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Measurement of Femtosecond Pulses in the Focal Point of a High NA Lens Using Two-photon Absorption

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There has been increasing interest in the application of femtosecond optical pulses in the field of microscopy (Curley et al. 1992). For instance, femtosecond pulses have been used for two-photon absorption (TPA) in microscopy on biological samples (Denk et al. 1990, Piston et al. 1992). The inherent sectioning properties of TPA allows 3D-imaging of biological samples, a facility which has been shown to be of great interest by confocal microscopy (Brakenhoff et al. 1985). It has been recognized that the strong focusing conditions of high NA lenses may distort the shape of the optical pulse (Horvath and Bor 1994). 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, where the interactions determining the two-photon imaging take place. For instance we found that a chirp-free input pulse of 47 femtosecond may broaden by ≈ 50% after focusing by a well compensated objective. We show that, with the actual pulse width information obtained by the presented methods, proper pre-chirp compensation can be applied to restore , the actual pulse width in the focus of such a lens system to (almost) its initial value.

The general principle of the autocorrelation technique for the pulse width measurements of ultra short optical pulses is schematically depicted in figure 1. An input beam is split by a 50% beam-splitter into two equal beams. One of the beams passes a variable delay line before it is recombined with the other beam. Moving the delay line changes the optical path of one of the beams with respect to the other. This spatial translation thus translates in a time delay. By mounting the variable delay line on a "shaker" -inducing a periodic change in the delay - the overlap of the pulses at the recombining beamsplitter is periodically varied. If some non-linear response is employed, usually Second Harmonic Generation (SHG) in a BBO crystal, the signal becomes a function of the amount of overlap in time of the two pulses. Note that in principle any non-linear interaction - that is also TPA - will fulfil the requirements for an autocorrelation measurement. We found, as illustrated in this contribution, that TPA autocorrelation can indeed be realised in the focal point of a high NA lens, thus providing a measurement of the temporal envelope of the pulse precisely at the point where the TPA induced fluorescence is generated. For the TPA autocorrelation experiments we used a number of different Rhodamine and Fluoresceine based samples.

Using the TPA autocorrelation response we found that it is not influenced by the specific non-linear medium in which the TPA takes place, indicating the possibility for a general application of this technique. Also it permits the measurement of the characteristic increasing penetration depth in the sample. This behavior is expected by considering the increase in spherical aberration that occurs for larger penetration depth in a medium with a refractive index that does not match that of the objective (Hell et al. 1993). A, not-unexpected but still satisfying observation, was that when the TPA autocorrelation signal is measured as a function of the pre-chirp compensation the minimal pulse width corresponding to the highest peak power, does indeed coincide with the maximum in the fluorescence signal amplitude.

Summarising we may say that the measurements presented in this paper show that it is possible to restore a pulse, broadened by propagation through the microscopic set-up, almost to its original transform limited width, by proper adjustment of the pre-chirp compensation and thus optimise the con-

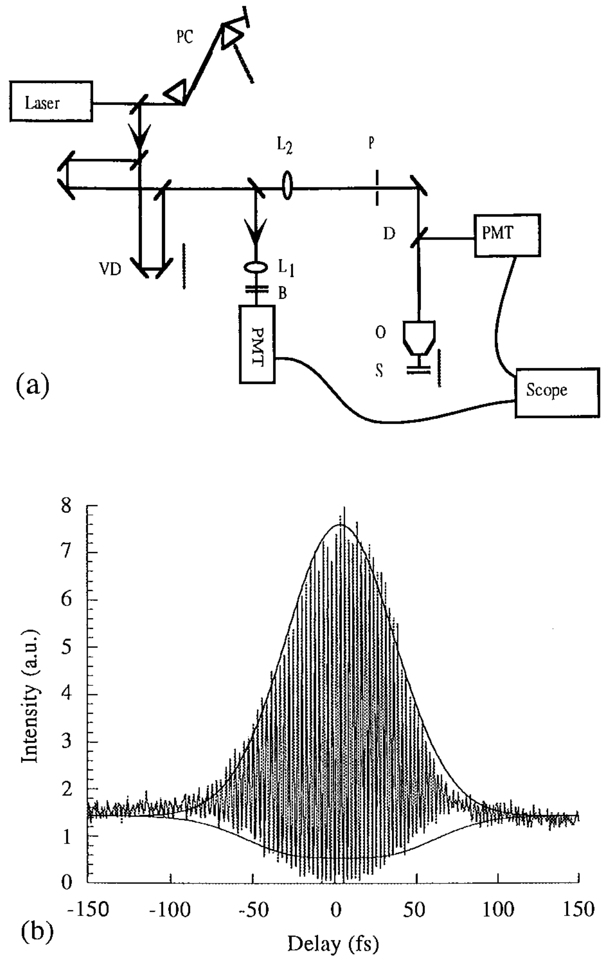

Fig. 1. a) Peinciple of autocorrelation measurement. The input beam is split into two equal parts by a 50% beamsplitter (BS). One of the beams passes a uariable delay line (VD) before being recombined with the other beam on a second - separate but identical -beamsplitter. The variable delay is mounted on a home-built "shaker". By applying a periodic movement of the variable delay, one of the pulses is scanned in time over the other. b) A two-photon autocorrelation measurement of a pulse width of 50.9 ± 1.2 fs in the focus of a high NA objective. The trace was obtained using a 10^{-3} pulse broadening induced by any objective used for a particular application. We observed a strong decrease in signal amplitude for M Rhodamine 6G solution as a medium after optimizing the pre-chirp conditions.

ditions for TPA imaging under the for the imaging relevant conditions.

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