Novel time domain ptychography, i^{2}PIE, for improved contrast in nonlinear microscopy

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Abstract: We present a novel nonlinear microscopy modality using a time-domain ptychographic phase measurement, i^{2}PIE, to compress 80 MHz supercontinuum pulses from an ANDi PCF used as excitation source, improving contrast at reduced average power.

OCIS codes: (110.0110) Imaging systems; (110.0180) Microscopy; (320.5520) Pulse compression; (300.0300) Spectroscopy

1. Introduction

Many nonlinear (NL) microscopy systems rely on ultrafast pico- or femtosecond lasers as these laser sources provide high peak intensities that drive the NL responses at relatively low average powers. Again, the fast repetition rates of these sources lead to a faster scan time with low energy deposition because of lower dwell time. The use of broadband supercontinuum (SC) light sources is currently gaining prominence in NL microscopy and spectroscopy [1] as it offers the possibility of tailoring the pulses for different applications. Its use in NL microscopy compliments the already numerous merits of such an imaging system such as increased penetration depth, 3-dimensional localized imaging etc. [2]. SC sources generated from all-normal dispersion photonic crystal fibres (ANDi-PCF), which generate SC mainly through self-phase modulation (SPM), have been shown to be more stable for pulse compression applications than SC generated from conventional anomalous dispersion fibres which rely on soliton dynamics [3]. Knowledge of the spectral phase of the SC can be used to correct phase distortions and compress the pulse to produce a near transform limited pulse using a spatial light modulator (SLM). The most common phase measurement technique used for pulse compression is the Multiphoton Intrapulse Interference Phase Scan (MIIPS) [4]. Recently, a new technique known as i^{2}PIE which is based on time domain ptychography has been developed [5]. i^{2}PIE has all the advantages of MIIPS including inline pulse characterization and compression, thus effectively correcting the phase at the focus of the pulse at the image plane and outperforms MIIPS in terms of fidelity of the reconstructed phase.
This work presents and highlights i^{2}PIE as a tool for high resolution nonlinear microscopy. We integrate this technique into a custom-built imaging system using an 80 MHz oscillator and a stable ANDi PCF generated supercontinuum and demonstrate the improvement in contrast and signal to noise of this technique when compared to other pulse compression techniques.

2. Experiment

The experimental setup consists of three sections: supercontinuum generation, pulse compressor and imaging microscope. An experimental polarization maintaining ANDi-PCF was pumped with a femtosecond titanium sapphire laser centred at 800 nm having a 13 nm bandwidth with 80 MHz repetition rate. This generated a broadband supercontinuum (SC) source spanning a spectral bandwidth of approximately 120 nm when pumped with an average power of 200 mW (2.5 nJ pulse energy). As the SC is temporally dispersed due to the material properties of the fibre, pulse compressions were carried out in two steps. The first included pre-compression with chirped mirrors to minimise the second order dispersions as a result of the SC travelling through the PCF. The second step included the use of a in house built 4f pulse shaper equipped with a 1D spatial light modulator (SLM) [6] allowing for spectral phase measurements using the novel i^{2}PIE and the standard MIIPS techniques. The measured spectral phases were then used to compress the pulses by adding a negative value of the recorded phase onto the SLM to create a near transform limited pulse in the sample plane. A second beam path was created to enable imaging with the pulse originating directly from the laser oscillator. The schematic diagram and full description of the setup is as shown in [7]. Both compressed and fundamental pulses were then used for second harmonic imaging of porcine dermal tissue using point-by-point scanning in a custom-built inverted microscope. Fresh pork devoid of cross contamination was sourced from a local butchery and hence eliminated the need for ethical clearance.

3. Results and Discussion

Figures 1 (a and b) show SC pulse overlayed with the spectral phases measured with (a) MIIPS and (b) the new i^{2}PIE, respectively. The MIIPS phase measured is consistent with the phase measured in [8]. Figure 1(c) shows the MIIPS and I^{2}PIE phases plotted together within the SC region of interest. The modulations in the i^{2}PIE phase profile confirms the improved ability in measuring the more structured phase profile of the SC pulse, which leads to better pulse compression [7].

