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# Rapid high-resolution four-dimensional NMR spectroscopy using the filter diagonalization method and its advantages for detailed structural elucidation of oligosaccharides

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**Abstract**

Four-dimensional nuclear magnetic resonance spectroscopy with high resolution of signals in the indirect dimensions is reported as an implementation of the filter diagonalization method (FDM). Using an oligosaccharide derivatized with $^{13}$C-labeled acetyl isotags, a four-dimensional constant-time pulse sequence was tailored for conjoint use with the FDM. Results demonstrate that high resolution in all dimensions can be achieved using a relatively short experimental time period (19 h), even though the spectrum is highly congested in the direct and all three indirect dimensions. The combined use of isotags, constant-time pulse sequences, and FDM permits rapid isolation of sugar ring proton spin systems in multiple dimensions and enables all endocyclic *J*-couplings to be simply measured, the key goal to assigning sugar stereochemistry and anomeric configuration. A general method for rapid, unambiguous elucidation of spin systems in oligosaccharides has been a long-sought goal of carbohydrate NMR, and isotags combined with the FDM now enable this to be easily performed. Additional general advantages of the FDM program for generating high-resolution 2D slices in any dimension from a 4D spectrum are emphasized.
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*Keywords:* Filter diagonalization method; Multi-dimensional NMR; Constant time; $^{13}$C labeling; O-acetylation; Oligosaccharides

## 1. Introduction

In NMR spectroscopy, one topic of fundamental concern and of great practical importance is signal processing. As higher-dimensional experiments are nothing more than sets of time-domain data judiciously combined, it is important to be cognizant that all points in free-induction decays are used in a transform to give some *estimate* of whatever frequencies may have contributed to the time-domain signal. The frequency estimate is then just a better representation of data for the human visual system that is mathematically equivalent to the time-domain spectrum, with the proviso that ubiquitous sinusoidal phenomena are actually under investigation. *All* points in one time-domain accumulation are also related to *all* points in other time-domain accumulations as a multi-dimensional spectrum accrues. Hence, methods that utilize orthogonal processing (i.e., treating each dimension separately) of some subset of the total number of points in the indirect dimensions will necessarily provide a much poorer estimate in these frequency domains than methods that utilize all the points.

These issues especially come to the forefront when correlations of four or more frequencies are sought. Spectra obtained using conventional methods (Fourier transform (FT) and mirror-image linear prediction

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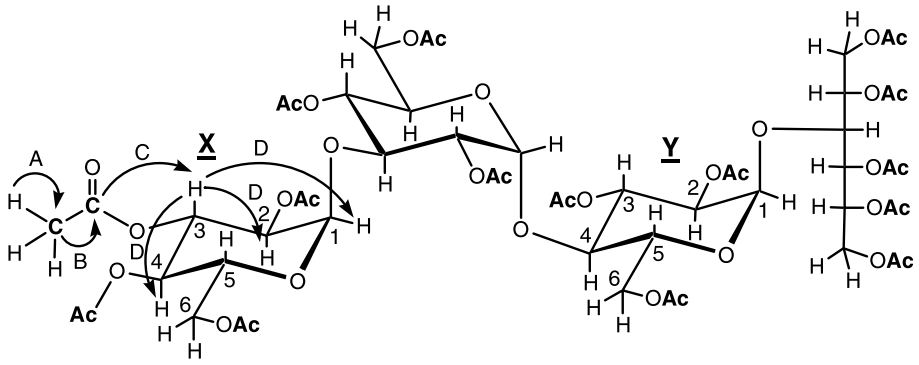

Fig. 1. The structure of the peracetylated nigeran oligosaccharide-alditol, having doubly $^{13}$C-labeled acetyl groups ($^{13}$C-labeled isotags are shown in bold letters). The numbering of positions on sugar spin systems X and Y are indicated. One acetyl group on spin system X is shown with arrows to illustrate magnetization transfers that occur during different steps of the 4D pulse sequence. Step A, INEPT-type transfer from $H_{Me}$ to $C_{Me}$; step B, INEPT-type transfer from $C_{Me}$ to CO; step C, HEHAHA transfer from CO to $H_{Ring}$; and step D, HOHAHA transfer among protons of the sugar ring.

(MI-LP)) often will not approach the natural linewidths of signals because they are limited by the data set actually used to estimate frequencies in the indirect dimensions. Relatively recently, procedures to circumvent this limitation in data use have been advocated [1] which include the filter diagonalization method (FDM) [2–4]. The FDM essentially utilizes the relationships between all the points in all free-induction decays, as it deals intrinsically with the data as complex multi-dimensional sinusoids, rather than being restricted to orthogonal sets. As a result, the amount of data that needs to be collected and the corresponding experimental time can be decreased dramatically in higher-dimensional experiments. While the FDM was originally presented as a general *n*-dimensional method, its practical implementation has been slower to achieve because developing the algorithm to accomplish the desired end required some new developments and extensive, methodical testing [5–8]. Here we present the application of the FDM to a 4D experiment that achieves high resolution in the direct and all indirect dimensions. A challenging example has been chosen from the carbohydrate field with considerable crowding of signals in all dimensions, and with narrower natural linewidths than proteins to illustrate the marked advantages of the FDM in estimating indirect and direct frequencies within very congested spectral precincts.

