6. Zewail, A. H., *Disc Faraday Soc.*, 1983, 75, 19; See also, Lambert, W M., Felker, P. M and Zewail, A. H, *J. Chem. Phys.*, 1981, 75, 5958–5960; For a summary, see Felker, P. M., in *Study of Fast Chemical Processes* (ed. Venkataraman, B), 1988, vol. 2, pp. 119–208 (Lecture note publication by TIFR, Bombay).

7. Chakraborty, T. and Chowdhury, M., *Chem. Phys. Lett.*, 1990, 171, 25–28.

8. Shin, Y D., Saigusa, H., Zgierski, M. Z., Zerbetto, F. and Lim, E. C., *J Chem. Phys*, 1991, 94, 3511–3516.

9 Guchhait, N., Dutta, R., Dutta, P. and Chakraborty, T., *Indian J. Phys.*, 1992, B66, 241–244.

10. Chakraborty, T. and Lim, E. C., *J. Chem. Phys.*, 1993, 98, 836.

11. Okuyama, K., Kakinuma, T., Fujii, M., Mikami, N. and Ito, M., *J. Phys. Chem.*, 1986, 90, 3948–3952.

12. Chakraborty, T., Nath, D. and Chowdhury, M., *J. Chem. Phys.*, 1992, 96, 6456–6463.

13. Suzuki, T., Mikami, N. and Ito, M., *J Phys. Chem.*, 1986, 90, 6431–6440.

14. Chakraborty, T. and Chowdhury, M., *Chem. Phys. Lett.*, 1991, 177, 223–228.

15. Okuyama, K., Mikami, N. and Ito, M., *J. Phys. Chem.*, 1985, 89, 5617–5625.

16. Chakraborty, T. and Chowdhury, M., *Chem. Phys*, 1992, 159, 439–448.

17. Chakraborty, T. and Chowdhury, M., *Spectrochim. Acta*, 1992, A48, 1683–1691.

18. Chakraborty, T., Oel Bene, J. E. and Lim, E. C., *J. Chem. Phys.*, 1993, 98, 8.

19. Guchhait, N., Chakraborty, T., Nath, D., Bera, P. and Chowdhury, M., *Chem. Phys. Lett.*, (communicated).

20. Chakraborty, T. and Lim, E. C., *J. Chem. Phys.*, (in press).

21. Ray, D. S., Bhattacharyya, K., Bera, S. C. and Chowdhury, M., *Chem. Phys. Lett.*, 1980, 69, 134–140.

22. Soyage, J. A., Felker, P. M. and Zewail, A. H., *J. Chem. Phys.*, 1984, 81, 4685–4705, 4706–4723; Khundkar, L. R. and Zewail, A. H, *Annu. Rev. Phys. Chem.*, 1990, 41, 15–60; See also, Zewail, A. H., *Sci. Am.*, 1990, 263, 76–82.

23. Knee, J. L. and Zewail, A. H, *Spectroscopy*, 1988, 3, 44 and references therein.

Ultrafast laser spectroscopy — A tool for chemists and biologists

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Application of ultrafast lasers to chemistry and biology has been an active area of research in the international scene for over a decade for physical and biophysical chemists. Perhaps, ultrafast laser spectroscopy is one of the most versatile tools available today to experimentally study structure and dynamics in the time domain of nanoseconds (10^{-9} sec) to femtoseconds (10^{-15} sec). In this article we attempt to highlight some of the recent developments in ultrafast laser spectroscopy with particular reference to vibrational spectroscopy, viz. infrared and Raman spectroscopy, in the above time domain.

Ultrafast processes

The definition of ultrafast may only mean subpicoseconds ($< 10^{-12}$ sec) from a technical purist point of view. However, any process that ranges from nanoseconds (ns) to femtoseconds (fs) is in general considered to be in the ultrafast regime. The dynamics in this time domain initiated by a photon can be classified into photochemical and photophysical processes. The photochemistry that can be investigated range from isomerization reactions to proton or electron transfer reactions, including rearrangement or cyclization reactions initiated by a photon. In the case of photophysical processes one is able to study processes such as vibrational relaxation, energy transfer, electronic relaxation, etc. Thus, biological systems do have limitations in the application of this technique in that, only systems that are photosensitive can be studied. Examples of such systems include bacteriorhodopsin, haemoglobin, photosynthetic bacteria, etc. In chemistry, one can investigate either inorganic, organic- or polymer-based molecular systems, in which the photochemistry or the photophysics play an important role in their reactivity. Due to the restricted nature of this article we shall only highlight some recent results.

Vibrational spectroscopy

Vibrational spectra provide information on the structural details of the molecule that is being investigated. In particular vibrational spectral analysis can lead to an

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understanding of molecular bonding characteristics, and in a time-resolved experiment one can also learn about architectural changes associated with photochemical reactions. Unlike in absorption and fluorescence experiments, where we only get information as a broad spectral profile with large energy spread relating to electronic transitions, vibrational spectrum contains fine structural details with energy separation within the vibrational energy spacings.

