

## **Pluri-IQ free software Instructions:**

### **How to cite:**

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[http://www.cell.com/stem-cell-reports/fulltext/S2213-6711\(17\)30269-2](http://www.cell.com/stem-cell-reports/fulltext/S2213-6711(17)30269-2) (Abstract / Free Full Text)

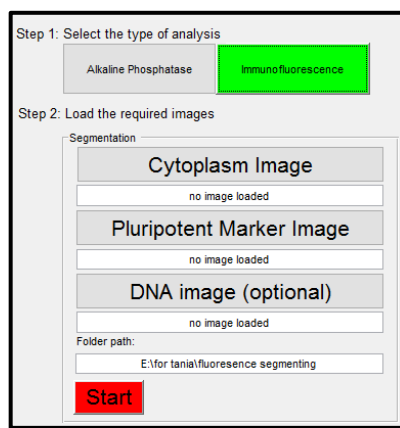
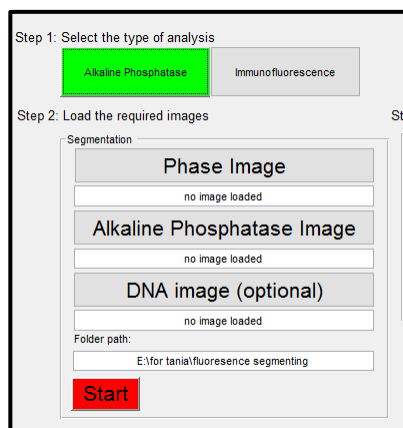
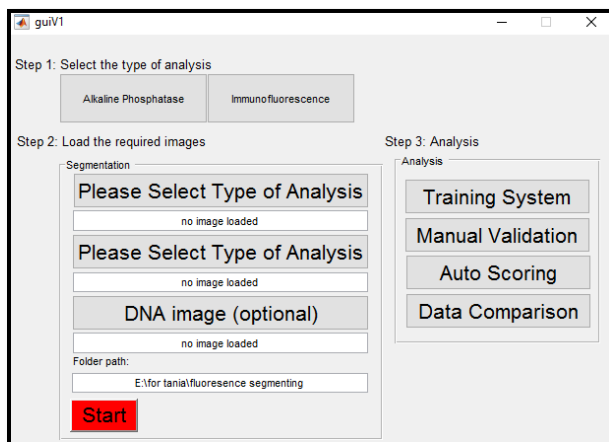
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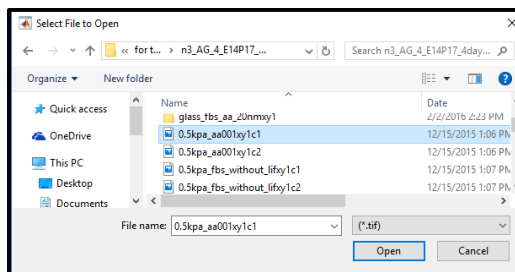
**Step 1: Select the type of analysis** – need to have all the channels in separate TIFF files.

- a. **Alkaline phosphatase (AP)** – implies a phase-contrast image taken to the colonies and a fluorescence image to the pluripotent marker.
- b. **Fluorescence** – implies that all the images are derived from fluorescent channels.



**Step 2: Load the required images for segmentation by clicking the buttons in the “Segmentation” section. The buttons will specify which image is needed depending on the selection in step 1.**

- a. A pop-up will appear and ask for the file selection. Navigate to the location of the file and *open* the image.

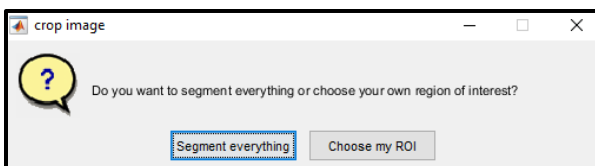


- i. Select the TIFF file for **the phase image** (for alkaline phosphatase analysis) or the TIFF file with **cytoplasm image**, e.g. actin or tubulin staining (for fluorescence analysis)
- ii. Select the TIFF file for **pluripotent marker image**

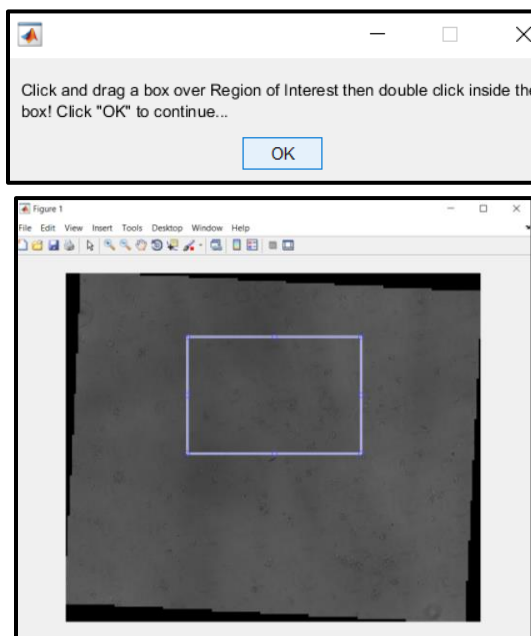
- iii. Select the TIFF file for the DNA image (optional)
- iv. Then click **Start**



