PROBE CLEANING PROTOCOL

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INTRODUCTION

This protocol describes the recommended ways to clean nanoindentation probes that are produced by Optics11 Life. This protocol is relevant for users who want to utilize a probe several times but believe to have accumulated dirt or sample residue on the sphere or cantilever between measurements. To prevent dirt from accumulating make sure to always rinse the probe after use, do this with isopropanol and distilled water, and then dry the probe before placing it back in its container (see Figures 3 and 4).

There are different methods to detect dirt on a probe, these are discussed in the first part of this protocol. Once the dirt is detected there are different effective cleaning procedures depending on the sample that the probe was used on. The second part of this protocol describes the best cleaning procedures distinguishing between biological sample residue and polymer-based residue.

DETECTING DIRT ON A PROBE

A contaminated probe could cause measurement values to be significantly different from the expected values. To verify that dirt is on the probe, follow these steps. First, examine the probe under a microscope to see if any dirt is visible. If the probe appears to be clean, the probe might still be contaminated yet the accumulation is not visible. The next step is to perform an indentation on a hard surface (e.g., hard glass as the bottom of a glass petri dish), this will display any dirt on the probe in the time-displacement graph. The next two sections will expand more on both procedures.

Visible residue on the sphere or cantilever

When a probe is expected to be contaminated first look at it under a microscope, to see if there is any dirt visible on the sphere, cantilever, or fiber. Relatively big pieces of residue should be detectable under a microscope, at least x40 magnification. If dirt is visible on the probe, try one of the cleaning methods listed below. Examine the probe again under the microscope after the cleaning procedures to make sure the dirt is gone.

Calibration on a hard surface

To detect smaller residue, that is not visible under a microscope, the user can do the calibration. If there is any sample residue attached to the sphere this will be visible in the time-displacement graph. First, get in contact with the surface by simply using the "find surface" function in the calibration tab. Check with 1 µm steps whether the surface was detected correctly. Calibrate the probe. For a clean probe, the loading and unloading lines of the cantilever signal should be straight and no relaxation visible during the holding step as shown in Figure 1 (left). Uneven loading and unloading lines of cantilever bending and relaxation during the holding step indicate the presence of dirt on the sphere (see Figure 1, right).

