PARAMETER ESTIMATION OF MULTIDIMENSIONAL NMR SIGNALS BASED ON HIGH-RESOLUTION SUBBAND ANALYSIS OF 2D NMR PROJECTIONS

I.Y.H. Gu[(a)], *M. Billeter*[(b)], *M.R. Sharafy*[(a)], *V.A. Sorkhabi*[(a)], *J. Fredriksson*[(b)], *D.K. Staykova*[(b)]

[(a)] Dept. of Signals and Systems, Chalmers University of Technology, Gothenburg, Sweden
[(b)] Dept. of Chemistry, University of Gothenburg, Gothenburg, Sweden
irenegu@chalmers.se, martin.billeter@chem.gu.se

ABSTRACT

NMR spectroscopy is a powerful technique used in protein research for comprehensive functional characterizations, e.g. structure determination at atomic resolution. Due to the molecular size (typically >1000 atoms), protein NMR spectra contain a large number of signal frequencies. Resolving these requires high-dimensional spectroscopy. However, when the number of frequency exceeds three, conventional approaches start to demand unrealistic long experiment time, and the data analysis becomes challenging. In this paper we explore a combination of novel methods: Data from 5D NMR experiments are recorded as a series of 2D projections, which are then subjected to 2D subband filters and 2D LS-ESPRIT for estimation of signal parameters. Based on the relations established between 5D NMR signals and their 2D counterparts, projection spectroscopy allows to extract highly similar information as what would be found in conventional 5D NMR experiments; however, while the latter would require months of experiment time, the recording of all necessary projections can be accomplished within 1-2 days. Preliminary results show the efficiency of the method with respect to accuracy and resolution of the parameter estimates as compared with conventional methods.

Index Terms— multidimensional NMR, projection spectroscopy, 2D ESPRIT, 2D subband, frequency estimation, protein structures.

1. INTRODUCTION

Proteins represent a highly complex class of molecules that play critical roles in almost every biological process. Their functional complexity relies on their immense structural variety. Nuclear magnetic resonance (NMR) is a spectroscopic technique that allows studies on proteins with atomic resolution: determination of 3D structures, characterization of dynamics at various time-scales and of interactions with other molecules [1]. Due to the size of proteins (often >1000 atoms) NMR spectra on these macromolecules contain thousands of signal frequencies, which can only be resolved in multidimensional spectra. However, adding a new dimension dramatically increases the experiment time. For example, with a time resolution of 64 sampled points in each dimension, a complete L-dimensional spectrum would require about 64^{L-1} seconds (>6 months of measurements for L=5!). To overcome this problem, modern NMR techniques reduce the experiment time typically by *non-uniform sampling*, or by *projection spectroscopy* (see review [2]). We shall deal with the latter technique in this paper.

Another concern is about frequency resolution: signals with close resonance frequencies need to be resolved. Despite this high-resolution requirement, FFT-based methods are still routinely used in multidimensional NMR analysis. Alternative approaches using maximum entropy or filter diagonalization principles are currently restricted to 2-3 dimensions [2].

This paper proposes a novel signal identification approach for multi-dimensional NMR, where for example 5D NMR signals are observed via a set of 2D projection spectra (Section 2). We then apply a high-resolution parametric estimation method, 2D ESPRIT, to the projected signals. To compensate for the small number of sampled data during the measurement intervals, and the large number of signal frequencies observed for proteins, a set of 2D subband filters is applied prior to the frequency estimation (Section 3). For the reconstruction of 5D NMR signals, the relations between the estimated parameters in 2D projections and the desired frequencies (chemical shifts) of the corresponding atomic nuclei are expressed as an over-determined set of linear equations (Section 4). Tests on synthetic data as well as on experimental 2D projections of 5D signals show good estimation results with high frequency resolution (Section 5).

