Contents

Chapter 1

Installation

ASTEC can run only on Linux system and was tested on:

- Ubuntu 14.04 64 bits

1.1 Requirements

In order to be able to compile the different source code you need to install several packages, mostly from the terminal application.

1.1.1 to run the different codes:

- python 2.7 or higher
  Installation:

  - Linux: Should be installed, refer with command line
    `python -V`
    to get the version or visit http://php.net

- and a C compiler
  Installation

  - Linux: Should be installed, refer with command line
    `dpkg --list | grep compiler`
    to get list of available C compiler version or install gcc with
    `sudo apt-get install gcc`

1.1.2 the install libraries are necessary:

- pip, an installer for python (https://pypi.org/project/pip/). Installation:

  - Linux: run command line
    `sudo apt-get install python-pip python-dev build-essential`
  - Others: see *Installation* from https://pypi.org/project/pip/

- cmake, a software to build source code (http://www.cmake.org)

  - Linux: run command line
    `sudo apt-get install cmake`
  - Others: see *Download* from https://cmake.org/

1.1.3 the following python libraries are necessary:

- numpy, scipy, matplotlib: different scientific packages for python (`http://www.numpy.org`, `http://www.scipy.org`, `http://matplotlib.org`).

  Installation

  - Linux: run command line
    `sudo apt-get install python-numpy python-scipy python-matplotlib ipython ipython-notebook python-pandas python-sympy python-nose`

- jupyter notebook: a open-source framework to share scientific code (`http://jupyter.org`)

  Installation:

  - Linux: run command line
    `sudo pip install jupyter`

- libhdf5-dev,cython,h5py a library to read/write hdf5 images (`https://www.hdfgroup.org/HDF5/`)

  Installation:

  - Linux: run the command lines for a global installation
    `sudo apt-get install libhdf5-dev`
    `sudo pip install cython`
    `sudo pip install h5py`
    or
    `pip install --user cython`
    `pip install --user h5py`

- pylibtiff a library to read/write tiff/tif images (`https://pypi.python.org/pypi/libtiff/`).

  Installation:

  - You can install the pylibtiff distributed with ASTEC
    `cd path/to/Package/ASTEC/CommunFunctions/libtiff-0.4.0/; sudo python setup.py install`
  - Alternatively:
    `sudo pip install libtiff`
    or
    `pip install --user libtiff`

1.1.4 the following libraries are necessary:

- `zlib`, a compression library (`http://www.zlib.net`)

  Installation (if not already installed)

  - Linux: run command line
    `sudo apt-get install zlib1g-dev`

- `libtiff`, `http://libtiff.org`

1.2 Installation

You need to clone the `morpheme-privat` project in your computer: run

`cd <morpheme-privat_parent_directory>`

`git clone git+ssh://<username>@scm.gforge.inria.fr/gitroot/morpheme-privat/morpheme-privat.git`

Please note that for this step, you may need to ask for an account on the inria gforge and for the access to the morpheme-privat project (for further information, contact `gregoire.malandain@inria.fr`)

Then you need to compile the dependencies LEMON, NIFTICLIB, TIFF in `<morpheme-privat_parent_directory>/e` by creating in each subrepository a build folder, going in it, and run:

`ccmake ..`

then configure, generate and quit (for NIFTICLIB, please take note that you should set the `CMAKE_C_FLAGS` to value '-fPIC' in the advanced mode ; you need therefore to press 't' to toogle advanced mode)

run `make -j<nb proc>` where `<nb proc>` is the number of processor require to compile the code Then go to `<morpheme-privat_parent_directory>/vt` directory and follow these instructions:

`mkdir build`

`cd build`

`ccmake ..`

Press 'c' to configure. At this step, set the links to the build folders of NIFTI, LEMON and TIFF. Press 'c' to re-configure. Press 'g' to generate and 'q' to quit.

Run `make -j<nb proc>` where `<nb proc>` is the number of processor require to compile the code

1.2.1 Previously

Since the vt library is compiled, you need to create the symbolic link of the folder `<morpheme-privat_parent_directory>/` to `ASTEC/CommunFunctions/cpp`. in a terminal:

`cd path/to/Package/ASTEC/CommunFunctions`

`ln -s <complete_path_of_vt_folder> cpp`

1.2.2 From version v2.0

Since the vt library is compiled, you need to create the symbolic link of the folder `<morpheme-privat_parent_directory>/` to `ASTEC/CommunFunctions/cpp`. in a terminal:

`cd path/to/Package/ASTEC/CommunFunctions`

`ln -s <complete_path_of_bin_folder> cpp`

1.3 Troubleshooting

Chapter 2

Tutorial

Before starting

It is advised to add to your `PATH` environment variable the paths to both the python and the C executable commands (the latter is important in case of non-standard installation). So, Astec commands can be launched without specifying the complete path to the command.

It can be done in a terminal (and will be valid only for this terminal)

```
$ export PATH=$PATH:/path/to/astec/src
$ export PATH=$PATH:/path/to/astec/src/ASTEC/CommunFunctions/cpp/vt/build/bin
```

or by modifying a setup file (e.g. `bashrc`, `.profile`, ...).

2.1   Tutorial data

The directory `/path/to/astec/tutorial/tuto-astec1/`, also denoted by `path/to/experiment/` or `<EXPERIMENT>`, contains the `RAWDATA/` and `parameters/` sub-directories and a `README` file

```
path/to/tuto-astec1/
├── RAWDATA/
├── README
└── parameters/
```

The `RAWDATA/` contains 21 time points (indexed from 0 to 20) of subsampled (for file size consideration) raw data from a 3D+t movie acquired by a MuViSPIM microscope.

```
RAWDATA/
├── LC/
│   ├── Stack0000/
│   │   ├── Time000000_00.mha.gz
│   │   ├── ...
│   │   └── Time000020_00.mha.gz
│   └── Stack0001/
│       ├── Time000000_00.mha.gz
│       ├── ...
│       └── Time000020_00.mha.gz
└── RC/
    └── Stack0000/
```

```
 1 PATH_EMBRYO = '.'
 2
 3 EN = '2019-Tutorial100'
 4
 5 begin = 0
 6 end = 20
 7
 8 acquisition_orientation = 'right'
 9 acquisition_mirrors = False
10 acquisition_resolution = (1., 1., 1.)
11
12 target_resolution = 1.0
```

Figure 2.1: Tutorial parameter file for the fusion step (lines are numbered).

```
    │   ├── Time000000_00.mha.gz
    │   ├── ...
    │   └── Time000020_00.mha.gz
    └── Stack0001/
        ├── Time000000_00.mha.gz
        ├── ...
        └── Time000020_00.mha.gz
```

where `LC/` and `LC/` stand respectively for the left and the right cameras.

2.2 Fusion

We assume that we are located in the directory `/path/to/astec/tutorial/tuto-astec1/`. Running the fusion is done with

`$ 1-fuse.py -p parameters/1-fuse-tutorial-parameters.py`

`1-fuse-tutorial-parameters.py` being the dedicated parameter file (figure 2.1).

- The variable `PATH_EMBRYO` is the path to the directory where the directory `RAWDATA/` is located. It can be either relative (as in the above example) or global (it could have been `/path/to/astec/tutorial/tuto-astec1/`).
- The variable `EN` is the prefix after which the result fusion images will be named.
- The variables `begin` and `end` set respectively the first and the last index of the input time points to be processed.
- The variables `acquisition_orientation` and `acquisition_mirrors` are parameters describing the acquisition geometry.
- The variable `acquisition_resolution` is the voxel size (along the 3 dimensions X, Y and Z).
- The variable `target_resolution` is the desired isotropic (the same along the 3 dimensions) voxel size for the result fusion images.

After processing, a `FUSE/` directory has been created

```
path/to/tuto-astec1/
└── FUSE/
```

7

```
    1 PATH_EMBRYO = '.'
    2
    3 EN = '2019-Tutorial100'
    4
    5 begin = 0
    6 end = 20
```

Figure 2.2: Tutorial parameter file for the sequence intra-registration step.

```
  └─ RAWDATA/
  └─ README
  └─ parameters/
```

The FUSE/ directory contains
```
FUSE/
└─ FUSE_RELEASE/
     └─ 2019-Tutorial100_fuse_t000.inr
     └─ ...
     └─ 2019-Tutorial100_fuse_t020.inr
     └─ LOGS/
```

The fused images are named after `<EN>_fuse<XXX>.inr` (where `<XXX>` denotes the value of the variable XXX) and indexed from `<begin>` to `<end>` (as the input data). The directory LOGS/ contains a copy of the parameter file (stamped with date and hour) as well as a log file (also stamped with date and hour) reporting information about the processing.

2.3 Sequence intra-registration (or drift compensation) [1]

We assume that we are located in the directory **/path/to/astec/tutorial/tuto-astec1/**. Running the sequence intra-registration is done with

`$ 1.5-intraregistration.py -p parameters/1.5-intraregistration-tutorial-parameters-fuse.py`

1.5-intraregistration-tutorial-parameters-fuse.py being the dedicated parameter file (figure 2.2).

- The variable PATH_EMBRYO is the path to the directory where the directory FUSE/ is located. It can be either relative (as in the above example) or global (it could have been **/path/to/astec/tutorial/tuto-astec1/**).
- The variable EN is the prefix after which the images are named.
- The variables begin and end set respectively the first and the last index of the input time points to be processed.

After processing, a INTRAREG/ directory has been created

```
path/to/tuto-astec1/
  └─ FUSE/
  └─ INTRAREG/
  └─ RAWDATA/
  └─ README
  └─ parameters/
```

The INTRAREG/ directory contains

```
INTRAREG/
└── INTRAREG_RELEASE/
    ├── CO-TRSFS/
    │   ├── 2019-Tutorial100_intrareg_flo000_ref001.trsf
    │   ├── ...
    │   └── 2019-Tutorial100_intrareg_flo019_ref020.trsf
    ├── FUSE/
    │   ├── 2019-Tutorial100_intrareg_fuse_t000.inr
    │   ├── ...
    │   └── 2019-Tutorial100_intrareg_fuse_t020.inr
    ├── LOGS/
    ├── MOVIES/
    │   └── 2019-Tutorial100_intrareg_fuse_t0-20_xy205.inr
    └── TRSFS_t0-20/
        ├── 2019-Tutorial100_intrareg_t000.trsf
        ├── ...
        ├── 2019-Tutorial100_intrareg_t020.trsf
        └── template_t0-20.inr
```

- The directory CO-TRSF/ contains the co-registration transformations.
- The directory FUSE/ contains the resampled fused images in the same geometry (images have the same dimensions along X, Y and Z), with drift compensation (the eventual motion of the sample under the microscope has been compensated).
- The directory MOVIES/ contains a 3D (which is a 2D+t) image, here 2019-Tutorial100_intrareg_fuse_t0-20_xy205 which the #205 XY-section of the resampled fused images for all the time points.
- The directory TRSFS/ contains the transformation of every fused image towards the reference one as well as the template image (an image large enough to including each fused images after resampling).

  The template image template_t0-20.inr is of size $422 \times 365 \times 410$ with a voxel size of 0.6 (the voxel size can be set by the variable intra_registration_resolution)

2.4   Segmentation of the first time point

We assume that we are located in the directory /path/to/astec/tutorial/tuto-astec1/. Segmenting the first time point is done with

```
$ 2-mars.py -p parameters/2-mars-tutorial-parameters.py
```

2-mars-tutorial-parameters.py being the dedicated parameter file (figure 2.3).

- The variable PATH_EMBRYO is the path to the directory where the directory FUSE/ is located. It can be either relative (as in the above example) or global (it could have been /path/to/astec/tutorial/tuto-astec1/).
- The variable EN is the prefix after which the images are named.
- The variable begin sets the index of the first input time point (to be processed).

After processing, a SEG/ directory has been created

```
path/to/tuto-astec1/
├── FUSE/
├── INTRAREG/
└── RAWDATA/
```

```
1 PATH_EMBRYO = '.'
2
3 EN = '2019-Tutorial100'
4
5 begin = 0
```

Figure 2.3: Tutorial parameter file for the segmentation of the first time point.

```
1 PATH_EMBRYO = '.'
2
3 EN = '2019-Tutorial100'
4
5 begin = 0
6
7 mancor_mapping_file='parameters/3-manualcorrection-tutorial.txt'
```

Figure 2.4: Tutorial parameter file for the segmentation correction of the first time point. See figure 2.5 for the <mancor_mapping_file> file.

```
├── README
├── SEG/
└── parameters/
```

The SEG/ directory contains

```
SEG/
└── SEG_RELEASE/
    ├── 2019-Tutorial100_mars_t000.inr
    ├── LOGS/
    └── RECONSTRUCTION/
```

2019-Tutorial100_mars_t000.inr is the segmented first time point of the sequence.

2.5   Correction of the first time point segmentation

We assume that we are located in the directory /path/to/astec/tutorial/tuto-astec1/. Segmenting the first time point is done with

$ 3-manualcorrection.py -p parameters/3-manualcorrection-tutorial-parameters.py

3-manualcorrection-tutorial-parameters.py being the dedicated parameter file (figure 2.4).

- The variable PATH_EMBRYO is the path to the directory where the directory SEG/ is located. It can be either relative (as in the above example) or global (it could have been /path/to/astec/tutorial/tuto-astec1/).
- The variable EN is the prefix after which the images are named.
- The variable begin set the index of the first input time point (to be processed).
- The variable mancor_mapping_file gives the file name containing the correction to be applied.

```
10 6
20 13
9 4
26 11
21 11
27 15
32 23
39 31
35 20
38 43
46 45
52 42
58 62
63 60
78 67
74 66
68 66
83 75
82 77
```

Figure 2.5: The segmentation correction file `3-manualcorrection-tutorial.txt` for the first time point. The first number is the line index (lines are numbered).

After processing, the `SEG/` directory contains

```
SEG/
└── SEG_RELEASE/
    ├── 2019-Tutorial100_mars_t000.inr
    ├── 2019-Tutorial100_seg_t000.inr
    ├── LOGS/
    └── RECONSTRUCTION/
```

`2019-Tutorial100_seg_t000.inr` is the corrected version of the segmentation obtained at the previous step.

2.6   Segmentation propagation

We assume that we are located in the directory `/path/to/astec/tutorial/tuto-astec1/`. Segmenting the first time point is done with

```
$ 4-astec.py -p parameters/4-astec-tutorial-parameters.py
```

`4-astec-tutorial-parameters.py` being the dedicated parameter file (figure 2.6).

After processing, the `SEG/` directory contains

```
SEG/
└── SEG_RELEASE/
    ├── 2019-Tutorial100_mars_t000.inr
    ├── 2019-Tutorial100_seg_lineage.test
```

11

```
 1 PATH_EMBRYO = '.'
 2
 3 EN = '2019-Tutorial100'
 4
 5 begin = 0
 6 end = 20
 7 delta = 1
 8 raw_delay = 0
 9
10 ## General parameters for segmentation propagation
11
12 astec_sigma1 = 0.6
13 astec_sigma2 = 0.15
14 astec_h_min_min = 4
15 astec_h_min_max = 18
16
17 ## Glace Parameters (if astec_membrane_reconstruction_method is set to 1 or 2):
18 ## membrane_renforcement
19
20 astec_sigma_membrane = 0.9
21 astec_sensitivity = 0.99
22 astec_manual = False
23 astec_manual_sigma = 15
24 astec_hard_thresholding = False
25 astec_hard_threshold = 1.0
26
27 ## Tensor voting framework
28
29 astec_sigma_TV = 3.6
30 astec_sigma_LF = 0.9
31 astec_sample = 0.2
32
33 ## Default parameters (for classical use, default values should not be changed)
34
35 astec_RadiusOpening = 20
36 astec_Thau = 25
37 astec_MinVolume = 1000
38 astec_VolumeRatioBigger = 0.5
39 astec_VolumeRatioSmaller = 0.1
40 astec_MorphosnakeIterations = 10
41 astec_NIterations = 200
42 astec_DeltaVoxels = 10**3
43 astec_nb_proc = 10
```

Figure 2.6: Tutorial parameter file for the segmentation propagation.

```
│
├── 2019-Tutorial100_seg_t000.inr
├── 2019-Tutorial100_seg_t001.inr
├── ...
├── 2019-Tutorial100_seg_t020.inr
├── 4-astec-tutorial-parameters.py
├── LOGS/
└── RECONSTRUCTION/
```

while a 2019-Tutorial100_seg_lineage.pkl file has been created in the path/to/tuto-astec1/ directory.

```
path/to/tuto-astec1/
├── 2019-Tutorial100_seg_lineage.pkl
├── FUSE/
├── INTRAREG/
├── RAWDATA/
├── README
├── SEG/
└── parameters/
```

2019-Tutorial100_seg_lineage.pkl is a pickle python file containing a dictionary (in the python sense). It can be read by

```
$ python
...
>>> import cPickle as pkl
>>> f=open('2019-Tutorial100_seg_lineage.pkl')
>>> d=pkl.load(f)
>>> f.close()
>>> d.keys()
['h_mins_information', 'lin_tree', 'volumes_information',
'sigmas_information']
```

In this pickle file, cells have an unique identifier $i * 1000 + c$, which is made of both the image index i and the cell identifier c within a segmentation image (recall that cells are numbered from 2, 1 being the background label).

2.7   Sequence intra-registration (or drift compensation) [2]

We assume that we are located in the directory /path/to/astec/tutorial/tuto-astec1/. Running the sequence intra-registration is done with

```
$ 1.5-intraregistration.py -p parameters/1.5-intraregistration-tutorial-parameters-seg.py
```

1.5-intraregistration-tutorial-parameters-seg.py being the dedicated parameter file (figure 2.7).

- The variable PATH_EMBRYO is the path to the directory where the directory FUSE/ is located. It can be either relative (as in the above example) or global (it could have been /path/to/astec/tutorial/tuto-astec1/).
- The variable EN is the prefix after which the images are named.
- The variables begin and end set respectively the first and the last index of the input time points to be processed.
- the variable EXP_INTRAREG set the suffix of the sub-directory of the INTRAREG/ directory to be created.
- the variable intra_registration_template_type set the images to be used to build the template. Here, since it is equal to "SEGMENTATION'', they are the segmentation images obtained at the previous step.

```
 1 PATH_EMBRYO = '.'
 2
 3 EN = '2019-Tutorial100'
 4
 5 begin = 0
 6 end = 20
 7
 8 EXP_INTRAREG = 'SEG'
 9
10 intra_registration_template_type = "SEGMENTATION"
11 intra_registration_template_threshold = 2
12 intra_registration_margin = 20
13
14 intra_registration_resample_segmentation_images = True
15 intra_registration_movie_segmentation_images = True
```

Figure 2.7: Tutorial parameter file for the sequence intra-registration step, segmentation images being used to build the template.

The variable `intra_registration_template_threshold` set a threshold to be applied to the template images to define the information to be kept: we want all the points with a value equal or greater than 2 to be contained in the template after resampling. Since cells are labeled from 2 and above, the template is designed to contain all labeled cells after resampling, so it is built as small as possible.

The variable `intra_registration_margin` allows to add margins (in the 3 dimensions) to the built template.
- The variable `intra_registration_resample_segmentation_images` indicates whether the segmentation images are to be resampled in the template geometry.
- The variable `intra_registration_movie_segmentation_images` indicates whether 2D+t movies have to be built from the resampled segmentation images.

After processing, a `INTRAREG/INTRAREG_SEG/` directory has been created and the `INTRAREG/` directory now contains

```
INTRAREG/
└── INTRAREG_RELEASE/
│   └── ...
└── INTRAREG_SEG/
    └── CO-TRSFS/
    │   └── 2019-Tutorial100_intrareg_flo000_ref001.trsf
    │   └── ...
    │   └── 2019-Tutorial100_intrareg_flo019_ref020.trsf
    └── FUSE/
    │   └── 2019-Tutorial100_intrareg_fuse_t000.inr
    │   └── ...
    │   └── 2019-Tutorial100_intrareg_fuse_t020.inr
    └── LOGS/
    └── MOVIES/
        └── 2019-Tutorial100_intrareg_fuse_t0-20_xy174.inr
```

14

```
     1 PATH_EMBRYO = '.'
     2
     3 EN = '2019-Tutorial100'
     4
     5 begin = 0
     6 end = 20
     7 delta = 1
     8 raw_delay = 0
     9
    10 postcor_Volume_Threshold=10000
    11 postcor_Soon=True
```

Figure 2.8: Tutorial parameter file for the segmentation propagation.

```
│
│  │__ 2019-Tutorial100_intrareg_seg_t0-20_xy174.inr
│__SEG/
│  │__ 2019-Tutorial100_intrareg_seg_t000.inr
│  │__ ...
│  │__ 2019-Tutorial100_intrareg_seg_t020.inr
│__TRSFS_t0-20/
   │__ 2019-Tutorial100_intrareg_t000.trsf
   │__ ...
   │__ 2019-Tutorial100_intrareg_t020.trsf
   │__ template_t0-20.inr
```

In addition to directories already described in section 2.3, the `INTRAREG_SEG/` directory contains

- The directory `SEG/` contains the resampled segmentation images in the same geometry (images have the same dimensions along X, Y and Z), with drift compensation (the eventual motion of the sample under the microscope has been compensated).
- In addition to a 2D+t movie made from the resampled fusion images, the directory `MOVIES/` contains a 2D+t movie made from the resampled segmentation images.
- The template image `template_t0-20.inr` in the directory `TRSFS/` is now of size $323 \times 265 \times 348$ with a voxel size of 0.6, which is smaller than the one computed in section 2.3, even with the added margins.

  Note that all resampled images (in both the `FUSE/` and the `SEG/` directories have the same geometry than the template image.

2.8 Segmentation post-correction

We assume that we are located in the directory `/path/to/astec/tutorial/tuto-astec1/`. Segmentation post-correction is done with

`$ 5-postcorrection.py -p parameters/5-postcorrection-tutorial-parameters.py`

`5-postcorrection-tutorial-parameters.py` being the dedicated parameter file (figure 2.8).

After processing, a `POST/` directory has been created

```
path/to/tuto-astec1/
│__ 2019-Tutorial100_seg_lineage.pkl
```

15

```
  ├── FUSE/
  ├── INTRAREG/
  ├── POST/
  ├── RAWDATA/
  ├── README
  ├── SEG/
  └── parameters/
```

The POST/ directory contains

```
POST/
└── POST_RELEASE/
    ├── 2019-Tutorial100_post_lineage.pkl
    ├── 2019-Tutorial100_post_lineage.test
    ├── 2019-Tutorial100_post_t000.inr
    ├── ...
    ├── 2019-Tutorial100_post_t020.inr
    ├── 5-postcorrection-tutorial-parameters.py
    └── 5-postcorrection.log
```

Chapter 3

User guide: command line interfaces

The Astec distribution contains 4 directories

```
path/to/astec/
├── documentation/
├── parameter-file-examples/
├── src/
└── tutorial/
```

- `documentation/` contains this documentation
- `parameter-file-examples/` contains examples of parameter files for the command line interfaces (CLIs) that are introduced below. These files are named after the CLI name.
- `src/` contains the command line interfaces (CLIs) and the code.
- `tutorial/` contains a tutorial (see chap. 2) along with a toy example.

Data organization

It is assumed that there will be one directory per experiment. This directory contains the acquired data, but will also contain the result data as depicted below.

```
/path/to/experiment/
├── RAWDATA/
│   └── ...
├── FUSE/
│   └── ...
├── SEG/
│   └── ...
└── POST/
    └── ...
```

`RAWDATA/` is assumed to contain the raw data (ie acquired images from the MuViSPIM microscope), while the other subdirectories will contain processing results.

3.1   1-fuse.py

The fusion is made of the following steps.

1. Optionally, a slit line correction. Some Y lines may appear brighter in the acquisition and causes artifacts in the reconstructed (i.e. fused) image. By default, it is not done.
2. A change of resolution in the X and Y directions only (Z remains unchanged). It allows to decrease the data volume (and then the computational cost) if the new pixel size (set by `target_resolution`) is larger than the acquisition one.
3. Optionally, a crop of the resampled acquisitions. It allows to decrease the volume of data, hence the computational cost. The crop is based on the analysis of a MIP view (in the Z direction) of the volume, and thus is sensitive to hyper-intensities if any. By default, it is done.
4. Optionally, a mirroring of the 'right' image. It depends on the value of `raw_mirrors` variable.
5. Linear registration of the 3 last images on the first one (considered as the reference). The reference image is resampled again, to get an isotropic voxel (whose size is given by `target_resolution`), i.e. the voxel size is the same along the 3 directions: X, Y, Z.
6. Linear combination of images, weighted by an ad-hoc function.
7. Optionally, a crop of the fused image, still based on the analysis of a MIP view (in the Z direction). By default, it is done.

3.1.1   `1-fuse.py` options

The following options are available:

`-h` prints a help message
`-p` <u>file</u> set the parameter file to be parsed
`-e` path set the `path` to the directory where the `RAWDATA/` directory is located
`-k` allows to keep the temporary files
`-f` forces execution, even if (temporary) result files are already existing
`-v` increases verboseness (both at console and in the log file)
`-nv` no verboseness
`-d` increases debug information (in the log file)
`-nd` no debug information

3.1.2   Important parameters in the parameter file

A simple parameter file for fusion is described in the tutorial section 2.2. A more comprehensive parameter file example is provided in the `parameter-file-examples/` directory.

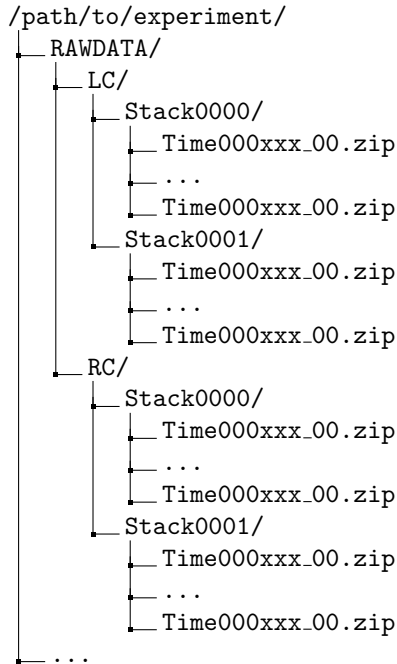Indicating the right values of the acquisition parameters is crucial; these parameters are

- `raw_ori` is a parameter describing the acquisition orientation of the acquisition of the second pair of images. Its value can be either 'left' (orientation of 270°) or 'right' (orientation of 90°).
- `raw_mirrors` is a parameter indicating whether the right camera images have to be mirrored or not. Its value is either `False` or `True`.
- `raw_resolution` is the voxel size (along the 3 dimensions X, Y and Z) of the acquired images.
- `target_resolution` is the desired isotropic (the same along the 3 dimensions) voxel size for the result fusion images.
- `begin` gives the index of the first time point to be processed.
- `end` gives the index of the last time point to be processed.

When one may not be sure of the `raw_ori` and `raw_mirrors` right values, it is advised to perform the fusion on only one time point (by indicating the same index for both `begin` and `end`), with the four possibilities for the variable couple (`raw_ori`, `raw_mirrors`), i.e. ('left', `False`), ('left', `True`), ('right', `False`), and ('right', `True`). It comes to write four parameter files that differ only for the parameters `raw_ori`, `raw_mirrors`, and `EXP_FUSE` (to store the fusion result in different directories, see section 3.1.4). For these first experiments, it is also advised to set `target_resolution` to a large value, in order to speed up the calculations.

3.1.3 Input data

Input data (acquired images from the MuViSPIM microscope) are assumed to be organized in a separate `RAWDATA/` directory in the `/path/to/experiment/` directory as depicted below.

- `RAWDATA/LC/Stack000` contains the images acquired at the first angulation by the left camera.
- `RAWDATA/LC/Stack001` contains the images acquired at the second angulation by the left camera.
- `RAWDATA/RC/Stack000` contains the images acquired at the first angulation by the right camera.
- `RAWDATA/RC/Stack001` contains the images acquired at the second angulation by the right camera.

```
/path/to/experiment/
  └─ RAWDATA/
       └─ LC/
            └─ Stack0000/
                 └─ Time000xxx_00.zip
                 ├─ ...
                 └─ Time000xxx_00.zip
            └─ Stack0001/
                 └─ Time000xxx_00.zip
                 ├─ ...
                 └─ Time000xxx_00.zip
       └─ RC/
            └─ Stack0000/
                 └─ Time000xxx_00.zip
                 ├─ ...
                 └─ Time000xxx_00.zip
            └─ Stack0001/
                 └─ Time000xxx_00.zip
                 ├─ ...
                 └─ Time000xxx_00.zip
  ├─ ...
```

where xxx denotes a three digit number (e.g. 000, 001, ...) denoting the time point of each acquisition. The range of time points to be fused are given by the variables `begin` and `end`, while the path `/path/to/experiment/` has to be assigned to the variable `PATH_EMBRYO`

Hence a parameter file containing

```
PATH_EMBRYO = /path/to/experiment/
begin = 0
end = 10
```

indicates that time points in $[0, 10]$ of the `RAWDATA/` subdirectory of `/path/to/experiment/` have to be fused.

3.1.3.1 Input data directory names

However, directories may be named differently. The variables `DIR_RAWDATA`, `DIR_LEFTCAM_STACKZERO`, `DIR_RIGHTCAM_STACK` `DIR_LEFTCAM_STACKONE`, and `DIR_RIGHTCAM_STACKONE` allow a finer control of the directory names. The images acquired at the first angulation by the left and the right cameras are searched in the directories

```
<PATH_EMBRYO>/<DIR_RAWDATA>/<DIR_LEFTCAM_STACKZERO>
<PATH_EMBRYO>/<DIR_RAWDATA>/<DIR_RIGHTCAM_STACKZERO>
```

while the images acquired at the second angulation by the left and the right cameras are searched in the directories

```
<PATH_EMBRYO>/<DIR_RAWDATA>/<DIR_LEFTCAM_STACKONE>
<PATH_EMBRYO>/<DIR_RAWDATA>/<DIR_RIGHTCAM_STACKONE>
```

where `<XXX>` denotes the value of the variable `XXX`. Then, to parse the following data architecture

```
/path/to/experiment/
└── my_raw_data/
    ├── LeftCamera/
    │   ├── FirstStack/
    │   │   └── ...
    │   └── SecondStack/
    │       └── ...
    └── RightCamera/
        ├── FirstStack/
        │   └── ...
        └── SecondStack/
            └── ...
└── ...
```

one has to add the following lines in the parameter file

```
DIR_RAWDATA = 'my_raw_data'
DIR_LEFTCAM_STACKZERO = 'LeftCamera/FirstStack'
DIR_RIGHTCAM_STACKZERO = 'RightCamera/FirstStack'
DIR_LEFTCAM_STACKONE = 'LeftCamera/SecondStack'
DIR_RIGHTCAM_STACKONE = 'RightCamera/SecondStack'
```

It has to be noted that, when the stacks of a given time point are in different directories, image file names are tried to be guessed from the directories parsing. It has to be pointed out that indexes have to be encoded with a 3-digit integer with 0 padding (i.e. 000, 001, . . .) and that has to be the only variation in the file names (within each directory).

3.1.3.2 Input data image file names

Images acquired from the left and the right cameras may be stored in the same directory, but obviously with different names as in

```
/path/to/experiment/
└── RAWDATA/
    ├── stack_0_channel_0
    │   ├── Cam_Left_00xxx.zip
    │   ├── ...
    │   ├── Cam_Right_00xxx.zip
    │   └── ...
    └── stack_1_channel_0
        ├── Cam_Left_00xxx.zip
        ├── ...
        ├── Cam_Right_00xxx.zip
        └── ...
```

The parameter file has then to contain the following lines to indicate the directory names.

```
DIR_LEFTCAM_STACKZERO = 'stack_0_channel_0'
DIR_RIGHTCAM_STACKZERO = 'stack_0_channel_0'
```

```
DIR_LEFTCAM_STACKONE = 'stack_1_channel_0'
DIR_RIGHTCAM_STACKONE = 'stack_1_channel_0'
```

In addition, to distinguish the images acquired by the left camera to those acquired by the right one, one has to give the image name prefixes, i.e. the common part of the image file names before the 3-digit number that indicates the time point. This is the purpose of the variables `acquisition_leftcam_image_prefix` and `acquisition_rightcam_image_prefix`. The parameter file has then to contain the following lines not only to indicate the directory names but also the image file name prefixes.

```
DIR_LEFTCAM_STACKZERO = 'stack_0_channel_0'
DIR_RIGHTCAM_STACKZERO = 'stack_0_channel_0'
DIR_LEFTCAM_STACKONE = 'stack_1_channel_0'
DIR_RIGHTCAM_STACKONE = 'stack_1_channel_0'
acquisition_leftcam_image_prefix = 'Cam_Left_00'
acquisition_rightcam_image_prefix = 'Cam_Right_00'
```

3.1.3.3 Multichannel acquisition

In case of multichannel acquisition, the fusion is computed for the first channel, and the computed parameters (e.g. transformations, etc.) are also used for the other channels.

For a second channel, the images acquired at the first angulation by the left and the right cameras are searched in the directories

```
<PATH_EMBRYO>/<DIR_RAWDATA>/<DIR_LEFTCAM_STACKZERO_CHANNEL_2>
<PATH_EMBRYO>/<DIR_RAWDATA>/<DIR_RIGHTCAM_STACKZERO_CHANNEL_2>
```

while the images acquired at the second angulation by the left and the right cameras are searched in the directories

```
<PATH_EMBRYO>/<DIR_RAWDATA>/<DIR_LEFTCAM_STACKONE_CHANNEL_2>
<PATH_EMBRYO>/<DIR_RAWDATA>/<DIR_RIGHTCAM_STACKONE_CHANNEL_2>
```

For a third channel, the images acquired at the first angulation by the left and the right cameras are searched in the directories

```
<PATH_EMBRYO>/<DIR_RAWDATA>/<DIR_LEFTCAM_STACKZERO_CHANNEL_3>
<PATH_EMBRYO>/<DIR_RAWDATA>/<DIR_RIGHTCAM_STACKZERO_CHANNEL_3>
```

while the images acquired at the second angulation by the left and the right cameras are searched in the directories

```
<PATH_EMBRYO>/<DIR_RAWDATA>/<DIR_LEFTCAM_STACKONE_CHANNEL_3>
<PATH_EMBRYO>/<DIR_RAWDATA>/<DIR_RIGHTCAM_STACKONE_CHANNEL_3>
```

3.1.4 Output data

The variable `target_resolution` allows to set the desired isotropic (the same along the 3 dimensions) voxel size for the result fusion images.

3.1.4.1 Output data directory names

The resulting fused images are stored in sub-directories FUSE/FUSE_<EXP_FUSE> under the /path/to/experiment/ directory

```
/path/to/experiment/
```

```
├── RAWDATA/
│   └── ...
└── FUSE/
    └── FUSE_<EXP_FUSE>/
        └── ...
```

where `<EXP_FUSE>` is the value of the variable `EXP_FUSE` (its default value is 'RELEASE'). Hence, the line

```
EXP_FUSE = 'TEST'
```

in the parameter file will create the directory `FUSE/FUSE_TEST/` in which the fused images are stored. For instance, when testing for the values of the variable couple (`raw_ori`, `raw_mirrors`), a first parameter file may contain

```
raw_ori = 'left'
raw_mirrors = False
begin = 1
end = 1
EXP_FUSE=TEST-LEFT-FALSE
```

a second parameter file may contain

```
raw_ori = 'left'
raw_mirrors = True
begin = 1
end = 1
EXP_FUSE=TEST-LEFT-TRUE
```

etc. The resulting fused images will then be in different directories

```
/path/to/experiment/
├── RAWDATA/
│   └── ...
└── FUSE/
    ├── FUSE_TEST-LEFT-FALSE/
    │   └── ...
    ├── FUSE_TEST-LEFT-TRUE/
    │   └── ...
    └── ...
```

This will ease their visual inspection to decide which values of the variable couple (`raw_ori`, `raw_mirrors`) to use for the fusion.

3.1.4.2 Output data file names

Fused image files are named after the variable `EN`: `<EN>_fuse_t<xxx>.inr` where `<xxx>` is the time point index encoded by a 3-digit integer (with 0 padding).

3.1.4.3 Multichannel acquisition

Variables `EXP_FUSE_CHANNEL_2` and `EXP_FUSE_CHANNEL_3` allows to set the directory names for the resulting fused images of the other channels.

3.1.5 Fusion parameters

3.1.5.1 Step 3: raw data cropping

For computational cost purposes, raw data (images acquired by the MuViSPIM microscope) are cropped (only in X and Y dimensions) before co-registration. A threshold is computed with Otsu's method [Ots79] on the maximum intensity projection (MIP) image. The cropping parameters are computed to keep the above-threshold points in the MIP image, plus some extra margins. Hyper-intense areas may biased the threshold computation, hence the cropping.

To desactivate this cropping, the line

```
raw_crop = False
```

has to be added in the parameter file.

3.1.5.2 Step 5: linear registration

To decrease the computational cost, images are normalized and cast on one byte before registration. While it generally does not degrade the registration quality, it may induce troubles when hyper-intensities areas are present in the image. In such a case, the useful information may then be summarized in only a few intensity values.

Intensity normalization in registration can be deactivated by adding the following line in the parameter file

```
fusion_registration_normalization = False
```

To verify whether a good quality registration can be conducted, the searched transformation type can be changed for a simpler one than affine. Adding the following line in the parameter file.

```
fusion_registration_transformation_type = translation
```

will search for a translation which is supposed to be sufficient, according that only translations relates the 4 acquisitions of the MuViSPIM microscope (in a perfect setting). If the search for an affine transformation (the default behavior) failed (the fusion looks poor) while the search for a translation is successful (the fusion looks good), a two-steps registration may help to refine the found translation by a subsequent affine transformation as explained below.

Hyper-intensities areas may also bias the threshold calculation used for the automatic crop (step 3 of fusion). In such cases, the iterative registration method may find a local minimum that is not the desired one, because the relative positions of the two images to be co-registered are too far apart. To circumvent such a behavior, a two-steps registration can be done. It consists on a first pre-registration with a transformation with fewer degrees of freedom (i.e. a 3D translation).

This pre-registration can be activated by adding the following line in the parameter file.

```
fusion_preregistration_compute_registration = True
```

It may be also preferable to deactivate the image normalization for both registration steps with

```
fusion_preregistration_normalization = False
fusion_registration_normalization = False
```

3.1.5.3 Step 7: fused data cropping

To save disk storage, fused images are cropped at the end of the fusion stage. To desactivate this cropping, the line

```
fusion_crop = False
```

has to be added in the parameter file.

3.1.6 Troubleshooting

- The fused images are obviously wrong.

  1. Are the values of the variable couple (`raw_ori`, `raw_mirrors`) the right ones? Conduct experiments as suggested in section 3.1.2 (see also section 3.1.4) to get the right values.
  2. The registration may have failed.
     (a) Deactivate the 1-byte normalization (see section 3.1.5.2).
     (b) Try to register with a simpler transformation type (i.e. translation) and/or with a two-steps registration (see section 3.1.5.2).

- The imaged sample is cropped by the image border in the fused image.

  1. Check whether the imaged sample was not already cropped in the raw data.
  2. The automated cropping may have failed. It is more likely to happen when cropping the raw data, so deactivate it (see section 3.1.5.1). If it still happens, try to deactivate also the fused image cropping (see section 3.1.5.3).

3.1.7 Parameter list

Please also refer to the file `parameter-file-examples/1-fuse-parameters.py`

- `DIR_LEFTCAM_STACKONE` see section 3.1.3
- `DIR_LEFTCAM_STACKONE_CHANNEL_2` see section 3.1.3
- `DIR_LEFTCAM_STACKONE_CHANNEL_3` see section 3.1.3
- `DIR_LEFTCAM_STACKZERO` see section 3.1.3
- `DIR_LEFTCAM_STACKZERO_CHANNEL_2` see section 3.1.3
- `DIR_LEFTCAM_STACKZERO_CHANNEL_3` see section 3.1.3
- `DIR_RAWDATA` see section 3.1.3
- `DIR_RAWDATA_CHANNEL_2` see section 3.1.3
- `DIR_RAWDATA_CHANNEL_3` see section 3.1.3
- `DIR_RIGHTCAM_STACKONE` see section 3.1.3
- `DIR_RIGHTCAM_STACKONE_CHANNEL_2` see section 3.1.3
- `DIR_RIGHTCAM_STACKONE_CHANNEL_3` see section 3.1.3
- `DIR_RIGHTCAM_STACKZERO` see section 3.1.3
- `DIR_RIGHTCAM_STACKZERO_CHANNEL_2` see section 3.1.3
- `DIR_RIGHTCAM_STACKZERO_CHANNEL_3` see section 3.1.3
- `EN` see section 3.1.4
- `EXP_FUSE` see section 3.1.4
- `EXP_FUSE_CHANNEL_2` see section 3.1.4
- `EXP_FUSE_CHANNEL_3` see section 3.1.4
- `PATH_EMBRYO` see section 3.1.3
- `RESULT_IMAGE_SUFFIX_FUSE`
- `acquisition_leftcam_image_prefix` see section 3.1.3
- `acquisition_mirrors` same as `raw_mirrors`
- `acquisition_orientation` same as `raw_ori`
- `acquisition_resolution` same as `raw_resolution`
- `acquisition_rightcam_image_prefix` see section 3.1.3
- `acquisition_slit_line_correction`
- `begin` see section 3.1.2
- `default_image_suffix`
- `delta`
- `end` see section 3.1.2

- `fusion_crop` see section 3.1.5.3
- `fusion_margin_x_0`
- `fusion_margin_x_1`
- `fusion_margin_y_0`
- `fusion_margin_y_1`
- `fusion_preregistration_compute_registration` see section 3.1.5.2
- `fusion_preregistration_lts_fraction`
- `fusion_preregistration_normalization` see section 3.1.5.2
- `fusion_preregistration_pyramid_highest_level`
- `fusion_preregistration_pyramid_lowest_level`
- `fusion_preregistration_transformation_estimation_type`
- `fusion_preregistration_transformation_type`
- `fusion_registration_compute_registration`
- `fusion_registration_lts_fraction`
- `fusion_registration_normalization` see section 3.1.5.2
- `fusion_registration_pyramid_highest_level`
- `fusion_registration_pyramid_lowest_level`
- `fusion_registration_transformation_estimation_type`
- `fusion_registration_transformation_type` see section 3.1.5.2
- `raw_crop` see section 3.1.5.1
- `raw_delay`
- `raw_margin_x_0`
- `raw_margin_x_1`
- `raw_margin_y_0`
- `raw_margin_y_1`
- `raw_mirrors` see section 3.1.2
- `raw_ori` see section 3.1.2
- `raw_resolution` see section 3.1.2
- `result_image_suffix`
- `target_resolution` see section 3.1.4

3.2 `1.5-intraregistration.py`

The sequence intra-registration procedure can be done either after the fusion step, or after the (post-)segmentation step. It aims at

- compensating for the eventual motion of the imaged sample with respect to the microscope
- resampling the fusion and/or the segmentation images into a common frame/geometry, so they can better be compared, and
- building 2D+t images made of 2D sections from either the fusion and/or the segmentation images, so that the quality of the fusion and/of the tracking step can be visually assessed.

The intra-registration procedure is made of the following steps:

1. Co-registration of pairs of successive fused images (section 3.2.3). This yields the transformations $T_{t+1 \leftarrow t}$. Fused images are located in `<EMBRYO>/FUSE/FUSE_<EXP_FUSE>`: the parameter `EXP_FUSE` is either set in the parameter file or is set at `RELEASE`. This step may be long.
2. Composition of transformations issued from the co-registration step. This step computes the transformations $T_{ref \leftarrow t}$ towards a reference image `ref` given by the parameter `intra_registration_reference_index`.
3. Computation of the *template* image (section 3.2.4). This *template* image dimension are computed so that the useful information of all resampled images fits into it. Useful information can be issued from either the fused sequence, the segmentation sequence or the post-segmentation sequence. It is indicated by the `intra_registration_template_type` which value can be either `'FUSION'`, `'SEGMENTATION'`, or `'POST-SEGMENTATION'`. This step may be long.

4. Resampling of either the fused or the segmentation images (section 3.2.4.1). Note that changing the parameters for this step will not require to re-compute the first steps.

5. Extraction of 2D+t images from the resampled sequences (section 3.2.5). Note that changing the parameters for this step (i.e. requiring extra movies) will not require to re-compute the first steps, with an eventual exception for the resampling step.

3.2.1 `1.5-intraregistration.py` options

The following options are available:

-h prints a help message
-p <u>file</u> set the parameter file to be parsed
-e <u>path</u> set the <u>path</u> to the directory where the `RAWDATA/` directory is located
-k allows to keep the temporary files
-f forces execution, even if (temporary) result files are already existing
-v increases verboseness (both at console and in the log file)
-nv no verboseness
-d increases debug information (in the log file)
-nd no debug information

3.2.2 Output data

The results are stored in sub-directories `INTRAREG/INTRAREG_<EXP_INTRAREG>` under the `/path/to/experiment/` directory where where `<EXP_INTRAREG>` is the value of the variable `EXP_INTRAREG` (its default value is 'RELEASE').

```
/path/to/experiment/
├── ...
└── INTRAREG
    └── INTRAREG_<EXP_INTRAREG>
        ├── CO-TRSFS
        ├── FUSE
        ├── LOGS
        ├── MOVIES
        ├── [POST]
        ├── [SEG]
        └── TRSFS_t<begin>-<end>
└── ...
```

`INTRAREG/INTRAREG_<EXP_INTRAREG>/FUSE` contains the fused images after resampling in a common geometry (i.e. all image have the same X, Y and Z dimensions)

```
/path/to/experiment/
├── ...
└── INTRAREG
    └── INTRAREG_<EXP_INTRAREG>
        ├── ...
        └── FUSE
            └── <EN>_intrareg_fuse_t<xxx>.inr .4 ...
└── ...
```

3.2.3 Co-registration parameters

Default registration parameters for the co-registration are set by:

```
# intra_registration_compute_registration = True
# intra_registration_transformation_type = 'rigid'
# intra_registration_transformation_estimation_type = 'wlts'
# intra_registration_lts_fraction = 0.55
# intra_registration_pyramid_highest_level = 6
# intra_registration_pyramid_lowest_level = 3
# intra_registration_normalization = True
```

Computed transformations are stored in `INTRAREG/INTRAREG_<EXP>/CO-TRSFS`. It may be advised to set the pyramid lowest level value to some higher value to speed up the co-registrations (recall that all pairs of successive images will be co-registered, i.e.

```
intra_registration_pyramid_lowest_level = 4
```

3.2.4 Template building parameters

```
# intra_registration_reference_index = None
# intra_registration_template_type = 'FUSION'
# intra_registration_template_threshold = None
# intra_registration_resolution = 0.6
# intra_registration_margin = None
```

The `intra_registration_reference_index` allows to choose the reference image (the one which remains still, i.e. is only displaced by a translation), by default it is the first image image of the series (associated to `begin`).

Depending on `intra_registration_template_type` ('FUSION', 'SEGMENTATION' or 'POST-SEGMENTATION', the two latter assume obviously that the segmentation has been done), the *template* image can be built either after the fusion or the segmentation images. If no threshold is given by `intra_registration_template_threshold`, the built template will be large enough to include all the transformed fields of view (in this case, the template is the same whatever `intra_registration_template_type` is).

If a threshold is given, the built template will be large enough to include all the transformed points above the threshold. E.g., the background is labeled with either '1' or '0' in segmentation images, then a threshold of '2' ensures that all the embryo cells will not be cut by the resampling stage. In this case, adding an additional margin to the template could be a good idea for visualization purpose. Last but not least, using a larger resolution than the `intra_registration_resolution` allows to decrease the resampled images volume (the default resolution i.e. voxel size, given by `target_resolution`, for the fusion step (see section **??**), is set to 0.3, while it is set to 0.6 here).

Thus, building a *template* image after the segmentation images can be done with

```
# intra_registration_reference_index = None
intra_registration_template_type = "SEGMENTATION"
intra_registration_template_threshold = 2
# intra_registration_resolution = 0.6
intra_registration_margin = 10
```

Computed transformations from the *template* image as well as the *template* image itself are stored in `INTRAREG/INTRAREG<EXP>/TRSFS_t<F>-<L>` where `<F>` and `L` are the first and the last index of the series (specified by `begin` and `end` from the parameter file).

3.2.4.1 Resampling fusion/segmentation images

The resampled fusion and segmentation images will be stored respectively in `INTRAREG/INTRAREG_<EXP>/FUSE`, `INTRAREG/INTRAREG_<EXP>/SEG` and `INTRAREG/INTRAREG_<EXP>/POST`. Resampling is done either if the following parameters are set to `True` or if movies are requested to be computed.

```
# intra_registration_resample_fusion_images = True
# intra_registration_resample_segmentation_images = False
# intra_registration_resample_post_segmentation_images = False
```

3.2.5   2D+t movies

For either visual assessment or illustration purposes, 2D+t (i.e. 3D) images can be built from 2D sections
extracted from the resampled temporal series. This is controlled by the following parameters:

```
# intra_registration_movie_fusion_images = True
# intra_registration_movie_segmentation_images = False

# intra_registration_xy_movie_fusion_images = [];
# intra_registration_xz_movie_fusion_images = [];
# intra_registration_yz_movie_fusion_images = [];

# intra_registration_xy_movie_segmentation_images = [];
# intra_registration_xz_movie_segmentation_images = [];
# intra_registration_yz_movie_segmentation_images = [];

# intra_registration_xy_movie_post_segmentation_images = [];
# intra_registration_xz_movie_post_segmentation_images = [];
# intra_registration_yz_movie_post_segmentation_images = [];
```

If `intra_registration_movie_fusion_images` is set to `True`, a movie is made with the XY-section lo-
cated at the middle of each resampled fusion image (recall that, after resampling, all images have the same ge-
ometry). Additional XY-movies can be done by specifying the wanted Z values in `intra_registration_xy_movie_fusion`
E.g.

```
intra_registration_xy_movie_fusion_images = [100, 200];
```

will build two movies with XY-sections located respectively at Z values of 100 and 200. The same stands for
the other orientation and for the resampled segmentation images.

3.2.6   Parameter list

Please also refer to the file `parameter-file-examples/1.5-intraregistration-parameters.py`

- EN
- EXP_FUSE
- EXP_INTRAREG
- EXP_POST
- EXP_SEG
- PATH_EMBRYO
- begin
- default_image_suffix
- delta
- end
- intra_registration_compute_registration
- intra_registration_lts_fraction
- intra_registration_margin
- intra_registration_movie_fusion_images
- intra_registration_movie_post_segmentation_images
- intra_registration_movie_segmentation_images

- intra_registration_normalization
- intra_registration_pyramid_highest_level
- intra_registration_pyramid_lowest_level
- intra_registration_reference_index
- intra_registration_resample_fusion_images
- intra_registration_resample_post_segmentation_images
- intra_registration_resample_segmentation_images
- intra_registration_resolution
- intra_registration_sigma_segmentation_images
- intra_registration_template_threshold
- intra_registration_template_type
- intra_registration_transformation_estimation_type
- intra_registration_transformation_type
- intra_registration_xy_movie_fusion_images
- intra_registration_xy_movie_post_segmentation_images
- intra_registration_xy_movie_segmentation_images
- intra_registration_xz_movie_fusion_images
- intra_registration_xz_movie_post_segmentation_images
- intra_registration_xz_movie_segmentation_images
- intra_registration_yz_movie_fusion_images
- intra_registration_yz_movie_post_segmentation_images
- intra_registration_yz_movie_segmentation_images
- result_image_suffix

3.3   2-mars.py

The name `mars` comes from [FDM⁺10] where `MARS` is the acronym of *multiangle image acquisition, 3D reconstruction and cell segmentation.*

    This method aims at producing a segmentation of a membrane cell image (e.g. a fused image) into a segmention image. This segmentation image is a integer-valued image where each integer labeled an unique cell in the image. By convention, '1' is the background label, while cells have labels greater than 2. It is is made of the following steps:

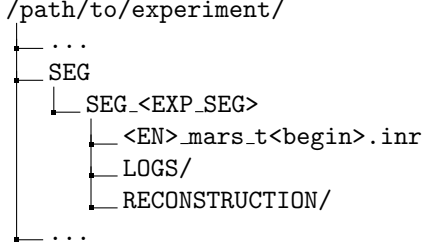1. Optionally, a transformation of the input image.
2. A seeded watershed.

3.3.1   2-mars.py **options**

The following options are available:

-h prints a help message
-p <u>file</u> set the parameter file to be parsed
-e <u>path</u> set the <u>path</u> to the directory where the RAWDATA/ directory is located
-k allows to keep the temporary files
-f forces execution, even if (temporary) result files are already existing
-v increases verboseness (both at console and in the log file)
-nv no verboseness
-d increases debug information (in the log file)
-nd no debug information

3.3.2 Output data

The results are stored in sub-directories `SEG/SEG_<EXP_SEG>` under the `/path/to/experiment/` directory where where `<EXP_SEG>` is the value of the variable `EXP_SEG` (its default value is 'RELEASE').

```
/path/to/experiment/
├── ...
├── SEG
│   └── SEG_<EXP_SEG>
│       ├── <EN>_mars_t<begin>.inr
│       ├── LOGS/
│       └── RECONSTRUCTION/
├── ...
```

3.3.3 Segmentation parameters

3.3.3.1 Input image for watershed computation

Before the watershed segmentation, the input image may be pre-processed. This pre-processing is controlled by the two variables.

- `mars_intensity_transformation` whose values are to be chosen in `None`, `'Identity'`, or `'Normalization_to_u8'`. Default is `'Identity'`.
- `mars_intensity_enhancement` whose values are to be chosen in `None` or `GACE`. Default is `None`.

Each of these variables, if not `None`, induce a transformation of the input (i.e. fused) image. If both values are not known, the input image for the watershed is the result of the maximum operator over the two images.

- `mars_intensity_transformation = 'Identity'`: the input image is not transformed.
- `mars_intensity_transformation = 'Normalization_to_u8'`: input images are usually encoded on 2 bytes. The choice transformed the input image in an 1-byte image by linearly mapping the input image values from $[I_{min}, I_{max}]$ to $[0, 255]$. I_{min} and I_{max} correspond respectively to the 1% and to the 99% percentiles of the input image cumulative histogram. Values below I_{min} are set to 0 while values above I_{max} are set to 255.
- `mars_intensity_enhancement = 'GACE'`: GACE stands for *Global Automated Cell Extractor*. This is the method described in [MGFM14]. It consists in

  1. extracting a centerplane image of the membranes,
  2. thresholding this centerplane image, and
  3. reconstruct the membranes through a tensor voting method.

If the input image is transformed before segmented, the transformed image is named `<EN>_fuse_t<begin>_membrane.in` and stored in the directory `SEG/SEG_<EXP_SEG>/RECONSTRUCTION/` if the value of the variable `mars_keep_reconstruction` is set to `True`.

3.3.3.2 Seeded watershed

The seed extraction is made of the following steps:

1. Gaussian smoothing of the input image, the gaussian standard deviation being given by the variable `watershed_seed_sigma`.
2. Extraction of the h-minima of the previous image, h being given by the variable `watershed_seed_hmin`.
3. Hysteresis thresholding (and labeling) of the h-minima image, with a high threshold equal to h and and a low threshold equal to 1. It then only selects the h-minima that have an actual depth of h.

Given the seeds, the watershed is performed on the smoothed input image (gaussian standard deviation being given by the variable `watershed_membrane_sigma`).

3.3.4 Parameter list

Please also refer to the file `parameter-file-examples/2-mars-parameters.py`

- `EN`
- `EXP_FUSE`
- `EXP_SEG`
- `PATH_EMBRYO`
- `begin`
- `default_image_suffix`
- `delta`
- `mars_begin`
- `mars_end`
- `mars_hard_threshold`
- `mars_intensity_enhancement`
- `mars_intensity_transformation`
- `mars_keep_reconstruction`
- `mars_manual`
- `mars_manual_sigma`
- `mars_sample`
- `mars_sensitivity`
- `mars_sigma_TV`
- `mars_sigma_membrane`
- `result_image_suffix`
- `watershed_membrane_sigma`
- `watershed_seed_hmin`
- `watershed_seed_sigma`

3.4 `3-manualcorrection.py`

The seeded watershed is likely to produce segmentation errors, even with a careful choice of parameters. It is advised to set the parameters to favour over-segmentations insted of under-segmentations since the former are much more easier to correct, which is the purpose of `3-manualcorrection.py`.

3.4.1 `3-manualcorrection.py` options

The following options are available:

`-h` prints a help message
`-p` <u>file</u> indicates the parameter file to be parsed
`-e` <u>path</u> indicates the <u>path</u> to the directory where the `RAWDATA/` directory is located
`-k` allows to keep the temporary files
`-f` forces execution, even if (temporary) result files are already existing
`-v` increases verboseness (both at console and in the log file)
`-nv` no verboseness
`-d` increases debug information (in the log file)
`-nd` no debug information
`-i` <u>input_image</u> set the <u>input_image</u> file to be corrected. Allows to skip the automated naming of files.
`-o` <u>output_image</u> set the resulting <u>ouput_image</u> file to be saved. Allows to skip the automated naming of files.
`-m` <u>mapping_file</u> set the <u>mapping_file</u> to be used for the correction.
`-nsc` <u>smallest_cells</u> set the number of the smallest cells to be displayed after correction. The smallest cells are the most likely to be issued from an over-segmentation.
`-nlc` <u>largest_cells</u> set the number of the largest cells to be displayed after correction. The largest cells are the most likely to be issued from an under-segmentation.

3.4.2 Output data

The results are stored in sub-directories `SEG/SEG_<EXP_SEG>` under the `/path/to/experiment/` directory where where `<EXP_SEG>` is the value of the variable `EXP_SEG` (its default value is 'RELEASE'). `<EN>_seg_t<begin>.inr` is the correction of the segmentation image `<EN>_mars_t<begin>.inr`.

```
/path/to/experiment/
├── ...
├── SEG
│   └── SEG_<EXP_SEG>
│       ├── <EN>_mars_t<begin>.inr
│       ├── <EN>_seg_t<begin>.inr
│       ├── LOGS/
│       └── RECONSTRUCTION/
└── ...
```

3.4.3 Segmentation correction parameters

`3-manualcorrection.py` parses a correction file whose name is given by the variable `mancor_mapping_file`. The syntax of this file is very simple. Lines beginning with `#` are ignored (and can be used to insert comments in the files). Non-empty lines should contain two numbers separated by a space, and `3-manualcorrection.py` will replace the first number by the second in the segmentation file.

E.g. a cell c is recognized to be over-segmented, and then is represented by two labels, says 9 and 10. Thus the line

```
10 9
```

will replace all 10's by 9's in the segmentation image, thus c will only be represented by 9's after correction. See also the tutorial section 2.5 for an other example.

3.4.4 Parameter list

Please also refer to the file `parameter-file-examples/3-manualcorrection-parameters.py`

- `EN`
- `EXP_SEG`
- `PATH_EMBRYO`
- `begin`
- `default_image_suffix`
- `delta`
- `mancor_input_seg_file`
- `mancor_mapping_file`
- `mancor_output_seg_file`
- `mars_begin`
- `mars_end`
- `result_image_suffix`

3.5 4-astec.py

The name `astec` comes from the Phd work of L. Guignard [Gui15] where `ASTEC` is the acronym of *adaptive segmentation and tracking of embryonic cells*.

3.6 5-postcorrection.py

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