Time-resolved 3D confocal fluorescence microscopy

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Application Note

Introduction

In conventional Confocal Laser Scanning Microscopy (CLSM) an excitation beam is projected onto a sample through a pinhole which is optically conjugate to a second pinhole immediately in front of a point detector. The use of these spatial filters suppresses out-of-focus and stray light allowing for greater depth discrimination and an increase in signal-to-noise ratios.
In order to enhance acquisition speed and system flexibility we replaced the pinholes with a spatial light modulator (DLP 4100, Texas Instruments) and the point sensor with an Andor EMCCD Camera (iXon Ultra DU888-UCS-BV, 1024 x 1024 x 13 µm pixels). By implementing a Moiré based alignment technique [1] each pixel of the EMCCD is aligned to a corresponding pixel on the spatial light modulator.
Due to high switching rates (up to 22 kHz) of the DLP up to 1000 individual scan patterns can be cycled within a single frame of the camera at video rate. This significantly reduces the average exposure time for each pixel, requiring a sensor with very low optical noise and high gain.

Experiment

Figure 1 shows a layout of the system employed for the experiment. The excitation light is reflected by a dichroic mirror onto the digital micro mirror device (DMD). Analogous to the CLSM only beams reflected along the objective axis are focused on the sample. Light emitted from these illuminated areas is reflected through the system by the same micro mirrors on the SLM array to reach the EMCCD-sensor.

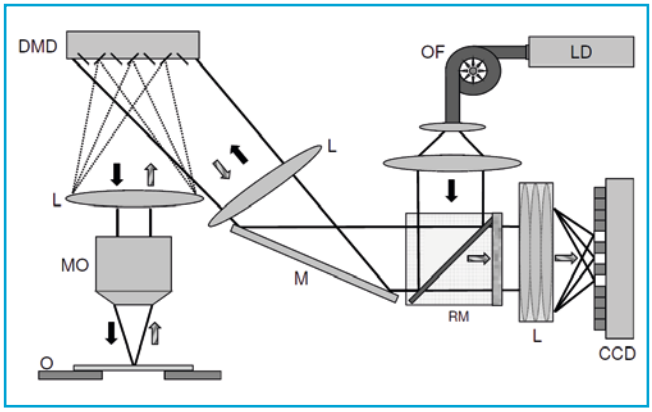

Figure 1. Optical layout of the DMD-confocal microscopy system. The black arrows represent excitation light. The striped arrows represent emitted fluorescence light. (LD: laser diodes, OF: optical fiber, RM: reflector module including dichroic mirror and fluorescence filter, M: mirror, L: lens, DMD: digital micromirror device, MO: microscope objective with piezo nanopositioner, O: sample, CCD: EMCCD camera iXon Ultra 888 (1024 x 1024 x 13 µm pixels)) [3]

Each frame of the camera corresponds to a single 2D slice of the 3D image. The object plane, within the sample, is axially shifted by moving the objective, via a piezo driven nanopositioner (PIFOC, PI).

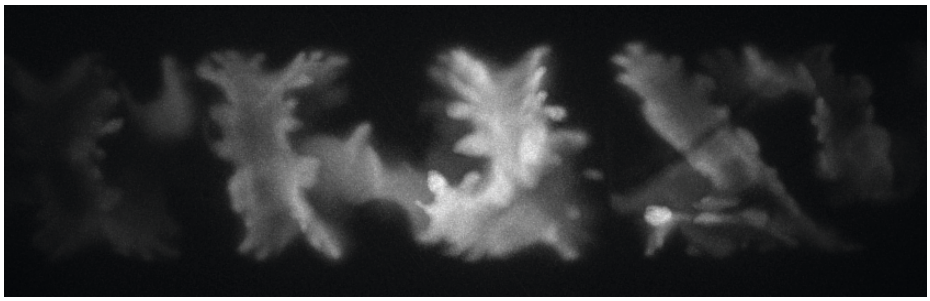

Figure 2 shows a 3D fluorescence image of a Spirogyra. Spirogyra is a genus species of green freshwater algae which form large mats of long filaments in eutrophic ponds. The impressive three dimensional structure makes the Spirogyra interesting specimens for observation.

Figure 2.
Stacked and projected 3D image of a Spirogyra algae using multi beam confocal fluorescence microscopy (λ_{ex} = 405 nm, λ_{em} = 650 nm, Zeiss Apochromat 100x oil immersion, NA: 1.4)

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The 3D image is made up of 102 2D slices each separated by 500 nm within the sample. The 3D image is rendered using the 3D project function with Fiji [3]. The acquisition time for this full 3D volume is 4.5 seconds. The total acquisition time can be further reduced by reducing the number of 2D slices, e.g. 22 2D slices result in one volume per second. The acquisition speed can also be increased by selecting a smaller region of interest on the camera, allowing up to 100 2D slices per second at a resolution of 512 x 512 pixels.

References

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[3] Schindelin, J.; Arganda-Carreras, I. & Frise, E. et al. (2012), "Fiji: an open-source platform for biological image analysis", Nature methods 9(7): 676-682, PMID 22743772 (on Google Scholar).

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