HIGH-SPEED SELECTIVE PLANE ILLUMINATION MICROSCOPY (SPIM)

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In the past the recording speed of a time lapse has ultimately been limited by the amount of light the specimen could tolerate. Lately, it has been shown that light sheet microscopy such as Selective Plane Illumination Microscopy (SPIM) reduces photo-toxic effects to a minimum. Due to the illumination of the sample in a thin volume around the focal plane no tissue outside the plane of interest is exposed and bleached. In addition, the fluorescence is collected with very high sensitivity cameras. In fact, SPIM benefits from the latest camera technology that becomes available and is therefore constantly improving in speed and sensitivity.

We observed that our implementations of SPIM have enabled time lapse recordings of superior speed and minimal phototoxicity. Experiments have become possible that run at full speed using the best possible hardware, without being limited by the fragility of the sample. High-speed 3D data can be recorded non-stop. The speed advantage of the SPIM over other fluorescence technique can be utilized to image rapid events in developing tissue or to record a large number of views for multi-view reconstruction. The large amount of data that is accumulated when modern cameras (EM-CCD, CMOS) are run at high-speed for hours or days is enormous and innovative data processing solutions are needed. Therefore, the challenges in microscopy are moving now from sample preparation and preservation to data storage and analysis.

We present some of our ideas on how to push the limits of high-speed 3D microscopy. Current applications include multi-dimensional imaging of the developing zebrafish larvae over extended periods of time. Especially the development of the cardiovascular system is of great interest and we present some of our solutions.