**Nature Methods**

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Dear Dr. Strack,

Following your kind invitation via Twitter, we are submitting our manuscript *“BigStitcher: Reconstructing high-resolution image datasets of cleared and expanded samples"* as a Brief Communication to Nature Methods.

Biology generates ever larger image volumes that are able to capture details with high resolution while preserving the entire global context of the sample. Prominent examples are **clearing and expansion microscopy**, which enable to image for example entire mouse brains at single cell resolution or an entire Drosophila larval nervous system with super-resolution. However, after rapid data acquisition using **light-sheet microscopy**, scientists are currently left with gigantic datasets that consist of hundreds of large three-dimensional images from one sample that are not aligned, suffer from optical disturbances and often cannot even be opened as a whole. **Coherent 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 can only partially reconstruct the data. BigStitcher fills this essential gap and **thereby makes powerful clearing and expansion microscopy datasets accessible for biological and medical research**. We developed a plethora of new and optimized algorithms that enable interactive handling and efficient reconstruction of multi-terabyte datasets. We illustrate the performance using multiple examples, benchmarks, and tests. Importantly, **BigStitcher is a versatile tool** that can also be applied to established microscopy technologies such as two- and three-dimensional widefield or confocal microscopy, which also allows to quickly adjust BigStitcher to new technological developments. Furthermore, it is **embedded into the Fiji ecosystem** where it interacts with all other image analysis tools, thereby shaping a **unique open access platform** for light-sheet microscopy.

With the rapid growth of clearing and expansion microscopy in biology and biomedical research labs, our work finally **enables scientists to focus on applying this technology** to solve complex biological problems. BigStitcher is already available as a Fiji plugin and used by many labs, which is highlighted by the overwhelming response to the bioRxiv pre-print (<https://www.biorxiv.org/content/early/2018/06/10/343954>). It reached an attention scoring higher than 99% of its contemporaries on bioRxiv of similar age, and it is in the 99th percentile of all research outputs ever tracked by Altmetric.

We are submitting the main manuscript text (1500 words), two Figures, one table, 17 Supplementary Figures, 5 Supplementary Videos and Supplementary Information as a brief communication.

We think that suitable reviewers with expertise both in lightsheet microscopy and sample preparation or software are Scott Fraser (USC), Raju Tomer (Columbia) and Jason Swedlow (Dundee University). Due to conflicts of interest we ask to exclude Giulio Iannello (University Rome) and Hanchuan Peng (Allen Institute).

We hope that you and the reviewers will find this manuscript suitable for publication in Nature Methods.

We are looking forward to your response.

Sincerely yours,

Stephan Preibisch