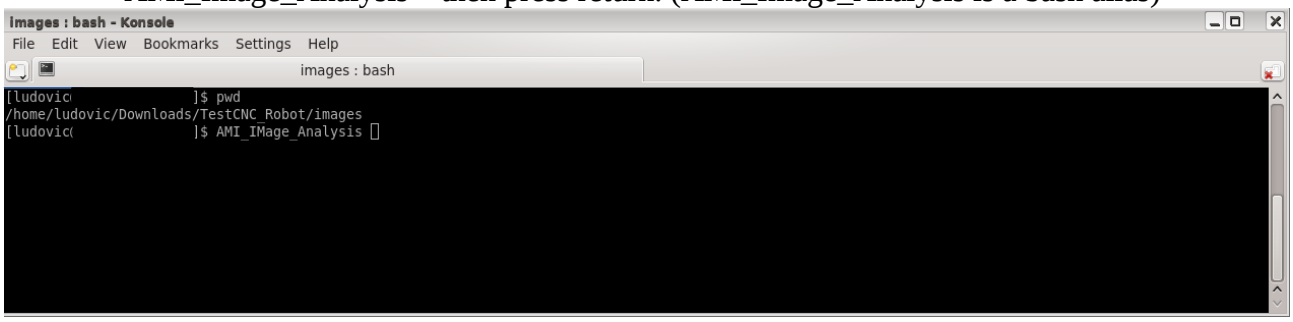
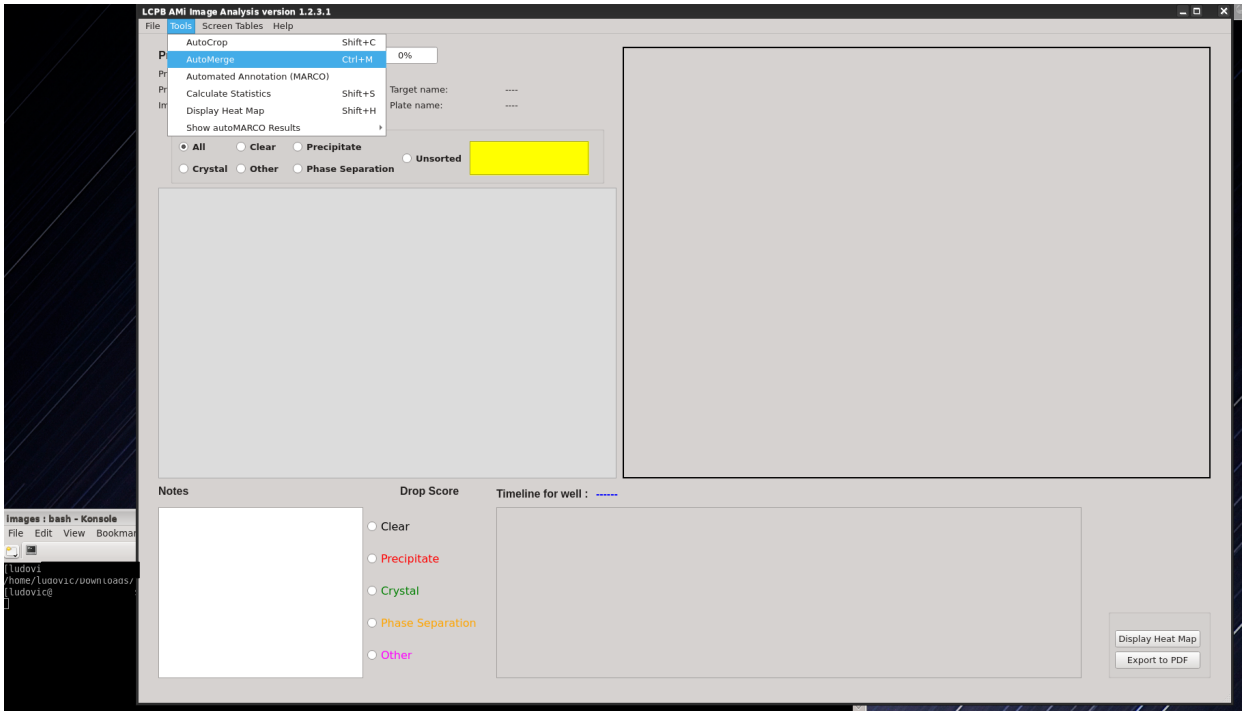
Manual of AMi_Image_Analysis