Carbohydrate NMR is fraught with several difficulties, and there are a number of important structural details in oligosaccharides that may only be possible to effectively establish through higher-dimensional methods. First, carbohydrate spectral overlap is severe due to the chemical similarity of the protons in all oligosaccharides. Second, the information needed to extract important stereochemical relationships among protons is crucial. This is inherent in their mutual *J*-couplings, which must be accurately measured in higher-dimensional experiments. Third, the information to assign linkage positions is required. Fourth, the vast majority of structures come from sources where isotopic labeling of the sugar backbone carbons is untenable. Recently, we developed a method to sidestep these difficulties that employs $^{13}$C-acetylation at all the hydroxyl groups of the oligosaccharide [9–11]. These labeled acetyl groups (“isotags”) increase the frequency range of the saccharide ring protons, and they afford a point of entry for magnetization to be transferred into the ring spin systems from the acetyl group nuclei. By employing a well-designed pulse sequence that allows evolution to be observed on each nucleus of the acetyl groups prior to their correlation to the sugar ring protons (shown schematically in Fig. 1), each spin system can be resolved in multiple dimensions. This enables the stereochemistry of individual sugars to be determined from the multiplet patterns of proton signals resolved in additional dimensions. Also, the positions of glycosidic linkages can be easily established on a sugar because the positions of acetyl group substitutions and the sites of linkage substitutions are mutually exclusive. Overlap of many signals in the direct and indirect dimensions, as well as the relatively large degeneracy of the spectrum, make this an ideal case for testing the applicability of the FDM.

## 2. Theory

The derivation of the filter diagonalization method (FDM) has been presented in detail for both one-dimensional and multi-dimensional cases [2–4]. However, a few of the key equations are presented here, as some of the issues are particularly relevant to this paper. The FDM is based on the assumption that a multi-dimensional data set:

$$c(n_1, n_2, \ldots, n_D) := c(n_1\tau_1, n_2\tau_2, \ldots, n_D\tau_D) = c(t_1, t_2, \ldots, t_D),$$

is composed of a sum of multi-dimensional sinusoids, with complex frequencies $(\omega_{1k}, \omega_{2k}, \ldots, \omega_{Dk})$ and complex amplitudes $(d_k)$. The dimensionality of the data set $(D)$ can be any size in principle, but for the purposes of this paper we will limit the discussion to four dimensions $(D = 4)$.

$$c(n_1, \ldots, n_4) = \sum_{k=1}^{K} d_k \prod_{l=1}^{4} \mathrm{e}^{-\mathrm{i}n_l\tau_l\omega_{lk}}. \quad (1)$$

In this case, the number of sinusoids $(K)$ may be any finite number that can be fit by the data size (for example if $n_l = 0, 1, \ldots, N_l - 1$, then $N_1 \times N_2 \times N_3 \times N_4 \geqslant 2K$). In this way, no prior knowledge of $K$ is required, so long as this inequality is satisfied. Eq. (1) is equivalent to the auto-regression assumption used in other NMR data processing schemes.

The multi-dimensional signal can be expressed in terms of a pseudo-time evolution operator $\hat{U}_l$ and initial state $|\Phi)$ such that application of $\hat{U}_l$ to $|\Phi)$ gives the next data point in the $l$-dimension:

$$(\Phi|\hat{U}_1^{n_1}\hat{U}_2^{n_2}\hat{U}_3^{n_3}\hat{U}_4^{n_4}|\Phi) = c(n_1, n_2, n_3, n_4). \quad (2)$$

Through Eq. (2) the signal is completely described by $\hat{U}_l$ and $|\Phi)$. The challenge is to extract the relevant parameters. By expressing Eq. (2) in terms of the eigenvalues $(u_{lk})$ and eigenvectors $(\Upsilon_k)$ of $\hat{U}_l$, and employing the assumption in Eq. (1), the relationship between the operators and the spectral parameters can be established:

$$\sum_{k=1}^{K}(\Phi|\Upsilon_k)\left[\prod_{l=1}^{4} u_{lk}^{n_l}\right](\Upsilon_k|\Phi) = \sum_{k=1}^{K} d_k \prod_{l=1}^{4} \mathrm{e}^{-\mathrm{i}n_l\tau_l\omega_{lk}}. \quad (3)$$

The spectral parameters can thus be extracted from the eigenvectors and eigenvalues:

$$\omega_{lk} = \frac{\mathrm{i}\ln(u_{lk})}{\tau_l}, \quad (4)$$

$$d_k = \sqrt{(\Phi|\Upsilon_k)}. \quad (5)$$

The multi-dimensional operator $\hat{U}_l$ is defined by the signal size and as such its diagonalization can be time consuming. The solution to this problem is to employ the Fourier basis for the construction of the matrix [2–4]. In this basis, the total size of the matrices is much less than the full data size, decreasing the computational effort required for the diagonalization substantially.

