Ultrafast Spectroscopy of Photosynthetic Systems

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Summary

This article discusses the use of fluorescence upconversion and transient absorption techniques for the study of photosynthetic systems. A description is given of the state-of-the-art laser systems available for ultrafast studies, along with examples of some common techniques for extending the wavelength range of these lasers. The experimental techniques of fluorescence upconversion and transient absorption are introduced, with the goal of describing the implementation, versatility, and limitations of these experiments. Recent experimental results are presented which illustrate applications of ultrafast spectroscopy to studies of energy transfer in light harvesting complexes and electron transfer in bacterial reaction centers.
I. Introduction

The elementary electron and energy transfer steps in photosynthesis occur on picosecond or subpicosecond timescales. Thus ultrafast spectroscopy has played, and continues to play, a key role in characterizing these highly efficient processes. In this chapter we focus on the two most common spectroscopic techniques: time resolved absorption and fluorescence spectroscopies. Over the past ten years or so, a formidable array of nonlinear spectroscopic techniques have been developed as have extensions into the infrared spectral region. The reader is referred to recent literature for details of these techniques (Diller et. al., 1992; Maiti et. al., 1993, Durrant et. al., 1994).

Solid state lasers, in particular Ti:sapphire lasers have greatly simplified the practice of, and extended the capabilities of ultrafast spectroscopy. We describe apparatuses based only on such lasers in the belief that dye laser-based systems will become obsolete within the next five to seven years.

One aspect of ultrafast spectroscopy that contrasts it with longer timescale spectroscopy should be mentioned. Femtosecond pulses are shorter than the periods of some molecular vibrations; in other words, "vibrationally abrupt" (Scherer et. al., 1993; Jonas et. al., 1994a,b). For example, a 50 fs pulse is abrupt with respect to a 100 cm$^{-1}$ vibration. In these cases, vibrational quantum beats may be observed in the pump probe or spontaneous emission signals. The beat frequencies allow measurement of vibrational frequencies in absorption spectra that are quite devoid of structure. It may, however, be quite tricky to assign the frequencies to the ground or excited state and to be sure that the fundamental frequency is being observed. Jonas et. al. (1994) and Scherer et. al. (1993) discuss these points in detail. The rate of disappearance of these beats reflects the timescale on which the environment perturbs the energy levels of the chromophore. A fascinating recent development in ultrafast photosynthesis research is the observation of coherent nuclear motion in bacterial reaction centers by Martin and coworkers (Vos et. al., 1993). The beats are assigned to the excited state and suggest that vibrational dephasing may be incomplete on the timescale of the primary charge separation.

The outline of this chapter is as follows. First, the newest laser sources are described. Various methods of extending the tuning range of these sources over the ultraviolet, visible, and
near-infrared regions are then outlined. Next, the principles of fluorescence upconversion are described, along with a discussion of the optical arrangements used for these experiments. Applications of fluorescence upconversion for monitoring the rates of energy transfer in bacterial and plant light harvesting systems, and measuring the rate of the primary charge separation in bacterial reaction centers are discussed. Finally, the experimental methodology of transient absorption is described, along with two applications of this technique to studies of bacterial reaction centers.

II. Laser Sources

A. Modelocked Ti:sapphire lasers

Modelocked Ti:sapphire oscillators and amplifiers represent an enormous simplification over the traditional femtosecond dye laser/amplifier arrangements. Additionally, modelocked Ti:sapphire oscillators can directly generate sub-20 fs pulses (Huang et. al., 1992a,b; Asaki et. al. 1993). This performance could only be achieved with a dye laser after amplification, continuum generation and pulse compression. The cavity arrangement of a typical self-modelocked Ti:sapphire laser, given schematically in Figure 1, shows the simplicity of these lasers. The oscillator is usually pumped by 5-8 Watts from a small frame argon ion laser, and modelocking is initiated by some mechanical disturbance of the cavity. Short pulse generation has been demonstrated from near 700 nm to above 1000 nm with essentially the same cavity design. These lasers produce pulses with 1-6 nJ energy at a repetition rate around 80 MHz, with pulses as short as 11 fs. Cavity dumping of these oscillators has also been demonstrated, giving 40-60 nJ pulse energies at hundreds of kHz repetition rate (Pschenichnikov et. al., 1994). No increase in pulse duration results from cavity dumping. Extremely low noise (< 0.1% RMS) and high long term stability combined with these desirable pulse durations make these lasers ideal sources for the study of near-IR photosynthetic systems, e.g. purple bacteria, green bacteria, and heliobacteria.

