SUPPLEMENTARY INFORMATION

Theoretical basis for Oliver-Pharr-based Matlab code

The script extracts peak indenter force, peak indenter displacement, and the slope of the unloading curve, and calculates the reduced modulus (E_r) according to the following equation:

$$S = \beta \cdot \frac{2}{\pi^{1/2}} \cdot E_r \cdot A^{1/2}$$

The stiffness (*S)* is calculated from the slope (dP/d*h*) of the upper portion of the retract curve (also known as contact stiffness). *A* is the contact area and β is a variable that takes into account non-axisymmetry of the indenter and large strains. Being close to unity, β has only a small influence compared to the overall experimental inaccuracy, and is therefore neglected in our model. However, *A* is calculated as:

$$A = \pi(2Rh_c - h_c^2)$$

Where *R* is the tip radius. $h_c = h_{max} - h_s$ where h_{max} is the maximum displacement (indentation depth), and $h_s = \epsilon \left(\frac{P_{max}}{S}\right)$ where P_{max} is the peak load and *S* is the slope of the retract curve (Fig. 4). In the script, epsilon (ϵ) is assumed as 0.75 which applies for spherical indenters.

The Young's modulus is then calculated from the reduced modulus (E_r) using the materials Poisson's ratio and assuming no deformation of the indenter. Currently the default is set to a Poisson's ratio *(v)* of 0.5 (although this can be adjusted). The Young's modulus (E) is then calculated from the following equation:

$$E = E_r \cdot (1 - \nu^2)$$

<u>Instructions for the code</u>

Program: Standard Matlab 2019a installation
– Make sure the Curve Fitting toolbox addon is installed

Code was developed on a Mac OS Sierra 10.12

System requirements for Matlab2019a ([Details for Windows listed here]()**):**

Operating systems
- macOS Mojave (10.14)
- macOS High Sierra (10.13)
- macOS Sierra (10.12)

Processors:
- **Minimum**: Any Intel x86-64 processor
- **Recommended:** Any Intel x86-64 processor with four logical cores and AVX2 instruction set support

Disk:
- **Minimum:** 3.3 GB of HDD space for MATLAB only, 5-8 GB for a typical installation
- **Recommended**: An SSD is recommended - A full installation of all MathWorks products may take up to 30 GB of disk space

Ram:
- **Minimum:** 4 GB
- **Recommended:** 8 GB For Polyspace, 4 GB per core is recommended

Graphics:
No specific graphics card is required.

Installation guidelines:
https://www.mathworks.com/help/install/

Instructions for user:

1) Ensure Matlab scripts (Oliver_and_Pharr_model.m and Contact_point.m) are located in the same location as the folder containing the force curves.
2) Within the Oliver_and_Pharr_model.m script
   a. Set ‘Number of Curves’ for the number of curves you want to process.
   b. Choose the percentage of the retract curve to take to calculate the slope (to start with we recommend using 25%).
   c. Ensure the ‘Starting row’ matches the row in the txt files where the data starts (This is usually 79).
   d. Ensure the ‘Folder’ name (e.g. Raw_curves) matches up with the folder where your extend and retract txt files are located.
   e. Set the bead radius ‘R’ (e.g. $5e^{-6}$ would be for a 10μm bead).

f. Set the Poisson's ratio 'V' (For hydrogel experiments we have used 0.5).

3) Within the Contact_point.m function, the differential difference used to identify the contact point can also be adjusted to suit the user's needs.

4) Click Run on the Oliver_and_Pharr_model.m script. This should generate 5 figures (*Extend curves, Retract curves, Full retract curve (Fmax-Fmin*), *Retract sectioned* and *Indentation depth*

5) At the end, an Excel sheet will be generated ('Youngs_modulus.cvs') which will contain all your calculated E values. An Excel sheet of the reduced modulus is also calculated which does not take into account the Poisson's ratio ('Reduced_modulus.cvs')

Note: Script should take ~ 2-5 minutes to run depending on the computer's processing power and how many curves you are processing.