MIAQuant: User manual

At first we might highlight that the images to be processed must have the following name format (which has been chosen for implementative reasons):

imgName _markerName_markerColor_0001.tif.

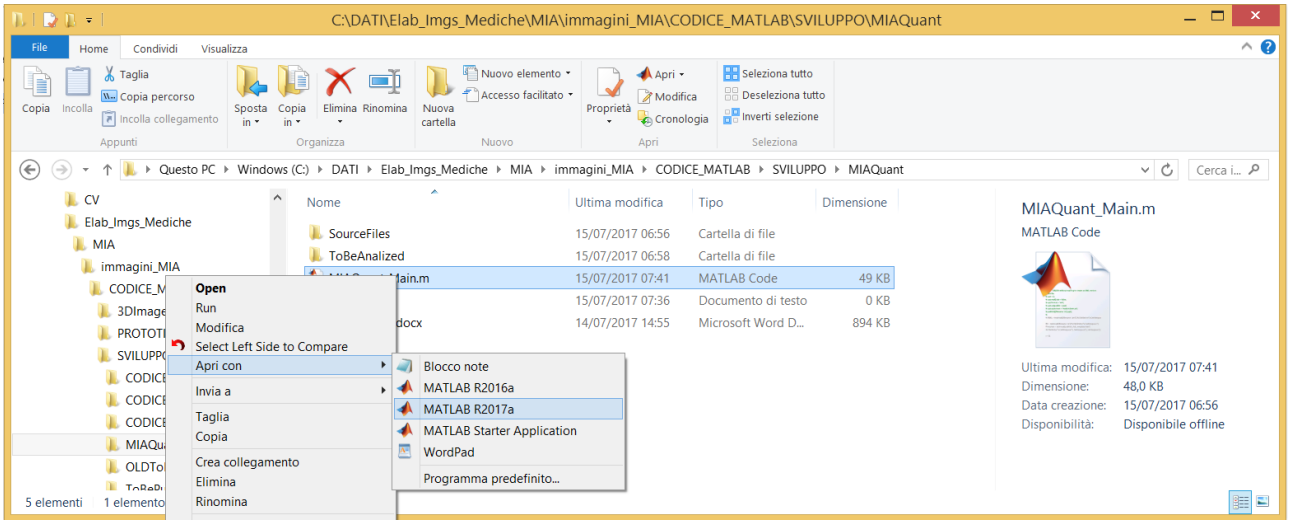
Specifically:

- **imgName** is the name of the sample image to be processed;
- **markerName** reports the name of the marker you decide to use;
- **markerColor** must equal:
    - _R_ if the color to be automatically extracted is reddish
    - _M_ if the color to be segmented is brown
- **_0001.tif** is the ending of your image name (the processed image format is .tif).