B. Ti:sapphire Amplifiers

For many applications, the tuning range of these lasers must be extended into the visible. There are several techniques for doing this, but higher pulse energies are usually necessary. Furthermore, lower repetition rate, higher pulse energy sources are often required for experiments in which the sample recovery time is longer than the modelocked frequency. Ti:sapphire
amplifiers are most easily categorized by their repetition rates. It should be noted that all three
types of amplifiers are available commercially.

The first category is that of Nd:YAG pumped amplifiers. The pump source is the second
harmonic of a Q-switched Nd:YAG laser. These amplifiers typically give hundreds of millijoules
to several joules of pulse energy, at 10-20 Hz repetition rate. Amplified pulses as short as 21 fs
have been obtained (Zhou et al., 1994). A pulse is selected from the oscillator, temporally
stretched (in order to prevent damage to the amplifier due to high peak power) by a grating,
routed into the amplifier cavity via electro-optic switching, and dumped out of the cavity in the
same way after several round trips. A grating compressor is used after amplification to restore
the pulse duration.

The second type of Ti:sapphire amplifier is pumped by the second harmonic of a Q-
switched Nd:YLF laser. These regenerative amplifiers operate at 1-10 kHz repetition rates and
typically utilize pre-amplification pulse stretching, electro-optic switching, and post-amplification
compression (Salin et al., 1991; Squier et al., 1993). These systems yield from tens of
microjoules to one millijoule pulse energies, with pulse durations down to 30 fs (Wynne et al.,
1994). The layout of a system designed and built in our laboratory which does not employ a
pulse stretcher is shown in Figure 2 (Joo et al., 1994).

The third type of amplifier system is the high repetition rate regenerative amplifier which
is pumped with 14-15 Watts from a CW ion laser (Norris, 1992). In this amplifier, the pulse is
injected and ejected acousto-optically (electro-optic devices are limited to 10 kHz or lower). Pulse
stretching prior to amplification may be employed, but more commonly the pulse is stretched by
dispersive elements within the amplifier cavity. As usual, the pulse is recompressed after
amplification. These amplifiers typically give 1-5 microjoule pulse energies, at repetition rates
from 50 kHz to 300 kHz. Pulses as short as 85 fs have been reported (Sosnowski et al., 1994).

C. Methods of extending the tuning range

A variety of methods may be employed for shifting the frequency of ultrafast laser
sources. One of the simplest methods is harmonic generation. Second and third harmonic
generation of Ti:sapphire wavelengths is commonly done with LBO and BBO crystals. The
wavelength range from 250 nm to 320 nm and 360 nm to 480 nm may be accessed in this way.
Another important technique is continuum generation. When an amplified femtosecond pulse
(pulsewidth < 200 fs and energy > 200 nJ) is focused into a transparent material (e.g. sapphire), the high intensity causes self focusing and subsequent self-phase modulation which creates a stable white light continuum extending across the entire visible spectrum. For pulse energies less than 2 microjoules, this produces a high-quality Gaussian beam at all wavelengths throughout the visible and near infrared. Typically, 1-5 nJ energy is produced for a given 10 nm bandwidth. The color may be selected with an interference filter, providing enough light to serve as the excitation pulse for a fluorescence upconversion experiment, or the probe pulse for a transient absorption experiment. Selected portions of the continuum may be amplified by optical parametric amplification (Reed et al., 1994). One way of doing this involves temporally and spatially overlapping a portion of the continuum into a BBO crystal, along with an intense (1 microjoule or more) pulse of second harmonic from an amplified Ti:sapphire beam. With a 250 kHz amplifier, it has been demonstrated that tens of nanojoules per pulse can be produced across the visible spectrum.

III. Fluorescence Upconversion

A. Technique

The principle of the fluorescence upconversion method is that a short pulse of light excites a sample whose fluorescence is focused into a nonlinear crystal along with a variably delayed 'gate' pulse, and the sum frequency is detected as a function of the delay between the two pulses (Figure 3). Rotation of the crystal determines the wavelength of fluorescence upconverted. The advantage of using this gating technique is that the time resolution of the experiment is determined by the width of the pulses (see below), not by the time resolution of the detection system. Detailed descriptions of the upconversion method were given by Shah (1988) and Kahlow et al. (1988).