Typically in a time-resolved infrared experiment one measures the change in absorption at a particular frequency of interest on photoexcitation. The time-dependent change in absorption is then related to dynamics of the system initiated by a photon. In the case of resonance Raman, the Raman scattering is observed from the intermediate species on excitation in resonance with a particular electronic transition. Thus, resonance Raman provides a facility by which to selectively study a specific part of a molecule where the electronic transition is centered. Of course, on appropriate time resolution one can then study the dynamics of the process using such a technique. Further, a careful analysis of the linewidth and relative intensity changes of the vibrational spectral profile may provide valuable clues relating to vibrational relaxation, energy transfer and also the presence of vibrationally hot intermediates.

Experimental methods

The characteristics that attract the use of lasers, in addition to their coherence, are (a) the ability to produce laser pulses of a given duration, viz. ns, ps or fs and (b) the availability of monochromatic laser source facilitating spectral resolution much less than the vibrational energy spacings. The spectral resolution is, however, within the limitations of Heisenberg's uncertainty principle, which implies that the shortening of the laser pulses (few ps to fs) to transform limited pulse widths will lead to energy spread (increase in spectral width of the laser) thus limiting experimental resolution. Although such limitations are forced by nature one can design ingenious experimental methods to enable an understanding of vibrational energy dynamics in the fs time domain.

In a typical experiment, one utilizes two laser beams. One laser (pump laser) is used to initiate the photoprocess and the second laser (probe laser) to follow the ensuing dynamics. The wavelength of these lasers is chosen such that the sample will absorb the laser light. In particular, for resonance Raman experiments one chooses a probe laser wavelength to be in resonance with the absorption envelope of the electronic transition of the intermediate state, generated by a pump laser. In a nanosecond experiment, one employs electronic gating of the detector to avoid

signals generated from the pump laser. But in the case of picosecond and femtosecond experiments, detecting method includes both either optical filtering of wavelengths or optical gating based on nonlinear processes. The latter method has been successfully used in time-resolved infrared experiments in the femtosecond time domain. Thus depending on the process that is being investigated one has to design an experimental set-up appropriate to the required time domains.

Chemical processes

In this section two specific examples are discussed very briefly, details are available in the references cited here. The two processes are namely, vibrational energy relaxation and electron transfer by picosecond and nanosecond resonance Raman spectroscopy respectively. In the case of vibrational energy relaxation, photodissociation of $Cr(CO)_6$ has been studied[1]. Transient absorption experiments have shown that the dissociation of $Cr(CO)_6$ leads to formation of a solvated $Cr(CO)_5$ in about 800 fs, whereas transient infrared experiments suggested formation of such products in a much slower time-scales. Further, it has been proposed that the observed time-dependent shift in the picosecond absorption experiment may be due to vibrational energy relaxation. These questions can be addressed unequivocally using a picosecond Raman experiment, described below.

In this experiment since both the precursor and the dissociated product absorb at ca. 266 nm, experiments were conducted with 266 nm excitation and probing. As shown in Figure 1, assume that we create a vibrationally excited species (in our case photodissociated $Cr(CO)_5$, one can also consider other electronically excited states) which can decay within the time-scale of our experiment to the lowest vibrational state, then one can study the anti-stokes and stokes Raman scattering. From an analysis of these spectra, that is, if the life time of decay of anti-stokes vibrational Raman intensities match with the rise time of the stokes Raman intensities,
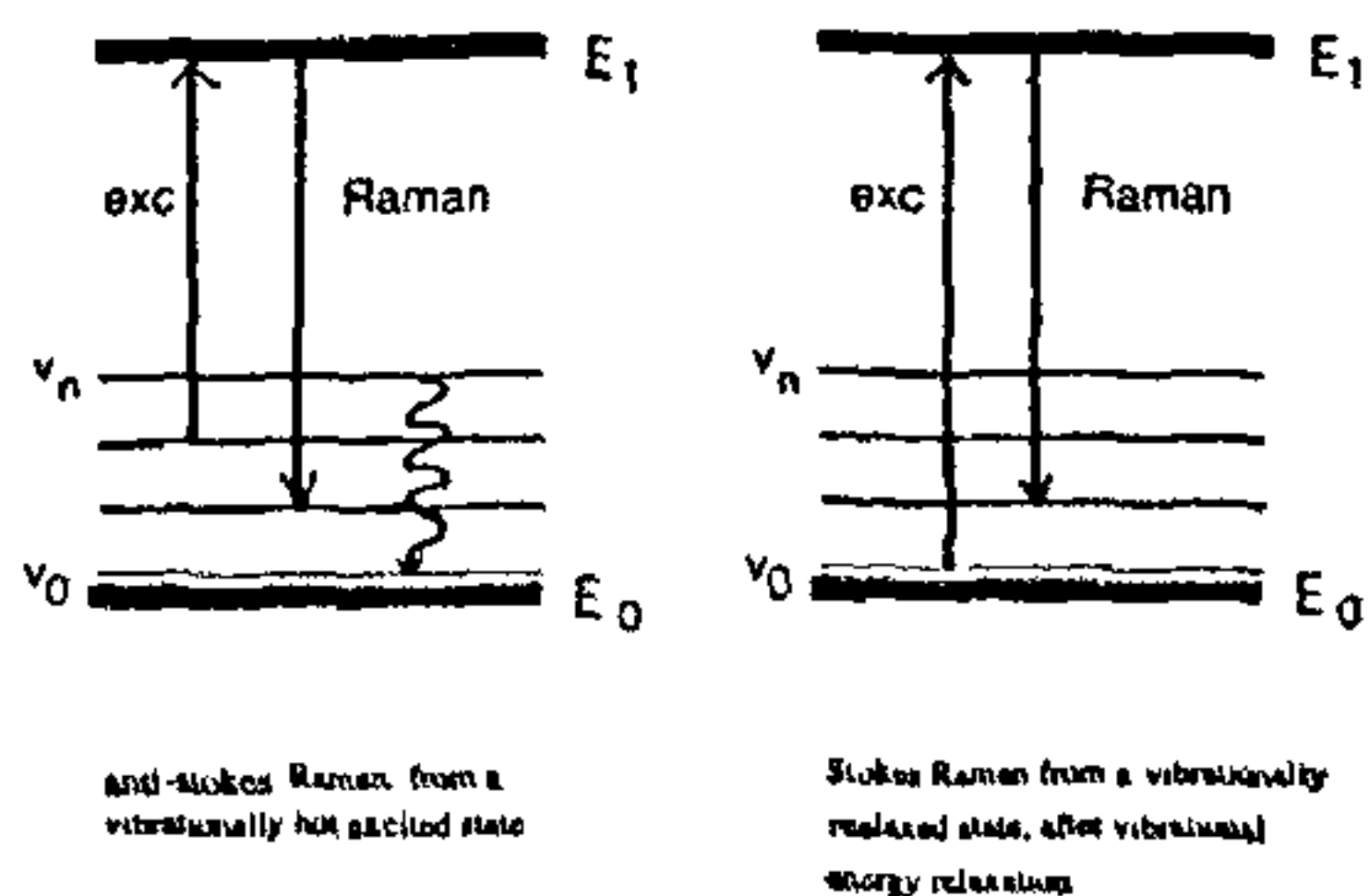

