

Magnetic Resonance Analogies in Multidimensional Vibrational Spectroscopy

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We survey the principles for the design of multidimensional vibrational spectroscopies that draw upon the analogy with liquid or solid state NMR and EPR spectroscopy. The much faster time-scales and the different energy regime in laser spectroscopy provide additional possibilities that lack magnetic spectroscopy counterparts. We also discuss the notion of through-bond vs through-space couplings between localized vibrations, which are of different nature than in NMR.

Multidimensional NMR spectroscopy has been a valuable and well established technique for over two decades. By manipulation of spin coherences in multiple pulse sequences it allows to probe structure and dynamics of molecules in great detail, exploiting spin–spin interactions through chemical bonds or directly through space.^{1,2} Optical spectroscopy has only recently reached a state where similar control over vibrational or electronic coherences is possible. This is due to the much shorter time-scales involved that require ultrafast pulse techniques to perform coherent multidimensional optical spectroscopy. Pulse shaping and phase control of femtosecond pulses has only been experimentally achieved over the last few years.³ Ultrafast pulses have been used to carry out multi-dimensional laser spectroscopy through the control and manipulation of vibrational and electronic coherences.^{4–16}

In this paper we propose vibrational analogues of various NMR pulse sequences that accomplish the same goals. Spectroscopic techniques based on the application of sequences of carefully shaped and timed femtosecond pulses provide a novel multidimensional view of molecular structure as well as vibrational and electronic motions, interactions and relaxation processes.¹⁷ Conventional spectroscopies such as linear absorption and spontaneous and coherent Raman give a one-dimensional (1D) projection of molecular interactions onto a single frequency (or time) axis. Multidimensional spectroscopies in contrast provide a wealth of additional information. An n 'th order heterodyne experiment involves the control of n time intervals and thus constitutes an n -dimensional (nD) spectroscopy.¹⁸ One is typically interested only in a comparatively low dimensional (2D, 3D) spectrum even if many more pulses are used to manipulate the coherences. This means that only a few of the possible time delays in a pulse sequence are varied and the decision which delays are varied should be based on the desired information and on a more detailed knowledge of the system to be studied. The intensities and profiles of new peaks give a direct signature of molecular structure (distances between chromophores) and dynamics (the spectral density of the chromophores' environments). These techniques may be developed into a standard diagnostic tool to

investigate hydrogen bonded complexes, molecular liquids, the secondary and tertiary structure of polypeptides, protein folding, and chromophore aggregates. Multiple pulse techniques have the capacity to prepare electronic and vibrational degrees of freedom in nonequilibrium states and monitor their subsequent dynamics, yielding femtosecond snapshots of dynamical processes, vibrational and electronic energy transfer pathways, charge transfer, photoisomerization, and chemical reactions. The observation of cross-peaks and the analysis of their magnitudes and lineshapes provide extremely powerful microscopic probes of local environments.

The theoretical description of optical multiple pulse spectroscopy was accompanied by developments in experimental techniques. The treatment of weak pulse experiments in terms of the density matrix in Liouville space¹⁷ has been successfully applied to propose new third- and fifth-order spectroscopies.¹⁹ An alternative description of third-order spectroscopy is given by the Nonlinear Exciton Equations (NEE) that provide a quasiparticle picture of the nonlinear response of large aggregates of strongly coupled three-level systems.^{20,21} The various possible techniques may be systematically classified and described using Liouville space pathways¹⁷ which represent the relevant sequences of population and coherence-periods that dominate the multiple-pulse nonlinear optical response of complex molecules.

Higher order optical vibrational response can be induced by various sources of nonlinearity such as the nonlinear dependence of the dipole μ on nuclear coordinates, anharmonic intermolecular and intramolecular potentials that contain terms cubic and higher in the coordinates, and relaxation and dephasing by nonlinear coupling to a bath. The various types of nonlinearities affect the response in a different manner and may thus be extracted from experimental signals.^{22,23} The nonlinear dipole creates inter-mode coherences instantaneously, each time the system interacts with the radiation field. In contrast, the effect of anharmonicity is not felt immediately because such a coherence may only be built during the evolution period between interactions. The resonances and phases of 2D signals contain distinct 2D signatures of all types of nonlinearities. If

the various vibrational modes do not interact (e.g. when they represent different components of inhomogeneous regions), the nonlinearities will be diagonal, and their contributions to the nonlinear response will be additive. Off-diagonal couplings will however result in cross-terms.

For simple systems, the harmonic and anharmonic parameters of the molecular potential energy surface (PES) and the dipole may be extracted directly from characteristic features in the spectra.^{13,14} In general, though, it is impossible to achieve the inversion of the spectroscopic data, i.e. the translation of possibly time-dependent peak positions, peak shapes,^{12,24} and intensities into molecular geometry and dynamic parameters, without extensive theoretical modeling. This is analogous to structure determination and dynamic studies by multidimensional NMR methods.^{2,25,26} In NMR the dynamics of the spectroscopic probes, the spins, does not affect the molecular dynamics that is studied and it is a good approximation to model the spectroscopic results by spin- $\frac{1}{2}$ systems coupled to classical degrees of freedom describing the molecular dynamics.²⁷ This separation is not generally possible in vibrational spectroscopy, since both the chromophores as well as the molecular dynamics are related to vibrational degrees of freedom. A practical way to overcome this difficulty is to use resonant weak field spectroscopy^{17,28} to study high frequency vibrations with chromophores that are given by vibrations that are well separated from low frequency modes. Even with this choice, the spectroscopic Hamiltonians are more complicated than their NMR counterparts, containing anharmonicities and high order coupling terms that yield more information about the system but also result in more complicated spectra requiring sophisticated pulse sequences.

Systems like synthetic polymers or biopolymers (proteins, RNA, DNA) that contain many similar or identical subunits typically contain bands that are derived from high-frequency stretching vibrations of functional groups in the monomeric units (e.g. carbonyl groups) and that are well separated from other vibrations (e.g. the amide bands in peptides²⁹).