Due to the non-orthogonal nature of the basis $(\mathbf{U}_0 \neq \mathbf{1})$ a generalized eigenvalue problem must be solved to obtain the eigenvalues and eigenvectors of $\mathbf{U}_l$:

$$\mathbf{U}_l\mathbf{B}_k = u_{lk}\mathbf{U}_0\mathbf{B}_k. \quad (6)$$

In practice, the matrices are ill-conditioned (they may be either over-determined or under-determined), therefore we apply a unique variant of regularization [5] to the problem prior to diagonalization:

$$\mathbf{U}_0^{\dagger}\mathbf{U}_l\mathbf{B}_k = u_{lk}(\mathbf{U}_0^{\dagger}\mathbf{U}_0 + q^2)\mathbf{B}_k. \quad (7)$$

This scheme is responsible for one of the chief adjustable parameters $(q^2)$ in the application of the FDM.

It is worthwhile to note that as the spectral parameters are extracted using FDM, there is no requirement for phase-sensitive data in construction of double absorption spectra. For a 4D experiment this means that only an eighth of the data (assuming that phase-sensitive data are collected in every indirect dimension) is actually used for the calculation. With an adjustment in the way the data are collected, however, the extra phase-sensitive data can be put to use in resolving power. If there is no decay of the signal with time, then the two data sets can be represented as one signal, effectively doubling the signal size. The requirement that the signal not decay is satisfied in the constant time (CT) [12] experiment where the total acquisition period in the indirect dimensions is held constant and the position of an inversion pulse in the period is arrayed. Thus, by using CT evolution periods in each of the indirect dimensions of a 4D experiment the resolving power of the FDM can be increased eight times over that of the FDM without using CT. This has been demonstrated previously in both two and three dimensions [6–8].

The CT experiment also improves the behavior of the signal for processing. As the signal does not decay in the indirect dimensions, the resulting lineshapes are ideal Lorentzians, satisfying the assumption of the FDM exactly. This means that the data can be fit by the FDM more efficiently, and correspondingly fewer data points are required in the fit. This does, however, come with the caveat that the imaginary parts of the resulting frequencies (they are not assumed to be zero by FDM) may be numerically imperfect, with arbitrary sign. This can result in unstable lineshapes in the resulting spectral estimate if a proper smoothing $(\Gamma)$ is not applied. The smoothing (usually on the order of the natural linewidth) is added to the width parameter prior to spectral generation. The CT experiment also means that the relative contribution of noise is the same throughout the FID, which is advantageous for processing methods. The constant-time intervals should be kept relatively short in order to take advantage of the larger signal-to-noise available during the initial evolution.

## 3. Experimental

A pulse sequence (Fig. 2) was developed to transfer magnetization through the nuclei of the acetyl groups, to the protons of the sugar rings. The pulse sequence

