



## ImatraNMR: Novel software for batch integration and analysis of quantitative NMR spectra

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### ABSTRACT

Quantitative NMR spectroscopy is a useful and important tool for analysis of various mixtures. Recently, in addition of traditional quantitative 1D <sup>1</sup>H and <sup>13</sup>C NMR methods, a variety of pulse sequences aimed for quantitative or semiquantitative analysis have been developed. To obtain actual usable results from quantitative spectra, they must be processed and analyzed with suitable software. Currently, there are many processing packages available from spectrometer manufacturers and third party developers, and most of them are capable of analyzing and integration of quantitative spectra. However, they are mainly aimed for processing single or few spectra, and are slow and difficult to use when large numbers of spectra and signals are being analyzed, even when using pre-saved integration areas or custom scripting features. In this article, we present a novel software, ImatraNMR, designed for batch analysis of quantitative spectra. In addition to capability of analyzing large number of spectra, it provides results in text and CSV formats, allowing further data-analysis using spreadsheet programs or general analysis programs, such as Matlab. The software is written with Java, and thus it should run in any platform capable of providing Java Runtime Environment version 1.6 or newer, however, currently it has only been tested with Windows and Linux (Ubuntu 10.04). The software is free for non-commercial use, and is provided with source code upon request.

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### 1. Introduction

Quantitative NMR spectroscopy is a valuable tool used routinely in the analysis of mixtures in many research and industrial applications. In addition to traditional quantitative <sup>1</sup>H and <sup>13</sup>C spectroscopy, in recent years several quantitative multipulse 1D and 2D sequences have been introduced for both liquid and solid-state NMR [1–9].

In order to use information found in quantitative spectra, the spectra has to be analyzed: signal areas are measured using integration or line-shape fitting, and the resulting values are used to determine concentration ratios of the mixture components. This kind of regular integration of 1D and 2D spectra is a basic feature found in practically all processing software. In addition to processing software provided by spectrometer vendors, numerous third party processing and analysis software exist, sometimes with emphasis on some special application, such as spectral analysis (PERCH [10]) or DOSY (DOSY Toolbox [11] and NMRnotebook [12]). Regular processing software performs well when dealing with relatively low number of samples and/or signals, which is usually the case in synthetic organic chemistry. However, when

dealing with large amounts of spectra, the existing tools can be cumbersome and inefficient, even when scripting or other automation features are available. This was exactly the situation we encountered when analyzing large numbers of oil products, containing vast numbers of signals, and which eventually lead us to the creation of the novel software ImatraNMR, presented in this article.

The goal of analyzing the aforementioned oil products was to analyze the immense amount of information present in a high-resolution, quantitative carbon spectrum with statistical models, as well as integrate well known signals from large amounts of spectra. As the carbon spectra of oil products contained tens or hundreds of signals, even analyzing a single spectrum with regular processing software is tedious, and when tens or hundreds of samples are being considered, it becomes just impractical. Thus the need for automatic analysis of this data was evident, and custom software aimed towards mass integration and analysis was developed.

While the need of this kind of software resulted from quite specific type of samples, it is definitely usable in various other scenarios as well. These include practically any situation when multiple similar type samples are being analyzed, such as any comparison of similar mixtures or screening of certain features. In some fields, such as metabolomics, these kind of situations are probably the

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norm rather than the exception. Also complex routine analysis, as a part of product quality or process control, could benefit from automation possible with ImatraNMR. In this article, we'll provide two examples for the use of ImatraNMR: simple integrations of 1D and 2D spectra obtained from oil products, and the analysis of  $^1\text{H}$  spectra obtained from blood plasma in conjunction with statistical analysis.

## 2. Imatra NMR workflow

The basic workflow of using ImatraNMR in quantitative analysis is presented on Fig. 1. The workflow consists of four basic stages, and each of these stages produces information, stored in files, which is passed to next stage. The only stage involving ImatraNMR software is the third stage, so any spectrometer, pulse sequence or processing software can be used in conjunction with ImatraNMR, as long as the resulting spectra is processed and saved in a file format that is compatible with ImatraNMR. It is also worth noting that ImatraNMR uses transformed spectra: it can not perform Fourier transform and relies on external processing software, which there are plenty.

The operation of ImatraNMR is thus simple: it reads processed spectra, does the analysis, and writes the results in (several) output files. The internal structure ImatraNMR can be visualized as a simple long pipeline, with each step consisting of a single operation to be done to all the spectra. This is in line with the batch processing nature of ImatraNMR. A simplified overview of the pipeline is presented on Fig. 2. All aspects of ImatraNMR are controlled by control files, which there are three kinds:

- **ISL-file (Imatra Spectrum List):** Determines which spectra to use.

- **ICF-file (Imatra Config File):** Determines the operations to perform on the spectra and their parameters.
- **IIL-file (Imatra Integral List):** Determines the integration regions to use.

All the control files are regular text files, with custom, but simple formatting. The separation of control information into three individual files allows, for example, to use same spectra but different integral area list (IIL-file). Processing is also separated into loading and analysis stages in the GUI (Fig. 3), which allows fast analysis without reloading spectra into memory every time analysis parameters or integration areas are modified. ImatraNMR also includes scripting support, which offers finer control over the analysis process.

