International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Review Article **CODEN: IJPNL6**

ULTRAFAST SPECTROSCOPY: A REVIEW

Rajeesha Surapaneni*, Rohini P, Santosh Kumar Vobbilireddi and Mounika G

University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Guntur, India

***Corresponding author e-mail:** rajeesha.surapaneni@gmail.com

ABSTRACT

Spectroscopy is the major tool used for the determination of quantum energy levels in atoms, molecules, semiconductor etc. Ultrafast spectroscopy is a very wide field of research from departments of physics, chemistry, electrical engineering, biology and material science. The field of ultrafast spectroscopy includes the spectroscopic measurements for which electronic detectors are not fast enough to allow direct measurement phenomena. These time scales presently range from about 10 fs to 100 ps. It is based on the use of light pulses that have very short temporal duration to interrogate matter. To illustrate the advantages of ultrafast spectroscopy and explore some of its implications, a quantum- mechanical formalism is required. Collisions in room-temperature liquids occur on a few-fs time scale, so nearly all processes in liquids are ultrafast. An ultrafast spectroscopic method using monolithic column high-performance liquid chromatography was evaluated for the simultaneous determination of a drug discovery. Ultrafast 2D-IRspectroscopy has been applied to study the structure and vibrational dynamics.

Keywords: Semi conductors, Femto second, Quantum, Vibrational Dyanamics, Ultrafast and Spectroscopy

INTRODUCTION

Spectroscopy in general is the study of the interaction between light and matter. It is the major tool used for the determination of quantum energy levels in atoms, molecules, semiconductor etc. For example, in addition to their internal electronic motion molecules possess vibrational and rotational degrees of freedom. Such motion is quantized i.e., only motion corresponding to discrete rotational or vibrational energies is allowed. Spectroscopy which excites transitions between these quantized states is the primary tool in elucidating the energy-level spacing. These in turn are useful in determining bond strengths and overall molecular structure. "Traditional" spectroscopy can be described as energy resolved because measurements associated with this type of spectroscopy involves spectrally narrow light that is tuned across discrete energy levels. These measurements can be carried out with pulsed light sources to provide time-resolved kinetic information about processes that are statistical in nature. "Ultrafast" spectroscopy, on the other hand involves temporally short (and therefore spectrally broad) light pulses, which are used to probe directly the dynamics of the system rather than the energy levels themselves. Rapid advances in laser technology over the past decade have resulted in the ability to produce pulses as short as 6 fs (I fs = 10-15 s) and pulses of width 60 fs are now produced routinely in many laboratories around the world. Ultrafast spectroscopy is a very wide field of research that is increasing in popularity. It is increasingly interdisciplinary covering research from departments of physics, chemistry, electrical engineering, biology and material science. The field of ultrafast spectroscopy includes the spectroscopic measurements for which electronic detectors are not fast enough to allow direct measurement phenomena. These time scales presently range from about 10 fs to 100 ps, a period that encompasses a wealth of interesting chemical processes. In this field, the techniques and their chemical applications are inextricably tied together and any thorough treatment must include both.

PRINCIPLE

Most events that occur in atoms and molecules occur on fs and ps time scales because the length scales are very small. Bond formation and dissociation is

ultrafast. Fluorescence occurs on a ns time scale, but competing non-radiative processes only speed things up because relaxation rates add: Biologically important processes utilize excitation energy for purposes other than fluorescence and hence must be very fast. Collisions in room-temperature liquids occur on a few-fs time scale, so nearly all processes in liquids are ultrafast.

INSTRUMENTATION

Ultrafast spectroscopy system: The instrument was designed for time resolved femtosecond absorption and emission spectroscopy measurements of liquid and solid samples. The system consists of four subsystems:

1. Generator of the femtosecond pulse (laser system)
2. Up-conversion instrument (emission time resolved spectroscopy)
3. Pump-probe transient absorption measurement system
4. Pump-probe transient reflectance measurement system

