Femtosecond Stimulated Raman Spectroscopy

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Abstract
Femtosecond stimulated Raman spectroscopy (FSRS) is a new ultrafast spectroscopic technique that provides vibrational structural information with high temporal (50-fs) and spectral (10-cm$^{-1}$) resolution. As a result of these unique capabilities, FSRS studies of chemical and biochemical reaction dynamics are expected to grow rapidly, giving previously unattainable insight into the structural dynamics of reactively evolving systems with atomic spatial and femtosecond temporal resolution. This review discusses the experimental and theoretical concepts behind FSRS, with an emphasis on the origins of its unique temporal and spectral capabilities. We illustrate these capabilities with vibrational studies of ultrafast electronic dynamics, as well as the direct structural observation of nonstationary vibrational wave-packet motion in small molecules and in complex biochemical reaction dynamics.
INTRODUCTION

Chemical change is the breaking and making of chemical bonds that transforms reactants into products. The timing and motion of the nuclei throughout the reaction occur along the reaction coordinate, a hypothetical construct consisting of one or more nuclear degrees of freedom, depending on the complexity of the system. In its simplest form, the reaction coordinate is nothing more than a molecular vibration taken to the extreme. In this picture, the breaking of a hydrogen bond, for example, is simply the extension of the corresponding hydrogen stretching vibration. In more complex systems, however, concerted or sequential displacement along many different nuclear coordinates may occur. This underlying connection between chemical reaction dynamics and molecular vibrations makes vibrational techniques the ideal tool for studying the origins, dynamics, and mechanisms of chemical change.

Even for molecular systems with limited complexity such as all-trans retinal in the photoactive protein bacteriorhodopsin, there are \( \sim 300 \) degrees of freedom, many of which are Raman active (Figure 1b). This inherent complexity makes the identification of critical coordinates in any type of reaction difficult. In addition, the relevant vibrational frequencies vary anywhere from 50 to over 3000 cm\(^{-1}\) and result from a variety of nuclear motions, ranging from reactive low-frequency delocalized backbone distortions and local double-bond torsions (<600 cm\(^{-1}\)), to structurally informative fingerprint modes such as C–C single-bond and double-bond stretches and hydrogen wags (900–1500 cm\(^{-1}\)), all the way to overtone bands in the region above 2000 cm\(^{-1}\), which give information on vibrational anharmonicity. Critically, the corresponding vibrational periods from 10 to 500 fs not only define the timescale of molecular vibrations, but, more importantly, also clock the nuclear dynamics occurring along the reaction coordinate. As a consequence, any spectroscopic technique used to study chemical reaction dynamics in real time must be capable of revealing the structure of the molecule on the fundamental timescale of atomic motion from 1 ps to 10 fs. This challenging goal drove the development of ultrafast laser sources (1–3), which have found application not only in studies of chemical reaction dynamics (4–8), but also in physics (9, 10) and biology (11–14).

Despite the wealth of information that can be obtained from the various implementations of most electronic pump-probe techniques (15–17), one must infer structural dynamics, except in simple circumstances (18, 19). This is mostly a consequence of the broad and unresolved nature of electronic absorption bands in the condensed phase and their relative insensitivity to molecular structure. Conversely, vibrational spectroscopies, such as infrared (IR) and Raman, are much more informative structural probes owing to the inherently narrow vibrational bands and their sensitive dependence on both electronic and molecular structure: Geometric changes of hundredths of an Angstrom can lead to easily detectable vibrational frequency shifts. Is it possible to extend these structurally sensitive vibrational techniques into the femtosecond time domain and study chemical reaction dynamics, as suggested in Figure 1a?

Time-resolved vibrational spectroscopy has traditionally been limited to the picosecond time domain, both in the common pump-probe configurations, as well as...
Comparison of a typical vibrational spectrum of a complex photoactive molecular system with the capabilities of current ultrafast vibrational spectroscopies. (a) Structurally sensitive vibrational techniques with subpicosecond time resolution. The width and horizontal arrangement define the available spectral window, whereas the vertical position indicates the best possible time resolution. The white striping represents the available bandwidth of femtosecond pulses in the infrared (IR), whereas the black striping marks achievable but not yet reported frequency regions. CARS, coherent anti-Stokes Raman spectroscopy. 

(b) Resonance Raman spectrum of the all-trans retinal chromophore in bacteriorhodopsin, exemplifying the structural diversity of molecular motion as a function of vibrational frequency. Both high- and low-frequency regions are magnified for clarity.

Impulsive stimulated Raman scattering (ISRS): the creation of coherent ground-state nuclear motion through an impulsive force caused by the interaction of a Raman-active medium with an ultrashort light pulse.

in nonlinear implementations such as coherent anti-Stokes Raman spectroscopy (20). Time-domain versions of Raman scattering have appeared in the form of impulsive stimulated Raman scattering (ISRS) (21, 22), which has the advantage of high time resolution but suffers from poor signal-to-noise ratios in the corresponding frequency domain spectra and works best with low-frequency modes (23). The recent availability of femtosecond pulses in the IR has revolutionized the field of time-resolved IR spectroscopy through the appearance of both traditional pump-probe experiments and the development of multidimensional techniques based on vibrational echoes (24–28). Despite the huge successes of these techniques, the time resolution, frequency range, and available bandwidth are limited (Figure 1a). As a consequence,
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One of the numerous critical advancements in the field of ultrafast spectroscopy over the past two decades involves the apparent circumvention of the uncertainty principle through femtosecond dynamic absorption spectroscopy (14). In this approach to pump-probe transient absorption spectroscopy, the broadband femtosecond probe pulse is dispersed onto a multichannel detector, resulting in measurement of the full pulse spectrum rather than just its intensity. The time resolution of this technique is only fundamentally limited by the durations of the two pulses initiating the macroscopic polarization in the sample that generates the detected signal. The frequency resolution, conversely, is determined by the lifetime of the induced polarization rather than the pulse duration because the detection step is not time resolved. The simultaneous high time and frequency resolution can then be used to observe electronic signatures of ultrafast chemical reaction dynamics with energy resolution much finer than the bandwidth of the femtosecond pulses used in the experiment.

the direct real-time observation of the structural changes associated with chemical reaction dynamics has remained an elusive task.