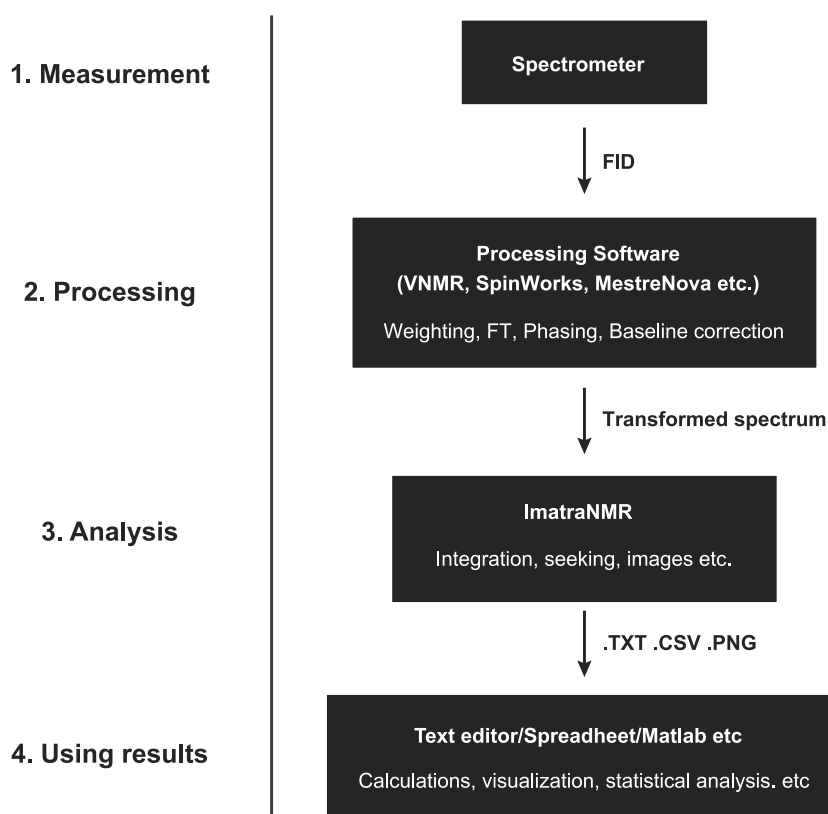
## 3. Features and limitations

We'll now briefly discuss what is possible to do with the software, before diving in to some concrete examples.

### 3.1. Supported spectrum formats

ImatraNMR can read spectra processed on **Bruker TopSpin**, **Varian VNMR/VNMRJ**, **SpinWorks**, **MestreNova**, **NMRPipe** or any other software capable of outputting spectra in the following formats:

- **Bruker/TopSpin:** Processed data from TopSpin software (pdata-directory) [13].
- **VNMR/VNMRJ:** Phased data copied from Varian VNMR/VNMRJ software [14].



**Fig. 1.** The basic workflow when using ImatraNMR in quantitative analysis: (1) Performing the (quantitative) NMR experiment itself and recording FID. (2) Transforming the FID with FT to a spectrum, and doing all associated processing and optimization, such as apodization, phasing, baseline correction, etc. (3) Using ImatraNMR to extract information from the spectra. (4) Using the information provided by ImatraNMR as such (plotting) or for further analysis, such as statistical methods.

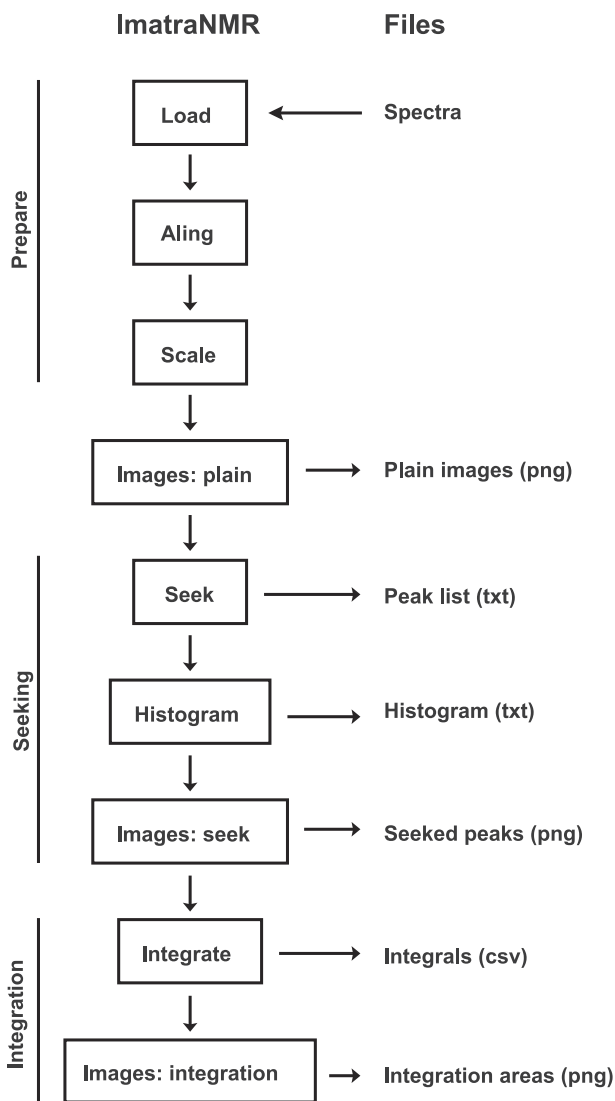


Fig. 2. The simplified pipeline of ImatraNMR software. At each stage, something is done or some information is extracted from every spectrum, until it's passed on to next stage.

- **SpinWorks:** SpinWorks fileformat, as produced by saving processed data in the same directory as the FID in SpinWorks [15].
- **NMRPipe:** Transformed data from NMRPipe processing tools, single binary file [16].
- **DmFit:** DmFit file format, as produced by various software. Single text file containing frequency-intensity pairs. Tested with SpinWorks. The frequency must be in ppm, not Hz, in order to work. Only 1D spectra is supported.
- **MestreNova:** Text file format, produced by saving spectrum in ASCII format in MestreNova [17]. Single text file containing frequency-intensity pairs. Only 1D spectra is supported.
- **ImatraNMR:** Native format of ImatraNMR. Directory which contains spectrum (binary) and parameters (text) files. Simple to read and write with external tools.

