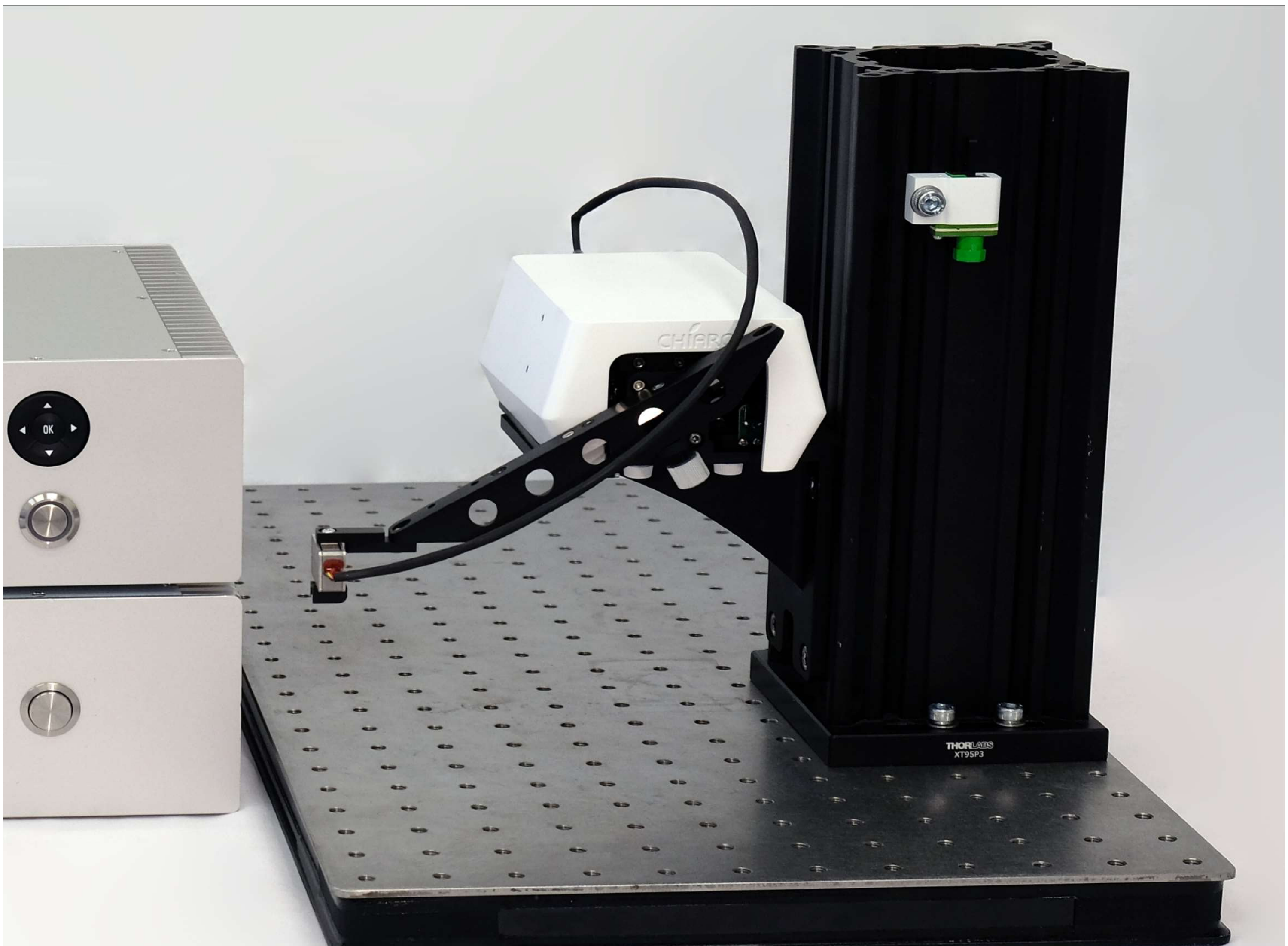
CHIARO USER MANUAL



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SAFETY, WARRANTY, AND LIABILITY

Safety

The OP1550 interferometer, part of the Chiaro Nanoindenter, is equipped with a class 1M laser. The laser light is coupled out via a fiber connector on the front panel and has a terminator on the back panel. Therefore, do not view directly into the beam with optical instruments.

The OP1550 is equipped with a 220V/110V plug. Disconnect the instrument before changing the fuse or switching from 220V to 110V (or vice versa). Do not open the box, as this might result in serious injuries.

Warranty

All of the electrical and mechanical parts of the Piuma or Chiaro Nanoindenter instruments as manufactured and installed by Optics11 B.V. are guaranteed to be free of defects for one year from the installation date. Within this period Optics11 B.V. will repair or replace the components at no charge.
All components included in the warranty are the Piuma or Chiaro indenter head, the controller box, the interferometer, and the laptop. Software updates are also part of the warranty. Probes and/or other consumables are not included in this warranty unless they are damaged during the shipment. After this period it is possible to extend your warranty by a desired amount of years.

Limitations of warranty

A warranty covers our products for regular use and does not cover the following types of damage to the components:

- (Internal) water damage by mishandling of the system.
- Improper operational damage or accident-related damage.
- Damage after repair services by third parties.

When a technical defect is present our support service team will inspect the origin of the fault. This inspection will be in the form of a (remote) appointment. When a repair is needed, repair costs will be charged accordingly. For more information about warranty and repair services, contact us.

INTRODUCTION

This document describes the installation and operation of the Chiaro Nanoindenter instrument, as developed by Optics11. Chapter 1 provides details of the Chiaro Nanoindenter installation. Chapter 2 describes the preparation of the Chiaro Nanoindenter for experiments. Chapter 3 explains in detail how to perform a measurement. Chapter 4 describes the optimization of sample preparation and measurement conditions. Chapter 5 deals with Chiaro Nanoindenter's working principle and software suite. . Chapter 6 explains how to operate the OP1550 interferometer. Finally, Chapter 7 provides a FAQ and alternative options for support.

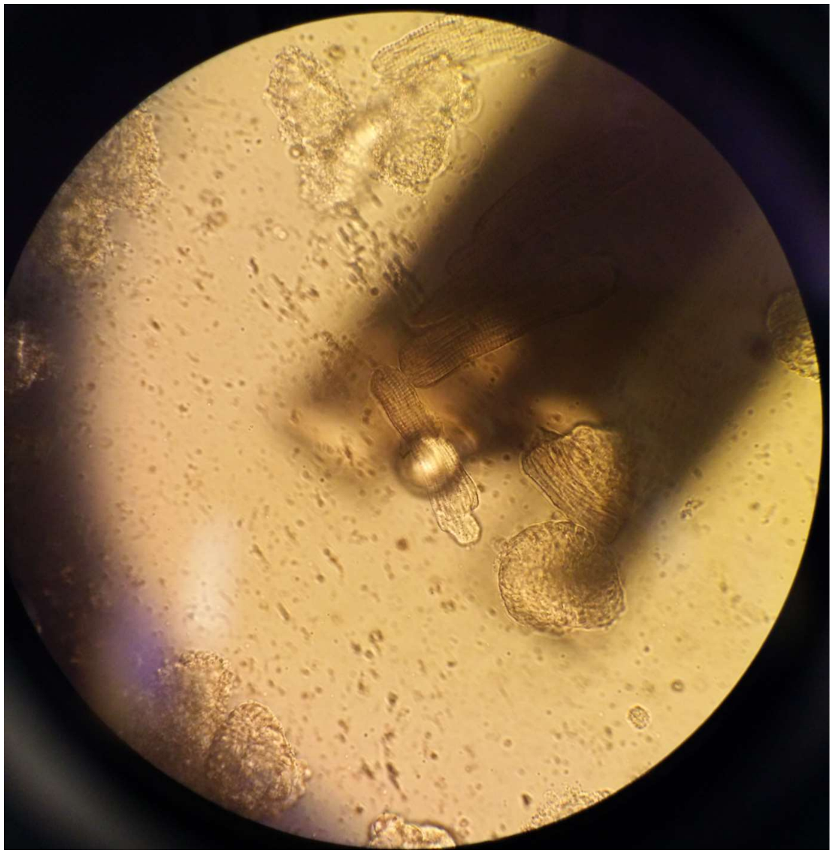

Figure 1: The Chiaro Nanoindenter in action: measuring adult rat cardiomyocytes.

1. INSTALLATION

This section describes the installation of the Chiaro Nanoindenter by providing an overview of the Chiaro Nanoindenter and its components, the considerations for finding a location to set up the system, the wiring scheme for connecting the individual parts, and the actions required to start up the Chiaro Nanoindenter. Also, a more detailed description of how to mount the mechanical parts of the Chiaro Nanoindenter to operate together with an inverted microscope can be found in the separate Chiaro Nanoindenter mounting guide.

1.1 The Chiaro Nanoindenter and its components

The Chiaro Nanoindenter consists of five components: (1) The Chiaro indenter head and (2) the patch cord adapter, (3) the Optics11 Life OP1550 interferometer, (4) the Chiaro controller box, (5) the PC running the Chiaro Nanoindenter software suite and (6) the Optics11 Life optical nanoindentation probes.

Figure 2: Picture of the Chiaro Nanoindenter setup showing the Chiaro indenter head (right), OP1550 interferometer, controller box, and PC.

The Optics11 Life OP1550 interferometer operates independently of the other components and can be switched on and off at any time. The Chiaro controller box powers the Chiaro indenter head and communicates with the software running on the PC and should not be switched off before exiting the Chiaro nanoindentation software suite. The software will automatically detect and connect to Chiaro components linked to the PC. For the best user experience, it is recommended to power on all peripheral components before running the software.

1.2 Mounting the Chiaro

The Chiaro Nanoindenter can be mounted on the side of any inverted microscope. The microscope should be placed on a breadboard or table with M6 mounting holes or equivalent. The microscope usually has rubber feet, but the Chiaro Nanoindenter requires a stiff connection between the microscope and the breadboard. It is, therefore, advised to place the microscope on hard contacts, such as screws or our additional mounting feet, if it does not already provide the option to mount it directly. Please note that three points of contact, rather than four, usually offer the most stable connection.

The Chiaro mounting post can be screwed onto the breadboard after aligning the position to the microscope. To obtain maximum stiffness, clean both surfaces and tighten the screws well. The Chiaro's head needs to be positioned such that the endpoint of the arm (the probe) can reach the optical axis of the objective and condenser of the microscope. It may be necessary to bring the condenser to a slightly higher position. Usually, a medium working distance condenser is advised. The arm that holds the probe at the end can slide and lock to the rest of Chiaro's head. This will provide different mounting configurations with respect for the microscope.

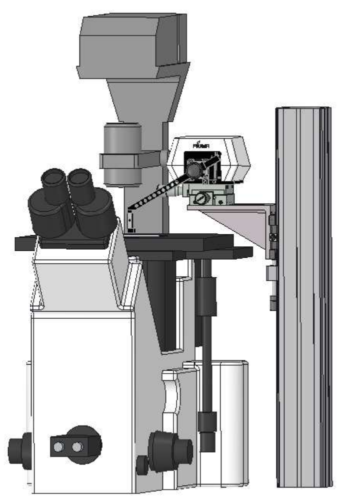

Figure 3: CAD picture (oscope. Ideally, both the microscope and the Chiaro mounting post are bolted onto a breadboard.

Together with the Chiaro, the following cables are provided:

- 1x BNC connector cable (previous Chiaro versions 2x BNC)
- 1x DVI signal cable
- 2x Power cable
- 1x PC power cable including adapter
- 2x USB (B-type) connector cable
- 50Ω Terminator
- 1x Fiber patch-chord & adapter

When installing the system, please carefully follow the scheme below (Figure 4).

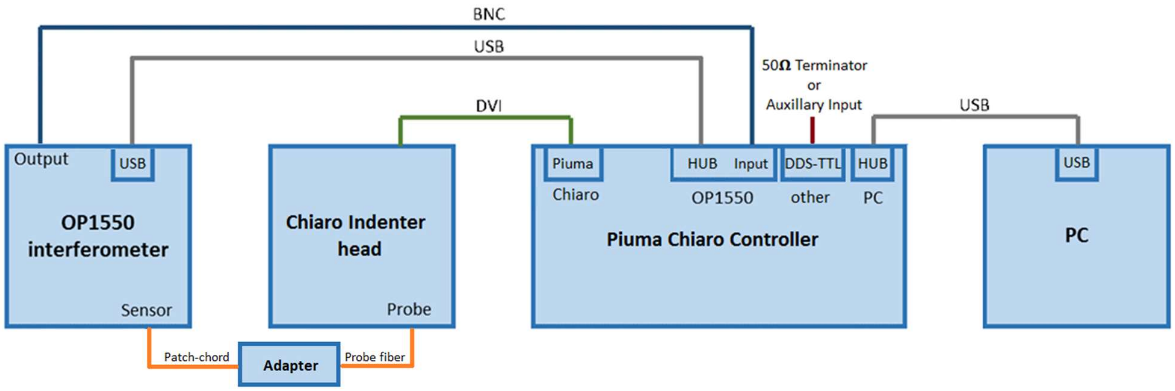

Figure 4: Schematic drawing of the connections required to set up the Chiaro Nanoindenter.

The controller box connects to the computer using one of the two USB 'HUB' connectors on the back of the Chiaro controller, and a free USB slot on the PC (it must be a USB3.0 port). The Chiaro indenter head plugs into the controller box using the original DVI cable supplied with the Chiaro system. The OP1550 readout plugs into the controller box using one BNC and USB cable. The BNC cable connects the 'Output' on the back of the OP1550 with the 'Input' behind the controller box, and the second USB cable connects the 'USB' output on the back of the OP1550 to the second 'HUB' connector behind the controller box.

The optional backlight module requires 5V to operate.Connect the USB cable to any free USB port on the PC or the controller box to power this module.

An optional camera requires 5V to operate and can be connected to the Chiaro through a free USB HUB port at the rear of the controller box or directly to a free USB port on the PC.

2. PREPARING THE SETUP FOR MEASUREMENTS

This section describes the steps to start and prepare the system for a measurement series.

2.1 Starting the system

Powering up the system

Before starting up the setup for the first time, ensure all cables are connected correctly, and the power switches on the back of the boxes are turned on. Switch on the devices in the following order:

1. PC
2. OP1550 interferometer
3. Controller box

The controller box automatically powers the Chiaro Nanoindenter head and the LED illumination in the indenter head turns on. After initialization, the interferometer shows a live measurement signal on the LCD screen.

Starting the software

Start the Chiaro Nanoindenter program by double-clicking the Chiaro software icon on the desktop. The software loads all devices while checking the hardware connection status, which should all be ‘Idle’ (Figure 5). As a safety check, the program asks to bring up the arm (also referred to as the manual stage) to the uppermost position. It prevents a probe collision with an obstacle while moving the X-Y stage to the home position. To move the arm up, release the grey knob on Chiaro’s and gently pull the arm up. There should always be some remaining friction to prevent the arm from falling. Next, press ‘OK’ in the software dialog box to confirm nothing can obstruct the Chiaro stages’motion.

Figure 5: Hardware check.

This action initiates the homing of the X-Y stage of the Chiaro Nanoindenter: The stage will move to the front-left corner, set the current ‘X’ and ‘Y’ coordinates to ‘0’, and subsequently move to the last coordinate set in the matrix scan parameters. After this, the system state will switch to 'ready', as indicated in the status bar on the home screen.

2.2 Mounting a probe to the Chiaro Nanoindenter

Selecting the right probe

Although a specific probe can measure a wide range of Young's Moduli, selecting a probe with suitable parameters that match the specific sample properties can enhance the quality of the measurement. For a softer sample, a probe with a less stiff cantilever is needed, and vice-versa, to ensure that significant cantilever bending as well as significant sample indentation is achieved during measurements. If the probe is either too stiff or too soft, there will be minimal cantilever bending or indentation respectively, resulting in a less optimal signal-to-noise ratio.

Advised cantilever stiffnesses provides a sample Young's modulus ranges as in the table below:

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

Sample Young's modulus	Advised cantilever stiffness range
10 Pa – 18 kPa	0.025 N/m
100 Pa – 180 kPa	0.25 N/m
2 kPa – 3.6 MPa	5 N/m
18 kPa – 36 MPa	50 N/m
89 kPa – 180MPa	250 N/m

These are estimated for a tip radius of 50 µm and an indentation depth of 1 µm. F14or smaller and larger tip sizes, slightly softer and stiffer cantilevers should be used, respectively. Indenting to larger depths requires stiffer probes, while indenting to lower depths expands the stiffness range that can be measured. In case of doubt, please do not hesitate to contact Optics11 Life for advice.

Installing the probe

After powering the Chiaro Nanoindenter system and running the software, open the indentation probe box. Carefully remove the probe from the box so that the probe clicks in the Chiaro indenter head in one go.

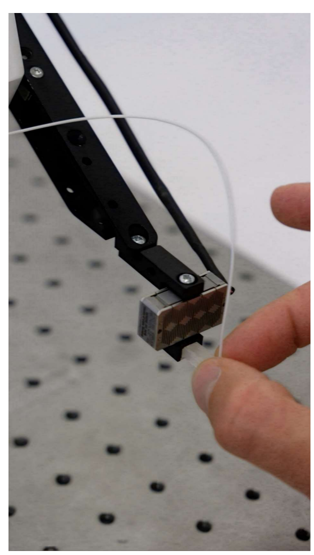

Figure 6: Probe and fiber wire inside the box (left), probe mounting to the indenter (right).

The probe fits into the Chiaro indenter head by pushing the probe with the holder in the probe mount (Figure 6). Ensure the cantilever points downwards, and the probe is pushed all the way to the most back-right position. When handling a probe, take special care to avoid contact with the cantilever. The glass cantilever at the end of the glass ferrule is extremely fragile, and almost any unintended contact will cause permanent damage. Therefore, when handling the probe, always hold it by the plastic probe holder, keep the optical fiber away from the cantilever and avoid touching the glass part. Given the size of the probe, it is advised to hold the probe between one's index finger and thumb whilst shielding it with the rest of the hand from the environment.

Connecting the probe

Our probes contain a new type of connector (E2000), which has a self-closing mechanism, making it less fragile and less susceptible to contamination. To connect the recently installed probe to the OP1550 Interferometer, the probe must connect to an adapter & patch-chord, which then goes directly to the OP1550 Interferometer.

Figure 7: Connecting the left end of the patch-chord to the OP1550, a notch (red circle) indicates the preferred orientation

To do so, first, connect the patch-chord to the OP1550, and keep it constantly connected, as the probe will only connect to the adapter. Next, remove the green safety cap from one end of the patch-chord and connect it to the 'Sensor' input on the OP1550 interferometer (Figure 7). This fiber connector is an FC/APC type connector, with a preferred orientation, indicated by the notch on the fiber connector and a groove in the OP1550 connector. Please note the center terminus of the connector is very sensitive to contamination or scratching; therefore, avoid direct contact with the connector. Then, take off the black cap of the adapter and connect the probe fiber to the adapter, as shown in Figure 8 left.

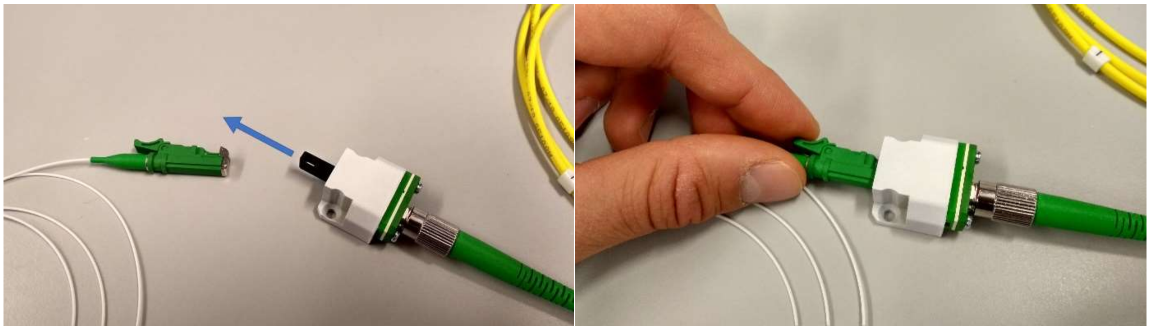

Figure 8: Removal of the black security cap to connect the probe fiber to the adapter.

Caution: Avoid bending the optical fiber sharply: this can break the glass fiber.

If a previous probe is present in the Chiaro, remove it before inserting the new probe. Disconnect the probe again by pushing the green lever at the connector and quickly putting on the black cap back into the adapter, to avoid further contamination. To prevent the fiber from touching the probe when packing the fiber and optical connector, always place the fiber first into the box and then the probe. For this, disconnect the optical connector from the OP1550, bundle the fiber wire, and put it into the box. Secure the optical connector in the box by placing a piece of tape on top of the connector. You can remove then the probe from the Chiaro indenter head holder by compressing the plastic connector and gently pulling it out. Carefully place the probe in the probe box at its designated location.

Caution: When installing the probe, always mount the probe first and then connect the fiber to the OP1550 via the adapter. When putting the probe back into the box, always place and secure the fiber wire first before placing the probe in the box.

Aligning the Chiaro probe tip

The optimal way to work with the Chiaro is to have the probe tip of the indentation probe in the middle, or to the side, of the field of view of a 20x or 40x objective. It is advised to start with a 4x or 10x objective to find the XY- and focal Z- position of a probe in the Chiaro. First, bring the Z motorized stage to its highest position by pressing ‘Z up’ in the stage controls of the main Chiaro software window (Figure 9). Then bring the Chiaro arm to the most extended position. The probe should now be a few millimeters above the objective lens. Use the motorized XY stages to bring the probe by eyesight to the middle position, directly centered above the objective lens. The XYZ stages can be controlled by entering a value in micrometers in the ‘Travel’ field and pressing one of the motor control buttons (Figure 9). Alternatively, one can use the keyboard arrow keys and the arrow keys at the numbering pad (‘Num Lock’ should be ‘off’) (Figure 9). Arrow keys stage movement can be activated in the ‘Advanced’ tab of the ‘Options’ menu (Figure 12). Now, use the microscope with the lowest magnification and bring the focal plane of the objective to the Z plane of the probe tip. If the working distance of the objective lens is too low to focus on the probe tip, you can use the Z stage to bring the tip down in the focal plane of the objective lens. It is recommended to use 10 µm-steps to avoid touching the objective lens with the tip! When the tip is in focus, you can reposition the probe by using the motorized XY stages to center the probe in the field of view (Figure 9).