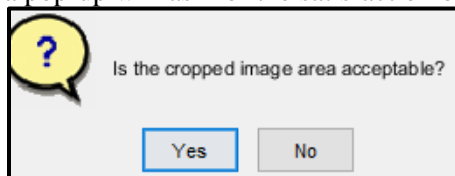
- b.** After the image is loaded, the region of interest (ROI) can be selected. The software can either segment the entire image or a specific ROI.



- i. Choosing a region of interest will require selecting an area in the image.

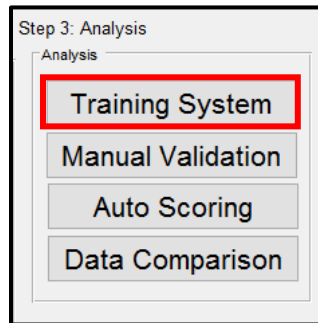


- ii. After cropping the ROI, a pop-up will ask for the satisfaction of the cropped area.

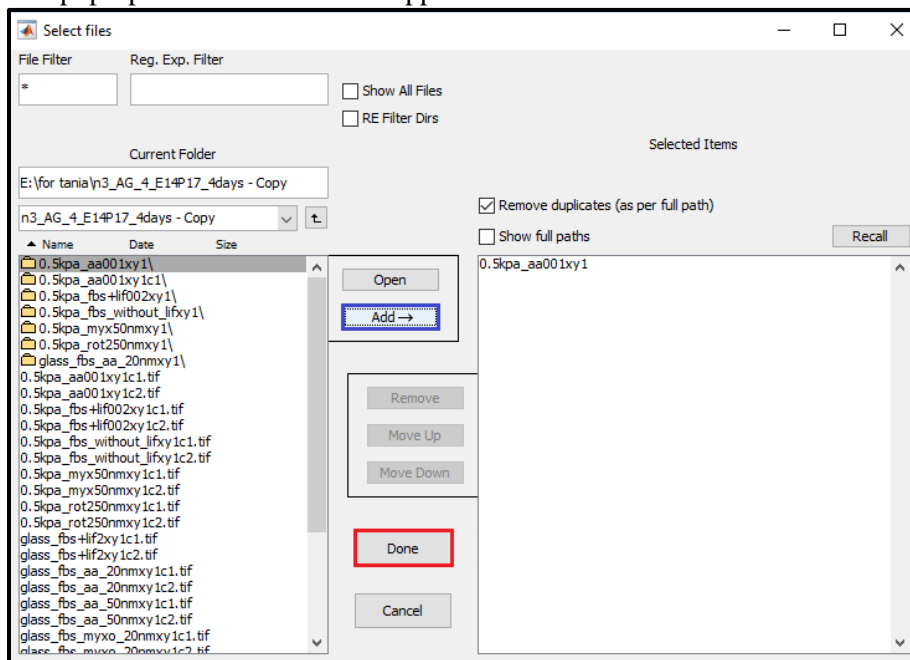


- iii. Clicking **YES** will proceed the segmentation, clicking **NO** will ask for reselection of the ROI. After clicking yes, please wait until all the processes is done. This can take a couple of minutes, depending on the size of the image.
      - iv. A folder will be created with new TIFF images, which allows colony segmentation and colony ID inspection.
      - v. Repeat Steps 2a and 2b for all images.

**Step 3: Training system** - Click on Training System to create the Random Tree Algorithm:



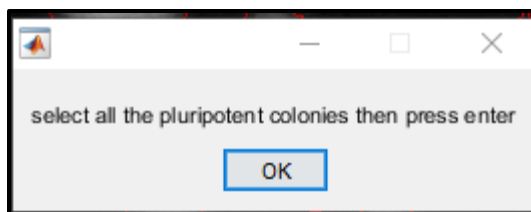
a. The pop-up folder selection will appear.



