Single-shot ultrabroadband two-dimensional electronic spectroscopy of the light-harvesting complex LH2

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Here we present two-dimensional (2D) electronic spectra of the light-harvesting complex LH2 from purple bacteria using coherent pulses with bandwidth of over 100 nm FWHM. This broadband excitation and detection has allowed the simultaneous capture of both the B800 and B850 bands using a single light source. We demonstrate that one laser pulse is sufficient to capture the entire 2D electronic spectrum with a high signal-to-noise ratio. At a waiting time of 800 fs, we observe population transfer from the B800 to B850 band as manifested by a prominent cross peak. These results will enable observation of the dynamics of biological systems across both ultrafast (<1 ps) and slower (>1 ms) timescales simultaneously. © 2011 Optical Society of America

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Femtosecond spectroscopy has successfully elucidated vibrational and electronic dynamics in a wide range of complex systems from semiconductors to proteins [1–3]. For systems consisting of multiple light-absorbing units or chromophores, disentangling effects of electronic or vibrational coupling, relaxation, and inhomogeneous broadening becomes increasingly difficult using one-dimensional methods. Following in the footsteps of nuclear magnetic resonance (NMR), two-dimensional (2D) optical spectroscopy has evolved over the last decade into a powerful probe of molecular structure, electronic coupling, energy transfer, and solute-solvent dynamics in the IR and visible regions of the electromagnetic spectrum [4–6]. Unfortunately, 2D coherent spectroscopy remains a specialized field because of the difficulty in maintaining the requisite phase stability among the optical pulses. Unlike magnetic resonance where waveform generation permits near-arbitrary phase and temporal control, multidimensional optical techniques require adjustments of optical path lengths to subwavelength accuracy. In the optical regime, small variations in path length due to environmental factors and mechanical noise lead to phase errors that are detrimental to the retrieved 2D spectrum. While several research groups have overcome these difficulties using specialized components such as pulse shapers [7,8], diffractive optics [9], and active feedback [10,11] among others, the field of multidimensional spectroscopy remains highly specialized.

Recently, we have demonstrated a method that circumvents the need for long-term phase stability by utilizing a temporal encoding of the field-matter interactions in space. This method, which we call gradient-assisted photon echo (GRAPE) spectroscopy, has allowed us to acquire the entire 2D spectrum of a laser dye 2–3 orders of magnitude faster than by "conventional" point-by-point methods without any sacrifice in signal and with lower overall noise [12]. In these initial reports, we averaged around 1000 laser shots on the CCD to generate a high signal-to-noise ratio (SNR). In this report, we demonstrate sufficient detection sensitivity to acquire the signal in a single laser pulse which completely eliminates all errors caused from phase instability. We demonstrate this capability by acquiring the 2D photon echo spectrum of the photosynthetic complex, LH2, from purple bacteria at room temperature. In addition, because of the single-shot nature of the GRAPE technique, we are able to utilize unstable coherent light sources to serve as both pump and probe pulses. Using supercontinuum generation, we extend the spectral bandwidth of our Ti:sapphire laser system to over 100 nm FWHM from 30 nm FWHM, sufficient to capture the dynamics between the B800 and B850 rings of the LH2 complex.

As shown in Fig. 1, the absorption spectrum of LH2 consists of two prominent bands at roughly 800 and 850 nm. These bands are attributed to two rings composed of bacteriochlorophyll molecules sandwiched between the alpha and beta apoproteins of the LH2 nonamer [13]. The B800 ring consists of nine BChls that lie parallel to the membrane plane, while the B850 ring consists of 18 strongly-coupled BChls that lie perpendicular to the

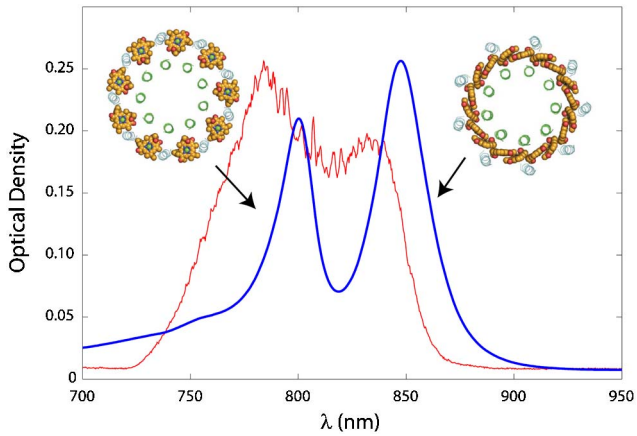

Fig. 1. (Color online) Linear absorption spectrum (blue) of LH2 complex from *Rh. sphaeroides* is shown with a spectrum of the continuum excitation pulse (red). Each band in the spectrum corresponds to one of two concentric rings consisting of BChl pigments sandwiched between protein subunits.

membrane plane. The LH2 complex serves as a broadband accessory antenna to absorb energy from the sun and transfer excitation to the reaction center (RC) where charge separation occurs. Light absorbed at 800 nm is funneled from the B800 ring to the B850 ring before transfer to the LH1/RC complex.