Femtosecond stimulated Raman spectroscopy (FSRS) provides a fundamental advance in the quest for real-time structural measurements of chemical change. The FSRS approach enables the recording of vibrational structural information with a time resolution comparable with or faster than the vibrational periods of the probed motions (29–34). In FSRS, the simultaneous interaction of a narrow-bandwidth picosecond Raman pulse and a broadband, femtosecond continuum probe pulse leads to the appearance of sharp vibrational gain features on top of the probe envelope (Figure 2). The principle conceptual advance of FSRS arises from the same disentanglement of time and energy resolution exploited by femtosecond dynamic electronic absorption spectroscopy (14): A broadband femtosecond pulse—in the presence of a picosecond Raman pulse that provides the background field in the stimulated Raman process—creates a macroscopic polarization with high time resolution, whereas the dispersed, non-time-resolved detection ensures high energy resolution. The two concepts are identical; the only difference is that FSRS drives vibrational rather than electronic coherence. Because vibrational dephasing times are much longer (up to picoseconds) than their electronic counterparts (<50 fs), the corresponding energy resolution is excellent (<10 cm⁻¹). In combination with a femtosecond optical actinic trigger, or pump pulse, the pump-probe time resolution can be 50 fs or better.

In this review, we present the theoretical concepts behind FSRS and illustrate its power in studying chemical reaction dynamics with a variety of photophysical and photochemical examples. We illustrate the advantage of following ultrafast reaction dynamics using unique vibrational fingerprints rather than potentially overlapping and confusing electronic signatures with FSRS studies of ultrafast electronic relaxation processes in polyenes and in transition-metal complexes. Direct structural
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Figure 2
Illustration of broadband vibrational probing employed in femtosecond stimulated Raman spectroscopy. The Raman pulse is a narrow-bandwidth, picosecond pulse (green), whereas the probe is a broadband femtosecond continuum pulse (purple). When both pulses are overlapped spatially and temporally in a Raman-active medium, photons are transferred from the high-intensity Raman pulse to the weak probe pulse at the vibrational resonances of the sample. A typical spectrum obtained in a single laser shot for cyclohexane is depicted. Division of probe spectra obtained in the presence (black) and absence (purple) of the Raman pulse produces the vibrational spectrum in the expanded trace (blue).

observation of ultrafast nuclear motion on a timescale much faster than the vibrational dephasing time is illustrated with studies of coherent vibrational motion in deuterio-chloroform. Our technique's ability to monitor chemical reaction dynamics in real time is exemplified by studies of the primary isomerization event in vision. These experiments illustrate the utility of FSRS as an ultrafast vibrational structural technique and point toward many future applications.

THEORY OF FEMTOSECOND STIMULATED RAMAN SPECTROSCOPY

Lee and coworkers (31) have previously described the theory of FSRS based on both classical coupled-wave theory and a quantum mechanical density matrix formalism. Here we focus on the concepts behind some of the more challenging aspects of FSRS. In particular, understanding the time resolution of FSRS is nontrivial because its time-frequency product, $\Delta t \Delta \nu$, of $\sim 500$ fs cm$^{-1}$ is an order of magnitude below what a priori appears to be demanded by the uncertainty principle (5000 fs cm$^{-1}$). To address this paradox, we first define what observables are actually measured by FSRS, followed by an introduction of a simple theory that can be used to predict and model FSRS spectra.

Figure 3 depicts the pulses and the timing commonly used in FSRS. An ultrashort ($<30$-fs) visible pump pulse initiates the photochemistry of interest. The structural evolution of the system is probed, after a time delay $\Delta T$, by two pulses that drive the stimulated Raman transition: a narrow-bandwidth Raman pulse (800 nm, 3 ps,
Figure 3

Schematic representation of time-resolved femtosecond stimulated Raman spectroscopy (FSRS). (a) Energy-level diagram for a typical time-resolved FSRS experiment. A femtosecond pump pulse (1) initiates photochemistry by promoting the system to an excited electronic state. The structural evolution is then probed by Raman (2) and probe (3) pulses driving stimulated Raman transitions after a variable time delay, $\Delta T$. The pulse durations and their relative timing are depicted in b. (c) Bra-ket energy-level diagram illustrating the FSRS process after Lee & Albrecht (35). Solid and dashed arrows represent bra and ket electric-field couplings. At $t = 0$, two field couplings with the pump pulse promote the system to the excited state $|n><n|$. After a specified time delay, Raman and probe pulses establish vibrational coherence on the excited state $|n+1><n|$ with high temporal precision, which decays with the characteristic vibrational dephasing time. An additional field coupling with the long-duration electric field provided by the Raman pulse at any time during the vibrational free-induction decay results in the collinear emission of a Raman-shifted photon along the probe beam.

50 nJ to 1 $\mu$J) and a broadband continuum probe pulse (830–960 nm, 20 fs, 5 nJ). The broad bandwidth of the probe pulse enables the simultaneous probing of vibrational features over a 1500-cm$^{-1}$ window (700–2200 cm$^{-1}$) with larger windows, including anti-Stokes coverage, possible.

Figure 3c depicts this process by the energy-ladder, bra-ket time-evolution diagram introduced by Lee & Albrecht (35). The visible pump promotes the system from $|0><0|$ to $|n><n|$, where $n$ is the $n$-th vibrational state on the excited electronic state. Following the visible pump, the system is allowed to evolve on the excited-state potential energy surface for a fixed amount of time, $\Delta T$, at which point the arrival...
of the probe pulse in combination with the long-duration Raman pulse initiates vibrational coherence, \(|n + 1\rangle < n\rangle\). We can then investigate the structural evolution of the system by changing the pump-probe time delay, \(\Delta T\). The FSRS process is completed during the vibrational dephasing time, \(T_{2}^{\text{vib}}\), of the mode in question by another coupling of the Raman pulse with the sample and subsequent emission of a photon. Importantly, FSRS is self-phase matched (\(k_{\text{FSRS}} = -k_{\text{Raman}} + k_{\text{Probe}} + k_{\text{Raman}} = k_{\text{Probe}}\)), resulting in the collinear emission of FSRS photons along the probe beam.

