Nonlinear microscopy pulse optimization at the sample plane using second-harmonic generation from starch

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ABSTRACT

In this paper we report the use of a starch as a non-linear medium for characterising ultrashort pulses. The starch suspension in water is sandwiched between a slide holder and a cover-slip and placed within the sample plane of the non-linear microscope. This simple arrangement enables direct measurement of the pulse where they interact with the sample.

Keywords: Nonlinear materials, ultrashort pulse characterisation, nonlinear microscopy, starch.

1. INTRODUCTION

The visualisation of the internal structure of biological organisms is essential for the advance of life science. Greater demands are placed upon the field of microscopy to develop techniques that are able to image ever more complicated structures. Techniques which are able to distinguish between different parts within cells are becoming increasing important and this is a reason why non-linear microscopy is a very dynamic area of investigation. Non-linear, fluorescence microscopy has generated much interest within the scientific community thanks to its ability to generate high-resolution, three dimensional images without having the restrictions that confocal microscopy possesses. Different non-linear processes have been utilised for fluorescence microscopy and include two-photon absorption, second harmonic generation, third harmonic generation and coherent anti-Stokes Raman Scattering. These non-linear processes all have the same fundamental advantage in that the signal comes solely from the focal point of the laser, helping eliminate the blurring of an image caused by scatter.

Recent research within the area of non-linear microscopy has shown how the temporal and spectral properties of the incident ultrashort pulse can have significant effect upon the efficiency [1] and selectivity [2] of an image. In the first of these publications, the phase of an ultrashort pulse was arbitrarily altered until the desired two-photon fluorescence signal was optimised. As yet, little to no information is known about this phase dependant optimisation process within a microscope and there is a great urgency to develop techniques that are capable of achieving this. For this to occur it is necessary to directly measure the phase information of the pulse at the sample plane of the microscope. Previously, pulse measurements within non-linear microscopy have consisted of either autocorrelation measurements [3] that give no direct phase information on the pulse or they are fully characterised before they enter the microscope. This information serves no practical use since the many dispersive optics within the microscope (specifically the objective lens) significantly alter the phase profile of the pulse, making the pulse incident upon the sample very different to that which enters the microscope.

In this paper we will describe, in section 2, a new and novel methodology to overcome the problems associated with the full characterisation of ultrashort pulses at the sample plane of a non-linear microscope. In section 3 we will demonstrate the effectiveness of this technique by fully characterising two differing ultrashort pulses at the sample plane of the microscope.

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2. METHODOLOGY

Second harmonic frequency resolved optical gating (SHG-FROG) is the most commonly used technique to fully characterise an ultrashort pulse. The technique is simply an extension of an SHG autocorrelation whereby the SHG signal is spectrally resolved as a function of delay. This operation is performed until a time-frequency representation of the pulse (ie. Spectrogram or SHG-FROG trace) is obtained. By passing the SHG-FROG trace through an iterative retrieval algorithm all information from the pulse can be gained.

Two main problems arise when trying to measure a SHG-FROG trace through a high numerical aperture (NA) objective lens.

1) A collinear geometry is imposed since the full NA of the objective lens has to be fully filled. SHG-FROG has traditionally always been carried out in a non-collinear geometry in order to avoid interference between DC terms. As a consequence, a FROG trace which has been acquired under a collinear geometry (CFROG), is very much different to that obtained under a non-collinear geometry and can not be directly used in current, optimised retrieval algorithms [4].

2) The choice of the nonlinear medium at the sample plane is very troublesome. The large acceptance angles introduced by the high NA objective lens, the large bandwidths associated with ultrashort laser pulses as well as polarisation sensitivity all combine to put great demands upon the non-linear medium, demands which previously have not been met.

In the following two sections (2.2 and 2.3) we will directly address these two problems respectively.

2.1. Collinear SHG-FROG (CFROG)

In this section we will first outline a new, general procedure to extract the non-collinear SHG-FROG term from a CFROG trace without the need to use complex and error prone cross-polarisation techniques that have been used in the past to avoid interference between cross terms [5]. This will enable the use of fast, commonly used SHG-FROG retrieval algorithms. We will then outline the criteria required for rapid acquisition of the CFROG trace.

As briefly outlined before, the collinear geometry, imposed within a microscope, causes difficulty when trying to measure a SHG-FROG trace. Unless the two interferometric arms are cross polarized to utilize Type II SHG signals [5, 6], the two beams will interfere to create interferometric fringes within the trace. Figure 1 outlines this idea schematically;