To capture population transfer between these two rings with 2D photon echo spectroscopy, our laser bandwidth must exceed the typical output of a Ti:sapphire regenerative amplifier system, which is roughly 30 nm FWHM. To increase the bandwidth of our excitation, we employ a supercontinuum light source from which we select the spectral range of 750–850 nm. Typically, such a light source would be detrimental to Fourier transform spectroscopy due to poor power and spectral stability. However, using the GRAPE approach, shot-to-shot fluctuations are eliminated by capturing the 2D spectra in one laser shot.

The principles of GRAPE spectroscopy are shown schematically in Fig. 2. A single 6 mm diameter pulse from the output of a Ti:sapphire laser (40 fs, 5 kHz, 800 nm) is focused into a flowing stream of argon gas with a backing pressure of approximately 5 psi to generate a supercontinuum of coherent light. After collimation, the continuum pulse is compressed to under 25 fs using chirped mirrors. The pulse is split into two beams by a beam splitter with a variable delay, T, introduced between the pulses by a standard delay stage. Each of these beams is further split using Fresnel reflections from uncoated glass wedges. The four resulting beams impinge on a mirror assembly in a distorted boxcar geometry. The energy density at the sample was measured at approximately 200 μJ/cm^2 per pulse to avoid multiexciton generation [14].

The timings between pulses were determined using spectral interferometry as described by Lepetit and Joffre [15]. Using nonresonant transient grating measurements, we found no evidence for spatial chirp, an important prerequisite for the GRAPES method. The timing of beam 1 with respect to beam 2 was set to create a temporal gradient from approximately −200 fs to 400 fs across the beam waist, corresponding to approximately 0.6 fs/pixel at the detector. We create a larger gradient across the beam waist than is required by the maximum rephasing time to avoid artifacts caused by the near-Gaussian intensity profile of the pulse wavefronts. Here, the intensity profiles of all beams are relatively flat near the 0 fs to 150 fs region of the spatially encoded temporal gradient. Beam 3 was aligned parallel to beam 2 to keep population time constant for each single-shot experiment. Finally, beam 4 was aligned to have an opposite gradient relative to the sample compared to beam 1, in accordance with the necessary phase-matched geometry. These four beams define the photon echo pulse sequence shown in the upper right portion of Fig. 2. The first pulse, $\mathbf{k}_1$, creates a single-quantum coherence between ground and excited states of the molecule. After a variable delay time, τ, a second pulse, $\mathbf{k}_2$, converts the quantum state of the system into a zero-quantum coherence that reflects population dynamics during a waiting time, T. Finally, a third pulse, $\mathbf{k}_3$, excites the system back into a single-quantum coherence but with opposite phase relative to the coherence created during the first evolution period. This particular phase matching choice in the $-\mathbf{k}_1 + \mathbf{k}_2 + \mathbf{k}_3$ direction creates a macroscopic polarization in the sample which is emitted as an echo signal. The signal is then interfered with a local oscillator pulse to retrieve the complex phase evolution during the coherence times. The interference pattern is then spectrally resolved and detected by a CCD camera. The signal, which is now emitted as a line, is imaged onto the slit of the spectrometer and a 2D map $S(\tau, T, \lambda)$ is recorded. After suitable data analysis that includes removing homodyne components of the signal, Fourier transformation about the coherence time axis results in a 2D photon echo spectrum $S(\omega_\tau, T, \omega_t)$.

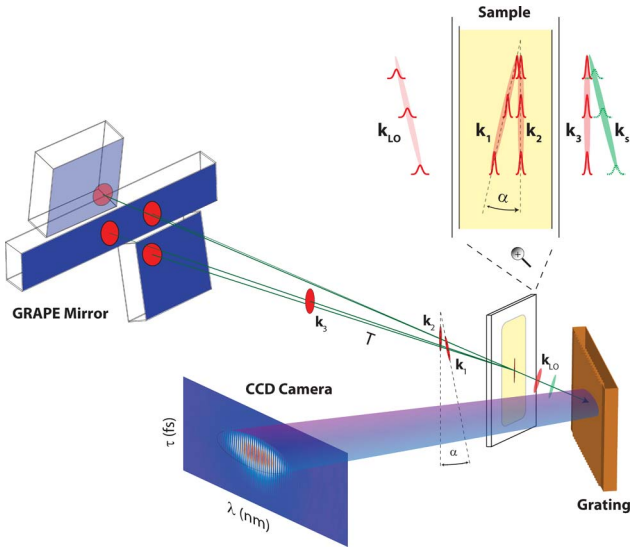

Fig. 2. (Color online) Schematic of the GRAPE apparatus. Four pulses are reflected off three mirrors and focused using a 250 mm cylindrical lens (not shown) onto a 200 μm thick sample cell. The spatially encoded 2D photon echo pulse sequence is shown in the upper right. The tilt of the wavefronts of each pulse defines the temporal gradient along the beam waist.

