Dear Editor,

Attached please find our manuscript *“BigStitcher: Efficient reconstruction of large multi-tile and multi-view image datasets"* that we submit as a Brief Communication.

With the recent advent of exciting new sample preparation methods such as clearing and expansion microscopy, it is now possible to image for example an entire mouse brains at single cell resolution or an entire Drosophila larval nervous system with super-resolution using standard lightsheet microscopes. These new techniques therefore allow to image with very high resolution while preserving the entire global context. The whole field is a phase of immense growth with enormous potential for cell and developmental biology as well as medically relevant studies based on the mouse model. However, after acquisition, scientists are currently left with terabyte-sized datasets consisting of hundreds of large three-dimensional images that are not aligned, suffer from optical disturbances and often cannot even be opened as a whole. Concepts, algorithms and efficient implementations to handle and reconstruct such data are missing. Therefore, labs typically repurpose old solutions to work on maximum intensity projections rather than the actual image data, without the possibility for proper reconstruction and analysis. Thus, working with these gigantic datasets is for most labs rather a theoretical concept than a real possibility. The BigStitcher is designed to fill that essential gap that to makes these powerful clearing and expansion microscopy datasets accessible for biological and medical research. It enables interactive handling and reconstruction of multi-terabyte datasets by building on top of state-of-the-art software frameworks BigDataViewer and ImgLib2. We developed efficient and precise algorithms covering the entire reconstruction process. This includes automatic selection of the best illumination direction, 100-fold faster multi-tile stitching, correction of chromatic aberrations, globally optimal placement of images, multi-view registration effectively doubling the sample size that can be imaged, real-time image fusion, deconvolution and easy data import. Importantly, users have the option to verify and interact with the reconstruction at any point, guiding potentially complicated image alignment tasks. We illustrate the performance of our approach on multiple examples, benchmarks, and tests. We believe that our work represents a substantial advance in the field of large-scale image reconstruction. It is embedded into the Fiji ecosystem where it easily interacts with all other available image analysis tools, thereby shaping a unique open access platform for light sheet microscopy. With the rapid growth of clearing and expansion microscopy in biological and biomedical research labs, our work will allow scientists to focus on applying this technology to solve complex biological problems.

We are submitting the main manuscript text (1499 words), two Figures, one table 17 Supplementary Figures, 5 Supplementary Videos and Supplementary Information

We think that suitable reviewers with expertise both in lightsheet microscopy and sample preparation are Scott Fraser (USC), Raju Tomer (Columbia), Emmanuel Reynaud (UC Dublin) and Rainer Heintzmann (Jena).

Due to conflict of interest we would like to exclude Ernst Stelzer (Goethe University Frankfurt) and Olaf Ronneberger (Google/Freiburg).

We are looking forward to your comments.

Stephan Preibisch