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Spectral Estimation of NMR Relaxation

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In this paper, spectral estimation of NMR relaxation is constructed as an extension of Fourier Transform (FT) theory as it is practiced in NMR or MRI, where multidimensional FT theory is used. *n*D NMR strives to separate overlapping resonances, so the treatment given here deals primarily with monoexponential decay. In the domain of real error, it is shown how optimal estimation based on prior knowledge can be derived. Assuming small Gaussian error, the estimation variance and bias are derived. Minimum bias and minimum variance are shown to be contradictory experimental design objectives. The analytical continuation of spectral estimation is constructed in an optimal manner. An important property of spectral estimation is that it is phase invariant. Hence, hypercomplex data storage is unnecessary. It is shown that, under reasonable assumptions, spectral estimation is unbiased in the context of complex error and its variance is reduced because the modulus of the whole signal is used. Because of phase invariance, the labor of phasing and any error due to imperfect phase can be avoided. A comparison of spectral estimation with nonlinear least squares (NLS) estimation is made analytically and with numerical examples. Compared to conventional sampling for NLS estimation, spectral estimation would typically provide estimation values of comparable precision in one-quarter to one-tenth of the spectrometer time when *S*/*N* is high. When *S*/*N* is low, the time saved can be used for signal averaging at the sampled points to give better precision. NLS typically provides one estimate at a time, whereas spectral estimation is inherently parallel. The frequency dimensions of conventional *n*D FT NMR may be denoted D_1, D_2, etc. As an extension of *n*D FT NMR, one can view spectral estimation of NMR relaxation as an extension into the zeroth dimension. In *n*D NMR, the information content of a spectrum can be extracted as a set of *n*-tuples ($\omega_1, \ldots \omega_n$), corresponding to the peak maxima. Spectral estimation of NMR relaxation allows this information content to be extended to a set of ($n + 1$)-tuples ($\lambda, \omega_1, \ldots \omega_n$), where λ is the relaxation rate.

Key Words: **spectral estimation; NMR relaxation; analytical continuation; Gaussian error propagation; nonlinear least squares.**

1. INTRODUCTION

Relaxation is a fundamental aspect of nuclear magnetic resonance. The relaxation rates of single-quantum transverse operators determine the linewidths of the resonances detected during the acquisition period of an NMR experiment.[2] Longitudinal relaxation determines the minimum recycle time. The relaxation rates of higher order operators during multidimensional experiments determine the linewidths of resonances in the indirectly detected dimensions and the sensitivity of the experiment. Relaxation provides experimental information on the physical processes governing relaxation, particularly of dynamics. Longitudinal and transverse NMR relaxation form a basis for MR imaging. However, very few methods are available for the estimation of the NMR monoexponential relaxation rate, with nonlinear least squares (NLS) estimation being the most common. Optimal experimental design in the context of NLS is not a trivial task (*1*) because NLS estimation is believed to be biased. Furthermore, the analysis provided by NLS becomes implicit in pulse program design (*2*). We have found that alternative concepts, like median estimation (*3*), have advantages over NLS estimation in NMR relaxation studies. However, a proper and widely based perspective of exponential analysis of general physical phenomenon was not available until recently (*4*). In that review, the authors observed that a Fourier transform (FT) approach gave estimates that correlated best with other physical measures. In this paper, we extend that FT approach into the context of NMR with a small number of sample points and into the domain of complex numbers.

2. SPECTRAL ESTIMATION

Let us focus on a monoexponential transient of the following form:

$$f(t) = Ae^{-Lt} + B, \qquad [1]$$

where initially we assume that all quantities are real and the relaxation rate, L, is positive. This form is general enough to encompass both longitudinal and transverse relaxation and situations similar to transverse relaxation in which B is different from zero due to instrumental effects. The one-sided continuous Fourier integral transform (*5, 6*) of Eq. [1] gives its transform as

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[2] Abbreviations used: NMR, nuclear magnetic resonance; MRI, magnetic resonance imaging; FT, Fourier transform; 2D, two dimensional; *n*D, *n* dimensional; pdf, probability density function; *S*/*N*, signal-to-noise ratio; *N*/*S*, noise-to-signal ratio; NOESY, nuclear Overhauser effect spectroscopy; NLS, nonlinear least squares.

