Ultrastructural analysis toolbox: User manual

Table of Contents

1. Introduction

The MATLAB Ultrastructural/computational toolbox contains scripts that can process coordinates data files extracted from two distinct types of filament arrangements. All scripts designated with "**Lamellipodia**", offer the possibility to visualize data and extract quantifiable parameters characterizing filamentous dendritic networks, such as actin filament meshwork in lamellipodia. All scripts designated with "**Filopodia**" allow the data visualization and extraction of parameters characterizing filaments aligned in quasi-parallel orientation or bundled filaments, such as those in filopodia/microspikes. Methods for deriving coordinate files from cryo-ET data are discussed in the following articles: (Rigort et al., 2012); (Winkler et al., 2012).

The toolbox is described in
Dimchev G, Amiri B, Fäßler F, Falcke M, Schur FKM, Computational toolbox for ultrastructural quantitative analysis of filament networks in cryo-ET data, Journal of Structural Biology (2021)

1.1. System Requirements

- MATLAB versions R2020a or newer (most toolbox modules also work on earlier versions)
- A computer with sufficient CPU/GPU-performance to run MATLAB-based visualization modules and scripts

2. Avoiding filament tracking issues

Given the usage of appropriately formatted input coordinate files, the ultrastructural toolbox presented here is blind to the upstream approach used for filament segmentation and tracking, as well as the composition, arrangement or dimensions of filaments. This provides increased flexibility to the users, who can implement a variety of upstream approaches and optimizations for increasing the accuracy of filament detection. Below we provide several considerations and tips, which could be helpful in maximizing the quality of the vectorized filament coordinate data in the workflow steps upstream of the toolbox:

1. If you are working with tomographic datasets, consider the orientation of filaments with respect to the tilt axis. Due to the influence of the missing wedge, filaments running perpendicular to the tilt axis might have lower SNR value and their detection could be less efficient (Jasnin and Crevenna, 2016). When applicable, a potential solution would be to perform dual-axis tomography.
2. Consider which pixel binning would be most appropriate for filament detection. For instance, when implementing neuronal networks/machine-learning approaches for automated filament segmentation, annotating tomograms at higher binning might provide to be more accurate for differentiating filaments from background due to increased SNR.
3. When training neuronal networks by manually annotating filaments for subsequent automated filament segmentation, make sure to annotate all types of unspecific background, including the hole edges when using holey carbon grids as specimen carriers. This will significantly reduce the amount of artifacts and potential false-positives.
4. Consider the properties of filaments, such as bendiness, when setting up the parameters used for template matching (e.g. Cylinder length in Amira-Avizo). If filaments are not straight, but are bent or wavy, choosing a high value of template length might not allow for accurate filaments detection as it reduces the sensitivity to curved contours. On the other hand, selecting a too low value could result in the increased detection of unspecific background.

5. Consider the spatial arrangement of filaments and the nature of the structure they form. Optimizing parameter values for minimum distance/proximity between filaments could be key in avoiding duplicates or false-positives.
6. Consider the dimensions of filaments, such as width or radius in order to avoid detection of unspecific background.
7. Verify manually the final outcome by overlaying selected examples with their respective original volume data and checking whether tracked contours correspond to real filaments, whether filaments are tracked twice or whether any false-positives are generated. Curation steps in our toolbox (see below) to reduce false-positives could involve eliminating filaments of certain size (e.g. below 30-50nm for actin filaments), filaments running in specific angles or parallel filaments running in closer proximity to each other (possible double-tracked single filaments).

3. Getting started

Download the package into the desired directory and unpack using either appropriate software (for windows users) or, for linux users:

$ sudo apt-get install unzip

and unzip using the command:

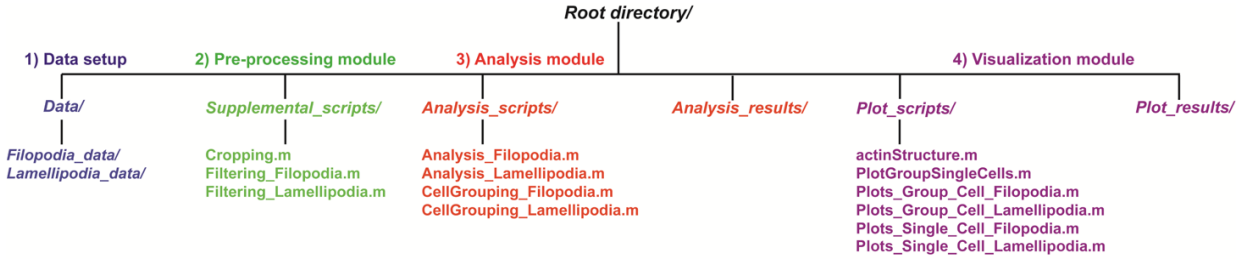
$ unzip filename.zip

or

$ unzip filename.zip –d /path/to/directory

3.1. Folder hierarchy

Upon download of the toolbox and unzipping the package you will find the following folders with respective scripts inside:



Below we provide a list of the default locations and functional organization of the various scripts included in the ultrastructural toolbox:

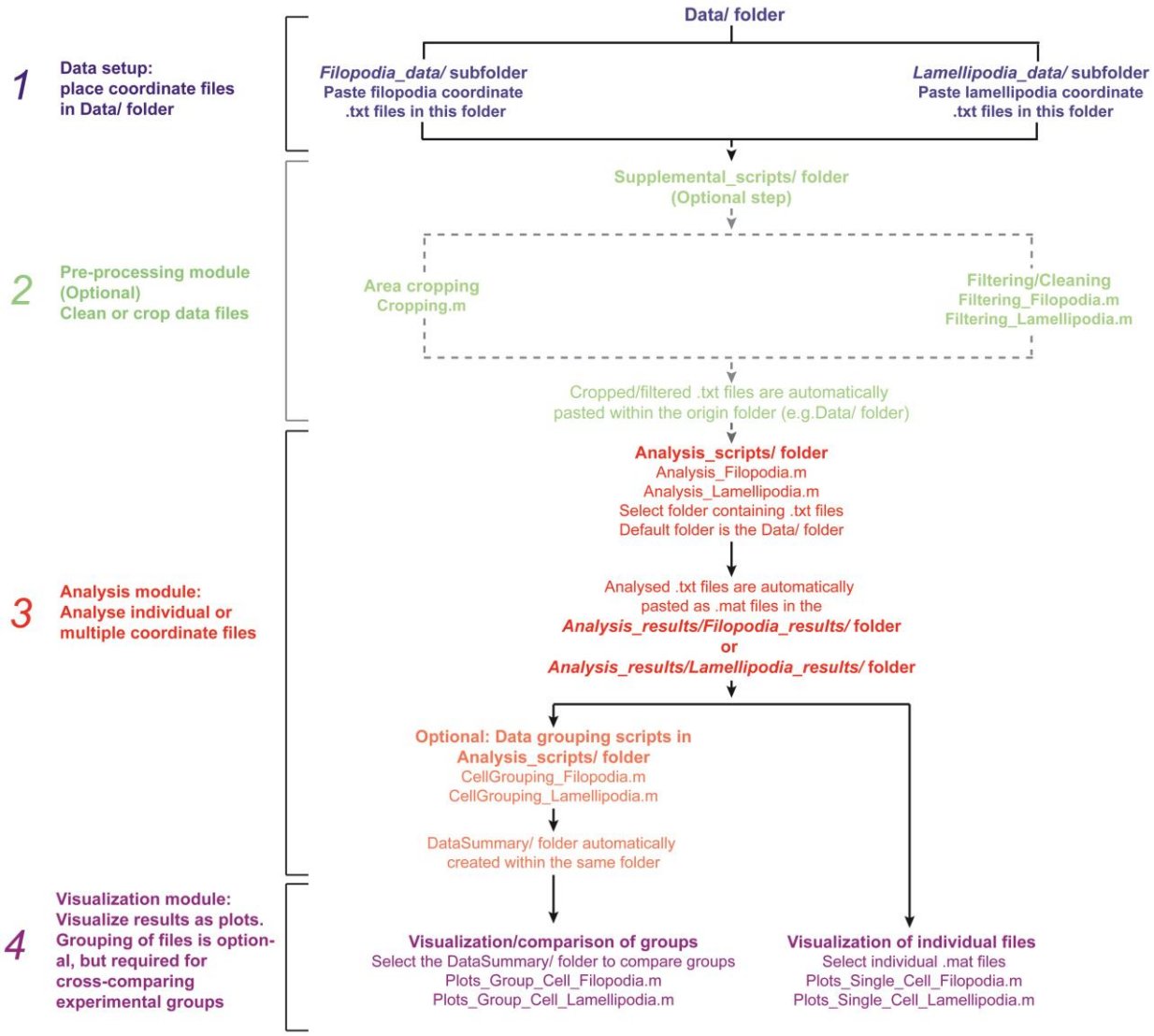
1. Pre-processing scripts (allowing coordinates data cropping and cleaning/filtering) are located in the subfolder “Supplemental_scripts”. Output (.txt format) of the scripts is copied within the input destination folder (when processing single files) or within a newly created folder in the input destination directory (when processing multiple files).
2. Analysis and Data grouping scripts are located in the “Analysis_scripts” folder. Upon running the main analysis script (Analysis_Filopodia.m or Analysis_Lamellipodia.m), user will be prompted to select data files from the “Data” directory by default, although data files can be loaded from any other destination. Analysed output is automatically copied in the “Analysis_results” folder in either “lamellipodial_results” or “filopodia_results” subfolders.

For single file analysis, the output is copied in a newly created subfolder named "Results_of_single_lamellipodium/filopodium analysis" within the "Analysis_results" folder.

3. Single plots can be saved manually. Plots from the single data file visualisation script, upon selecting the "save 3D plot" or "save cross section" options, are also automatically saved in the "Plot_results" folder.
4. The "Plot_scripts" folder contains all plots described here.

3.2. Workflow

A schematic summary of the workflow steps is provided below. From it the user can derive a better idea of how the processing of coordinate data is performed within the ultrastructural analysis toolbox.