Other than the file format, there are few restrictions for the input data. For fast (in-memory) analysis, all spectra must fit into memory at the same time, which limits the size or number of spectra somewhat. However for large datasets, such as detailed 2D spectra, queued mode can be used, in which only single spectrum must fit the memory at a time.

### 3.2. Supported operations

- **Alignment/Referencing:** Uses selected signal to align the chemical shift scale in the spectrum in order to accurately synchronize the scales of all processed spectra.
- **Scaling:** Normalizes spectra so integrals are reported in relation to the reference signal (e.g. signal from internal or external standard).
- **Peak seeking:** Finds peaks from 1D/2D spectra.
- **Peak histogram:** Classifies found peaks into bins using their chemical shift. The found signals are classified into same bin, if they are close enough (within given offset). List of the groups, containing area where the signals included in the group exist, is outputted. The list can be then used as integration area list (IIL-file) to integrate the defined regions, yielding a list that contains integral for any area that has signal present in any of the analyzed spectra.
- **Integration:** Integration of peaks or areas, with various options, including automatic peak area detection and “searchable” integration areas, which alter the exact integration area on spectrum-by-spectrum basis.
- **Imaging:** Creating images of the spectra, with marking found peaks or integrals as an option. Various options, such as image size and limiting the image area (cropping) are also provided.
- **Reshaping and Resampling:** Spectrum resampling to fewer datapoints, cropping to include specific region only, or slicing to include multiple regions. The slices can also be aligned separately.
- **Exporting:** Exporting spectra in ImatraNMR or text format. This can be used to save reshaped/resampled spectra.

### 3.3. Output file formats

- **Integrals:** Standard CSV-file (Comma-Separated Values), loadable in various spreadsheet and mathematical software.
- **Peak lists:** Custom text format, human readable, entries can be used as integral areas in integral area lists in ImatraNMR (in IIL-files).
- **Peak histogram:** Custom text format, human readable, can be used as integral area list in ImatraNMR
- **Images:** Bitmap graphics saved in standard PNG file format.
- **Spectra:** Native ImatraNMR or txt format. Simple and easily readable in other analysis software.

### 3.4. Additional features

- **Scripting and console interfaces:** In addition to the standard minimal GUI, ImatraNMR offers basic scripting and limited console interface, which provides additional flexibility.
- **Integration area list conversion:** While ImatraNMR can only read it's native integration list format (IIL-file), it includes conversion tool for converting SpinWorks integral list into IIL-format. The distribution also includes script for MestreNova for saving integral areas in IIL format. These features enable the use of SpinWorks or MestreNova GUI to mark the integration areas for ImatraNMR.
- **Automatic IIL-file creation:** While it can be feasible to create input files manually and whole directories containing spectra can be loaded quite easily, IIL-files can be also created automatically for any directory containing spectra, to be used as such or as a templates for further customization.

## 4. Examples

Two examples are provided to illustrate what can be achieved with ImatraNMR software. The first example demonstrates the

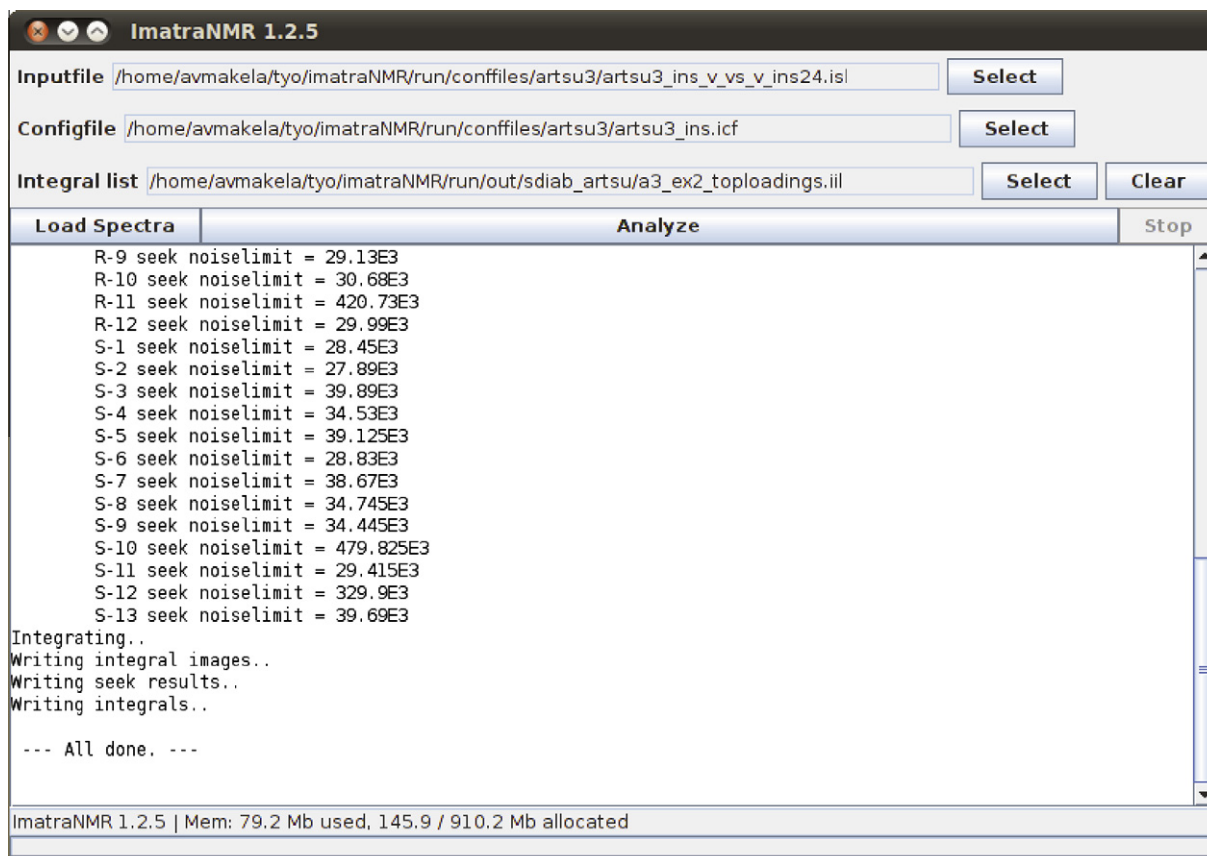


Fig. 3. The simple GUI of ImatraNMR, shown here after successful analysis.