2. MULTIDIMENSIONAL NMR AND PROJECTIONS

Spins in a magnetic field: NMR is about the interaction of magnetic fields with the spins of atomic nuclei [1]. For spin $\frac{1}{2}$ nuclei, the larger population of the lower energy state (out of two possible states) leads to an equilibrium magnetization oriented along the magnetic field. This equilibrium can be disturbed, resulting in a magnetization tilted with respect to the magnetic field, which then starts to precess with a frequency Ω around it. Observation of the magnetization along a given direction perpendicular to the magnetic field yields an

oscillating function in time, which is exponentially dampened due to relaxation towards the equilibrium. Each nucleus of a protein has its own precession frequency, and thus one observes a sum of dampened oscillations, which is called a FID (Free Induction Decay). Fourier analysis of this FID yields for each nucleus a precession frequency Ω and a relaxation rate constant λ.

Multidimensional NMR: A 2D NMR experiment is like a series of 1D experiments acquired according to the scheme of Fig.1(a). After a first pulse the magnetizations are allowed to precess during an evolution time t_1. Interaction between neighboring spins may transfer part of the magnetization from one nucleus to another. During the acquisition time t_2, following a second pulse, a FID is observed with the frequencies of the nuclei receiving magnetization. Variation of the evolution time t_1 in a series of experiments provides an identification of the spins where the magnetizations originate. The result is a series of FID measurements (Fig.1(b)), whose Fourier spectrum shows signals connecting the frequencies of neighboring nuclei (Fig.1(c)). Generalizing to L dimensions, the

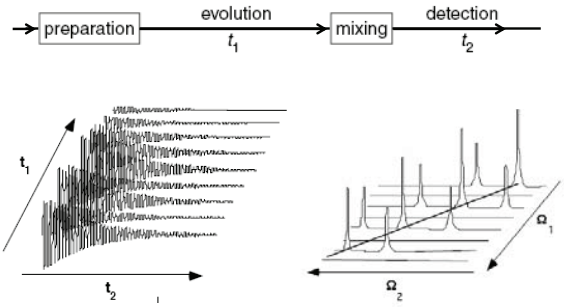

Fig. 1. (a) Schematic description of a 2D experiment; (b) FID measurements as a function of t_1 and t_2; c) Fourier spectrum of the signal in (b).

NMR data can be described by a L-dimensional matrix form,

$$\mathbf{y}(t_1, \cdots, t_L) = \sum_{k=1}^{K} a_k \mathbf{f}_1^k(t_1) \otimes \mathbf{f}_2^k(t_2) \cdots \otimes \mathbf{f}_L^k(t_L) + \mathbf{w} \quad (1)$$

where $\otimes$ is the Kronecker product, a_k are amplitudes, t_1, $\cdots, t_{L-1}$ are evolution times, and t_L is the acquisition time. The sum over k reflects the presence of many signal components. The vectors $\mathbf{f}_l^k$, $l = 1, \cdots, L$, $k = 1, \cdots, K$, contain discrete samples for complex exponentials in the form $e^{(i\Omega_l - \lambda_l)t_l}$ with resonance frequencies Ω_l, and relaxation rates λ_l. The L-D residual matrix $\mathbf{w}$ is required due to measurement noise. The vectors $\mathbf{f}_l^k$ provide an optimal characterization of the NMR data set $\mathbf{y}$ when the residual $\mathbf{w}$ is minimal.

Projection spectroscopy: Consider a modified 5D NMR experiment where a set of 2D projections are recorded instead of a 5D data matrix. Given the projection angles, a L-D signal can be related to its 2D projections. For example, a 5D signal can be projected along the axes Ω_3 and Ω_4, and also along a diagonal with respect to the axes Ω_1 and Ω_2. The new coordinates (ω_1, ω_2) in the projected 2D plane are now related to the 5D coordinates by $\omega_1 = \Omega_1 + \Omega_2$ and $\omega_2 = \Omega_5$. Typically, about 30-50 projections with different angles are recorded [3].