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$$F(\omega) = A(L - i\omega)/(L^2 + \omega^2) + 2\pi B\delta(\omega), \qquad [2]$$

and hence the decay rate L can be extracted from the real and imaginary parts of $F(\omega)$ as

$$L = -\omega \mathrm{Re}(F(\omega))/\mathrm{Im}(F(\omega)) \qquad [3]$$

for any nonzero angular frequency ω. In practise, the discrete Fourier transform (*5*) must be used with sampled data. This method is advocated as the best method for the determination of a monoexponential decay rate (*4*). This suggests that Eq. [3] may provide relaxation estimates, which are of minimum variance and minimum bias. The relationship in Eq. [3] is a very good approximation when the discrete Fourier transform is used with long records of 256 points or more but is a poor approximation when short data records are collected, like in NMR relaxation. Nevertheless, for many exact relationships involving the continuous FT there exist similar exact relationships involving the DFT.

Assume a sample period of dt, so that we have relaxation data, $\mathbf{f}_1, \mathbf{f}_2, \mathbf{f}_3, \mathbf{f}_4, \ldots$ sampled at times $0, dt, 2dt, 3dt, \ldots$. We use the general convention that bold symbols refer to random variables and unbold symbols refer to their mean values. According to Eq. [1], the mean values of the first four sampled relaxation data are

$$f_1 = A + B; \quad f_2 = Ae^{-Ldt} + B;$$
$$f_3 = Ae^{-2Ldt} + B; \; f_4 = Ae^{-3Ldt} + B. \qquad [4]$$

Now, for a DFT of order N, the knth element can be represented as $1/N\, e^{-2\pi ikn/N}$, hence when $k = N/2$, coefficients are $1/N\, e^{-\pi ik}$, $k = 0, \ldots, N-1$. Thus, substitution from [4] shows that for a short record DFT, the decay rate can be extracted from data as

$$Ldt = \ln(\mathrm{Re}(F(\omega))/\mathrm{Im}(F(\omega))), \qquad [5]$$

where ω is selected to be at the half Nyquist angular frequency, $\omega_N/2$. Consequently, N must be a multiple of four. Expression [5] could be evaluated within NMRpipe (*7*), particularly if it was the end result of a multidimensional FT calculation. The DFT can be thought of as a matrix of complex values, hence this latter expression can be used to construct a more rational expression for the extraction of L from data. Therefore, L can be extracted from data as follows:

for 4 points, $f_1, \ldots, f_4$,

$$Ldt = \ln((f_1 - f_3)/(f_2 - f_4))$$

for 8 points, $f_1, \ldots, f_8$,

$$Ldt = \ln((f_1 - f_3 + f_5 - f_7)/(f_2 - f_4 + f_6 - f_8))$$

for 12 points, $f_1, \ldots, f_{12}$,

$$Ldt = \ln((f_1 - f_3 + f_5 - f_7 + f_9 - f_{11})/(f_2 - f_4 + f_6 - f_8 + f_{10} - f_{12})). \qquad [6]$$

These formulas provide a family of estimators each giving a noniterative estimate of L which is independent of any assumption concerning the error distribution within the data and without the necessity to simultaneously estimate the other parameters. In the context of FT NMR, the data values $f_1, f_2, \ldots$, are numbers which come from a (possibly multidimensional) Fourier transform and typically are block exponent 32-bit integers. It is generally assumed that these high-precision numbers are contaminated by Gaussian error.

3. IN THE LIMIT OF SMALL ERROR

For any function F of x_i, the error (variance) of F can be calculated according to the Gaussian error propagation technique as

$$\langle \delta F^2 \rangle = \sum_{i,j} \left(\frac{\partial F}{\partial x_i} \cdot \langle \delta x_i \delta x_j \rangle \cdot \frac{\partial F}{\partial x_j} \right), \qquad [AA]$$

where $\langle \delta x_i \delta x_j \rangle$ is the error (covariance) matrix element. Measurement error is usually assumed to be independent, in which case off-diagonal elements of the covariance matrix are zero. At this point we need make no assumptions about the error distribution function, except that the error is assumed to be small. A careful numerical study (*8*) of Gaussian error propagation in the limit of small error of this family [6] of estimators and of related divisor formulas (*4*) reveals that it is the four-point spectral estimator Eq. [6] which is of minimum variance. If we assume that each "measured" data value has error of the same standard deviation, σ, then, in the limit of small error, each partial derivative contributes error of the amount $\partial L/\partial f_i \sigma$, and the total estimation error (standard deviation) is the root squared sum of all contributions. In the limit of small error, L is unbiased and each of $f_1, \ldots, f_4$ can be substituted for its expression derived from Eq. [4]. The sum of terms can then be expressed conventionally as a rational form to give an expression for the standard deviation of the estimate, L:

$$\sum = \frac{\sigma}{dt} \sqrt{\frac{2}{(f_1 - f_3)^2} + \frac{2}{(f_2 - f_4)}}$$
$$\sum = \frac{\sigma}{|A|dt} \frac{\sqrt{2}\sqrt{1 + e^{2Ldt}}}{(1 - e^{-2Ldt})}. \qquad [7]$$

This last expression, Eq. [7], can be optimized in Ldt. It is minimal when

$$Ldt = \frac{1}{2} \ln\left(\frac{3}{2} + \frac{1}{2} \sqrt{17} \right) = 0.635. \qquad [8]$$

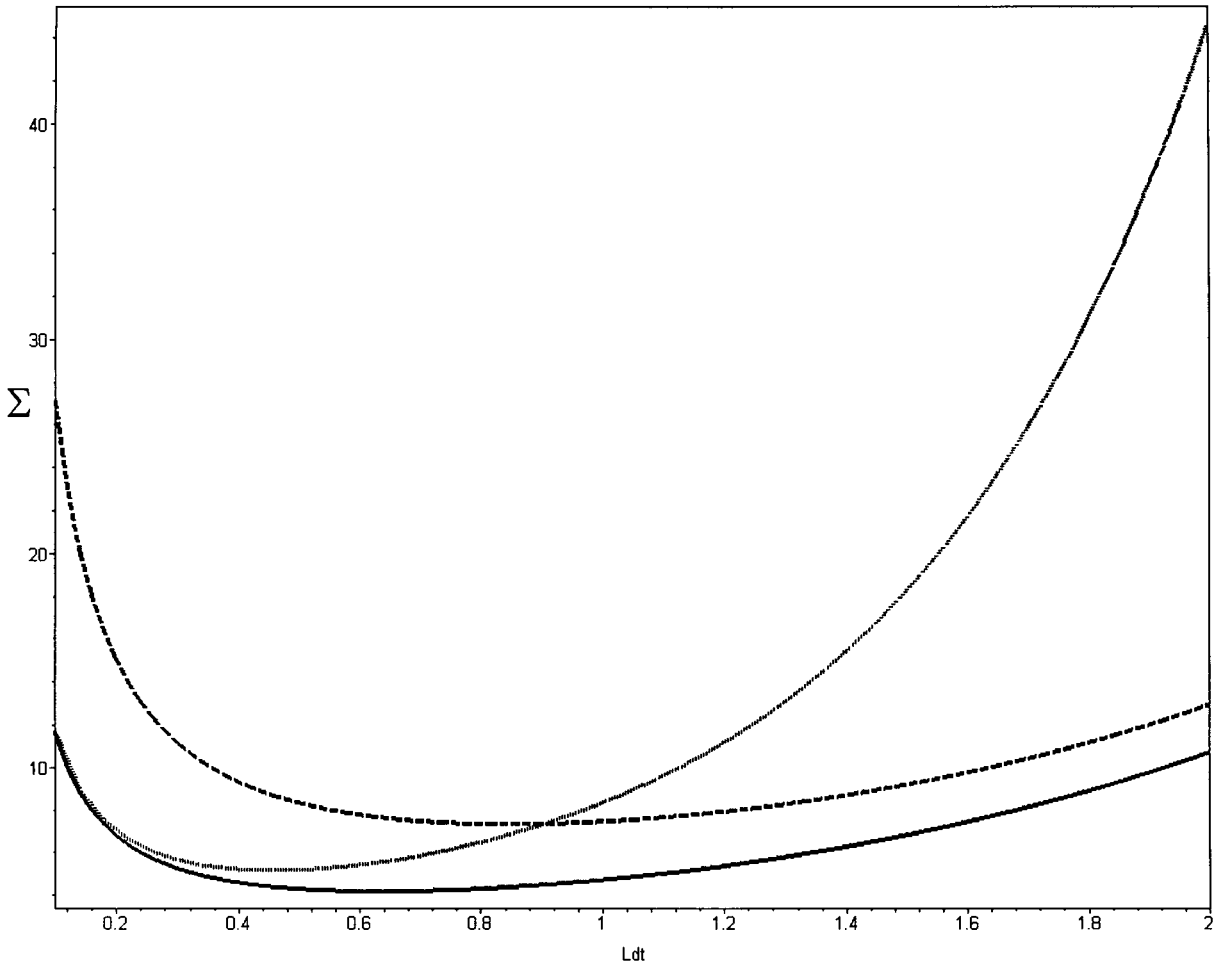

FIG. 1. $\Sigma(Ldt)$, standard deviation of estimate in units of $\sigma/|A|dt$ for three estimators. The dark curve at bottom is for $\ln((f_1 - f_3)/(f_2 - f_4))$, four-point spectral estimator at $\omega_{N/2}$, the dotted line is for spectral estimator $0.5 \ln((f_1 - f_2)/(f_3 - f_4))$ (we are indebted to the reviewer for suggesting this estimator) and the dashed line is for the divided difference formula $\ln((f_1 - f_2)/(f_2 - f_3))$ (*4*).

Thus, if we have a prior estimate L of the relaxation rate, we can obtain an estimate of minimal variance if we choose dt, the sample period such that $Ldt = 0.635$. Sampling at the nearby half-life would imply that $Ldt = \ln(2) = 0.693$, given the prior estimate of L. A plot of Σ as a function of Ldt is shown in Fig. 1. Σ is very flat at the bottom. It can be seen that we will have nearly optimal variance when $0.5 < Ldt < 0.8$. A/σ can be identified with the signal-to-noise ratio, S/N. Thus, the estimation variance can be reduced linearly with decrease in N/S. **L** gives a point estimate. Interval estimation can be obtained by a separate measurement of S/N.