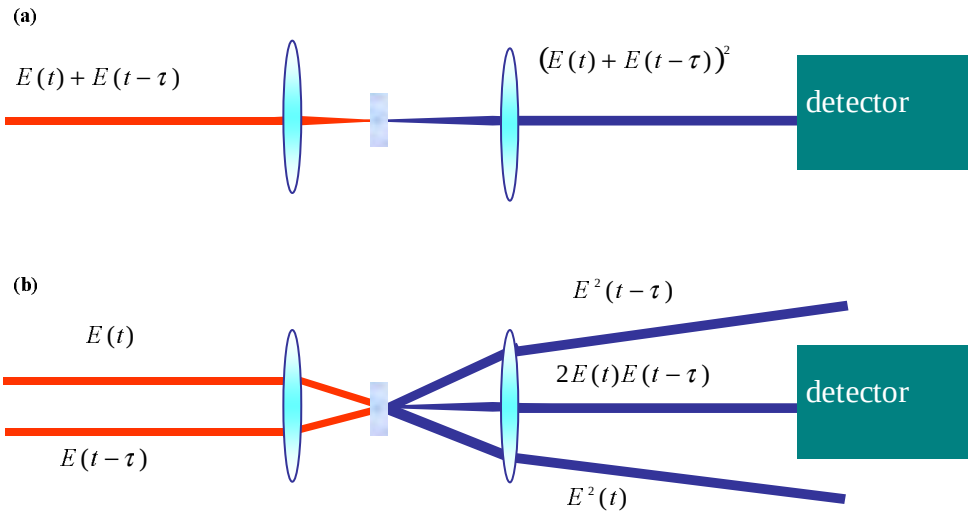

Figure 1. Schematic representing the differences within the detected (a) collinear and (b) non-collinear signal.

By Fourier analysis, the generated signal of the collinear term, given in Figure 1(a) can be written as:

$$\begin{aligned} I_{CFROG}^{SHG}(\tau, f) \propto & \quad 2I_{SHG}(f) \\ & + \quad 2I_{SHG}(f)\cos\left(2\pi\left(2f_0 + f\right)\tau\right) \\ & + \quad 4\mathrm{Re}\left\{E_{SHG}^{*}(f)E_{FROG}^{SHG}(\tau, f)\left(\exp\left(-j2\pi f_0\tau\right) + \exp\left(j2\pi(f_0 + f)\tau\right)\right)\right\} \\ & + \quad 4I_{FROG}^{SHG}(\tau, f) \end{aligned} \tag{1}$$

The first two terms in Equation (1) correspond to the intensity resulting from the linear interference between the SHG of the pulse and the delayed one. The first term is the inherent background associated with a normal interference autocorrelation trace while the second term is the exact same background but modulated at $2f_0$. The third term is a mixture of many terms, all of which are modulated at f_0. Finally, the last term is exactly the same as that measured under non-collinear conditions and is thus the term needed to be unwrapped for our purposes. Figure 2 gives an example of what is observed when the Fourier Transform of a CFROG trace is taken

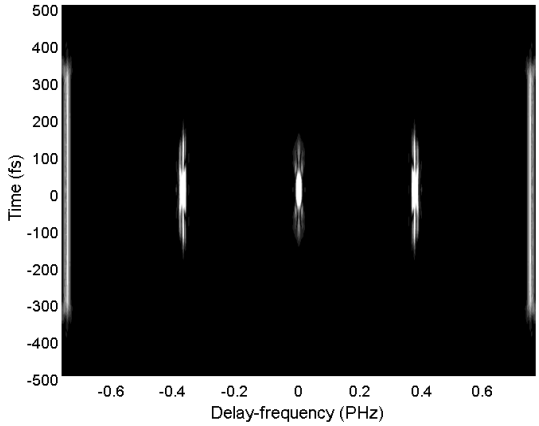

Figure 2. A Fourier Transformed CFROG trace.

It can be seen from this trace that a relaxed cut-off frequency for filtering can be applied without major loss of information since the interferometric components at f_0 and $2f_0$ are well separated from DC. After filtering, the only non desired term that remains in Equation (1) is the first one. This component, the SHG spectrum, overlaps with the DC term. However, it can be easily removed by either measuring it or taking the average from several samples at the edges of the delay axis and then subtracting it from the trace. After all this process, the remaining DC term contains the same information as a typical non-collinear SHG-FROG trace and thus a normal retrieval algorithm can be employed. It should be noted that when using fast Fourier transforms (FFT) there is an assumption of periodicity within the CFROG trace. This is a condition that is impractical to carry out when experimentally acquiring the trace. As a consequence, an error will be introduced in the form of modulation components in the frequency direction. This error however has been found to be insignificant if a two-dimensional Fourier Filter is applied rather than the expected one-dimensional Fourier Filter [4].

When dealing with ultrashort pulses that include many of optical cycles, a large number of sample points will be needed if the Nyquist criterion is to be met. As a consequence, a large and clumsy data set will be generated that is both difficult and time consuming to acquire and analyse. It has been shown however that Nyquist does not have to be obeyed so long as two criteria are met [4].

Criterion 1

The first criterion involves choosing an appropriate delay step ($\Delta\tau$) and is outlined in equation (2)

$$\Delta\tau = \frac{n \pm \frac{1}{3}}{f_0} < \frac{\tau_{IA}}{10} \tag{2}$$

where f_0 is the frequency of the optical carrier, n is an integer and τ_{IA} is the full width at 15% of the maximum of the interferometric autocorrelation trace.

