**Installation**

Note: ALIAS 2.4 has been tested only on macOS 12 and Python 3.9, compatibility issues may be present using other operating systems or Python versions. For best compatibility, it is recommended to run the ALIAS application using the latest version of Anaconda as the Python distribution, and PyCharm (JetBrains) as the integrated development environment.

1. Install latest version of Anaconda Python distribution (<https://www.anaconda.com/products/distribution>)
2. Install latest community version of PyCharm (<https://www.jetbrains.com/pycharm/download>)
3. Download ALIAS 2.4 as a ZIP file from GitHub (<https://github.com/PierangeloGobbo1986/ALIAS>) and extract ALIAS2.4.py to a suitable location
4. Open ALIAS2.4.py using PyCharm (select a suitable distribution of Python if necessary and allow all necessary packages to be downloaded)
5. Run ‘ALIAS2.4’

**Operation**

Note: ALIAS 2.4 has been developed specifically for the data format exported by an FT-MTA03 indenter. A sample folder is provided of a example dataset from this instrument. If data from another instrument is used, code modification will be needed depending on the exact format of data input.

*Data organization:*

1. Click ‘Extract Files’ under ‘Extract and reorganize original data’
2. Select folder containing ‘data.txt’ files (each in their own subfolder) - This function will save a copy of the original data in a separate folder and new txt files with the experiment names

*Analysing individual indentation measurements:*

1. Click ‘Find curves’ under ‘Analysis of individual indentation curves’
2. Select the experiment txt file you wish to analyse – This function will divide each measurement within the file into individual txt files
3. Click ‘Txt to Csv and plot curves’
4. Select the folder containing the individual measurement txt files to be analysed – This function will plot a force-displacement curve for each file and convert the data to csv
5. Input Probe’s diameter (in µm)
6. Input Sample’s Poisson’s ratio
7. Input ‘Baseline data points’ (fraction of data which will be used to determine a baseline – a good starting value is 0.125)
8. Input ‘Contact point threshold constant’ (multiple of baseline datapoints standard deviation which will be used to determine the contact point – a good starting value is 10)
9. Input ‘Datapoints per fitting segment’ for loading/unloading data (the number of individual datapoints per fitting segment used by the error-minimisation algorithm – a good starting range is 10-20)
10. Select a fitting model using the drop-down menu
11. Click ‘Fit curves!’
12. Select all the csv files to be analysed – This function will apply the data processing method (baseline subtraction and contact point determination) then fit the data to the chosen model
13. Results obtained from fitting will be displayed in the dialog window and can be saved as a txt file using the ‘Save report as \*.txt file’ button
14. Plots showing the fitting for each curve are saved in a new ‘Fitting results’ folder with a csv file for each measurement containing all data and fitting results

*Analysing arrays of indentation measurements:*

1. Click ‘Find arrays’ under ‘Analysis of an array of indentation curves’
2. Select the experiment txt file you wish to analyse – This function will divide each array measurement within the file into individual txt files
3. Click ‘Txt to Csv and plot curves’
4. Select the txt file containing the array measurement to be analysed – This function will plot a force-displacement curve for each measurement within the array and convert the data to csv files
5. Input Probe’s diameter (in µm)
6. Input Sample’s Poisson’s ratio
7. Input ‘Baseline data points’ (fraction of data which will be used to determine a baseline – a good starting value is 0.125)
8. Input ‘Contact point threshold constant’ (multiple of baseline datapoints standard deviation which will be used to determine the contact point – a good starting value is 10)
9. Input ‘Datapoints per fitting segment’ for loading/unloading data (the number of individual datapoints per fitting segment used by the error-minimisation algorithm – a good starting range is 10-20)
10. Select a fitting model using the drop-down menu
11. Click ‘Generate 3D maps!’
12. Select all the csv files to be analysed – This function will apply the data processing method (baseline subtraction and contact point determination) then fit the data to the chosen model
13. Results obtained from fitting will be displayed in the dialog window and can be saved as a txt file using the ‘Save report as \*.txt file’ button
14. Plots showing the fitting for each curve are saved in a new ‘Fitting results’ folder with a csv file for each measurement containing all data and fitting results
15. Plots showing the spatial mapping of derived properties and a summary of all output data with X/Y coordinates are saved in a new ‘3D Plots’ folder