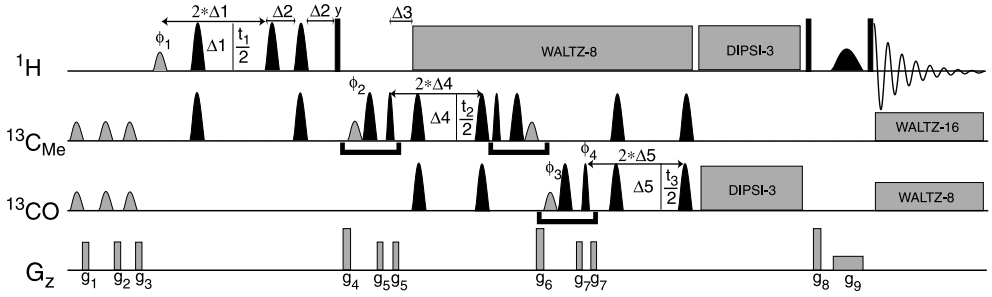


Fig. 2. Pulse sequence for the transfer of magnetization through the labeled nuclei of acetyl "isotags" to saccharide ring protons. The sequence is constant time [12] in all indirect dimensions. The black rectangles are hard 90° pulses, the shaded rectangles indicate application of gradients. The small gray shaded curves represent shaped 90° pulses. All black curves represent shaped 180° pulses. The series of three shaped pulses (bracketed) represent hyperbolic secant ABSTRUSE pulse trains [14]. All other 180° shaped pulses are BIP pulses [13]. The three shaped pulses on carbon at the beginning of the sequence are hyperbolic secant pulses designed to remove native magnetization. The shaped 90° pulse on proton is an e-BURP pulse [17] applied to the methyl protons. The delays used are as follows: $\Delta 1 = 11.5$ ms, $\Delta 2 = 1.9$ ms, $\Delta 3 = 1.5$ ms, $\Delta 4 = 20.8$ ms, and $\Delta 5 = 29.2$ ms. The DIPSI-3 [18–20] mixing sequence is applied at 2 kHz at the carbonyl and ring proton frequencies (9440 and −50 Hz, respectively) for 265 ms. These values were optimized empirically to affect the largest transfer of magnetization. After the mixing sequence, zero-quantum artifacts were removed using a simultaneous 180° BIP pulse and gradient *z*-filter [15]. These are applied at 65 kHz for 30 ms. WALTZ decoupling is applied on the carbon channel during acquisition. The decoupling is modulated to apply 1.375 kHz to the methyl frequency and 0.687 kHz to the carbonyl frequency. On the diagram it has been represented as separate WALTZ-16 and WALTZ-8 sequences applied at each frequency. The gradients used in this sequence have the following values: $g_1 = 2.3$ G/cm, $g_2 = 2.3$ G/cm, $g_3 = 2.3$ G/cm, $g_4 = 23.2$ G/cm, $g_5$=2.3 G/cm, $g_6$=20.9 G/cm, $g_7$=3.9 G/cm, $g_8 = 16.2$ G/cm, and $g_9 = 10.2$ G/cm. Durations of the gradients are: $g_1 = 500$ μs, $g_2 = 705$ μs, $g_3 = 860$ μs, $g_4$–$g_8 = 400$ μs, and $g_9 = 30$ ms. Phase cycling is accomplished by alternating $\phi_4 = (x, y)$ and rec $= (x, -x)$. Complex data are acquired using States-TPPI [21] incrementation of $\phi_1$, $\phi_2$, and $\phi_3$.

is a direct extension of the 3D constant-time pulse sequence presented previously [8] employing an indirect evolution period on the methyl protons, and a heteronuclear Hartmann–Hahn transfer from the carbonyl carbons to the sugar ring protons. It is the 4D constant-time version of the sequence presented previously [10], with significant improvement in overall magnetization transfer through the use of broadband inversion (BIP [13]) and ABSTRUSE [14] pulses, in addition to a newer zero-quantum dephasing procedure [15]. The carbohydrate selected was nigeran tetrasaccharide (Sigma N7263, α-D-Glc-[1 → 3]-α-D-Glc-[1 → 4]-α-D-Glc-[1 → 3]-α-D-Glc). The compound was reduced with sodium borohydride to the alditol form before being acetylated with doubly $^{13}$C-labeled acetic anhydride according to Bendiak et al. [11]. Data were collected on a Varian Inova 500 spectrometer equipped with a triple resonance gradient probe. The sample concentration was approximately 8 mM in $CDCl_3$ saturated with $D_2O$. The high sample concentration allowed the data to be collected using only two transients but reasonable results have been obtained with lower concentrations as well. In the methyl proton dimension, 10 complex points were collected with a spectral width of 400 Hz, 8 points were collected in the methyl carbon dimension with a spectral width of 200 Hz and 18 points were collected in the carbonyl carbon dimension with a spectral width of 300 Hz. In the direct dimension, a spectral width of 1200 Hz was used and 1024 data points were recorded. The data collection took 19 h 17 min. Data were processed using NMRPipe [16] for mirror-image linear prediction (MI-LP) calculations, and FDM software written in-house to apply the FDM algorithm to 4D data sets. All calculations were performed on an Apple PowerMac G5 with dual 2.0 GHz CPUs and 2 Mb RAM. The FDM calculation, using a single basis function per window in the direct dimension, took 22 min with this configuration. A further detailed study of the FDM applied using this single basis function scheme with model and experimental signals will be the topic of a forthcoming paper. The remaining parameters for the FDM calculation were optimized at: $q^2 = 1 \times 10^{-3}$ and $\Gamma = 1$ Hz. In this case $\Gamma$ was set to the approximate natural linewidths of the peaks, and the optimization of $q^2$ was accomplished using a manual, iterative procedure. In practice, the method is fairly insensitive to adjustments of $q^2$ over a large range, and arriving at an optimal value is straightforward for moderately experienced users. The default parameter set from NMRPipe was used for the MI-LP calculation, with cosine-squared weighting applied in all dimensions.

## 4. Results and discussion

All two-dimensional projections from the 4D spectrum of the peracetylated nigeran oligosaccharide-alditol having doubly $^{13}$C-labeled acetyl groups are presented in Fig. 3. It should be pointed out that these planes represent all the 4D data compressed to 2D planes for the frequency coordinates indicated. The current FDM program is able to generate all six 2D correlations possible from the 4D experiment, as well as slices of any width from any set of 3D-correlated nuclei. The data from the sugar spin system protons can be better conceived as running along a rod or dowel that runs

