PROBE CALIBRATION: PIUMA AND CHIARO

OPTICS11 life

CONTACT INFORMATION

Optics11 Life
+31 20 809 6000
info@optics11.com
support@optics11.com
www.optics11life.com

COMPANY ADDRESS

Optics11 Life
Hettenheuvelweg 37-39
1101 BM Amsterdam
The Netherlands

COMPANY INFORMATION

Optics11 B.V.
KvK/CC: 52469417
VAT: NL850459734B01
Amsterdam, NL

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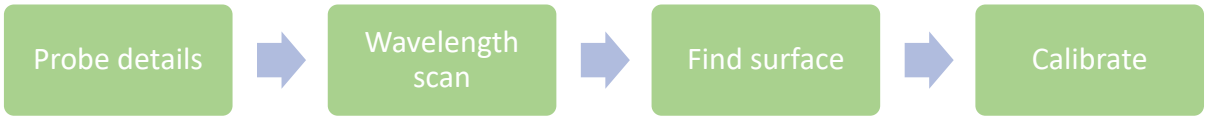

1. Input the probe parameters in the software suite to obtain meaningful measurement results. Probe parameters that need to be set in the program are the cantilever spring constant k (N/m) and probe tip radius R (µm). These parameters can be entered in the 'Initialize' (calibration menu), see Figure 1. 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. 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.[1]

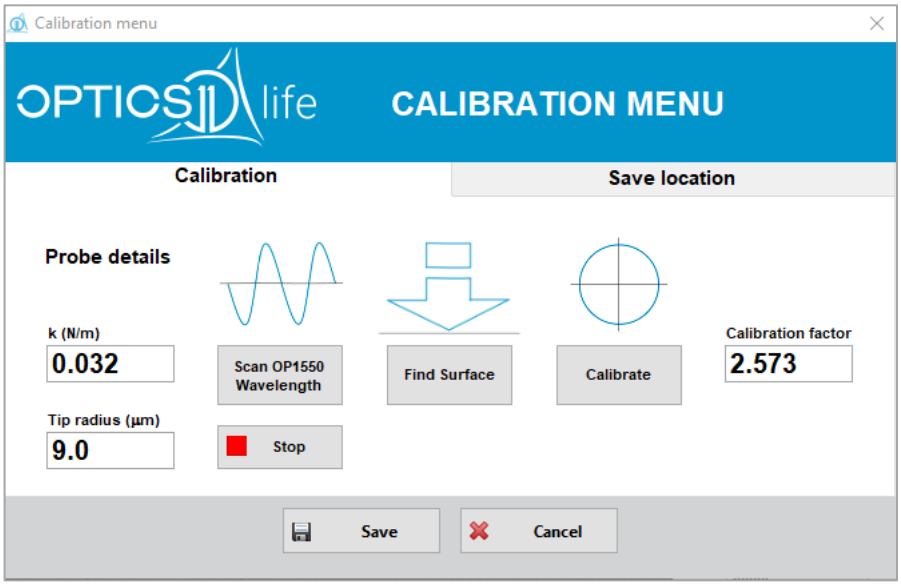

Figure 1: Probe calibration menu.

2. Move the probe to the calibration dish – a glass petri dish.
3. Make sure that the probe is prewetted.
4. Now you should move down the probe until the probe is clearly below the liquid level. If needed, fill the petri dish with more medium so that the probe is clearly submerged but not too close to the bottom of the well.

[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.

5. Wait a few minutes so that the probe can adjust to the new environment. When performing experiments with temperature control, the calibration should be accomplished at the same temperature as the measurement temperature.
6. 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 2 B (zoom in if needed). Wait until that is finished and check if no error (Figure 2 D) is reported on the interferometer screen or software. You can also check the result of the "Wavelength scan" in the corresponding interferometer window Figure 2 C.