Criterion 2

The second constraint is used to define a sufficient delay span τ_{span} to give a negligible error:

$$\tau_{span} \geq 2 \cdot \tau_{IA} \tag{3}$$

By following the criteria given above, it is possible to dramatically reduce both acquisition and data processing time with insignificant loss in accuracy. This point is highlighted in Figure 3 which shows the RMS g-error between experimentally obtained non-collinear and collinear measurements using a BBO crystal with different number of sampling points (all of which obey the criteria in equations (2) and (3))

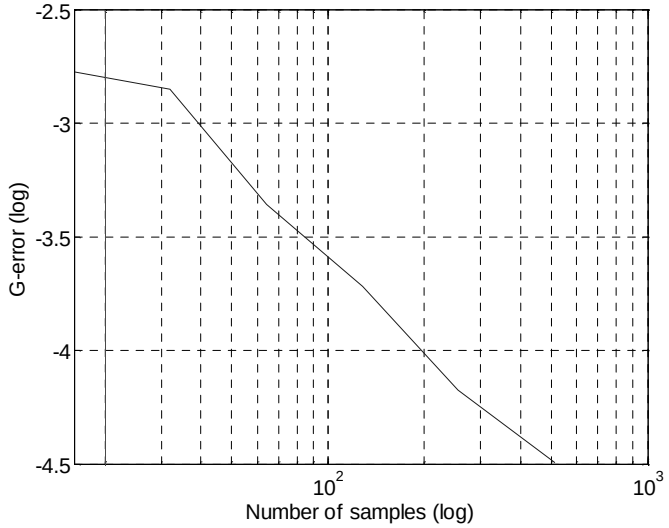

Figure 3. Experimental G-error from the FROG trace and the CFROG trace depending on the number of samples.

We can see from Figure 3 that with 512 samples there is a very small error of G = 3.1·10-5. As the number of samples reduce the error increases. However even with only 16 sampling points a relatively low error of G = 1.6·10-3 is produced. This sampling rate is in fact below our sampling limit (Eq. (2-3)), but for well-behaved pulses the criteria may be relaxed.

2.2. Starch as the non-linear medium

In the previous section we have outlined a methodology that will allow, by way of a collinear geometry, a full characterisation of pulses at the focal plane of a high NA objective lens. However, the choice of a non-linear medium for characterising an ultrashort laser pulse passing through a high NA objective lens is still problematic. There are a number of reasons for this:

1) There is an intrinsically large range of incident angles at the focal plane. As a consequence, the efficiency of the generated SHG signal, ideally, needs to be angle independent.

2) The large frequency bandwidth associated with ultrashort laser pulses require the phase matching bandwidth of the non-linear medium to be large (ie. there is a need for a thin non-linear crystal).

3) The extremely steep convergence angles modify the polarization of the outer beam [7] and will thus cause errors with polarisation sensitive non-linear media.

All of the above problems make the use of traditional non-linear crystal error prone. This is emphasized by Fittinhoff et al [5] where the use of a 'thin' non-linear crystal at the focus of a high NA objective lens was not able to yield a satisfactory 8:1 ratio interferometric autocorrelation trace. There are other alternatives such as measuring the photocurrent generated by two-photon absorption (TPA) photodiode [3] and by measuring fluorescence emission by TPA in a dye [8]. In the first technique they were able to obtain reliable autocorrelation traces but a more complex set up is needed to be able to recover amplitude ad phase of the pulses. The second technique could be used for obtaining a FROG trace, but it will be subject to photobleaching. Another available technique relies on the SHG from protein polymer chains such as bacteriorhodopsin (bR) [9]. However, this technique relies on complex preparation for the nonlinear medium. In this section, we will demonstrate the use of a starch suspension (see Figure 4) as a novel type of non-linear medium whose natural characteristics provide a solution for the problems described above. This medium will be used to reliably generate a FROG trace for complete pulse characterisation at the sample plane of a nonlinear microscope.

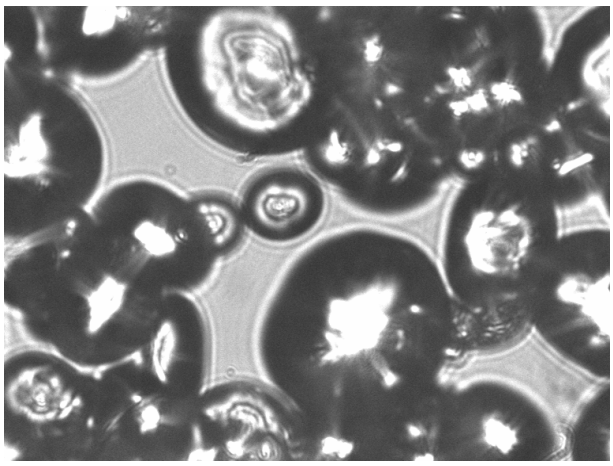

Figure 4. Photograph of starch granules.