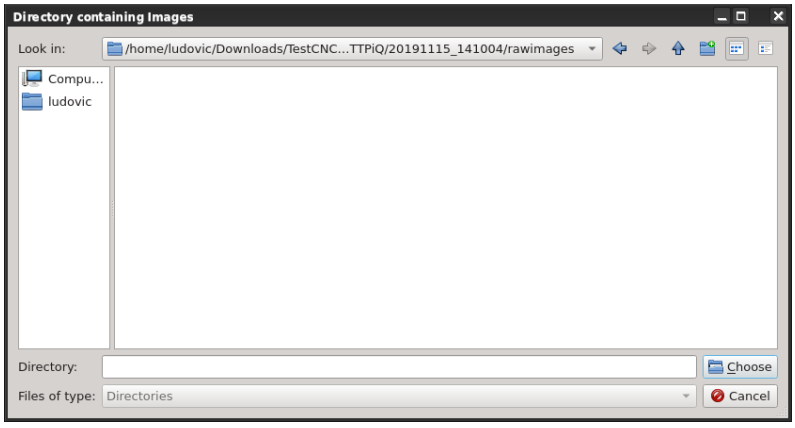
1. To start the program, open a terminal window and change directory to the folder « images » or even downstream (changing directory is not mandatory but may prove more efficient later).

