The MIIPS method for simultaneous phase measurement and compensation of femtosecond laser pulses and its role in two-photon microscopy and imaging

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ABSTRACT

A number of nonlinear imaging modalities, such as two-photon excitation and second harmonic generation, have gained popularity during the last decade. These, and related methods, have in common the use of a femtosecond laser in the near infrared, with the short pulse duration making the nonlinear excitation highly efficient. Efforts toward the use of pulses with pulse duration at or below 10 fs, however, have been a great challenge, in part due to the fact that shorter pulses have been found to cause greater sample damage. Here we provide a brief review of the MIIPS method for correction of phase distortions introduced by high numerical aperture objectives and the introduction of simple phase functions capable of preventing three-photon induced damage, reducing autofluorescence, and providing selective probe excitation.

Keywords: two-photon microscopy, spectral phase correction, FROG, SPIDER

1. INTRODUCTION

Nonlinear imaging modalities, such as two-photon microscopy, introduced by Denk and Webb more than a decade ago, have proliferated because of their ability to provide high resolution images through thick biological samples with minimal damage[1]. The efficiency of the nonlinear excitation depends on the peak intensity of the laser pulses; hence, shorter pluses should lead to better images. However, efforts towards the use of shorter pulses immediately were met by greater phase distortions, some inherent to the laser system, but the majority caused by the microscope objective. A number of methods have been introduced to try to pre-compensate at least the linear chirp, the most common and easy to use being a prism pair compressor[2]. In 2003, our research group introduced the multiphoton intrapulse interference phase scan (MIIPS) method to automatically characterize and correct arbitrary order phase distortions. Unlike other methods, MIIPS is a single beam technique that requires no autocorrelation or interferometric interference between two beams. Here we review the method briefly, and highlight its advantages for two-photon microscopy and imaging. MIIPS has proven to be extremely powerful for the accurate and reproducible demonstration of selective microenvironment probing,[3] multiphoton microscopy,[4] functional imaging[5,6] and molecular recognition[7,8] using ultrashort phase-shaped pulses.

2. EXPERIMENTAL IMPLEMENTATION

A detailed description of the MIIPS method and its theory has been presented elsewhere[9,10]. Briefly, MIIPS is a straightforward technique, needing only a thin SHG crystal, a spectrometer, and a pulse shaper (see Fig. 1). MIIPS requires no moving parts, nor do beams need to be overlapped in time or space. The mode of operation has been likened to the Wheatstone Bridge in electronics, where a known resistor is used to measure an unknown resistance[11]. In MIIPS, a series of known spectral phase functions are introduced to measure the unknown spectral phase distortions. The MIIPS algorithm analytically retrieves the spectral phase and introduces a correction. Typically within three to five iterations (a couple of minutes), all orders of phase distortion are removed and the pulses are ready to be used for imaging. The experiments presented here were carried out with a titanium sapphire oscillator (K&M Laboratories) that produced 10 fs pulses (100 nm FWHM) centered near 800 nm, with a repetition rate of 97 MHz and average power of 250 mW. Essentially, MIIPS can be carried out with a wide range of laser pulse durations, energies and wavelengths.

The only component that needs to be properly aligned is the shaper. Shapers programmed to provide automated MIIPS pulse correction will become available early in 2006.

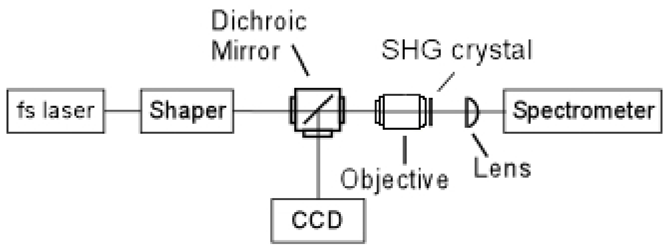

Fig. 1. Schematic of the setup for MIIPS and image acquisition.