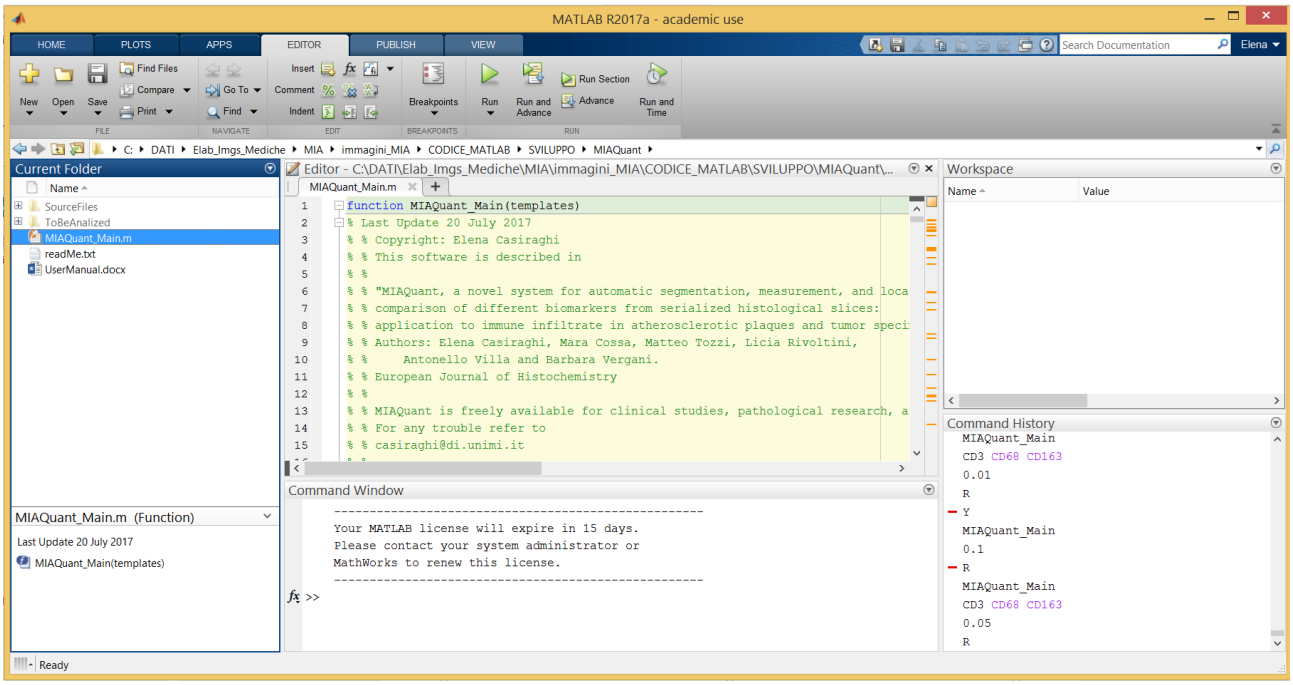
For a detailed explanation of the name format see the last section, where it is exhaustively described.

Run MIAQuant Software

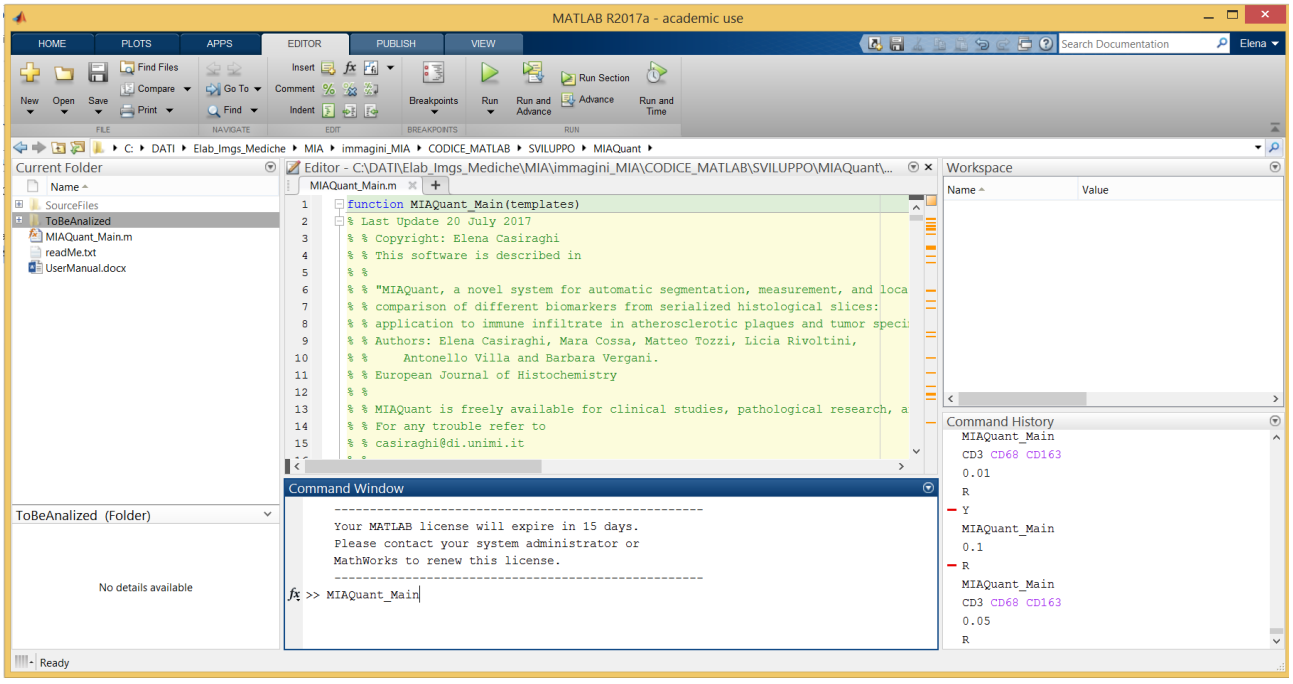
To run the MIAQuant software, open the file “MIAQuant_Main.m” (saved in the MIAQuant folder) with the latest MATLAB release (see image below).



