Short communication

FRAME (Force Review Automation Environment): MATLAB-based AFM data processor

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ABSTRACT

Data processing of force–displacement curves generated by atomic force microscopes (AFMs) for elastic moduli and unbinding event measurements is very time consuming and susceptible to user error or bias. There is an evident need for consistent, dependable, and easy-to-use AFM data processing software. We have developed an open-source software application, the force review automation environment (or FRAME), that provides users with an intuitive graphical user interface, automating data processing, and tools for expediting manual processing. We did not observe a significant difference between manually processed and automatically processed results from the same data sets.

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1. Introduction

An atomic force microscope (AFM) is capable of measuring a wide range forces ($5-10^6$ pN) applied on a cantilever probe by recording the deflection of the cantilever (Alessandrini and Facci, 2005; Muller et al., 2009). Controlled vertical displacement is applied to the probe by piezoelectric actuators in order to produce force–displacement curves. Atomic force microscopy has several applications. In particular, resultant force–displacement curves have been used to measure the elastic modulus of biological samples (Dimitriadis et al., 2002) and the unbinding force between specific receptor-ligand bonds (Muller et al., 2009). Manually processing these data requires researchers to separately review each measurement. This is very time consuming and susceptible to user error or bias. Few free software applications for automatic processing of AFM force measurements have been developed, and only two such programs have seen some use (Roduit et al., 2012; Sandal et al., 2009). OpenFovea processes unbinding measurements by curve-fitting either the Worm Like Chain (WLC) or the Freely Jointed Chain (FJC) models in order to identify unbinding events, while Hooke uses a fuzzy logic algorithm to identify unbinding events based on conformity with specific, pre-defined, characteristics. However, in AFM experiments that probe cellular surfaces directly, and in single cell force spectroscopy (SCFS) experiments, both aforementioned methods cannot be implemented since cellular surface deformation and multiple

simultaneous ligand-receptor bonds alter the force–displacement curve shape (Evans and Calderwood, 2007; Maciaszek et al., 2014). As a result, most AFM data are still manually processed, and no clear standard has emerged in the automated processing of both Young's moduli and ligand-receptor unbinding measurements.

We have created an open-source software application based on MATLAB, the force review automation environment or FRAME, to greatly increase the speed at which AFM data are processed. FRAME is intuitive and easy to operate, even for the novice MATLAB user. It features a number of algorithms to streamline automated processing and a graphical user interface (GUI) to expedite manual processing and review of the data. Even if FRAME is only used in manual mode it can greatly accelerate data processing, especially for large sets of data. The results can be displayed in FRAME with MATLAB plotting tools, as well as exported as a Microsoft Excel file, for further statistical analysis. FRAME, the user manual, and a demonstration video can be found online at the Cellular Mechanics Laboratory webpage (http://www.engr.uconn.edu/~gelyko/frame.html). FRAME is shared both as a Windows executable and as MATLAB code under the open-source BSD license.

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2. Methods

FRAME can be used to compute Young's moduli and ligand-receptor unbinding forces. In particular, the Hertz contact model for spherical and pyramid shape cantilever tip has been implemented (Sneddon, 1965; Bilodeau, 1992). Manual processing of data produced in a contact experiment requires the user to identify, on the force–displacement curve, the point where the cantilever tip comes in contact with the sample's surface. FRAME uses a least-squares minimization for the curve-fit of the

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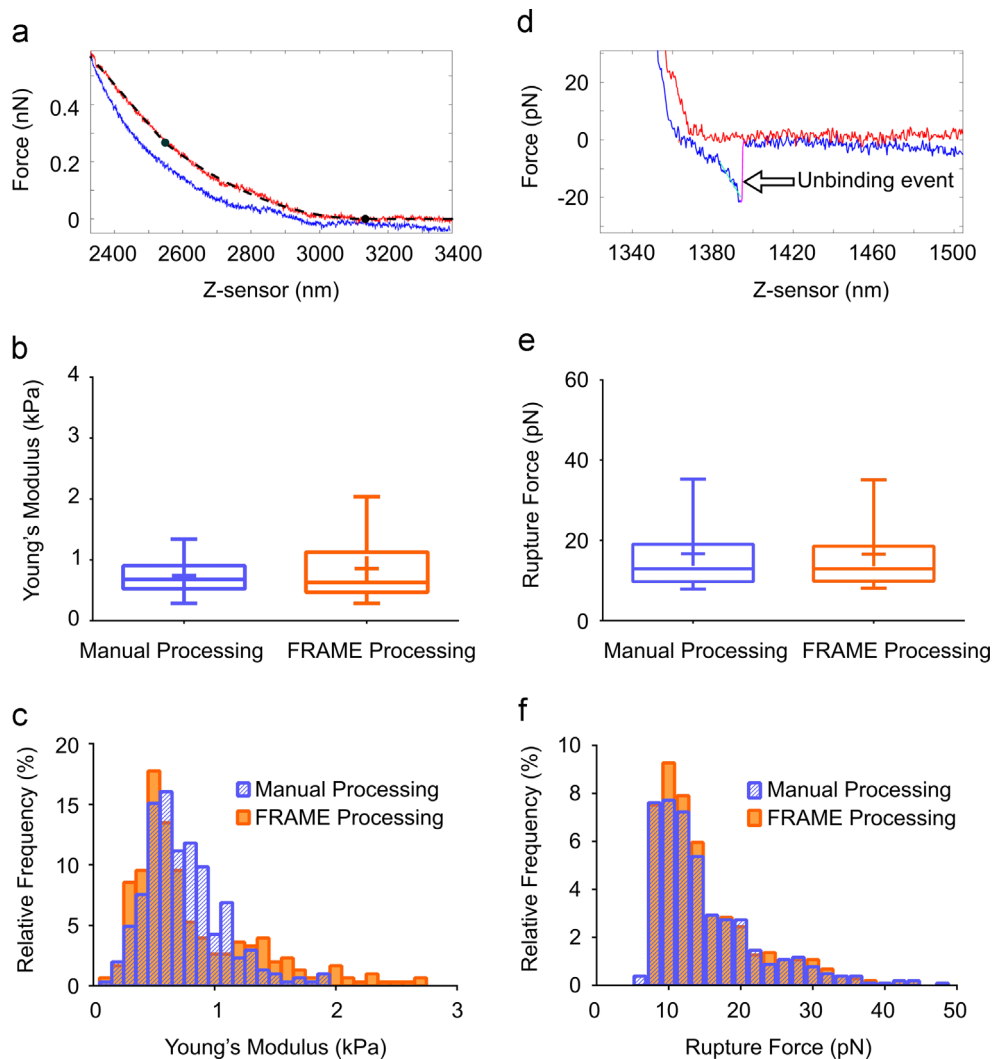*E-mail address:* gelyko@engr.uconn.edu (G. Lykotrafitis).

