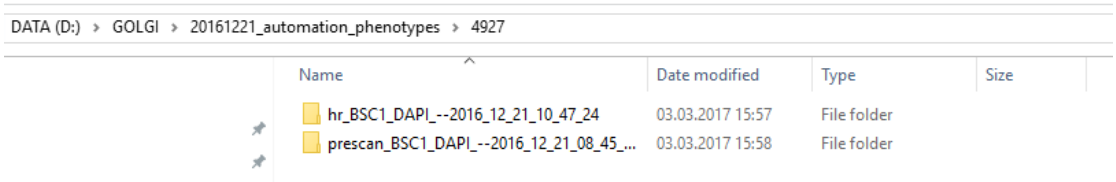
PROCESSING Light Microscopy images for MSite CLEM

Instructions for LM

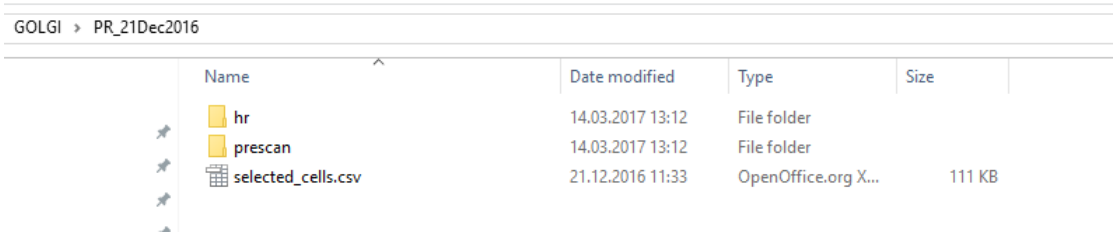
1. Create the following folders:
    - Project folder where all operations will happen (E.g. PR_Name_Date\\Renamed). Then inside create:
        - A folder called **prescan** with the images used for the software to find the selected phenotypes.
        - A folder called **hr** (high resolution) with the final images (Z stack, channel for organelle, nucleus, spots, transmitted light and reflected light).

THE STRUCTURE MUST BE .*renamed**prescan* and .*renamed**hr*. DO NOT REPLACE THE NAMES BY OTHER NAMES, because the scripts will search for this structure.

Search the folder with the images (usually a number, like 4927) and then RENAME the prescan and high resolution respectively. This step is not mandatory, but helps to identify the folders later. If you have naming problems with Windows(name is too long to be copied), you can also rename the previous directories or copy the folder with images to a new directory closer to root, e.g. *C:\Project_name\hr* and *C:\Project_name\prescan*



Copy the .csv file containing all the information from the scans (usually in a file called *selected_cells.csv*) inside the folder of the project.



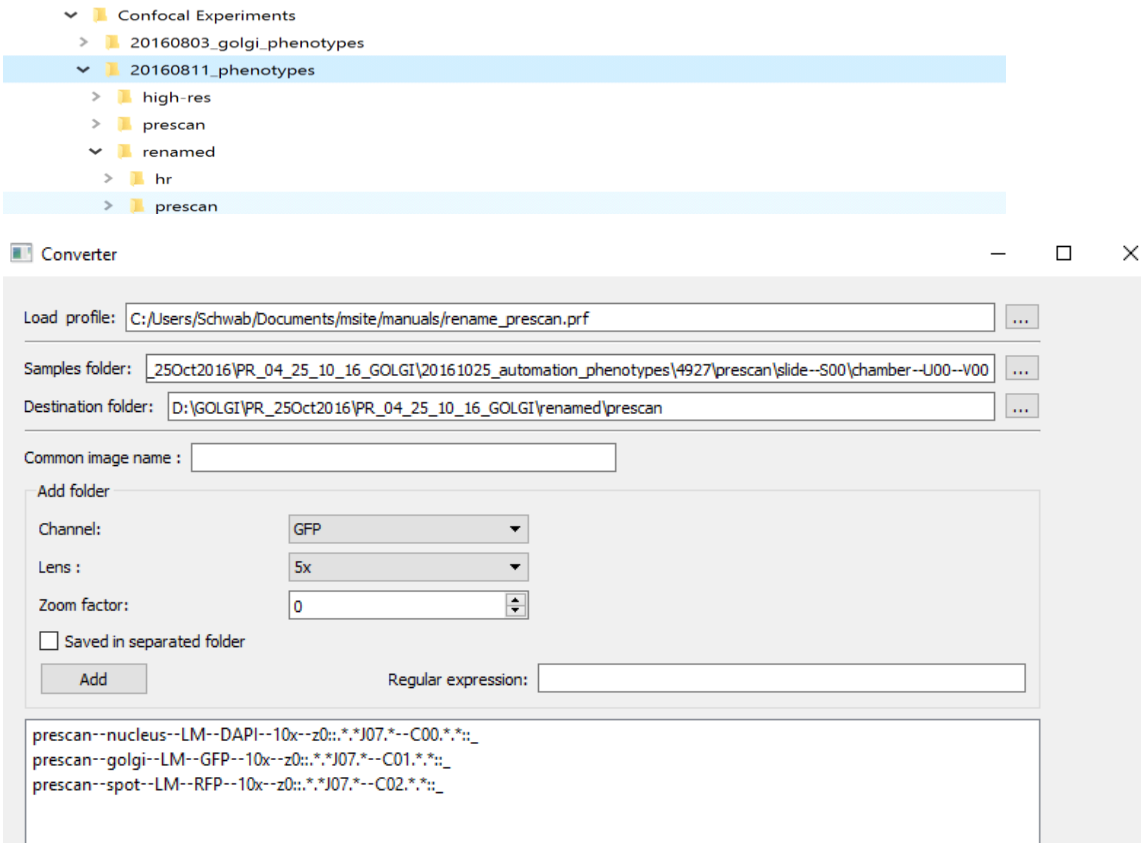
This file has to contain essential information: for each prescan image the coordinate of the region of interest (usually the center of gravity of the object computed in a software like CellProfiler).