Starch has been shown to have naturally high χ^2 coefficient [10, 11]. SHG signals have previously been generated from starch with ultrashort laser pulses with wavelength between 700 nm and 1300 nm [12]. These results are highly significant since they demonstrate the extremely large spectral bandwidth that starch possesses. Furthermore, since starch is an isotropic medium, its χ^2 coefficient is both polarisation insensitive and its conversion efficiency is angle independent. All of these properties make starch an ideal non-linear medium for use with a high NA objective lens.

As well as the advantages gained by the natural physical properties of starch it is also important to highlight the more practical advantages that starch possesses. For example, it is ideally suited for work within a microscope since starch water suspension can simply be sandwiched between a slide holder and a cover slip and placed directly within the sample plane of the non-linear microscope. The difference in cost between a thin, nonlinear crystal and starch is also extremely significant. Futhermore, starch is non-toxic, easy to store, non-photo bleaching, easy to obtain and easy to handle.

As an initial test to verify that starch can be used successfully for pulse characterisation we used it to measure the interferometric autocorrelations of a pulse passing through a 0.3 NA objective lens. This pulse was centred at a wavelength of 810nm. We compared this trace with those obtained with two, more traditional, non-linear media – a BBO crystal and a GaAsP two-photon detector. The traces are given in Figure 5.

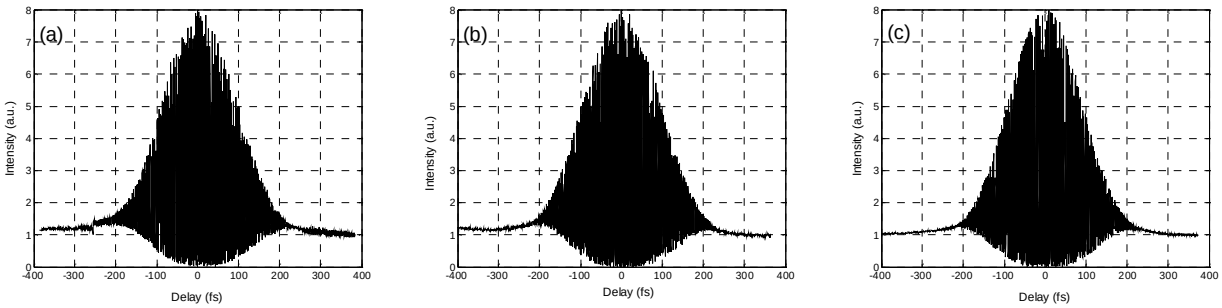

Figure 5. Interferometric autocorrelation traces produced from (a) Starch, (b) a BBO crystal and (c) a GaAsP two-photon detector.

From the previous figure we see that the traces compare remarkably well with each other by producing identical pulse durations of 150 fs as well as giving the required 8:1 ratio. The results verify that starch can be successfully used as a direct replacement for a traditional SHG nonlinear crystal within an autocorrelator.

In the next section we will demonstrate the use of starch suspensions to obtain a complete characterisation of the pulses at the focal plane of high NA objective lens by means of the CFROG technique. Thus, by using starch as a nonlinear medium, two different CFROG traces will be acquired under different conditions. The results obtained will help to demonstrate both, the versatility of starch as nonlinear medium and the flexibility of the CFROG technique.

3. EXPERIMENTAL RESULTS

The general optical arrangement to measure a starch-based SHG-CFROG trace remained the same throughout both measurements, the schematic of which is given in Figure 6.

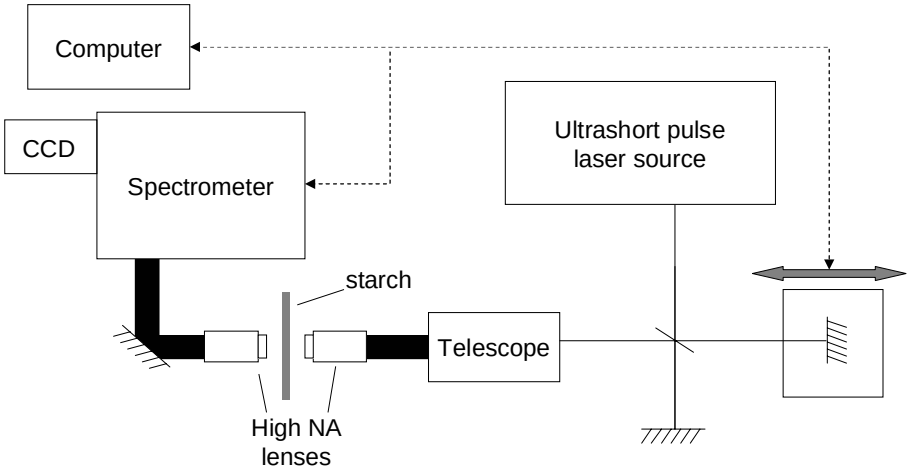

Figure 6. Schematic of the CFROG optical arrangement.

