DATAVIEWER V2

User manual

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1. DOWNLOAD AND INSTALLATION

Downloading the software package

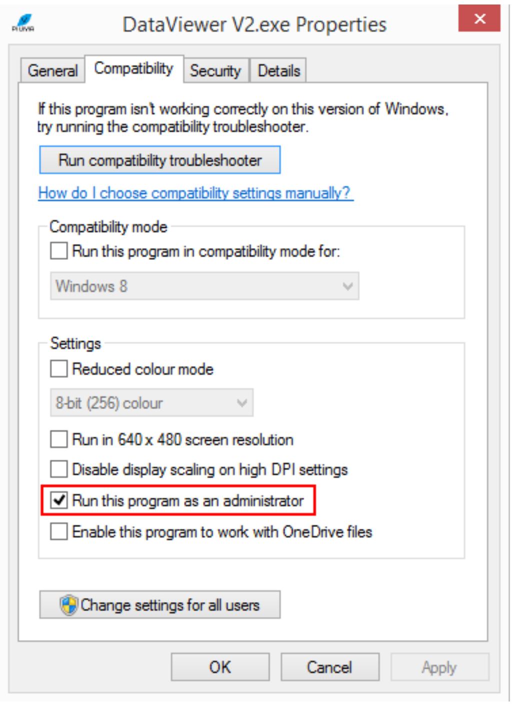
Download the latest DataViewer V2 software package from **https://optics11life.com/resources /** and extract the zip file.

Installation

Run Installer/Setup.exe to start the installation. Click **next** and install it in C:\Program Files. Let the installer finish.

Open software

Run *DataViewer V2.exe* as administrator located on the location chosen during installation. The default location is C:\Program Files\DataViewer V2\DataViewer V2.exe.



If this program isn't working correctly on this version of Windows, try running the compatibility troubleshooter.

Compatibility mode

☐ Run this program in compatibility mode for:

Windows 8

Settings

☐ Reduced colour mode

8-bit (256) colour

☐ Run in 640 x 480 screen resolution

☐ Disable display scaling on high DPI settings

☑ Run this program as an administrator

☐ Enable this program to work with OneDrive files

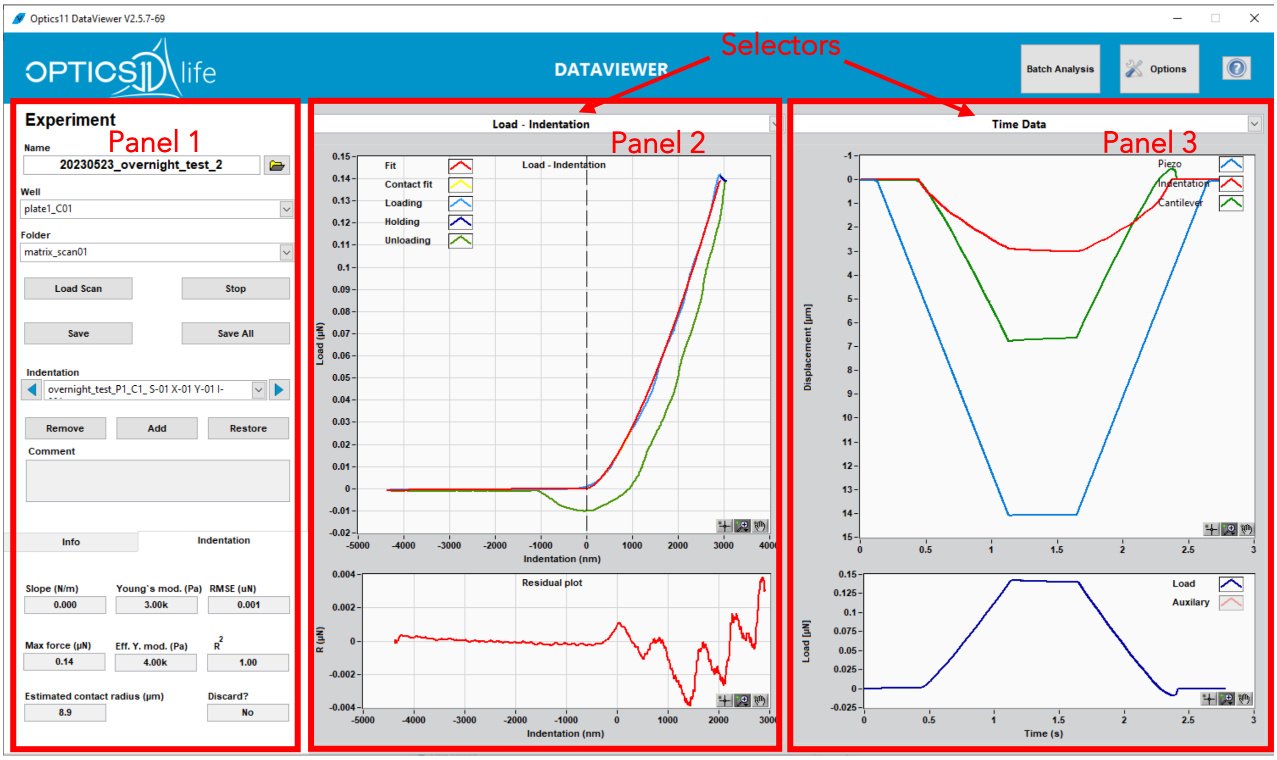
Figure 1: DataViewer V2 properties.

To make sure the software is set to run as administrator, right-click *DataViewer V2.exe* and open properties. In the **Compatibility** tab, make sure **Run this program as an administrator** is checked.

2. SOFTWARE LAYOUT

Panel explanation

The software consists of three panels and **Options** (see Figure 2).



Optics11 DataViewer V2.5.7-69

Experiment

Name: 20230523_overnight_test_2

Well: plate1_C01

Folder: matrix_scan01

Indentation: overnight_test_P1_C1_ S-01 X-01 Y-01 I-

Comment:

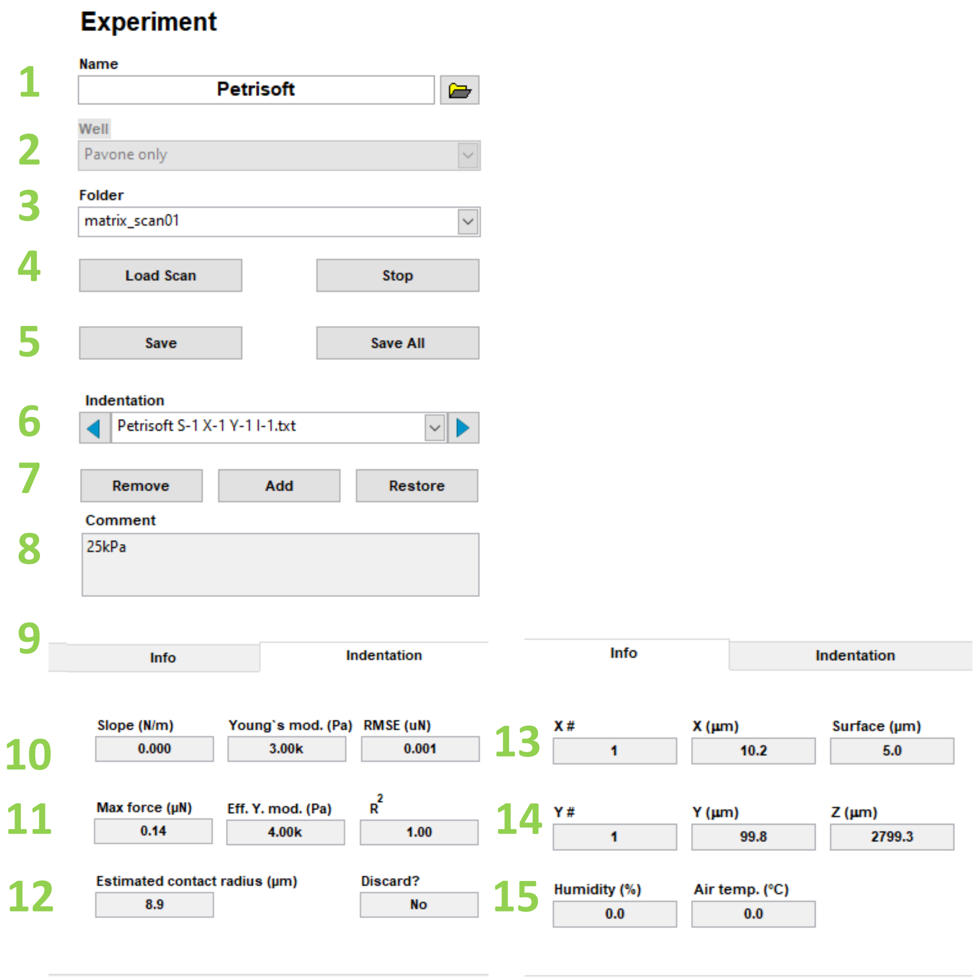
Slope (N/m)	Young`s mod. (Pa)	RMSE (uN)
0.000	3.00k	0.001

Max force (µN)	Eff. Y. mod. (Pa)	R^2
0.14	4.00k	1.00

Estimated contact radius (µm)	Discard?
8.9	No

Figure 2: Main front panels.

2.1 Panel 1



Experiment

Name: Petrisoft

Well: Pavone only

Folder: matrix_scan01

Indentation: Petrisoft S-1 X-1 Y-1 I-1.txt

Comment: 25kPa

Slope (N/m): 0.000
Young`s mod. (Pa): 3.00k
RMSE (uN): 0.001
Max force (μN): 0.14
Eff. Y. mod. (Pa): 4.00k
R^2: 1.00
Estimated contact radius (μm): 8.9
Discard?: No

X #: 1
X (μm): 10.2
Surface (μm): 5.0
Y #: 1
Y (μm): 99.8
Z (μm): 2799.3
Humidity (%): 0.0
Air temp. (°C): 0.0

Figure 3: Panel 1, navigation between different indentation data, information on the currently selected indentation file: fit results and stage position.

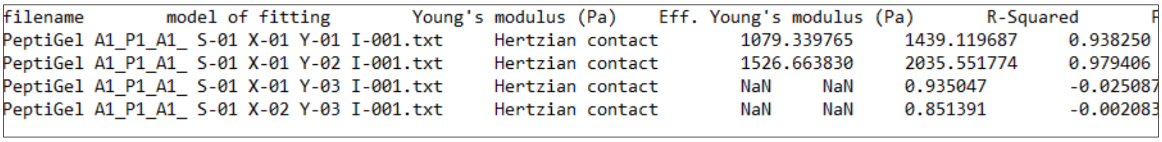
Explanation of Panel 1 (see green numbers in *Figure 3*):

1. **Name**: browse for the folder *SampleData* with the *Indentations* and *matrix_scan_01* folders inside. The name of the folder can be changed and will be updated after pressing **Save** or **Save All**.
   Browse for the sample data found in the DataViewer V2 extracted zip file by pressing the browse button. The default location is Downloads\DataViewerV2\SampleData\

2. **Well (Pavone only):** Select which well plate the sample was measured in. There should be no other folders than the ones named as *plate1_A1, plate1_B1, etc.*
3. **Folder**: select the subfolder for the analysis: *Indentations* or *matrix_scan_01.* Do not change these folder names, only the name of the higher folder.

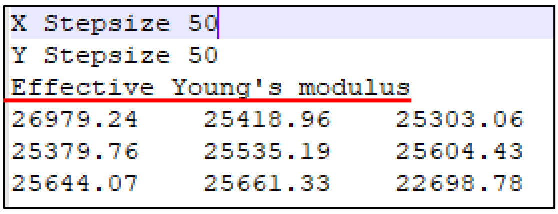
4.
    - If *matrix_scan_01* is selected in point 2, press **Load Scan** to load all the indentation files which will be analyzed according to fitting settings in **Options**.
    - Press **Stop** to stop loading the scan.