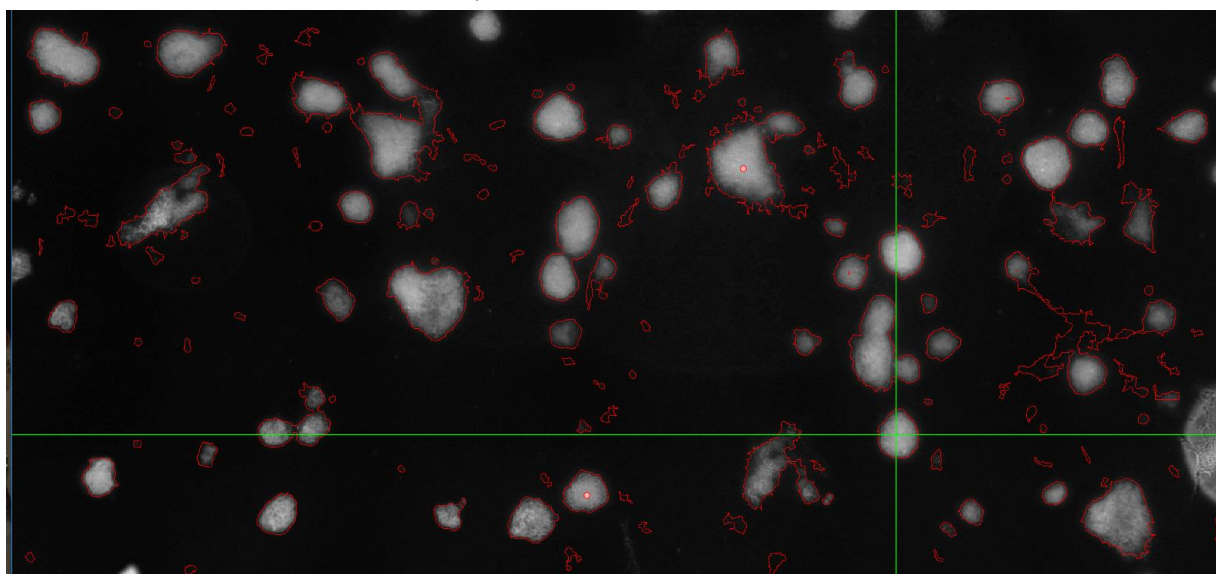
- i. Select the folders with data to analyze [in the *current folder space*]
    - IMPORTANT: select the folder created in *step 2 b iv* and not the images per se.
  - ii. Click “Add” to add the folders to analyze
    - There is no limit of folders that can be selected to train the system
- b. When all the folders were selected and “add”, click “done” to start the training.

*\* Please note, select folders from only one type of analysis (“alkaline phosphatase” or “immunofluorescence”) as these analysis work in two different classification systems (please, see the paper for more detail).*

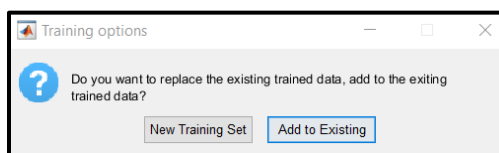
c. Follow the instructions:



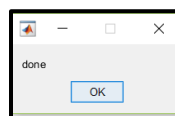
- vi. “select the pluripotent colonies” with the mouse. After selecting all the pluripotent colonies click “enter” on the keyboard.
- vii. “select the mixed colonies” with the mouse. After selecting all the mixed colonies click “enter” on the keyboard.
- viii. “select the differentiated colonies” with the mouse. After selecting all the mixed colonies click “enter” on the keyboard.



- d. After all the training, a new box will pop up: “Do you want to replace the existing training data, add to the existing trained data?”

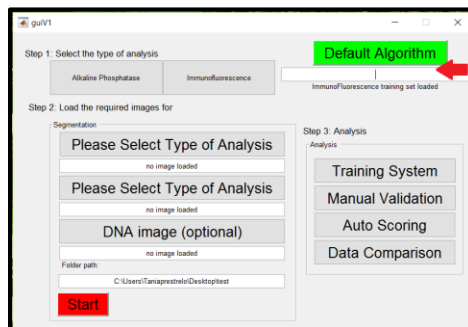


- e. To add the training set to a previous one please click “add to existing” and select the previous trained data.
- f. To create a new training set please click “new training set”.
- g. Please wait until the entire process stop and the pop up ‘done’ shows up.



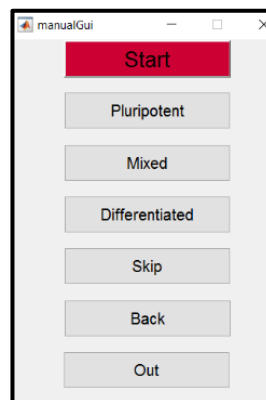
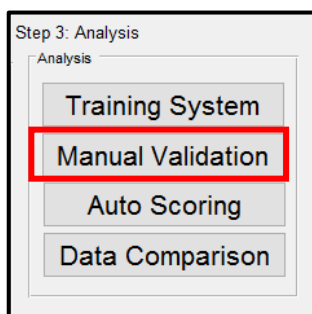
#### Step 4: Change the “default algorithm”.