basic integration capabilities of ImatraNMR, used to integrate complex 1D and 2D spectra obtained from base oil samples. As mentioned earlier, analysis of these kind of samples was the initial motivation to develop ImatraNMR. In the second example,  $^1\text{H}$  spectra recorded from blood plasma samples is analyzed with ImatraNMR, demonstrating the use of automatic peak seeking and histogram functions to perform statistical analysis on NMR spectra.

In the examples, some the technical details, such as the construction of input (ISL) and integration (IIL) files are omitted, as these topics are covered in-depth in the manual of the software (see supplementary material). However, the creation of configuration files (ICF) is described, and the full configuration files are listed in appendices. Additionally, the relevant configuration file parameters under discussion are listed in parenthesis.

#### 4.1. Example #1: using 1D/2D NMR and ImatraNMR in the analysis of base oils

Base oils are hydrocarbon mixtures which form the basis of many automotive oils and other lubricants. Modern base oils are made from crude oil with extensive refinement processes or synthetically. Thus, most modern base oils are very pure products, containing practically only saturated hydrocarbons. However, in the molecular level, even synthetically made oils still have much structural diversity, as hydrocarbon chain lengths and branch positions vary. Because the oils are a complex mixtures of structurally similar molecules with similar chemical shifts, NMR spectroscopy becomes challenging. In practice the structural similarity renders  $^1\text{H}$  NMR almost useless, as proton signals are concentrated on two major areas ( $\text{CH}_3$  and  $\text{CH}_2/\text{CH}$ ). On the other hand, carbon spectra provides a lot of information, since it usually can resolve hundreds of signals

(Fig. 4). High quality, quantitative carbon spectra is also usually obtainable relatively easily due to generous amount of substance available, which enables preparation of highly concentrated NMR-samples. Thus, quantitative  $^{13}\text{C}$  NMR is probably the most used method when analyzing base oils and similar oil products. However, even in high-resolution carbon spectra, some signal overlap is present, as the aliphatic carbon signals exist in the relatively narrow region of about 10–50 ppm. With 2D  $^1\text{H}$ - $^{13}\text{C}$  correlation experiments, even more resolving power can be obtained, enabling the integration of practically every signal.

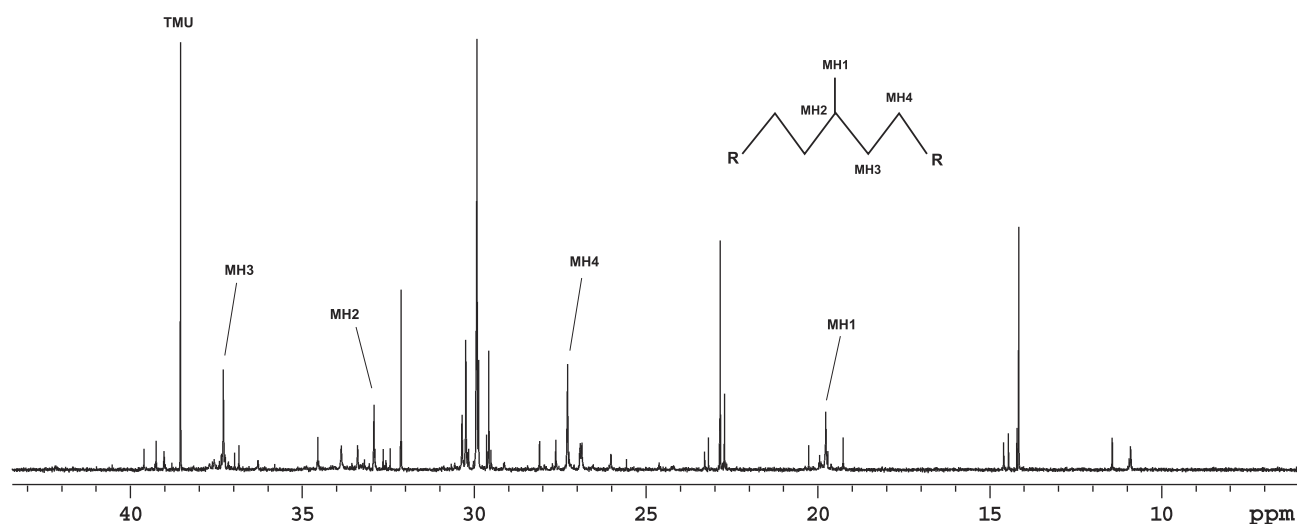
Base oil samples were analyzed with traditional quantitative  $^{13}\text{C}$  spectra and additionally with 2D  $^1\text{H}$ - $^{13}\text{C}$  correlation experiment, HSQC. We used quantitative  $^{13}\text{C}$  spectra to demonstrate simple 1D integration with ImatraNMR, and HSQC to demonstrate the use of 2D integration to resolve overlapping signals in the  $^{13}\text{C}$  spectra. In this example we use limited numbers of samples and spectra for sake of simplicity, but much larger sets have been used and analyzed with the software.