Figure 1. Energy level diagrams of anti-stokes and stokes Raman scattering from vibrationally hot and relaxed species, respectively

vibrational energy relaxation can be followed. This experiment was performed on the above system by following the metal-CO stretch vibration of the $Cr(CO)_6$ and $Cr(CO)_5$ and has been shown that, indeed, vibrational relaxation occurs in a time-scale of about 100 ps.

In the case of electron transfer studies, we look at interfacial electron transfer studies on a colloidal semiconductor surface[2]. It has been shown that in a photocatalytic splitting reaction of water (into hydrogen and oxygen) on colloidal TiO_2, one of the factors that determine the efficiency of the splitting process is the photosensitization of the TiO_2 by a sensitizer. We have studied such a sensitization process using nanosecond resonance Raman spectroscopy. The photosensitization process involves photoexcitation of the ruthenium tris (2,2'-bipyridyl-4, 4'-dicarboxylate) which transfers an electron from its metal to ligand charge transfer triplet excited state to the conduction band of the TiO_2 semiconductor. This process has been studied by recording the Raman spectra of the triplet excited state and also the charge transferred oxidized ruthenium complex at different times (as seen in Scheme 1), along with other more efficient sensitizers in order to understand the relation between the structure and electron transfer efficiency. From such a study it was concluded that when the ligands form part of the solvation layer of the semiconductor colloidal particle the electron transfer is very fast and efficient.

Biological processes

Numerous examples are available in the literature on time-resolved vibrational spectroscopy of bacteriorhodopsin, haemoglobin, chlorophyll, photosynthetic systems, etc. Here we take bacteriorhodopsin as an example, which, on photoexcitation undergoes isomerization at the retinal chromophore thereby effecting proton pumping across the cell membrane. This light-driven proton pumping cycle has been found to have a number of intermediates with life times ranging from few hundred femtoseconds to milliseconds. In order to understand the structure of these intermediates in relation to their function it is necessary to learn about the vibrational structural details. Resonance Raman
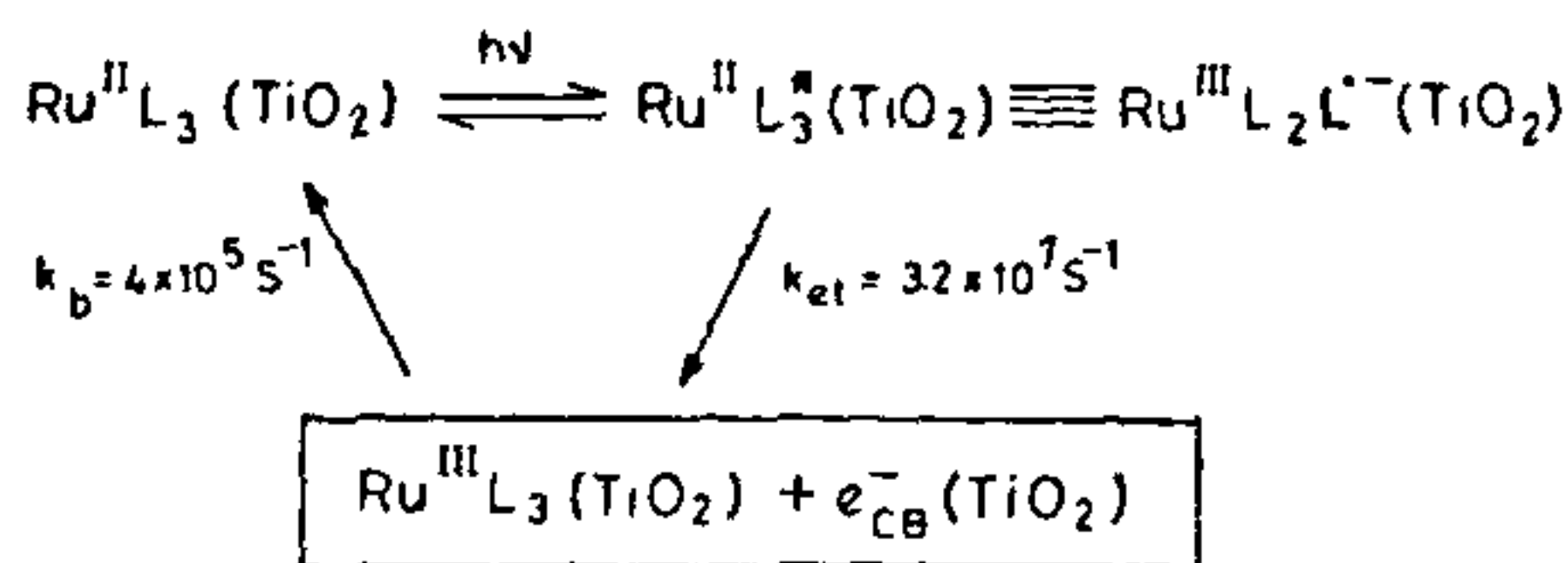
spectra of most of these intermediates have been recorded[3] as shown in Figure 2. However, still a number of questions remain regarding the very early time (femtoseconds) dynamics.