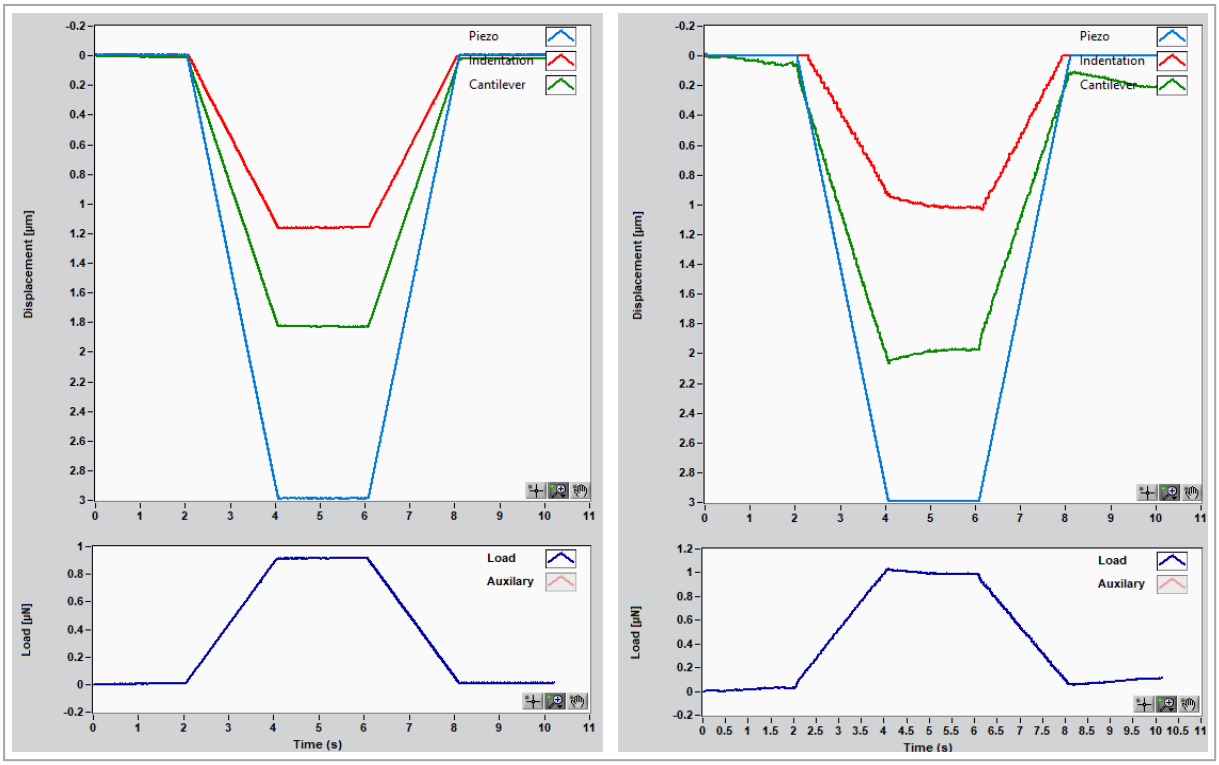

Figure 1: Calibration with a clean probe (left) and with dirt on the sphere (right).

Furthermore, when the calibrated geo-factor is deviating from the expected value of more than 10%, it can be an indication of dirt. The geo-factor after calibration should be the geo-factor that is given on the box divided by the refractive index of the medium in which the calibration is done (1 for air). If this number deviates by more than 10%, do an indentation on the hard surface from the contact to the same distance as during calibration, e.g., 3000 nm and inspect the curves. Try one of the cleaning procedures below and then try to calibrate the probe again.

CLEANING PROCEDURES

When dirt is found on either the sphere or cantilever, this residue most probably originates from the sample that has been measured previously. The most effective way of cleaning the probe depends on the type of residue. Here we distinguish between standard cleaning procedures, the cleaning of biological and polymer residues.

Standard cleaning with 70% Isopropanol (IPA)

One of the reasons for the wrong geometrical factor is dirt on the sphere. Furthermore, you can also have a failed wavelength scan or low signal (Gain in "Range" "High") because the probe is stuck to the optical fiber (see Figure 2). This happens when the probe is directly placed into the box after use while it should be first cleaned, then dried and the cantilever position inspected (unstuck with the tissue if it is bent towards the fiber). Cleaning the probe of any residue is very important and it is recommended to be done before and after each measurement session. In this regard, demineralized water and isopropanol can be used to clean the probe (see 3).

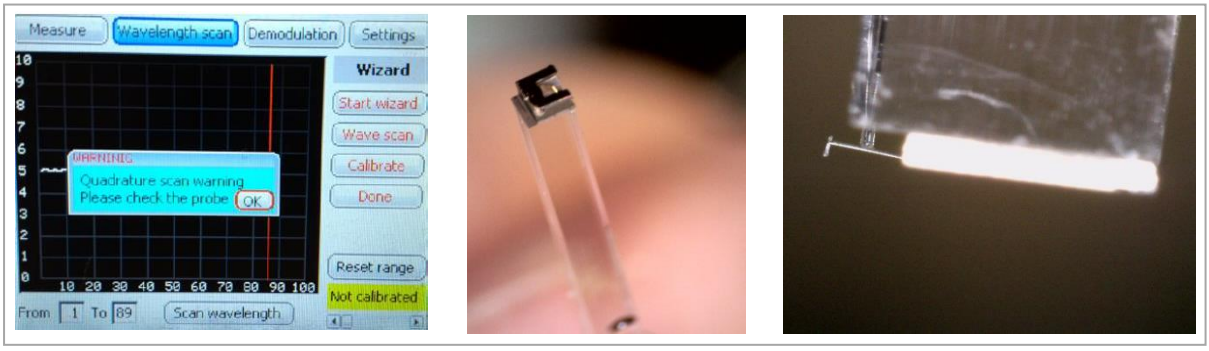

Figure 2: Failed wavelength scan and stuck cantilever of MEMS probe and ribbon-type probe.