5. Press **Save** to generate the result files of the currently loaded data, both for *Indentations* and *matrix_scan* folders. A new folder with the name *SampleData_corrected* will be created containing *SampleData Result01.txt* with all the information about the fitting in columns which can be used for further statistical analysis or data sorting. Every time you press **Save** – a new text file will be created. This is convenient to use when various fitting options are applied e.g. fitting to different depths to show nonlinearity of the material. For the easier reading of the file, copy the information into an excel table.



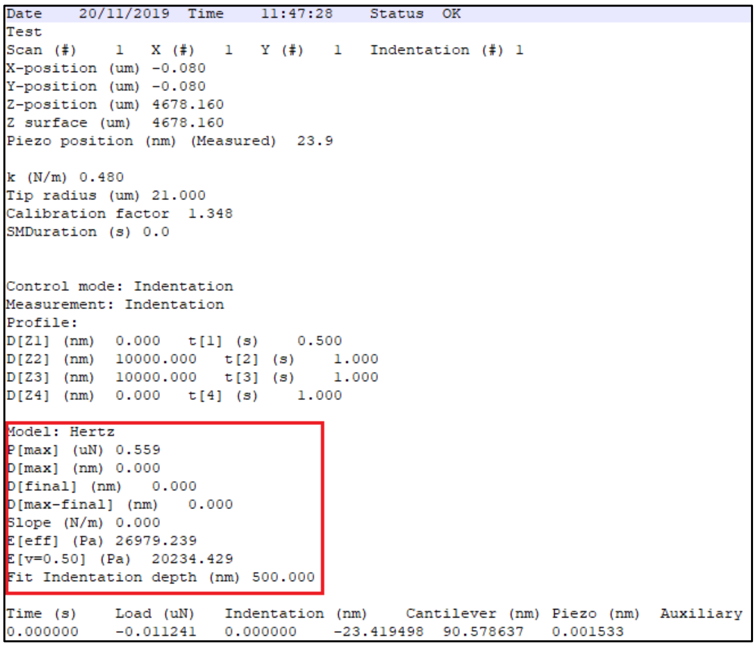
```
filename        model of fitting        Young's modulus (Pa)    Eff. Young's modulus (Pa)       R-Squared       F
PeptiGel A1_P1_A1_ S-01 X-01 Y-01 I-001.txt     Hertzian contact        1079.339765     1439.119687     0.938250
PeptiGel A1_P1_A1_ S-01 X-01 Y-02 I-001.txt     Hertzian contact        1526.663830     2035.551774     0.979406
PeptiGel A1_P1_A1_ S-01 X-01 Y-03 I-001.txt     Hertzian contact        NaN     NaN     0.935047        -0.025087
PeptiGel A1_P1_A1_ S-01 X-02 Y-03 I-001.txt     Hertzian contact        NaN     NaN     0.851391        -0.002083
```

For the *matrix_scan* folder, *an Additional file is created: SampleData S-1 result vs XY position.txt* to show mapping results. Values in this file are updated according to what is plotted in **3D Plot** or **Histogram** panel (Young's, Eff. young's, Storage modulus, Loss modulus, topography).



```
X Stepsize 50
Y Stepsize 50
Effective Young's modulus
26979.24     25418.96     25303.06
25379.76     25535.19     25604.43
25644.07     25661.33     22698.78
```

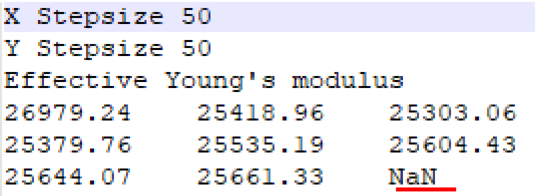
- Press **Save All** to reload and save all the indentation files containing new fitting results in the header of the text file and to generate the two result files.



```
Date    20/11/2019  Time    11:47:28    Status  OK
Test
Scan (#)    1   X (#)    1   Y (#)    1   Indentation (#) 1
X-position (um) -0.080
Y-position (um) -0.080
Z-position (um) 4678.160
Z surface (um)  4678.160
Piezo position (nm) (Measured)  23.9

k (N/m) 0.480
Tip radius (um) 21.000
Calibration factor  1.348
SMDuration (s) 0.0


Control mode: Indentation
Measurement: Indentation
Profile:
D[Z1] (nm)  0.000   t[1] (s)    0.500
D[Z2] (nm)  10000.000   t[2] (s)    1.000
D[Z3] (nm)  10000.000   t[3] (s)    1.000
D[Z4] (nm)  0.000   t[4] (s)    1.000

Model: Hertz
P[max] (uN) 0.559
D[max] (nm) 0.000
D[final] (nm)   0.000
D[max-final] (nm)   0.000
Slope (N/m) 0.000
E[eff] (Pa) 26979.239
E[v=0.50] (Pa)  20234.429
Fit Indentation depth (nm) 500.000

Time (s)    Load (uN)   Indentation (nm)    Cantilever (nm) Piezo (nm)  Auxiliary
0.000000    -0.011241   0.000000    -23.419498  90.578637   0.001533
```

6. **Indentation**: select the indentation file to be loaded, or use the arrows to scroll through them.

7.
   - **Remove** the current indentation file from the matrix scan. **3D plot** and **Histogram** panels will be updated after you scroll through all indentation points. Press **Save All** if you want to save new results and removed data points will be replaced with NaN values in *SampleData S-1 E-eff vs XY position.txt*.



```
X Stepsize 50
Y Stepsize 50
Effective Young's modulus
26979.24    25418.96    25303.06
25379.76    25535.19    25604.43
25644.07    25661.33    NaN
```

   - **Add** a previously removed indentation file to the matrix scan.
   - **Restore** all removed indentation files to the matrix scan.

8. **Comment** section

9. Selector between **Stage** and **Indentation** information

10.

- **Slope (N/m):** slope value from Oliver & Pharr fit (see **Options**).
- **Young's mod. (Pa):** Young's modulus from fitting Hertz, Oliver & Pharr or JKR model (kPa, MPa or GPa). During the fitting procedure, effective Young's modulus E_{eff} is obtained while Young's modulus E is: $E = E_{eff}(1-\nu^2)$, where Poisson's ratio ν is set by the user in **Options** (0.5 for incompressible materials).
- **RMSE (µN):** root means square error of the fit (standard error of the residuals), lower values mean a better fit (as it is an absolute number, compare it to **Load (µN)**).

11.

- **Max force (µN)**: peak force applied to the sample (the peak of **Loading** curve)
- **Eff. Y. mod. (Pa)**: effective Young's modulus $E_{eff} = \frac{E}{(1-\nu^2)}$ (in the literature it is also called plane strain modulus, reduced or marked with the asterisk *).
- R^2: R-squared of the fit. The closer the value to 1 – the better the fitting.

12.

- **Estimated contact radius (µm):** contact radius a=√(h*R) at the indentation depth of the fit (peak of Fit) where h is indentation depth.
- **Discard?** The Discard Curve feature is a fully automatic feature that helps the user to evaluate the output data and whether to keep it or not. Its output is either Yes or No. Please see the Appendix for more information on how the data is evaluated.

13. **X#** - step number along X-axis, **X (µm)** – position of the x-stage and **Surface (µm)** distance to the surface, calculated by the contact fit.

14. **Y#** – step number along Y-axis, **Y (µm)** - position of y-stage and **Z (µm)** the position of the Z-stage

15. **Humidity (%)** – humidity and **air temp (°C)** - temperature measured by the Pavone.

2.2 Panels 2 and 3

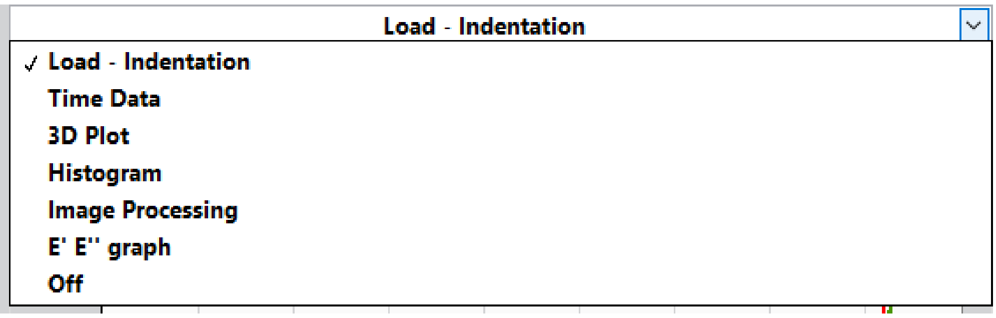


Figure 4: Dropdown menu to select graph visible in the panel.

Panels 2 and 3 are for plotting the data. Use the panel selectors to switch between different graphs:

1. Load – Indentation
2. Time data
3. 3D Plot
4. Histogram
5. Image processing (Pavone only)
6. E’ E’’ graph
7. Off

2.2.1 Load – indentation

Load – Indentation selection shows two graphs (see green numbers in Figure 5):

1. The plot of **Load (µN**) versus **Indentation (nm)** where different indentation steps are separated by colors identified in the top left corner:
    - Fit – red
    - Contact fit – yellow
    - Loading – light blue
    - Holding – dark blue
    - Green – unloading

   Assigned colors can be changed by pressing with the right-click on the color. The results of the fit are given in Panel 1. The range of fitting and model can be changed in **Options**.
2. The bottom graph shows the residual plot of the fit. RMSE and NRMSE values are given in Panel 1.

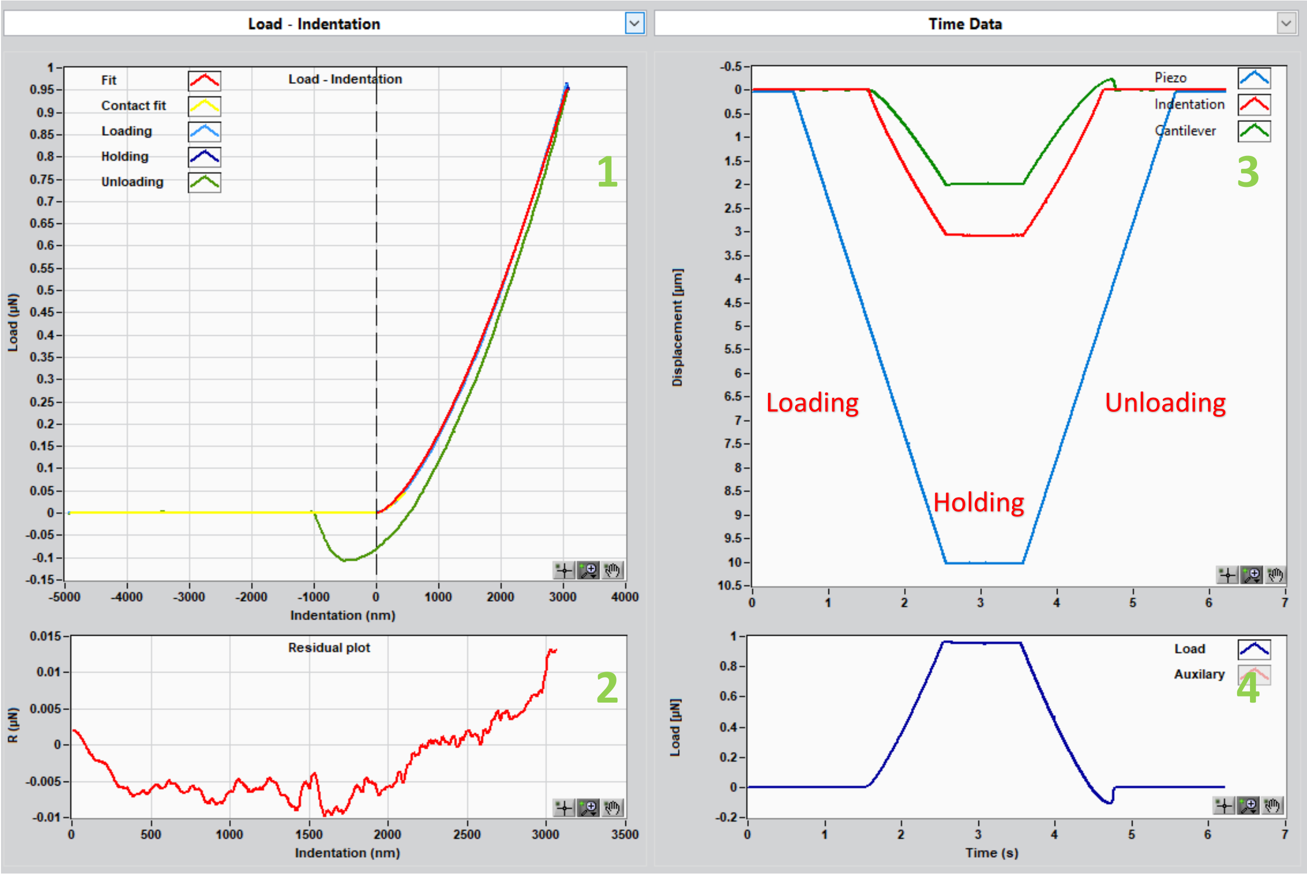


Figure 5: Selector of Load - Indentation and Time Data.