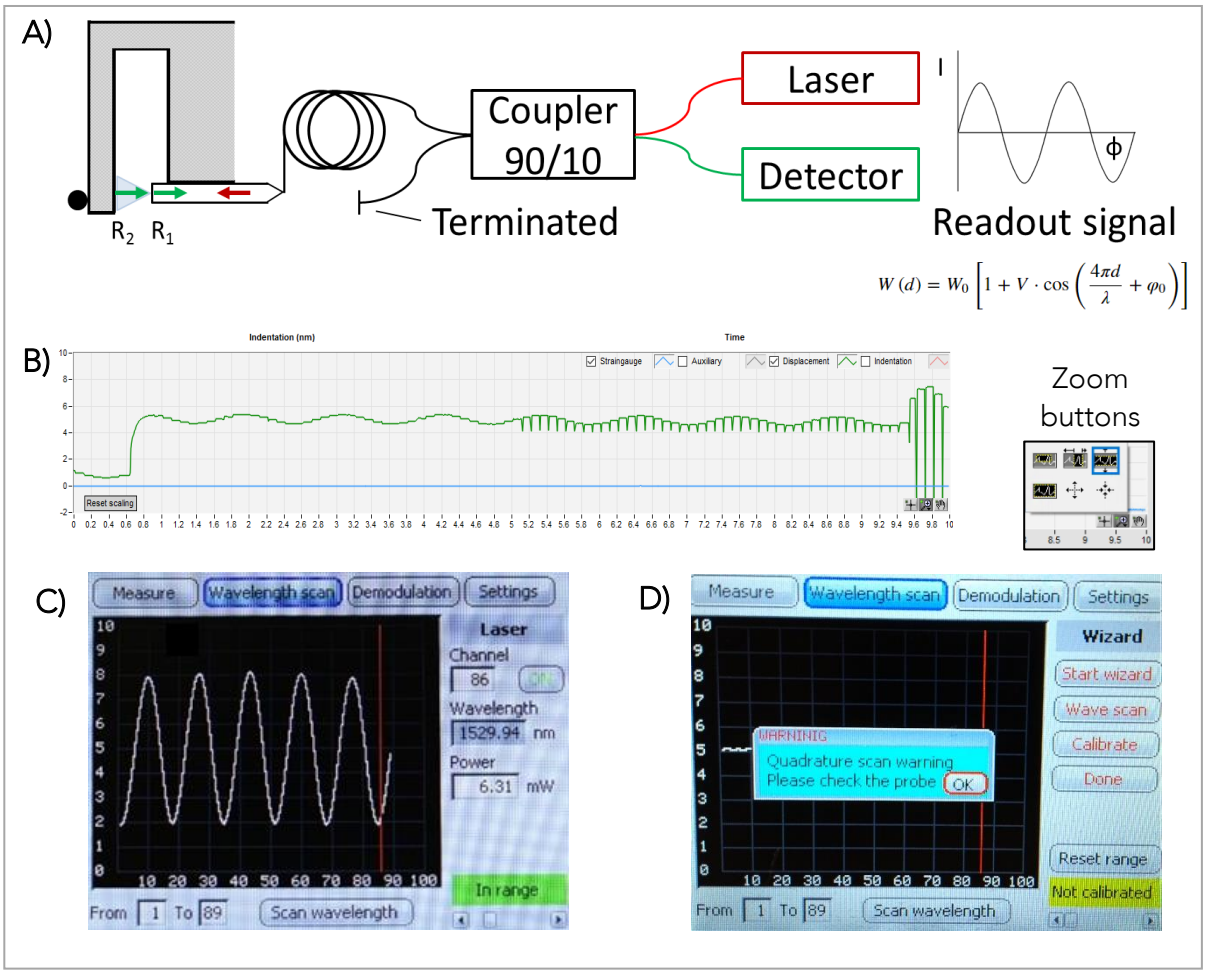

Figure 2: A) Working principle of the interferometer. B) Live wavelength scan signal, use buttons on the right bottom corner to zoom in and out. C) Good wavelength scan and D) failed one.

