Published in final edited form as:

Opt Lett. 2018 April 15; 43(8): 1750–1753. doi:10.1364/OL.43.001750.

Compressed Supercontinuum Probe for Transient Absorption Microscopy

Kevin Higgins and Tessa R. Calhoun*

Department of Chemistry, University of Tennessee, Knoxville. 1420 Circle Dr., Knoxville TN 37996

Abstract

Here we combine three optical advancements to transient absorption microscopy in order to access the photodynamics in systems requiring stringent spatial and temporal resolution criteria. First, a broadband visible probe is generated by a commercial photonic crystal fiber. Second, a spatial light modulator-based pulse shaper is incorporated to reduce the pulse dispersion and improve temporal resolution. Third, 1.4 numerical aperture objectives for excitation and light collection provide optimal spatial resolution. The result of these improvements is a probe beam that spans 115 nm across the visible region yet maintains a ~100 fs instrument response at the sample position. We demonstrate the capabilities of this microscope by imaging polystyrene beads in a solution of IR-144 dye revealing aggregated species at the bead surfaces.

Keywords

(190.4370) Nonlinear optics, fibers, (180.4315) Nonlinear microscopy; (320.5520) Pulse compression, (320.7100) Ultrafast measurements

The ability to measure ultrafast processes governing excited state evolution has dramatically aided in our understanding of a wide range of chemical and physical phenomena including solvation,¹ photosynthetic energy transfer,² and the photophysics of new materials.^{3–5} Within the last decade, this capability has been adapted onto a microscopic platform allowing the imaging of nonfluorescent species in biological systems,^{6–10} direct visualization of energy flow in solar materials,^{11–12} and characterization of pigments in historic artwork,¹³ to name just a few applications. Universal to these applications of transient absorption microscopy (TAM) are the use of two ultrafast laser pulses, the first to promote a molecule of interest to an excited state and the second to probe the transient populations. By varying the delays between the two pulses one can measure the state's lifetime and track energy and decay pathways.^{14–17} The resulting information provides insight not only into the electronic structure of the system itself, but also reflects changes in the system's interaction with its local environment. This makes TAM a powerful approach for chemical imaging where changes in local chemical composition or interactions can be mapped using intrinsic ultrafast events as chemical contrast.^{14–15, 17}

*Corresponding author: Tessa R. Calhoun, trcalhoun@utk.edu.

In most ensemble TA spectroscopy experiments, a relatively narrowband pump pulse is used to excite a single electronic transition while a broadband probe, often produced via sapphire supercontinuum, interrogates a wide energetic window. The resulting signals are spectrally isolated and simultaneously detected using a spectrometer. While this approach has been successfully applied to microscopy,¹⁸ it is not without limitations. Most importantly, pushing the limits of spatial resolution in order to understand complex, heterogeneous systems requires the use of high numerical aperture (NA) objectives and increasingly small sample volumes. This necessitates operating in the MHz repetition rate regime with lock-in detection to obtain an acceptable signal-to-noise on the resulting images.^{16–17} As such, it is most common in TAM measurements to use optical parametric oscillators (OPOs) to produce a tunable probe pulse. The use of OPOs, however, introduces the requirements that the laser be separately tuned to probe different transient states, in contrast to ensemble spectroscopy, and restricts the ultimate temporal resolution limit due to their narrowband output. OPOs also add significant cost to the experimental apparatus.

As an alternative to OPOs, supercontinuum generated by photonic crystal fibers (PCF) has been proven to be a useful probe for transient absorption spectroscopy^{19–21} and microscopy.²² PCFs have been shown to be capable of producing octave-spanning supercontinuum with low energy input that can be provided with a high repetition rate laser, making them ideal for use with readily available and low cost ultrafast oscillators.²³ The previous applications of PCFs to transient absorption experiments, however, used the raw output of the fiber which is many ps long, and limits the temporal resolution of the instrument. In parallel, multiple groups have demonstrated the ability to compress PCF supercontinuum in microscopes with high NA objectives, but their fibers were limited to near-infrared (IR) wavelengths, which restricts the systems that can be studied.^{24–25} All-normal dispersion PCFs have been introduced to provide compressible supercontinuum pulses,²¹ but they are either custom fibers or again limited to the near-IR. There are multiple advantages to utilizing a commercially available, sealed PCF: ease of mounting and alignment, optically cleanable facets, reduced cost, and increased longevity of the fiber compared to bare PCF fiber. Here we demonstrate the compression of a PCF-generated *visible*, broadband supercontinuum probe at the focus of a high NA objective and its application to resolving spatial heterogeneities with transient absorption microscopy using temporal responses as chemical contrast.