2.2.2. Time data

Time Data selection shows two graphs (see green numbers Figure 5):

3. The plot of **Displacement (µm)** versus **Time (s)** where the type of data is separated by colors:
    - Piezo – light blue, piezo displacement over time;
    - Indentation – red, indentation-depth over the time, obtained by subtracting **Cantilever** bending from **Piezo** displacement;
    - Cantilever - green, cantilever bending over time.
4. The plot of **Load (µN)** versus **Time (s)** is obtained by multiplying **Cantilever** bending with the spring constant of the cantilever k (N/m).

2.2.3. 3D plot

3D Plot selection shows indentation data of the matrix scan plotted as a 3D surface (see green numbers in Figure 6).

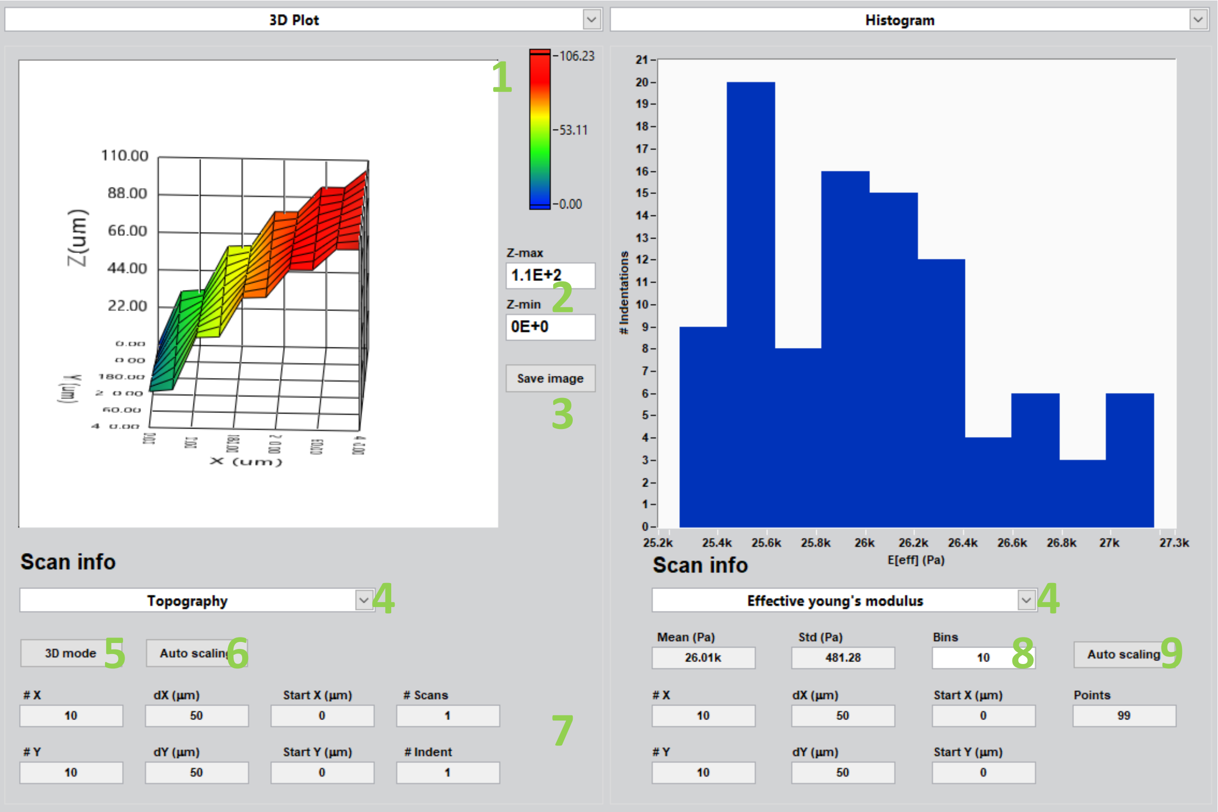
1. Colour bar of modulus values in Pascals. Press right-click to change **3D plot properties**.

2. **Z-min** and **Z-max** are minimum and maximum values of the Z-axis. You can type the number to change it e.g. to see mechanical features at certain stiffness range.

3. Press **Save image** to save the graph as a .jpg image. The location of the saved image is set in Options-> Advanced-> Save location.

4. Selector for the data to be plotted:
    - Young's
    - Eff. young's
    - Storage modulus
    - Loss modulus
    - Topography

5. Press **2D mode** to switch from 3D to the 2D mode and the other way around. Notice orientation of X and Y axis, the scan was started from 0,0 position.



Z-max 1.1E+2
Z-min 0E+0

Scan info
Topography
X 10
dX (µm) 50
Start X (µm) 0
Scans 1
Y 10
dY (µm) 50
Start Y (µm) 0
Indent 1

Scan info
Effective young's modulus
Mean (Pa) 26.01k
Std (Pa) 481.28
Bins 10
X 10
dX (µm) 50
Start X (µm) 0
Points 99
Y 10
dY (µm) 50
Start Y (µm) 0

Figure 6: Selector of the 3D plot and Histogram in panel 2 or 3.

6. Press **Auto scaling** to automatically scale Z-axis.

7. **Scan Info**: provides information about the matrix scan:
   - **#X** – number of indentations along the X-axis
   - **dX (µm)** – distance between indentation locations (step size)
   - **Start X (µm)** – stage position at the start of the scan
   - **# Scans** – number of performed matrix scans
   - **#Y** – number of indentations along the X-axis
   - **dY (µm)** – distance between indentation locations (step size)
   - **Start Y (µm)** – stage position at the start of the scan
   - **# Indent** – number of indentations per location

2.2.4 Histogram

The histogram gives the distribution of indentation results (modulus values) where the Y-axis gives the number of indentations within the bin and the X-axis the modulus values (see green numbers in Figure 6).

8. **Bins:** the total range of indentation results (from minimum to maximum) is divided into equal parts – bins. Each indentation value is then placed into the corresponding bin.

9. Press **Auto scaling** to automatically scale X-axis.

2.2.5 Image processing (Pavone only)

A sequence of TIFF files is converted into a .avi file. Press zoom to fit to see the whole image. Images need to be in the folder with the same name as indentation file in order to load them into DataViewer.

2.2.6 E', E'' graph

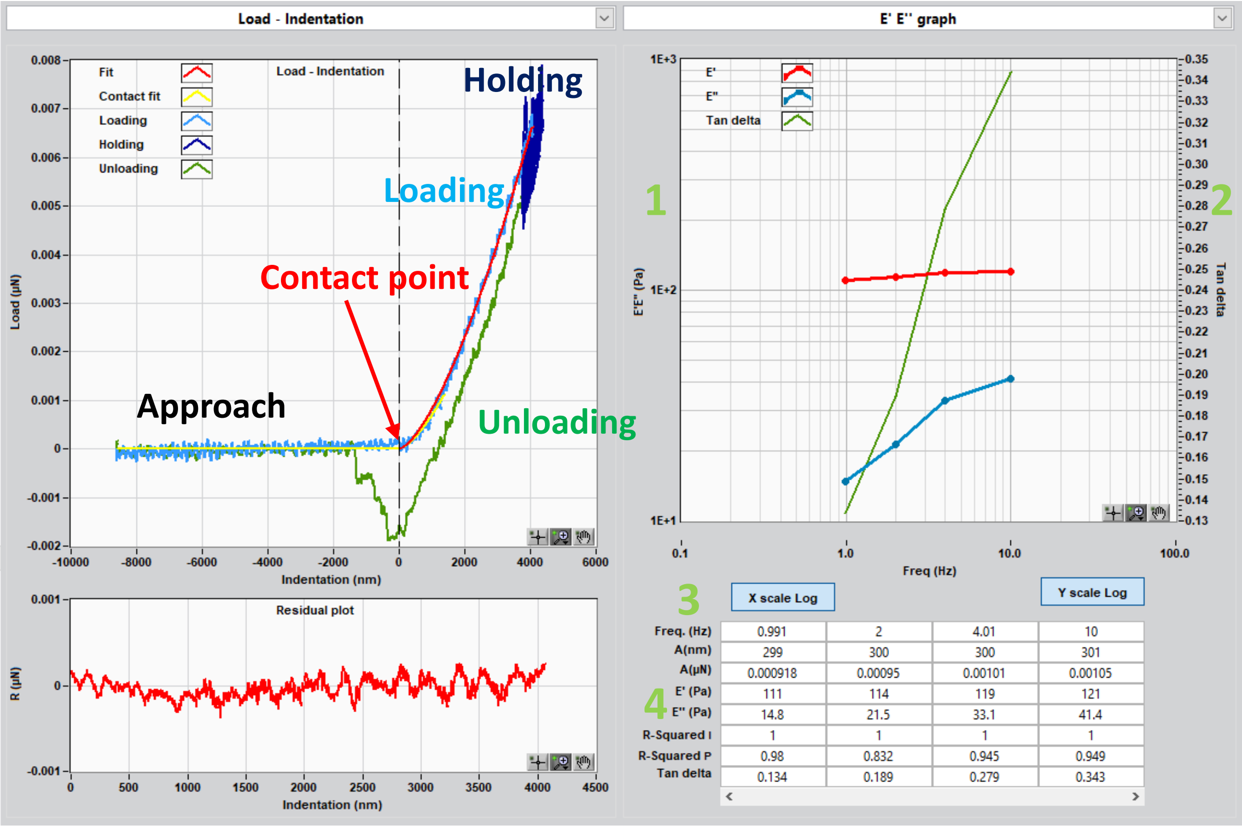
Load the *Indentations* folder and select the *Test Indentation_001.txt* file which contains data from a DMA measurement in I-mode. E', E'' graph contains (see green numbers in Figure 7):

1. The left axis is the scale of storage modulus E' and loss modulus E'' expressed in Pascals (Pa) as a function of oscillation frequency in Hertz (Hz). The red curve is storage modulus E', the blue curve is loss modulus E''. Press with right-click to change the colors or other graph parameters.

2. The right axis is for Tan delta which is the ratio between loss and storage modulus.

3. Press **X scale Log** and **Y scale Log** to change between linear and logarithmic scales.

4. Table with values plotted in the E', E'' graph as well as the fitting parameters such as the amplitude and R-squared values.



Freq. (Hz)	0.991	2	4.01	10
A(nm)	299	300	300	301
A(µN)	0.000918	0.00095	0.00101	0.00105
E' (Pa)	111	114	119	121
E'' (Pa)	14.8	21.5	33.1	41.4
R-Squared I	1	1	1	1
R-Squared P	0.98	0.832	0.945	0.949
Tan delta	0.134	0.189	0.279	0.343

Figure 7: Results from DMA curves (left side) plotted at E′ E′′ graph (right side). Arrows indicate good Hertz fit for the determination of the contact point.

In order for the fitting of DMA to work, indentation must be performed in a correct manner.

1. The minimum initial relaxation time should be 5 s. However, fitting of oscillations which are on an angle rather than horizontal might fail, thus, if the sample shows a long relaxation duration or temperature drift, the time should be chosen so that equilibrium is reached before DMA starts.

2. Amplitude should be chosen as small as possible due to the assumption that the contact area does not change during oscillations and material nonlinearity is not sensed. Check that your material is not sensitive to changes in amplitude by performing DMA with different amplitudes.

