Available online at www.sciencedirect.com
ScienceDirect
Optics Communications 281 (2008) 1796–1805
OPTICS COMMUNICATIONS
www.elsevier.com/locate/optcom

Simultaneous spatial and temporal focusing in nonlinear microscopy

M.E. Durst *, G. Zhu, C. Xu

School of Applied and Engineering Physics, Cornell University, Ithaca, NY 14853, United States

Received 18 January 2007; received in revised form 2 May 2007; accepted 10 May 2007

Abstract

Simultaneous spatial and temporal focusing (SSTF), when combined with nonlinear microscopy, can improve the axial excitation confinement of wide-field and line-scanning imaging. Because two-photon excited fluorescence depends inversely on the pulse width of the excitation beam, SSTF decreases the background excitation of the sample outside of the focal volume by broadening the pulse width everywhere but at the geometric focus of the objective lens. This review theoretically describes the beam propagation within the sample using Fresnel diffraction in the frequency domain, deriving an analytical expression for the pulse evolution. SSTF can scan the temporal focal plane axially by adjusting the GVD in the excitation beam path. We theoretically define the axial confinement for line-scanning SSTF imaging using a time-domain understanding and conclude that line-scanning SSTF is similar to the temporally-decorrelated multifocal multiphoton imaging technique. Recent experiments on the temporal focusing effect and its axial confinement, as well as the axial scanning of the temporal focus by tuning the GVD, are presented. We further discuss this technique for axial-scanning multiphoton fluorescence fiber probes without any moving parts at the distal end. The temporal focusing effect in SSTF essentially replaces the focusing of one spatial dimension in conventional wide-field and line-scanning imaging. Although the best axial confinement achieved by SSTF cannot surpass that of a regular point-scanning system, this trade-off between spatial and temporal focusing can provide significant advantages in applications such as high-speed imaging and remote axial scanning in an endoscopic fiber probe.

Keywords: Two-photon excitation fluorescence microscopy; Temporal focusing; Axial scanning; Medical and biological imaging; Ultrafast nonlinear optics

1. Introduction

Multiphoton microscopy (MPM) has become a powerful tool for imaging biological samples due to its ability to perform optical sectioning [1,2]. In two-photon excited fluorescence (TPEF) microscopy, for example, the signal depends quadratically on the excitation intensity, thus substantially decreasing the out-of-focus background [1,3]. TPEF also depends inversely on the temporal pulse width of the excitation pulse, but in standard TPEF microscopy, the temporal pulse width is a constant throughout the sample. Thus, the spatial focusing due to the objective lens determines the axial confinement achieved in conventional TPEF microscopy.

Recent papers have proposed and demonstrated simultaneous spatial and temporal focusing (SSTF) as a way of further enhancing the axial confinement in MPM [4,5]. An extra degree of confinement can be obtained by creating a temporal focus where the shortest pulse width is only achieved at the focal point. SSTF works by spatially separating the frequencies of a short pulse with a grating [4,5], collimating these beams with a lens, and then recombining them with an objective lens (Fig. 1). A temporal focus occurs because the different frequency components only overlap within the focal region of the objective lens, and thus the pulse width is shortest only at the focal plane. In an alternative explanation, the grating is imaged at the focal plane of the objective lens [4]. By Fermat's principle, the light rays emerging from different points on the grating travel the same optical path length to the focal plane, and so a short pulse at the grating will be imaged as a short

* Corresponding author.
E-mail address: med43@cornell.edu (M.E. Durst).

0030-4018/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.optcom.2007.05.071

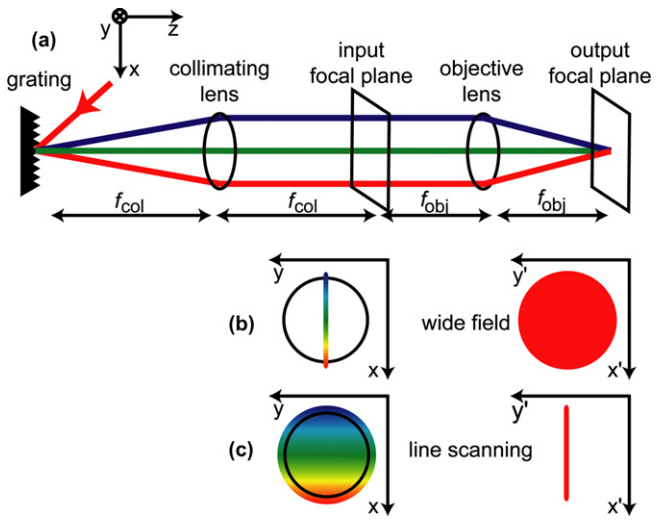

Fig. 1. (a) A typical SSTF setup. (b) Beam profile for wide field SSTF. On the left is a cross-section at the input focal plane of the objective. On the right represents the beam shape at the focal plane. (c) Beam profile for line scanning SSTF.

pulse at the focus. This is not true for points outside of the focus, however, for different rays will follow paths of different lengths, thus broadening the pulse [4,6].