3. RESULTS

3.1 Spectral phase correction and its reproducibility

To test the reproducibility of MIIPS, the laser output was corrected ten times consecutively. The retrieved phases were analyzed for reproducibility. As shown in Fig. 2, the reproducibility was excellent: the solid line shows the average of all ten retrieved phases, while the error bars show ±1 standard deviation. As the left panel indicates, there is an extremely high degree of reproducibility of a range greater than the FWHM. Notice as well that the maximum phase distortion achieved was less than 0.1 radians within 90% of the pulse intensity. This level of performance cannot be obtained by simple correction of linear dispersion with a prism pair, and, in fact, cannot be presently obtained by other methods.

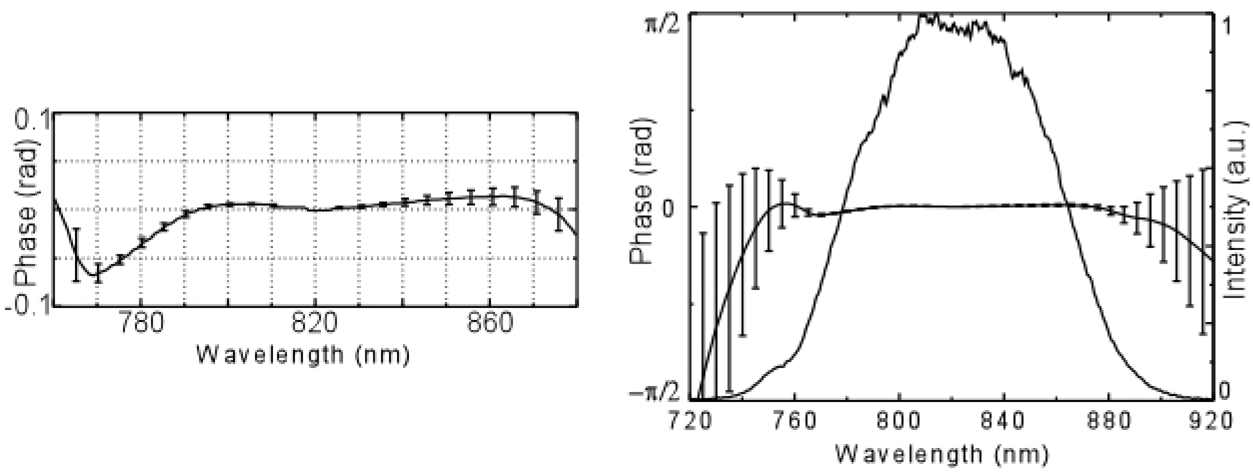

Fig. 2. Reproducibility of phase retrieval. The solid line shows the average of the ten retrieved phases. The error bars indicate ± 1 standard deviation.

A quantitative comparison of MIIPS with well known methods for phase characterization such as FROG[12-17] and SPIDER[18-21] was carried out by calculating the statistical phase error of the collected data. The results are shown in Table 1. The full data set refers to the collection of all ten retrieved phases, while the reduced data set refers to a five scan subset, to match the methodology put forth by Gallman, et.al.'s analysis of FROG and SPIDER.[22] Additionally, the statistical phase error was calculated over the FWHM, a range acceptable for many purposes. The data, presented below, leads us to conclude that MIIPS is a method with high reproducibility, exceeding that of FROG and SPIDER, and with unprecedented reproducibility over the FWHM.

Table 1. Reproducibility of MIIPS, FROG and SPIDER:
Statistical Phase Error (in rad)

Method	Full Data Set	Reduced Data Set	Full Data Set (FWHM)
MIIPS	0.013	0.011	0.0028
FROG[22]	0.122	0.048	
SPIDER[22]	0.044	0.017	

3.2 Correction of phase distortions introduced by high numerical aperture objectives using MIIPS

The quantitative performance of multiphoton imaging modalities has been hindered by the significant amount of phase distortion introduced by high numerical aperture (NA) microscope objectives. Until MIIPS, the characterization of the phase distortions associated with sub-10 fs pulses had been reported for objectives with NAs up to 0.85.[2] The upper limit of NA is increased to 1.30 if longer pulses are used.[23-26] In some of these cases, the compensation of the quadratic term of the characterized distortions was achieved via pre-compensation. Higher order phase distortions, introduced by the objectives and the prisms necessary for pre-compensation, were not compensated, nor were the distortions quantified. Here, we show that MIIPS can characterize, quantify and compensate for all orders of phase distortion introduced into sub-10 fs pulses by high NA microscope objectives.