A telescope arrangement was used at the output of the interferometer and before the microscope objective to ensure the beam filled the whole input aperture of the objective lens. A starch suspension in water was prepared and sandwiched between two cover slips. These were placed at the focus of the high NA lens and oil refractive index matching was used to ensure full use of the NA of the lens. Another lens was used to collimate the generated frequency doubled signal that was sent to the spectrometer. Both the delay stage (in the interferometer) and the spectrometer were controlled by the computer in order to acquire the results quickly and efficiently. A back thinned CCD linear array (Hammamatsu HC230-1007), operated in vertical binning mode, was attached to the spectrometer to record the spectrum of the generated SHG.

In what follows (section 3.1 and 3.2), two separate CFROG pulse characterisation measurements are presented. These were taken using different pulse sources, different high NA objective lenses, different delay spans and different sampling rates. These changes were purposely carried in order to demonstrate not only the flexibility and robustness of this new CFROG characterisation technique but also to demonstrate the use of starch as an ideal nonlinear medium for performing such measurements.
In particular, in section 3.1 we acquire a CFROG trace at a sampling rate which obeys the Nyquist criteria. This allows us to resample to a lower number of points in order to investigate the effect (if any) that undersampling has on the retrieved result. In section 3.2 however we directly undersample while at the same time characterising a pulse which has a vastly differing wavelength. This enables us to highlight the phenomenal bandwidth which starch possesses.

3.1. Pulse characterisation through high NA lenses.

In this section, the pulses at the focal plane of an oil-immersion objective lens with a NA = 1.25 are characterised. These pulses were generated from a Kerr-lens modelocked Ti:Sapphire laser that was operating at the central wavelength of λ = 820nm. The pulses entering the objective lens had an average power of 15mW. The CFROG trace (shown in Figure 7) was then acquired at Nyquist sampling rate.

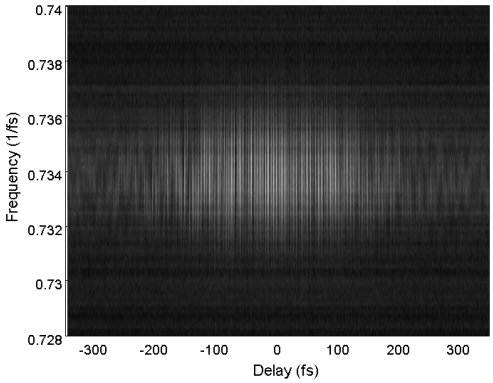

Figure 7. Experimentally acquired starch-based SHG-CFROG trace using a NA = 1.25, at λ = 820nm.

Since a Nyquist criterion was used, the integration in time of this trace yields the interferometric autocorrelation (see next figure). This can be used to help verify the validity of the acquired data.

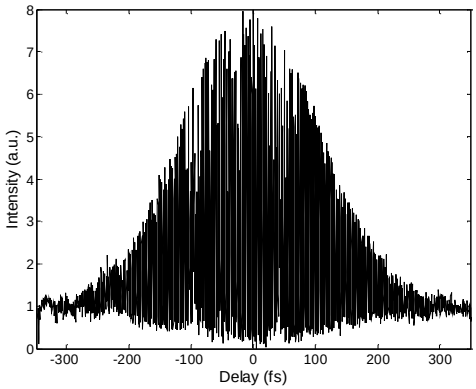

Figure 8. The interferometric autocorrelation trace obtained at the focus of a N.A.=1.25 lens.

Figure 8, clearly shows the required 8:1 ratio of the interferometric autocorrelation. This result in itself demonstrates the effectiveness of starch as a non-linear medium given the failure of traditional non-linear crystals to obtain this ratio when using high NA lenses. The CFROG trace (in figure 7) was then filtered using the process outlined in section 2.1 and retrieved using a traditional SHG-FROG retrieval algorithm [13]. The experimental and retrieved traces are presented in Figure 9 along with the retrieved pulse and spectrum.