Projections are achieved by correlating several evolution times; when t_1 to t_{L-1} are correlated, the number of dimensions of the projection is two, and (1) becomes [4]

$$\mathbf{y}^m(t, t_L) = \sum_{k=1}^{K} a_k \prod_{l=1}^{L-1} \mathbf{f}_l^k(t_l, c_{l,m}) \otimes \mathbf{f}_L^k(t_L) + \mathbf{w}'_m \quad (2)$$

where $\mathbf{y}^m$ is a projected NMR data set and m indicates what type of projection was chosen. The correlated evolution times t_1 to t_{L-1} yield a single signal dimension, while the detection time t_L is left alone to form the second dimension in the projection. Thus, the left-hand side of (2) represents now a 2D data set, where a new time variable t is used to replace the correlated times t_1 to t_{L-1}. The vectors $\mathbf{f}_1^k$ to $\mathbf{f}_{L-1}^k$ carry a second, formal argument that reflects the effect on the vector of the projection type chosen. Again, the equal sign is only warranted if noise is taken care of by a residual $\mathbf{w}'_m$. The need to consider all 2D projections simultaneously and the high ($\sim \mathbf{5}$) dimensionality of the final data make at least partial automation a necessity.

3. ESTIMATION OF PAIRED FREQUENCIES FROM 2D PROJECTED NMR: 2D-ESPRIT IN SUBBANDS

For each 2D projected NMR with a known projection angle, (2) can equivalently be described as damped complex exponentials in white noise,

$$y(m, n) = \sum_{k=1}^{K} A_k e^{s_{1k} m + s_{2k} n} + w(m, n) \quad (3)$$

where $s_{ik} = \alpha_{ik} + j\omega_{ik}$, i=1,2, $w(m, n)$ is 2D white noise, $A_k = a_k e^{j\phi_k}$ is a complex amplitude, $a_k \in \mathbb{R}^+$ is a shared amplitude and ϕ_k is a shared initial phase, $0 \leq m \leq M-1$ and $0 \leq n \leq N-1$. To estimate the unknown parameters in (3), FFT-based methods, due to its simplicity, are still routinely used. The main disadvantage is the low frequency resolution. On the other hand, parametric methods (e.g. ESPRIT [5]) offer high resolution but require more computation. Applying 2D ESPRIT (Estimation of Signal Parameters Via Rotational Invariance Techniques) to a projected 2D NMR, as compared with its 1D version, has several tricky parts. For example, the two frequencies ω_{1k} and ω_{2k} in each harmonic term of (3) are coupled. Also, each harmonic term shares one power and one initial phase value. Therefore, 2D ESPRIT should not be implemented as the tensor product. The relations between matrices concerning the first and second dimensions need to be employed for jointly estimating each pair of frequencies and damping factors. We apply the 2D ESPRIT method in [6] to projected NMR signals, which is briefly summarized as follows.

Given 2D projected NMR data samples, a Hankel *block* matrix containing $P \times (M - P + 1)$ blocks, is formed for the 1st dimension,

$$\mathbf{Y}_1 = \begin{bmatrix} \mathbf{Y}_{(0)} & \mathbf{Y}_{(1)} & \cdots & \mathbf{Y}_{(M-P)} \\ \mathbf{Y}_{(1)} & \mathbf{Y}_{(2)} & \cdots & \mathbf{Y}_{(M-P+1)} \\ \vdots & \vdots & & \vdots \\ \mathbf{Y}_{(P-1)} & \mathbf{Y}_{(P)} & \cdots & \mathbf{Y}_{(M-1)} \end{bmatrix} \quad (4)$$

where each block matrix is of size $Q \times (N-Q+1)$, $\mathbf{Y}_{(m)} = \begin{bmatrix} y(m,0) & y(m,1) & \cdots & y(m,N-Q) \\ y(m,1) & y(m,2) & \cdots & y(m,N-Q+1) \\ \vdots & \vdots & & \vdots \\ y(m,Q-1) & y(m,Q) & \cdots & y(m,N-1) \end{bmatrix}$. $\mathbf{Y}_1$ can be decomposed into signal subspace (the 1st term) and noise subspace (the 2nd term) by using the SVD, as follows,

$$\mathbf{Y}_1 = \mathbf{U}_{S1}\mathbf{D}_{S1}\mathbf{V}_{S1}^H + \mathbf{U}_{N1}\mathbf{D}_{N1}\mathbf{V}_{N1}^H \quad (5)$$

