Title
Continuous Wave Diode Laser Induced Two-Photon Fluorescence Excitation of Three Calcium Indicators

Permalink
https://escholarship.org/uc/item/2ds1x494

Journal
Japanese Journal of Applied Physics, 36(12A)

ISSN
0021-4922

Authors
Zhang, Zhanxiang
Sonek, Gregory J
Wei, Xunbin
et al.

Publication Date
1997-12-01

DOI
10.1143/jjap.36.l1598

Copyright Information
This work is made available under the terms of a Creative Commons Attribution License, available at https://creativecommons.org/licenses/by/4.0/

Peer reviewed
Continuous Wave Diode Laser Induced Two-Photon Fluorescence Excitation of Three Calcium Indicators

Zhanxiang Zhang1,2, Gregory J. Sonek1,2, Xunbin Wei2, Michael W. Berns2 and Bruce J. Tromberg2
1Department of Electrical and Computer Engineering, University of California, Irvine, Irvine, CA 92612, USA
2Beckman Laser Institute and Medical Clinic, University of California, Irvine, Irvine, CA 92612, USA

(Received October 7, 1997; accepted for publication October 28, 1997)

Continuous wave (CW) diode laser induced two-photon fluorescence spectra have been measured for calcium indicators of Indo-1, Oregon-green, and Fluo-3. The near 2.0 log-log power-dependence of fluorescence intensity versus incident laser power indicates that the fluorescence from these three calcium indicators was excited by two-photon absorption process of 809-nm laser light. Calcium-dependent emission spectra of Indo-1 and Oregon-green for two-photon excitation under wavelength of 809-nm have also been measured.

KEYWORDS: Two-photon fluorescence, calcium indicator, diode laser

1. Introduction

Since Webb and co-workers1,2) and others3-5) have shown that two-photon excitation can be used in fluorescence microscopy to provide localized excitation in the focal plane and increased the spatial resolution, two-photon microscopy and two-photon imaging techniques have attracted considerable interest in recent years. Calcium probes, which show a spectral response upon binding Ca2+, have enabled researchers to investigate changes in intracellular free Ca2+ concentrations.6) Lakowicz et al. have studied three-photon induced fluorescence of calcium probe Indo-1 and the possibility of multiphoton calcium imaging using Indo-1 under the excitation of picosecond pulsed lasers.8) However, to the best of our knowledge, there has no study focused on two-photon fluorescence excitation and two-photon imaging about calcium indicators by using continuous wave diode lasers. In this paper, we report two-photon fluorescence excitation of three calcium indicators, Indo-1, Oregon-green, and Fluo-3 by using a continuous wave diode laser with wavelength 809-nm as the exciting laser source. Two-photon fluorescence spectra have been measured for Indo-1, Oregon-green, and Fluo-3, and they were peaked around 475-nm, 525-nm, and 530-nm, respectively. The slopes of the log-log power dependencies for these three probes were all near 2.0 indicating that the fluorescence was excited by two-photon absorption process. Ca2+-dependent two-photon fluorescence emission spectra of Indo-1 and Oregon-green were also measured.

2. Materials and Methods

The samples we used were Indo-1 (AM Easter), Oregon-green (potassium salt), and Fluo-3 (potassium salt) ordered from Molecular Probes, Inc. For each indicator, we made two samples, one was made by adding 20-μL calcium free ringer solution into 20-μL indicator solution with concentration of 1-mM, the other one was made by adding 20-μL ringer solution with calcium concentration of 1-mM into 20-μL indicator solution with concentration of 1-mM.

Our experimental setup was similar to the one previously described.9) An optical trapping beam was derived from an AlGaAs laser diode (SDL 2352-H1) with maximum optical output power of 500-mW at wavelength 809-nm. The laser beam was first collimated by a special collimating lens, then circularized by a pair of anamorphic prisms. Passing through another lens and a long pass (λ > 780-nm) filter the beam was deflected by a dichroic beamsplitter, and then focused to its diffraction-limited spot-size (2ω0 ~ 0.8-μm) onto a sample using an oil-immersion, 100×, 1.25 N.A. microscope objective (Zeiss Neofluar). The fluorescence excited by this highly focused laser beam was collected by the same objective lens, passed through a dichroic mirror and a pinhole aperture, collimated with a beam expansion optics, passed through a short pass filter (λ < 750-nm), and directed onto a 300-groove/mm diffraction grating, which dispersed the optical signal. The signal was then focused onto an electrically CCD array. Spectra data, collected over 300-nm bandwidth, was acquired and analyzed using a personal computer.

3. Results and Discussion

Figure 1 shows the fluorescence spectra took from Indo-1 in calcium free ringer solution, Oregon-green in calcium saturated solution, and Fluo-3 in calcium saturated solution. The fluorescence spectra of Indo-1, Oregon-green, and Fluo-3 were peaked around 475-nm, 525-nm, and 530-nm, respectively. It is impossible for these spectra to be excited by one-photon absorption process of laser light with wavelength 809-nm, and they should be excited by two-photon absorption process or other higher nonlinear processes.

To further characterize the emissions of these three calcium indicators with 809-nm excitation we measured the log-log power dependencies of fluorescence intensity versus incident power. The slopes of log-log power dependence for Indo-1 in calcium free solution, Oregon-green in calcium saturated solution, and Fluo-3 in calcium saturated solution were 2.2, 1.9, and 2.0, respectively, and they were all near 2.0 indicating that the fluorescence from these three calcium indicators were excited by two-photon absorption process of 809-nm laser light. The results were shown in Fig. 2. The log-log power dependencies for Indo-1 in saturated calcium solution, Oregon-
peak intensity of Oregon-green in free calcium solution. The integration time for CCD array were all 25-s for the measured samples. Spectrum shapes have no clear difference for Oregon-green in calcium free solution and in calcium saturated solution, they both were peaked around 530-nm. However, the two-photon fluorescence spectrum of Indo-1 in saturated calcium solution had blue shift comparing to Indo-1 in free calcium solution. As discussed in reference, two-photon fluorescence spectrum of Indo-1 should have peaks around 400-nm and 475-nm, the peak at 400-nm would increase with the increasing of calcium concentration under the excitation of 590-nm laser light. Under our experimental conditions of 809-nm excitation, there was no clear peak around 400-nm, as 400-nm wavelength is shorter than half wavelength of 809-nm, two-photon fluorescence can not be excited through 809-nm laser light.

4. Conclusion

In conclusion, we have demonstrated that we could observe two-photon fluorescence spectra from calcium indicators Indo-1, Oregon-green, and Fluo-3 by using a cw diode laser as the exciting source. Fluorescence intensity changes for Indo-1 and Oregon-green with different calcium concentrations have been measured. If we could find suitable cw laser sources with half emission wavelengths just locating at the peak regions of one-photon absorption spectra of these indicators, the two-photon fluorescence signals from them will be enhanced a lot. As a result, a single cw diode laser beam can be used as a trapping source and a two-photon exciting source simultaneously to trap the biological samples and detect the calcium concentration changes of these trapped samples. Also, the above studies have suggested that it will be easy to take two-photon calcium images from biological samples by using pulsed laser sources with wavelength around 809-nm as the pulsed laser source has higher peak power.

Acknowledgement

This work was finished in The LAMMP of Beckman Laser Institute and Medical Clinic and supported by
The National Institute of Health under grants: R01-RR06961-01A2 and 5P41-RR01192-5, The Office of Naval Research (N00014-91-C-0134), and The Department of Energy (DE-FG03-91ER61227).