It is of interest to consider the spectral implications of this insight. **L** is an imaginary frequency. In the complex plane it is close to some real frequencies, hence there is some optimal sample period. Since a relaxation decay dies away quickly, only a limited number of samples are needed. Rather than oversampling, it is better to put effort into signal averaging at the optimal four points so as to reduce N/S. The four-point spectral estimator can be thought of as a kind of digital filter which ignores irrelevant information. If the quantity (A/σ) can be identified with the signal-to-noise ratio, S/N, then the number of significant bits provided by this S/N is $\log_2(S/N) = n$. Given a prior estimate of exponential relaxation rate, L, then the optimal variance is obtained if we select the sample period, dt, such that $Ldt = 0.635$. Then, at the optimum, the estimation standard deviation, Σ, will be calculated to be $4.2(\sigma/Adt)$. This says that the number of significant bits $\log_2(L/\Sigma)$ in the estimate L will be $n - 2.73$. Thus, a minimum of three significant bits is required of S/N in order to have any significance in an estimate L, even at the optimum.

4. UNBIASED ESTIMATION

The bias of an estimator **L**, can be determined by evaluation of its expectation value, $E(\mathbf{L})$ (*9*). It is reasonable to assume a normal, i.e., Gaussian, probability density function for FT error at this stage. As a random variable we write the spectral estimator as

$$\mathbf{L}dt = \ln((\mathbf{f}_1 - \mathbf{f}_3)/(\mathbf{f}_2 - \mathbf{f}_4)). \qquad [9]$$

