

# Whole-brain functional imaging at cellular resolution using light-sheet microscopy

Misha B. Ahrens<sup>1,\*</sup>, Michael B. Orger<sup>2</sup>, Drew N. Robson<sup>3</sup>, Jennifer M. Li<sup>3</sup> and Philipp J. Keller<sup>1,\*</sup>

<sup>1</sup>*Howard Hughes Medical Institute, Janelia Farm Research Campus*

<sup>2</sup>*Champalimaud Neuroscience Programme, Champalimaud Centre for the Unknown*

<sup>3</sup>*Department of Molecular and Cellular Biology, Harvard University*

\*Correspondence: [ahrensm@janelia.hhmi.org](mailto:ahrensm@janelia.hhmi.org) (M.B.A.), [kellerp@janelia.hhmi.org](mailto:kellerp@janelia.hhmi.org) (P.J.K.)

## *Supplementary Software*

Image processing and analysis of whole-brain functional recordings

This software package contains custom tools for registration,  $\Delta F/F$  calculation and analysis of high-speed light-sheet microscopy recordings of zebrafish larval brains expressing a genetically-encoded calcium indicator. All algorithms were developed using the Matlab computer language (version R2012b, The Mathworks). In addition to the Matlab core installation, the Parallel Computing Toolbox is recommended for multi-threaded execution of the code. Software compatibility was only verified for PCs with a Windows 7 64-bit operating system.

Software modules (listed in the order of their application):

- **runRegistration.m**

*Note:* This program performs non-linear spatial registration of a raw whole-brain time-lapse microscopy data set, using a pre-defined reference time interval. The script enables multi-threaded execution of this task, allowing up to 12 parallel threads. Execution requires the auxiliary functions *registerImages.m*, *registerStacks.m* and *warpImages.m*.

- **createReference.m**

*Note:* This program operates on the registered time-lapse microscopy data set and generates the volumetric reference stacks required for  $\Delta F/F$  calculation. The reference stacks are

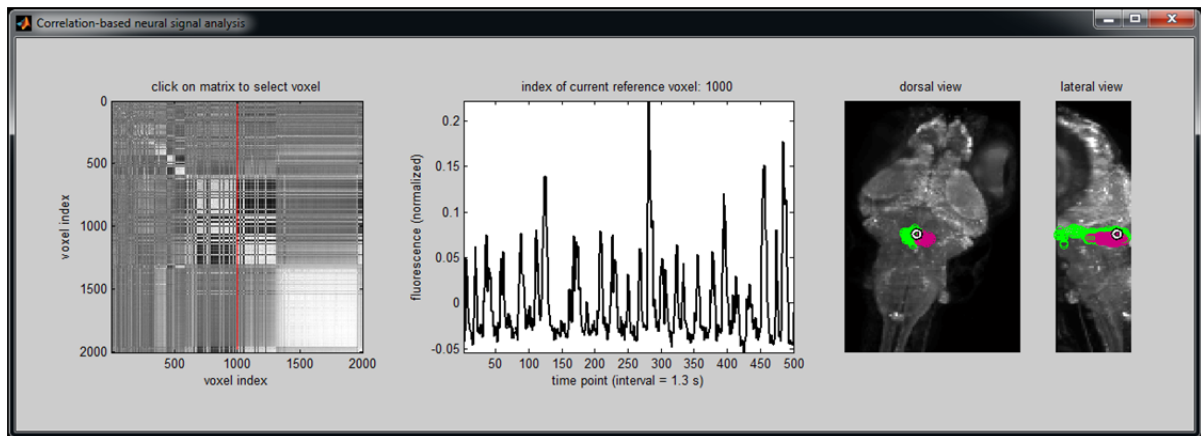
obtained by using a spatial median filter and temporal percentile computation based on a sliding-window approach. The script enables multi-threaded execution of this task, allowing up to 12 parallel threads.

- **extractSignal.m**

*Note:* This program operates on the registered time-lapse microscopy data set and performs the volumetric  $\Delta F/F$  calculation, using the reference stacks generated by *createReference.m*. The script enables multi-threaded execution of this task, allowing up to 12 parallel threads.

- **selectTrace.m**

*Note:* This program operates on the fluorescence traces in super-voxel representation, which are extracted from the  $\Delta F/F$  stacks generated by *extractSignal.m*, and performs fluorescence trace ranking based on the power spectrum to generate a correlation matrix of the best hits for a pre-defined frequency range. Using the graphical user interface of *selectTrace.m*, individual voxels can be selected from this correlation matrix to determine a functional anatomy preview of highly correlated or anti-correlated fluorescence traces.



Screenshot of the graphical user interface of *selectTrace.m*

- **makeReferenceMontage.m**

*Note:* This program generates a scaled multi-slice visualization of the reference stacks computed by *createReference.m*, which is used together with the output of *makeMontage.m* to inspect and present the functional activity information captured in the whole-brain time-lapse recordings (see **Supplementary Video 3**). The script enables multi-threaded execution of this task, allowing up to 12 parallel threads.

- **makeMontage.m**

*Note:* This program generates a scaled multi-slice visualization of the  $\Delta F/F$  stacks computed by *extractSignal.m*, which is used together with the output of *makeReferenceMontage.m* to inspect and present the functional activity information captured in the whole-brain time-lapse recordings (see **Supplementary Video 3**). The script enables multi-threaded execution of this task, allowing up to 12 parallel threads.

- **rotateReferenceMIP.m**

*Note:* This program generates a projection-based visualization of the reference stacks computed by *createReference.m*, which is used together with the output of *rotateMIP.m* to inspect and present the functional activity information captured in the whole-brain time-lapse recordings (see **Supplementary Video 4**). The script enables multi-threaded execution of this task, allowing up to 12 parallel threads.

- **rotateMIP.m**

*Note:* This program generates a projection-based visualization of the  $\Delta F/F$  stacks computed by *extractSignal.m*, which is used together with the output of *rotateReferenceMIP.m* to inspect and present the functional activity information captured in the whole-brain time-lapse recordings (see **Supplementary Video 4**). The script enables multi-threaded execution of this task, allowing up to 12 parallel threads.

### Input data structure:

The first computational module of the pipeline (*runRegistration.m*) expects raw input data to be provided as 3D image stacks in TIF format, using the following naming scheme and directory structure (*ttttt* = time point, *x* = camera index):

[Root]/SPM00/TMttttt/ANG000/SPC00\_TMttttt\_ANG000\_CMx\_CHN00\_PH0.tif