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Measurement of femtosecond pulses in the focal point of a high-numerical-aperture lens by two-photon absorption

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We demonstrate a method for the measurement of femtosecond optical pulses in the focal point of a high-NA lens, using interferometric autocorrelation through two-photon absorption. A chirp-free input pulse of 47 fs is found to broaden by $\approx 50\%$ after focusing by a well-compensated objective. With proper prechirp compensation, the actual pulse width in the focus of such a lens system can be restored to (almost) its initial value. The unique value of the presented two-photon autocorrelation technique is its capability of measuring the actual pulse width at the focal point of a high-NA lens, an aspect that is of direct importance to two-photon imaging approaches, for example.

There has been increasing interest in the application of femtosecond optical pulses in the field of microscopy.¹ For instance, femtosecond pulses have been used for two-photon absorption (TPA) in microscopy on biological samples.²⁻⁴ The inherent sectioning properties of TPA permit three-dimensional imaging of biological samples, an ability that has been shown to be of great interest in confocal microscopy.⁵ It has been recognized that the strong focusing conditions of high-NA lenses may distort the shape of the optical pulse.⁶ We demonstrate the use of TPA for the actual measurement of the pulse width in the focal point of a high-NA lens. This technique uses a standard interferometric autocorrelation setup, with which the nonlinear interaction takes place through TPA in a medium with no intermediate-level resonances.

In our experimental setup (Fig. 1), the output of a Ti:sapphire laser is sent through a prechirp unit of a pair of prisms,⁷ which permits both positive and negative chirp compensation. The beam is then split by a 50% beam splitter, and one of the beams passes through a variable-delay line, which is mounted on a home-built shaker. The two beams are recombined on a second—separate but identical—beam splitter, ensuring that both beams encounter the same dispersive elements. To monitor the autocorrelation traces through second-harmonic generation (SHG) and TPA simultaneously, we split the beam into two, each with an average power of ≈ 20 mW, using another 50% beam splitter. One part is focused by a 50-mm lens on a 0.1-mm β -barium borate crystal (type I) for SHG. The other part is focused by a 80-mm lens on a 100 μ m pinhole, passes a dichroic mirror, and is focused, taking into account proper

tube-length conditions, by a high-NA objective on the sample, which is mounted on a variable-height translation stage. The fluorescence is monitored in the backscattering direction, reflecting off the dichroic mirror and passing a filter to block scattered light at the Ti:sapphire frequency. Measurement of the transmission characteristics of the dichroic mirror confirmed that the spectrum of the Ti:sapphire pulse is not affected by the mirror. The SHG and TPA signals are detected by a photomultiplier tube, the output of which is digitized on an oscilloscope (Tektronix 2440, 500 Msamples/s). For the TPA experiments, a number of different samples are used: a gel doped with Rhodamine 6G

Fig. 1. Schematic of experimental setup for SHG and TPA autocorrelation measurements. PC, prechirp unit; VD, variable-delay line mounted on a home-built shaker; L₁, 50-mm lens; L₂, 80-mm lens; B, 0.1-mm β -barium borate crystal (type I); P, 100- μ m pinhole; D, dichroic mirror; O, objective; S, sample on a variable-height translation stage; PMT, photomultiplier tube; Scope, Tektronix 2440 (500 Msamples/s) digital scope.

Fig. 2. (a) Autocorrelation trace of the laser pulse through SHG in a 0.1-mm β -barium borate crystal (type I). The envelope is the best fit obtained by assuming a Gaussian pulse of 46.6 ± 0.5 fs. (b) Autocorrelation trace of the laser pulse through TPA in a 10^{-3} M solution of Rhodamine 6G, using the Student objective (see text). The envelope is the best fit obtained by assuming a Gaussian pulse of 50.9 ± 1.2 fs.

(10^{-4} M), 10^{-3} and 10^{-4} M solutions of Rhodamine 6G in distilled water, and a 2.5×10^{-3} M solution of fluorescein in distilled water. The solutions are contained in 0.2-mm microslides (Vitro Dynamics, Rockaway, N.J.).

The spatial uniformity of the prechirp compensation has been checked, and the pulses from the Ti:sapphire laser have been optimized for minimal pulse width. The interferometric method permits an actual count of the optical oscillations, which, in combination with the spectrum of the pulse, permits calibration of the time scale. Such calibration is required, because small variations in the frequency of the shaker may easily account for variations in the time scale of 10 fs or more. The data have been checked for possible aliasing caused by under-sampling. An additional advantage of the interferometric technique is that it ensures equal focusing conditions for both pulses.

