This software determines a ratio between the signal on the PM and intra-cellular regions. Once the image is prepared, initiate the program in Matlab and follow the on-screen prompts. A graphic user interface (GUI) is loaded where the user can enter a threshold value to segment the individual cells based on filling the intra-cellular region. Then the overall ratio is calculated on the cells which the user has selected. The results are displayed in the Matlab window and saved as a mat file.

Requirements

This software requires a requires an installation of Matlab with the imaging processing toolbox also installed.

It has been tested on Windows, Mac (Mojave) and Ubuntu with Matlab 2013b, 2016a and 2019a. Also for Mac (Catalina) with Matlab 2019b *.

The fmUptakeAnalysis folder containing the scripts should be copied to the Matlab path directory. For further information about this, see this link;

https://uk.mathworks.com/help/matlab/matlab_env/add-remove-or-reorder-folders-on-the-search-path.html

Images should be 8-bit tif files.

* Note that for Catalina, and if using git to import plantCMEMethods repository it is necessary to approve all files in the terminal using this code;

```
> sudo xattr -dr com.apple.quarantine plantCmeMethods-master
```

Data Analysis

Enter the program name into the Matlab command line;

```
>> fmUptakeAnalysis
```

Select the image file for analysis

Enter a suitable threshold value for segmentation and press apply

Select (by mouse click) the cells for analysis and press enter

Viewing the Analysis

This program will allow you to check the cells which you selected for the analysis, and give an option to save an example cell with the segmented areas.

Enter the program name into the Matlab command line;

```
>> fmDataGUI
```

Browse through cells with the next and previous buttons, and use the save button to save the displayed images.

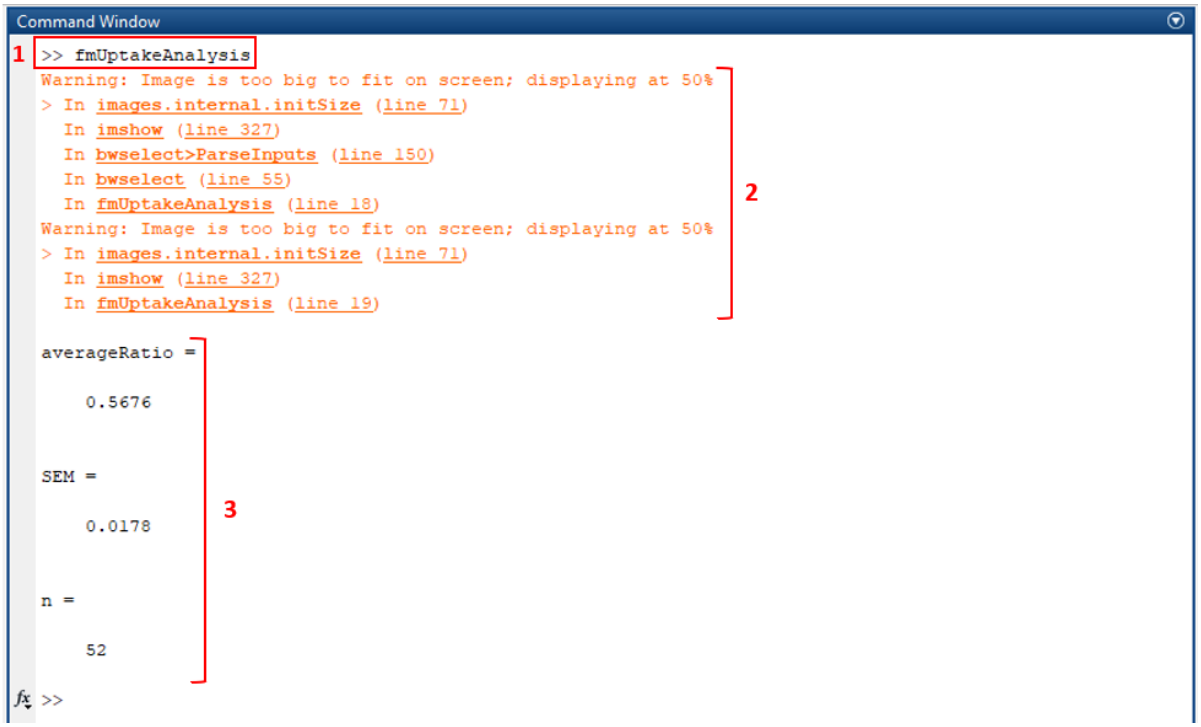
Example Data

The example data is of an 8-bit tif image of an *Arabidopsis* epidermis acquired on an Zeiss 880 inverted LSM (001.tif). A 40x objective was used.

First run (command window figure, point 1);

```
>> fmUptakeAnalysis
```

An orange message will appear to tell you that the image has bene scaled to fit in the Matlab figure window (command window figure, point 2).



