Research Highlight #146
MDIFF NARS of Cu (II) in Frozen Solution

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Introduction and Methods: Non-adiabatic rapid sweep (NARS) of a spin-label was introduced by A.W. Kittell *et al* [1]. In NARS, the spectrum is divided into segments, each of which is scanned repetitively about a million times at a rate of 10,000 sweeps per second and time-averaged. Averaged spectral segments are aligned to produce a complete pure absorption spectrum.

Results: NARS has been applied by us to doubly labeled proteins [2] and to a frozen solution of Cu(II) at 1.9 GHz [3]. The upper spectrum of the figure shows the pure absorption of Cu(II). Hyperfine and Zeeman interactions are very evident in this display. Application of a moving difference (MDIFF) filter, which is defined in the figure, results in the center spectrum. Further application of a moving "difference-of-differences" digital filter results in the lower spectrum. Both low frequency noise and broad spectral features are filtered by these transformations, and the nitrogen ligand superhyperfine features are elegantly revealed. The middle spectrum was found to have higher SNR by a factor of about 6 compared with field-modulation EPR for equal acquisition times.

NARS-EPR of $Cu^{II}(imidazole)_4$

MDIFF, $I_{n-\Delta H/2} - I_{n+\Delta H/2}$
$\Delta H = 5$ G
($\times 10$)

$2I_n - I_{n+\Delta H} - I_{n-\Delta H}$
$\Delta H = 5$ G
($\times 10$) $\nu_{mw} = 1.9$ GHz

200   400   600   800   1000
Magnetic Field (G)

Implications: Ernst [4] observed that a transformation such as MDIFF transforms and filters the spectrum. He comments that these are identical processes but with different purposes. An alternative approach known as "pseudomodulation" was introduced by us some years ago [5]. It convolutes a sinusoid with the spectrum, which mathematically mimics the effect of magnetic field modulation in EPR spectroscopy. The value of the sinusoidal amplitude in pseudomodulation and the value of ΔH in MDIFF play similar roles. This paper discussed the transformation between first- and second derivative-like displays. We recognized the dual role of the pseudomodulation algorithm: filtering and transformation, and recommended that if the 2nd derivative-like display were desired, it is preferable to acquire the 1st harmonic-like display and then apply pseudomodulation. NARS acquisition now permits application of either pseudomodulation, MDIFF, or moving difference-of-differences directly to the pure absorption spectrum. These algorithms filter low frequency noise and improve the signal-to-noise ratio. If desired, filtering of high frequency noise can be accomplished in a separate step by application of a smoothing filter.

Discussion: These filters can be applied to simulated spectra as well as experimental spectra. They enhance slight inflections in the spectrum and suppress broad features, and therefore can be useful in computer-driven fits of theoretical models to data. A fundamental difference between pseudomodulation and MDIFF is that every point in the MDIFF display is derived from just two experimental points, whereas all points between two preset experimental values are used to produce a single point in pseudomodulation. NARS spectroscopy has opened new opportunities for computer-based post processing analysis of EPR spectra. These opportunities arise for several reasons: 1) The signal to noise ratio, assuming dominant Gaussian noise, is intrinsically higher by a factor that is in the range of five to ten compared with conventional magnetic field modulation. 2) Noise that has a 1/f character is suppressed by time averaging. 3) To a high approximation, there is no instrumental distortion of the spectrum, whereas conventional field modulation introduces considerable distortion. And 4), alternative digital transformations of spectra to enhance relative sensitivity to particular spectral features are readily compared. These opportunities have collectively been called "Information Geometry", which is the subject of Collaborative Project 2 of the current funding period of the EPR Center.
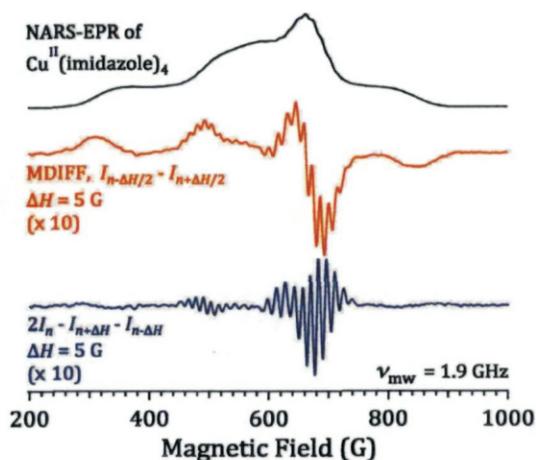
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