Spectroscopy Analysis in Scanning Probe Microscopy

New Tools for Accessibility and Throughput

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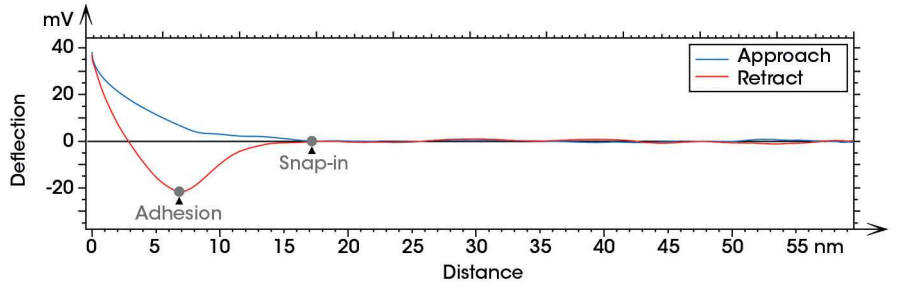
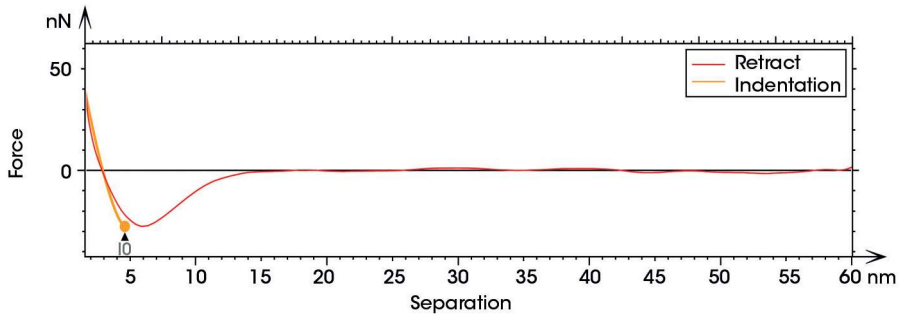
Force spectroscopy is a popular technique in scanning probe microscopy (SPM) for single-point measurements of materials, primarily focused on probing nanomechanical properties such as stiffness, deformation, and adhesion. Analysis of the single-point measurements, commonly known as force curves, poses various challenges, including the selection of the appropriate contact mechanics models and being time-consuming. MountainSPIP software has introduced new tools to streamline force curve analysis that address these challenges. In addition, the software has begun to integrate machine learning into this analysis workflow to enhance force curve validation.

Introduction to Force Spectroscopy

While most of scanning probe microscopy (SPM) data involves images, some of the most powerful data is actually spectroscopic. In the world of nanomechanical measurements, where SPM provides uniquely powerful capabilities of probing properties such as adhesion, deformation, and modulus on the nanoscale, force spectroscopy or force curves are the go-to measurements. While there are a number of sophisticated ways in which to make an SPM-based nanomechanical measurement, the simplest – and most frequently conducted – is one in which the tip is brought into contact with the surface, pushed against the surface, and then retracted away from the surface. As this motion progresses, the cantilever deflection is measured as a function of the Z piezo position. After calibrations of the cantilever spring constant and photodetector sensitivity, that measurement is ultimately transformed into a force vs. displacement curve that can be analyzed via different models to extract relevant mechanical properties of the sample.

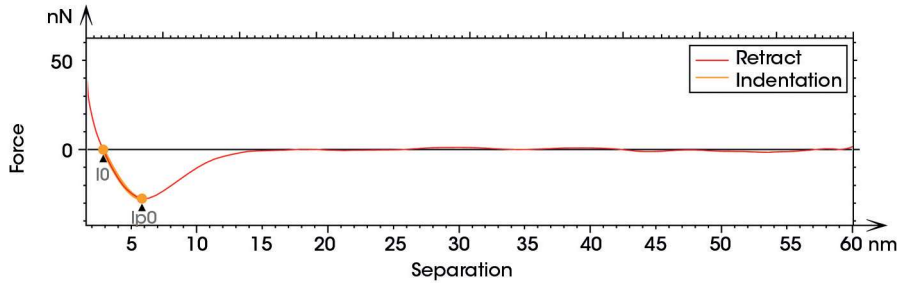


Force curve paramenters	Value	Unit
Cantilever spring constant	29.2	N/m
Photodiode sensitivity constant	44.1	nm/V