3. The frequency range can be chosen between 0.05 Hz up to 50 Hz in closed-loop operation and 75 Hz in open-loop operation. However, the performance might differ depending on PID settings set in the 'Maintenance'. Therefore, initial tests need to be performed to evaluate system performance for the specific sample/probe combination. Check whether the amplitude of oscillations in indentation is constant over all frequencies (for DMA in I-

mode), if not – you can try to increase I-term in the Maintenance menu when performing indentations. Get in contact with support@optics11life.com to get more information and training.

4. The number of periods should be chosen so that oscillations take at least 1s, for instance when using 0.5 to 5Hz, the number of periods should be 5 while for higher frequencies, the number of periods should match the frequency, e.g., 75 Hz - 75 periods.

5. Relaxation time between oscillations should be 2 s in order to separate frequencies correctly.

6. Fitting success will also depend on data segmentation such as loading should be marked in light blue, holding in dark blue, and unloading in green. Segmentation can be shifted in Options by changing Approach duration.

7. Make sure that Hertz fit is well fitted for the initial load-indentation curve (see red arrows in Figure 8). The position of 0, also called contact point, of the **Indentation (nm)** axis, is determined based on this fit and is needed for the correct calculation of storage and loss modulus. See Figure 8 for an example of a bad fit and wrong estimation of the contact point.

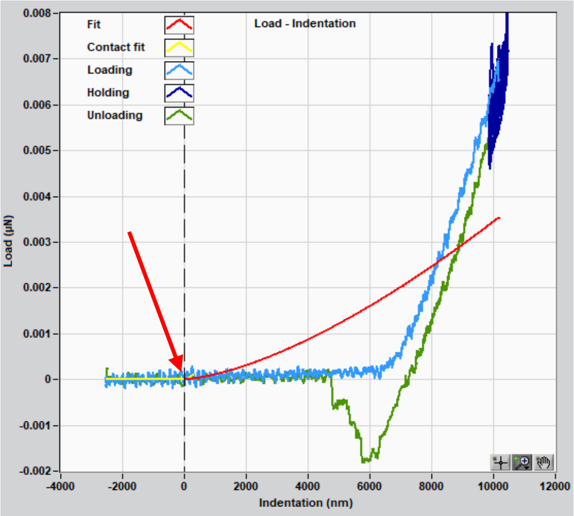


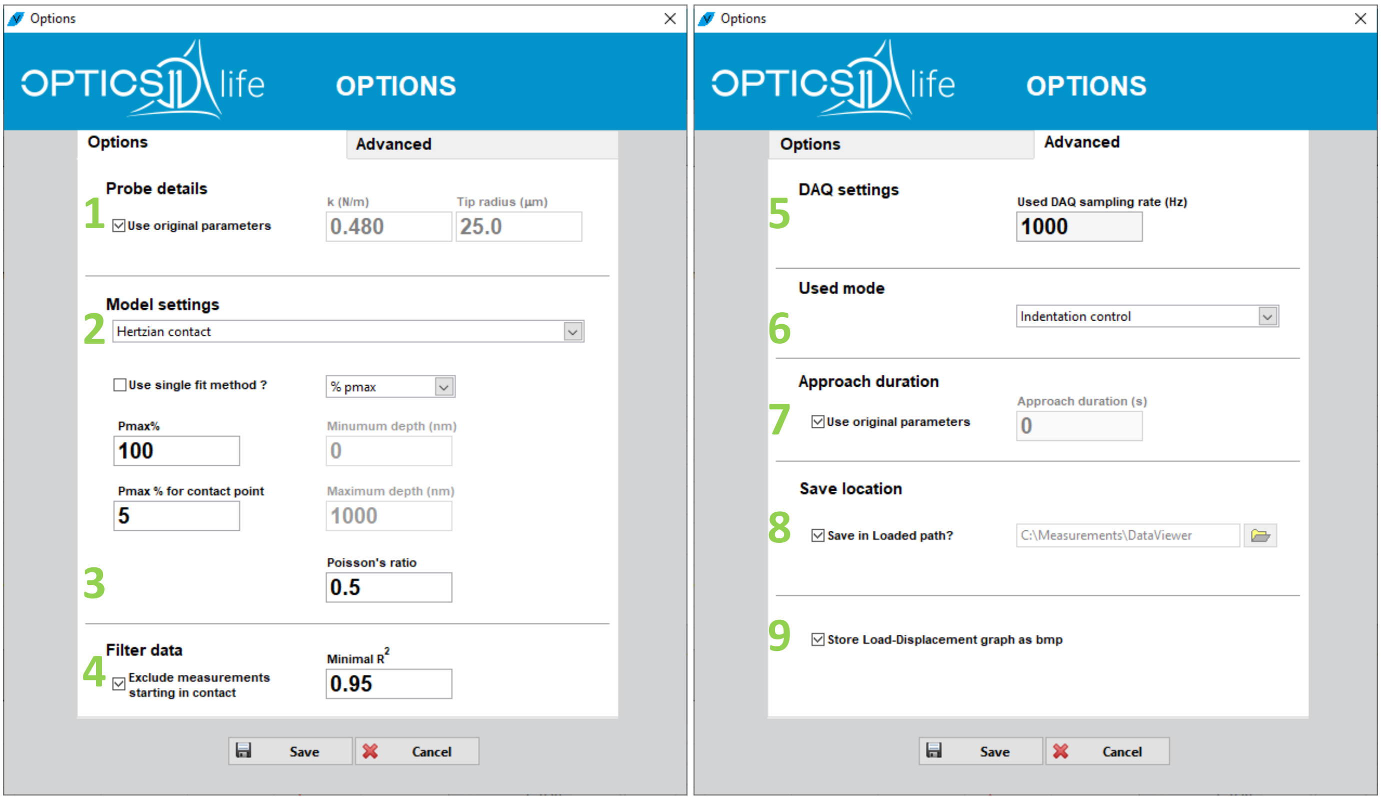
Figure 8: Example of a bad Hertz fit and a wrong location of the contact point.

2.3 Select model parameters: Options

Open the Options menu by clicking on the Options button in the top right corner. The options menu allows changing of settings, the contact mechanics models and fitting parameters (see green numbers in Figure 9):

1. **Probe details**: shows stiffness of the probe **k (N/m)** and **Tip radius (µm)** which was set during the experiment. If the probe details set during the experiment were wrong, you can uncheck **Use original parameters** and change it. The data will be recalculated according to new probe details:
   - probe stiffness k (N/m) changes load data as $P = k \times cantilever\ deflection$
   - tip radius changes estimation of contact radius $a\ (R)$ and, thus, Young's modulus for all models.

2. **Model settings** – select the desired model, **Oliver & Pharr**, **JKR, Hertzian contact or Hertz for hemispherical samples** and press **Save** to initiate analysis with the new settings. Decrease the value in **Pmax % for contact point** until a good fit of the initial load-indentation curve is achieved (see Figure 7 and Figure 8 for good and bad fit). Using the **Single fit method** for **Hertzian contact** will disable **Pmax % for contact point** and use one fit to calculate both the contact point and the Young's modulus.



Probe details
☑ Use original parameters
k (N/m): 0.480
Tip radius (µm): 25.0

Model settings
Hertzian contact

☐ Use single fit method ?
% pmax
Pmax%: 100
Minumum depth (nm): 0
Pmax % for contact point: 5
Maximum depth (nm): 1000
Poisson's ratio: 0.5

Filter data
☑ Exclude measurements starting in contact
Minimal R^2: 0.95

DAQ settings
Used DAQ sampling rate (Hz): 1000

Used mode
Indentation control

Approach duration
☑ Use original parameters
Approach duration (s): 0

Save location
☑ Save in Loaded path?
C:\Measurements\DataViewer

☑ Store Load-Displacement graph as bmp

Figure 9: Options menu: Options and Advanced.

Models used for data fitting

Hertz model is used to extract Young's modulus for linear elastic materials. Furthermore, the material is assumed to be infinite half-space (flat and thick), homogenous, isotropic, and non-adhesive. However, due to its simplicity, it is also commonly used to obtain contact point and extract Young's modulus from complex viscoelastic and nonlinear materials with some limitations. Hertz model fits two sections of load-indentation curves: approach and loading. During the approach, the load is zero while piezo is moving down towards the sample which is expressed as the negative indentation of the load-indentation curve. From the contact point, load start to increase as $F \sim h^{3/2}$ which is nonlinear relationship between the two variables and gives the specific shape to the load-indentation curves. During fitting, contact point (0 point in indentation graph) and effective Young's modulus is determined from the Hertz model[1] when fitting the initial load-indentation curve:

---

[1] Über die Berührung fester elastischer Körper, J. für die Reine Angewandte Math. (Crelle's J.) (2009), pp. 156-171, 1882

$$F = \frac{4}{3} E_{eff} \sqrt{R} \cdot h^{3/2}$$

$$E = E_{eff}(1 - \nu^2)$$

where F is load, R is the tip radius, h is indentation depth, ν is Poisson's ratio, E_{eff} is effective Young's modulus and E is Young's modulus which can be estimated when Poisson's ratio is known. If the fitting has failed, the wrong contact point will be drawn in the graph.

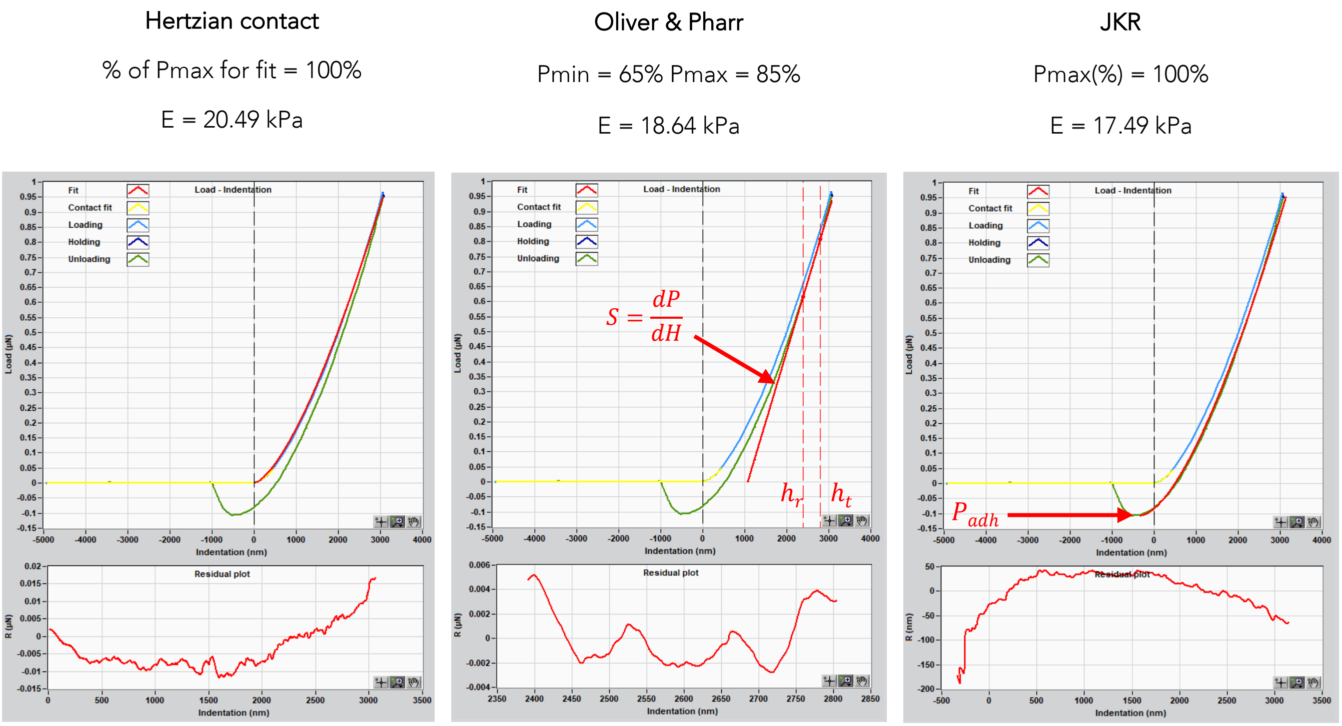
Fitting to Pmax(%)
Double fitting
Fitting to set load
Fitting to set indentation-depth

Oliver & Pharr model is used for elastic-plastic materials, where the initial unloading part in the load-indentation curve is assumed to be purely elastic and used to calculate Young's modulus (see Figure 10). The range used for fitting can be set as the minimum load $\boldsymbol{P_{min}}(\%)$ and maximum load $\boldsymbol{P_{max}}(\%)$. Typically used values are between 65% and 99%.