##### 4.1.1. Samples and processing

Thirteen base oils, obtained from Neste Oil were measured with quantitative  $^{13}\text{C}$  spectra. Seven of these oils were also measured with HSQC. Tetramethylurea (TMU) was used as internal standard in all samples. After acquisition, measured  $^{13}\text{C}$  spectra were processed using Varian VNMR 6.1 C software and saved in phasefile format, while the 2D HSQC spectra were processed using SpinWorks 3.1 and saved in native SpinWorks file format.

##### 4.1.2. 1D Integration

To integrate the quantitative  $^{13}\text{C}$  spectra with ImatraNMR, all spectra were first loaded to ImatraNMR using automatically



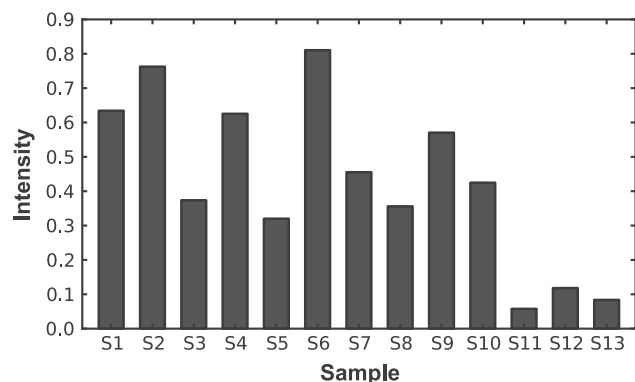
**Fig. 4.** Typical quantitative  $^{13}\text{C}$  spectra of a base oil, obtained in this case from the sample S7. The vast amount of signals is evident. The methyl branch and its associated chemical shifts, including the MH2 signal used in example, are marked in the spectrum.

created spectrum list file (ISL). Then, the ImatraNMR configuration parameters were determined in sequence (the final configuration file used can be found at Appendix A). First, the spectra were referenced and scaled using the TMU methyl signal, found at 38.58 ppm (parameters *align* and *scaleto*). Then, a signal list was created by enabling the seek function, using automatic noise level from the region of 8–10 ppm which should be clean from any signals (*seek*, *seek\_autonoise* and *seek\_autonoise\_mult*). Using the obtained signal list as a reference, integration list was constructed manually, which was used to integrate spectra (*integrate*). During the process, images were created to monitor the integration regions, cropping them to the regions of interest (*image\_plain*, *image\_integrals*, *images\_width*, *images\_height*, *images\_ticwidth* and *images\_crop*).

As a simple example, the signal of methyl branch in a chain, “MH2” (Fig. 4), was integrated, with results presented on Fig. 5. In addition, some methyl signals were integrated, which are used for comparison with the HSQC spectra in the next section.

#### 4.1.3. 2D integration

To integrate the HSQC spectra, very similar process to the 1D version was taken. First, all spectra were loaded into ImatraNMR using automatically created ISL-file. Spectra was also referenced and scaled with the TMU methyl signal, found at 2.72/38.58 ppm



**Fig. 5.** Integration results for the MH2 signal from the quantitative  $^{13}\text{C}$  spectra. As seen on the figure, methyl content varies greatly among samples, e.g. sample S5 has less than half of the methyl branches compared to sample S6. The last three samples (S11, S12 and S13) have the lowest number of methyls, which is explained by their synthetic production method, yielding few methyl branches.

$^1\text{H}/^{13}\text{C}$  shifts respectively (*align* and *scaleto*). Signal list was created with the peak seek function (*seek*), using the area 2–3 ppm proton and 8–10 ppm carbon for obtaining noise level automatically (*seek\_autonoise* and *seek\_autonoise\_mult*). Using the signal list as a reference, integration list was manually constructed and signals integrated (*integrate*). During the process, images were obtained and used to confirm the integration areas (*image\_integrals*, *images\_crop* and *images\_crop\_id* and other image parameters).

To demonstrate benefits of 2D integration, methyl signals from at 14–15 ppm were chosen. In this area, signals from both methyls at the end of carbon chains (BL1, Me1 and Me3) and signals from methyl branches in the middle of carbon chain (Me2) are present (Fig. 6). These are easily distinguished from the triplet and doublet structure visible in the HSQC. Two of these signals overlap nearly perfectly at 14.5 ppm in the carbon spectra (Me1 and Me2), but the methyl branch has (Me2) slightly lower proton shift, enabling HSQC to differentiate the two signals.