Laser system: The laser system produces femtosecond pulses (50-70 fs) in a wide spectrum range.The primary Ti:sapphire generator (TiF-50, CDP Corp.) is pumped by Nd CW laser (Verdi-6, Coherent Inc.). It produces 50 fs pulses at repetition rate of 90 MHz. The generator can be tuned in 750-950 nm wavelength range (covered by two sets of mirrors). The generator emission can be used to carry out up-conversion emission measurements. The femtosecond pulses are amplified by a multipass Ti:sapphire amplifier (CDP Corp.) pumped by Q-switched Ng:YAG laser (LF-114, SOLAR TII). The amplifier utilizes stretcher-pulse picker-amplifier-compressor scheme. The output pulse energy of the amplifier is ~1 mJ at 10 Hz repetition rate. Typical pulse width is 60-70 fs. These pulses can be use to generate white continuum (probe in pump-probe experiments), and second harmonic at 400 nm (e.g. to be used as pump pulse in pump-probe experiments). An optical parametric amplifier (CDP2017, CDP Corp.) utilizes the pulses from the amplifier and implements a two pass scheme. A typical tuning range for the signal beam of the parametric amplifier is 1100-1600 nm and for the idle beam is 1600-3000 nm. Mixing signal with fundamental harmonic (sum frequency generation) provides pulses in the blue-green range (460-530 nm), and for idle-fundamental harmonic in the green-red range (540-630 nm) with typical pulse energy 10-30 μJ. Additionally, one can utilize the second harmonic of the signal (550-780 nm) or idler (820-1500 nm) beams respectively. The up-conversion instrument (FOG-100, CDP Corp.) is used to study dynamics of the photo-induced emission in femto- picosecond time domain. An example of the emission decay measurerements is presented in Fig. 3. The figure shows a rapid fluorescence decay of phytochlorin-fullerene dyad due to a fast intramolecular electron transfer reaction from the phyotochlorin to fullerene moiety.

Transient absorption measurement: The absorption pump-probe system is designed to measure time resolved spectra at single shot or averaging hundreds of shots. Typically the spectra are measured in a range of delay times to provide both the spectrum and time dependences of the transient absorption. After the measurements the data are analyzed using global fitting routines to gain information on the lifetimes and spectra of transient states.

Transient reflectance measurement:T he primary goal for pump-probe transient reflectance measurements is characterization of saturable absorber mirrors based on semiconductor quantum well and dot structures. For this application the excitation (pump) and monitoring (probe) wavelengths are the same. This type of measurements is usually referred to as mono-color pump-probe. The system is under development and testing stage at present.

Analysis of the measurements: A set of homemade programs is provided for the quantitative analysis of the time resolved measurements. The fitting programs can be used to fit single decay curves to acquire a global data fit in the whole measured spectrum range. Specifically for measurements in sub picosecond time domain the global fitting algorithm can account for the group velocity dispersion and provide the spectra with compensated delay dispersion.

Femto Biology:

1. The interaction between a protein and a photon lasts from 1 – 106 fs A Laser that emits ~100 fs pulses can investigate such a process.
2. Typically both a Pump and a Probe pulse separated by some femtoseconds or picoseconds in time are used.
3. Femtosecond pulses may be used to alter biochemical processes.

Technology needed in order to carry on Femtochemistry -Laser Choice: We need a laser emitting light in very short high intensity pulses each pulse being ~ 100 femtoseconds. The most widely used laser is based on Titanium Sapphire. Many different procedures have been developed. Some common methods are:

1. Ultra-fast transient absorption (TA)
2. Time-correlated single photon counting (TCSPC)
3. Time-resolved photo-electron spectroscopy (TRPES)

All methods must take into consideration for the quantum-mechanical nature of absorption and fluorescence, specifically that individual molecules, even in pure samples, will not emit their photons simultaneously even though they are excited simultaneously, and the rate of decay between the two statesis related to the difference in energy between them.

