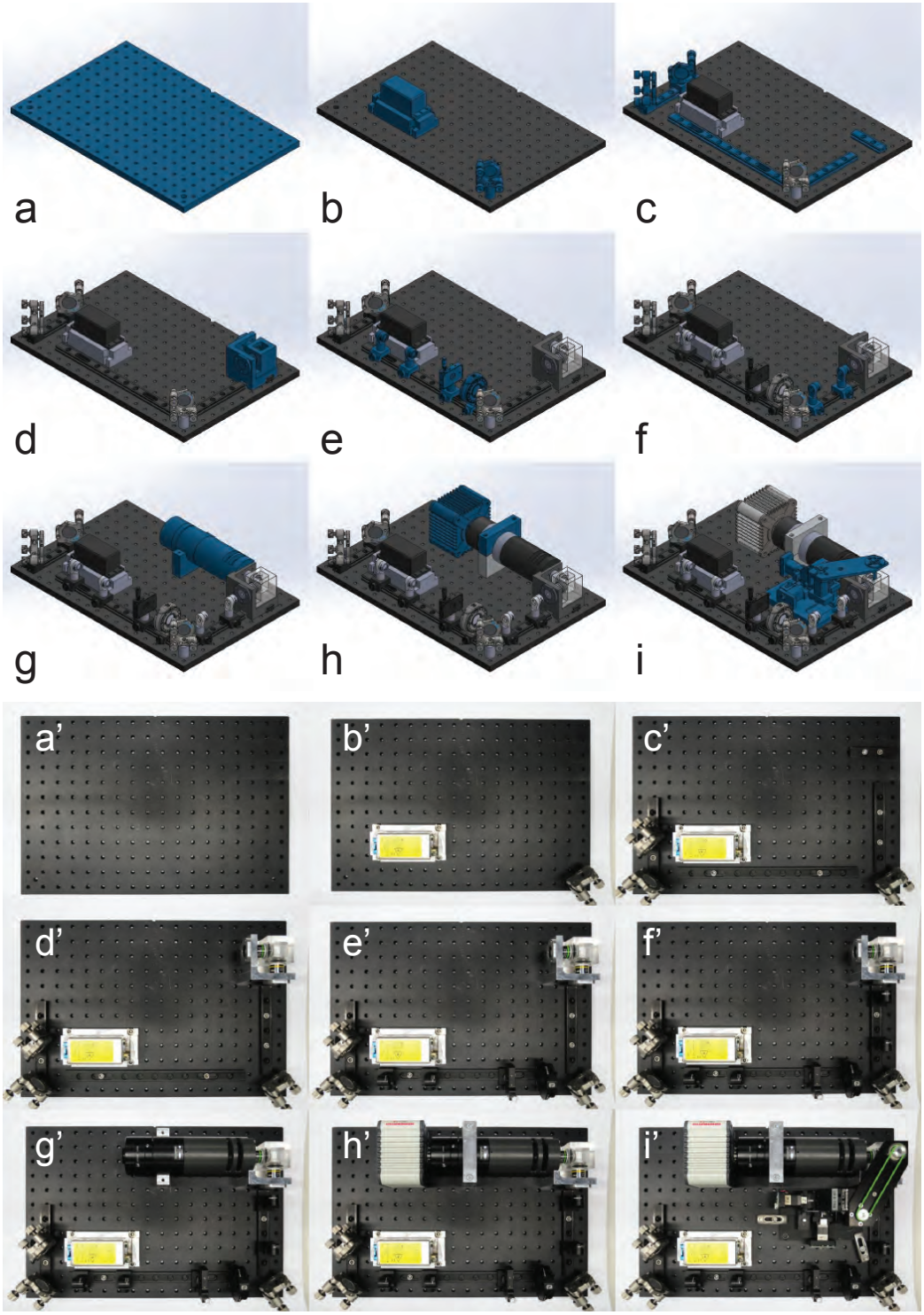
Supplementary Figures



Supplementary Figure 1: *OpenSPIM assembly.* (**a-i**) SolidWorks rendering of the steps necessary to assemble the OpenSPIM, (**a**) Optical breadboard of the OpenSPIM setup, (**b**) laser on a custom made heat sink and corner mirror, (**c**) dovetail optical rails and mirrors that invert the laser beam, (**d**) OpenSPIM sample chamber, (**e**) light sheet formation optics including two beam expander lenses, vertical slit