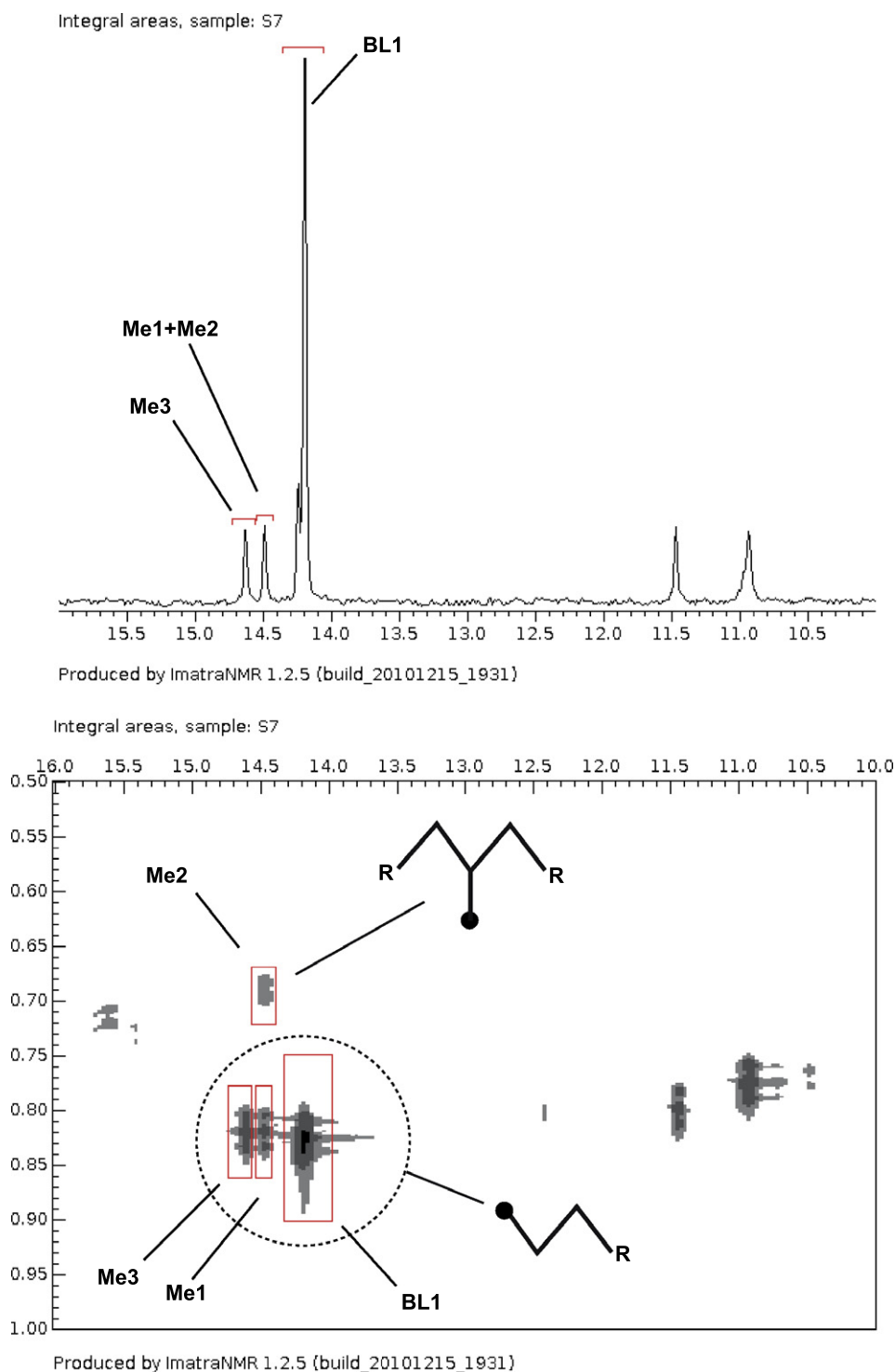
The four signals from 14–15 ppm were integrated from both quantitative  $^{13}\text{C}$  and HSQC spectra, and compared. It's worth noting that the obtained normal HSQC spectra is not quantitative. However, because the  $J_{\text{CH}}$ -coupling varies relatively little in these kind of aliphatic molecules (values are within 1–2 Hz of 125 Hz), the signals are all methyls and the molecules are similar in size, semi-quantitative results can be obtained for demonstration purposes. In a real scenario, Q-HSQC [4,5] or some other quantitative technique should be employed. The integration results are presented on Fig. 7, where it can clearly be seen that both Me1 and Me2 signals contribute significantly to the signal observed at 14.5 ppm in 1D carbon spectra.

#### 4.2. Example 2: statistical analysis of blood plasma samples

In this example,  $^1\text{H}$  NMR was used to study changes occurring in blood plasma samples between fasting state and after hyperglycemic clamp to increase plasma glucose concentration. ImatraNMR was used to automatically pick signals, create histogram of the signals, and integrate the resulting areas. Results were subsequently transferred to Matlab software for PCA (Principal Component Analysis).

##### 4.2.1. Samples and processing

Blood plasma samples of 10 healthy volunteers were obtained from a previous project [18,19]. All volunteers signed a written



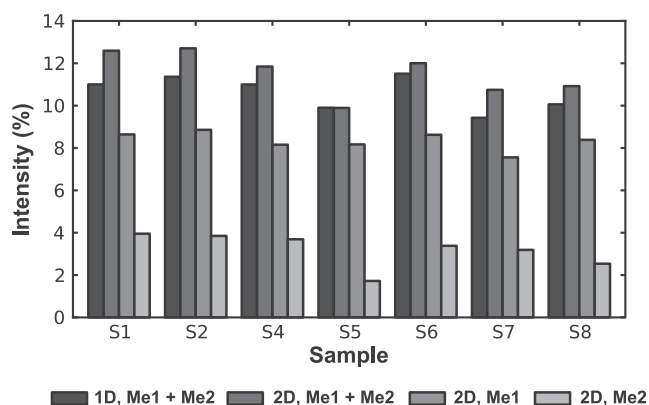
**Fig. 6.** Base oil sample S7, showing the area between 10 and 16 ppm from both 1D quantitative  $^{13}\text{C}$  and 2D HSQC spectra. As it can be clearly seen from the HSQC spectrum, signal at 14.5 is actually two overlapping signals (Me1 + Me2). The signal names and integrated areas are represented in the images. The original spectrum images with integration markings were created by ImatraNMR during the integration process.

informed consent. Two samples for each volunteer were used: a fasting state sample and hyperglycemic clamp sample with elevated glucose concentration. Hyperglycemic clamp was performed by blocking endogenous insulin secretion using somatostatin analogue and simultaneous intravenous controlled glucose infusion to elevate glucose concentration by  $\sim 12$  mM. Detailed description can be found from recent articles [18,19].  $^1\text{H}$  NMR data of plasma samples were recorded using CPMG-sequence to suppress

