Fast Multidimensional NMR Spectroscopy by the Projection–Reconstruction Technique

The authors describe a new method for making faster measurements of multidimensional NMR spectra. The technique involves acquiring a small number of projections and using them to reconstruct the entire spectrum.

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Great strides were made in high-resolution nuclear magnetic resonance (NMR) spectroscopy with the introduction of two-dimensional (2-D) Fourier transformation (1, 2), which spreads the important information into two frequency dimensions. For the first time, spectroscopists were presented with a simple graphical picture of the correlations among different nuclear sites within a molecule — evidence for spin–spin coupling, cross-relaxation effects, chemical exchange, and many other internuclear interactions. This pictorial approach proved popular particularly with organic chemists and biochemists whose prime concern is molecular structure determination, because the 2-D charts provide a clear and essentially unambiguous map of the interesting interactions. With the advent of higher and higher magnetic fields the quest was extended to spectra of greater complexity, notably those of biomolecules, and, in particular, proteins. However, it soon became clear that very crowded spectra required extension to three or even four frequency dimensions to resolve ambiguities.

These additional frequency dimensions inevitably increase the extent of data gathering and hence the duration of a three-dimensional (3-D) experiment, often translating to several hours of spectrometer time. This is because they monitor all the elements of a 3-D array one step at a time, rather like examining every book on every shelf of every stack in a library. Higher-dimensional spectroscopy demands even more extensive sampling, involving measurements that can take many days to complete. Soon NMR spectroscopists began to ask whether there might be a quicker way to gather the data without compromising resolution or degrading the quality of the spectra in some other manner.

Suppose we think of a 3-D NMR spectrum as being made up of tiny bright “stars” sparsely distributed in otherwise empty space. This certainly is how the NMR results look when displayed on a monitor. If we view this array from two or three different perspectives we should be able to pinpoint all these “stars” and get a clear map of all their positions, relying upon the human eye and brain to complete the reconstruction. Indeed, we sometimes display a 3-D NMR spectrum on the pages of a scientific journal as two stereoscopic views to create the 3-D effect.

This “image reconstruction” concept already has proved its worth in other scientific fields where the object is continuous and therefore far more demanding — for example, a patient in an x-ray scanner. Even the very complex spectra of proteins should be susceptible to this approach, but when we take into account the possibility that some resonances might be eclipsed by others in both perspective views, we need to acquire some
additional projections, although still a rather small number in total. This is the essence of projection–reconstruction NMR (PR-NMR) (3). By gathering only a very limited amount of raw experimental data, we can speed up the measurement by a large factor (roughly one order of magnitude for 3-D NMR). This greatly increases the scope of such experiments and opens up possibilities for studying time-dependent phenomena, such as relaxation or chemical exchange.

**Projection of 3-D Spectra**

In practice, we easily can record a projection of a 3-D spectrum onto a suitable plane. There is a Fourier transform theorem (2, 3) that relates a section through the origin of a 2-D time–domain signal $S(t_1, t_2)$ at some inclination $\alpha$, to the projection of the corresponding frequency–domain spectrum $S(F_1, F_2)$ onto a line inclined at the same angle $\alpha$. It is only a small step to extend this concept to the Fourier transform of time–domain data acquired while we increment two time parameters ($t_1$ and $t_2$) jointly, generating a projection of the 3-D spectrum onto a tilted plane in frequency space (see Figure 1). We derive differently-tiled plane projections simply by changing the relative rates of incrementation of the time parameters $t_1$ and $t_2$, thus varying the “point of view” defined by the angle $\alpha$. The choice of projection angle $\alpha$ usually is not critical. A program can be written to search for values of $\alpha$ that avoid instances during the spectrometer set up.

A program can be written to search for values of $\alpha$ that avoid instances during the spectrometer set up. This is the only occasion where one peak is eclipsed by another. In practice, spectroscopists routinely employ the special cases $\alpha = 0^\circ$ and $\alpha = 90^\circ$ during the spectrometer set up. These “orthogonal projections” provide two additional viewpoints that can be used in the reconstruction.

