ViscoIndent Documentation

Yuri Efremov

August 2020

1 Introduction

ViscoIndent is a library written in the Python programming language for simulation of the force versus indentation (time) curves of soft viscoelastic materials (Section 2). It also allows fitting of the experimental force versus indentation (time) curves with the selected viscoelastic models (Section 3). The algorithm for the numerical simulation of curves was previously used and described in the works [1, 2, 3] and based on the numerical solution of the equations presented by Dr. T.C. Ting [4, 5].

2 Simulation of the force versus indentation curves

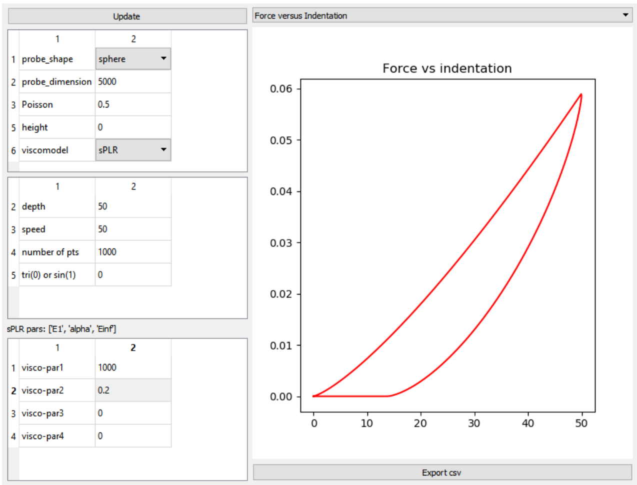

Figure 1: The graphical user interface

The graphical user interface (GUI) for the simulation of the indentation experiment has following functionality:

1. “Update” button at the top left - press it to update the graph with the current parameters in the field below

2. Table with the general parameters. Probe shape: sphere, cone or cylinder. Probe dimension: radius for the sphere and cylinder (nm), half-opening angle for the cone (degrees). Poisson’s ratio of the sample. Height(thickness) of the sample (for the bottom effect correction). Viscoelastic model - select one of the models from the list.

3. Table with the parameters that define the indentation history. Maximum indentation depth (nm), speed (nm/s), number of points in curve, triangular (0) or sinusoidal (1) displacement.

4. Table with the viscoelastic parameters. The meaning of the parameters is presented in the text above the table (after the viscoelastic model is selected)

5. Top right - selector for the graph representation, the calculated force versus indentation or the force versus time. Press “update” button to apply the changes.

6. The graph with force versus indentation or force versus time curve [nN vs nm or nN vs].

7. “Export csv” button - exports current data into the .csv file in the same folder where the python files are located.

The main parameters are:

- “probe_shape” – currently available probe (tip) shapes are: sphere (Hertz model), cone (Sneddon model), cylinder;
- “probe_dimension” – probe radius in [nm] (for sphere/cylinder) or angle in [degrees] (for cone);
- “Poisson” – Poisson’s ratio of the sample;
- “height” – local height/thickness of the sample, if known, a value in [nm]. Leave “0” if it is assumed to be infinite. The model with bottom effect correction is currently available only for certain probe shapes in a test mode;
- “viscomodel” – viscoelastic model. Please see the description of the viscoelastic models in [1].

The indentation parameters are:

- “depth” – maximum indentation depth [nm];
- “speed” – indentation speed [nm/s];
- “number of pts” – number of points in curve;
- “tri(0) or sin(1)” – select between triangular (0) or sinusoidal (1) displacement profile.

3 Processing (fitting) of the experimental data

The fitting of the experimental force versus indentation (time) curves is achieved by using the nonlinear optimization algorithms. There are, however, several steps that are required to transform the data to the appropriate format. Here, an example of the AFM force volume experiment will be considered that shows the basic pre-processing and processing steps.

The GUI for the processing of the experimental data has a modular structure, including the modules for the data import, conversion, and selection of the special regions on the AFM maps, and the modules for the data processing and visualization. the main module is called by the “” function.After calling the function, first, the module for the import of the data is called. It both imports and transforms the data from the format used by the equipment manufacturer to the format used in the Python workspace. Here, the import of the file generated by the Bruker Nanoscope software will be considered that represents the result of the force volume experiment. Basically, the file contains the set of force curves and some additional parameters that might be used for the data processing.