Figure 3: Demineralized water, isopropanol, and pipetting from Petri dishes.

The cleaning procedure is recommended as follows:

First, pour two wells of the well plate (for Pavone) or two glass dishes (for Piuma and Chiaro) with demineralized water and isopropanol. Then use a pipette to rinse the probe with demineralized water, isopropanol, and again demineralized water. For this, you can run a full pipette of demineralized water and isopropanol over the glass part of the probe and finish the cleaning procedure by running again a full pipette of demineralized water over the probe. You can repeat these steps in the same order a few times to make sure that the probe is completely clean of any residue. Note that the probe should not be soaked in isopropanol for a long time since the isopropanol can damage the glue that is used to assemble probe components. For Pavone users, the cleaning procedure can be set automatically via the Pavone software interface (please see the Pavone manual for more details).

As explained before, always dry the probe after the cleaning procedure before packing it back into its container (see Figures 4 and 5). After cleaning and drying the probe, it could happen that the cantilever is stuck to the optical fiber. To release the cantilever from the optical fiber, you can gently touch the cantilever with a wipe as it is shown in Figure 6.

Cleaning biological residue

To clean biological residue the enzyme-based solution Trypsin is recommended. Most residue should be removable with a 0.25% solution. If the contamination is strongly adhesive increase the concentration steps wise to up to 2.5% for a stronger effect.

First, carefully rinse the probe with the Trypsin solution and then soak the probe in the Trypsin solution. It is recommended to leave the probe in the solution for approximately 15 min. If residue is still present it might help to leave the probe in for a little longer. It is not advised to leave the probe in for more than an hour, any additional time will not improve the cleaning. Rather, the water of the solution will start to evaporate effectively changing the concentration of the solution. Significant evaporation of water can cause condensation of the enzymatic substances on the sphere and cantilever. This condensation should be prevented as it worsens the contamination of the probe.

Use the Piuma, Chiaro or Pavone probe holder for the rinsing and the soaking (see Figure 4). Always rinse the probe with distilled water after cleaning and dry the probe before placing it back in its container (see Figures 4 and 5).

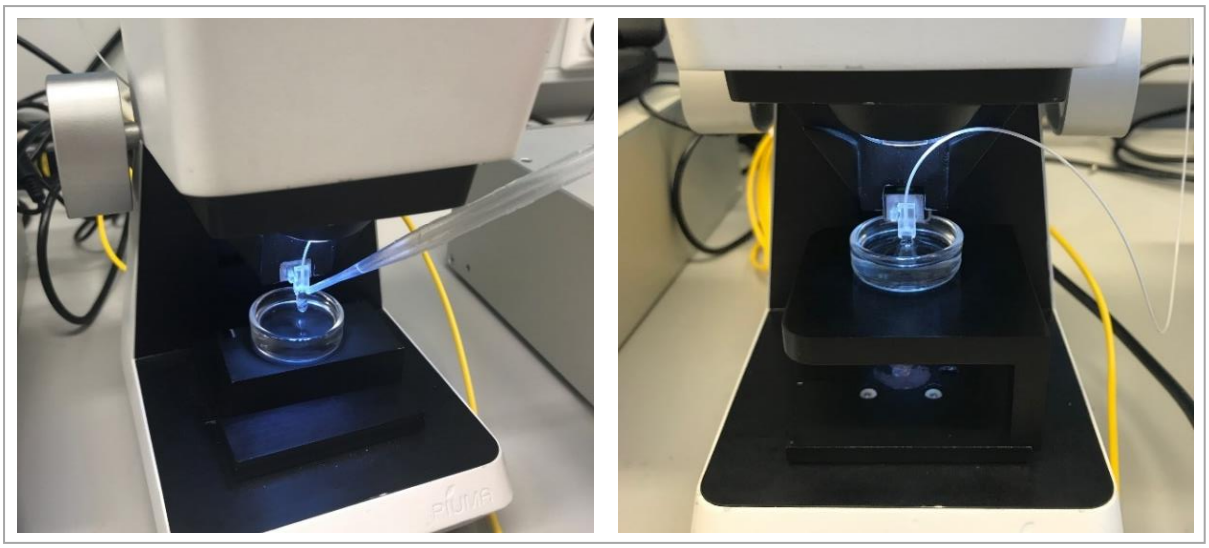

Figure 4: Left: Rinsing the probe. Right: Soaking the probe.

