Transient absorption microscopy

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Introduction

We report our initial experimental findings for using tightly focussed, short pulse length laser beams to obtain transient absorption spectra from microscopic interaction volumes. The laser systems are traditionally associated with studying ultrafast processes on macroscopic samples, in bulk solutions. We wish to extend this to enable the study of molecular transformations such as protein folding and DNA transformation in real time. Here, the focussing of the laser beams to micron-scale beam waists is achieved using conventional microscope objective lenses.

The advantages of the apparatus are that we can significantly reduce sample volumes and decrease integrated laser power loading on the sample both factors of increasingly high importance for high value and synthetically challenging chemical and biological specimens. One area in particular that we would like to apply the technique is in extending our recent studies probing the spectroscopic signals from optically trapped aerosol droplets[1,2]. Here it may be possible to follow fast reaction photochemistry and begin to explore cavity effects within these microdroplets using ultrafast spectroscopic techniques.

Experimental

Transient states are excited by pumping the samples at 400 nm and following the time-resolved evolution of these states with a white light continuum probe (400 – 700 nm). The probe beam is focussed into the sample and the transmitted light is collected using two lenses as detailed in Figure 1 whilst the pump beam is focussed in a counter-propagating direction through the collection lens.

The transient absorption apparatus has been described in detail elsewhere in this annual report[3]. Briefly the WLC (white light continuum) probe beam was generated by focussing ~3 μJ of the output from a SpectraPhysics Spitfire regenerative amplifier (800 nm, 200 fs, 1 kHz) into a 2 cm quartz cell containing water. The polarisation was set at horizontal using an 800 nm polarisation rotator. After supercontinuum generation, the residual 800 nm fundamental was removed using a dichroic filter. The continuum was then collimated and split using a silvered plate to 2:3 reference and probe arms. The visible probe FWHM spot diameter in the sample attained using different focusing optics is discussed below. The reference and probe beams were re-collimated and then focused onto the reference and probe silicon diode arrays[3] after dispersion from a 600 l/mm 500 nm blazed diffraction grating (Thorlabs) set ~30 cm from the arrays.

The WLC probe beam was initially focussed using a ×10 microscope objective lens as shown in Figure 1. The beam diameter was measured at the sample using a knife edge and piezo electric stage (Physik Instrumente Piezo Flexure

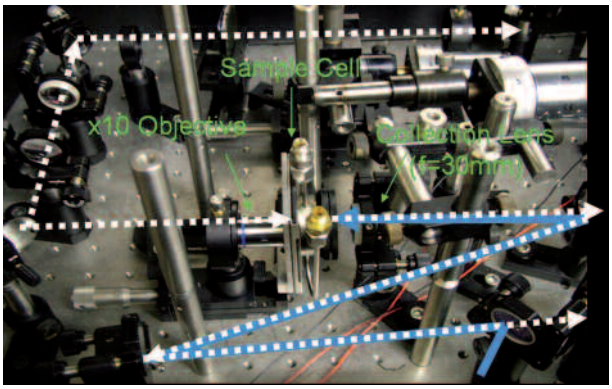

Figure 1. Optical configuration at sample. Dashed white line indicates the probe and reference paths of the WLC, the blue line indicates path of the pump beam.

Stage P517.3CL with capacitive sensor position control) and a plot of percentage transmission versus knife edge position was fitted to a spline function for individual wavelengths.

The beam diameter at $1/e^2$ was found to increase with wavelength from 1.7 μm at 475 nm to 4.4 μm at 675 nm. The anomalies in the measured values at 450 nm and 700 nm are attributed to the sharp cut-off of the filters used at the high and low energy side of the spectra to remove the residual visible pump and WLC generation pump beams respectively. (This can be clearly seen from the shape of the probe spectrum overlaid in Figure 2). The pump beam was measured in a similar way using the piezo mounted knife edge and a Molectron J3-5 energy meter to be 17 μm diameter at focus.

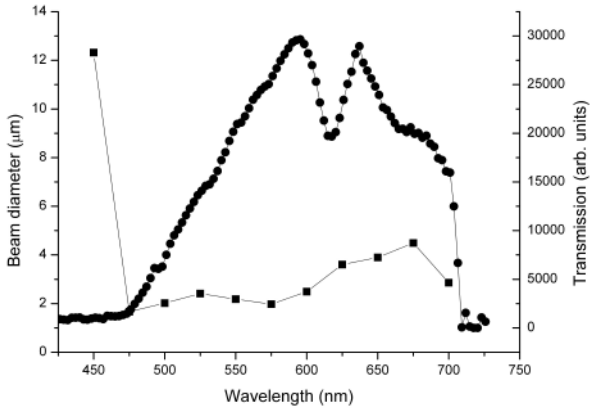

Figure 2. The beam diameter of the WLC is shown vs wavelength. The probe spectrum is also displayed (scale on right) to show interference from filters at either end of spectral range.