The upconverted signal is usually detected by a photomultiplier tube used with a photon counter. A double monochromator is very helpful because despite the non-collinear geometry, the signal is contaminated with doubled gate beam and doubled excitation beam, both of which are orders of magnitude more intense than the upconverted signal. A prism may also be used for dispersing the upconverted light prior to the monochromator. The use of nonlinear crystals which require orthogonally polarized interacting beams (Type II phase-matching), are also helpful in this sense, in reducing the background due to one beam signals. Two optical layouts for the
upconversion experiment are shown in Figure 4. The setup with the elliptical mirror is the easiest
to align. However, the scheme with the parabolic mirrors is more flexible since there is enough
space to insert a small cryostat. In either arrangement, the excitation beam may be aligned so that
it does not hit the reflector, and fluorescence is collected at a small angle off-axis from the
excitation beam. This technique makes it easier to measure fluorescence wavelengths near the
excitation wavelength without upconverting the excitation beam.

The time resolution of the upconversion experiment is determined by the instrument
response function (IRF), which is approximately given by the cross-correlation of the excitation
pulse with the gate pulse:

\[ I_{IRF}(\tau) = \int I_{EXC}(t) * I_{GATE}(t-\tau) dt \]

where \( I_{EXC}(t) \) and \( I_{GATE}(t) \) are the intensity profiles of the excitation and gate pulses, respectively.
Operationally, the IRF is measured by angle-tuning the crystal to upconvert transmitted or
scattered excitation light. Utilizing the setup of Figure 4b, Du, Xie and coworkers (1992) have
achieved a 70 fs IRF.

For pulses appreciably shorter than 100 fs, crystals much thinner than 1 mm are required.
The sum-frequency is generated at all points through the thickness of the nonlinear crystal where
the gate pulse and fluorescence (or excitation pulse) are temporally and spatially overlapped. But
the group velocity mismatch between the fluorescence and gate beam wavelengths causes
broadening of the IRF. For example, Du, Xie and coworkers (1992) calculate the group velocity
mismatch for their 0.4 mm LiIO, crystal to be 115-130 fs when upconverting 940 nm
fluorescence with a 608 nm gate pulse. This broadening needs to be added to the measured cross-
correlation in considering the time resolution of the measurement (Shah, 1988).

Thus, limitations of the upconversion technique make experiments with IRFs of
significantly less than 100 fs difficult, even with the availability of shorter pulses. Furthermore,
the necessity of using thin crystals with large upconverted bandwidths causes a difficulty in
discriminating fluorescence from scattered or transmitted excitation beam. This contamination of
the signal can be very difficult to overcome when measuring fluorescence much closer than 30-40
nm from the excitation wavelength. Fluorescence upconversion measurements with high time resolution (<100 fs) are most readily performed on samples with large Stokes shifts. Unfortunately, this is not usually the case for chlorophylls in, for example, light harvesting systems.

Many fluorescence upconversion measurements resolve the isotropic emission from a sample in order to monitor the appearance or lifetime of an emitting species. In contrast, depolarization measurements can be used to resolve dynamics within a fluorescence band, such as energy transfer. Performing these measurements requires the addition of a polarizer to each beam and a waveplate for rotating the polarization of the excitation beam. In addition, it must be verified that the optical arrangement preserves the anisotropy of the fluorescence by ensuring that the anisotropy of a standard system (such as Rb. sphaeroides R26 reaction centers) is 0.4. A further complication in these studies is the presence of singlet-singlet and singlet-triplet annihilation processes whenever high excitation densities and/or high repetition rates are used (van Grondelle, 1985). These processes shorten the fluorescence lifetime of pigment-protein complexes, and may affect depolarization measurements when the systems are highly ordered.

Low temperature fluorescence upconversion studies have not yet become common. Signal averaging considerations make fluorescence upconversion most convenient with medium and high repetition rate laser sources. But biological samples are easily damaged, and the sample must be moved throughout the experiment. The challenge of refreshing the sample volume excited by the beam has not been easily met. Stanley and Boxer (1994) have measured fluorescence decays of bacterial reaction centers at 80 K by mounting a light (4 oz.) Joule-Thompson refrigerator (MMR Technologies) on an audio speaker, in an optical arrangement similar to that shown in Figure 4b.

