Chiaro Nanoindentation and Data Analysis V.1

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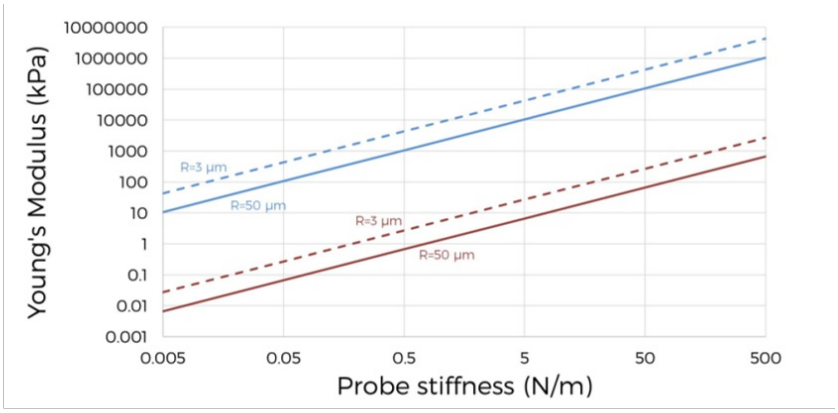
Set-up and choosing the tip

1 Make sure everything is ON: Turn on the laptop first (1) and the equipment afterwards (2) and (3). Finally, turn on the EVOS microscope.

2 To choose the right tip, refer to the advised stiffness range table below (Table1).



Sample Young's modulus	Advised cantilever stiffness range
10 Pa – 10 kPa	0.05 N/m
1 kPa – 500 kPa	0.5 N/m
500 kPa – 10 MPa	5 N/m
10 MPa – 100 MPa	50 N/m
100 MPa – 1 GPa	500 N/m

Table 1: Advised stiffness ranges for expected Young's Moduli.

3 Insert tip first into the tip holder without placing any sample under the cantilever (make sure it does not contact anything, the tip is extremely sensitive).

4 Centre the probe into the optical axis (so that you can see it in the screen) Start software in laptop: **PIUMA v2**

Using the system

5 The interface might ask 'do you want to centre the stage?', answer yes if you want to get your x,y and z values = 0. Keep in mind that clicking '*yes*' will maybe move the arm in ways you do not expect. Therefore, I recommend clicking '*no*' and sticking to careful and gentle manual movements (done by slightly unscrewing the arm).

6 Calibrate the tip into the same solution the sample you are trying to measure is in i.e. if trying to indent hydrogels in a glass slide in a petri dish with PBS, add PBS to a petri dish with a glass slide on it and use this as a calibration sample.

7 To place the tip in the sample sometimes it is easier (faster) to manually approach the tip to the sample, and once very close **add a few drops of the sample solution** to the tip (using a Pasteur Pipette) to help it connect to the liquid surface; if many bubbles are forming around the tip, a few drops of 70% EtOH might also help this step.

8 Finally, to save your measurements, make sure you have the right folder selected. The programme will save your measurements automatically, just ensure each sample's name is explicit.

Calibration

9 Click on the '**Configure Probe'** Option. Here, you should include the stiffness of the probe **K (N/m)**, the tip radius **(µm)** and the calibration factor, which should be the same as the **Geo Factor** seen on the tip box.

10 Then, click '**Calib**' on the main interface, which will open the '**Calibration Menu'**

11 To start the calibration, click '**Scan OP1550 Wavelength'**; this should provide a new calibration factor that accounts for the solution the sample is placed in. This new factor should not greatly vary from the previously input value (± 50% of the previous value).

12 Next, click **'Find the Surface'**. The system lowers the tip a few µm at a time (you can input how much you wish to move the probe using the stage controls). This will take a few seconds or minutes depending on the distance and will put the probe in contact with the surface it plans to indent.

13 Finaly, you can click **'Calibrate'**, when the tip has found a surface. Let the system go up and down and calibrate.
To ensure the calibration was successful you can check the ***'Demodulation'*** window in the interferometer (instrument no. 2). This should show a wave that follows the shape of the red circle. Furthermore, the wavelength should show a sinusoidal wave in the '***Wavelength*** 'menu.