Force curve paramenters	Value	Unit
Cantilever spring constant	29.2	N/m
Photodiode sensitivity constant	44.1	nm/V

Indentation paramenters	I0	Unit
Tip shape	Spherical (DMT model)	
Tip radius	125	nm
Young's modulus	771	MPa
Coefficient of determination R^2	0.981	



Force curve paramenters	Value	Unit
Cantilever spring constant	29.2	N/m
Photodiode sensitivity constant	44.1	nm/V

Indentation paramenters	I0	Unit
Tip shape	Spherical (JKR model)	
Tip radius	125	nm
Young's modulus	421	MPa
Coefficient of determination R^2	0.998	

Figure 1: (a) Individual force curve on PCL with (b) DMT analysis and (c) JKR analysis.

Figure 1a shows a basic force curve on a sample of a polystyrene (PS)/polycaprolactone (PCL) blend chosen as a model sample. This force curve was conducted on the PCL component. Note the two curves where the blue segment shows the tip approaching the surface, snapping into contact, and being in repulsive contact with the sample, followed by the red segment showing the tip pulling away from the surface. In the retraction portion, there is a labelled dip resulting from adhesion between the tip and the sample as the tip is withdrawn.

The two most sought-after parameters from SPM curves are adhesion and elastic modulus, a measure of stiffness. While the former is fairly straightforward to measure directly off the force curve, the stiffness or modulus measurements are more complicated. The rest of this article focuses on the analysis and calculation of SPM force curves that are used for modulus.

The calculation of modulus from SPM force curves requires the modeling of the force curve with a contact mechanics model. The various contact mechanics models that are available simulate the tip-sample contact and account for the tip-sample adhesion in various ways. One of the challenging parts of the analysis of force curves is understanding which model to use. Calculation of a dimensionless parameter known as the Tabor coefficient, μ_T, can help guide contact mechanics model selection, but few commercial analysis software packages offer this calculation.

Figure 1b and Figure 1c show the retraction curve measured by the DMT model and JKR model, respectively, two of the most common contact mechanics models used in the analysis of force curves; the other popular model is the Hertz model. While the Hertz model does not incorporate any adhesion into the tip-sample contact, the DMT model typically accounts for weaker adhesion outside the tip-sample contact, with the yellow line in Figure 1b showing the fit and an R^2 value of 0.981. The JKR model incorporates stronger adhesion inside the tip-sample contact area, resulting in a much better fit with an R^2 value of 0.998 in Figure 1c. With easy implementation and side-by-side comparison, it is quickly established that the JKR model is the appropriate one for this dataset on PCL.

Workflow for Large Throughput Analysis of Force Curves

Force spectroscopy is typically a high-volume measurement in order to get good statistics on the sample. Multiple force curves can either be collected at individually selected points or over the entire image, in what is popularly known as force volume. Thus, a streamlined workflow for the analysis of a large number of force curve measurements is critical.

Furthermore, force curve analysis can be complicated on heterogeneous samples. In many cases, different models are needed to analyze the different components present within a single image. The ability to differentiate force curves based on the appropriate model and analyze accordingly is a process currently handled manually, and which can be very tedious. To address these challenges, MountainSPIP software has developed a 5-step workflow as shown in Figure 2.

Machine Learning Validation of Force Curves

The powerful functionality of the software's workflow begins in the second step of validating curves. The quality of force curves remains a serious challenge for force spectroscopy. Similar to instrumented nanoindentation, both these techniques require large datasets to get accurate measurements. Identification of force curves as artifacts or outliers, which should ideally be removed, can be tedious and time-consuming. Sometimes, force curves may fall on a feature edge or on anomalous region of the sample. Identifying "good" vs "bad" force curves is an appropriate task for machine learning, which is starting to be integrated into MountainSPIP for force curve selection.

