

BigStitcher: Reconstructing high-resolution image datasets of cleared and expanded samples

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Continuous advancements in microscopy and sample preparation methods such as clearing or expansion allow for the investigation of ever larger samples at high resolution. This entails increasingly large datasets that may consist of hundreds of images of one sample that are not aligned, suffer from optical disturbances and often cannot even be opened as a whole, which can pose a serious bottleneck to scientific inquiries. With terabyte-sized datasets becoming more and more common, development of software tools that make handling and analysis of large and complex image data available to the broader scientific community is an urgent issue.

To allow efficient handling and reconstruction of large multi-tile and multi-view image data, we developed the BigStitcher software. It enables import from most file formats, interactive handling, fast and precise alignment, as well as deconvolution and real-time fusion of large image datasets. We additionally support the alignment of multi-tile acquisitions taken from different orientations, effectively doubling the size of objects that can be imaged. We also enable the correction of a variety of optical distortions, e.g. via automatic illumination selection, flat-field correction and interest-point-based correction of chromatic aberrations.

We implemented BigStitcher using the state-of-the-art frameworks ImgLib2 and BigDataViewer. By combining multi-resolution data representation and sub-pixel accurate registration algorithms, even very large datasets can be reconstructed on conventional, off-the-shelf hardware. In an effort to make large sample reconstruction available as a routine task, we provide a user-friendly graphical user interface (GUI) to manually guide the alignment and interactively display the intermediate results using BigDataViewer. BigStitcher is open-source and provided as a Fiji-plugin, making it a powerful, scalable tool for automated processing of very large image datasets.

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