Two-frequency Fluorescence Laser Scanning Confocal Microscope

Yung-Chin Chung¹, Jheng-Syong Wu¹, Chien Chou¹,²*

1. Graduate Institute of Electro-Optical Engineering, Chang Gung University, Taoyuan 333, Taiwan
2. Research Center for Biomedical Engineering, Chang Gung University, Taoyuan 333, Taiwan

E-mail: echou@mail.cgu.edu.tw, cchou01@gmail.com

ABSTRACT

Our previous research [1,2] verified that a two-frequency linearly polarized confocal laser scanning microscope (TF-PCLSM) shows the abilities of simultaneously reducing the scattering effect and the spherical aberration induced by refractive-index mismatch in specimen both in imaging formation theory and experimental demonstration. This is due to the features of common-path propagation of p- and s-polarized waves in specimen while the spatial coherence gating, polarization gating and spatial filtering gating are produced intrinsically and simultaneously following with the heterodyne synchronized detection. In this study, a two-frequency linear polarized fluorescence confocal laser scanning microscope (TF-FCLSM) is proposed and setup. The working principle and features of TF-FCLSM are based on TF-PCLSM and the fluorescence intensity axial response in TF-FCLSM demonstrates the ability of cancellation of induced spherical aberration as well. Those features are due to the common path propagation of the linear polarized paired photons in two-frequency laser beam and the heterodyne detection. In this experiment, the axial response of fluorescence intensity was measured whereas the performance of axial response in TF-FCLSM better than conventional fluorescence laser scanning confocal microscope (FCLSM) was demonstrated. As results, an improvement on sectioning image in conventional FCLSM is anticipated by using a two-frequency orthogonal linear polarized laser beam instead of using a single frequency laser beam when probing a thick specimen. Moreover, different performance of axial response of TF-FCLSM among using (a) orthogonal, (b) parallel and (c) non-polarized two-frequency laser beam are compared and discussed.

References