CRAFT
Complete Reduction to Amplitude-Frequency Table

Spectrum to Spreadsheet: Automated Extraction of NMR Data

April 2013
CRAFT
Complete Reduction to Amplitude-Frequency Table

Converting an NMR spectrum into a useable, tabular format has traditionally been a challenge for researchers who want to use NMR to investigate large numbers of samples.

CRAFT is a new utility built into VnmrJ 4 Software that automatically extracts the information inherent in an NMR data set and converts the data into a simple spreadsheet. The result is a simple, rapid, and automated method for interpreting NMR data with high reproducibility and fidelity.
Current NMR Analysis Workflow

The NMR workflow for data analysis is primarily based on manual processing and interpretation of each individual spectrum.

This is a slow, tedious, and error-prone process.

There are various drawbacks to interpretation based on a processed spectrum: significant issues can be introduced by the Fourier Transform, including baseline issues and phase distortions.

Spectral Deconvolution

What do we mean by deconvolution?

When we talk about “deconvolution” we mean extracting individual peaks from a complex spectrum.
Spectral Deconvolution

What do we mean by deconvolution?

What we really want is to convert a human-readable NMR spectrum into a data format that can be used for computer-based data manipulation.

This means converting the data into a spreadsheet.
Spectral Deconvolution

What do we “really, really” want from deconvolution?

By analyzing the data directly in the time domain, all the issues induced by using a Fourier Transform are avoided.

Phase and baseline corrections are no longer relevant, and overlap is based solely on acquisition time and decay constant.
CRAFT
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Spectrum to Spreadsheet
CRAFT – Deconstructing a Spectrum

The CRAFT workflow is simple. Once data are collected, Regions of Interest (ROI) are selected interactively, and then CRAFT does the work.

The result is a spreadsheet containing all the information that was captured in the original experiment.
Once data have been captured in the spreadsheet, analysis is essentially complete.

Most users want to see how well the data reduction step worked. CRAFT includes a complete set of tools to allow visual inspection of the results.
The common method for displaying CRAFT data is to show the experimental NMR spectrum after Fourier Transform, the CRAFT spectrum reconstructed from the final spreadsheet, and the residual signal, or the “difference between the two” spectrum.

This step is for user convenience; it is not required for analysis!
An initial proof-of-concept study used eleven samples containing prednisone and ibuprofen made in a range of concentrations. After data collection (3x of each conc., 2 spectra per sample, 66 spectra total), CRAFT deconstructed the spectra.

The result table was used to generate concentration values for each component.
The CRAFT workflow for a whole collection of spectra is simple:

1. Collect the data
2. Create a study cluster
3. Assign cluster ROIs
4. CRAFT
5. Extract library components
6. Generate final results table
7. Export for analysis
CRAFT – Fermentation Broth

Even complex data sets are easily handled. Broad resonances can be excluded based on user preferences.
This is another way to visually inspect CRAFT results. A stacked plot was created showing each line extracted from an expansion of the complete data set.
CRAFT ~2.3 Min/Spectrum

Overlap in the frequency domain is not a problem.

Individual models

Residual

CRAFT Experimental
A major pharmaceutical customer collaborated with us to test CRAFT against a skilled human analyst.
CRAFT Analysis vs. The Human Expert

Results were performed on each spectrum by the analyst and plotted against those recovered by CRAFT’s fully automated workflow.

It took 10 hours of human interpretation to get the same results that CRAFT provided automatically in minutes.
For many applications, spectral alignment of NMR resonance is required before meaningful analysis can be accomplished. This is due to the sensitivity of some NMR signals to the local chemical environment.

CRAFT provides a robust and intuitive alignment tool.
CRAFT and Metabolomics

CRAFT is a widely applicable utility that can be used by many different types of NMR users on many different kinds of analysis problems.

The nature of Metabolomic and Food Science studies implies spectra collected on a large number of nominally equivalent samples.

Agilent Technologies is a market leader in these areas, and CRAFT interfaces seamlessly with Agilent’s downstream data analysis solution, Mass Profiler Professional.
NMR Metabolomics Workflow

Acquisition

Identify & Quantify

Alignment & Statistics

Enrich & Visualize

NMR

CRAFT VnmrJ 4

Mass Profiler Professional

Pathway Architect
# CRAFT – MPP Compatible Output

One of the default methods for exporting a CRAFT analysis is an MPP-compatible .cef file format.

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<thead>
<tr>
<th>Profiled Data Relative Concentrations</th>
<th>Export date</th>
<th>Thu May 10 14:38:55 PDT 2012</th>
</tr>
</thead>
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<td>Alanine</td>
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<td>Choline</td>
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</table>
The combination of CRAFT and MPP allows users to exploit the full range of statistical analysis tools available in MPP.
To demonstrate the power of CRAFT, we analyzed three over-the-counter soy dietary supplements.
Analysis of Soy

Three different soy supplements were investigated; 15 replicates of each sample were used for the analysis.

These spectra are representative of the results from each sample.
Analysis of Soy – Targeted for Isoflavonoids

Isoflavonoids are the class of compounds generally associated with the dietary benefits of soy.

Those aromatic resonances representing the isoflavonoid compounds in the soy extracts were selected for analysis.
Targeted Analysis

Soy dietary supplement extract

CRAFT analysis of the targeted resonances was straightforward and results were exported.

Principal Component Analysis (PCA) readily discriminated between the three different supplements.

Soy dietary supplement extract + CRAFT
(Avg. CRAFT’ing time: ~1.7 min/spectrum)

Soy supplement CRAFT + MPP (PCA Analysis)
Untargeted Analysis

CRAFT analysis was also performed on the non-aromatic resonances, primarily representing lipids, sugars and amino acids. No attempt was made to identify the individual components. Results were exported for analysis.

PCA readily discriminated between the three different supplements.
Once a data set has been CRAFT’ed, the CRAFT table can extract useful information from the data set.

For example, those resonances associated with relevant compounds can be used to recreate spectra containing only the peaks of interest.
CRAFT – Extract Only the Data You Need
CRAFT’ing a Needle From a Haystack

CRAFT can simplify analysis of a very complicated spectrum.

Tetracycline is known to bind to endogenous proteins in plasma. NMR can be used to directly measure the concentration of unbound tetracycline using CRAFT analysis of a fingerprint peak.

Smriti Khera, David Russell & Krish Krishnamurthy (2012)
Redefining “Overlap”

CRAFT analysis is based on time-domain data so peak overlap in the frequency-domain is not an issue. CRAFT was used to extract a peak from a strongly congested region with 100% fidelity.

Reaction Monitoring
MRI Chemical Shift Imaging data is often broad, but CRAFT can cleanly extract useful chemical shift and concentration data.

Here, an internal/external mouse muscle cell lipid ratio can be a potential biomarker for muscle atrophy. The ratio would be totally intractable based on standard 1D deconvolution techniques.
Conclusions

✓ NMR is a valuable tool for Metabolomics research.

✓ CRAFT allows automated reduction of very complex spectra to a spreadsheet result with high fidelity. The requirement for a human to analyze each data set is no longer a research bottleneck.

✓ Once converted to a spreadsheet, NMR data can be imported into Agilent’s MPP software suite for analysis.

✓ The CRAFT technique can provide answers for a wide variety problems.
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