For this setup, a periscope was used to direct the laser beam into the rear port of a Nikon TE2000-U inverted microscope. The beam was focused by the objective onto the SHG crystal. Compensation of Nikon Plan Fluor ELWD 20x/0.45 NA, Nikon Plan Fluor ELWD 40x/0.60 NA and Plan Apo TIRF oil immersion 60x/1.45 NA objective was achieved.

For the 20x/0.45 NA and 40x/0.60 NA objectives, MIIPS alone was sufficient to compensate for the phase distortions present. (Results not shown). For the 60x/1.45 NA objective, a pair of SF10 prisms was used to pre-compensate and reduce the linear chirp contributions (~10^4 fs^2) before MIIPS subsequently compensated the remaining linear dispersion as well as all higher-order distortions. Results are shown in Fig. 3. The solid line shows the average phase residue from five independent measurements. The error bars show ±1 standard deviation. The phase distortions were corrected to within 0.1 rad over the entire bandwidth of the pulse (top panel). This narrow standard deviation indicates the efficacy of MIIPS even when highly dispersive optics are used. It is also of note that the distortions were characterized, quantified and compensated at the focus of the objective. Consequently, the phase of a pulse can now be known at the position of the sample without autocorrelation or interferometry.

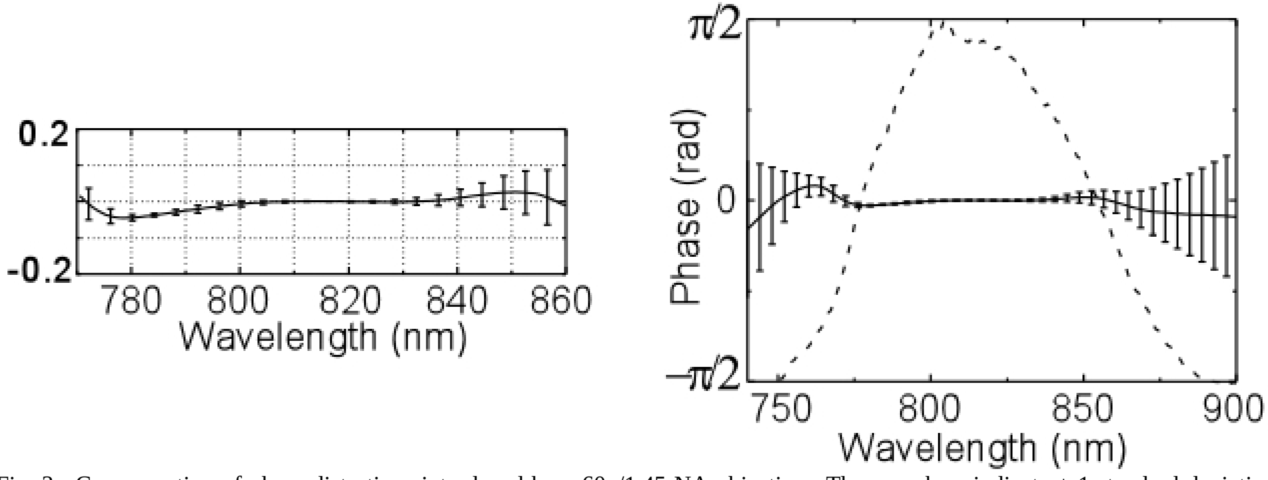

Fig. 3. Compensation of phase distortions introduced by a 60x/1.45 NA objective. The error bars indicate ± 1 standard deviation. The right panel shows the full range of data, while the left panel shows the same data over the FWHM of the pulse.