3.3. Input data

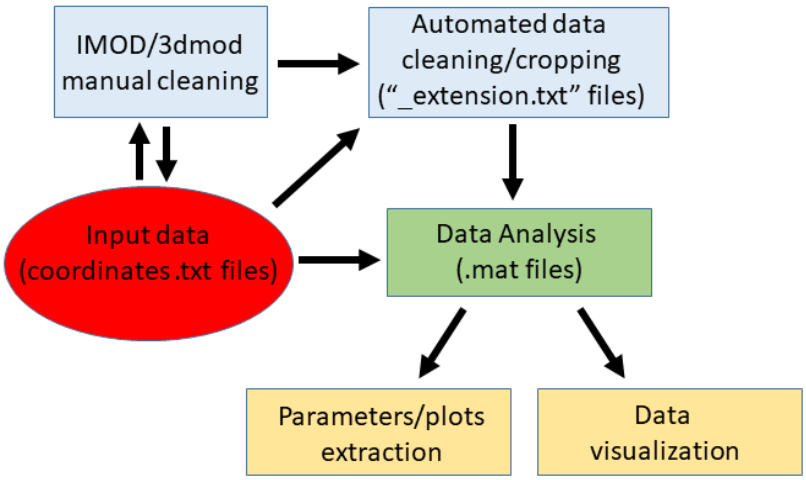
The toolbox requires .txt coordinate files as an input and provides two different types of output:

1) **.txt files** in the same format as the input files, obtained through optional preprocessing (cleaning/cropping)

AND/OR

2) **.mat files** (analysis output files) from which various parameters or plots can be extracted.

The formatting style of the input/output **.txt** files also allows their transformation into IMOD-compatible 3D point coordinate **.mod** files via the "*point2model*" function (more info here), enabling additional manipulations, such as manual exclusion of single objects/filaments. A simplified graphical depiction of the workflow is provided below:



3.3.1.Input data format

The scripts use as input a tab-delimited .txt file, formatted as shown:

1	173	24	9
1	169	27	8
1	163	31	7
1	157	36	7
1	147	46	8
1	137	57	9

Column1: Filament/object identifier

Column2: X coordinate for a specific point of the filament/object

Column3: Y coordinate for a specific point of the filament/object

Column4: Z coordinate for a specific point of the filament/object

3.4. Re-formatting Amira-Avizo output files

Filament coordinates derived from the "Trace correlation lines" module of the Amira-Avizo software package can be transformed to a version compatible as an input with the here described computational toolbox. We provide a script to facilitate this step, located in the main folder (amira_reformat_to_coordinates.m). The script requires 2 types of input files extracted from Amira-Avizo (in tab delimited .txt format): 1) A points file containing "Point ID", X, Y and Z coordinates and 2) a segments file containing only the "Point IDs". As annotated within the script, the user needs to place both files in the script directory, modify the file names accordingly within the annotated lines

in the code and run the script. The output of the script is a newly created .txt file, fully compatible with the computational toolbox.

4. PreProcessing *(OPTIONAL)*

4.1. Data cleaning and cropping

Scripts located in the "Supplemental_scripts" folder allow to either filter data files according to user-defined parameter ranges or crop the coordinate data files by pre-defined X-Y dimensions. The user can typically test various filtering ranges for e.g. filament length, bendiness or angular distribution. This generates filtered output as .txt files. To visualize the output, a .txt file needs to be processed with the main analysis scripts (Analysis_Filopodia.m or Analysis_Lamellipodia.m) to generate a .mat file, which can then be displayed with the visualization module. Given that the user prefers not to pre-process the data files, a limited set of filtering options is also preserved within the main data analysis scripts.

4.1.1. Pixel size notes

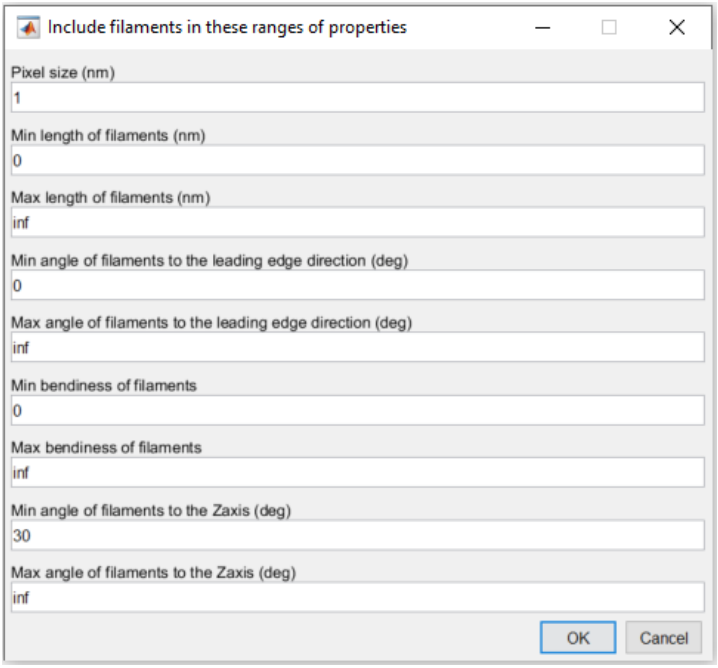
The .txt output files of data processed with either the filtering or cropping scripts, in which pixel size other than 1 was selected by the user, are rescaled for the newly defined pixel size. It is important to remember that when processing these rescaled .txt output files with any other downstream script, such as the main analysis script, the original pixel size should not be re-defined again. Instead, a pixel size of "1" should be used as input.

4.2. Data filtering

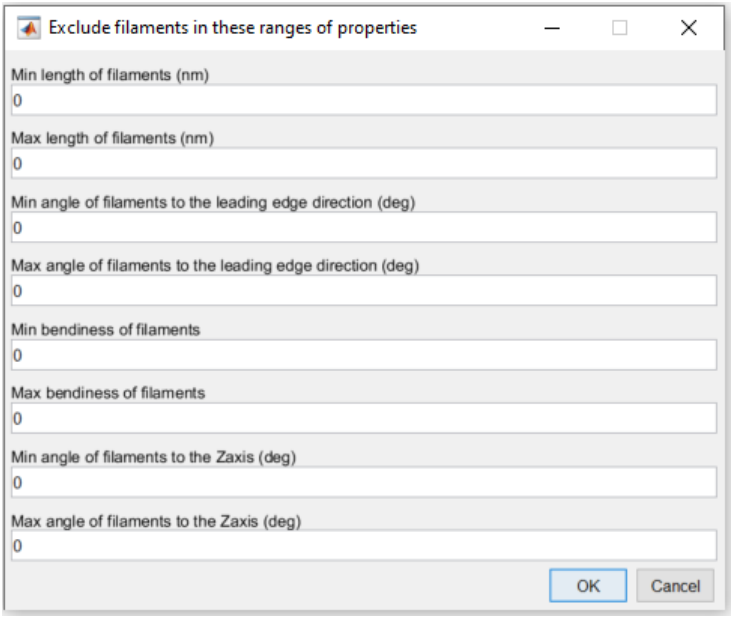
Used scripts: Filtering_Filopodia.m; Filtering_Lamellipodia.m
Default location: "Supplemental_scripts" folder

Depending on the quality of the data, it may sometimes be necessary to clean unspecific background tracks or otherwise undesirable filaments/objects. Filtering parameters include length, bendiness and angle to XY- or Z-axis. Upon running the Filtering_Filopodia.m or Filtering_Lamellipodia.m scripts, one can choose to process either single or multiple files. After selecting the data containing directory, the script allows the following options:

1. Only INCLUDE filaments in specific ranges. The pixel size can be specified in the first field.



2. Only EXCLUDE filaments in certain ranges

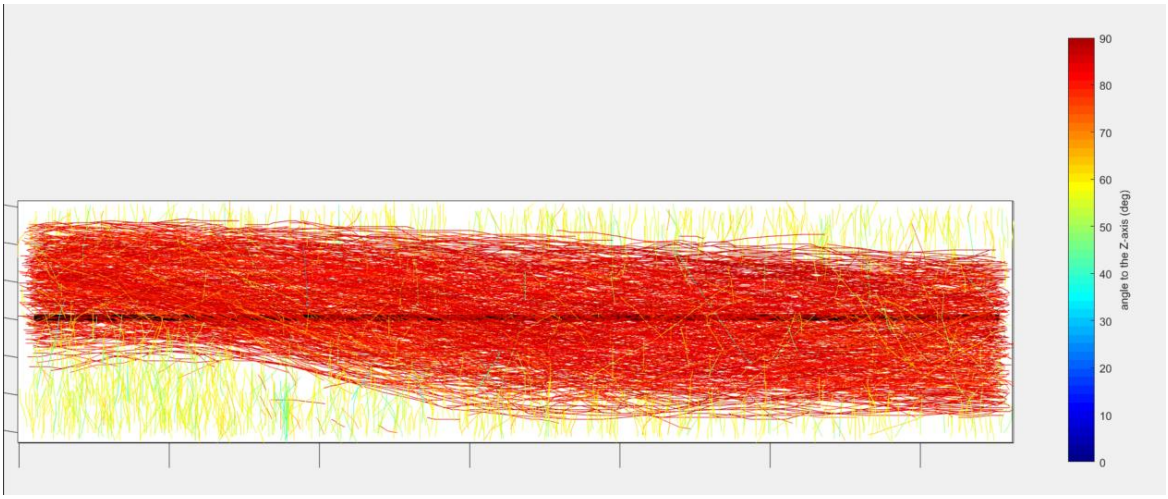


The script requires manually defining the direction/orientation axis (for lamellipodia) or a point close to the filopodium base. Please note that setting the orientation axis at this stage is only required for data filtering of angular ranges, which uses the orientation axis of each structure as a reference. The user will be prompted to input the orientation axis again when using the analysis scripts at a later stage.

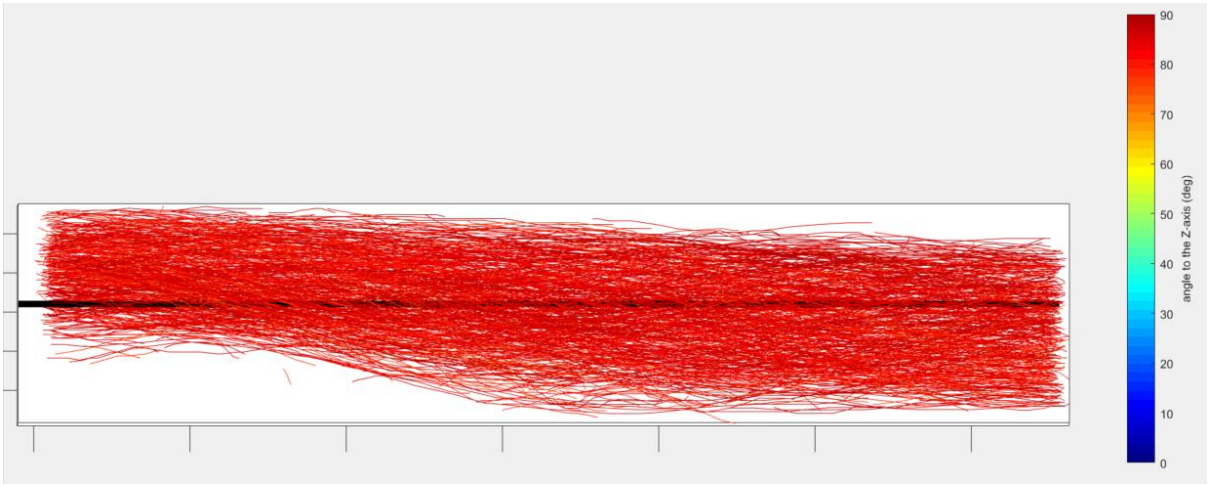
Examples:

1. Data file displayed in Z-axis view, the black line indicating the X-axis orientation. Color coding represents the angular distribution of filaments in Z (with 0 degrees being equivalent to filaments parallel to the Z-axis and 90 degrees equivalent to filaments perpendicular to the Z-axis). Unspecific background is visualized as yellow contours. The coloring indicates tacks being oriented predominantly at angular ranges of 60-75 degrees. *Please note that for acquiring a more accurate*

visual idea of the angular distribution of filaments, the structure has to be rotated around the Z axis.



2. Filtering_Lamellipodia.m script was used to exclude filaments with the following properties: 1) <100nm in length and 2) within ranges of 0-75 degrees in Z.



Data examples: *For testing the script, the user can find a .txt file located in "Data/1-Filtering-example/Lamellipodium-filter.txt" and could use the filtering settings described above to clean unspecific background (e.g. exclude filaments of <100nm and between 0-75 degrees in Z). Note that the scale of the coordinates file is still in Angstrom units, thus for working in nm scale, a pixel size of 0.1nm has to be set. To compare the filtered vs the unfiltered data, one can use the Analysis_Lamellipodia.m script to generate output (.mat) files for both filtered and unfiltered coordinate files (info here) and visualize them with the Plots_Single_Cell_Lamellipodia.m script (info here).*

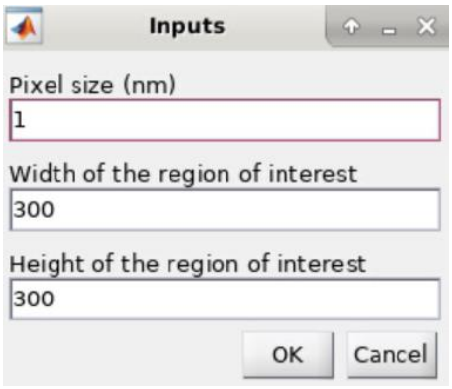
4.3. Data cropping

Used script: Cropping.m
Default location: "Supplemental_scripts" folder

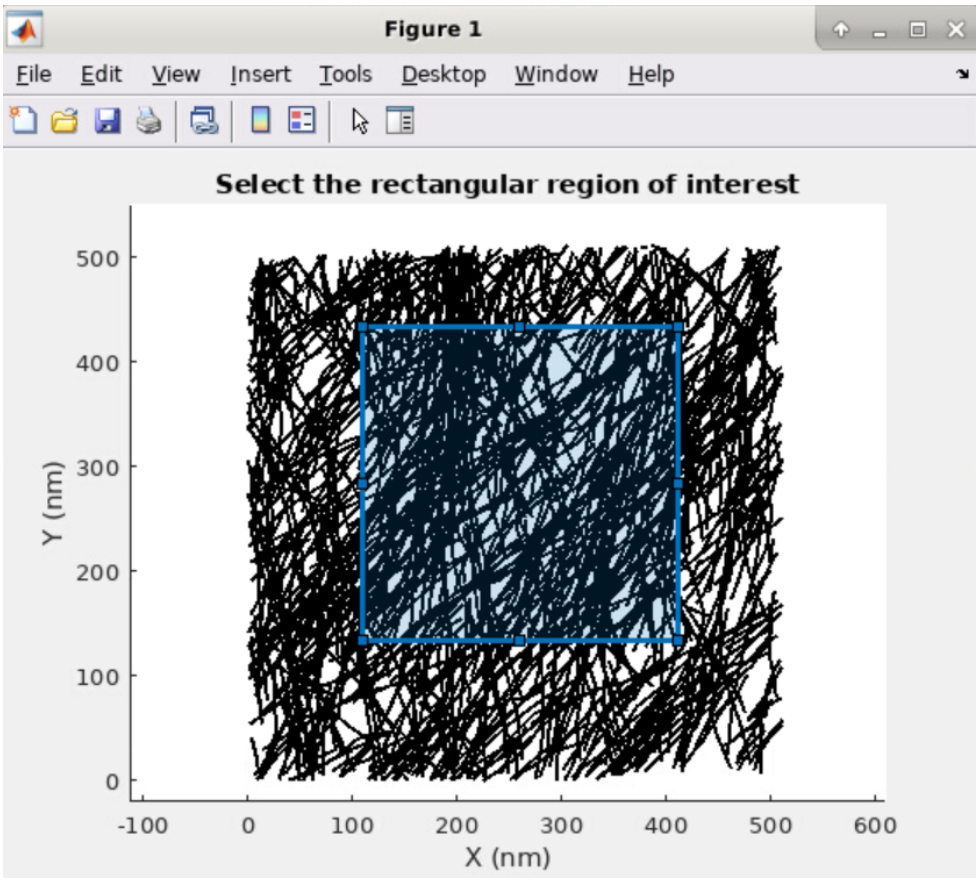
Working with large datasets and comparing different experimental groups, sometimes acquired at different pixel sizes, might require normalizing the dimensions of the regions to analyse and compare. Cropping.m script facilitates that by allowing the user to input the pixel size and to set a rectangular

region of certain dimensions. Coordinate points outside of the region will be excluded. Upon running the script:

1. The user will be prompted to input pixel size and width/height of the region of interest:



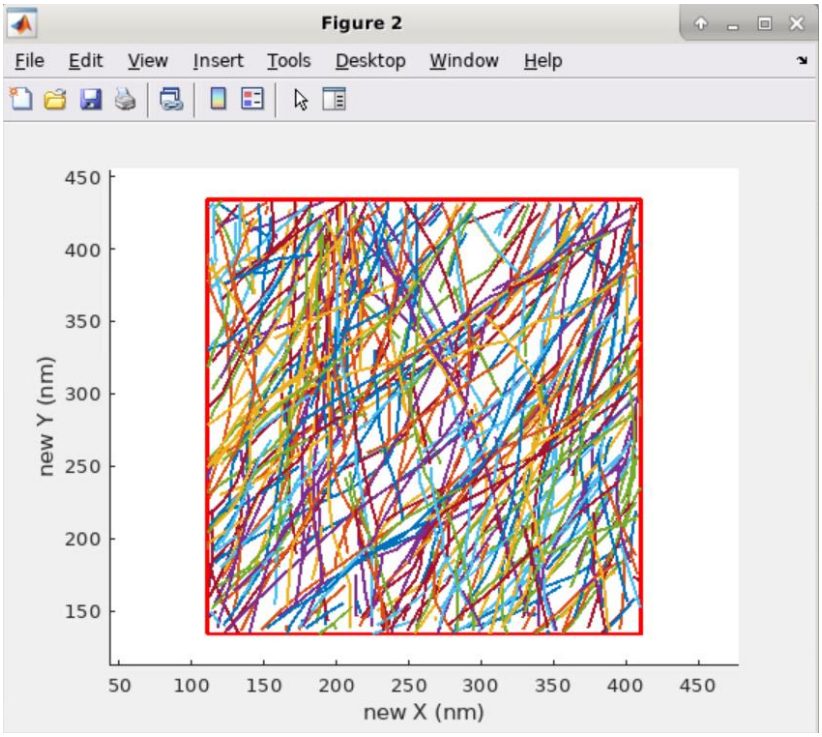
2. A region of interest can be dragged and rotated in order to position it at the desired location. Dimensions of the region can also be changed manually by pulling the corners of the blue rectangle. The user should double-click on the region to proceed.



Note: For optimal performance of the cropping script, it is desirable to use MATLAB versions 2020 or higher. We have experienced issues with earlier versions of the software when the defined region (blue rectangle in the image above) might not be visible. If this occurs, the following fix sometimes works: select the hand icon in the top right corner of the image, move the image slightly and deselect the hand tool. The region should re-appear.

3. The region selection can be modified (if a user error was made) or the user could choose to proceed further with the other dataset files.

4. Once confirmed, a window will appear with the cropped region displayed. Double-click on the keyboard (e.g. Enter or Space) to continue with other data files or to terminate the process.



A new .txt file with the extension "_cropped.txt" will be generated for each file in the input directory. The output file now contains only the coordinate points within the cropped region of interest.

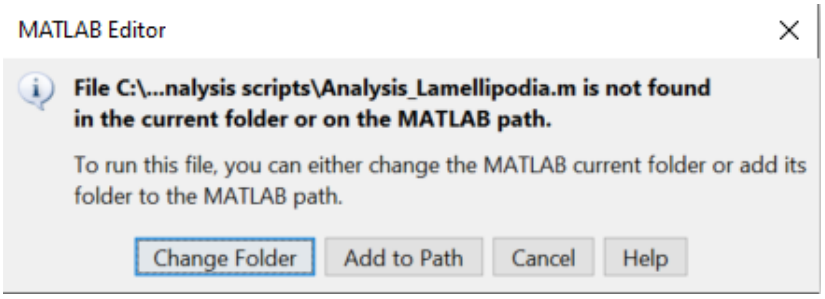
Data examples: *For testing the script, the user can find a .txt file located in "Data/2-Cropping-examples/To-crop.txt". Note that the scale of this coordinates file is still in Angstrom units. Thus, for cropping a region of size 800x800nm, a pixel size of 0.1nm has to be set.*

5. Data analysis

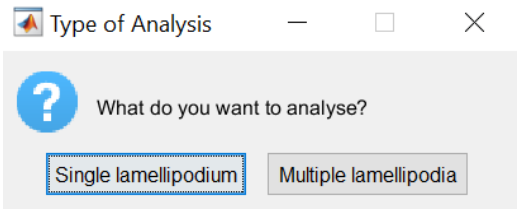
5.1. Data analysis of Lamellipodium/Filopodium

Used scripts: Analysis_Filopodia.m; Analysis_Lamellipodia.m
Default location: "Analysis_scripts" folder

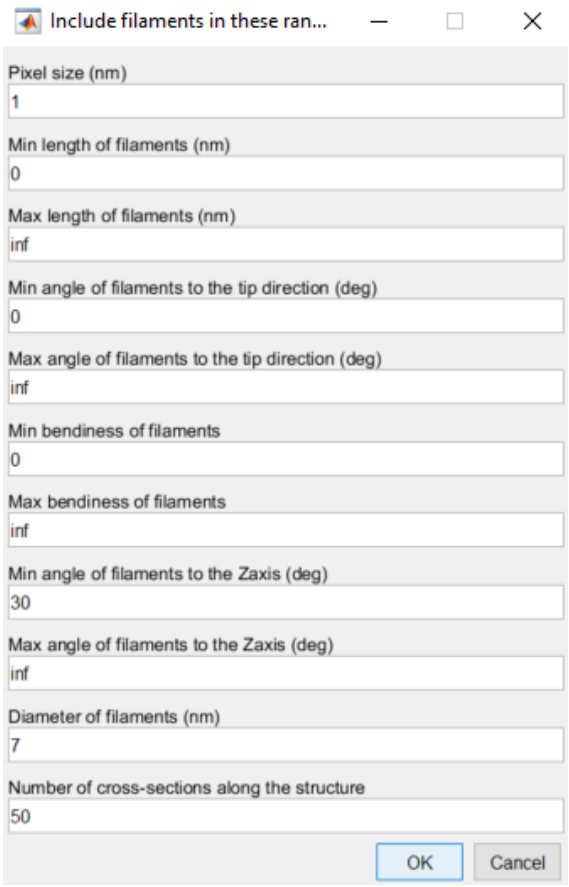
1. Run the Analysis_Lamellipodia.m or the Analysis_Filopodia.m script. Make sure you go to the directory where the script is located. Alternativey, upon running the script (right click on the script and select "run"), when prompted, click on "Change folder"



and select either a single or multiple data files.



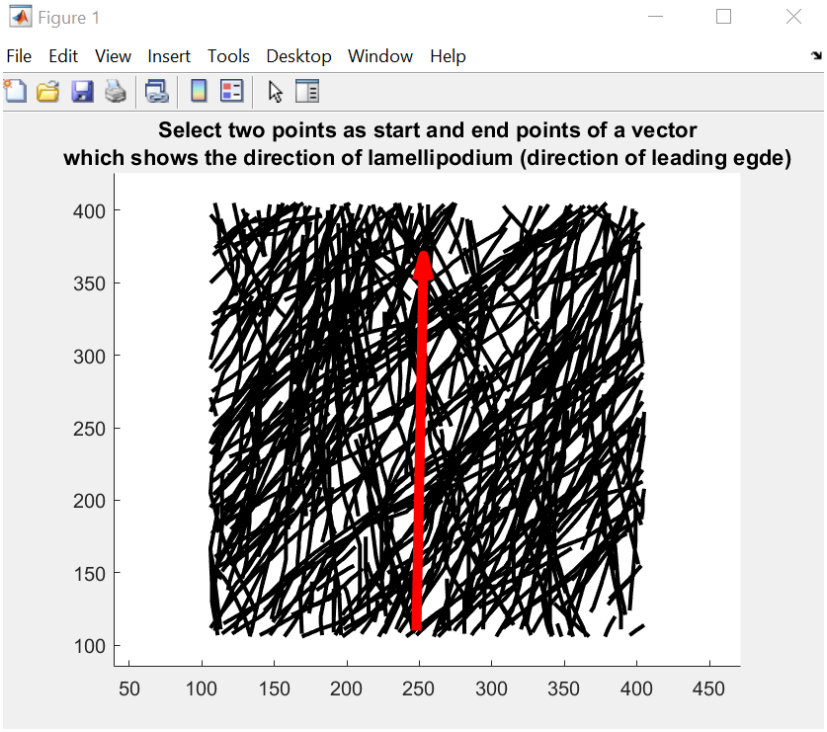
2. The user will be prompted to input pixel size and (optionally) filtering parameter ranges (i.e. only filaments within defined ranges for length, bendiness or angles will be considered for analysis). In addition, an option for defining diameter of filaments exists. The input is set to 7nm by default, as this is approximately the diameter of an actin filament. This value is required for calculating some of the volume-related parameters and the option is integrated to allow the analysis of filament data of other properties/dimensions (e.g. microtubules). An option to select the number of cross-sections along the structure might be an important step to consider, depending on its length and the pixel size. Selecting an optimal number of cross-sections for longer structures, such as filopodia, might improve the sensitivity of downstream plots based on cross-sectional parameters. As explained in section 6.3.3, the toolbox still allows the comparisons of experimental groups even if the individual files they contain were analysed using a different number of cross-sections.



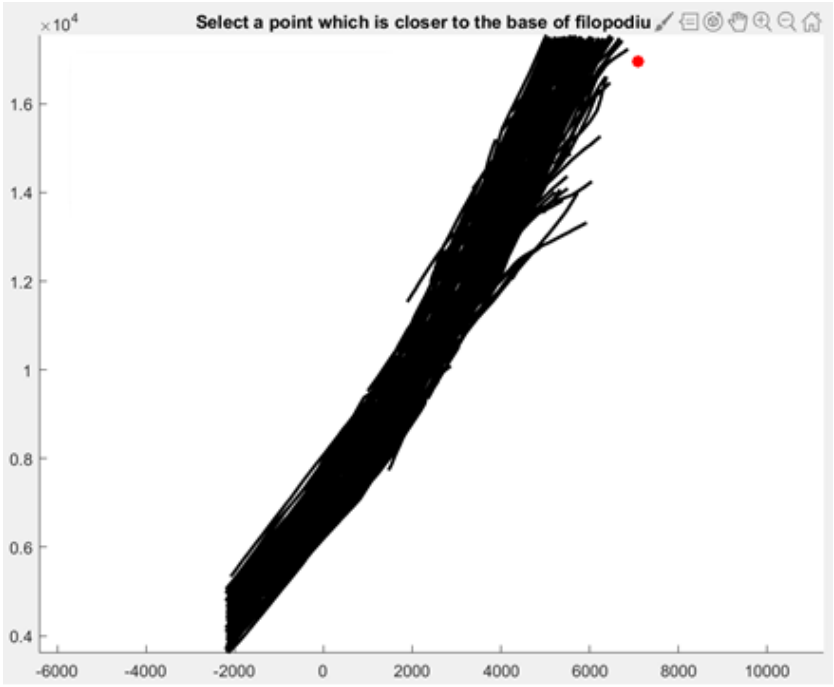
As mentioned above, if data was previously processed once with any of the pre-processing scripts (cropping or filtering) and pixel size was already defined, set the pixel size in the analysis script as "1" and do not input the original pixel size again. This would re-scale the data once more and thus lead to inaccurate dimensions.

Example: Coordinate files extracted by processing raw header-containing .rec files with the Amira-Avizo package are by default formatted in an Angstrom scale (unless otherwise specified). For extracting information in the nm scale for those files, set the pixel size to 0.1nm.

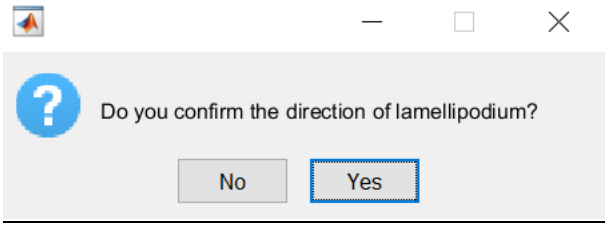
3. Define the orientation of the X-axis towards the cell edge in lamellipodia by selecting start and end points with the left-mouse click.



4. For defining X-axis orientation of a filopodium, choose any point close to the base of the structure (the precise location is irrelevant, it only needs to be in proximity to the base).



5. If user errors were made, the action can be repeated for each data file.



From all processed input (.txt) files, output (.mat) files will be generated, which are automatically copied into the "Analysis_results" folder, in either "filopodia_results" or "lamellipodia_results" subfolders. Each .mat file contains information on dimensions, orientation axis and calculated parameter values, which can be plotted together with results generated from other files for averaging or comparison.

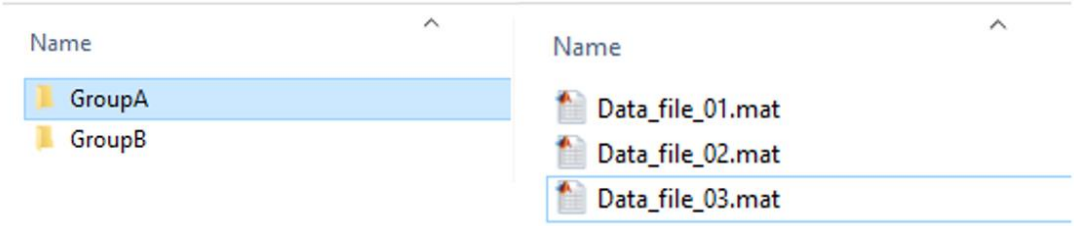
Data examples: *For testing the scripts, the user can find 2 folders in the "Data" directory ("Filopodia_data" and "Lamellipodia_data") containing .txt coordinate files. Each folder contains two additional subfolders/groups to compare.*

5.2. Data grouping

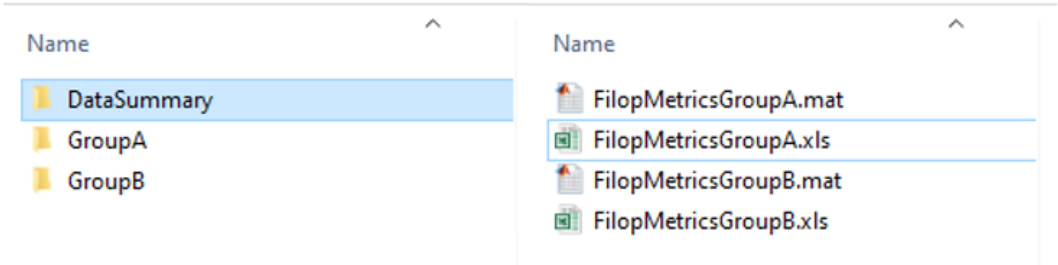
Used scripts: CellGrouping_Filopodia.m; CellGrouping_Lamellipodia.m
Default location: "Analysis_scripts" folder

Multiple output (.mat) files can be assigned to a group, which allows the comparison of different experimental conditions.

1. The user can create a folder with subfolders, each containing the .mat files belonging to an assigned group:



2. By running the "CellGrouping_Filopodia.m" or "CellGrouping_Lamellipodia.m" scripts and selecting the destination directory, a new "DataSummary" subfolder will be created in the selected directory. It contains all parameter data for each experimental group in the form of a .mat file and an .xls file:



The .mat files within the "DataSummary" folder are required for the various plotting options, allowing the averaging of parameter values and calculating of standard deviations for each group. The .xls file contains the individual parameter values for each data file in a group. The .xls file is not required for

any downstream script, but might be useful for extracting the raw parameter values to perform various statistical tests.

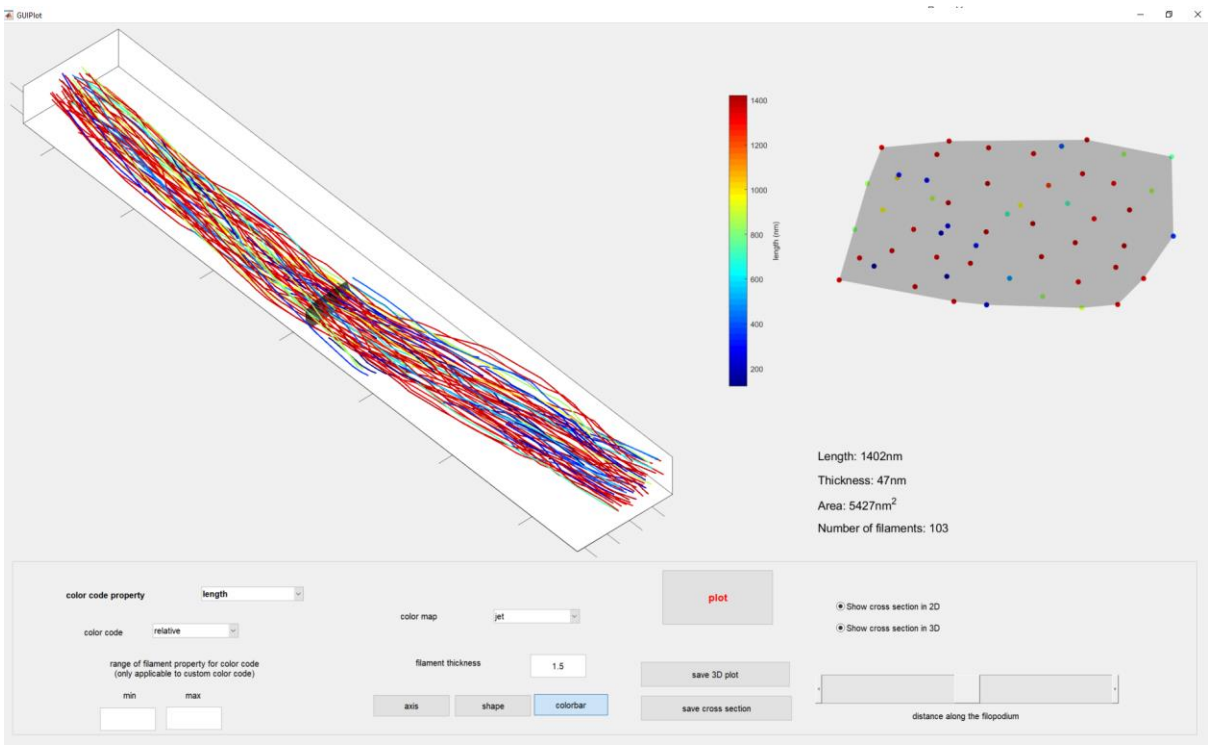
Script examples: *The user can find an example of already analyzed and grouped subfolders for filopodia and lamellipodia, including a "DataSummary" subfolder for each, located in the "Analysis_results/1-Analyzed/" directory*

6. Data visualization

6.1. Visualization of individual data files

Used scripts: Plots_Single_Cell_Filopodia.m, Plots_Single_Cell-Lamellipodia.m; PlotGroupSingleCells.m
Default location: "Plot_scripts" folder

"Plots_Single_Cell_Filopodia.m" or "Plots_Single_Cell-Lamellipodia.m" scripts allow the visualization of individual output (.mat) files. Note that not yet analyzed raw files (.txt) cannot be visualized with this module since they do not contain information on axis orientations and parameter values. Upon running the script and selecting an individual .mat file, a GUI window will open with various visualization options:



The following options are available, with on/off effects activated by the red "plot" button:

- 360 degrees view of the displayed structure
- Color-coding the structure by properties, such as length, bendiness or angular orientation of filaments (X- or Z-axis). The color code range can be adjusted by setting min or max values in the "custom" mode.
- Various color maps
- Adjusting the filament thickness
- Overlaying objects, such as orientation axis or shape
- Cross-section visualization with a slider for moving the cross-section position along the axis

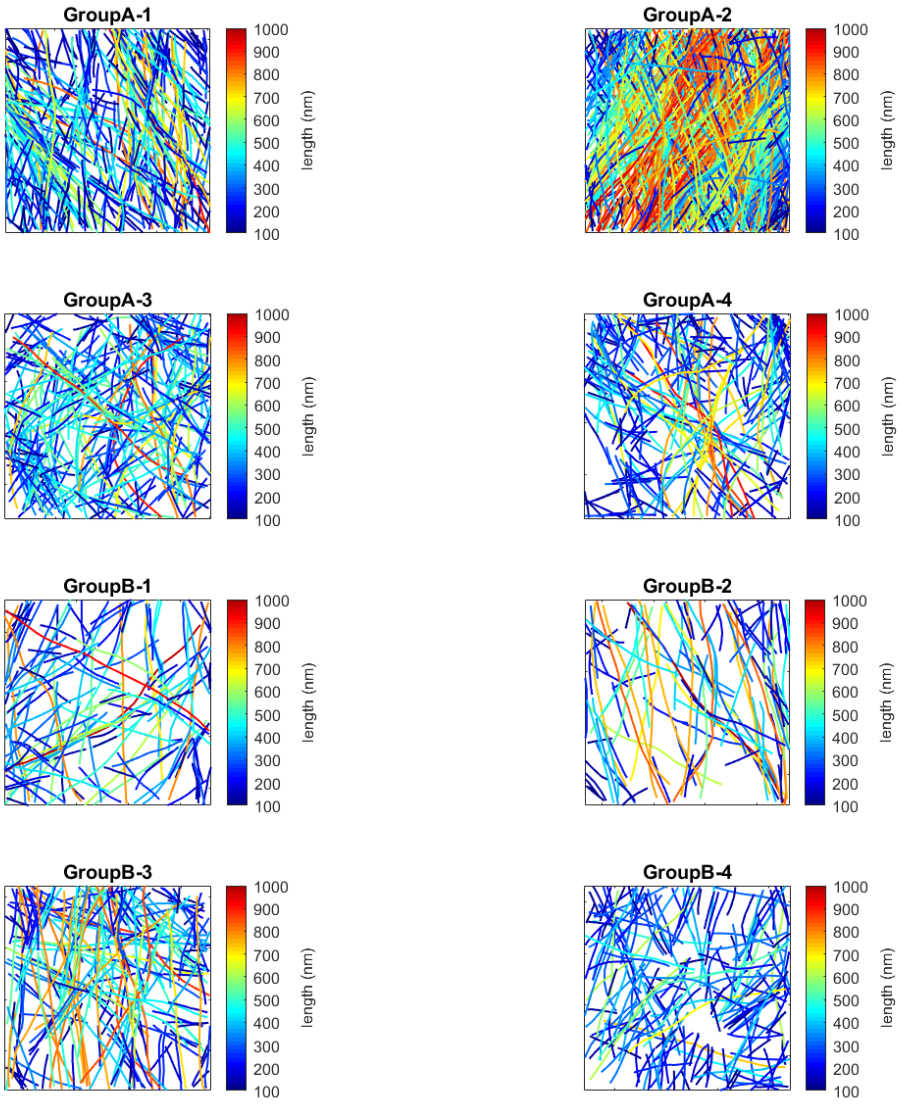
- Information on the main characteristics of the structure, such as length, thickness, area and number of filaments
- Option to save the structure or cross-section area as images (Automatically saved in the “Plot results” folder)

Script examples: *For testing the visualization module, the user can find two subfolders (for Filopodia and Lamellipodia) containing individual .mat files located in the “Analysis_results/1-Analyzed/” folder*

6.2. Gallery view

Used scripts: PlotGroupSingleCells.m
Default location: “Plot_scripts” folder

A gallery view allows the quick visualization of multiple files in the same window by running the “PlotGroupSingleCells.m” script in the “Plot_scripts” folder. The user is prompted to select the directory containing all individual files, which are to be displayed (.mat format). Different color codes can be selected for bendiness, length and angular distribution of filaments. The number of rows/columns, the thickness of filaments and the range of values are also customizable.

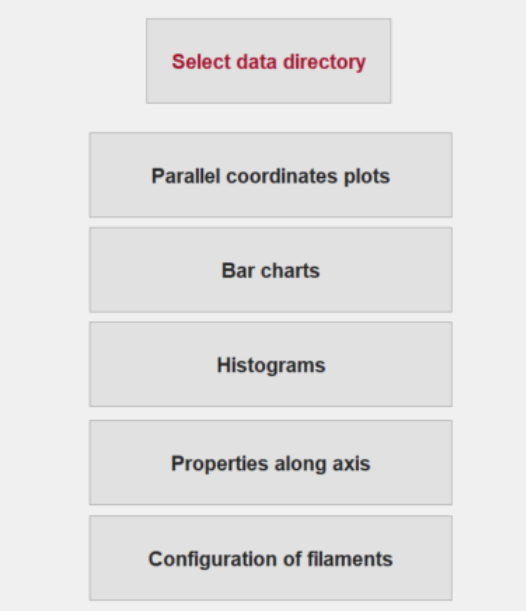


Script examples: *For testing the script, the user can find examples of already analyzed and grouped data in the "Analysis_results/1-Analyzed/" directory, containing individual .mat files that can be grouped and displayed together*

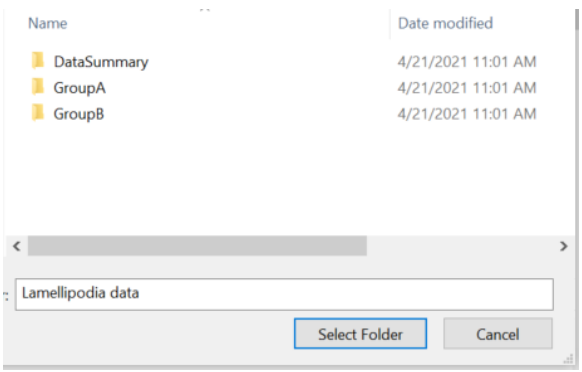
6.3. Cross-comparison

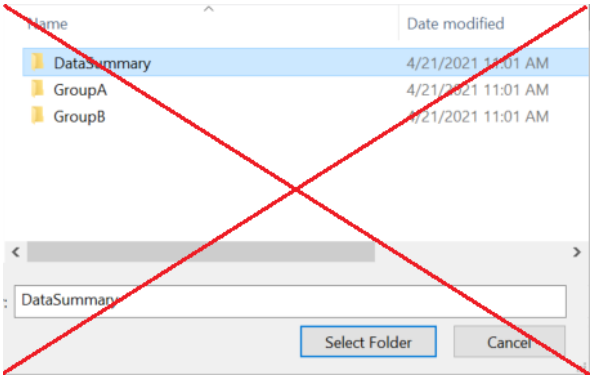
Used scripts: Plots_Group_Cell_Filopodia.m; Plots_Group_Cell_Lamellipodia.m;
Default location: "Plot_scripts" folder

Various plots are available in the "Plot_scripts" folder allowing the comparison of analyzed experimental groups. Upon running the "Plots_Group_Cell_Filopodia/Lamellipodia.m", the user should first define the location of the directory containing the "DataSummary" subfolder generated by the grouping script (as explained here). This can be done after clicking the red "Select data directory" button:



Note: In order to avoid errors, upon clicking on the red "Select data directory" button, only go to the location of the "DataSummary" folder and press enter or click on "Select folder". Do not select (single-click) or open the "DataSummary" folder or the individual .mat files in it.

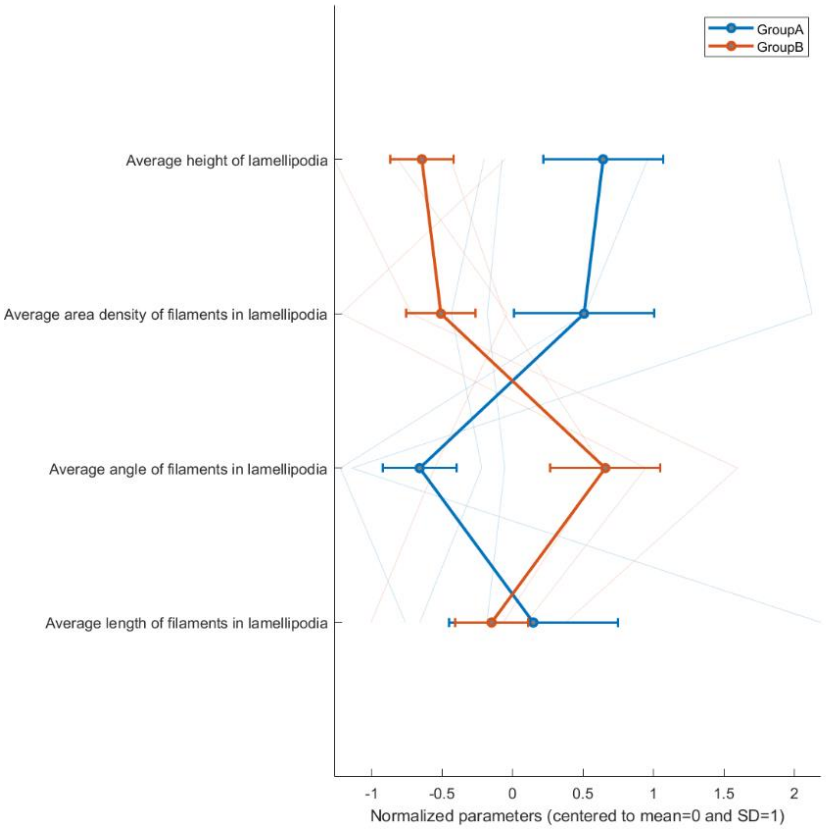




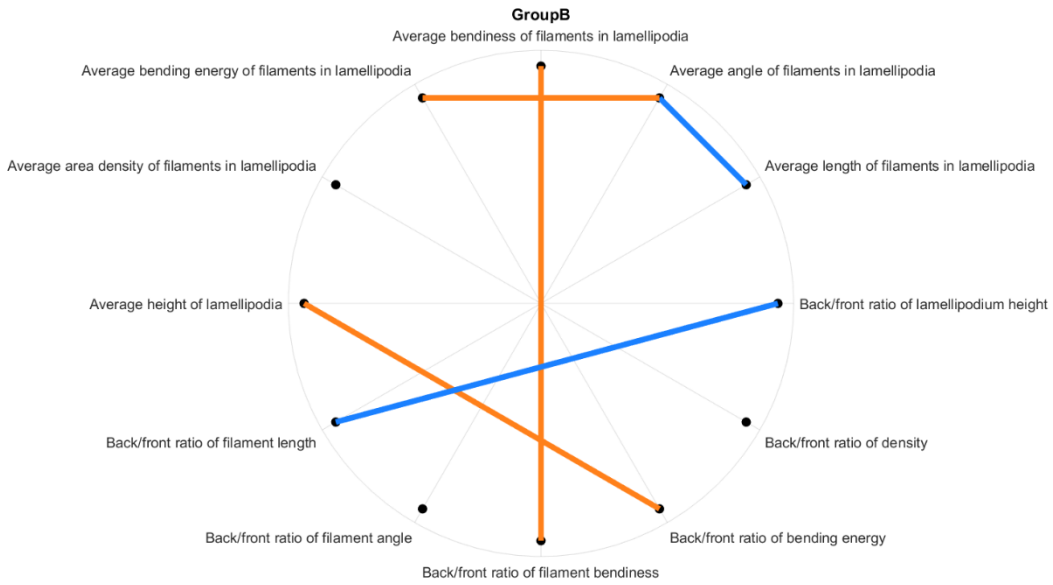
After defining the "DataSummary" location, various plotting options can be selected from the menu. Plots and figures can be manually saved.

6.3.1.Parallel coordinate plots

A linear visualization plot allows for a quick and convenient overview of differences in multiple parameters between 2 or more groups. Upon running the script, the user will be prompted to select one or more parameters of interest. For facilitating their visualization, parameters are normalized by centering them to mean=0 and SD=1. Thus, differences between groups are all relevant and not represented in absolute values. Parameter values are plotted on the X-axis, with their value increasing towards the right (when applicable). This type of plot allows the quick determination of whether differences in any parameter are observed between groups. The user can then choose to further dissect differences in individual parameters by plotting them with another of the below described plots. *(E.g. Observed differences in filament angles can be examined in more detail by plotting a Histogram plot to find which specific angular populations show larger differences between groups).* Note that the list of parameters differs between filopodia and lamellipodia since not all parameters are applicable to both structures.

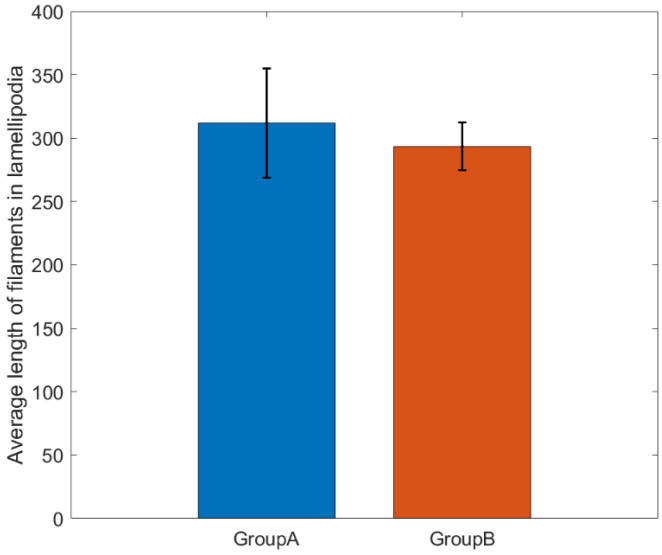


This script also includes the visual representation of correlations between parameters separately for each group, with blue and orange lines corresponding to positive or negative correlations, respectively.



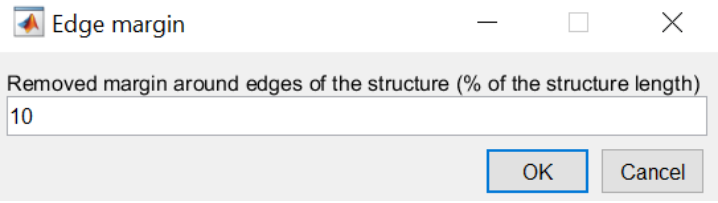
6.3.2.Bar charts

This option creates a bar chart comparing the average values of two or more groups. Upon running this script, the user will be prompted to select the parameter of interest. Note that the list of parameters differs for filopodia and lamellipodia since not all parameters are applicable for both structures. The error bars in the chart represent the SD for each group.



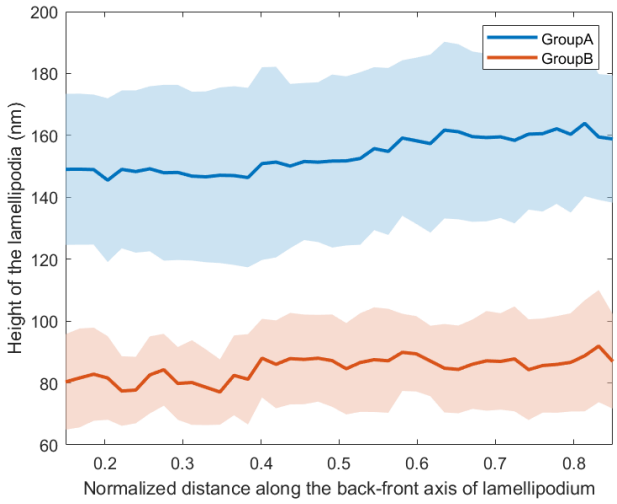
6.3.3.Properties along axis

The module compares parameter values between 2 or more groups. The Y-axis displays parameter values for each cross-sectional plane and X-axis displays the distance from the base of the structure (left) to the tip/edge (right). Upon running this script, the user will be prompted to select the parameter of interest. Note that the list of parameters differs for filopodia or lamellipodia since not all parameters are applicable for both structures. The user can also define margins around edges of the structure, which excludes filament coordinate points beyond those margins:



This might sometimes be necessary due to potential issues arising from unequal cross-sectional areas at the very edges of the structure, particularly in cases where structures are cropped in a way that sharp edges are present.

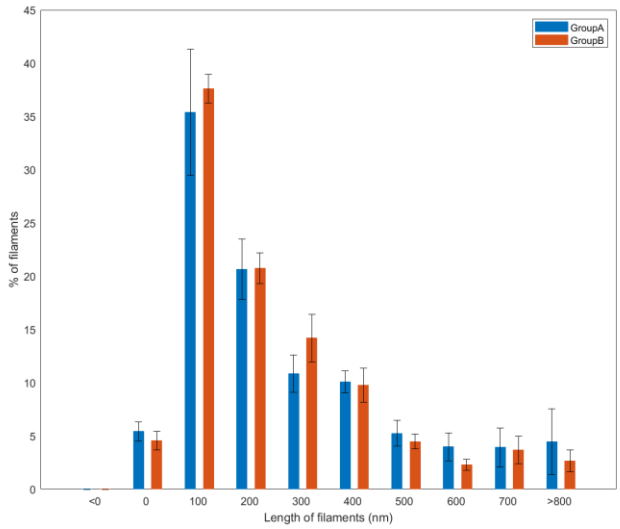
In the resulting plot, the color-coded areas around the solid lines represent SD for each group.



The number of cross-sections can be defined again at this step, independently of the number of cross-sections selected during the generation of individual .mat files with the analysis script (see Section 5.1). When the number of cross-sections is chosen to be different than the number previously defined during the analysis step, an interpolation procedure is performed. This allows to plot and compare experimental groups containing multiple .mat files with varying number of cross-sections encoded in each file.

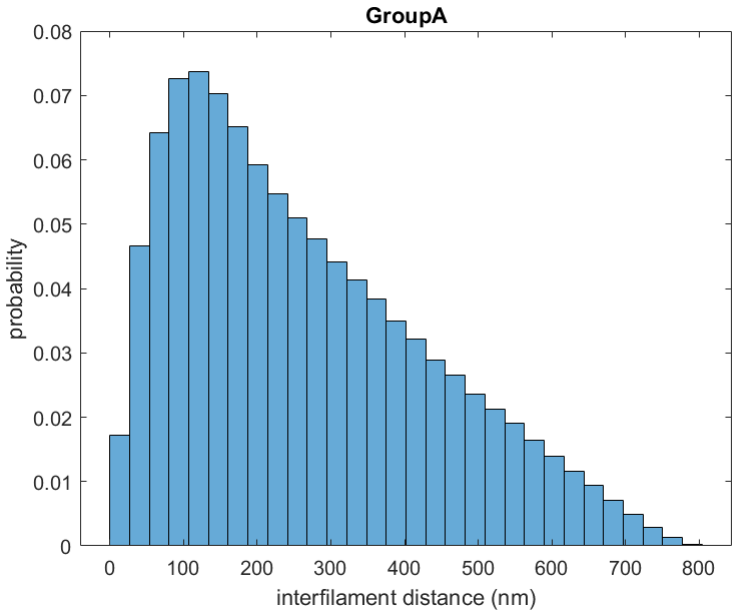
6.3.4.Histograms

This option plots a histogram chart comparing the average parameter values of 2 or more groups by separating them into bins. Upon running this script, the user will be prompted to select the parameter of interest, as well as the width, first and last edge of binning. Note that the list of parameters differs for filopodia or lamellipodia since not all parameters are applicable for both structures. The error bars in the chart represent the SD for each group and binning. In order to use this plot, more than one .mat file per group needs to be present to allow the calculation of SD and the display of error bars.

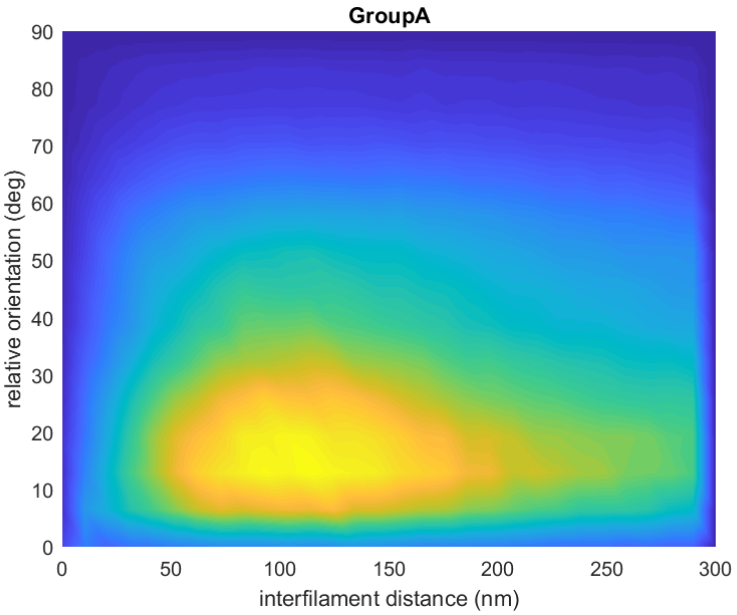


6.3.5.Configuration of filaments

Allows visualisation of the interfilament organization by plotting the distribution of interfilament distances against the relative orientation of the neighbors for each filament. Results are plotted as both a histogram (probability that a number of filaments have neighbors within certain distance) and as a 2D intensity map. For the 2D plot, the user is prompted to select ranges for min and max distance between filaments and interfilament angles, as well as number of bins for both parameters. The parameter is adapted from Jasnin et al. (Jasnin et al., 2013).



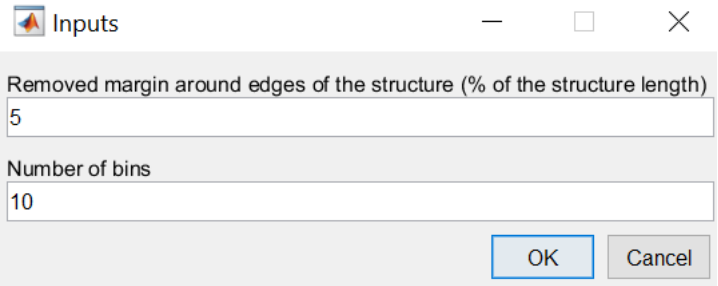
The plot above also allows to display median and mean values of interfilament distance or relative orientation (deg).



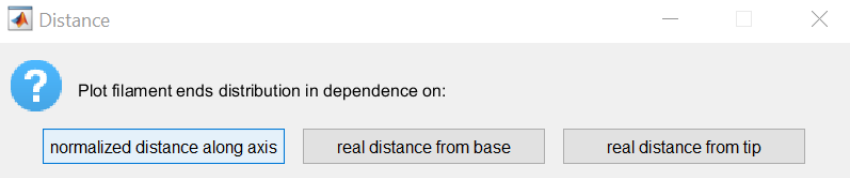
6.3.6. Density of pointed/barbed ends

Upon running the Plots_Group_Cells_Filopodia/Lamellipodia.m script, in the "Properties along axis" plot, it is also possible to derive the density of barbed and pointed ends of actin filaments, i.e. start and ending point of filaments.

Upon selecting the "Barbed/Pointed Ends" parameter, the user will be prompted to select the number of bins and the percentage of length to be removed around the edges of the structure. The latter option might be important for cropped areas, where artificial filament starting/ending points might be introduced around the edges of the cropped area.



The user will then be prompted to select 3 plotting options:

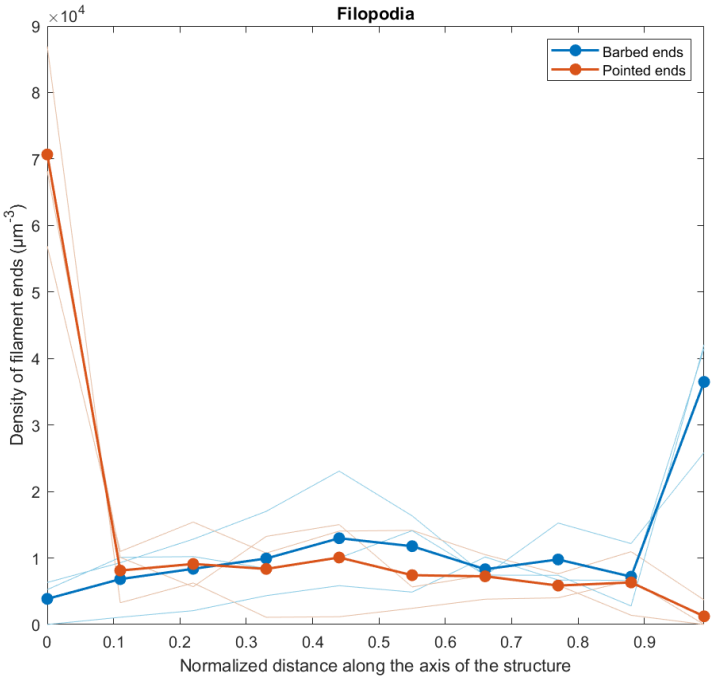


1. "Normalized distance along axis" will average the values of all cells in an experimental group based on normalized distance (min=0, max=1). For instance, the density of barbed ends will be averaged for all cells at normalized distance=0.3.

2. “Real distance from base” will average values from all cells in an experimental group based on the distance (in nm) of barbed/pointed ends along the axis.
3. “Real distance from tip” will average values from all cells in an experimental group along the axis based on the distance (in nm) of barbed/pointed ends from the tip.

Note: Be aware that the “Real distance from base/tip” parameters would ideally require a dataset with uniform distribution of structure length.

An example of the visual output of this parameter is shown below for a small dataset of filopodia for which the difference in the distribution of pointed and barbed ends is clearly distinguishable towards the base and tip of the structure, respectively.

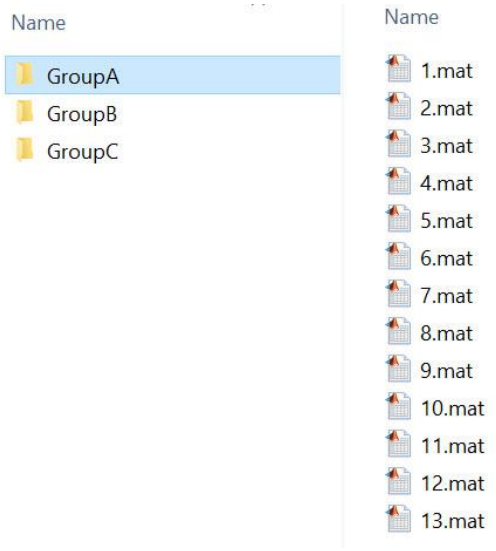


Script examples: *For testing the scripts, the user can find examples of already analyzed and grouped data in the “Analysis_results/1-Analyzed/” directory, containing “DataSummary” subfolders for filopodia or lamellipodia, as well as individual .mat files*

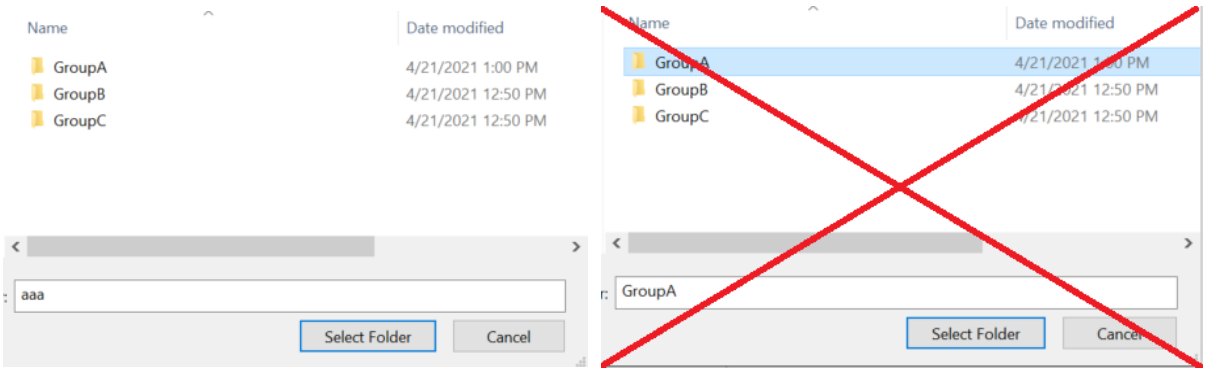
6.4. Parameter correlations and PCA plots

Used scripts: actinStructure.m
Default location: “Plot_scripts” folder

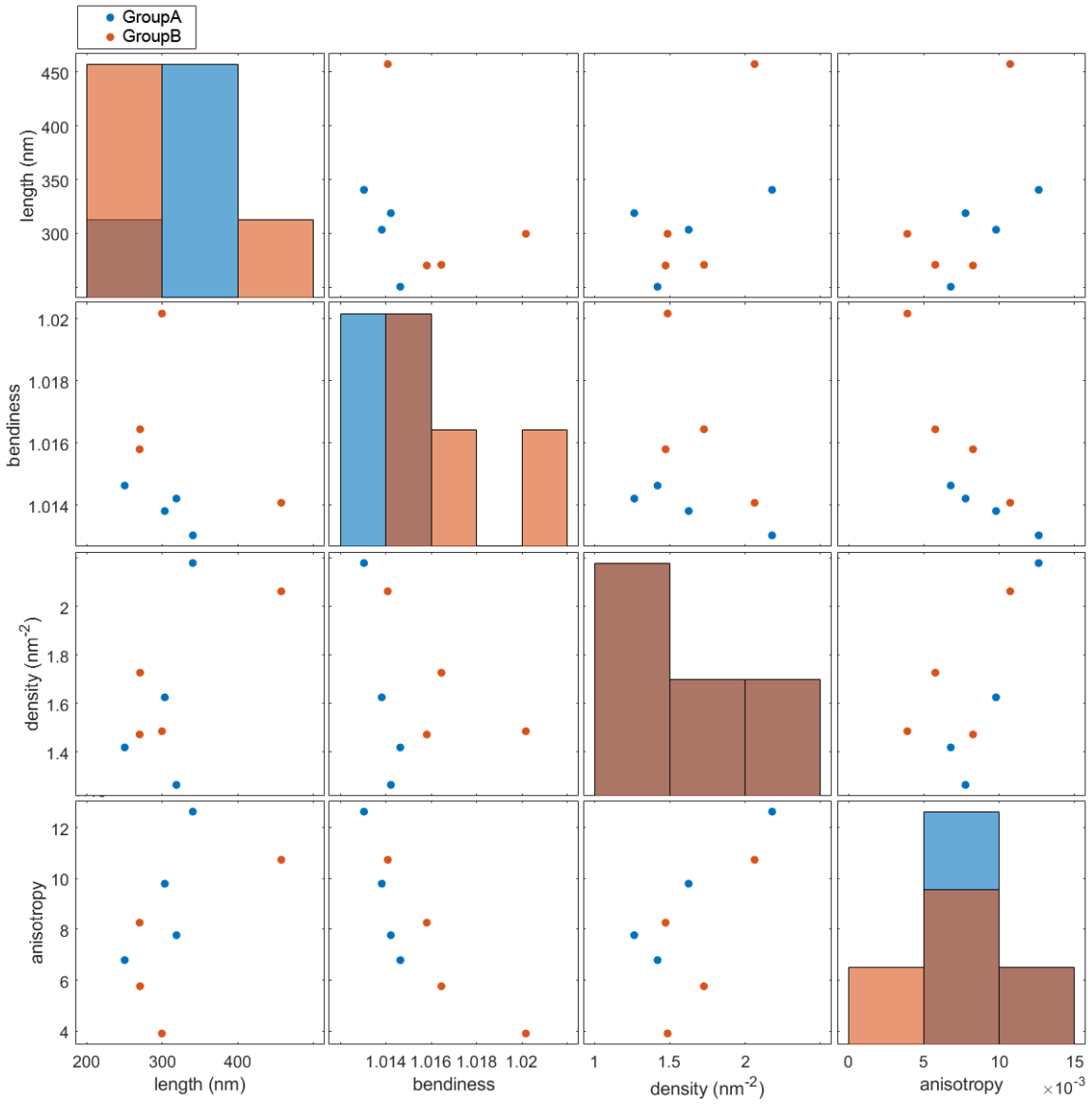
Apart from the correlation plots between parameters for individual groups in the Parallel coordinate plots option, the Ultrastructural toolbox offers the opportunity to plot the relationship between 2 parameters in a XY coordinate system containing multiple individual files regardless of their filament architecture. In this plot, *individual .mat files* of both dendritic networks and parallel filament arrays can be plotted together. This is performed by running the actinStructure.m script in the “Plot_scripts” folder and selecting the directory containing all files (.mat format) of interest. Since the script considers data files assigned to different groups, .mat files should be copied in their respective subfolders:



Note: In order to avoid errors, when prompted to select data files, only go to the location of the destination directory with the individual subfolders and click enter or "Select folder". Do not select (single-click) or open any individual folders or the .mat files located in them.

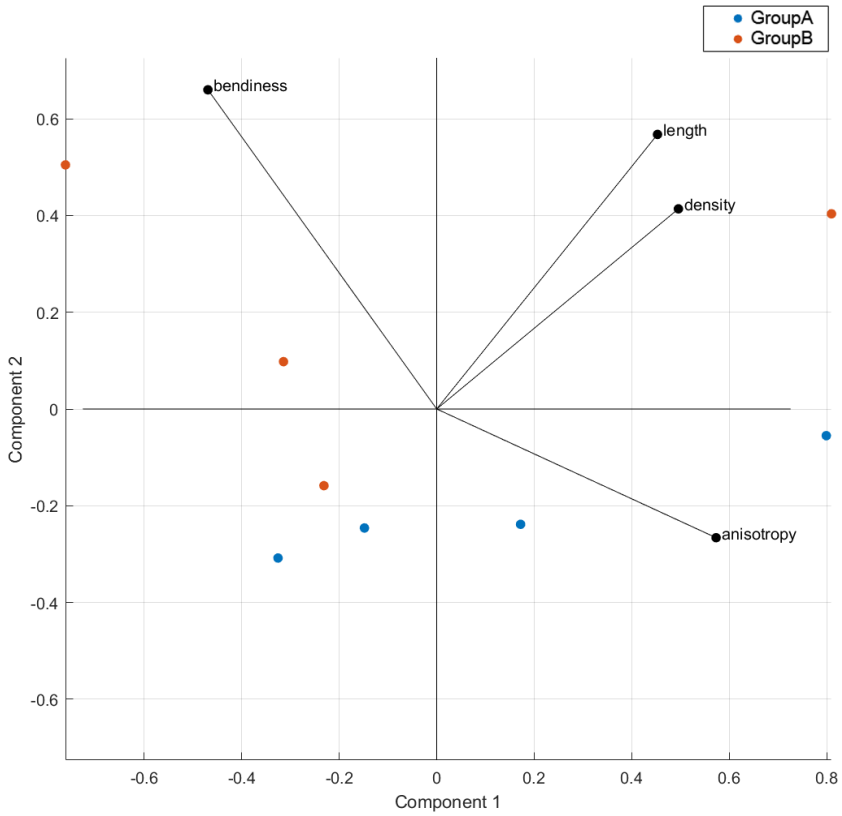


As shown below, four parameters are compared across eight *individual .mat files* originating from two groups. The results are plotted in a coordinate system with the values of one parameter displayed on the Y-axis and the values of the other parameter on the X-axis. The off-diagonal plots are scatter plots. The diagonal plots of the matrix show grouped histograms of parameters. These types of plots allow the user to deduct whether there are obvious correlations between the values of one vs another parameter and whether they are linked to a specific phenotype associated with an experimental group or treatment.



The outcome of the same script also provides a principal component analysis (PCA) plot of a user-selected dataset (see example below). This provides an idea of the tendencies of individual data samples to display certain characteristics, manifested by the likeliness of those samples to be influenced by the changes in a given parameter.

The outcomes of the actinStructure.m script are easier to interpret with datasets larger than the one shown above.



6.5. Extracting numerical values for performing statistics

The toolbox does not offer options to perform statistical tests on the extracted data. In order to allow users more flexibility to select a statistical approach of their choice, numerical data of each analyzed group, following data grouping (instructions here), is easily accessible in an .xls format in every "DataSummary" folder. The .xls files contain the averaged value for each of the analyzed parameters and displayed for every individual analyzed file.

7. References

Jasnin, M., Asano, S., Gouin, E., Hegerl, R., Plitzko, J.M., Villa, E., Cossart, P., Baumeister, W., 2013. Three-dimensional architecture of actin filaments in Listeria monocytogenes comet tails. Proc. Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.1320155110

Jasnin, M., Crevenna, A.H., 2016. Quantitative Analysis of Filament Branch Orientation in Listeria Actin Comet Tails. Biophys. J. https://doi.org/10.1016/j.bpj.2015.07.053

Rigort, A., Günther, D., Hegerl, R., Baum, D., Weber, B., Prohaska, S., Medalia, O., Baumeister, W., Hege, H.C., 2012. Automated segmentation of electron tomograms for a quantitative description of actin filament networks. J. Struct. Biol. https://doi.org/10.1016/j.jsb.2011.08.012

Winkler, C., Vinzenz, M., Small, J.V., Schmeiser, C., 2012. Actin filament tracking in electron tomograms of negatively stained lamellipodia using the localized radon transform. J. Struct. Biol. https://doi.org/10.1016/j.jsb.2012.02.011