FRAME: Force Review Automation Environment User Manual

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Introduction

This user manual provides in-depth descriptions for the all features and menus in the Force Review Automation Environment, or FRAME. This software expedites the processing of AFM force-displacement curves by providing automation algorithms and a manual processing environment. For a brief overview of the program, please see the accompanying demonstration video.

When working in FRAME, it is generally recommended to keep each scan separate, since different scans, even if conducted using the same probe, could require different parameters for proper automation. It is a good idea to try several parameter setups based on a few representative curves before automatically processing, or reprocessing, an entire scan. While fully manual processing is an option, it is highly recommended that users make as much use of the processing algorithms as possible. All scans should always be checked to satisfaction by the user. Currently, only Asylum Research AFM data files (.ibw) are supported. Four unaffiliated open-source functions, available on Mathworks' File Exchange, are a part of FRAME. These are "IBWread.m," "readIBWbinheaders.m," "readIBWheaders.m" and "getAllFiles.m" and their respective licenses are be bundled with all versions of FRAME.

Note that FRAME was written in MATLAB 2014b, and may not function as indented in older versions.

Installation

To install FRAME as a Windows application, please launch "FRAME Installer.exe" and follow the instructions in the installation menus. Users will be prompted by the installer to download the MATLAB runtime before completing the installation.

To run FRAME through MATLAB place all relevant source files in a folder, and set MATLAB's "Current Folder," or working directory, as this folder. Run "FRAME_GUI.m" and make sure to maintain the previously set "Current Folder" or the graphical user interface (GUI) will no longer function. To run "FRAME_GUI.m" simply type "FRAME_GUI" in the command prompt and hit enter.

Loading Panel
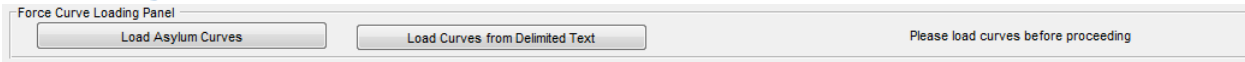
Load Asylum Curves

The loading curves operation must be done before any processing can be conducted. The "Load Asylum Curves" button loads ".ibw" force-displacement curves into the graphic user interface (GUI).Users will be prompted to refer to a directory in a new window. This directory, and all of its subdirectories will be scanned for all ".ibw" files which will be loaded in alphabetical order based on the hierarchy of folders. Any ".ibw" files that are not force-displacement curves will be ignored by FRAME. A sample of ".ibw" curves is available with this manual for users.

Load Curves from Delimited Text

The loading curves operation must be done before any processing can be conducted. The "Load Curves from Delimited Text" button loads force-displacement curves into the graphic user interface (GUI) from a delimited text file saved with the ".txt" ".csv" or ".dat" extensions. Users will be prompted to refer to a file in a new window. In order to be loaded properly into FRAME the delimited text files must conform to the follow formatting specifically:

1. Each column of data must alternate z-sensor data and the corresponding force response data, and the first column of each such pair must be z-sensor data.
2. The force response data column and the corresponding and preceding z-sensor data column must be of equal length.
3. Columns must be separated by the "tab" character, referred to as '/t' in MATLAB functions.
4. The first field in every column should be a string (it is suggested that these strings describe the current curve pair of z-sensor to force data).
5. Besides the first field, all other values in each column must be values
6. Unrelated column pairs do not need to be of equal length
7. Data should be provided in meters and newtons.

A sample ".txt" file of 32 curves (and thus 64 columns) is provided with this manual. Users can examine this file with Microsoft Excel for a clearer picture of how it should be formatted.

It may sometimes be easier to change the way in which MATLAB imports these delimited text files rather than directly formatting each data set. Refer to "fTxtDataLoader.m" lines 9 through 36. Specifically, line 14 "`F=importdata(Fullpath,'\t',1);`" should be of interest to users, and MATLAB documentation on this function should be referenced. If text headers are difficult to institute, both lines 14 and 32 will need to be changed to save any arbitrary string for every curve.

