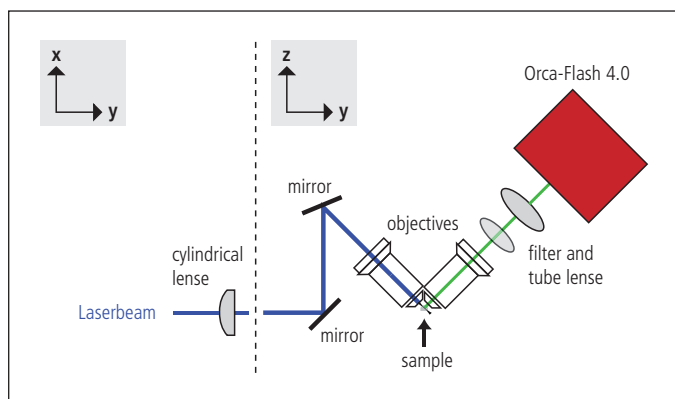


## Monitoring early embryogenesis via light sheet Microscopy

Single-plane illumination microscopy (SPIM) is a fluorescence imaging technique that combines rapid wide-field detection with optical sectioning. By reducing phototoxicity induced by bleaching, SPIM is capable of providing a long-term, three-dimensional and time-resolved in vivo imaging of specimen.

We have built and automated a SPIM setup specifically designed for imaging the early stages of embryogenesis of the small nematode *Caenorhabditis elegans*. Philipp Struntz, graduate student in the group of Prof. Matthias Weiss (Dept. of Experimental Physics I, University of Bayreuth). The setup consists of the widened beam of a 491.5 nm DPSS-laser, which is focused in one dimension by a cylindrical lens onto the back-focal plane of a water-dipping objective to form an elliptically deformed Gaussian beam that serves as light sheet (minimum waist of 2.8 mm FWHM). Fluorescence from the sample is collected perpendicular to the illumination light sheet by a second water-dipping objective. Fluorescence light is filtered and focused onto the sensor of an ORCA-Flash 4.0 sCMOS-camera (Hamamatsu Photonics). The lateral and axial extension of the setup's point-spread function was determined with FluoSpheres (diameter 20nm) to be 460 nm and 1.4 mm in the light sheet waist.

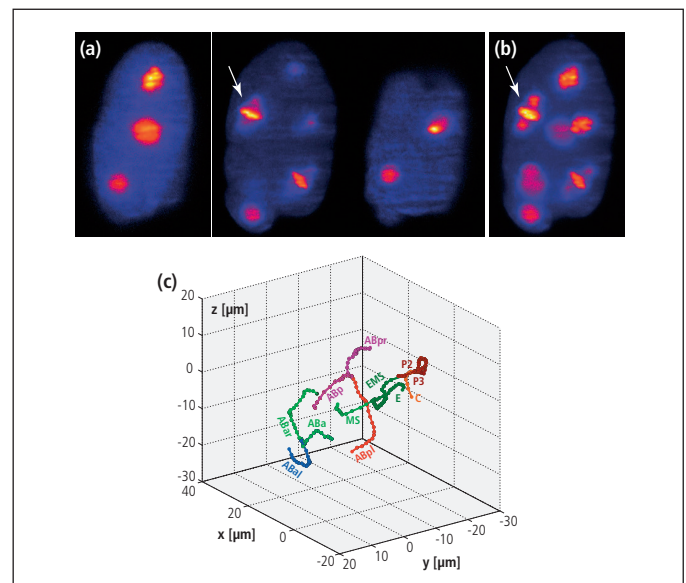
As can be seen from figure 1, objectives are arranged in an angle of 45° degree with respect to the optical table. The sample is moved with respect to the light sheet by two motorized stages in xy-direction. A nanopositioner allows for moving the sample through the light sheet with a precision of a few nm, hence enabling the acquisition of three-dimensional image stacks of the sample.



**Figure 1: SPIM-Setup: Sketch of the beam path of the custom-made SPIM. All parts left from the dashed line are shown from top whereas all parts on the right are shown from the side.**

The setup is controlled via a custom-made LabVIEW program that also has an interface to the HOKAWO imaging software (Hamamatsu Photonics) that controls the camera. Post-processing of raw images, i.e. aligning individual sections to obtain three-dimensional data, is done with a custom-made Matlab code. With this approach, nuclei can be tracked in the developing embryo and even division axes during mitotic events can be assessed.

Philipp Struntz explains the experiment conditions: We repetitively acquired 51 optical sections (separated by a distance of 2 μm) of a *C.elegans* embryo with an exposure time of 50 ms per layer. Stack acquisition was repeated every 60 s to cover early developmental processes. Representative individual sections of this imaging are shown in figure 2a, the maximum projection obtained from the three-dimensional data is depicted in figure 2b. Since cell lineages in *C. elegans* are invariant, not only trajectories but also the type of individual cells could be identified in three dimensions over time. An example is shown in figure 2c.



**Figure 2: (a) Different layers of a *C. elegans* embryo (long axis 50 μm) in the 8-cell stage. (b) Maximum projection of all 51 sections of the embryo in (a), showing all 8 nuclei positions with a high contrast. (c) Example of three-dimensional cell trajectories. Cells are color-coded and named. Cell divisions are visible as branching points of trajectories.**

Based on our data, we observed in all cases a planar cell arrangement in 4-cell embryos, and cell movements and division axes were strikingly similar between embryos, states Philipp Struntz. It is tempting to conclude on this basis that mechanical cues support the cell arrangement in early *C. elegans* embryogenesis [1]. The obtained time lapse images allowed us to quantitatively monitor individual cell positions and division axes within the developing embryo. These data build a valuable starting point for a deeper understanding of the spatiotemporal dynamics of developing tissues.

Prof. Matthias Weiss concentrates in his research on challenging problems at the interface of physics and biology. His group focuses on a quantitative understanding of intracellular transport processes, on elucidating interactions of proteins and membranes, and on the dynamics and self-organization of eukaryotic cells from the organelle to the organismal level. For further information see the website of the Dept. of Experimental Physics I, University of Bayreuth, <http://www.ep1.uni-bayreuth.de/weiss/en/index.html>

[1] Fickentscher, Struntz and Weiss, Mechanical cues in the early embryogenesis of *Caenorhabditis elegans*, to appear in *Biophys. J.* 105(8), (2013).