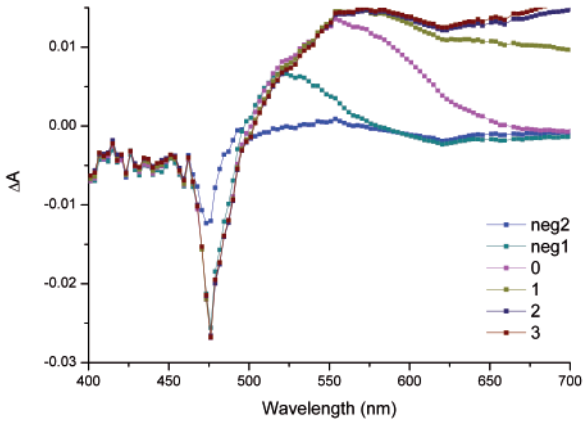

Figure 3. Transient Absorption spectra of ruthenium tris bipyridine recorded using ×10 objective at -2, -1, 0, 1, 2, and 3 ps after excitation. Acquisition time 10s.

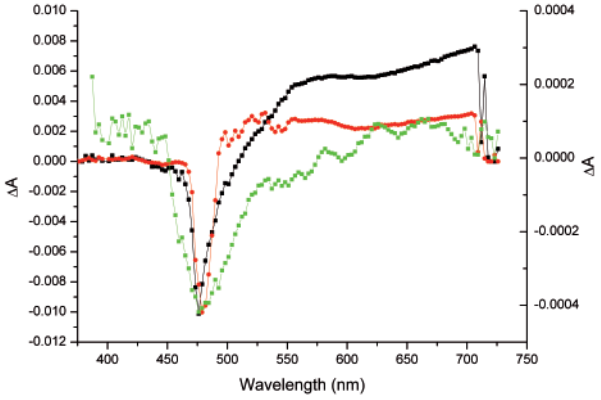

Figure 4. Transient Absorption spectra of a saturated solution of $[Ru(bpy)]^{2+}$ in CH_3CN recorded using ×10 objective at 50 ps after excitation with 100 µm and 6 µm spacers (black and red traces respectively). The spectrum from the doped beads is shown in green (scale on right).

In the 500 to 700 nm wavelength range the observed beam diameter is close to the diffraction limit expected for a TEM_{00} beam with this microscope objective (~ 2.4 µm at 500 nm). The variation of beam diameter observed stems from a combination of the wavelength dependence of beam divergence seen in white light continuum generation and chromatic and optical aberrations in the beam transport optics, such as the beamsplitter angled at 45° and the microscope lens.

Wavelength dispersion in the water cell and subsequent optics leads to temporal chirp of the beam at the sample. This was measured about time zero, Figure 3, and is around 1.3 ps per 100 nm.

Transient absorption spectra of a saturated solution of ruthenium tris bipyridine $[Ru(bpy)_3]^{2+}$ in acetonitrile at 50 ps after excitation with an exposure time of 10 s are shown in Figure 4 below. The pathlength was varied by using a Harrick cell with 100 and 6 µm Teflon spacers. The optical density of the sample was ~ 11 in 1 mm. The spectra show a characteristic ground state bleach at 460 nm and a broad featureless transient above 500 nm [3] as observed with the conventional TA setup (discussed in reference [2]). The signal-to-noise ratio did not significantly deteriorate on going to the shorter pathlength cell due to the high optical density and because the pump probe overlap remains good over this range. The changes in shape in the spectrum between 100 and 6 µm spacers is thought to be due to inner filter effects.

To apply this in a microscopic scenario, we prepared samples of dry 5 µm polystyrene beads doped with $[Ru(bpy)_3]^{2+}$ solution on the Harrick cell window. After careful manipulation of the cell to overlap with a bead, the spectrum shown in Figure 4 was obtained (ΔA scale shown on right hand side). The spectrum is the averaged signal from 5 beads with 10 s exposure from each bead. The broadening of the bleach signal from the doped bead is attributed to increased scattering of the transmitted light and the averaging process.

Discussion

Work is underway to implement this optical setup on a microscope for future experiments. A number of important considerations from the results presented above will need to be addressed in a forthcoming beamtime. Publications reporting TA spectra from a single a-perylene crystal obtained using a microscope and single point photodiode detectors [4,5] used pump energy densities of ~20 mJ cm^{-2} and in this study we operated at ~100 mJ cm^{-2} (pump) and ~10 mJ cm^{-2} (probe). Although we did not see evidence of sample damage over the 10 second accumulation times, signal strengths for the bead measurements were found to decrease at longer exposure times. Given the peak power of the pump laser, we might have expected greater damage. We suspect that the pump beam may in fact have been bigger than the measured value of 17 µm due to a slight mismatch between the focal planes at the sample since we only took into account the effect of the thick windows in the Harrick cell for the probe and not the pump beam. We anticipate that the proposed use of this optical arrangement on a microscope will significantly improve signal acquisition through precise alignment of the pump-probe focal cross-sections to within a micron both in pointing positions and in focal overlap. This will also be coincident with the object of interest and should allow us to reduce the laser energies from those used previously and to reduce the likelihood of sample damage.

We have demonstrated the feasibility of combining the USL transient absorption capability with the microscopic techniques employed in the Tweezers Laboratory to collect transient spectra from very small sample volumes. The ability to reduce sample volumes and laser interaction volumes without compromising signal to noise has been shown to be feasible. By reducing the beam diameter to <5 µm there is a potential reduction in average power loading on the sample of up to 400 times for thin samples over those used in typical TA experiments. This work will be extended to various novel targets later this year and could be applied to future very high resolution pump probe instruments employing 10's of kHz to MHz repetition rate light sources.

References

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