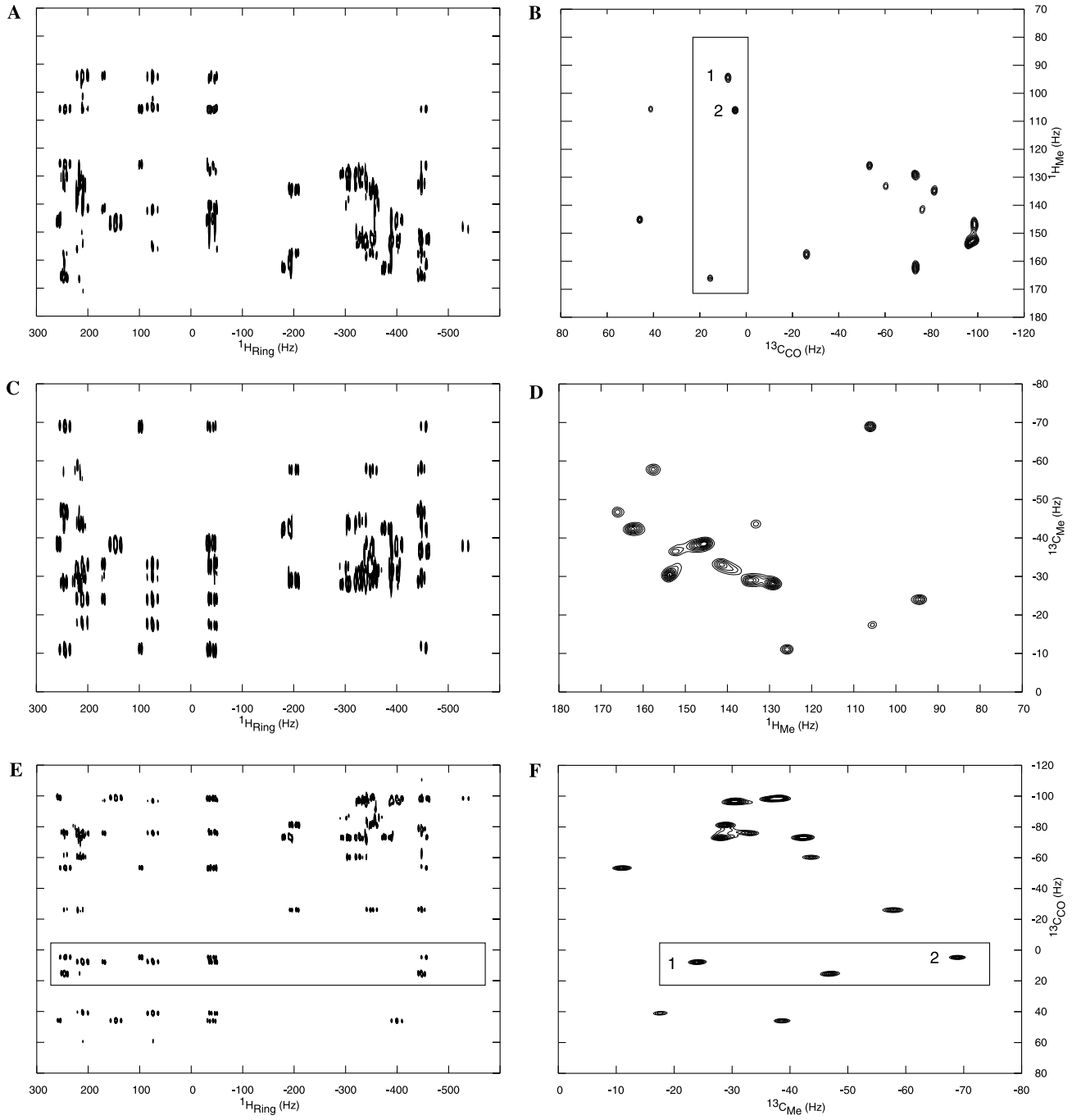


Fig. 3. Two-dimensional projections of the 4D experiment conducted on the peracetylated nigeran oligosaccharide alditol having doubly $^{13}C$-labeled acetyl groups, processed with the FDM. The projections on the left (A, C, and E) show correlations between the direct dimension ($^{1}H_{Ring}$) and each of the indirectly detected dimensions ($^{1}H_{Me}$, $^{13}C_{Me}$, and $^{13}CO$). The projections on the right (B, D, and F) show correlations among the indirect dimensions only. Note that all the data in the 4D spectrum are essentially compressed onto each 2D projection. Indicated in boxes are a set of signals in a significantly overlapped region of the directly detected spectrum, that are completely resolved in two independent dimensions that correlate with the acetyl group frequencies. Frequencies of nuclei of acetyl groups associated with two different spin systems, X and Y (Fig. 1) are indicated as 1 and 2, respectively, in the indirect projections. Note the high-resolution frequency estimates in the indirect dimensions even though only 10, 8, and 18 points were used in each dimension ($^{1}H_{Me}$, $^{13}C_{Me}$, and $^{13}CO$, respectively). These views of the resolved indirect projections enable specific regions of the 4D spectral continuum to be rapidly selected for slicing to any desired thickness.

orthogonal to all three coordinates of acetyl group nuclei. Hence, as long as the frequency of any one of the three acetyl group nuclei are different from those of another acetyl group, protons of that sugar ring spin system are fully resolved. It is evident from Fig. 3 that all the dimensions are well resolved using the FDM. Despite the fact that there were only 8 increments in the $^{13}C_{Me}$ dimension, and this dimension also exhibits the

largest density of peaks, high resolution was afforded by the input of all points from all free-induction decays. Hence, whether or not the evolution period for this specific nucleus has many increments does not impede the FDM from generating a high-resolution frequency estimate in this dimension.