LS ESPRIT can be used to estimate the frequencies and damping factors as follows

$$\Phi_1 = diag_{1\le k\le K}\ e^{(\alpha_{1k}+j2\pi f_{1k})} = \mathbf{T}_1\mathbf{F}_1\mathbf{T}_1^{-1}$$

where $\mathbf{F}_1 = \underline{\mathbf{U}}_{S1}^{-}\overline{\mathbf{U}}_{S1}$, and $\underline{\mathbf{U}}_{S1}$ is a matrix by removing the last Q rows of $\mathbf{U}_{S1}$, and $\overline{\mathbf{U}}_{S1}$ is by removing the first Q rows, $\underline{\mathbf{U}}_{S1}^{-}$ is the pseudo inverse of $\underline{\mathbf{U}}_{S1}$.

For the 2nd dimension, the 2nd Hankel block matrix $\mathbf{Y}_2 = \begin{bmatrix} \mathbf{Y}^{(0)} & \mathbf{Y}^{(1)} & \cdots & \mathbf{Y}^{(N-Q)} \\ \mathbf{Y}^{(1)} & \mathbf{Y}^{(2)} & \cdots & \mathbf{Y}^{(N-Q+1)} \\ \vdots & \vdots & & \vdots \\ \mathbf{Y}^{(Q-1)} & \mathbf{Y}^{(Q)} & \cdots & \mathbf{Y}^{(N-1)} \end{bmatrix}$, where the block matrix is $\mathbf{Y}^{(n)} = \begin{bmatrix} y(0,n) & y(1,n) & \cdots & y(M-P,n) \\ y(1,n) & y(2,n) & \cdots & y(M-P+1,n) \\ \vdots & \vdots & & \vdots \\ y(P-1,n) & y(P,n) & \cdots & y(M-1,n) \end{bmatrix}$.

Exploiting the paired sinusoidal frequency relations in two dimensions, computing the SVD of $\mathbf{Y}_2$ is no longer needed. Using the following relation,

$$\Phi_2 = diag_{1\le k\le K}\ e^{(\alpha_{2k}+j2\pi f_{2k})} = \mathbf{T}_2\mathbf{F}_2\mathbf{T}_2^{-1} = \mathbf{T}_1\mathbf{F}_2'\mathbf{T}_1^{-1}$$

where

$$\begin{aligned} \mathbf{F}_2' &= (\underline{\mathbf{E}_1\mathbf{U}_{s1}})^{-}(\overline{\mathbf{E}_1\mathbf{U}_{s1}}) \\ \mathbf{E}_1 &= \textstyle\sum_{k=1}^{Q}\sum_{l=1}^{P}\mathbf{E}_{k,l}^{Q\times P}\otimes\mathbf{E}_{l,k}^{P\times Q} \end{aligned} \quad (6)$$

and $\mathbf{E}_{k,l}^{Q\times P}$ is a matrix of size $(Q \times P)$ with value 1 for k,l element and 0 otherwise.

A common transform $\mathbf{T}$ can be used to diagonalize two matrices $\mathbf{F}_1$ and $\mathbf{F}_2'$, and using the linear combination,

$$\beta\mathbf{F}_1 + (1-\beta)\mathbf{F}_2^T = \mathbf{T}^{-1}\mathbf{D}\mathbf{T} \quad (7)$$

where β is a scalar value.

