

Quantification / qNMR

PERCH NMR Software Course

Turku - 16- 17.5.2006

Spectral Parameter Prediction



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Overview

- Methods
- Linear & Non-Linear Approaches
- Prior Knowledge
- Applications
- Examples



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Spectroscopic Methods

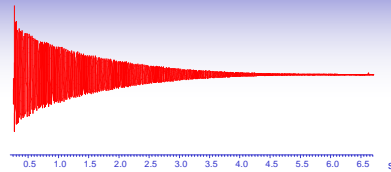
- UV, VIS, IR, Fluorescence
- HPLC, GC, LC
- MS
- **NMR**



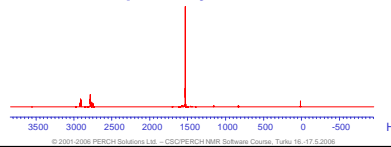
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Time Domain



Frequency Domain



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Time vs. Frequency Domain

- Time Domain
 - Raw data
 - No processing artifacts
 - Difficult to implement prior knowledge
 - Difficult to comprehend
- Frequency Domain
 - Processed data (windowing, phasing)
 - Processing artifacts
 - Easy to implement prior knowledge
 - Easy to visualize



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Linear & Non-Linear Approaches

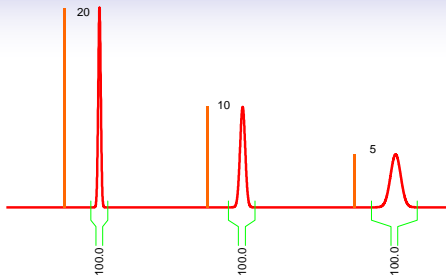
- Linear
 - Linear prediction (time domain)
 - Integration (freq. domain)
 - Histograms
- Non-Linear
 - Non-linear fitting in the time-domain (VARPRO) - Total-Line-Shape Fitting (freq. domain)
 - Other iterative approaches



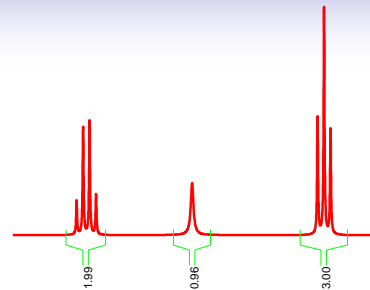
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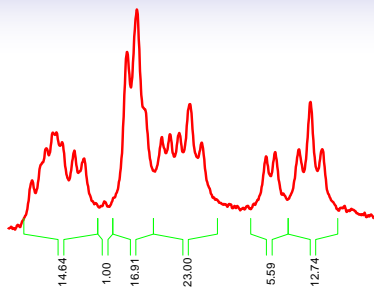
Discrete - Continuous Lines



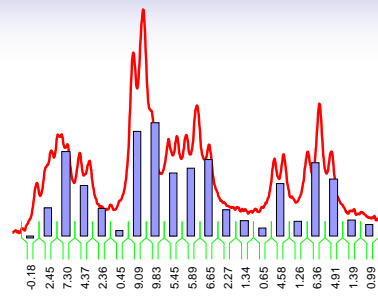
Integration



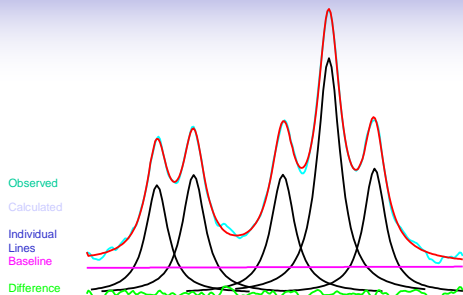
Overlapping Signals



Histograms (Buckets)

