

Using GaAsP photodiodes to characterise ultrashort pulses under high numerical aperture focusing in microscopy

Andrew C. Millard *, David N. Fittinghoff † and Jeffrey A. Squier ‡

* Department of Chemistry and Biochemistry

† Institute for Nonlinear Science

‡ Department of Electrical Engineering and Computer Science

University of California at San Diego

9500 Gilman Drive MS 0339

La Jolla, CA 92093-0339, USA

Michiel Müller

BioCentrum Amsterdam, Institute for Molecular Cell Biology

Kruislaan 316, 1098 SM Amsterdam, The Netherlands

Alexander L. Gaeta

School of Applied and Engineering Physics

Cornell University, Ithaca, NY 14853, USA

Keywords: autocorrelation, dispersion, high numerical aperture (NA),
photodiode, pre-chirping, two-photon, ultrashort pulse

Correspondence to: Andrew C. Millard; tel: +1 619 534 0290; fax: +1 619 534 7654;
e-mail: acm@chem.ucsd.edu

Summary

We demonstrate for the first time that the two-photon response of GaAsP photodiodes can be used to conveniently characterise the spatial and temporal profiles of ultrashort pulses at the focus of a high numerical aperture (NA) system.

In most applications of ultrashort pulse methods to microscopy, it is important to accurately characterise the temporal and spatial profiles of the pulse at the focus of the microscope. This is especially true, for instance, in cases where the pulse is used to generate a harmonic signal (Müller *et al.*, 1998a) or to excite a fluorophore either through multi-photon absorption (Denk *et al.*, 1990) or through single photon absorption with the possibility of intra-pulse, pump-dump processes (Buist *et al.*). In addition, a well characterised focus is necessary for a quantitative measure of the exposure conditions at the specimen. This is helpful for optimising viewing and for accurately assessing intensity levels that may result in damage to the specimen.

Conventionally, ultrashort pulses are measured through auto-correlation in a second-harmonic generating (SHG) crystal and detection with a photo-multiplier tube (PMT). Since this approach becomes less feasible under high NA focusing conditions (Fittinghoff *et al.*), new techniques have been developed — notably two-photon absorption autocorrelation (TPAA) (Müller *et al.*, 1995) — which record the induced fluorescence following two-photon absorption in a fluorophore. In this communication we report a technique, using GaAsP photodiodes, which has several advantages over early TPAA methods. These include a broad

invariance to excitation wavelength (much broader than that of a dye) and excellent signal-to-noise; the diodes are also inexpensive, non-toxic and simple to manipulate.

The Hamamatsu G1117 diffusion diode exhibits a linear response in the spectral range 300 – 680 nm, with a peak response at 660 nm, allowing for a two-photon response in the range 600 – 1360 nm. This response covers the entire tuning range of short pulse lasers commonly used in microscopy, such as Ti:sapphire, including those systems which have extended tuning ranges through the addition of non-linear frequency conversion. In previous studies (Ranka *et al.*, 1997), the maximal quantum efficiency for the two-photon response, defined as the number of photoelectrons per incident photon, was measured to be $\sim 2 \times 10^{-4}$, comparable with that of a 100 μm BBO crystal and PMT combination. However, the spectral response in the range 720 – 950 nm was found to be considerably more uniform than that calculated for a 100 μm BBO crystal. For pulse measurements in those studies, autocorrelations measured with the photodiode were noticeably less noisy than those obtained with the crystal and PMT. As a final comparison, most commonly used fluorophores for TPA in microscopy have bandwidths of only $\sim 30 - 50$ nm, requiring multiple dyes to cover this

same 720 – 950 nm wavelength range. In addition, this large bandwidth ensures the ability to measure ultrashort pulses as short as 6 fs (Ranka *et al.*, 1997).

For the experiments described in this paper, we used a home-built Ti:sapphire oscillator capable of producing 20 fs pulses centered at 795 nm. The average power of the oscillator is 150 mW, and the repetition rate is 96 MHz. The pulses first double pass a fused silica prism sequence, which pre-chirps the pulses. The negatively chirped pulses are recompressed by the positive material dispersion present in all subsequent optics. The magnitude of the negative chirp is chosen such that the pulses are essentially transform-limited after passing through the final imaging optic. After the pre-chirper, the pulses are passed through an autocorrelator, as shown in Fig. 1, and then through the test objective (or lens) of choice to the photodiode positioned at the focus of the final optic. It is important to note that, for this application, the photodiode as provided by the manufacturer did not feature the usual clear protective resin normally coating the diode surface, or the plastic window which is included in the diode housing. This must be specified, and it is imperative that they are removed — otherwise, the photodiode cannot be used in conjunction with high numerical aperture

optics. The protective coatings are simply too thick in relation to the general working-distance of high NA microscope objectives.

