# MULTICOLOR 3D LIVE IMAGING OF BRAIN TISSUES AND SPHEROIDS BY SPECTRAL IMAGE SCANNING MICROSCOPY (SPECTRAL-ISM).

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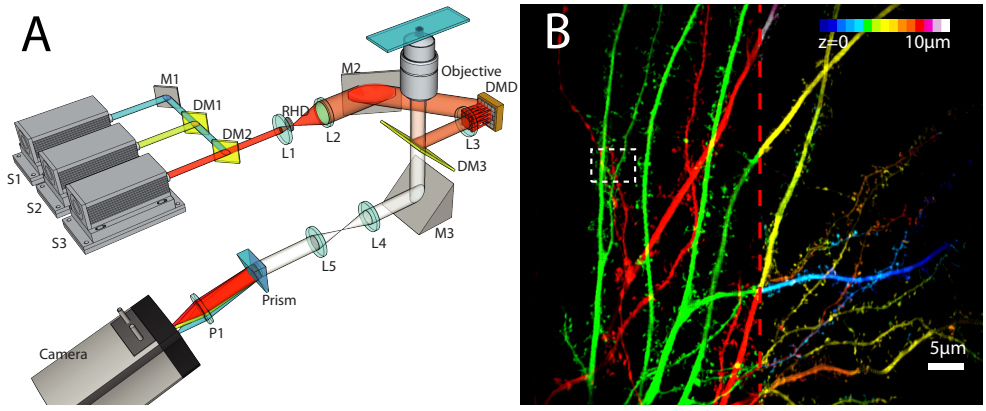
So far, the gold standard for multicolor 3D fluorescence imaging of live biological samples is the spinning disk microscope, which allows for fast video-rate diffraction limited sectioned imaging. Yet this technique has two major limitations: (1) an important background signal due to pinhole crosstalk (2) a poor photon collection efficiency. These limitations become severe for multidimensional time-lapse imaging of living samples such as brain slices, or cell spheroïds where phototoxicity can dramatically hinder the image acquisition rate. To overcome these limitations, we developed an original implementation of multipoint image scanning microscopy [1]which we call **Spectral-ISM**. It allows for the simultaneous acquisition of multiple fluorophores at the highest lateral and axial resolution allowed by state of the art confocal microscopes (200nm lateral, 400nm axial). Spectral-ISM relies on a multipoint and multicolor illumination generated by a digital micromirror device (DMD). The sample is illuminated with a sparse grid of points which is digitally scanned over the sample to cover the entire field of view. The fluorescence emission spectrum of the sample is dispersed at each illumination spot of the grid by a prism and recorded for each position of the grid on a fast sCMOS camera. After spatial filtering on each camera image and wavelength assignment, a sectioned multicolor image of the sample is computationally reconstructed. We image living samples at one multicolor plane per second. In our hands samples labelled with 5 distinct fluorophores could be imaged simultaneously. The hyperspectral imaging capability of our method allows for spectral quantification of biological processes as well as linear unmixing of fluorescent species with known overlapping spectral signatures. We demonstrate long term 3D multicolor imaging in 150 um thick brain slices and 50 um diameter brainbow labelled HEK cells spheroids.




A) Schematic of the Spectral-ISM microscope including a multi-laser (S1, S2, S3)wide field illumination spatially modulated by a DMD (digital micromirror device). (B) Two color (GFP/TdTomato) maximum projection image of a living brain slice with z color coding on the right half image

[1]Müller, C. B. & Enderlein, Image Scanning Microscopy J. *Phys. Rev. Lett.* **104,** 198101 (2010)