The capability of MIIPS to accurately compensate for phase distortions and then accurately deliver phases has allowed us to achieve selective functional imaging through biological tissue.[6] Additionally, simulations[27] indicate that, by smoothing MIIPS data before analysis, spectral phase can be retrieved with high precision for signal to noise ratios as low as one. This resistance to noise and independence from mode quality are significant advantages of MIIPS, based on "quasi-local" phase compensation. In a number of cases we have been able to make a MIIPS measurement after the beam has propagated through half a millimeter of scattering biological tissue[6].

3.3 Accurate Retrieval of a Synthetic Phase Distortions

MIIPS was further tested by applying pre-defined phases to a transform-limited (TL) corrected pulse by a second MIIPS setup. Two synthetic phase functions were tested: a double Gaussian (results in Fig. 4(a)) and a sine function (results in Fig 4(b)). The applied phase is given by the solid line, while the retrieved phase is indicated by the dots. The good agreement indicates that MIIPS is capable of accurately retrieve and correct arbitrary phase distortions that could arise from optics in the beam path of a pulse.

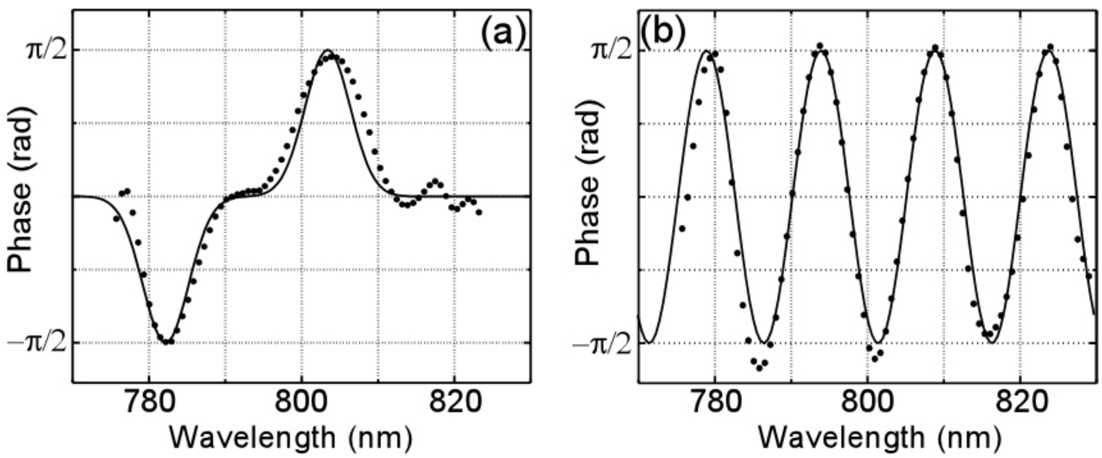

Fig. 4. Ability of MIIPS to retrieve the phase of a pulse. The solid lines are the phase applied to a TL pulse, while the dots show the retrieved phase.

3.4 Effective Delivery of Phase Functions for Controlling Multiphoton Processes

The ability of MIIPS to accurately compensate phase distortions and then deliver accurate phase functions with the goal of controlling multiphoton excitation was tested by comparing the resulting output spectrum of a thin second harmonic crystal experimentally acquired after the application of a phase function with the spectra theoretically predicted by multiphoton intrapulse interference[28, 29] if the phase function was applied to a TL pulse. The results for two functions (a binary function and a sine function) are shown below. Fig 5(a) and (c) exhibit the excellent match between the experimentally observed spectra (dots) and the theoretically predicted spectra (solid line). In contrast, Fig 5(b) and (d) show the resulting experimentally acquired spectra if the same phase functions are applied to an uncompensated pulse. It is clear that phase distortions in the pulse cause significant, deleterious changes in the nonlinear optical properties of the pulse. If theoretical simulations are to be used to predict nonlinear optical properties, or if results are to be reproducible from lab to lab, or even week to week in the same system, it is clear that the distortions must be compensated. The results seen in Fig. 5 indicate that MIIPS is capable of compensating for these distortions.