The TA microscope used in this study is depicted in Fig. 1. It uses a Ti:sapphire oscillator (Mai Tai, Spectra Physics) to produce pulses centered at 800 nm with a 80 MHz repetition rate. The output is split into two arms, a pump and probe, where the pump beam is modulated using an electro-optic modulator to 26.7 MHz for lock-in detection. The supercontinuum probe beam is generated by focusing 3.5 nJ of the 800 nm pulses into the PCF (SCG-800-CARS, Newport) using a 40X objective. The broadband output is collected and collimated using an off-axis parabolic mirror. Probe compression is achieved with both a prism line and a liquid-crystal spatial light modulator (SLM)-based pulse shaper.²⁶ This pulse shaping line has been adapted from previous work with some modifications.^{27–28} Briefly, the line consists of four optics; a volume phase holographic transmission grating, a folding mirror, a cylindrical mirror ($f = 100$ mm), and a reflective SLM. The SLM consists of a 600×800 array of liquid-crystals with a pixel size of $20 \mu\text{m}$ (Hamamatsu). This

configuration, with a two-dimensional SLM array, allows for both amplitude and phase shaping although the experiments reported here rely exclusive on the application of phase. The pump and probe beams are finally combined using a dichroic mirror before being sent into the microscope where they are focused with a 100X 1.4NA oil immersion objective (Olympus) onto the sample. The samples are raster scanned using a scanning microscope stage. Signal is collected in a transmitted direction using a second objective, spectrally isolated using a combination of filters, and sent to a single pixel avalanche photodiode. The signal is finally isolated using a lock-in amplifier and read out on a computer using custom LabVIEW codes.

The generation of blue frequencies from PCFs is a longstanding challenge^{29–31} and the PCF used in our work is one of the few commercially available fibers that produces supercontinuum spectrum extending significantly into the visible region. Theoretically, the output of the PCF has a spectral range of <600 nm to >1200 nm, largely dependent on the input parameters of pulse width and energy.³² For our experiments, 115 nm of visible wavelengths, 560 nm to 675nm (Fig. 2C), are selected from the fiber supercontinuum and introduced into the microscope. The nonlinear processes taking place in the fiber to produce the spectrum introduce a large amount of dispersion leaving the output pulse at nearly 1 ps (Fig. 2A) even after prism compression. In addition to its visible spectrum output, this fiber also has the benefit of not relying on soliton fission for spectral broadening.³² This property reduces the noise of the output and retains the pulse coherence necessary for compression.
32–33

To characterize the temporal and spectral characteristics of our supercontinuum pulses, cross-correlation frequency resolved optical gating (XFROG)³⁴ experiments were performed at the sample position in the microscope using potassium dihydrogen phosphate (KDP) crystalline powder grains as a nonlinear up-conversion media. Figure 2A shows an XFROG trace collected with zero phase applied to the SLM. The sum frequency generation signal between the pump and probe pulses was collected with a 40X 0.5 NA UVB objective (LMU-40X-UVB ThorLabs), and spectrally resolved with a USB spectrometer (Maya, Ocean Optics). The 800 nm pump pulse was compressed to 85 fs at the sample position with a prism pair as confirmed by cFROG³⁵ (data not shown). The >1000 fs duration of the signal in Fig. 2A therefore predominantly arises from the uncompensated phase on the probe pulse. The prism compression line corrects a majority of the second order dispersion leaving a large third order component. In order to extract the exact phase needed for compression, the data in Fig. 2A was processed with the FROG algorithm.^{34, 36} This phase is then fit to a high order polynomial which is in turn applied to the SLM. The use of a polynomial fit eliminates any discontinuities in the extracted phase which can induce unwanted aberrations in the pulse if applied to the SLM directly. 2B shows the XFROG data collected when this extracted phase is corrected using the SLM. The full width at half maximum (FWHM) of this data is reduced from over 1000 fs to <105 fs (Fig. 2D) with the remaining major contribution due to the pump pulse width of 85 fs. In fact, the duration of the probe pulse alone is estimated to be 53 fs as extracted from the FROG algorithm. The remaining deviation between this value and the transform limit likely arise from the inability of the polynomial fit to reproduce the exact phase necessary for correction. It is expected that further optimization could be achieved by the addition of phase corrections extracted from

data in Fig. 2B. Figure 2C shows that the entire 115 nm of bandwidth introduced to the sample through the 1.4 NA objective (red trace) is preserved after the addition of the phase (blue trace) and recovered by the FROG algorithm when applied to the compressed XFROG data.