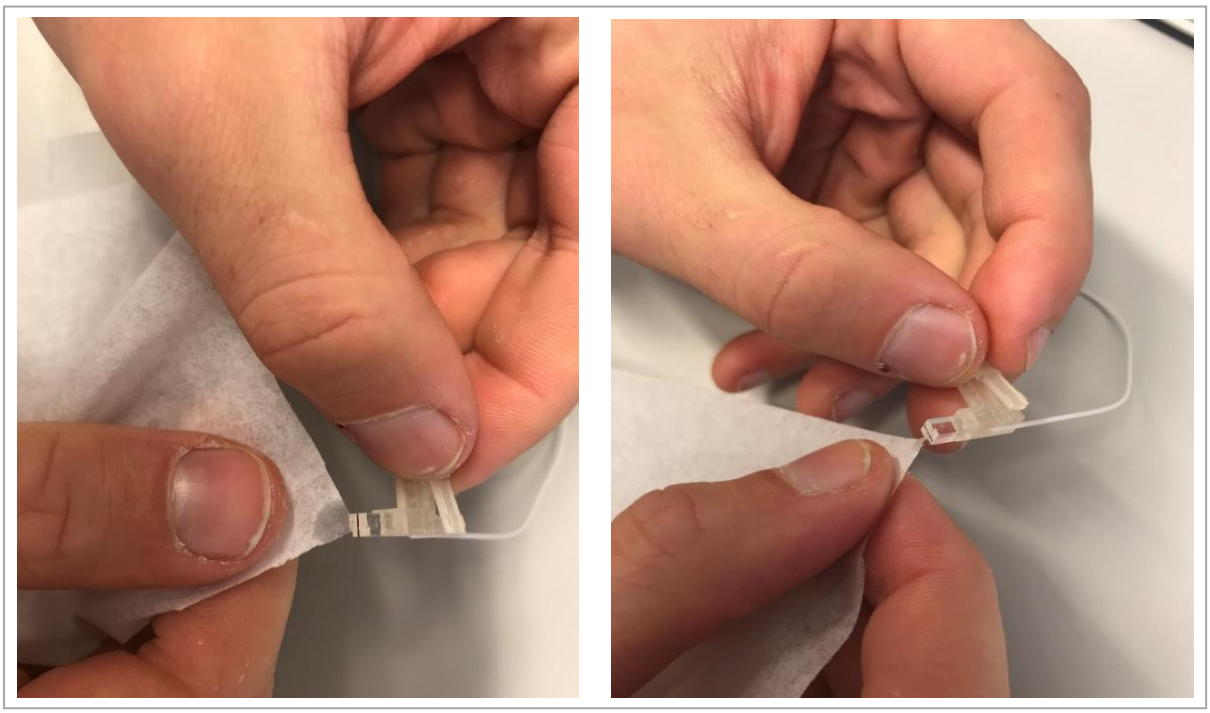

Figure 5: Drying residual liquid of the probe by carefully placing a wipe next to the cantilever.

After cleaning and drying the probe, it could happen that the cantilever is stuck to the optical fiber. By gently touching the cantilever with a wipe you can release the cantilever from the optical fiber (see Figure 6).