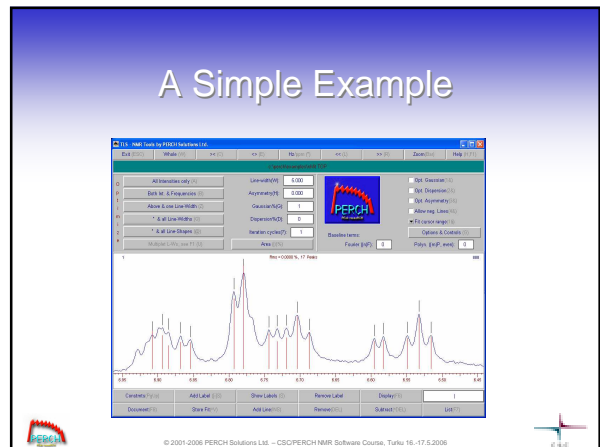
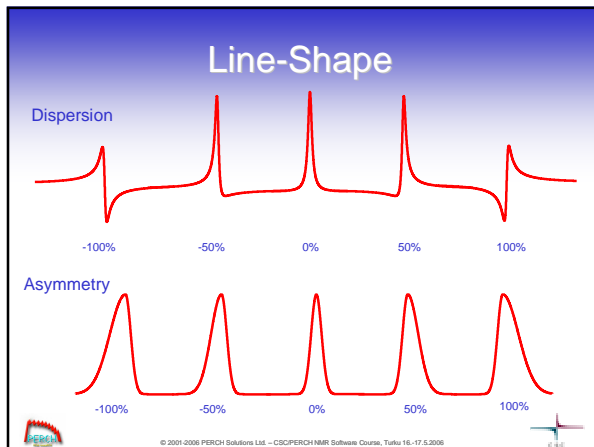
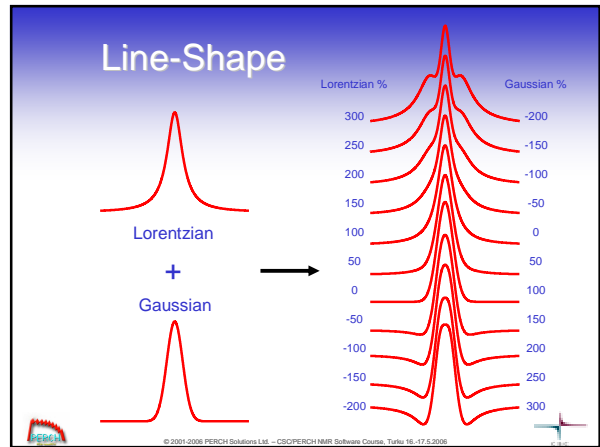
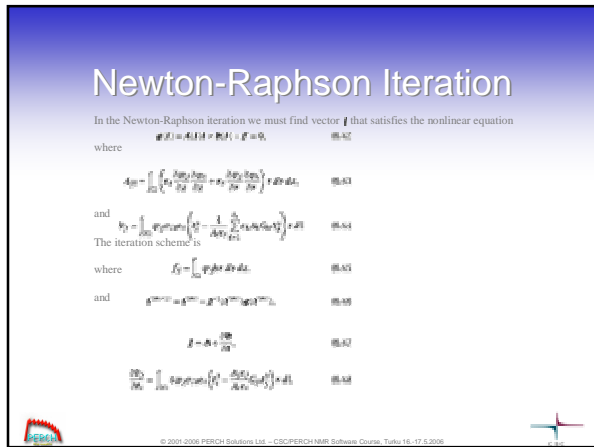
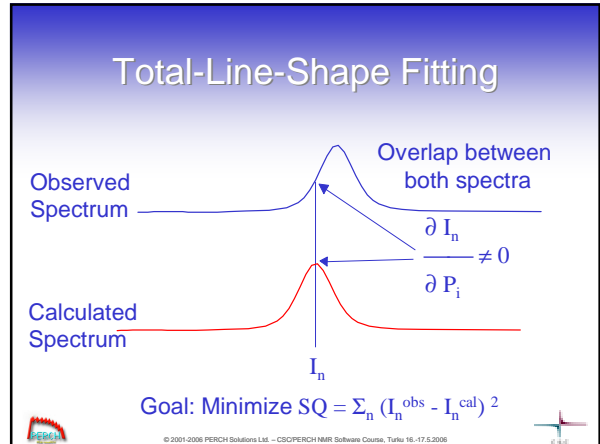
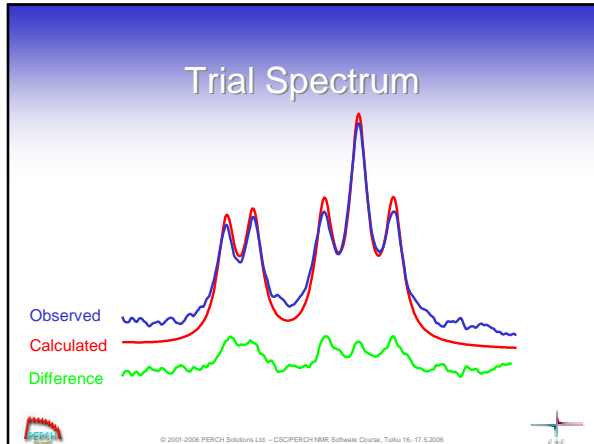