The modes in these bands are usually delocalized over several units and are appropriately described as *vibrational excitons* using the Frenkel exciton model where the delocalization is due to couplings between a set of local oscillators.³⁰ The IR and Raman response of peptides and small proteins has been successfully calculated from vibrational Exciton Hamiltonians.^{23,31,32} Multidimensional vibrational spectroscopies of the amide bands in proteins could become as powerful and versatile as heteronuclear NMR experiments of fully labelled (¹H, ¹³C, ¹⁵N) proteins.

Vibrational Analogues of Magnetic Resonance Spectroscopies

The design of multidimensional optical experiments can benefit from the transfer of ideas from NMR spectroscopy. A typical 2D NMR pulse sequence involves four distinct blocks: preparation, evolution, mixing, and detection.³³ The detection period constitutes one time domain and incrementing the evolution period yields the other time domain. The preparation sequence contains all pulses that are applied to the system before the first time domain that gets incremented in consecutive experiments. The time delays between pulses in the preparation block are not varied and can be optimized to prepare the system in a specific initial non-equilibrium state. Similarly, the mixing sequence consists of all pulses between the evolution and the detection period and is also fixed. It serves to map the coherence pattern that evolved during the evolution period into a different pattern at the beginning of the detection period, thus revealing correlations in the system that is studied. The full power of 2D spectroscopy becomes apparent if preparation and/or mixing consist of multiple pulses, creating and manipulating coherences in very specific ways, thus uncovering chromophore interaction topologies.

Fundamental differences between NMR (EPR) and optical methods should be accounted for to allow the transfer of pulse sequences (see Table 1): in NMR kT is usually much larger than the transition frequencies (ω_0). In this high temperature

Table 1. Comparison of Multidimensional Infrared and NMR Techniques

Infrared	NMR
• anharmonic vibrational Hamiltonian ➡ many parameters, inversion of signals is complex • weak pulses ➡ only few molecules are excited - sequences with few pulses possible ➡ susceptibilities and response functions • directly manipulate relevant degrees of freedom • $\lambda \ll$ sample size, $k \cdot r \gg 1$ ➡ signal is highly directional ➡ coherence transfer pathway selection by spatial phase matching • temperature low compared to frequencies: $kT \ll \hbar\omega_0$ ➡ calculations more complex • linear polarization • varying dipoles, arbitrary orientation ➡ many independent parameters for the dipole • femtosecond time-scales	• spin Hamiltonian ➡ few parameters, spectroscopic data easier to invert • strong saturating pulses ($\pi, \frac{\pi}{2}$) ➡ all spins excited - long pulse sequences possible ➡ Bloch picture • manipulate spins of certain isotopes (mainly spin-$\frac{1}{2}$) • $\lambda \gg$ sample size, $k \cdot r \ll 1$ ➡ signal is isotropic ➡ coherence transfer pathway selection by phase cycling techniques • temperature high compared to frequencies: $kT \gg \hbar\omega_0$ ➡ simplifies calculations • circular polarization • all dipoles are equal and aligned ➡ gyromagnetic ratio • microsecond time-scale, picosecond time-scales observed indirectly

limit the one-dimensional (single-quantum) spectra are very complex and they become simpler for multi-quantum transitions (since there are fewer of them). High frequency vibrations are closer to the opposite, zero temperature, limit: Here the single-quantum spectrum is simple and multi-quantum spectra are more complex. Another difference is that in weak-field optical techniques the signal's intensity decreases rapidly with the number of pulses since only a small fraction of molecules is affected by the application of each successive pulse. In contrast strong-field NMR and EPR techniques manipulate the entire ensemble and there is no loss of intensity as the number of pulses is increased. Consequently vibrational techniques are limited to a few (~ 3) pulses while complex sequences with hundreds of pulses are possible in NMR. In NMR, strong fields only affect the spin dynamics and due to the weak spin-vibrational coupling and the low pulse energies it is possible to observe unperturbed native molecular dynamics with strong fields. Strong fields in laser spectroscopy directly affect the vibrational dynamics of interest, which can be used to control the dynamics. In addition vibrational dynamics at high intensities becomes increasingly more complex: high anharmonic and reactive regions of phase space become accessible complicating the interpretation of such experiments. To obtain information about unperturbed dynamics from laser spectroscopy we must resort to weak field techniques. We will therefore not consider strong optical pulses with finite flip angles^{34–36} but rather consider the limit of small perturbations by the pulse fields.

The closest analog to spin- $\frac{1}{2}$ NMR is optical spectroscopy of coupled two-level systems. These can be realized for electronic transitions (visible) or, approximately, for strongly anharmonic vibrational transitions (infrared). Models for the interaction between optical two-level systems in molecular crystals and aggregates are usually based on the resonant J -coupling Hamiltonian which only contains the first excitonic off-diagonal coupling term in Eq. 1. Here $\mathbf{B}_\alpha$ ($\mathbf{B}_\alpha^\dagger$) is the exciton annihilation (creation) operator describing the localized oscillator α .

$$\mathbf{H}_{J,\text{res}} = J_{\alpha\beta} (\mathbf{B}_\alpha^\dagger \mathbf{B}_\beta + \mathbf{B}_\alpha \mathbf{B}_\beta^\dagger) + J'_{\alpha\beta} \mathbf{B}_\alpha^\dagger \mathbf{B}_\beta^\dagger \mathbf{B}_\alpha \mathbf{B}_\beta \quad (1)$$

The off-diagonal coupling J has a noticeable influence on the spectra only if the transition frequency difference of the chromophores is small or comparable to the coupling strength ($\Delta\omega \approx J$). This case is called *strong coupling* in NMR and yields a change in line shape known as the *roof effect*.¹ The name is derived from the fact that the isotropic liquid state NMR coupling Hamiltonian contains diagonal (J' , second term in Eq. 1) as well as off-diagonal couplings with the same magnitude and the intensity of the peripheral peaks in coupling multiplets is always reduced with respect to the central lines. For the high-temperature limit of NMR the diagonal (non-resonant) coupling yields the splitting of the resonances in multiplets while the intensities in the multiplets depend on the off-diagonal coupling. For weak coupling only the splitting is observed and the off-diagonal coupling can be neglected, yielding the *weak coupling* Hamiltonian which is the standard model of the NMR product operator formalism.^{1,28,37} For these non-resonant couplings the perturbative expansion in J and the use of product

states of localized basis states for the chromophores allows a real-space interpretation of the action of the pulse sequence. This considerably simplifies the design of specialized pulse sequences for the extraction of specific system parameters like transition frequencies and J -couplings and yields a more intuitive picture than possible using the global eigenstates. In general we need to address both coupling terms in Eq. 1 as well as possibly other higher order anharmonic coupling terms. We have recently analyzed a vibrational model which includes diagonal couplings $J_{\alpha\beta} \mathbf{I}_z^\alpha \mathbf{I}_z^\beta$ similar to the full scalar coupling Hamiltonian of liquid state NMR.²⁸