3.1 Short tutorial

In this tutorial, the processing of the force volume data acquired over REF52 living fibroblasts will be performed. These are the data acquired in a routine experiment on cells growing in a plastic Petri dish with a complete cell medium at 37°C. The AFM measurements were performed with a commercial Bioscope Resolve atomic force microscope (Bruker, USA) combined with an Axio Observer inverted optical microscope (Carl Zeiss, Germany). The PeakForce QNM-Live Cell cantilever (PFQNM-LC-A-CAL, Bruker AFM Probes, USA) with a pre-calibrated spring constant (0.069 N/m) and a 70 nm tip radius was used. Nanomechanical map was acquired in the Fast Force Volume mode with a size of 100x100 μm and 52x52 measurement points. A vertical ramp distance of 3 μm, a vertical piezo speed of 183 μm/s, and a trigger force of 0.5-1 nN were used

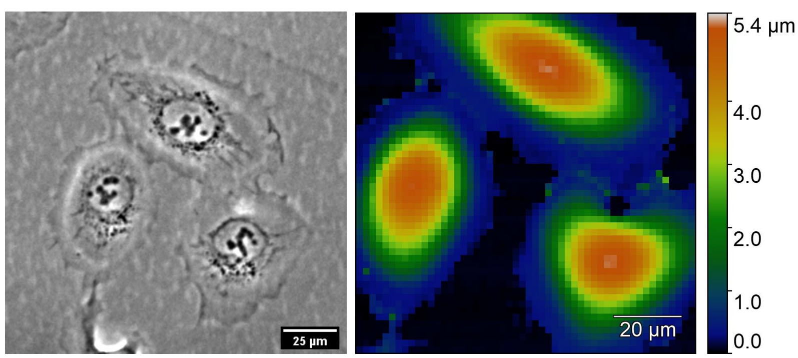

Figure 2: The optical (phase contrast) image and AFM topography (from force volume) images of the cells which are used in this tutorial

1. launch Viscoindent_dataGUI. You should see the data selection window:

Note: if you are using Spyder, the window might appear under the main Spyder window. Press “Open” button to select a data file. Select the file “Bruker_forcevolume_cells.spm” in the “examples” folder.

2. The next window will ask if you want to process the data as a force

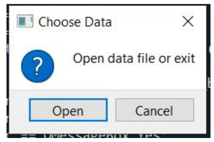

Figure 3: The data selection window

volume map or not. In this case, since the data are the output of the force volume experiment, the better answer is “Yes”. This will allow to extract additional parameters from the force volume data such as local height (required for the bottom effect correction) and to analyse distribution of the mechanical parameters over the mapped region.

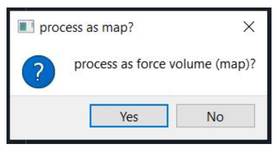

Figure 4: The force volume selection window

3. After pressing the “Yes” button, the next window will appear which allows performing topography correction. The main purpose of this step is to locate the zero height level, from which the local cell thickness will be further estimated. That generally also requires subtraction of the tilt from the image. The window allow selection of the options for the Bottom effect correction (BEC) strategy and Zero height level location strategy. To use the latter options, select option “locate zero height level” in the former menu. Then select “plane by 3 points” in the “Zero height level location strategy” menu. The idea here is to select (at least) three points on the substrate (not on cells) that will be used to estimate both the tilt and the zero height, which is zero level plane. This plane will be subtracted from the original image, and the resulted (processed) image will be presented in the right window. To perform the zero leveling by three points, select three or more points on the original image. The points should be outside of the cell borders and far away from each other covering most regions of the image. Use left mouse button (LMB) for the selection of the points. After selection, press the middle mouse button. Then, press “Continue” button or “Re-select points” button

4. At the next step, the region of interest (ROI) selection window will appear. Click “Yes” to move forward to the ROI selection if needed. The selecting of one of several ROIs will allow to exclude the force curves acquired on the hard surface from the analysis and to collect the curves only from the specific cells. To achieve the first task it is helpful to use the specific height level (“h-level”) below which all curves will be ignored. The second task is achieved by a freehand selection tool (“draw option”). Press and hold LMB (left mouse button) to draw a region around the specific cell. h-level and the number of