Deconvolution



Spectral Parameters

- Frequency
- Intensity
- Line width
- Line shape
- Baseline



Prior Knowledge

- Frequency
- Intensity
- Line-width
- Line-shape
- Areas
- NMR multiplets
- and any linear combination of the above



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Quantification by Deconvolution

^{13}C - ^2H Lactate Isotomers: 33 Lines



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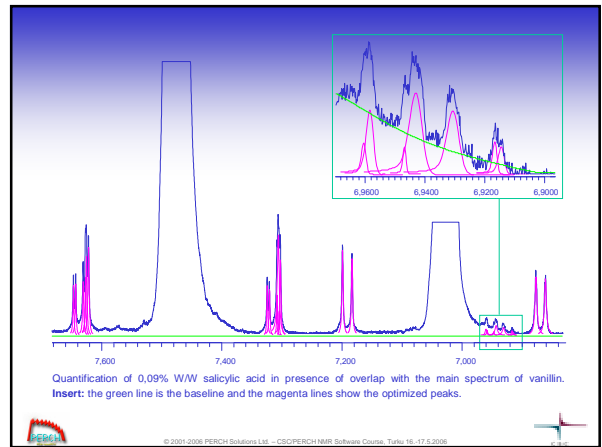


Application of qNMR

- Drug impurity analysis
- Bio-Fluids (Medical Diagnostics)
- Metabonomics
- Protein-ligand interactions
- Lipid peroxidation
- T1 Measurement



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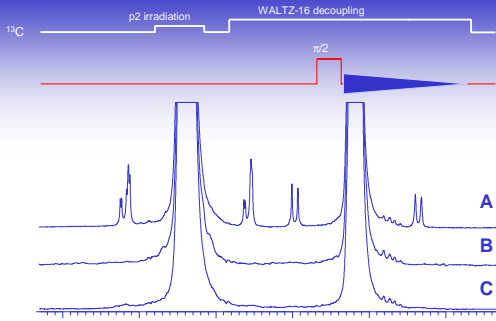


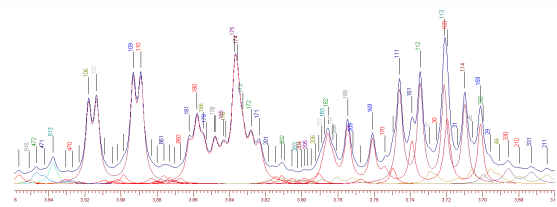
Figure 1. A) Standard ^1H NMR spectrum. B) WALTZ- ^{13}C -decoupled (offset for decoupling 126.5 ppm) ^1H NMR spectrum C) ^1H NMR-spectrum using pulse sequence described at the top of the picture. Offset for p2 and WALTZ was altered so that the aromatic carbon region was swept through during 64 scans.



