

Quantitative High-Speed Imaging of Entire Developing Embryos with Simultaneous Multi-View Light Sheet Microscopy

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Supplementary Software

SiMView module for high-throughput multi-view image registration

This archive contains our custom software tools for registration and fusion of simultaneous multi-view (SiMView) image data. Two different versions of the code are included (sub-folders “1p-SiMView” and “2p-SiMView”), for processing one-photon SiMView data sets (asynchronous bi-directional illumination) and two-photon SiMView (synchronous bi-directional illumination) data sets, respectively.

All algorithms were developed and tested in the Matlab computer language (version R2011b, The Mathworks). In addition to the Matlab core installation, the Image Processing Toolbox is required to execute the programs. Multi-threaded execution through the job management scripts listed below furthermore requires the Parallel Computing Toolbox. Software compatibility was only verified for PCs with a Windows 7 64-bit operating system.

Software modules:

- **multiFuse.m**

Note: This program performs four-view fusion of time-lapse or single-run data sets recorded with bi-directional illumination and two cameras. The workflow of this module is described in **Online Methods** and requires the auxiliary MEX file *ba_interp2.mexw64* from the MathWorks File Exchange repository.

- **runMultiFuse.m**

Note: This program is a job management script for multi-threaded execution of *multiFuse.m* for large data sets (allowing up to 12 parallel threads).

- **timeFuse.m**

Note: This program performs four-view fusion of time-lapse data sets recorded with bi-directional illumination and two cameras, for which pre-sampled transformation parameters and transformation mask data are already available from a preceding *multiFuse.m* run. Prior to *timeFuse.m* execution, the *multiFuse.m* transformation data has to be post-processed with *analyzeParameters.m* in order to obtain an interpolated look-up-table for the complete time-lapse recording. The workflow of this module is described in **Online Methods** and requires the auxiliary MEX file *ba_interp2.mexw64* from the MathWorks File Exchange repository.

- **runTimeFuse.m**

Note: This program is a job management script for multi-threaded execution of *timeFuse.m* for large data sets (allowing up to 12 parallel threads).

Note: The required amount of memory depends substantially on the size of the data sets that are processed. If the images are stored as *uint16*, each thread requires an amount of memory equal to ~5 times (for one-photon recordings) or ~3 times (for two-photon recordings) the size of the final fused image stack. For example, processing a typical *Drosophila* one-photon SiMView recording from our study requires approximately 4 GB of memory per parallel thread.

Input data structure:

Raw input data should be provided as single 2D images in TIFF or J2C format, using the following naming scheme and directory structure:

[Root]/SPM00/TM $tttt$ /ANG000/SPC00_TM $tttt$ _ANG000_CM x _CHN yy _PH0_PLN*.*

$tttt$ = time point, x = camera index, yy = channel index (one per light sheet in 1p-SiMView)