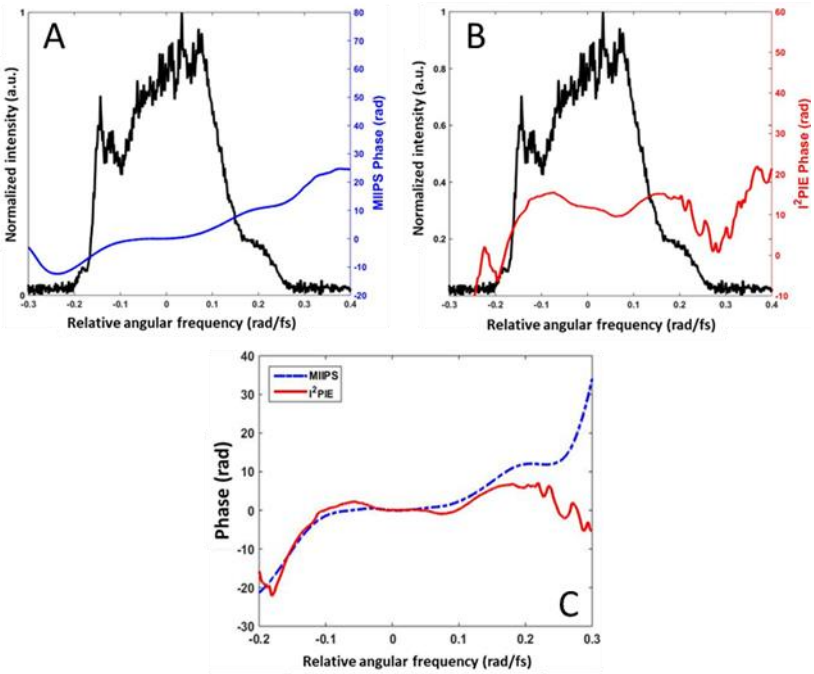

Figure 1: Spectral phases measured with (a) MIIPS and (b) the i^{2}PIE plotted over a SC pulse generated with a 200 mW coupling power. A comparison of MIIPS and i^{2}PIE phases plotted in (c).

The application of i^{2}PIE in second harmonic imaging of biological tissue is shown in figure 2 with the images of porcine dermal tissue taken with 25 pJ energy pulses (2 mW power at the focus of the imaging objective).