Principle: Laser light (~1550nm) is coupled to the single-mode fiber. The light is reflected from the two interfaces: the end of the fiber and the cantilever. The back-reflected light interferes, and the signal is measured in the detector. The interference signal is described by the formula in Figure 2 A where the signal is a cosine function of the gap size (between the cantilever and the fiber) and wavelength. It means that whenever the cantilever is bending or the wavelength of the laser is changing over time, interference fringes (cosine function) will be observed.

During the wavelength scan, the laser will sweep its wavelength rapidly from ~1565nm to ~1525nm and create an interference pattern. As the photodiode's output voltage depends on the intensity of the interference pattern, an automated offset and gain setting will be set,

which optimizes the photodiode output for the 0-10V range. When changing the medium or probe, a new wavelength scan needs to be performed to optimize the laser settings. [2]

Reasons for an error during wavelength scan:

- *Noise – get rid of any noise sources close to the instrument;*
- *No connection – check the fiber connections (unplug-plug);*
- *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 by moving the probe out of the medium and running medium over it;*
- *Cantilever stuck at the tip fiber – clean the probe or get it unstuck by touching with the tissue.*

Tip: for checking the probe, you can start by running the wavelength scan in the air before submerging it in liquid. If there is no signal in the air, proceed to the troubleshooting steps. For using ribbon-like probes in air, decrease the laser power to 6.31mW to avoid a saturated signal (Section 6.1, Pavone manual).

Repeat the wavelength scan until it's successful.

7. The next step is to press 'find surface'. The system will now start to continuously move down the probe stage while the piezo is fully extended and operates in a closed-loop. Once the cantilever bends by a threshold value set in the 'Options' menu (Figure 4), the stage will stop and the piezo will retract so that the probe is in contact with the surface. You can check if 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 when the cantilever is in contact with the sample (Figure 3). 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.

[22] Chavan, D. C., Watering, T. C. van de, Gruca, G. L., Rector, J. H., Heeck, K., Slaman, M. J., & Iannuzzi, D. (2012). Ferrule-top nanoindenter: An optomechanical fiber sensor for nanoindentation. *Review of Scientific Instruments*, *83*(115110). https://doi.org/10.1063/1.4766959

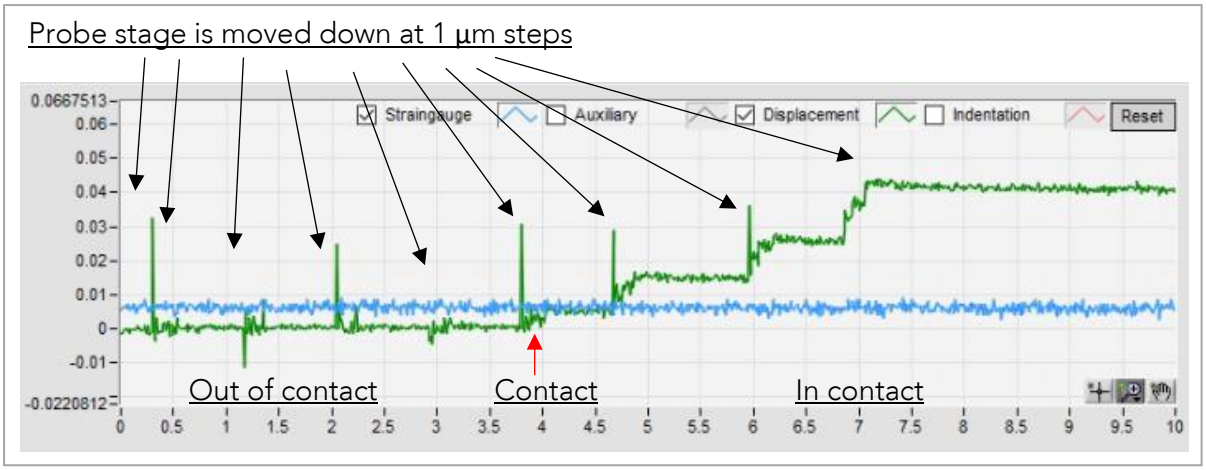

Figure 3 Manual finding of the surface position by pressing down the probe stage at 1 µm steps.