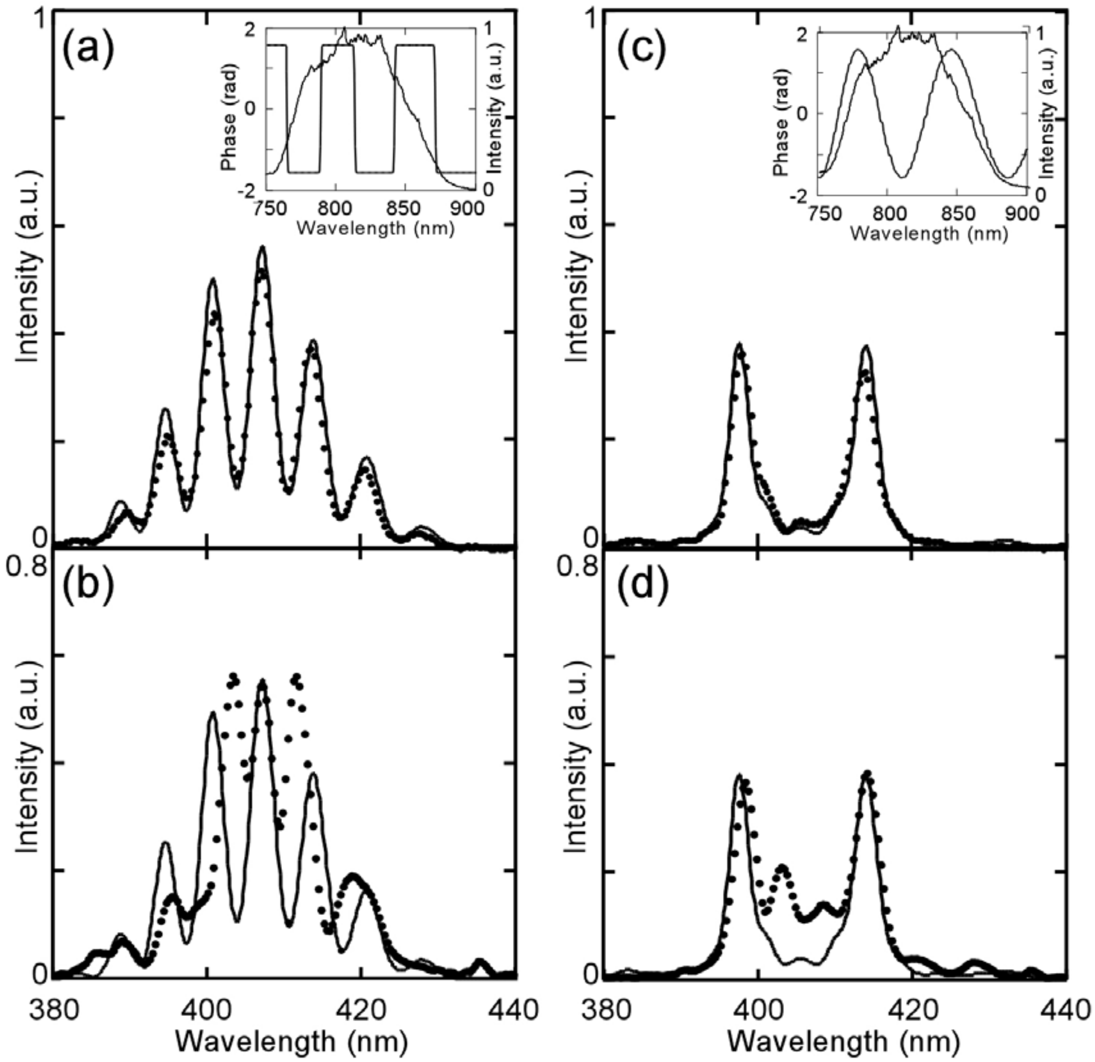

Fig. 5. Effective compensation of inherent phase distortions. The solid line in each panel shows the theoretical spectrum predicted for the application of a particular binary phase (panels (a) and (b)) or a particular sine function (panels (c) and (d)). The dotted lines correspond to the experimentally measured SHG spectrum for each case. Panel (a) shows the experimental result of the application of a binary phase mask (inset) to a pulse compensated by MIIPS. (b) shows the result of the application of the same phase mask to an uncompensated pulse. (c) and (d) show the corresponding information using a sine function (inset).

