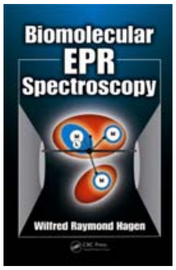
BIOMOLECULAR EPR SPECTROSCOPY



The book *Biomolecular EPR Spectroscopy* by W.R. Hagen (CRC Press / Taylor and Francis Group 2009, ISBN 978-1-4200-5957-1) is accompanied by a set of stand-alone application programs in which the theory worked out in the book is implemented in order to carry out simulations and manipulations of solution and frozen solution EPR spectra. Each program is a single document exe file. The present document briefly describes the different programs and links them to specific chapters or sections of the book.

DESCRIPTION OF THE PACKAGE

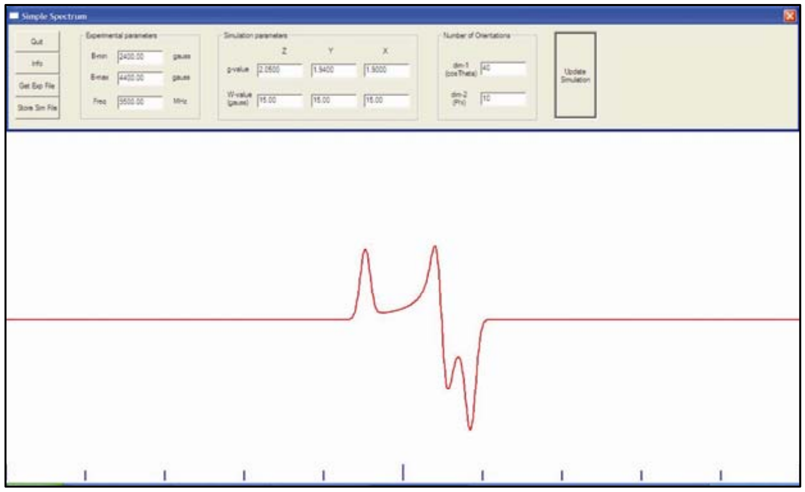
Table-1: Overview of Programs

Class	name	application area
Introduction	SimpleSpectrum	teaching/learning tool
Work horses	Hyperfine Spectrum	metal+ligand splittings
	Gee Strain 5	multicomponent spectra with g-strain
	Visual Rhombo	half-integer high-spin (also: rhombograms)
Manipulation	EPR File Converter	conversion to standard format
	EPR Editor	baseline correction and spin counting
Special cases	Isotropic Radicals	radicals in solution
	Single Integer Signal	integer-spin (parallel/normal mode)

The minimal system requirements to run these programs are: off-the-shelf PC with Windows XP and a monitor with (preferably) 1280 pixels resolution in the x-dimension.

The used file format (input and output) is: bare Ascii files with 1024 amplitude entries (program EPR File Converter can be used to convert from other file formats to this standard).

As a general philosophy this set of eight programs is intended to cover the most common problems in numerical analysis (simulation and manipulation) of cw biomolecular EPR powder spectra. The design of the programs is towards maximization of "ease of use": each program is set up around a standardized single-window user interface that should be intuitive and (nearly) self explanatory. Choices of mathematical and numerical approaches are automatic and thus completely screened off from the user.



The standard user window consists of two adjacent (non-overlapping) sub windows: at the top is a permanent dialog box for entering commands and values, at the bottom is a spectral window to display an overlay of an experimental spectrum and a simulation.

