User Guide for smLD Analysis Software

Tyler Camp

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1 Introduction

This software can be used to analyze trajectories from our single-molecule linear dichroism measurements. Before using this software, ensure that trajectories have been identified and saved into a MATLAB matrix object (file extension .mat). Critically, the software will only work for trajectories in which the incident polarization has been flipped "instantaneously" or nearly so.

2 UI Layout

Below is an image of the app. Each UI element is described in detail below.

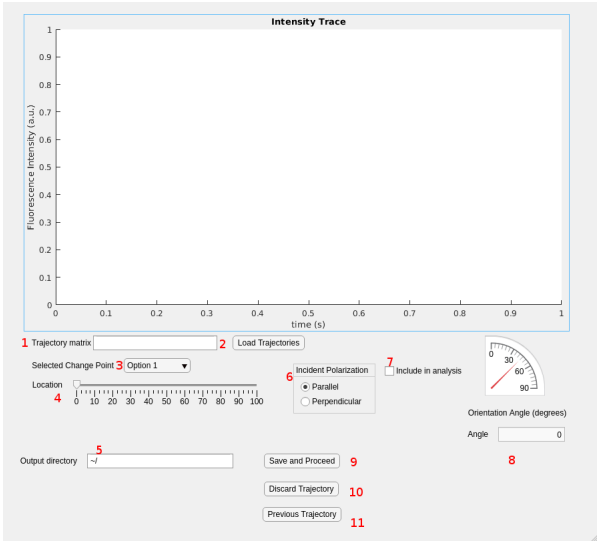

Figure 1: The user interface of the analysis software.

1. **Trajectory matrix**: this holds the path to the .mat file containing your trajectories.

2. **Load Trajectories**: this button loads the trajectories from the .mat file into the software.

3. **Selected change point**: a drop-down menu that allows the user to select a change point to modify.

4. **Location**: this slider allows the user to modify the location of the change point selected with the "Selected change point" drop-down menu.

5. **Output directory**: this field allows the user to specify the location of the output directory, which is created by the program.

6. **Incident polarization**: these radio buttons determine which polarization is assigned to the region of the trace given by the currently selected change point (see item 3 and below for further discussion of calculations).

7. **Include in analysis**: a check box to allow the user to exclude certain regions from the calculations.

8. **Orientation angle display**: these fields display the orientation angle of the current trajectory numerically and on a gauge.

9. **Save and Proceed**: writes the current annotated trace to disk and saves the orientation data in program memory to be written later.

10. **Discard Trajectory**: Ignores the current trajectory and loads the next one.

11. **Previous Trajectory**: Allows user to return to a previously saved/loaded trajectory.

3 Quick Start Guide

Once you have the app installed and running, follow the steps below to begin analyzing data:

1. Enter the path to the .mat file in the "Trajectory matrix" text box.

2. Click the "Load Trajectories" button. You will see the first trace appear on the screen. It should be annotated with change points, and the orientation angle should be displayed in the "Orientation angle display" text box and gauge.

3. Check that the software has correctly identified change points and has correctly identified the incident polarization for each region.

4. Experiment with changing some of the UI element values:

   (a) Change the location of the currently selected change point by dragging the "Location" slider. Notice that the orientation angle automatically updates each time you move the slider.

(b) Uncheck the "Include in analysis" check box. Notice that the region of the plot between the previous and current change point changes to black, and that the orientation angle has changed.

(c) Experiment with adjusting the incident polarization and note how the computed orientation angle changes.

5. When you have finished adjusting the plot, click "Save and Proceed". A copy of the trace will briefly appear as a separate window (this is so MATLAB can save the figure). Then the next trajectory will load.

6. Analyze the rest of the trajectories.

7. Once you have analyzed the last trajectory, the software will display a histogram of the computed orientation angles. At this point, all data have been saved to the output directory and you can close the program.

Below is an image of an annotated trajectory. Notice the regions near the change points are colored differently. These data points are not included in the calculations. See the "Miscellaneous" section for details.

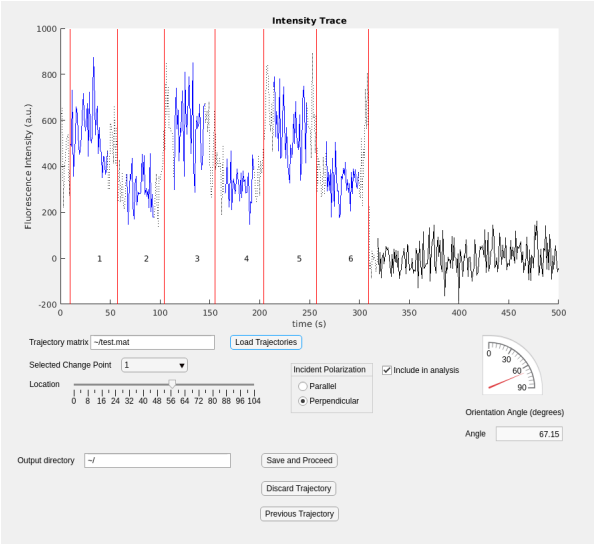

Figure 2: An example trajectory, annotated with change points. Some regions (colored black and/or dotted) are not included in the calculation of the orientation angle, which is displayed below the plot on the bottom-right.

4 Data Analysis Overview

Each trajectory is analyzed in the following steps:

1. Identify the change points in the trajectory
2. Assign the incident polarization to each region between change points:
   (a) The first region is assigned "Perpendicular"
   (b) Subsequent regions are assigned using an alternating sequence of "Parallel" and "Perpendicular"
3. Calculate the orientation angle as the average intensity of each polarization state over ALL included regions

5 Data Analysis in Detail

1. **Identify the change points in the trajectory.** The script "findcp.m" (3) is applied to the trajectory using a minimum state length of 30 frames. Hence the software will attempt to throw out change points that occur after less than 30 * (acquisition rate). Our integration time is typically 60 ms - 100 ms, and thus the software will exclude change points that appear within, at most, 3 seconds of each other.
2. **Assign the incident polarization to each region between change points.** The n change points divide the trajectory into $n+1$ regions. Region n runs from the $(n-1)^{th}$ change point (or the start of the trajectory if $n = 1$, the first change point) to the n^{th} change point. The final region is not currently included in the analysis. Most molecules bleach well before the end of the trajectory.
3. **Calculate the orientation angle.** For a given trajectory, the average intensity under parallel illumination is computed as the average of all regions of the trajectory for which the sample undergoes parallel illumination. For example, if regions 1, 3, and 5 are "Parallel" regions, then the average parallel intensity will be computed as $\langle I_{\parallel} \rangle = \langle I_1, I_3, I_5 \rangle$ where angle brackets denote averaging. The same is done for perpendicular illumination. The equations that relate the measured intensities to the orientation angle of the sample are given in Paul Bohn's paper (1).

6 Miscellaneous

1. **Buffering.** The software will include "buffering" regions near the change points which are not included in the calculations. This is done to ensure that no data is included near the time when I was manually rotating the half-wave plate. The rotation usually takes 10-20 frames, and the current buffering size is 10 frames on either side of a given change point.
2. **Output.** The software creates the following outputs:

(a) A CSV containing the index of each trajectory and its orientation angle (or -1 if the trajectory was discarded)

(b) A .tif image of each trajectory, fully annotated with change points, included regions highlighted, and orientation angle

(c) A histogram of the orientation angles, normalized as a probability distribution

3. **Source code.** The source code is stored on GitHub. The repository URL is listed in the "References" section (2).

References

[1] D. Cropek and P. Bohn. Surface Molecular Orientations Determined by Electronic Linear Dichroism in Optical Waveguide Structures. J. Phys. Chem. 1990, Vol. 94, 16:6452-6457.

[2] GitHub repository located at `https://github.com/stationarysalesman/single-molecule-LD`

[3] H. Li and H. Yang. Statistical Learning of Discrete States in Time Series. J. Phys. Chem. B 2019 123 (3), 689-701