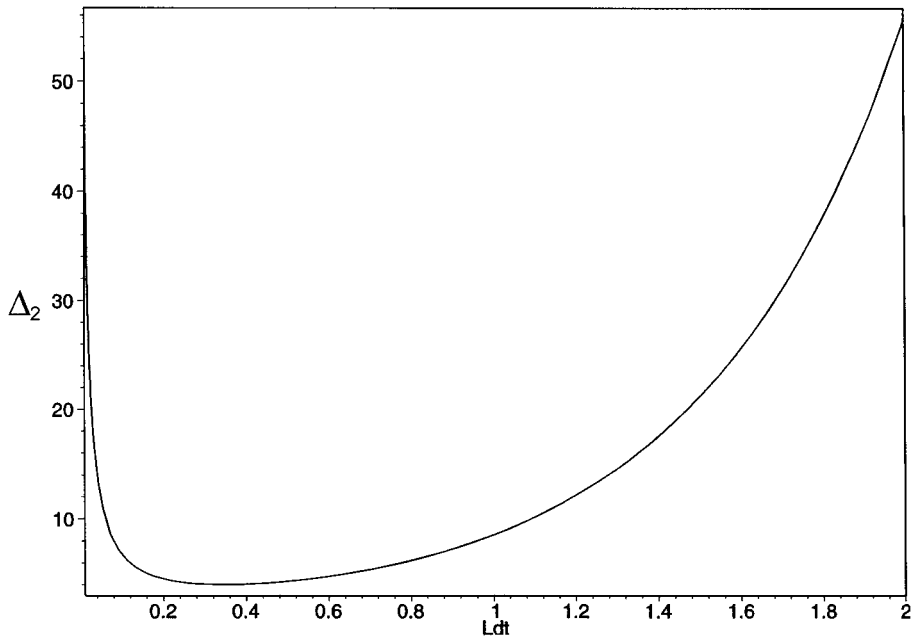

FIG. 2. $\Delta_2(Ldt)$, the second-order bias, in units of σ^2/A^2dt.

For our four-point spectral estimator, **L**, we can combine variation terms in the numerator and denominator and express its expectation value as

$$E(\mathbf{L})dt = \frac{1}{2\pi}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\ln\left(\frac{(f_1 - f_3 + \sigma' x)}{(f_2 - f_4 + \sigma' y)}\right) \times e^{-(x^2+y^2)/2}dxdy, \qquad [10]$$

where $\sigma' = \sqrt{2}\sigma$. The logarithm can be expanded as an asymptotic expansion (*10*) (not a convergent expansion) in σ' and the integral evaluated term by term (*11*). All of the terms in odd powers of σ' evaluate to zero.

$$E(\mathbf{L})dt = \ln\left(\frac{(f_1 - f_3)}{(f_2 - f_4)}\right) + \frac{\sigma'^2}{2}\left(\frac{1}{(f_2 - f_4)^2} - \frac{1}{(f_1 - f_3)^2}\right) + \mathrm{O}(\sigma'^4) \qquad [11]$$

In an asymptotic expansion the remainder term does not necessarily grow smaller with higher order. The bias expression may not be well behaved for large σ. However, there may be some insight derived from summing such a series. The coefficients, C_k, of the even order terms can be computed to higher order. The exponential generating function $1/(\sqrt{1-2x}(1+\sqrt{1-2x}))$ and the general coefficient $C_k = (2k-1)!/(2^k k!)$ can be computed (*12*). This allows the bias to be expressed asymptotically in closed form:

$$\Delta \approx \Psi\left(\frac{\sigma'^2}{(f_2 - f_4)^2}\right) - \Psi\left(\frac{\sigma'^2}{(f_1 - f_3)^2}\right), \qquad [12]$$

where

$$\Psi(X) = X\,\mathrm{hypergeom}([3/2, 1, 1], [2], 2X)/2, \qquad [13]$$

in which "hypergeom" is the Barnes's extended hypergeometric function. In Eq. [11], the first term on the right, $\ln((f_1 - f_3)/(f_2 - f_4)) = Ldt$, gives the true value of L, and the other terms are the bias terms. Thus, the four-point spectral estimator alone is a biased estimator of relaxation rate, L. This may be an unavoidable aspect of many kinds of nonlinear estimation, which is seen to have the potential to rectify noise power into bias. However, with a separate measurement of noise statistics we can calculate a reasonable estimate of the bias and subtract away the bias to give an unbiased estimate of L. Further insight can be derived by focusing on the form of the second-order bias term, Δ_2. After substitution from [4], it can be expressed as

$$\Delta_2 = \frac{\sigma^2 e^{2Ldt}}{A^2 dt(1 - e^{-2Ldt})} \qquad [14]$$

and plotted as a function of Ldt in Fig. 2. This function has a minimum at $Ldt = \frac{1}{2}\ln(2) = 0.347$. Thus, it can be seen that the objectives of minimum variance and minimum bias are contradictory experimental design objectives. It is probably better to aim for minimum or near minimum variance and to subtract off the bias.

Spectral estimation of NMR relaxation is an exact treatment. Other treatments are not exact. The purpose of an exact treatment of NMR relaxation is to allow the relaxation estimates to be used as if they were measurement data, for the purposes of further mathematical modeling (*13*). Such mathematical modeling generally falls into the category of nonlinear estimation and may involve nonlinear least squares or some other advanced techniques like median estimation (*3*). For this purpose it is necessary that the values estimated be unbiased and that reasonable estimates of the standard deviation of each estimate be available. Spectral estimation of NMR relaxation provides a rigorous mathematical framework in which this can be achieved.