This unified description of resonant multiple-pulse experiments in coupled spin- $\frac{1}{2}$ systems in NMR spectroscopy and two-level systems in optical spectroscopy accounts for all of their differences. We assume resonant well-separated, time-ordered pulses and the relationship $\Delta\omega \ll \omega_1 \ll \omega_0$, where $\Delta\omega$ is the width of the spectrum, ω_1 the Rabi frequency, and ω_0 the central transition frequency. In resonant spectroscopy the theoretical description is simplified considerably since only few of the Liouville space pathways contributing to the nonlinear response functions dominate the response and should be retained within the rotating wave approximation. The theoretical tool that is used to describe resonant multiple-pulse NMR spectroscopy is the product operator formalism.^{1,37} In this formalism the evolution under pulses and the system Hamiltonian are described as mappings acting on an initial density matrix and yielding a transformed density matrix. The density matrix itself is expanded in a basis of product states in which the action of pulses is especially simple to describe. The transformation rules are given for all possible basis states. We have developed the necessary operator mappings to describe resonant optical multiple pulse spectroscopy of weakly J -coupled two-level systems and studied the similarities and differences between optical spectroscopy of two-level systems and NMR spectroscopy of spin- $\frac{1}{2}$ systems. We showed which coherence manipulations commonly used in NMR can be performed in the weak field limit. This includes phase cycling and coherence transfer pathway selection, which are effects achievable by macroscopic observation and therefore closely related to phase-matching and directional detection.³⁸

We will illustrate the connection between the NMR product operator formalism and the Liouville space pathways in optical spectroscopy by showing how the information obtained in various strong field two and three pulse NMR experiments can be extracted from weak fields with controlled phases. These results allow the design of sequences of weak optical pulses that accomplish the same goals as multidimensional NMR techniques.

Two Dimensional Optical Analogs of NMR Pulse Sequences. The simple two- and three-pulse strong field experiments like COSY (2D correlation spectroscopy), NOESY (2D Nuclear Overhauser Effect spectroscopy), EXSY (2D exchange spectroscopy), or double quantum spectroscopy are of great importance in liquid state NMR spectroscopy and yield detailed information about weakly J -coupled spin- $\frac{1}{2}$ systems. The same type of information can be obtained with weak pulses for optical two-level systems. Two-pulse experiments do not yield an observable signal in the weak field limit. Optical three-pulse experiments can be described in close analogy to

NMR using the operator transformation rules and optical weak field techniques that contain the same information as their NMR analogs can be identified. Comparison of the resulting density matrix evolution and responses with the corresponding NMR experiments reveals the close analogies between weak- and strong-field experiments.

We consider for example the COSY $\frac{\pi}{2} - \tau_1 - \beta - \tau_2$ experiment which is the basic 2D correlation spectroscopy technique and employs a single mixing pulse with flip angle β to induce coherence transfer. The frequency domains Ω_1 and Ω_2 are obtained by a 2D Fourier transformation of the signal with respect to the τ_1 and τ_2 time domains:

$$S(\Omega_1, \Omega_2) = \int_0^\infty d\tau_2 \int_0^\infty d\tau_1 \exp(i(\Omega_1\tau_1 + \Omega_2\tau_2)) S(\tau_1, \tau_2) \quad (2)$$

The evolution during τ_1 as well as τ_2 depends on both transition frequencies and couplings, giving rise to complicated mul-

tiplet patterns in both Ω_1 and Ω_2 . The coherence transfer yields off-diagonal peaks between multiplets that correspond to coupled spins. This allows the identification of coupled spins and the interpretation of the observed coupling multiplets. We were able to identify the optical analog of a three-pulse variant, the double quantum filtered COSY, in which the mixing block consists of two pulses with fixed delay during which the system evolves in a double quantum coherence. The signal for this technique is given by the Liouville space pathway S_{III} in Fig. 1 (with time domains τ_1 and τ_3 and mixing time $\tau_m = \tau_2$) and can be observed in the given phase matched direction. The resulting spectrum for a pair of coupled two-level system is compared to the corresponding NMR COSY spectrum in Fig. 2. The different multiplicities in the Ω_1 domain are due to the different initial conditions (high vs zero temperature approximation) but otherwise both spectra exhibit the same information content in terms of cross peaks and multiplet posi-

