PART 3: GUI Preparation

3.1 Background:

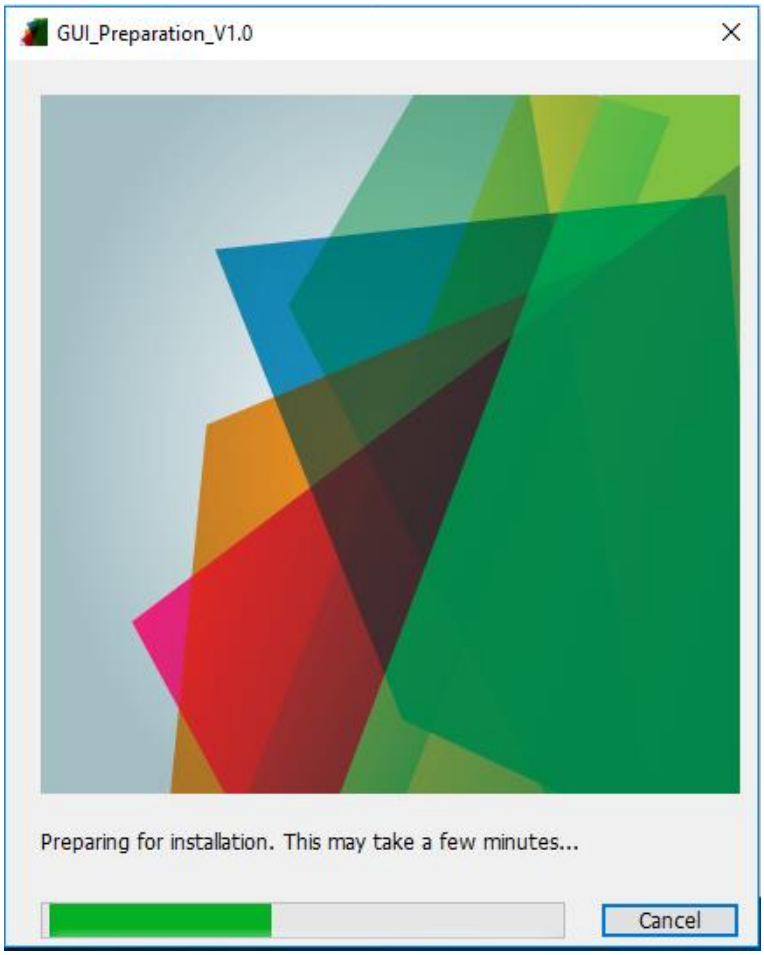
This GUI actually helps to preprocess the hyperspectral data with respect to supporting reference images. In each experiment, a set of reference images (e.g. calibration, water images, and dark images) is taken using the hyperspectral microscope system to pre-process the sample images. The pre-processing steps include image equalisation, removing undetectable pixels and outliers (spikes), removing background fluorescence and flattening the images. ***GUI_Preparation*** preprocess the data by removing the undetectable spikes and dips, removing the background images and flattening the image.

