Journal of Photochemistry and Photobiology B: Biology 141 (2014) 128–138

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Short Review

Advances and challenges in label-free nonlinear optical imaging using two-photon excitation fluorescence and second harmonic generation for cancer research

Giju Thomas [a,c,*], Johan van Voskuilen [b], Hans C. Gerritsen [b], H.J.C.M. Sterenborg [a]

[a] Department of Biomedical Engineering and Physics, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands
[b] Department of Molecular Biophysics, Utrecht University, 3508 TA Utrecht, The Netherlands
[c] Centre for Optical Diagnostics and Therapy, Erasmus Medical Centre, Post Box 2040, 3000 CA, Rotterdam, the Netherlands

ARTICLE INFO

Article history:
Received 20 May 2014
Received in revised form 20 August 2014
Accepted 23 August 2014
Available online 16 September 2014

Keywords:
Cancer diagnostics
Nonlinear optical imaging
Two-photon excitation fluorescence
Second harmonic generation
Label-free
Endogenous optical fluorescence

ABSTRACT

Nonlinear optical imaging (NLOI) has emerged to be a promising tool for bio-medical imaging in recent times. Among the various applications of NLOI, its utility is the most significant in the field of pre-clinical and clinical cancer research. This review begins by briefly covering the core principles involved in NLOI, such as two-photon excitation fluorescence (TPEF) and second harmonic generation (SHG). Subsequently, there is a short description on the various cellular components that contribute to endogenous optical fluorescence. Later on the review deals with its main theme – the challenges faced during label-free NLO imaging in translational cancer research. While this review addresses the accomplishment of various label-free NLOI based studies in cancer diagnostics, it also touches upon the limitations of the mentioned studies. In addition, areas in cancer research that need to be further investigated by label-free NLOI are discussed in a latter segment. The review eventually concludes on the note that label-free NLOI has and will continue to contribute richly in translational cancer research, to eventually provide a very reliable, yet minimally invasive cancer diagnostic tool for the patient.

Contents

* Corresponding author at: Centre for Optical Diagnostics and Therapy, Erasmus Medical Centre, Post Box 2040, 3000 CA, Rotterdam, the Netherlands. Tel.: +31 643461473.
E-mail address: gijuthomas82@gmail.com (G. Thomas).

http://dx.doi.org/10.1016/j.jphotobiol.2014.08.025
1011-1344/

1. Introduction

Nonlinear optical imaging (NLOI), based on the nonlinear excitation of fluorophores has taken rapid strides in the field of biomedical imaging since the last two decades. Technological innovations and research have steadily progressed in trying to develop NLOI as a diagnostic tool for the patient in a clinical environment [1–7]. Simultaneously NLOI based research has also been able to provide fundamental researchers with new perspectives in cancer research, especially with regard to studying pathogenesis of cancer and drug development for cancer therapy [8–14]. Numerous NLOI studies have given deep insights about cancer progression and the steps involved in angiogenesis and metastasis [8,9,15–26]. These studies however have been performed by administering exogenous fluorophores that enhanced contrast. Nonetheless NLOI can also be performed relying solely on endogenous fluorescence provided by the biological sample itself [27–32]. This review will thus cover cancer research studies that rely on label-free NLOI using only endogenous optical fluorescence.

The key aim of this review is to evaluate the advances made and challenges faced in label-free NLOI, as researchers attempt to use this technology for cancer diagnosis in a clinical scenario. The authors have tried to highlight the accomplishments and assess the limitations of the reviewed studies. This review eventually raises certain pertinent questions that have not been investigated by researchers till date, which gives scope for further studies.