B. Results

The first example of a fluorescence upconversion experiment concerns the dynamics of primary charge separation in the reaction centers of purple bacteria such as Rhodobacter capsulatus and Rhodobacter sphaeroides. The timescale for electron transfer from the primary donor, an excitonically coupled dimer of bacteriochlorophyll a (denoted P*) to the acceptor bacteriopheophytin is around 3 picoseconds at room temperature. This rate was measured by a number of groups by monitoring the decay of stimulated emission from P* (for example, Martin
et. al., 1986). In measurements of the decay of P* by stimulated emission, most workers have made measurements at or near the isosbestic point in the spectrum consisting of ground state bleaching and absorption of the radical cation of P (P*) and P*. However, such a procedure makes it difficult to observe longer decay components in the stimulated emission. Fluorescence upconversion measurements performed by Du and coworkers clearly showed behavior which had been difficult to observe in pump-probe experiments. After exciting the special pair's Q_x or Q_y bands and time resolving the P* emission at 940 nm, Du, Rosenthal and coworkers (1992) observed non-exponential decay kinetics. An example of a P* decay is shown in Figure 5. Attempts to explain this observation have ranged from considerations of heterogeneity in the protein environment of the reaction center to models based on protein fluctuations on the time scale of the electron transfer. (Gehlen et. al., 1994; Jia et. al., 1994)

A second application of fluorescence upconversion to photosynthesis is to the study of energy transfer in light harvesting systems. Energy transfer amongst differently oriented but otherwise similar or identical chromophores is conveniently monitored via fluorescence depolarization. In a series of polarized light experiments, Du and coworkers (1993, 1994) have measured the depolarization of fluorescence from membranes of PSI-only and LHC-II-only strains of Chlamydomonas reinhardtii. The average single-step energy transfer in PS-I was observed to occur in 200 fs. The depolarization process in LHC-II, on the other hand, was observed to be highly nonexponential, spanning 2 orders of magnitude from hundreds of femtoseconds to tens of picoseconds (see Figure 6).

As a final example of time resolved fluorescence studies of photosynthetic systems, we consider the energy transfer processes in bacterial light harvesting complexes. The purple photosynthetic bacteria contain two types of light harvesting complexes which channel excitations to the photochemical reaction center. In Rhodobacter sphaeroides, these complexes are thought to be built from dimers of bacteriochlorophyll a, along with the carotenoid spheroidene. The LH-I complex is closely associated with the reaction center in a ringlike structure, and the LH-II complexes form pools which connect LH-I/RC structures. Recently, depolarization measurements have resolved the single-step hopping of electronic excitation amongst the bacteriochlorophylls in LH-I (van Mourik et. al., 1994). The anisotropy of 940 nm fluorescence decays from a value around 0.3 to 0.1 with a 350 fs exponential time constant. The low value of the initial anisotropy
(0.3 as opposed to 0.4) may be a result of a distribution of energy transfer rates which result in a non-exponential depolarization process. As an example of an unusual anisotropy decay, Figure 7 shows the decay of anisotropy from the bacteriochlorophyll fluorescence upon excitation of spheroidene in LH-I (Bradforth et al., 1994). This type of decay (starting near zero and going to a negative value) can result from energy transfer when the long axis of the spheroidene is tilted 30 degrees and 46 degrees with respect to the normal of the plane containing the transition moments of two bacteriochlorophyll dimers.

IV. Transient Absorption

A. Technique

Transient absorption methods are applicable to a far wider range of systems than fluorescence upconversion. The fact that several electronic states may contribute to the signal and that the temperature dependence of the signals can be rather subtle has recently been discussed by Jonas et al. (1994a,b). Conceptually, the experiment involves monitoring the intensity of a weak probe beam transmitted through a sample subsequent to the passage of a much stronger pump beam. The differential absorbance of the sample is monitored by a detector, and this signal is plotted as a function of delay between the pump and probe pulses.

An experimental arrangement for pump-probe spectroscopy is shown in Figure 7. The transmitted probe beam may be detected by a diode, or multi-channel detection may be used in order to frequency resolve the signal. Lock-in detection may be used with high repetition rate sources, detection being referenced to a chopper placed in the pump beam. Experiments with low repetition rate sources cannot efficiently utilize lock-in detection, so this type of measurement can suffer from high background levels. Shot-by-shot normalization of the signal to compensate for fluctuations in the laser power can also be employed, and is useful both for low repetition rate experiments, and for experiments in which a portion of white light continuum serves as the probe beam. Multi-color pump probe experiments require care to ensure that the pump and probe beams are focussed at the same spot in the sample. Pump-probe experiments can easily be performed at low temperatures (with low repetition rate lasers), and in contrast to fluorescence upconversion, many studies have been reported at cryogenic temperatures.