The resolution of the FDM in the indirect dimensions far surpasses that which can be obtained using FT

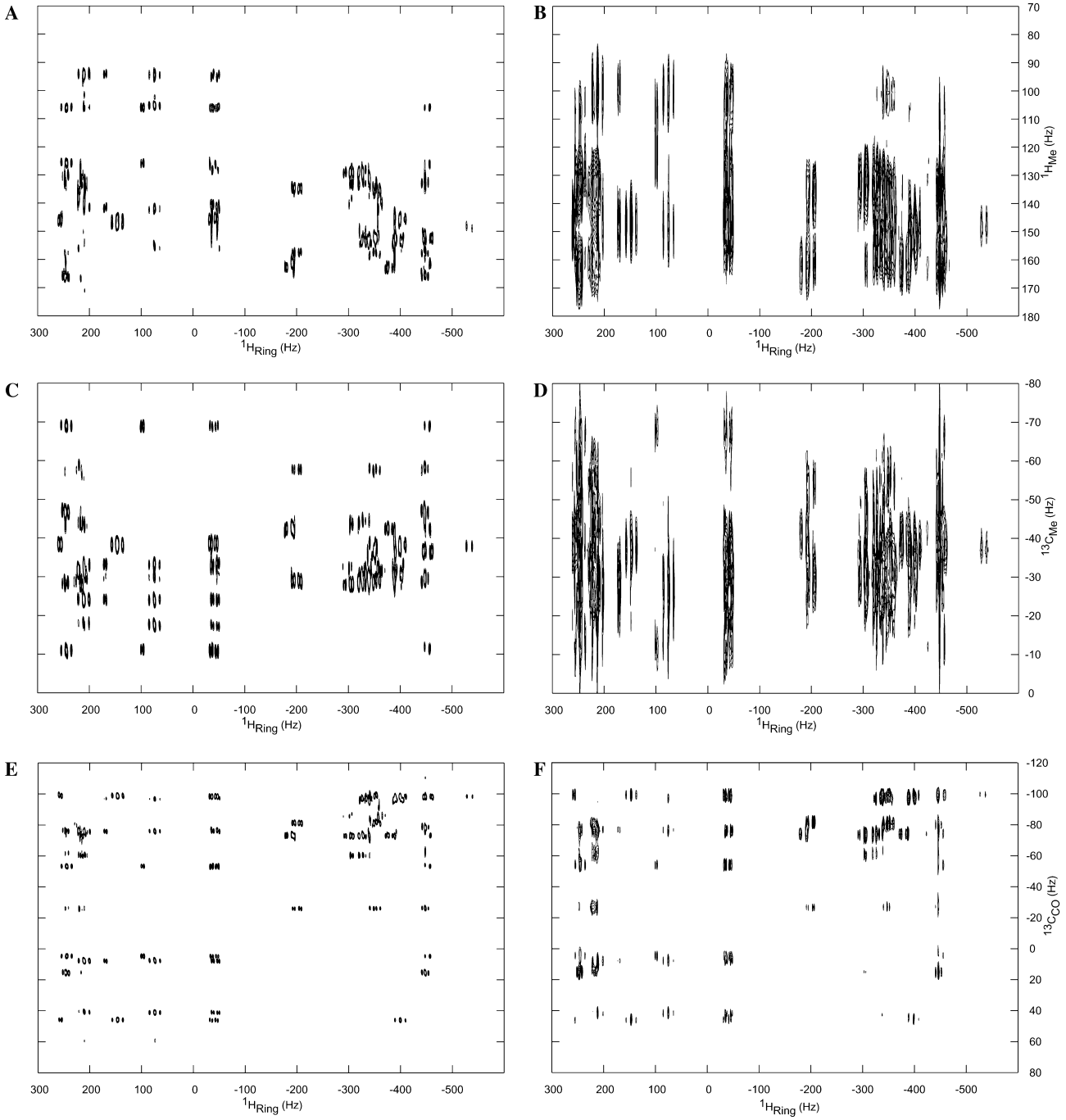


Fig. 4. A direct comparison between the FDM and mirror-image linear prediction for processing data obtained from the 4D spectrum of the $^{13}C$-acetyl-labeled nigeran oligosaccharide-alditol. Two-dimensional projections are shown using the identical 4D data set processed on the left (A, C, and E) with the FDM, and on the right (B, D, and F) by MI-LP. The 2D projections show a compression of the 4D data set onto one plane that includes the directly detected dimension in each panel. Note that in dimensions where few points have been collected frequency estimates in the indirect dimensions are conspicuously superior using the FDM ($^1H_{Me}$, 10 points, compare panels (A) and (B), or $^{13}C_{Me}$, 8 points, compare panels (C) and (D)). As the FDM uses all points of all time-domain data to estimate four-dimensional frequencies, it provides superior frequency estimates than orthogonal methods.