As with all machine learning, the key is having an effectively trained model. A supervised machine learning model using a random forest algorithm was trained on 100,000 curves, primarily on various polymeric materials (homopolymers and polymer blends) to rate them as a 0, 1, or 2, a classification of "poor", "medium", or "good", respectively. Factors such as baseline flatness, approach/retract hysteresis, and ratio of maximum force/adhesion were all incorporated.

Figure 3a shows a 4µm x 4µm force volume image of a blend of PS/PCL where each point is the adhesive force at a 3 nm z distance as measured from the retract segment; a zoomed-in portion for further analysis focusing on one spherical domain of PS with surrounding PCL matrix is shown in Figure 3b. The machine learning model was applied to uncorrected force curves from Figure 3b, resulting in the plot in Figure 3c, which rated all the force curves with a "0" or the poorest quality force curve. This result is unsurprising as force curves generally do need some correction, as noted above, for artifacts like baseline flattening or separation. A simple second-order polynomial baseline correction applied to these force curves greatly improved their quality, as shown in Figure 3d where the model was rerun on the corrected force curves, showing that the vast majority of force curves were now in the "1" or medium range. Thus, the curves that were deemed poor quality or "0" can be filtered out in step 3 of the workflow, thereby improving the analysis and workflow.

One of the key benefits of using machine learning models is the customizability of the datasets used to train the models, in addition to the ability to share and pool datasets. While this trained model described above is available on MountainSPIP, users will be able to train their own models with their own datasets in a capability that is in the process of being implemented. Ultimately, they will be able to provide their own training datasets and assign a "grade" to representative force curves defined using principal component analysis (PCA). Then the model could be trained for analysis of subsequent curves. Thus, researchers whose work focuses on a particular kind of sample (e.g. living cells, hydrogels, elastomers, nanocomposites) will be able

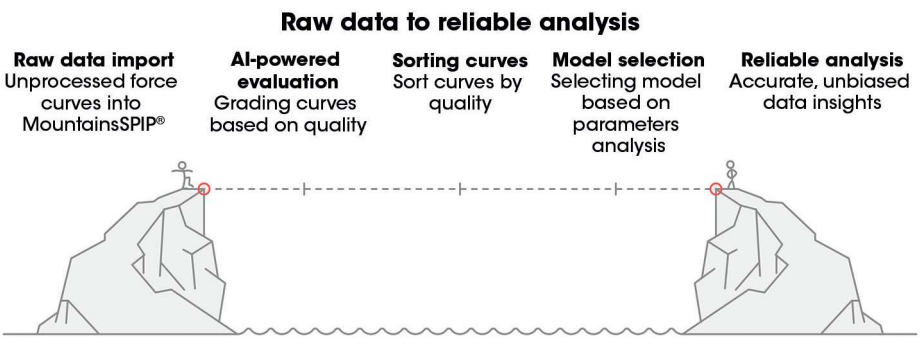


Figure 2: Five-step workflow for analysis of force curves in MountainSPIP.

to create a customized machine learning model to classify force curves on their specific samples to optimize analysis. This is useful as different samples provide different challenges for force curve analysis, and a "one size fits all" approach may not necessarily be appropriate. Finally, users will not be limited to just their datasets. If a group of users is all producing measurements on a similar type of sample (e.g. a living cell), they will be able to combine all their force curves into 1 training dataset to improve the quality of the model ultimately used to analyze the remaining untrained dataset.

Removing User Bias from Contact Mechanics Model Selection

Once the curves have been validated, step 4 involves the selection of the model to be used for analysis. There are multiple approaches to model selection. Currently, users intuitively base their decision on the sample-tip interactions (e.g. How much adhesion is there? Is it a biological sample imaged in physiological conditions, which tends to use Hertzian model analysis?) and fit individual curves to assess which model is best. MountainSPIP provides robust approaches that remove the user bias through either a comparison of the degree of model fit (R^2 values) or a calculation based on the Tabor coefficient.