5. THE ANALYTICAL CONTINUATION OF SPECTRAL ESTIMATION

Now let us assume that the quantities A, B, and f in Eq. [1] are complex. Since spectral estimation is constructed as an extension of Fourier transform theory in which all quantities are complex, we may expect analytical continuation (*14*) of spectral estimation of NMR relaxation to provide an extension into its natural domain. Phase invariance is seen to be self-evident from the divisor structure of the estimator, Eq. [6]. In the presence of an exponentially decaying transient, L is an estimator for a real positive quantity and so any imaginary part can be identified as due to error and ignored. We may write (*14*)

$$\begin{aligned} Ldt &= \mathrm{Re}(\ln((f_1 - f_3)/(f_2 - f_4))) \\ &= \ln(|(f_1 - f_3)/(f_2 - f_4)|) \end{aligned} \quad [15]$$

when $\mathrm{Im}(\ln((f_1 - f_3)/(f_2 - f_4))) < \textit{threshold}$, zero otherwise.

Determination of the expectation value of the estimator now requires evaluation of a quadruple integral for bivariate complex Gaussian error,

$$\begin{aligned} E(\mathbf{L})dt = \frac{1}{4\pi^2} \int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} \\ \times \ln\left(\frac{f_1 - f_3 + \sigma'(u + iv)}{f_2 - f_4 + \sigma'(r + is)}\right) \\ \times e^{-u^2+v^2+r^2+s^2/2} du dv dr ds. \end{aligned} \quad [16]$$

As before, the logarithm can be expanded as a power series in σ' and integrated term by term. This is a convergent sum with only two nonzero terms.

$$E(\mathbf{L})dt = \ln\left(\left|\frac{f_1 - f_3}{f_2 - f_4}\right|\right) - \frac{i\pi}{2}\left(\mathrm{signum}\left(\frac{f_1 - f_3}{f_2 - f_4}\right) - 1\right) \quad [17]$$

In the presence of an exponentially decaying transient, the expectation of the imaginary part is zero and the real part is unbiased. This is much simpler than in the case of real error. Determination of the variance about the mean of the real part requires explicit construction of the quantity in Eq. [15]. Let

$$\begin{aligned} P_r &= \mathrm{Re}(f_1 - f_3), \quad P_i = \mathrm{Im}(f_1 - f_3), \\ Q_r &= \mathrm{Re}(f_2 - f_4), \quad Q_i = \mathrm{Im}(f_2 - f_4). \end{aligned} \quad [18]$$

We wish to evaluate the quantity $E((\mathrm{Re}(\mathbf{L}))^2)$, the variance about zero. We write

$$(\mathrm{Re}(L))^2 dt^2 = \left[\ln\left(\sqrt{\frac{(P_r + \sigma' u)^2 + (P_i + \sigma' v)^2}{(Q_r + \sigma' r)^2 + (Q_i + \sigma' s)^2}}\right)\right]^2. \quad [19]$$

As before, the quantity on the right-hand side of Eq. [19] can be expanded in a power series in σ' and integrated term by term using the pdf weighted integral of Eq. [16]. Evaluated to second order, this gives

$$\begin{aligned} &E((\mathrm{Re}(\mathbf{L}))^2)dt^2 \\ &\quad = \left[\ln\left(\left|\frac{f_1 - f_3}{f_2 - f_4}\right|\right)\right]^2 \\ &\qquad + \left(\frac{1}{P_r^2 + P_i^2} + \frac{1}{Q_r^2 + Q_i^2}\right)\sigma'^2 + O(\sigma'^4). \end{aligned} \quad [20]$$

On the right side of Eq. [20], the first term is the square of the mean value of the estimator. The second term is the second-order contribution to the variance. Substituting from Eq. [18], the latter can be expressed as a function of σ:

$$\begin{aligned} &2\left(\frac{1}{|f_1 - f_3|^2} + \frac{1}{|f_2 - f_4|^2}\right)\frac{\sigma^2}{dt^2} \\ &\quad = 2\left(\frac{1}{(1 - e^{-2Ldt})^2} + \frac{1}{(e^{-Ldt} - e^{-3Ldt})^2}\right)\frac{\sigma^2}{|A|^2 dt^2}. \end{aligned} \quad [21]$$

If we take the square root of Eq. [21] and simplify, we get precisely the same form as is shown in Eq. [7] and plotted in Fig. 1. However, in this instance the $|A|$ is the complex modulus and accounts for a greater portion of the signal. Hence, the variance and standard deviation are reduced.

