An automated workflow for parallel processing of large multiview SPIM recordings

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Abstract:

Multiview light sheet fluorescence microscopy (LSFM) allows to image developing organisms in 3D at unprecedented temporal resolution over long periods of time. The resulting massive amounts of raw image data requires extensive processing interactively via dedicated graphical user interface (GUI) applications. The consecutive processing steps can be easily automated and the individual time points can be processed independently, which lends itself to trivial parallelization on a high performance computing (HPC) cluster. Here we introduce an automated workflow for processing large multiview, multi-channel, multi-illumination time-lapse LSFM data on a single workstation or in parallel on a HPC cluster. The pipeline relies on snakemake to resolve dependencies among consecutive processing steps and can be easily adapted to any cluster environment for processing LSFM data in a fraction of the time required to collect it.

Availability:

The code is distributed free and open source under the MIT license http://opensource.org/licenses/MIT. The source code can be downloaded from github: https://github.com/mpicbg-scicomp/snake-make-workflows. Documentation can be found here: http://fiji.sc/Automated_workflow_for_parallel_Multi-view_Reconstruction.

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1 Introduction

The duration and temporal resolution of 3D fluorescent imaging of living biological specimen is limited by the amount of laser light exposure the sample can survive. LSFM alleviates this by illuminating only the imaged plane thus reducing photo damage dramatically. Additionally LSFM achieves fast acquisition rates due to sensitive wide-field detectors and in Selective Plane Illumination Microscopy (SPIM), sample rotation enables complete coverage of large, non-transparent specimen. Taken together, LSFM allows imaging of developing organisms in toto at single cell resolution with unprecedented temporal resolution over long periods of time (Huisken et al., 2004; Keller et al., 2008).
This powerful technology produces massive, terabyte size datasets that need computationally expensive and time-consuming processing before analysis. Existing software solutions implemented in Fiji (Preibisch et al., 2010, 2014; Preibisch, unpublished; Schmied et al., 2014) or in ZEISS ZEN black are performing chained processing steps on a single computer and require user inputs via a GUI. As the spatial and temporal resolution of the light sheet data increase, such approaches become inconvenient since processing can take days.

In controlled experiments, SPIM image processing is robust enough to be automated and key steps are independent from time point to time point. HPC is inherently designed for such time consuming and embarrassingly parallel tasks that require no user interaction. Therefore, we developed an automated workflow with minimum user interaction that is easily scalable to multiple datasets or time points on a cluster. In combination with the appropriate computing resources it enables for the first time processing of SPIM data that is faster than the total acquisition time required for collecting the raw images.

2 Processing workflow

The Fiji SPIM processing pipeline uses Hierarchical Data Format (HDF5) as data container for the originally generated TIFF or CZI files by custom made (Pitrone et al., 2013) or commercial SPIM microscopes (Fig. 1A,B). Following format conversion, multiview registration aligns the different acquisition angles (views) within each time point (Fig. 1C), and subsequent time-lapse registration stabilizes the recording over time (Preibisch et al., 2010) (Fig. 1D). Fusion combines the registered views of one time point into a single volume by averaging or multiview deconvolution (Preibisch et al., 2010, 2014) (Fig. 1E,F). The result is a set of HDF5 files containing registered and fused multiview SPIM data that can be examined locally or remotely using the BigDataViewer (Pietzsch et al., 2015).

All steps are implemented as plugins (Preibisch et al., 2010, 2014; Preibisch, unpublished; Pietzsch et al., 2015), in the open-source platform Fiji (Schindelin et al., 2010). We use these plugins by executing them from the command line as Fiji beanshell scripts (Suppl. Fig. 1). To overcome the legacy dependency of Fiji on the GUI we encapsulate it in a virtual framebuffer (xvfb) that simulates a monitor in the headless cluster environment (Suppl. Fig. 1).

To map and dispatch the workflow logic to a single workstation or on a HPC cluster, we use the automated workflow engine snakemake (Köster and Rahmann, 2012). The workflow is defined using a Snakefile containing the name, input and output file names of each of the processing steps and python code calling the beanshell scripts (Suppl. Fig. 1). Upon invocation, the snakemake rule engine resolves the dependencies between individual processing steps based on the input files required and the output files produced during the workflow. It also creates the command that fits the input/output rule description and the template command as defined in the Snakefile. Most importantly, if single tasks on individual files are discovered to be
independent, they are invoked in parallel (Suppl. Fig. 2). Each instance of \textit{snakemake} for one dataset is independent and thus the workflow can be applied simultaneously to multiple dataset.

The required parameters for processing are collected by the user during GUI processing of an exemplary time point and entered into a \textit{.yaml} configuration file (Suppl. List 1). The workflow is executed by passing the \textit{.yaml} file to \textit{snakemake} on the command line (Suppl. Fig. 1). Importantly, from the user perspective the launching of the pipeline on a HPC cluster and on a local workstation appears identical and require a single command (Suppl. List 2). If the parameters are chosen correctly and the local or HPC resources are sufficient (Suppl. Table 1 and 2) no further action from the user is necessary.