Fig. 1. Comparison of data processed manually and using FRAME. (a) FRAME displays the contact fit (dashed black line) used to compute a sample's Young's modulus, the contact point (black circle) and the end of fit point (green circle) based on the force–displacement curve (red and blue line plots). (b) Box-whisker plots showing the results of Young's modulus measurements on Human Embryonic Kidney (HEK293T) cells as processed from the same data set manually and using FRAME. The two results do not pass the test of significant difference (nonparametric Mann–Whitney test, $p > 0.8$). (c) Histograms showing the results of Young's modulus measurements on HEK293T cells as processed from the same data set manually and using FRAME. (d) FRAME detection of an unbinding event (purple line plot noted by arrow) between $\alpha_v\beta_3$ and ICAM4 receptor expressed on the surface of a red blood cell (SS-RBC) from a patient with sickle cell disease (SCD) and an approximation of the curve behavior before the unbinding event (teal line plot) on a force–displacement curve (red and blue line plots). (e) Box-whisker plots of unbinding forces between $\alpha_v\beta_3$ attached on a cantilever tip and ICAM4 receptor expressed on the surface of a SS-RBC. When processed manually and using FRAME, the results show no significant difference (nonparametric Mann–Whitney test, $p > 0.84$). (f) Histograms of unbinding forces between $\alpha_v\beta_3$ attached on a cantilever tip and ICAM4 receptor expressed on the surface of a SS-RBC as processed manually and using FRAME. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Hertz contact model to identify both the Young's modulus of the sample and the contact point on the curve. Before any force–displacement curve is fit with a contact model, it is first "flattened," a process in which the curve's force response before contact with the sample is defined to have an average force value of zero. In order to curve-fit the extension portion of force–displacement curves to the contact model and locate the contact point, FRAME effectively uses a least-squares minimization of the following expression: $F(z) = g(z-z_1) * [H(z-z_1) - H(z-z_2)]$. In this expression z is the vertical displacement, $F(z)$ is the force detected by the cantilever, H is the Heaviside function, $g(z)$ represents the contact model, z_1 is the contact point and a design variable, and z_2 is the end the curve-fit range which can be either defined by the user or be a design variable. The initial conditions for the minimization are determined from the flattening process (see Supplementary information). Other contact models can be easily implemented by the user through a simple guided modification of the MATLAB script. The step by step instructions for using custom contact models can be found in the user manual. The quality of the curve-fit, based on the root-mean-squared of the curve-fit residuals, can also be employed to ignore low quality curves from data sets. FRAME also provides users with the tools to manually identify the contact point and the curve-fit range on individual curves while retaining automated processing functionality on all others.

In addition, FRAME provides users with a set of algorithms to detect and quantify ligand-receptor unbinding events based on a number of user provided parameters. Potential unbinding events are selected by the algorithms based on

force thresholds provided by the user. The unbinding event detection algorithms verify that high amplitude noise is not misinterpreted as an unbinding event by confirming that the abrupt curve shift at the location of a possible unbinding event is permanent. FRAME can fit the curve before an unbinding event to the WLC model and produce the persistence and contour lengths associated with this event. Because the software treats each unbinding event on an individual basis and because users may alter many relevant parameters for unbinding event detection, these algorithms are applicable to any force range. FRAME is equipped with several functions and a user friendly GUI that allow users to very quickly review preliminary results and modify parameters based on a few representative curves per set of data in order to assure consistent performance. Users also have access to tools that allow them to manually select unbinding events on individual curves while retaining automated processing functionality on all others.

3. Results

We compared results derived from manual and automatic processing of data collected in receptor-ligand unbinding and in elasticity experiments.