6. COMPARISON WITH NLS

Clearly, there is no natural extension of NLS (*15*) to complex data. For analytical comparison we start by considering the case of four points of real data collected at equal time increments of dt. It was Oberlander (*16*) who first recognized that NLS may have an analytical solution. If we take the

expressions in Eq. [4] and form the sum of squared deviations expression, then differentiate with respect to A, B, and L and set these expressions to zero, we obtain a system of three equations which can be solved in L to obtain

$$L = -\frac{1}{dt}\ln(\text{RootOf}((f_2 - 2f_3 + f_1)Z^4 + (2f_4 - 2f_2 + 4f_1 - 4f_3)Z^3 + 3(f_4 - f_3 - f_2 + f_1)Z^2 + (4f_4 - 4f_2 + 2f_1 - 2f_3)Z - 2f_2 + f_3 + f_4)). \quad [22]$$

It can be seen that this expression has similarity to that of the four-point spectral estimator and might yield the same value under some circumstances. However, since a quartic with real coefficients may have up to four real roots, there may be uncertainty regarding whether the global minimum has been achieved via numerical optimization. NLS needs to estimate all of the parameters, which is more than we desire. It can be seen from [22] that analysis of error propagation or of bias as we have done for spectral estimation is an intractable problem for NLS even with just four points.

NLS relies on approximation. For statistical analysis the covariance matrix can be calculated from the inverse of the Hessian (*15*), yet generally, in NLS, the Hessian is only approximated. The approximations used in NLS are good approximations when S/N is high, when the number of points used is large, and when convergence is good. However, poor S/N defines the experimentally interesting situation (*2*). In NMR we wish to minimize machine time so a small number of points is advantageous. Spectral estimation does not require iteration so convergence is not an issue nor is the question (*2*) of whether to use a two-parameter fit or a three-parameter fit.

For numerical comparison, Fig. 3 of Ref. (*2*) is worth considering. A total of 512 SR scans were used to achieve $S/N = 3000$. For a two-parameter fit they report $T_1 = 4.95 \pm 0.15$ s, $M_0 = 99.4 \pm 1.1$, and $\chi^2 = 69923$, while for a three-parameter fit $T_1 = 4.561 \pm 0.007$, $M_0 = 98.01 \pm 0.05$, $\alpha = -0.0397 \pm 0.004$, and $\chi^2 = 121$ for NLS analysis of 20 points unequally spaced in time.

It is worth noting the 9% error between NLS T_1 estimates from two-parameter and three-parameter fits. Visually, the two fits appear to be very similar. This illustrates a limitation of the concept of data fitting to an exponential function and then visualizing the data. Good visualization requires more data which can be counterproductive and wasteful. Also, an exponential function is an extreme test of the mathematical assumptions of NLS that the error in data is small. Error may be relatively small over part of the range but not over the whole range.

Unfortunately, the authors failed to use software which reports the covariance of estimation error. We have found there may be a large correlation between estimation errors of variables. Our 4-point spectral estimator is an analytical expression which is orthogonal in estimation to other parameters. The authors measured 20 points spanning 20 s. An optimal sample period for SE would be 0.635*4.561 = 2.896 s. Starting with the first point at $t = 0$, optimal 4-point SE would use equally spaced points with the last point at $t = 8.869$ s. NLS requires points at longer delays because of the need to accurately estimate baseline offset. SE represents a large experimental time saving. SE with 512 SR scans is likely to require less than $\frac{1}{4}$ the instrument time.

Given $S/N = 3000$ and assuming four-point SE estimation is done at the optimal sampling period, 2.896 s, a calculation of Gaussian error (*8*) gives an estimation error standard deviation of ± 0.0054 compared to ± 0.007 for three-parameter NLS. Hence, we provide better precision with 1/4 the work. In our theoretical formulation we advocate the estimation of relaxation rate as in kinetics. When $S/N = 3000$, the second-order estimation bias in relaxation rate is only 1.5E-7 1/s.

It has been reported that when $S/N = 100$, NLS can achieve a precision of 2% in exponential estimation. In this case, the estimation error standard deviation for four-point spectral estimation is 6.6%. Assuming a saturation recovery experiment and neglecting other contributions to experiment time, the estimated time saved can be calculated.

