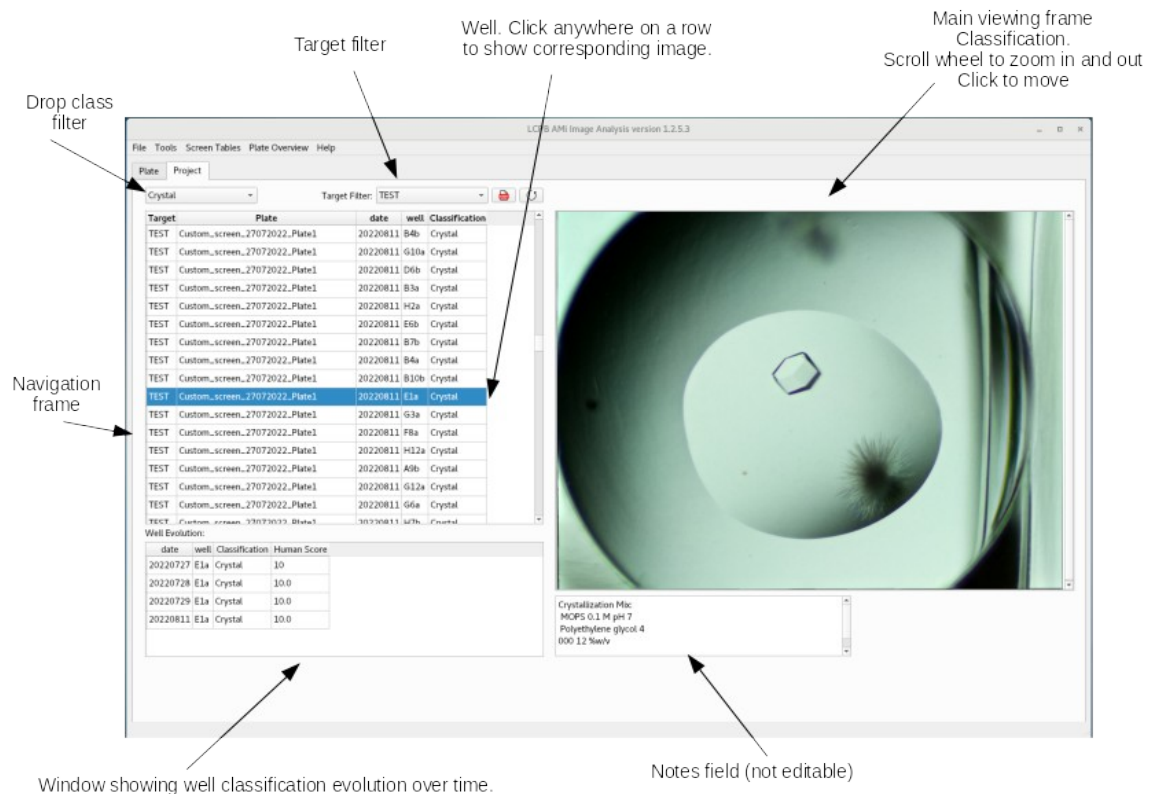# Manual of AMi_Image_Analysis

GUI Overview

Main viewing frame
Classification.
Scroll wheel to zoom in and out
Click to move

Information log

Project details

Viewing filters

Navigation frame

kit selector

Notes frame

Drop classification

autoMARCO current well

Timeline frame

Display heatmap

Click to copy Crystallisation mix to Notes

Drop Score
(e.g as in Fig 1
https://www.nature.com/articles/nprot.2007.198)

Main viewing frame
Classification.
Scroll wheel to zoom in and out
Click to move

Target filter

Well. Click anywhere on a row to show corresponding image.

Drop class filter

Navigation frame

Window showing well classification evolution over time.