**Figure 4** illustrates the factors affecting the time and energy resolution of FSRS. The time-dependent electric fields of the Raman and probe pulses and their energy-domain equivalents are presented in Figures 4a,b. As mentioned above, the arrival of the probe pulse initiates vibrational coherence in the sample, which decays with its characteristic vibrational dephasing time, \(T_{2}^{\text{vib}}\). This can be described as an impulsive driving of the Raman-active modes of the system by the beat frequency of Raman and probe-pulse frequencies. The finite duration of the vibrational coherence results in a limited bandwidth in the frequency domain (**Figure 4c**). The induced coherent vibrational motion modulates the macroscopic polarization of the sample at the frequency of the vibration. This effect can be observed in **Figure 4d** in which modulations of the Gaussian envelope are apparent after the arrival of the probe pulse. Detection of the radiation generated by the sample polarization in the frequency domain results in three Lorentzian peaks. The main band at \(\omega_{R}\) is a result of Rayleigh scattering of the Raman pulse. Two additional features appear shifted by \(\omega_{0}\) to the high- and low-frequency side of the central peak and correspond to the Stokes and anti-Stokes features observed in spontaneous Raman spectroscopy. The width of these features is determined by the convolution of the duration of the Raman pulse and the vibrational dephasing time. For a 2-ps Raman pulse and a 700-fs vibrational dephasing time, it is 17 cm\(^{-1}\), as indicated. Thus, for the commonly employed picosecond Raman pulses, the energy resolution of FSRS is dominated by the vibrational dephasing time of the probed mode (i.e., by the properties of the system).

The phase-matching conditions for FSRS result in an intrinsically heterodyne-detected signal. The FSRS peak appears on top of the probe-pulse envelope (**Figures 2 and 4e**). The temporal precision of FSRS is, however, uncoupled from these energy limitations because the photons contributing to the FSRS signal can be emitted by the sample at any time during the vibrational dephasing time. Nevertheless, the initiation of the vibrational coherence can be resolved with a precision approaching the duration of the probe pulse, which in our current setup is on the order of 20 fs.

The temporal evolution of the macroscopic polarization of the system is entirely determined by the state of the system at the time of the arrival of the probe pulse. The wave packet launched by the probe pulse therefore evolves differently in time for different pump-probe delays because it begins its journey in a different region of the potential energy surface. Despite the arrival of the FSRS photons at the detector throughout the vibrational dephasing time, the polarization of the sample responsible for their emission is different for different pump-probe delays, which can be determined with femtosecond precision. This principle leads to the
\[ I(\omega) = \left| \int_{-\infty}^{\infty} f(t) e^{i\omega t} dt \right|^2 \]

- **a** Raman pulse
  - \( E_r(t) \)
  - \( \sim 2 \text{ ps} \)
  - \( \sim 8 \text{ cm}^{-1} \)

- **b** Probe pulse
  - \( E_p(t) \)
  - \( \sim 20 \text{ fs} \)
  - \( \sim 800 \text{ cm}^{-1} \)

- **c** Coherent vibration
  - \( Q(t) \)
  - \( T_2 \sim 700 \text{ fs} \)
  - \( \sim 15 \text{ cm}^{-1} \)

- **d** Macroscopic polarization
  - \( P_{\text{TOTAL}}(t) \)
  - \( \sim 17 \text{ cm}^{-1} \)

- **e** Detected field
  - \( E_p(t) \)
  - \( E_{\text{FRRS}}(t) \)

- **f** Signal
  - \( S_{\text{FRRS}}(\omega) \)
  - \( \omega_p, \omega_m, \omega_p^2 \)
disentanglement of time and energy resolution and is completely equivalent to the scenario encountered in femtosecond dynamic electronic spectroscopy in which the time and energy resolution are independent of each other because of the dispersed detection (14).

**VIBRATIONAL STRUCTURAL STUDIES OF ULTRAFAST ELECTRONIC DYNAMICS**

Now that the theoretical foundations of FSRS have been laid, how do these principles manifest themselves in actual experimental results? We focus first on the most straightforward application of FSRS: monitoring ultrafast electronic kinetics by observing changes in vibrational structure and intensity. Although electronic kinetics can be precisely determined with techniques such as transient absorption, the broad and potentially overlapping nature of electronic absorption and emission signatures makes it often difficult to unambiguously assign the molecular origin of the observed features. A key advantage of the FSRS approach lies in the measurement of specific molecular vibrational structure.

Carotenoids, whose electronic manifold and electronic relaxation pathways have been hotly debated in the recent past (36), provide an excellent example of the power of measuring transient vibrational structure rather than electronic kinetics on the femtosecond timescale. In \( \beta \)-carotene (Figure 5a), the existence of additional intermediate excited electronic states between \( S_2 \) and \( S_1 \) has been proposed on the basis of theoretical calculations (37), Raman excitation profiles (38), and transient absorption experiments (39).

