**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.* Visualization of the simulation process used for **Fig 2b-e**. Left side shows the maximum intensity projection of both lightsheets as they pass through the sample (see **Fig. 2b,c**). The middle shows the same view, but only light that actually excites the sample is shown. On the right the corresponding acquisition (orthogonal to left, middle) by the virtual camera is shown.

**Supplementary Video 8:** *Quality measurement by relative Fourier Ring Correlation (single image tile).* Overlays the results of the relative Fourier Ring Correlation computed in 128x128 pixel blocks and a z-spacing of 10 (magenta) with the image tile of a cleared sample (green). Compare with **Suppl. Fig. 7**.

**Supplementary Video 9:** *Quality measurement by relative Fourier Ring Correlation (large sample).*Visualization of the relative Fourier Ring Correlation on a multi-tile, dual-illumination, multi-view dataset (compare with **Suppl. Fig. 8** and **Fig. 3d**) that illustrates that the reconstruction of such a dataset is able to entirely cover a large sample such as this adult mouse brain with good quality. The left side shows the rFRC of a single illumination and single view of the mouse brain in *xy* orientation, the middle depicts all both illuminations and views overlaid in *xy* orientation, and the right side shows the overlay in *xz* orientation. The bottom right drawing explains the color code in *xz* orientation.