Efficient striping artefacts suppression in LSFM by flexible AOD based beam pivoting

Vladislav Gavryusev[1,2,+], Giuseppe Sancataldo[1,2,+], Giuseppe de Vito[2,3], Lapo Turrini[1,2], Massimiliano Locatelli[1,2], Chiara Fornetto[1,2], Natascia Tiso[5], Francesco Vanzi[2,4], Ludovico Silvestri[2,3], Francesco Saverio Pavone[1,2]

[1]University of Florence, Department of Physics and Astronomy, Sesto Fiorentino, Italy
[2]University of Florence, European Laboratory for Non-Linear Spectroscopy, Sesto Fiorentino, Italy
[3]National Institute of Optics, National Research Council, Sesto Fiorentino, Italy
[4]University of Florence, Department of Biology, Sesto Fiorentino, Italy
[5]University of Padova, Department of Biology, Padova, Italy

[+] equal contribution   **E-mail: gavryusev@lens.unifi.it**

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Abstract: Light-sheet fluorescence microscopy (LSFM) [1] is used in many biological and biomedical research fields, like live studies of murine and zebrafish neural activity or of cell division and growth. By illuminating selectively a single plane, it provides intrinsic optical sectioning and fast image recording, while minimizing out-of-focus fluorescence background, sample photo-damage and photobleaching. However, LSFM is more affected by absorption or scattering artefacts than point scanning methods [2], leading to un-even illumination.
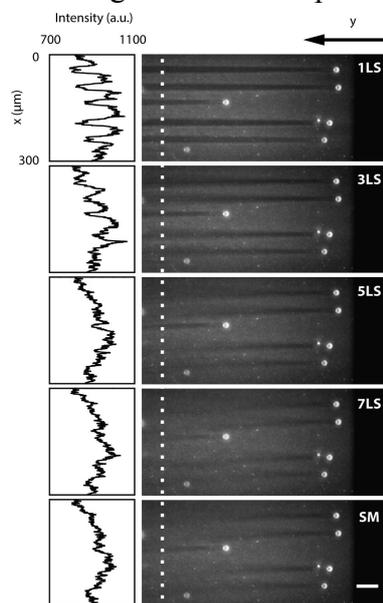
We present here an easily implementable and cost-efficient method, based on acousto-optical deflectors (AOD), to overcome this obstacle [3]. We report the advantages provided by flexible and fast AODs in generating simultaneous angled multiple beams from a single laser beam and in fast single light sheet pivoting and we demonstrate the suppression of illumination artefacts. We benchmark our method's performance on bead samples and on a typical biological application of imaging neuronal brain activity in zebrafish larvae. We show that un-even illumination may induce in the spatial and temporal correlation maps spurious features that can be mistaken for biological activity and we demonstrate their removal by our AOD based pivoting approach.

Fig.1 LSFM images acquired by illuminating with different light-sheet configurations (1, 3, 5, 7 static sheets at different angles and single-plane sweeping mode) a single plane of a sample of 15 μm polystyrene beads embedded in a 1% agarose gel and immersed in water. the The background becomes more uniform with an increasing number of illumination directions as shown in the inset by the fluorescence intensity profiles along the y-axis, averaged over 6 μm around the white dotted line. White scale bar of 50 μm.

References

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