and cylindrical lens, (**f**) telescope optics to image the back focal plane of the illumination objective onto an adjustable mirror, (**g**) infinity space tube, tube lens and camera mount, (**h**) CCD camera, (**i**) 4d USB motor system. Newly added components in (**a-i**) are colored blue. (**a-i**) Equivalent steps of the OpenSPIM assembly represented by real photographs of the set-up.

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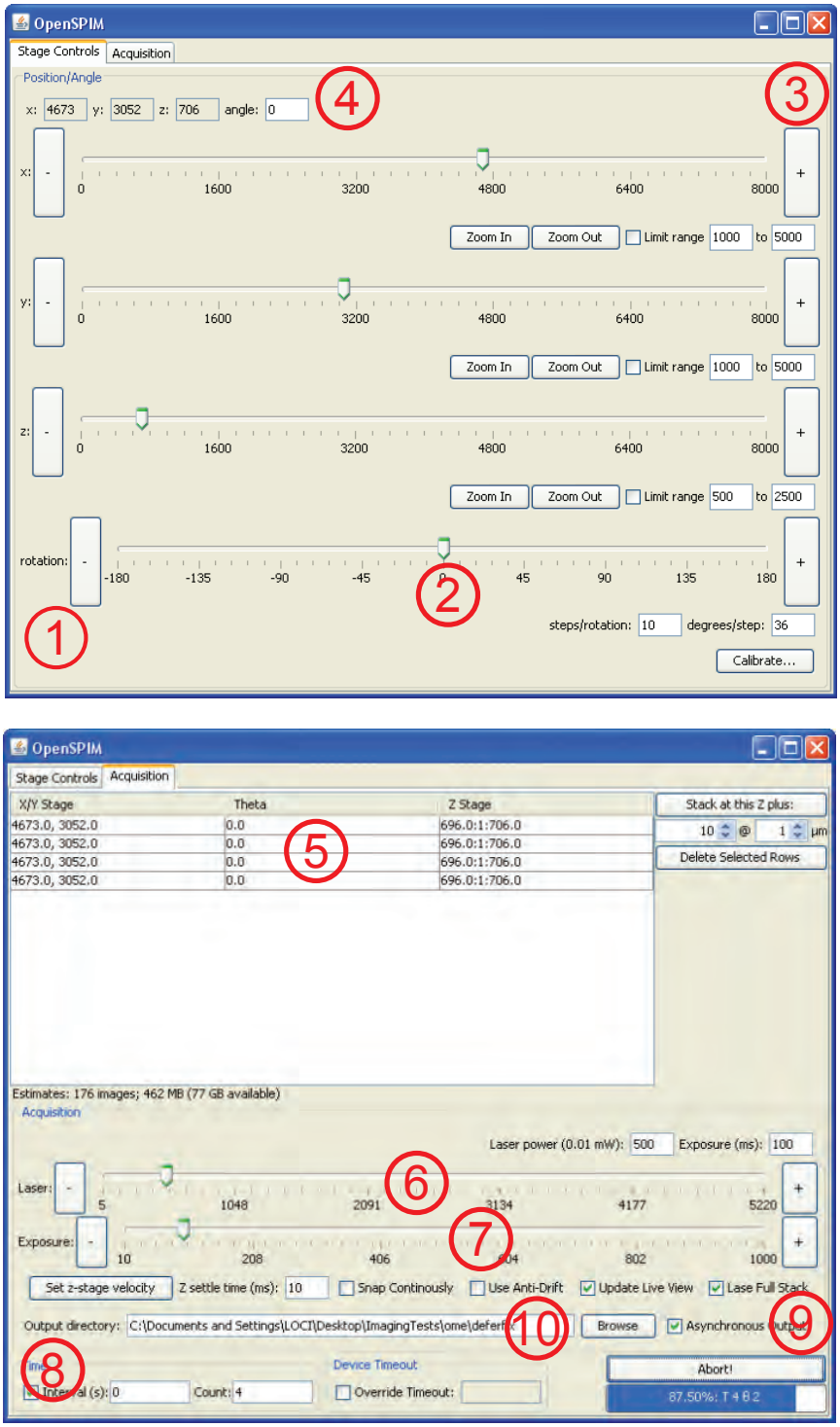

