Real-time high dynamic range fluorescence microscope using spatially-selective excitation with a digital micro-mirror device

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The luminance range captured in a single image is determined by the dynamic range of the detector. In order to obtain a wider range of luminance in a single image than the limited dynamic range of a camera, various applications have been developed by employing numerical calculation or by improving hardware. One of the most popular HDR imaging methods is reconstructing a single HDR image with multiple images acquired by different amount of exposure [1]. Additional equipment is not necessary for acquiring HDR image with this method. However, multiple acquisition is required which increases the total imaging time accordingly. Due to this drawback, multiple exposure fusion is not appropriate for observing dynamic processes because the imaging target should be fixed during the multiple acquisition.

To overcome such limitations, HDR imaging could be achieved by improving experimental schemes. Active illumination can enhance dynamic range of scanning microscopies with modulating the laser power adaptive to the detected signal intensity with real-time negative feedback [2]. Another HDR imaging method enabled real-time acquisition of HDR image, using multiple beam splitters in detection parts for acquiring different signal ranges simultaneously [3]. Although these methods can achieve real-time HDR image acquisition by adding simple optics in illumination/detection parts, they can be adopted only to laser point scanning imaging systems. To incorporate active illumination in widefield geometry, a method was proposed to use a digital micro-mirror device (DMD) to modulate illumination intensity for suppressing saturated regions [4]. The feedback process generates an illumination pattern iteratively, based on the detection of saturated pixels in acquired images. However, the iteration process requires typically 3-4 images until none of the pixels are saturated and is still limted in imaging dynamic samples. Here, we developed a real-time HDR imaging method based on structured illumination (SI) using a DMD to modulate illumination intensity for spatially-selective excitation. We modulated the intensity of excitation light spatially, with parallel computing algorithm adaptive to time-varying fluorescence distribution or wide field of view. Using this formalism, we demonstrate fast HDR imaging of temporally changing fluorescent samples.

Reference

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