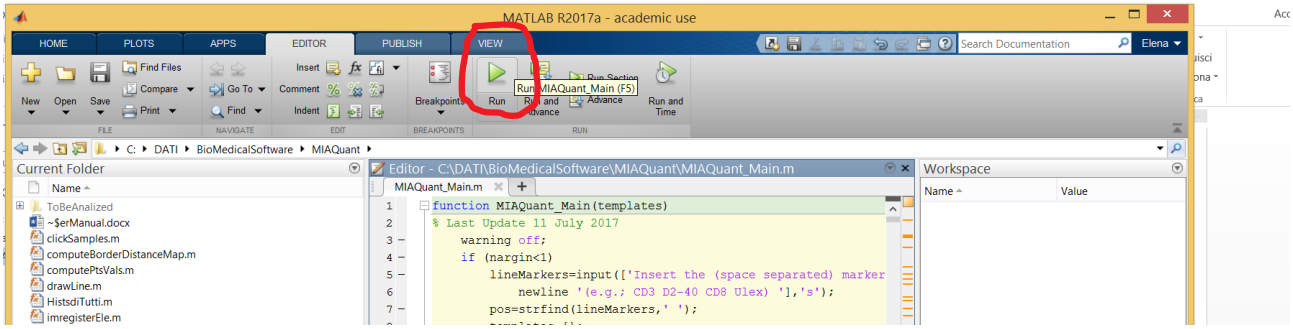
This will open MATLAB platform and set folder MIAQuant as the current processing folder (see image below).



To run the code, in the command window write MIAQuant_Main and press ENTER (see image below)



Otherwise, press the Run button (the green triangle on the top of the MATLAB window - see image below).



After running the main function, the program starts processing and requires the user to make some choices.

Precisely, in the command window, the user is requested to:

```
>> MIAQuant_Main

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Insert the (space separated) marker Names (e.g. CD3 CD68 CD163)
```

The required list must report the name of markers to be analized; this list must report the marker names as they are written in the names of the images (the abovementioned markerName). Referring to our examples, we insert the list: “CD3 CD68 CD163” (see below) and press enter.

```
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Insert the (space separated) marker Names (e.g. CD3 CD68 CD163)
CD3 CD68 CD163
```

Note that the list of markers has a random order; the ordering is not important. **Be aware that each tissue sample in the folder must have one slice image per marker**.

MIAQuant generally processes images in the folder ToBeAnalized. However, if you have created the same folder structure in another folder elsewhere (e.g. if you use an external disk for too high memory space needed for storing the images), MIAQuant lets you choose if you want to work in another folder (see image

```
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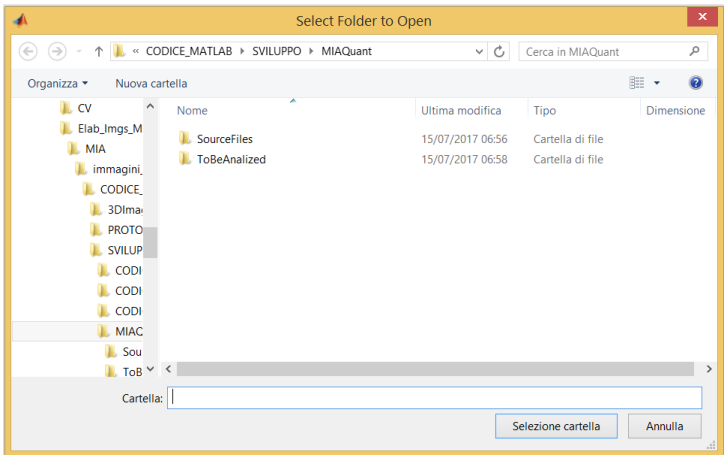
MIAQuant will process images in folder
.ToBeAnalized
do you want to select another folder? Y/N Y
```

below)