Supplementary Figure 2: *Screenshots of OpenSPIM plug-in.* Top screenshot shows the 4d (xyz and rotation (1)) stage control of the OpenSPIM plugin; sample can be moved by dragging the slider (2), clicking the +/- buttons (3) or typing in the position (4). Bottom screenshot shows the multi-view time-lapse setup window of the OpenSPIM plugin; the microManager position list includes rotation settings (5), laser power (6) and exposure time (7), delay and number of time points (8) can be set; laser can be on for the duration of stack or synchronized with the camera (9), anti-drift option (10) uses image processing and active feedback to motors to keep the sample in the field of view.

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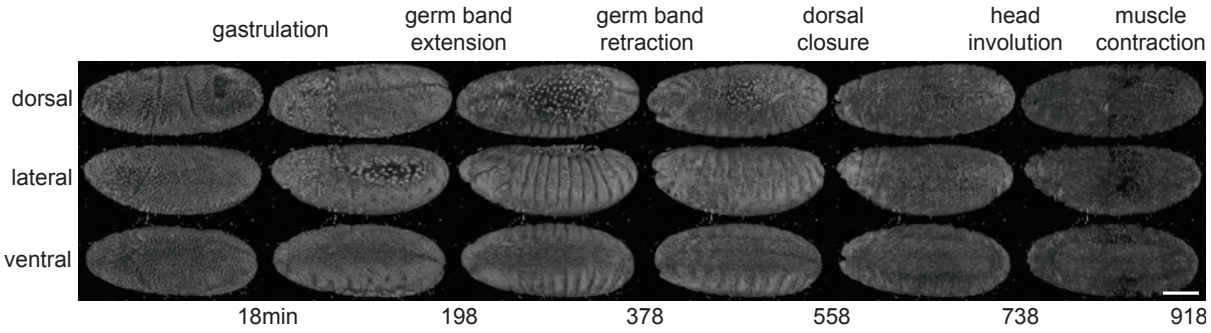

Supplementary Figure 3: *OpenSPIM recording of* Drosophila *embryogenesis.* 3d rendering Drosophila embryos, expressing His-YFP in all cells, imaged from 5 angles every 6 minutes from gastrulation until embryo movement prevents further meaningful imaging. Each view consists of 50 slices 6 µm apart covering the entire embryo. During the acquisition on-the-fly image processing was used to center the imaged specimen in the microscope's field of view by comparing two adjacent time point. This approach compensates robustly for the sample drift caused by agarose contraction and mechanical instabilities during extended long-term time-lapse.