In order to demonstrate the spatial and temporal capabilities of the compressed PCF based TA microscope, we have imaged polystyrene particles in a solution of IR-144 dye. Commercial polystyrene particles (0.7 – 0.9 μm) coated with biotin were purchased from Fischer Scientific and subsequently diluted 1:1000 in a phosphate buffer solution. Particles were then immobilized on a commercial avidin coated coverslip. This coverslip with the immobilized particles is applied as the bottom half of our homebuilt flow cell with a second coverslip as the top half. The TA signal is collected using a 60X 1.4 NA oil immersion objective (Nikon). The dye solution was flowed over the beads in order to dissipate any local heating damage caused by the incident laser beams.

IR-144 has previously been studied via transient absorption spectroscopy³⁷ and its aggregation at interfaces has been reported.³⁸ Figure 3, however, shows the first application of transient absorption microscopy to this system directly linking the spatial and temporal behavior in a single experiment. A 200 μM solution of IR-144 in methanol was flowed through the sample cell. The path length of the flow cell is estimated to be $\sim 140 \mu\text{m}$ based on thickness of the tape used between the coverslips. A 375 pJ/pulse pump beam at 800 nm was used to excite from the ground state to the first excited state of IR-144. The ground state bleach signal was then monitored with 12.5 pJ/pulse probe beam centered at 600 nm. TAM images are shown in Figure 3A at an interpulse time delay, τ , of 2 ps and took approximately 10 min to obtain. This image clearly shows distinct signal arising from IR-144 around the beads (white), and the bulk solution (grey), whereas the polystyrene beads appear as black regions since they do not have an optical response in this spectral region. To further confirm the source of the signal, a TAM image has also been acquired at a τ of -12 ps (Fig. 3B) when the probe precedes the pump. As expected, the contrast arising from the transient states of IR-144 is no longer apparent. A line profile across the bead edge is shown in Fig. 3C. A sigmoidal fit of this data is shown and estimates the lateral resolution of our instrument as 414 nm.³⁹

It is evident in Fig. 3A that there is an accumulation of dye molecules, or an enhanced optical response, at the surface of the polystyrene beads. Based on previous ensemble spectroscopy work, it was surmised that aggregation at the particle interface results in notably different dynamics of IR-144 as opposed to species in the bulk solution at the same concentration. To more directly illustrate that the species at the surface are aggregated, we collected the ultrafast temporal dynamics at two spatial regions in Fig. 3A. This information is only available using the intrinsic ultrafast time resolution in conjunction with high spatial resolution afforded by the compressed PCF based TA microscope. The time traces are shown in Fig. 3D. Each decay measurement took approximately 20 minutes to acquire and the high signal-to-noise suggests that the supercontinuum stability is suitable for these types of experiments. The blue 'x'-markers were obtained from the marked region in Fig. 3A at the surface of the particle where the dye is appearing to accumulate. The response from the bulk solution away from the particles, shown in red, displays notably different dynamics,

indicating different local chemical environments, mostly likely due to different aggregation states. By fitting each of the curves to a single exponential we are able to extract the excited state lifetimes from these different environments. For the monomeric solution of IR-144 the time decay results in a 321 ps lifetime while the dye near the edge of the particle is significantly shorter at 189 ps.

In addition to facilitating the use of PCFs for TAM experiments requiring optimal temporal resolution, the approach developed in this work can easily be expanded to other nonlinear microscopy techniques in the visible spectrum with minimal instrumental modifications. For example, the pulse shaper can be used to generate two different colored pulses from the supercontinuum for pump-dump-probe experiments⁴⁰ on spatially heterogeneous samples. Similarly, pulse trains could be generated for optical Kerr effect measurements⁴¹ or the probe polarization itself can be shaped.⁴² It is expected that with the flexibility afforded by a whitelight probe pulse and pulse shaper in a high NA microscope, that these advances will impact a broad range of fields beyond those demonstrated here.