Status Display Text

This text provides information to the user about what FRAME is currently working on, whether that is loading curves, processing curves, or what input the program is waiting for.

Main Menu
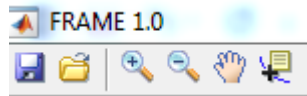
Save GUI

The "Save Figure" button, represented by a floppy-disk icon, saves totally the state of the GUI as a new file. This includes data that has been loaded into the GUI, parameters, and saved results. Note that these files should be accessed from FRAME via the "Load" button, not from Windows Explorer. If these ".fig" files are opened in MATLAB, the "Working Folder" should include the FRAME code.
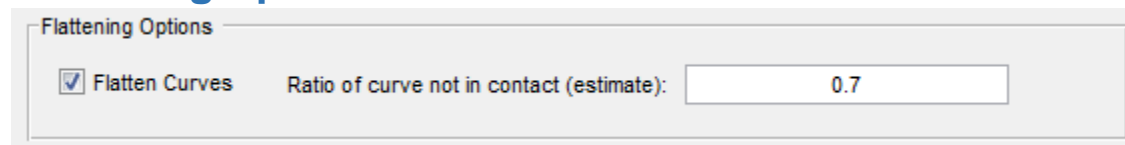
Load GUI

The "Open File" button, represented by a folder with an arrow pointed outward, allows FRAME to open the ".fig" files created by the "Save" button. This button is the only way to properly load saved GUI states, including loaded data, parameters, and saved results.

Plotting Tools

The Zoom tools, Pan tool, and Data Cursor tool, located next to the save and load buttons, are extra features that allow users to inspect their curves with greater detail. The Zoom tools allow manual zooming on a displayed curve. The Pan tool allows users to pan across a particular plot of a displayed curve. The Data Cursor tool allows users to identify specific data points on a displayed curve.

Flattening Options

Flatten Curves Checkbox

When the "Flatten Curves" checkbox is activated, all curves will be "flattened" based on a linear fit of some portion of extension curve. The linear fit is subtracted from the data thus flattening the curve. The portion used by this algorithm is defined in the "Ratio of curve that is not in contact (estimate)" box by the user if stiffness is not computed, and is provided by the stiffness fit otherwise.

Ratio of curve that is not in contact

The "Ratio of Curve that is not in contact (estimate)" box is a user estimate for the domain of data in which the cantilever is not in contact with the sample. This value is predominantly used by the flattening algorithm if stiffness is not computed. Despite appearing in the "Flattening Options," this user value is also used by other parts of the code. Specifically, this value is used in the calculation of the amplitude of noise when noise sensitivity is employed for unbinding event detection.

Stiffness Options

Compute Stiffness Checkbox

Checking the "Compute Stiffness" checkbox will curve-fit every curve to the Indentation Model selected in the "Indentation Model Menu." If this is checked along with the "Flatten Curves" checkbox, this fit will be performed twice, and the first fit will be used to flatten the curve. FRAME uses a least-squares minimization to find both the contact point and the Young's Modulus of the sample.

Ignore Bad Curves Checkbox

If the "Ignore Bad Curves" and the "Compute Stiffness" checkboxes are both enabled, FRAME will not collect any data (both stiffness and unbinding events) from specific curves if the root-mean-squared of the residuals of the curve-fit are higher than the threshold set by the user.

Contact Model Menu

The "Contact Model Menu" allows the user to choose between two different contact models when computing stiffness. The first option is the Hertz rigid pyramid to elastic half-space contact model, and the second is the Hertz rigid sphere to elastic half-space contact model.

Contact Distance Menu

The "Contact Distance Menu" allows users to define the domain on which the contact model is fit. If "Ratio of Contact Max Fit" is select than the "Ratio of Contact Max Fit" input box is available to be edited and the user defined ratio is used by FRAME to determine an upper bound for the contact model fit distance.

If "Contact Fit Distance" is selected, than the "Contact Fit Distance" input box is available to be edited and users may define an exact value for the contact model fit distance.

Nue Value

The input box labeled "Nue Value" allows the user to define the Poisson's ratio of the sample. Note that the indenter is assumed to be rigid, with a Poisson's ratio of 0.5.

