

Force-Extension Analyzer

This document provides a tutorial on how to operate the Force-Extension Analyzer software suite. The tutorial is organized into four different sections:

1. Launching the Force-Extension Analyzer Suite
2. Overview of the Graphical User Interface (GUI)
3. General workflow for analyzing force-extension curves
4. Some special use cases

1) Launching the Force-Extension Analyzer Suite

- Download the **force_extension_analyzer.egg** file and save it to any directory. The file will be saved to the Desktop for this tutorial
- Navigate to the file's directory in the terminal:

```
cd ~/Desktop/
```

- Launch the Force-Extension Analyzer suite with the following command:

```
python force_extension_analyzer.egg
```

Required to run:

python 2.7; numpy; matplotlib; scipy; pandas

These python libraries may be installed using the command:

```
pip install numpy; pip install matplotlib; pip install scipy; pip install pandas
```

Recommended to run:

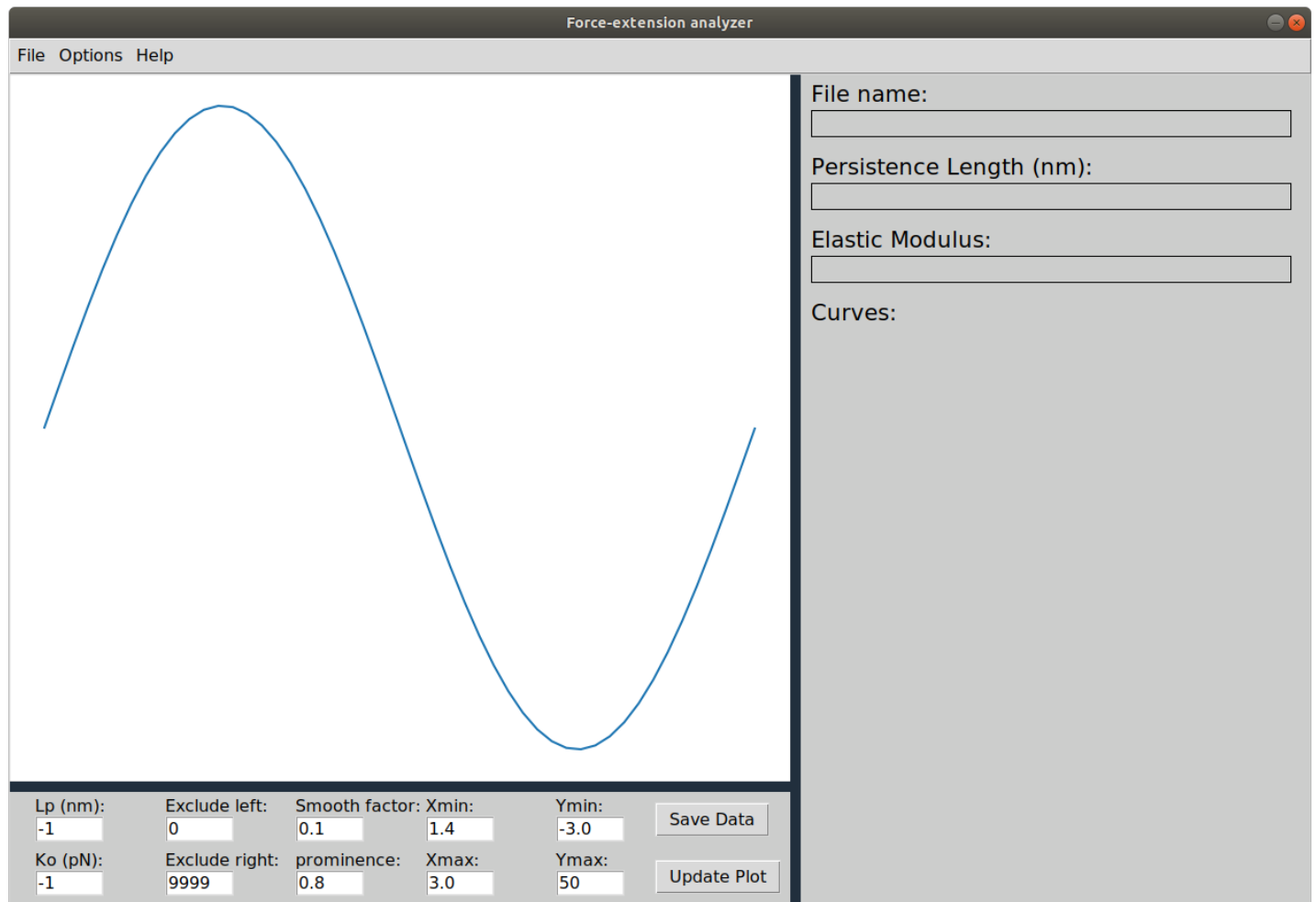
-It is recommended to run the software in your operating system's base python installation. You may also use Anaconda or other python installations, but fonts may not display correctly.