When you press Y (as in the example) a dialog box lets you choose the working folder:



Be aware that the chosen folder must contain images whose name has the format described above, and, if needed, a folder named “manualLandmarks”, containing images with manually signed landmark points. After choosing the desired folder, MIAQuant notifies your choice, and proceeds asking:

The required **reduction factor** is needed to speed up computation when images are too big. This is the scaling factor, which allows to down-sample images at the inserted scale. It is expressed as a real valued number

```
--------------------
If wanted insert the reduction factor
e.g: 0.1 for reduction at 10% image size, 0.5 to halve the image size,...
```

which must be bigger than zero and less or equal than 1 (0<factor<=1). A scaling factor equal to 0.2 (as the one in our example) down-samples the images so that their pixel-dimensions are the 20% of the original dimensions.

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MIAQuant will process images in folder
.ToBeAnalized
do you want to select another folder? Y/N Y
Selected Folder: D:\RIVOLTINI\IMMAGINI

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If wanted insert the reduction factor
e.g: 0.1 for reduction at 10% image size, 0.5 to halve the image size,...
0.2

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Do you want REGISTRATION of images + Biomarker Segmentation (press R)
or ONLY Biomarker Segmentation (press S)? R/S
```

As shown in the image above the user is then requested if processing must be restricted to biomarker segmentation and density estimation, or registration should also be performed.

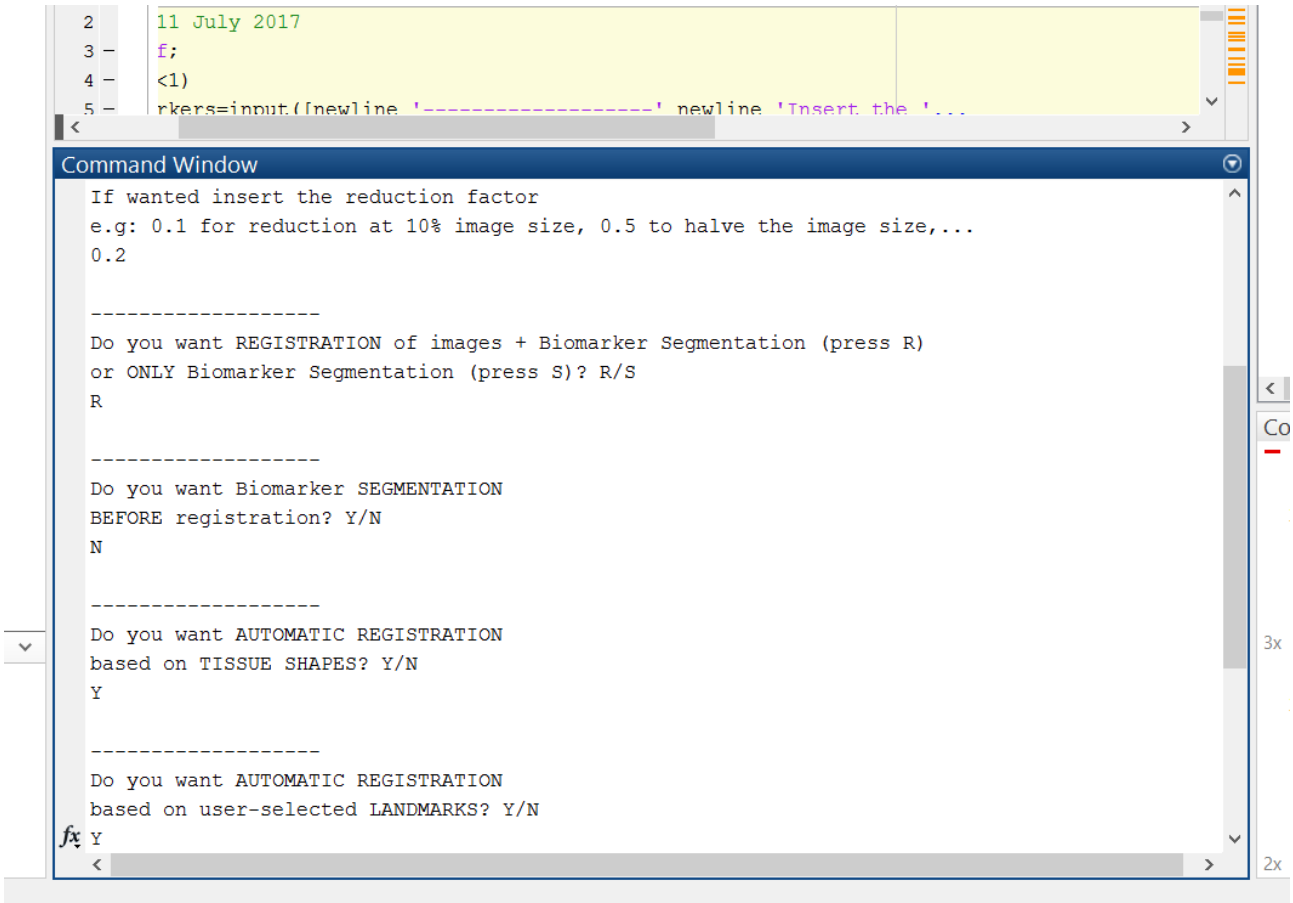
If the user prompts S, only biomarker segmentation is performed, otherwise, the user is requested to choose whether the registration steps must be:

- only multiscale-hierarchical registration based on tissue shapes;

- only hierarchical registration based on user-selected landmarks (this choice should be performed either when the tissue covers the entire image and has no borders, or when the user is interested in registering a specific region in the image);
- multiscale-hierarchical registration based on tissue shapes, followed by hierarchical registration based on user-selected landmarks (this obviously is expected to produce the best results).

The image below shows how to enter these preferences. In this example we have preferred to:

- ✓ down-sample images at their 20% pixel size;
- ✓ perform registration without requesting marker segmentation and density estimation before registration;
- ✓ perform both automatic multiscale-hierarchical registration based on tissue shapes, and automatic hierarchical registration based on user selected landmarks.



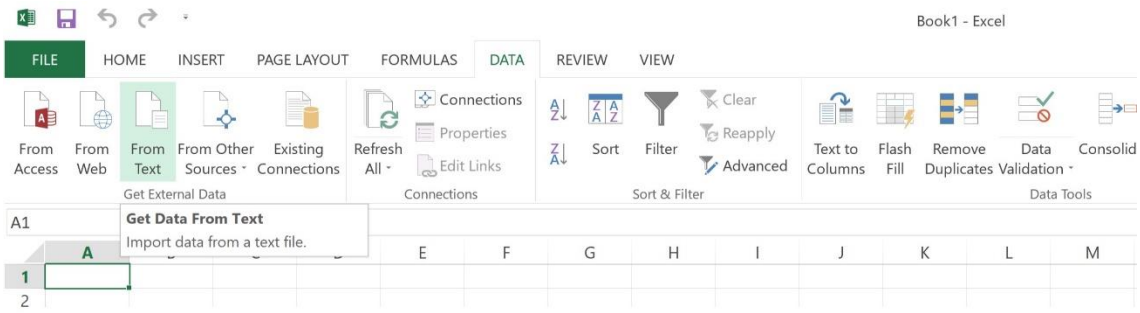
After processing ends, the user finds saved results in folders created in "ToBeAnalized". The name of the folder is depending on the requested tasks. Anyway, it reports the date of execution (An example of result folder is MHReg_PolyReg_20Reduction_12-Jul-2017).

This folder is automatically created with the commands prompted in the figure above.

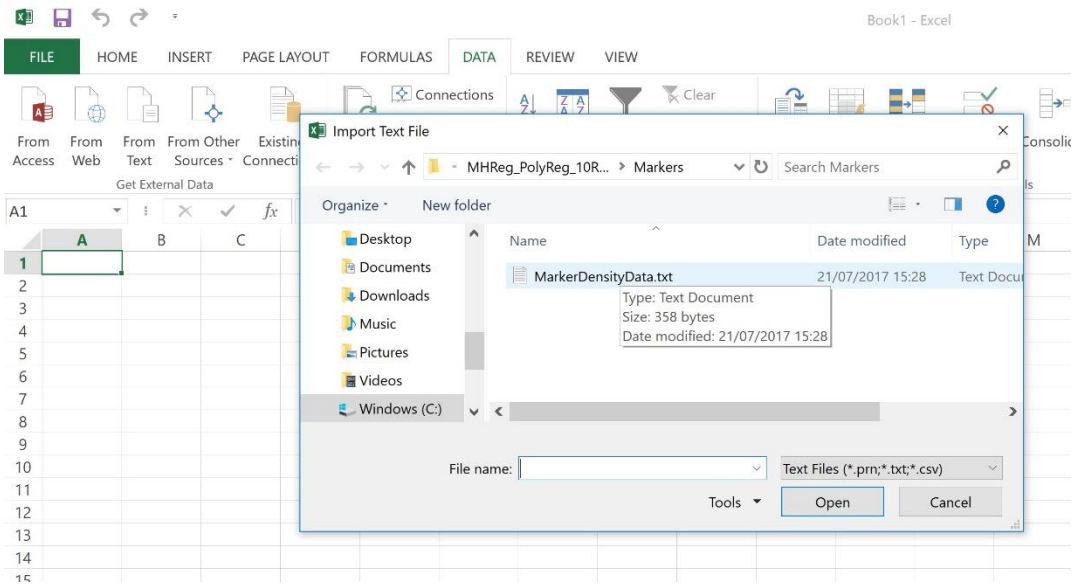
This folder contains some Matlab data stored during processing, plus "*_RegsF.jpg" images, which show, for each slice, the segmented tissue area and the polygon whose vertexes are the manual Landmarks (if they are inserted by the user). Results can be found in two subfolders.

Subfolder "Markers" contains all the segmented markers results. Precisely it contains images "*_BINmarker*.jpg" showing the binary result of segmented markers, images "_RGBmarker*.jpg" where the

