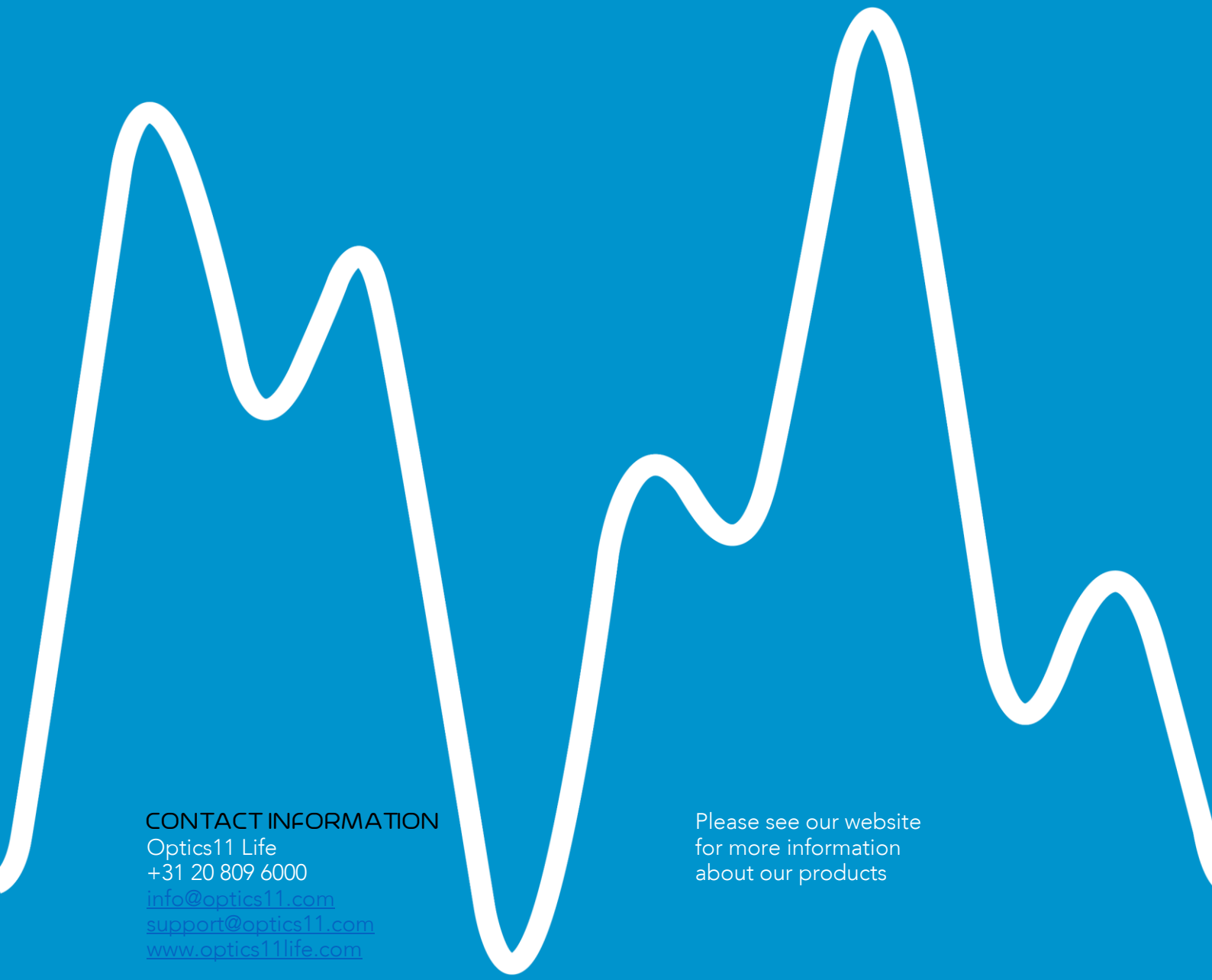
QUICK STARTUP INDENTATION

OPTICS11 life



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STARTUP STEP BY STEP

1. Turn on devices: PC → OP1550 → controller and then the software "Piuma" and check that all devices are working - at the bottom of the software it should say "Idle".