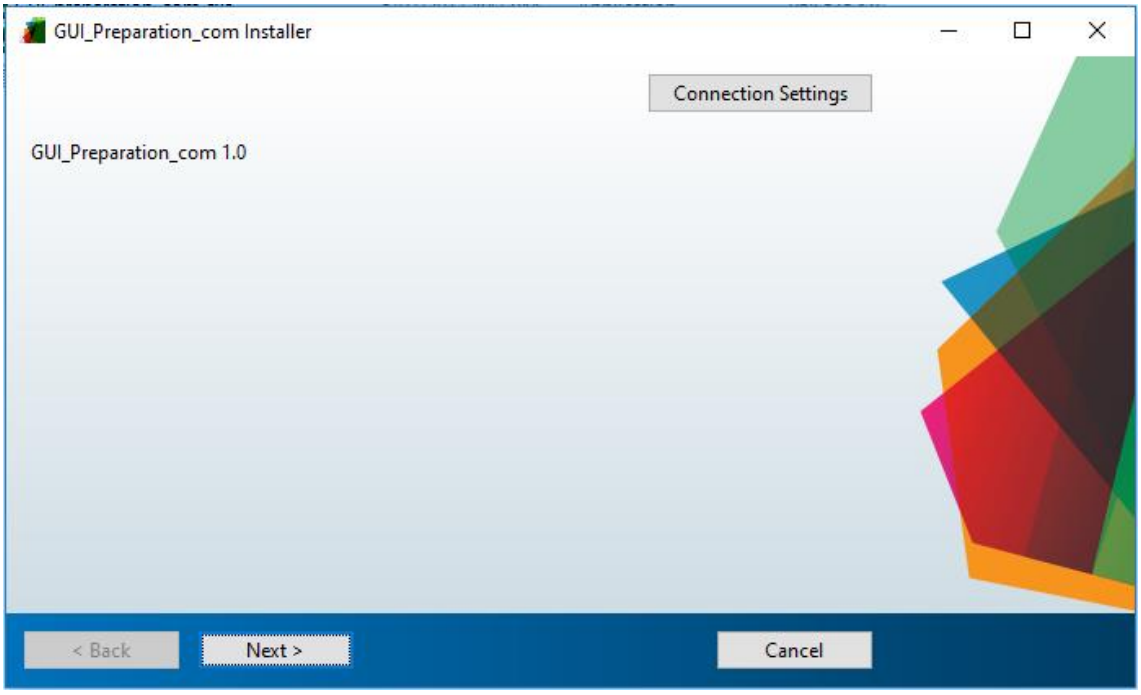
3.2 Install the software

An application (GUI_preparation_com.exe, size 968 MB) file has been made available for installing this GUI. The operating platform (e.g. pc or laptop) does not need any special software. All the necessary files are already included in this package, so the user does not need any internet connection at all.

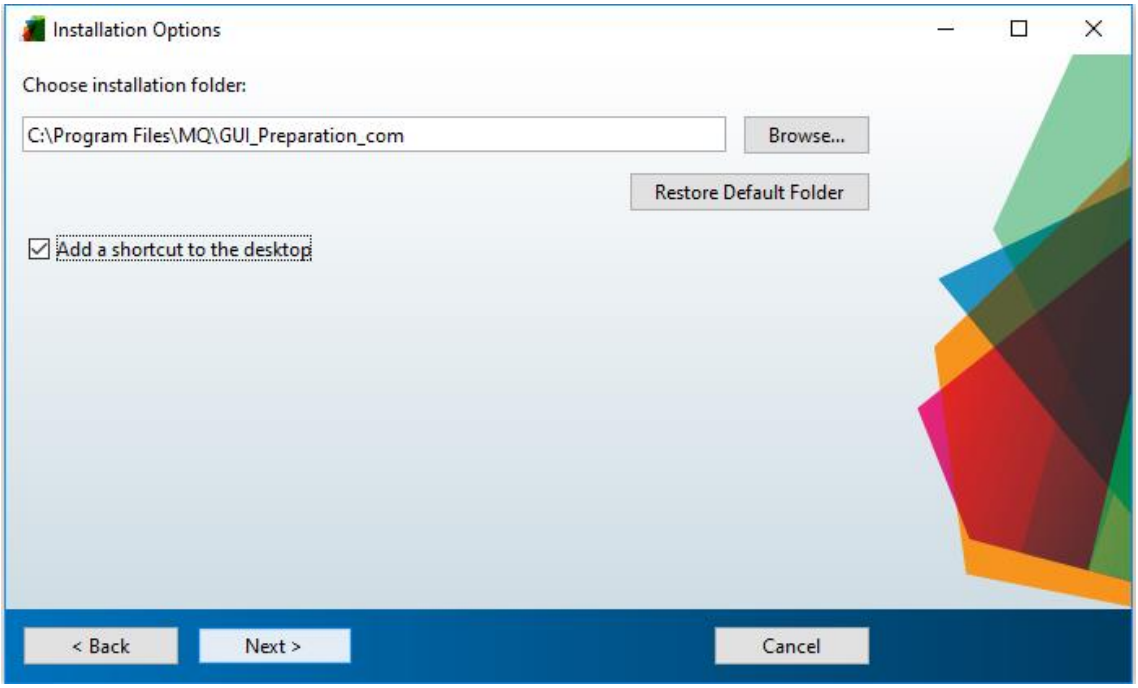
3.2.1 Installation Process:

- Minimum requirement to use the GUI. → Processor: i3, Ram: 4GB, free space: 2 GB and operating system: windows 8 or higher with x64-based processor.
- Double Click on the GUI_preparation_com.exe
- It will ask for the user's permission through the User Account Control window to allow the following program. Choose the option for YES
- After few second, a popup window GUI_preparation_com installer will appear. Click Next.