In Figure 3 are shown the potential energy surfaces and various photophysical processes of the bacteriorhodopsin photocycle. Recent transient absorption and. fluorescence studies (ref. 4 and references therein) have shown the presence of vibrationally excited intermediates BR' and K' as shown in Figure 3. These intermediates can be identified by anti-stokes resonance Raman studies. In this experiment, the probe laser is tuned to a wavelength which is selected by subtracting the vibrational frequency from the absorption maximum of the electronic state in which the vibrationally hot intermediate is being probed. For example, if we are interested in investigating the vibrationally hot state of K' along a $C=C$ stretching coordinate with a frequency $1525\ cm^{-1}$, in the electronic state which has an absorption maximum at 570 nm, then one chooses the wavelength 624 nm ($17544\ cm^{-1}$ (570 nm)$-1525\ cm^{-1}$ $=16019\ cm^{-1}$ (624 nm)) as a resonance excitation for the anti-stokes spectrum of the hot species. It is important to recognize that in addition to recording the Raman spectrum, for a sensible comparison with stokes
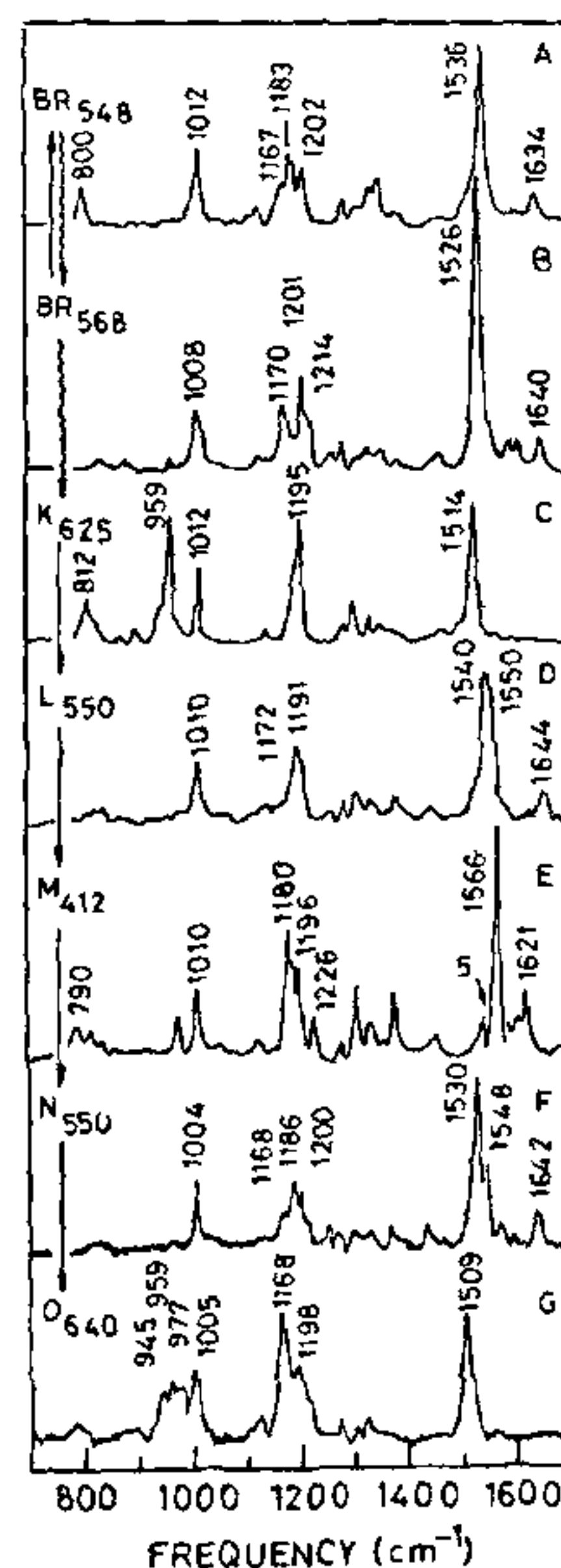

