

Supplementary Information for

π SPIM: high NA high resolution isotropic light sheet imaging in cell culture dishes

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Supplementary Movies

Supplementary Movie 1 | *In toto* imaging of endocytosis in yeast. π SPIM dual-channel time-lapse movie of endocytosis in yeast capturing all endocytic events for about 1 min at a speed of ~ 2 stacks/s (567 ms interval and 104x104x100 nm voxels) and a roughly isotropic resolution of about 300 nm. Sla1-EGFP and Abp1-mCherry were used (see Results and Discussion).

Supplementary Movie 2 | Long term *in toto* imaging of endocytosis in yeast. π SPIM dual-channel time-lapse movie of endocytosis in yeast capturing all endocytic events for about 7 min at a speed of ~ 2 stacks/s (435 ms interval and 104x104x500 nm voxels). Sla1-EGFP and Abp1-mCherry were used (see Ref¹⁵ in main manuscript).

Supplementary Movie 3 | Microtubule dynamics in HEK293T cells. π SPIM dual-channel time-lapse movie (700 ms interval) of HEK293T cells. π SPIM dual-channel time-lapse movie (100 stacks at 700 ms interval) of HEK293T cells. The maximum intensity projection of 35 planes (500nm spacing) through a HEK293T cell shows microtubules (in green) by visualizing the plus-end-binding protein EB3 (EB3-GFP) relative to the ER marker (CD3 δ -mCherry).

Supplementary Movie 4 | Dynamics of filopodia in HeLa cells. π SPIM dual-channel 30 minute time-lapse movie (400 stacks at 4.5 s interval) of HeLa cells with stable integration of lifeact-GFP and H2B-mCherry. Maximum intensity projection of 25 planes through a HeLa cell expressing lifeact-GFP shows the dynamics of its filopodia.