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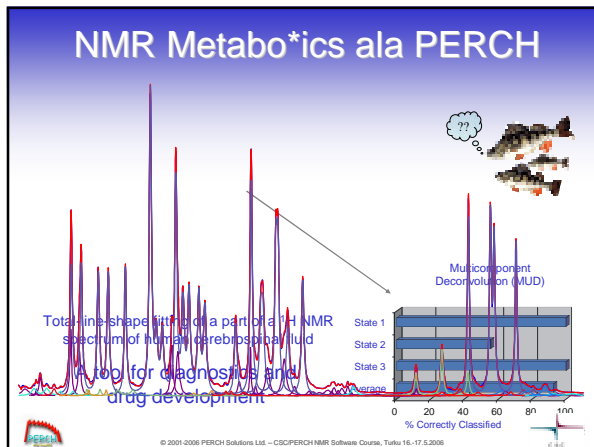
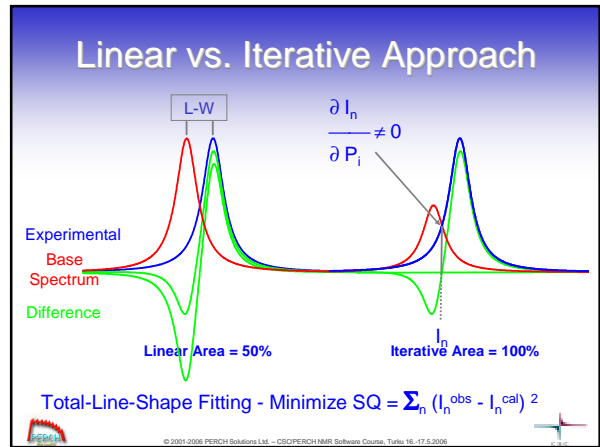
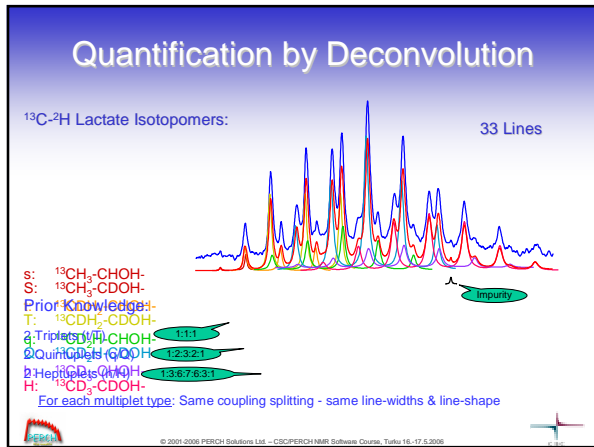
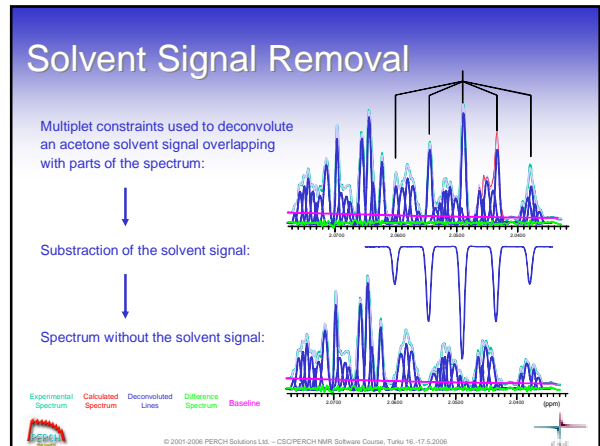
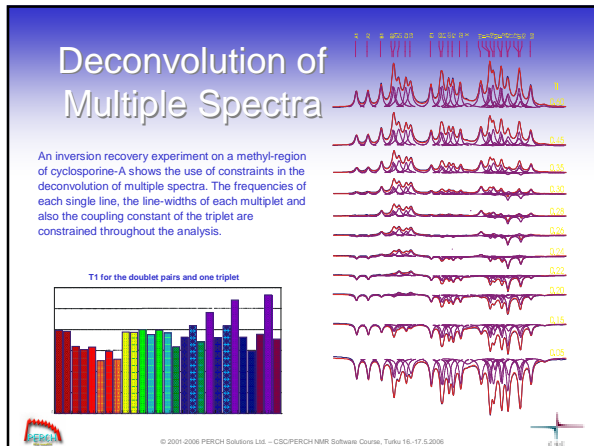