JKR model is used for elastic adhesive materials and allows a better estimation of Young's modulus in the presence of adhesion forces that changes the contact area. The unloading curve is fitted from the minimum load (negative) up to the maximum load which is set as $\boldsymbol{P_{max}}(\%)$.

Hertz for hemispherical samples: This model can be used to obtain the Youngs modulus of hemispherical samples with a known radius. This model also works for indentations beyond 8% strain.

For more information about the equations used, please check the appendix.



Hertzian contact

% of Pmax for fit = 100%

E = 20.49 kPa

Oliver & Pharr

Pmin = 65% Pmax = 85%

E = 18.64 kPa

JKR

Pmax(%) = 100%

E = 17.49 kPa

Figure 10: Example of Hertz, Oliver & Pharr and JKR fits.

3. **Poisson ratio**: Poisson ratio ν relates the effective Young's modulus and Young's modulus E:

$$E_{eff} = \frac{E}{(1-\nu^2)}$$

4. **Filter data:** Removes fitted data from the histogram and 3D plot based on a minimum R-squared value or when measurement started in contact.
5. **DAQ settings:** Shows data acquisition sampling rate in Hertz. For example, 100 Hz means there is 100 data points per second or each data point is obtained every 10 ms. DAQ settings can be changed in the Piuma software.
6. **Used mode**: Allows choosing which indentation mode was used (for data obtained with older versions of the software).
7. **Approach duration**: If the segmentation of the data is not correct in load-indentation curves, you can introduce here the time delay which shifts the segmented parts of the data.

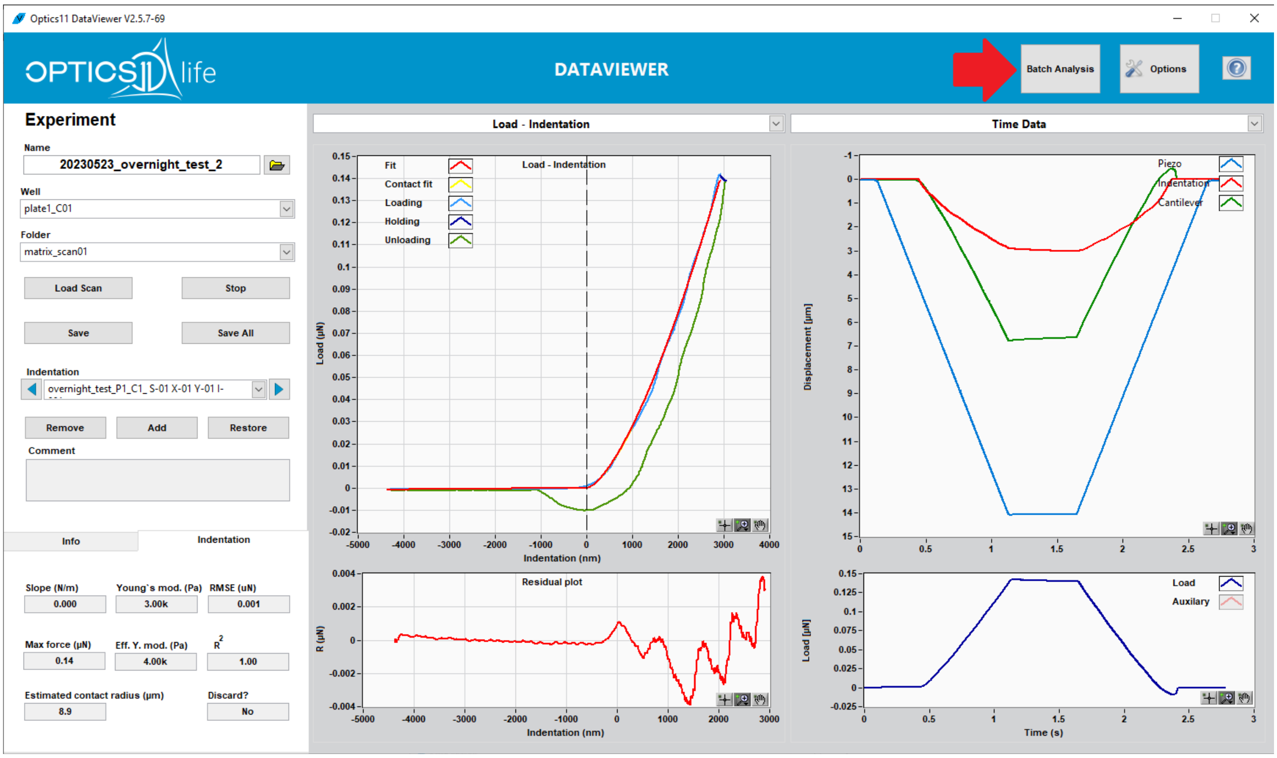
8. **Save location**: The path to the location where 3D graphs will be saved can be set here.

9. **Store load-displacement curves as .bmp.**

2.3. Batch analysis

The Batch Analysis is a feature allowing the user to pick a number of indentation files from various experiments and run a batch fitted by one of the available varieties of fitting models. The software processes all of the selected files in one go. The output contains both the file details and the fitting results, which will be saved as a result file in the selected directory. Moreover, the algorithm aims to identify failed indentations that are recommended to be discarded, such as for instance, out of contact or in contact measurements (flat lines or short baselines). For more information about how the Discard feature works, please see the Appendix.

1. To use the batch analysis feature, click on the "Batch Analysis" button next to the Options as shown by the red arrow on Figure 11.



Name: 20230523_overnight_test_2
Well: plate1_C01
Folder: matrix_scan01
Indentation: overnight_test_P1_C1_ S-01 X-01 Y-01 I-

Slope (N/m)	Young`s mod. (Pa)	RMSE (uN)
0.000	3.00k	0.001

Max force (μN)	Eff. Y. mod. (Pa)	R^2
0.14	4.00k	1.00

Estimated contact radius (μm)	Discard?
8.9	No

Figure 11: New GUI of the DataViewer v2.6.0 with the Batch Analysis button next to the Options.

2. The batch analysis menu will pop up. Click on "Add" to add indentation files.

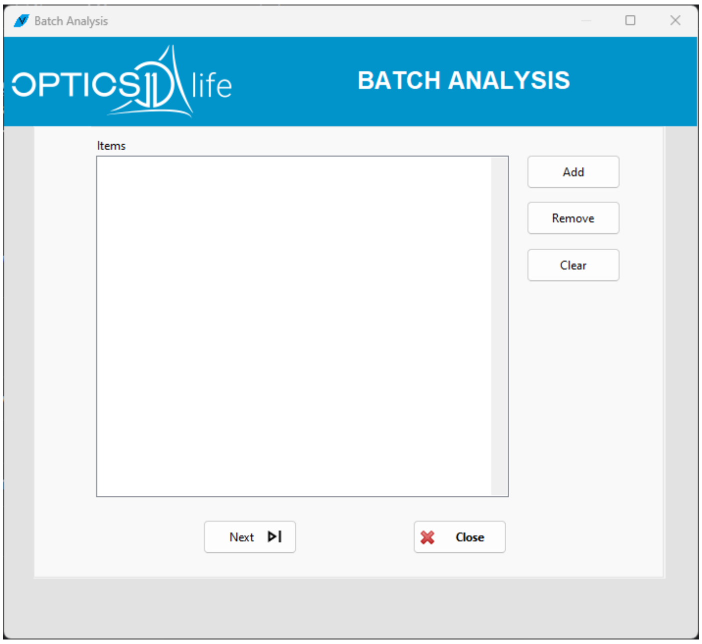
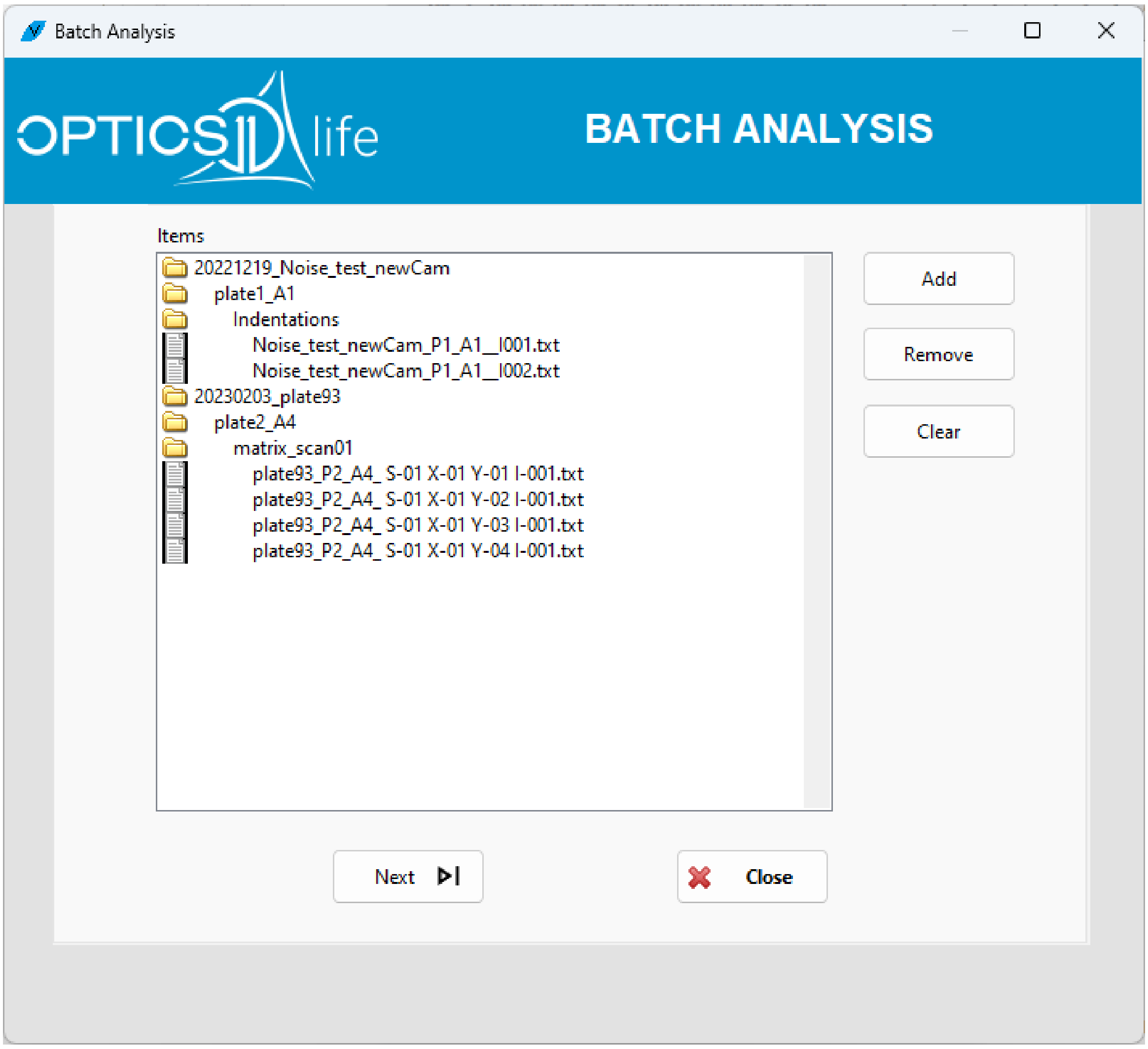


Figure 12: Batch analysis menu.

3. The folder/file selection window will open. Here the user can choose any file or folder containing measurement data. Once the selected files are added, the items box shows the files including their directory.