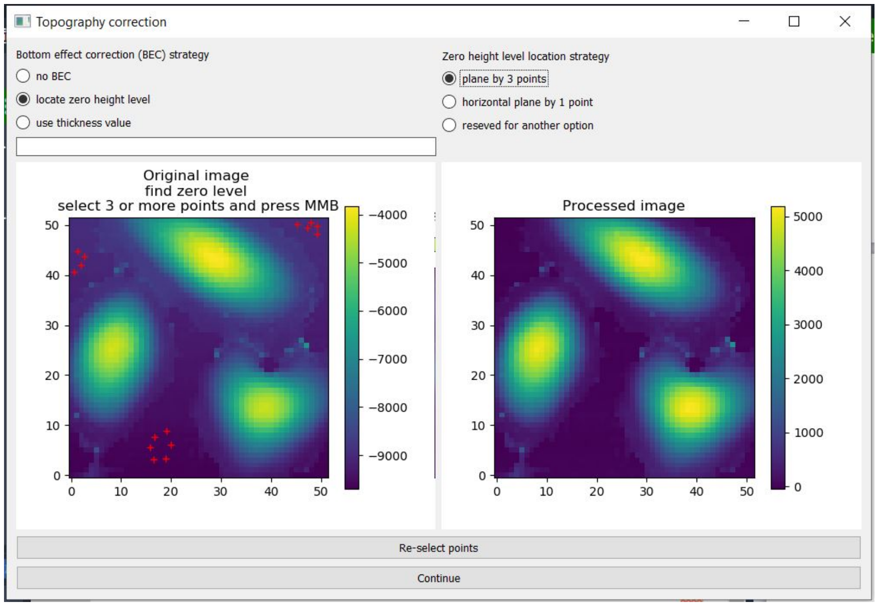

Figure 5: Topography correction window. The red crosses on the left image are the points selected ob the surface outside of the cell borders that are used to locate the zero height level (plane). The right image (Processed image) is obtained by subtraction of the zero height level from the original image

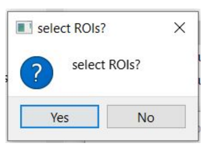

Figure 6: The force volume selection window

the ROIs should be inserted in the corresponding fields. By selecting the option "draw and select above h-level", the curves below h-level will be automatically excluded from the freehand selection region.

5. After the ROI selection stage, the force curve processing window will open. The windows demonstrate main processing parameters on the left, results of the processing on the right, and raw or processed force curves in the middle. Initially, only unprocessed (raw) curves will be shown. The processing stage is launched with the "Start processing" button. Before that, the parameters of the experiment might be checked and modified by calling the corresponding menu with the "Modify parameters" button. The range of the curves for the processing is selected with two fields above this button (default values - complete set of the curves). On the right panel, there are buttons "Show maps" to check the results in the form of maps and "Save" to save the results in a file for the

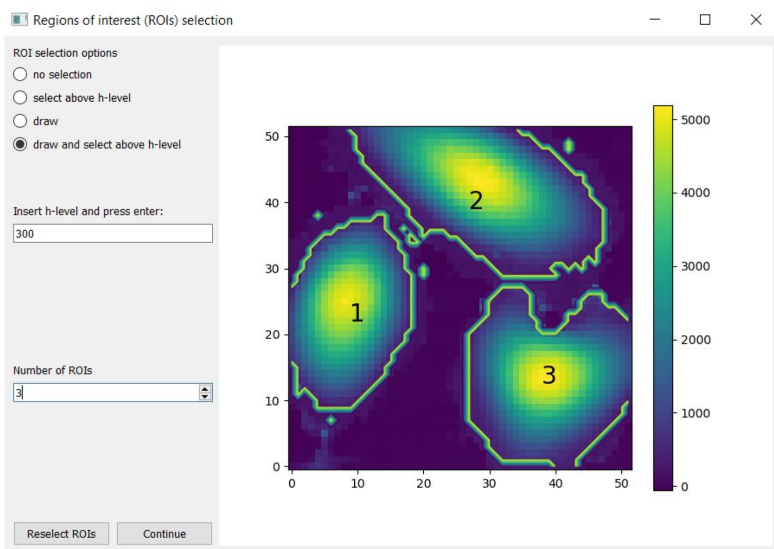

Figure 7: The ROI selection window. Here, three ROIs were selected that corresponds to three cells. The h-level of 300 nm was used to exclude force curves from the surface and thin cell regions.

processed data storage.