Subband filters: The rank of the data matrix (or, the Hankel block matrix) used by the 2D ESPRIT is equal to the rank of the signal subspace plus the noise subspace. This requires that the observation data window size P and Q in the Hankel block matrices satisfy:

$$K \le P \le M-K+1,\ \ K \le Q \le N-K+1 \quad (8)$$

However, due to the short length of our measured 2D projection signals, (8) is not satisfied. One solution is to re-measuring these NMR projections with higher sampling rate, thus longer data. However, this is not always easy as it requires the use of NMR equipment, cooling materials and laboratory experts. To tackle this problem, the projected 2D NMR signal is first split into a set of subbands by using a set of 2D subband filters (e.g. using linear-phase Parks-McClellan equiripple FIR filters). The bandwidth of subband filters are selected such that (8) is satisfied to the subband filtered projection signal.

Computing powers and initial phases: Once the frequencies and damping factors are estimated, the powers and initial phases of sinusoids can be estimated by using the LS estimation by plugging in the estimated frequencies and damping factors as the true values. Consider a simple case of 1D signal $y(n) = \sum_{k=1}^{K} A_k e^{s_k n} + w(n)$ as an example, or its vector form $\mathbf{Y} = \mathbf{H}\theta + \mathbf{W}$, where $s_k = -\alpha_k + j\omega_k$. The LS criterion can be formed as $J(\mathbf{A},\theta) = (\mathbf{H}\theta+\mathbf{W})^T(\mathbf{H}\theta+\mathbf{W})$, where $\theta = [A_1\cdots A_K]^T = [a_1e^{j\phi_1}\cdots a_Ke^{j\phi_K}]^T$, and $\mathbf{H} = \begin{bmatrix} 1 & \cdots & 1 \\ e^{s_1} & \cdots & e^{s_K} \\ \vdots & \ddots & \vdots \\ e^{s_1(N-1)} & \cdots & e^{s_K(N-1)} \end{bmatrix}$. The LS solution is $\hat{\theta} = (\mathbf{H}^T\mathbf{H})^{-1}\mathbf{H}^T\mathbf{Y}$. Estimation in the 2D case is performed as follows. First, a set of 1D signals are obtained from the 2D signal by setting e.g. $y(m,1), \cdots, y(m,N)$. Each data sequence can be considered as one realization of the measured signal. Parameters can hence be estimated by applying 1D LS estimation followed by the ensemble averaging. Table 1 summarizes the algorithm.

Giving 2D FID measurements $y(m,n)$, size $M \times N$, from a 2D projection, and assuming $y(m,n)$ consists of K sinusoids in white noise:

1. Split 2D measured signal by applying 2D subband filters:

1.1. Applying subband filters (e.g. Parks-McClellan equiripple FIR filter) to 2D signal $y(m,n)$;

2. Estimation of paired frequencies and dampings by 2D ESPRIT:

2.1. Create a Hankel block matrix $\mathbf{Y}_1$ of size $P \times (M-P+1)$ related to the observation window $P \times Q$ for the first dimension using (4);

2.2. SVD decompose $\mathbf{Y}_1$ using (5);

2.3. Obtain $\underline{\mathbf{U}}_{s1}$ by removing the last Q rows, and $\overline{\mathbf{U}}_{s1}$ by removing the 1st Q rows from $\mathbf{U}_{s1}$.

2.4. Compute $\mathbf{F}_1 = \underline{\mathbf{U}}_{S1}^{-}\overline{\mathbf{U}}_{S1}$.

2.5. Compute $\mathbf{F}_2'$ by using (6).

2.6. Compute $\mathbf{T}$ from the linear combination of $\mathbf{F}_1$ and $\mathbf{F}_2'$ using (7);

2.7. Diagonalize $\mathbf{F}_1$ and $\mathbf{F}_2^T$ from a same transform matrix $\mathbf{T}$, using (7);

2.8. Find frequencies and damping factors from the diagonal of $\mathbf{\Phi}_1 = \mathbf{T}\mathbf{F}_1\mathbf{T}^{-1}$ and $\mathbf{\Phi}_2 = \mathbf{T}\mathbf{F}_2'\mathbf{T}^{-1}$.