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

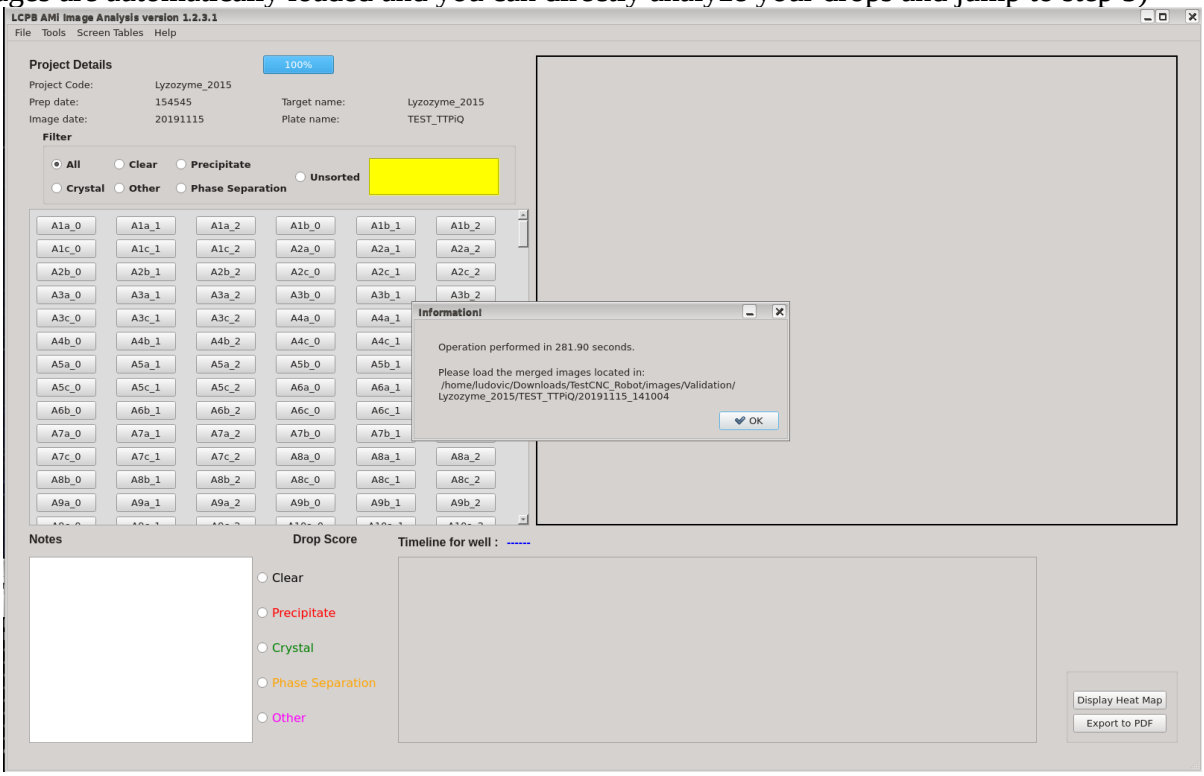




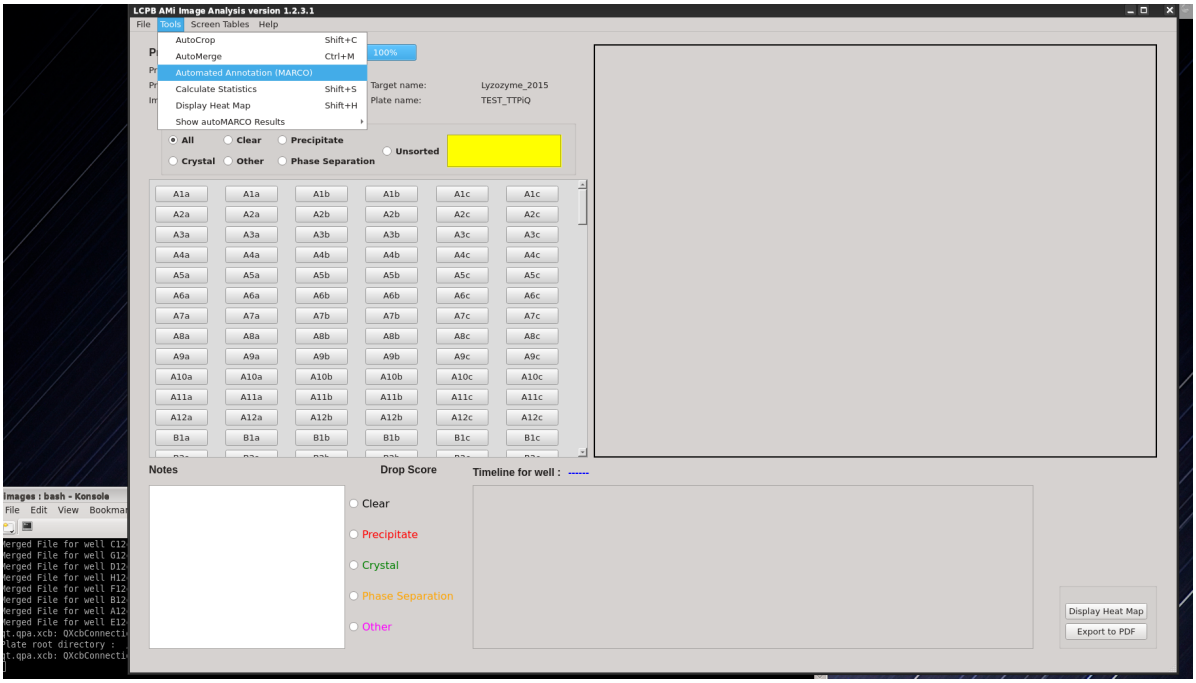
then press « Choose »

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.

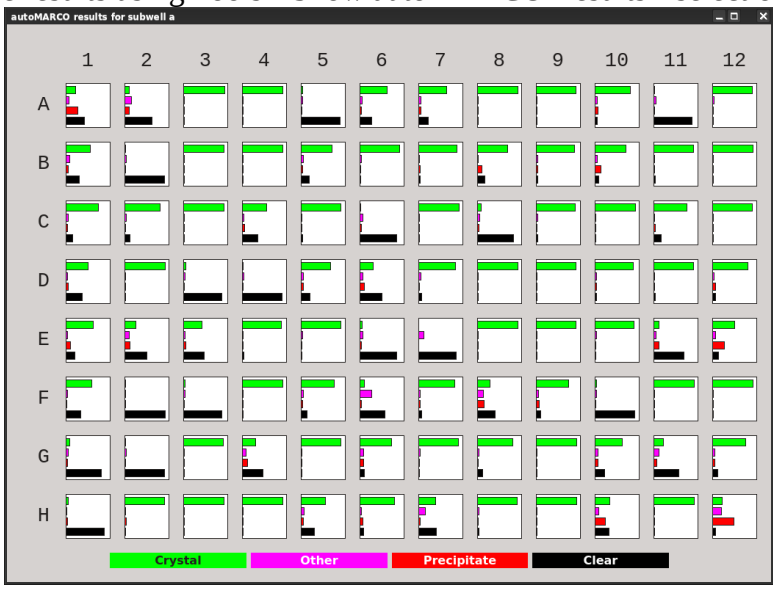
At the end, you will be prompted to load the new set of images. (as of version 1.2.3.5, merged images are automatically loaded and you can directly analyze your drops and jump to step 5)



