1   Overview

`zlineDetection` is a program written in MATLAB that quantifies z-line architecture in 2D images of α-actinin stained cardiac tissues. It has the option to calculate and report the following aspects of z-line architecture, which are selected from a user friendly graphical user interface (GUI):

- Z-line fraction

- Continuous z-line lengths

- Z-line orientational order parameter (OOP)

- Actin OOP

`zlineDetection` can also combine and summarize these features for a coverslip, and can compare between coverslips and conditions.

1.1   System Requirements

`zlineDetection` has been developed and tested on Matlab 2018b on Windows 10. The required Matlab version and Toolboxes:

- MATLAB Version ≥ 9.5

- Image Processing Toolbox Version 10.3

- Statistics and Machine Learning Toolbox Version 11.4

2   Retrieve `zlineDetection` from Github

1. Navigate to the Cardiovascular-Modeling-Laboratory Organization account and select the "zlineDetection" repository.
   `https://github.com/Cardiovascular-Modeling-Laboratory/zlineDetection`

2. Select **"Download ZIP"** from the **"Clone or Download"** dropdown menu.

3. Locate and unzip the zipped "zlineDetection" file on your computer.

3   Prepare Data for Analysis

It is recommend that all fields of view for a single coverslip should be in the same folder and each coverslip should have its own folder (Fig 1). In order to use actin to guide segmentation, fields of view stained for actin and α-actinin should be contained within the same folder and have similar file names with the only major distinction being the name of the stain (Fig 1).
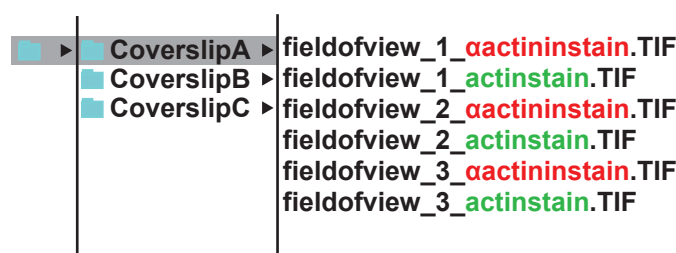
Fig 1. File organization for `zlineDetection`. Each Coverslip (A,B,C) has its own directory and within a coverslip directory each field of view is labeled by number and stain.

4   Run zlineDetection

1. Open MATLAB.

2. Navigate to the location of zlineDetection, by browsing for the directory.

3. Once you have opened the directory containing zlineDetection, type zlineDetection in the command line and press enter. A window titled "Z-line Detection & Analysis" will open (Fig 2).
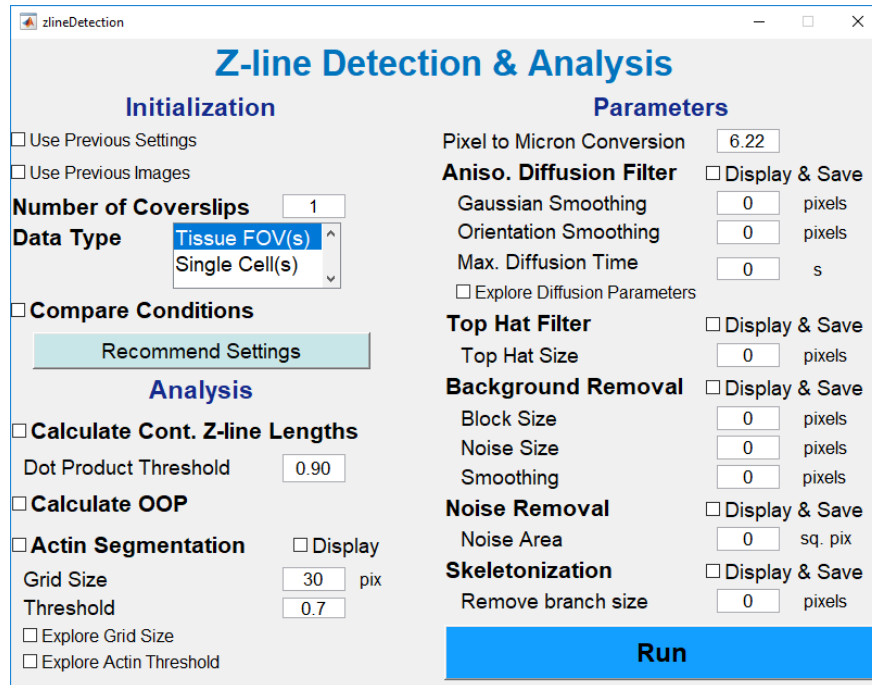

Fig 2. GUI for zlineDetection in MATLAB.

