Dear Dr. Stack,

As discussed, we are submitting our manuscript *“BigStitcher: Reconstructing high-resolution image datasets of cleared and expanded samples"* as a Brief Communication, which is already available as bioRxiv submission: <https://www.biorxiv.org/content/early/2018/06/10/343954>

Biology constantly pushes sample-preparation and microscopy technology to generate ever larger image volumes that are able to capture images with very high resolution while preserving the entire global context. Prominent examples are clearing and expansion microscopy, which allow to image for example an entire mouse brains at single cell resolution or an entire Drosophila larval nervous system with super-resolution, and modern light-sheet microscopy is capable of scanning these volumes rapidly.

However, after acquisition, scientists are currently left with gigantic datasets that consist 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 or inadequate solutions that work on maximum intensity projections or only partially reconstruct the data. BigStitcher is designed to fill that essential gap to make these powerful clearing and expansion microscopy datasets accessible for biological and medical research. We developed a plethora of new and optimized algorithms to enable interactive handling and efficient reconstruction of multi-terabyte datasets, which are implemented on top of state-of-the-art software frameworks BigDataViewer and ImgLib2. We illustrate the performance of our approach on multiple examples, benchmarks, and tests.

Importantly, BigStitcher is a very versatile tool that can be applied to any established microscopy technologies such as two- and three-dimensional widefield and confocal microscopy, as well as any lightsheet microscopy acquisition that can contain multiple tiles, angles, channels, and illuminations. This also allows to quickly adjust BigStitcher to new developments. It is further 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.

BigStitcher is already available as Fiji plugin as is used by many labs, which is highlighted by the overwhelming response to the release of the pre-print on bioRxiv. It reached an attention scoring higher than 99% of its contemporaries on bioRxiv of similar age, and it is in the 98th percentile of all research outputs ever tracked by Altmetric.

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 the Giulio Iannello (University Rome) and Hanchuan Peng (Allen Institute).

We are looking forward to your comments.

Stephan Preibisch