**Intermodulation**

A 3-D experiment involves two consecutive evolution periods ($t_1$ and $t_2$). A typical signal evolving at a chemical shift $\Omega_A$ during $t_1$ modulates the signal in the second evolution period $t_2$. We can arrange for this to be either sine or cosine modulation. Similarly, evolution at a new chemical shift $\Omega_B$ during $t_2$ generates a doubly modulated signal during the detection stage $t_3$. We acquire four scans, involving signals described by four different intermodulation expressions:

\[
S(1) = \cos(\Omega_A t_1) \cos(\Omega_B t_3) \quad [1]
\]

\[
S(2) = \sin(\Omega_A t_1) \sin(\Omega_B t_3) \quad [2]
\]

\[
S(3) = \sin(\Omega_A t_1) \cos(\Omega_B t_3) \quad [3]
\]

\[
S(4) = \cos(\Omega_A t_1) \sin(\Omega_B t_3) \quad [4]
\]

We increment the evolution times $t_1$ and $t_2$ jointly at different rates, according to:

\[
t_1 = t \cos \alpha \quad [5]
\]

\[
t_2 = t \sin \alpha \quad [6]
\]

Then we combine the modulation terms [1] through [4] in pairs, and standard trigonometrical functions give the expressions:

\[
S(1) - S(2) = \cos[\Omega_A \cos \alpha + \Omega_B \sin \alpha] t \quad [7]
\]

\[
S(1) + S(2) = \cos[\Omega_A \cos \alpha - \Omega_B \sin \alpha] t \quad [8]
\]

\[
S(3) + S(4) = \sin[\Omega_A \cos \alpha + \Omega_B \sin \alpha] t \quad [9]
\]

\[
S(3) - S(4) = \sin[\Omega_A \cos \alpha - \Omega_B \sin \alpha] t \quad [10]
\]

We see that Fourier transformation as a function of $t$ generates the sum and difference frequencies (scaled according to the tilt angle $\alpha$):

\[
(\Omega_A \cos \alpha + \Omega_B \sin \alpha) \text{ and } (\Omega_A \cos \alpha - \Omega_B \sin \alpha)
\]

Because these two expressions differ only in the sign of $\alpha$, the projections always are obtained in symmetrically related pairs tilted at ±$\alpha$. This is the only consequence of chemical shift intermodulation. A related technique, recently named G-matrix Fourier transform-NMR (GFT-NMR) [5–7]), separates the sum and difference frequencies algebraically, avoiding differential scaling by incrementing $t_1$ and $t_2$ at the same rate ($\alpha = 45^\circ$).

We can apply the same principles to spectra in higher dimensions (3) where the time-saving factor increases roughly by one order of magnitude for each new frequency dimension. We can imagine the four-dimensional problem in 3-D “evolution space” defined by $t_1$, $t_2$, and $t_3$, and choose between three modes of operation:

(a) We can vary just one of the three evolution parameters, holding the other
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Figure 1. (a) When the two time parameters \( t_1 \) and \( t_2 \) are incremented jointly, the acquired NMR data are restricted to the tilted plane outlined by the bold lines. (b) Fourier transformation of this data set generates a projection of the 3-D spectrum onto the corresponding tilted plane in the frequency domain.

Samples with good sensitivity. For simplicity, we focus on the 3-D problem as an illustrative example; higher-dimensional spectra rely upon exactly the same principles. Good intrinsic sensitivity permits reconstruction of the spectrum from just a small number of plane projections. The goal is to find the only spatial distribution of cross-peaks compatible with this information. We start with the pair of orthogonal projections onto the \( F_1F_3 \) and \( F_2F_3 \) planes, processing one \( F_2F_3 \) plane at a time, until the complete \( F_1F_2F_3 \) matrix has been reconstructed. In a typical \( F_2F_3 \) plane, the projections onto the \( F_1 \) and \( F_3 \) axes do not contain enough information to locate the correlation peaks unequivocally. However, they do define a provisional map representing all conceivable cross-peak positions in that plane, as if every response along the \( F_3 \) axis were correlated with every response along \( F_1 \). We calculate this provisional map by scaling the \( F_1 \) projection according to the amplitudes in the \( F_3 \) projection, essentially multiplying the two traces (see Figure 2a). Some of the resulting peaks later turn out to be genuine correlations, while others are false.

We then convert the provisional map into a true map by using information gleaned from tilted projections. Although, as shown above, we record these in symmetrical pairs tilted at \( \pm \alpha \), for the sake of illustration we can consider a single tilted projection at \( +\alpha \). We back-project this to create a 2-D mask made up of parallel ridges running across the \( F_1F_2 \) plane (see Figure 2b). Because genuine correlation peaks must lie under these ridges, we superimpose the mask on the provisional map and compare intensities at corresponding pixels, retaining only the lower value at each location. In this manner we eliminate the majority of the “false” correlation peaks. For example, in Figure 2c we have reduced 16 provisional peaks to only four genuine correlation peaks. The second projection at \( -\alpha \) normally completes the process. For particularly complex spectra, we might need to acquire a further pair of tilted projections to resolve remaining ambiguities. The key is to work with the minimum amount of data gathering, keeping the duration of the measurement as short as possible; the processing time itself need not tie up the NMR spectrometer.