This work demonstrates the compression of visible supercontinuum from a commercially available PCF using an adaptive pulse shaper for use in high repetition rate transient absorption microscopy. Through optimal pulse compression, a temporal resolution of ~100 fs is achieved at the sample position of a high NA microscope. We demonstrate the utility of having both this high temporal resolution and probe wavelengths in the visible spectral window by imaging the aggregation and dynamics of IR-144 at polystyrene nanoparticles. These images reveal that aggregation of IR-144 at the surfaces produces distinct dynamics, and show that TAM is uniquely sensitive to imaging differences in chemical environments. Continued work using compressed PCF-based TAM is aimed at imaging nonemissive species in complex matrices leveraging the high joint temporal and spatial resolutions of the present instrument.

Acknowledgment.

The authors would like to thank Dr. Benjamin Doughty for helpful discussions.

Funding. National Institutes of Health (NIH, NIGMS R15 GM119111); The University of Tennessee (UT), Knoxville.

References

1. Joo T; Jia Y; Yu JY; Lang MJ; Fleming GR, *J. Chem. Phys* 1996, 104 (16), 6089–6108.
2. Berera R; van Grondelle R; Kennis JTM, *Photosynth. Res* 2009, 101 (2), 105–118. [PubMed: 19578970]
3. Link S; El-Sayed MA, *Annu. Rev. Phys. Chem* 2003, 54 (1), 331–366. [PubMed: 12626731]
4. Dawlaty JM; Shivaraman S; Chandrashekhar M; Rana F; Spencer MG, *Appl. Phys. Lett* 2008, 92 (4), 042116.
5. Bakulin AA; Rao A; Pavelyev VG; van Loosdrecht PHM; Pshenichnikov MS; Niedzialek D; Cornil J; Beljonne D; Friend RH, *Science* 2012, 335 (6074), 1340–1344. [PubMed: 22362882]
6. Fu D; Ye T; Matthews TE; Chen BJ; Yurtserver G; Warren WS, *Opt. Lett* 2007, 32 (18), 2641–2643. [PubMed: 17873920]
7. Li J; Zhang W; Chung T-F; Slipchenko MN; Chen YP; Cheng J-X; Yang C, *Sci. Rep* 2015, 5, 12394. [PubMed: 26202216]