The utility of the photodiode is demonstrated in the next two figures. Fig. 2 is the autocorrelation trace as measured using a 10 mm focal length singlet. The diode is simply dialed to the focus of the lens, and, at the appearance of the autocorrelation trace, the relative lens/diode position is adjusted to maximise the autocorrelation signal amplitude. Fig. 3 shows an autocorrelation trace taken with a Zeiss CP-Achromat 100x, 1.25 NA, oil-immersion, infinity-corrected microscope objective. The diode is index-matched to the objective by placing a drop of immersion oil directly on the diode surface. Both traces show approximately the same full-width half-maximum (FWHM), assuming a Gaussian pulse shape, after optimising the setting of the prisms that pre-compensate for dispersion before each measurement. The autocorrelation for the singlet fits better to the eight-to-one ratio (peak to baseline) that is expected for a perfect interferometric autocorrelation than does the objective (Fittinghoff *et al.*). The slight broadening of the pulse-width in the case of the objective is due to the increased prism separation which results in a higher residual third-order dispersion from the prism pre-compensator (Müller *et al.*, 1998b).

Notably, all measurements are made without filters (blocking the fundamental wavelength) in front of the detector (as required with PMT detection for instance), and are performed with full room lighting. The same diode can easily be used with both low NA and high NA focusing conditions, as demonstrated here. The average power necessary for making these measurements was only several milliwatts, which is at or below the average powers used in making typical images in multiphoton microscopy.

Finally, Fig. 4 shows how the same diode can be used to characterise the axial sectioning of the microscope objective. In this case, one of the beams in the autocorrelator is blocked, and the photodiode signal is measured as a function of the axial position of the photodiode, which is scanned through the focus. For this measurement the focusing optic was a Zeiss C-Apochromat 40x, 1.2 NA, water-immersion, infinity-corrected microscope objective. The photodiode was index-matched to the objective by placing a coverslip, with a drop of water on both sides, directly on the diode. Clearly the optical set-up used here shows residual aberration as manifested in a less than diffraction-limited axial sectioning: that is, a distance of $\sim 2.7 \pm 0.5 \mu\text{m}$ instead of $\sim 1.1 \mu\text{m}$ between the 17% and 83% intensity levels.

In conclusion, we have shown that GaAsP photodiodes can be used to characterise ultrashort pulses both temporally and spatially under a variety of focusing conditions. They have been used with a singlet (low NA) and with high NA oil- and water-immersion objectives. These diodes offer a very simple and practical method of pulse characterisation in microscopes, featuring excellent signal-to-noise and very broad wavelength range characteristics.

References

- Buist, A., Müller, M., Brakenhoff, G.J., Squier, J., Bardeen, C., Yakovlev, V. & Wilson, K.R. (in press) Probing microscopic chemical environments with high intensity chirped pulses. *Opt. Lett.*
- Denk, W, Strickler, J.H. & Webb, W.W. (1990) Two-photon laser scanning fluorescence microscopy. *Science* **248**, 73 – 76.
- Fittinghoff, D.N., Millard, A.C., Squier, J.A. & Müller, M. (in press) Frequency resolved optical gating measurement of ultrashort pulses passing through a high numerical aperture objective. *J. Quantum Electron.*
- Müller, M., Squier, J. & Brakenhoff, G.J. (1995) Measurement of femtosecond pulses in the focal point of a high numerical aperture lens using two-photon absorption. *Opt. Lett.* **20**, 1038 – 1040.
- Müller, M, Squier, J., Wilson, K.R. & Brakenhoff, G.J. (1998a) 3D microscopy of transparent objects using third-harmonic generation. *J. Microsc.* **191**, 266 – 274.
- Müller, M., Squier, J., Wolleschensky, R., Simon, U. & Brakenhoff, G.J. (1998b) Dispersion pre-compensation of 15 femtosecond optical pulses for high numerical aperture objectives. *J. Microsc.* **191**, 141 – 150.