processing. In Fig. 4, comparisons were made, using the identical 4D data set, between the FDM and the more widely used data processing scheme for constant-time data, MI-LP (which generally provides better resolution than FT). Strictly speaking, this is not a fair comparison because LP is an orthogonal method with resolution limited in any given dimension by the number of increments in that dimension. Nevertheless, it is useful to compare the results so that the practical advantage of the FDM for four-dimensional data processing can be illustrated. As shown side-by-side in Fig. 4, by comparing those panels processed by FDM on the left with their counterparts processed by MI-LP on the right, the superiority of the FDM at providing high-resolution frequency estimates in the indirect dimensions becomes patently apparent.

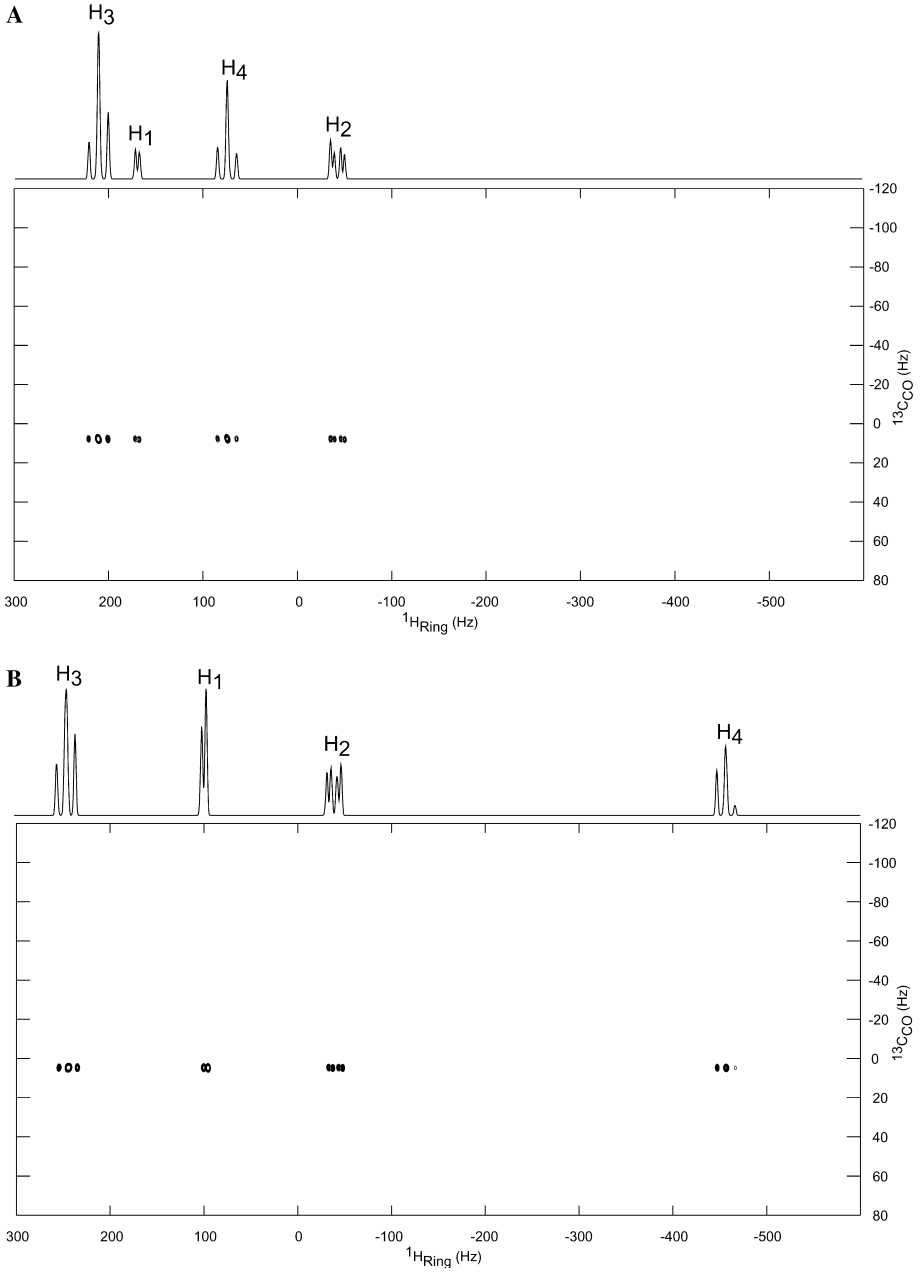


Fig. 5. Sugar ring proton spin systems completely isolated from the 4D FDM spectrum through correlations to the acetyl group nuclear frequencies. Shown in panel (A) is the spin system corresponding to the sugar ring X (Fig. 1), derived from narrow slices of the 4D spectrum corresponding just to the regions defined by the peaks marked 1 in the $^{1}H_{Me}$ (Fig. 3B) and $^{13}C_{Me}$ (Fig. 3F) dimensions. A 1D trace of the signals in panel (A) is shown above it with the multiplets from H-1 to H-4 of that sugar ring indicated. Illustrated in panel (B) is the spin system of the sugar ring Y (Fig. 1), defined by narrow slices corresponding to the regions delineated by the peaks marked 2 in the $^{1}H_{Me}$ (Fig. 3B) and $^{13}C_{Me}$ (Fig. 3F) dimensions. The 1D trace of the signals in panel (B) is presented above it along with the H-1 to H-4 multiplets assigned within that sugar ring. The $^{3}J_{H,H}$ determined from these multiplets are: for sugar ring X, $J_{1,2} = 4.0$ Hz, $J_{2,3} = 10.7$ Hz, $J_{3,4} = 9.6$ Hz, and $J_{4,5} = 10.5$ Hz, and for sugar ring Y, $J_{1,2} = 4.3$ Hz, $J_{2,3} = 10.6$ Hz, $J_{3,4} = 9.2$ Hz, and $J_{4,5} = 10.0$ Hz. They both define the spin system characteristic of an α-glucopyranoside.

In NMR spectroscopy of complex carbohydrates, one crucial set of measurements is the monosaccharide H-1 through H-5 3-bond *J*-couplings, which define the stereochemical relationships of endocyclic protons around the sugar rings. The FDM permits interesting regions of a multi-dimensional spectrum that may be congested in the directly detected dimension to be evaluated in other indirect frequency domains. For example, with this oligosaccharide, there is a region boxed in Fig. 3E from about 0 to 20 Hz on the $^{13}$CO frequency scale that shows significant crowding of sugar ring proton signals. However, the same frequency region in the adjacent $^{13}$CO–$^{13}C_{Me}$ frequency correlation (Fig. 3F, boxed region) clearly shows the same three $^{13}$CO frequencies correlated to three completely independent $^{13}C_{Me}$ frequencies. Also, as indicated in the boxed region in Fig. 3B, the $^{1}H_{Me}$ frequencies that corresponded to this set of $^{13}$CO frequencies are also completely resolved. Two of these frequency correlations, derived from individual acetyl groups (shown as 1 