Insert the path to the training set created on step 3 by clicking in “default algorithm”

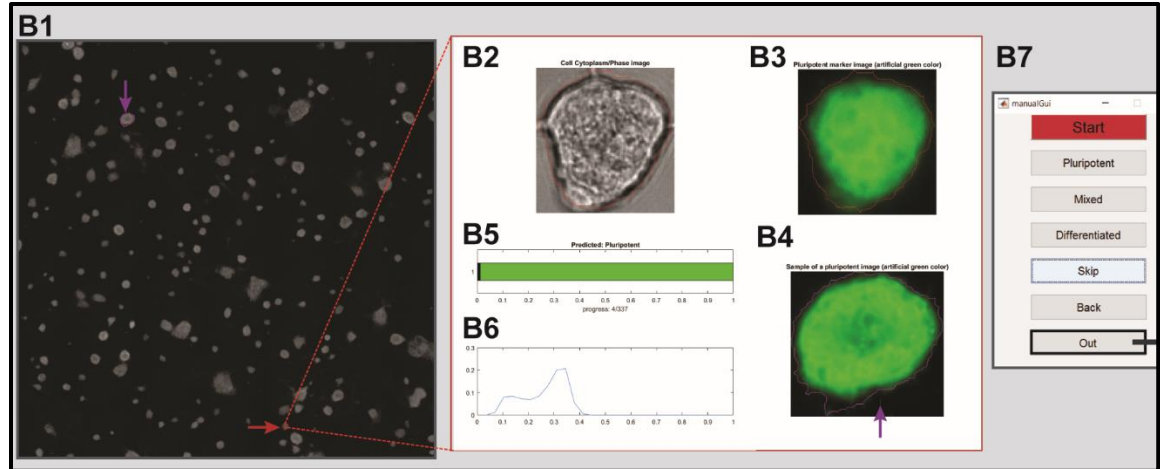


#### Step 5: Manual Validation

- a. Click on “Manual Validation”, then select *start* on the popup to select the folder created by image segmentation for manual validation of the analysis.

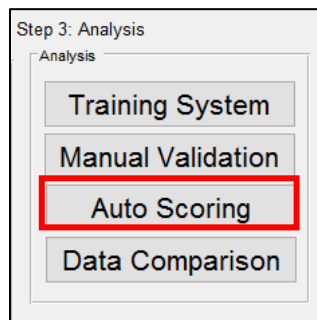


- b. Repeat steps 3 a – 3b.
- c. To evaluate each colony that is popping up, click “*Pluripotent*”, “*Mixed*” or “*Differentiated*”.



- i. (B1) Image overview. Purple arrow shows an example of a pluripotent colony. Red arrow shows the colony picked to validate the classifier prediction. (B2) Phase-contrast image and (B3) pluripotent marker image of the colony picked to validate the classifier prediction. (B4) Example of a pluripotent colony. (B5) Classifier prediction and progress bar with the total number of colonies present in the image and the number of colonies already validated. (B6) Normalized number of pixel versus pluripotent marker intensity.
  - ii. The program allows to go back to a previous colony and overwrite the previous classification (back button), to skip a colony (skip button).
- d. To finish the manual validation before analyzing all colonies, click “out”.
- i. An excel file named “*validation data #.xls*” will be stored with colony information, program score and score given by the user. In the excel sheet, the score “1” stands for a differentiated colony, the score “2” for a mixed colony, and the score “3” for a pluripotent colony.
  - i. A pop-up will display the accuracy of the algorithm.
  - ii. The algorithm will be retrained after every validation.

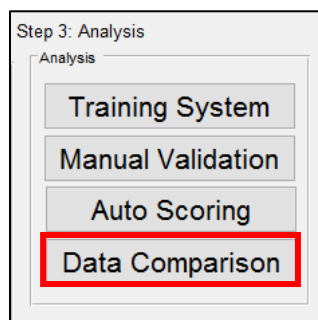
## Step 6: Automatic score



- a. Click “Auto scoring”
- b. Repeat steps 3 a – 3b.
  - ii. The program will automatically score all the colonies and an excel file will be generated

named “*AllDataOut #.xls*”. In the excel sheet, the score “1” stands for a differentiated colony, the score “2” for a mixed colony, and the score “3” for a pluripotent colony.

## Step 7: Data comparison



- Click “Data comparison”
- Repeat steps 3 a – 3b.
- The program will automatically compare all the conditions and an excel file will be generated with the percentage of pluripotency, number of pluripotent, mixed and differentiated colonies, among other parameters. The user will then be asked where they prefer to store a copy of the comparison data spreadsheet.

## Summary