3.5 The Use of MIIPS Corrected Phase Functions to Enhance Multiphoton Imaging

As indicated in the beginning, the efficiency of multiphoton excitation depends on the pulse duration. When 10 femtosecond pulses transmit through a high NA objective, a significant degradation to the signal occurs. MIIPS can be used to correct the distortions and to optimize all nonlinear excitation processes, resulting in greatly enhanced signal. When imaging living organisms, however, optimization of three-photon excitation, for example, results in increased cell death. It is possible to introduce phase functions that optimize two-photon excitation while minimizing three-photon induced damage[3]. Similarly, it is possible to achieve high efficiency two-photon excitation at a selected wavelength within the bandwidth of the pulse while suppressing excitation elsewhere. Selective two-photon excitation based on MII has been used in our group to probe microenvironment pH[3,4], and by Ogilvie et al[30] to greatly reduce cell autofluorescence. An image of *zygocactus* pollen, acquired with a pulse compensated to TL (zero phase) by MIIPS, is shown in Fig. 6. The compensation, and any subsequent application of phase, is accomplished through the introduction of computer instructions. The laser beam is never tweaked or scanned, reducing the potential for error and the ease of use, as both actions require a significantly higher level of expertise and resulting in changes of the phase distortions and, in some cases, diminished registration between multiple images.

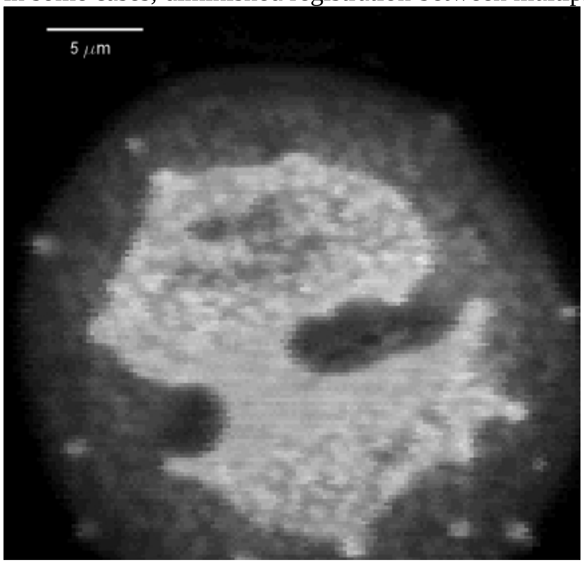

Fig 6. Two-photon image of *zygocactus* pollen taken using pulses compensated by MIIPS.

4. CONCLUSION

We provide a brief review of the advantages of MIIPS for two-photon microscopy and imaging. The demonstrations are based on a series of quantitative tests of the reproducibility and accuracy of the MIIPS method. MIIPS accomplished phase characterization, compensation of all orders of phase distortions, and accurate phase delivery. Because MIIPS can be carried out at the location of the sample, dispersions introduced by optics, including high NA microscope objectives can be compensated.

Our data show that the method is highly reproducible and accurate with precision exceeding that of other phase measurement methods, and is capable of retrieving arbitrary phases. The compensation and delivery of accurate spectral phase functions at the sample using MIIPS was confirmed by the excellent agreement between the experimentally recorded and the theoretically predicted SHG spectra for a continuous sine function and a discontinuous binary phase function. When the pulses were not compensated by MIIPS, significant deviations in the spectrum were observed.

MIIPS can be used on diffuse beams and under very poor signal to noise conditions because it is a single-beam method that requires no overlap of beams in space and time. MIIPS does not use a retrieval algorithm; it is based on an analytical formula which directly provides the second derivative of the spectral phase. The integrated pulse shaper in MIIPS can simultaneously measure and compensate spectral phase distortions at the target. We are presently developing a method to obtain a MIIPS trace in a single laser shot which will provide valuable real-time characterization information. We conclude that MIIPS is an excellent choice for scientific and industrial applications involving femtosecond lasers.

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