6. "Modify parameters" button is calling a panel which allow user to modify parameters that are used during the processing. Some of the parameters might be extracted from the loaded .spm file, e.g. cantilever stiffness, tip radius, in the case if these parameters were set up during the AFM experiment. Some other parameters are set up during the previous steps (BEC), and some are default parameters.

The parameters that are used at the processing step are:

• InvOLS - inverse optical lever sensitivity; also known as deflection sensitivity. Could be in units [nm/V] or [nm/A] depending on the AFM. NOTE This value is required for conversion of raw deflection data to deflection in [nm]. You might insert data in the "Data" Array directly in [nm] format, then InvOLS=1 should be used (this is the case for the files from Bruker Nanoscope).

• Indenter stiffness (k) – cantilever spring constant with units [nN/nm] ([N/m])

• Probe (tip) shape – available: 1 – sphere (Hertz model), 2 – cone (Sneddon model), 3 - pyramid (Bilodeau model), 4 – cylinder

• Radius/Angle (probe_dimension) – probe radius in [nm] (for sphere/cylinder) or angle in [degrees] (for cone/pyramid)

• Poisson's ratio – Poisson's ratio of the sample

• dT – Sampling time, [s]. Equals to (Sampling Frequency)$^{-1}$

• Viscoelastic model – currently available: 1 – "elastic" (no viscoelastic model), 2 – "SLS", 3 – "sPLR", 4 – "sPLReta" (sPLR with Newtonian viscosity

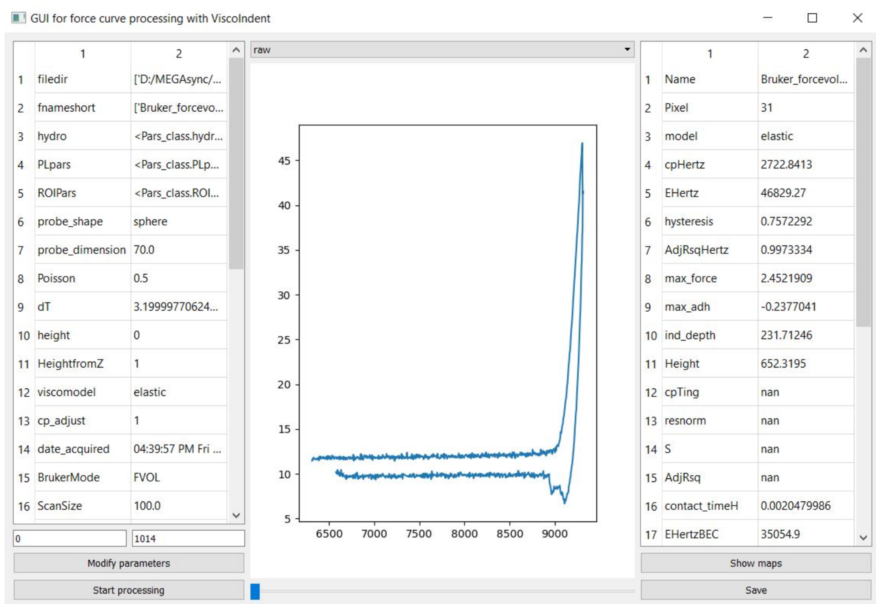

Figure 8: The force curve viewer window.

