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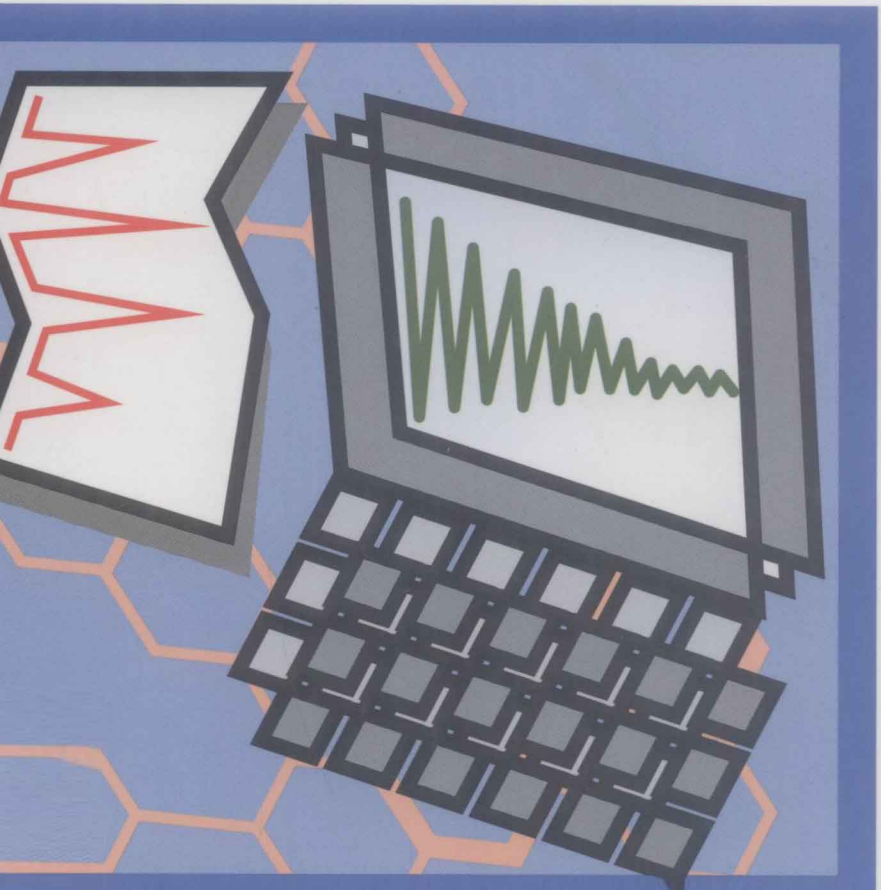
Christian Schorn, Brian Taylor

NMR Spectroscopy: Data Acquisition

Second, Completely Revised and Updated Edition



Spectroscopic Techniques: An Interactive Course

Christian Schorn
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NMR Spectroscopy: Data Acquisition

Second, Completely Revised
and Updated Edition

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A CD-ROM containing a teaching version of the program NMR-SIM (© Bruker Analytik GmbH) is included with this book. Readers can obtain further information on this software by contacting:
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Ursula Weber, Herbert Thiele
NMR Spectroscopy: Modern Spectral Analysis
1998, ISBN 3-527-28828-7

Ernö Pretsch, Jean-Thomas Clerc
Spectra Interpretation of Organic Compounds
1997, ISBN 3-527-28826-0

Preface to 2nd Edition

Since the publication of the first edition of this book, there have been many important developments in the field of NMR spectroscopy. These developments have included the award of two Nobel prizes: in 2002 to Kurt Wüthrich for his major contributions to biomolecular NMR spectroscopy and in 2003 to Paul C. Lauterbur and Sir Peter Mansfield for their work on MRI, both awards confirming the scientific importance of the general method and its wide application. Consequently, this second edition has been extended to incorporate a number of these pulse sequence developments. Nevertheless, to understand these sophisticated methods it is still necessary that students and newcomers start with the basic experiments and proceed on a step-by-step basis. In this context NMR-SIM is an outstanding, user-friendly simulation program that may be used by both the novice and the expert as an efficient training tool. Therefore, it is no surprise that BRUKER have included NMR-SIM in their latest spectrometer software package TOPSPIN.