4. After selecting files and folders, click on the "Next'' button. The settings page will open.



20221219_Noise_test_newCam
plate1_A1
Indentations
Noise_test_newCam_P1_A1__I001.txt
Noise_test_newCam_P1_A1__I002.txt
20230203_plate93
plate2_A4
matrix_scan01
plate93_P2_A4_ S-01 X-01 Y-01 I-001.txt
plate93_P2_A4_ S-01 X-01 Y-02 I-001.txt
plate93_P2_A4_ S-01 X-01 Y-03 I-001.txt
plate93_P2_A4_ S-01 X-01 Y-04 I-001.txt

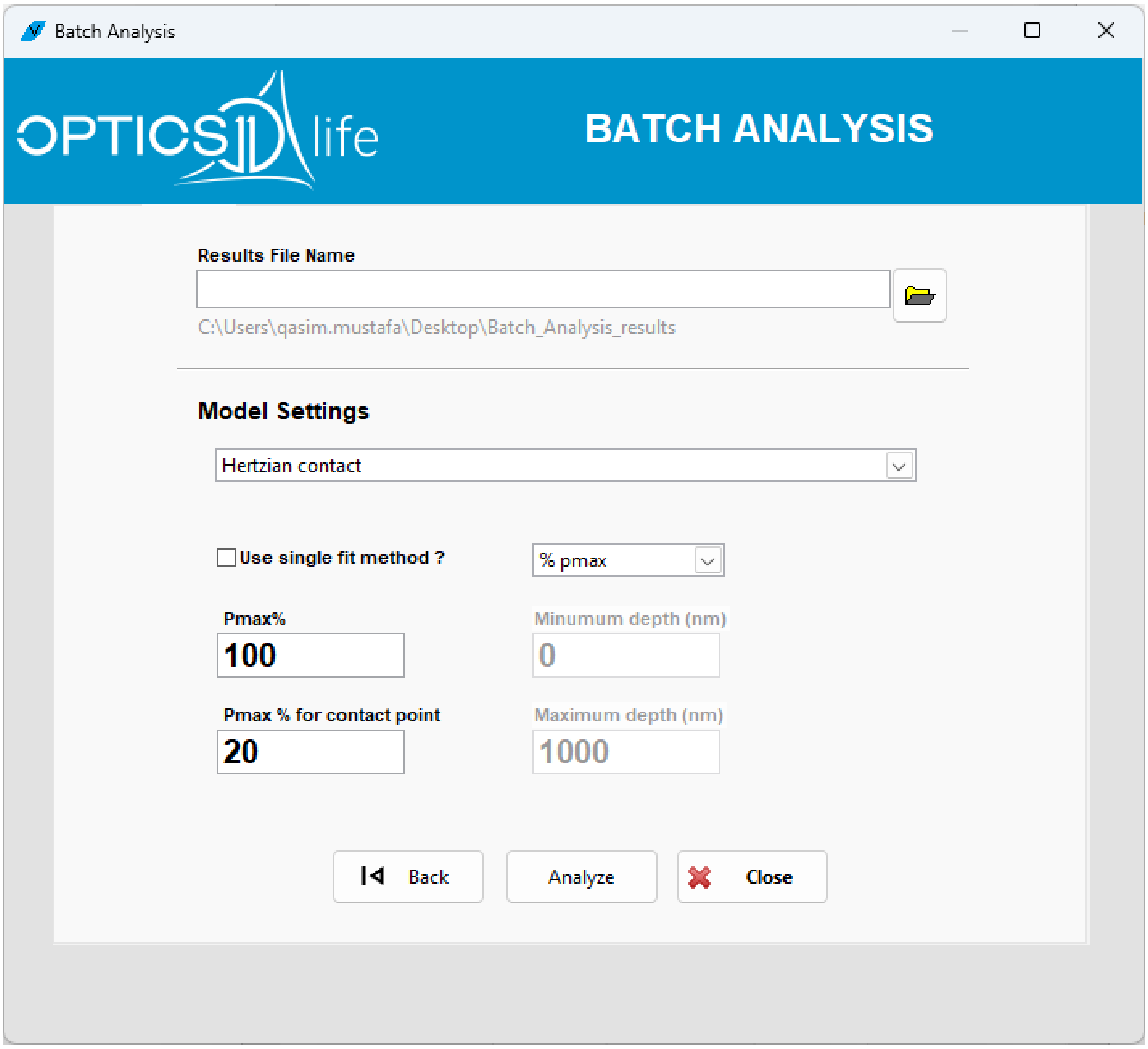
Figure 13: Folders containing the measurement data can be added, removed, or completely cleared.

5. In the Results File Name box, enter a name for the file containing the Batch analysis results.

6. Click on the folder icon beside the Results File Name box to select a save directory for the results file.
   Note: If a save path has not been selected, the software will take the following default save path: C:\Users\<user>\AppData\Local\DataViewerV2\logs\batch_analysis_results

7. Select the fitting model and parameters.

8. Click on the "Analyze" button.



BATCH ANALYSIS

Results File Name

C:\Users\qasim.mustafa\Desktop\Batch_Analysis_results

Model Settings

Hertzian contact

☐ Use single fit method ?

% pmax

Pmax%: 100

Minumum depth (nm): 0

Pmax % for contact point: 20

Maximum depth (nm): 1000

Figure 14: Parameters and model settings for the batch analysis.

9. Once the "Analyze" button is clicked, the software begins the batch analysis and a pop-up with a progress bar will show the progress of the analysis.
   Note: The ''Cancel'' button can be used at any moment during the analysis to stop the operation and return to the batch analysis menu.

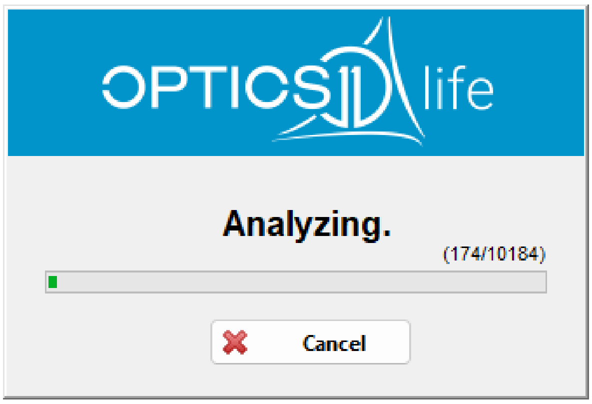
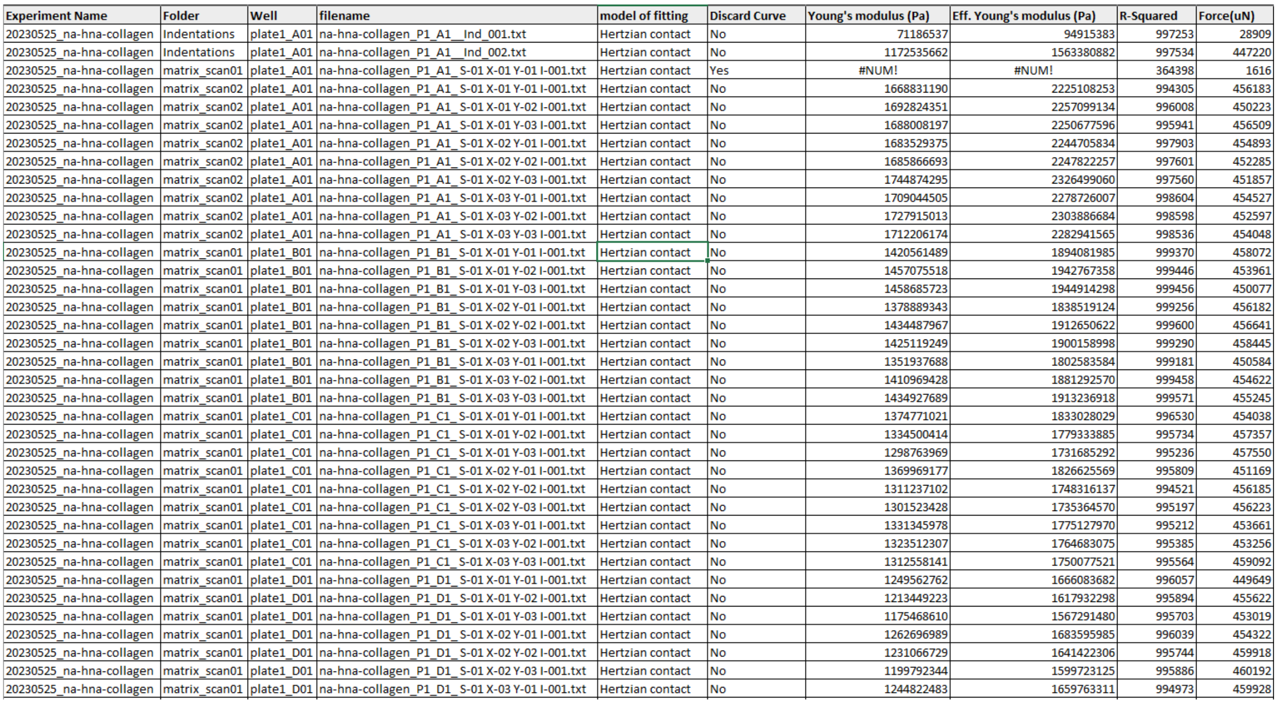


Figure 15: Loading menu for the Analysis. Clicking on Cancel will stop the operation and return to the batch analysis menu.

10. Once the analysis is complete, the software returns to the Main GUI. The "Indentation" drop-down list will now contain the indentations that were analyzed for easy screening and checking. The results file saved in the location chosen in step 5 or the default folder contains details such as the experiment name, the directory folder, well, and flag to indicate if the curve is discarded.