1.1. Physics of nonlinear optical imaging

1.1.1. Two-photon excitation fluorescence (TPEF)

In TPEF, two near-infrared (NIR) photons are absorbed simultaneously, where each photon provides half of the energy, which is normally required to excite the fluorophore into a higher electronic state as seen in Fig. 1. Therefore, emission of fluorophores in wavelengths that fall in visible light or UV region can be induced with low-energy NIR photons. The NIR excitation spectra is unique as the linear absorption and scattering coefficients contributed by cells and tissues is low in this wavelength range, which leads to a high light penetration depth. In addition, the two-photon absorption occurs only in the plane of focus, minimizing background scatter from regions outside focus. Though Göppert-Meyer had formulated the theory of TPEF in 1931 [33], it was only observed three decades later by Kaiser and Garrett [34] and Abella [35]. Eventually it was put into application by Denk et al. who built the first TPEF microscope and observed intracellular fluorescent probes [36]. TPEF, however, is achieved only at very high photon concentration in space and time, requiring extremely high NIR laser intensities [34]. However the development of ultra-short pulsed lasers [37] can now provide transient intensities of GW/cm^2 in a pulsed form, with the pulse duration ranging in the femto-seconds and at a high pulse frequency of 80–90 MHz. As a result TPEF signals can be generated effectively at average laser powers lower than 5 mW [38] incident on the sample.

1.1.2. Second harmonic generation (SHG)

SHG is a nonlinear optical process where two photons combine together to produce a new photon with twice the energy or half the wavelength emission of the incident photons as shown in Fig. 1. Unlike in TPEF, there is no non-radiative energy loss involved in SHG. SHG was first demonstrated in 1961 by Franken et al. [39] and a year later Bloembergen and Pershan described the formulation of SHG [40]. In order to obtain SHG, an intense laser beam from ultra short pulsed NIR laser should pass through materials with a specific molecular orientation. These materials are generally composed of non-centrosymmetric molecular structures. Certain biological materials such as collagen, microtubules (tubulin), and muscle myosin are highly polarisable, as these materials are assembled from fairly ordered, large non-centrosymmetric structures. Therefore the secondary, tertiary and quaternary structure of proteins that involve specific folding of the proteins into its unique 3-dimensional conformation play a major role in determining the polarisability of biological materials and its ability to produce SHG. Alteration of biological materials at the secondary, tertiary or quaternary structural levels in a diseased state will therefore affect the level of SHG obtained from the imaged tissue, making it a useful optical property for diagnostic purposes by NLOI.

1.2. Comparison of NLOI over other linear imaging methods

NLOI provides distinct advantages over other linear imaging methods as:

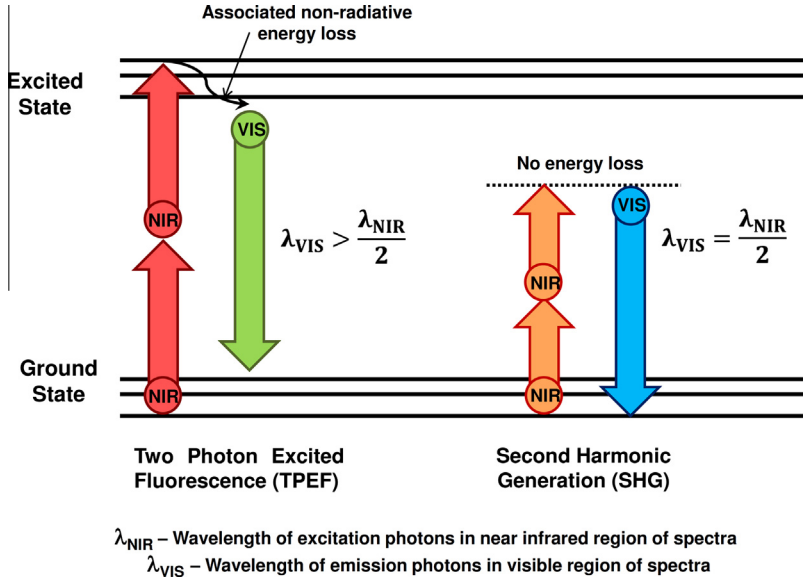

Fig. 1. Jablonski energy diagram showing the process involved for two photon excited fluorescence (TPEF) and second harmonic generation (SHG). TPEF requires the existence of an actual excited state, while SHG does not.