To address this issue, we have used FSRS to explore the temporal evolution of \( \beta \)-carotene’s vibrational structure with femtosecond time resolution (40). Immediately following excitation to the one-photon-allowed second excited electronic state (\( S_2 \) or \( 1\text{Bu}^+ \)), we observed broad, intense features that transform within 500 fs into the well-known vibrational spectrum of the optically forbidden first 2Ag excited state (\( S_1 \), Figure 5b). The assignment of the initial broad features to the \( S_2 \) state is confirmed by (a) kinetic analysis of the temporal evolution of the band intensities that matches the electronic kinetics and by (b) the considerable resonance enhancement.
Figure 5

Monitoring ultrafast electronic dynamics using femtosecond stimulated Raman spectroscopy (FSRS) as exemplified by studies of β-carotene (a–c) and Ru(bpy)$_3^{2+}$ (d–f). (a) Energy-level diagram depicting FSRS probing of ultrafast electronic relaxation in β-carotene. (b) Ground-state and femtosecond time-resolved FSRS spectra of β-carotene at selected time delays. (c) Contour plot of time-resolved FSRS spectra obtained in 25-fs steps from −100 to 650 fs, suggesting the presence of only two excited electronic states in the relaxation pathway. (d) Schematic representation of ultrafast intersystem crossing in Ru(bpy)$_3^{2+}$. (e) Ground-state and selected time-resolved FSRS spectra of Ru(bpy)$_3^{2+}$ following excitation by a 30-fs pulse at 480 nm. Rapid growth of unique vibrational structural features attributable to the $^1$MLCT (metal-to-ligand charge-transfer) state is observed with a $\sim$110-fs time constant (f). The kinetics of these features correspond well to the rise of a weak transient absorption signal owing to the $^1$MLCT state in the near infrared (870 nm).
of these features compared with those of the ground and first excited states. The S2 bands are significantly broadened beyond the instrumental resolution of FSRS (15 cm\(^{-1}\)), mostly owing to the limited lifetime (160 fs) of the S2 state, which leads to the truncation of vibrational coherence by electronic decay. The precision with which the vibrational structure can be determined is thus limited by the properties of the molecular state, itself, rather than by the vibrational resolution of the experimental technique. The high signal-to-noise ratios commonly obtained with FSRS in combination with rapid data acquisition times make it possible to construct contour plots such as the one given in Figure 5c. The figure clearly shows the presence of only two distinct vibrational signatures attributable to the S2 and S1 states, suggesting that the originally proposed electronic model in long polyenes involving only two excited states is correct (41).

An equally illuminating example is provided by time-resolved FSRS studies of transition-metal complexes. Ru(bpy)\(_3^{2+}\) is a model compound for transition-metal sensitizers employed in Graetzel-type solar cells (42). Its ultrafast intersystem crossing dynamics is extremely important in partitioning the photon energy between electron transfer and intramolecular relaxation (43). Previous electronic absorption studies (44) provided indirect evidence that intersystem crossing from the initially populated 1MLCT (metal-to-ligand charge transfer) to the 3MLCT state is complete in 300 fs (Figure 5d). Because of the extremely short lifetime of the 1MLCT state, no characteristic vibrational features owing to the singlet state are observable at early times as a result of the expected large lifetime broadening (∼500 cm\(^{-1}\)) (45). Instead, a delayed, rapid growth of unique vibrational bands is observed that is complete by 300 fs (Figure 5e). This spectrum then remains unchanged until 10 ps and is identical to that obtained by traditional picosecond and nanosecond time-resolved spontaneous Raman studies of the long-lived 3MLCT state.

These observations demonstrate that the initial dynamics in Ru(bpy)\(_3^{2+}\) is in fact a result of ultrafast intersystem crossing. The observed 3MLCT vibrational bands are sharp because the nanosecond lifetime of the 1MLCT does not produce a truncation of vibrational coherence through electronic decay; the spectral resolution thus approaches the instrumental resolution of FSRS. Nevertheless, the ability of FSRS to time resolve the initiation of vibrational coherence on the femtosecond timescale allows for precise determination of the 1MLCT rise time (Figure 5f) and a clear structural proof that it is the 1MLCT state that is formed within 300 fs (45).

VIBRATIONAL STRUCTURAL STUDIES OF NONSTATIONARY NUCLEAR DYNAMICS

Although electronic dynamics is critical in many optically induced processes, nuclear dynamics is responsible for facilitating chemical change. We consider here how FSRS can be used to study nonstationary nuclear dynamics with unprecedented spectral and temporal resolution. To aid the understanding of these new concepts and observations, we first provide two examples of commonly encountered coherent nuclear motion and their effects on FSRS spectra, followed by experimental observations of these phenomena.
Anharmonic coupling: breakdown of orthogonality between nuclear degrees of freedom leading to variation of the energy of one mode when another is excited

Reporter mode: vibrational mode that reports on the structural rearrangement of a system through anharmonic coupling with other reactive degrees of freedom

Figure 6a depicts schematically an example of the changes in vibrational structure associated with chemical reaction dynamics. For simplicity, we consider a simple two-mode system: a low-frequency reaction coordinate—which in reality may be multidimensional—and an orthogonal but anharmonically coupled high-frequency reporter mode. In this case the structural evolution of the system can thus be directly followed by monitoring the frequency of the reporter mode that is coupled to, and therefore dependent on, the position of the system along the reaction coordinate as a function of time. If the system is probed at a time delay \( t_1 \), for example, the frequency of the reporter mode, \( \nu_1 \), is much lower than at time delay \( t_2 \). For a reaction that takes place on a timescale comparable with the vibrational dephasing time of the reporter mode (i.e., \( T_2^{\text{vib}} \sim t_2 - t_1 \)), the vibrational frequency changes during the vibrational free-induction decay, resulting in a time-dependent frequency of the vibrational amplitude, \( Q(t_1,t) \) (Figure 6b). The corresponding FSRS lineshape is dispersive with a negative feature on the high-frequency side. The physical basis of the dispersive lineshape is that the phase of the final vibration, occurring with vibrational period \( \tau_2 \), is not locked to the phases of the Raman and probe fields; it is determined by the shift in frequency and thus phase owing to motion along the reaction coordinate. If, however, vibrational coherence is initiated at \( t_2 \) when the reaction is complete, the vibrational frequency and phase remain constant over the vibrational dephasing time, and the resulting FSRS band is purely Lorentzian and positive definite (Figure 6c).