Both DMT and JKR analyses were applied separately to all the force curves in the small area of the PS/PCL blend in Figure 3b. In order to ascertain the accuracy of these two models, the subtraction of R^2 values for the DMT fit minus the R^2 values for the JKR fit is shown in figure 4a so that values above zero (pink) show points which have a higher R^2 value for the DMT model, while points below zero (black) have an R^2 value that is higher for the JKR model. Figure 4a shows that the PS should be modeled with the DMT, while the PCL leans towards JKR. The resultant Young's modulus mapping for the entire image (Fig. 3a) is shown in Figure 4b with the PS modeled with DMT and the PCL with JKR, yielding an overall R^2 value for the model fit of 0.980. Implementing the reverse analysis – of modeling the PS with the JKR model and PCL with the DMT model – results in an overall R^2 value of 0.923, which is considerably worse. Thus, calculations of the R^2 value for different models for the various components enable a straightforward optimization of contact models for components within one image.

A second approach to contact mechanics model selection relies on the calculation of the Tabor parameter, μ_T. Tabor determined that DMT and JKR models are simply different ends of the same spectrum as defined by a ratio of the range of adhesion relative to the elastic deformation caused by the forces and the parameter given by:

$$\mu_T = \left(\frac{RW_{adh}^2}{E^{*2}z_0^3}\right)^{\frac{1}{3}}$$

Where R = tip curvature radius, W_{adh} = work of adhesion, E*=reduced modulus, and z_0= equilibrium spacing of the surface. A value of less than 0.1 means the DMT model should be used, and a value above 5 suggests the JKR model. The MountainSPIP results for the transition parameter calculation, derived from the Tabor parameter, are shown in figure 4c and then the corresponding model from the parameter in Figure

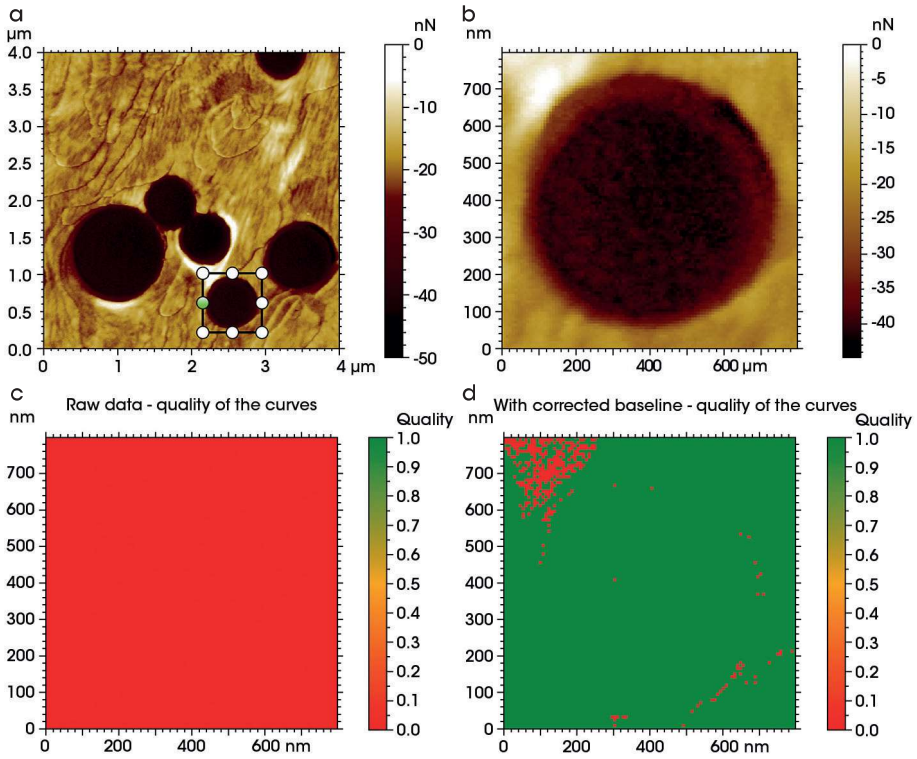


Figure 3: a) Force volume on a blend of PS/PCL where each point is the adhesive force at a 3nm z distance; PS are the spherical domains and PCL is the matrix b) Zoomed-in portion of (a) that includes spherical PS domain with some surrounding PCL matrix for additional analysis c) Machine learning random forest model applied to figure 3b with no correction to force curves. d) Machine learning random forest model applied to Figure 3b with second-order polynomial baseline correction of force curves.