Open MSite2 LM-SEM-> I have a LM dataset and move to **Settings->Rename LM files**.

- Load a profile or write a new one. If you don´t know how to do it, use the **Converter manual** instructions in this page.
- Rename the prescan files: the way to rename them is up to the user, **but it is important to remember that prescan pictures must have the suffix prescan in front of them**. The destiny folder must be \\renamed\\prescan

- Repeat for hr, again inside the renamed folder into hr. It can happen that the profile used as example does not apply for your dataset. Have a look, sometimes just changing the Job Number (using a text editor)

- We need to end up with a replica of the other folders but completely renamed.



Here you have a possible example here and also in the folder of this manual with the .prf extension. Load the profile (or create a new one), select first folder where you copied your data and the destiny should be your renamed folder:

Prescan renaming

```
[
  {
    "name": "prescan--nucleus--LM--DAPI--10x--z0",
    "re": ".*.*J07.*--C00.*.*",
    "separated": false
  },
  {
    "name": "prescan--golgi--LM--GFP--10x--z0",
```

```
    "re": ".*.*J07.*--C01.*.*",
    "separated": false
  },
  {
    "name": "prescan--spot--LM--RFP--10x--z0",
    "re": ".*.*J07.*--C02.*.*",
    "separated": false
  }
]
```


HR renaming


```
[
  {
    "name": "nucleus--LM--DAPI--40x--z2",
    "re": ".*.*--J20--.*--C00.*.*",
    "separated": true
  },
  {
    "name": "golgi--LM--GFP--40x--z2",
    "re": ".*.*--J20--.*--C01.*.*",
    "separated": true
  },
  {
    "name": "nucleus--LM--DAPI--10x--z1",
    "re": ".*.*--J21--.*--C00.*.*",
    "separated": false
  },
  {
    "name": "golgi--LM--GFP--10x--z1",
    "re": ".*.*--J21--.*--C01.*.*",
    "separated": false
  },
  {
    "name": "grid--LM--RL - Reflected Light--10x--z1",
    "re": ".*.*--J21--.*--C02.*.*",
    "separated": false
```

```
    },
    {
      "name": "cells--LM--TL -Transmited Light--10x--z1",
      "re": ".*.*--J21--.*--C03.*.*",
      "separated": false
    },
    {
      "name": "spot--LM--RFP--10x--z1",
      "re": ".*.*--J21--.*--C04.*.*",
      "separated": false
    }
  ]
```

In the next step prescan images have to be copied inside **hr** . This will allow the registration script to work properly. However, we have more prescans than hr samples, so the script will select first from the prescan the names that match with the hr jobs.

To proceed, with all the files renamed and ready, open a python editor (pycharm) and execute the following script (in this folder ..\msite\manuals):

```
import regscan
# Folder where your original data is located
main_folder= "D:\\GOLGI\\PR_25Oct2016\\PR_04_25_10_16_GOLGI"
regscan.copyPrescanToHR(main_folder)
```

main_folder is the folder that contains the "renamed" folder and the file *selected_cells.csv*

- Now we need to generate a file with the information of where the center of mass of your organelle of interest is situated. This information is inside the .csv file.