Disease Status Prediction from ^1H NMR Spectra

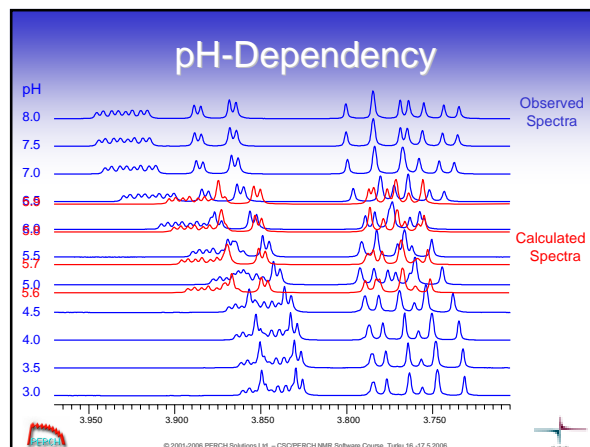
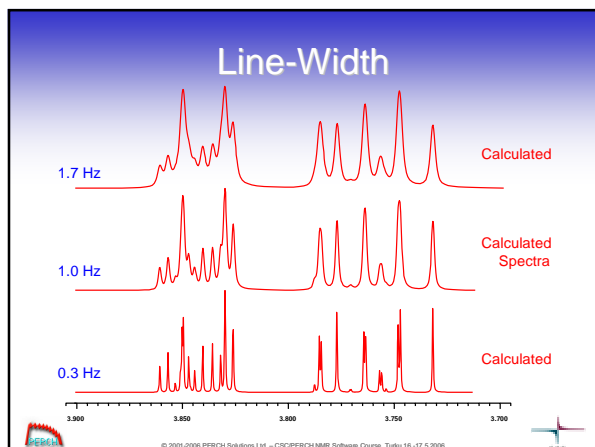
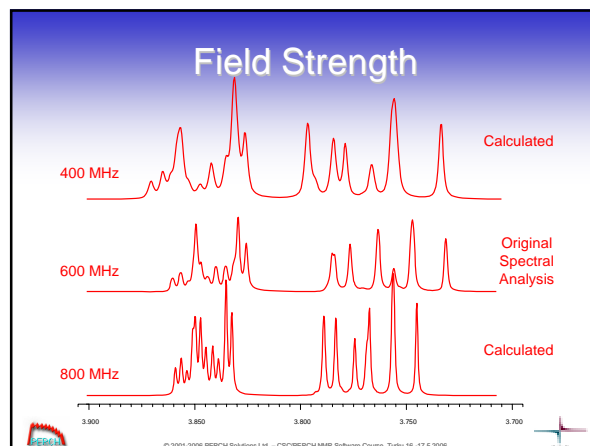
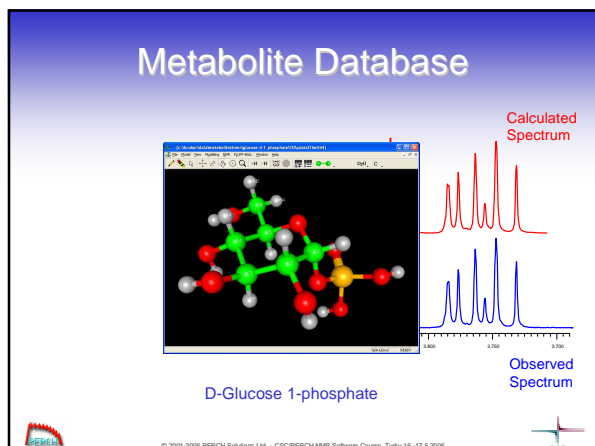


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- ## qNMR Strategies
- Linear Approach
 - Spectral Libraries, Buckets
 - Constraint Total-Line-Shape Fitting (CTLTS)
 - Frequencies, Intensities, Signal Areas
 - Couplings (Multiplets)
 - Line-Shape Parameters
 - Any linear combination of the above
 - Quantum Mechanical TLS Fitting (QM-TLS)
 - Adaptive Spectral Libraries: Field, pH,...
 - Integral Transforms
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Adaptive Spectral Database

- Completely assigned & verified
- Free of artefacts, impurities etc.
- Flexible adaptable to any parameterized condition (field-strength, solvent, pH, etc.)
- Minimum storage space (just parameters)
- Fast calculation

Applications?

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CONCLUSIONS

- NMR is a versatile quantification tool with many potential applications in chemistry and biosciences (both in-vitro & in vivo).
- With modern instrumentation & software, the method rivals the traditional methods like HPLC also in economy – extensive calibration is not necessary.

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