**Supplementary Videos**

**Supplementary Video 1:** *Interactive walk through cleared sample.* The video visualizes the quality of the reconstruction and the power of interactive visualization on a reconstructed adult mouse BsxH2BGFP coronal slice encompassing the hypothalamus. The movie was recorded on a MacBook Pro in the BigStitcher after stitching and ICP refinement. It highlights that very large datasets can be handled freely in three dimensions independently of the size of the data.

**Supplementary Video 2:** *Interactive link verification.* The movie shows a screencast of how individual links can be optionally interactively inspected before global optimization. This ensures that the user is able to reconstruct even very complex datasets.

**Supplementary Video 3:** *Quality of multi-view registration on the expanded sample.* Slicing of an image stack of the 7.5-times expanded *Drosophila* first instar larval nervous system. Green and magenta colors represent two orthogonal views, each consisting of multiple, stitched tiles (**Supplementary Fig. 17**). The stack is slices along the rotation axis of the lightsheet microscope, resulting in elongated, orthogonal point spread functions (PSFs). Crossing of the PSFs highlights the quality of the multi-view registration of the multi-tile views.

**Supplementary Video 4:** *3d maximum intensity projection of the expanded sample.* Three-dimensional maximum intensity projection of the reconstructed, the 7.5-times expanded *Drosophila* first instar larval nervous system. It highlights the isotropic resolution of the final image.

**Supplementary Video 5:** *3d maximum intensity projection of the reconstructed C. elegans dauer.* Three-dimensional maximum intensity projection of the multi-view, multi-tile deconvolved *C. elegans* dauer expressing tagRFP in all neuron nuclei, co-stained with DAPI.Posterior neurons show a higher expression level, thus anterior neurons are shown less bright to avoid saturation (compare with **Fig. 2f**)

**Supplementary Video 6:** *Low resolution overview of reconstructed mouse brain.*Video of a stack of an entire BsxH2B-GFP/+ adult mouse brain at low resolution that was reconstructed from a 2.24TB multi-tile, multi-view acquisition as shown in **Fig. 2d**.

**Supplementary Video 7:** *Simulations of light propagation in tissue.*

**Supplementary Video 8:** *Quality measurement by relative Fourier Ring Correlation (single image tile).*

**Supplementary Video 9:** *Quality measurement by relative Fourier Ring Correlation (large sample).*