Optics Communications 281 (2008) 6139–6144

Contents lists available at ScienceDirect

Optics Communications

journal homepage: www.elsevier.com/locate/optcom

Out-of-focus fluorescence collection in two-photon microscopy of scattering media

A. Leray [a,*], C. Odin [b], Y. Le Grand [b,*]

[a] Institut de Recherche Interdisciplinaire, Equipe Biophotonique Cellulaire Fonctionnelle, CNRS USR3078, 1 rue du Professeur Calmette, BP 447, 59021 Lille Cedex, France
[b] Institut de Physique de Rennes, Equipe Biophysique, IPR UR1/CNRS UMR6251, Université de Rennes 1, Campus de Beaulieu, 35042 Rennes Cedex, France

ARTICLE INFO

Article history:
Received 9 July 2008
Received in revised form 2 September 2008
Accepted 10 September 2008

Keywords:
Monte carlo simulations
Scanning microscopy
Two-photon microscopy
Scattering

ABSTRACT

Imaging depth in two-photon microscopy is ultimately limited by the out-of-focus fluorescence background which is collected indistinctly with the useful in-focus signal in scattering samples. We report on a complete Monte Carlo analysis of the collection process in two-photon imaging of turbid media. Epifluorescence collection efficiency of the microscope is shown to vary significantly from point to point inside the scattering medium, to the detriment of the in-focus signal and in favor to the background, lowering the signal-to-background ratio in the image. Moreover we found that this ratio is almost independent of the collecting path field of view for situations where the background overcomes the signal. Assuming that the out-of-focus background can be subtracted to the image, the signal-to-noise ratio in two-photon microscopy is forecast to benefit from enlarging the collection field of view, with a gain roughly proportional to this enlargement for deep imaging. Asymptotic behaviors of the Monte Carlo simulations are quantitatively interpreted from ballistic and diffusive approximations.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

Two-photon excitation fluorescence (TPEF) microscopy [1] has become a well-established technique to image deeply into turbid media. For example, reaching an imaging depth of 500 μm in brain tissue [2,3] is possible. This depth penetration improvement compared to confocal microscopy is mainly achieved by combining the intrinsically spatially localized fluorescence produced by two-photon excitation and the wide-field fluorescence detection which allowed to collect both scattered and ballistic (unscattered) photons. Recently it has been shown that the available excitation power is not the limiting factor to perform deep imaging, insofar as the laser peak intensity can always be increased to compensate for the excitation loss induced by scattering [4,5]. In fact, an imaging depth limitation arises when the out-of-focus fluorescent background, which is mainly generated by the superficial layers, becomes on the same order of magnitude as the useful intra-focal fluorescence signal. This limitation has already been described analytically [4], and recently pointed out experimentally by us [5]. In this latter work, we interpreted the axial profile of the TPEF intensity measured in a tissue-like optical phantom using time-resolved Monte Carlo simulations. The simulations also showed how the signal-to-background ratio could benefit from a pulse shortening in the femtosecond excitation regime. However the collection efficiency of the microscope has not been taken into account in this study, or was assumed to be spatially homogeneous [4]. Rejection of this background has also been realized experimentally by using differential aberration imaging [6,7], or other techniques [8,9]. However, even in that case, the signal-to-noise ratio (SNR) is expected to be limited by the noise produced by the background itself for situations where this latter overcomes the signal. It is then of paramount importance to evaluate precisely how the fluorescence background is collected by the microscope for scattering conditions relevant to TPEF microscopy.

Many efforts have been done to describe the collection properties of the in-focus fluorescence of two-photon microscopes operating in scattering media [10]. Unfortunately the fraction of fluorescence collected by a spatio-angular apertured system can be rigorously determined only in the diffusive limit, that is for imaging depths z_0 much larger than the photon transport mean-free-path $l_t = l_s/(1-g)$. This characteristic length is the average path length over which photons lose memory of their initial launching conditions, whereas l_s is the scattering mean-free-path or the average path between two scattering events. The anisotropy factor g is the mean cosine of the scattering angle. For a non scattering medium, the epicollected fraction of fluorescence emitted from the objective focal volume is well known to be roughly proportional to the square of the numerical aperture angle θ_{NA}^{obj} of the objective. When the medium is scattering, and $z_0 \gg l_t$, this collection efficiency is lowered by a factor proportional to the square of the field of view (fov) θ_{fov} of the collecting path, or equivalently the effective field of view of the objective [10]. Wide-field non-descanned (NDS) modality is then preferred to the low-field

* Corresponding authors. Tel.: +33 3 20 87 10 59 (A. Leray), tel.: +33 2 23 23 62 11; fax: +33 0 2 23 23 67 17 (Y. Le Grand).
E-mail addresses: aymeric.leray@ibl.fr (A. Leray), yann.legrand@univ-rennes1.fr (Y. Le Grand).