Critically, the final FSRS lineshape exhibits varying degrees of spectral dispersion when vibrational coherence is initiated at different time delays, \( t \), where \( t_2 < t < t_1 \). The temporal precision with which the lineshape can be measured in FSRS is the instrumental time resolution defined by the cross-correlation between pump and probe pulses. This example demonstrates the possibility of measuring vibrational structural change with FSRS with what appears to be sub-Heisenberg precision: The observed FSRS signal is emitted by the sample over several hundreds of femtoseconds, making high spectral resolution possible, but the polarization giving rise to the emitted radiation changes on the femtosecond timescale, and these ultrafast changes can be...
Heterodyne detection: detection of the radiation emitted by a macroscopic polarization through interference with an additional applied electric field.

Another example of nonstationary behavior that can be studied with FSRS is vibrational coherence created by ISRS (21). In ISRS, a broadband femtosecond pulse whose spectral bandwidth exceeds the energy of a particular molecular vibration, impulsively excites wave-packet motion along this coordinate by resonant or nonresonant impulsive Raman scattering. The wave packet then oscillates back and forth in the potential well, as indicated in Figure 6d for picoseconds. As in the previous example, the motion of the nonstationary state can be followed by monitoring the frequency of an orthogonal, but weakly coupled, high-frequency reporter mode. In the given example, the frequency of the reporter mode is assumed to oscillate between \( \nu_0 \) and \( \nu_1 \), as the low-frequency mode oscillates from \( Q_0 \) to \( Q_2 \) (i.e., as the system oscillates along the low-frequency coordinate). Figure 6e depicts this oscillatory behavior schematically in which the vibrational frequency of the high-frequency mode is plotted as a function of time. It oscillates about the equilibrium value, \( \nu_1 \), with a frequency equaling the vibrational period of the impulsively excited low-frequency mode. In most cases, this modulation occurs on a timescale much faster than the vibrational dephasing time of the high-frequency mode, resulting in modulations of the frequency and thus phase of the coherent high-frequency vibration.

The FSRS lineshapes resulting from such nonstationary dynamics can be calculated and are depicted in Figure 6f. The spectrum consists of a main peak that appears at the equilibrium position of the high-frequency mode and two side bands displaced equally to lower and higher energy. The side bands are displaced by the energy of the low-frequency vibration and exhibit revealing phase behavior as a function of time. The phase of the side bands is preserved at time delays equaling integer multiples of the low-frequency vibrational period, as can be deduced intuitively from Figure 6e: Initiating vibrational coherence at any point in time, \( t_0 \), is indistinguishable from initiating it at any time \( t = t_0 + n \tau_{\text{low}} \) and thus results in an identical experimental spectrum. Importantly, one can use the phase of the side band to determine the absolute phase of the low-frequency nuclear wave packet with high precision through lineshape analysis (46).

The spectra in Figure 6 are simulated using the heterodyne-detection picture of FSRS, in which the probe field and the field emitted by the sample polarization are treated individually. In this approach, the output field is defined as \( E_{\text{FSRS}}(z,t) = E_P(z,t) + E_{\text{RO}}(z,t) \), where \( E_P(z,t) \) is the probe field, and \( E_{\text{RO}}(z,t) \) is the field generated by the polarization initiated by the interaction of the Raman pulse with the coherent vibrational amplitude:

\[
E_{\text{RO}}(z,t) = c N E_R(z,t) \left[ \alpha_0 + \sum_i \alpha'_i Q_i \right].
\]

\( E_{\text{FSRS}}(z,t) \) is established by the known Raman and probe-pulse durations and wavelengths, as well as any Raman-active coordinates, \( Q_i \), and the magnitude of \( c N \alpha'_i \) is chosen to match the experimentally observed FSRS intensities. By introducing detected in the lineshape owing to the high time resolution with which vibrational coherence can be initiated. The instrumental capabilities are thus by no means violating the Heisenberg uncertainty principle: They just circumvent it in an elegant fashion.
time-dependent behavior through amplitude modulations of the polarizability or changes in the vibrational frequency of the coherent vibration, we can investigate the effects of nonstationary dynamics on FSRS spectra. The final spectra are calculated as a Raman gain in the same way as in the experiment by evaluating the modulus squared of the Fourier transform of $E_{FSRS}(z,t)$ in the presence of the Raman pulse divided by the same quantity in the absence of the Raman pulse:

$$\text{Gain}(\omega) = \frac{I_{FSRS}(\omega)}{I_P(\omega)} = \frac{|E_{FSRS}(\omega)|^2}{|E_P(\omega)|^2} = \frac{\int e^{i\omega t'} \cdot E_{FSRS}(t')dt'|^2}{\int e^{i\omega t'} \cdot E_P(t')dt'|^2},$$

where $t' = z/c + t$.

**TIME-RESOLVED OBSERVATION OF ANHARMONIC COUPLING IN DEUTERIO-CHLOROFORM**

The earliest manifestations of nonstationary nuclear dynamics appeared in ISRS experiments by Nelson and coworkers (21). ISRS is an ideal tool to study the effects of nonstationary vibrational motion on FSRS spectra because it can be used to explore the fundamental concepts with a system of limited complexity. We use an off-resonant, broadband femtosecond pump pulse to create vibrational coherence in the low-frequency bending modes of deuterio-chloroform. The structural evolution of the system is subsequently interrogated in real time by following the time dependence of the anharmonically coupled high-frequency C-D stretch reporter mode (46).