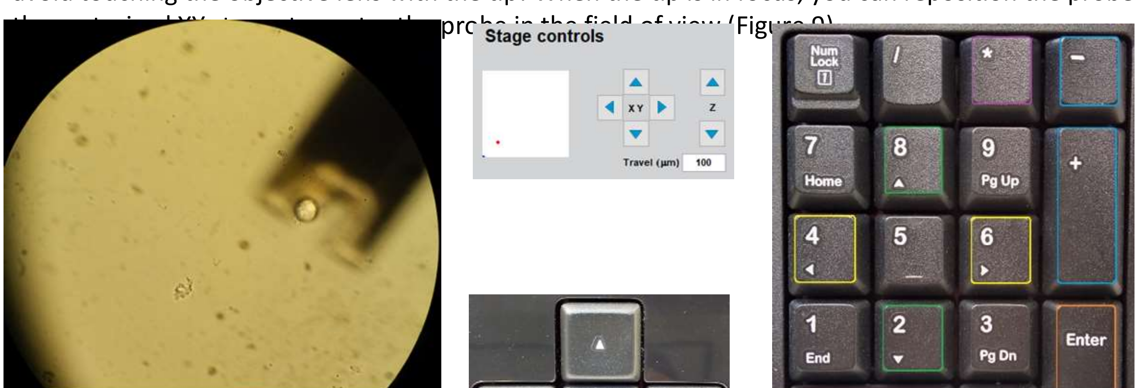

Figure 9: Aligning the probe tip to the optical axis by using the motorized XYZ stages in the stage controls of the main software window or by arrow keys (X stage, Y stage, Z- up, Z+ down, Stop). Please note that the Chiaro was deliberately placed at an angle in this example.

Front panel window

The front panel contains all the controls required to operate the Chiaro stages, set up experiments, and displays the system's camera feed and actual status.

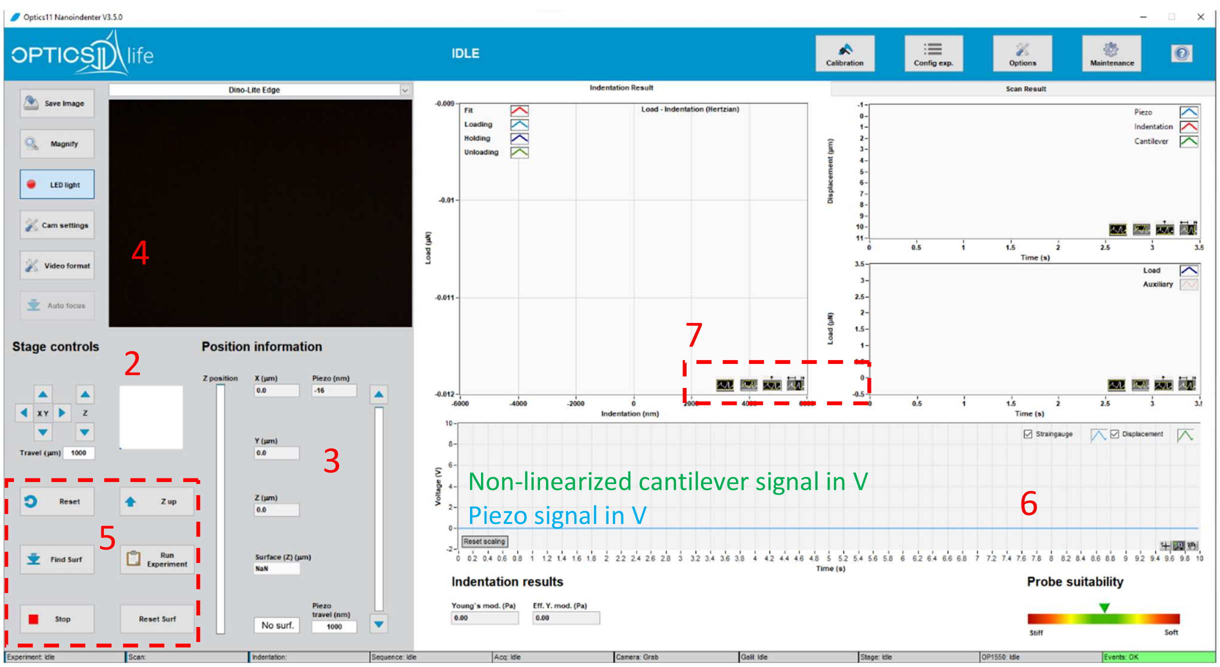

Figure 10: The home screen of the Chiaro Nanoindenter software.

Short description of main window controls (see figure 8)

1. **Top window selection:**
   - **Initialize –** set probe details and probe calibration.
   - **Options –** settings for Find Surface and fitting of data.
   - **Config. exp. –** set up indentation protocol and other steps.
   - **Maintenance –** additional settings.

2. **Probe stage controls:**
   - **Single arrow –** controls the step size of the probe stage and allows to move it in all three dimensions.
   - **Travel (µm) –** step size of the step in the selected direction.
   - **White square –** the relative position of XY stages.

3. **Position information:**
   - **X(µm) –** X stage coordinate.
   - **Y(µm) –** Y stage coordinate.
   - **Z (µm) –** probe stage coordinate.
   - **Z position –** relative position to the full range (left bar)
   - **Piezo (µm) –** current position of the piezo together with the right bar.

4. **Camera:**
   - **Camera output –** Camera image of the top view Chiaro camera
   - **LED light –** turns on LED.
   - **Cam settings –** camera settings for changing brightness, grain, etc.

5. **Run experiment:**
   - **Reset** – resets all the hardware (including homing of the stages).
   - **Z up** – move the Z stage to its upper position.
   - **Find surface** - Finds the surface of your sample with a determined cavity between the sample and tip (Z above the surface).
   - **Stop** – stops experiment or any other function.
   - **Run experiment** – runs experiment set in "Config. exp"
   - **Reset Surface –** resets the surface position to NaN.

6. **Live signals**
   - **In blue** – piezo position signal in Volts.
   - **In green** – non-linearized cantilever position in Volts (the same as in the interferometer "Measure" window)

7. **Plot visualization buttons**

From left to right
   - **Reset scaling** – scales axes to default view
   - **Scale to square** – scales axes to fit data in a square
   - **.Scale to Y** – scales axes to maximise Y value
   - **Scale to X** – scales axes to maximise X value

Configuring the probe in the software

It is important to correctly input the probe parameters in the software suite to obtain meaningful measurement results. Probe-specific parameters are used in the software to multiply the cantilever deflection with the calibration factor, convert it into load by multiplying with spring constant k (N/m), and calculate indentation depth which depends on tip radius (um). Probe parameters that need to be set in the program are the cantilever spring constant (in N/m) and probe tip radius (in µm). These parameters can be entered in the probe configuration menu, by accessing the 'Configure Probe' item in the main software window (Figure 11, left). The numbers can be found on the side of the probe packaging box and are unique for each probe. If you accidentally forget to update the probe details after changing the probe, you can still change those in the DataViewer software while analyzing the obtained data

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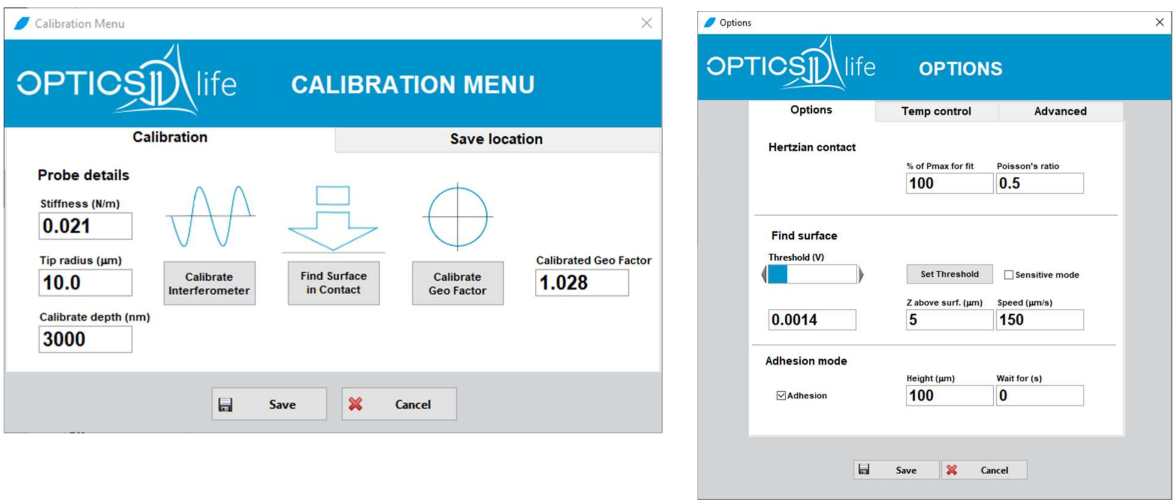


Figure 11: Configure probe menu (left), Options menus (right).

In most cases, the probe must be calibrated first, which will result in a measurement of the factor that describes the geometrical factor of the probe. As this is unique for each probe, leave the factor 1.000 in the configuration panel and see paragraph 2.4: "Calibrating the geometrical factor" in this manual on how to obtain this value through the automated calibration procedure. For this procedure, a value of 3000 nm for the calibration depth is set by default. You can change this value in the 'Options' menu, shown in Figure 11.

The "Model parameters" settings sectiondisplays the 'Oliver & Pharr' and 'Hertzian contact' buttons, allowing one to select the contact mechanics model of choice to be fit over the load-indentation curve after each measurement. If the Oliver & Pharr model is selected, the 'Pmax %' and 'Pmin %' fields control the section of the unloading curve, expressed in a percentage of the maximum load in the unloading part of the curve, used to make the slope fit. If the Hertzian model is selected, the 'Pmax %' field controls the part of the curve to be fitted, expressed in a percentage of the maximum load in the loading part of the curve. One can also choose not to apply a model by clicking whichever model is highlighted a second time, which will grey out both model parameters. The data can be reanalyzed and fitted with more models in DataViewer.

Setting the correct indenter head

The correct indenter head, here the Chiaro, must be selected in the 'Advanced' tab in the 'Options' menu (Figure 12).

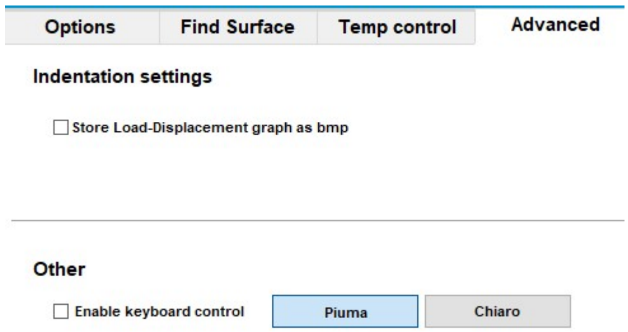


Figure 12: Chiaro selection in the "advanced" options menu.

A tick-box 'Store Load-Displacement graph as BMP' can be ticked to store each load-displacement curve as BMP in addition to the tab-separated text file containing the raw data.

This section is only relevant for Chiaro Nanoindenter systems V2.X (acquired before July 2019)

Inserting the corresponding XYZ stage values (only necessary when using two indenter heads with previous Chiaro versions)

Since the XYZ stage travel values of the Chiaro differs for previous Chiaro versions, you need to update the right values corresponding to the preferred indenter head. This can be accomplished in the 'XYZ stages' tab of the maintenance menu by pressing 'Calib. all' (Figure 13). You can enter this menu with the password 'showme' after clicking the 'Maintenance' button in the main software window. This procedure will take a few minutes since all XYZ values are being calibrated.

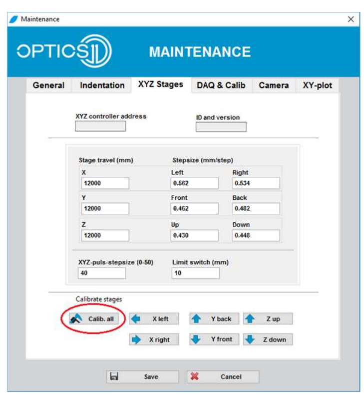
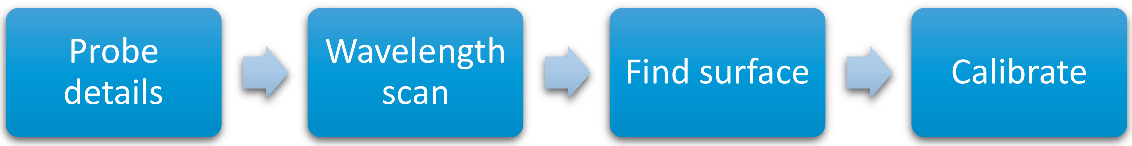


Figure 13: XYZ stage calibration when switching between Chiaro Indenter head (for previous Chiaro versions).

3. INSTRUMENT AND PROBE CALIBRATION

1.1 Calibration procedure

Before using the probe, the laser inside the interferometer and the geometrical factor of the probe must be calibrated as it is unique for each probe and medium. This process must be performed for each new medium/probe combination: to measure multiple samples in a solution of which the refractive index is not significantly different, this procedure does not have to be executed again. The steps contained in the following procedure must be followed sequentially.



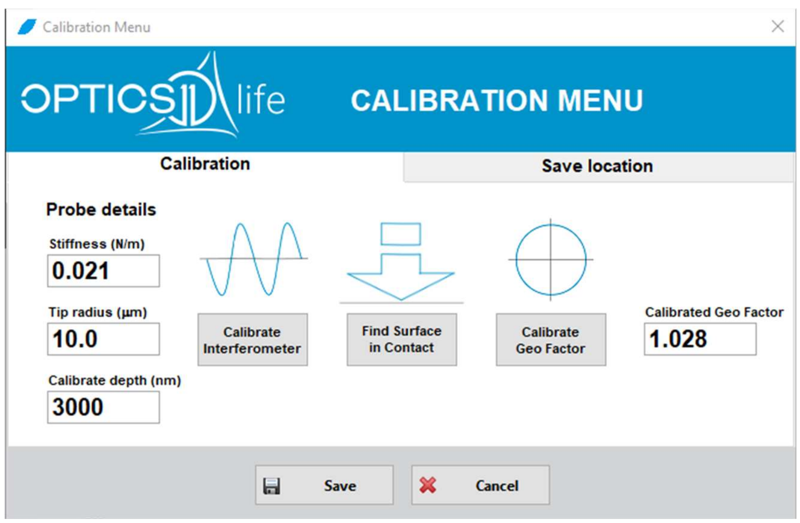


Figure 14 Probe calibration menu.

1. Open the calibration menu by clicking on the Initialize button in the top section of the software interface, the window shown in Figure 12 will pop up.
2. Input the probe parameters in the software suite and the calibration depth. The probe parameters that need to be set in the software are the cantilever spring constant k (N/m) and probe tip radius R (µm). These parameters can be entered in the 'Initialize' (calibration menu). The numbers can be found on the side of the probe packaging box and are unique for each probe. They are calibrated in the air by indenting on a scale[1]. If you accidentally forget to update the probe details after changing the probe, you can still change those in the DataViewer software while analyzing the obtained data. The calibration

[1] Beekmans, S. V., & Iannuzzi, D. (2015). A metrological approach for the calibration of force transducers with interferometric readout. *Surface Topography: Metrology and Properties*, *3*(2), 025004. https://doi.org/10.1088/2051-672X/3/2/025004

depth by default is set to 3000 nm, which is a value suitable for most applications. In case of necessity to change the calibration depth, contact technical support.

3. Place a clean Petri dish on the stage. Fill it with the same solution in which the sample is immersed in case of wet measurements.
4. In wet measurements, ensure the probe is prewetted with the same solution in which the sample will be immersed. It is essential to calibrate the optical signal in the same solution because the interference pattern analyzed in the OP1550 depends on the refractive index of the medium surrounding the probe.
5. Move down the probe using the manual stage knob and ensure it is fully submerged in the solution. Pay attention to avoid meniscus effects in case of partial submersion of the probe. Ensure that the probe does not come into contact with the bottom surface of the Petri dish by eyeballing the distance. In case of calibration in air, bring the probe a 1-2 mm distance from the sample surface.
6. Wait a few minutes so the probe can adjust to the new environment in wet conditions. When performing experiments with temperature control, the calibration should be accomplished at the same temperature as the measurement temperature. This is because the refractive index of the medium is dependent on the temperature.

Caution: The probe is sensitive to the medium's refractive index and temperature; thus, the probe needs to be calibrated in the same medium as the sample and at a stable temperature.

7. Click the “Scan OP1550 Wavelength” button once. The interferometer screen will now show a progress bar. The live signal window on the screen show oscillations; see Figure 13 B (zoom in if needed). Wait until that is finished and check if no error (Figure 13 D) is reported on the interferometer screen or software. You can also check the final result of the “Wavelength scan” in the corresponding interferometer window Figure 13 C. If an error message states Quadrature Scan Failed check the section Calibration troubleshooting.

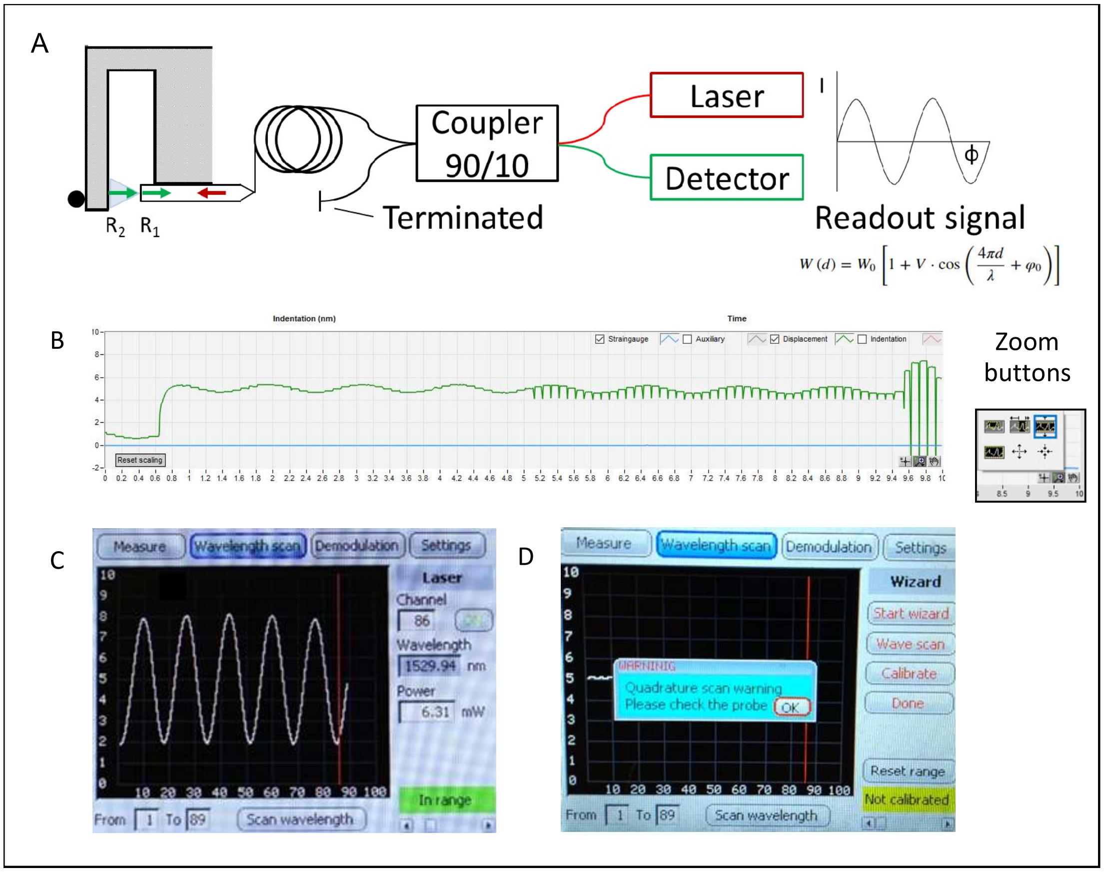


Figure 15 A) Working principle of the interferometer. More details are explained in section 3.2. B) Live wavelength scan signal, use buttons on the right bottom corner to zoom in and out. C) Good wavelength scan. D) Failed wavelength scan

8. Press the button Find surface in the initialize menu. The system will now start continuously moving down the probe stage to approach the surface of the glass. The piezo is fully extended and operates in a closed loop constantly checking the distance between the surface and the cantilever. Once the cantilever bends by a threshold value set in the ‘Options’ menu, the stage will stop and the piezo will retract so that the probe is in contact with the surface.