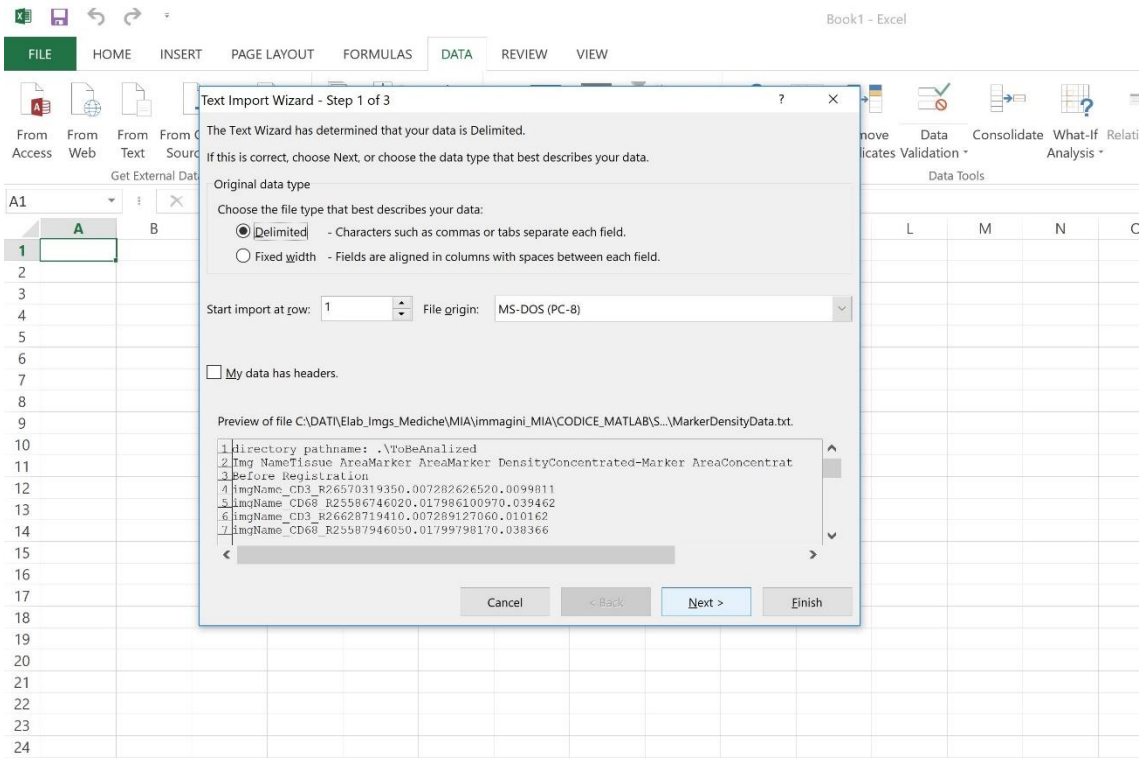
segmented markers are represented in their original color, and a file “MarkerDensityData.txt” containing the ‘tab’ separated marker density data (one line per image). We suggest to import this file in an excel sheet by using the “import data from text” command, which is found in the tab Data in the Excel ribbon (see the screenshot below).



After clicking on the “From text” command, browse your pc to select the file “MarkerDensityData.txt” …



And then keep clicking next buttom as shown below



Subfolder “ResBeforeAfterReg” contains the overlapped results, before and after registration.

MIAQuant Folder description

This folder contains all the source files (*.m) and a folder named “ToBeAnalized”.

This folder contains the images to be processed to perform:

- biomarker segmentation (of markers stained in red or brown color) and density estimation (as described in section “***Marker Segmentation via Rule Based System and K-NN classifier”*** of the article);
- biomarker segmentation (of markers stained in red or brown color), followed by serial slice registration and biomarker comparison (as described in section “***Registration and marker comparison”*** of the article); this second step obviously includes biomarker segmentation and biomarker visual comparison of registered slice images.

To provide an example, the folder ToBeAnalized contains a set of serial slices to be processed.

Image Name Format

Each image is identified by an **image name**.
NOTE THAT in folder “ToBeAnalized” the images are split into two/three vertical blocks. Each block is identified by the end of the name format (0001, 0002, 0003,…); this choice is used to deal with huge images (Matlab memory storage limits do not allow to open images bigger than 1.5 Gb).