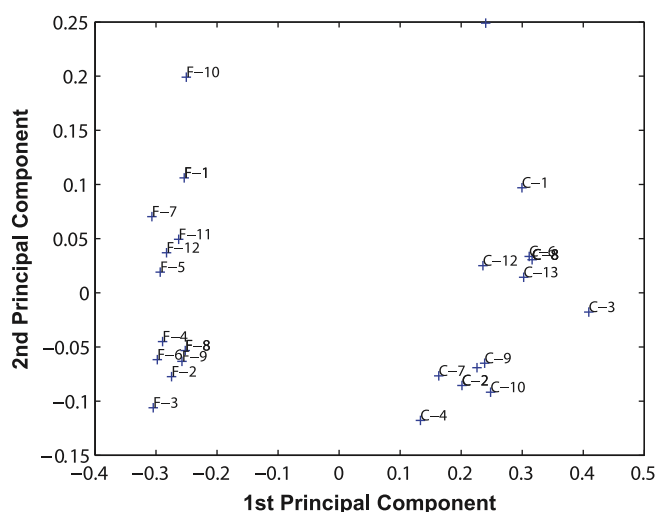
macromolecule signals via  $T_2$ -relaxation. After acquisition, all spectra were processed with SpinWorks software, saving them in native SpinWorks format for ImatraNMR.

#### 4.2.2. Analysis

All spectra were loaded to ImatraNMR by first creating the ISL -file automatically, like in previous cases. The spectra were aligned and scaled using the TSP-signal at 0.0 ppm (*align* and *scalet*



**Fig. 7.** Integration results from the quantitative  $^{13}\text{C}$  1D and semi-quantitative 2D HSQC spectra. The intensity unit is percents of all methyl signals in 14–15 ppm (BL1 + Me1 + Me2 + Me3). As seen on the figure, 1D and 2D integral results differ somewhat, but still its evident from the 2D integration results that the Me1 + Me2 signal observed in the carbon spectra has significant components from both signals.



**Fig. 8.** The results of the Principal Component Analysis (PCA) performed on the blood plasma samples. The first principal component cleanly separates the samples into two groups, fasting state (F) and after hyperglycemic clamp (C).

parameters). Then peak seeking was enabled (*seek*), and after initial testing, region between 11–12 ppm was selected to serve as a noise reference (*seek\_autonoise*). As noise level was quite low, signals larger than five times the baseline noise were selected (*seek\_autonoise\_mult*). To filter unwanted signals, only signals between 0.5–8 ppm were included, and residual water signal was ignored (parameters *seek\_include* and *seek\_exclude*). Also the spectrum images were cropped to the 0.5–8 ppm region (*image\_s\_crop*). Using the found peaks, a peak histogram was created, grouping signals within 0.01 ppm apart from each other (*seek\_histogram\_tole*). The final histogram had 279 distinct groups/regions, and it was then used as such to integrate the regions automatically from every spectra. The configuration file used can be found at [Appendix C](#).

The resulting integrals were loaded from the produced CVS-file into Matlab software for performing PCA (the script used to do this is found in supplementary material). The analysis cleanly separates the samples into two groups (Fig. 8), with principal component #1 explaining 89.0% of the variance. The loadings of the 1st principal component point out the main signals differing between the two groups. Unsurprisingly, these signals are related to glucose (Fig. 9).

## 5. Experimental

### 5.1. Example 1

Both experiments (Quantitative  $^{13}\text{C}$  and HSQC) were conducted at 27 °C on a Varian UNITYINOVA 500 MHz spectrometer using 5 mm triple resonance ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ) pulsed field gradient probehead. High concentration samples were prepared by mixing base oil and solvent in 1:1 (wt.) proportion, with the solvent being 1:9 (wt.) mixture of  $\text{CDCl}_3$  and TMU.

For quantitative  $^{13}\text{C}$  experiments, the limiting  $^{13}\text{C}$   $T_1$  time of  $\sim 5$  s was determined for the TMU methyl carbons. To account for small carbon signals with possibly larger  $T_1$  relaxation times, the acquisition delay was set to 28 s, while the acquisition time was 2 s. Inverse gated  $^1\text{H}$ -decoupling and  $45^\circ$  excitation pulse were used to ensure quantitative conditions. The number of transients recorded was 512. Spectral width was 13.0 kHz (103.5 ppm), and the transmitter was positioned at 29.9 ppm. The duration of  $90^\circ$  pulse was  $\sim 15$   $\mu\text{s}$ . All FIDs were apodized using an exponential function (0.5 Hz line broadening) prior to Fourier transform.

For 2D HSQC experiments, a standard, vendor provided pulse sequence was used. Phase sensitive spectra were obtained using two transients and 1024 or 2048 increments. Relaxation delay was 1 s while acquisition times of 0.25–1 s were used. The duration of  $90^\circ$  pulse was  $\sim 6.5$   $\mu\text{s}$  for proton and  $\sim 15$   $\mu\text{s}$  for carbon. Spectral width was 2.0 kHz (4.0 ppm) for proton and 7.5 kHz (60.1 ppm) for carbon. Transmitter carrier frequency was at 1.45 ppm (proton), while the decoupler was at 27.5 ppm (carbon), respectively. All FIDs were apodized using a Gaussian weighting function prior to Fourier transform.

### 5.2. Example 2

All experiments were conducted at 27 °C on a Varian UNITYINOVA 500 MHz spectrometer using 5 mm triple resonance ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ) pulsed field gradient probehead.