Measuring your sample

14 Now, change the sample. Every time a new sample is placed, be careful to not touch the tip and move the arm up (do this manually as it will save a lot of time – but with caution!). Anytime you start a new measurement, click '**use x and y**'; if you do not do this, the system assumes a random x and y and moves to random points when activated.

15 Click '**Find surface'** again, so it finds the new sample.

16 Click '**Indent**' to indent the sample. You can see the load-displacement curve on the '**Load indentation**' tab as well as the signal monitor. These should indicate whether your indentation was successful. If the resulting curve does not resemble the curve in Figure 1, then lift the tip from the

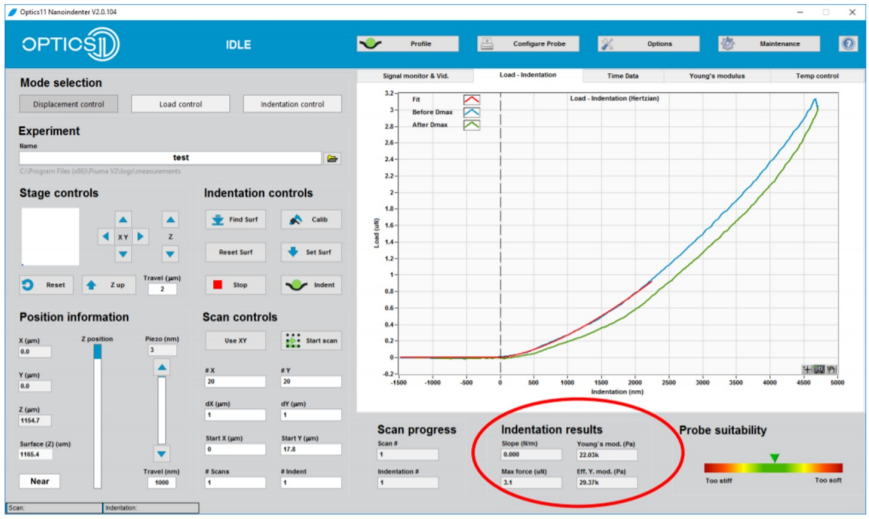


Figure 1

17 Now, if you want to automate the various measurements you want to make, you can choose a measurement map using the **'Scan Controls'** interface. To start, find the surface and determine the step lenght in each direction and the number of indetations you intend to perform. i.e. for MSC's single cell measurements, 10 cells in each condition were measured. On each cell, 9 indentations were performed between a 'square' of 3x3 µm (dX and dY).

18 Each scan will create its own folder of results with a .txt file for each indentation. On the software you will also be able to save a histogram of the various indentations along with a Young's Modulus 3D map. You can save any of these, although data analysis is recommended for showing more accurate results.

Finishing and Cleaning the equipment 10m

19 Once you are done, soak the tip in 70% EtOH for 10 min. 10m

20 Save all your data, turn off all the instruments and once the tip is clean in EtOH, place it back in its box carefully, as it was before use.

Data Analysis

21 To analyse the data you obtain with the Chiaro equipment we use the following software created by Dr. Massimo Vassalli (University of Glasgow).