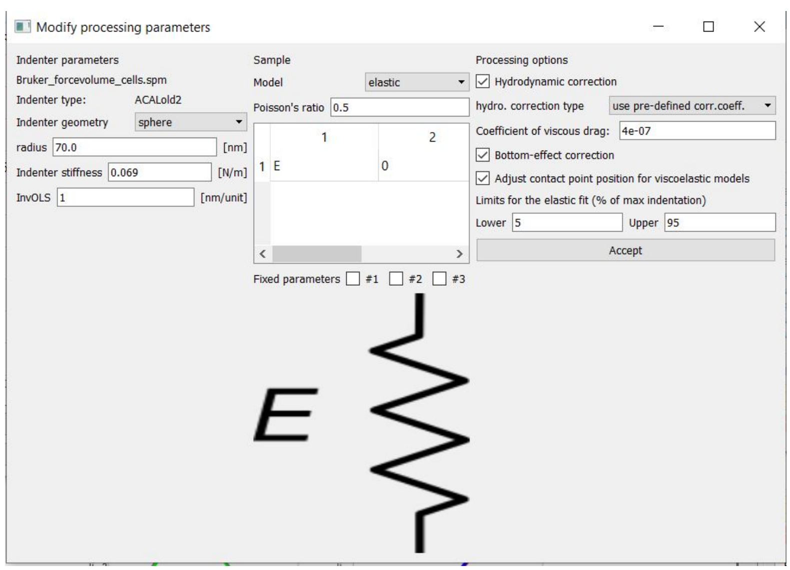

Figure 9: “Modify parameters” window.

term eta η).

• fixed visco-parameters – viscoelastic parameters that will be fixed during the fitting procedure (up to two parameters could be fixed). The values for the fixed parameters (E0/E1 [Pa], alpha/tau [- or s], Einf (or η) [Pa]) should be inserted in the table. Default value: all parameters are not fixed.

• Hydrodynamic correction – (recommended for high piezo speeds). See the original SciRep and Soft Matter papers for details [2, 3]. Two sub-options are avilable, namely "use pre-defined correction coefficient" and "find corr. coeff. automatically" (test mode). If the coefficient of the viscous drag for the used cantilever is known, insert it in the below field, the units are $nN \cdot s \cdot nm^{-1}$.

• Adjust contact point position for viscoelastic models (Contact point adjustment) – no adjustment (faster), or with adjustment (slower, but better fit, recommended). See the original SciRep paper for details [3].

• Height/Thickness – local height/thickness of the sample, if known, a value in [nm]. Leave "0" if it is assumed to be infinite. The model with bottom effect correction is currently available only for the spherical probe based on (Garcia PD, Garcia R (2018) Determination of the Elastic Moduli of a Single Cell Cultured on a Rigid Support by Force Microscopy. Biophys J 114(12):2923–2932.). (case for the bonded sample).

• Downsampling – 0 - none - disable downsampling (could be slow calculation speed, but highest accuracy); 1 - moderate - allow downsampling (default parameter, few points removed, faster calculation speed); 2 - aggressive - aggressive downsampling (many points removed, fastest calculation speed, decrease in accuracy). If allowed, when there are many data points in the curve (>2000), for example due to small sampling time and slow piezo speed, part of the data points will be removed (e.g. each 2nd, or each 2nd and 3rd, etc.) to decrease the calculation time, also the moving average filter will be applied. Decimated data will replace the original data in the "Data" array, the adjusted sampling time dT will be used.

7. The processing using viscoelastic models might take a substantial amount of time, check the progress in the console. It is recommended to use the procedure described in a step 10 to process large datasets with the use of the multiprocessing package.

8. "Show maps" window will demonstrate the results of the processing in the form of maps if the input data were from a force volume experiment, as in the case of this tutorial. Most of the parameters acquired during the processing can be presented in the maps. The maps of "Topography" (as obtained from the trigger position in the force curves) and "Corrected topography" (same as previous but after plane subtraction if applicable) can be shown even without force curve processing.The "Show ROIs" checkbox will overlay the selected ROIs on the image.