Ranka, J.K., Gaeta, A.L., Baltuska, A., Pshenichnikov, M.S. & Wiersma, D.A. (1997) Autocorrelation measurement of 6 fs pulses based on the two-photon-induced photocurrent in a GaAsP photodiode. *Opt. Lett.* **17**, 1344 – 1346

Figures

Figure 1: Schematic of the autocorrelator, shown with a high NA, oil-immersion objective.

Figure 2: Autocorrelation obtained using a singlet with 10 mm focal length.

Figure 3: Autocorrelation obtained using a Zeiss CP-Achromat 100x, 1.25 NA, oil-immersion, infinity-corrected microscope objective.

Figure 4: Axial sectioning measurement, using a Zeiss C-Apochromat 40x, 1.2 NA, water-immersion, infinity-corrected microscope objective.

The measured axial distance between the 17% and 83% intensity levels is $2.7 \pm 0.5 \mu\text{m}$.

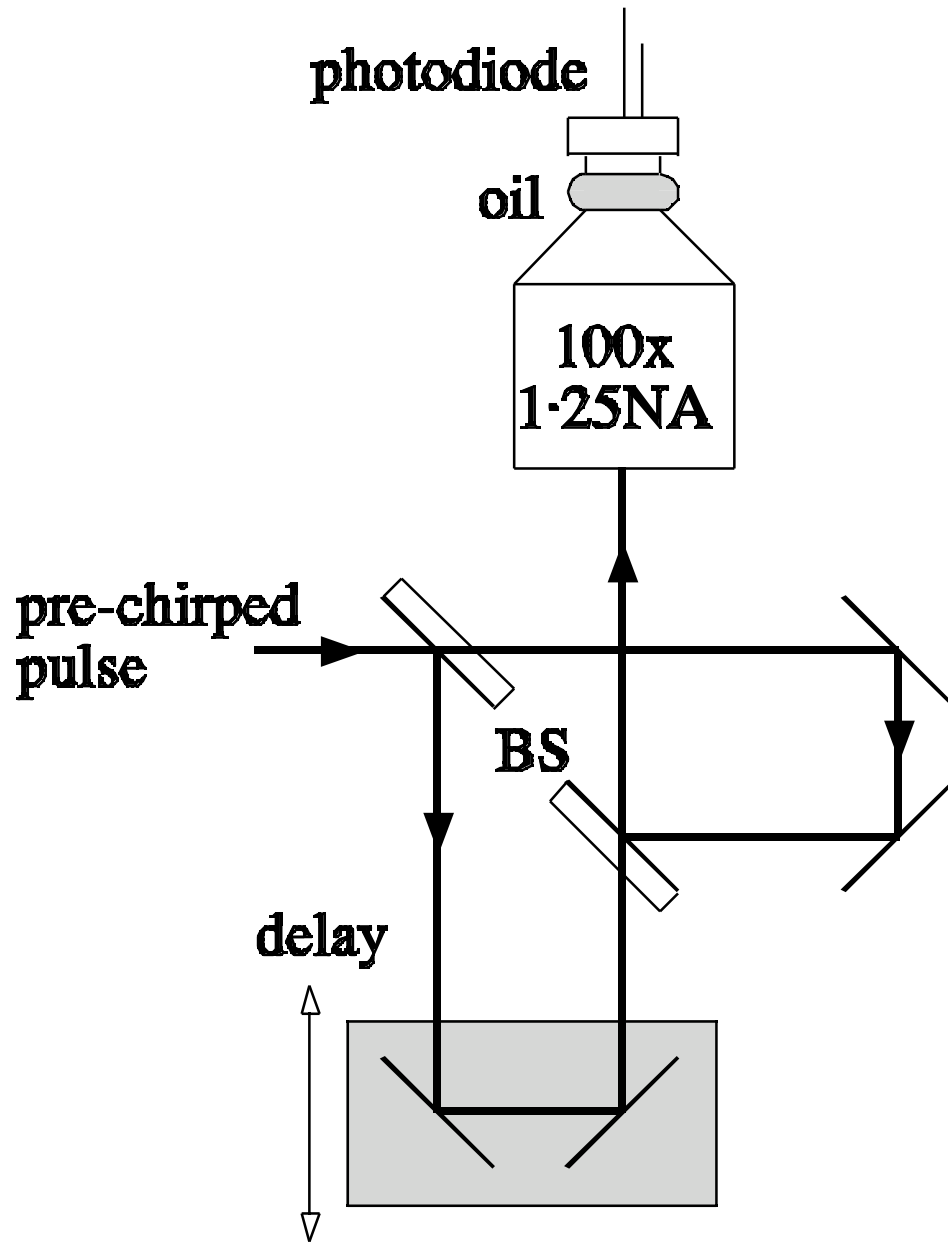


Figure 1

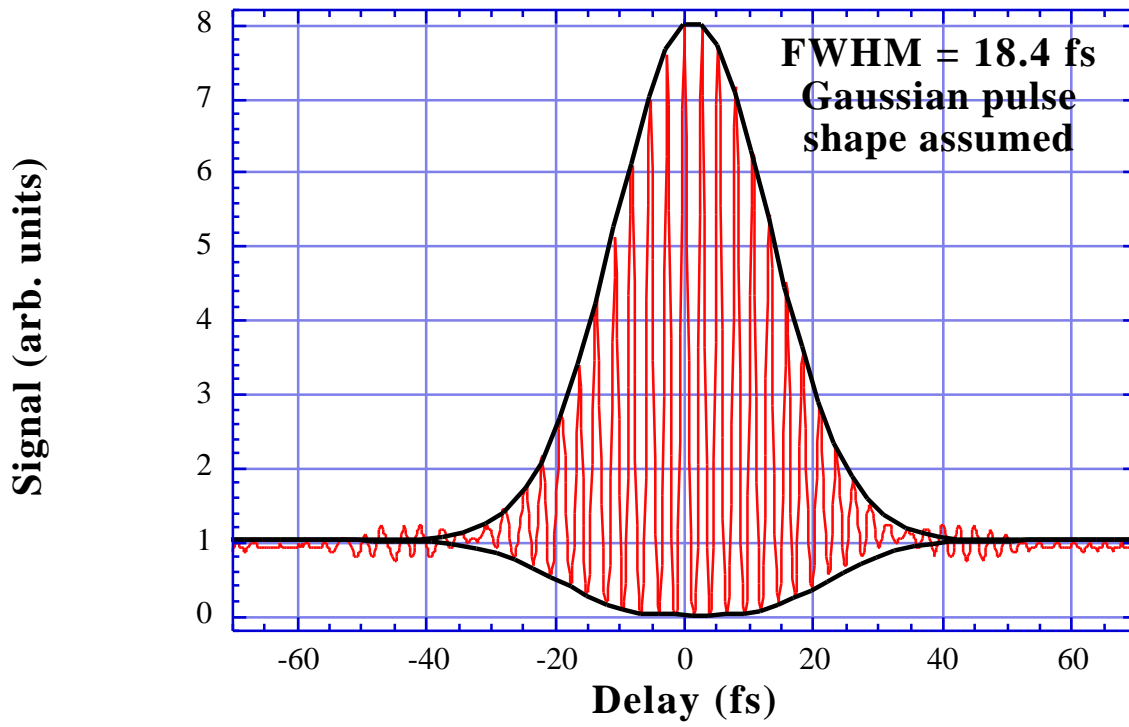


Figure 2

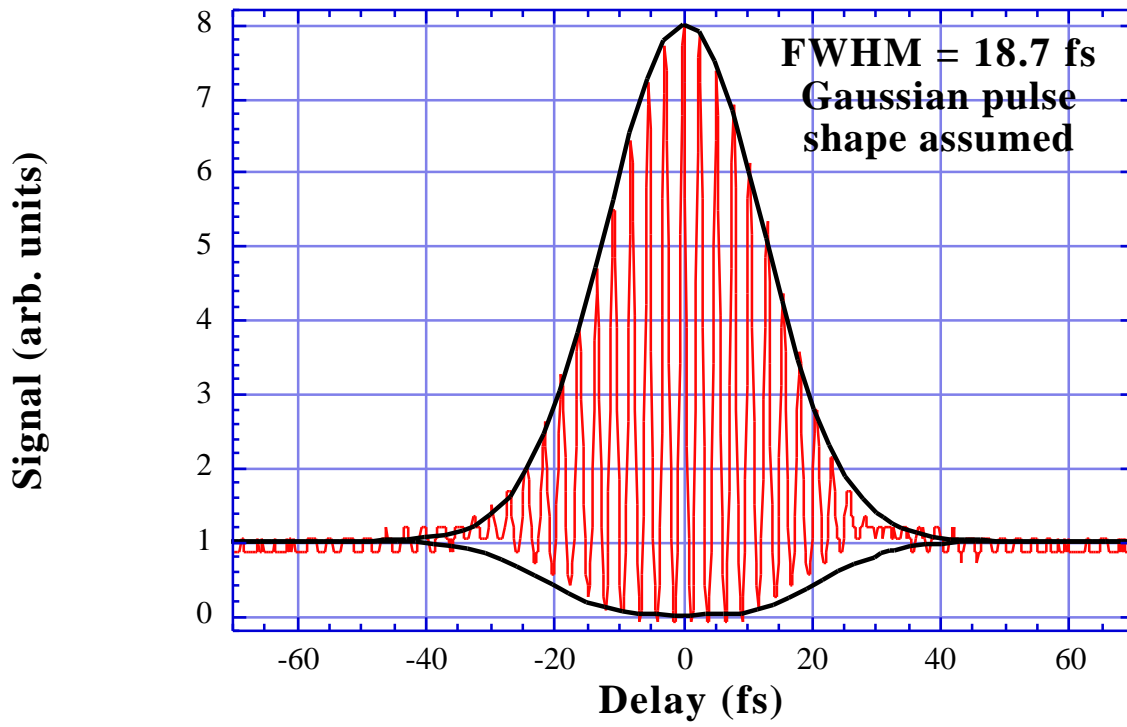


Figure 3

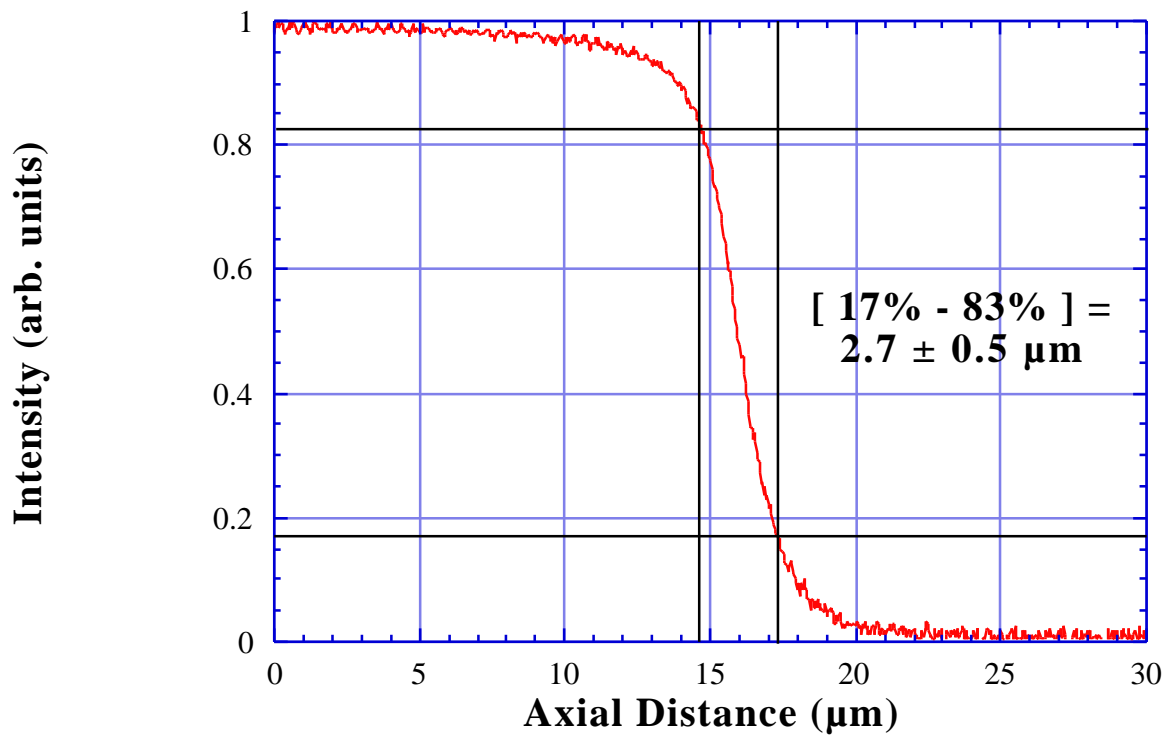


Figure 4