4.1   Initialize Input Data

1. Under the heading "Initialization", enter the number of coverslips you would like to run next to the text box "Number of Coverslips".

2. In the drop down menu next to "Data Type" select either Tissue FOV(s) or Single Cell(s).

3. If you would like to analyze multiple coverslips that have different conditions (i.e. treated vs. control), check the box next to "Compare Conditions".

4. By checking "Use Previous Settings" or "Use Previous Images", you will be able to load and use the parameter and analysis settings chosen previously and/or the images you have previously analyzed.

4.2   Analysis Options

Pushing the button "Recommend Settings" will auto-fill recommended analysis options and parameters.

zlineDetection has the following analysis options found under the "Analysis" heading:

- Check "Calculate Cont. Z-line Lengths" to plot and calculate the continuous z-lines. Enter the dot product threshold next to the text "Dot Product Threshold."

- Check "Calculate OOP" to report the OOP of the z-lines.

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- Check "Actin Segmentation" to perform actin guided segmentation and calculate the z-line fraction. You will be asked for the grid size and threshold values.

4.3　Set Parameters

Pushing the button "Recommend Settings" will auto-fill recommended analysis options and parameters. Parameters were chosen for images with the resolution 6.22 µm per pixel. A detailed description of parameters can be found in Section 7.

4.4　Start Analysis and Select Images

1. Push "Run" to start the analysis.

2. If you selected "Compare Conditions" you will be asked for the number of conditions, and the name of the conditions in popup dialog boxes.

3. You will be prompted for a "base directory." This is where your coverslips are located, and where you would like to save summary files.

4. You will then be prompted for the name of your summary file.

5. After entering the name of your summary file, you will be asked to choose the images stained for z-lines (and then actin if selected) for each coverslip.

6. If you provided images stained for both actin and z-lines, you will be asked if the files are matched correctly.

7. After selecting all the z-line images (and actin if applicable) for a coverslip, you will be asked to select the condition for the current coverslip if you checked "Compare Conditions". The name of the coverslip selected with output on the MATLAB command line. You will be asked to approve the selection, or re-select (and re-label) the coverslip.

After all of the data has been selected, each field of view for each coverslip will be analyzed without the need for user input.

5　Results of Analysis

5.1　Each Field of View

1. For each field of view, a new folder will be created with the same name of that field of view. This folder will contain a `OrientationAnalysis.mat` file with the results of the analysis. See Section 8 for more detailed information.

2. If you did continuous z-line length analysis, a histogram of continuous z-line lengths and the plotted continuous z-lines (.fig and .tif) will also be contained in this folder.

3. (**Single Cells Only**) If "Single Cell(s)" was selected, a summary pdf will be saved for each image (Fig. 3) in a folder "SingleCell_RESULTS_YYYYMMDD" in the directory chosen to store summary files.

5.2　Each Coverslip

The results of each coverslip will be contained in a .mat file with name, directory_name + CS_Summary + YYYYMMDD. See Section 8 for more detailed information on variable names and their descriptions.
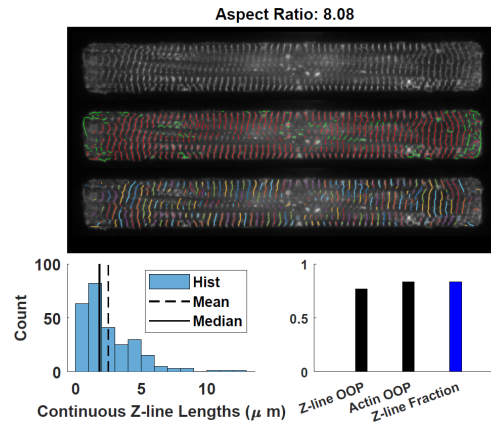
Fig 3. Representative single cell summary .pdf. Each summary pdf contains images of the z-lines (top), the actin segmented image (middle), and plotted continuous z-lines (bottom). Additionally, it has a histogram of continuous z-line lengths, the z-line OOP, actin OOP, and z-line fraction.

See Section 8 for more detailed information.

5.3   Coverslip Comparison

If you checked "Compare Conditions", coverslips conditions will be compared.

1. In the folder you specified there will be a MATLAB .mat file, an excel file, and a folder created with your summary filename.

2. The .mat file contains all the analysis, which is summarized in the excel file.

3. Plots summarizing your analysis will be in the folder with the name of your summary file + _RESULTS. The plots will include the z-line fraction (if filtered with actin), continuous z-line lengths (if requested), and the OOP for actin and z-line (if requested).

6   Comparing Multiple Coverslips Post Analysis

In progress...

7   Detailed Parameter Explanation

To be added... table and sections

8   Detailed Output Explanation

To be added... table with descriptions