ANALYZING THREE-DIMENSIONAL BIOLOGICAL SPECIMENS WITH LIGHT SHEET MICROSCOPY AT NEAR-NATURAL CONDITIONS

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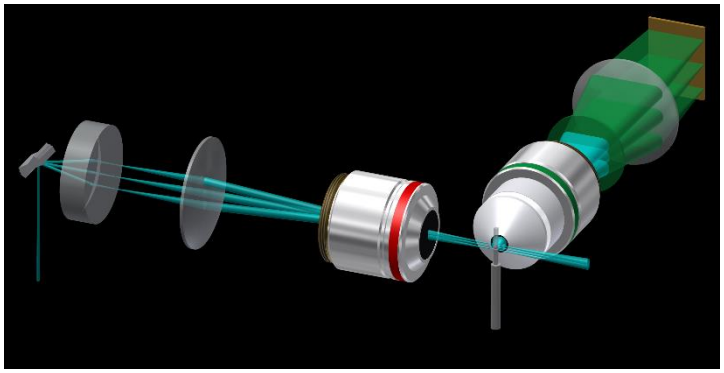
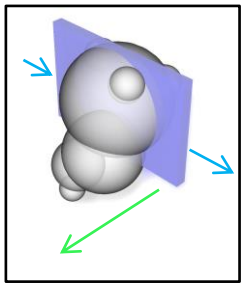
A major objective of the modern life sciences is to perform experiments in the context of near natural conditions (NNC), i.e. to rely on three-dimensional biological specimens such as cysts, organoids, spheroids, embryonic bodies, tissue sections and small model organisms. Scientific projects relate to developmental biology, including embryogenesis and tissue formation, as well as to cell biology, e.g. investigating specific pathways in three dimensions rather than in cell culture systems that rely on hard and flat surfaces.



This talk concentrates on three-dimensional light microscopy and image processing pipelines that are capable of handling millions of large-scale images as well as various different applications, which stem from our own research and our collaborations.

In general, fluorescence microscopy provides a high contrast, since only specifically labelled cellular components are observed while all other structures remain "dark". Fundamental issues of fluorescence microscopy that have to be addressed are: 1) Excitation light degrades endogenous organic compounds and bleaches fluorophores. 2) Specimens provide only a finite number of fluorophores, which limits the number of collectable emitted photons. 3) Organisms are adapted to a solar flux of 1.4 kW/m^2. Thus, irradiance should not exceed a few mW/mm^2 or nW/µm^2 in live imaging assays.



Furthermore, the optical sectioning capability is fundamental for dynamic three-dimensional imaging. One of the very few instruments, with this property is light sheet-based fluorescence microscopy (LSFM). LSFM makes the most sincere effort to address the NNC-related issues by decoupling the excitation and emission light pathways. The significance of LSFM's illumination-based optical sectioning property is that the viability and the fluorescence signal of a living specimen are retained while millions of images are recorded for days or even weeks.

Particular benefits of LSFM are: (i) good axial resolution, (ii) imaging along multiple directions, (iii) deep tissue penetration due to the low numerical aperture of the illumination objective lens, (iv) high signal-to-noise ratio, (v) unrestricted compatibility with fluorescent dyes and proteins, (vi) reduced fluorophore bleaching and (vii) photo-toxicity at almost any scale, (viii) millions of pixels recorded in parallel, (ix) excellent specimen viability and (x) compatibility with many functional imaging approaches (FCS, FLIM, FRET etc.).

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