Starch-based second-harmonic-generated collinear frequency-resolved optical gating pulse characterization at the focal plane of a high-numerical-aperture lens

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We report the use of starch as an ideal nonlinear medium with which to perform collinear frequency-resolved optical gating measurements of ultrashort pulses at the focal plane of a high-numerical-aperture (NA) lens. We achieved these measurements by simply sandwiching starch granules (suspended in water) between two coverslips and placing them within the focal plane of a high-NA lens. The natural nonlinear characteristics of starch allow the correct phase matching of pulses at the focal plane of a high-NA lens at different wavelengths. This elegant arrangement overcomes all the complexity and problems that were previously associated with pulse characterization within a multiphoton microscope.

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The visualization of the internal structure of biological organisms is essential for the advance of life science. Great demands are placed on the field of microscopy to develop techniques that are able to image increasingly more complicated structures. Nonlinear microscopy has generated much interest within the scientific community because of its ability to generate high-resolution, three-dimensional images. Recent research within the area of nonlinear microscopy has shown how the temporal and spectral properties of the incident ultrashort pulse can have significant effects on the efficiency[1,2] and selectivity[3] of an image. In that research the phase of an ultrashort pulse was arbitrarily altered until the desired two-photon fluorescence signal was optimized. Yet little is known about this phase-dependent optimization process, and there is a great urgency to develop adequate tools to help in understanding it. Direct phase measurement of pulses at the sample plane of a microscope is thus required. Two main problems arise when one tries to fully characterize the pulses going through a high-numerical-aperture (NA) objective lens. First, a collinear geometry is imposed because the NA has to be completely filled.[4,5] Second, severe requirements are imposed on the nonlinear medium. The collinear geometry generally restricts the pulse measurements within nonlinear microscopy to the use of interferometric autocorrelation.[6–8] This, however, provides no direct phase information on the pulse. Other pioneering research based on the well-known frequency-resolved optical gating (FROG) technique[9] showed how to characterize pulses at the sample plane by means of type II phase-matching collinear geometry.[4,5] Unfortunately, several difficulties are present when one tries to measure such a trace through a high-NA lens by using conventional nonlinear crystals. First, at the focal point the polarization of the fundamental beam is modified owing to the extremely steep convergence angles.[10] Second, there is an intrinsically large range of incident angles at the focal plane. Finally, the large frequency bandwidth associated with ultrashort laser pulses requires the phase-matching bandwidth of the nonlinear medium to be large. All the problems cited above make the use of traditional nonlinear crystals error prone. To overcome these difficulties, the use of other nonlinear media has been explored. One of these options is the use of nonlinear fluorescent dyes.[11] These dyes, however, are subject to photobleaching. It has also been reported that protein polymer chains, such as bacteriorhodopsin, have been used to generate the required second-harmonic signal.[12] This technique, however, requires complex preparation of the medium.

In this Letter we outline a simple and elegant technique with which to characterize a pulse at the focal plane of a high-NA objective lens, solving the problems that we described above. This technique is based on the use of starch granules (suspended in water) as a novel nonlinear medium and on the use of a recently reported data processing procedure to successfully transform a collinear FROG (CFROG) trace into a standard noncollinear FROG trace.[13]

Starch has been shown to have a naturally high χ^2 coefficient.[14,15] Second-harmonic signals were previously generated from starch with ultrashort laser pulses with wavelengths of 700–1300 nm.[16] These results are highly significant because they demonstrate the extremely large spectral range in which starch can be used. In this sense, the small sizes of these granules (approximately 5–10 μm) provide them with a naturally large bandwidth. Furthermore, as starch

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is an isotropic medium, its χ^2 coefficient is polarization insensitive and its conversion efficiency is angle independent. Together with these natural physical properties, it is also important to highlight a practical advantage: Starch is ideally suited for working within a microscope because a drop of the starch suspension in water can simply be sandwiched between two coverslips and placed directly within the focal plane of a high-NA lens (or at the sample plane of the nonlinear microscope). All these properties make starch ideally suited for the high demands that are placed on a nonlinear medium that is being used to characterize pulses through a high-NA objective lens.

To verify that starch can be successfully used for characterizing ultrashort pulses we proceeded to obtain a CFROG trace of pulses at the focal plane of high-NA objective lens. The general optical arrangement for measuring a starch-based second-harmonic generation (SHG) CFROG trace is shown in Fig. 1.