-The software was written to run on a 1920x1080 monitor. If the aspect ratio of your computer is different than 1920x1080, it is recommended that when you launch the program, you click "Options >> Screen Resolution" in the top menu.

The software has been tested and works on Ubuntu 18.04, Ubuntu 16.04, CentOS 7, Windows 10, and macOS Mojave with different screen resolutions.

2) Overview of the Graphical User Interface (GUI)

Upon launching the software, the GUI should open to its default screen:



Menu Bar:

- File:** user may select a trace to analyze or close the program. Traces currently must be from the LUMICKS CTRAP and be in .asc format. Minimal changes are needed to import an arbitrary file, but as they are specific to each instrument, I will only make these changes if there is interest.
- Options:** user may change the window dimensions (if needed) or enable/disable certain features
- Help:** displays a help menu with helpful tips on operating the software

Mutable fields:

- Lp, Ko:** user may set a persistence length and/or elastic modulus instead of fitting these parameters based on data. If the field values are -1, the appropriate parameter will be fit
- Exclude left/right:** allows the user to remove data points at the beginning (left) or end (right) of the trace. These fields may be useful to change in niche cases, but primarily only exclude right is used for excluding points after the DNA strand breaks
- Smooth factor, prominence:** these values affect the segmentation of the trace, explained below
- Xmin, Ymin, Xmax, Ymax:** these fields specify the plotting axes. They do not affect the results; just how the displayed graph looks

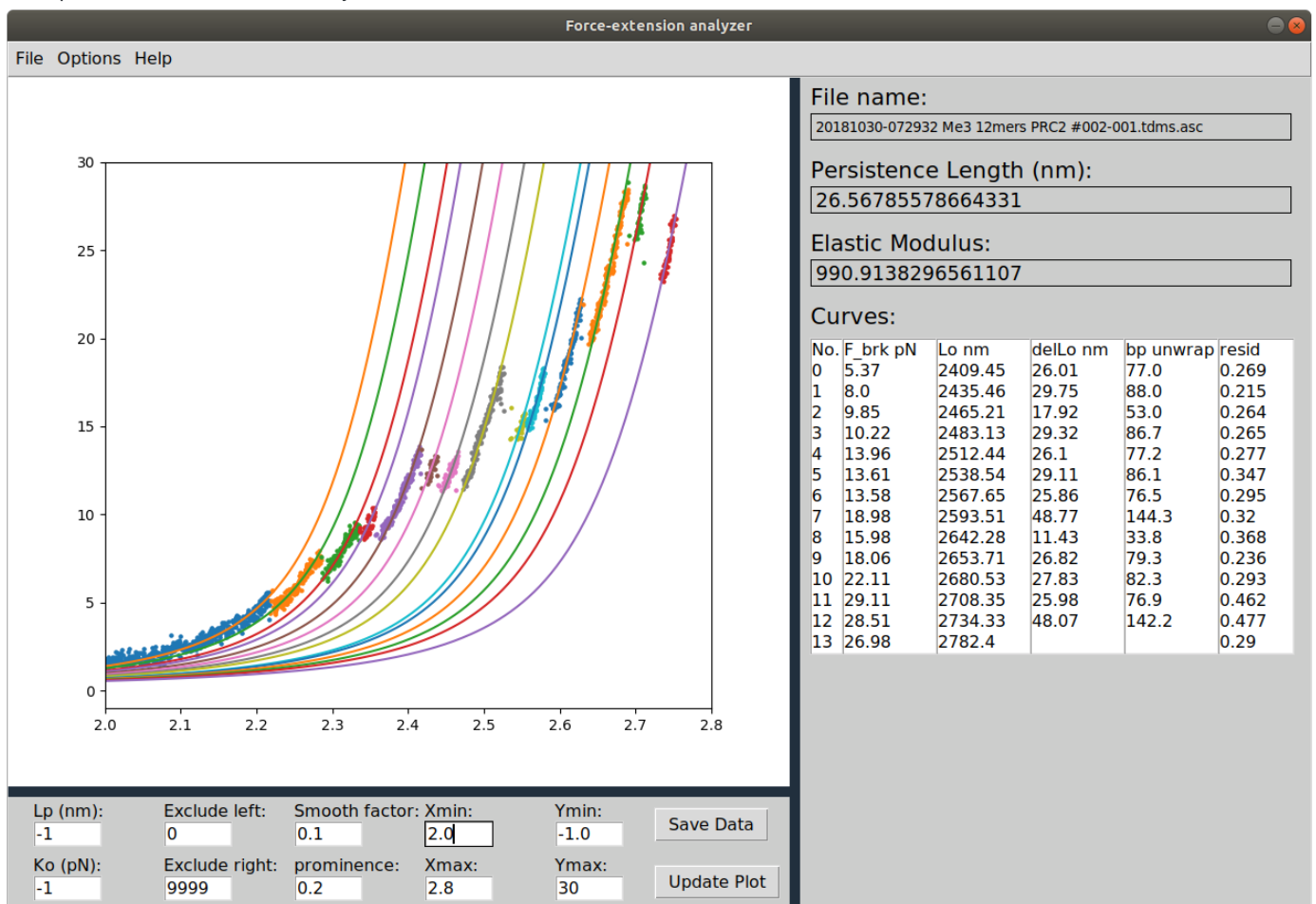
Results Display:

- File name:** the name of the file currently being operated on.
- Persistence length:** the fit persistence length.
- Elastic Modulus:** the fit elastic modulus.
- Curves:** results from the curves. If delete mode is on, by clicking on a curve, the user may delete that curve (by setting results to -100, which is then removed in later processing). Deletion mode may be disabled in the Options tab.

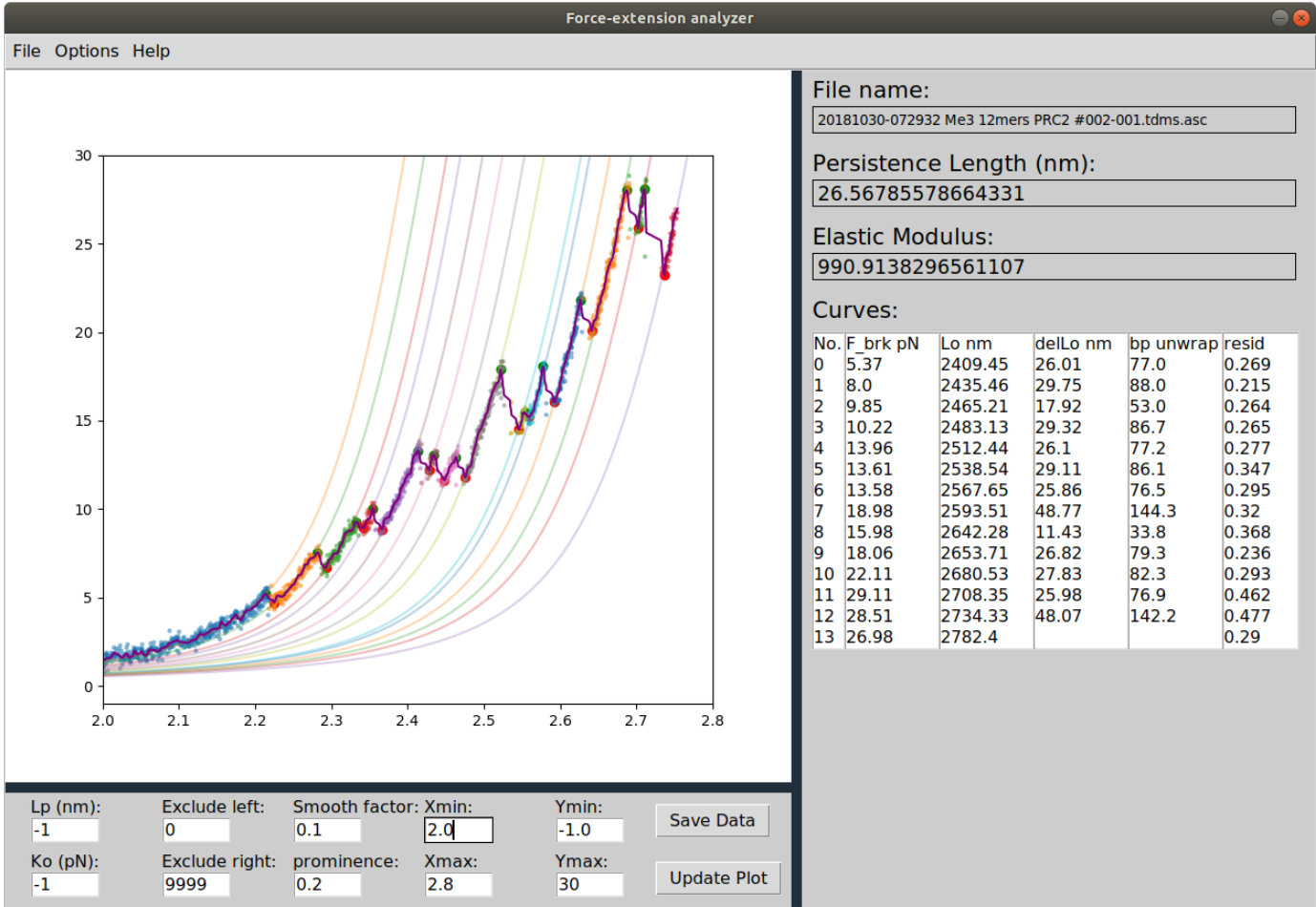
3) General workflow for analyzing force-extension curves

- Open file of interest using "File >> New File"; this will remain the current file until a new file is chosen
- For the first time plotting the data, leave all values to the default and click "Update Plot"
- Tweak the **Xmin**, **Ymin**, **Xmax**, **Ymax** fields until the trace occupies the full image window. This does not affect the curve fitting or results, but it does allow the user to manually inspect the trace
- Change the **Exclude right** field entry to be the extension distance of the last data point before the DNA breaks
- In many traces, the segmentation will be good with the default parameters. If the segmentation is not good, the smooth factor and prominence may be adjusted. The filtered trace used for segmentation depends on the smooth factor, and it may be visualized in debug mode, under the Options tab.
- If desired, erroneous curves may be removed (although this may have downstream consequences on analysis. It is recommended to segment the curves correctly instead of deleting curves.
- Once the fitting is successful, the data may be saved by clicking "Save Data". For the first analyzed trace of a given condition, a new csv file will be created. For subsequent traces, they will be appended to this csv file, as specified by the user. Analysis on relevant parameters like breaking force, contour length, persistence length, elastic modulus, and changes in contour length are printed in this output file, and it may be further processed.

Example trace of 12-mer DNA array.



Example trace in debug mode.



4) Some special use cases

- If for some reason the first segmented curve does not fit correctly, and the determined **Lp** and **Ko** poorly fit the other curves, you may want to temporarily exclude the first curve using the **Exclude left** field. Once properly measured values of persistence length and elastic modulus are obtained from the second curve, these values may be entered into the fields in the bottom left of the GUI, and **Exclude left** may be set back to zero.
- For the 12-mer nucleosome array studied on this particular instrument, transitions below 5 pN were ignored because occasionally noise could outweigh signal in the low-force regime, and the transitions we were studying typically occurred around 15 pN or higher. The user may study transitions in this low force regime by activating the low force mode in the Options tab. Alternatively, the user may not want to exclude any transitions, or the user's force regime of interest could be at a lower force i.e. 3 pN to 20 pN. If this is the case, the source code may be easily changed by altering lines 34 and 39 in the helper_methods.py script.