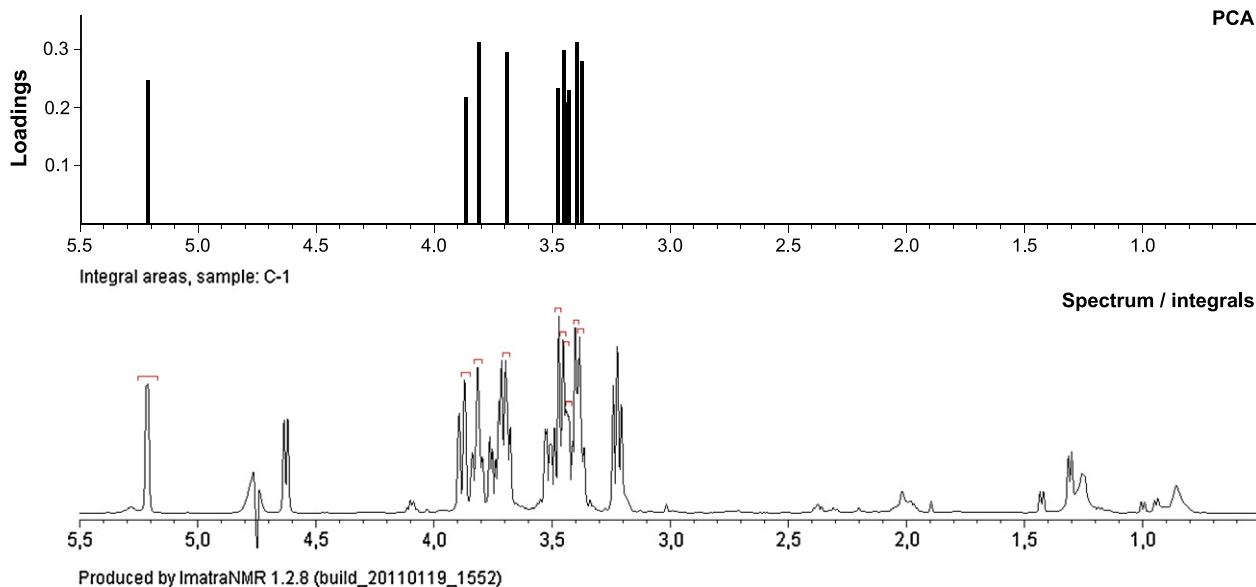
Samples were prepared by measuring 550  $\mu\text{l}$  blood plasma into a 5 mm NMR-tube. A 2 mm O.D. coaxial insert containing 40 mM TSP- $d_4$   $\text{D}_2\text{O}$  solution was positioned within NMR-tube, to serve as external standard and to provide lock signal. To measure  $^1\text{H}$  spectra from blood plasma, a CPMG sequence was used to suppress signals from macromolecules with short  $T_2$  values. The duration of CPMG-based  $T_2$ -filter was 200 ms with 0.5 ms interpulse delays. Acquisition time was 2 s. Relaxation delay of 8 s contained 5 s period of water presaturation. The number of transients was 256, with four steady-state transients pulsed prior to starting the recording of the FID.

All FIDs were apodized using an exponential function (0.5 Hz line broadening) prior to Fourier transform, and baseline was corrected using the Least Squares method implemented in the SpinWorks software [15].

## 6. Conclusions

We have introduced novel software, ImatraNMR, and demonstrated its use to analyze quantitative NMR data. The software can be used to significantly accelerate the processing and analysis of large NMR datasets. While the software might not be extremely userfriendly or easy to use as it requires manual preparation of text files, the batch processing and scripting interfaces make it simple, customizable and powerful tool. Also the software is still under development, so additional features or even full GUI interface might be implemented.

The software is available freely for academic and non-commercial use, with the source code also available on request [20].



**Fig. 9.** Blood plasma sample C1. The integral regions corresponding to the top 10 largest loadings of the 1st principal component of the performed PCA (bottom) and the actual loading coefficients presented as a simple “spectrum” (top). All of the top 10 loadings were positive, so as the amount of 1st principal component increases, signals in these regions become larger (see Fig. 8).

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## Appendix A. ImatraConfigFile for Example 1, 1D spectra

```
# general
output_path = out/
output_prefix = a3_ex1_1d

# align/scale to TMU signal
align = 37 -> 39 @ 38.58
scaleto = 38.38 -> 38.78

# seeking
seek = y
seek_autonoise = 8.0 -> 10.0
seek_autonoise_mult = 1

# integrate
integrate = y

# images
image_integrals = 1
images_crop = 31 -> 34
images_width = 600
images_height = 400
images_ticwidth = 0.1
```

## Appendix B. ImatraConfigFile for Example 1, 2D spectra

```
# general
output_path = out/
output_prefix = a3_ex1_2d
```

```
# scale to TMU signal
align = 2 -> 3.2 @ 2.72 | 37 -> 39 @ 38.58
scaleto = 2.68 -> 2.77 | 38.38 -> 38.78

# seeking
seek=y
seek_autonoise = 2 -> 3 | 8.0 -> 10.0
seek_autonoise_mult=1

# integration
integrate=y

# images
image_integrals=y
images_crop = 0.5 -> 1
images_crop_id = 10 -> 16
images_width=600
images_height=400
images_2d_flip=y
images_2d_colors=bw
images_2d_limitfactor=0.002
images_ticwidth=0.01
images_ticwidth_id=0.1
```

## Appendix C. ImatraConfigFile for Example 2

```
# general settings
output_path = out/
output_prefix = a3_ex2

align = -1 -> 1 @ 0.0
scaleto = -0.25 -> 0.25

# seek
seek=y
seek_autonoise = 11.0 -> 12.0
seek_autonoise_mult = 5
```

(continued on next page)



```

seek_include = 0.5 -> 8
seek_exclude = 4.67 -> 4.9
seek_histogram_tole = 0.01

# integration
integrate=y

# images
image_seek=y
image_integrals=y
images_crop = 0.5 -> 5.5
images_width = 1200
images_height = 400
images_ticwidth = 0.1

```

## Appendix D. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmr.2011.05.012.

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