Time-correlated single photon counting: This method is used to analyze the relaxation of molecules from an excited state to a lower energy state. Since various molecules in a sample will emit photons at different times following their simultaneous excitation, the decay must be thought as having a certain rate rather than occurring at a specific time after excitation. By observing how long individual molecules take to emit their photons, and then combining all these data points, an intensity vs. time graph can be generated that displays the exponential decay curve typical to these processes. However, it is difficult to simultaneously monitor multiple molecules. Instead, individual excitation-relaxation events are recorded and then averaged to generate the curve. This is done by splitting a pulsed laser beam into two paths. A pulse along one path travels to a photomultiplier tube (PMT), while another path travels through the sample. The first pulse is detected by a photomultiplier tube, which activates a time-to-amplitude converter (TAC) circuit. This circuit begins to build a charge on a capacitor which will only be discharged once the PMT sends another electrical pulse to the circuit. This electrical pulse comes after the second laser pulse excites the molecule to a higher energy state, and a photon is eventually emitted from a single molecule upon returning to its original state. Thus, the longer a molecule takes to emit a photon, the higher the voltage of the resulting pulse. The central concept of this method is that only a single photon is needed to discharge the capacitor. Thus, this experiment must be repeated many times to gather the full range of delays between excitation and emission of a photon. After each trial, a pre-calibrated computer converts the voltage sent out by the TAC into a time and records the event in a histogram of time since excitation. Since the probability that no molecule will have relaxed decreases with time, a decay curve emerges that can then be analyzed to find out the decay rate of the event. A major complicating factor is that many decay processes involve multiple energy states, and thus multiple rate constants. Though non-linear least squared analysis can usually detect the different rate constants, determining the processes involved is often very difficult and requires the combination of multiple ultra-fast techniques. Even more complicating is the presence of inter-system crossing and other non-radiative processes in a molecule. A limiting factor of this technique is that it is limited to studying energy states that result in fluorescent decay.

Ultra-fast transient absorption: This method is typical of 'pulse-probe' experiments, where a pulsed laser is used to excite a molecule's electrons from their ground states to higher-energy excited states. A probing light source, typically a xenon arc lamp, is used to obtain an absorption spectrum of the compound at various times following its excitation. As the excited molecules absorb the second pulse, they are further excited to even higher states. After passing through the sample, the light from the arc lamp continues to an avalanche photodiode array, and the data is processed to generate an absorption spectrum of the excited state. Since all the molecules in the sample will not undergo the same dynamics simultaneously, this experiment must be carried out many times, and the data must be averaged in order to generate spectrums with accurate intensities and peaks. Unlike TCSPC, this technique can be carried out on non-fluorescent samples.

Time-resolved photo-electron spectroscopy: This method is very similar to Ultra-fast transient absorption, the difference being that the second laser pulse ionizes the molecule. The kinetic energy of the electrons from this process are then detected, through various different methods including energy mapping, time of flight measurements etc. As above, the process is repeated many times, with different time delays between the probe pulse and the pump pulse. This builds up a picture of how the molecule relaxes over time. A variation of this method looks at the positive ions created in this process, and is called time-resolved photo-ion spectroscopy (TRPIS)

The usage of lasers falls into two categories:

1. The first uses lasers as a convinient and highly concentrated source of photons
2. The second ultilizes the highly coherent nature of the light beam.

An advantage of using lasers for biophotonics is the use of time resolution provided by laser pulses.

Lasers: A new light source

- For many biophotonics applications a laser is simply an intense light source that is

- Monochromatic (one color or wavelength)
- A highly directional beam with low divergence
- Capable of being focused into a very small spot
- Capable of producing short bursts of light of very high intensity (in less than a trillionth of a second)

APPLICATIONS

In addition to explosives, many benign materials simulating explosives in their visible characteristics have been studied for comparison.

[1] Pharmaceutical applications: Generally **narcotic** drugs present in plasma are analysed through ultrafast spectrophotometric me explosives and potential confusant materials.

Determination of a drug candidate and its metabolite in plasma samples using ultrafast spectroscopy in combination with monolithic column high-performance liquid chromatography: An ultrafast spectroscopic method using monolithic column high-performance liquid chromatography was evaluated for the simultaneous determination of a drug discovery compound and its metabolite in plasma. Baseline separation of the two compounds was achieved with run times of 24 or 30 s under isocratic or gradient conditions, respectively. The monolithic column-spectroscopic system offers shorter chromatographic run times by increasing flow rate without sacrificing separation power for the drug candidate and its biotransformation product (metabolite). In this work, the necessity for adequate chromatographic resolution was demonstrated because the quantitative determination of the drug-related metabolism product was otherwise hampered by interference from the dosed drug compound. The chromatographic performance of a monolithic silica rod column as a function of HPLC flow rates was investigated with a mixture of the drug component and its synthetic metabolite. The assay reliability of the monolithic column HPLC/UFS system was checked for matrix ionization suppression using the post-column infusion technique. The proposed methods were successfully applied to the analysis of study plasma samples for the simultaneous quantitation of both the dosed drug and its metabolite. The analytical results obtained by the proposed monolithic column methods and the 'standard' silica particle-packed HPLC column methods were in good agreement, within 10% error.