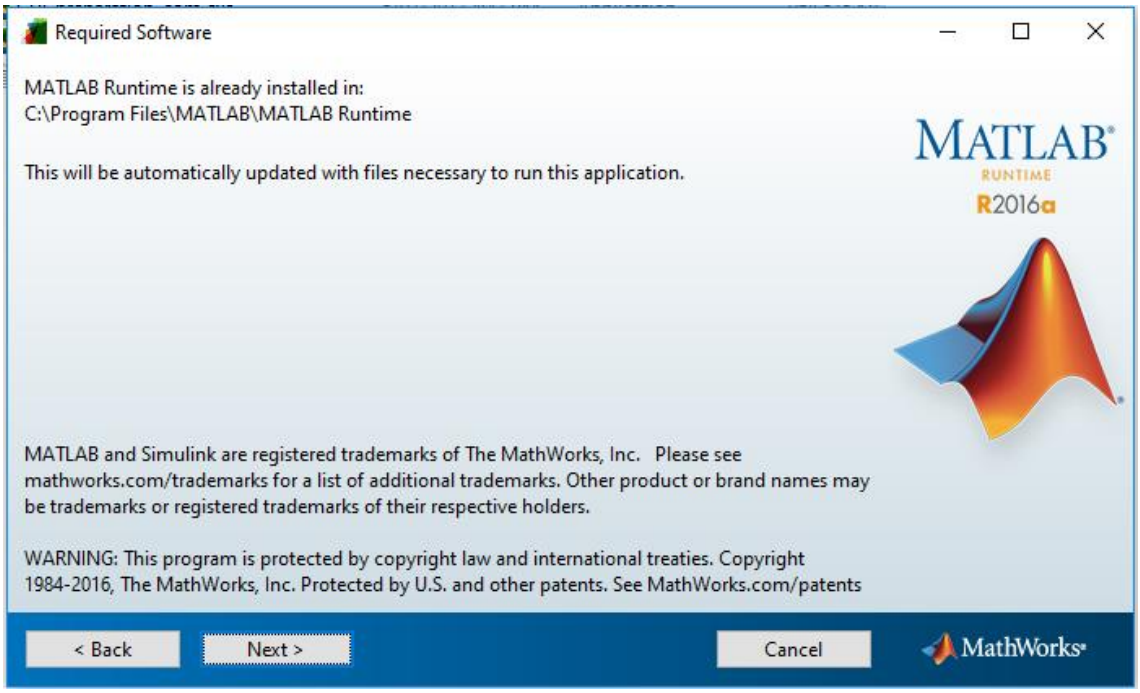




- Choose the installation folder and make sure you have at least 2 GB free space to operate the GUI.
- Additionally you can add a desktop shortcut for the GUI.