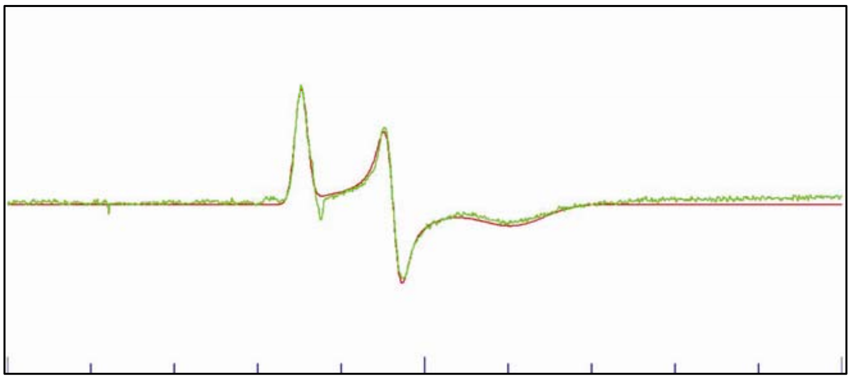
For the simulation programs the dialog windows are set up along a standard, pattern of modules: from left to right each starts with a decision module containing a set of four knobs to (i) stop the program; (ii) get some basic information on its application range; (iii) to read an experimental spectrum; (iv) to save a simulation. Next is a module to define the experimental boundary conditions of magnetic-field range (in gauss) and of the microwave frequency (in megahertz). Then follows the key module to enter the simulation parameters (including the number of molecular orientations). The fourth module consists of a single button to initiate the calculation.

Buttons are activated by a left mouse click. Entering a parameter is by moving the mouse to the specific window that displays its present value and then typing over this value.

Some of the programs (the three "work horses") additionally contain one or more 'progress bars' that indicate how far the calculation has progressed. Simple simulations will be generated in real time (in less than a second), but more demanding simulations encompassing several spectral components or distributed conformations may take up to several minutes. If the filling up of the progress bar is so slow as to indicate computation times of tens of minutes or hours, then one either has an extremely demanding problem at hand or a typing error has been made in the simulation parameters{, and a program abort is indicated}.

As indicated in table 1, the eight programs can be divided into four categories: an introductory, practicing tool, three number crunchers for a variety of anisotropic spectra, two programs to modify experimental and/or simulated spectra, and two programs for the special cases of radicals in solution and for integer-spin systems with strong zero-field interactions.

Program ‘Simple Spectrum’ is mainly intended as an introductory, hands-on learning tool for those who are inexperienced in EPR spectral simulation. It is, for example, instructive to start the program, hit the ‘Update simulation’ button to generate an example spectrum with pre-set simulation parameters, and then to increase the rhombicity (e.g., change from gz=2.05 to gz=2.30) to see the ‘mosaic ripples’ artifact produced by computing too low a number of orientations. Increasing dim-1(cosTheta)=40 to 400 will dissolve the artifact. The basics of simulation an EPR powder pattern are treated in section 6.3 of the book. An experimental example spectrum (simpleexample.asc) is provided to be read in by ‘Simple Spectrum’ for practicing the simulation of an actual spectrum (note: the experimental parameters have to be changed to bmin= 2600, bmax = 4600, frequency= 9322.9).



Note that the baseline of the experimental spectrum is not straight; this can be fixed with the program ‘EPR File Converter’ (see below). With a fixed baseline the fit will still not be perfect, because this spectrum (the Rieske [2Fe-2S] protein in the yeast bc_1 complex) is subject to g-strain. g-Strain spectra can be generated with the program ‘Gee Strain-5’ (see below).

Program ‘Hyperfine Spectrum’ works along the same lines as ‘Simple Spectrum’, but the number of simulation parameters that can be entered has drastically increased because now the sum spectrum of two different components can be calculated, and furthermore each component can have central (metal) hyperfine splittings and also ligand superhyperfine splittings.
Also, for each component a ‘checkbox’ can be ticked for the program to use the natural isotope distribution for one of the elements Cu, Mo, or W. Two blue-filling column bars indicate the progress of the calculation of the simulation for each component. The theoretical background for this program can be found in chapter 5 and section 9.5 of the book.

Program ‘GeeStrain-5’ can generate sum spectra of up to five different components subject to broadening by g-strain. This situation is typically encountered in respiratory-chain complexes and in complex enzymes with multiple electron-transfer prosthetic groups. In a g-strained spectrum the linewidth is no longer described by three components wx, wy, wz, along the molecular axes, but by six components of a symmetrical g-strain tensor. Each component can be positive or negative. g-Strain results in characteristic patterns of skewed and asymmetric features in the powder spectrum. g-Strain is a reflection of protein conformational distributions. The theory and math is explained in chapter 9 of the book.