The total time for four-point spectral estimation in SR is

$$0 + dt + 2dt + 3dt = 6dt.$$

Now at optimum,

$$Ldt = 0.635,$$

or

$$dt = 0.635T_1,$$

$$6dt = 3.81T_1.$$

For a typical NLS SR experiment assume 16 equally spaced times out to a maximum of $5T_1$, for a total time of

$$0 + 1/3T_1 + 2/3T_1 + \cdots + 4\ 2/3T_1 + 5T_1 = 15*16*1/2*1/3 = 40T_1.$$

This implies a time saving of 40/3.81 = 10.5 times. This time savings can be used to signal average at the four points with an increase in S/N of sqrt(10.5) = 3.24 times, since estimation error standard deviation is linear in S/N this implies that it is reduced by 6.6%/3.24 = 2%.

NLS is believed to be biased but the nature of bias is not well understood. We have shown a bias that is proportional to $(N/S)^2$, so it is not wise to ignore bias in the experimentally interesting case of poor S/N. In general (*15*), bias is proportional to $1/n$, where n is the number of points used, but the

proportionality constant is unknown. The need to use a large number of points in NLS to mimimize bias is wasteful of time. Reference (*17*) provides a method by which bias can be reduced to a value which is proportional to $1/n^2$ but this method is tedious and has never been used in NMR.

In summary, efficiency and rigorous mathematical statistics (*9*) requires that we use four-point spectral estimation rather than NLS for the estimation of NMR relaxation.

7. THE BASEPLANE

Consider what happens when we apply the spectral estimator in a region in which there is no signal. The mean values of f_1, f_2, f_3, and f_4 are now zero. So we need to consider the properties of the random variable,

$$\mathbf{L} = \ln\left(\frac{\mathbf{x}}{\mathbf{y}}\right), \qquad [23]$$

where $\mathbf{x}$ and $\mathbf{y}$ are each random variables from a complex Gaussian probability distribution. Now the argument or phase angle of $\mathbf{x}$ and of $\mathbf{y}$ is uniformly distributed in the range $[0, 2\pi]$, therefore, the argument of the quotient is also uniform in $[0, 2\pi]$. The expected magnitude,

$$E\left(\ln\left|\frac{\mathbf{x}}{\mathbf{y}}\right|\right) = \ln\left(\frac{E(|\mathbf{x}|)}{E(|\mathbf{y}|)}\right) = \ln\left(\frac{E(|\mathbf{x}|)}{E(|\mathbf{x}|)}\right) = 0,$$

and, since each of $\mathbf{x}$ and $\mathbf{y}$ is of unit variance or standard deviation, so is $|\mathbf{x}/\mathbf{y}|$. Now since $\partial \ln(z)/\partial z|_{z=1} = 1$, $\ln|\mathbf{x}/\mathbf{y}|$ has unit variance or standard deviation about zero. This is much greater error than that in the presence of an exponentially decaying transient. Hence, we should choose *threshold* sufficiently high that points on the baseplane are coerced to zero. Nor do we need worry about the concept of a lineshape function. With *threshold* sufficiently high, the information content is contained in the nonzero points which remain.

8. DISCUSSION

The property of phase invariance is an extremely important property of spectral estimation. Not only does this allow us to dispense with a lot of the work associated with phasing but also it allows the dispensing of a lot of the computational machinery. Hypercomplex data storage is used in *n*D FT NMR to separate the imaginary parts of FT spectra by frequency dimension so that proper phase can be determined and applied. With spectral estimation there is a savings in storage required since only complex FT data are needed. Thus, the parts of signal strength that are lost as mixed real and imaginary parts are retained. This adds to sensitivity. We have discussed spectral estimation as a generalization of conventional NMR. Spectral estimation can be applied to conventional NMR relaxation pulse sequences in a straightforward manner. In 2D NOESY the cross-peak volumes give a rough approximation of the off diagonal elements of the cross-relaxation matrix. If expanded in one more dimension with four slices at equally spaced mixing times, spectral estimation will give estimates of the NOE build up rates as the end result of a *n*D FT calculation. Many kinds of *n*D pulse sequences could be likewise expanded by appending a (possibly heteronuclear) Carr–Purcell sequence. Similar considerations may apply to MRI.

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