B. Results
Extensive measurements of the rates of primary charge separation in reaction centers from *Rhodobacter capsulatus* have been performed at room temperature, and low temperatures, by Jia and coworkers (1994). A series of mutants was constructed in which the amino acid residues at sites L181 and M208 were modified in order to change the free energy of the electron transfer reaction. The redox potential of each mutant was measured electrochemically, and the rates of electron transfer were measured via monitoring the stimulated emission from P*. The fastest rate measured was for a mutant containing a tyrosine in place of the wild type pheophytin at position L181 (< 3 ps) and the slowest rate was for the Thr(L181)-Thr(M208) mutant (around 30 ps). An example of their data is displayed in Figure 9. The temperature dependence of the rates was also studied. A model based on a distribution of electron transfer rates arising from a distribution in free energy gaps was used to fit the data in order to estimate the reorganization energy arising from low-frequency protein and intermolecular modes coupled to the electron transfer.

A remarkable series of pump-probe measurements by Martin and coworkers (Vos et. al., 1993) suggest the need to re-examine standard models of electron transfer for the primary charge separation step. Using the D_{LL} mutant of *Rhodobacter capsulatus* Martin and coworkers observed oscillations in the 77 K pump-probe signals (Figure 10) which they interpret as arising from vibrational wavepackets in the excited state. These oscillations persist for many picoseconds, and indicate that vibrational coherence persists on the timescale of the primary charge separation. The D_{LL} mutant does not undergo electron transfer due to the absence of the acceptor pheophytin, but in more recent studies, the same authors have shown that these oscillations are present in pump-probe signals from *Rhodobacter sphaeroides* reaction centers in their native membrane environment (Vos et. al., 1994). Observation of an oscillatory contribution to the signal calls into question the conventional assumption that vibrational dephasing and relaxation occur on much shorter timescales than does the electron-transfer step. A full description of electron transfer incorporating a realistic model of the protein dynamics is still an active area of theoretical development (Jean et. al., 1992; Skourtis et. al., 1993).

V. Concluding Remarks

Improved precision in ultrafast spectroscopy will allow more sophisticated data analysis such as singular value decomposition (Woodbury et. al., 1994) to determine the number of components in a transient spectrum. With improved precision, decays will in many cases no
longer be satisfactorily fit as single exponential processes and more complex models will be required to fit data and extract underlying system parameters. It seems likely that four and six wave mixing techniques such as the various echo methods (Nibbering et. al., 1991; Cho et. al., 1993, 1994) will be increasingly applied to photosynthetic systems, to reveal dephasing timescales and the dynamics of spectral diffusion over wide dynamic ranges.

References


Figure Captions

Figure 1  A typical layout for a self-modelocked Ti:Sapphire oscillator. The pump beam enters through one of the curved mirrors around the laser crystal. The gain medium is from 4-20 mm length, and two prisms are placed in the cavity to compensate the dispersion of the laser rod, thus controlling the pulse duration. A high reflector (HR) is at one end of the cavity, and a 5-10% output coupler (OC) at the other end.

Figure 2  A medium repetition rate regenerative amplifier designed and built in our laboratory (Joo, et. al., 1994). The pump laser provides 5-10 mJ of 537 nm light, at 4 kHz. Pulses from the oscillator are temporally stretched by the 4 prisms inside the amplifier cavity during the amplification process. The pockels cell (PC) switches the amplified pulse out of the cavity after 15-20 round trips. The Faraday rotator (FR) ensures that the amplified pulse is diverted by the polarizing beamsplitter cube (PBC) into the grating compressor, where it is compressed to 60 fs duration. CM: curved mirrors, L: lens, TFP: thin film polarizer, RR: retro-reflector.

Figure 3  Schematic of the fluorescence upconversion process. The emission from the sample (the exponential decay) begins at time $t_1$, subsequent to short-pulse excitation. At later times ($t_2$ or $t_3$) the sum frequency is generated only during the time that the delayed gate pulse is present. The upconverted signal (the shaded area) is proportional to the intensity of the fluorescence at that time delay. Thus, the fluorescence is sampled with a time resolution comparable to the pulse width.

