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Imaging proteins, cells, and tissues dynamics during embryogenesis with two-photon light sheet microscopy

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Light sheet microscopy has gained widespread recognition in recent years due to its distinct advantages for the 3-dimensional imaging of living biological samples. Light sheet microscopy, also known as selective plane illumination microscopy, uses a planar sheet of light to illuminate a sample, generating fluorescence over an optical section of the sample that is collected by a wide-field microscope camera oriented orthogonal to the light sheet. The orthogonal geometry between the illumination and detection pathways enables massive parallelization in both illumination and detection. Furthermore, it allows light illumination to be confined to essentially only the optical section that is being interrogated, minimizing undesired interaction of light with the biological sample. Because of these features, light sheet microscopy significantly outperforms standard imaging modalities in imaging speed, photodamage, and signal to noise in many imaging applications. We recently applied two-photon excitation to light sheet microscopy to improve its penetration depth, allowing long-term imaging of cells deep inside of live embryos. We present a comparison of two-photon light sheet microscopy with other conventional imaging modalities in live imaging of embryos to demonstrate its ability to simultaneously achieve high penetration depth, high acquisition speed, and low photodamage. We also present a selection of applications where two-photon light sheet microscopy is utilized to study the spatio-temporal organization and control of proteins, cells, and tissues during embryogenesis.