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Communication

Post-processing of EPR spectra by convolution filtering: Calculation of a harmonics' series and automatic separation of fast-motion components from spin-label EPR spectra

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Abstract

This communication reports on post-processing of continuous wave EPR spectra by a digital convolution with filter functions that are subjected to differentiation or the Kramers–Krönig transform analytically. In case of differentiation, such a procedure improves spectral resolution in the higher harmonics enhancing the relative amplitude of sharp spectral features over the broad lines. At the same time high-frequency noise is suppressed through filtering. These features are illustrated on an example of a Lorentzian filter function that has a principal advantage of adding a known magnitude of homogeneous broadening to the spectral shapes. Such spectral distortion could be easily and accurately accounted for in the consequent least-squares data modeling. Application examples include calculation of higher harmonics from pure absorption echo-detected EPR spectra and resolving small hyperfine coupling that are unnoticeable in conventional first derivative EPR spectra. Another example involves speedy and automatic separation of fast and broad slow-motion components from spin-label EPR spectra without explicit simulation of the slow motion spectrum. The method is illustrated on examples of X-band EPR spectra of partially aggregated membrane peptides.

Keywords: EPR; Data processing; Filtering; Spin-labeling EPR; Data modeling; Membrane peptides

1. Introduction

One common experimental problem of site-directed spin-labeling continuous wave (CW) EPR experiments is the presence of multiple spectral components such as a fast-motion component that would overlap with the rest of the spectrum. The former component could be present for some undesirable reasons related to the sample preparation including residual free label that was not removed by dialysis and/or contamination from a denatured protein. In other studies the balance between the fast motion and immobilized components is of specific interest as an indication of two conformational states. Examples include protein folding and equilibrium between aggregated and monomer viral peptides in lipid bilayers. In both cases accurate separation of spectral components is highly desirable but difficult, especially for only partially resolved lines. The latter often requires rigorous simulations and least-squares optimization of spectral shapes corresponding to multiple species.

It is well-documented that resolution of CW EPR spectroscopy could be improved by the use of higher harmonics detected experimentally by phase-sensitive detection or obtained at the post-processing stage (e.g. see [1] and references therein). One could find the post-processing approach to be more convenient in practice as it does not require any new hardware and can be used for any digitized spectra. For this purpose Hyde and coworkers developed a pseudomodulation—a computer-based strategy for resolution enhancement [1–3]. In brief, this strategy is based on computer simulations of the effect of a sinusoidal magnetic

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field modulation on EPR spectra. Modulation amplitude could be varied and higher harmonics are calculated even if only the first harmonic has been originally measured [1]. Subtraction and addition of various amounts of even harmonics to the original spectrum resulted in the resolution enhancement [1]. In their studies Hyde and coworkers arrived to several practical conclusions. Among those was the observation that addition of harmonics greater than the second is not worthwhile as it adds considerable amount of noise.

Indeed, because of noise considerations, only the first harmonics of the resonance signal are typically detected and displayed in CW EPR although second-derivative displays have been used to improve resolution between partially-resolved spectral components of spin probes partitioned between aqueous and hydrocarbon phases of micelles [4]. In some cases echo-detected field-swept EPR spectra are differentiated digitally in order to obtain a more convenient for an EPR spectroscopist first-derivative display (e.g. [5]). Digital differentiation of experimental spectra could be achieved by using the above-mentioned pseudomodulation technique [1] or, by applying various differentiation filters (e.g. see [6] and reference therein).

Although the software for digital differentiation of CW EPR spectra is easily available, literature survey shows that this approach has been rarely employed for calculating harmonics higher than the second. One reason for this is that the digital differentiation enhances high-frequency contributions leading to an increase in noise level. The high-frequency noise could be suppressed by increasing the amplitude of magnetic field modulation (either at the experimental or the post-processing stages) but this comes at the cost of distorting CW EPR line shapes. For first derivative EPR spectra exhibited by spin labels in fast motion limit such distortions could be accurately accounted for through spectral simulations using the model of Robinson and coworkers [7,8]. More recently, Nielsen et al. analyzed resolution-enhancing effects of magnetic field modulation through solving Bloch equations [9]. The Robinson model has been also applied for analyzing EPR oximetry data from probes exhibiting spectra of Lorentzian shape [10,11].

Here another strategy for digital differentiation EPR and other spectroscopic signals is presented. Rather than simulating effects of sinusoidal field modulation, differentiation of digitized spectra is replaced by a convolution with a filter function of a chosen derivative form. Such a procedure improves spectral resolution in the higher harmonics that enhance relative amplitude of sharp spectral features over the broad lines. At the same time raising high-frequency noise is suppressed through filtering. These features are illustrated on an example of a Lorentzian filter function that has a principal advantage of adding a known magnitude of homogeneous broadening to the spectral shapes. Such spectral distortion could be easily and accurately accounted for in the consequent least-squares data modeling. Moreover, existing convolution-based fitting EPR software [12] could be utilized for all the spectral manipulations. Application examples include calculation of higher harmonics from pure absorption echo-detected EPR spectra and resolving small hyperfine coupling that are unnoticeable in conventional first derivative EPR spectra. Another example involves resolving overlapping spin-label EPR spectra from slow (broad) and fast (sharp) spectral components. It is shown that the fast component could be digitally filtered out in a higher harmonic display, least-squares simulated to determine all the parameters, and precisely back-calculated as it should appear in the normal first-derivative display before any filtering. Such a procedure leads to fast and automatic separation of sharp spectral component without explicit simulation of the slow motion spectrum. The method is illustrated on examples of X-band EPR spectra of partially aggregated membrane peptides.

2. Theory and methods

Post-processing of digital signals usually involves filtering that could be easily accomplished in frequency domain by multiplying Fourier transform image $P(\omega)$ of the signal $p(B)$ by a window function $F(\omega)$

$$I(\omega) = P(\omega) \cdot F(\omega) \tag{1}$$

yielding the filtered signal $I(\omega)$. The window $F(\omega)$ could be chosen to suppress undesirable frequencies and/or to select the signals occurring only within specific frequency window [13]. In the field domain Eq. (1) corresponds to a convolution integral

$$I(B) = \int p(B - B')f(B)\mathrm{d}b = p(B) \otimes f(B), \tag{2}$$

where $\otimes$ is the convolution symbol.

One useful property of the convolution integral is that differentiation of the filtered signal $I(B)$ requires taking derivative of only one of the functions in the right-hand part of Eq. (2)

$$I'(B) = p'(B) \otimes f(B) = p(B) \otimes f'(B). \tag{3}$$

Thus, instead of differentiating the signal $p(B)$ and then filtering the noise one could differentiate the filter function $f(B)$ instead. Advantage of the latter is that such a differentiation could be easily carried for the filter function in the analytical form.

While many filter functions $f(B)$ could be applied for differentiation of EPR spectra using Eq. (3), let us consider the case of a Lorentzian filter

$$f(B) = \frac{2A}{\pi} \frac{\Delta B_{1/2}^2}{\Delta B_{1/2}^2 + 4(B_0 - B)^2}, \tag{4}$$

where A is the value of the Lorentzian line integral, B_0 is the center of the line, and $\Delta B_{1/2}$ is the width at half-height. It is easy to show that convolution of two Lorentzian functions $f_1(B)$ and $f_2(B)$ with corresponding widths $\Delta B_{1,1/2}$ and $\Delta B_{2,1/2}$ yields a Lorentzian function with width