



Seminar/Talk

Hyperspectral three-dimensional widefield microscopy in living zebrafish and fruit fly embryos

Wiebke Jahr

MPI-CBG, Dresden

Host: Johann Georg Danzl

A major goal in biological imaging is to visualize interactions of different tissues, often fluorescently labeled, during dynamic processes. Only a few of these labels fit into the available spectral range without overlap, but can be separated computationally if the full spectrum of every single pixel is known. In medical imaging, hyperspectral techniques show promise to identify different tissue types without any staining. Yet, microscopists still commonly acquire spectral information either with filters, thus integrating over a few broad bands only, or point-wise, dispersing the spectra onto a multichannel detector, which is inherently slow. Light sheet fluorescence microscopy (LSFM) and optical projection tomography (OPT) are two techniques to acquire 3D microscopic data fast, photon-efficiently and gently on the specimen. LSFM works in fluorescence mode and OPT in transmission. Both are based on a fast widefield detection scheme where a 2D detector records the spatial information but leaves no room to acquire dispersed spectra. Hyperspectral imaging had not yet been demonstrated for either technique. I present a line-scanning hyperspectral LSFM and an excitation scanning OPT to acquire 5D data and show how the performance of both setups can be optimized to minimize acquisition times without sacrificing image contrast, spatial or spectral information. We implemented and assessed different evaluation pipelines to classify and unmix relevant features from the hyperspectral data. We demonstrated the efficiency of the workflow by acquiring up to five fluorescent markers and the autofluorescence in zebrafish and fruit fly embryos on the hyperspectral LSFM. Both concentration maps and spectra for each of the fluorophores were extracted using linear unmixing and spectral phasor analysis. The same methods were applied to investigate the transmission data acquired on the spectral OPT, where we found evidence that OPT image formation is governed by refraction, whereas scattering and absorption only play a minor role.

Wednesday, March 8, 2017 04:00pm - 05:00pm

Mondi Seminar Room 1, Central Building



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