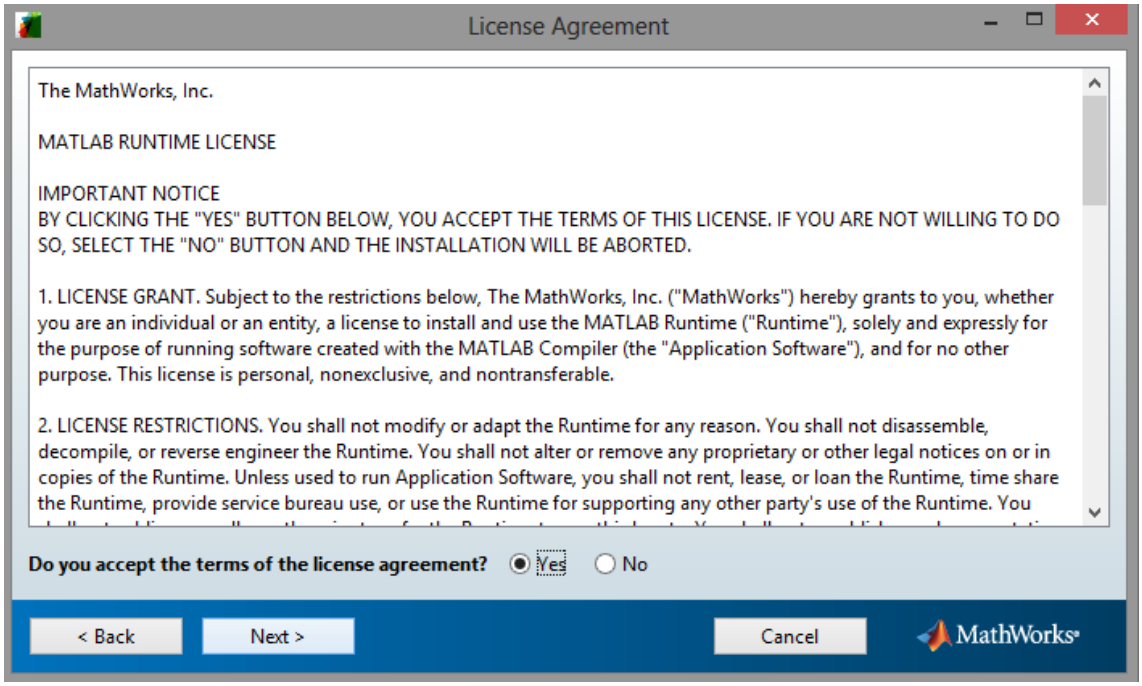


- After selecting Next, another popup window Required Software will appear which will notify the requirement of Matlab Runtime. But it is already installed in the package. So, the user can simply choose the Next option.

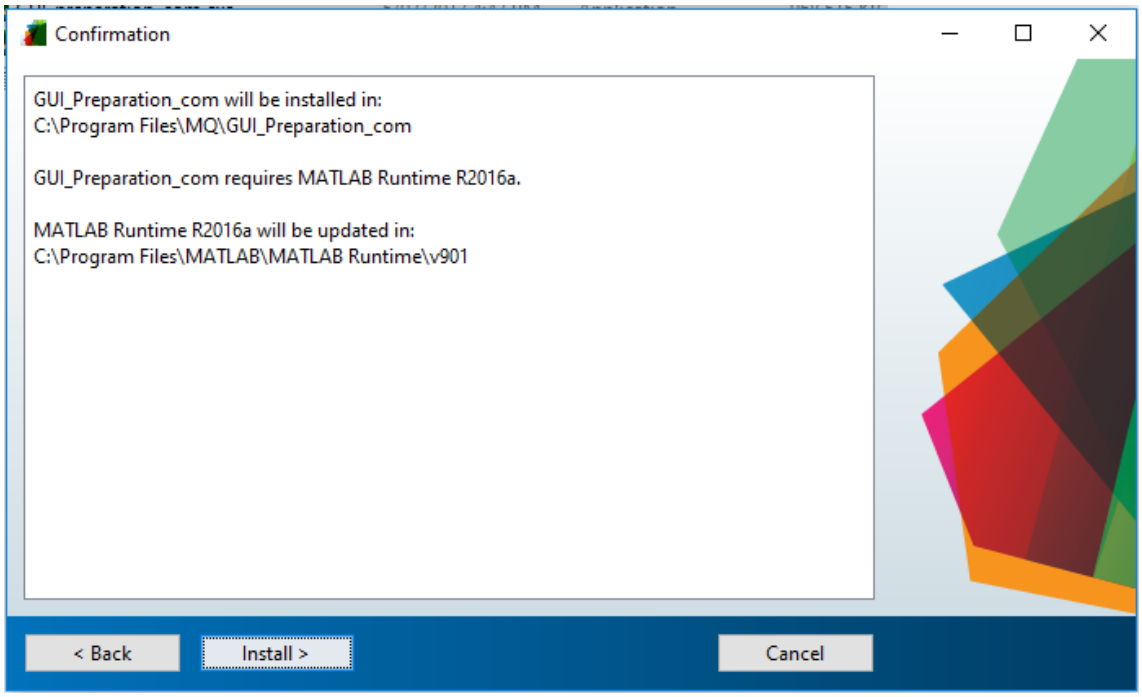


- But, if the user has a problem downloading the Windows 64-bit version of the MATLAB Runtime for R2016a, it is recommended to visit the MathWorks Web site by navigating to http://www.mathworks.com/products/compiler/mcr/index.html