Looking at this second edition in a little more detail; chapter 5.3.1 has been extended to include simulations of multiple offset selective pulse experiments whilst chapter 5.5.2 examines ACCORDION-principle based HMBC experiments. Multiple offset selective pulse experiments are now an important application in LC-NMR and biomolecular NMR spectroscopy whilst the discussion of the CIGAR and the IMPEACH-HMBC experiment in chapter 5.5.2 are an invitation to use NMR-SIM to trace complex pulse sequences back to their origin. There is now a more comprehensive discussion of filter elements, which are now a vital element in the latest pulse sequences, in chapter 5.8.2. Finally the new chapter 5.9 is subdivided into two subsections. The first subsection 5.9.1 is a collection of some of the latest published ideas to improve existing sequences. Section 5.9.2 is dedicated to spin-state selective experiments; spin-state selective experiments using transverse relaxation optimized spectroscopy (TROSY) have given some spectacular results enabling the measurement of signals from very large biomolecules which, hitherto, it has not been possible to detect.

April 2004

Christian Schorn
Brian Taylor

Acknowledgements

It would not have been possible to produce this volume in such a short time without the assistance of several individuals who took part in the proof-reading of the manuscript, the software development and the testing of the enclosed *Check its*.

We would like to thank Dr. Pavel Kessler (BRUKER, Karlsruhe, FRG), who wrote the NMR-SIM software and the enclosed teaching version of the program. He helped considerably by modifying the program and incorporating our suggestions. We have enjoyed this very fruitful co-operation.

Furthermore we have to express our gratitude to Prof. Dr. Peter Bigler (University of Bern, CH) for stimulating discussions and ideas.

To all the authors who were so kind to allow the citation of their papers, lectures or books and who give us help on request, we have to express many thanks.

Since the first volume was created during his post-doctorate at the University of Bern (CH), CS is grateful to the Department of Chemistry and Biochemistry of the Kanton Bern and to his former colleagues in the NMR group at the University of Bern.

For assistance and the confidence in us in completing the task in hand, we are extremely grateful to the staff at BRUKER AG (Karlsruhe, FRG) and Wiley-VCH (Weinheim, FRG).

Table of Contents

1 Introduction

1.1 Scope and Audience

The aim of this book is to illustrate the use of the NMR-SIM simulation program; in common with the other volumes in the series *Spectroscopic Techniques: An Interactive Course* a teaching version of NMR-SIM is included with this book. Thus the basics of NMR as described in manifold monographs [1.1 – 1.14] can be visualized. It is necessary to draw a distinction between the program WIN-DAISY covered in *NMR Spectroscopy: Modern Spectral Analysis* [1.11] and NMR-SIM, both are simulation programs but both have very different uses in NMR spectroscopy. Essentially WIN-DAISY uses chemical shifts and coupling constants to simulate a NMR spectrum using standard quantum mechanical calculations whilst NMR-SIM uses experimental parameters in conjunction with pulse sequences to simulate the experimental 1D and 2D NMR data which may then be processed to produce the appropriate NMR spectrum. NMR-SIM assumes a perfect spectrometer and certain sample specific variables such as probe matching, probe tuning, shimming and temperature control cannot be incorporated into the simulation. However other experimental variables such as the effect of non-optimized pulse angles, delays and a non-uniform excitation range due to limited transmitter power (off-resonance effects) may be simulated. In these cases the effect of the experiment parameters in a specific pulse sequence can be studied and their influence on the overall result evaluated. NMR-SIM may also be used to study and optimize pulse sequences published in the literature; in this way new pulse sequences may be completely debugged before implementing the new pulse sequence on an NMR spectrometer saving a considerable amount of spectrometer time. The applications of NMR-SIM can be summarized as follows:

- To demonstrate and understand the basic principles of pulse sequences by means of the resulting NMR spectra.
- To analyze the dependence of particular parameters, such as spin systems variables, on the experiment result
- To visualize the effect of new pulse sequences on complex spin systems
- To check the performance of a real spectrometer and to assist in evaluating experimental errors

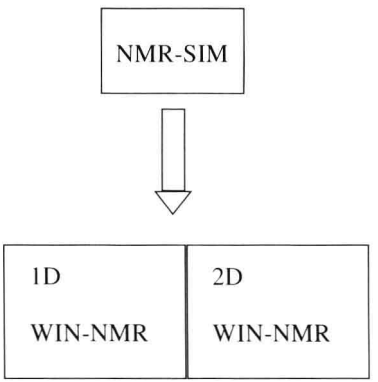
The focus of this book is data acquisition using NMR-SIM to simulate the raw experimental data. In common with the other volumes in this series the emphasis is on "learning by doing" and there are many simulations to help the reader become familiar with the simulation program. This book will be of use to all NMR spectroscopist ranging from the newcomer to the more experienced user. The newcomer can use this book and

software to understand how pulse sequences work and to check and evaluate the flood of new pulse sequences appearing in the literature prior to testing a proposed pulse sequence on a spectrometer. This approach is a much more efficient use of spectrometer time because the pulse sequences used in NMR-SIM can be used directly on a BRUKER DRX spectrometer with only minor modifications.

The advanced NMR spectroscopist may use this book in a different manner using NMR-SIM for teaching and demonstration purposes. They may also use it in the evaluation or the design of new pulse sequence particularly when applied to a complex spin system where analysis using mathematical methods is cumbersome, less obvious and time-consuming compared to the visual results of NMR-SIM.

1.1.1 Simulation Environment

The NMR-SIM program: NMR-SIM is based on a density matrix approach to generate data that is as comparable to real experimental data as possible. Although the calculation is based on an ideal spectrometer and ignores effects such as magnet field inhomogenity, several parameters can be set to study the impact of non-optimum conditions in a spectrum. There are also a number of other constraints in the current version of NMR-SIM aimed at reducing computer resources. Relaxation effects are implemented using the Bloch equations and can be "switched off" or restricted to only the acquisition period instead of the whole experiment. Transverse relaxation is defined for the linewidth calculation. Furthermore transverse and longitudinal relaxation can be taken into account in the simulation to reproduce relaxation artefacts. Due to this simple approach relaxation related processes and phenomena such as cross-relaxation and NOE effects which are used experimentally to detect and measure spatial proximity among nuclear spins, are neglected.



Generation of raw data for 1D and 2D NMR experiments

Transformation of 1D and 2D raw data into corresponding spectra to analyse and understand experiments and to study influences of experimental or spin system parameters

Fig. 1.1: Flow of raw data simulation and data processing.

Similarly incoherent magnetisation transfer due to chemical exchange is also not part of the current version of NMR-SIM. Only chemical shift and coupling evolution that

occur during the execution of a pulse sequence are considered whilst relaxation processes are simplified. Finally in this teaching version of NMR-SIM the simultaneous execution of a selective pulse and a hard or selective pulse on a second rf channel is not enabled.

The Reader: It is assumed that the reader understands the fundamental principles of pulse Fourier transform spectroscopy (PFT) and is proficient in using the MS-Windows operating system and Windows based programs. The references at the end of section 1.4 list both introductory and comprehensive texts on the main principles of NMR spectroscopy. Furthermore the reader should be familiar with computer environments, as working on a spectrometer inevitable requires computer management skills.

The Personal PC: The NMR-SIM program can be run on any IBM-compatible PC with an 80486 or higher processor. The operating system has to be MS-Windows NT 4.0 or MS-Windows 2000, NMR-SIM will not install on a PC running MS-Windows 9x. For the correct operation of the teaching version of both NMR-SIM and the WIN-NMR the operating system must be installed properly. The basic hardware configuration is set by the requirements of the operating system but the program requires a minimum of 32 MB RAM memory and a free hard disk partition of approximately 300 MB for temporary files and stored data. To ensure that the calculation times are no longer than two minutes all the *Check its* have been optimized and the simulations tested on a PC equipped with a 300 MHz Intel Celeron processor and 128 MB RAM memory.

The full version of the NMR-SIM program can be purchased either as the Windows NT/Windows 2000 version or the UNIX version from BRUKER. These versions use the same program set-up and commands as the Teaching version or though by necessity the directory structure of the UNIX version is different. For further details the reader is invited to contact BRUKER or their local representative directly, the contact addresses are listed on BRUKER's worldwide web homepage [1.13, 1.14].

1.1.2 Book Content

The book is separated into five main chapters with the overall layout designed to cater for both the new and the experienced NMR spectroscopist. Chapter 1 describes the installation of NMR-SIM and the processing software packages 1D WIN-NMR and 2D WIN-NMR, it also contains a brief description of NMR-SIM. Chapter 2 uses NMR-SIM to illustrate the theoretical background of the NMR experiment instead of the normal mathematical approach and shows that even when using complex pulse sequences NMR-SIM can be used to simulate realistic experimental data. The concepts introduced in this chapter form the basis for understanding the pulse sequences discussed in chapter 5 as well as the pulse sequences that appear in the literature. Chapter 3 briefly discusses data processing using 1D WIN-NMR and 2D WIN-NMR. Chapter 4 is essentially a reference chapter and using *Check its* extensively looks in detail at various aspects of using NMR-SIM such as editing spin systems, the pulse programming language and using the Bloch simulator. Chapter 5 is the main part of the volume and examines the pulse sequences routinely used for studying non-biopolymer molecules in a modern NMR laboratory. In

many cases the differences between the standard sequence and its variants are demonstrated. This chapter also looks in detail at the building blocks that often occur in pulse sequences such as hard and shaped pulses and filter elements.

1.2 Software

The CD-ROM enclosed with this book contains the special teaching version of the commercially available simulation program NMR-SIM and the NMR data processing programs 1D WIN-NMR and 2D WIN-NMR. The versions of the WIN-NMR programs are the same as included with the books in this series *Processing Strategies* and *Modern Spectral Analysis* [1.10, 1.11].

The NMR-SIM program is based on the full commercial version of NMR-SIM 2.8.5. Both versions will only run under MS Windows NT 4.0 or Windows 2000 operating system. There are some minor differences between the teaching and full program version:

- the type of experiment that can be studied is restricted to two rf channels
- the number of pre-defined nuclei is reduced to a minimum and the examples used in this volume have taken this into account
- the number of magnetically non-equivalent spins which form a coupling system is reduced to 9 spins
- all data which is generated by the NMR-SIM teaching version can only be processed by the teaching versions of WIN-NMR.

If the teaching versions of 1D WIN-NMR and 2D WIN-NMR have not been installed already as part of *Spectroscopic Techniques: An interactive Course* they must be installed as outlined below. The CD-ROM also contains the NMR-SIM, 1D WIN-NMR and 2D WIN-NMR manuals as pdf files plus a copy of Adobe Acrobat Reader required to read pdf files. Finally the CD-ROM contains a file ***result.pdf*** containing additional information and results appertaining to the various *Check its* in this book.

1.2.1 Installation

All the programs and files required to perform the *Check its* in this book are contained on the CD-ROM which is enclosed in this book. The CD-ROM is configured with an autorun function such that program installation starts automatically after the CD-ROM has been inserted in the CD-ROM drive. A self-instructing menu guides the reader through the whole installation procedure. If the autorun facility has been disabled or is not available the programs must be installed using the Run command and Windows Explorer as shown in *Check its 1.2.1.1* and *1.2.1.2*. The default pathnames used by the installation procedure are also used by the *Check its* in this book, if the default pathways are altered in any way the files used in the *Check its* must be modified accordingly. Users who already have the full version of NMR-SIM installed should take care during the installation procedure not to overwrite the existing executable program and related files.

It must be emphasized that the simulation results calculated with the teaching version of NMR-SIM can only be processed with the teaching version of 1D WIN-NMR and 2D WIN-NMR.

1.2.1.1 Check it in WINDOWS

Insert the software CD-ROM in the CD-ROM drive. If the autorun facility is available the introductory window will appear and it is only necessary to follow the dialogue. If the autorun facility is not available use the **Run** option of the **Start** pop-up menu in the bottom menu bar. In the pop-up window select *[CD-ROM drive letter]:SETUP.EXE*. Confirm the introductory window with the **Next** button. In the Product Selection window select the programs WINNMR, NMRSIM and AcroRead 4.0. Click the **Next** button to start the installation. During the installation procedure a number of different windows appears as shown in the flow diagram below. Most of these windows can be passed unchanged by clicking the **Next** button. During the installation of the Adobe Acrobat Reader several user-friendly and self-explanatory windows will appear.

Windows ⇒ User interaction

"Welcome" ⇒ **Next**

"Custom Options Sections" ⇒ **Next**

"Choose Destination Location":
1D C:\TEACH\WIN1D etc. ⇒ **Next**

"Choose Destination Location":
Spectra C:\TEACH\NMRData etc.
⇒ **Next**

"Choose Destination Location":
Other Files C:\TEACH\ etc. ⇒ **Next**

"Select Components":
make no selection ⇒ **Next**

"WIN-NMR Teaching Version Folder":
BRUKERTeach
⇒ **Next** or enter the *new name*

"Information":
"Setup Complete" ⇒ **Finish**

1D / 2D WIN-NMR installation

Windows ⇒ User interaction

"Welcome"
⇒ **Next**

"Select the installation type"
⇒ choose *User installation*, **Next**

"Choose Destination Location":
C:\Teach\NMR-SIM
⇒ **Next** or **Browse** (to select a new path)