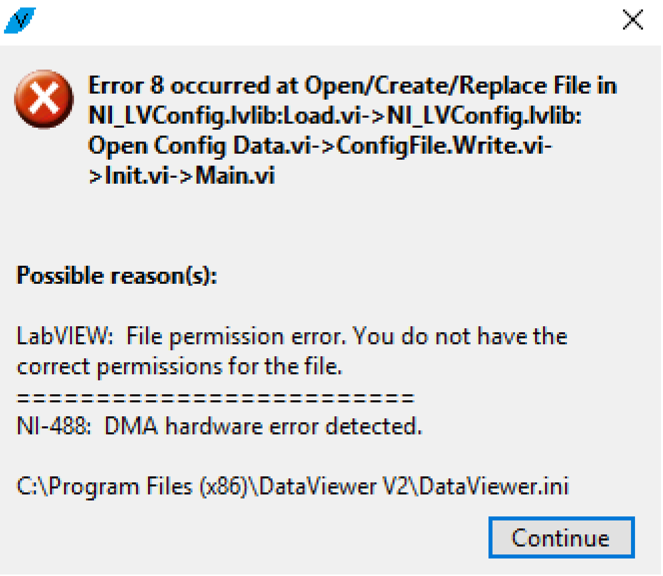
Experiment Name	Folder	Well	filename	model of fitting	Discard Curve	Young's modulus (Pa)	Eff. Young's modulus (Pa)	R-Squared	Force(uN)
20230525_na-hna-collagen	Indentations	plate1_A01	na-hna-collagen_P1_A1__Ind_001.txt	Hertzian contact	No	71186537	94915383	997253	28909
20230525_na-hna-collagen	Indentations	plate1_A01	na-hna-collagen_P1_A1__Ind_002.txt	Hertzian contact	No	1172535662	1563380882	997534	447220
20230525_na-hna-collagen	matrix_scan01	plate1_A01	na-hna-collagen_P1_A1_ S-01 X-01 Y-01 I-001.txt	Hertzian contact	Yes	#NUM!	#NUM!	364398	1616
20230525_na-hna-collagen	matrix_scan02	plate1_A01	na-hna-collagen_P1_A1_ S-01 X-01 Y-01 I-001.txt	Hertzian contact	No	1668831190	2225108253	994305	456183
20230525_na-hna-collagen	matrix_scan02	plate1_A01	na-hna-collagen_P1_A1_ S-01 X-01 Y-02 I-001.txt	Hertzian contact	No	1692824351	2257099134	996008	450223
20230525_na-hna-collagen	matrix_scan02	plate1_A01	na-hna-collagen_P1_A1_ S-01 X-01 Y-03 I-001.txt	Hertzian contact	No	1688008197	2250677596	995941	456509
20230525_na-hna-collagen	matrix_scan02	plate1_A01	na-hna-collagen_P1_A1_ S-01 X-02 Y-01 I-001.txt	Hertzian contact	No	1683529375	2244705834	997903	454893
20230525_na-hna-collagen	matrix_scan02	plate1_A01	na-hna-collagen_P1_A1_ S-01 X-02 Y-02 I-001.txt	Hertzian contact	No	1685866693	2247822257	997601	452285
20230525_na-hna-collagen	matrix_scan02	plate1_A01	na-hna-collagen_P1_A1_ S-01 X-02 Y-03 I-001.txt	Hertzian contact	No	1744874295	2326499060	997560	451857
20230525_na-hna-collagen	matrix_scan02	plate1_A01	na-hna-collagen_P1_A1_ S-01 X-03 Y-01 I-001.txt	Hertzian contact	No	1709044505	2278726007	998604	454527
20230525_na-hna-collagen	matrix_scan02	plate1_A01	na-hna-collagen_P1_A1_ S-01 X-03 Y-02 I-001.txt	Hertzian contact	No	1727915013	2303886684	998598	452597
20230525_na-hna-collagen	matrix_scan02	plate1_A01	na-hna-collagen_P1_A1_ S-01 X-03 Y-03 I-001.txt	Hertzian contact	No	1712206174	2282941565	998536	454048
20230525_na-hna-collagen	matrix_scan01	plate1_B01	na-hna-collagen_P1_B1_ S-01 X-01 Y-01 I-001.txt	Hertzian contact	No	1420561489	1894081985	999370	458072
20230525_na-hna-collagen	matrix_scan01	plate1_B01	na-hna-collagen_P1_B1_ S-01 X-01 Y-02 I-001.txt	Hertzian contact	No	1457075518	1942767358	999446	453961
20230525_na-hna-collagen	matrix_scan01	plate1_B01	na-hna-collagen_P1_B1_ S-01 X-01 Y-03 I-001.txt	Hertzian contact	No	1458685723	1944914298	999456	450077
20230525_na-hna-collagen	matrix_scan01	plate1_B01	na-hna-collagen_P1_B1_ S-01 X-02 Y-01 I-001.txt	Hertzian contact	No	1378889343	1838519124	999256	456182
20230525_na-hna-collagen	matrix_scan01	plate1_B01	na-hna-collagen_P1_B1_ S-01 X-02 Y-02 I-001.txt	Hertzian contact	No	1434487967	1912650622	999600	456641
20230525_na-hna-collagen	matrix_scan01	plate1_B01	na-hna-collagen_P1_B1_ S-01 X-02 Y-03 I-001.txt	Hertzian contact	No	1425119249	1900158998	999290	458445
20230525_na-hna-collagen	matrix_scan01	plate1_B01	na-hna-collagen_P1_B1_ S-01 X-03 Y-01 I-001.txt	Hertzian contact	No	1351937688	1802583584	999181	450584
20230525_na-hna-collagen	matrix_scan01	plate1_B01	na-hna-collagen_P1_B1_ S-01 X-03 Y-02 I-001.txt	Hertzian contact	No	1410969428	1881292570	999458	454622
20230525_na-hna-collagen	matrix_scan01	plate1_B01	na-hna-collagen_P1_B1_ S-01 X-03 Y-03 I-001.txt	Hertzian contact	No	1434927689	1913236918	999571	455245
20230525_na-hna-collagen	matrix_scan01	plate1_C01	na-hna-collagen_P1_C1_ S-01 X-01 Y-01 I-001.txt	Hertzian contact	No	1374771021	1833028029	996530	454038
20230525_na-hna-collagen	matrix_scan01	plate1_C01	na-hna-collagen_P1_C1_ S-01 X-01 Y-02 I-001.txt	Hertzian contact	No	1334500414	1779333885	995734	457357
20230525_na-hna-collagen	matrix_scan01	plate1_C01	na-hna-collagen_P1_C1_ S-01 X-01 Y-03 I-001.txt	Hertzian contact	No	1298763969	1731685292	995236	457550
20230525_na-hna-collagen	matrix_scan01	plate1_C01	na-hna-collagen_P1_C1_ S-01 X-02 Y-01 I-001.txt	Hertzian contact	No	1369969177	1826625569	995809	451169
20230525_na-hna-collagen	matrix_scan01	plate1_C01	na-hna-collagen_P1_C1_ S-01 X-02 Y-02 I-001.txt	Hertzian contact	No	1311237102	1748316137	994521	456185
20230525_na-hna-collagen	matrix_scan01	plate1_C01	na-hna-collagen_P1_C1_ S-01 X-02 Y-03 I-001.txt	Hertzian contact	No	1301523428	1735364570	995197	456223
20230525_na-hna-collagen	matrix_scan01	plate1_C01	na-hna-collagen_P1_C1_ S-01 X-03 Y-01 I-001.txt	Hertzian contact	No	1331345978	1775127970	995212	453661
20230525_na-hna-collagen	matrix_scan01	plate1_C01	na-hna-collagen_P1_C1_ S-01 X-03 Y-02 I-001.txt	Hertzian contact	No	1323512307	1764683075	995385	453256
20230525_na-hna-collagen	matrix_scan01	plate1_C01	na-hna-collagen_P1_C1_ S-01 X-03 Y-03 I-001.txt	Hertzian contact	No	1312558141	1750077521	995564	459092
20230525_na-hna-collagen	matrix_scan01	plate1_D01	na-hna-collagen_P1_D1_ S-01 X-01 Y-01 I-001.txt	Hertzian contact	No	1249562762	1666083682	996057	449649
20230525_na-hna-collagen	matrix_scan01	plate1_D01	na-hna-collagen_P1_D1_ S-01 X-01 Y-02 I-001.txt	Hertzian contact	No	1213449223	1617932298	995894	455622
20230525_na-hna-collagen	matrix_scan01	plate1_D01	na-hna-collagen_P1_D1_ S-01 X-01 Y-03 I-001.txt	Hertzian contact	No	1175468610	1567291480	995703	453019
20230525_na-hna-collagen	matrix_scan01	plate1_D01	na-hna-collagen_P1_D1_ S-01 X-02 Y-01 I-001.txt	Hertzian contact	No	1262696989	1683595985	996039	454322
20230525_na-hna-collagen	matrix_scan01	plate1_D01	na-hna-collagen_P1_D1_ S-01 X-02 Y-02 I-001.txt	Hertzian contact	No	1231066729	1641422306	995744	459918
20230525_na-hna-collagen	matrix_scan01	plate1_D01	na-hna-collagen_P1_D1_ S-01 X-02 Y-03 I-001.txt	Hertzian contact	No	1199792344	1599723125	995886	460192
20230525_na-hna-collagen	matrix_scan01	plate1_D01	na-hna-collagen_P1_D1_ S-01 X-03 Y-01 I-001.txt	Hertzian contact	No	1244822483	1659763311	994973	459928

Figure 16: Results file.

3. TROUBLESHOOTING

File permission error

The following error occurs.



Error 8 occurred at Open/Create/Replace File in NI_LVConfig.lvlib:Load.vi->NI_LVConfig.lvlib: Open Config Data.vi->ConfigFile.Write.vi->Init.vi->Main.vi

Possible reason(s):

LabVIEW: File permission error. You do not have the correct permissions for the file.
=========================
NI-488: DMA hardware error detected.

C:\Program Files (x86)\DataViewer V2\DataViewer.ini

This occurs when the software is not set to ‘Run as an Administrator’. See Figure 1, page 4.

APPENDIX

DMA theory

DMA results are obtained by fitting cosine function over oscillations at each frequency which gives amplitudes h_0 and F_0, frequency and phases. From there, phase difference δ is calculated between oscillations in load and in indentations. The frequency-dependent storage modulus, E', and loss modulus, E'', which represent elastic and viscous components respectively, are calculated with the following equations[2]:

$$E'(f) = \frac{1}{2}\frac{F_0}{h_0}\cos(\delta)\frac{(1-\nu^2)}{\sqrt{hR}}$$

$$E''(f) = \frac{1}{2}\frac{F_0}{h_0}\sin(\delta)\frac{(1-\nu^2)}{\sqrt{hR}}$$

$$\tan(\delta) = \frac{E''}{E'}$$

where δ is the phase lag between oscillations of indentation and load (see Figure 17). Tan(δ) is the dissipation (damping) factor which is the ratio between loss and storage modulus. Materials with a higher damping ratio than 1 are considered to be viscoelastic fluids and below 1, viscoelastic solids. Also, viscoelastic solids have a higher storage modulus than loss modulus at low frequencies which switches at higher frequencies while it is the other way around for viscoelastic fluids. Complex modulus can be calculated according to this formula:

$$E^* = \sqrt{E'^2 + E''^2}$$

If the amplitude of the input oscillation is small enough, the measurements can be considered within the material linear viscoelastic region (LVR). Within the LVR, the response is assumed to be independent of the input amplitude and sinusoidal. Furthermore, when selecting the amplitude of oscillations, the depth should be considered due to the curvature of the sphere, as it is assumed that the contact area does not change during oscillations, so that $\frac{h_0}{h} \ll 0.25$. The performance of the DMA can be evaluated in the DataViewer by comparing the amplitude which was measured with the amplitude which was set in the experiment and by looking at the R-squared values of cosine fits.

---

[2] E.G. Herbert, W.C. Oliver, G.M. Pharr, Nanoindentation and the dynamic characterization of viscoelastic solids, J. Phys. D Appl. Phys., 41 (2008), 074021

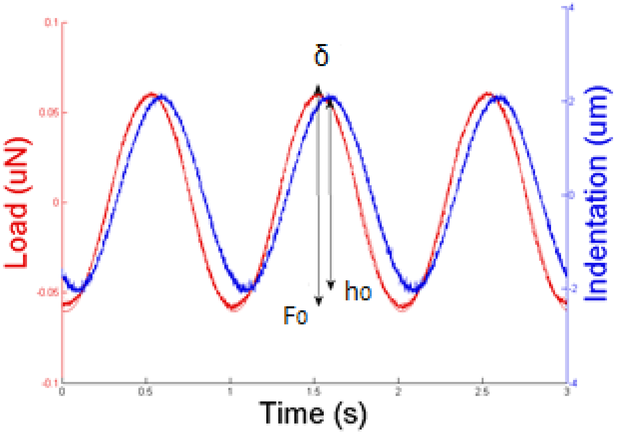


Figure 17: Sinusoidal stress is applied, and the reaction of the material is measured. Viscoelastic material materials show a phase shift δ.

Quasi-static indentation data fitting

Hertz model is used to extract Young's modulus for linear elastic materials. Furthermore, the material is assumed to be infinite half-space (flat and thick), homogenous, isotropic, and non-adhesive. However, due to its simplicity, it is also commonly used to obtain contact point and extract Young's modulus from complex viscoelastic and nonlinear materials with some limitations. Hertz model fits two sections of load-indentation curves: approach and loading. During the approach, the load is zero while piezo is moving down towards the sample which is expressed as the negative indentation of the load-indentation curve. From the contact point, load start to increase as $F{\sim}h^{3/2}$ which is nonlinear relationship between the two variables and gives the specific shape to the load-indentation curves. During fitting, contact point (0 point in indentation graph) and effective Young's modulus is determined from the Hertz model[3] when fitting the initial load-indentation curve:

$$F = \frac{4}{3} E_{eff} \sqrt{R} \cdot h^{3/2}$$

$$E = E_{eff}(1 - \nu^2)$$

---

[3] Über die Berührung fester elastischer Körper, J. für die Reine Angewandte Math. (Crelle's J.) (2009), pp. 156-171, 1882

where F is load, R is the tip radius, h is indentation depth, ν is Poisson's ratio, E_{eff} is effective Young's modulus and E is Young's modulus which can be estimated when Poisson's ratio is known. If the fitting has failed, the wrong contact point will be drawn in the graph.