Command window figure

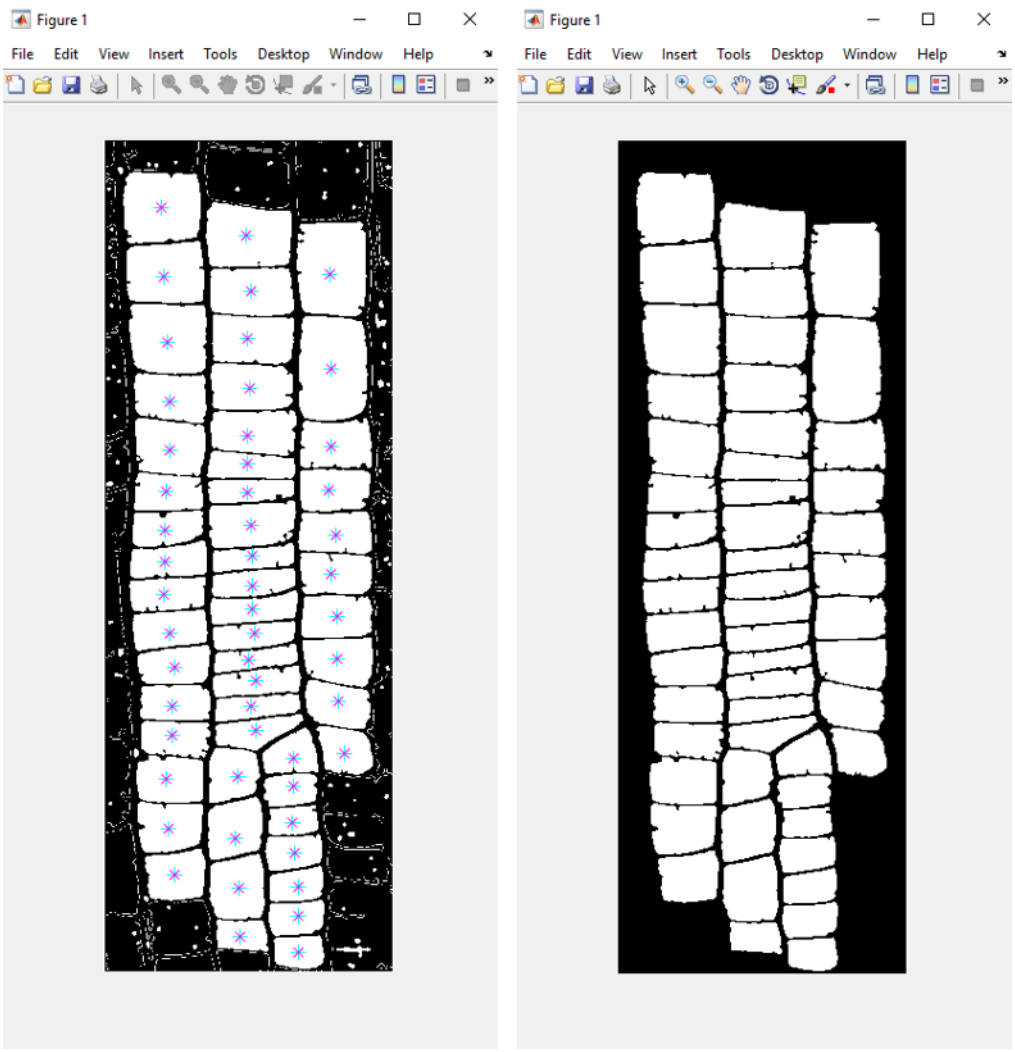
A GUI will open with the raw image and a 1st attempt at segmentation

To change the segmentation threshold, enter a value between 0-1 and press the apply button (GUI figure, point 1). The example dataset uses a value of 0.3.

When you are happy with segmentation, prese the save button (GUI figure, point 2).

FM GUI figure

A window with the segmented cells will be displayed. Select each cell you want to include with analysis using a mouse click. Selected cells will display a blue/purple * (Segmented figure, left). And press enter, and just the selected cells will be displayed (Segmented figure, right).



Segmented cell figure

Save the results when prompted. This will save all the image information, cell segmentations and results in a mat file.

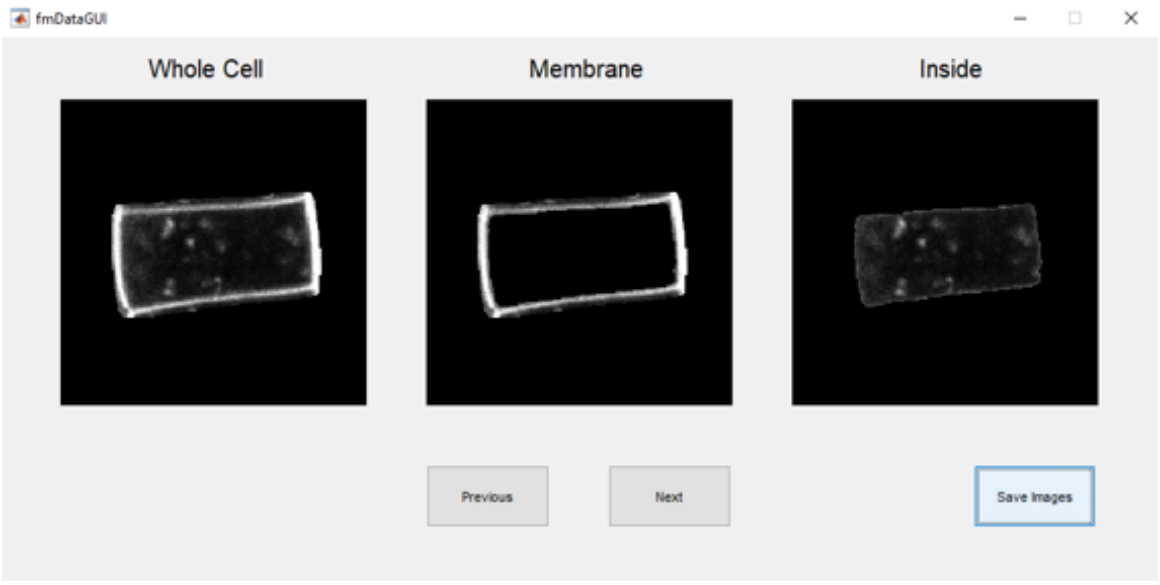
The command window will display the average ratio, SEM and the number of cells counted (command window figure, point 3).

To check each cell and save an example cell's segmentation areas, run;

```
>> fmDataGUI
```

Load the results (the mat file saved from the above script).

Browse the cells using the previous and next buttons (Data viewer figure). To save the segmented cell images, press save.



Data Viewer Figure