0030-4018/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.optcom.2008.09.032

descanned (DS) one to maximize the collection efficiency for optimal in-depth imaging [2,10]. However the imaging depth z_0 in two-photon microscopy is still limited to a few mean-free-paths l_s, that is roughly l_t for living tissue where forward scattering prevails. Indeed the useful fluorescence has to be produced by ballistic excitation photons at the objective focal volume. As the imaging depth z_0 increases, the fluorescence drops like $\exp(-2z_0/l_s)$ while the out-of-focus background counterpart decreases only as z_0^{-2} [3]. Unfortunately no analytical expression of the collection efficiency exists for such middle scattering conditions ($z_0 \approx l_t$) and numerical approaches have to be used to determine precisely the signal-to-background ratio as a function of the imaging depth for usual DS and NDS collection modalities. Among these methods, Monte Carlo analysis has proved to be well suited to simulate both photon propagation in scattering media and collection by a spatio-angular apertured system [11,12].

The aim of this work is thus to study the role of the collection on the signal-to-background and signal-to-noise ratios in TPEF microscopy based on Monte Carlo simulations. In particular, a detailed comparison of the DS and NDS collection modalities is reported. In a first section we introduce the Monte Carlo analysis used to simulate the spatial distribution of the collection efficiency of a two-photon microscope operating in a turbid medium. The axial profiles of the fluorescence signal collected through the DS and NDS ports of a two-photon microscope imaging through a model scattering sample for relevant excitation and collection conditions are then derived. The second section deals with the simulation of the signal-to-background ratio as a function of the imaging depth for the DS and NDS microscope ports. In the third section, we compare the signal-to-noise ratio measured with these ports, assuming that the fluorescence background can be subtracted from the images.

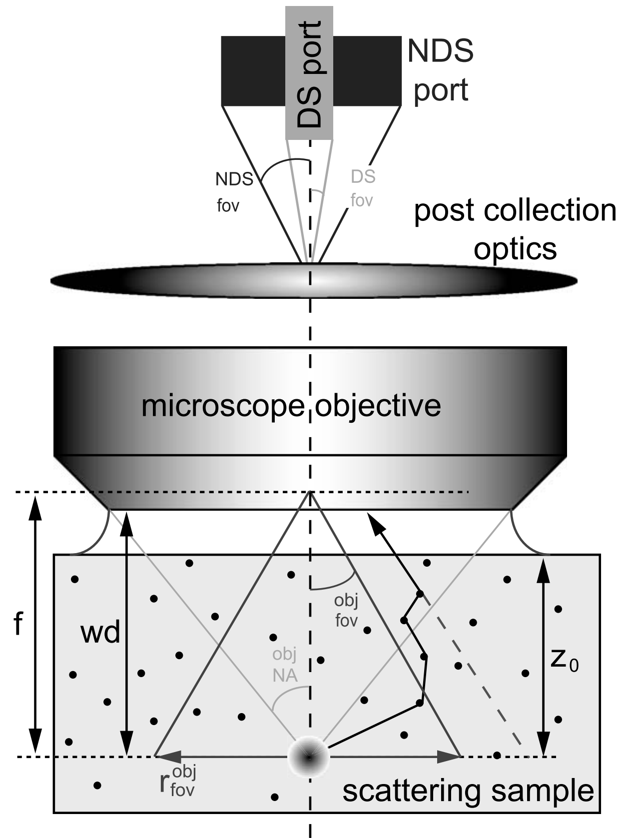

Fig. 1. Geometry of the collection path used in the Monte Carlo simulations and useful parameters. f: focal length of the objective; wd: working distance; r_{OFA}: objective front aperture; θ_{NA}^{obj}: numerical aperture angle; r_{fov}^{obj} and θ_{fov}^{obj}: linear (radius) and angular fields of view of the objective; θ_{fov}^{DS} and θ_{fov}^{DS}: angular fields of view of the descanned (DS) and non-descanned (NDS) ports respectively.