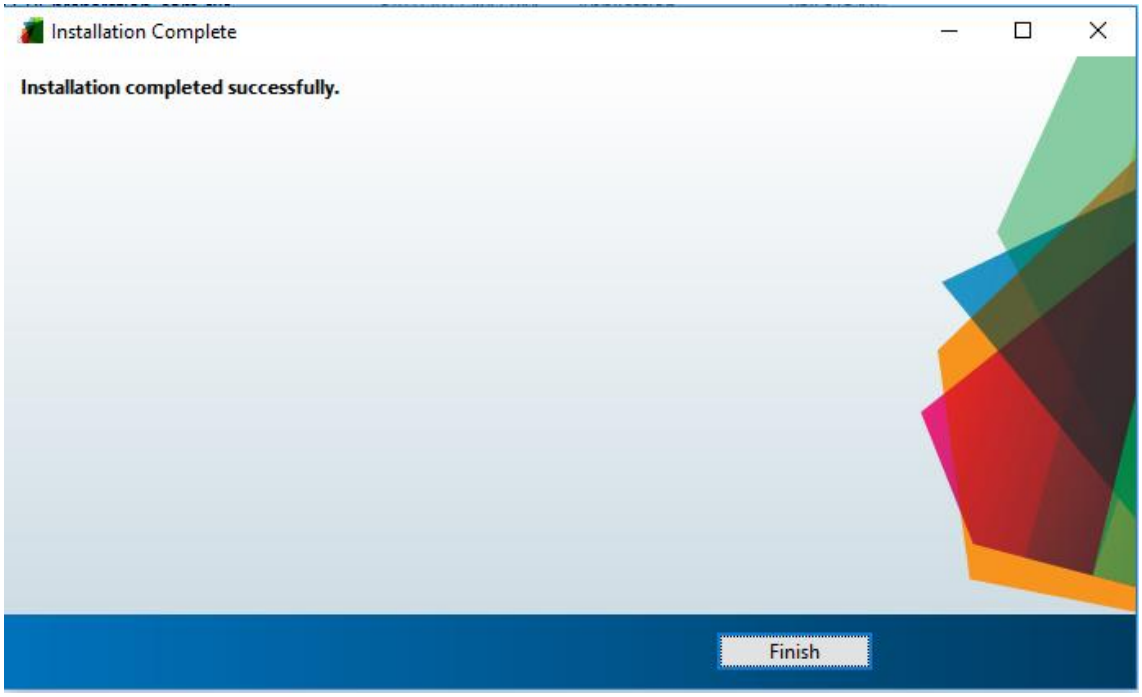
- The user needs to agree to the licence agreement with Mathworks. If you already have Matlab Runtime in the same specific folder, the package automatically discards the installation process for Matlab Runtime.



- After that confirmation is needed to start install the GUI.

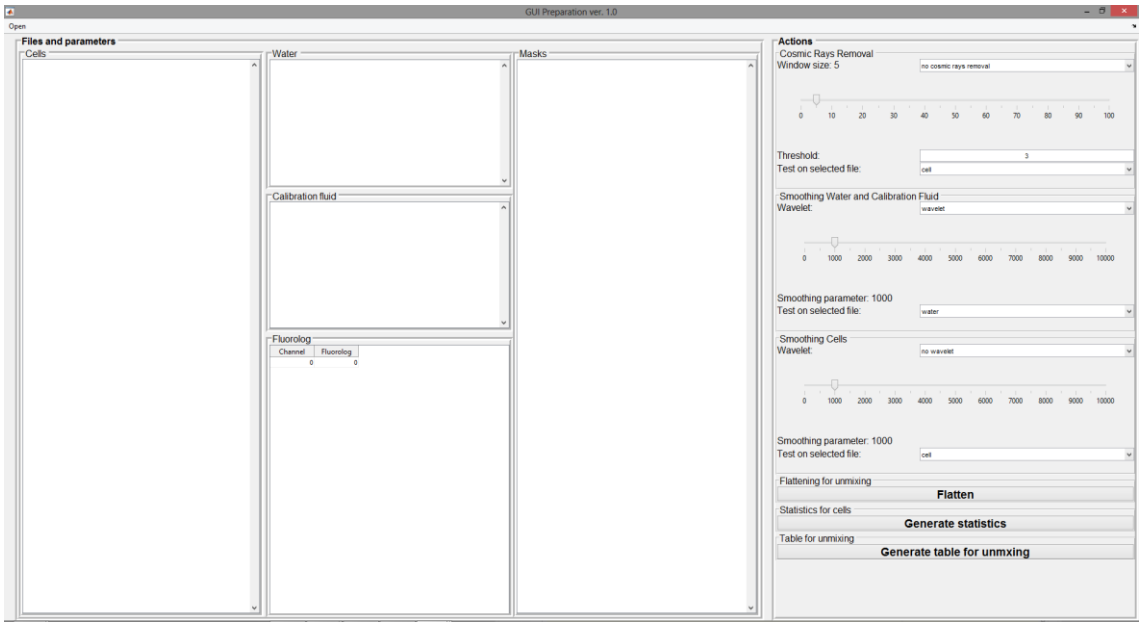


- Installation time may vary from two minutes to 10 minutes depending on the performance of the user's computer.