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 early. This early triggering of the surface is caused by a noisier cantilever signal during the stage movement due to the high force sensitivity of these probes. Decreasing the 'Speed (µm/s)' of 'find surface' will decrease the noise level as well. Settings can be found in "Options".

Caution: When the probe is calibrated in liquid, the functions such as "find-surface" and "Run Experiment" do not work when the probe is not immersed in liquid.

8. Next, the linearization of the interferometer signal (demodulation circle) and calibration of the cantilever arm, also called the geometrical factor, needs to be performed. Those two steps are accomplished in one calibration procedure. To perform this step, press the final button 'Calibrate' in the initialization menu.
9. During the signal linearization, the interference signal measured at the detector (Volts over time) is transformed into a linear signal of cantilever bending (µm over time), using the unit circle as a linearization tool. You can find the unit circle or so-called demodulation circle in one of the tabs of the LCD screen of the interferometer (Figure 4 E). Sensitivity is already set in the interferometer (µm/V) and used to translate Volts to µm.
10. The geometrical factor called the 'calibration factor' originates from the mismatch in the spherical tip position and the readout fiber position (Figure 4 A). By indenting on a stiff surface, the distance is measured by the fiber can be compared with the distance that piezo displaced. Taking the ratio between them gives a geometrical factor that is used to correct the cantilever signal by multiplying it.