  Execute:

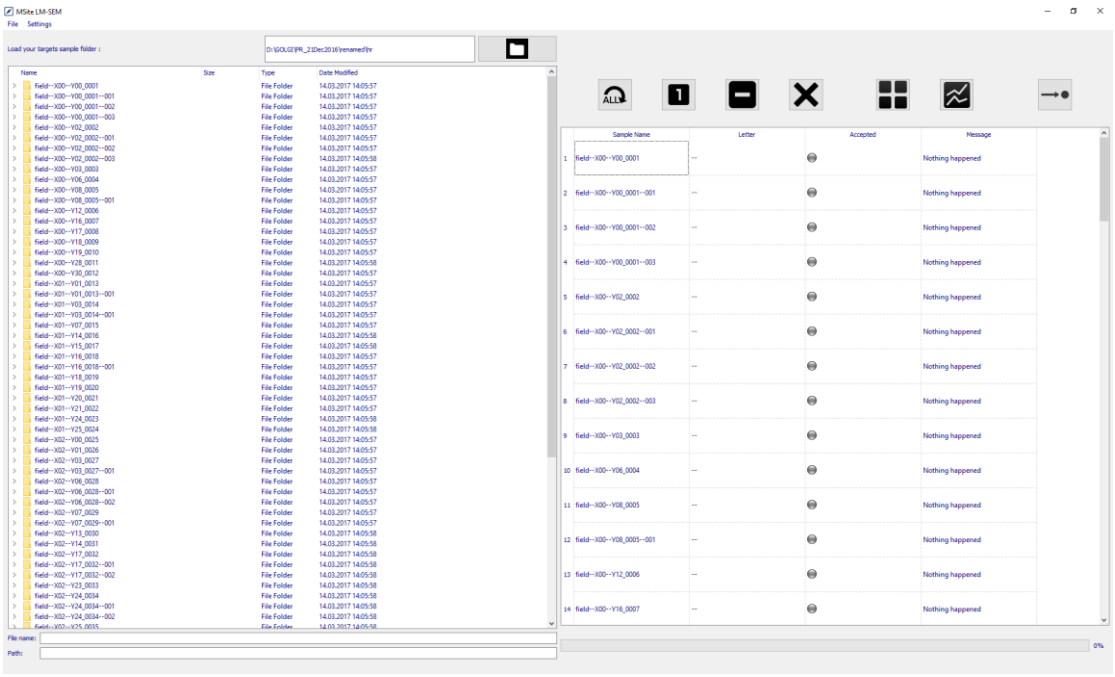
```
shift_folder = main_folder+"\\renamed"

regscan.savePrescanShift(main_folder,shift_folder,separator=',', organ_name
="golgi")
```

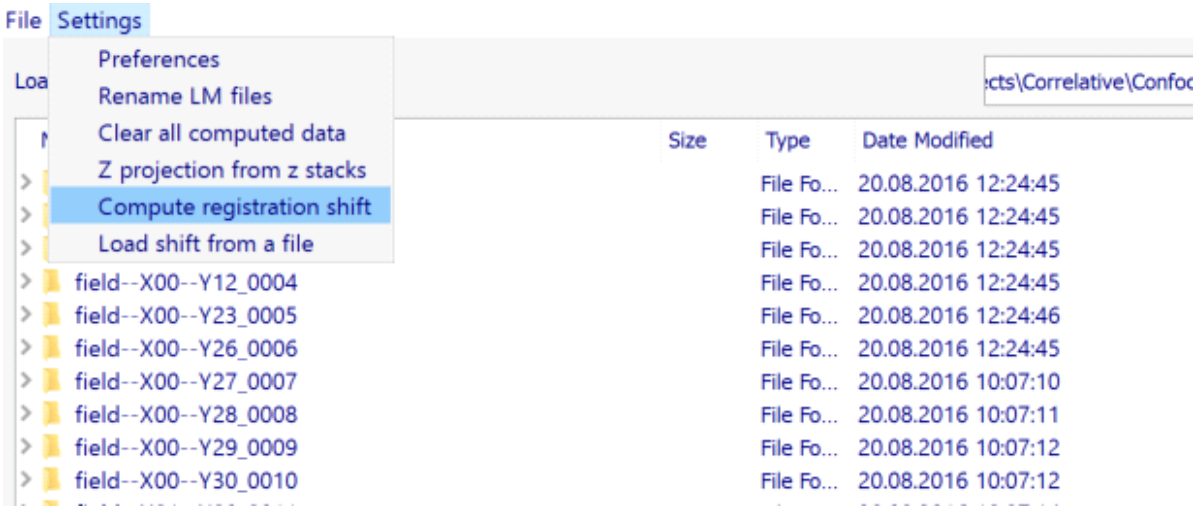
At the end, the folder *renamed* should contain *center.json* and *center_golgi.json*

Note: In case you have problems, check your separator (, or ;, can be indicated with the field separator="") or the syntax of your file. You can also rename your organelle to something different to golgi.