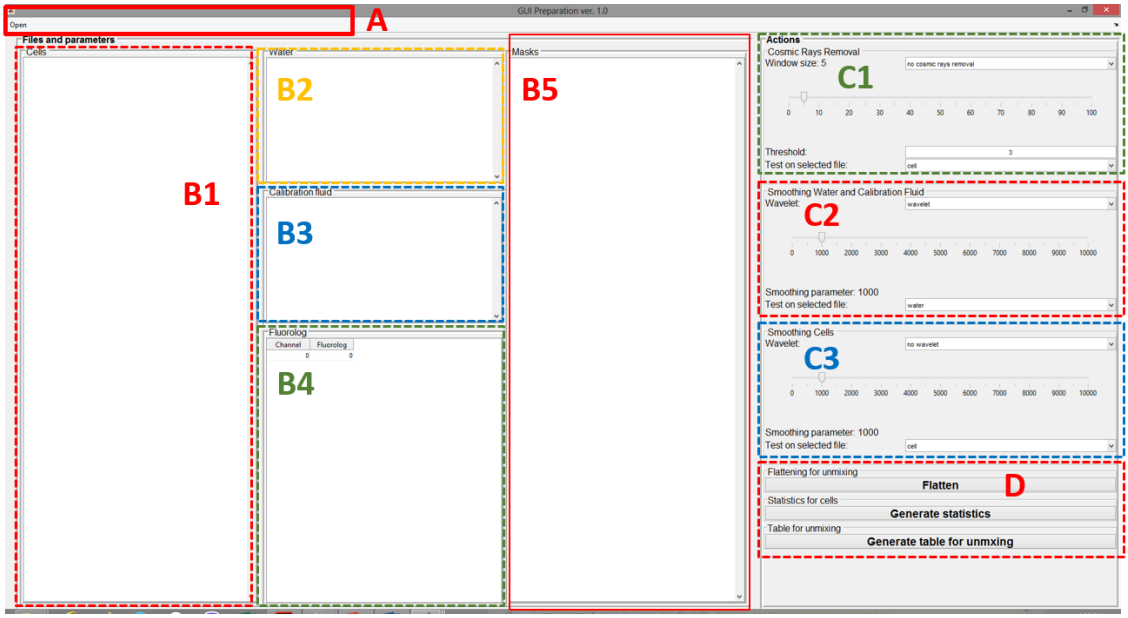
3.3 How to open GUI_Preparation_com

1. Open the GUI_Preparation_com. And it will start with popup GUI interface like this,



2. Cross the help window (if any)
3. Click “Open” and select folder with *.mat files.

Before going further we need to divide the GUI window for better understanding.

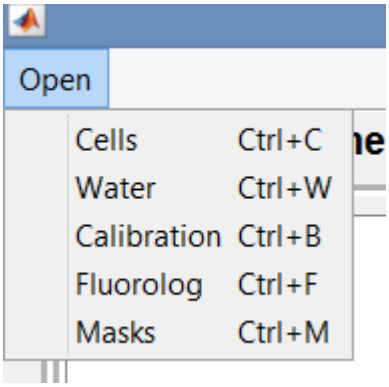


This window has roughly four columns, which are indicated by the different panels. The first column consists of only one panel, Panel B1 and the second column is shared by three panels; Panel B2, Panel B3 and Panel B4. Similarly, the third column only includes Panel B5. All these three panels (B1-B5) indicate the working files, whereas these files need to be defined manually by the user with the open menu bar.

The forth column is the working panel for the different processes. Below all the panes are described with their respective working procedures.

3.3.1 Panel A

Open panel A by simply clicking the Open button from the top menu bar. This will create a drop down list like the following Figure.



Corresponding files should be selected by the user.

- Cells for cell images
- Water for water images
- Calibration for calibration images
- Fluorolog for calibration files taken at eclipse or Fluorolog.

