The pipeline presented here is comparable in functionality and performance particularly to the pipeline developed by Fernando Amat at the Janelia Research Campus (Amat et al. 2015). While the Amat pipeline is faster owing to the full reliance on GPU processing, it is limited to processing of datasets generated using the SimView microscope (or similar) where precisely aligned orthogonal views are acquired. The Amat pipeline relies on Matlab R2013b and the following toolboxes: Curve Fitting, Image Processing, Statistics, Optimization, Signal Processing, Parallel Computing. This limits its deployment on a large computer cluster, which is nevertheless possible and has been implemented. The Amat pipeline replaces a previous version used in Tomer et al. 2012.

Additionally, several groups implemented dedicated fusion algorithms. Rubio Guivernau et al. 2012 introduces wavelet fusion running in MATLAB. It is not integrated into any SPIM processing pipeline and the source code cannot be found. Schmid and Huisken 2015 implemented a fast 2D approximation of 3D multi-view deconvolution in Fiji. In the near future it will be integrated into Multiview Reconstruction Fiji plugins and thus will be fully available to our cluster-processing pipeline.

Finally, Zeiss Microscopy GmbH implemented Preibisch et al. 2010 bead-based registration in their proprietary software along with custom fusion and multi-view deconvolution algorithms. Since the details of these algorithms are not publicly accessibly, we do not compare our approach to them in the context of an academic paper. They are however fully functional for processing of Lightsheet Z.1 data.

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|  | **used algorithms** | **available software** | **parallelization** | **license** | **analyzed datasets** | **processing times** | **advantages** | **disadvantages** |
| Preibisch et al. 2010, 2014, upublished, Schmied et al. 2015 | Bead-based registration, Sample feature registration, content based multiview fusion, Richardson- Lucy 3D Multiview deconvolution | Fiji plugins | Yes | GNU GPL version 2 | Drosophila Zebrafish PlatynereisParhyale | See manuscript and Supplementary Tables 1 and 2 | Versatile, graphical user interface (GUI), provides compression, fast visualization via HDF5 and BigDataViewer, automated parallel processing, deals with commercial data formats, GPU acceleration | Relies on beads embedded in the rigid mounting medium or the ability to segment blobs in the sample (via the Difference of Gaussian detector). |
| Fernando Amat et al. 2015 | Content-based registration and fusion | Matlab scripts | Yes (requires multiple MATLAB licenses) | open source undeclared license,  MATLAB + toolboxes  licenses | Drosophila  Zebrafish  Parhyale  Platynereis | processes image data at a rate faster than the data acquisition rate of the light-sheet microscope, i.e. in the order of seconds. | Independent of embedding and beads, provides compression, uses web based CATMAID for visualization, fast GPU accelerated processing | Works specifically with orthogonal views of the SimView microscope, cannot register arbitratry views, i.e. Lightsheet Z1 data and similar |
| Tomer et al. 2012 | Works specifically with orthogonal views of SimView, its an older version of the pipeline described in Amat et al. 2015 | | | | | | | |
| Rubio Guivernau et al. 2012 | Content based registration,  Wavelet-based image fusion | Matlab scripts | No | Claims open source however no source code is available,  MATLAB license | Sea urchin  Zebrafish | Registration: 24 minutes compared to 1.8 minutes using Preibisch et al. 2010 method  Fusion:  64 minutes compared to 1.5 minutes using Preibisch et al. 2010 method | Unique fusion method not available elsewhere | Lack of integration with other aspects of SPIM data processing.  No source code. |
| Schmid and Huisken 2015 | Richardson- Lucy 2D multiview decovnolution | Fiji Plugin | Currently No, but can be implemented | GNU GPL version 3 | Zebrafish | 25 to 75 times faster compared to Preibisch et al. 2014 | Very fast plane-wise deconvolution, with GPU acceleration allows real time processing | Based on 2D approximation of 3D PSF, works only on systems where the rotation axis lies within the image plane |
| ZEN Black | Bead-based registration, content based multiview fusion, Richardson- Lucy 3D multiview deconvolution | Proprietary commercial platform | No | hardware or software license key | Drosophila, Zebrafish, Parhyale  Platyneries | Commercial implementation of registration algorithm in Preibisch et al. 2010, custom multiview deconvolution. We do not benchmark against closed source commercial solutions | Applicable to Lightsheet Z.1 data, fully integrated | Closed. |