Theta Value

This is the nominal angle of the indenter, used by the Hertz "rigid pyramid" model.

R-value

This is the radius of the spherical indenter, used by the Hertz "rigid sphere" contact model.

E-RMS Threshold

This user defined arbitrary value sets a threshold for curves to be ignored based on the root mean squared of the residuals of the curve-fit.

Ratio of Contact Max Fit

The "Ratio of Contact Max Fit" input box allows users to define a ratio (with a value between of 0 to 1). The input value indirectly computes the upper limit for the contact model's curve fit domain. The input value refers to the ratio of force response between the "zero" value that is calculated from the contact point and the maximum recorded force. The displacement of the last point within the ratio is taken as the upper limit for the contact fit. This option is only available when the "Contact Distance Menu" is set to the "Ratio of Contact Max Fit" option.

Contact Fit Distance

The "Contact Fit Distance" input box allows user to define the exact domain in nanometers along which the contact model will be curve fit to the data. This domain will begin at the contact point and extend the exact distance provided in this field, if the "Contact Distance Menu" is set to the "Contact Fit Distance" option.

Unbinding Event Detection Options

Detect Unbinding Events Checkbox

The "Detect Unbinding Events" checkbox will prompt FRAME to check each curve for potential unbinding events. FRAME identifies every upward jump in the data separately to see if it is higher than a minimal value, makes sure the this rise is steep enough to be an unbinding event, and finally identifies the linear behavior before and after every potential unbinding event to make sure that a change in the values does, in fact, occur.

Minimum Force Menu

Through the "Minimum Force Menu" the user defines the method FRAME uses to gauge whether a rise in force in the retraction curve is a potential unbinding event. If "Noise Sensitivity" is selected, the "Noise Sensitivity" input box is available for input. During the noise sensitivity mode, FRAME will smooth out the retraction curve by using a high order zero-phase digital filtering (MATLAB's "filtfilt" function). The root-mean-squared of the difference between the two is multiplied by the "Noise Sensitivity" from the respective input box and is set as the minimum threshold for a rise to be considered a potential unbinding event. The domain where this value is taken is between the non-contact ratio (as defined by the "Ratio of curve that is not in contact (estimate)" input box) and the end of the retraction curve.

If "Minimal Force" is selected, the "Minimum Unbinding Force" input box is available. In this mode, FRAME uses the input minimal force as the threshold for a rise to be considered a potential unbinding event. Note that when switching between the two options, the input values are neither stored separately nor updated.

Multiple Unbinding Event Handling

The "Multiple Unbinding Event Handling" menu gives users options when dealing with more than one unbinding event in a single retraction curve. If "Ignore Curves w/multi. Unbinding

Events" is selected, a curve that, after all filtering, exhibits more than one unbinding event will be computed as having no unbinding events.

If "Save Unbinding Event w/Greatest Force" is selected, only the unbinding event with the greatest force will be computed.

If "Save Total Force" is selected, FRAME will compute the unbinding force as the difference between the lowest force among all unbinding events, and the highest force among all unbinding events. All other parameters will be taken from the first unbinding event.

If "Save All Unbinding Events" is selected, information pertaining to all unbinding events will be computed.

K-eff Handling Menu

The "K-eff Handling Menu" provides the user with options regarding the slope of the linear behavior of the curve before each unbinding event (k-eff). It is recommended by the authors that curves be flattened if this functionality is used. If "Ignore k-eff" is selected, unbinding events will not be ignored based on the linear behavior of the curve before each unbinding event.

If "Must be positive" is selected, unbinding events with a negative k-eff will be ignored by FRAME.

If "Must be positive and below threshold" is selected, unbinding events with a negative k-eff, or with a k-eff that is too high (based on the "Max k-eff (in pN/nm):" input box) will be ignored by FRAME.