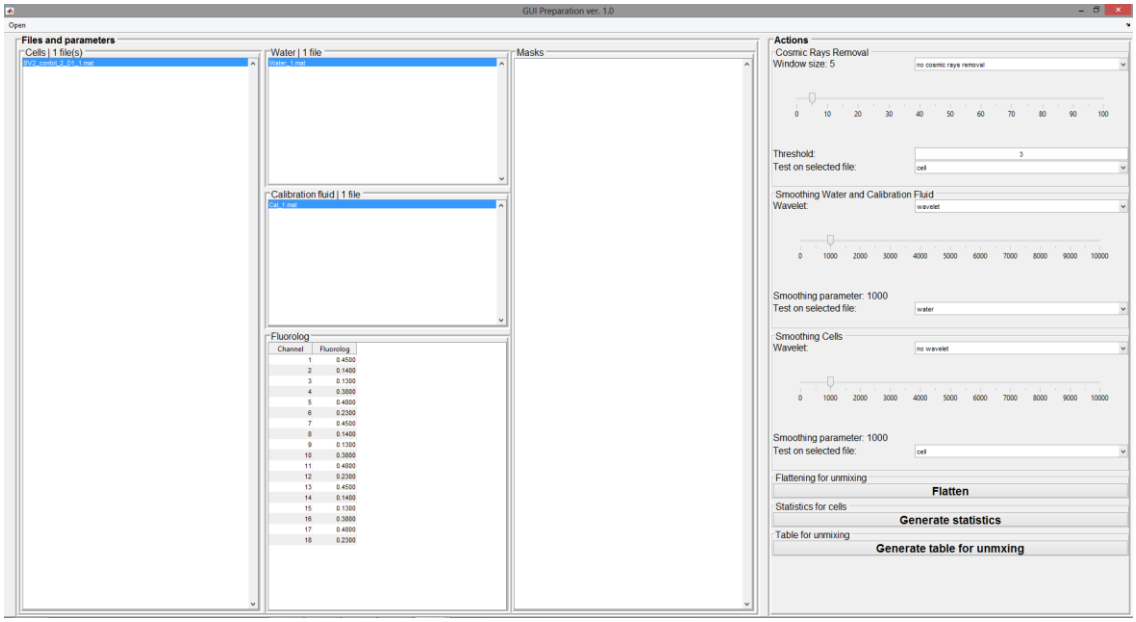
NOTE: More than 1 image in the same category could be open at the same time.

Mask is for importing mask, which is used for further mathematical analysis, and is not necessary for flattening purpose. It is important for the simple statistical analysis with respect to the sample intensity ratio.

3.3.2 Panel B1 to Panel B5

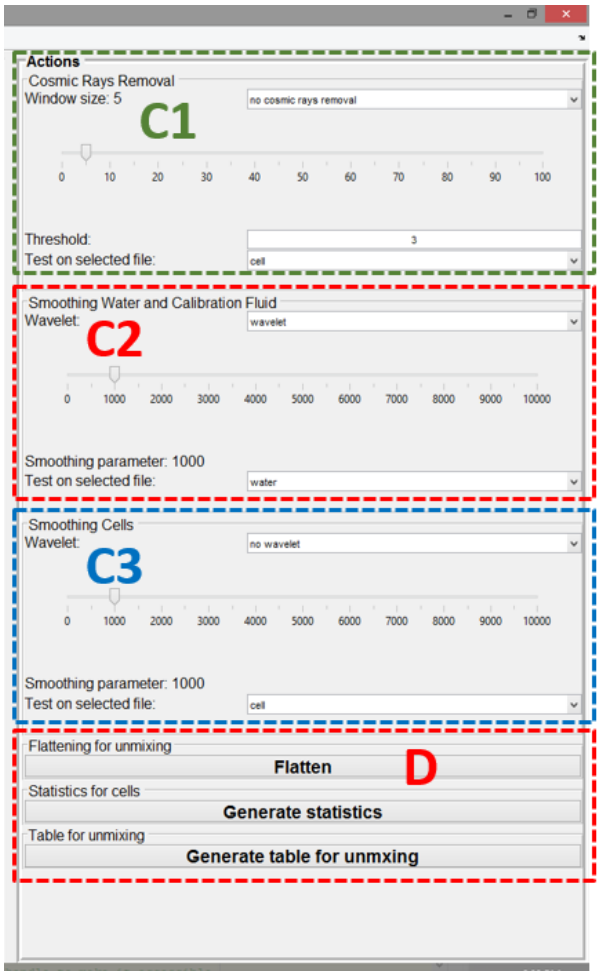
- Section B1 shows opened files for cell images.
- Section B2 shows opened files for water images.
- Section B3 shows opened files for calibration fluid images.
- Section B4 shows opened files for Fluorolog files.
- Section B5 shows opened files for mask files. These files can be prepared from the GUI visual inspection.