8. Liu J; Irudayaraj JMK, *Nanoscale* 2016, 8 (46), 19242–19248. [PubMed: 27883134]
9. Min W; Lu S; Chong S; Roy R; Holtom GR; Xie XS, *Nature* 2009, 461 (7267), 1105–1109. [PubMed: 19847261]
10. Tong L; Liu Y; Dolash BD; Jung Y; Slipchenko MN; Bergstrom DE; Cheng J-X, *Nat. Nanotechnol* 2012, 7 (1), 56–61.
11. Guo Z; Manser JS; Wan Y; Kamat PV; Huang L, *Nat. Commun* 2015, 6.
12. Simpson MJ; Doughty B; Yang B; Xiao K; Ma Y-Z, *J. Phys. Chem. Lett* 2015, 6 (15), 3041–3047. [PubMed: 26267200]
13. Villafana TE; Brown WP; Delaney JK; Palmer M; Warren WS; Fischer MC, *Proc. Natl. Acad. Sci* 2014, 111 (5), 1708–1713. [PubMed: 24449855]
14. Davydova D. y.; de la Cadena A; Akimov D; Dietzek B, *Laser Photonics Rev* 2016, 10 (1), 62–81.
15. Grumstrup EM; Gabriel MM; Cating EEM; Van Goethem EM; Papanikolas JM, *Chem. Phys* 2015, 458, 30–40.
16. Min W; Freudiger CW; Lu S; Xie XS, *Annu. Rev. Phys. Chem* 2011, 62 (1), 507–530. [PubMed: 21453061]
17. Fischer MC; Wilson JW; Robles FE; Warren WS, *Rev. Sci. Instrum* 2016, 87 (3), 031101. [PubMed: 27036751]
18. Schnedermann C; Lim JM; Wende T; Duarte AS; Ni L; Gu Q; Sadhanala A; Rao A; Kukura P, *J. Phys. Chem. Lett* 2016, 7 (23), 4854–4859. [PubMed: 27934055]
19. Léonard J; Lecong N; Likforman J-P; Crégut O; Haacke S; Viale P; Leproux P; Couderc V, *Opt. Express* 2007, 15 (24), 16124–16129. [PubMed: 19550901]
20. Carroll EC; Hill MP; Madsen D; Malley KR; Larsen DS, *Rev. Sci. Instrum* 2009, 80 (2), 026102. [PubMed: 19256678]
21. Heidt AM; Hartung A; Bosman GW; Krok P; Rohwer EG; Schwoerer H; Bartelt H, *Opt. Express* 2011, 19 (4), 3775–3787. [PubMed: 21369202]
22. Massaro ES; Hill AH; Grumstrup EM, *ACS Photonics* 2016, 3 (4), 501–506.
23. Dudley JM; Genty G; Coen S, *Rev. Mod. Phys* 2006, 78 (4), 1135–1184.
24. von Vacano B; Wohlleben W; Motzkus M, *Opt. Lett* 2006, 31 (3), 413–415. [PubMed: 16480226]
25. Tu H; Liu Y; Turchinovich D; Boppart SA, *Opt. Lett* 2011, 36 (12), 2315–2317. [PubMed: 21686005]
26. Weiner AM, *Rev. Sci. Instrum* 2000, 71 (5), 1929–1960.
27. Vaughan JC; Hornung T; Feurer T; Nelson KA, *Opt. Lett* 2005, 30 (3), 323–325. [PubMed: 15751899]
28. Calhoun TR; Davis JA; Graham MW; Fleming GR, *Chemical Physics Letters* 2012, 523 (Supplement C), 1–5.
29. Genty G; Lehtonen M; Ludvigsen H, *Opt. Lett* 2005, 30 (7), 756–758. [PubMed: 15832929]
30. Jiang X; Joly NY; Finger MA; Babic F; Wong GKL; Travers JC; Russell PSJ, *Nature Photonics* 2015, 9, 133.
31. Kudlinski A; George AK; Knight JC; Travers JC; Rulkov AB; Popov SV; Taylor JR, *Opt. Express* 2006, 14 (12), 5715–5722. [PubMed: 19516740]
32. Hilligsøe KM; Andersen TV; Paulsen HN; Nielsen CK; Mølmer K; Keiding S; Kristiansen R; Hansen KP; Larsen JJ, *Opt. Express* 2004, 12 (6), 1045–1054. [PubMed: 19474920]
33. Hartung A; Heidt AM; Bartelt H, *Opt. Express* 2011, 19 (8), 7742–7749. [PubMed: 21503084]
34. Linden S; Giessen H; Kuhl J, *physica status solidi (b)* 1998, 206 (1), 119–124.
35. Amat-Roldán I; Cormack IG; Loza-Alvarez P; Gualda EJ; Artigas D, *Opt. Express* 2004, 12 (6), 1169–1178. [PubMed: 19474935]
36. Trebino R, *Frequency-Resolved Optical Gating: The Measurement of Ultrashort Laser Pulses* Springer US: New York, 2000; p 425.
37. Mohanty J; Palit DK; Mittal J, *Proceedings of the National Academy of Sciences, India Section A* 2000, 66 (2), 303–315.
38. Shang X; Benderskii AV; Eienthal KB, *J. Phys. Chem. B* 2001, 105 (47), 11578–11585.

39. Curtin AE; Skinner R; Sanders AW, *Microsc. Microanal* 2015, 21 (3), 771–777. [PubMed: 26007244]
40. Kee TW, J. *Phys. Chem. Lett* 2014, 5 (18), 3231–3240. [PubMed: 26276338]
41. Robles FE; Fischer MC; Warren WS, *Opt. Lett* 2014, 39 (16), 4788–4791. [PubMed: 25121875]
42. Brixner T; Gerber G, *Opt. Lett* 2001, 26 (8), 557–559. [PubMed: 18040384]

Fig. 1. Experimental layout of the transient absorption microscope. EOM – electro-optic modulator, Obj – objective, PCF – photonic crystal fiber, OAP – off-axis parabolic mirror. Top Right inset, microscope sample position with high NA focusing and collection objectives on either side of a flow-cell.

Fig. 2. XFROG traces of the uncompressed (A) and compressed (B) PCF supercontinuum output. Spectra at the sample position (C) with (blue) and without (red) phase applied to the SLM and the recovered spectrum from FROG (gray). Temporal profile of the compressed XFROG with a FWHM of ~ 100 fs (D).

Fig. 3. Transient absorption images of 200 μM IR-144 solution flowing over 0.7 – 0.9 μm polystyrene particles. (A) TAM image taken at $\tau = 2$ ps (B) TAM image of the same region with $\tau = -12$ ps (C) Line profile taken from image A as shown by the yellow line. Data are represented by dots while the solid trace is the fit. (D) TA time-decay of IR-144 and the indicated areas from the image in A. Scale bars are 2 μm.