Fitting to Pmax(%)
Double fitting
Fitting to set load
Fitting to set indentation-depth

Oliver & Pharr model is used for elastic-plastic materials, where the initial unloading part in the load-indentation curve is assumed to be purely elastic and used to calculate Young's modulus according to this equation (see Figure 10):

$$E_{eff} = \frac{E}{1-\nu^2} = \frac{dP}{dh}\frac{1}{2\sqrt{R(h_t + h_r)}}$$

h_t is the total indentation depth, h_r – residual indentation depth, $\frac{dP}{dh} = S$ is slope with dP – load difference, dh - indentation depth difference. The range used for fitting can be set as the minimum load $\boldsymbol{P_{min}}(\%)$ and maximum load $\boldsymbol{P_{max}}(\%)$. Typically used values are between 65% and 99%.

JKR model is used for elastic adhesive materials and allows a better estimation of Young's modulus in the presence of adhesion forces which changes the contact area. The equation used for the fitting:

$$h - h_0 = \frac{a^2{}_0}{R}\left(\frac{1+\sqrt{1-\frac{P}{Padh}}}{2}\right)^{\frac{4}{3}} - \frac{2}{3}\frac{a^2{}_0}{R}\left(\frac{1+\sqrt{1-\frac{P}{Padh}}}{2}\right)^{\frac{1}{3}}$$

$$P_{adh} = -\frac{3}{2}\pi\Delta\gamma R$$

$$a_0^3 = \frac{9\pi R^2\Delta\gamma}{2E_{eff}}$$

where h - indentation depth, h_0 - the contact point, a_0 - the contact radius at zero load, R - the tip radius of the indenter, P- the load and P_{adh} is the pull-off force (minimum load), $\Delta\gamma$ – work of adhesion. h_0 and a_0 are fitting parameters[4].

---

[4] Ebenstein, D. M., & Wahl, K. J. (2006). A comparison of JKR-based methods to analyze quasi-static and dynamic indentation force curves. *Journal of colloid and interface science*, *298*(2), 652-662).

The unloading curve is fitted from the minimum load (negative) up to the maximum load which is set as $\boldsymbol{P_{max}}(\%)$.

Hertz for hemispherical samples: This model can be used to obtain the Youngs modulus of hemispherical samples with a known radius. This model also works for indentations beyond 8% strain. The equation used for the fitting:

$$P = \frac{4}{3} E_{eff} R^{\frac{1}{2}} h^{\frac{3}{2}} (1 + a\frac{h}{R})$$

$$\frac{1}{R} = \frac{1}{R_s} + \frac{1}{R_i}$$

$$a = 1.1e^{(-2.5/\beta)} + 0.55e^{(-0.4/\beta)} - 0.15$$

$$\beta = \frac{R_i}{R_s}$$

Where Ri - the tip radius of the indenter, Rs - the radius of the sample, R – the equivalent radius, $\boldsymbol{a}$ – the correction factor for large strains, h – indentation depth, P- the load. E_{eff} is the fitting parameter[5].

Discard Curve feature

In the Batch Analysis feature, the "Discard Curve" algorithm is used to recommend the user to discard an indentation curve if the quality of the acquired data suggests a failed indentation. The Discard Curve feature is a fully automatic feature that helps the user to evaluate the output data. You can find the Discard Curve recommendation in the Indentation tab (see Figure 18).

Note: The algorithm aims to identify failed indentations that are recommended to be discarded; see *the table below for possible causes of rejection.*

---

[5] Ding, Y., Xu, G. K., & Wang, G. F. (2017). On the determination of elastic modulus of cells by AFM based indentation. *Scientific reports, 7, 45575.*

Experiment

Name: 20230523_overnight_test_2

Well: plate1_C01

Folder: matrix_scan01

Indentation: overnight_test_P1_C1_S-01 X-01 Y-01 I-

Comment:

Slope (N/m): 0.000

Young's mod. (Pa): 3.00k

RMSE (uN): 0.001

Max force (µN): 0.14

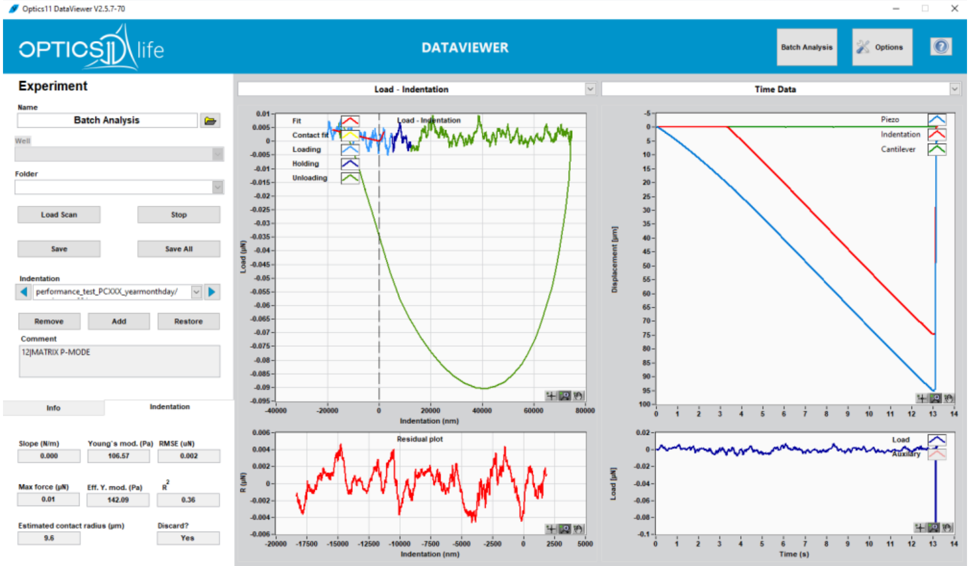
Eff. Y. mod. (Pa): 4.00k

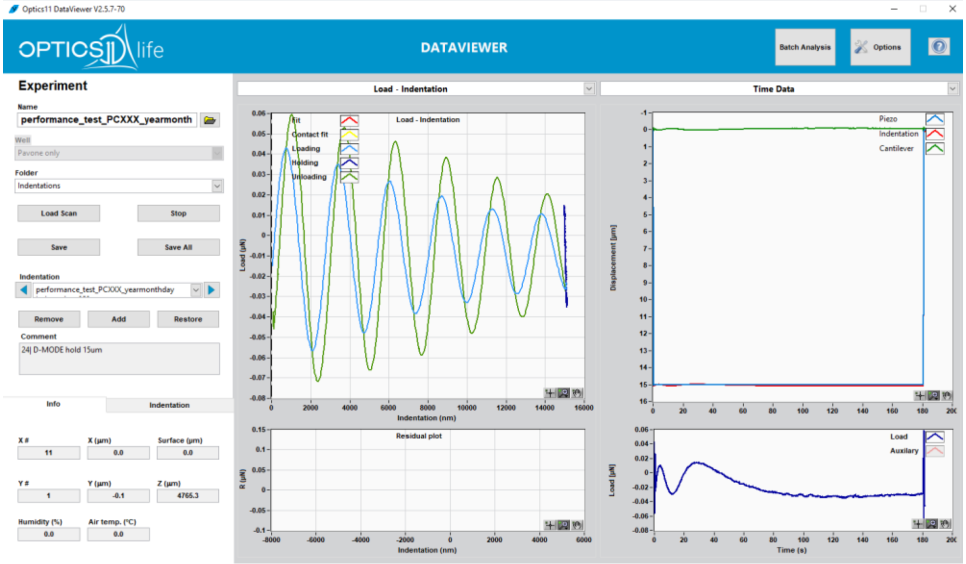
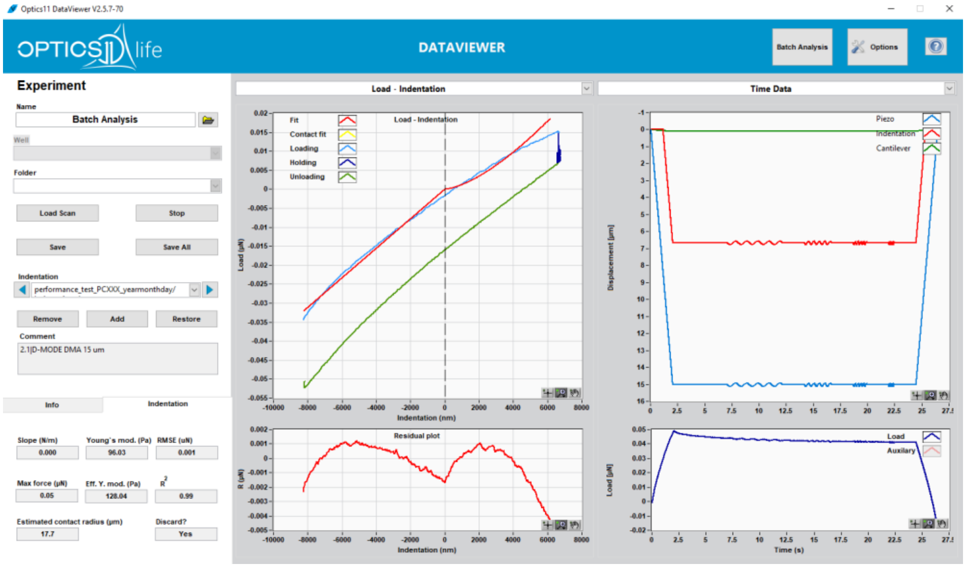
R^2: 1.00

Estimated contact radius (µm): 8.9

Discard?: No

Figure 18: Discard options is indicated on the bottom left part of the GUI.

Artifact	Possible cause(s)
Flat line	The probe is out of contact  Optics11 DataViewer V2.5.7-70 **Experiment** Name: Batch Analysis Well: Folder: Indentation: performance_test_PCXXX_yearmonthday/ Comment: 12|MATRIX P-MODE Slope (N/m): 0.000 Young's mod. (Pa): 106.57 RMSE (uN): 0.002 Max force (µN): 0.01 Eff. Y. mod. (Pa): 142.09 R^2: 0.36 Estimated contact radius (µm): 9.6 Discard?: Yes

Oscillations	PID tuning issues of probe stage  Optics11 DataViewer V2.5.7-70 Name: performance_test_PCXXX_yearmonth Well: Pavone only Folder: Indentations Indentation: performance_test_PCXXX_yearmonthday Comment: 24| D-MODE hold 15um X #: 11 X (µm): 0.0 Surface (µm): 0.0 Y #: 1 Y (µm): -0.1 Z (µm): 4765.3 Humidity (%): 0.0 Air temp. (°C): 0.0
Short baseline	Dirt on the probe, probe not raised high enough, irregularities or extensive roughness of sample surface.  Optics11 DataViewer V2.5.7-70 Name: Batch Analysis Indentation: performance_test_PCXXX_yearmonthday/ Comment: 2.1|D-MODE DMA 15 um Slope (N/m): 0.000 Young's mod. (Pa): 96.03 RMSE (uN): 0.001 Max force (µN): 0.05 Eff. Y. mod. (Pa): 128.04 R^2: 0.99 Estimated contact radius (µm): 17.7 Discard?: Yes

Note: The algorithm has a recall of > 90% (i.e. <10% false negatives) and a precision of > 80% (i.e. <20% of selected items are false positives).

REVISION HISTORY

Version 0.1	New document	E. Labordus	08 Feb 2018
Version 0.2	Added sections	E. Labordus	20 Feb 2018
Version 0.3	Written for V2.1.11	N. Antonovaite	06 Dec 2019
Version 0.3.1	Updates	N. Born	27 Feb 2020
Version 1.0	Updated for V2.4.0	J. Nieuwenhuis	07 Oct 2020
Version 1.1	Updated for V2.5.0	J. Nieuwenhuis	27 Oct 2021
Version 1.2	Data fitting standards	N. Antonovaite	01 April 2022
Version 1.3	Updated for V2.6.0	S. Fontaine	19 Jul 2023