

Fast nearly isotropic imaging of large samples with light sheets beyond the diffraction limit

Monday, 13 August 2018 12:00 (15)

We were able to generate extremely long thin sheets of light in the one micron range and a vastly increased Rayleigh length by breaking the diffraction limit of light sheets of low numerical aperture. We measured the thickness of the light sheets with different methods including standard point spread function measurement with fluorescent beads. By using these light sheets in our ultramicroscope fast 3D imaging of whole mouse brains with objectives with a large field of view was possible. Due to the extremely low divergence of the light sheets mouse brains could be reconstructed from a single stack of optical sections with nearly isotropic resolution. The light sheets used were essentially non-Gaussian generated by new optics we developed. Compared to a Gaussian light sheet of the same NA our new light sheet is much thinner. Thus the diffraction limit which holds also for low NA Gaussian light sheets was significantly surpassed. These optics will allow the application of ultramicroscopy to ever increasing samples beyond the whole mouse brain range including human cancers.

Besides mouse brains we imaged also cleared whole adult drosophilae. We were able to get good transparency for all developmental stages of the insect from larvae to adult animals with fully preserved GFP signal. We showed that also dualview imaging of cleared adult drosophilae is possible and allows easy isotropic resolution also with standard light sheet microscopy for such kind of specimens.

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Session Classification : Light sheet hardware 2

Track Classification : Light sheet fluorescence microscopy