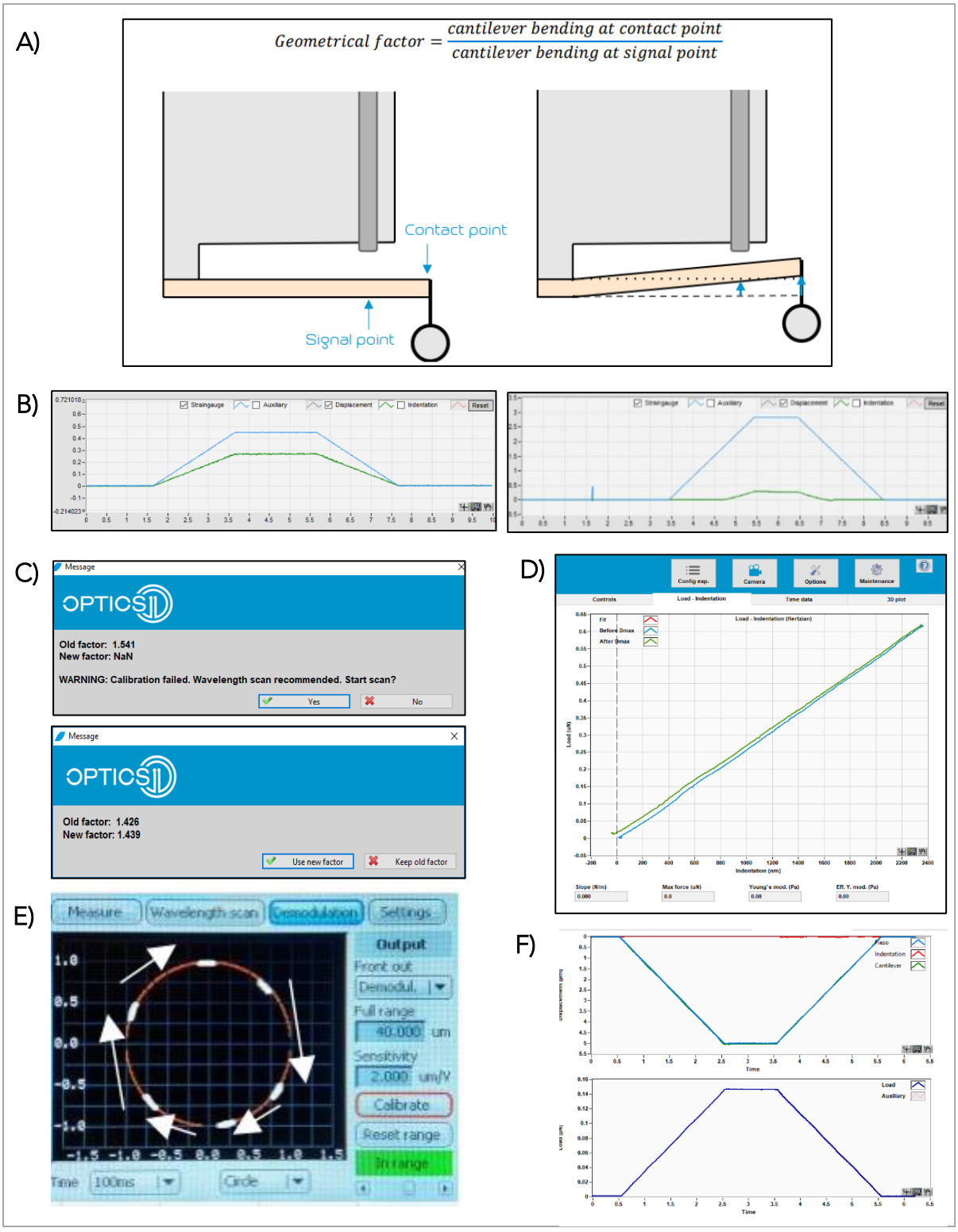

Figure 4: A) Schematic drawing of the meaning of geometrical factor. B) Live piezo and cantilever signals during calibration when probe is in contact with the hard surface (left) and when it is out of contact (right). C) Successful (top) and failed (bottom) calibration messages. D) Calibration curve. E) Signal movement in time on demodulation circle after it is calibrated. F) There is no indentation on a hard surface after calibration (red line is flat).

11. During the calibration step, 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 6.2, Pavone manual). Check that in the live signal window both piezo and cantilever signals move up at the same time as shown in Figure 4 B, left. If there is a mismatch in time (Figure 4 B, right), it means that the probe is close to contact but not fully in contact when the piezo starts to move. 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, it means that the probe is far away from the sample. Repeat the step.

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

12. 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 (Figure 4 C). 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 small variation is expected <5%. If calibration has failed, see below for the possible reasons. You can also check in Load-Indentation data that loading and unloading data overlap and are straight slopes (Figure 4 D). A small mismatch is expected due to drift and hysteresis in piezo movement.

Reasons for failed calibration:

- *The tip is not in contact with the surface during calibration (go to step 7).*
- *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 (*Figure 2 *D).*
- *Cantilever stuck at the tip fiber – dry it and unstuck it with a piece of paper (*Figure 2 *A-C).*

Repeat the wavelength scan before calibrating again.

13. Next, check whether the demodulation circle was calibrated correctly. Either tap on the body of Piuma/Chiaro to induce a sufficient amount of noise to see the whole white signal around the red demodulation circle or move down the probe at small steps e.g., 1 µm. **In both cases, the white signal should overlap with the red circle.**

Caution: If during the operation of the instrument, you observe that the white signal is not on the red demodulation circle or you get a warning from the system about it, you need to recalibrate the demodulation circle. This can be done in two ways: 1) continuously tap on the body of Piuma/Chiaro to induce one circle of noise and press the "calibrate" button on the interferometer, 2) go in contact with the sample and press calibrate from the "Initialize" menu, do not save calibration factor. However, if the signal is not just slightly displaced from the demodulation circle but rather became very small, it means that the cantilever got stuck to the fiber. To fix it, you can try one of these methods: 1) lift the probe up out of the well, prewet the probe and move down, 2) perform cleaning procedure, 3) dry the probe with the tissue, 4) get the cantilever unstuck by gently touching it from below with the tissue.