Figure 2. Resonance Raman spectra of various intermediates of bacteriorhodopsin (adapted from ref. 3).
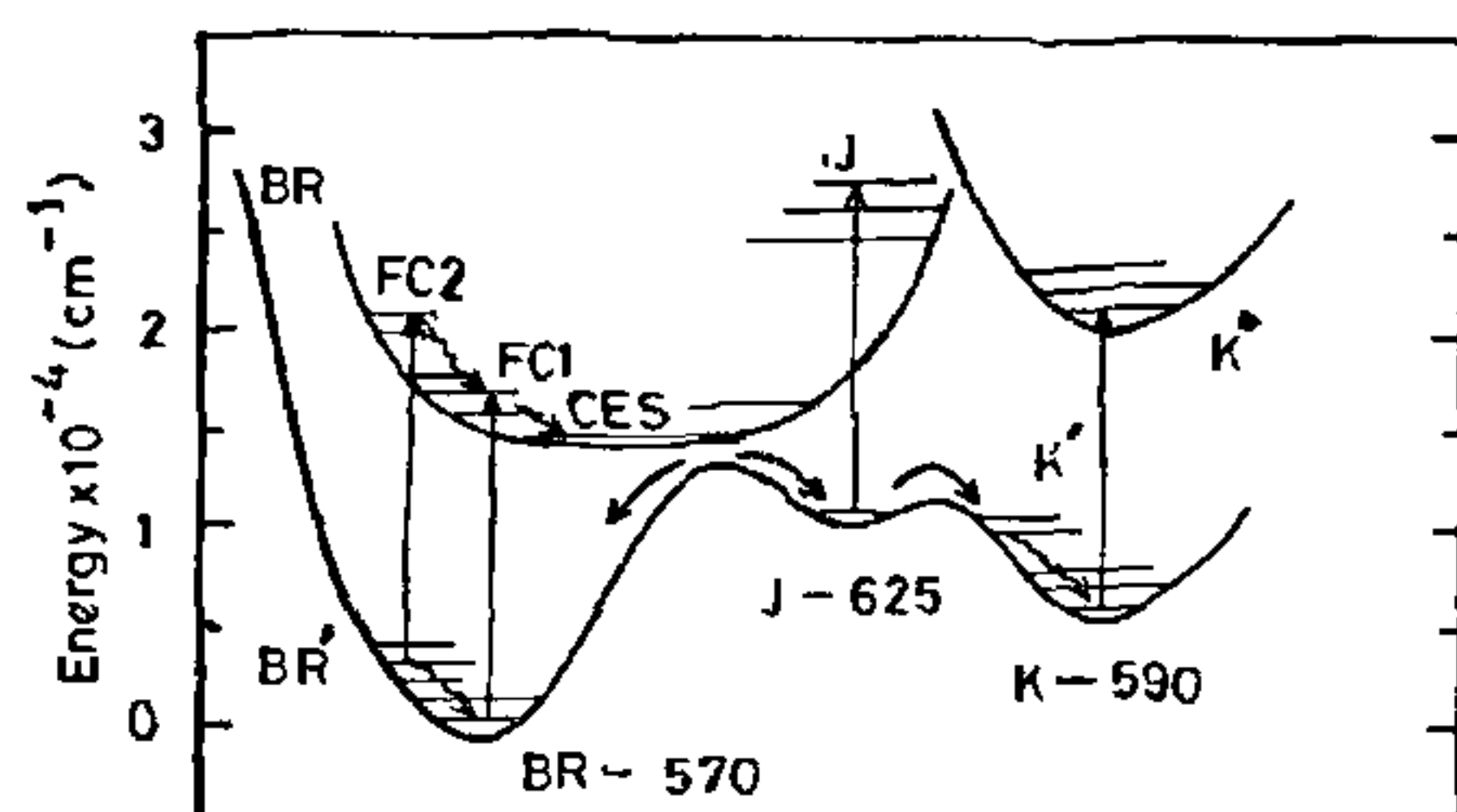
$$Ru^{II}L_3 (TiO_2) \underset{h\nu}{\rightleftharpoons} Ru^{II}L_3^*(TiO_2) \rightleftharpoons Ru^{III}L_2L^{\cdot-}(TiO_2)$$

$$k_b = 4 \times 10^5\ s^{-1} \qquad k_{et} = 3.2 \times 10^7\ s^{-1}$$

$$Ru^{III}L_3 (TiO_2) + e^-_{CB}(TiO_2)$$

Scheme 1.

Figure 3. Potential energy surfaces of bacteriorhodopsin photocycle indicating the various intermediates (adapted from ref. 4).

resonance Raman spectrum one has to consider the relative change in Raman cross sections for both the excitations. Such an experiment has been performed on the bacteriorhodopsin system and the results obtained are interpreted to show the presence of the vibrationally hot intermediates.