Figure 4  Two possible optical arrangements for fluorescence upconversion. (a) An elliptical reflector is used to focus the emission. The 1 mm path length sample cell is placed at one focus of the ellipse, the upconversion crystal at the other focus. The foci are typically separated by 10 cm. The excitation beam copropagates with the fluorescence. (b) Two off-axis parabolic mirrors are used to collect the fluorescence. The first parabolic mirror collects the fluorescence and collimates it, and the second parabolic reflector focuses the fluorescence into the crystal. The excitation beam does not hit the reflector. In either arrangement, the sample is either flowed, or stirred by a platinum wire attached to an electric toothbrush.

Figure 5  Spontaneous emission decay from deuterated reaction centers of Rb. Sphaeroides (R26) excited at 850 nm. The decay of P* emission reflects the depopulation of due to electron transfer to the bacteriopheophytin. This experiment was performed with a modelocked Ti:sapphire laser (Coherent Mira 900F). The data shown is the sum of 5 scans. Fit parameters (Rosenthal et. al., unpublished): 2.7 ps (80 %), 12 ps (20%).
Figure 6  Fluorescence depolarization of LHC-II with excitation at 650 nm and detection at 710 nm. The solid lines are fitted curves and dots represent data. The top panel shows the raw anisotropy data and the fit, and the bottom panel shows parallel (top) and perpendicular (bottom) decay data (dots) and the fitted curves (solid lines). Fit parameters (from Du et al., 1994) \( r(t) = 0.15 \exp(-t/0.25 \text{ ps}) + 0.10 \exp(-t/11 \text{ ps}) + 0.05 \).

Figure 7  Fluorescence depolarization of 910 nm emission from LH-I. Excitation of the spheroidene pigment at 505 nm was provided by a 20 nm segment of a white light continuum (0.5 nJ/pulse) at 250 kHz. The gate pulse (820 nm) energy was 200 nJ. The experiment was performed with an optical arrangement similar to that of Figure 4a. Fit parameters:

Figure 8  A pump-probe apparatus based on a medium repetition rate Ti:sapphire regenerative amplifier. In the arrangement shown, the second harmonic is used to create the species of interest from a precursor. The photoproduct is studied with an IR pump pulse, and with sections of a white light continuum for the probe pulse. The continuum is generated in a 1 cm thick spinning quartz disk (CG), and the color is selected with a variable interference filter (CVF). The pump-probe signal is normalized to the pump and probe pulse intensities. P polarizer, PC Pockels cell, BS beam splitter, λ/2 half wave plate, FR Faraday isolator, PD photodiode. (Figure courtesy of PF Barbara, Univ. of Minnesota)

Figure 9  Stimulated emission decay of P* from the Thr(L181)-Thr(M208) mutant of Rb. Capsulatus. Fit parameters (from Jia et al., 1993): 8.10 ps (52%), 56.3 ps (48%). The experiment was performed with a 20 Hz amplified dye-laser system.

Figure 10  Decay of the excited state of the special pair of the Dll mutant, produced by stimulated emission from a probe beam, shows that no electron transfer occurs in this mutant. The oscillations result from vibrational wavepackets created by the excitation pulse. Because of the coherent vibrational motion, the probability for interaction with the probe light shows a periodic modulation whose phase and amplitude depend on the wavelength of the probe light. The 80 fs pump pulse and 30 fs probe pulse were derived from amplified sections of white light continuum produced by an amplified 20 Hz dye laser system. (Figure courtesy of JL Martin, ENSTA Palaiseau)
FLUORESCENCE UP-CONVERSION

\[ S(\tau) = \int_{0}^{\tau} F(t)P(t - \tau) \, dt \]

\[ F(t) = \int_{0}^{t} f(t')P(t' - t) \, dt' \]
Fluorescence Upconversion with Elliptical Reflector

Excitation Beam

Gate Beam

1 mm Path Length Cell

$\omega_1$

Upconversion Crystal

Collimating Lens

$\omega_1 + \omega_2$

Double Monochromator

PMT
Off-Axis Collection Employing Off-Axis Parabolic Reflectors
LHI raw anisotropy, 600 fs decay

$R(t)$ vs. Time (ps)

-0.10
-0.05
0.00
0.05
0.10

0.0  0.5  1.0  1.5  2.0  2.5  3.0