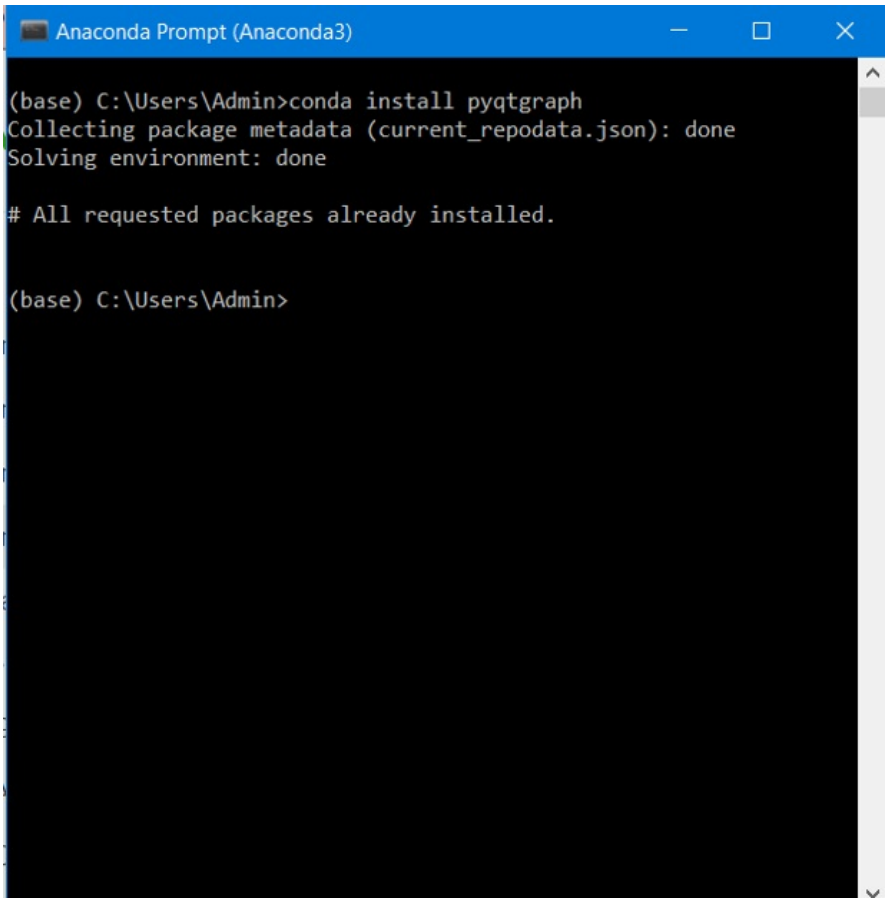
Available to download on GitHub.

22 No installer is necessary for obtaining the programme, but the following are needed to open it:
a Python 3 environment with the following packages: **PyQt5, numpy, scipy** and **pyqtgraph**.

23 For example, you can run Anaconda and open the Spyder Environment (included in the Anaconda package)

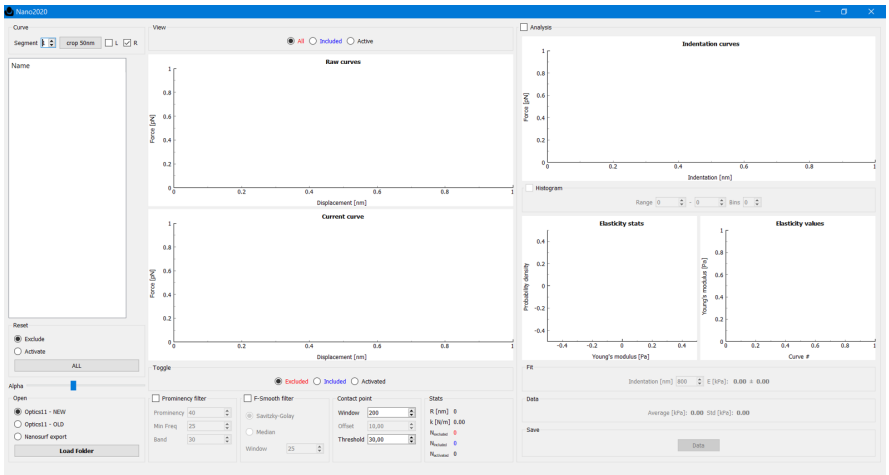
To add pyqtgraph open '**Anaconda promp command line**' and type *'conda install pyqtgraph'*; this will add the

package in a few seconds. Below is an example of this and what happens when the package is already installed.



24 In Spyder (Python 3.7) you can run the file '**nano.py**' (To run nano.py, you should open the downloaded folder in the Spyder environment and run the file from there).

This will open the following user interface **(nano2020)**:



25 Load the .txt files or folders to the '**Name**' tab in order to visualise the nanoindentation curves. You can exclude any outliers and personalise the parameters of your analysis here.