Reference document: Tracking Cells and Analyzing images

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1 Installation

All the guidelines here apply to Windows 7 system, but similar procedures could be followed in other operational systems. To run the tracking software described in [1], a PC with a GPU with CUDA capabilities[1] is required. If you have this, install the CUDA toolkit, available in `https://developer.nvidia.com/cuda-toolkit-archive`.

1.1 Download the C program

1. Download the ZIP file in `http://www.janelia.org/sites/default/files/Labs/Keller%20Lab/TGMM_Supplementary_Software_1_0.zip`
2. Unzip the file into a desired folder
3. Copy the file `$C_FOLDER$/data/TGMM_configFile.txt` to any folder you want, so that you can edit it while keeping the original[2]
4. It is highly recommended that you read the `$C_FOLDER$/README.txt` file

*1st version by Eduardo Olimpio

[1]More information found in `http://docs.nvidia.com/cuda/cuda-c-programming-guide/index.html#axzz3ejDLnsIT`

[2]We refer to `$C_FOLDER$` as the folder where you extracted the C software

1.2 Download GitHub

If you do not have GitHub installed, download it from `https://windows.github.com/`. Be familiar with the simple git functions, it is indeed very simple and useful. The task here is to clone a repository (where the analysis code is) to your machine, in a folder that we will refer as `$GIT_FOLDER$`. Is you want to do it as fast as possible, follow the simple instructions (up to step 3) in `https://help.github.com/articles/getting-started-with-github-for-windows/`. The URL of the repository is `https://github.com/epolimpio/image_extraction.git`.

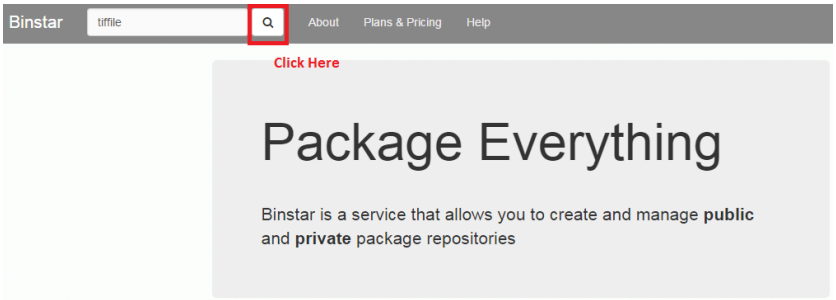
If you want to start to change the code from this version, just fork the project to your Desktop. This option is the best if you want to keep developing the code using the functionalities of GitHub. The instructions to do it are found in `https://guides.github.com/activities/forking/`.

1.3 Install Python

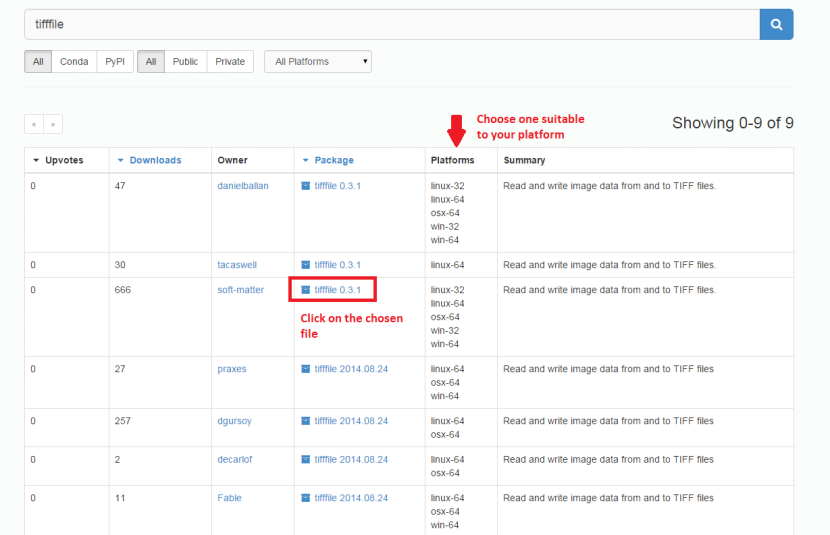
All the analysis are done in Python, so if you do not have Python installed, it is time to do so. For Windows I recommend the Anaconda distribution, but feel free to install any distribution you want. We will need some extra packages, which I explain below. The easiest way install the packages is by using the command line[3] and type the command:

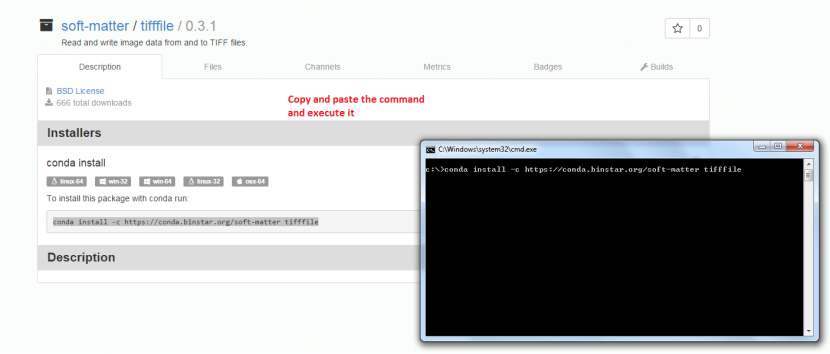
```
conda install <package_name>
```

If the package is not available, the easiest way to get it is going to `https://binstar.org/` and search for the package in the top left of the page. In the example below we show how to do it to install the *tifffile* package.



[3] Accessible in Windows by running `cmd.exe` in the run prompt (Windows+R in the keyboard)





1.3.1 Necessary Packages

There are several extra packages needed to run the analysis. The packages marked with * come with the basic Anaconda installation.

1. tifffile
2. lxml
3. numpy*
4. matplotlib*

1.4 Running a test of the C software

To figure out if the tracking software is properly working, you need to change the configuration file and run an example (already provided with the software) and check if there are results coming out from it.

1.4.1 Changing the configuration file

The configuration file contains many parameters used to run the tracking software. It is important to be familiar with how to use this, as explainend in `https://www.janelia.org/sites/default/files/Labs/Keller%20Lab/TGMM_UserGuide.v2.pdf`. Here we will teach the basics on how to run the tracking program.

1. Open the copy of the configuration file you made during installation of the tracking software, in the path that we call `$CONFIG_PATH$`
2. Change the line containing imgFilePattern to imgFilePattern=`$C_FOLDER$/data/data/TM?????_timeFused_blending/SPC0_CM0_CM1_CHN00_CHN01.fusedStack_?????`
3. Change the line containing debugPathPrefix to debugPathPrefix=`$RESULTS_FOLDER$`

Here, replace `$C_FOLDER$` by the folder where you extracted the tracking program and `$RESULTS_FOLDER$` by a folder of your choice created to handle the results of the tracking program. Each run of the program will generate a new folder inside the chosen one. Remember that the folder of the variable imgFilePattern MUST use slash and for debugPathPrefix you MUST use backslash.

1.4.2 Changing the batch file

The batch files are used to make it easier to run the tracking software. There are three different batch files in the folder `$GIT_FOLDER$`:

1. `segmentation.bat`: Used to run the pre-Track program, required to run the main program.
2. `bayesian_pos.bat`: Runs the main program

3. `seg_analysis.bat`: Used to run the segmentation analysis in debug (with report) if the segmentation program fails.

We need to change these files to provide them with the right path. To edit these files, right click on them and select "Edit". The changes you must provide in all of them are the same. You need to change the line with `c_folder` to include the path you extracted the tracking software (referred here as `$C_FOLDER$`) and the line containing the set of `config_path` should be changed to `$CONFIG_PATH$`.

1.4.3 Running the software

To run the program now, just execute the file `segmentation.bat` (double/click or use command line) and use initial frame 0 and final frame 30. If the program has sucessfully run, you will get the message *"Hierarchical segmentation ran sucessfully"*. Then you can run the file `bayesian_pos.bat`. If everything runs OK, you will find a new folder inside the `$RESULTS_FOLDER$` in a format similar to `GMEMtracking3D_2015_7_10_8_11_23` and inside it you will have two folders with the XML and the binary (`.svb`) files, and a `.txt` with the log of your run.

References

[1] Fernando Amat, William Lemon, Daniel P Mossing, Katie McDole, Yinan Wan, Kristin Branson, Eugene W Myers, and Philipp J Keller. Fast, accurate reconstruction of cell lineages from large-scale fluorescence microscopy data. *Nature methods*, 11(July), 2014.