The acquisition of millisecond-scale optical signals in biological samples is extremely useful in the study of fast biological events, such as neurons signalling or hemodynamics. However, the most used techniques for optical imaging in thick biological samples, such as confocal and two photon microscopy, are not capable of providing reliable millisecond-scale signals on a wide field of view. The main limiting factor for confocal acquisition rate is the use of galvanometric mirrors for raster scan beam displacement.

In order to allow effective parallel fast acquisition, the presence of multiple confocal excitation volumes is required. This can be achieved through the use of a Spatial Light Modulator (SLM), which can divide the coherent excitation light in multiple diffraction limited focal points across the field of view. Reported implementations of SLM based confocal microscope [1] rely on a standard galvanometric scanner for the acquisition of an image of the sample, on which locations of interest are selected to be illuminated with Spatial Light Modulation.

We present an alternative approach, allowing for the acquisition of complete three dimensional confocal images through the use of an SLM and a pixelated detector. This dramatically reduces the cost and complexity of the setup, as the microscope works without any mechanical moving part, and allows for placement of excitation volumes in locations of interest for fast parallel signal acquisition with sub-micrometer precision. Spatial light modulation – two photon microscopy (SLM-2PM) image acquisition is based on the use of the SLM to illuminate the sample with a grid of diffraction limited excitation volumes, and move such grid according to a raster scan pattern. A pixelated detector acquires the fluorescence signals of each excitation volume separately during the scan sequence, and a custom software creates the confocal image. The same process can be executed on different focal planes through the use of the SLM, without the need for mechanical movement of the objective.

Images acquired with SLM-2PM have comparable quality to the ones obtained through a standard two photon microscope, and the method was successfully employed for millisecond scale calcium imaging in acute cerebellar slices.


Acknowledgement: This work was supported by grants from European Union to Egidio D’Angelo (CEREBNET FP7-ITN238686, REALNETFP7-ICT270434) and by grants from the Italian Ministry of Health to Egidio D’Angelo (RF-2009-1475845).
Fig. 1: Example of high frequency acquisition: Image on the left shows granular layer in a cerebellar slice loaded with Fura-2 AM. Multiple focal points are placed on the cells indicated by red circles, obtaining the image on the center on a fast CMOS camera. Calcium signals from each cell are acquired at frequencies up to 1KHz (example on the right).

Fig. 2: SLM-2PM 3d image of a Purkinje cell, obtained without any mechanical moving part. Panel A: three confocal images acquired at planes 4 μm apart. Panel B: Projection of a total of 10 confocal images of planes.