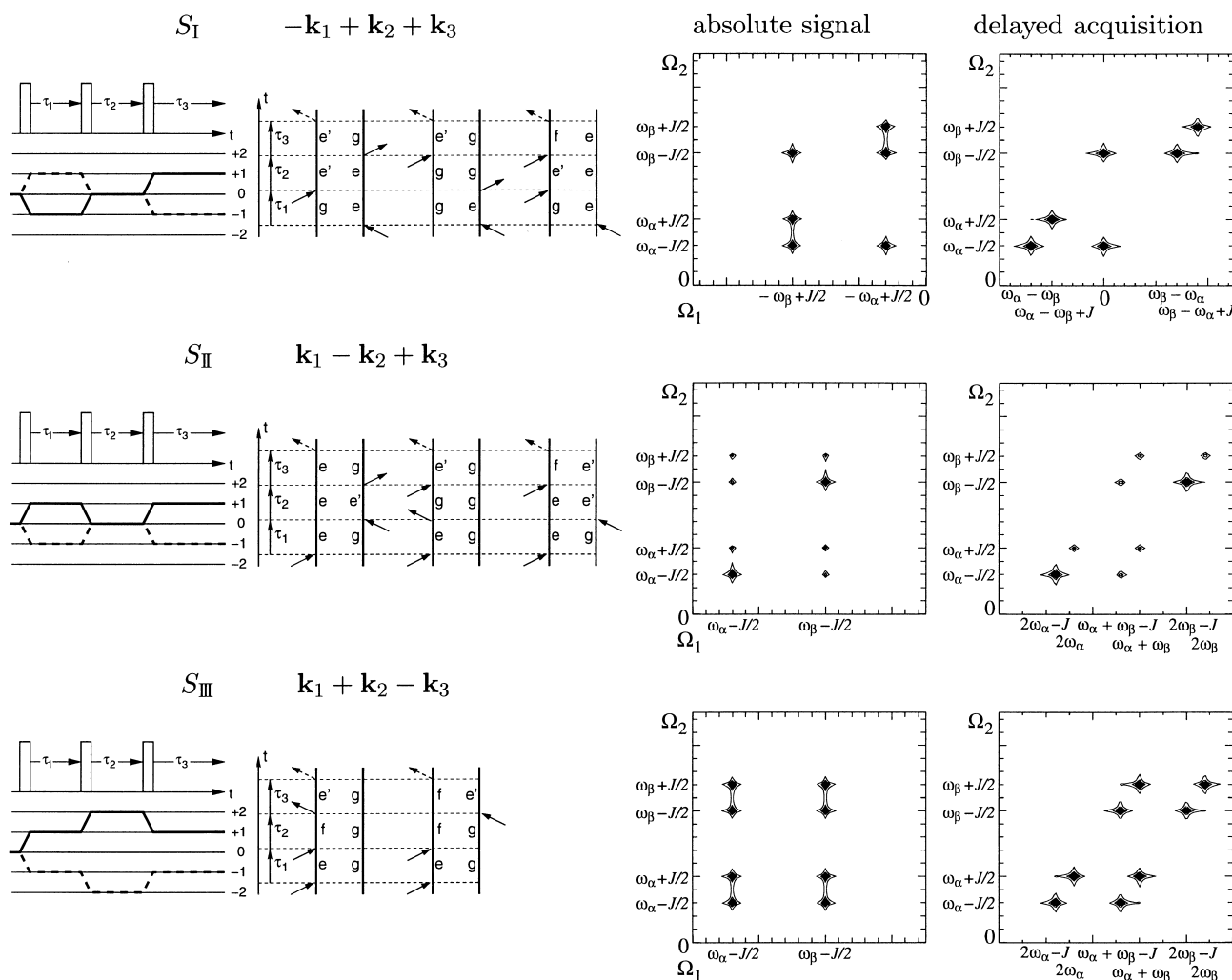


Fig. 1. The coherence order diagrams and double sided Feynman diagrams of the possible weak field signals from optical three-pulse experiments are shown along with the corresponding 2D absolute value spectra for a dimer of two-level systems. The frequency domains of the spectra are obtained by Fourier transformation of the time-delays τ_1 and τ_3 where $\tau_2 = \tau_m$ is a short mixing time. The last column shows the signals for delayed acquisition with acquisition starting after an additional delay of τ_1 after the third pulse. This is analogous to the detection in J -resolved spectroscopy and results in a skew transformation of the observed signals.

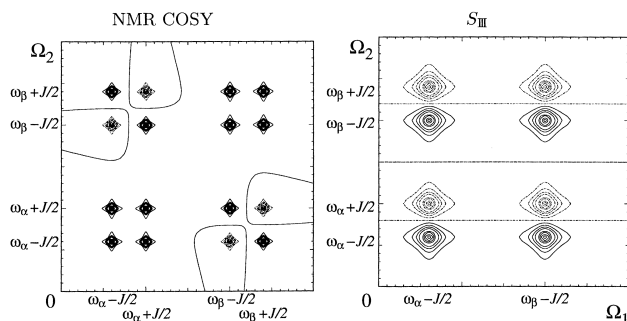


Fig. 2. Comparison of the NMR COSY experiment with the vibrational S_{III} three pulse signal that contains analogous information.

tions and splittings.

If instead of fixing τ_2 we hold τ_1 constant and therefore have now a preparation block that consists of two pulses and a single pulse for mixing, we obtain the optical analog of the simplest double quantum experiment in NMR. In this case the system evolves in a double quantum coherence during the evolution period and the frequency domain Ω_1 will contain overtone and combination frequencies which are hard to observe in simple 1D experiments. The value chosen for the fixed delay τ_1 can be additionally adjusted to favor the double quantum coherences involving chromophores with a coupling of a specific magnitude.

A similar analysis reveals that the remaining pathways S_I and S_{II} in Fig. 1 together are observed in NMR NOESY or EXSY experiments. The pulse sequence of both of these techniques is given by $\frac{\pi}{2} - \tau_1 - \frac{\pi}{2} - \tau_m - \frac{\pi}{2} - \tau_2$ where the mixing time τ_m is the time during which cross relaxation or chemical exchange are active.¹ The system is in a population state after the first two $\frac{\pi}{2}$ -pulses and cross peaks are observed when polarization is transferred between spins that are connected by cross-relaxation in the case of NOESY or between sites involved in chemical exchange in EXSY. The nuclear cross-relaxation (in liquids) observed in NOESY is caused by mutual spin flips in pairs of dipolar-coupled spins which are induced by motional processes. Early 1D measurements were mostly concerned with steady state Overhauser effects observed by irradiating one of the coupled spins and measuring the intensity change in a different resonance line due to cross relaxation.³⁹ More specific information can be obtained from transient Overhauser effects where the redistribution of magnetization is studied as a function of time after selectively inverting of one spin.^{39–41} The 2D NOESY is closely related to these transient experiments with the advantage that all transfer paths can be detected in one experiment. The detection of cross peaks is also more sensitive than the observation of the NOE or exchange as intensity changes in 1D saturation experiments. Vibrational three-pulse echo experiments that are analogous to NOESY have recently been performed⁴ and theoretically analyzed using the NEE for a two chromophore model.⁴²