The interferometric autocorrelation of a chirp-free, transform-limited optical pulse is characterized by a fully symmetric curve, with a peak-to-background ratio of the constructive interferences of 8:1.⁸ The SHG autocorrelation of our pulses is shown in Fig. 2(a). It shows a proper 8:1 ratio and slight asymmetry. We obtain the best fit of the envelope by assuming Gaussian input pulses of $46.6 (\pm 0.5)$ fs. The FWHM of the spectrum of the pulse—centered

at 770.9 nm—is 1.06×10^{13} Hz, giving an almost transform-limited time–bandwidth product of 0.49 (compared with 0.44 for Gaussian pulses). A slight asymmetry of the spectrum confirms that the pulses are not perfectly Gaussian. For the analysis of the data we calibrate the time scale of the autocorrelation by taking the Fourier transform of the data. The envelope of the constructive interferences is subsequently fitted to the interferometric autocorrelation function of Gaussian input pulses.

To demonstrate the unique utility of the TPA autocorrelation technique, we investigated the influence of the objective on the pulse width in the focal point for two different high-NA objectives. The first objective, denoted Student, is a simple three-element compound objective (Leitz: oil 100, NA = 1.25). The second objective (Nikon: oil plan 100, NA = 1.25), denoted Nikon in the following, has better wide-field correction properties and therefore contains a substantially higher count (approximately 9) of optical elements with different refractive indices. Figure 2(b) shows the TPA autocorrelation measured with the Student objective in the 10^{-3} M solution of Rhodamine 6G. Like the SHG autocorrelation, the trace is almost symmetric, with an 8:1 ratio of constructive interferences to background. Assuming Gaussian-shaped pulses, the fit yields a pulse width of 50.9 ± 1.2 fs, an increase of $\approx 10\%$ resulting from propagation through the objective. For the Nikon objective the pulse broadening is even more pronounced: to 71 ± 2 fs (i.e., an increase of $\approx 52\%$) for the same sample. In this case, the amplitude of the signal has dropped considerably, which probably is due to the higher losses of the Nikon objective.

The TPA autocorrelation technique provides interactive pulse-width control to attain, through prechirp compensation by a pair of prisms, the minimum pulse width under the strong focusing conditions of a high-NA lens. Figure 3 shows the dependence of the pulse width and amplitude of the autocorrelation signal for the Student and Nikon objectives on the induced prechirp. For both objectives the original pulse width could be almost restored, which shows that the induced dispersion is mainly linear. A larger negative prechirp compensation is required for the Nikon objective (≈ 450 fs²) than for the Student objective (≈ 300 fs²), which indicates that the former induces a larger positive dispersion. Because the minimum pulse width that can be obtained for the Nikon objective is still larger than the initial pulse width, we suspect that there is some residual chirp—possibly nonlinear—left in the pulse, which could not be compensated. An important observation and internal check on the results obtained is that the pulse width and amplitude show complementary behavior. This is expected from the square dependence of the TPA fluorescence intensity on the power of the excitation field. One can quantitatively model the dependence of the amplitude on the prechirp compensation by varying the pulse width of a Gaussian input pulse, while keeping its energy content constant, and then calculating the corresponding autocorrelation function.⁸ The result of this calculation is shown in Fig. 3(b).

Fig. 3. (a) Measured pulse width and (b) the measured and calculated amplitude of the two-photon autocorrelation signal as a function of the prism distance when translated in the direction normal to the base. Approximately 450 fs^2 of group-velocity dispersion is compensated for in the Nikon case, and 300 fs^2 of group-velocity dispersion for the Student objective (see text).

We obtained identical results for all samples used. This, together with the fact that the induced pulse-width broadening could be compensated almost completely, is strong evidence that there is no contribution by molecular properties of the dyes used to generate the TPA signal. We found a sharp decrease in the amplitude of the TPA autocorrelation signal as a function of penetration depth ($0 \rightarrow 60 \mu\text{m}$) in the sample. This decrease is probably due to the increase in spherical aberrations that results from the refractive-index mismatch.⁹ The pulse width is found to remain almost constant with increasing

penetration depth, which is consistent with the fact that dispersion in a watery medium is expected to be negligible for path lengths $\leq 60 \mu\text{m}$.

Interferometric TPA autocorrelation permits the measurement of ultrashort optical pulses in the focal point of a high-NA lens. The pulse width in the focus can be controlled—and reduced almost to its original width—by inducing a negative chirp on the pulse. This shows that the pulse broadening is due mainly to group-velocity dispersion and that pulse front distortions caused by chromatic and spherical aberrations are negligible. The measurements show that it is possible to use high-NA lenses in combination with ultrashort optical pulses down to at least 50 fs. After optimal prechirp compensation, there remains some broadening and asymmetry of autocorrelation trace. This may be due to a nonlinear chirp induced by propagation through the lens system. Full determination and quantification of the pulse shape and asymmetry are currently under investigation for a wider range of objectives than the two presented in this Letter.

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