2. Spatial distribution of the collection efficiency of a two-photon microscope

In order to determine the signal-to-background ratio in two-photon microscopy, one has first to derive the spatial distribution $I^{coll}(\mathbf{r})$ of the TPEF intensity collected by the microscope from a fluorescent and scattering medium. $I^{coll}(\mathbf{r})$ depends on both excitation and collection processes. As already mentioned, two-photon excitation of fluorescence by short laser pulses in turbid media is a time-dependent problem. The subsequent collection of this fluorescence is however a linear process that can be considered independently of both excitation pathway and time. $I^{coll}(\mathbf{r})$ is then simply written as

$$I^{coll}(\mathbf{r}) = C(\mathbf{r}) \times I^{exc}(\mathbf{r}), \tag{1}$$

where $C(\mathbf{r})$ is the collection efficiency of the microscope with regard to the TPEF intensity $I^{exc}(\mathbf{r})$ emanating from point $\mathbf{r}$ of the scattering medium.

Our numerical analysis of $C(\mathbf{r})$ was based on a Monte Carlo method developed for modeling photon transport in living tissue [13,14] that we already adapted to perform the simulations of $I^{exc}(\mathbf{r})$ [5]. Details of the simulations are reported in the Appendix. The collection scheme considered in our Monte Carlo analysis is represented in Fig. 1. It is based on a high numerical aperture (NA = 0.9) and long working distance (wd = 2000 μm) water-immersion 60× objective of focal length f = 3 mm (Olympus LUMPLFL 60x/0.9W). The radius of the objective front aperture was taken to be $r_{OFA} = \text{wd} \times \tan\theta_{NA} = 1840$ μm, where $\theta_{NA} = \sin^{-1}(\text{NA}/n)$ and $n = 1.33$ the refraction index of water.

Moreover the effective field of view radius of our 60x objective apertured by DS and NDS post-collection optics were previously measured to be respectively, $r_{fov}^{DS} = 14$ μm and $r_{fov}^{NDS} = 320$ μm [15]. The corresponding DS and NDS angular fields of view were then found to be respectively, $\theta_{fov}^{DS} = 0.27^\circ$ and $\theta_{fov}^{NDS} = 6.09^\circ$ using $\theta_{fov} = \tan^{-1}(r_{fov}/f)$. The DS and NDS fields of view represent respectively about 3.5% and 80% of the 60× objective field of view which was measured to be $r_{fov}^{obj} = 394$ μm or $\theta_{fov}^{obj} = 7.48^\circ$ [2]. Note that we designed the NDS port so that to maximize its field of view. Consequently, the NDS-to-DS collection ratio of our microscope is expected to be of $(\theta_{fov}^{NDS}/\theta_{fov}^{DS})^2 \approx (r_{fov}^{NDS}/r_{fov}^{DS})^2 = (320/14)^2 \approx 500$ in the diffusive limit.

To better emphasize the spatial variations of the collection efficiency in two-photon microscopy, we first performed simulations for the maximal objective imaging depth ($z_0 = \text{wd} = 2000$ μm). A 3D representation of the simulated collection efficiency $C(\rho, z)$ of the NDS port of our microscope is presented in Fig. 2. The corresponding curve for the DS port was not reported since it exhibited a similar profile with higher statistical noise due to lower collection efficiency (see below). From Fig. 2, it clearly appears that the collection efficiency of the microscope strongly depends on both the depth z of the fluorescence source within the turbid medium and its distance ρ from the optical axis. In particular, the collection efficiency for the useful intra-focal fluorescence produced 2000 μm beneath the surface of the scattering medium is almost three-fold lower than the maximum observed for points located around 400 μm beneath this surface. In other words, epifluorescence collection is expected to greatly accentuate the contribution of the background to the detriment of the useful signal at high imaging depth. Similarly, the on-axis collection is favored to the detriment of the off-axis counterpart.

In order to derive the signal-to-background ratio associated to the DS and NDS collection modes of our microscope, one has to

Download English Version:

https://daneshyari.com/en/article/1539604

Download Persian Version:

https://daneshyari.com/article/1539604

Daneshyari.com