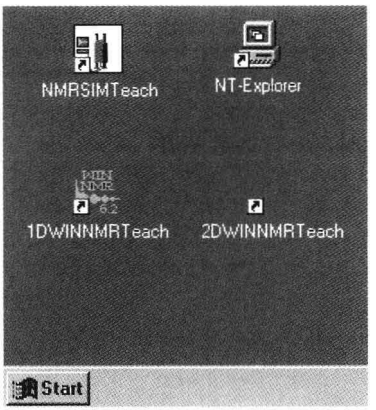
"Choose Program Folder":
BRUKERTeach
⇒ **Next** or enter the *new name*

"Information": "NMR-SIM setup is complete"
⇒ **Ok**

NMR-SIM installation

The configuration files used in the *Check its* can be used with the full version of NMR-SIM but the experimental data can only be processed using the full version of 1D

WIN-NMR and 2D WIN-NMR or by another processing software package after file conversion from the BRUKER format. It is convenience to have the icons for each program of the teaching software displayed on the main Windows desktop for fast access. *Check it 1.2.1.2* describes the procedure for setting up these shortcuts and gives the recommended icon names which will also be used in the later *Check its*.



Windows desktop

1.2.1.2 Check it in WINDOWS

Open NT-Explorer and if necessary resize the window so that part of the Desktop is also visible. Locate the file *C:\Teach\NMR-SIM\nmrsim_w.exe*. Select the file by holding down the right-hand mouse button and then drag the file onto the Desktop. Release the right-hand mouse button and in the on-the-fly-pop-up menu choose the **Create Shortcut(s) Here** option. To rename the icon click on the icon using the right-hand mouse button and select the **Rename** command from the on-the-fly pop-up menu. Using the keyboard enter the new name NMRSIMTeach followed by **RETURN**. Repeat the procedure for the 1D and 2D WIN-NMR programs using the file and path names *C:\Teach\Win1d\Demo1D.exe* and. *C:\Teach\Win2D\Demo2D.exe* respectively to create the icons named 1DWINTeach and 2DWINTeach.

In *Check it 1.2.1.3* the program manual files and the *result.pdf* file are installed.

1.2.1.3 Check it in MS-Windows

Using Windows NT Explorer create in the directory *C:\Teach* the subdirectories *Manuals* and *Results*. Copy the corresponding pdf-files from the CD-ROM to the newly created directories. On the CD-ROM the manuals and the result file are stored in the directories *\NMR-Sim\Manuals* and *\NMR-Sim\Results* respectively. To open the pdf-files move the cursor onto the filename in the Explorer directory tree and double click with the left-hand mouse button.

Nmrsim.pdf manual of NMR-SIM (Version 2.8)

Winnmr1d.pdf manual of 1D WIN-NMR

Winnmr2d.pdf manual of 2D WIN-NMR (16bit)

result.pdf documented simulation results of the *Check its*

Fig. 1.2 shows the directory structure generated by the software installation using the recommended directory. The main TEACH directory contains the program subdirectories Getfile, NMR-Sim, Win1D and Win2D plus the data subdirectory Nmrdata. The subdirectories Manuals and Results contain the software documentation for the programs and the additional information and results for most of the *Check its*.

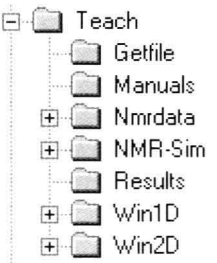


Fig. 1.2: Directory structure - Software, data and documentation subdirectories installed according *Check its 1.2.11* and *1.2.1.3*.

1.2.2 The User Interfaces of NMR-SIM, 1D WIN-NMR and 2D WIN-NMR

The programs NMR-SIM, 1D WIN-NMR and 2D WIN-NMR are started using a double left-hand mouse button click on the appropriate icons on the Windows desktop.

The main window of NMR-SIM is subdivided in the **title bar** with the program name, the **menu bar** with the pull-down menu commands **File**, **NMR-Wizard**, **Edit** etc., the **rf channel option bar** with two accessible rf channel combo boxes and the **main status** window, see Fig. 1.3. Each command in the menu bar opens a pull-down menu that may contain sub-menus or commands that can be selected and opened/executed. The rf channel option bar uses isotope identifier to assign a specific nucleus and hence NMR frequency to the F1 or F2 channel. The main status window is built up in line order and shows the pulse program name, spin system name and other optional files associated with the current simulation.