**Analysis of microtubule arrays**

**Supervisor:** Anne Straube (http://mechanochemistry.org/Straube/)

**Introduction:** Microtubules are structural components, long-distance transport tracks as well as force generators within the cell. Microtubules form the mitotic spindle during cell division and support morphological changes such as the outgrowth of neurites and directional cell locomotion. To fulfil this variety of functions, microtubules self-organise into characteristic arrays. This self-organisation is achieved by the dynamic assembly and disassembly of the microtubules themselves, the sliding of microtubules relative to each other by motor proteins and the crosslinking by motors and non-motorised microtubule-associated proteins. Here we study the self-organisation mechanisms of microtubules in differentiating skeletal muscle cells. The formation of skeletal muscle fibres is accompanied and dependent upon the reorganisation of microtubules into a paraxial array. The array is thought to be organised by the cooperation of bifunctional motor proteins and passive crosslinkers (Mogessie et al, eLife 2015). As the microtubule array in differentiating muscle cells is very dense, the analysis with light microscopy is challenging. We have recently employed expansion microscopy (Chen et al., Science 2015) to achieve a 4-fold increase in resolution. We have now acquired datasets for cells expressing different RNA interference constructs targeting bifunctional motor proteins implicated in sliding microtubules (dynein, KIF15 and Eg5).

**Aims:** The aim of the project is to develop an image and data analysis pipeline and apply it to analyse microtubule array organisation at different stages of myoblast differentiation and when bifunctional motor protein are depleted.

**Methods:** The starting point are overlapping 3D image stacks of fluorescently labelled microtubules in expanded cells. You will need to stitch the imaging stacks to cover the entire volume of the cells of interest, which are after expansion too large to be captured in one field of view. There are existing plugins in ImageJ that you could use for that.
You will then segment microtubules in the 3D volumes. A potential approach is to adapt, optimise and validate the existing python-based software SOAX (Xu et al. Scientific Reports, 2015) for this.
Once you obtained the location data for all microtubules, you will perform downstream data analysis to report paraxial microtubule alignment and the curvature of microtubules in a spatially resolved manner, i.e. as a function of distance to the nucleus / tip of the cell / cell membrane. If time permits, you will integrate multichannel imaging and the location of microtubule end markers to refine the extracted microtubule array.

**References:**


1. A novel isoform of MAP4 organises the paraxial microtubule array required for muscle cell differentiation. Mogessie et al. eLife, 2015
2. SOAX: A Software for Quantification of 3D Biopolymer Networks. Xu et al. Scientific Reports, 2015.
3. Expansion microscopy. Chen et al. Science, 2015


**PhD:** The project is suitable as a PhD project to analyse the dynamic rearrangement of microtubules and generate a mathematical model / simulations that recapitulate microtubule array and morphological changes grounded in your data and those already available in the literature. To do this, we will provide you with timelapse movies of 3D stacks with different fluorescent markers to label microtubules along their length or just their ends obtained using a lattice light sheet microscope. Stefan Grosskinsky would be available as co-supervisor to help with the modelling part. A potential project partner is Intelligent Imaging Innovations (3i), the manufacturer of the lattice light sheet microscope.