Notes field (not editable)
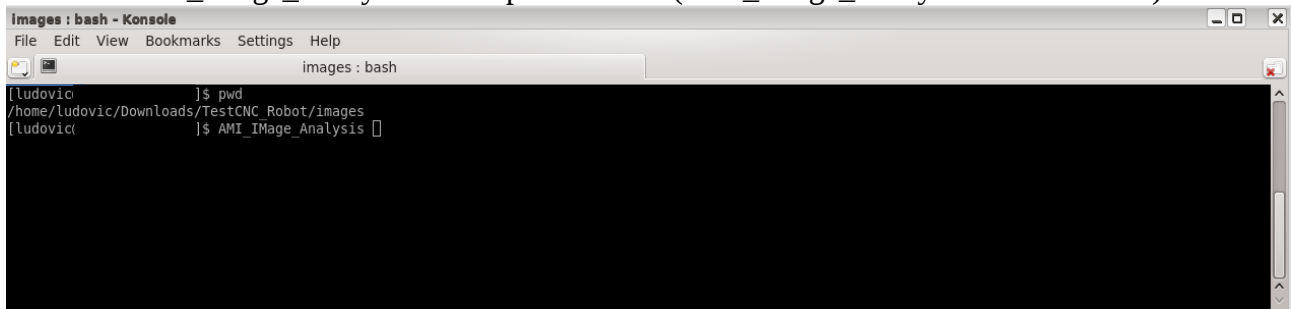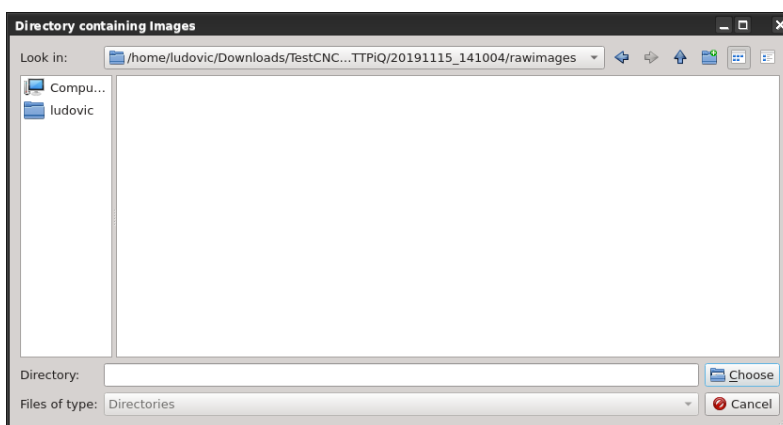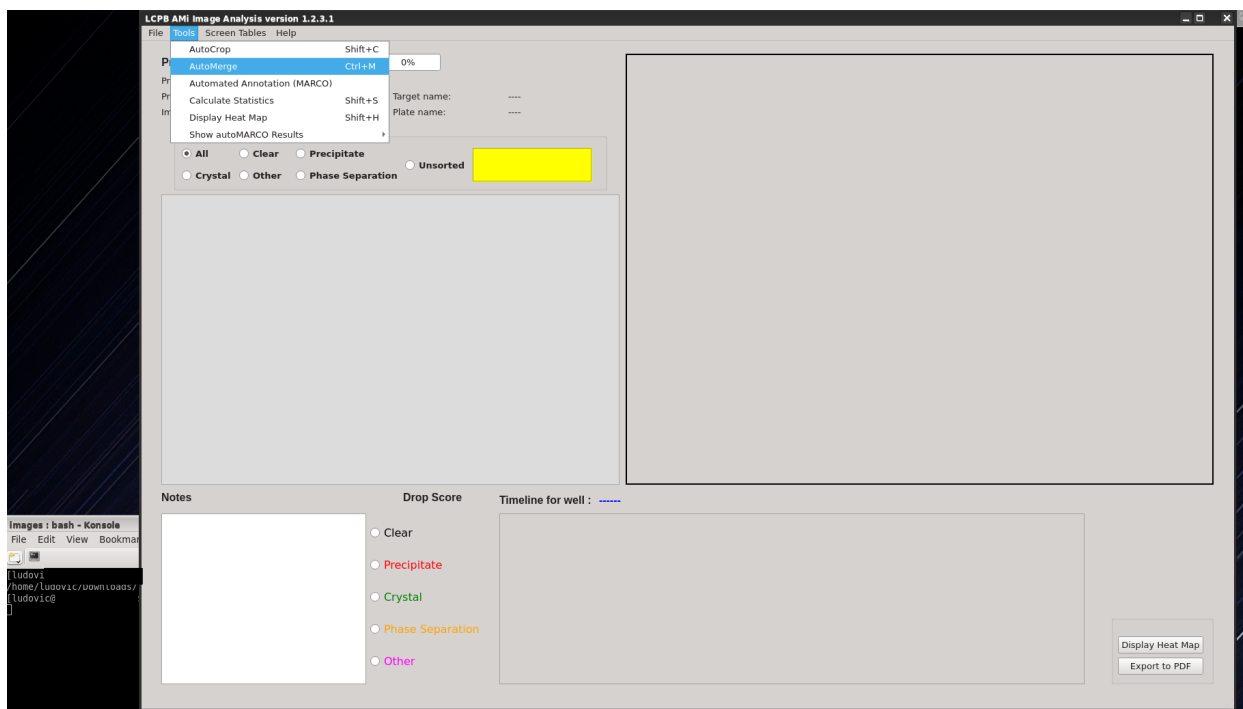
1. To start the program, open a terminal window and change directory to the folder « images » or even downstream (changing directory is not mandatory but may prove more efficient later).