LH2 was isolated from *Rhodobacter sphaeroides* cultures as described by Frank *et al.* [16]. Sufficiently pure samples suitable for spectroscopic investigation required two sequential runs through a DEAE-Sephacel column and eluted between 500–600 mM NaCl. Samples were subsequently concentrated down to 0.2–0.3 OD at 850 nm in a 200 μm cuvette. A single pulse from the regenerative amplifier was triggered by manually firing the output Pockels cell inside the amplifier cavity. The camera shutter was open for 1 s to allow capture of the signal with only manual triggering.

The 2D rephasing spectrum of LH2 is shown in Fig. 3 at two waiting times, 100 fs and 800 fs. Qualitatively, these spectra are in good agreement with those obtained under lower power and with 100 ms of averaging at 5 kHz. The peaks along the diagonal of the spectrum are representative of the linear absorption spectrum of LH2 convolved with the laser spectrum. A slight shift of the peaks away from the diagonal is representative of a Stokes shift. Note that the coherence frequency is measured in the rotating frame such that uncertainty in the carrier frequency ω_0 leads to uncertainty in the position of the diagonal of the 2D spectrum. Use of a well-characterized sample such as Rb vapor [7] should provide an unambiguous determination of the coherence frequency axis, and future application of a reference should remove this uncertainty.

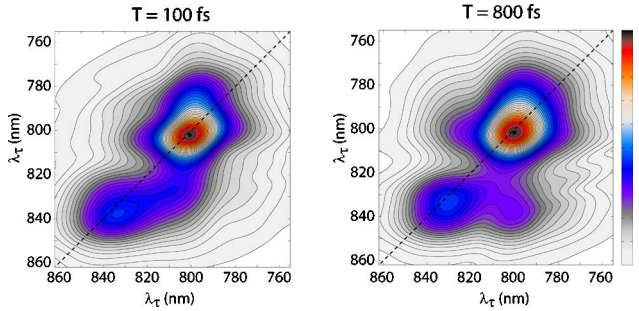

Fig. 3. (Color online) Rephasing 2D electronic spectra of LH2 recorded at $T = 100$ fs and $T = 800$ fs in one laser shot. Absolute value spectra are shown. With spectrally resolved pump-probe measurements (not recorded here), the absorptive and dispersive portion of the response could be separated. The coherence axis is recorded in the rotating frame.

Consistent with transient gradient measurements on LH2 from *Rh. sphaeroides* [17], we observe energy transfer between the two rings, B800 and B850, in 0.8 ps as manifested by the appearance of a prominent cross peak in the lower right of the 2D spectra. While we could not perform a quantitative SNR comparison with other 2D acquisition methods protocols because of the drastically different experimental arrangement of the GRAPE apparatus, the spectrum appears clean and free of any significant artifacts that would otherwise be expected with a point-by-point acquisition protocol. It should also be noted that the SNR could be significantly improved by shortening the exposure time to its minimal value. This change would decrease the noise significantly and allow more moderate pulse powers to be used. While only the rephasing spectrum was recorded in these experiments, in principle, GRAPES is capable of recording the nonrephasing spectrum in parallel by utilizing another area of the CCD detector and a separate local oscillator in the appropriate phase-matched direction.

In conclusion, we report on the first 2D photon echo spectrum capturing both the B800 and B850 bands of LH2 simultaneously. The use of GRAPE spectroscopy allows for the acquisition of the spectrum in only one laser shot, completely eliminating the need for phase stability and opening up the opportunity of excitation by continuum-generated pulses. Such broadband excitation will allow a host of new applications where broad linewidths have precluded the use of multidimensional optical spectroscopy to date. Furthermore, GRAPE spectroscopy requires no specialized optical components such as diffractive optics, pulse shapers, and active or passive phase stabilization. We see this simplification as a large step in making multidimensional spectroscopy accessible to a broader scientific community. Future work will focus on using fast frame rate cameras to capture 2D spectra in real time to observe changes of electronic structure during photodegradation on a millisecond timescale.

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