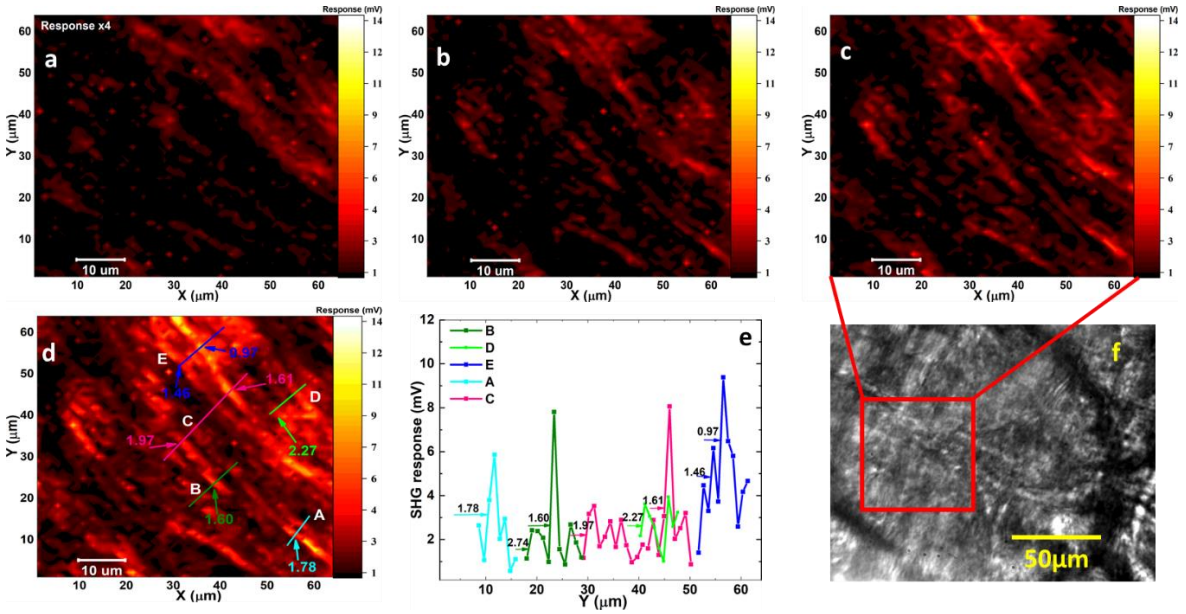

Figure 2: SHG from the dermal tissue of porcine skin imaged under four configurations: (a) fundamental laser pulse (signal enhanced by a factor of 4), SC with only chirped mirror (CM) compression (b), SC with CM and MIIPS compression (c) and SC with CM and i^{2}PIE compression (d). All images taken under same conditions with pulse energy of 25 pJ. The estimated line thickness of the collagen fibrils highlighted in (d) are shown in (e) while the micrograph showing the scanning region is shown in (f). The image is taken through a 60x microscope objective.

For comparison, we show images obtained from 4 different imaging options with the same imaging pulse energy i.e. imaging with the pulse directly from the laser without SC generation (fig. 2a) which is typically what one would expect in basic NL microscopy. Figures 2(b-d) shows SC light source-based imaging with only chirped mirror (CM) compression (b), CM with MIIPS compression (c) and CM with i^{2}PIE compression (d).

It is observed that i^{2}PIE provides greater signal strength and greater details than the other techniques. Imaging with SC light sources also provides greater signal strength over non-SC sources as evident from the low signal and level of detail in fig 2(a). Figure 2(e) shows an estimation of the thickness of the collagen (in μm) across fibrils of interest shown in (d). The average thickness is found to be (1.8 ± 0.6) μm which is within the diameter of collagen [9]. The region of scanning is highlighted in the micrograph in fig. 2(f). Table 1 provides the contrast and signal-to noise (SNR) ratios calculated for figure 2(a-d) based on techniques discussed in [7].

Table 1. Contrast and signal-to-noise ratio measurements for SHG in dermis of porcine skin.

Technique	Fundamental	CM only	CM + MIIPS	CM + i^{2}PIE
Contrast	33.1±2.2	60.1 ± 3.0	70.1 ± 3.5	116.3 ± 3.1
SNR	8.4± 0.5	9.2 ± 0.5	10.6 ± 0.3	26.0 ± 0.7

These results show that not only does the new i^{2}PIE technique provide greater image details, but it also provides better image contrast and signal-to-noise than other phase measurement and pulse compression techniques. In comparison to the more established MIIPS, i^{2}PIE offers a significant increase of contrast and SNR while it can be implemented with the same experimental setup, requiring only a software update. This makes the technique extremely relevant and applicable in high resolution microscopy as greater detail can be extracted while maintaining low pulse energies. This ability to work with lower pulse energies also imply that specimens can be studied for a long period of time with a reduced risk of photodamage. Despite the use of point-by-point scanning in this work which makes imaging relatively slow, samples were able to withstand multiple exposures. It is therefore worth noting that use of scanning mirrors promises to provide a faster scanning as well as wider scanning areas for enhanced image acquisition facilitated by the stable compressed 80 MHz SC pulse repetition rate.

4. Conclusion

We have shown the application of a new time domain ptychographic phase reconstruction technique that can be used for pulse compression of 80 MHz supercontinuum pulses from an oscillator pumped ANDi PCF in SHG imaging in porcine dermal tissue. This i^{2}PIE technique has proven to provide higher contrast and signal-to-noise ratios at the same input pulse energy than other well established pulse characterization techniques investigated.

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