Many packing materials also have transmissive properties, including plastics and cardboard which would allow for non-destructive imaging of packages and containers. Materials investigated include simple crystalline forms of amino acids, carbohydrates and polypeptides as well as short-chain polypeptides and serine and cystine.

[2] Probing the effect of the solution environment on the vibrational dynamics of an enzyme model system with ultrafast 2D-IRspectroscopy: Ultrafast 2D-IRspectroscopy has been applied to study the structure and vibrational dynamics of (μ-$C(CH_3)(CH_2S)_2(CH_2S(CH_2)_2Ph)Fe_2(CO)_5$, an organometallic model of the active site of the FeFe[hydrogenase]enzyme. 2D-IRspectra have been obtained in solvents ranging from non-polar to polar and protic. The influence of the solvent bath on vibrational relaxation, including rapid intramolecular population transfer has been characterized. In addition, the temporal dependence of the 2D-IR lineshape has been used to extract information relating to hydrogen bond-mediated spectral diffusion via the frequency–frequency correlation function. Comparisons with previous 2D-IR studies of hydrogenase model systems offer insights into the dependence of the rate of population transfer upon vibrational mode separation and solvent environment, with important implications for the composition and reactivity of the active site of the enzyme.

[3] Inorganic, organic, physical and analytical chemistry: The field of ultrafast spectroscopy includes the spectroscopic measurements for which electronic detectors are not fast enough to allow direct measurement of phenomena. These time scales presently range from about 10 fs to 100 ps, a period that encompasses a wealth of interesting chemical processes. In this field, the techniques and their chemical applications are inextricably tied together, and any thorough treatment must include both. In his monograph, Graham Fleming accomplishes the challenging task of fully describing both the technological and the chemical ends of ultrafast spectroscopy. Fleming is a recognized leader in the area of ultrafast spectroscopy who has made important contributions to techniques and chemical studies. The large number of chemical applications is covered in relaxation processes in vapours, in liquid phases, and in solid phases.

[4] Ultra fast Spectroscopy of Semi Conductors and Nanostructures: Ultrafast spectroscopy of semiconductors and semiconductor nanostructures is currently one of the most exciting areas of research in condensed-matter physics. Remarkable recent progress in the generation of tunable femtosecond pulses has allowed direct investigation of the most fundamental dynamical processes in semiconductors. This presents

the most striking recent advances in the techniques of ultrashort pulse generation and ultrafast spectroscopy it discusses the physics of relaxation, tunneling and transport dynamics in semiconductors and semiconductor nanostructures following excitation by femtosecond laser pulses.

[5] Liquids: Over the past few years numerous theoretical and experimental studies on photoexcitation and photodissociation in the liquid phase have been performed. In the gas phase, accurate measurements of the reactant and product in terms of how energy is distributed among electronic, vibrational and rotational degrees of freedom is at least in principle possible. However in liquids, where the solvent continuously interacts with the solute molecule in question, such detailed information is impossible. Nevertheless ultrafast spectroscopy has been successfully used to learn about dynamical and energy- transfer processes between the solute and solvent and within the solute molecules themselves. Detailed femtosecond studies of HgI2 photodissociation in various solvents have also been performed.

[6] Solids: Observation of molecular motions in the solid phase has also been achieved with femtosecond pulses. The example chosen to illustrate these observations is the impulsive stimulated Raman scattering (ISRS) of an organic crystal (a-perylene). The technique involves a sudden (impulse) driving force exerted by a short pulse on a Raman-active vibrational mode of the crystal. The force initiates coherent vibrational oscillations that are monitored by a delayed probe pulse notice the great similarity with gas and liquidphase experiments.

[7] Biological: Femto second pulse technology has made in biology as well. Ultrafast processes such as electron transfer in photosynthetic systems can now be studied; including the dynamics of primary events in photobiological systems such as the conversion of solar energy to chemical energy .One of the major goals in these studies is to learn how the energy is distributed both within the molecule and in the solvent. These studies are an important complement to structural studies of photosynthetic reaction centers, and, in particular, femtosecond studies of the vibrational transients show how the process of electron transfer and vibrational nuclear dynamics are related. Another important process in biological systems is proton transfer. Again as with electron transfer, this process is strongly dependent on the sub-picosecond solute-solvent interactions, which can now be studied with ultrafast pulse techniques. Other applications of ultrafast spectroscopic techniques to biological molecules include observation of the cis-trans photoisomerization of rhodopsin, which has been studied by examining the transient absorption spectra of an excited wave packet created by a 35-fs pump pulse.