9. Confirm that the probe is in contact with the surface by pressing the probe stage up and down in 1 µm steps. The green signal in the live window of the software will change its baseline with each step if the cantilever is in contact with the sample (Figure 1**Error! Reference source not found.**4). If it does not, it means that the cantilever is not in contact and you should either increase the 'threshold' value in the 'Options' menu and repeat the 'find surface' step or manually bring down the probe to contact in small 1 µm steps by acting on the Z stage controls

   Caution: For the softest probes of k = 0.025N/m, the 'threshold' value most likely will have to be increased so that, during the 'find surface' stage movement, the probe does not stop too

Probe stage is moved down at 1 µm steps

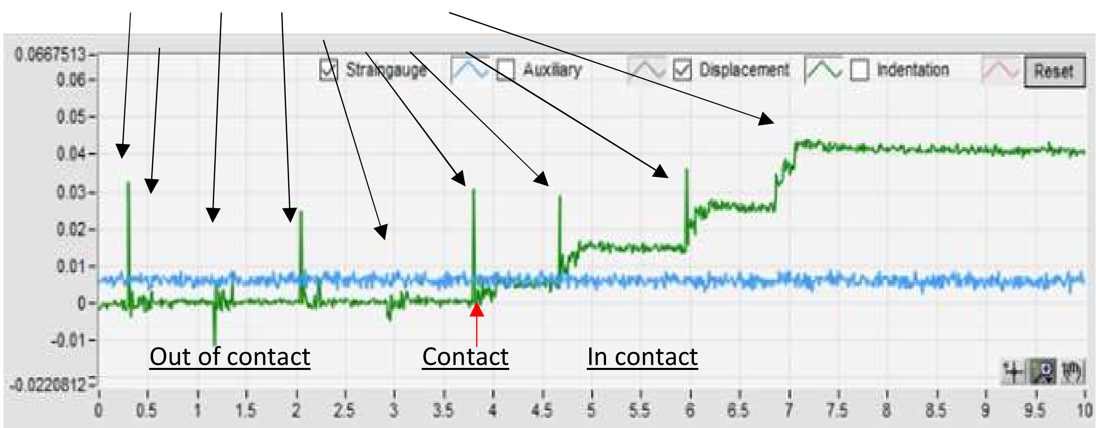


Figure 16 Manual finding of the surface position by pressing down the probe stage at 1 µm steps.

10. Press Calibrate in the initialize menu to start the last calibration step. Next, the linearization of the interferometer signal (demodulation circle) and the calibration of the cantilever arm (also called the geometrical factor) must be performed. Those two steps are accomplished in one calibration The geometrical factor called the 'calibration factor' originates from the mismatch in the spherical tip position and the readout fiber position. When indenting on a stiff surface, the distance measured by the fiber can be compared to the distance that the piezo displaced. Taking the ratio between them gives a geometrical factor to correct the cantilever signal.

$$Geometrical\ factor = \frac{cantilever\ bending\ at\ contact\ point}{cantilever\ bending\ at\ signal\ point}$$

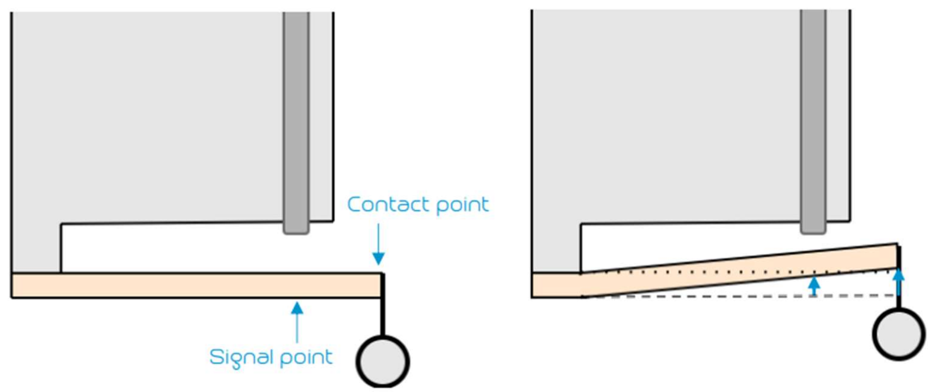


Figure 17: Schematic drawing of the meaning of geometrical factor

11. After pressing Calibrate, the piezo will move down twice by the amount set in the 'Options' menu 'Calibration depth (nm)'. We recommend using the value between 5000 and 10000 nm. If 'live calibration' is used (you can check it in maintenance), decrease 'Calibration distance' to 3000 nm (explained in Section).
12. Check piezo and cantilever signals move simultaneously in the live signal window, as shown in Figure 16 B(on the top). If there is a mismatch in time (Figure 16 B, on the bottom), the probe is close to contact but not entirely in contact with the surface. Manually move down the probe with steps of 1um until you see that the baseline of the cantilever signal has changed. If the cantilever signal does not change at all during calibration, the probe is far from the sample. Repeat the procedure from the 'Find Surface' step.

Caution: During the calibration step, ensure the probe is in contact with a stiff surface.

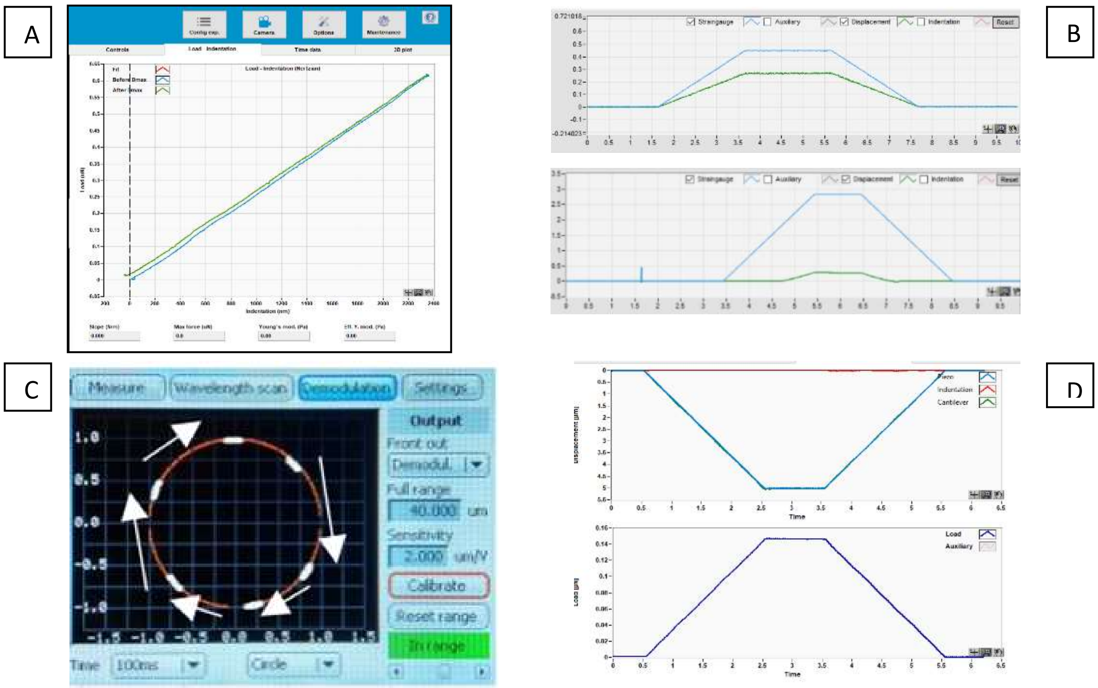
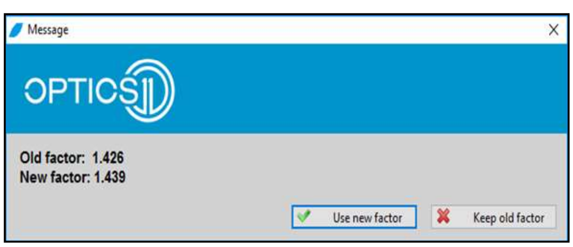
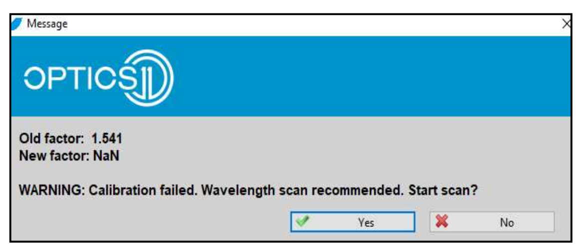


Figure 18 A) Calibration curve B) Live piezo and cantilever signals during calibration when the probe is in contact with the hard surface (left) and when it is out of contact C) Signal movement in time on demodulation circle after it is calibrated D) Final check of calibration. After calibration, there is no indentation on a hard surface (red line is flat).

13. When calibration is completed, 'New factor' is given. By pressing 'Use new factor' the software automatically saves the new 'Calibration factor' in the probe configuration menu. The calibration factor should be ~1.33 times lower in the medium than it is in the air which is given on the box of the probe, e.g. if the number on the box is 3.2, then the geometrical factor in the medium should be 3.2÷1.33≈2.4. When repeating geometrical factor calibration, only a slight variation is expected < 5%. You can also check in Load-Indentation data that loading and unloading data overlap and are straight slopes (Figure 16 D). The slight mismatch is expected due to drift and hysteresis in piezo movement. **If calibration has failed, check the troubleshooting section in this manual.**





Troubleshooting Calibration: “Quadrature Scan failed”

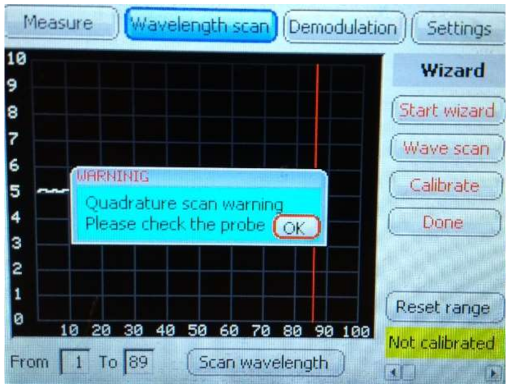


Figure 19: Error message – quadrature scan warning. Please check the probe.

During the optical calibration procedure, the OP1550 interferometer stimulates an interference pattern and automatically adjusts the gain and offset of the signal. If the OP1550 detects a little or no signal, the system will react with an error message “Quadrature scan failed” . Please press “OK” to visualize the resulting wavelength scan (see Chapter 7.1). Please be aware that this error message pops up due to a reason, which might be one of the following issues and can be solved by corresponding actions:

- Noise – *Get rid of any noise sources close to the instrument*
- No connection – *Check the fiber connections (Chapter* ***Error! Reference source not found.*** *& Chapter* ***Error! Reference source not found.****)*
- No cantilever – *Exchange probe*
- Dirty cantilever – *Clean the probe with demi water & Isopropanol & water again*
- Air bubble between the cantilever and tip fiber – *Get rid of the air bubble (Chapter* ***Error! Reference source not found.****)*
- Cantilever stuck at the tip fiber – *Clean the probe with water & Isopropanol & water again*

After performing the actions mentioned above, please pre-wet the probe before inserting it back into the measuring solution. Repeat the wavelength scan and check the result at the OP1550 display. If the error message pops up again, please repeat one of the procedures or contact us via support@optics11life.com

Troubleshooting Calibration: New factor NaN

- The tip is not in contact with the surface during calibration –
  Repeat the calibration procedure from step 7
- Air bubble between the cantilever and tip fiber – Get rid of the air bubble
  Lift the probe out of the well, prewet the probe, and move down
- Cantilever stuck at the tip fiber – *Dry it and unstuck it with a piece of paper as depicted in picture 18. Repeat the wavelength scan before calibrating again.*

Troubleshooting Calibration: New Factor not coherent with specs

- Ensure the probe immerses in the same medium as the sample
- Attractive forces between tip and surface – snap-on behavior results in the calibration of the over-bended cantilever – clean probe and the surface or use Teflon surface for calibration.
- Dirty tip or surface – clean the probe with demi water & Isopropanol & water again.

- Air bubble between the cantilever and tip fiber – Get rid of the air bubble
  Repeat the wavelength scan before calibrating again.

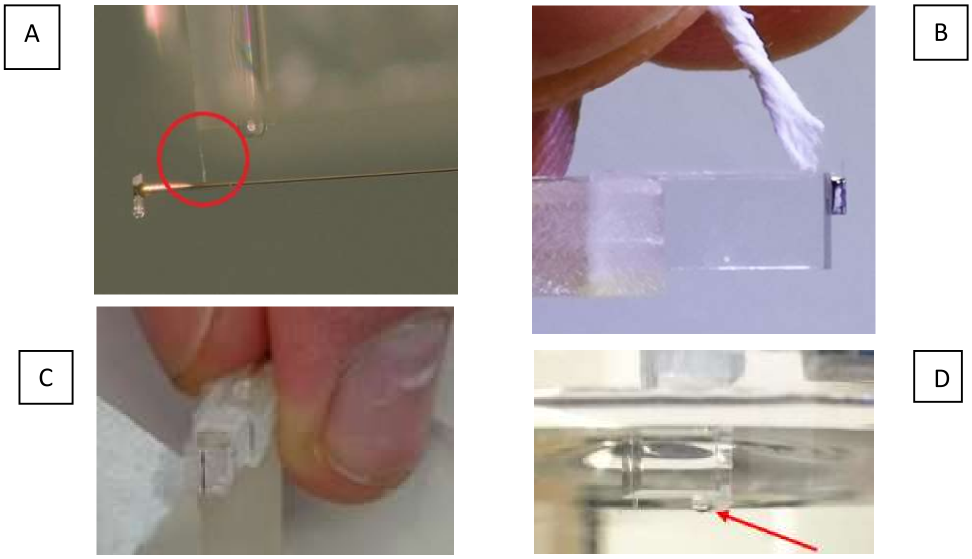
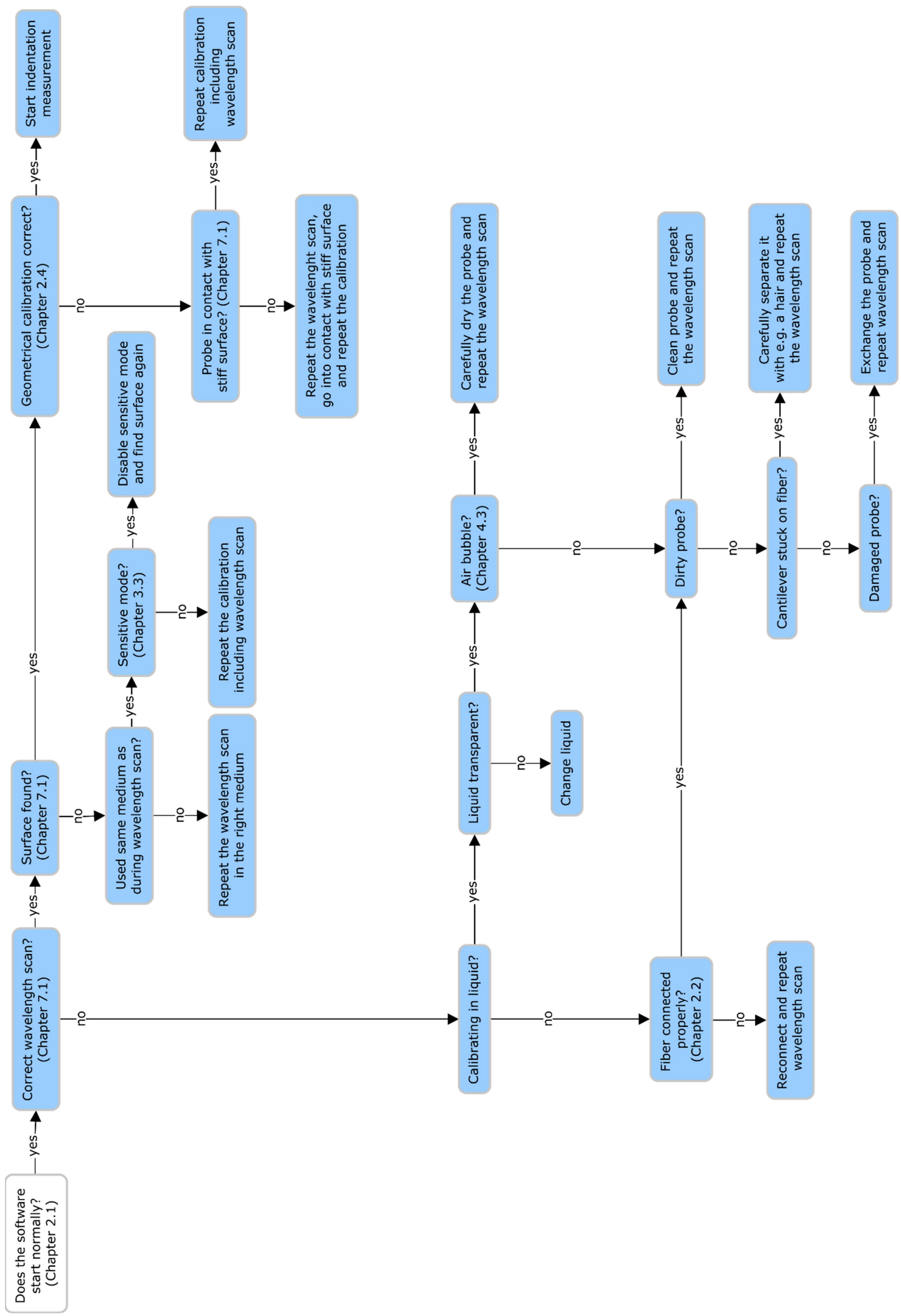


Figure 18 A) Cantilever stuck to fiber. B) Releasing the cantilever with the tissue. C) Drying the probe with the tissue. D) Air bubble stuck to the cantilever.

After performing the actions mentioned above, repeat the calibration procedure and check the result on the interferometer display. If the error message appears again, please repeat one of the procedures or contact us via support@optics11life.com.

Flowchart - Calibration

Most issues while measuring with the Chiaro Nanoindenter are related to the instrument's calibration. A troubleshooting flowchart is presented on the next page to guide you through possible issues and solutions, to ensure a safe calibration of the Chiaro Nanoindenter and, therefore, , correct instrument functionality.

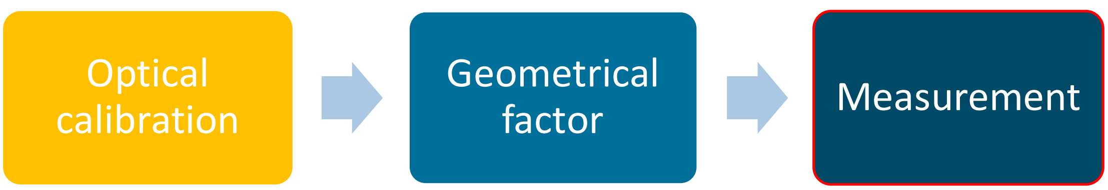


Ensure the optical calibration was performed for the same medium/probe combination for which you would like to perform the calibration of the geometrical factor!

Figure 20: Example of calibrating the probe in a medium droplet on top of a regular microscope sample slide.

4. USING THE CHIARO NANOINDENTER

This section describes the use of the Chiaro Nanoindenter after having prepared the instrument for a measurement (Chapter 3).



4.1 Stage controls

The motorized stages of the Chiaro, each having a maximum travel path of 12000 µm, can be controlled in the Chiaro Software in X, Y, and Z directions. To correctly position the area of interest under the probe tip, start by bringing the probe into the field of view, following the indications in Chapter 2.1. The desired alignment can be reached by controlling the position of Chiaro's arm and the movement of the stages. The Z stage can be controlled by inserting a value in micrometers in the Travel field present in the Stage control area (see figure 27) and then pressing one of the motor control buttons above and below the z character. The Z-Stage, then, will be moving in the selected direction. The white bar in the Position Information area of the software represents the whole 12000 µm movement range for the stage. By moving the Z stage in the down direction, the bar will be gradually filled in blue color.
Once the blue bar is filled, the Z stage reaches its lowest position. Therefore, it will not be possible to move further in that direction.

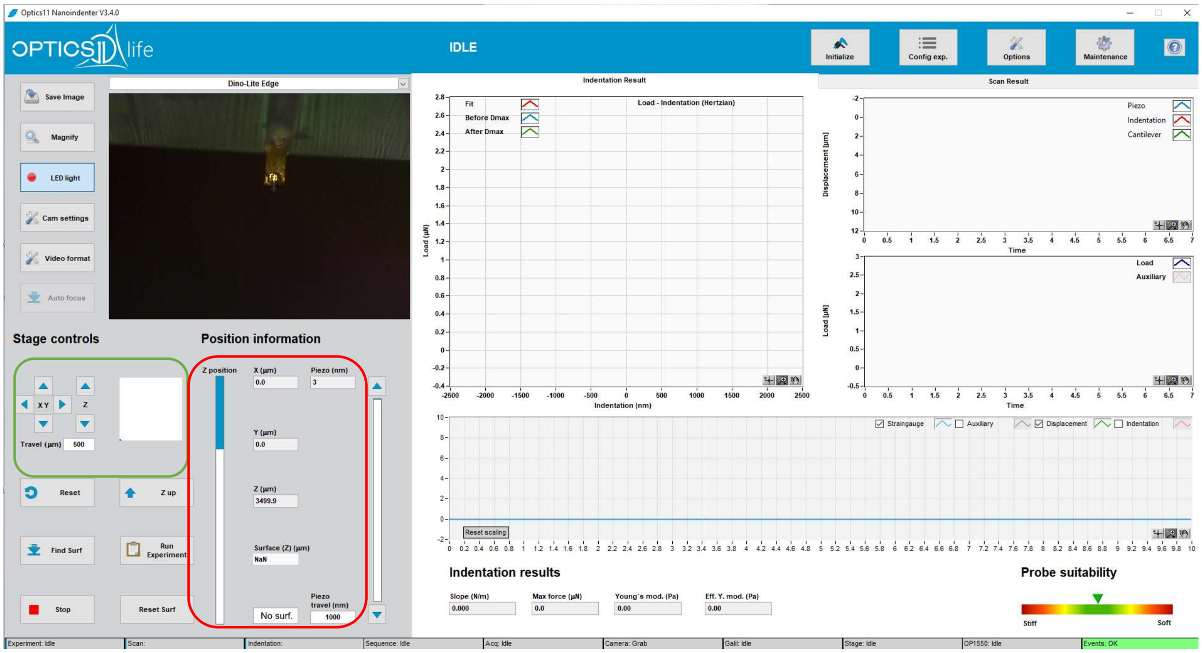


Figure 21: Main software window: Stage controls (green), Piezo controls/Position information (red). The probe is brought into the camera image, close to the focal plane, using the down arrow of the Z-stage.