and 2 in Figs. 3B and F), can be selected from the 4D spectrum to reconstruct narrow slices. These slices (shown in Fig. 5) correspond to frequency ranges that just bound the $^{13}C_{Me}$ and $^{1}H_{Me}$ intensities for each acetyl group. The 4D spectrum along with the FDM program permitted two different sugar ring proton spin systems to be independently isolated from a crowded region, without searching through numerous slices to find the regions of interest. As there was no overlap with other slices (each slice may be constructed with any desired thickness), 1D traces could be made directly through the 2D spectrum shown in Fig. 5. Essentially, this moves the spectrum from each individual sugar ring proton spin system into its own three-dimensional space defined by the acetyl $^{13}$CO, $^{13}C_{Me}$, and $^{1}H_{Me}$ frequencies that correlate to it. As indicated in the traces seen in Fig. 5, the multiplet structures from H-1 to H-4 are evident. While the amplitudes of the multiplets can be more difficult to accurately estimate in very overlapped regions of the FDM spectrum, the frequencies are well defined. The pulse sequence was designed to eliminate zero-quantum effects though a recent zero-quantum dephasing technique [15] that enables *J*-couplings to be read directly from multiplet structures. Inherent in these multiplet structures are the values of all the endocyclic sugar ring proton *J*-couplings that unambiguously define the sugars' stereochemistries: $J_{1,2}$, $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$. The 4D experiment, therefore, enables many spin systems to be rapidly "teased apart" into independent dimensions. We are only beginning to examine just how many spin systems can be simultaneously resolved using acetyl isotags in this way. One additional advantage is that most sugar spin systems correlate to more than one acetyl group, so the spin system multiplet structures are typically seen with redundancy at other acetyl $^{13}$CO, $^{13}C_{Me}$ and $^{1}H_{Me}$ frequencies as well.

Although the FDM has been applied for the first time here in four dimensions to dramatically improve the independent isolation of some very congested sugar spin systems, it can, of course, be applied to any molecules having appropriately arranged nuclei. It is a generally applicable *n*-dimensional processing scheme that, although more advantageously applied to constant-time experiments, can be applied to any conventional data sets having multiple frequency correlations. For 3D or 4D experiments, the FDM should provide a definite advantage for reprocessing older sets of data that were previously deemed too poorly resolved in the indirect dimensions to be of much use. In addition, new experiments that may have been considered too time-consuming for the resolution afforded by four dimensions may now become viable options. The advantage of being able to select slices of any desired frequency width between any two correlated dimensions within a 4D spectrum also cannot be overemphasized. This is far less onerous than searching through numerous slices only available as stacks in one direction.

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