2. Start the program by typing
« AMi_Image_Analysis » then press return. (AMi_Image_Analysis is a bash alias)


3. If you want to merge Z-stacks

 then press « Choose »

The program will now stack images and save the result to a new file in the parent directory (i.e. one directory up « rawimages »).

During processing, the GUI will not respond, don't worry, you can see the progress in the terminal window. It typically takes ~5 minutes with 8 cores at 2 Ghz for a 96 well plate with 3 subwells and 5 images at different focus height.

At the end, you will be prompted to load the new set of images. (as of version 1.2.3.5, merged images are automatically loaded and you can directly analyze your drops and jump to step 5)
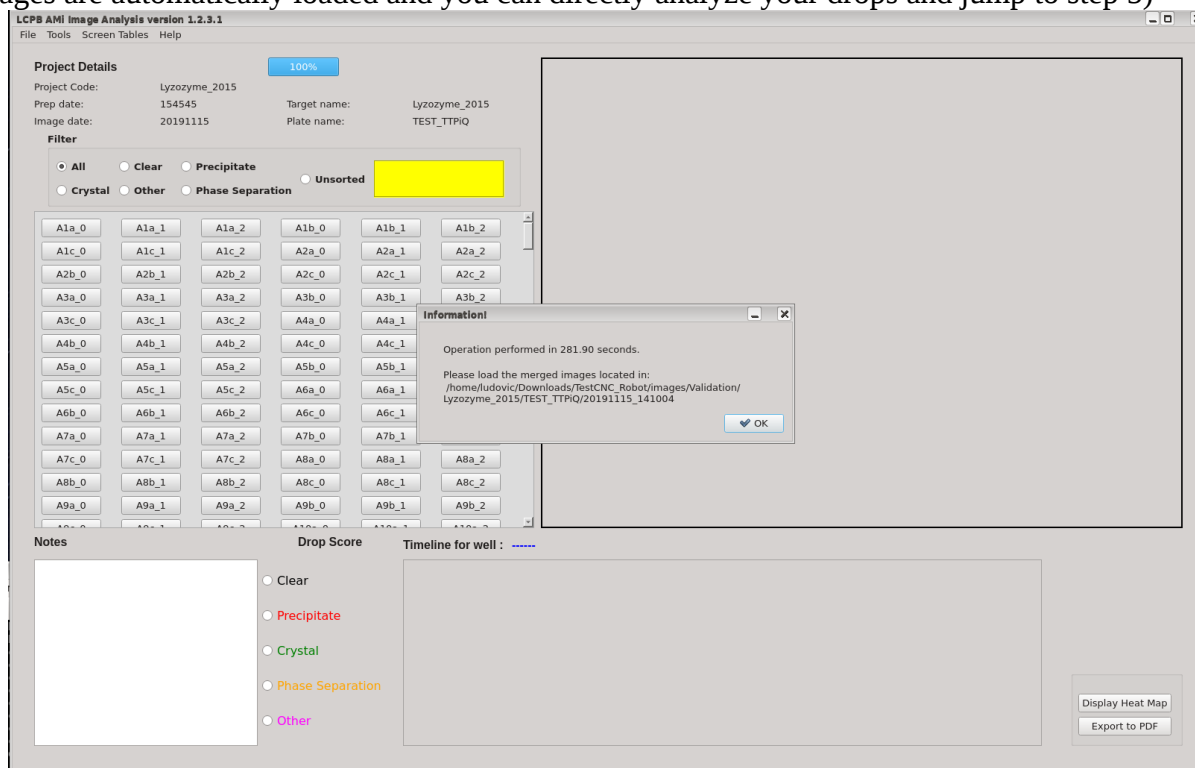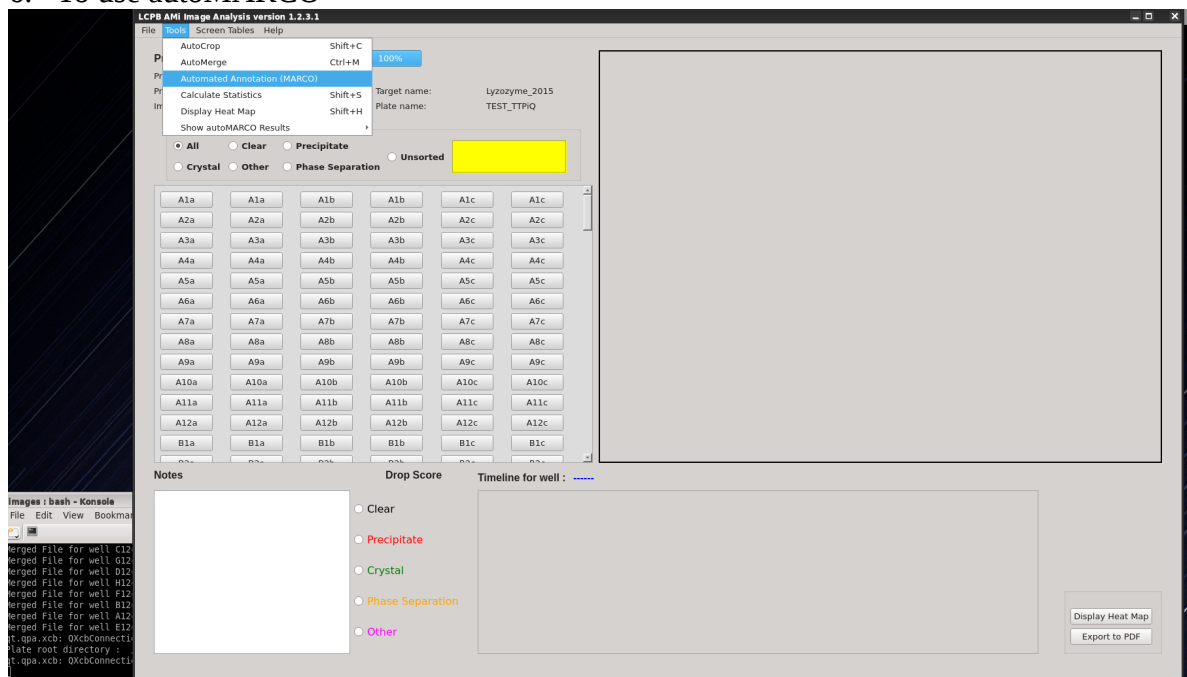