L-Distance Starting Point

The detachment length of an unbinding event (L-distance) depends on the starting point used. The "L-Distance Starting Point" menu provides options for choosing said starting point. Whenever an appropriate option is not selected in this menu, FRAME will use the first point of the retraction curve. If the "Retraction Curve "Zero" Value" is selected, FRAME will select the first point on the retraction curve that is below "zero" force after flattening. FRAME will flatten the curve in case flattening isn't enabled to compute only this function.

If "Stiffness Model Fit End" is selected, FRAME will use the final point of the stiffness model as the starting point.

If "Stiffness Contact Point" is selected, the contact point of the extension curve, detected during the curve fit of the contact model, is used as the starting point.

If "Stiffness-Retraction Switch" is selected, the first point of the retraction curve is used as the starting point. This, however, may invalidate the L-Distance measurements.

If "Halfway Through Fit" is used, the midway point between the model fit end and the contact point is set as the L-distance starting point.

Minimum Unbinding Force

The "Minimum Unbinding Force" input box sets the minimal value for FRAME to use when deciding if a rise is a potential unbinding event.

Noise Sensitivity

The "Noise Sensitivity" input box allows the users to indirectly define the minimum threshold for a rise to be considered a potential unbinding event. During the noise sensitivity mode, FRAME will smooth out the retraction curve through a high order zero-phase digital filtering (MATLAB's "filtfilt" function). The root-mean-squared of the difference between the two curves is multiplied by the "Noise Sensitivity" from the respective input box and is set as the minimum threshold for a rise to be considered a potential unbinding event. The domain where this value is taken is between the non-contact ratio (as defined by the "Ratio of curve that is not in contact (estimate)" input box) and the end of the curve. The authors recommend a noise sensitivity between 5 and 6.

Minimum Steepness

Using the "Minimum Steepness" input box, the user defines the minimum steepness (in pN/nm) of a potential unbinding event. Any potential unbinding events that are not steeper than this value will be ignored by FRAME.

Max L-Distance Limit

The "Max L-Distance Limit" input box gives the user additional tools for sorting out unwanted unbinding events. Any unbinding event with a computed L-Distance higher than the provided value will be ignored by FRAME. To disable this feature, simply input a number outside the possible curve or input "Inf" as the value.

Min L-Distance Limit

The "Max L-Distance Limit" input box gives the user additional tools for sorting out unwanted unbinding events. Any unbinding event with a computed L-Distance lower than the provided value will be ignored by FRAME. This functionality accepts negative values. To disable this feature, input a number outside the possible curve or input "-Inf" as the value. Another method to disable this feature would be to set the "L-Distance Starting Point" menu to "Stiffness-Retraction Switch" and setting the value of "Max L-Distance Limit" to 0 or a negative value. The latter method may invalidate the actual L-Distance value.

Linear Behavior Fit Distance

The value input into the "Linear Behavior Fit Distance" input box refers to calculations of k-eff as well as confirmation that potential unbinding events are not noise. FRAME applies a linear fit to the curve immediately before the unbinding event, up to the linear behavior fit distance provided in this input box. During unbinding event detection, FRAME confirms that a potential unbinding event is not noise by applying a linear fit immediately before and after a potential unbinding event up to the distance provided in this input box. A potential unbinding event is considered an actual unbinding event if the evaluation from the two fits and the respective ends of the unbinding event have a difference of at least half of the minimum threshold as well as half of the unbinding event itself.

Max Unbinding Force

In the "Max Unbinding Force" input box, the user can supply a maximum unbinding event force in order to ignore unbinding events with particularly high forces. This functionality can be disabled by setting "Inf" as the value.

Max k-eff

The "Max k-eff" input box allows the user to set a maximum k-eff, or slope of the linear curve fit immediately before an unbinding event. If the "K-eff Handling Menu" is set to "Must be positive and below threshold", then any unbinding events below the value set in this input box will be ignored by FRAME.
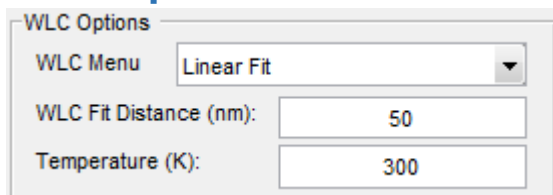
Max Combine Length/Max Combine Ratio

Certain unbinding events may sometimes exhibit small noises. Alternatively, consecutive unbinding events may be connected by a single, relatively small, drop in the force response. These small and relatively small forces may be combined back into their respective unbinding events. The "Max Combine Length" input box sets the maximum length that an unbinding event may have before being considered independent. The "Max Combine Ratio" sets the maximum relative size of an unbinding event between itself and both of its neighbors before it's considered to be independent. To disable this functionality, and consider the data in an unaltered way, set both values to 0.

Save Parameters/Load Parameters/Save Parameters As/ Save Custom Parameters

The four buttons labeled "Save Parameters," "Load Parameters," "Save Parameters As," and "Save Custom Parameters," provide users the option to save their parameters for future use. The "Save Parameters" and "Load Parameters" buttons allow the user to save and load the settings and values input by the user to a default file that is read at the launch of the program. The "Save Parameters As" and "Load Custom Parameters" buttons provide the user with a dialogue window to create and load new files for storing and loading set parameters, without altering the default file.

WLC Options

WLC Menu

"WLC Menu" selects how FRAME processes the data before each unbinding event and how the linear slope of the curve before each unbinding event (the "k-eff") is calculated. If "Linear Fit" is selected, FRAME will fit the data immediately before each unbinding event up to the distance specified in the "Linear Fit Distance" input box or up to the end of the nearest unbinding event, whichever is shorter.

If "WLC Model" is selected, FRAME will curve fit the data immediately before each unbinding event up to the distance specified in "WLC Fit Distance" or up to the end of the nearest unbinding event, whichever is shorter, with the worm-like chain model (WLC model). FRAME will use the "L-distance starting point" as the force-displacement "zero" when fitting this model using a least-squares minimization. The parameters returned by this curve fit are the persistence length and the contour length of each unbinding event. The "k-eff" is determined by the average slope of the WLC curve immediately before the unbinding event and up to the distance specified in the "Linear Fit Distance" input box, ignoring other unbinding events. In the case of a single unbinding event or multiple unfolding events, the program will provide the user with approximate values of the persistence length and the contour length for each event. It is highly recommended by the author that when using the WLC model, both curves are flattened by checking the "Flatten Curves" checkbox and that the "L-Distance Starting Point" is set to "Retraction Curve "Zero" Value" option.

WLC Fit Distance

The value input into the "WLC Fit Distance" input box refers to the curve fit of the WLC model. When the "K-eff Menu" is set to the "WLC Model" option, FRAME applies a least squares minimization of the WLC model to the curve immediately before the unbinding event, up to the WLC fit distance provided in this input box, or up to the nearest unbinding event, whichever distance is shortest.

Temperature

The value input into the "Temperature" input box will set the temperature, in Kelvin, used in the WLC curve-fit.

The Processing Port
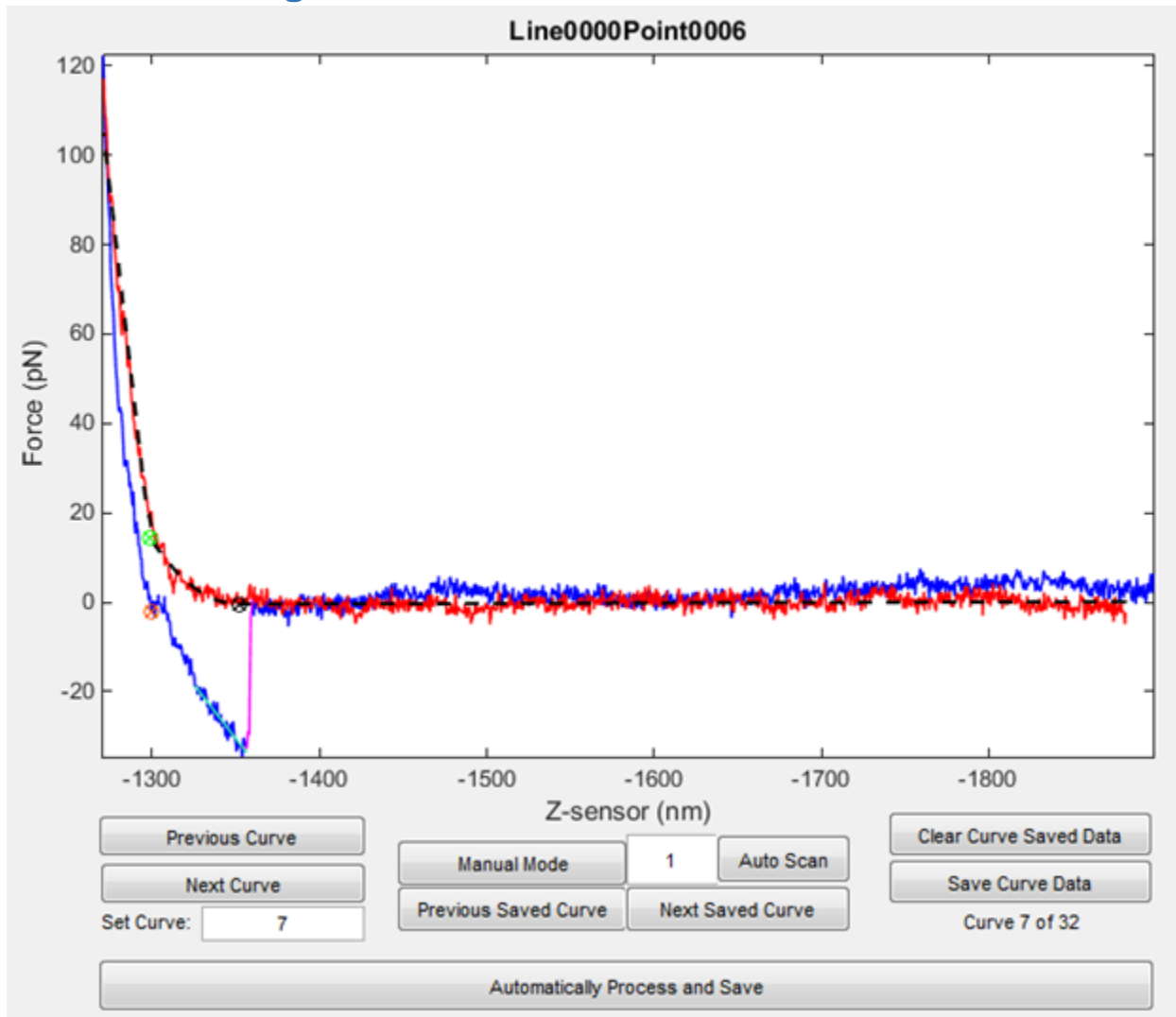
Curve Plot

The figure plots the extension curve as red, the retraction curve as blue, all unbinding events as magenta, the "L-Distance starting point" as an orange point, the contact point as a black point, the contact model fit end as a green point, the line behavior of the curve before each unbinding event as a light blue line, and the contact model fit as a black dashed curve.

Previous Curve/Next Curve/Set Curve/Previous Saved Curve/Next Saved Curve

The three controls, the "Previous Curve" button, the "Next Curve" button, and the "Set Curve" button, allow the user to browse through any curves loaded into FRAME. Whenever a parameter is changed or a new curve is displayed, the curve is processed again by FRAME and displayed on the axes. These computations are not saved. The "Previous Curve" button processes and displays a curve that was read and loaded before the currently displayed one. The "Next Curve" button processes and displays a curve that was read and loaded after the currently displayed one. The "Set Curve" input box allows the user to jump directly to a specific

curve in the set. The "Previous Saved Curve" button allows the user to jump to the previous curve with a saved unbinding event. The "Next Saved Curve" button allows the user to jump to the next curve with a saved unbinding event.

Manual Mode

This mode allows the user to manually process specific curves for both unbinding events and contact model fitting depending on which parameters are set. When this mode is entered on a curve for the first time, all automated computation will cease on that curve unless the information is cleared by the "Clear Curve Saved Data" button. If the "Manual Mode" button is pressed and the "Compute Stiffness" checkbox is enabled, the user will be prompted to select the contact point by clicking on the plot. At all similar inputs, the code will select the point on the relevant curve closest to the user selection. The user will then be prompted to select a second point between contact point and the end of the curve to terminate the fit. If the "Flatten Curves" checkbox is enabled, the extension curve data before the selected contact point will be used to flatten the data via a linear regression.

If the "Flatten Curves" checkbox is enabled but the "Compute Stiffness" checkbox is not, the user will be prompted to select a point along the "non-contact" region of the extension curve data the first time the user enters Manual Mode. The extension curve data before the selected point will be used to flatten the data via a linear regression.

If the "Manual Mode" button is pressed and the "Detect Unbinding Events" checkbox is enabled, the user will be prompted to select an "L-Distance Starting Point" by clicking on the plot the first time that they enter Manual Mode. The code will then ask to select both ends of an unbinding event. If the manual mode is selected again the user will be able to select more unbinding events. For each unbinding event, FRAME will compute the unbinding force, the linear behavior before the unbinding event or apply a WLC curve fit and relevant parameters, the steepness of the unbinding event, the L-Distance, and the unbinding event length. Note that if the plot is zoomed in on an unbinding event when the "Manual Mode" button is pressed, unbinding event selection will be conducted within this zoomed region. It is suggested that, when saving multiple unbinding events, users do so going from the contact region to towards the non-contact region (as plotted from "left" to "right"). This will prevent the linear behavior fit or WLC curve fit from being altered by unbinding events in the data.

Whenever a specific curve has been edited using "Manual Mode" it will not be processed by FRAME, even when processing all curves automatically. To revert a specific curve back to a state where it will be processed automatically, select the "Clear Curve Saved Data" button while displaying said curve.

Auto Scan/Auto Scan Timer

The "Auto Scan" toggle button allows the viewer to see all curves from the current to the final loaded curve, one at a time. The next curve is loaded after the number of seconds set into the "Auto Scan Timer" unlabeled input box after processing and plotting has concluded. It is not recommended that the user adjust any settings or any other buttons while the auto scan is running. The auto scan may be disabled by toggling to the "Auto Scan" toggle button back to its original position. Note that when doing so, the next curve is usually displayed.

Clear Curve Saved Data/Save Curve Data

The buttons, "Clear Curve Saved Data" and "Save Curve Data" allow users to pick which curve data will be saved. Pressing the "Clear Curve Saved Data" button will set all tracked values to 0. If the curve has been edited via "Manual Mode," pressing this button will revert the curve back to being automatically processed by FRAME. Pressing the "Save Curve Data" button will save the currently computed and displayed results into FRAME. Note that FRAME only plots and exports saved data.

Automatically Process and Save

Pressing the "Automatically Process and Save" button will make FRAME go through all loaded curves and save the calculated results. Where data is not computed or not found, FRAME will set a value of 0. Note that when data is first loaded, all of these values are set to an empty matrix to help users distinguish unprocessed data sets.

Curve Data

```
Curve Data
  Calculated Data:
  Youngs Modulus (Pa): 0
  Unbinding Force (pN): 24.5108
  K-eff (pN/nm): 0.5199
  Persistence Length(nm): 0.22375
  Contour Length (nm): 116.039
  Steepness (pN/nm): 18.0452
  L-Distance (nm): 58.0195
  Breaklength (nm): 1.3583




  Curve Notes:




  Saved Data:
  Youngs Modulus (Pa):
  Unbinding Force (pN): 24.5108
  K-eff (pN/nm): 0.5199
  Persistence Length(nm): 0.22375
  Contour Length (nm): 116.039
  Steepness (pN/nm): 18.0452
  L-Distance (nm): 58.0195
  Breaklength (nm): 1.3583
```

This viewport informs the users of the currently computed results, any particular notes, and the information saved about this curve. Any manually processed data will be displayed here as well. The currently computed results refer to the currently plotted information such as the identified unbinding events and the curve fit. The curve notes bring attention to important factors that the user should be aware of about this curve. Most notably, the curve notes explain why particular unbinding events were not considered to be unbinding events or were ignored.