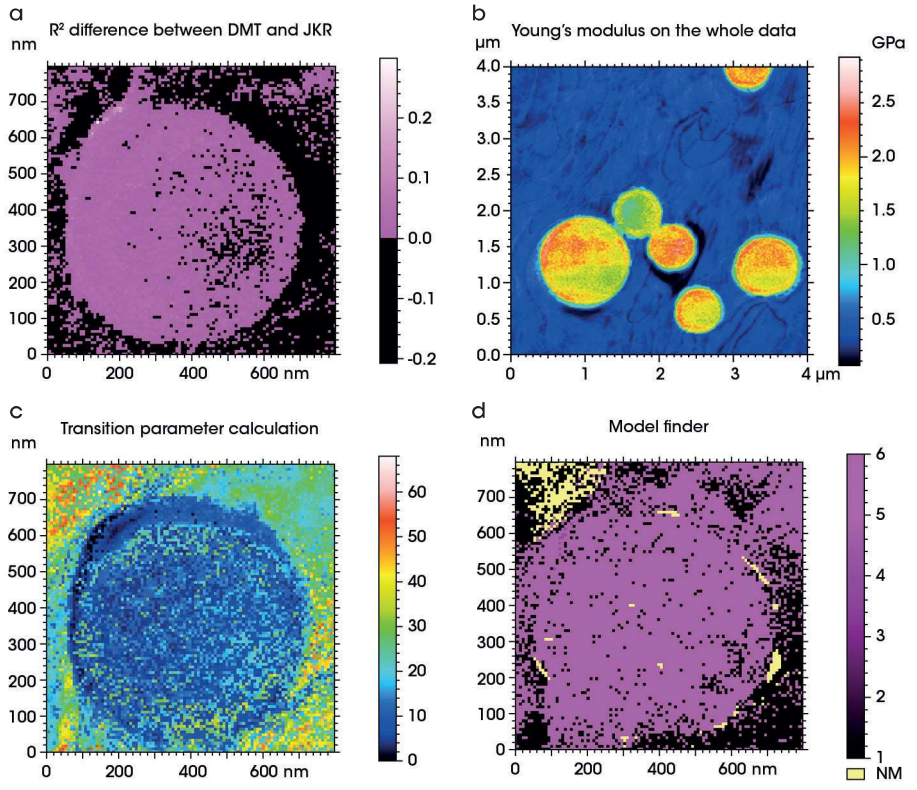


Figure 4: a) subtraction of R2 values for 2 different models that are fit to all the force curves in Figure 3b: R2 from DMT model fit subtracted from JKR model b) Resultant map using DMT model to fit PS and JKR model to fit PCL c) Transition parameter fit and d) Corresponding model from Tabor parameter fit where DMT is "3" and JKR is "2".

4d where "3" indicates DMT and "2" indicates JKR (note the points in yellow are "non-measurable" points due to a poor-fitting force curve). This shows that the R^2 analysis (Fig. 4a) and the transition parameter calculation (Fig. 4d) are consistent with suggesting DMT for the PS domain and JKR for the PCL component.

Conclusion

Analysis of SPM force curves can be tedious, challenging, and subject to user bias. The described software offers unique workflows for this task, incorporating a 5-step workflow that includes data validation, sorting, correction, and model selection. Recently, machine learning models have also been included to facilitate the data validation step; these models provide powerful customizability for one's own samples as well as the ability to pool datasets to improve the model training. For model selection, software users can either analyze their data with different models and optimize via best-fit R^2 value or use the Tabor parameter to guide the model selection, enabling the most appropriate contact mechanics model to be used for the modulus calculation at each pixel (i.e. each force curve). These new capabilities will help make force curve analysis more accessible, faster, and user-friendly for scanning probe microscopists of all backgrounds.

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