The X and Y stages can be controlled similarly: by entering a value in micrometers in the 'Travel' field and pressing one of the motor control buttons. Additionally, a white field with a blue dot is displayed, simulating the (theoretical) X-Y movement range and the current position of the tip with respect to the moving stage. Alternatively, one can use the keyboard arrow keys and the arrows keys at the numbering pad ('Num Lock' should be 'off') (Figure 22). Arrow keys stage movement can be activated in the 'Advanced' tab of the

Figure 22: Keyboard stage controls (X stage, Y stage, Z- up, Z+ down, Stop).

'Options' menu. The controller box can be reset by clicking the 'Reset' button in the lower-left corner. In the next window, which automatically will pop up, you can reset the X, Y, and Z stages to their home position by clicking 'ok'

4.2 Piezo controls

The Piezo stack generally used for indenting can also be controlled manually. By providing a number in the "Travel" field below the Piezo status bar, the piezo can be commanded to make relative steps. The range of the Piezo stack is ~100 000 nm, and a manual offset can be set anywhere in this range. In normal operations, this feature is not used. However for specific experiments that require more precise control of the starting position of the indentation, this feature can be helpful.

4.3 The Find surface procedure

The tip of the probe needs to be near the sample surface to perform indentation. The Chiaro Nanoindenter features an automated find-surface approach for best accuracy and probe safety. This approach combines the motorized Z stage and the indentation piezo. To safely and accurately bring the cantilever near to the sample surface using the automated find-surface routine, the following steps should be taken:

1. Approach the sample surface by 1-2 mm with the manual stage, using eyesight or the onboard camera. Safely use the 'Find surface' function of the Chiaro Nanoindenter software. Click on the 'Find Surface' button on the main screen once the probe is submerged in the medium.

Caution: Do not use the find surface button in the initialize menu.

In the 'Options' menu (Figure 23 left) the settings of the automated find-surface procedure can be adjusted:

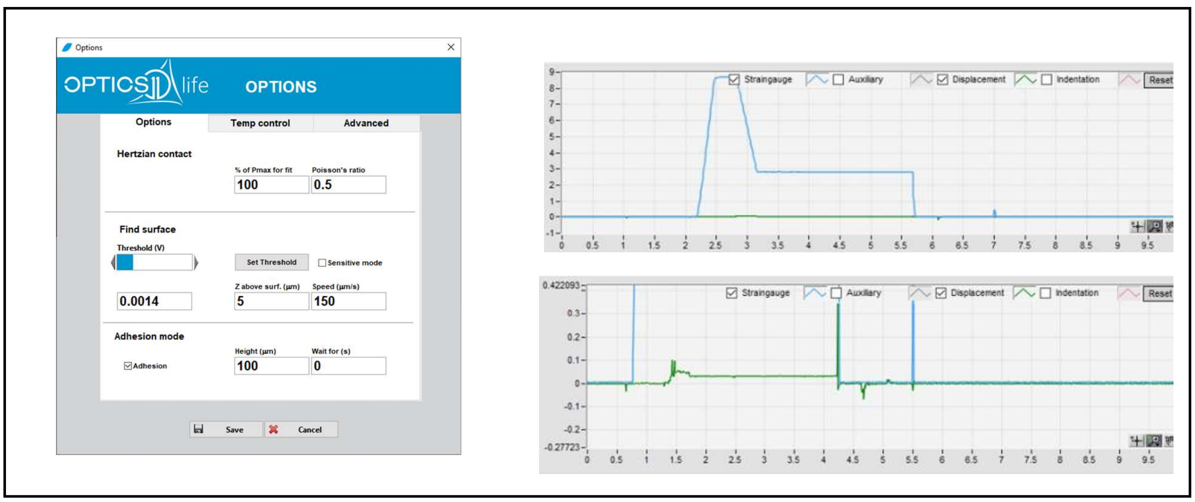


Figure 23: Find Surface options window. Piezo and cantilever signals during the "Find Surface" step

- ***Threshold*** defines the threshold value of cantilever deflection when the surface is detected. Units are arbitrary. The higher the noise level, the higher the value will be needed. The **Set threshold** function calculates the appropriate threshold value according to the noise level detected by the system.
- ***Speed (µm/s)*** regulates the approach speed of the downwards movement of the Z stage during Find Surface. A speed of 200 µm/s is recommended for stiffer probes and <100 µm/s for the softest probes of 0.025 N/m because higher speed causes higher noise and the possibility of early triggering when the probe is not yet in contact with the surface.

- ***Z above surf. (µm)*** is the distance that the probe is retracted after the surface is found. If this distance is too small, the measurements will start in contact with the sample as shown in Figure 23 E, F, which could be due to 1) not ideal match between the probe and sample stiffness (probe is too stiff), 2) threshold is too high, 3) sample is adhesive. For load-indentation curves that start in contact, the contact point between the sample and the probe is unknown and is placed at the beginning of the load-indentation curve. For the correct contact point estimation, an approach distance to the sample is needed to fit the Hertz model (red line). This is shown in Figure 23**Error! Reference source not found.** D from which the contact point is given as one of the fitting parameters.

  Caution: The maximum distance of the piezo extension is ~100um. Keep that in mind when selecting the distance above the surface.

- ***Adhesion mode*** is used when the sample is sticky/adhesive which results in in-contact measurements. To eliminate contact, increase the **height (µm)** value to which the probe is retracted to get out of contact with the sample and the time value in the field "**wait (s)**" after which the probe is brought back to Z-above surface position. For example, if the sample is found at stage position 23 500 µm and height is set to 1000 µm, then after the Find Surface step, the probe will be retracted to 22 500 µm position and then brought back to 23 480 µm when Z-above surface is set to 20 µm.
- ***Sensitive mode*** improves the reliability and precision of the detected surface

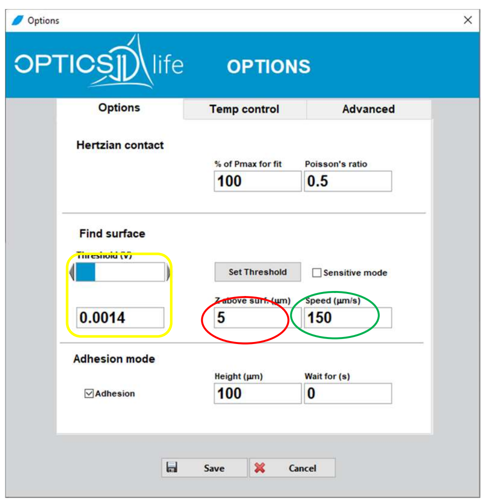


Figure 24: Options menu with 'Z above the surface' setting (red), speed adjustment (green), and threshold settings (yellow).

In addition to setting the height above the sample surface, the speed of the coarse Z-motor and the threshold can be selected from the Find Surface tab. The automatic procedure starts after clicking 'Find Surf' in the main software window. During find surface, the piezo fully extends and controls the cantilever to a bending of approximately 10 nm; when the piezo retracts beyond the threshold to control the bending to this amount, it triggers the coarse Z-stage to stop and then resets to be above the sample, per the adjustment in the Options menu. Depending on the environment's noise and the probe's stiffness and sample , the speed may influence how accurate the find surface settings are. If the stage regularly stopsfarther away from the surface, changing the speed to a slower speed can improve the performance (Figure 24, green). After finding the surface, the tip should be in focus, together with the sample surface. It is recommended to raise the tip a few micrometers away from the sample surface (in the Z direction) to avoid damage to the tip while moving the sample in the XY direction. It is important to perform a wavelength scan, as discussed in the previous chapter, performing a find surface routine. The software has some limits to prevent you from attempting the automated find surface routine if the wavelength scan has not been completed or the calibration has not been completed. If the software prevents you from performing an automated find surface routine, you may need to check that the optical signal is good and whether you need to complete a new wavelength scan or calibration.

Information on the automated find-surface function for Chiaro Nanoindenter systems installed before August 2019

For older versions of the Chiaro hardware (installed before September 2019), the automated find surface routine works in a slightly different method. In this method, the point of contact is determined by monitoring the bending of the cantilever while stepping the motorized Z-stage according to the large and small stepsizes set in the 'Find Surface' tab of the Options menu, with default stepsizes of 10 µm (large) and 1 µm (small) (Figure 25).

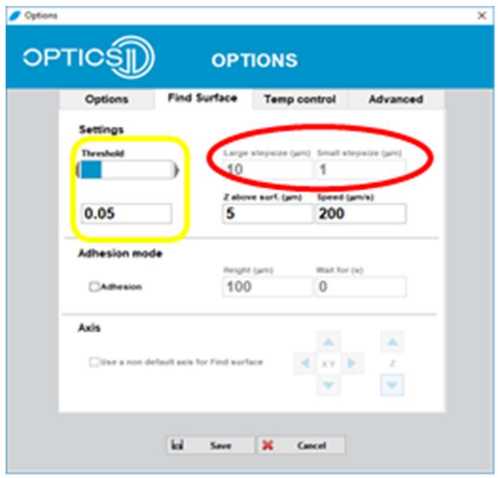


Figure 25 In the older version of the Chiaro software, the large and small step sizes can be changed (red), and the threshold settings refer to the voltage change on the cantilever signal when the surface is found.

The stepping of the motorized Z-stage can be confirmed by the 'beeping' sound, which is the sound of the motor moving. In case the find-surface routine is aborted and the message 'surface not found' is displayed, the 'Find-surface' function can be restarted. When using very soft cantilevers (< 0.4N/m), you might need to decrease the threshold (**Error! Reference source not found.**, yellow) if the surface is "found" too early. If the probe ends up in contact with the sample, you might need to increase the threshold. Please find a more detailed description for adjusting the find surface threshold settings in our technical note, which you can find online via www.optics11life.com.

In the older software versions, the find surface approach works in disabled Sensitive (or Standard-) mode or enabled Sensitive mode, which can be tuned in the 'General' tab in the Maintenance.

Please note that a wrong cantilever stiffness – sample stiffness combination can cause the find-surface routine to fail. Therefore, always verify the state of the cantilever by looking at the interferometer screen during finding the surface procedure (see Chapter 7.1).

4.4 Configure experiment

The Chiaro Nanoindenter can perform simple indentations or run any sequence of indentations, matrix scans, moves, and the find surface procedure. In all cases, indentation sequences are configured using the configure experiment menu. You can find this by clicking on the Config exp. button from the main menu (Figure 26).

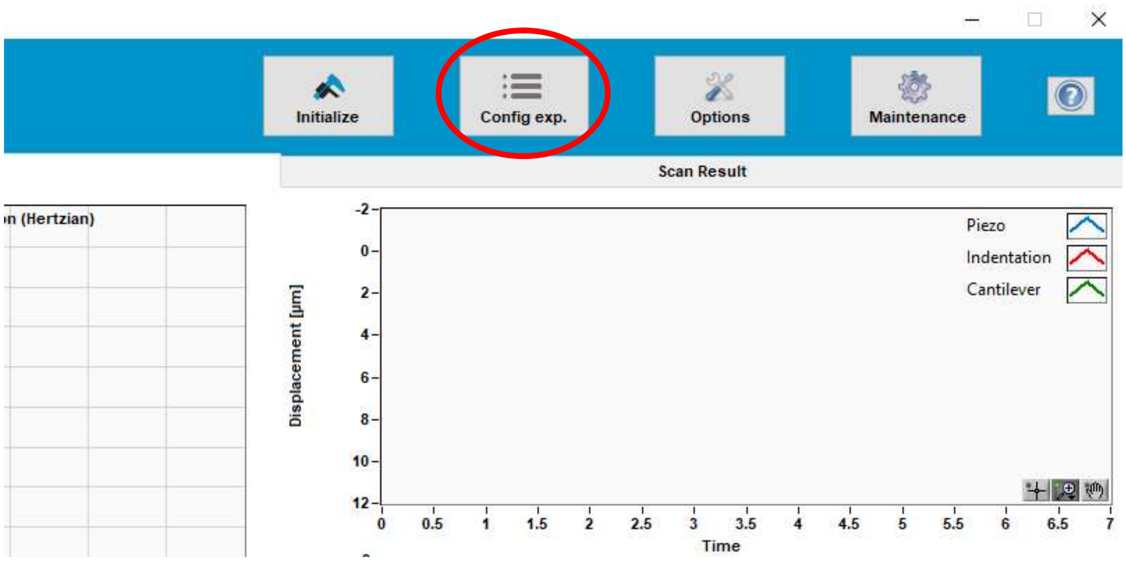


Figure 26: Open the Configure Experiment section by clicking on 'Config exp.' from the main window.

The Configure Experiment window can add, remove, or change steps within an experiment. The window shows details about the experiment, including the different steps in the current experiment, the location where the experiment file is saved, and the information about the details of the current step.
To begin creating an experiment, click the ‘Add’ button to add a step. This will create a blank step, and you can choose what you want to include in the experiment. The current choices are ‘Move’, ‘Find Surface’, ‘Indentation’, and ‘Matrix Scan’. You can also open a previously saved experiment by clicking on the folder next to the ‘Experiment Path’ bar (Figure 27).

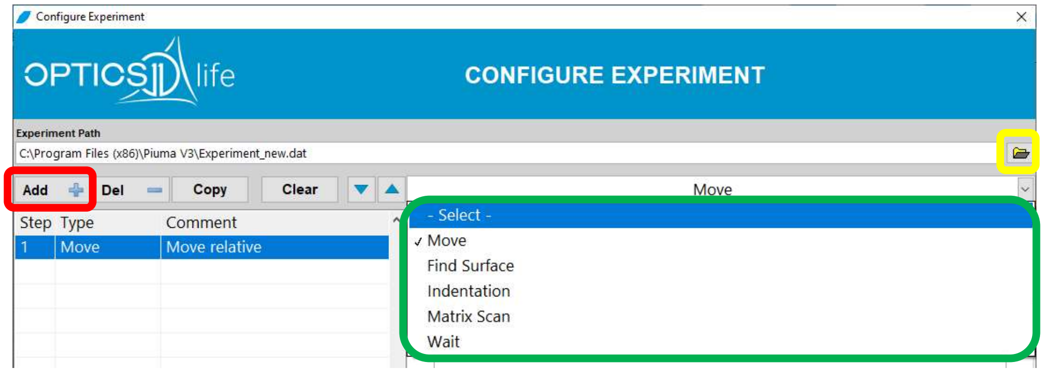


Figure 27: To add a new step, Click the 'Add' button (red) to add a new step. When a step is highlighted, the function of the step can be chosen from the dropdown menu (green). A previously saved experiment can also be chosen by clicking on the folder icon next to the dialog box labeled 'Experiment Path' (yellow).

Multiple different steps can be added to an experiment. Each step can be highlighted, and the parameters can be individually set. More details will be discussed about each option for Configure Experiment steps.

Find Surface

A find surface step can be called during the experiment. The only parameter that can be set separately from the parameters in the 'Options' menu is the speed of the Find Surface (Figure 28,**Error! Reference source not found.** green). All the information previously described regarding Find Surface still applies while doing this find surface. This means that the parameter 'Z above surf' is defined by the value set in the 'Options' menu.

Users with the automated find-surface function for Chiaro Nanoindenter systems installed before August 2019:

The 'large stepsize' and 'small stepsize' can be defined in the find surface step (Figure 28, orange), yet the 'Find Surface threshold' and the 'Z above surface' are defined in the 'Options' menu.

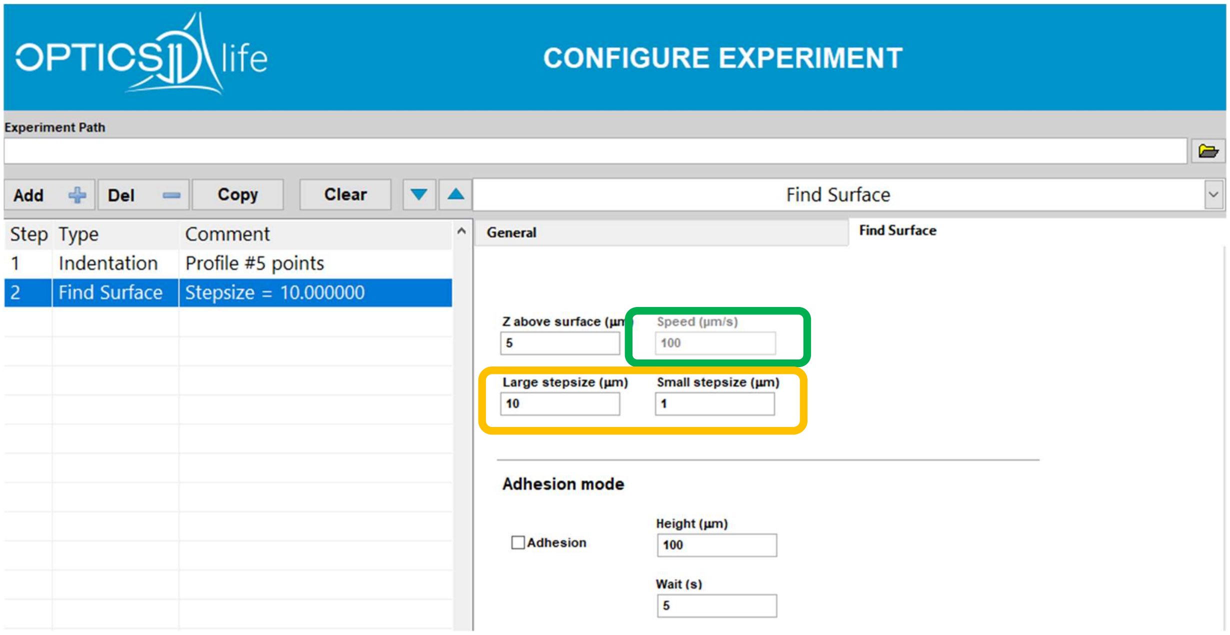


Figure 28: A 'Find Surface' step can be configured as part of an experiment. Here, for systems installed before August 2019 the large and small stepsize (orange) and otherwise, the find surface speed (green) can be adjusted.

Wait

The wait step can be added to the experiment to introduce a break. The break duration can be either relative or absolute in the tab. A relative waiting time corresponds to a timeframe (hours, minutes, or seconds). An absolute waiting time refers to a break created until a set time of the day (see Figure 29).

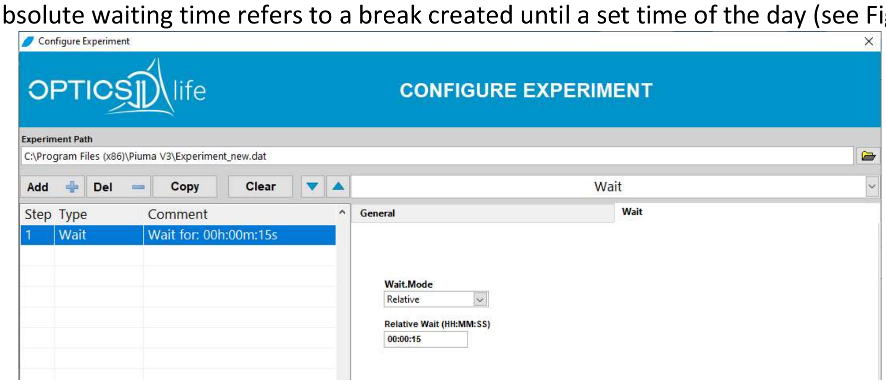


Figure 29: Select the wait step to create a break in the experiment. The waiting time can be set as a duration in relative mode or an absolute waiting time until a specific time of the day.

Move

The 'move' step allows one to make either a relative or absolute move of the stage in X, Y, and Z. The distance to travel, or the absolute stage location is recorded in µm. If an absolute move is desired, then the current location of the stages can be loaded into the dialog boxes for X, Y, and Z using the 'Use stage pos.'

button (Figure 30). The stages move in all directions simultaneously, unless the moves are split into different 'move' steps. A Z-direction move is positive if it moves the probe closer to the sample and negative if it moves the probe away from the sample.

Users with the automated find-surface function for Chiaro Nanoindenter systems installed before August 2019:

Due to hardware specifications, 'Move' in Z-direction must be separated from 'Move' in XY-direction. Therefore, two move steps must be implemented for a movement in XYZ.

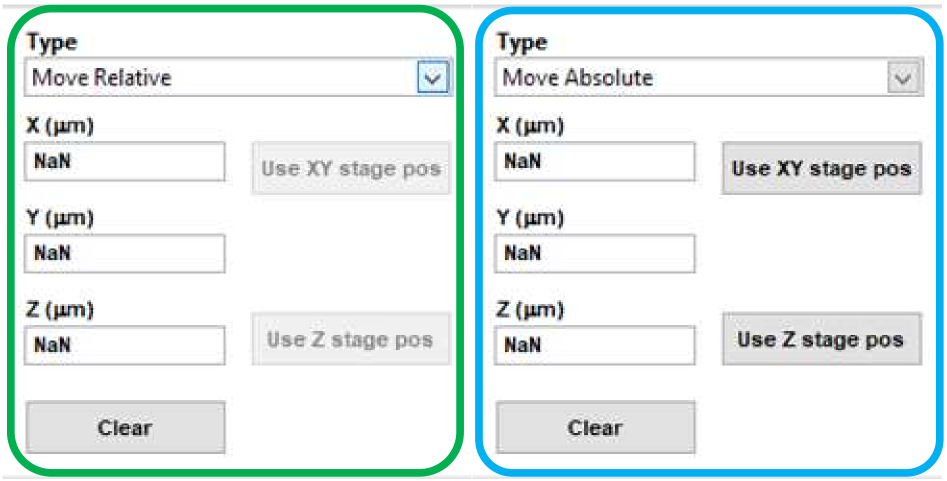


Figure 30: A move in either relative (left, green) or absolute (right, blue) coordinates can be specified as a 'Move' step in the Configure Experiment window. All stages move simultaneously. Z-direction moves are positive to move closer to the sample and negative to move away from the sample.

Please note that a positive Z value moves the piezo down, and a negative Z value moves the piezo up! No movement is achieved by inserting '0' or 'NaN'! Out of precaution, move the piezo up relatively before moving to an absolute position!

