ECM-fiber-graph MATLAB GUI

The pipeline including Gabor detection, graph-based fiber representation and parametric maps analysis can be tested through a MATLAB-based graphical user interface (GUI), (Figure 1), which can run on MATLAB versions as early as 2015a version. It allows testing of intermediate steps of the methods of one sample image, and additional parallel testing of multiple images. Results are stored as either .png files or .csv files, as detailed below.

Graphical user interface, application

Description automatically generated

Figure 1. Graph-based fiber analysis GUI application

**Start: select image**

Test sample images are found in the *fiberGraphApp/sample\_img* directory which can be selected once Start:select image button is pressed and directory explorer is enabled. The user is then presented with a selection prompt (Figure 2) to indicate the type of image previously selected. (E.g. fluorescent or SHF microscopy images typically show a darker background with lighter structures of interest, other stainings can show a light background with the main objects at lower levels of color/intensity).

Graphical user interface, text, application, chat or text message

Description automatically generated

Figure 2. Image type selection

**Fiber extraction pipeline**

The fiber extraction pipeline is divided into multiple steps (Figure 3) and can be tested on one sample image that has been previously loaded with ‘Start:select’ image button. The results of these steps will be directly stored as .png files in a pre-created directory, *fiberGraphApp/stats/img/’file\_name’*.

1. First step is represented by fiber enhancement with Gabor filters (with predefined filters to cover a wide range of fiber features).
2. Step 2 refers to the creation of a fiber morphological skeleton and its associated skeleton graph network (collection of nodes connected by edges; nodes can represent crossing fibers (2D representation) or fiber ends).
3. Step 3 is entirely optional, depending on the quality of skeletonization observed in during step 3. Briefly, the principle behind is to reconnect fibers that might have been mis-represented before, within a certain predefined radius. This step will attempt to mitigate the artefacts arising from previous step, and will typically result in a less fragmented, more connected network, but will be time-consuming. This is important to remember when consider the batch processing step.
4. The fourth step will assign a simplified graph network to the previous skeleton, where fibers are represented here through straight lines connecting previously detected nodes.

**Fiber features and parametric maps**

The second panel can be accessed once fiber analysis has been performed, and relevant features can be extracted.

1. By selecting Gabor skeleton and Gabor graph-based features buttons, two different maps will be created respectively. These represent the local fiber thickness and orientation of the morphological skeleton and graph-based representation. Additionally, graph-based local properties of each fiber will be stored in a .csv file, located at *fiberGraphApp/stats/graph/’file\_name.csv’*.
2. Selecting pore features will save various morphological shape characteristics of pores, such as area, eccentricity, perimeter, orientation, in a .csv file, located at *fiberGraphApp/stats/pores/’file\_name.csv’*.
3. The parametric maps panel creates the extrapolated and dense fiber length and pore isotropy maps, such as described in the main paper. The resuls are stored in *fiberGraphApp/stats/img/’file\_name’*.

**Batch processing**

To apply the fiber extraction - feature extraction - map analysis pipeline to multiple images in parallel, the user can press ‘select files’ button and subsequently select a folder, not a file (e.g. *fiberGraphApp/sample\_img/sample\_batch*.) by opening it from the directory explorer. The test folder sample\_batch contains a few images already; the user can create a different directory or simply place all images intended for analysis in this one. Ideally, images should be of the same type, as the user will be asked to confirm the acquisition type.

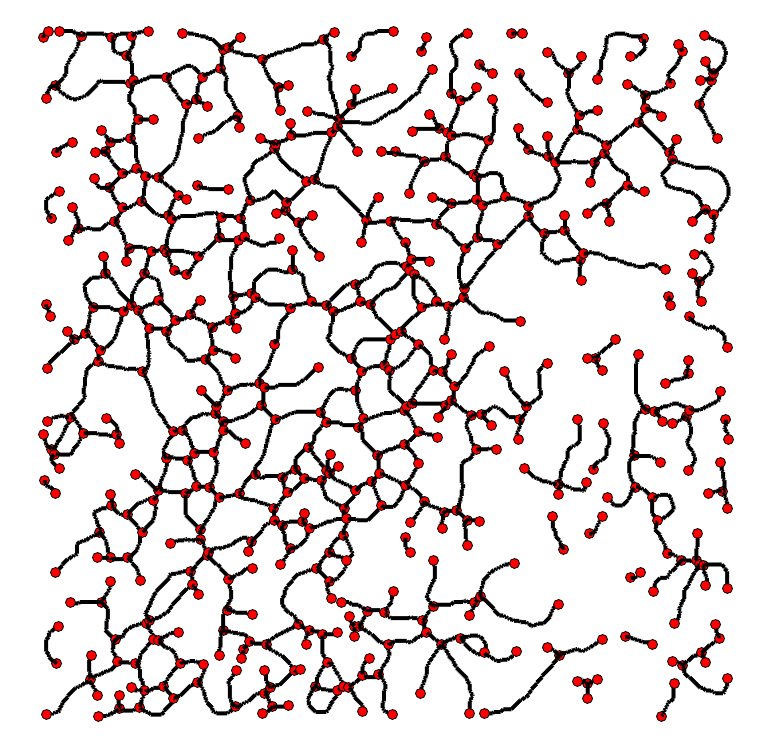
To restart the analysis and reset the data values, the user can press ‘reset’. This will close all figures and re-open the GUI application.

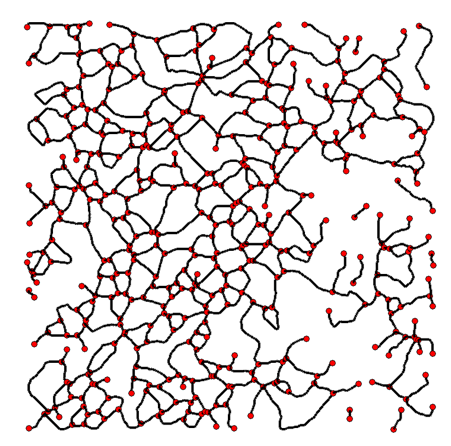
A picture containing calendar

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Graphical user interface, application

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Chart, map

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Chart, map

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Figure . Results of the pipeline test to one image sample. First row depicts the test sample and fiber detection with Gabor filters after Step1. Second row shows the morphological skeleton and associated graph (Step2). Third row shows the reconnected fibers results (Step3) and associated simplified graph-based representation (Step4). Fourth row illustrates the parametric fiber length and pore isotropy maps. Fifth and sixth rows illustrate fiber thickness and local fiber orientation map for both the morphological skeleton and the graph-based representation of fibers.