High-speed fluorescence lifetime imaging microscopy for multimodal visualization of biomedical characteristics using a commercial microscope platform

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Fluorescence lifetime imaging microscopy (FLIM) is one of the most powerful technique to visualize various photo-physical characteristics of the specimen [1]. Since it is sensitive to various chemical properties such as pH, ion concentration, or molecular interactions, FLIM has been applied to analyze various biomedical effects [2]. However, the application of FLIM into live specimen is usually limited by its slow image acquisition speed. To enhance the image acquisition speed of FLIM instrument, including both signal acquisition and data processing, we adopted analog mean-delay (AMD) method, which has recently been introduced [3], [4]. The AMD method utilizes photon flux composing a pulsed output of the laser instead of the single photon of conventional time-correlated single-photon counting (TCSPC) techniques. Therefore, the signal acquisition speed of the AMD method is much faster than that of the TCSPC method. By using the AMD method, a custom-built fluorescence lifetime imaging microscope system which can acquire three-modal images within 0.3 seconds was developed. It includes the image of confocal laser scanning microscopy (CLSM), near-infrared fluorescence microscopy (NIRF), and FLIM. In addition, to enhance the data processing speed of the AMD method, we designed a parallel processing algorithm using an open source template library called threading building blocks (TBB, Intel) [5]. Finally, we reconstructed CLSM and NIRF imaging by using the back-scattered and fluorescence signals, which were generated from the specimen and were utilized for calculating fluorescence lifetime [6]. In this paper, we introduces our multimodal FLIM system which has recently been combined with the commercial microscope system (DMi8, Leica microsystems) to utilize the existing imaging modalities including phase contrast microscopy, wide-field microscopy, and multi-channel fluorescence microscopy.

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