Single indentations

The configured displacement, load, or indentation profile is executed by choosing an 'Indentation' from the drop-down menu of a step in the 'Configure Experiment' window (Figure 27). The indentation parameters for each of the three modes of operation are described in more detail below in Section 3.5 Modes of operation.

Matrix indentations

Instead of performing a single indentation, a series of indentations can be programmed to perform an automated scan over an area of the sample. The indentation matrix is defined by selecting a 'Matrix Scan' from the drop-down menu when determining a step (Figure 27). The Profile of the Matrix Scan can be set using either the Profile tab or the DMA tab as described below in Section 4.5 Modes of operation. The parameters of the scan are placed in the 'Matrix Scan' tab, such as the number of points in the X and Y directions (#X, #Y), the distance between each point in the X and Y directions (dX (µm), dY (µm)), the starting

location (Start X (µm), Start Y (µm)), whether or not to use Auto find surface (checkbox), the height above the sample to use when moving the XY stage (Z at XY move (µm)), and the height above the sample on which to start each indentation if 'auto find surface' is used (Z above surf. (µm)). Too use the current XY position as the starting position of the scan, click 'Use stage pos', and the current location will be filled into the Start X and Start Y boxes. The grid the scan will perform is shown in Figure 31 blue. Here, the maximum grid size is 12 x 12 mm. The red dot represents the position an indentation will be performed.
If the 'Auto find surface' checkbox (Figure 31, green) is checked, then, before continuing to a new point, including the first indentation of a matrix scan, the probe moves up to a safe transportation height and subsequently transfers to the indentation location. After reaching this location, the find-surface procedure is initiated. Using the find-surface again for each point in a grid scan allows the Chiaro to scan samples with higher surface topography.

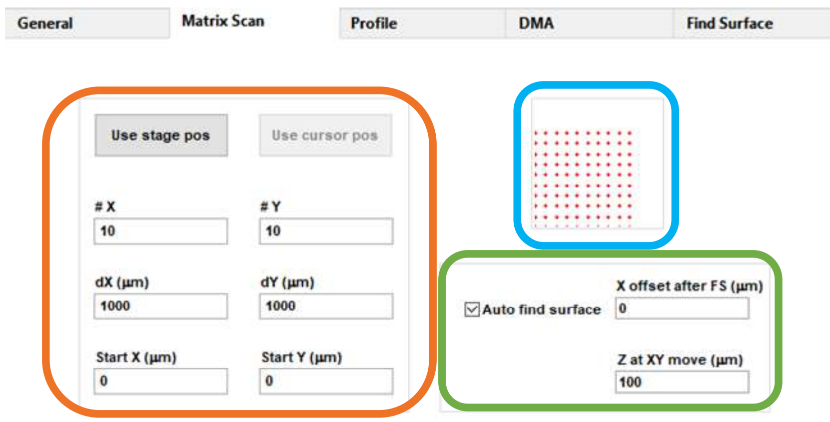


Figure 31: Defining the parameters of a Matrix Scan (orange), the automated find surface approach (green), and the select grid (blue) in the Configure Experiment window.

The scan data is saved in a generated 'Matrix_scan_X' folder in the directory specified in the General Tab of the Matrix Scan step. In this folder, a file is created for each indentation, as well as one file containing the effective Young's moduli per coordinate in a single grid.

The following points should be considered when initiating a matrix scan over an area. First, since a positive point-to-point pitch is added to the starting position, the first point will always be displayed on the bottom left in the program's probe position field. Because the stages move the sample and not the probe, the indentation area will be on the bottom left of the camera image to the first point of the matrix scan. To measure another quadrant, the pitch can be made negative.

Second, setting a correct transportation height (the Z at X-Y move value) is important. If a large point-to-point pitch is chosen, please set such a value for the transportation height so that the probe cannot hit the sample during repositioning to a new coordinate. For large pitches and rough samples, it is advised to set the transport height so that it is at least half the size of the point spacing.

Saving configure experiment file

After setting up all the experiment steps, you can save the experiment step by either overwriting the current file if one exists by clicking on 'Save', or creating a new file by clicking on 'Save As…'. Clicking on 'Save' if a file with the same name already exists will show a popup confirming that you want to overwrite the experiment file. Clicking on 'Save As…' will create a popup that allows you to choose a location to save the experiment. The experiment will then be saved as a file with a .dat extension that can be opened by selecting the folder icon next to the Experiment Path dialog box.

4.5 Modes of operation

The Chiaro Nanoindenter can operate in three different modes:

- Piezo displacement control: *D-mode*
- Load or Force control: *P-mode*
- Indentation depth control: *I-mode*

All three modes of operation monitor piezo movement, load (cantilever bending), and indentation depth. The modes are different in their controlling parameters and operational loop with the sample, as shown in the following table. It is very important to understand that the piezo displacement is not the same as indentation depth since a part of the probe displacement is converted into cantilever bending and material deformation. This means that the indentation depth is the difference between the piezo displacement and the cantilever bending minus the approach. In all operational modes the electronic working principle of the piezo, which regulates the probe displacement, is always in closed-loop with the strain gauge, which permanently measures the absolute extension of the piezo.

	D-mode	**P-mode**	**I-mode**
Control	*Probe movement*	*Load*	*Indentation depth*
Controlled parameter	*Piezo displacement*	*Cantilever bending*	*Piezo displacement- cantilever bending*
Electronic circuit	*closed-loop*	*closed loop*	*closed loop*
Sample circuit	*open-loop*	*closed-loop*	*closed loop*

Table 2: Comparison of the three modes of operation

The differences in modes allow you to define measurement designs, dependent on the parameter which can be held constant during one measurement. For this, a basic understanding of the working principle of the operational modes is crucial.

D-mode

In D-mode, the user defines a piezo displacement profile, resulting in a specific load and corresponding indentation depth. The indentation depth depends on the displacement profile, the cantilever ratio , and sample stiffness. Since the piezo is not receiving any feedback signal from the sample (cantilever bending), it is working in an open loop with the sample.

To define such displacement profile of the probe for an experiment in D-mode, open the Configure Experiment window, add an Indentation or Matrix Scan step, select the 'Profile' tab of the step, and then choose 'Displacement control' in the 'Mode Selection' section (Figure 32, blue). In the window, a graph represents the displacement profile, of which the displacement (nm) and speed (nm/s) variables can be customized for each profile segment.

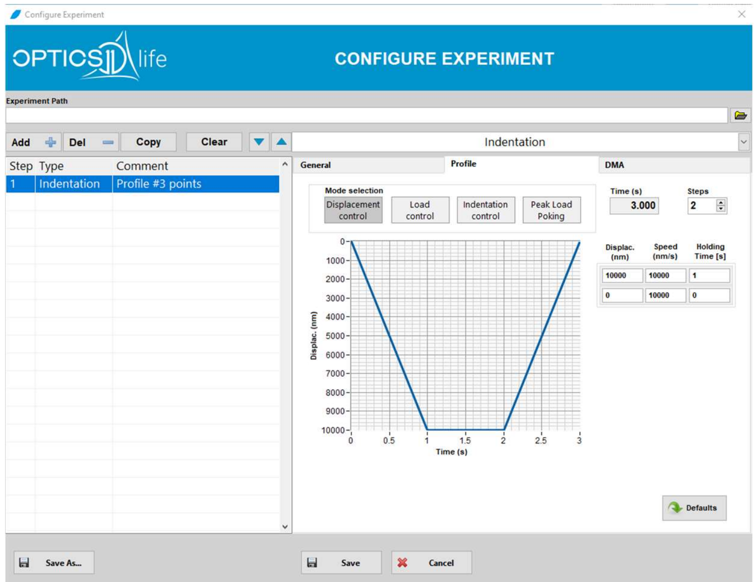


Figure 32: Mode selection: Displacement control

P-mode

The P-mode of operation allows you to set a specific load, which can be kept constant over a certain time interval. For this, the probe needs to be in contact with the sample. A velocity-limited approach of sample surface sensing monitors the threshold of cantilever bending when approaching the surface. The piezo receives a new feedback signal from the sample, which feeds the piezo to keep the intended load constant. Therefore, the P-mode of operation is in a closed loop with the sample. The threshold at which the cantilever bending triggers the indentation profile to start can be set, along with the approach and retraction speeds. It is best to match the approach and retraction speeds with the start and end speed of the profile to smooth out results. The threshold may need to be changed if a setup is particularly noisy, which causes a profile to start before the cantilever touches the surface.

To set a load profile, switch to 'Load control' in the 'Mode Selection' part of the 'Profile' tab. The load profile with three default segments can now be adjusted, according to the desired load (µN) and time (s) (Figure 33).

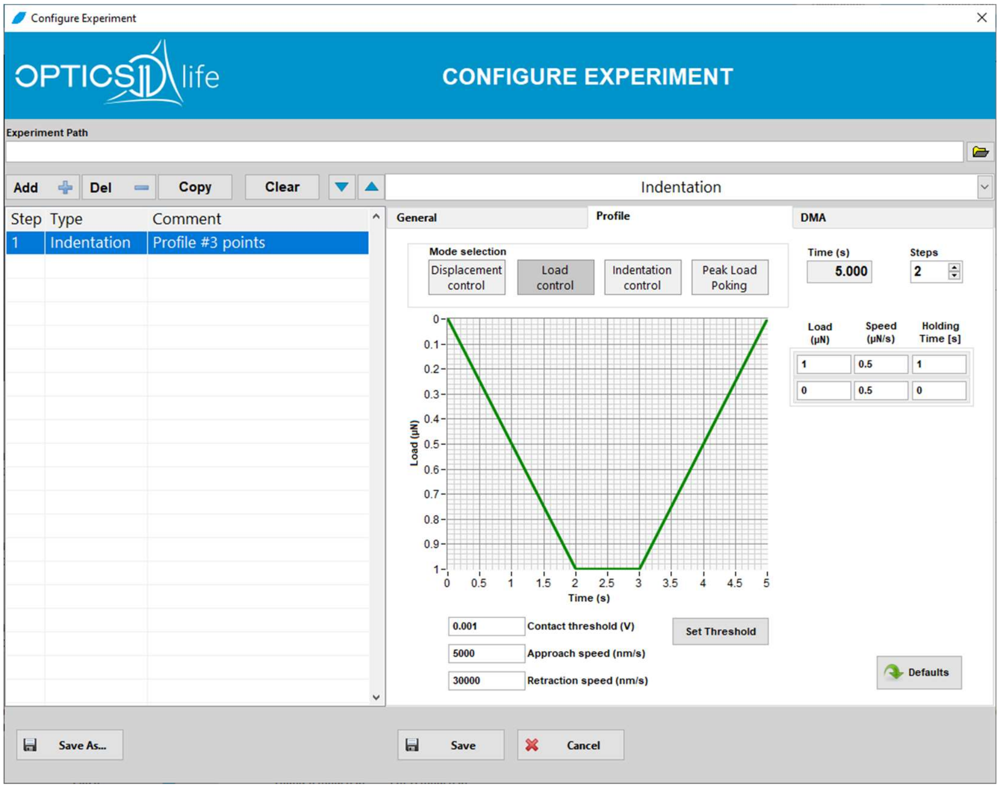


Figure 33: Mode selection: Load control

I-mode

Operating in I-mode gives you the possibility to set an exact indentation depth. Like in the previously discussed P-mode, the piezo stroke is permanently updated by a feedback signal from the cantilever after getting into surface contact. Using this feedback signal, the piezo adjusts continuously to control the sample indentation depth. Similarly, to the previously discussed P-mode, the threshold of cantilever bending triggers the start of the profile and the approach, and the retraction speed can be set for each indentation. It is best to match the speed of approach and retraction to the speeds used during the profiles.

To set an indentation profile, switch to 'Indentation Control' in the 'Mode Selection'. The indentation profile with three default segments can be defined by an exact indentation depth (µm) over a specific time interval (s), and adjusted to the appropriate number of segments (Figure 34).

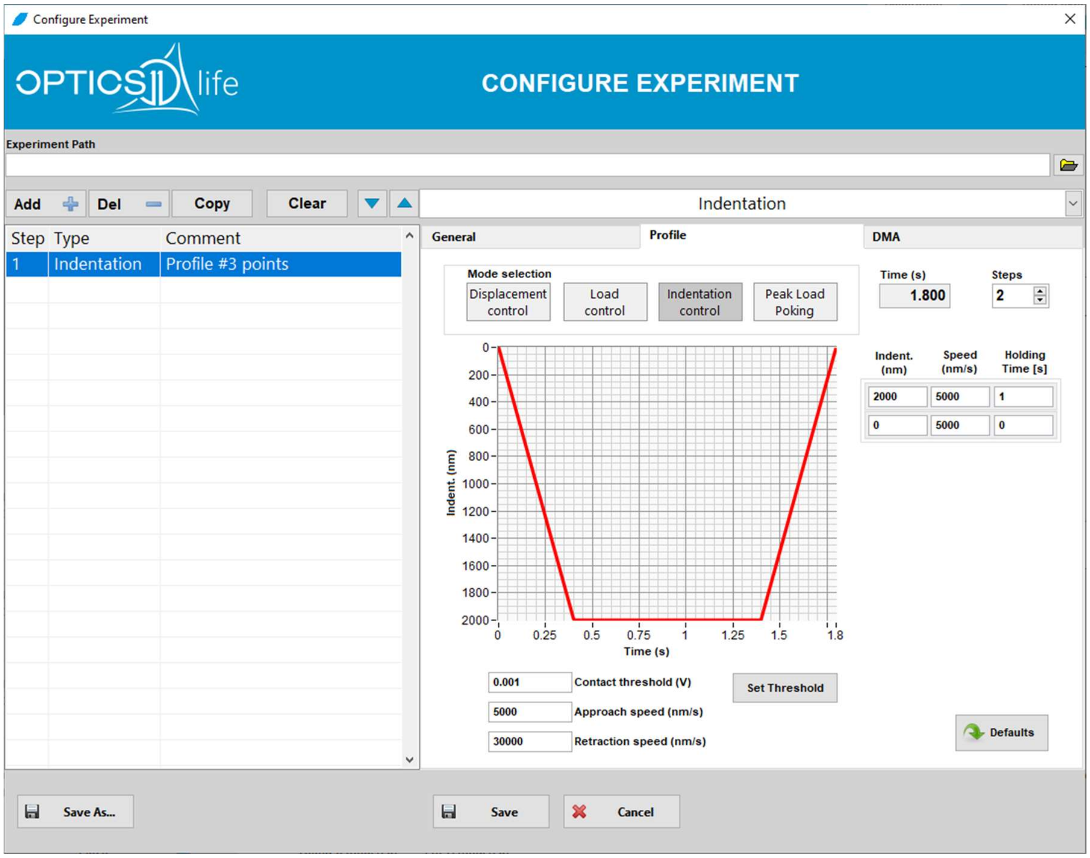


Figure 34: Mode selection: Indentation control

Peak load poking – PLP mode

The Poking-mode of operation allows you to set a maximum load and piezo-speed at which it is reached in an open-loop operation meaning that indentation-speed, load-speed or indentation-depth are not controlled. Therefore, this mode can be described as a mode between the Displacement control (D-mode) and Load control (P-mode) modes. The open-loop operation is the main difference between Peak Load Poking mode (PLP) and Load control (P-mode). Hence, this mode can be used when many experiments need to be performed quickly and when the Hertz model is sufficient for extracting Young's modulus. "Max load (µN)" shows the maximum load that needs to be reached with poking the sample and "Piezo speed (µm/s)" defines how fast the piezo moves down to reach the sample (see -d). To set a peak load profile, click "Peak load poking" in the 'Mode Selection' part of the indentation configuration window and define the values accordingly.

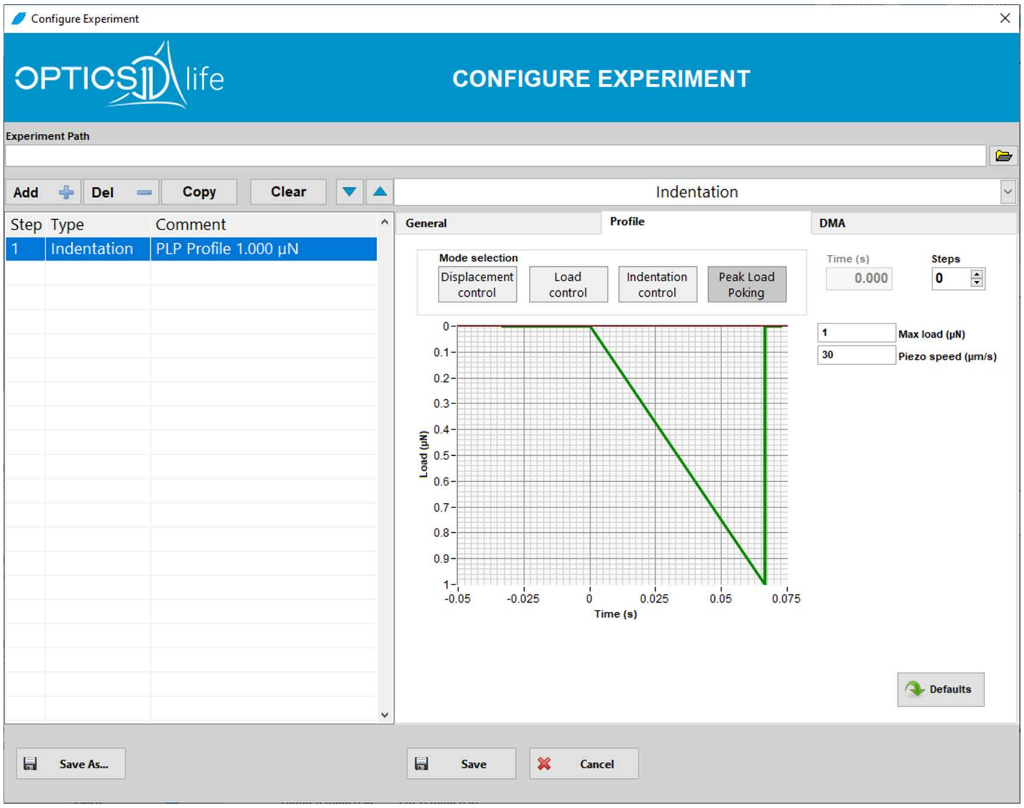


Figure 35 Peak load poking profile

4.6 Dynamic mechanical analysis – DMA

The Chiaro Nanoindenter features, besides the quasi-static operation, a dynamic operational mode. This DMA mode allows mechanical oscillations in all three modes of operation while indenting in a sample. It can be switched 'On' in the DMA tab of the corresponding step information window in the Configure Experiment window. Oscillation parameters like frequency, periods, amplitude and relaxation time are freely adjustable to provide a flexible measurement design (Figure 35). However, to guarantee a smooth DMA measurement choose the measurements following the minimum requirements in Table 5.

Min. initial relaxation time	5s
Min. relaxation time	2s
Min. amplitude	2x noise level
Lowest frequency possible	0.01 Hz
Highest frequency possible	20 Hz closed loop, 75Hz open loop
Min. oscillation time	1s

Table 3 Minimum requirements for DMA analysis

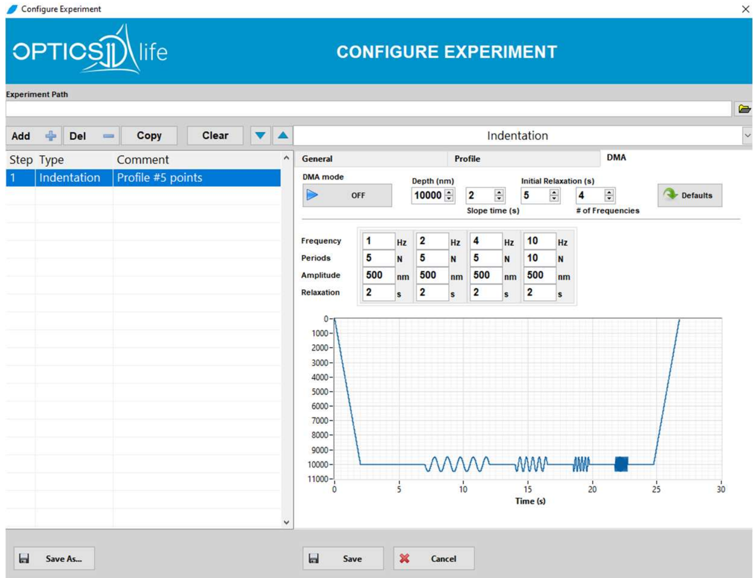


Figure 35: Configure displacement DMA profile.

Dynamic Mechanical Analysis (DMA) uses a cyclic motion with frequency, f, while controlling indentation-depth or load with an amplitude of h_0 or F_0, respectively, if used in closed-loop. In an open-loop, only the displacement of piezo is controlled, thus, h_0 and F_0 will depend on the relationship between sample and probe stiffnesses. The frequency-dependent storage modulus, E', and loss modulus, E'', which represent elastic and viscous components respectively, are calculated with the following equations:

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

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

Where δ is the phase lag between oscillations of indentation and load (see right of **Error! Reference source not found.**). 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 viscoelastic fluids and below 1, are 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. Therefore, 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 sphere's curvature, 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 may be assessed in the DataViewer by comparing the amplitude to the amplitude specified in the experiment and by looking at the R-squared values of cosine fits (figure 36)

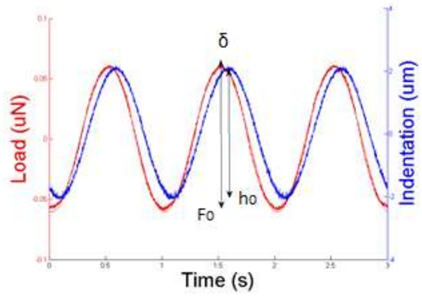


Figure 35 phase relation between indentation and load

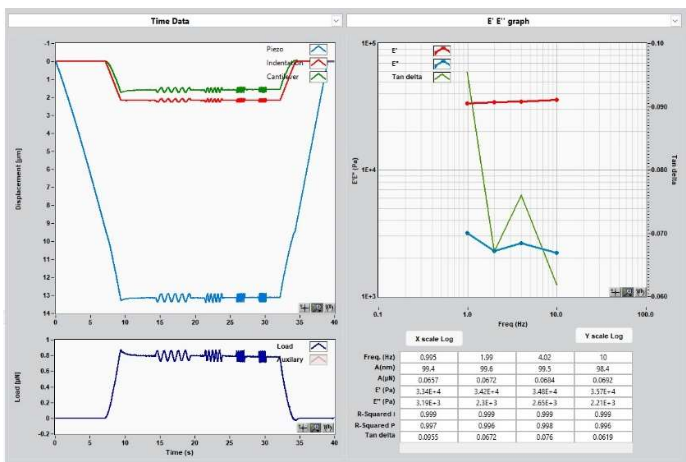


Figure 36 DMA Analysis in Dataviewer

4.7 Maintenance menu and PID settings and PID tuner

PID settings and PID tuner can be found in the “Maintenance” menu (Figure 37). The maintenance panel is protected by a password, as any change in these settings may compromise the performance of the Chiaro Nanoindenter. Please note that changing parameters in the maintenance menu can cause unexpected behavior and potentially break the hardware on the Chiaro. Use caution when changing parameters.