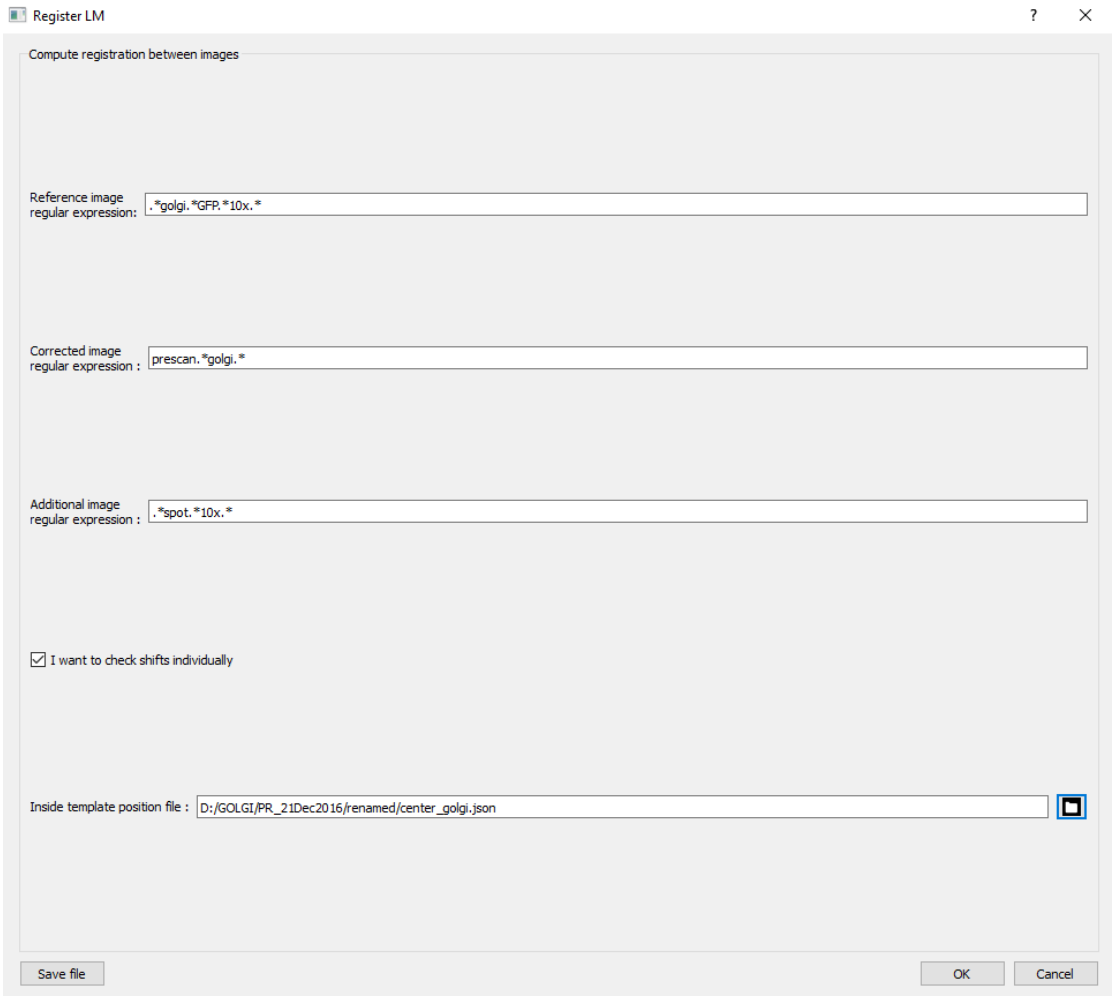
- Open again the MSite2 Bridge and load your targets folder (*renamed/hr*).



Click on **Settings-> Compute registration shift**:



Introduce the two images that you are going to register for all the samples, in a general way.



REFERENCE IMAGE : smallest magnification (10x), to be superimposed to the other channels and RL.

CORRECTED IMAGE: The image from the prescan with bigger magnification respect your reference.

ADDITIONAL IMAGE : Image used for visualizing if your cells are inside the spot of transfection or not.

It is important to click on to check shifts individually, so each image will be valued by the user individually, otherwise, the registration will be taken as good without checking.

Note:

It is better if you first inspect your folder with the viewer. Remember that .* is the wild card for any expression. You can use to generalize the names. E.g. .*golgi.*10x.*

The previous script that you executed saved a list with organelle names and the correspondent position inside the prescan image. You will be prompted now if you have such a file. If you don't want, the shift will be shift respect the center of the prescan image. Otherwise, this position will be calculated and considered your point of interest.

Click on save. This will save a list of computed shifts. If you click compute shifts individually, then each shift will be saved for each picture, otherwise the median and median absolute deviation will be saved for all.