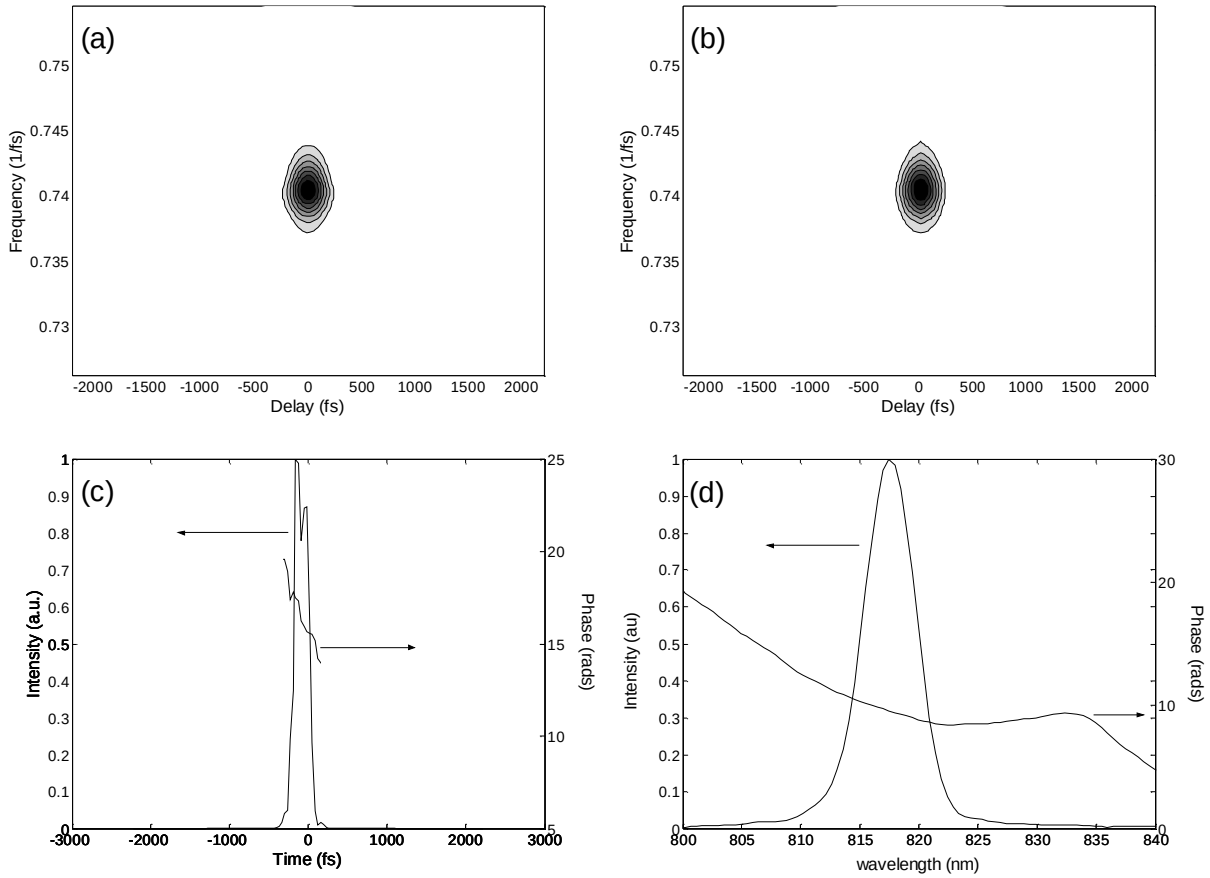

Figure 9. Experimentally retrieved pulses at the focal plane of a NA = 1.25 objective lens. a) Fully filtered CFROG trace, b) retrieved trace, c) amplitude and phase and d) spectrum and phase of of retrieved pulse.

By comparing the two traces, we found a low G-error of $G = 8 \cdot 10^{-5}$. We further checked the validity of this retrieved data by comparing the experimentally obtained and retrieved intensity autocorrelation traces. These are shown in Figure 10. where it is possible to see an excellent agreement between them. This indicates that stach appears to be a good option when characterising ulrashort pulses at the focal plane of a high NA lens.

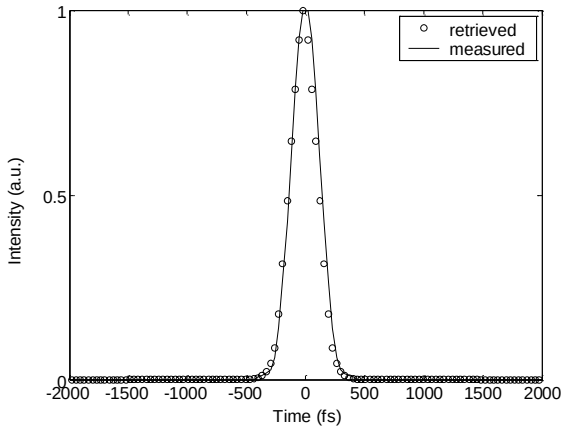

Figure 10. Comparison of experimentally obtained (solid line) and retrieved (circles) intensity autocorrelation traces.

Since a Nyquist criterion was carried out during this set of results we have been able to test the effectiveness or our undersampling criteria. We have achieved this by comparing how the retrieved, fully sampled trace (figure 9(b)) compares with retrieved traces taken from undersampled CFROG data. The results of this investigation are outlined in Figure 11

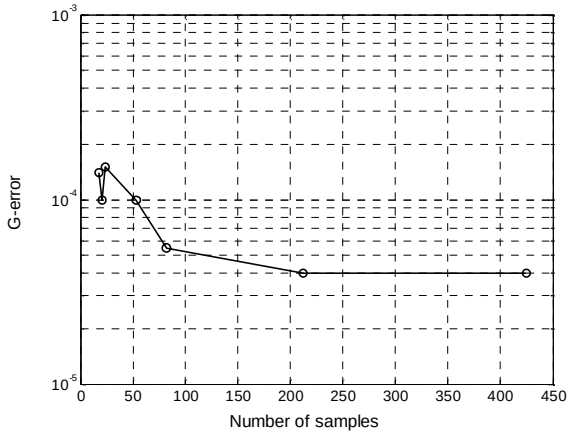

Figure 11. RMS G-error of the Nyquist sampled retrieved data (Fig. 9(b)) with undersampled experimental data.