All signals corresponding to S_I and S_{II} are observed simultaneously in NMR due to the $\mathbf{k} = 0$ limit where the sample size is much smaller than the wavelength of the field and can only be separated using phase cycling techniques (see Table 1). Due to phase matching these signals are observed separately in

different directions in optical spectroscopies which allows us to linearly combine them in new ways to simplify the spectra and extract different types of information about the system. For example, the following combination of signals

$$S_D = S_{\text{II}}(\Omega_1, \Omega_2) - S_{\text{III}}(\Omega_1, \Omega_2) \quad (3)$$

yields a spectrum that contains only the diagonal peaks and

$$S_{\text{JRS}} = S_I(-\Omega_1, \Omega_2) - S_{\text{II}}(\Omega_1, \Omega_2) + S_{\text{III}}(\Omega_1, \Omega_2) \quad (4)$$

can be considered an analog of J -resolved spectroscopy (see Fig. 3), since in the Ω_1 dimension the signal depends only on the transition frequencies while it depends on both frequencies and couplings in the Ω_2 dimension. The NMR J -resolved spectroscopy is an example of a pulse sequence that employs a π -pulse $\frac{\pi}{2} - \frac{\tau_1}{2} - \pi - \frac{\tau_1}{2} - \tau_2$ to refocus the Zeeman evolution at the end of the time period given by τ_1 . It is therefore closely related to the Photon Echo technique (pathway S_I in Fig. 1) of optical spectroscopy. Placing a pulse in the middle of the evolution period and delaying the detection of the signal after the last pulse is known as *delayed acquisition* in NMR. The signals obtained with this technique for the vibrational three-pulse experiments are given in the right column of Fig. 1 and can be described by the Fourier transformation

$$S(\Omega_1, \Omega_2) = \int_0^\infty d\tau_2 \int_0^\infty d\tau_1 \exp(i(\Omega_1\tau_1 + \Omega_2\tau_2)) S(\tau_1, \tau_1 + \tau_2) \quad (5)$$

A comparison to the column with regular detection shows the effect of the skew transformation which in the case of S_I shifts the diagonal peaks to zero frequency. For these peaks any inhomogeneous broadening will be canceled in a photon echo experiment. The differences between the NMR J -resolved spectra and S_I with delayed acquisition stem from the different initial conditions (high vs low temperature regime, see Table 1) which yield different cancellation effects for the diagonal peaks and the cross-peak multiplets in both cases.

From the example of J -resolved spectroscopy it is clear that signals detected in different directions can be linearly combined to simplify the resulting 2D spectra, yielding techniques that resemble results from NMR pulse sequences involving strong π -pulses. The combination of directional detection, linear combination of signals from different spatial directions, and cycling of pulse phases gives a high degree of control over the selection of coherence transfer pathways. To gain this control it is necessary to measure the full complex signal by heterodyne detection (which is common practice in NMR) and not only the absolute value spectrum.

A further advantage of heterodyne detection is that for well-designed pulse sequences that do not generate mixed absorptive and dispersive peaks by selecting certain combinations of coherence transfer pathways, it is possible to obtain pure absorptive spectra after appropriate phase correction and data processing. These spectra have considerably higher resolution than absolute value spectra.¹ The respective choices of pulse phases (combinations of $\pm x$ - and $\pm y$ -pulses) and phase cycling schemes for consecutive measurements have been analyzed for most commonly used NMR pulse sequences. The separation of absorptive and dispersive contributions to the

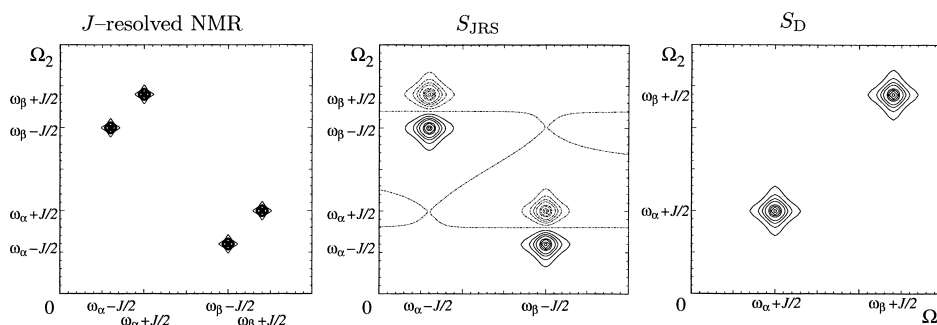


Fig. 3. Comparison of the NMR J -resolved experiment (without delayed acquisition) with the vibrational combination signal S_{JRS} that contains analogous information. In the complementary combination signal S_D only the diagonal peaks are retained.

spectra is based on the assumption of clearly distinct time-scales for homogeneous and inhomogeneous broadening which is usually valid for the microsecond time-scale of NMR spectroscopy.^{1,27} The experimentally observed homogeneous lineshapes in liquid state NMR are either absorptive or dispersive Lorentzians and only a very limited number of combined 2D lineshapes has to be considered.¹ The typical inhomogeneously broadened lineshape of solid state NMR are the well known Pake patterns which arise from the orientational distributions of the chemical shift anisotropy (CSA) tensors in a crystalline powder with respect to the external field.^{27,43,44} The situation in vibrational spectroscopy is more complicated due to the existence of a continuous range of intermediate relaxation regimes that are on time-scales similar to the experiment.¹⁷ This yields a larger variety of possible 2D lineshapes depending on the spectral densities of the bath.¹²

It will be necessary to carefully design pulse sequences using pulse phases, phase cycling techniques, combinations of different phase matched signals, and field polarizations⁴⁵ to obtain spectra with simplified, localized peak shapes yielding increased resolution. Only techniques with the highest resolutions that make use of the available information about relaxation mechanisms will allow the study of large biomolecular systems. An important current NMR example for a pulse sequence that is designed to take advantage of information about relaxation is the transverse relaxation-optimized spectroscopy (TROSY) technique used to increase resolution for large biomolecules.^{46–50} TROSY is an approach for suppression of fast transverse relaxation which in large biomolecules is dominated by dipole–dipole coupling and CSA contributions that are modulated by rotational molecular motions. It is based on constructive use of interference between the two active relaxation mechanisms to reduce linewidths by about 50% and is applicable to a large class of medium to large proteins that exhibit homogeneous broadening of this type.

Infrared Analogs of Heteronuclear NMR Coherence Transfer Experiments. Most of the pulse sequences mentioned in the last section can be employed in either a *homonuclear* (one-color) or *heteronuclear* (two-color) fashion. In NMR the term homonuclear describes a system that consists of spins with transition frequencies that are all covered by the pulse bandwidth and therefore all excited unselectively by the pulse. In a heteronuclear system it is possible to selectively excite one kind of spins at one frequency and the other kind at

their well separated transition frequency. In the heteronuclear analogs of the simple two-pulse experiments for example the first pulse is applied to only one kind of spins while the second pulse is applied simultaneously at both frequencies.

Heteronuclear pulse sequences beyond the simple two and three pulse examples are of great importance for studying complicated molecules like biopolymers by multidimensional NMR.^{1,2} These experiments provide further increase in resolution and more advanced possibilities to simplify the spectra by specific coherence transfer manipulation. Analogous techniques will be needed for studying biomolecules by optical spectroscopy. These macromolecular systems are typically built from a set of similar repetitive units. The vibrational spectrum of a macromolecule thus contains bands that can be described in terms of localized vibrations of the basic units and couplings between them. The analog of heteronuclear NMR spectroscopy can be achieved making use of frequency resolution during specific time intervals in a multidimensional pulse sequence. The frequency resolution is not used to address individual chromophores but only to separate different vibrational bands. Such multi-color multidimensional IR experiments have the potential to yield more detailed information on the vibrational motions in polypeptides and to achieve higher resolutions than one-color experiments, in analogy to heteronuclear NMR. At the same time they offer high temporal resolution and are time-domain rather than frequency domain measurements due to the large pulse bandwidth and short pulse duration needed to cover a whole vibrational band. A prerequisite for multi-color IR analogs of heteronuclear NMR experiments is the existence of several spectrally well resolved but rather broad and intense vibrational bands in the spectrum of the macromolecule, like e.g. the amide-I, amide-II, and amide-A,B bands in proteins.

The basic principle in many heteronuclear pulse sequences is to use a specific heteronuclear coherence transfer to simplify the spectra considerably.^{1,2} This transfer requires a coupling between the different chromophores and the delays in the transfer sequence can be chosen to maximize transfer for certain pairs of chromophores that are connected with a coupling of a specified magnitude. The correct choice of the pulse phase (i.e. x- or y-pulses) becomes crucial for these experiments since only the combination of pulses with a certain fixed relative phase allows to maximize the transfer across a specific coupling for a given intermittent evolution period that is tuned

to this coupling. The resulting spectrum will then ideally only contain peaks that correspond to pairs that fulfill the transfer condition. This can be used to obtain information about the bonding topology in a molecule, which is for example the basis of the sequential NMR protein assignment techniques. In these complex pulse sequences the knowledge about characteristic sizes of couplings along the protein backbone are used in consecutive coherence transfer steps, allowing the sequential assignment of all peaks along the backbone. These assignment sequences are good examples for the design of complicated liquid state NMR pulse sequences. Building blocks achieving coherence transfer, refocusing, and spin decoupling are combined to realize a very specific coherence transfer across several connected chromophores.²

Once each peak is assigned to a specific site, it is possible to use this information in more complicated relaxation experiments to obtain data about molecular structure and dynamics.⁴⁹ This additional information is frequently obtained by heteronuclear methods that contain the simpler coherence transfer schemes as building blocks that provide simplified well-resolved spectra. In this case additional delays are introduced in the pulse sequence during which auto- and cross-relaxation is active and a series of 2D spectra is recorded. The changes in the peak intensities as a function of the relaxation delay can be described theoretically with models for the relaxation active coupling mechanism, like dipolar or CSA (chemical shift anisotropy) interactions, and spectral density functions for the bath. Dynamical time-scales and order parameters can be extracted from the time dependence of the peak intensities. Relaxation measurements thus provide an indirect way to observe dynamical processes that are faster than the experimental time-scale given by the pulse duration.

The indirect observation of motional processes by relaxation experiments extends the time-scales accessible by NMR from microseconds to picoseconds. It is possible to determine the spectral density function of the bath that induces relaxation from a set of measurements of relaxation rates at different transition frequencies if the spectral density only contains a few model parameters. If a detailed model for the dynamical process is known e.g. from MD simulations^{26,51} or if a simple analytical form for the bath correlation functions can be assumed (e.g. in the Lipari-Szabo approach^{52,53}) this data can be inverted and the dynamical time-scales of the bath can be extracted which are on the order of the transition frequencies and not restricted by the pulse duration. NMR measurements are usually performed at different magnetic field strengths since the Zeeman term and thus the Larmor frequency depend on the external field strength. A variation of transition frequencies in vibrational experiments can be achieved by isotopic substitution making vibrational relaxation experiments feasible that have close analogs in NMR spectroscopy.

Structural parameters are obtained for example from the strong distance dependence of the dipolar cross relaxation (NOE). Another use of heteronuclear coherence transfer in more complicated pulse sequences is the indirect observation of transitions with small dipole moments through their coupling to transitions with strong dipole moment (INEPT). This technique is frequently used to enhance the sensitivity of carbon or nitrogen spectroscopy by coherence transfer from pro-

tons. It is then possible to manipulate the coherences of the less sensitive spins and finally either detect them directly or transfer the coherence back to the more sensitive species for detection.

We will analyze vibrational analogs of heteronuclear NMR coherence transfer experiments (Figs. 4A and 4B), the closely related INEPT (Insensitive Nuclei Enhanced by Polarization Transfer) experiment (Fig. 4C), and the heteronuclear multiple quantum correlation (HMQC) experiment (Fig. 4D). These techniques are by themselves important 2D NMR experiments but constitute furthermore frequently used building blocks in more complicated pulse schemes. The key concepts on which the NMR pulse sequences are based are transferable and allow us to devise analogous IR pulse sequences. We present the pulse sequences in terms of two frequency bands A and B for both NMR and IR techniques. In NMR these bands are traditionally named the I and S spins in a heteronuclear experiment. For IR spectroscopy of peptides they represent for example the amide-I and amide-II bands.