RESEARCH ACTIVITIES OF ULTRAFAST SPECTROSCOPY

[1] Structure and dynamics of aqueous salvation shells: The dynamics of water molecules in aqueous solvation shells of ions can be studied with unprecedented selectivity using nonlinear femtosecond mid-IR spectroscopy. With this technique we observed that the hydrogen bonds between water molecules and halogenic anions fluctuate with a characteristic time constant of 10-25 ps (depending on the ion), which is 20-50 times slower than the hydrogenbond fluctuations in bulk liquid water. We also found that the water molecules in the solvation shells reorient on a time-scale that is ~3 times slower than in bulk. This reorientation is not due to rotation of the water molecules within the shell, but to orientational diffusion of the complete solvation structure. These results show that the first solvation shell of ions forms a surprisingly rigid and stable structure. In contrast, we have observed that the water dynamics outside the first solvation shell of the ion are only negligibly affected by the presence of ions. This result shows that ions do not act as structure breakers or makers of the hydrogen-bond structure of liquid water.

[2] Anomalous temperature dependence of the vibrational relaxation of water: We found that the OH stretch vibrations of water show a very rapid relaxation with a time constant of 260 femtoseconds at 300 K. With increasing temperature, this relaxation becomes slower a phenomenon which is now being acknowledged as one of the 41 anomalous properties of liquid water.

[3] Energy dynamics of single embedded water molecules: We observed that a single water molecule embedded by acetone forms two fluctuating hydrogen bonds with the C=O groups of two neighbouring acetone molecules. We found that these hydrogen-bond fluctuations can tune the O-H vibrations of the water molecule into resonance, thereby enabling energy transfer between the two OH groups.

[4] Dynamics of water in nanodroplets: We studied the dynamics of water in nanodroplets with a diameter of 1-10 nanometers (10-10.000 molecules). For a droplet with a diameter of ~1 nm, the OH-vibrational

lifetime increases by a factor of 4 to ~1 picosecond compared to the bulk. We also found that the water layer at the surface of the nanodroplet forms a very rigid structure, whereas the water in the core of even a small nanodroplet shows the same orientational mobility as bulk liquid water. This result shows that the centre of a nanodroplet of water does not show an ice-like structure as was commonly believed.

[5] Proton-transfer reactions in liquid water: We studied the mechanism of acid-base reactions in water with femtosecond visible-pump mid-IR probe spectroscopy on an aqueous system of a photoacid and an accepting base. The conventional view of this reaction is that the acid and the base have to diffuse into direct contact to enable proton transfer. However, we found that proton transfer occurs primarily via., Grotthuss conduction, through a hydrogen-bonded 'water wire' of 2-4 water molecules which connects the photoacid with the base. We also found that the excitation of the second excited state of the OH stretch vibration leads to a strong delocalization of the hydrogen atom along the O-H...O hydrogen bond between two water molecules. An important implication of this finding is that this second excited vibrational state forms, energetically, the most favourable transition state for the autodissociation of water, i.e. the process in which two water molecules split spontaneously into H3O+ and OH-.

SUMMARY AND CONCLUSION

In the present, there is always need to develop a new technique to achieve results accurate and fast. Ultrafast Spectroscopic technique was developed considering the fact for time resolution. Molecules possess vibrational and rotational degrees of freedom in adition to their internal electronic motion. Spectroscopy is the primary tool in elucidating the energy-level spacing. Ultrafast spectroscopy, on the other hand involves temporally short light pulses which are used to probe directly the dynamics of the system rather than the energy levels themselves. It resulted in the ability to produce pulses as short as 6 fs (I fs = 10-15 s), and pulses of width 60 fs. This technique includes research from departments of physics, chemistry, electrical engineering, and biology and material science. Its time scales presently range from about 10 fs to 100 ps. The femtosecond pulses are amplified by a multipass Ti:sapphire amplifier. The absorption pump-probe system is designed to measure time resolved spectra at single shot or averaging hundreds of shots. A set of homemade programs is provided for the quantitative analysis of the time resolved measurements. The interaction between a protein and a photon lasts from 1-106fs. A laser that emits ~100fs pulses can investigate such a process. Detailed femtosecond studies of HgI2 photodissociation in various solvents have also been performed. Structural, Dyanamic and Vibratiational parameters of aqeous solvation shells are uder research.