Two important points can be taken from this result. Firstly, unless extreme undersampling is applied there is insignificant error introduced into the retrieved data. Secondly, if extreme undersampling is applied, the error / noise that is introduced into the trace is largely compensated for by the retrieval algorithm.

3.2. Pulse characterisation at different wavelengths

To further investigate on the use of starch, we proceed to characterise the output pulses from a synchronously pumped optical parametric oscillator (OPO) operating at 1100 nm. To do this, an objective lens with a NA = 0.85 was used. In this case, the acquired CFROG trace was purposely undersampled, obeying the criteria outlined in section 2.1. In this case we used the delay step of 42.6 fs and producing 96 samples. The resulting CFROG trace, showing broading interference filters as a result, is given in Figure 12.

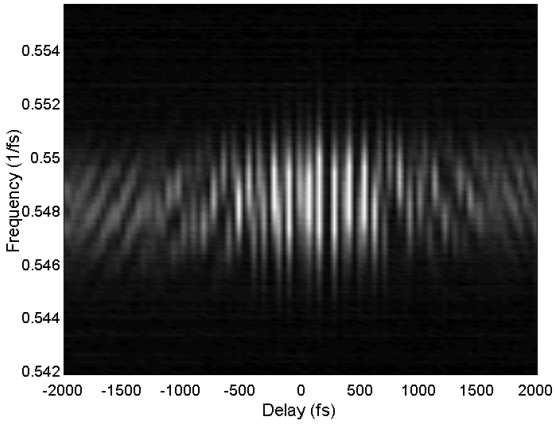

Figure 12 Experimentally acquired starch-based SHG-CFROG trace using a NA = 0.85, at λ = 1095 nm.

As before, the trace was again filtered to produce a non-collinear SHG-FROG trace and then retrieved. The results of this retrieval is shown in Figure 13.