Program Visual Rhombo is for half-integer high-spin systems with strong zero-field interaction, which frequently occur in a variety of iron proteins and in high-spin cobalt proteins. It can be used in two ways. Firstly, entering a value for the rhombicity η=E/D will generate a complete set of effective g-values, which may be traced back in experimental spectra. Secondly, spectra (sums of all intradoublet sub spectra) can be generated, where realistic simulation of experimental data frequently requires the rhombicity to be distributed ('D-strain'), and one has to specify the width and the number of digital steps of the simulation. This option is usually computationally demanding, and a progress bar is in place to tell the user about the program's progress (or lack of it). Consult sections 5.6 and 7.4 and in chapter 12 of the book for background information on rhombograms and the strong-field limit.

Program 'EPR File Converter' is a tool to convert experimental data files to the standard (1024 ascii amplitude values) used by the other programs. As an extra option 'EPR File Converter' can also produce the first integral (the EPR absorption spectrum) or the second derivative of an experimental or a simulated spectrum.

Program 'EPR Editor' is to normalize two experimental spectra to the microwave frequency of one of them, to allow for subsequent construction of difference spectra, as explained in section 6.4 of the book. 'EPR Editor' also provides the numerical value of the second integral of a spectrum (or a difference spectrum) calculated between two limits defined by the user. The second integral is required for quantification (or: spin counting) as explained in section 6.2 of the book. 'EPR Editor' can also read-out g-values from experimental data.

Program 'Isotropic Radicals' is for radicals in solution that tumble so fast as to completely average out any anisotropy in the g- and A-values. Two typical applications are envisioned. The first case is an organic radical (e.g., flavin, viologen) possibly in an organic solvent (to decrease linewidth) and subject to multiple hyperfine interactions due to delocalization of the spin over many nuclei. The second case is that of a spin trap whose complex (some say: erratic) chemistry usually produces several different metastable radical species when the trap reacts with a single short-lived radical. Spin trap spectra are usually dominated by hyperfine interaction with one N and one H: 'Isotropic Radicals' can generate up to four species each with two hyperfine interactions. See also chapter 10 of the book.

Program 'Single Integer Signal' is for integer spin systems subject to strong zero-field interaction, e.g. Fe(II) complexes, reduced 3Fe-4S clusters or reduced 2Fe oxo clusters, which typically exhibit only a single transition within their highest non-Kramers doublet detectable in parallel-mode EPR and, with increased linewidth and reduced intensity, in normal-mode EPR. 'Single Integer Signal' generates these two spectra simultaneously (parallel=bright red, normal= dark red). The shape is a function of three parameters only: an effective g-value and two linewidth parameters that model rhombic D-strain. The theory of these spectra is explained in section 12.5 of the book.

ADDITIONAL INFORMATION

Note that the following type of problems are not covered by any of the here provided programs: (i) systems with non-colinear tensors (except for g-strain broadening); (ii) intermediate-field high-spin systems (i.e. systems with electronic Zeeman and zero-field interactions of comparable strength); (iii) data from pulsed techniques. It is not easy to design universally applicable simulators for these problems, whose solution is anyway sufficiently complex as to call for either teaming up with a specialist or, alternatively, to become a specialist oneself and to develop dedicated code for specific cases.

The programs presented here are for a considerable part based on FORTRAN77 code that was developed in the late 70s, the 80s and the 90s in Amsterdam, Ann Arbor, and Wageningen, with help from SPJ Albracht and from WR Dunham. The present programs were written in Intel Visual Fortran (an extension of FORTRAN90/95) using the Windows Visual Studio developer environment.

All programs are the intellectual property of the author. Future corrections, improvements, and extensions may be stimulated by user remarks to w.r.hagen@tudelft.nl.

CITATION

In referencing, please cite:

Hagen, W.R. *Biomolecular EPR Spectroscopy*, CRC Press, Boca Raton, Fl, USA, 2009.

and for accompagnying software:

http://www.bt.tudelft.nl/biomolecularEPRspectroscopy

UPDATES

There are no updates at this time.

WRH 20090109
End of document 'Instructions for use'.