14. Finally, calibration can be verified by performing an indentation directly after the calibration. Load or make an experiment file, using the default displacement mode settings and changing to a calibration distance to displace the probe again on the stiff substrate. When 'Run experiment', check the demodulation circle in the interferometer window. The white signal should be on top of the red circle during indentation. The results in 'Time data' should show that the piezo displacement (blue line) is equal to the cantilever bending (green line) because the indentation starts in contact and no material deformation is expected (Figure 4 F). If both lines do not superimpose, check the reasons above for failed calibration.

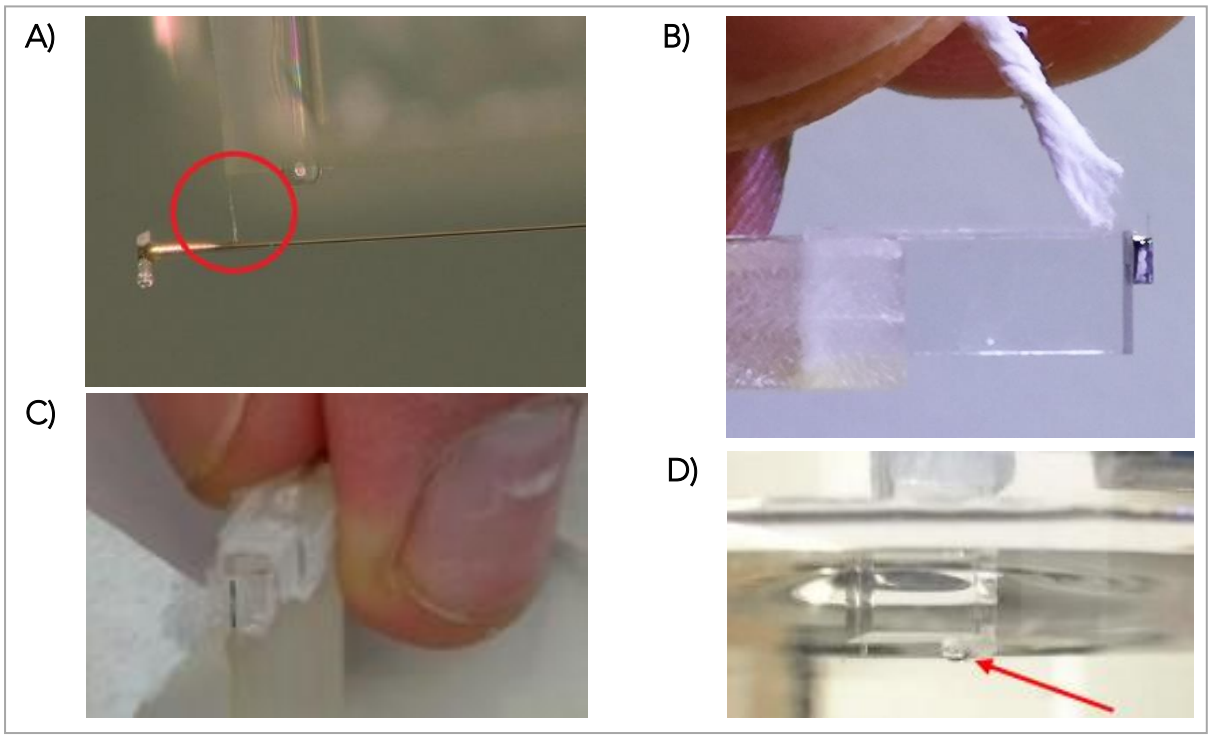

Figure 2 A) Cantilever stuck to fiber. B) Releasing cantilever with the tissue. C) Drying the probe with the tissue. D) Air bubble stuck to cantilever.