9. In the Results, the following parameters are presented:

• EHertz - the apparent elastic modulus;

• cpHertz - the contact point position (Z-coordinate) obtained from the elastic model fit;

• hysteresis - approach-retraction hysteresis, or the normalized hysteresis area, is the area enclosed between the approach and retraction curves divided by the area under the approach curve;

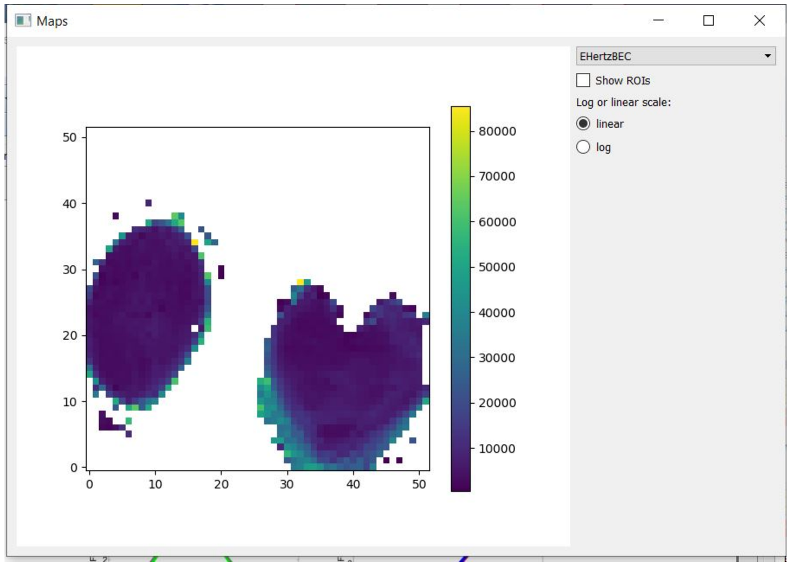

Figure 10: “Maps” window.

• AdjRsqHertz - adjusted coefficient of determination, Adjusted R2, for the elastic model fit;

• max_force, max_adh, ind_depth - maximum force, maximum adhesion force, and maximum indentation depth for the curve;

• Height - the height calculted from the contact point position and zero-level position, if applicable;

• cpTing - the contact point position (Z-coordinate) obtained from the viscoelastic model fit;

• E0, Einf, alpha/tau - extracted viscoelastic parameters;

• resnorm, S, AdjRsq - the goodness-of-fit parameters obtained from the viscoelastic model fit (norm of residuals = sum of squared residuals, S = standard error of the regression, adjusted coefficient of determination);

• contact_timeH - contact time (duration of the approach part of the indentation region);

• EHertzBEC, E0BEC, EinfBEC, alpha/tauBEC - parameters obtained with the bottom-effect correction;

• contact_timeT - contact time (full indentation region);

• Freq, Estor, Eloss - effective frequency, components of the complex Young's modulus (storage and loss Young's moduli);

10. For the processing of large datasets with the viscoelastic models, the following procedure is recommended. First, use the GUI to perform the topography correction and ROI selection. Then, perform the elastic fit on 10-20 curves to check the correctness of the processing parameters (especially the hydrodynamic drag coefficient). Then, select the viscoelastic model and save the

partially processed file. Now, you can launch the script for the data processing with the multiprocessing package that allows to fully engage multiple processors on a given machine and shorten the processing time. After the processing (which still might take a substantial amount of time), the same file will be rewritten with a completely processed dataset. The data might be checked using the "Show maps" module.

References

[1] Yuri Efremov, S.L. Kotova, and P.S. Timashev. Viscoelasticity in simple indentation-cycle experiments: a computational study. *bioRxiv*, 2020.

[2] Yuri M. Efremov, Anastasia I. Shpichka, S. L. Kotova, and Peter S. Timashev. Viscoelastic mapping of cells based on fast force volume and PeakForce Tapping. *Soft Matter*, 15:5455–5463, 2019.

[3] Yuri M. Efremov, Wen-Horng Wang, Shana D. Hardy, Robert L. Geahlen, and Arvind Raman. Measuring nanoscale viscoelastic parameters of cells directly from AFM force-displacement curves. *Scientific Reports*, 7(1):1541, 2017.

[4] T. C. T. Ting. Contact Problems in the Linear Theory of Viscoelasticity. *Journal of Applied Mechanics*, 35(1):248, 1968.

[5] T.C.T. Ting. The contact stresses between a rigid indenter and a viscoelastic half-space. *Journal of Applied Mechanics*, 33(4):845–854, 1966.