Time resolved Raman spectroscopy facility at IISc

The experimental set up involves the use of a SPEX double monochromator, an intensified CCD, and two nanosecond pulsed Nd-YAG lasers with frequency doubled output. This set up facilitates time-resolved experiments in the nanosecond time domain. A part of the experiment facility is shown in Figure 4 (see photo).

Experiments have been performed to characterize the system, by recording the triplet excited state Raman spectrum at 20 ns after excitation for benzophenone and chloranil. These two systems were chosen in order to reproduce Raman data already available in the literature and also because these systems are suited for laser wavelengths available in the laboratory.
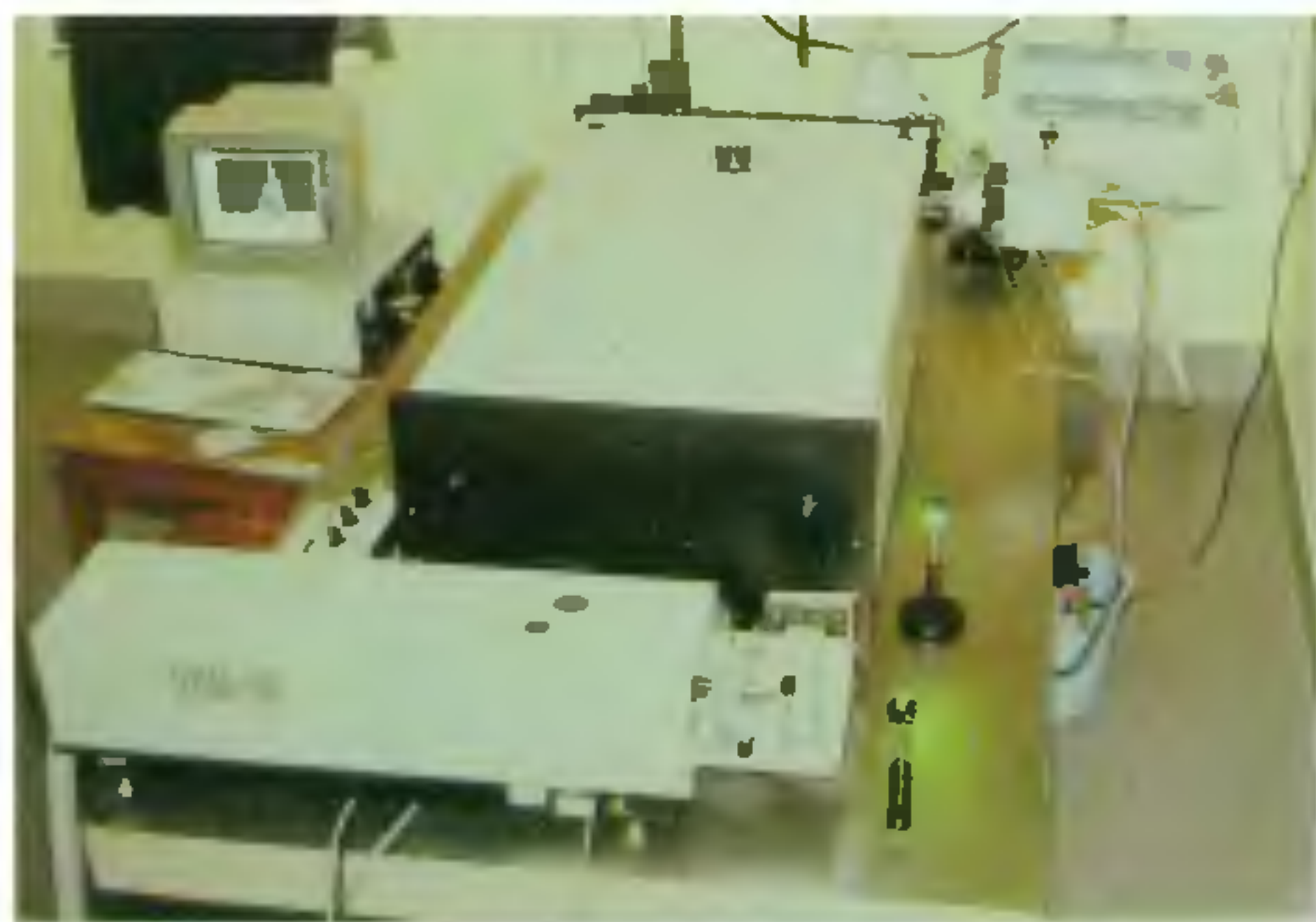
Figure 4. Experimental set up at the Indian Institute of Science In view are the SPEX double monochromator, a Nd-YAG laser and a CCD detector.

Benzophenone triplet excited state has attracted attention due to its reactivity and highly electrophilic nature[5]. The life time of the triplet has been reported to be longer than a few microseconds with an absorption maximum at 530 nm. The triplet state can be produced by direct excitation, using a 355 nm laser pulse, of benzophenone, which first forms a singlet excited state and then by inter system crossing it forms the triplet state in a few picoseconds. The time-resolved resonance Raman spectra were recorded for the ground state and after 20 ns for the triplet excited state as shown in Figure 5.