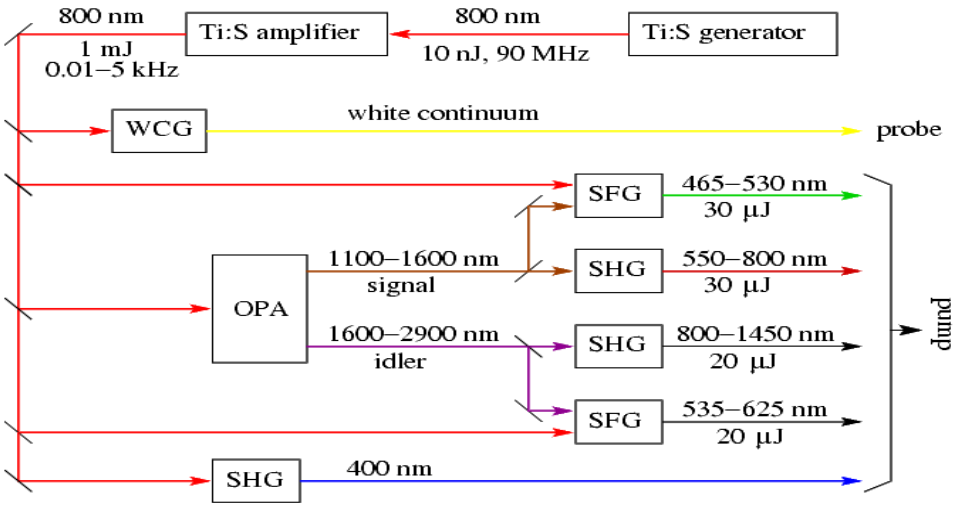

Figure 1: Scheme of the laser system. WCG - white continuum generator, OPA - optical parametric amplifier, SHG - second harmonic generator, SFG - sum frequency generator

Up-conversion instrument:

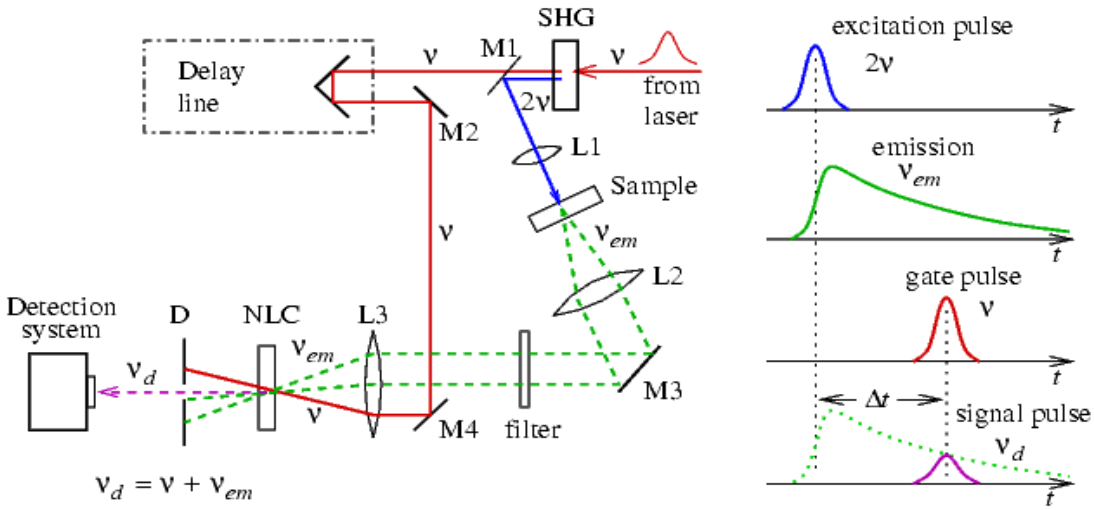

Figure 2: Optical scheme of the up-conversion instrument. The input pulses at the system entrance are fundamental radiation of Ti:sapphire generator (see laser system), which can be tuned in the wavelength range 750-950 nm, thus providing excitation in the range 375-475 nm after second harmonic generator (SHG).