**Figure 7a** presents a ground-state spontaneous Raman spectrum of deuterio-chloroform. The bandwidth of the pump pulse indicates that it will impulsively excite two low-frequency vibrations of deuterio-chloroform. The two-dimensional diagram in **Figure 7b** represents this process in which we consider only one of the two impulsively excited bending motions for simplicity. The high time resolution of FSRS makes it possible to differentiate initiation of high-frequency coherence for different positions of the wave packet on the ground-state surface. High-frequency coherent vibrational motion created at $t_1$ differs from that starting at $t_2$, resulting in different time-dependent macroscopic polarizations and thus FSRS signals. **Figure 7c** presents a typical FSRS spectrum of the spectral region surrounding the C-D stretch at 2255 cm$^{-1}$ for a 777-fs time delay. Side bands on the C-D fundamental appear both red- and blue-shifted by the energy of the low-frequency bends. The phase of the bands is inverted about the origin defined by the C-D stretching band. **Figure 7d** shows the temporal evolution of the side bands as a function of pump-probe delay. The general behavior of these bands is well illustrated by focusing on the feature at 2622 cm$^{-1}$ that is a result of coupling between the coherently excited 365 cm$^{-1}$ bend and the C-D stretch. As the time delay is increased from 777 fs, the feature initially becomes dispersive and then purely positive by 822 fs. This process is repeated to produce a feature at 867 fs identical to that of the initial band observed at 777 fs. The phase of the peak is thus preserved every 90 fs, which is the vibrational period of a 365-cm$^{-1}$ vibration. At first glance, the corresponding peak at 2519 cm$^{-1}$ appears to undergo the same transformation, but close inspection reveals that the phase...
changes more slowly; here the phase recurs every ~130 fs, which is the period of a 262-cm\(^{-1}\) mode. All the side bands disappear at long time delays (5 ps) because by then vibrational dephasing has destroyed all low-frequency coherence. This observation suggests that the origin of these features is indeed the nonstationary nuclear dynamics along the impulsively excited low-frequency coordinates.

The observed spectra are examples of the features predicted previously in Figures 6d–f. Using the heterodyne-detection picture of FSRS, it is possible to simulate the observed spectral evolution as demonstrated in Figure 7e. The approach to generating these spectra is identical to the one described earlier by introducing an oscillatory modulation of the high-frequency vibrational period owing to anharmonic coupling with the low-frequency bends. Mathematically, this is expressed as

\[
\tau_{CD}(t) = \tau_0 \left[ 1 + e^{-t/\Gamma} \cdot \left( \lambda_A \cdot \sin \left( \frac{2\pi \cdot t}{\tau_A} \right) - \lambda_E \cdot \sin \left( \frac{2\pi \cdot t}{\tau_E} \right) \right) \right],
\]

where \(\tau_0\) is the equilibrium vibrational period of the C-D stretch, \(\Gamma\) is the vibrational dephasing time for the two bends, \(\lambda_A\) and \(\lambda_E\) represent the magnitude of the modulation of the period induced by wave-packet motion, and \(\tau_A\) and \(\tau_E\) are the vibrational periods of the two low-frequency bends. This simple model reproduces exactly the experimental spectra and their dynamics (Figure 7e).

These results demonstrate clearly the ability of FSRS to detect vibrational structural changes in molecules that occur much faster than the vibrational dephasing time. In this case, oscillatory motion with periods as low as 90 fs is clearly resolved by initiating vibrational coherence in the C-D stretch, which has a dephasing time of picoseconds! Despite the arrival of the photons created by the macroscopic polarization at the detector at any time during this long vibrational free-induction decay, the final spectra show obvious differences for coherence initiation time delays that differ by as little as 15 fs.
Rhodopsin: Seven α-helical trans-membrane protein containing an 11-cis retinal prosthetic group responsible for vertebrate vision

WATCHING CHEMICAL REACTION DYNAMICS: THE PRIMARY VISUAL EVENT

The primary event in vision is the photochemical cis-trans isomerization of 11-cis retinal in the seven α-helical trans-membrane protein called rhodopsin found within retinal rod- and cone-cell pigments (Figure 8b). Because of its unique reactive properties, this apparently simple photochemical isomerization reaction has attracted much experimental and theoretical attention since its discovery almost 40 years ago (47–51). Early ultrafast transient absorption studies have shown the first step to be complete in only 200 fs, making it one of the fastest chemical reactions known (11). Calorimetric measurements demonstrated that more than 60% of the incoming photon energy is stored in the primary thermodynamically stable intermediate, called bathorhodopsin (52, 53). In addition, the photoisomerization inside the protein is highly specific with an isomerization quantum yield of 0.65, almost an order of magnitude above the yield observed in the absence of the highly constraining protein environment (54, 55).

Figure 8a depicts the course of the reaction schematically. Electronic excitation of the system by a photon is followed by rapid nonstationary motion out of the Franck-Condon region, resulting in ultrafast 50-fs excited-state decay. To elucidate the nature and timing of the atomic motions producing bathorhodopsin, we used FSRS to follow the structure of the chromophore as it moves along the reaction coordinate. Because of the ultrafast excited-state decay, this probing is performed on the initial ground-state photoproduct transients. This reactive study is an example of the general scenario outlined in Figure 6a.

Figure 8c presents selected femtosecond time-resolved FSRS spectra of retinal in rhodopsin following a 30-fs pump pulse centered at 500 nm. The depicted spectral region contains three major structural features: (a) the ethylenic C= tether stretch at \( \sim 1550 \text{ cm}^{-1} \), (b) C-C single-bond stretching and C-H rocking modes in the so-called fingerprint region from 1100 to 1300 cm\(^{-1}\), and (c) hydrogen-out-of-plane (HOOP) modes between 800 and 1000 cm\(^{-1}\). Scheme 1 depicts the high-frequency in-phase A\(_n\) HOOP mode.

Figure 8

Direct structural observation of the primary photoisomerization in vision. (a) Schematic potential energy surfaces involved in the cis-trans isomerization of 11-cis retinal. Following excited-state decay, the formation of bathorhodopsin is monitored using femtosecond stimulated Raman spectroscopy (FSRS) as the system relaxes in the photoproduct potential well. (b) Crystal structure of bovine rhodopsin. The retinal chromophore is indicated in red. (c) Selected time-resolved FSRS spectra of rhodopsin following excitation by a 30-fs pulse. Conventional resonance Raman spectra of the rhodopsin reactant and the bathorhodopsin photoproduct are included for comparison. Theoretical simulations of the hydrogen-out-of-plane (HOOP) features are indicated in red. (d) Transient hydrogen-wagging frequencies obtained from the spectral simulations of the HOOP region depicted in (c). (e) Structure of retinal in rhodopsin and photon-induced structural changes in the polyene backbone along the reaction coordinate predicted by modeling the observed time-dependent vibrational frequencies (58).
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**Figure a** shows the energy levels of Rhodopsin and Bathorhodopsin, with a multidimensional reaction coordinate. The figure illustrates the transition between the 11-cis and all-trans states, with 0 fs to 50 fs, 200 fs, and 500 fs reaction times.

**Figure b** presents a structural representation of Rhodopsin and Bathorhodopsin, highlighting the conformational changes during the transition.

**Figure c** displays Raman shift spectra for Batho and Rho at various time delays (0 fs, 1 ps, 500 fs, 375 fs, 300 fs, 225 fs, 200 fs). The spectra show shifts in frequencies, with peaks at 875, 850, 920, 969 cm⁻¹.

**Figure d** illustrates the change in H-wag frequency with time delay (0.5, 1.0, 2.0 ps) for Batho, Rho, and Photorhodopsin.

**Figure e** provides structural details of Rhodopsin, Photorhodopsin, and Bathorhodopsin at 0 fs, 200 fs, and 1 ps, respectively, with specific angles labeled (+25°, +25°, +31°, +21°, +35°, -144°).
Hydrogen-out-of-plane (HOOP) wagging motion.

Comparison of reactant (Rho) and product (Batho) resonance Raman spectra shows small but significant changes in frequency and intensity in both the ethylenic and fingerprint modes. Larger changes are observed in the HOOP region, in which the spectrum evolves from a single peak in rhodopsin at 970 cm$^{-1}$ due to C$_{11}$H=C$_{12}$H HOOP motion depicted in Scheme 1 to three bands at 920, 875, and 850 cm$^{-1}$ in bathorhodopsin attributable to isolated C$_{11}$–H, C$_{10}$–H, and C$_{12}$–H hydrogen-wagging motion, respectively (56). Close inspection of the presented FSRS spectra reveals a rapid narrowing and blue shift in the ethylenic band with increasing time delay, as expected from vibrational cooling, as well as a relatively slow formation of the photorhodopsin fingerprint pattern by $\sim$1 ps originating from a pattern at 200 fs that appears to be midway between the 11-cis reactant and the trans product.

The most dramatic changes, however, are observed in the HOOP region in which an unexpected intensity and peak pattern evolve toward the bathorhodopsin spectral features by 1 ps. Critically, the HOOP features at early time delays ($\leq$300 fs) are dispersive with considerable negative intensity. This observation is reminiscent of our discussion above of FSRS features resulting from vibrational frequency shifts that occur faster than the vibrational dephasing time (Figures 6a–c). This spectral evolution is simulated with the coupled-wave theory of FSRS using three isolated hydrogen-wagging modes, whose frequencies increase by $\sim$100 cm$^{-1}$ with a $\sim$325-fs time constant (Figure 8d). Excellent agreement between experiment and theory is found as demonstrated by the simulated spectra in Figure 8c.

The ability of FSRS to measure transient vibrational frequencies with femtosecond time resolution has tremendous implications for real-time structural studies of chemical reaction dynamics because of the close relationship between molecular and vibrational structure. As a consequence, we can now use the observed HOOP frequencies to determine the actual structural transformation of retinal throughout the reaction because the dispersive vibrational bands originate from vibrations in the photochemically active region of the retinal backbone. The key fact is that the frequency of hydrogen-wagging motion is highly sensitive to the restoring force provided by the retinal backbone. Out-of-plane distortion of the backbone results in a decrease of conjugation and a concomitant frequency drop in the hydrogen wags.

Scheme 1

Hydrogen-out-of-plane (HOOP) wagging motion.
Resonance enhancement:

This intuitive picture, we used density-functional theory to elucidate the dependence of hydrogen-wagging frequency on backbone distortion and thereby identified likely structures of retinal as a function of time. Figure 8 presents the optimized structures for the 200-fs photorhodopsin transient and the 1-ps bathorhodopsin intermediate. These structures provide a more complete temporal and structural picture of the primary isomerization event in vision.

Importantly, these results reveal a number of critical properties of rhodopsin that make it such an effective catalyst for retinal isomerization. The overall structure of retinal in photorhodopsin resembles more the reactant than the product, which is expected given its extremely rapid formation, but rather surprising in the light of the common one-dimensional isomerization model indicated in Figure 8a. Accordingly, a large fraction of the structural rearrangement leading to bathorhodopsin actually takes place on the ground-state surface along slow torsional coordinates, whereas rapid excited-state decay is mediated largely by fast nontotally symmetric coordinates, such as the C11H=C12H A2 HOOP. This separation of timescales is required because the protein’s tight binding pocket prevents a rigid global rotation of the backbone but accommodates a rapid local C11=C12 torsion as long as it is compensated by adjacent single- and double-bond twists to minimize the overall shape change. Rapid excited-state decay (τ ~ 50 fs) is the most critical factor in ensuring rhodopsin’s exceptional energy storage because it dominates other energy-relaxation processes, such as intramolecular vibrational relaxation, which commonly occur at least an order of magnitude more slowly (57). In addition, this rapid decay ensures that virtually all the incoming photon energy is used to drive the large-scale conformational changes in retinal, leading to bathorhodopsin formation.

The protein’s role in catalyzing the isomerization is thus threefold: (a) It primes the system by distorting the ground-state chromophore along nontotally symmetric torsional and HOOP coordinates, thereby enabling rapid excited-state motion toward a conical intersection; (b) it accelerates excited-state decay by using a tight binding pocket to retain the overall shape of the chromophore; and (c) it efficiently captures the high-energy bathorhodopsin intermediate through intermolecular strain with the residues in the binding pocket (58).