4. Load the stacked images using File-> Open Directory and choose the correct directory.
5. You can now analyse your drops.
   You can use the MARCO artificial intelligence model to automatically classify your drops.
   Do not expect wonders, it is prone to error probably linked to the light source we have.
6. To use autoMARCO

7. Check autoMARCO classification (autoMARCO result is kept if probability is > 0.6).
   You can view the results using Tools->Show autoMARCO Results->selection

8. In the main window, navigate through your wells using the mouse or keyboard shortcuts to check and modify classification. You can change the classification by clicking on the relevant type in the « Drop Score » section or using shortcuts.
    You can navigate through specific classification by selecting the relevant type in the « Filter » section.
    You can zoom and move the picture. Zoom using the scroll wheel.
    You can write some notes in the section « Notes ».

If you have taken pictures of the same plate multiple times, you should see a timeline of the selected well in the « Timeline » section. If you click on the image in the « Timeline » section, it will appear in a new viewing window for further inspection and comparison.

9. When you are happy with your classification results, you can calculate statistics on your plate. Go to Tools->Calculate Statistics
    A table will appear and can be saved as a csv file for archiving.
    You can also display a heatmap of your plate and save a picture for your lab book.
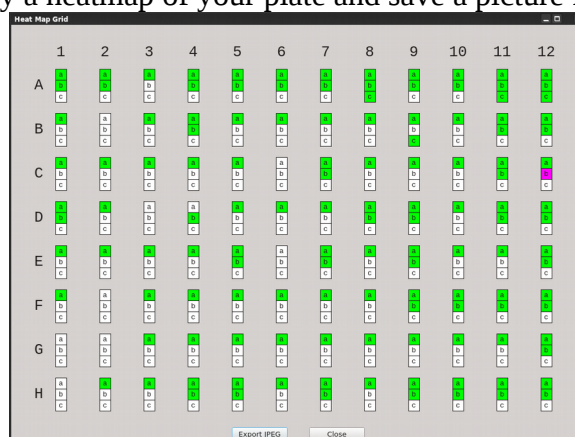
10. If you see promising conditions, you can check the composition of the well by going to Screen Tables->Provider->crystallization kit.
11. You can save a report for an interesting condition by either clicking on the button « Export to PDF » or going to File->Export to PDF or keyboard shortcut.
12. You can view/export an overview of your plate (per subwell) by using the menu « Plate Overview »

13. When you are sure you are happy with the stacked images, you can go to File->Delete « rawimages » and File -> Delete « cropped » to save **a lot** of disk space (~85%).
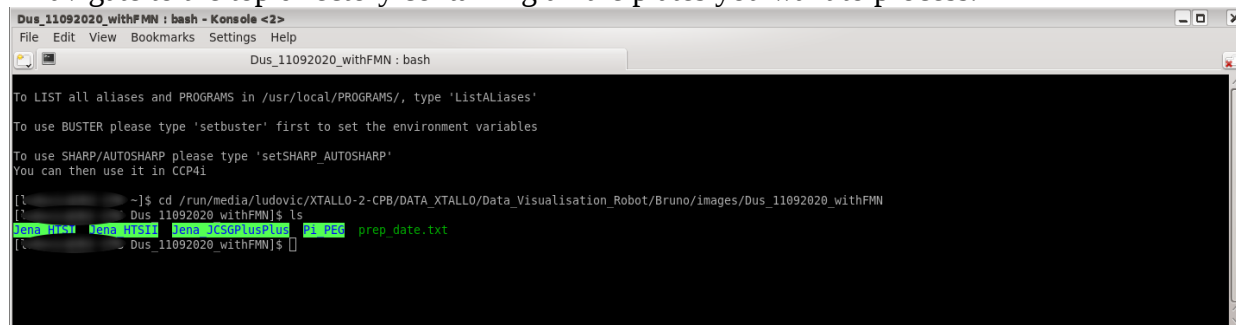
Other useful Notes:
- A command line tool is available "Merge_AllNewPlates.py". This tool is intended to be used when you have taken pictures of several plates and you want to merge the Z-stacks for all your plates automatically not within the GUI. This is really a time saver.
  To do so:
  1 Open a terminal if you don't have one already opened
  2 navigate to the top directory containing all the plates you want to process.

  3 Start the utility with the following command :
  "/whereveryouinstalled/bin/Merge_AllNewPlates.py"
  Type "/whereveryouinstalled/bin/ Merge_AllNewPlates.py -h or –help" for more details.

The tool will iterate over the directory tree to find all the subdirectories named "rawimages" and will process the data.

- A command line tool is available "SaveDiskSpace.py". This tool is intended to be used to delete all the directory "rawimages" when data have been merged and you are happy with the results.
  To do so:
  1 Open a terminal if you don't have one already opened
  2 navigate to the top directory containing all the plates you want to process.
  3 Start the utility with the following command:
  "/whereveryouinstalled/bin/SaveDiskSpace.py"
  Type "/whereveryouinstalled/bin/SaveDiskSpace.py -h or –help" for more details.
  The tool will iterate over the directory tree to find and remove all the subdirectories named "rawimages" only if data have been merged (i.e. presence of a file named "DONE" in the parent directory of "rawimages").

- A command line tool is available "Create_json.py". This tool is intended to create the JSON file for a plate **that has already been analyzed** (i.e. files data_*.txt exist) from an old project.
  To do so:

1 Open a terminal if you don't have one already opened
2 navigate to the directory containing the images (note: not rawimages).
3 Start the utility with the following command:
"/whereveryouinstalled/bin/Create_json.py"
Type "/whereveryouinstalled/bin/ Create_json.py -h or –help" for more details.
Note: if the plate has not been already analyzed, the script will just crash.