MA932: **Study Group on "Seeing is believing – unravelling biological dynamics from the next generation of light microscopes"**

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Light microscopy has been going through a revolution over the last decade, highlighted by the 2014 Nobel prize being awarded for surpassing the limitations of the light microscope. These developments are driving a new frontier in biology, observing detail that was previously lost within the noise and limited spatial resolution (limited to the wavelength of light ~500nm, visible spectrum). This has raised many mathematical and statistical challenges, primarily extracting information from these images (feature detection, segmentation, tracking, visualisation) and modelling the new dynamics that is now observable.

This project will look at the emerging light sheet technology (LLSM, diSPIM) and challenges associated with this new imaging data type. The light sheet microscope uses a thin sheet of light to illuminate a sample; this differs from scanning microscopes where a laser beam is rastered across the sample. Techniques you may need include (but not limited to) programming (MatLab, ImageJ primarily), geometry (curves, surface coordinate systems, curvature), differential equations, optimisation, statistical distributions (mixtures of Gaussians) and statistics (basic tests such as t-tests).

Possible directions for the project are:

1. ***Tracking non-point-like objects***. Tracking spots using Gaussian mixture models is fairly well established (Chenouard et al., 2014, Armond et al., 2016), but tracking non-point like objects (distorted spots, comets) presents new challenges. Such objects have structure, e.g. orientation and shape. Tracking is an optimisation problem where the optimal tie up for objects between frames is found, possibly incorporating predictions for an object's location in the next frame. *Experimental data:* Chromosomes (diSPIM/LLSM, McAinsh), Microtubule end-tracking (LLSM, Straube).
2. ***Tracking curved polymers*** (such as microtubules) using for instance active contour models (Kass et al., 1987). Here, instead of a spot moving in time, you have a curve, that may change shape and/or length. *Experimental data:* MTs in muscle cells (3D SIM, Straube), MTs in zebra fish embryos (diSPIM, Mohan; low time resolution so more of a pattern detection of different types of cell division mechanisms).
3. ***Disentangling multiple objects***. Images with a large number of multiple objects are notoriously difficult to track. Tracking may be improved by utilising object information (speed, shape, intensity) and more sophisticated noise and movement models. *Experimental data:* Microtubules (LLS/3D SIM, Straube).

4. ***Extracting and parametrising cell surface dynamics.*** A further generalisation to 2D surfaces; requires techniques from geometry, curvature and elasticity. *Experimental data:* Dictyostelium cells labelled for F-actin, (diSPIM, Bretschneider).
5. ***Using GPUs to accelerate processing*** (requires expertise with CUDA). Processing the large data sets from light sheet microscopes pose significant challenges highlighting clever algorithms and faster processing platforms.

Data from 3 biological systems will be available:

1. *Microtubule tracking* (LLSM, 3D SIM). Microtubules are dynamic polymers that are a principle part of the cell cytoskeleton, performing both structural functions and transportation functions. Their growth and shrinkage is highly regulated and can be monitored by labelling either their ends (plus-end with EB3, LLSM) or the microtubules themselves (dual-colour data of microtubules and minus end marker (CAMSAP2) is also available, 3D SIM).

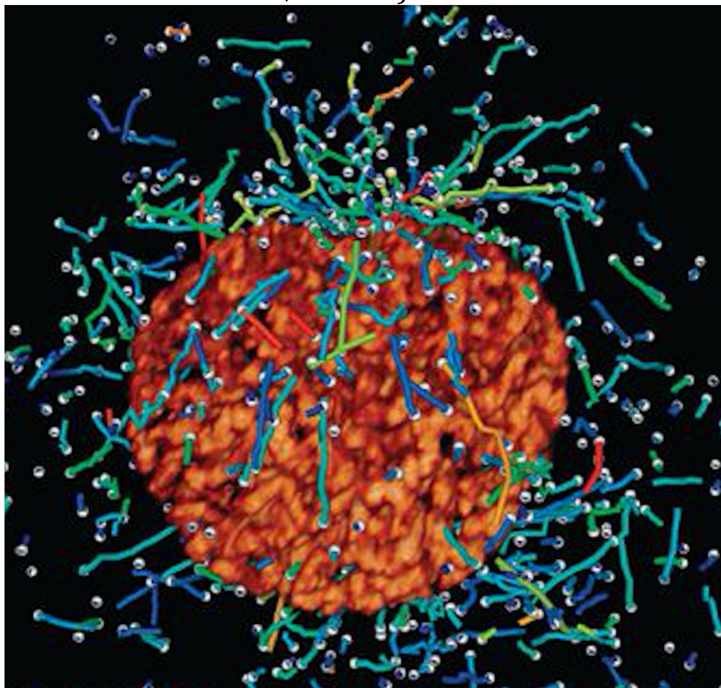

Figure: Intracellular dynamics in three dimensions from LLSM showing chromosomes (histones) and 3D tracks of growing microtubule ends. **Bi-Chang Chen et al. Science 2014;346:1257998**

2. *Chromosome dynamics* (diSPIM, LLSM). During cell division duplicated chromosomes are held in a holding pattern near the cell equator, waiting until all chromosomes are in position before triggering separation of duplicated chromosomes to the two daughter cells. Chromosomes can be tracked by labelling their kinetochores, the protein complex that attaches the chromosomes to microtubules, dynamic microtubules repositioning

the chromosomes. Labelled kinetochores are observed to undergo oscillations across the equator.

3. *Cell surface tracking* (diSPIM). The cell surface is comprised of a lipid bilayer membrane and an actin cell cortex below the cell membrane. Actin is a polymer that makes cross-linked networks that function as rapidly adapting stress bearing media that shapes, and provides support to the cell surface. Moving the surface thus entails reworking the cortex.

Microscope platforms.
A number of microscope platforms will be available (i) diSPIM (this microscope uses two interleaved light sheets to improve resolution, giving an isotropic (equal x,y,z) point spread function (PSF) after deconvolving the two sheets), (ii) lattice light sheet microscopy (or Bessel beam, LLSM), a form of structured illumination (this is a super resolution technique so achieving spatial detail below the wavelength of light), (iii) 3D structured illumination (3D SIM).

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