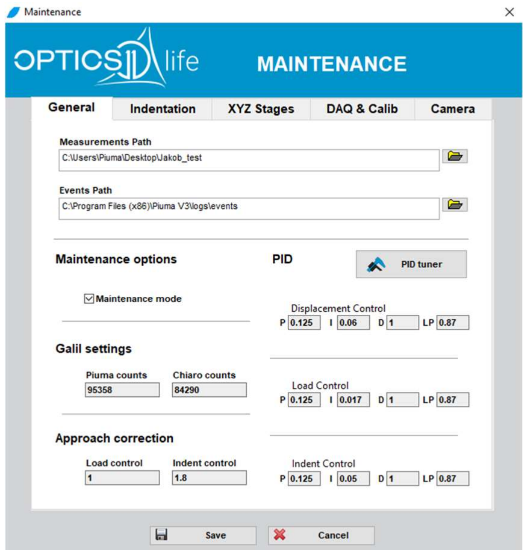


Figure 37: Maintenance menu

PID settings can be changed manually. Usually, adjustment of the I term is sufficient to improve the precision of the feedback loop. Try slightly increasing or decreasing it to see the effect on the indentation profile, or consult our Application Specialists.

PID tuner is a new feature that is still under development. Use the PID tuner to tune the PID parameters live while a block generator is running. First, select the mode to be tuned in the top left. PID parameters from this mode will be loaded in the indicators. Next, configure the block diagram parameters (offset, amplitude, and time between iterations) on the right side. Then, run the block generator by pressing the ‘Run’ button. Optionally, hide the plots not being tuned by right-clicking the plot legend and disabling ‘Plot visible’. While the block generator is running, use the sliders to change the integral or derivative terms of the PID controller. It is currently not possible to change the proportional term from this menu. Use the control in the “Maintenance” menu for that. The integral term will have the most effect. Use the graph to determine whether the tune is sufficient visually. EG overshoot after motion, no drifting in straight sections. Use the graph controls to optimize the visual (zoom, drag, etc.).

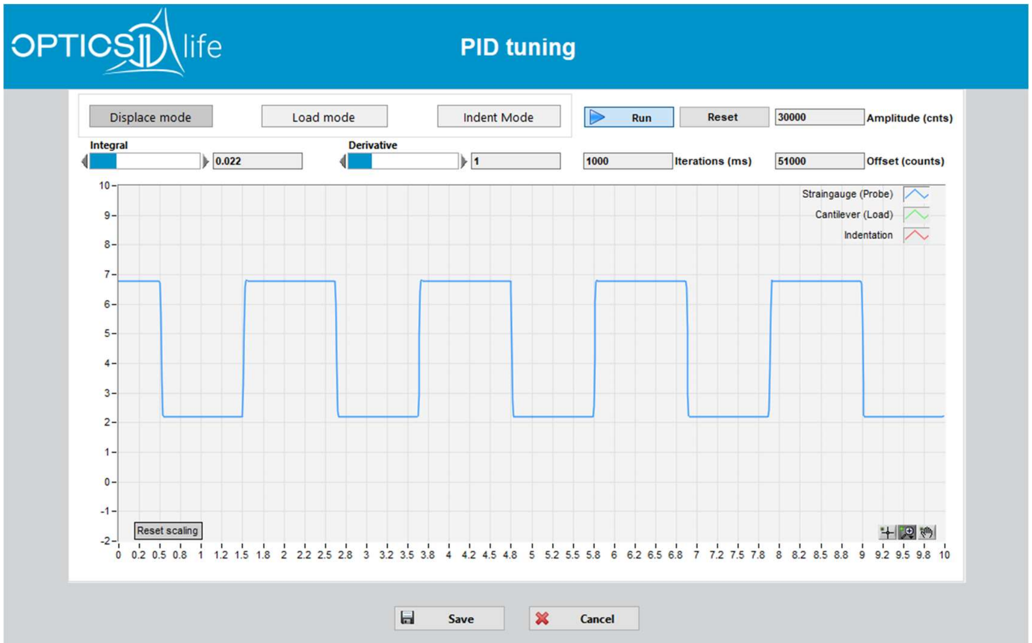


Figure 38: PID tuning window with block generator running in Displacement control

4.8 Saving measurements

Each Indentation or Matrix scan, independent of operational modes, can be saved in a dedicated folder. Create a folder in the browser dialog in the chosen location by clicking the 'General' tab of the Indentation or Matrix Scan (Figure 39). Each Indentation or Matrix Scan can be given a different name in the dialog box (as well as a different folder location). The Chiaro software will automatically create a subfolder with this name, in which the data will be saved. You should avoid changing the saved file name afterward in Windows Explorer. This may vary for different indentation or matrix scan steps. A comment can also be added about the indentation or matrix scan performed in the comment box (Figure 39). These comments are saved along with the data.

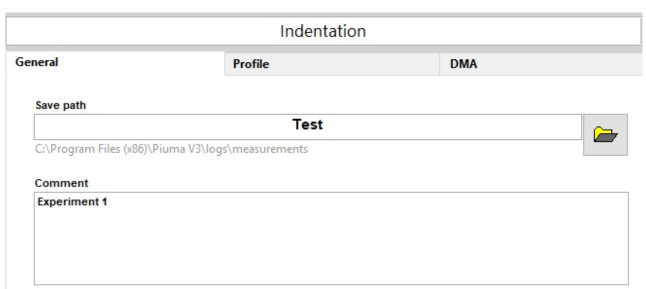


Figure 39: The General Tab of an experiment step allows you to change the save location of an Indentation or Matrix Scan step and t comment about the step performed.

4.9 Performing measurements

After configuring all parameters for the experiment in the configure experiment window, collecting of the data is relatively straightforward. Any saved or loaded experiment can be run by pressing the 'Run Experiment' button in the main window (Figure 40). Below, details on each of the different experiment steps are described in further detail. The measurement process remains the same for all three modes of operation.

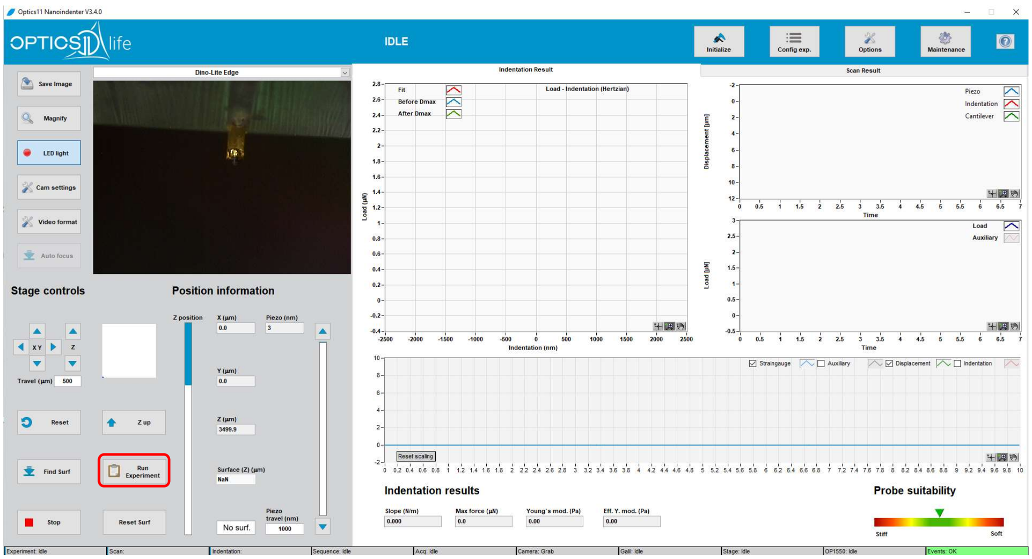


Figure 40: A sequence of steps specified in the Configure Experiment window can be run by pressing the 'Run Experiment' button from the main window.

4.10 Results

Indentation results tab

The indentation results section describes the results derived from the latest indentation. The parameters displayed here are slope, the maximum applied force, effective Young's Modulus, and the bulk Young's Modulus using a pre-configured Poisson's ratio (Figure 41).

To the right of the 'Indent results', an indicator shows the suitability of the probe for the sample: the indicator shows if the probe's cantilever stiffness matches up well with the sample (green), is suboptimal (yellow), or when you might need to consider using another probe (red).

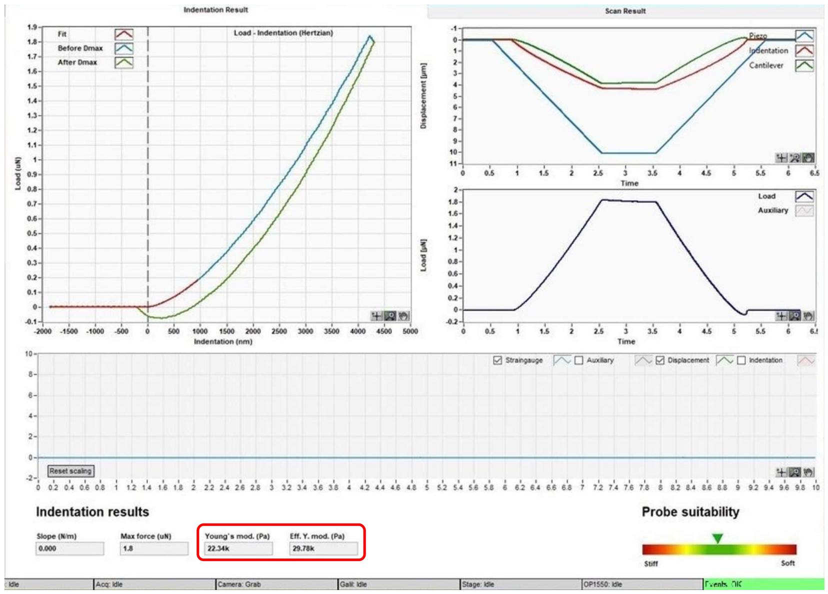


Figure 41: Indentation results after indenting a sample in D-mode.

Each graph can be saved directly to the hard disc by right-clicking on the graph and selecting 'Export' and 'Export simplified image'. Follow the instructions and the image can be saved.

The main window displays a live feed of the optical cantilever signal and the video camera feed provided by the microscope camera. An image of the video feed can be saved to the hard disc by clicking 'Save image'. The image can be magnified on the screen by clicking 'Magnify'. This feature is only available if the microscope camera feed is compatible with the Chiaro software. Otherwise, external visualization and acquisition software is needed to get images and video footage of the sample , usually provided with the microscope.

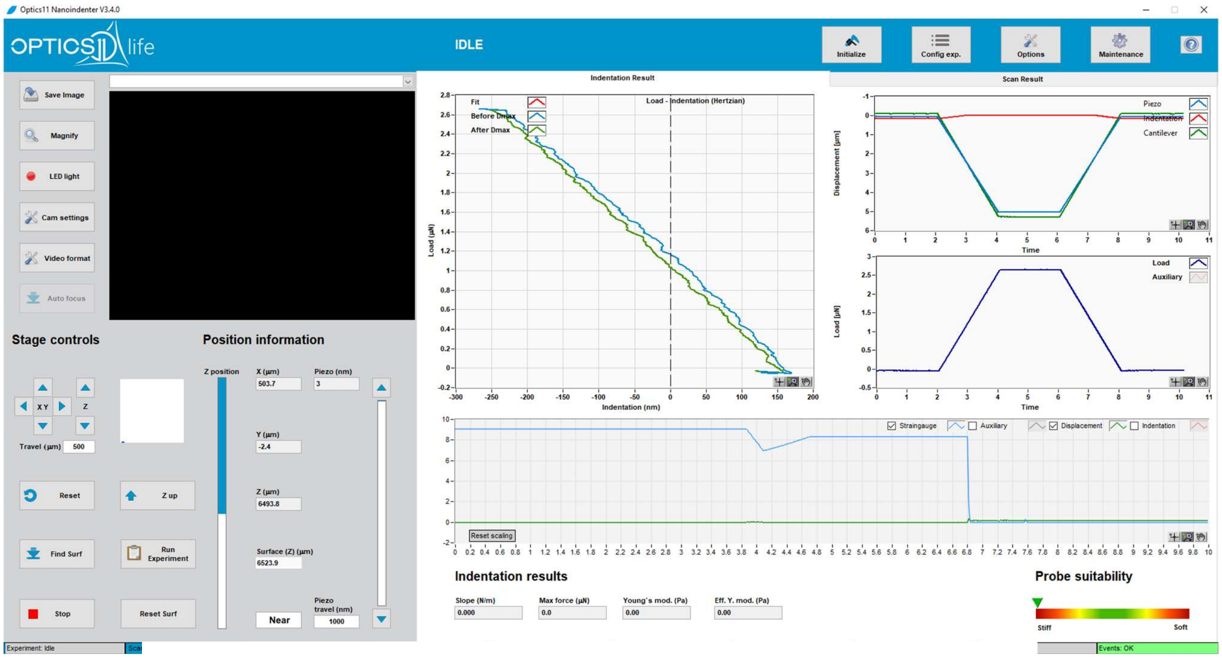


Figure 42: Signal monitoring during find surface.

The load-displacement window shows the load-displacement curve of the last indentation, in Displacement mode (Figure 41). The blue line shows the loading curve, and the green line shows the unloading curve. The red line indicates the polynomial fit used in the Hertzian model.

You can find the time data on the right side of the load-displacement window. The top graph contains probe displacement, cantilever bending, and indentation depth as a function of time. Following the displacement (Piezo) curve down, the cantilever starts bending at some point. This indicates the indentation starts of contact, which is preferred. Also, after the point of contact, the slope of the blue line should be distributed over the slope of the green and red lines as the initial displacement can only result in an approach of the sample, sample deformation, and cantilever bending.

The bottom graph shows the applied load as a function of time (Figure 41). Additionally, there is a possibility to activate an auxiliary graph by left-mouse-clicking on the 'Auxiliary' icon and enabling 'Plot visible'. This function is handy when an external signal source is used in parallel to the nanoindentation experiment.

Scan results tab

When a matrix indentation is performed, the 'Young's Modulus' tab shows a map representation of the Young's Modulus per coordinate. This map can be shown in 2D or 3D, and the minimum and maximum Z-axis values can be manually adjusted (Figure 43 and 44). The 'Show Young's button allows converting the effective Young's Modulus to the bulk Young's Modulus using the preconfigured Poisson's ratio. The histogram displays the matrix scan results, and subsequent single indentations, if any. The number of bins can be adjusted manually to gather a better overview of the distribution of the results.

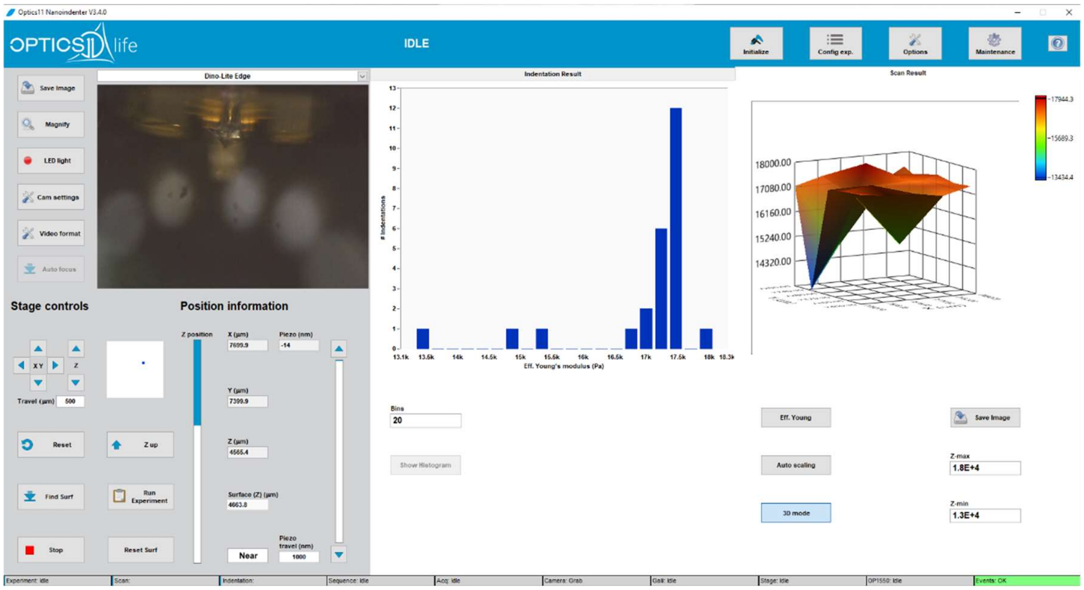


Figure 43: The scan results tab shows a 5x5 2D plot and a histogram.

Figure 44: Results tab showing a 5x5 D plot and a histogram.

4.11 Data processing and analysis

All the load-displacement data is stored in a tab-separated text file for every single indentation and indentation in a matrix scan. By using this text file, the load-displacement data can be imported into any program for post-processing. Also, the Chiaro Dataviewer program quickly load all data and make minor adjustments where necessary.

For each matrix scan, a tab-separated text file is saved with a matrix of the Effective Young's Modulus per coordinate. This allows one to quickly recreate Young's Modulus map in any other software program. Additionally, per indentation, a separate text file is saved that contains all information for this indentation. The header of the file contains the absolute coordinates, the automatically defined surface position, the probe parameters used, the calibration factor applied, the displacement profile used, and some variables that are extracted from the load-displacement curve, such as maximum load (P_{max}), maximum displacement (D_{max}) and (Eff.) Young's Modulus.

```
Date    27/02/2020      Time    11:28:55        Status  OK
Petrisoft
Scan (#)        1       X (#)   1       Y (#)   1       Indentation (#) 1

X-position (um) 0.000
Y-position (um) 0.000
Z-position (um) 6662.281
Z surface (um)  6662.281
Piezo position (nm) (Measured)  0.4

k (N/m) 0.450
Tip radius (um) 10.000
Calibration factor      0.902
SMDuration (s) 9.8

Device: Piuma
Software version: V3.1.2

Control mode: Indentation
Measurement: Indentation

Profile:
D[Z1] (nm)      1000.000        t[1] (s)        2.000
D[Z2] (nm)      1000.000        t[2] (s)        1.000
D[Z3] (nm)      0.000   t[3] (s)        2.000

Model: Hertz
P[max] (uN)     0.148
D[max] (nm)     0.000
D[final] (nm)   0.000
D[max-final] (nm)       0.000
Slope (N/m)     0.000
E[eff] (Pa)     19034.223
E[v=0.500] (Pa) 14275.668
Comment:

Time (s)        Load (uN)       Indentation (nm)        Cantilever (nm) Piezo (nm)      Auxiliary
0.000000        0.002676        0.000000        5.947185        33.473133       1.398156
0.001000        0.002624        0.000000        5.830179        33.657609       1.397520
0.002000        0.002606        0.000000        5.790796        34.088746       1.397997
0.003000        0.002373        0.000000        5.272859        34.921451       1.397997
0.004000        0.002282        0.000000        5.070264        35.872202       1.397758
0.005000        0.001901        0.000000        4.224580        36.799542       1.397679
0.006000        0.001794        0.000000        3.985675        37.912261       1.397440
0.007000        0.001520        0.000000        3.377546        39.169951       1.398553
0.008000        0.001523        0.000000        3.384614        40.238303       1.398394
0.009000        0.001347        0.000000        2.992670        41.221446       1.397679
```

Figure 45: Typical indentation file showing the header lines and the start of the five data columns describing the indentation curve.

5. OPTIMIZING INSTRUMENTAL SETUP

Optimizing the experiment conditions and sample mounting can significantly improve the quality of the indentation. During an indentation, environmental stability, sample stability, and probe suitability define the quality of the experimental dataset. If the indentation results show irregularities or high noise, it is worth considering the factors described below.

5.1 Environment stability

The Chiaro Nanoindenter operates independently from the environment. Nonetheless, it is still possible for environmental conditions to influence and compromise measurement results.

Temperature

Sudden room or medium temperature changes should be avoided when measuring to minimize the measurement error due to temperature drift. Verify the Chiaro is mounted away from any air-conditioning, heating, or ventilation vent.

Vibrations

To minimize measurement error due to mechanical disturbances, such as ground vibrations from other labs, other equipment, or appliances, ensure the Chiaro Nanoindenter is mounted on a stable bench, or isolated optical table and all the interfaces from the probe to the microscope stage are as stiff as possible. Preferably the bench kept fromother vibrating machines such as vortexes, pumps, or large fans. For best performance, install the Chiaro Nanoindenter with the microscope on top of a stabilization platform, such as an air table or spring table.