\textit{Snakemake} supports multiple back ends to perform the command dispatch: local, cluster and \textit{Distributed Resource Management Application API (DRMAA)} (Köster and Rahmann, 2012). The local back end creates a new sub-shell and calls the command(s) required. The cluster back end is a general interface to HPC batch systems based on string substitution. \textit{DRMAA} specifies a system library that interfaces all common batch systems based on a generalized task model, thus multiple batch systems are supported through one interface.

### 3 Results

| Step                          | 1 TP local | 90 TPs local | 90 TPs cluster |
|-------------------------------|------------|--------------|----------------|
| Resave to hdf5                | 3          | 262          | 15             |
| Detection and registration    | 2.5        | 221          | 15             |
| Average fusion                | 7          | 661          | 47             |
| Deconvolution (GPU)           | 21         | 1874         | 740            |
| Resave output                 | 3          | 286          | 7              |
| Total with average fusion     | 23 h 56 min| 1 h 30 min   |                |
| Total with deconvolution (GPU)| 44 h 08 min| 13 h 10 min  |                |

\textbf{Table 1:} Processing time comparison. Time (minutes) for key processing steps that are parallelized on a cluster. Total processing time including non parallel processing steps on the example dataset using either average fusion or deconvolution.

We compared the performance of the pipeline on a 175 GB, single channel SPIM recording of a \textit{Drosophila} embryo consisting of 90 time points and 5 views, processed either on a single computer or on a HPC cluster (Table 1). The processing using average fusion takes almost precisely one day on a single powerful computer. In contrast, using the full cluster resource the dataset can be processed in 1 h 31 min, which represents a 16-fold speedup in processing. Since the time-lapse covers 23 hours of \textit{Drosophila} embryonic development the processing becomes real time with respect to the acquisition. Using deconvolution on a cluster with only 4 GPUs (Suppl.
Figure 1: Automated workflow for Multiview processing. Workflow for SPIM image processing (A-E) using parallelization (B, C and E). Shown on the right yz–slices in the BigDataViewer of a Drosophila embryo expressing histone H2Av-mRFPruby raw (A) registered (C) and deconvolved (E). Results of deconvolution with xy–, xz– and xz–slices through the fused volume of the same embryo (F). Scale bars represent 50 µm.
Table 1) still brings a more than 3-fold speed up (Table 1). A dataset of 2.2 TB in size with 715 time points (Schmied et al., 2014) would take an estimated week to process on a single computer. Using this method the processing is reduced to only 15 h with typical cluster workload from other users.

4 Conclusion and Outlook

The biologist’s goal is to analyse, for instance, cellular behaviour using time-lapse SPIM recordings. The steps between data acquisition and analysis are of rather technical interest. Our pipeline leverages HPC to reduce the notoriously difficult and time-consuming SPIM data processing to a single autonomous command. Future improvements of the workflow will provide greater accessibility to novice users by using the UNICORE GUI framework (Almond and Snelling, 1999). Ultimately, we aim for a completely unsupervised automated processing similar to grid computing practiced in fields facing similar big data challenges such as particle physics and molecular simulation (Bird, 2011; Gesing et al., 2012)

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Supplementary data

Suppl. Fig. 1: Conceptual architecture for processing a multiview dataset. Time-lapse recording of Histone-YFP expression during *Drosophila melanogaster* embryogenesis with 5 views. The parameters are determined prior to the automated processing and stored in a .yaml configuration file (A). These parameters are passed to a Snakefile, which contains the logic of the workflow (B). Upon execution of snakemake and presence of the input files (e.g. images) snakemake dispatches the jobs which call Fiji beanshell scripts to carry out the processing using Fiji (C). The output generated by the workflow triggers the next batch of jobs once the input rules of the next step are fulfilled. Additionally, the processing writes log files and the cluster error and output files (D).
Suppl. Fig. 2: Dependency graph of the snakemake workflow. Example of a directed acyclic graph (DAG) for processing a dataset with 5 time points. Snakemake resolves the file dependencies (arrows) between the different processing steps (boxes, each step with different colour). Jobs are dispatched when the input rule of the first processing step is fulfilled (A). The next batch is sent when all outputs of the processing step are created and the input rule of the next step is fulfilled (B-E). Independent tasks in the same processing step are dispatched in parallel, i.e. parallel processing of time points (B, E).
1. Processing switches
2. Define dataset
   2.1. General Settings
       hdf5_xml_filename: "dataset",
       ntimepoints: 90,
       angles: "0,72,144,216,288",
       channels: "green",
       illumination: "0",
   2.2. Settings for .czi files
       first_czi: "name.czi",
   2.3. Settings for .tiff datasets
       image_file_pattern: "img_TL(((t))_Angle({a}).tiff",
       multiple_channels: "NO (one channel)"
3. Detection and registration
   3.1 Channel settings
       reg_process_channel: "All channels",
       source_channel: "red",
       target_channel: "green",
       reg_interest_points_channel: "beads",
       type_of_detection: "Difference-of-Gaussian"
3.2 Settings for Difference-of-Mean
3.3 Settings for Difference-of-Gaussian
       sigma: "1.3",
       threshold_gaussian: "0.025"
4. Time-lapse registration
   reference_timepoint: "45",
5. Weighted-average fusion
   downsample: "1",
   minimal_x: "274",
   minimal_y: "17",
   minimal_z: "423",
   maximal_x: "1055",
   maximal_y: "1928",
   maximal_z: "480"
6. Multiview deconvolution
   6.1. External transformation
       external_trafo_switch: "_trafo",
       matrix_transform: "0.5, 0.0, 0.0, 0.0, 0.0, 0.5, 0.0, 0.0, 0.0, 0.0, 0.0, 0.0, 0.5, 0.0"
   6.2. Deconvolution settings
       iterations: "15",
       minimal_x_deco: "137",
       minimal_y_deco: "58",
       minimal_z_deco: "211",
       maximal_x_deco: "527",
       maximal_y_deco: "964",
       maximal_z_deco: "240"
   detections_to_extract_psf_for_channel: "beads"
7. Resave output
8. Software directories
9. Fiji resource settings
10. Advanced settings

