

Hyperspectral, multimodal light microscopy

Complex developmental processes cannot be captured in single-color recordings. More data can be extracted from a single sample using multiple labels and contrasts. Implementing several modalities in one instrument provides comprehensive information from a single specimen without the need for registration and synchronization.

Selective Plane Illumination Microscopy (SPIM [1]) and Optical Projection Tomography (OPT [2]) are highly suited for 3D imaging of embryonic development and provide complementary optical contrasts: one fluorescence, the other transmission. We demonstrate three examples how SPIM and OPT were combined and extended to collect more data from a single sample:

1) OPT requires large depths of field in contrast to high NAs desirable for fluorescence microscopy [3]. We implemented a spiral acquisition OPT to enhance depth of field on any standard SPIM setup. The 3D transmission data provides morphological context to the sparse fluorescence [4].

2) We added a descanned detection and an imaging spectrograph to a scanned light sheet microscope to acquire spectrally resolved fluorescence data. Up to five overlapping fluorophores and autofluorescence were distinguished in living zebrafish and fruit fly embryos [5].

3) We equipped an OPT setup with supercontinuum source and AOTF to sweep through the wavelengths used for transmission. We found strong evidence that OPT image formation in living zebrafish embryos is governed by refraction, whereas absorption and scattering play minor roles.

By harvesting spectral information with two modalities we have extended the capabilities of a single instrument. Ideally all modalities will benefit from each other when combined. If care is taken that imaging speed matches sample development, detailed insights into the dynamic processes are obtained.

[1] Huisken, J et al, Science 2004

[2] Sharpe, J et al, Science 2002

[3] Mayer, J et al, Opt. Lett. 2014

[4] Bassi, A et al, Development 2015

[5] Jahr, W et al, Nat. Commun. 2015

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