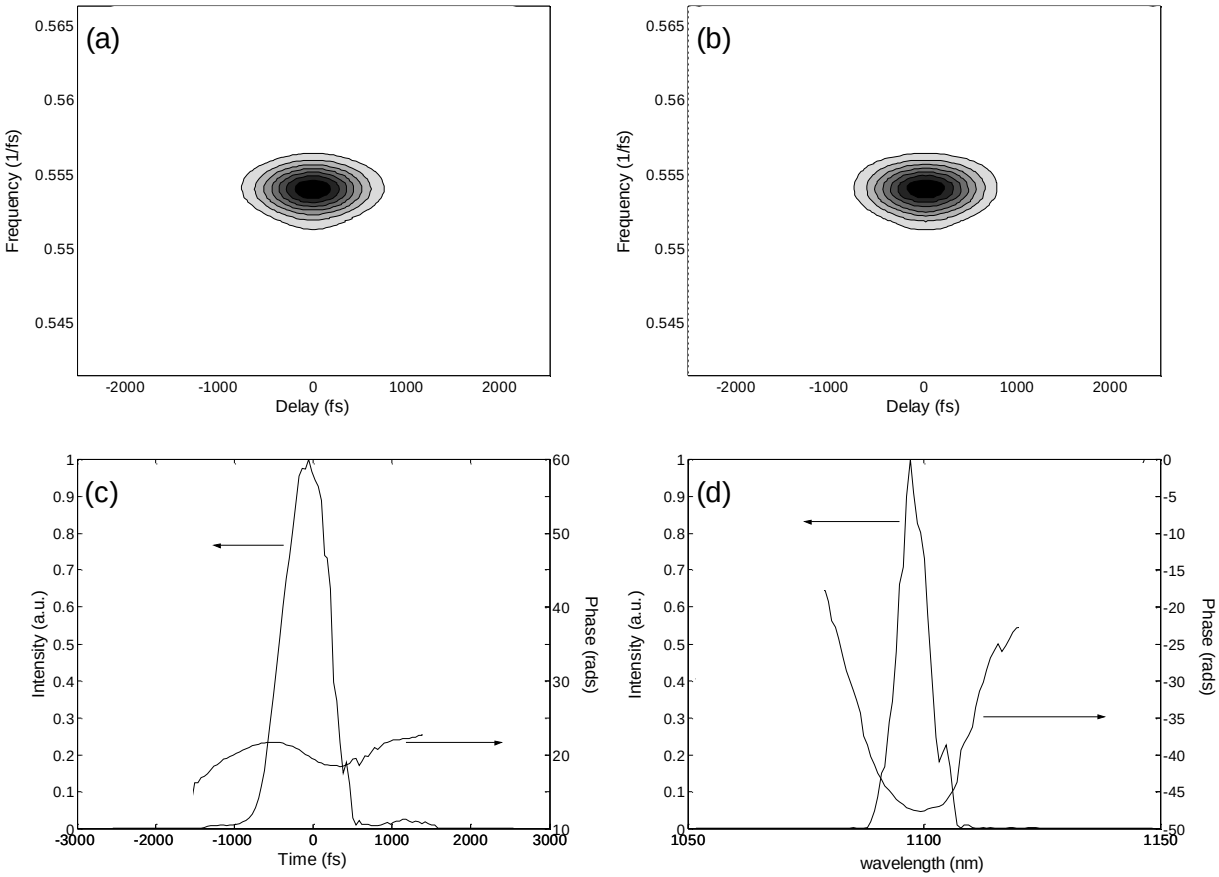

Figure 13. Experimentally retrieved pulses at the focal plane of a NA = 0.85 objective lens. a) Fully filtered CFROG trace, b) retrieved trace, c) amplitude and phase and d) spectrum and phase of of retrieved pulse.

These retrieved results are validated by comparing them with externally measured data. Figure 14 shows the excellent agreement obtained.

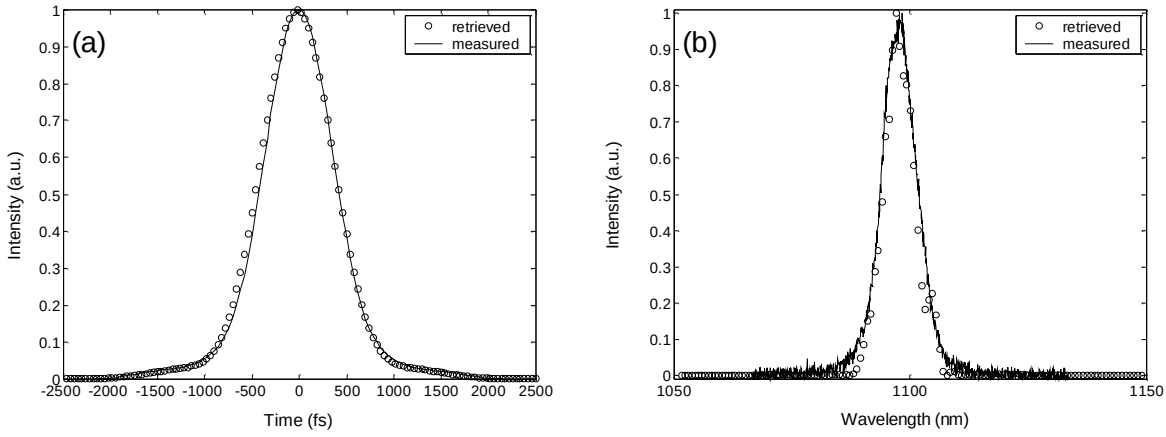

Figure 14 Comparison of experimental and retrieved data. a) Measured (solid line) and retrieved (circles) intensity autocorrelation. b) Measured (solid line) and retrieved (circles) spectrum.

From the previous result we can confirm that starch can be successfully used to characterise pulses with different characterisctics (pulse durations, wavelengths, NA,etc…), at the focal plane of a nonlinear microscope in an easy a quick way by means of the CFROG technique.

4. CONCLUSIONS

Within this paper we have demonstrated a successful full characterisation of ultrashort pulses at the sample plane of a high NA objective lens. We have achieved this goal in two steps. Firstly, by allowing the use of a collinear geometry and secondly, by introducing the use of a starch suspension as a nonlinear medium.

We have shown that it is possible to obtain a conventional SHG-FROG trace using a collinear geometry (CFROG). This technique, based on a filtering process, allows experimental data to be rapidly acquired by means of undersampling with negligible error being introduced into the retrieved data.

Additionally the the use of a starch suspension has shown to be very suitable to characterise pulses at the sample plane of high NA lenses. This is possible because the generation of the nonlinear signal in such medium is polarization and angle independent. Moreover, we have shown its broad bandwidth by characterising pulses at vastly different wavelengths. This importantly overcomes the inherent difficulties associated with thin nonlinear crystals. Finally, the use of this material represents a simpler, non-toxic, easy-to-store, non-photobleaching, easy-to-obtain, far cheaper and easier-to-handle medium for the full characterisation of ultrashort pulses at the microscope sample plane.

The barriers we have eliminated within this paper will allow scientists to gain a fuller understanding of the interaction between light and an object at sample plane of a microscope. The ability to accurately measure the phase of the pulse will additionally open up the possibility to tailor or ‘shape’ the exact pulse profile of a pulse in order, for example, to optimise or select the generated multi-photon signal.

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