The Selective Plane Illumination Microscope (SPIM) is a new type of fluorescence microscope that allows fast three-dimensional imaging of biological specimens with high resolution [1]. Optical damage to the sample is reduced by orders of magnitude compared to confocal microscopy, since by using a diffraction-limited light sheet the SPIM illuminates only the part of the sample that is being imaged.

Microtubules (MTs) are cytoskeletal filaments which play a fundamental role in imparting polarity to the cell, determining the plane of symmetry in cell division, and regulating cell movements and shape. MTs are highly dynamic structures which self-assemble from the dimeric protein tubulin and continuously undergo stages of growth and shrinkage, in a process called “dynamic instability”. In living cells, MTs usually radiate from a microtubule organizing center (centrosome) toward the cell cortex, forming star-shaped structures (MTs asters).

Up to now, the dynamic instability of MTs could only be measured in 2D by squeezing MTs asters in shallow chambers and observing them with epi-fluorescence microscopy. Consequently, some important aspects of dynamic instability and MT interactions are still missing. In this work, we study the dynamics of MT assembly in 3D, taking advantage of the unique imaging features of the SPIM. We are performing the experiments under controlled conditions in vitro, using *Xenopus laevis* mitotic egg extract as a physiological environment.

MT asters are imaged and single MTs radiating from the centrosome are tracked by an automated custom routine written in our lab. By monitoring the length change of MTs over time, all parameters describing dynamic instability can be acquired. We also show that by tracking the relative 3D movements of centrosomes, the interactions between asters can be measured. The characterization of these interactions is important for elucidating the mechanism of spindle assembly during mitosis.

**Figure 1.** Asters in *Xenopus* egg extract recorded with the SPIM: (a) automated tracing of MTs, (b) aster projections of an image stack.