A telescope arrangement was used at the output of the interferometer and before the microscope objective to ensure that the beam filled the entire input aperture of the objective lens. A starch suspension in water was prepared and sandwiched between two coverslips. As the second-harmonic is generated from a single starch granule, precision in the preparation is not critical (a pinch of starch and a drop of water). The suspension was then placed at the focus of a high-NA (NA 1.25) lens. Refractive-index matching oil was used to ensure full use of the NA of the lens. Another lens was used to collimate the generated frequency-doubled signal, which was then sent to the spectrometer after passing through a BG39 filter. The interferometer and the spectrometer were both controlled by the computer. A backthinned CCD linear array (Hamamatsu HC230-1007), operated in vertical binning mode, was attached to the spectrometer to record the spectrum of the SHG signal.

The pulses were generated from a Kerr-lens modelocked Ti:sapphire laser (repetition rate, ~76 MHz) that was operating at a central wavelength of $\lambda = 835$ nm. The pulses entering the objective lens had an average power of 15 mW. The second-harmonic signal was generated only when the pulses were focused upon a granule of starch. We then acquired the CFROG trace [shown in Fig. 2(a)], obeying the undersampling criteria outlined in Ref. 13 (220 sample points with delay steps of 7.58 fs). It should be noted that the CFROG trace is in fact an undersampled frequency-resolved interferometric autocorrelation. Consequently, by integrating the CFROG trace in time [Fig. 2(b)] we can obtain the envelope of the interferometric autocorrelation and verify its validity by ensuring that there is the required 8:1 ratio. This result in itself demonstrates the effectiveness of starch as a nonlinear medium when high-NA lenses are used. The CFROG trace was then filtered[13] and retrieved with a traditional SHG FROG retrieval algorithm.[17] The experimental and retrieved traces are presented in Fig. 3, along with the retrieved pulse and spectrum. We further checked the validity of the retrieved data by comparing them with the time marginal and the experimentally measured spectra. These spectra are shown in Fig. 4, where excellent agreement can be observed.

To further investigate the use of starch as a nonlinear medium we proceeded to characterize the output pulses from a synchronously pumped optical parametric oscillator operating at 1100 nm. To do this, we used an objective lens with a NA of 0.85. In this case we used a delay step of 42.6 fs and produced 96 samples. The resultant CFROG trace was again filtered to produce a noncollinear SHG FROG trace and then retrieved. The result of this retrieval is shown in Fig. 5. As above, to validate the data we compared the retrieved results with externally measured data. Figure 6 shows the excellent agreement obtained.

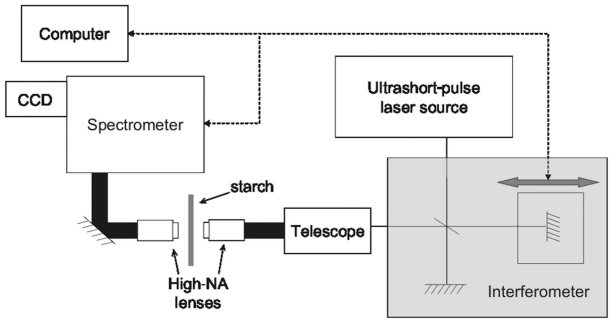

Fig. 1. Schematic of the CFROG optical arrangement.

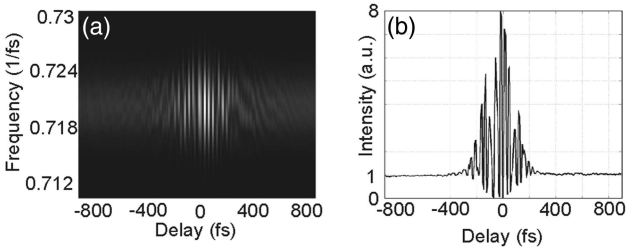

Fig. 2. (a) CFROG trace and (b) its time marginal spectrum obtained at the focus of a 1.25-NA lens.

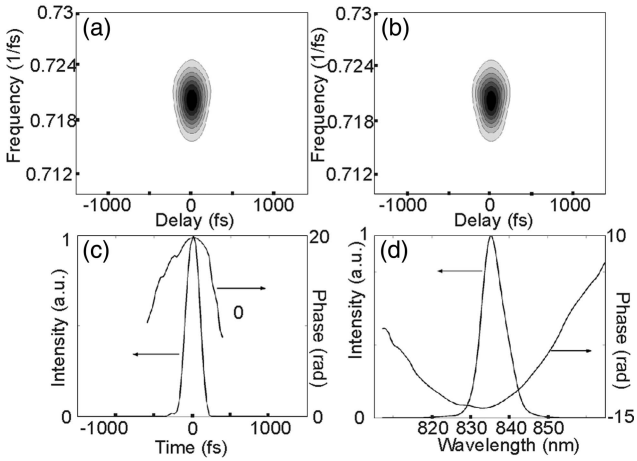

Fig. 3. Experimentally retrieved pulses at the focal plane of a 1.25-NA objective lens. (a) Fully filtered CFROG trace, (b) retrieved trace, (c) amplitude and phase and (d) spectrum and phase of the retrieved pulse. The rms error of the traces is $G = 8 \times 10^{-5}$.