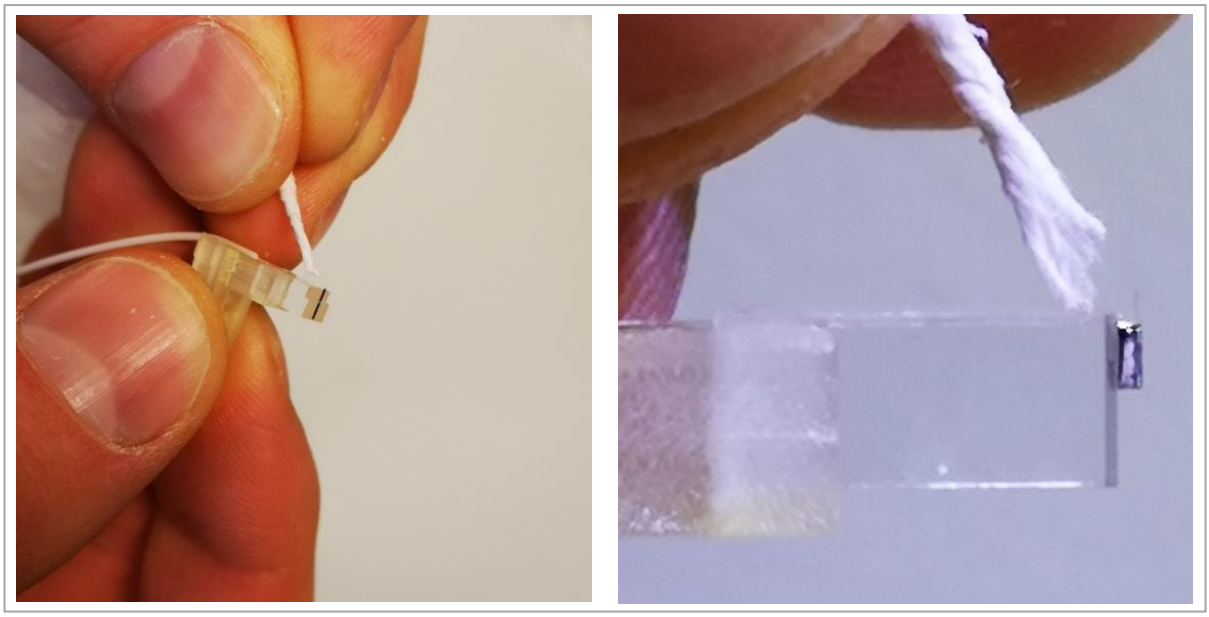

Figure 6: In the cantilever is stuck to the fiber, use the wipe to gently touch the cantilever from underneath.

Cleaning polymer residue

The most effective and simple method to remove any excess polymer residue is to rinse and soak the probe in hot water. Most polymer-based samples will soften when exposed to high temperatures. Therefore, rinse the probe with hot water and afterward soak the probe in the hot water. Rinse the probe a couple of times during and after the soaking. As the temperature of the water decreases quickly this procedure will only take a couple of minutes.

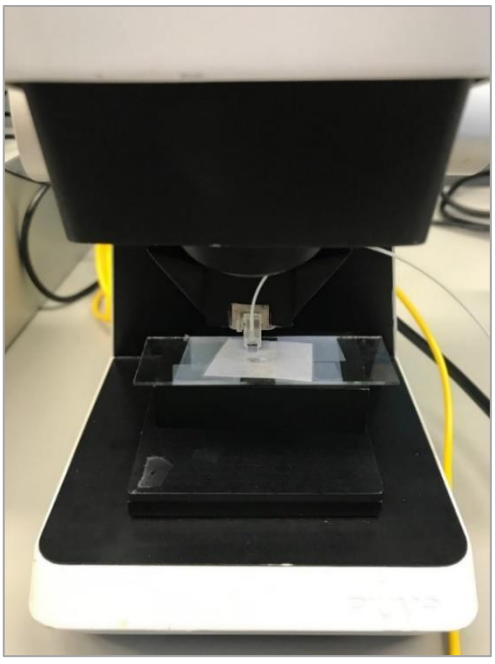

Figure 7: Rubbing dirt of the probe with the use of a tissue and optionally with small amount of isopropanol (if really sticky dirt, acetone).

If there is still residue left after this procedure, try to carefully mechanically rub the residue off. Do this by placing a tissue on the worktable of the instrument (see Figure 6). Get in contact with the surface by simply using the "find surface" function in the calibration tab. Then move the XY-stage in all directions in steps of approximately the size of the radius of the sphere. For strongly adhesive residue the user can decide to add small amounts of isopropanol or acetone to the tissue. Importantly, special caution is needed when applying acetone to the wipe by verifying that only the tip of the probe is in contact. Iterate the cleaning procedures multiple times if needed. Rinse the probe with distilled water after cleaning it and dry the probe before placing it back in its container.