Suppl. List 1. Parameter List for processing. List of the key input parameters in the .yaml file for multiview, multi-channel multi-illumination side processing of time-lapse SPIM recordings. The parameters are sorted to match the logic of the processing workflow: define and resave to hdf5 (2) > detect and register (3) > time lapse registration (4) > fuse (5 + 6) and resave output (7). The key parameters (highlighted in red) are recorded during manual processing of the reference time point in the GUI.
Local back end:
/path/to/snake_make/snake_make -j 1 -d /path/to/data/

To specify the configuration script for the queuing system:
--cluster-config ./cluster.json

For DRMAA back end add:
--drmaa " -q {cluster.lsf_q} {cluster.lsf_extra}"

For LSF backend add:
--cluster "bsub -q {cluster.lsf_q} {cluster.lsf_extra}"

Flag for number of jobs run in parallel:
-j <number of jobs>

Flag for specifying data location and config file:
-d /path/to/data/

Flag for dry run of snake_make:
-n

Force the execution of a rule:
-R <name of rule>

To save error and output files of cluster add:
--drmaa " -q {cluster.lsf_q} {cluster.lsf_extra} -o test.out -e test.err"
--cluster "bsub -q {cluster.lsf_q} {cluster.lsf_extra} -o test.out -e test.err"

Suppl. List 2. List of commands for the snake_make workflow.
### A Workstation

| Component          | Specification                                                                 |
|--------------------|-------------------------------------------------------------------------------|
| Processor          | 2x hexa-core Intel Xeon E5-2630 @ 2.30 GHz                                   |
| Memory             | 128 GB (16x8GB) @ 1600 MHz, DDR3 ECC RDIMM                                    |
| GPU                | 2x NVIDIA Quadro 4000, 2 GB of Memory, 256 CUDA cores                         |
| Hard Drive         | 4x 4 TB SATA disks in RAID 5 configuration                                     |

### B MPI-CBG Cluster "Madmax"

| Component          | Specification                                                                 |
|--------------------|-------------------------------------------------------------------------------|
| Processor          | 44x Nodes with 2x hexa-core Intel Xeon E5-2640 @ 2.50 GHz                     |
| Total No. of cores | 528                                                                            |
| Memory per node    | 128 GB (16x8GB) @ 1333 MHz                                                    |
| GPU                | 4x nodes with NVIDIA Tesla M2090 Fermi Generation @ 1.3GHz, 6GB GDDR5 Memory @ 1.85GHz, 512 CUDA cores |

### Lustre storage server

| Component          | Specification                                                                 |
|--------------------|-------------------------------------------------------------------------------|
| Storage            | Fully redundant Lustre volume with 200 TB of usable space                     |
| Storage architecture| 2 metadata servers in active/passive configuration with a shared disk enclosure and 4 object storage servers with 3 disk enclosures delivering 2.5 GB/s each and 10 GB/s aggregated |
| Interconnect       | InfiniBand QDR 40Gbps fully non-blocking fat tree topology                    |

**Suppl. Table 1. Hardware used for processing.** Parameters of the computer hardware used for the automated processing. Standalone desktop machine (A) and HPC cluster (B).
| Job name                  | Average memory (MB) | Average CPU time (sec) | #jobs |
|--------------------------|---------------------|------------------------|-------|
| Define dataset           | 2315                | 908                    | 1     |
| Define hdf5 dataset      | 2158                | 39                     | 1     |
| Resave to hdf5           | 2827                | 530                    | 90    |
| Detection and registration| 7189                | 1388                   | 90    |
| Merge xml                | 3                   | 43                     | 1     |
| Time lapse registration  | 2534                | 953                    | 1     |
| Average fusion           | 7761                | 3806                   | 90    |
| Deconvolution GPU        | 27171               | 7485                   | 90    |
| Define output            | 3                   | 23                     | 1     |
| Define hdf5 output       | 2                   | 32                     | 1     |
| Resave output to hdf5    | 4918                | 534                    | 90    |

**Suppl. Table 2. Cluster processing resource summary.** Average resources per job were determined on the 175 GB example dataset with 90 time points.