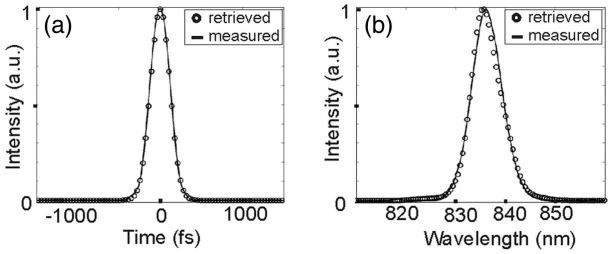

Fig. 4. Comparison of experimental and retrieved pulses (λ = 835 nm) at the focal plane of a 1.25-NA lens. (a) Time marginal and retrieved intensity autocorrelation. (b) Measured and retrieved spectra.

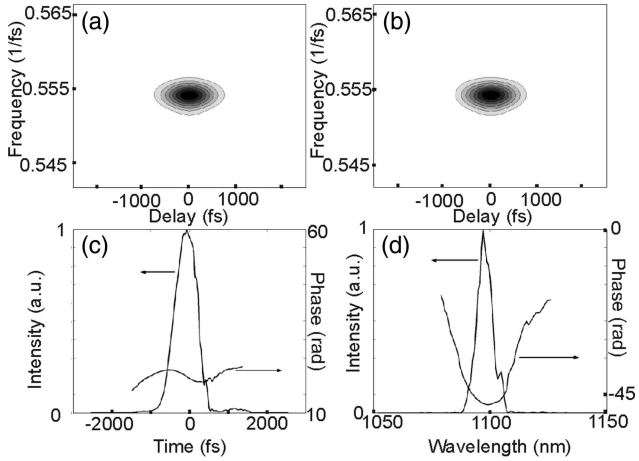

Fig. 5. Experimentally retrieved pulses at the focal plane of a 0.85-NA objective lens. (a) Fully filtered CFROG trace, (b) retrieved trace, (c) amplitude and phase, and (d) spectrum and phase of the retrieved pulse.

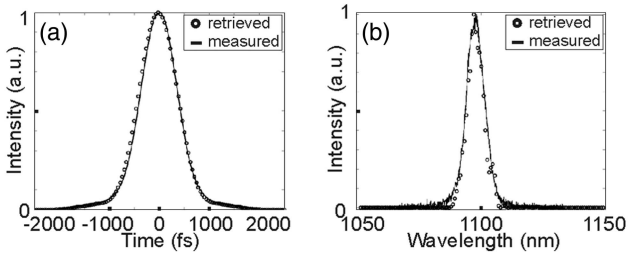

Fig. 6. Comparison of experimental and retrieved pulses (λ = 1100 nm) at the focal plane of a 0.85-NA lens. (a) Measured time marginal and retrieved intensity autocorrelation. (b) Measured and retrieved spectra.

The results of both experiments confirm that starch can be successfully used with a wide range of wavelengths to characterize pulses with different characteristics at the focal plane of a high-NA lens in an easy and quick way.

In conclusion, we have demonstrated a successful full characterization of ultrashort pulses at the focal plane of a high-NA objective lens. We achieved this goal in two steps: first, by the use of a recently reported method that permits the use of collinear geometry[13] and second, by introducing the use of starch as a nonlinear medium. The use of a starch-in-water suspension has been shown to be highly suitable for the characterization of pulses at the focal plane of high-NA lenses. This is possible because the generation of the nonlinear signal in such a medium is polarization and angle independent. Moreover, we have shown the large bandwidth of starch by characterizing ultrashort pulses at two different wavelengths, confirming earlier reports that it can be successfully used from 700 to 1300 nm.[16] This procedure importantly overcomes all the inherent difficulties associated with the thin nonlinear medium used in previous techniques. Finally, this material is a simple, nontoxic, easy-to-store, nonphotobleaching, easy-to-obtain, cheap, and easy-to-handle medium for the full characterization of ultrashort pulses at the sample plane of a nonlinear microscope.

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