CONFOCAL MULTI-LINE SCANNING MICROSCOPE FOR EFFICIENT 3D FLUORESCENCE IMAGING

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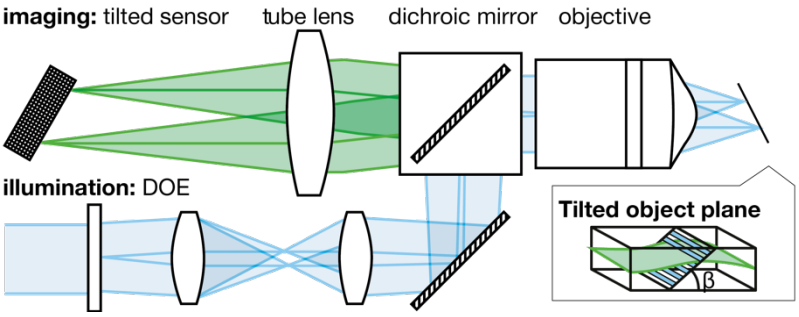
In the current era of big data analysis, hardware tools to generate massive amounts of (image) data are in demand. For biological exploration, or for novel computer aided medical diagnoses, there is need for fluorescence imaging of tissue over large fields of view (~few mm), in 3D (up to hundred layers of μm thickness), and at cellular resolution (~1μm). This can be used, for example, in immunofluorescence or FISH studies. In recent work, we showed that fluorescence line scanning microscopy can be implemented by using widefield LED illumination [1]. This is an uncomplicated and low-cost solution, but it is intrinsically slow because of the limited illumination power in the objective etendue.

These limitations can be overcome by laser illumination, which is not etendue limited. The laser is focused into a single line inside the sample, optically conjugate to the line image sensor to have confocal illumination [2]. This has the advantage of a high optical sectioning, very high efficiency, as well as low bleaching compared to wide field illumination.

We present a novel optical architecture for scanning with multiple laser illumination lines at different depths in parallel. The basic idea is to place the image sensor tilted in the light path and generate a set of illumination laser lines inside the sample conjugate to pixel lines in the sensor plane (see Figure). This 3D laser line illumination scheme is a form of illumination PSF engineering, and can be designed using diffractive optics principles. In experiment we use a Spatial Light Modulator (SLM) shape the illumination PSF and correct aberrations in the illumination path. In the presentation we will give an overview of the underlying principles, provide implementation details of the design, and show scan results of samples stained with immunofluorescence.



Novel scanning concept for multi-line 'push broom' scanning. Only two lines are drawn for clarity. By using a tilted sensor, we create a tilted object plane (see inset). A diffractive optical element (DOE) is used to create a multi-line illumination pattern matching the sensor.

References

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