Import the files for preparation, which requires one Fluorolog file, at least one water file, at least one calibration files and intended cell files need to be prepared. Now the GUI_preparation is ready to use. After importing, the file the window will look like this,



3.3.3 Panel C and D

This panel consists of four different subpanels, indicated by different colour codes. This panel plays important role to collect the user defined paearmeter. On the basis of input data and command this right most panel is grouped by Actions panel (C1-C3) and the Procedure (panel D). The top subpanel (C1) (colour coded green) is for removing the spikes.



3.3.3.1 Subpanel C1: Cosmic rays removal

- The user can either chose to remove the cosmic ray removal or skip the step from this Subpanel 1, by choosing the proper step from the option menu.
- The user can chose the parameter (which is window size) for cosmic rays removal by either entering the value for threshold or sliding the parameter bar.
- Then chose “test on selected file:”option which removes the spikes from the samples.

3.3.3.2 Subpanel C2: Smoothing Water and Calibration Fluid

- Chose the value for wavelet filter by sliding the parameter bar.
- At this stage this selected value is 1000.

- Then chose which file needs to be smoothed based on water or calibration fluid.

3.3.3.3 Subpanel C3: Smoothing cells

- Chose the wavelet option from the drop down parameters that will be used for smoothing the cell.
- Chose the value for wavelet filter by sliding the parameter bar.
- At this stage this selected value is 1000 (need more option).
- Also chose the cell for the test on the selected file.

3.3.3.4 Flattening for unmixing:

- The Flatten button is used to flatten the cell images using the water files, calibration fluid files and the parameters set before.

3.3.3.5 Subpanel D: Generating table for unmixing

- Select "Open" and "Cells" and select *.mat files with cells
- Select "Open" and "Water" and select *.mat file with water
- Select "Open" and "Calibration" and select *.mat file with calibration fluid
- Select "Open" and "Fluorolog" and select *.xls file with fluorolog data. See TEST/fluorolog.xls for structure of *.xls file
- Select "Open" and "Masks". Each *.mat file with cell has to have an equivalent mask file *.png or *.tif. White color means 1, black means 0.
- Three sets of parameters have to be decided before creating the table for unmixing: Cosmic Rays Removals, Smoothing Water and Calibration Fluid and Smoothing Cells.
- Select "Flatten" button
- Select "Generate table for unmixing"

Selecting "Generate table for unmixing" performs the following set of operations:

1. It reads *_prepared_smoothed.mat or *_prepared.mat files with cells and imposes masks on them to select pixels for unmixing.
2. All pixels and other pieces of information like name of files and their types, position of pixels in respective images and fluorolog data are stored in three tables: tableFile, tableFQ, tablePixel in pixel.mat

Note that values in tablePixel are not normalized (to 1).

3.3.3.6 Subpanel D: Statistics for cells

- Select "Open" and "Cells" and select *.mat files with cells

- Select “Open” and “Water” and select *.mat file with water
- Select “Open” and “Calibration” and select *.mat file with calibration fluid
- Select “Open” and “Fluorolog” and select *.xls file with fluorolog data. See TEST/fluorolog.xls for structure of *.xls file
- Select “Open” and “Masks”. Each *.mat file with cell has to have equivalent mask file *.png or *.tif. White color means 1, black means 0.
- Select “Generate statistics”

Selecting “Generate statistics” performs the following set of operations:

1. It reads *.mat files with cells and imposes masks on them.
2. Area and average is calculated for each white region (based on mask)
3. File stats.xls is generated with all calculated statistics.
4. Masks *_numbered.png (or tif) are created with numbered regions so it is possible to connect statistics from stats.xls to the respective white regions in the masks files.
5. Masks need to be imported in this step. Order of the masks files must match the order of the cell files.
    - Section one, two, three will NOT be used if the images are already flattened.
    - Otherwise flatten procedure needs to be carried out as above.
    - Using the corresponding button for statistics or unmixing for statistics or unmixing.