2. Home stages – make sure that the probe doesn't hit anything during this step.

3. Put the probe in the holder and plug in the fiber – make sure you hear the click sound.

4. *Optional*: Lower the probe down by moving the slider (make sure it is really at the lowest position of the slider) and position the probe in the air in the center of the camera by moving the stage motors in X and Y. Make sure that the probe is going to be able to reach the surface that you want to measure (you can check by moving the Z stage). You can also do positioning of the probe to the center of the objective or camera view at the later point (either in calibration dish or your sample dish) but you should be VERY CAREFUL to don't hit the edge of the dish with the probe.

5. Move the probe up with the slider and place the glass dish with the medium (the same one as your sample's medium) at the room temperature above the objective (thick glass petri dish).

6. Approach the glass dish – pipet to avoid trapping the air bubbles and submerge until you don't see capillary forces around the probe. Recommendation: add more medium into the dish to avoid hitting the bottom of the dish at this step.

7. Go to "Probe": fill in stiffness value from the box into k(N/m) and tip radius (um), not a geometrical factor.

8. Go to "Calib": press "wavelength scan". After the scan is finished – check how interference fringes look like in interferometer screen. If there are no fringes – the probe is probably broken or you did one of the previous steps wrong. If there are fringes, check whether "Range" is "low" or "high" in interferometer screen. If it is high, increase the "laser power" to 25mW and repeat the "wavelength scan".

9. Press "Find surface" in "Calib" – if it finds the surface too early (triggers on the noise), increase the threshold in "Options". Press down and up in 1um steps to check that the surface was really found (green cantilever line should make steps).

10. Press "Calibrate" – check that the piezo movement and cantilever deflection starts at the same time. If not – either increase the threshold in "Options" for "Find Surface" or manually move the piezo down at 1um steps until the probe is in contact (you can also see it by following the signal in the circle) and then press "calibrate".

11. Indent afterward on the glass (3000nm) to check that indentation depth is 0, cantilever and piezo overlaps. If not – go to "Options" and check that "calibration depth" is set to 3000nm, check that the surface and the tip are clean, check that only the tip is touching the surface.

12. The geometrical factor should be between 0.8 and 2.5. Divide the calibration factor written on the probe box by the refractive index of medium that you are measuring in, to get an approximate value. For example, in water: 1.5/1.33=1.12.

13. Press "Z up", pull up the slider, place the sample above objective (do this quickly- don't let the probe to dry in the air while changing the sample), pull down the slider without hitting the edge of your dish (you might have to move microscope stage with the sample) and submerge the probe. Press "Find surface" (check the setting of "Z above surface" in "Options"). If you don't see the probe in your field of view, move the probe up to be in the safe height and move the probe around in X and Y until you see it but be carefull to don't hit anything while moving around.

14. Position the tip of the probe above the area that needs to be measured. This will require moving the probe up and down from the focus plane. Make sure that only the tip will touch the sample during indentation and not the cantilever or probe holder or piezo.

15. "Configure experiment" set up the **displacement mode** profile to assess whether the stiffness of the probe is suitable for your sample. Make sure that your displacement is set to more than "Z above surface" in "Options". If the probe is too soft – cantilever bends as much as piezo moves and indentation depth is very low. If the probe is too stiff – cantilever doesn't bend and indentation depth is high. The bar in the right bottom corner gives you indication of probe suitability for the chosen indentation depth. If your load-indentation curve starts in contact – use "adhesion mode" or increase "Z above surface".

16. After the first assessment, you can work in **indentation or load control mode** by setting up the indentation depth or load to the values which were possible to reach during displacement mode. You can decrease the "contact threshold" value if measurements are overshooting the set depth/load and you can increase "contact threshold" value if feedback triggers on the noise (doesn't indent the sample).

17. During the measurements make sure that your signal is on the demodulation circle. If not, manually calibrate the circle by pressing "calibrate" on the interferometer window and tapping on the table "one circle of noise". If the signal is still not on the circle (tap table to check how the full circle looks like) – you need to tap stronger or less strong. If it is still not on the circle – recalibrate the probe on the hard surface. If one side of the circle is a line, you need to repeat the "wavelength scan" and this step. This might happen during temperature changes in your medium.

Few other notes:

18. If you are measuring very sticky samples and have an issue of signal not being on the circle, which gives a wobbly load-indentation curve – turn off "live calibration" in the maintenance (password: showme) and use stiffer probe so that you only need small cantilever bending for your measurements (below 5um).

19. If you notice any bugs in the software or you have some suggestions, please let us know. We will be very happy with your feedback.