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 an 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 \rightarrow 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
 - \rightarrow Interferogram





Interferogram

• Sum of cosine signals of all frequencies



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

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:
 - <u>Multiplex-Advantage</u>: Capability of a complete wavelength scan at a time
 - Reduced background noise
 - Less scan time
 - <u>Throughput-Advantage</u>: Optical throughput is ten times higher
 - <u>Connes-Advantage</u>: Stable wavenumber because of internal reference (He-Ne-Laser)

Interpretation

- Typical absorbance positions:
 - "Lipids"
 - =CH₂: 3100-3000
 - -CH₂-, -CH₃: 3000-2850
 - Protein Amide I:
 - 1690-1600
 - Protein Amide II:
 - 1575-1480
 - Nucleic Acid:
 - -PO₂⁻: 1225; 1084



Amide I & Amide II

- Amide I:
 - -C=O (stretch)
 - prim. Amids
 - sec. Amids
 - tert. Amids
- Amide II:
 - -C-N (stretch); -C-N-H (deformation)
 - prim. Amids
 - sec. Amids
- Secondary structure of proteins (α-Helix, ß-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
 - γ 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
 - Operater has to give more "information"

Example



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"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