Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 328 (2005) 78–84
BBRC
www.elsevier.com/locate/ybbrc

Two-photon induced fluorescence of Cy5-DNA in buffer solution and on silver island films

Joanna Lukomska*, Ignacy Gryczynski, Joanna Malicka, Slawomir Makowiec, Joseph R. Lakowicz, Zygmunt Gryczynski

Center for Fluorescence Spectroscopy, Department of Biochemistry and Molecular Biology, University of Maryland at Baltimore, 725 West Lombard Street, Baltimore, MD 21201, USA

Received 24 November 2004
Available online 5 January 2005

Abstract

We report the observation of a strong two-photon induced fluorescence emission of Cy5-DNA within the tunable range of a Ti:Sapphire laser. The estimated two-photon cross-section for Cy5-DNA of 400 GM is about 3.5-fold higher than it was reported for rhodamine B. The fundamental anisotropies of Cy5-DNA are close to the theoretical limits of 2/5 and 4/7 for one- and two-photon excitation, respectively. We also observed an enhanced two-photon induced fluorescence (TPIF) of Cy5-DNA deposited on silver island films (SIFs). In the presence of SIFs, the TPIF is about 100-fold brighter. The brightness increase of Cy5-DNA TPIF near SIFs is mostly due to enhanced local field.

Keywords: Two-photon excitation; Cy5; Silver islands

Cyanine dyes are frequently used in genomic analysis [1,2]. The common practice is to label the cDNA pool from the experimental and control samples with two different fluorophores such as Cy3 (*N*,*N*′-(dipropyl)-tetramethylindocarbocyanine) and Cy5 (*N*,*N*′-(dipropyl)-tetramethylindodicarbocyanine) [3,4]. The relative fluorescence signals of these two dyes from each location on the DNA array correspond to the relative amount of each mRNA present in the experimental and control samples.

In this manuscript, we describe two-photon induced fluorescence of Cy3 and Cy5 labeled DNA. Two-photon excitation became a preferable tool in confocal microscopy [5–7]. This was possible because of a development of robust Ti:Sapphire femtosecond lasers. The long wavelength and localized excitation provide less unwanted scattering and less overall sample photodamage. In the last decade, many commonly used fluorophores have been characterized with multiphoton excitation [8–13]. Also, several compounds have been designed to possess large two-photon absorption cross-sections [14–18]. Two-photon excitation has been already employed with array techniques and high throughput screening (HTS) [19].

We examined Cy3- and Cy5- labeled DNA (23-mer) using Ti:Sapphire femtosecond laser within its tunable range (740–900 nm). Cy-labeled DNA was present either in a buffer solution or deposited as a monolayer on silver island films (SIFs). It is already well established that the presence of silver particles on the surface can enhance the fluorescence of deposited fluorophores by an order of magnitude or more [20–25]. This enhancement depending on fluorophore–SIF distance [26] is a result of two factors: increased local field near metal particles and increased radiative rate—the phenomenon we called radiative decay engineering (RDE) [27–29]. RDE effect is due to interaction of excited molecule with the metal

* Corresponding author. Fax: +1 410 706 8408.
E-mail address: joanna@cfs.umbi.umd.edu (J. Lukomska).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.12.148