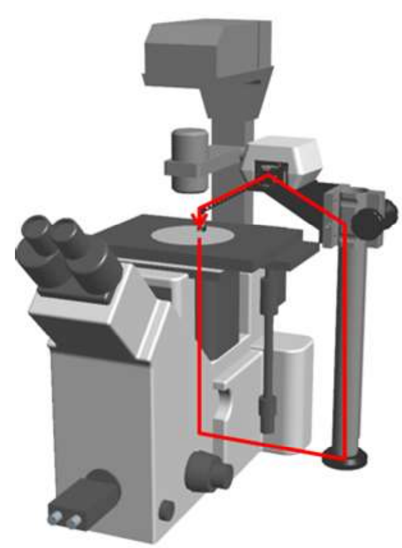


Figure 46: Chiaro measurement loop

Mechanical instability can also originate from the sample container or sample substrate contact with the microscope stage. To get the best measurement possible, the complete 'measurement loop', so everything between the probe and the microscope stage with the sample container on top should be as stiff and stable as possible (this can be achieved by ensuring good and stable contact between the sample substrate or container and the microscope stage, eliminating soft interfaces such as the rubber feet of a microscope and by using stiff substrates and containers. Plastic sample containers, such as Petri dishes, may be used, but it is advised to check the stability of the dish when placing it on the microscope stage: If the dish rocks when pushing gently on edge, it is unstable, and an alternative sample mounting is advised.

5.2 Sample stability

Additional to the environment, the sample itself can also introduce a measurement error.

Sample temperature and integrity

When attempting to measure samples that come fresh out of the incubator, let the sample temperature stabilize to room temperature before initiating the measurement.

Also, the sample itself can have unstable mechanical properties. Mainly this is caused by a structure of the sample material. Therefore, when measuring a highly heterogeneous sample, such as decellularized ECM, porous scaffolds, or 3D-printed structures, extra care should be taken in selecting the appropriate cantilever stiffness, tip size, and indentation settings. For example, when working with samples with porous surface, selecting a tip diameter that is much smaller or larger than the average pore size avoids having the tip trapped in the sample.

Sample immobilization

Next to a stable sample, it is important to immobilize the sample to eliminate lateral and height drift during and between indentations. Indenting drifting or floating samples results in unreliable indentation measurements. Especially when performing automated matrix scans ensure the sample is immobilized correctly.

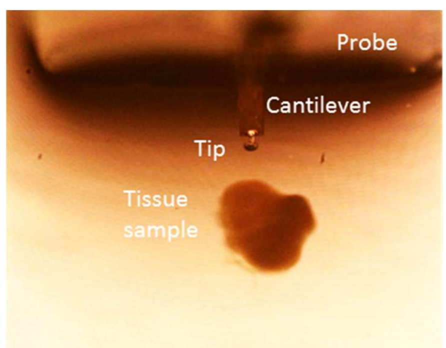


Figure 47: Microscope camera view of a living immersed micro-tissue on a microscope slide. Extra care should be taken when measuring samples that cannot be glued or weighed down.

5.3 Probe stability

The probe may be unstable if subjected to sudden temperature changes or if an air bubble is trapped near or on the cantilever upon immersion in a liquid.

Solution temperature

When immersing the probe in a liquid of a different temperature, such as refrigerated liquids or liquids obtained from the incubator, wait a few minutes upon immersion to let the probe adjust to the new temperature. This can be observed by the interferometer signal: if the voltage changes a lot when the probe is immersed, this is a sign the probe is still adjusting to its new environment.

Air bubble

As the probe and cantilever are made of glass, it can occur that an air bubble gets trapped between them. This will result in a failed probe calibration or excessive noise in the measurements. An air bubble on the probe can be detected by observing the probe through the side of a petri-dish while immersed in a transparent medium (Figure 48).

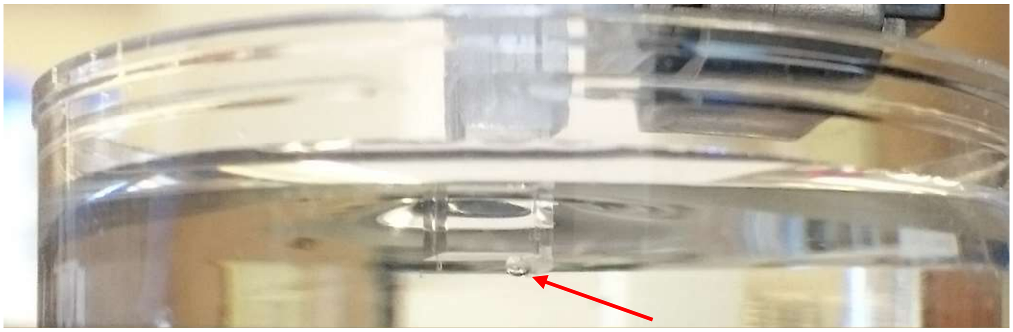


Figure 48: Side-view of a Chiaro probe while immersed in a transparent medium. As indicated by the red arrow, an air bubble is present at the base of the cantilever.

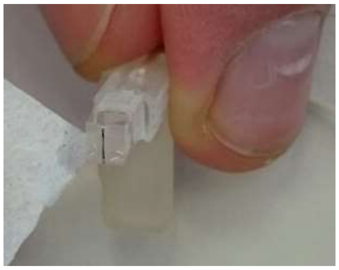


Figure 49: Removing an air bubble by drying the probe.

To remove an air bubble, lift the probe out of the medium and carefully take it out of the probe mount. Now draw the liquid out of the cantilever-probe gap by holding a tissue to the side of the probe. Once the bubble pops, the surface tension is released, and when immersed again, the air bubble will not form. Note that you should avoid directly touching the cantilever. The cantilever is very fragile and can easily break when touched. Holding the tissue at a safe distance will also allow you to dry the probe without touching the cantilever (Figure 49). How to avoid trapping an air bubble when inserting the probe into a solution is being described in the next chapter 5.3.

Suitable probe cantilever stiffness

To perform successful indentation experiments, a probe cantilever with a suitable stiffness compared to the sample is necessary. When the probe cantilever is too soft or too stiff, there will be too little indentation or cantilever bending, respectively, likely resulting in inaccurate measurements. The probe indicator in the bottom-right corner of the software indicates the suitability of the probe in use. Please see the probe selection guide for more information on selecting the appropriate probes for your experiments.

Suitable probe tip size

Choosing an appropriate tip size is related to the structure of the sample and the kind of experiment you wishe to perform. For example, when measuring the global elasticity of a sample, a larger tip would be more suitable since each indentation's applied and recorded force would effectively average over a larger area than for a small tip. On the other hand, for measuring small spatial features or a very rough sample, a smaller tip could be more suitable. Tip size should be carefully considered when working with porous materials. To determine the overall structural properties of a porous material, select a tip size several times larger than the largest pore. To examine local features within one pore, select a tip size several times smaller than the pore diameter. A probe selection calculator is available on request as

Adhesion

Adhesion between a sample and the indentation tip could interfere with the find-surface procedure or affect the measurement result. There are three solutions for measuring adhesive samples: (1) immerse the sample in a liquid; (2) passivate the sample using non-adhesive coating, for example, bovine serum albumin (BSA); or (3) use the 'Adhesion mode', to adjusted in the Chiaro software's 'Advanced' tab within the Option' menu. It pulls the cantilever free from the surface to a desired height and duration after detecting the surface using the find-surface function (see figure below).

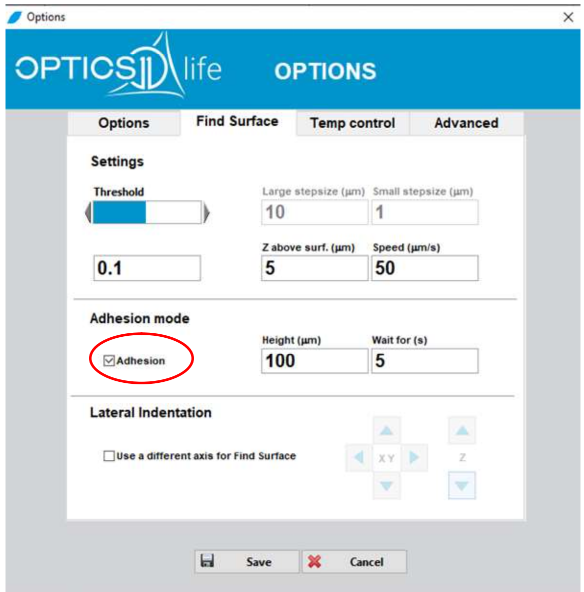


Figure 50: Adhesion mode – with adjustable height and time to get out of contact with an adhesive sample surface.

6. WORKING PRINCIPLE OF THE CHIARO NANOINDENTER

The goal of performing indentation experiments with the Chiaro is to measure the mechanical properties of a sample under study. These properties include Young's Modulus (or modulus of elasticity) and visco-elastic properties. These experiments are performed by gradually deforming the sample while accurately measuring the reaction force. The mechanical properties are derived using mechanical models from the load-displacement curves, where the load is plotted against the sample deformation.

6.1 Principle of operation

The Chiaro Nanoindenter uses proprietary optical probes in combination with the Optics11's OP1550 interferometer, to precisely detect sample deformation by detecting cantilever bending. The Chiaro's unique probes are at the heart of what makes the Chiaro instrument so easy to operate and provide unparalleled accuracy and precision at the same time. The next paragraphs briefly describe the physics behind this optical probe design.

Interferometric displacement sensing

The Chiaro interferometer contains a laser source, of which light is coupled into the optical fiber. The laser wavelength, 1550nm, is highly defined (a narrowband source). The light is trapped inside the optical fiber by a refractive index difference between the core and the cladding. As the fiber terminates in the probe, a sudden change in refractive index (fiber-to-air/medium) occurs. This causes the interface to reflect a part of the light into the fiber. The remaining light is coupled to the air or medium and is reflected by the next interface, the cantilever. This reflection is also coupled back into the fiber.

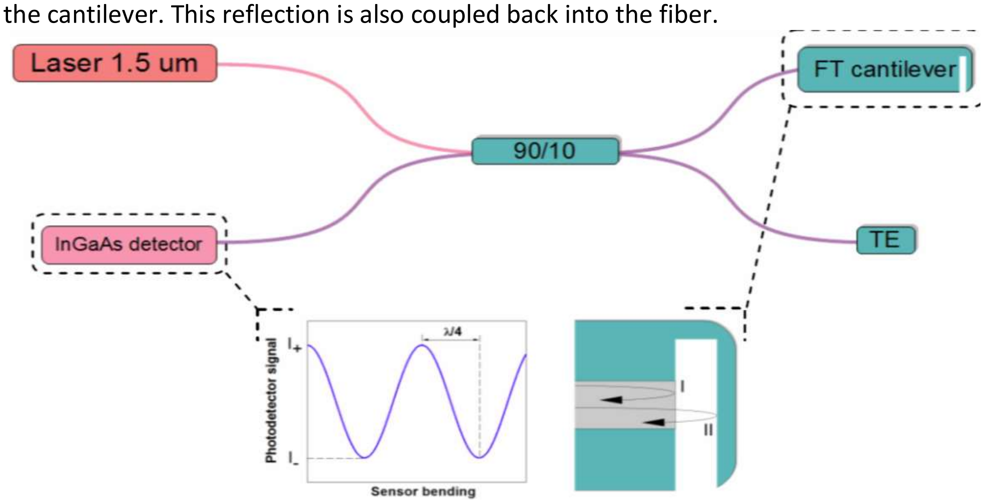


Figure 51: Simplified optical scheme of the Chiaro Nanoindenter.

The two back-propagated waves interfere with each other, of which the photodiode detects the resulting intensity. Whenever a phase shift between the two reflecting interfaces occurs, the resulting interference will also change, resulting in a higher or lower total intensity at the end of the photodetector. A sinusoidal intensity pattern at the end of the photodetector can be observed for a continuous phase shift of these two interfaces. Read more about it in this publication: https://pubs.rsc.org/--/content/articlehtml/2016/sm/c6sm00300a

The Chiaro optical indentation probes

The optical probes used in the Chiaro Nanoindenter are manufactured in Amsterdam and consist of an optical connector, an optical fiber, a probe holder, and an indentation probe, all in one piece. The cantilever stiffness and tip size of the probe are calibrated at Optics11 Life, and the values are provided on the probe's container.

Since the optical probe, the cantilever, and the tip consist of glass with resistant glue interfaces, the whole probe can be immersed in any solution that does not affect glass or HT-epoxy. Usually, before bringing the probe into first contact with the solution in which the calibration is going to be performed, it is highly recommended to pre-wet the probe. Applying a few droplets from around 1 cm above the cantilever significantly reduces the chance of trapping an air bubble, as described in the previous chapter(Figure 52, right).

Often applications involve salt solutions in which a sample, such as a cell-seeded scaffold, is immersed and needs to be characterized in that state. The Chiaro can perform this experiment, as the whole probe can be submersed, eliminating the need to compensate for surface tension or medium interface effects. In addition, this enables cleaning the probe with demi-water or solvents, such as isopropyl alcohol or ethanol, to sterilize and re-use the probes

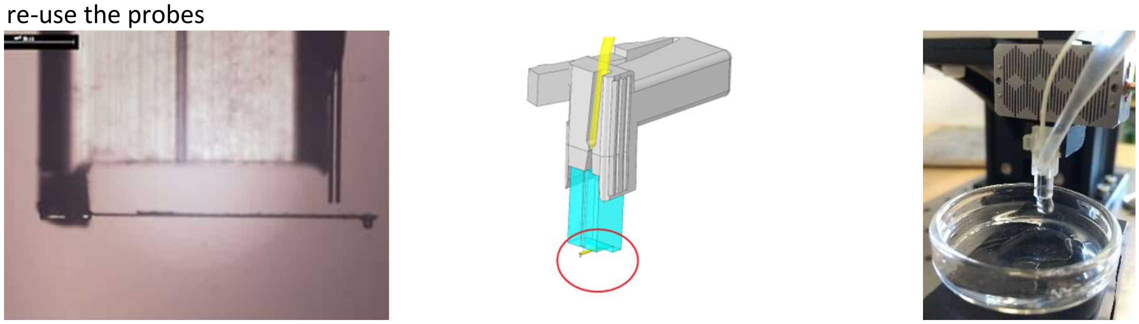


Figure 52: Side-view of a probe (left) with schematic picture, sensitive part (middle, red), and indentation probe being pre-wetted before brought into contact with the measuring liquid (right).

6.2 The measurement and calculation of the Young's modulus

The determination of Young's modulus relies on the measurement of load-indentation curves, which can be analyzed by different material models. For example, for elastoplastic materials, the unloading part of the curve can be fitted by software with the so-called *Oliver&Pharr model*[2,3,3], which excludes any plasticity bias

[2] W.C. Oliver, G.M. Pharr, An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation J.Mater. Res. Vol. 7 No. 6 1564 (1992)
[3] W.C. Oliver, G.M. Pharr, Measurement of hardness and elastic modulus by instrumented indentation: Advances in understanding and refinements to methodology J.Mater. Res. Vol. 19 No. 1 3 (2004)
[3] Fischer-Cripps, A. C. "A Review of Analysis Methods for Sub-Micron Indentation Testing." *Vacuum* 58, no. 4 (September 1, 2000): 569–85. https://doi.org/10.1016/S0042-207X(00)00377-8.

from the results. However, a more direct *Hertzian contact model* can be used for purely (visco-) elastic materials.

Note regarding maximum indentation depth:

1. The maximum indentation depth should be at most 16% of the tip radius[4]. This is the Hertz model assumption of the parabolic indenter used to calculate the contact area during indentation: a=sqrt(h*R), a - contact radius, h - indentation depth, R - tip radius.
2. The maximum indentation depth should be 5-10% of the sample thickness to not sense the substrate underneath the sample. Furthermore, the substrate should be much stiffer than the sample itself.
3. The sample is also assumed to be an infinite half-space; thus, tip size should be chosen so that this assumption is true.
4. When performing indentation mapping, the step size should be at least two times the contact radius a to avoid oversampling, a=sqrt(hR) where h is indentation depth and R – sphere radius.

Regarding the tip size, you should balance between the spatial resolution of the mapping, which depends on the heterogeneity of the sample, and the scale of the measurement in terms of depth. Smaller indentation depth is desirable if you want to sense surface properties. To sense more bulk/averaged properties, you should consider measurements at larger depths with a larger radius. However, when using larger spheres, step size needs to be higher, and thus, you lose spatial resolution during indentation mapping.

Oliver and Pharr / Field and Swain

This method derives Young's modulus from the slope of the unloading part of the -load-indentation curve, where deformation during unloading is assumed to be elastic, indenter tip radius and final indentation depth using the following formula:

$$E_{eff} = \frac{S/2}{\sqrt{R_i} \cdot \sqrt{h_{max} + h_r}}$$

[4] Lin, David C., David I. Shreiber, Emilios K. Dimitriadis, and Ferenc Horkay. "Spherical Indentation of Soft Matter beyond the Hertzian Regime: Numerical and Experimental Validation of Hyperelastic Models." *Biomechanics and Modeling in Mechanobiology* 8, no. 5 (October 2009): 345–58. https://doi.org/10.1007/s10237-008-0139-9.

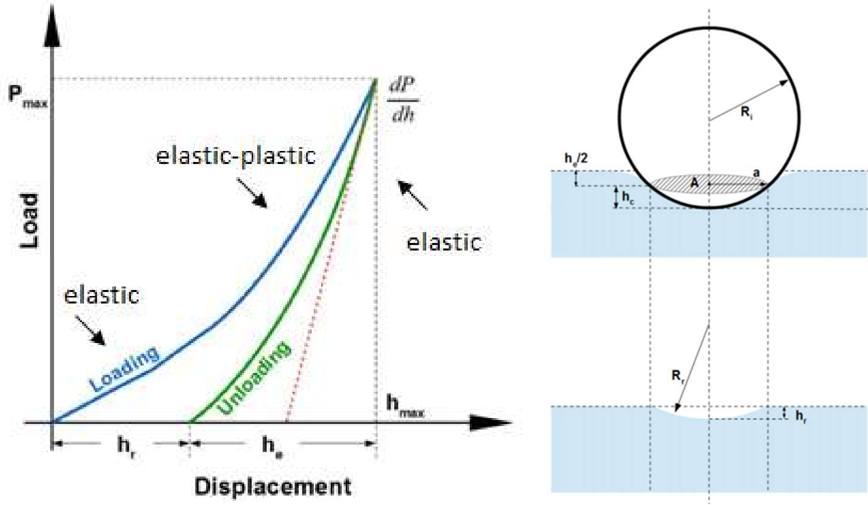


Figure 53: Typical load-displacement curve of an indentation (left). The 'unloading' curve i derives Young's modulus by applying the Oliver and Pharr method. Schematic imprint made by a spherical indenter after the loading and unloading cycle (right).

E_{eff} represents the effective Young's modulus; R_i is the spherical tip radius; S is the slope at the maximum indentation; and h_{max} and h_r are the maximum indentation depth and final contact depth, respectively. To optimize the retrieval of Young's modulus from the unloading curve, the software allows one to set the range of data points to be included in the slope estimate relative to the maximum load in the unloading phase (default setting = 60% to 80% of the maximum load on the unloading curve). For more details, please find our whitepaper on nanoindentation on our website.

Hertzian contact

The calculation of the effective Young's modulus by considering the Hertzian contact model follows the fit of the loading curve to the following equation:

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

Hertz's model assumes material to be purely linear elastic. The software evaluates the goodness of the fit by calculating the root mean square error (RMSE) and normalized root mean square (NRMSE).

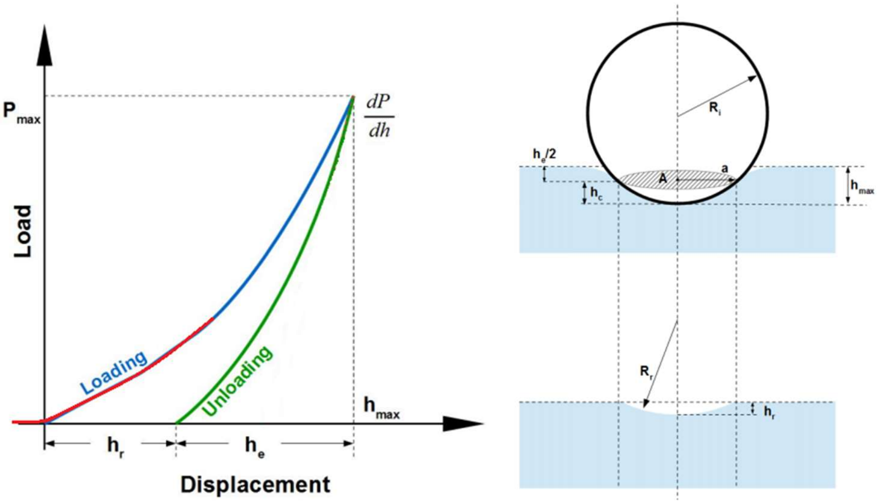


Figure 54: The loading curve derives the effective Young's modulus from the Hertzian contact (left). A sphere of radius R_i indents an elastic half-space to depth h_{max}, and thus creates a contact area of radius $a = \sqrt{R_i h_{max}}$ (right).

Effective vs. bulk Young's Modulus

In both models, the analysis returns the effective or reduced Young's modulus (E_{eff}), in which the Poisson ratio has not been considered. The Chiaro software also enables directly calculating the 'bulk' Young's Modulus (E), for which a Poisson's ratio ν can be set in the software (default is 0.5 for perfect incompressible materials). If the Poisson's ratio of the sample is unknown, it is advised to report the effective Young's modulus (E_{eff}) since the Poisson's ratio can have a slight impact on the calculated Young's modulus (E).