3. LS estimation of powers and initial phases of sinusoids:

3.1. Estimate powers and initial phases for $y(m,1),\cdots,y(m,N)$ using 1D LS formulation;

3.2. Apply ensemble average to obtain 2D estimates.

Table 1. Pseudo Algorithm.

4. RELATIONS BETWEEN PARAMETERS IN 2D PROJECTIONS AND IN L-D SIGNALS

For each signal from a 2D projection, the above analysis provides frequencies ω_r that are linear combinations of the desired frequencies (chemical shifts) Ω_l of the atomic nuclei $l = 1,\cdots,L$. The relation between observed and true frequencies can be expressed as a set of linear equations:

$$\mathbf{A}\Omega \approx \omega \quad (9)$$

where Ω is a vector of size L containing the desired chemical shift frequencies, ω is a vector of size R containing the signal frequencies from all projections, and $\mathbf{A}$ is a matrix of size

$R \times L$ (rank L), describing linear relations according to the selected projections and their angles. Due to the presence of noise, the number of projections should exceed the theoretical minimum, i.e. $R > L$, and determination of ω according to (9) is an over-determined problem. Since the matrix $\mathbf{A}$ cannot be inverted, a pseudo inverse $\mathbf{A}^-$ is obtained from the SVD of $\mathbf{A}$ (i.e., $\mathbf{A} = \mathbf{UDV}^T$), $\mathbf{A}^- = \mathbf{VD}^{-1}\mathbf{U}^T$. It can be shown that the optimal estimation of $\hat{\Omega}$ in (9) is

$$\hat{\Omega} = \mathbf{A}^- \omega \tag{10}$$

5. SIMULATION AND EXPERIMENTAL RESULTS

Tests on simulated data: The 2D ESPRIT algorithm was first tested on several synthetic data. As an example, Fig.2 shows the the ground truth frequencies, the estimated frequencies from 2D ESPRIT, and the equal energy contours from 2D FFT spectrum for a synthetic signal consisting of 33 sinusoids in white noise. From Fig.2 one can see that 2D ESPRIT has yielded more accurate frequency estimation with higher frequency resolution as compared with that in the FFT.

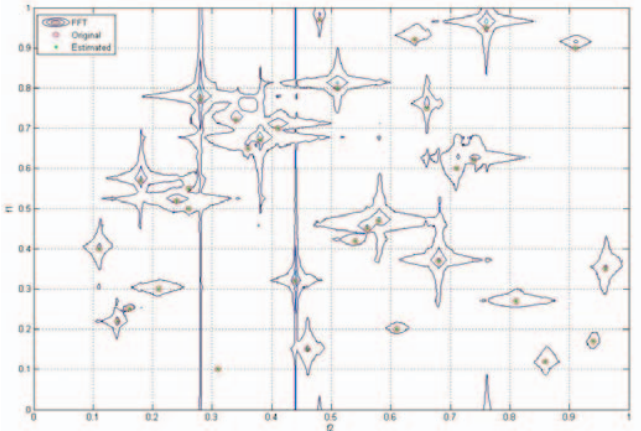

Fig. 2. 2D ESPRIT vs. 2D FFT: estimation of the frequencies from synthetic 2D signals with 33 damped sinusoids in white noise. Red: ground truth; Green +: from 2D ESPRIT; Blue: equal energy contours from 2D FFT.