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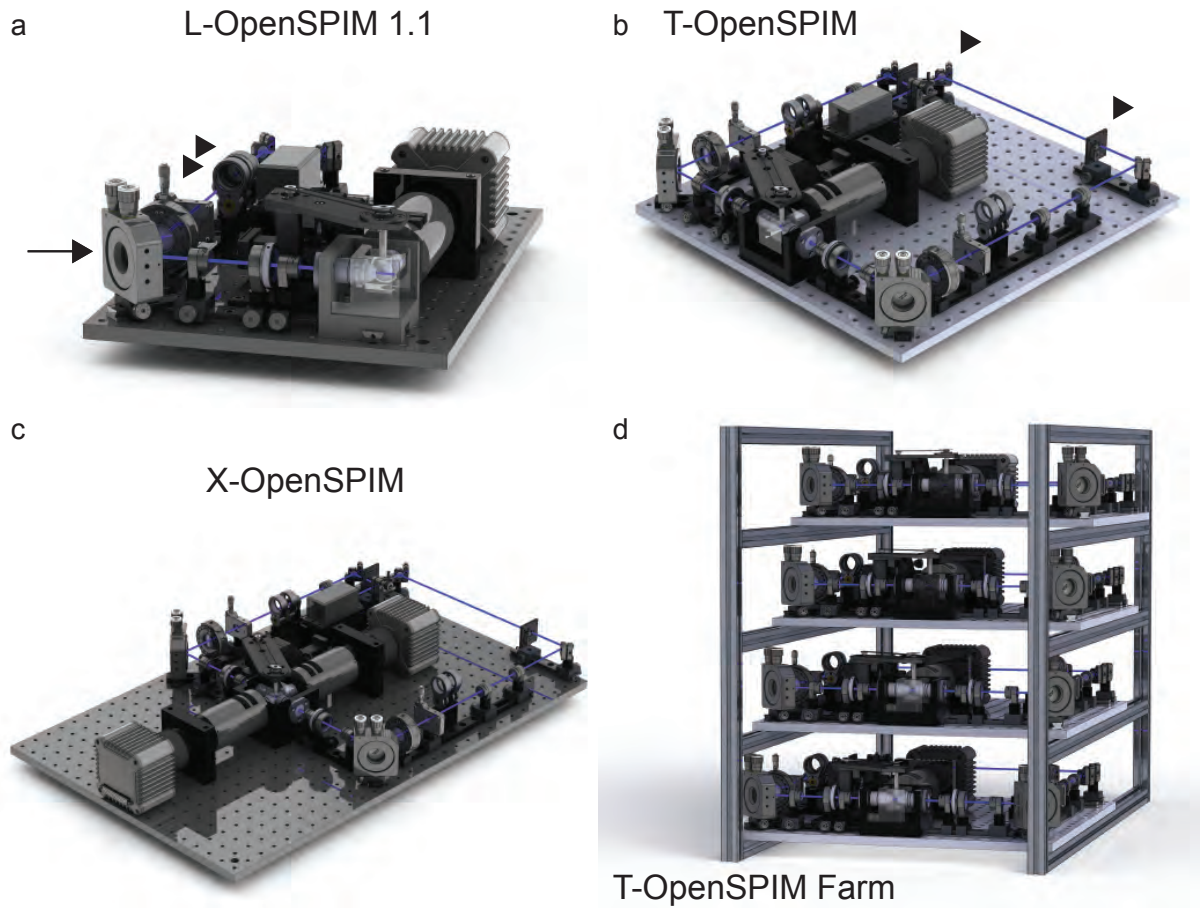

Supplementary Figure 4: *OpenSPIM concept configurations.* (**a**) OpenSPIM 1.1 - a small, incremental evolution of the current OpenSPIM 1.0 setup adding a gimbal corner mirror mount (arrow) for better control of light sheet focussing and removable neutral density and cleanup filters (arrowheads) including 4d position system for sample rotation. One-sided illumination and one-sided detection form an L-OpenSPIM (**b**) Two-sided illumination and one-sided detection T-OpenSPIM configuration with single laser line and shutters for both illumination arms (arrowheads). (**c**) X-OpenSPIM with two-sided illumination and two-sided detection. (**d**) T-OpenSPIM farm of four parallel setups mounted in a rack with a single laser serving the left and right illumination arm of each unit. The racks can be moved individually for convenient sample chamber access.

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