This is the documentation for code to run three-dimensional (3D) and two-dimensional (2D) waviness analysis on images of fiber-like structures.

**1. Software**

The code runs in the MATLAB programming language. To install, download MATLAB from: <http://www.mathworks.com/products/matlab/>

**2. Data format**

Theoretically, any format that can be read or loaded by MATLAB is suitable to de tested by the code.

**3. Run the analysis**

Here are totally 9 ATLAB files, where ‘wavinessmain\_2D.m’ and ‘wavinessmain\_3D.m’ are the main programs for 2D and 3D context respectively, and the others are functions that will be called during the running of the main programs. The explanations for the variables within the code, as well as the ideas in organizing each part of the code, have been detailed in the main program. The possible parameters that should be modified accordingly to your data sets have been highlighted as ‘modify x’ (x refers to the numbering).

Generally, the main program can be divided into 6 parts:

1. **Load images of fiber-like structures**

Here the 2D images are loaded, and for 3D case, images are stacked up and form a 3D stack for waviness and orientation analysis.

1. **Acquire the voxel-wise (pixel-wise for 2D case) orientation**

Here the voxel-wise (pixel-wise for 2D case) orientation of the fiber structures is acquired. The method is described in our previous papers (*Biomed. Opt. Express* **6**, 2294–2310 (2015);

1. **Create the binary mask selecting the fiber-only regions**

Here a binary mask will be created mainly based on the signal intensity. The fiber-only regions will be identified by this mask, which will be used in acquiring the waviness map.

1. **Calculate the voxel-wise (pixel-wise for 2D case) waviness**

Here the voxel-wise (pixel-wise for 2D case) waviness of the fiber structures is acquired. The width of the window for calculation is about 5-6 times the fiber diameter to get optimal results. Of course, the window size can be defined personally according to the research question.

1. **Perform post-processing**

Here we generate ‘pretty’ images of orientation and waviness. To acquire these images, the raw intensity image is used to provide the contrast of fiber features, and the orientation or waviness maps are labeled by different colors to show the orientation or waviness information.

**4. Example**

Images of collagen fiber as examples to test waviness code are saved in the folder ‘Example’. Here are one of the example fiber image and corresponding waviness map calculated with the initial parameter set in the code.