This splitting system allows Matlab to separately open the vertical-block sub-images, compose the original region-sub-image, and process it. In folder ToBeAnalized, we saved images splitted into vertical blocks as an example, since the original images are small enough to be processed.

When only biomarker segmentation + density estimation is needed (NO serial slice registration and comparison) the folder must contain all the images of tissue samples (*.tif format) to be segmented. It is important that the images be named according to the image-name protocol described above.

When serial slice registration is also needed, this folder must contain, **for each tissue sample to be quantified**, a set of images representing N serial slices (*.tif format) of that tissue sample, one slice for each marker. It is important that the images be named according to the image-name protocol described above.

In this case, if the user wants the images to be registered based on manual landmark points, the folder ***"manualLandmarks"*** must contain the same images contained in "ToBeAnalized", where a number n>=3 of user selected points (landmarks) have been signed with BIG dots with the green RGB color, that is [0,255,0].

IMPORTANT NOTICE: given a set of serial slices, the same set of manual landmarks must be placed on each slice. The manual landmarks must be enough (at least 3), they must be placed in each image on "corresponding and meaningful" locations, the polygon they limit as vertexes should not be a regular shape (a square, equilateral triangle).

A set of serial slices of a tissue sample is provided in folders "ToBeAnalized" and "manualLandmarks", to allow a better understanding.