCs represents the amount of secondary structure if related to the 2nd derivative peak positions