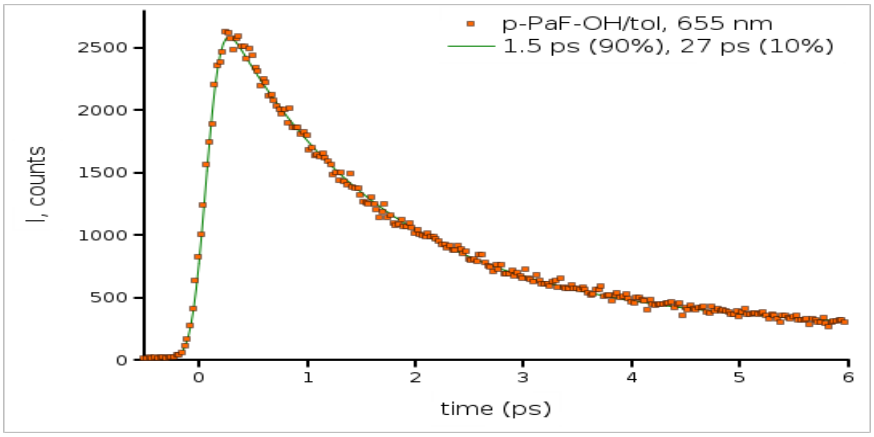

Figure 3: Emission decay of a phytochlorin-fullerene dyad in toluene. The sample was excited at 410 nm and emission was monitored at 655 nm. The dots present measured data points and solid line the bi-exponential fit.

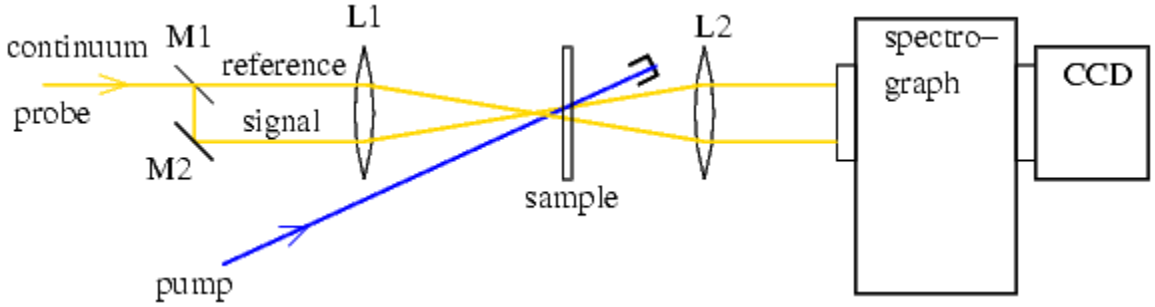

Figure 4: Optical scheme of the pump-probe absorption system. The pump pulse can be the second harmonic of fundamental pulses (λ_{ex} = 380-420 nm) or pulses generated by OPA (see Fig. 1). The white continuum for probe and reference is generated using a part of fundamental radiation.

REFERENCES

1. Bartels R, *et al*. Shaped-pulse optimization of coherent emission of high-harmonic soft X-rays.Nature, 2000; 406: 164-6.
2. Mittleman DM, Jacobsen RH, Neelamani R, Baraniuk RG, Nuss MC. Gas sensing using terahertz time-domain spectroscopy. Appl. Phys. B: Lasers Opt., 1998; 67: 379-90.
3. Manceau JM, *et al*. Terahertz pulse emission optimization from tailored femtosecond laser pulse filamentation in air. Optics Lett., 2009; 34: 2165-7.
4. Rohwetter P, *et al*. Remote LIBS with ultrashort pulses: characteristics in picosecond and femtosecond regimes. J.Anal. Atom. Spectrom., 2004; 19: 437-44.
5. Korter TM, Balu R, Campbell MB, Beard MC, Gregurick SK, Heilweil EJ. Terahertz spectroscopy of solid serine and cysteine. Chem. Phy. Lett., 2006; 418: 65-70.
6. Beard, M.C.; Turner, G.M.; Schmuttenmaer, C.A. Terahertz spectroscopy. J. Phys. Chem. B 2002,106, 7146-7159.
7. Dutta P, Tominaga K. Terahertz time-domain spectroscopic study of the low-frequency spectra of nitrobenzene in alkanes. *J. Mol. Liq.*, 2009; *147*: 45-51.
8. Zewail AH. Femtochemistry-Ultrafast Dynamics of the Chemical Bond, Singapore: World Scientific, 1994.
9. Hashe T, Ashworth SH, Riedle E, Woener M, Elsaesser T. Chem. Phys. Lett. 1995; 244.