It is necessary for all two-color coherence transfer experiments that a coupling between chromophores in bands A and B exists that will produce density matrix elements that are A–B correlated. The coupling between chromophores belonging to bands A and B in two-color experiments is typically off-resonant and can be described in analogy to the weak J -coupling limit in NMR if it is much smaller than the difference in transition frequencies of bands A and B. Our exciton Hamiltonian also contains the resonant coupling term (see Eq. 1) which will yield additional contributions but the main features of the spectroscopic technique can be understood based on the weak coupling approximation in which the coupling is described by an $I_z^A I_z^B$ operator term in NMR or a fourth order term in the exciton Hamiltonian (Eq. 1), respectively.

The basic heteronuclear coherence transfer scheme in NMR consists of a single pulse on band A, followed by two coincident pulses of which one is resonant with band A and the other with band B (see Fig. 4A).¹ The first pulse creates a coherence of the A chromophores. The coupling between A and B chromophores then leads to the formation of an A–B correlated state during the period τ_1 . The two coincident pulses perform a transfer of the coherence from the A to the B chromophores. It is not necessary for the transfer that the two pulses k_2^A and k_2^B are phase coherent. It is possible to derive several pulse schemes (e.g. 'shift correlation spectroscopy' shown in Fig. 4B) that result in simplified spectra from the simple third order correlation sequence by introducing further pulses and delays to control the evolution of coherences in more detail. The important INEPT sequence (see Fig. 4D) can also be derived from the general correlation scheme. It is commonly used in longer pulse sequences to achieve coherence transfer between coupled groups of chromophores where one kind has a considerably smaller dipole moment ("insensitive") than the other.

All pulse sequences discussed so far were solely based on populations and single quantum coherences. It is also possible to create higher order coherences in hetero systems and use combinations of transitions to obtain frequency correlation maps. This is for example the idea of the HMQC (Hetero-Nuclear Multiple-Quantum Coherence) pulse sequence given in Fig. 4C.² In the vibrational analog the central NMR π -pulse is

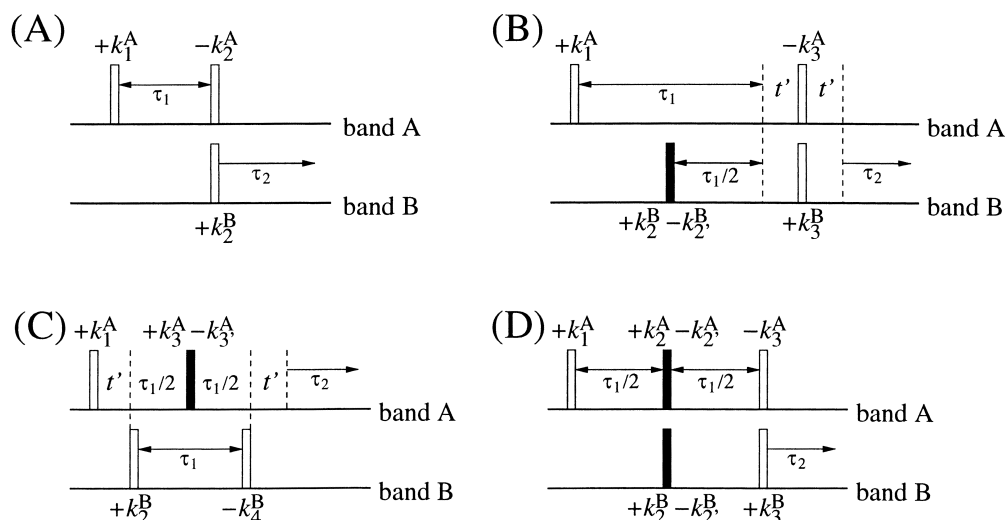


Fig. 4. Pulse sequences of important heteronuclear NMR experiments and building blocks for which optical analogs can be devised.

(A) shows the basic coherence transfer sequence which is closely related to heteronuclear COSY. (B) is derived from (A) and simplifies the spectra by refocusing the evolution during the evolution period. If decoupling is applied during detection, the experiment is known as 'shift correlation spectroscopy.' (C) shows the HMQC pulse sequence, (heteronuclear multiple quantum correlation technique). (D) is the INEPT building block used for sensitivity enhancement by coherence transfer.

substituted by two time coincident pulses on band A. The resulting experiment is fifth order in the pulse fields and two-dimensional with respect to the time domains given by τ_1 and τ_2 . The pulse sequence does not produce any observable signal for band B alone and the experiment looks like a reverse transient grating experiment²¹ if only band A is considered. The multiple quantum coherence transfer yields a modulation of the signal of band A with the double quantum frequencies.

Analogies with EPR and Solid State NMR Spectroscopy.

High resolution liquid state NMR spectroscopy is based on the fact that the NMR timescale is long compared to rotational diffusion of moderately sized molecules in solution. All observable properties are thus rotationally averaged and effectively isotropic. In EPR (performed in frozen solutions or solids at cryogenic temperatures) and solid state NMR spectroscopy the molecular orientation is fixed and the orientational distribution with respect to the external field yields inhomogeneously broadened spectra. All contributions to the Hamiltonian contain a real space part which depends on the sample orientation with respect to the external fields multiplied by a spin space part. Typical contributions in solid state NMR are the chemical shift anisotropy (CSA) tensor, dipole-dipole coupling, and, for nuclei with spin $S > \frac{1}{2}$, quadrupole interaction.²⁷ In EPR the g factor, as well as hyperfine and dipolar couplings are orientation dependent.⁵⁴ An increase in resolution and simplification of spectra can be achieved by averaging the different rank tensor operators by performing fast rotations in either real or spin space. The first idea leads to techniques like magic angle spinning (MAS) while the second concept is the basis of multiple pulse experiments.⁴³ In multiple pulse experiments blocks with complicated pulse trains are used to obtain the evolution of the spin system that corresponds to an effective Hamiltonian that is different from the free evolution Hamiltonian and can be calculated using average Hamiltonian theory.¹ Such building blocks are used to effectively remove dipolar couplings or qua-

drupolar field effects or to average CSA contributions over all possible orientations and thus increase resolution. Other uses are in cross-relaxation experiments or for spin-spin decoupling during certain periods in an experiment. It is also possible to generate sequences of echo signals as for example in the classic Carr-Purcell sequence and perform stroboscopic sampling of the decaying echo intensity during observation delays between the pulse blocks to study relaxation.