Plotting Options

Exporting Options/Export All into Excel

Plotting Options

For each relevant parameter the user may input the lowest value of interest, highest value of interest, and the number of bins for the frequency histograms. The lowest and highest values are set by FRAME after automatically processing and saving all curves. The results may also be plotted on 3D histograms that may be viewed as a top down map of the values.

Topography Options

The topography recorded by the AFM may be plotted by FRAME and overlaid, or colored, by the Young's Modulus, the unbinding event forces, or the topography itself at each point as a 3D image. If the "Use Computed Contact Point" checkbox is enabled, the topography of the surface will be altered to use the contact point evaluated during stiffness computation instead of the maximum indentation distance.

Scan Dimensions

The "Scan Dimensions" input boxes labeled "x" and "y" allow the user to input the number of points along the two axes that the AFM traverses. When correctly provided, these values allow proper plotting of the maps of all relevant saved data, as well as the topography. If the values do not agree with the total number of force-curves (i.e. 32 by 32 should produce 1024 force curves) the topography mapping will attempt to approximate values to smooth out (or roughen) the resultant 3D surfaces.

Exporting
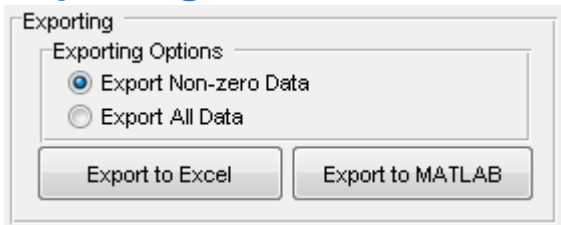
Export to Excel

Pressing the "Export to Excel" button provides the user with a dialogue window to create a new Excel spreadsheet with all saved data. If "Export All Data" is selected, new sheets for all data are created, including separate sheets for the saved data where zero values are not stored. If "Export Non-zero Data" is selected, only non-zero saved data are stored in the new Excel spreadsheet.

Export to MATLAB

Pressing the "Export to MATLAB" button provides the user with a dialogue window to create a new MATLAB data file with all saved data in a structure.

Custom Contact Model

In order to input a custom contact model into the automation algorithm, the code will have to be modified directly. The GUI itself should be modified to include a new option in the "Contact Model Menu."

1. Launch GUIDE using the "guide" command, and navigate to the "FRAME_GUI.fig" file.

2. Double click the menu under "Contact Model Menu" and add a new line to the "String" field named after the new custom contact model. When done, save the GUI. Note the number of the line you've added.

3. Open "fPlotting.m" for editing. There are two "if" statements that need to be amended: originally they start on line 156 and 281.

4. In each relevant "if" block, add a new "elseif" statement with the condition "handles.D.stffmodel==3" or another value that's equal to the number noted in step 2.

5. Copy and paste the code from the "elseif handles.D.stffmodel==1" code block in the newly created blocks.

6. In both new blocks, adjust the "func=@(x,xdata)..." lines (for both of the contact distance menu options) to include your custom contact model. Specifically the second term that originally is "…x(4).*(xdata-x(3)).^(2)…" as well as the second part of the third term "…(x(4).*((x(5)-x(3)).^2))…" should be made to contain the new contact model.
   > NOTE: the xdata variable inside of the function definition refers specifically to the probe displacement, while the "x" variable refers to a matrix of the 6 parameters required for a proper fit. The "func" function refers to the force experienced by the probe at a position of "xdata" with the parameters set at "x". If more parameters are added to the "x" matrix, the variables "x0" (initial guess for the parameters), "lb" (lower bounds for the parameters), and "up" (upper bounds for the parameters) will need to be adjusted to include the relevant information. Please see the documentation on MATLAB's "lsqcurvefit" function for more information.

7. In the same blocks, modify the "parameter" and "YoungsMod" variables such that the "YoungsMod" variable stores the computed Young's Modulus from the contact model fit.

8. Save and close all files.