This “lower-value algorithm” is a non-linear process, the main consequences being the possible introduction of a small amount additional noise on the correlation peaks arising from noise on the mask, and some suppression of baseplane noise in regions not covered by the ridges. A more serious criticism is the fact that the signal-to-noise does not increase as we introduce more tilted projections. Consequently we reserve this algorithm for spectra of good inherent sensitivity.

Samples with marginal sensitivity. We adopt an alternative processing scheme if the signal-to-noise ratio is only marginal. We use a rather larger number of tilted projections, and the algorithm ensures that each new projection contributes to the overall intensity of the correlation peaks. The method is related to the inverse Radon transform (8, 9), a widely used scheme for reconstructing images in ultrasonics, x-ray tomography, and magnetic resonance imaging (MRI). We acquire a set of plane projections, usually taken at regular inclinations around a circle, and back-project them to form a set of parallel ridges running across the \( F_1F_2 \) plane. We add together the intensities from the various back-projections so that the amplitudes of the correlation peaks increase in proportion to the number of projections. In this way we ensure that the signal-to-noise ratio improves in the same manner as in multiscan averaging.

Figure 3 sketches the case where only two projections have been employed, generating just two sets of ridges with peaks at all the intersections. This creates a provisional map; we will need additional projections to identify genuine correlation peaks. In the general case of several different projections, there are two...
kinds of undesirable artifacts in the reconstruction — a set of low-intensity ridges criss-crossing the $F_1F_2$ plane, and weak false peaks where some (but not all) of the ridges intersect. As we increase the number of independent projections, the genuine correlation peaks grow stronger while the artifacts become less and less obtrusive, eventually sinking below the base-plane noise (which is quite prominent in this mode). Genuine correlation peaks occur where all the ridges intersect, whereas the false peaks always involve fewer intersections. In principle we could apply a pattern-recognition algorithm to make this distinction.

Note that we erode the speed advantage of PR-NMR as we increase the number ($N$) of independent projections, but we can still achieve a net gain in speed provided that $N$ is less than the number of evolution increments employed in the conventional mode. We could consider hybrids of the lower-value algorithm with the additive scheme, with a view to balancing the adverse effects of artifacts and noise. Alternatively we could use an iterative algorithm to find the only distribution of correlation peaks that is consistent with the projections.

**Applications of the PR-NMR Method**

It is interesting to note that the idea of a tilted projection can prove useful in itself, without any reconstruction stage (10). A...
2-D spectrum of a protein can be so complex that several resonances overlap. If our goal is to measure relaxation times or nuclear Overhauser effects, then we cannot evaluate these parameters for the overlapped peaks. The conventional solution would be to separate the peaks in a third frequency dimension, but this involves a significantly longer experimental measurement. Alternatively, we can record the 2-D spectrum again in the form of a slightly tilted projection, derived by Fourier transformation of time-domain data obtained by slow incrementation of the new evolution parameter in step with the normal incrementation of t1. We now can resolve the troublesome responses, allowing their relaxation parameters to be evaluated.

We illustrate the application of the projection-reconstruction method by recording a 900-MHz 3-D (constant-time) HNCO experiment performed on a 1.7 mM aqueous solution (10% D2O) of the isotopically enriched 187-residue protein HasA (11). As the first step we obtained the orthogonal N–H and C–H planes, using 128 complex data points, each requiring 20 min of data acquisition. To obtain better sensitivity and lineshape, we “borrowed” the NH plane from a 2-D HSQC experiment (11). We then recorded projections of the 3-D HNCO experiment for planes tilted at 30° and 60°, using 100 complex data points, requiring 30 minutes each. For the purpose of illustration, we show a typical plane from the reconstructed 3-D spectrum — an $F_1,F_2$ plane taken at a proton frequency of 8.40 ppm, comprising seven C–N correlation peaks (see Figure 4). Total time for the measurement was 1 h 40 min. We estimate that an equivalent experiment run in the traditional mode, with 32 complex data points in $t_1$ and in $t_2$ would require at least ten times longer. With this level of digitization the conventional experiment would have involved a significant compromise in terms of resolution; by contrast PR-NMR can afford to use more evolution increments (sacrificing part of its speed advantage), to achieve better resolution. It is clear that many different multidimensional NMR experiments can benefit from the projection–reconstruction approach.

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References


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