Analysis of experimental 2D projections: From a set of 30 projections, resulting from two 5D experiments, the spectrum corresponding to a ^{15}N-HSQC was chosen as a test example. The data were collected for a 2mM solution of the protein ubiquitin on a 600 MHz instrument at 303 K [3]. Each projection requires about 30 minutes of measurement time. For the parameter estimation, the data file is arranged as a matrix of size M=60 and N=955. The estimated number of sinusoids is K=76 (derived from the protein size). Since $M = 60$ is too short, which limits the use of the 2D ESPRIT, subband filters are used to split the 2D signal into subbands, each of which containing a smaller number of sinusoids. After that, a 2D ESPRIT is applied to each subband filtered signal. Fig.3 shows the estimated frequencies from a lowpass filtered 2D NMR projected signal, where a 1D lowpass filter was applied along the direction of the first frequency axis ω_1. For comparisons, the equal energy peak contours of the corresponding FFT-spectrum are also included. Observing the results obtained from the measured data in Fig.3, one can see that 2D ESPRIT has resulted in estimated frequencies agreeing well the FFT spectral peak contours. Further, the former clearly shows high frequency resolution hence able to resolve frequencies that a conventional FFT spectrum cannot separate.

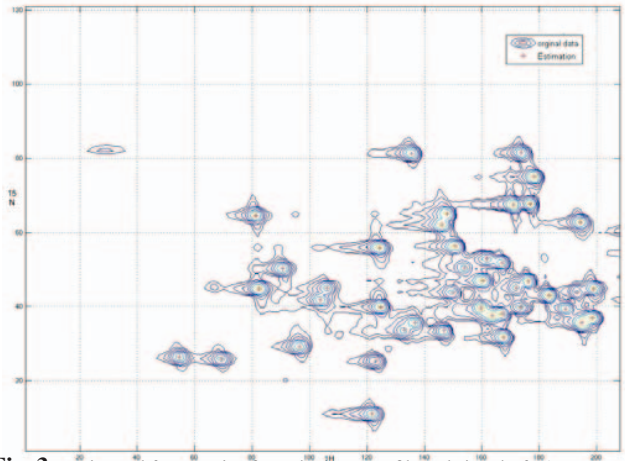

Fig. 3. Estimated frequencies from the lowpass filtered signal of a 2D NMR projection data set. Red +: the estimated frequencies from the 2D ESPRIT; Blue curves: equal energy contours of the peaks in the FFT spectrum.

6. CONCLUSION

This paper employed a combination of methods for improving the estimation of resonance frequencies of 5D NMR through the use of projection spectroscopy, where the most commonly used analysis methods are still FFT-based. Multiple 2D projections of a 5D NMR signal from a protein were measured and analyzed. Tests have been conducted on synthetic data as well as projected NMR measurements by applying subband filters and 2D parametric estimation. Results showed clear improvement of estimation accuracy with enhanced frequency resolution. Mathematical formulations for reconstructing the desired frequencies (i.e., the chemical shifts) of atomic nuclei from the estimated frequencies in multiple 2D projections are also given. Further NMR measurements are planned for extensively testing the proposed method and evaluating the performance.

7. REFERENCES

[1] J.Cavanagh, W.J.Fairbrother, A.G.Palmer III, M.Rance, N.J.Skelton, Protein NMR Spectroscopy, Elsevier, 2007.

[2] D.Malmodin, M.Billeter, "High-throughput analysis of protein NMR spectra", Progr. Nucl. Magn. Reson. Sepctrosc., vol.46, pp. 109-129, 2005.

[3] D.K.Staykova, J.Fredriksson, W.Bermel, M.Billeter, "Assigment of protein NMR spectra based on projections, multiway decomposition and a fast correlation approach", J. Biomol. NMR, vol.42, pp. 155-158, 2008.

[4] D.Malmodin, M.Billeter, "Multiway decomposition of NMR spectra with coupled evolution periods", J. Amer. Chem. Soc., vol.127, pp. 3486-3487, 2005.

[5] Y.Li, J. Razavilar, K.J.R.Liu, "A high-resolution technique for multi-dimensional NMR spectroscopy", IEEE Trans. Biomedical Engineering, vol. 45, pp.78-86, 1998.

[6] S.Rouquette, M. Najim, "Estimation of frequencies and damping factors by two-dimensional ESPRIT type methods", IEEE Trans. Signal Processing, vol.49, pp.237-245, 2001.