From earlier photochemical studies on chloranil it is known that the triplet excited state of chloranil has a
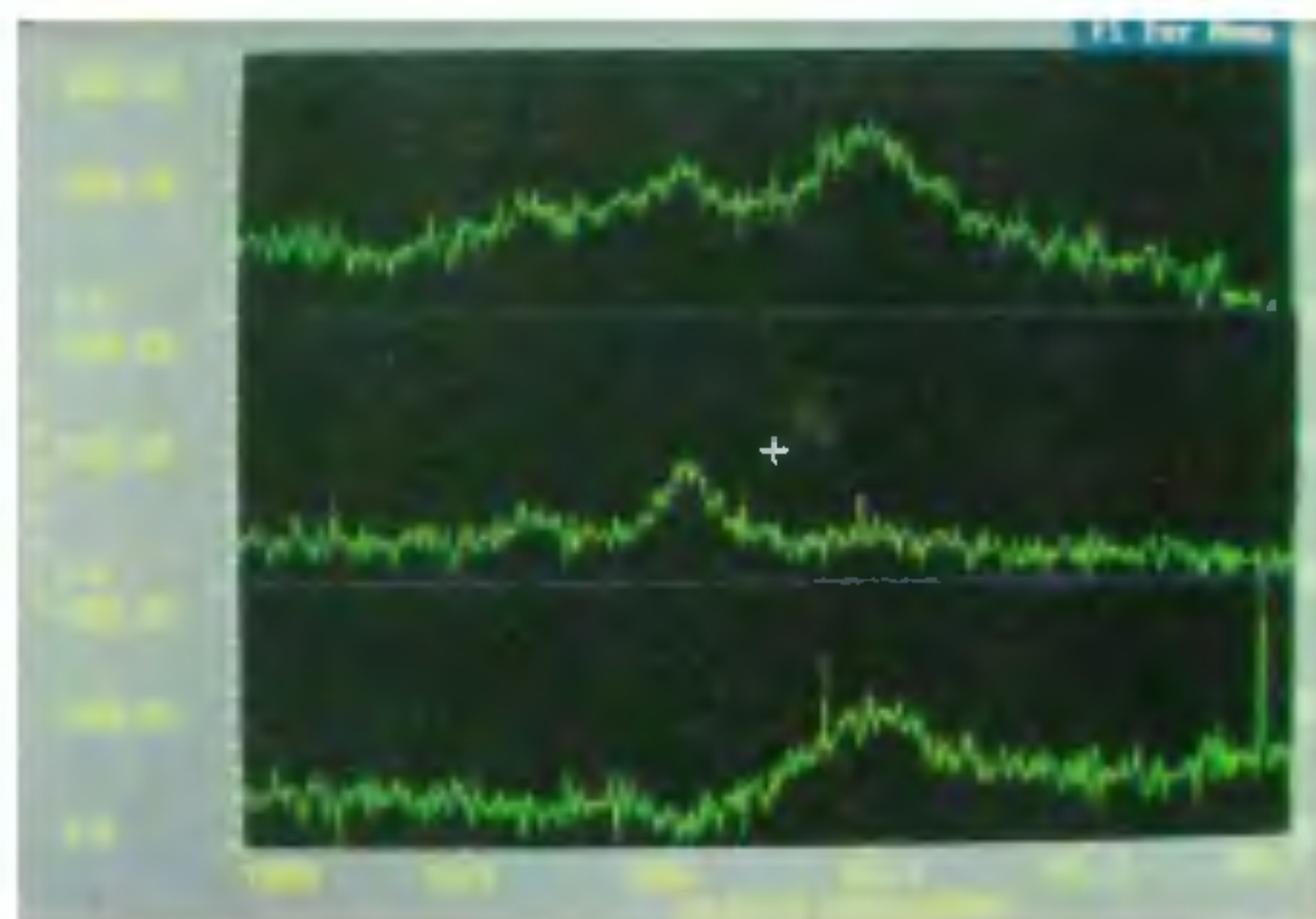