The situation in optical spectroscopy is similar to solid state NMR in so far that the molecular dipoles can have arbitrary orientation with respect to the applied fields and that the molecular orientation seems frozen on the fast time-scale of ultrafast laser experiments. The response functions therefore have a tensor character and need to be averaged over all possible molecular orientations. While an analog of multiple pulse sequences is conceivable as optical pulse shaping techniques advance, it seems impossible to achieve anything similar to MAS. Yet, optical spectroscopy has another easily controllable degree of freedom, given by the field polarization. The correct choice of field polarizations for consecutive pulses can suppress peaks in multidimensional spectra.⁴⁵ The combination of variation of field polarizations with phase cycling and averaging over spectra observed in different directions allows to strongly simplify multidimensional spectra much as in multiple pulse sequences in NMR.

Very exciting developments have been made in the field of vibrational IR and Raman CD using circularly polarized light.^{55,56} Combining these with femtosecond techniques should be very promising.

All concepts for optical spectroscopy discussed so far are based on the assumption of extremely weak fields with infinitely small flip angles. EPR operates in many cases in an intermediate regime where flip angles are small but finite. Pulse sequences for multidimensional EPR with small flip angle pulses and echo detection have been developed⁵⁷⁻⁶⁰ and can

provide guidance in the development of optical pulse sequences with moderately strong pulse fields.

Couplings in Vibrational and NMR Spectroscopy

Couplings in NMR and EPR spectroscopy can be classified as being either *through space* or *through bond*. The former are direct dipole–dipole interactions between different spins and their strength is determined by the spin geometry in the external field. The latter are mediated by the indirect influence of the spin dipoles on the electron density of the molecule (most importantly by the Fermi contact interaction) that influences the resonance frequency of another spin in the same molecule. These couplings are calculated as the second derivative of the electronic energy of the molecule with respect to the two dipole moments of the coupled spins. The application of sum-over-states perturbation theory for the calculation of these derivatives yields the Ramsey formula for the magnetic spin-spin couplings.^{61–63} The calculation of couplings from these formulas requires generally the knowledge of excited electronic states with high precision and is thus based on high level *ab-initio* calculations that can only be performed for small molecules. The recent development of sum-over-states and finite perturbation methods for the DFT calculation of magnetic properties allows to calculate couplings and chemical shielding tensors (as well as EPR quantities) at a high level of accuracy with a rather small computational effort.^{64–68} It is now possible to calculate magnetic couplings for larger molecules and fragments of biopolymers with an accuracy compatible with NMR experiments.^{69,70} The sum-over-states DFT formalism uses determinants constructed from Kohn–Sham orbitals as a basis for the perturbation expansion instead of the true excited electronic states of the molecule. A heuristic functional for the excitation energies provides the corresponding energy denominators in the perturbation series. In the finite perturbation calculation one of the coupled sites is perturbed in an additional DFT calculation and the derivative is obtained as a finite difference. Magnetic couplings are therefore obtained from essentially two ground state DFT calculations with little additional numerical effort.

The distinction between through space and through bond couplings is less obvious in vibrational spectroscopy since both depend on the same mechanism. Such distinction is implicitly the basis for the different terms in classical MD force fields. In most commonly used protein force fields, for example, hydrogen bonds are described by purely electrostatic interactions between the partial charges on donor and acceptor atoms.^{71–73} On the other hand, for the description of energy transport along hydrogen bonded peptide planes in α -helical proteins, a third order coupling term was introduced in a force field by Clarke et al. to describe the mode coupling between the amide I and amide A vibrations across hydrogen bonds.⁷⁴ The transition dipole coupling model for the amide I band is another case where couplings are described approximately as through space electrostatic interactions.^{75–77} Most of these approximations are of an *ad hoc* nature and should better be based on a clear criterion based on the electron density.

Within the Born–Oppenheimer approximation, the nuclear configuration determines the electron density and the potential governing nuclear motion can be calculated from this. A cou-

pling between two local oscillators therefore exists if the electron density around one oscillator changes depending on the coordinate of the other oscillator. This is the reason for through space as well as through bond couplings. We can define a criterion for distinguishing the two coupling cases based on the electron density. We denote a coupling through space if there exists a surface separating the two local oscillators at which the change in the density is negligible for small excursions of either of the coupled oscillators from its local equilibrium position. In this case the coupling can be described as purely electrostatic due to the polarization of the charge density around one oscillator by changing the charge distribution around the other oscillator during vibrations. If no surface with negligible change exists, we have to describe the coupling interaction as through bond.

Conclusions

The development of new pulse sequences that carefully account for the differences between NMR and optical spectroscopy is a widely open area with enormous potential. The two-color experiments described in this paper are one interesting possibility and pulsed experiments that are tailored to certain classes of compounds (proteins, polymers) could be developed.² The systematic transfer of ideas from NMR pulse sequence design and their adaption to arbitrary multi-level systems should not pose fundamental difficulties and will enable the treatment of a large variety of systems, including vibrational systems with moderate anharmonicities. Another topic that should be studied is the description of pulse sequence blocks in terms of average Hamiltonians that yield effective transformations for the whole pulse train. This approach has been effectively used in solid state NMR^{1,43} and can become important in dealing with the arbitrarily oriented dipoles in optical spectroscopy. Techniques that effectively deal with small pulse flip angles, offset effects, and echo detection have been developed in multidimensional EPR spectroscopy^{59,60} and might stimulate further development of optical pulse techniques.

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