In this paper, we review the derivation of the beam propagation to the focal volume in wide-field SSTF using Fresnel diffraction in the frequency domain (Section 2.1). In Section 2.2, we show that changing the GVD of the system results in axial scanning of the temporal focal plane. In Section 2.3, we present a new derivation for the axial confinement of line-scanning SSTF in the time domain. In Sections 3.1 and 3.2, we review experimental results of the temporal focusing effect as well as the capability of scanning the temporal focus axially by tuning the GVD. In Section 3.3, we present new experimental results on the axial confinement of SSTF. In Section 4, we discuss in detail the axial confinement of SSTF in an MPM setup and the possibility of a multiphoton fiber probe with remote axial scanning capability.

2. Theoretical analysis of SSTF

Fig. 1 shows a schematic of a typical SSTF setup. There are two imaging modalities for SSTF: wide-field and line scanning. In conventional wide-field imaging, the beam incident upon the input focal plane of the objective lens is small, resulting in a loosely focused spot at the focal plane. In the SSTF wide-field case, however, the beam shape at the input focal plane of the objective is a thin line, where the width in the x-direction is the spreading of the monochromatic components due to the grating, and the height in the y-direction is the monochromatic beam size s (see Fig. 1). Wide-field illumination in SSTF is achieved by using a spherical lens as the collimating lens (Fig. 1) to focus the spatially-chirped beam to a small height s in the y-dimension at the input focal plane of the objective lens. Because the radius s of each monochromatic beam is small, the objective lens will not focus the monochromatic beams tightly, creating a large spot at the focal plane (Fig. 1b). In contrast with conventional imaging, however, wide-field imaging with SSTF allows for optical sectioning due to the evolution of the temporal pulse width within the sample. In the SSTF line-scanning case, the beam has a circular cross-section at the input focal plane of the objective lens, such that the y-component of each monochromatic beam is large, but the x-component of each monochromatic beam remains the small width s. Thus, each monochromatic beam will focus to a large x-dimension but a small y-dimension, forming a line at the focus of the objective lens (Fig. 1c). By using a 1D scanner to sweep the line across the sample, 2D images can be obtained [7].

2.1. Temporal pulse-width evolution

The theoretical understanding of SSTF follows closely the published theory for wide-field SSTF with a chirped incident pulse [8]. Our frequency-domain analytical model uses Gaussian beam propagation under the paraxial limit [9]. We assume that the input beam profile, $A_1(x,\omega)$, at the input focal plane of the objective lens can be written as a superposition of many monochromatic, spatially transform-limited Gaussian beams whose center positions are linearly displaced according to their wavelengths. We further assume that the optical spectrum of the input waveform has a second-order chirp and has a Gaussian spectral profile. For one monochromatic beam with frequency ω, the beam amplitude at the input focal plane of the objective lens is:

$$A_1(x,\omega) = A_0 e^{-\frac{\omega^2}{\Omega^2}} e^{-\frac{(x-\alpha\omega)^2}{s^2}} e^{i\beta\omega^2} \tag{1}$$

where A_0 is a normalization constant, $\sqrt{2\ln 2}\cdot\Omega$ is the full-width half maximum (FWHM) of the frequency spectrum of the pulse, $\sqrt{2\ln 2}\cdot s$ is the FWHM of each monochromatic beam in space, α is a constant proportional to the groove density of the grating and the focal length of the collimating lens [10], $\alpha\omega$ is the linear displacement of the monochromatic beam of frequency ω, and 2β is the GVD. The imaginary term in Eq. (1) represents the second-order chirp (quadratic spectral phase) of the input pulse.

In order to analytically describe the field at the output, we follow the paraxial approximation used in Ref. [9] to propagate the beam to the focal volume (for detailed calculations, see the Appendix in Ref. [8]). The spatially-chirped beam, $A_1(x,\omega)$, is incident upon the input focal plane of the objective lens. First, the Fresnel diffraction formula is applied to A_1 to propagate the beam to the objective lens. After adding the quadratic phase due to the objective lens, the Fresnel diffraction formula is used again to propagate it a distance z toward the focal plane. Fourier transforming back into the time domain, the field distribution at the focal volume is: