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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 --get-selections | 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 toggle 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'
```

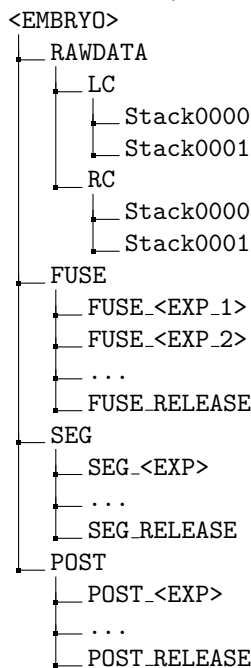
```
ln -s <complete_path_of_bin_folder> cpp
```

1.3 Troubleshooting

Chapter 2

Running Astec

From version 2.0, the data architecture should be as follows



<SOMETHING>.RELEASE sub-directories should content the "last version" validated by an expert. <SOMETHING>.<EXP_x> are folders containing the different experiments conducted at one given step (with different parameters/methods). After inspection of the results, one of them is supposed to be renamed as <SOMETHING>.RELEASE and the others can be deleted.

Particular files:

nomenclature.py: file fixing the set of naming rules in working directories

- this file should not be modified.

parameters.py: file defining the set of parameters useful for all the process steps (parameters are all prefixed with respect to the step they are used in).

- it is a "template" file which should be duplicated and whose copy can be modified by the user to its convenience (only the parameter values should be modified, not their name...).

The scripts of steps 1-fuse.py, 2-mars.py, 3-manualcorrection.py, 4-astec.py, 5-postcorrection.py are executables, so that each astec step calling can be made as described here: For example, in order to launch the fusion step on an embryo called "171107-Karine-St8" one should:

1. Duplicate the file <astec-package>/parameters.py and rename it as a new file <arbitrary-path>/parameters_karine.py
2. Edit this new file <arbitrary-path>/parameters_karine.py to specify the desired value of each parameter related to the fusion step
3. In a terminal,


```
$ cd <astec-package> # in order to be in the astec directory
(/media/DATA/Codes/astec-package on Hermione)
$ ./1-fuse.py --parameters <arbitrary-path>/parameters_karine.py
--embryo-rep /media/DATA/171107-Karine-St8/
(or equivalently, a shorter format)
$ ./1-fuse.py -p <arbitrary-path>/parameters_karine.py -e
/media/DATA/171107-Karine-St8/
```

At each Astec step execution, a copy of the parameters file as well as a log file are automatically generated in the target working directory.

For each Astec step, it is possible to display the help relative to the corresponding script by launching the script with the option '--help'. For example, for the fusion step:

In a terminal, launch the command line:

```
$ <astec-package>/1-fuse.py --help
```

The terminal displays the following message:

```
Usage: 1-fuse.py [options]
Options:
-h, --help show this help message and exit
-p FILE, --parameters=FILE
python file containing parameters definition
-e PATH, --embryo-rep=PATH
path to the embryo data
-q, --quiet don't print status messages to stdout
```

2.1 The parameters-example.py file

[Pay attention] The file included below may be not up-to-date with respect to the software package. Please refer to the file parameters-example.py of the ASTEC distribution.

```
1
2
3 # File for parameters and nomenclature settings
4
5 PATH_EMBRYO=''
6
7 # Must be the path to the embryo data
8 # eg: '/media/DATA/171107-Karine-St8'
9 # Can also be set by providing option '-e' in the command line
10 EN=''
```

```

10             # Embryo Name
11             # CRBM naming format is YYYYDD-SaintOfTheDays-Stage
12 # eg: '171107-Karine-St8'
13 # (automatically extracted from PATH_EMBRYO if not provided)
14
15
16 EXP_FUSE=''
17             # Workspace for the considered step; this workspace
18 # is included in the repository corresponding to the
19 # ASTEC step ([FUSE|SEG|POST]) and prefixed by this
20 # repository name (eg. FUSE_EXP_NO_CROP or SEG_RELEASE)
21 # ----> use replaceDIR_EXP_FUSE(path_fuse_exp[...], EXP_FUSE)
22 # method from <astec-package>/nomenclature.py to get the right
23 # path for the given fusion experience
24 EXP_REG=''
25             #
26 EXP_MARS=''
27             #
28 EXP_SEG=''
29             #
30 EXP_POST=''
31             #
32
33
34 #TIME=''# Time-point of an embryo snapshot
35 #TIMEREF=''# For registration, time-point of reference snapshot
36 #TIMEFLO=''# For registration, time-point of floating snapshot
37
38 #####
39 ### GENERAL PARAMETERS ###
40 #####
41
42 begin=1
43             # First time point
44 end=5
45             # Last time point
46 delta=1
47             # Delta between two time points (if one does not want
48 # to deal with every single time point) (default = 1)
49 target_resolution = .3
50             # Isotropic resolution of the final fused and
51 # segmented images (default = 0.3)
52
53
54 #####
55 ### RAWDATA DEFINITION ###
56 #####
57
58 raw_ori = 'left'
59             # if im2 angle - im1 angle < 0 => right
60 raw_resolution = (.17, .17, 1.)

```

```

61                                     # Resolution of the raw images (here are the
62 # known values for 140317-Patrick-St8)
63 #raw_resolution = (.21, .21, 1.)
64                                     # Resolution of the raw images for Karine
65 raw_delay = 0
66                                     # If the time stamps in the folder are not the
67 # actual time stamps in the global movie
68 raw_mirrors = False
69                                     # Depends on the acquisition protocol, value
70 # can be set to True or False
71 # - the standard value of this parameter is
72 #   False
73 # - in case of axial symmetry between left
74 #   and right cameras, then set to True
75
76
77
78
79 #####
80 ### FUSION PARAMETERS ###
81 #####
82
83 fusion_margin_x_0 = 40
84                                     # margin_x_0 [default=40]: parameter for margin of the
85 # bounding box computed for the cropping of the
86 # resampled image in 'left' x direction
87 fusion_margin_x_1 = 40
88                                     # margin_x_1 [default=40]: parameter for margin of the
89 # bounding box computed for the cropping of the
90 # resampled image in 'right' x direction
91 fusion_margin_y_0 = 40
92                                     # margin_y_0 [default=40]: parameter for margin of the
93 # bounding box computed for the cropping of the
94 # resampled image in 'top' y direction
95 fusion_margin_y_1 = 40
96                                     # margin_y_1 [default=40]: parameter for margin of the
97 # bounding box computed for the cropping of the
98 # resampled image in 'bottom' y direction
99 fusion_crop = True
100                                     # crop [default=True]:
101                                     # if False, then the resampled image is not cropped ;
102 # if True, then image is cropped
103
104
105
106
107 #####
108 ### MARS PARAMETERS ###
109 #####
110
111 # Modules choice

```



```

112 mars_method=1
113             # 1 for 'Classic' method
114     # 2 for 'Gace' method
115 # General parameters for MARS segmentation
116 mars_sigma1 = 0.6
117             # sigma 1 (0.6um) in real coordinates
118 mars_sigma2 = 0.15
119             # sigma 2 (0.15um) in real coordinates
120 mars_h_min = 4
121             # H min initialisation to ease correction
122 # Gace Parameters (if mars_method is set to 2):
123 # membrane_reinforcement
124 mars_sigma_membrane=0.9
125             # membrane enhancement parameter (in real units, a
126 # priori 0.9 um is a good choice for data like
127 # Patrick/Ralph/Aquila)
128 # anisotropicHist /\ critical step
129 mars_sensitivity=0.99
130             # membrane binarization parameter, /\ if failure,
131 # one should enter in "manual" mode of the function
132 # anisotropicHist via activation of 'manual' option
133
134 mars_manual=False
135             # By default, this parameter is set to False. If
136 # failure, (meaning that thresholds are very bad,
137 # meaning that the binarized image is very bad),
138 # set this parameter to True and relaunch the
139 # computation on the test image. If the method fails
140 # again, "play" with the value of manual_sigma...
141 # and good luck.
142 mars_manual_sigma=15
143             # Axial histograms fitting initialization parameter
144 # for the computation of membrane image binarization
145 # axial thresholds (this parameter is used iif
146 # manual = True).
147 # One may need to test different values of
148 # manual_sigma. We suggest to test values between 5 and
149 # 25 in case of initial failure. Good luck.
150
151 mars_hard_thresholding=False
152             # If the previous membrane threshold method
153 # failed, one can force the thresholding with a
154 # "hard" threshold applied on the whole image.
155 # To do so, this option must be set to True.
156 mars_hard_threshold=1.0
157             # If hard_thresholding = True, the enhanced
158 # membranes image is thresholded using this
159 # parameter (value 1 seems to be ok for
160 # time-point t001 of Aquila embryo for example).
161
162 # Tensor voting framework

```

```

163 mars_sigma_TV=3.6
164             # parameter which defines the voting scale for membrane
165 # structures propagation by tensor voting method (real
166 # coordinates).
167 # This parameter should be set between 3 um (little cells)
168 # and 4.5 um(big gaps in the binarized membrane image)
169 mars_sigma_LF=0.9
170             # Smoothing parameter for reconstructed image (in real
171 # coordinates). It seems that the default value = 0.9 um
172 # is ok for classic use.
173 mars_sample=0.2
174             # Parameter for tensor voting computation speed
175 # optimisation (do not touch if not bewared)
176
177
178
179 #####
180 ### MANUAL CORRECTION PARAMETERS ###
181 #####
182
183 mancor_seg_file=''
184             # segmentation file to be manually corrected
185 # If not provided, then looking for the output of MARS
186 mancor_mapping_file=''
187             # path to mapping file for manual correction of the
188 # mars segmentation. See above the syntax of this file.
189 # - 1 line per label association
190 # - background label has value 1
191 # - the character '#' denotes commented lines
192 # See file "mapping.txt" in the astec project to get an
193 # example
194 '''
195 # EXAMPLE OF mancor_mapping_file CONTENT:
196 # here the input label 8 will be mapped with new value 7, etc...
197 8 7
198 9 2
199 4 64
200 29 23
201 # ... etc ...
202 # background labels
203 30 1
204 89 1
205 '''
206
207
208
209 #####
210 ### SEGMENTATION PROPAGATION PARAMETERS ###
211 #####
212
213

```

```

214 # Modules choice
215
216 astec_membrane_reconstruction_method=0
217 # Membrane reconstruction module choice
218 # 0 for 'Classic' method
219 # 1 for 'Glace' method
220 # 2 for 'Gace' method
221 # If not set or set to 0, the input fused image is not processed for
222 # membrane structures enhancement.
223 # If set to 1, the GLACE reconstruction method is going to be called
224 # If set to 2, the GACE reconstruction method is going to be called
225
226 astec_fusion_u8_method=0
227 # Selection of the method which converts the fused image into a 8 bits
228 # images for the segmentation propagation.
229 # If set to 0 (default), calling the historical "to_u8" method
230 # If set to 1, calling the mc-adhocFuse program which enhances the fused
231 # image while converting it to u8 knowing the semgnetation propagation from
232 # previous time point
233
234 astec_flag_hybridation=False
235 # If set to True and if the membrane_reconstruction_method parameter is
236 # provided and not equal to 0, then the reconstructed gray level image
237 # used for the segmentation propagation framework is goind to be an hybridation
238 # between the original fused image and the result of image reconstruction by
239 # the specified method.
240
241 astec_keep_reconstruct_files=False
242 # Set it to True in order to keep a copy
243 # Flag enabling to keep a copy of graylevel files provided to the watershed
244
245
246
247 # General parameters for segmentation propagation
248 astec_sigma1 = 0.6
249                                     # sigma 1 (0.6um) in real coordinates
250 astec_sigma2 = 0.15
251                                     # sigma 2 (0.15um) in real coordinates
252 astec_h_min_min = 2
253                                     # H min initialisation to ease correction
254 astec_h_min_max = 18
255                                     # H min initialisation to ease correction
256
257 # Glace Parameters (if astec_membrane_reconstruction_method is set to 1 or 2):
258 # membrane_reinforcement
259 astec_sigma_membrane=0.9
260                                     # membrane enhancement parameter (in real units, a
261 # priori 0.9 um is a good choice for data like
262 # Patrick/Ralph/Aquila)
263 # anisotropicHist /\ critical step
264 astec_sensitivity=0.99

```

```

265             # membrane binarization parameter, /\ if failure,
266 # one should enter in "manual" mode of the function
267 # anisotropicHist via activation of 'manual' option
268
269 astec_manual=False
270             # By default, this parameter is set to False. If
271 # failure, (meaning that thresholds are very bad,
272 # meaning that the binarized image is very bad),
273 # set this parameter to True and relaunch the
274 # computation on the test image. If the method fails
275 # again, "play" with the value of manual_sigma...
276 # and good luck.
277 astec_manual_sigma=15
278             # Axial histograms fitting initialization parameter
279 # for the computation of membrane image binarization
280 # axial thresholds (this parameter is used iif
281 # manual = True).
282 # One may need to test different values of
283 # manual_sigma. We suggest to test values between 5 and
284 # 25 in case of initial failure. Good luck.
285
286 astec_hard_thresholding=False
287             # If the previous membrane threshold method
288 # failed, one can force the thresholding with a
289 # "hard" threshold applied on the whole image.
290 # To do so, this option must be set to True.
291 astec_hard_threshold=1.0
292             # If hard_thresholding = True, the enhanced
293 # membranes image is thresholded using this
294 # parameter (value 1 seems to be ok for
295 # time-point t001 of Aquila embryo for example).
296
297 # Tensor voting framework
298 astec_sigma_TV=3.6
299             # parameter which defines the voting scale for membrane
300 # structures propagation by tensor voting method (real
301 # coordinates).
302 # This parameter should be set between 3 um (little cells)
303 # and 4.5 um (big gaps in the binarized membrane image)
304 astec_sigma_LF=0.9
305             # Smoothing parameter for reconstructed image (in real
306 # coordinates). It seems that the default value = 0.9 um
307 # is ok for classic use.
308 astec_sample=0.2
309             # Parameter for tensor voting computation speed
310 # optimisation (do not touch if not beware)
311 astec_rayon_dil=3.6
312             # dilatation ray for propagated ROI from time t to t+1
313 # (default: 3.6, in real coordinates)
314
315 # Fused image conversion parameters (if astec_fusion_u8_method is set to 1)

```

```

316 astec_min_percentile=0.01
317             # mc-adhocFuse parameter of type %f (default: 0.01)
318 astec_max_percentile=0.99
319             # mc-adhocFuse parameter of type %f (default: 0.99)
320 astec_min_method='cellinterior'
321             # mc-adhocFuse param. (default: 'cellinterior')
322             # taken in global|cell|cellborder|cellinterior|voxel
323 astec_max_method='cellborder'
324             # mc-adhocFuse parameter (default: 'cellborder')
325             # taken in global|cell|cellborder|cellinterior|voxel
326 astec_sigma_hybridation=5.0
327             # mc-adhocFuse parameter of type %f (default: 5.0)
328
329 # Default parameters (for classical use, default values should not be changed)
330 astec_RadiusOpening=20
331             # (using the approximation of a sphere of radius 20
332             # voxels as a structuring element)
333 astec_Thau= 25
334             # s(c)=h2+(c).N2(c) >t identical
335 astec_MinVolume=1000
336             # Miss Suppressing cells with too small volumes (not
337             # yet in supdata)
338 astec_VolumeRatioBigger=0.5
339             # If a cell in St+1 is at least 50% bigger than its
340             # progeny in St+1,
341 astec_VolumeRatioSmaller=0.1
342             # Cells in St+1 that are 10% or smaller than their
343             # equivalent in St+1 are tagged for correction
344 astec_MorphosnakeIterations=10
345             # Then, an active contour algorithm is applied
346             # using the dilated shape of c, obtained by
347             # iterating 10 times
348 astec_NIterations=200
349             # The algorithm is applied up to stability
350             # (at th voxels) or after n iterations
351             # (in our case th = 103 and n = 200).
352 astec_DeltaVoxels=10**3
353             # y (at th voxels)
354 astec_Volum_Min_No_Seed=100
355             # Then, if the volume of c is greater than 100
356             # voxels (2.7 um3)
357 astec_nb_proc=10
358             # Number of processor ...
359 astec_nb_proc_ace=7
360             # number of processors for ACE (7 is recommended)
361
362
363
364 #####
365 ### POST-CORRECTION PARAMETERS ###
366 #####

```

```

367
368 postcor_Volume_Threshold=10000
369             # volume low threshold for final cells
370 # (in voxels)
371 postcor_Soon=True
372             # True if the cell life span has to be
373 # taken into account
374 postcor_ShortLifespan=25
375             # (length < SL time points, in our case
376 # SL = 10)
377 postcor_PearsonThreshold=0.9;
378             # If they are anticorrelated (Pearson
379 # correlation under -0.9)
380
381
382

```

2.2 List of command line interfaces

2.2.1 1-fuse.py

2.2.1.1 Basic use

Assume that the raw data are organized as follows

```

180927-Vincent-Nodal-St8
├── RAWDATA
│   ├── LC
│   │   ├── Stack0000
│   │   │   ├── Cam_Left_00000.zip
│   │   │   ├── Cam_Left_00001.zip
│   │   │   └── ...
│   │   ├── Stack0001
│   │   │   ├── Cam_Left_00000.zip
│   │   │   ├── Cam_Left_00001.zip
│   │   │   └── ...
│   └── RC
│       ├── Stack0000
│       │   ├── Cam_Right_00000.zip
│       │   ├── Cam_Right_00001.zip
│       │   └── ...
│       ├── Stack0001
│       │   ├── Cam_Right_00000.zip
│       │   ├── Cam_Right_00001.zip
│       │   └── ...

```

Then a simple parameter file (see `astec-parameters-examples/parameters-fusion-vincent-1.py`) allows to perform the fusion

```
# path to the embryo data
```

```
PATH_EMBRYO = '/Users/greg/COLLABORATIONS/DIGEM/DATA/TEST/180927-Vincent-Nodal-St8'
```

```
# Embryo Name
```

```

EN = '180927-Vincent-Nodal-St8'

# time points to be processed
# begin: first time point
# end: last time point
# delta: Delta between two time points (if one does not want
#       to deal with every single time point) (default = 1)

begin = 0
end = 0
delta = 1

# output image format
#
# default_image_suffix is for all images (including auxiliary ones)

default_image_suffix = 'mha'

# raw_ori: image orientation, value is in {'right','left'}
#   if im2 angle - im1 angle < 0 => raw_ori = 'right'
# raw_mirrors: value is in {True, False}
#   - the standard value of this parameter is False
#   - in case of axial symmetry between left
#     and right cameras, then set to True
# raw_resolution: acquisition voxel size
# raw_delay: increment to to be added to the time values
#   (values in range [begin,end]) when generating the
#   fused image names (default is 0)

raw_ori = 'left'
raw_mirrors = False
raw_resolution = (.195, .195, 1.)
raw_delay = 0

# Isotropic resolution of the final fused image

target_resolution = .3

```

2.2.1.2 Other organization of data

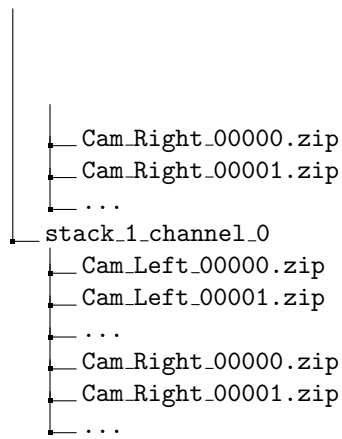
In case of the data organization does not follow the one described in section 2.2.1.1, the variables `DIR_RAWDATA`, `DIR_LEFTCAM_STACKZERO`, `DIR_RIGHTCAM_STACKZERO`, `DIR_LEFTCAM_STACKONE`, and `DIR_RIGHTCAM_STACKONE` allow a finer control of the directory names, while the variables `acquisition_leftcam_image_prefix` and `acquisition_rightcam_image_prefix` allow a finer control of the file names.

For instance, assume that the data are organized as below:

```

180927-Vincent-Nodal-St8
├── RAWDATA
│   └── stack_0_channel_0
│       ├── Cam_Left_00000.zip
│       ├── Cam_Left_00001.zip
│       └── ...

```



Then, fusion can be achieved by adding the following lines to the parameter file of section 2.2.1.1 (see `astec-parameters-examples/parameters-fusion-vincent-2.py`).

```
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',
```

2.2.2 1.5-intraregistration-GC.py

2.2.3 1.5-intraregistration.py

2.2.4 2-mars.py

2.2.5 3-manualcorrection.py

2.2.6 4-astec.py

2.2.7 5-postcorrection.py

2.2.8 6-named.py

2.2.9 7-virtualembryo.py

Chapter 3

Tutorial

Before starting

It is advised to add to your PATH environment variable the path to the astec package. 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-package
```

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

3.1 Starting with a toy embryo

Say we have an embryo named 160708-Tutorial with four time points (these are the four first time points of the embryo 160708-Aquila-St8). Thus at the beginning, we only have the raw data in the <EMBRYO>=160708-Tutorial file tree structure.

```
<EMBRYO>=160708-Tutorial
├── RAWDATA
│   ├── LC
│   │   ├── Stack0000
│   │   │   ├── Cam_Left.00001.zip
│   │   │   ├── Cam_Left.00002.zip
│   │   │   ├── Cam_Left.00003.zip
│   │   │   └── Cam_Left.00004.zip
│   │   ├── Stack0001
│   │   │   ├── Cam_Left.00001.zip
│   │   │   ├── Cam_Left.00002.zip
│   │   │   ├── Cam_Left.00003.zip
│   │   │   └── Cam_Left.00004.zip
│   └── RC
│       ├── Stack0000
│       │   ├── Cam_Right.00001.zip
│       │   ├── Cam_Right.00002.zip
│       │   ├── Cam_Right.00003.zip
│       │   └── Cam_Right.00004.zip
│       └── Stack0001
│           ├── Cam_Right.00001.zip
│           └── Cam_Right.00002.zip
```

```

├─ Cam_Right_00003.zip
├─ Cam_Right_00004.zip

```

3.2 Fusing data

Let start by fusing data. I advised to create a **PARAMETERS** repository under the root **<EMBRYO>=160708-Tutorial** to store all the parameters file we will use. Then we copy there the **parameters.py** file and rename it as **parameters-fuse1.py**. I added **-fuse** to easily recognize it as a parameter file for fusing, and also add the numbering 1 to handle the case of several parameters files for fusing (if fusion parameters have to be changed for a few time points).

```

$ cd /path/to/160708-Tutorial
$ mkdir PARAMETERS
$ cp /path/to/astec-package/parameters.py PARAMETERS/parameters-fuse1.py

```

Then, this file **parameters-fuse1.py** is edited to be adapted to our experiment at hand. Here, the modification are as follows. Note that we only have the following changes with respect to the template version of the parameter file (see section 2.1).

```

...
5 PATH_EMBRYO='/Users/greg/COLLABORATIONS/DIGEM/TUTO-ASTEC/160708-Tutorial'
...
9 EN='160708-Tutorial'
...
16 EXP_FUSE='EXP1'
...
44 end=4
...
58 raw_ori = 'right'
...
60 raw_resolution = (.21, .21, 1.)
...
68 raw_mirrors = True
...

```

We have then the following file tree.

```

<EMBRYO>=160708-Tutorial
├─ RAWDATA
│   └─ ...
├─ PARAMETERS
│   └─ parameters-fuse1.py

```

Now we can run the fusion

```

$ cd /path/to/160708-Tutorial
$ 1-fuse.py -p PARAMETERS/parameters-fuse1.py

```

After completion of the fusing stage, the tree structure is

```

<EMBRYO>=160708-Tutorial
├─ RAWDATA
│   └─ ...
├─ PARAMETERS
│   └─ ...

```

```

├── FUSE
│   └── FUSE_EXP1
│       ├── 1-fuse.log
│       ├── 160708-Tutorial_fuse_t001.inr
│       ├── 160708-Tutorial_fuse_t002.inr
│       ├── 160708-Tutorial_fuse_t003.inr
│       ├── 160708-Tutorial_fuse_t004.inr
│       └── parameters-fuse1.py

```

The folder FUSE_EXP1 not only contains the fused images, but also a copy of the parameter file (hence the importance that all parameter files have different names) as well as a log file (`1-fuse.log`) that keeps trace of all the Astec launched commands. However, it won't keep trace of the other commands (e.g. the user erases a file), thus it is still important to keep all this elsewhere.

3.3 Segmenting the first time point

The segmentation of the first time point consists in labeling each and every cell of the embryo. By convention, the background has the 1 label (0 is used when label images are resampled). Thus cell labels are always larger or equal than 2.

Although any method can be used to obtain this segmentation, the script `2-mars.py` provide a means to use the MARS method [?].

Again the parameter file `parameters.py` is copied and renamed to eventually edit it.

```

$ cd /path/to/160708-Tutorial
$ cp /path/to/astec-package/parameters.py PARAMETERS/parameters-seg-first1.py

```

Then, this file `parameters-seg-first1.py` is edited. The changes with respect to the template version of the parameter file (see section 2.1) are

```

...
5 PATH_EMBRYO='/Users/greg/COLLABORATIONS/DIGEM/TUTO-ASTEC/160708-Tutorial'
...
9 EN='160708-Tutorial'
...
16 EXP_FUSE='EXP1'
...
26 EXP_MARS='EXPMARS1'
...

```

Note that the line `#16` indicates where to find the fused images. The segmentation is done through

```

$ cd /path/to/160708-Tutorial
$ 2-mars.py -p PARAMETERS/parameters-seg-first1.py

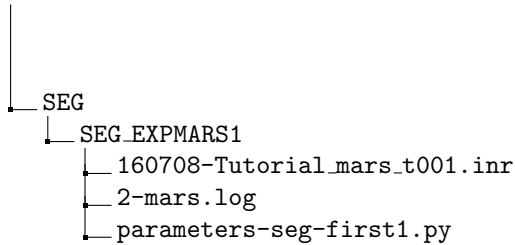
```

After completion of the segmentation stage, the tree structure is

```

<EMBRYO>=160708-Tutorial
├── RAWDATA
│   └── ...
├── PARAMETERS
│   └── ...
├── FUSE
│   └── FUSE_EXP1
│       └── ...

```



Note the name of the result image with the `_mars` suffix. Such a naming convention is mandatory and has to be followed in case of segmentation by other means.

3.4 Correcting the first time point segmentation

As the sequence segmentation proceeds by propagation, the first segmentation must be cured. Under-segmentations may be corrected either by changing segmentation parameters and to relaunch the `2-mars.py` script or by manual edition (then be careful not to use an already used label). Over-segmentations can be automatically corrected (after identification). They have to be listed into a file whom lines contains two numbers, the labels to be merged (more precisely, only the second label will be kept) . E.g.

```
43 54
38 20
```

means that cells of labels 43 and 54 will be merged together (forming a cell of label 54), as well as cells of labels 38 and 20 (forming a cell of label 20). The file `/path/to/astec-package/mapping.txt` is a template for this merging.

Again, copy the parameter template file, as well as the merging template file (cells to be merged have to be identified beforehand).

```
$ cd /path/to/160708-Tutorial
$ cp /path/to/astec-package/parameters.py PARAMETERS/parameters-seg-correction1.py
$ cp /path/to/astec-package/mapping.txt PARAMETERS/mancor_mapping_file.txt
```

and edit the two files. The second file contains only the two lines indicated above. The changes with respect to the template version of the parameter file are

```
...
5 PATH_EMBRYO='/Users/greg/COLLABORATIONS/DIGEM/TUTO-ASTEC/160708-Tutorial'
...
9 EN='160708-Tutorial'
...
16 EXP_FUSE='EXP1'
...
26 EXP_MARS='EXPMARS1'
...
28 EXP_SEG='EXPSEG1'
...
186 mancor_mapping_file='PARAMETERS/mancor_mapping_file.txt'
...
```

Note that the line `#186` gives the (here relative) name of the file containing the cells to be merged. The MARS result is supposed to be found in the `SEG/EXPMARS1` folder (given by the variable `EXP_MARS`) while the correction result will be placed into the `SEG/EXPSEG1` folder (given by the variable `EXP_SEG`)

```
$ cd /path/to/160708-Tutorial
$ 3-manualcorrection.py -p PARAMETERS/parameters-seg-correction1.py
```

After completion of this stage, we have:

```

<EMBRYO>=160708-Tutorial
├── RAWDATA
│   └── ...
├── PARAMETERS
│   └── ...
├── FUSE
│   └── FUSE_EXP1
│       └── ...
├── SEG
│   ├── SEG_EXPMARS1
│   │   └── ...
│   └── SEG_EXPSEG1
│       ├── 160708-Tutorial_seg_t001.inr
│       ├── 3-manualcorrection.log
│       ├── mancor_mapping_file.txt
│       └── parameters-seg-correction1.py

```

Note the name of the result image with the `_seg` suffix.

3.5 Segmentation propagation

```

$ cd /path/to/160708-Tutorial
$ cp /path/to/astec-package/parameters.py PARAMETERS/parameters-astec.py

```

```

...
5 PATH_EMBRYO='/Users/greg/COLLABORATIONS/DIGEM/TUTO-ASTEC/160708-Tutorial'
...
9 EN='160708-Tutorial'
...
16 EXP_FUSE='EXP1'
...
28 EXP_SEG='EXPSEG1'
...
44 end=4
...

```

```

$ cd /path/to/160708-Tutorial
$ 4-astec.py -p PARAMETERS/parameters-astec.py

```