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



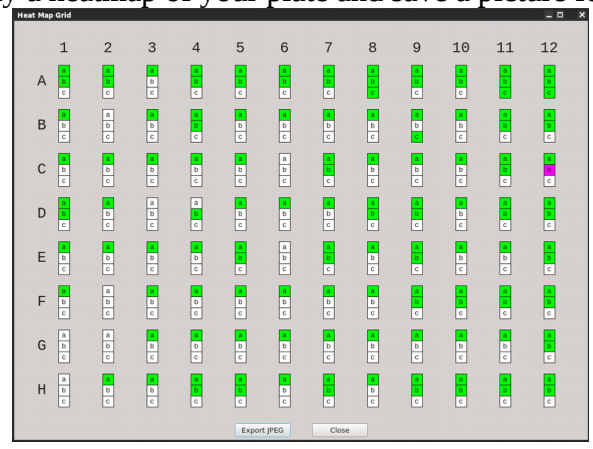
7. Check autoMARCO classification (autoMARCO result is kept if probability is > 0.6).
   You can view the results using Tools->Show autoMARCO Results->selection



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 the main 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.

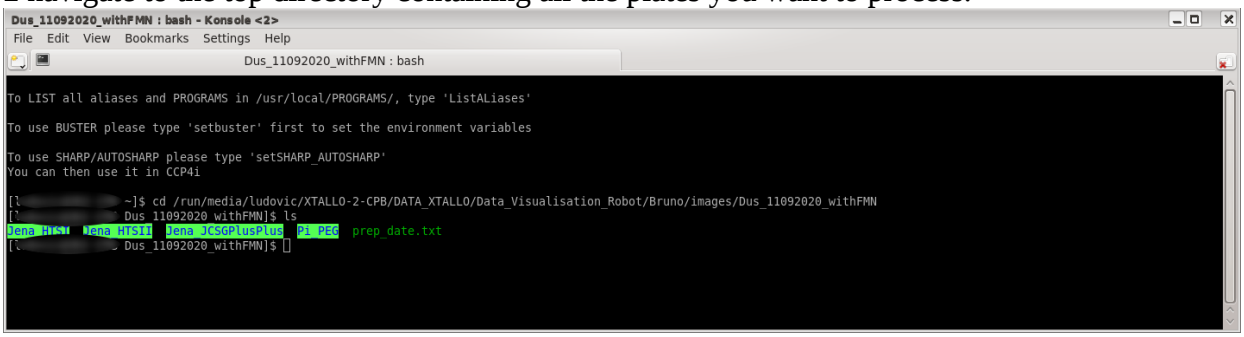


10. If you see promising conditions, you can check the composition of the well by going to Screen Tables->Provider->crystallization kit.
11. You can save a report for an interesting condition by either clicking on the button « Export to PDF » or going to File->Export to PDF or keyboard shortcut.

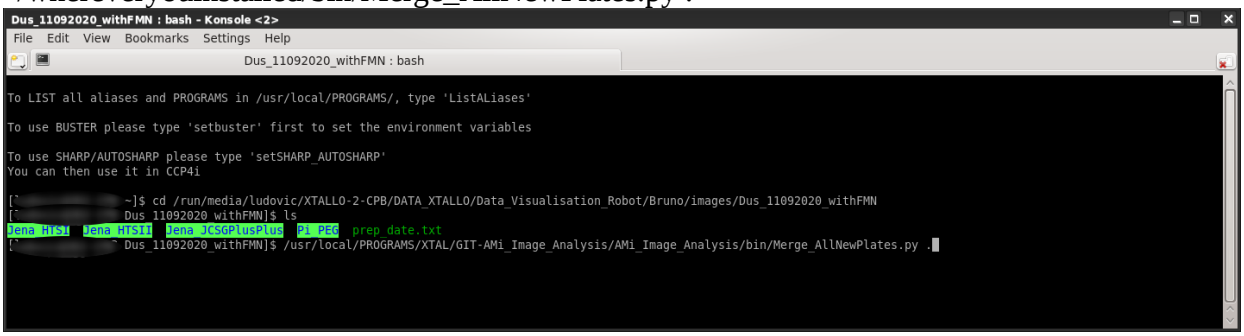
12. When you are sure you are happy with the stacked images, you can go to File->Delete « rawimages » and File -> Delete « cropped » to save **a lot** of disk space (~85%).

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 (without the quotes and do not forget the “.”): “/whereveryouinstalled/bin/Merge_AllNewPlates.py .”



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