SUMMARY AND PROSPECTS

These successful applications of FSRS demonstrate that it is an excellent tool for structural studies of ultrafast chemical and biochemical reaction dynamics. The most straightforward improvement over the current setup is being realized by extending the possible wavelength range of the stimulated Raman process from the near IR to the visible (59) and to the near UV (33). Bluer and tunable probe wavelengths have the advantage of (a) increased resonance enhancement for improved signal-to-noise ratios, (b) the avoidance of interfering nonlinear signals such as Raman initiated by nonlinear emission that become dominant for near-IR probing of excited- to ground-state dynamics (60), and (c) the observation of anti-Stokes FSRS signals that could provide exceptional insight into the real-time flow of energy through molecules (61).
Resonance Raman intensity analysis: determination of excited-state geometry changes through simultaneous analysis of Raman excitation profiles and electronic absorption spectra

The initial experiments on CDCl₃ also indicate the possibility of developing multidimensional implementations of FSRS. This could be achieved by simply replacing the impulsive excitation step in the current experiment by a shorter broadband ISRS pump pulse that initiates vibrational coherence over a broader 1500-cm⁻¹ window. By changing the time delay between the femtosecond pulse initiating vibrational coherence and the one probing it, investigators should be able to reveal the time dependence of vibrational coherence and coupling, similar to recent two-dimensional IR experiments on chemical exchange (62, 63), with the advantages of (a) a much larger spectral window compared with the limited bandwidth of femtosecond IR pulses (<200 cm⁻¹) and (b) the possibility of interrogating low-frequency degrees of freedom (<1000 cm⁻¹) currently inaccessible in the IR. We believe that these experiments will be successful as a result of the very high intrinsic signal-to-noise ratios achievable with FSRS and the ease of producing the required sub-20-fs near-IR pulses.

The observation of nonstationary nuclear dynamics with FSRS has so far been limited to the ground electronic state, as demonstrated in deuterio-chloroform and rhodopsin. The corresponding excited-state studies promise to give detailed structural information about the potential energy surfaces on which photochemical reactions occur that can be combined with resonance Raman intensity analysis (64) to create a detailed structural understanding of chemical reaction dynamics and mechanisms. The concept of these experiments is similar to that introduced for the aforementioned ground-state studies (Figure 9). Here, the initial nonstationary state is created by electronic excitation rather than impulsive Raman scattering or ultrafast photochemical change. On formation, the excited-state wave packet evolves along displaced nuclear coordinates on the reactive excited-state surface on its way toward an excited-state minimum, a conical intersection, or a photochemical product. This evolution can be monitored in real time with FSRS to determine the properties of the reactive portion of the multidimensional potential energy surface in exquisite detail.

These future FSRS experiments studying reactive potential energy surfaces will benefit from (a) improved resonance enhancement provided by a tunable light source for both Raman and probe pulses, resulting in superior signal-to-noise ratios for excited-state spectra, (b) the potentially large transient excited-state populations for excited states with lifetimes that exceed typical vibrational dephasing times (>1 ps), and (c) the much larger excited-state anharmonicities that determine the magnitude of both vibrational side bands, as well as the sensitivity of the reporter mode to the system’s evolving structure.

In this review, we give a conceptual introduction to FSRS based on the coupled-wave theory, and we use the close connection between the decoupling of time and energy resolution in FSRS and ultrafast electronic spectroscopy in the dispersed detection regime to rationalize the exceptional instrumental capabilities of FSRS. These concepts and their manifestation in various experimental scenarios through differing vibrational lineshapes and dynamics have demonstrated the ability of FSRS to study dynamic vibrational structure on a timescale faster than most chemical...
Figure 9
Schematic representation of femtosecond stimulated Raman spectroscopy (FSRS) studies of reactive potential energy surfaces. The motion of an optically created nuclear wave packet along a complex multidimensional reaction path is monitored at different locations in the reactive phase space using FSRS probing. Initiating vibrational coherence in one or more coupled high-frequency reporter modes at different times results in different vibrational free-induction decays (green versus red), which reveal detailed molecular vibrational structural information along the reaction coordinate.

transformations and vibrational dephasing times. We anticipate that the unique capabilities of FSRS demonstrated by these studies in combination with potential future experimental improvements will make FSRS a widely used tool in studies of chemical reaction dynamics. The resulting experimental data are likely to spark advanced theoretical efforts to transform the time-dependent FSRS frequencies and lineshapes into detailed pictures of the potential energy surfaces on which the reactive dynamics occur.

SUMMARY POINTS
1. Femtosecond stimulated Raman spectroscopy provides vibrational structural information on reacting systems with high temporal (50-fs) and spectral (10-cm$^{-1}$) resolution.
2. The unique instrumental capabilities of FSRS are a result of the disentanglement of time and energy resolution based on dispersed detection of broadband femtosecond pulses.
3. Monitoring vibrational structural rather than electronic features as a function of time is a dramatic advance in chemical and biochemical reaction dynamics.
4. Structural changes that occur faster than vibrational dephasing are manifested in FSRS through unique vibrational lineshapes.

5. The dynamic evolution of HOOP lineshapes in rhodopsin reveals that much of the torsional rearrangement that initiates vision occurs on the ground-state potential energy surface.

**FUTURE ISSUES**

1. Development of visible and near-UV FSRS setups will make possible studies of a huge variety of photochemical and photobiological systems by improving signal magnitudes through resonance enhancement.

2. Multidimensional implementations of FSRS will provide a wealth of information on solvation dynamics, vibrational coupling, and structural change on the femtosecond timescale.

3. Monitoring nonstationary nuclear dynamics with FSRS will be used to structurally map the multidimensional shape of reactive excited electronic state surfaces in real time.

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**LITERATURE CITED**


8. Presents an overview of current state-of-the-art experiments in ultrafast chemistry and biology.

11. Demonstrates the first use of broad-band electronic femtosecond probing to determine the 200-fs timescale of the primary step in vision.

14. Presents a theoretical treatment of femtosecond dynamic absorption spectroscopy; provides a quantitative basis for the disentanglement of time and energy resolution.