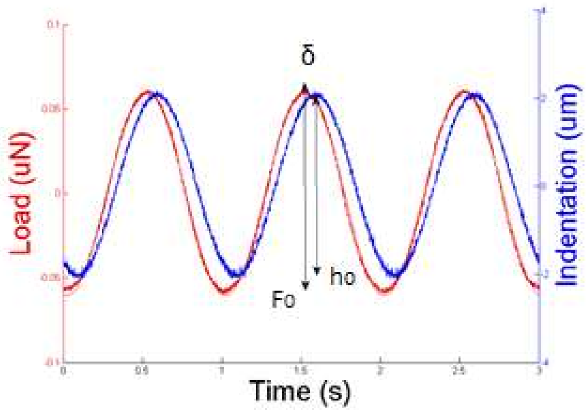
$$E = E_{eff}(1 - \nu^2)$$

DMA Analysis

Dynamic Mechanical Analysis (DMA) uses a cyclic motion with frequency, f, while controlling displacement or load with an amplitude of h_0 or F_0, respectively. The frequency-dependent storage modulus, E', and loss modulus, E'', are calculated with the following equations:

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

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



where δ is the phase lag between oscillations of indentation and load. tan(δ) is the dissipation factor, which is the ratio between loss and storage modulus. 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, so that $\frac{h_0}{h} \ll 0.25$. The phase lag is 0° for a strictly elastic material (tan(δ)=0), 90° for a purely viscous material (tan(δ)=undefined), and in-between 0° and 90° for a viscoelastic material. For most biological materials, tan(δ) is between 0 and 1 (δ between 0° and 45°), which means that the material behaves as a viscoelastic solid rather than a viscoelastic liquid. After gathering the frequency-dependent storage and loss moduli, they can be fit to lumped parameter rheological models to derive material viscoelastic constants.

7. THE OP1550 INTERFEROMETER

The interferometer operates as a stand-alone device and can be switched on or off whenever the Chiaro Nanoindenter system is on stand-by. The OP1550 interferometer contains a tunable laser source, modulation options, a high-speed photodiode, and data acquisition electronics.

The OP1550 interferometer has five buttons on the front panel that allow you to navigate the menus and select and alter menu items. To select a field or button on the OP1550 screen, move the cursor over the button or field and press the center button (enter). If a field is selected, the field turns blue and the up and down buttons can be used to alter the value the cursor highlights.

The interface is built from two menus. The first menu (Measurement, Wavelength scan, Demodulation, and Settings) can be accessed using the buttons on the top of the screen. The second menu (Laser, ADC, Output, Modulation, and Maintenance) can be accessed by scrolling from left to right while keeping the cursor on the right-hand side of the screen.

Figure 55: The OP1550 interferometer.

7.1 Using the OP1550 interferometer

The Measurement, Wavelength, Demodulation, and Settings menu

The ‘Measure’ tab displays the intensity that reaches the photodiode, which converts this intensity into a voltage.

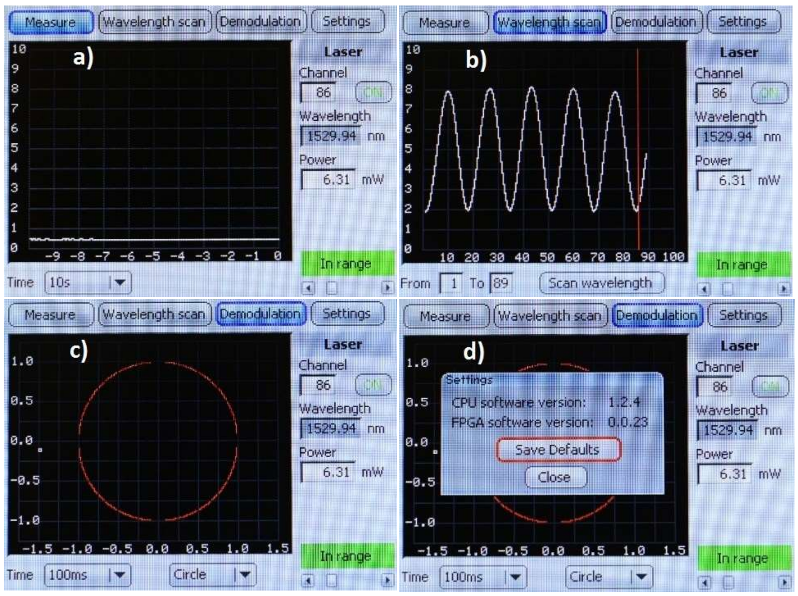


Figure 56: Measurement (a), Wavelength scan (b), Demodulation (c), and Settings menu (d) of the OP1550 interferometer.

The ‘Wavelength scan’ menu shows the resulting interference after a wavelength scan in which the laser can be tuned over ~50 nm. This automatic tuning is performed by pressing the ‘Scan wavelength’ button at the bottom of the screen in the ‘Wavelength scan’ menu. A correct wavelength scan results in a sinusoidal curve as shown in Figure 56b).

The ‘Demodulation’ tab displays the interferometric linearization, by aligning the collected data points to a unity circle. It can be handy for observing the cantilever bending, while manually approaching the surface of a sample or a stiff surface during the calibration procedure. When the tip touches a surface, the datapoint changes its position along the unity circle (Figure 57). When calibrating the geometrical factor, the probe must be in contact with a stiff surface; otherwise, the calibration fails. If the probe is not in contact after the find surface procedure, you can manually move the Z-stage in 1µm-steps down to bring the probe into contact.

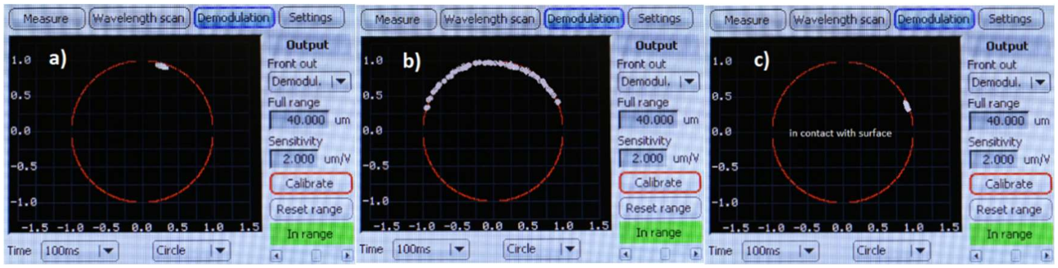


Figure 57: Datapoint changing its position after the tip is getting in contact with a surface: probe out of contact (a), noise caused by probe moving during automated find surface procedure or by manually moving the Z-stage (b), probe in contact with surface resulting in datapoint position change (c).

The 'Settings' menu allows changing all current parameters as default values. When restarting the OP1550 interferometer the last saved values will be loaded.

The Laser, ADC, Output, Modulation, and Maintenance menu

The right side of the screen is reserved for the Laser, ADC, Output, Modulation, and Maintenance menus. To scroll through the menus, move the cursor to the right section of the graph area, and press the left or right arrow.

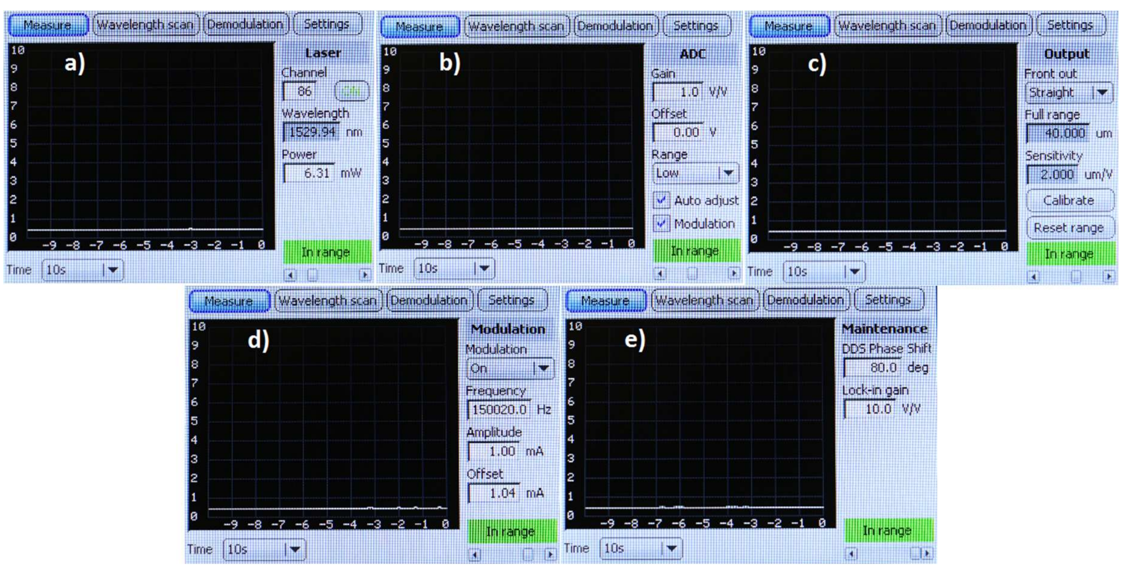


Figure 58: Laser (a), ADC (b), Output (c), Modulation (d) and Maintenance menu (e) of the OP1550 interferometer.

The 'Laser' menu displays the laser controls. Always operate channel 86 of the laser: this corresponds to 1530nm, which is also set in the maintenance software menu.
The 'ADC' menu displays the photodiode and data acquisition settings. Using the 'Scan wavelength' feature in the 'Wavelength scan' menu, the Offset and Gain values for converting the raw photodiode voltage to fit the 0-10V range are automatically performed. If the automatic procedure fails, the ADC menu allows the adjustment of the offset and gain settings manually. Also, the automatic adjustment of the offset and gain settings can be switched off by unticking the 'Auto adj.' box. If this is done, the 'Scan wavelength' function in

the Modulation menu will tune the laser without setting offset and gain values, which can be convenient for manually setting offset and gain values. Always ensure the 'Modulation' box is ticked before the experiment.

In the 'Output' menu, you can adjust the signal output at the 'Front out' either as a 'Straight', which displays the modulated signal, or as a demodulated by switching to 'Demodul.' The range and sensitivity parameters can be changed in the software maintenance's menu in the software, but keeping them at the default settings is recommended.

The 'Modulation' menu contains the controls to induce a small modulation on top of the laser output signal by quickly and continuously modulating the laser wavelength. This modulation is required for the Chiaro to function properly and should always be turned on when performing indentations. The modulation is active when the mode is set to 'On'; the menu will show the controls to change the frequency, amplitude, and offset of the modulation signal. In regular operation, these will not have to be changed.

In the 'Maintenance' menu the modulation phase shift and the lock-in amplification can be adjusted to certain parameters. However, the default settings are 80.0 degrees for the 'DDS Phase shift' and 10.0 V for the 'Lock-in gain' and should not be changed.

Adjusting Laser Power to improve the signal to noise ratio

The default setting of the laser power is 6.31 mW. In case the "Range" of gain is "High", you can increase the laser power manually which will decrease the range of gain to "low". As a result, the signal-to-noise ratio will be improved. See the video:

- https://youtu.be/coKxhkDsS6Q

Live calibration

You can turn on the "live calibration" function in the "Maintenance". This enables the real-time correction of scaling factors of the demodulation circle so that the signal stays on the demodulation signal and load-indention curves do not look wavy at large cantilever bending. This function is required when the wavelength scan is not horizontal but at angle like the image below.

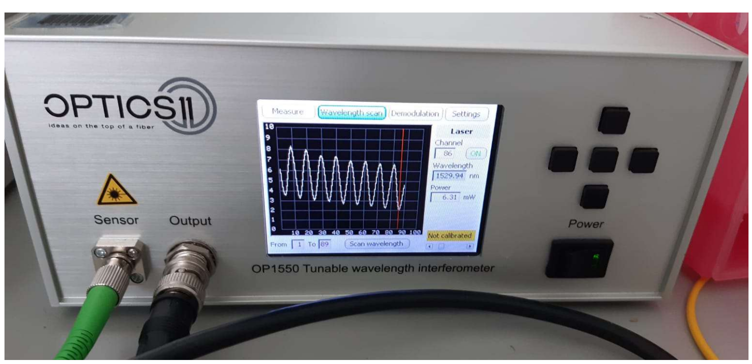


Figure 59: Wavelength scan with

"Live calibration" should be enabled before starting the calibration procedure. Furthermore, calibration should be done to no more than 3 µm piezo displacement (you can change that in Options).

Do not use “live calibration” if the samples are adhesive as it will cause a reset of scaling factors to an over-bended position. In general, better to use a stiffer probe for a very sticky sample or contact us for ideas on how to decrease the stickiness.

Please see the videos on how the demodulation signal looks with and without “live calibration”:

- https://youtu.be/1VL6cXOqMsE - live calibration on
- https://youtu.be/IPfTWs02yQ8 - live calibration off

8. FAQ & TROUBLESHOOT

Q1: The automated find-surface function fails to detect the surface automatically.
A1: The Chiaro looks for a voltage difference when using the find-surface function. If any problems occur with the find-surface function, please verify that the signal amplitude on the OP1550 is enough (absolute values 3-7 V minimum, or amplitude of 4V or higher). In addition, please check whether the piezo BNC cable is connected properly. If the problem persists, verify that the probe is not too stiff for the sample and probe contamination under a (stereo) microscope.

Q2: I only get 'noise' data after performing an indentation.
A2: Check if the 'Modulation' box in the ADC menu of the OP1550 is ticked. , Check the probe for contamination under a (stereo) microscope, if the problem persists.

Q3: I get more cantilever bending than displacement in the results (a backward sloping load-displacement curve).
A3: Verify that the probe is calibrated in the medium the probe is used in. In case this is uncertain, recalibrate the probe on a stiff surface. Also, please ensure the tip hits the sample prior to any other part of the cantilever hitting the sample. In case the problem persists, check the probe for contamination under a (stereo) microscope.

Q4: The Chiaro stage motors keep moving during the initial 'homing' procedure when starting the software.
A4: If the X, Y-stages fail to reach starting positions during the homing procedure, something obstructs the stage. Take off the protector stage or backlight module and check whether the X-Y stage is free to move.

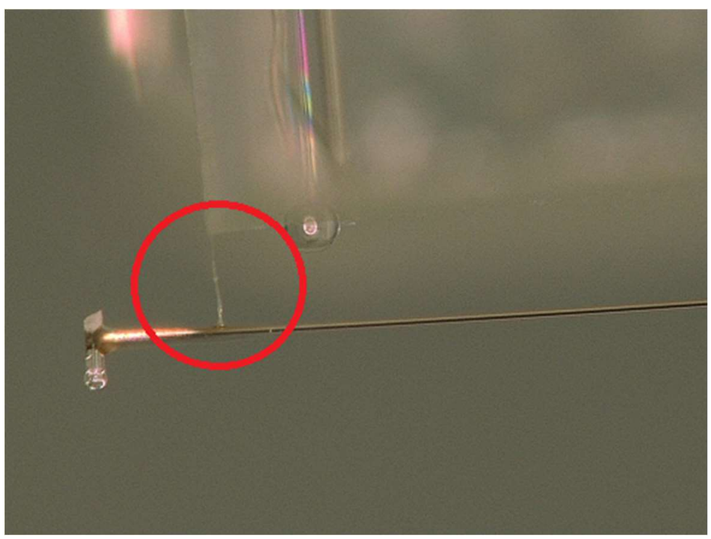


Figure 60: Example of contamination that compromises cantilever bending.

Changing fuses

The recommended fuse rating for 115V is 1A T (Tine delay type) Fuses marked F (Fast) will not work. Always replace both fuses if one is blown.

- US(115V): https://nl.farnell.com/eaton-bussmann-series/s505-2-5-r/fuse-antisurge-2-5a/dp/1123116?st=fuse%202.5A
- Europe(230V): https://nl.farnell.com/schurter/0034-3118/fuse-antisurge-glass-1-25a/dp/1360811

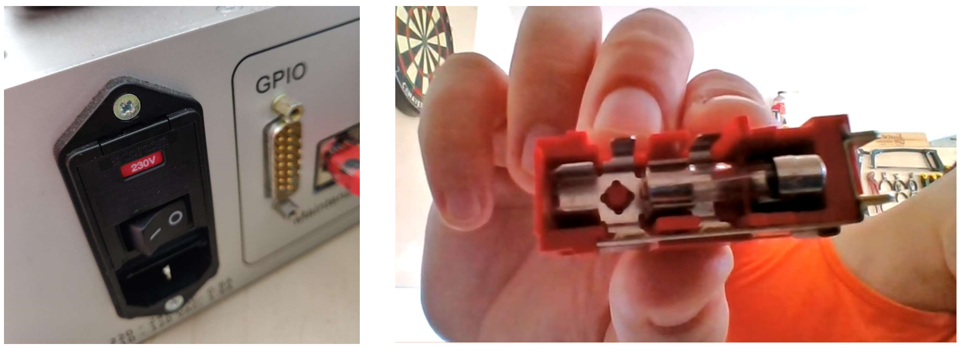


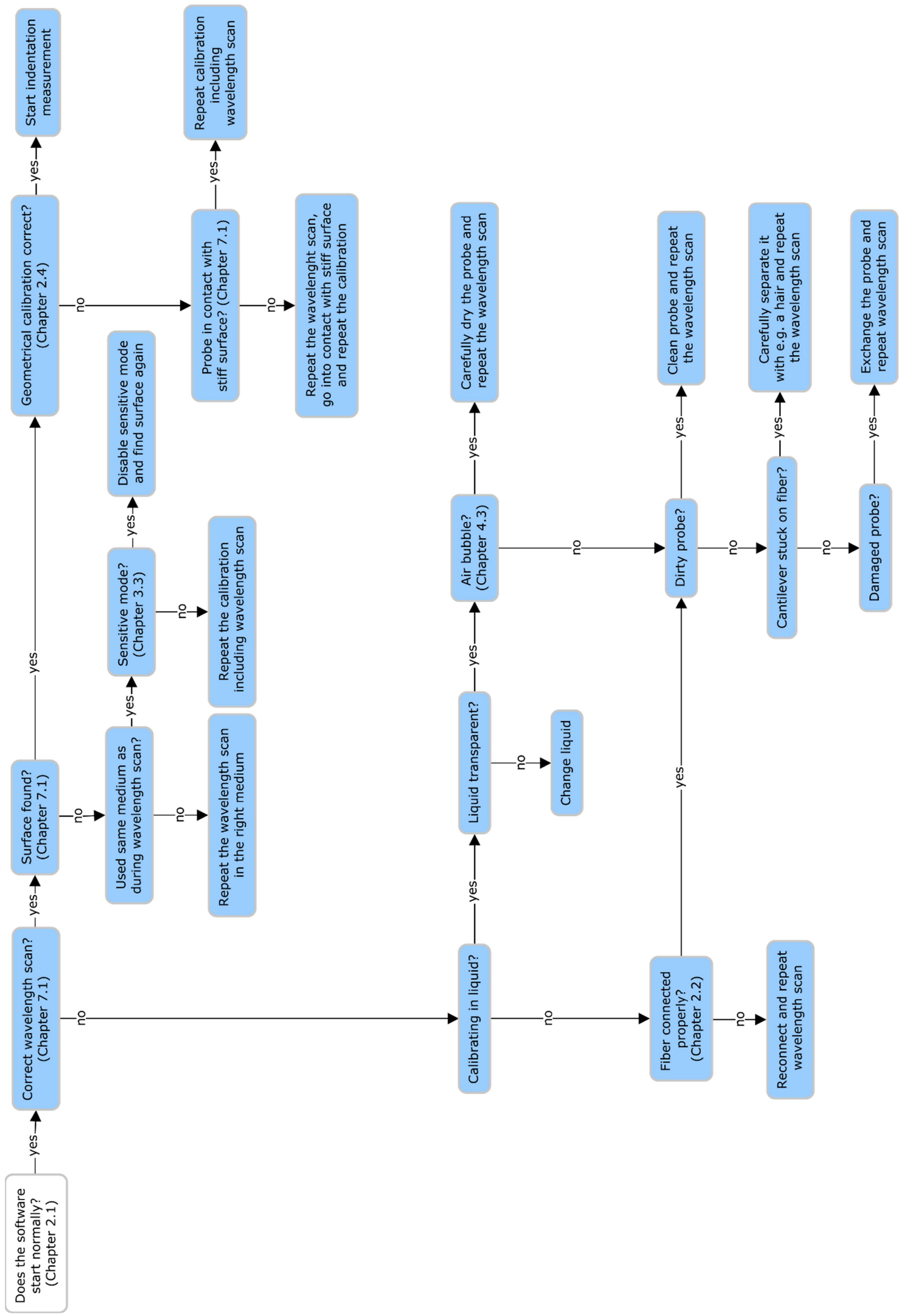
Figure 61: A 230V plug and a fuse.

Ensure to select the right voltage when putting the fuses back.

Flowchart - Calibration

Most issues while measuring with the Chiaro Nanoindenter are related to the instrument's calibration. A troubleshooting flowchart is presented on the next page to guide you through possible issues and solutions, to ensure a safe calibration of the Chiaro Nanoindenter and, therefore, correct instrument functionality.

Flowchart – Calibration



For troubleshooting, support or questions, while working with the Chiaro Nanoindenter, please visit us online at www.optics11life.com or contact us at:

Tel.: +31 20 5987917
E-mail: support@optics11.com

Office hours are between 8h and 18h, CET.

8. WARRANTY CONDITIONS

Warranty duration

All Optics11 Life instruments ship with at least 1-year of included warranty which can be extended purchasing of optional Service plans. For more information contact your local sales representative

Hardware Warranty

Warranty DOES cover the replacement and working hours spent repairing hardware defects and malfunctioning derived by causes that are clearly attributable to Optics11 Life. Optics11 Life will perform hardware repairs only at Optics11 Life premises, and instruments will have returned for diagnosis and repair.

Warranty DOES NOT cover damage caused to probes or derived by misuse of the equipment. Examples of damage derived by improper use of the equipment include and may not be limited to breakage of the probe cantilever and stage malfunctioning caused by water contact.

Software Warranty

Optics11 Life commits to support its proprietary software with frequent updates and bugfix patches which will be provided free of charge following the purchased service plan. Therefore a customer relying on the product warranty will receive software updates for 1 year while silver and gold customers will get the update for 3 and 5 years respectively.
Bug reporting can be issued via the website or by email at support@optics11.com. Each report will be handled individually and remote support will be provided. Remote support is available from 9 a.m. to 6 p.m. CET. In addition, specific arrangements can be put in place for customers in different time zones.

For any inquiries on warranty conditions, contact support@optics11life.com