Documentation Fingerprint enhanced method code package

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Introduction

The fingerprint-enhanced method is a MATLAB (The MathWorks Inc.) tool for automatically extracting the fibrillar collagen network, and analyzing them, from a stack of second harmonic generation (SHG) microscope images. It also works when the collagen fibers are fluorescently labeled. The method has been discussed in detail in:

- Clara Manesco, Thierry Cloitre, Marta Martin, Yannick Nicolas Gerber, Florence Evelyne Perrin, Oscar Saavedra-Villanueva and Csilla Gergely. (2025) "Undergrowth collagen fibers analysis by fingerprint enhancement method" Biol. Cell. https://doi.org/10.1111/boc.70001.

The software execution requires MATLAB and MATLAB image processing toolbox and was extensively tested in MATLAB 2022b. However, it should run on older MATLAB

versions. In case of any questions or problems, please contact Oscar Saavedra-Villanueva (saavedrav.oscar@gmail.com).

Software use:

The software is designed to read the metadata directly from the TIFF image file. Ensure that your TIFF images have the image size properly set in their metadata. You can check it by opening your stacks in Fiji (ImageJ) and looking for the information on image size and pixels highlighted in a red box (Figure 1). If you only see the image in pixels, you must add the image scale in Fiji (Analyze -> Set Scale...) and save them in the image stack. The

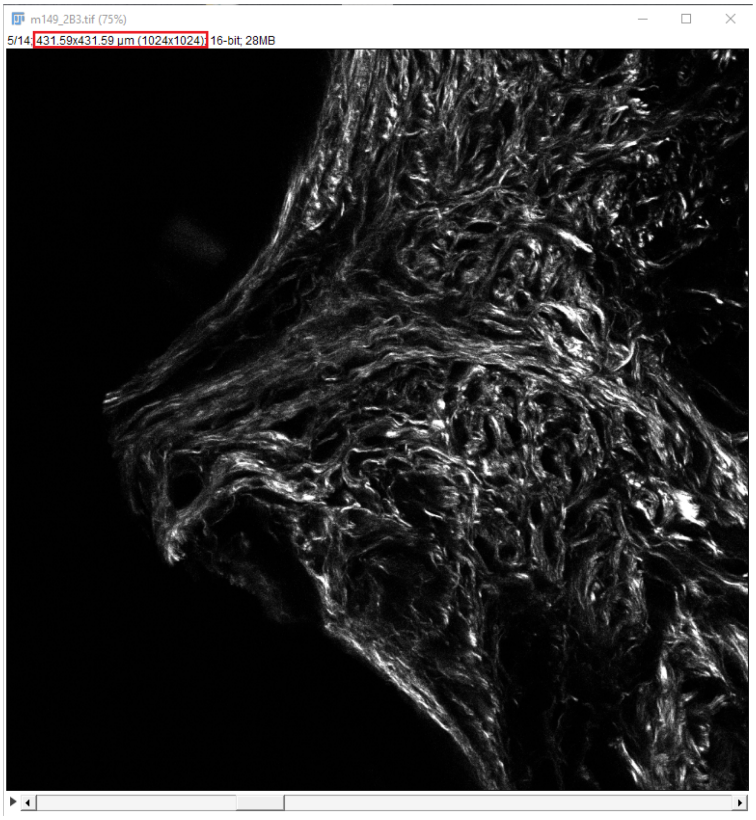

Fig. 1: Image stack opened in Fiji. Image size in μm and pixels is shown in the red box.

fingerprint-enhanced method works properly with 16-bit tiff images. If the images are in 12- or 16-bit provided by the instrument, you do not need to convert them into 8-bit images. The code starts by defining the important parameters the user needs to tune to extract and

analyze the fibrils properly. It is recommended to start from a single z-plane stack image, and not from the full stack, to first tune these parameters and save computational time during the fitting. These parameters are three:

a) Scale factor ($scale\_factor$): This parameter re-scales the input image to generate more space where to define fibrils and separation between the fibrils. The input image will scale as a multiplication of the image dimensions by this number, e.g. if the input image is 1024×1024 pixels and the scale factor is 2, the output image will be 2048×2048 pixels. Please note that the larger the factor, the longer the calculation time, and the noise added to the image due to the pixels interpolation. We found that 2 is a good compromise for this factor in an image of 0.42 $\mu m/px$.

b) FingerPrint threshold (FP_thresh): This threshold is used to define whether a region is considered a ridge-like region or not. This parameter is defined in the interval $(0, 1]$. However, in our calibration tests, it was always in the interval $(0, 0.2]$. A higher number results in a lower number of ridges as fewer regions of the image will be considered ridge-like.

c) Reliability threshold ($reliability\_thresh$): This threshold helps to remove fake positive ridge-like regions by assigning a reliability value to each of them. This parameter is defined in the interval $[0, 1]$. If the value is 0, all detections are trusted. When the value rises, fewer regions are accepted as ridge-like regions.

After defining these parameters, the path and filename of the image stack should be added as $path$ and $filenames$, respectively. The code will automatically read the image metadata from the TIFF file, and calculate the binary map of features (BW_map) and the skeleton map ($Skel\_map$). The results of the previous calculations will be automatically displayed by the function $fibers\_visualization$ where the last input parameter corresponds to the delay time, in seconds, between different stack planes (only valid for image stacks). An example of the visualization is shown in Fig. 2. If the visualization of the results is not desired, please comment this command line. Subsequently, the different metrics described in detail in (REF) are evaluated by function $fiber\_metrics$. This function will return:

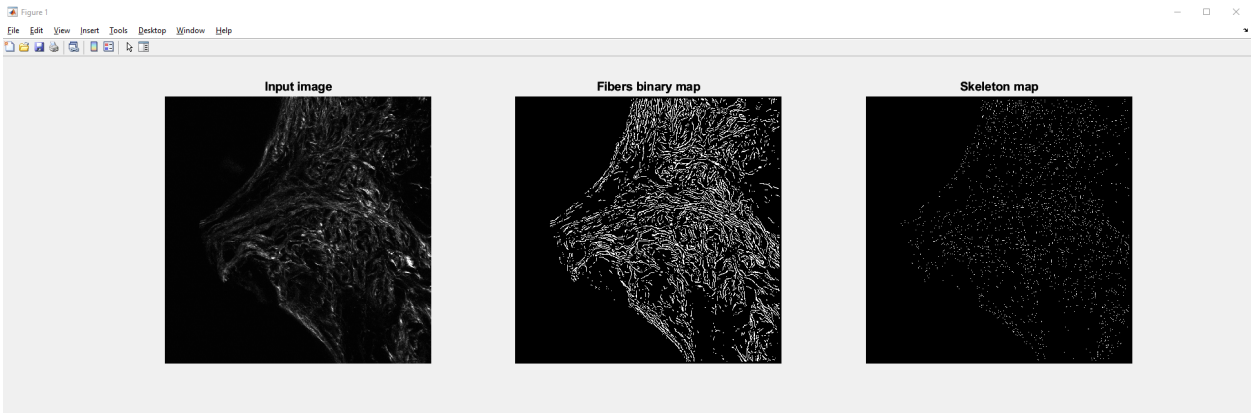

Fig. 2: Visualization of the FingerPrint method segmentation. Input image (left), binary feature map (center), and skeleton map (right). The discontinuity of the skeleton map is due to how MATLAB displayed larger images. The skeleton can be visualized in detail by zoom-in or displaying it on another window.

- Total fibers' length ($T\_fibersLength$) as a single scalar element per image slide.
- Tortuosity ($Tortuosity$) as a cell array with one element per stack image. Each cell element will contain the tortuosity of each fiber detected in the respective image of the stack as a scalar element. Then, a N-length vector will contain the tortuosity of the N-fibers detected in the image slide.
- Entropy (Statistical) as a single scalar element per stack image. The software evaluates the statistical entropy using two equivalent forms: $Entropy\_01$ and $Entropy\_10$ using an angular bin width of 1° and 10° respectively.
- Variance map (Var_map) is a map where the local organization of fibers is evaluated in a window of $Var\_size \times Var\_size$. It needs to be defined in μm. Var_map will be calculated with a 50 % of overlapping, i.e. for $Var\_size = 20\ \mu m$ will generate a Var_map of 20 μm window size evaluated every 10 μm. Only the Var_size (see line 6 of Listing 1) should be entered by the user.

Finally, the results found from the FingerPrint enhanced method will be saved in the directory $pathSave$ and $filenameSave$ as saved file name.

Listing 1: FingerPrint running code

```
%% Fingerprint methods parameters

scale_factor = 2;
FP_thresh = 0.08; % Threshold to define when a region is
   considered a ridge-like region
reliability_thresh = 0.2; %
Var_size = 20;

%% Define image stack to open and process it
path = '.\'; % full path of the image to analyze
filename = 'm149_2B3.tif'; % name of the image to analyze
full_path = ['' path filename ''];

metadata = read_tiff_metadata(full_path);

[IM, BW_map, Skel_map] = FP_segmentation(full_path, metadata,
   scale_factor, ...
                              reliability_thres, FP_thresh);

%% Results visualization

fibers_visualization(IM, BW_map, Skel_map, 1) % comment this
   line to avoid segmentation visualization

%% metric calculations

```

```
24 [T_fibersLength, Tortuosity, Entropy_01, Entropy_10, Var_map] =
       fiber_metrics(Skel_map, radius, metadata, scale_factor);
25
26 %% Save results
27
28 pathSave = '.\'; % full path where the results will be saved
29 filenameSave = 'm149_2B3_SavedTest'; % name of the file to be
       saved
30 full_path_Save = ['' pathSave filenameSave ''];
31
32 save([full_path_Save '.mat'], 'Skel_map','Var_map', '
       T_fibersLength', 'Tortuosity', 'Entropy_01', 'Entropy_10')
```


Acknowledgements

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List of external dependencies

- *bbspline*, Peter Kovesi.
- *catstruct*, MATLAB Central File Exchange.
- *derivative5*, Peter Kovesi.
- *freqest*, Peter Kovesi.
- *gaussfilt*, Peter Kovesi.
- *ini2struct*, MATLAB Central File Exchange, Andriy Nych.
- *normalise*, Peter Kovesi.

- *plotridgeorient*, Peter Kovesi.
- *ridgefilter*, Peter Kovesi.
- *ridgefreq*, Peter Kovesi.
- *ridgeorient*, Peter Kovesi.
- *ridgesegment*, Peter Kovesi.
- *skeletonOrientation*, GitHub, Stavros Tsogkas.

Citation

If you use our code to analyze your data. Please cite our publication as: Clara Manesco, Thierry Cloitre, Marta Martin, Yannick Nicolas Gerber, Florence Evelyne Perrin, Oscar Saavedra-Villanueva and Csilla Gergely. (2025) "Undergrowth collagen fibers analysis by fingerprint enhancement method" Biol. Cell. https://doi.org/10.1111/boc.70001