Figure 5. Time-resolved resonance Raman spectral data of benzophenone. *Top,* time resolved data at 20 ns delay between the pump and probe lasers, *middle,* probe only spectrum = ground state Raman spectrum of benzophenone, *bottom,* resonance Raman spectrum of the triplet excited state of benzophenone, obtained by subtracting middle spectrum from the top spectrum.
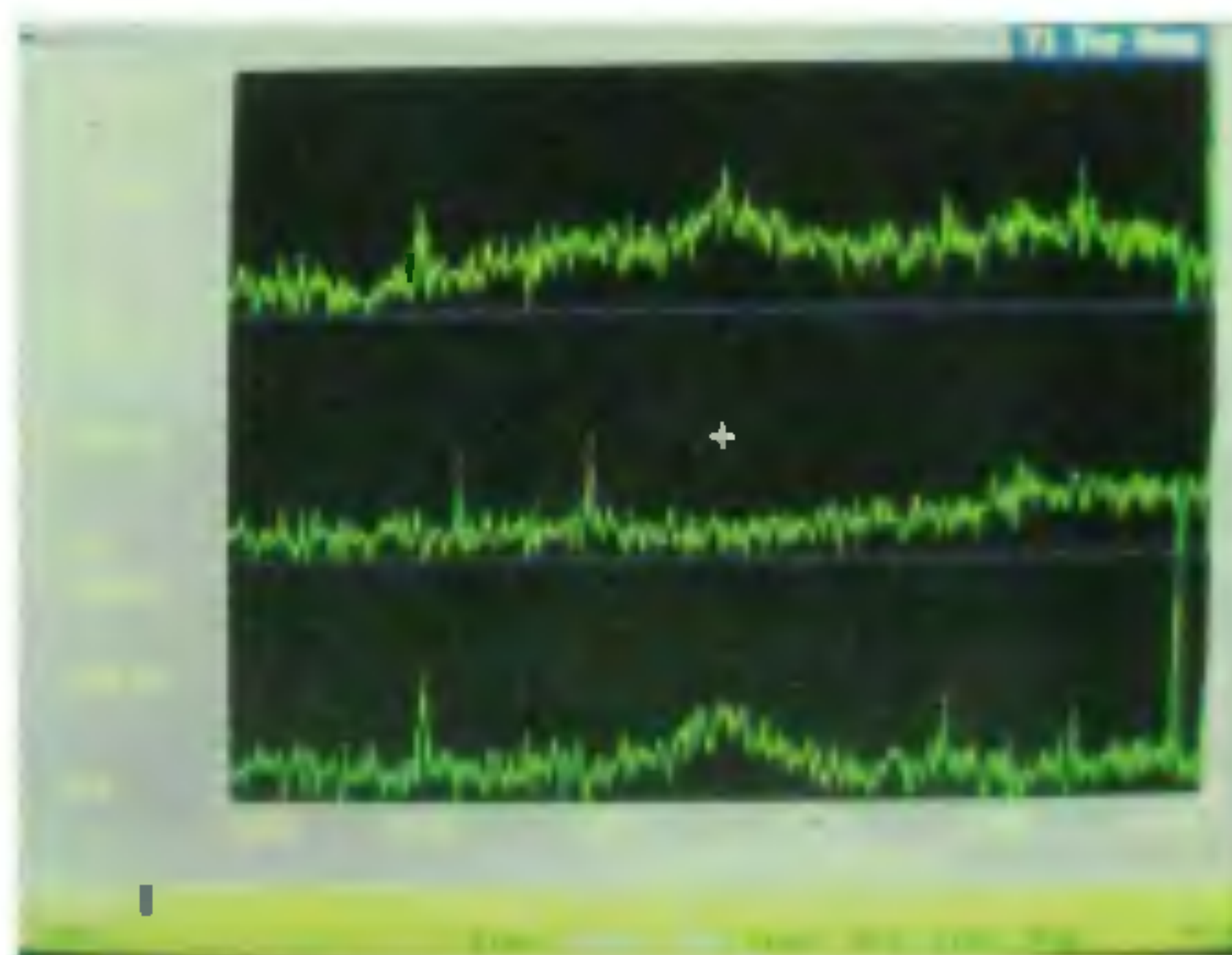

Figure 6. Time-resolved resonance Raman spectral data of chloranil. *Top,* time resolved data at 20 ns delay between the pump and probe lasers, *Middle,* probe only spectrum = ground state Raman spectrum of chloranil, *bottom,* resonance Raman spectrum of the triplet excited state of chloranil, obtained by subtracting middle spectrum from the top spectrum

life time of 1 10 ps in deoxygenated solution and in presence of oxygen the life time is reduced to about few hundreds of nanoseconds. The triplet-triplet absorption has a maximum at 520 nm. This facilitates resonance Raman excitation at 532 nm (frequency doubled output from a Nd-YAG laser) to probe the triplet state. The spectra recorded are shown in Figure 6. The spectrum at the bottom of the frame is the Raman spectrum recorded at 20 ns delay with respect to photoexcitation.

Where do we go from here?

With the versatility and use as is today, this method stands unique in learning about structural changes that are associated with very short time dynamics in any system. Time-resolved vibrational spectroscopy is developing further in order to reach a level of simplicity, in terms of both experimental methods and also interpretation of data, so as to enable this technique to become an analytical tool like most other forms of simple spectroscopies. It is particularly important to appreciate the fact that under resonance Raman conditions the intensities and to a limited extent even the peak positions, in time resolved data, are very difficult

quantities to predict with the existing theoretical knowledge. Thus, sometimes the interpretations are more qualitative than one would wish, however, a careful analysis of the spectra can form a basis for an understanding of dynamic information needed in very short time-scales which is not available by any other technique.

1 Yu, S., Xu, X, Lingle Jr R. and Hopkins, J. B, J Am. Chem. Soc., 1990, 112, 3668-3669
2. Umapathy, S., Cartner, A M., Parker, A W. and Hester, R. E, J Phys. Chem., 1990, 94, 8880-8885.
3. Mathies, R A., Proc. Indian Acad Sci (Chem Sci), 1993, 103, 283-293.
4. Brack, T. L. and Atkinson, G H., J. Phys. Chem., 1991, 95, 2351-2356
5 Tahara, T, Hamaguchi, H and Tasumi, M., J Phys. Chem., 1987, 91, 5875-5880.