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Water — A Clean "Glue" to Attach Hydrophilic Plates to an AFM Sample Stage

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Attaching a sample to the instrument's sample stage is commonly the first step in the operation of an AFM. For our commercial Digital Instruments Multimode AFM, a sample is usually attached to a magnetic sample puck by double-sided tape, the sample puck is then placed on the instrument's magnetic sample stage, and analysis can begin.

The use of double-sided tape, however, has unpleasant consequences. First, the surface of the sample puck becomes quite sticky due to residual glue from the double-sided tape — removing the sticky glue is not a happy job. One cannot just throw a soiled sample puck away! Second, double-sided tape does not always give satisfactory mechanical stability during AFM operation, especially when high resolution imaging is being carried out. Sometimes the tape-mounted sample will creep slowly, which can cause significant drift of AFM images. This is because the tapes and their adhesive films are made of pressure sensitive materials that tend to change shape when pressure and/or temperature vary. Third, and the most annoying fact, is that these tapes may render one's precious samples totally unusable due to residual sticky glues on the back of the sample. I have been dealing with some transparent sapphire samples. After AFM analysis, I found that it is extremely difficult to remove the residual mounting glue from the sample. These glues seem to be quite resistant to organic solvents, strong acids and bases, and UV light radiation.

My solution for a replacement for double-sided tape is to use plain liquid-water as a no-cost and convenient glue to mount hydrophilic smooth AFM samples onto a magnetic sample puck. This idea originated from the observation (everybody may have the same experience, see Fig. 1) that the strong capillary force formed between two, clean, glass slides forms a bond that is extremely resistant to lateral movement, while at the same time, the two slides can be easily separated by applying a vertical force (sometimes a wedge like a knife edge is required to do so). The most important thing is: after separation, the two surfaces are as clean as they were before mounting. If one wishes to repeat the analysis, just wipe the surfaces and remount.

Figure 1

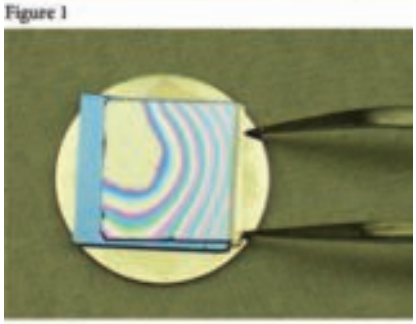

Figure 2

My water-glue AFM sample mounting protocol is as follows:

1. Clean both a piece of smooth glass slide (or silicon, silica plate) and the sample to be studied, by whatever methods that are appropriate to the specific purpose, then dry both surfaces. The cleanliness of the surfaces may be checked by observing if water will smoothly sheet across the surfaces.
2. Semi-permanently glue the glass slide to a magnetic sample puck via any solvent-soluble glue of your choice. Apply pressure to ensure good bonding.
3. Apply a small drop of water onto the surface of the glass slide. Note that the drop should be as small as possible since the final volume of water film between the two surfaces is extremely small.
4. Put the sample on the glass slide. Be sure that some part of the sample extends out over the edge of the glass slide (see Fig. 2 where a transparent sapphire sample is mounted on a silica surface). Providing a bit of an overhang facilitates removing the sample. Press against the four corners of the sample to squeeze out as much excess water as possible from between the two surfaces. After the sample is externally dry, check the bond by trying to move the sample to make certain that it has formed a strong bond with the glass slide. Oftentimes partial bonding between the two surfaces, as shown in Fig. 2, is satisfactory for AFM mounting.
5. To remove the sample, use a pair of tweezers to gently pull the overhanging edge of the sample to break the water bond.

The sample puck with the glass slide can be used repeatedly for mounting other samples; the only requirement is that the surface of the glass slide should be clean. I recommend wiping the glass slide with ethanol and drying with UV light. The glass slide may be replaced if it becomes soiled or damaged by dissolving the glue bonding it to the sample puck.

Protect Your Detectors

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Excessive light to detectors can be very harmful. Too much light, either monochromatic or wide band, can easily destroy photomultiplier tubes (PMTs). Charge-coupled devices (CCDs) cameras, of modern design, are typically made with cut-outs so that saturation beyond several pixels will cause them to shut off. Unfortunately, the detectors in the back of your eyes do not have automatic shut-off mechanisms, and can be easily destroyed with excessive light.

As a review of filters and cubes, keep in mind that the primary job for the excitation filter is to block all light other than the desired band being used to excite the fluorochrome. The primary job of the emission filter is to block the excitation source. If these two optics are chosen correctly and inserted in the beam path, the only light reaching the detector is the fluorescence emission from the fluorochrome. Nothing from the source should make it to the detector. This very weak emission intensity has no chance of harming the detectors. If these two optics are not perfectly matched, the excitation light may reach the detector and cause all sorts of problems.

State-of-the-art cameras have sensitivities well into the near infrared (NIR), so many systems now require some sort of blocking optic for the longer wavelengths, such as one which blocks 750-2000 nm or so. These same cameras may be damaged by strong UV light as well; therefore those wavelengths should also be blocked, typically by the excitation filter.

PMTs should never be hit with excitation light nor strong emission light for "long" periods of time. Note that long is a very relative term here. A few milliseconds of direct laser illumination can certainly destroy a PMT.

The human detector (eye) can be irreparably damaged by a few seconds of very strong UV and/or NIR light. In the early days of microscopy, filter sets were designed such that it was very difficult to expose detectors to the excitation light from the lamp source. The filters and

mirrors were typically glued into a cube/holder device, and blanks were used so that the light from the mercury or xenon lamp source could not get to any detector (including the eye) without at least going through the emission filter. Mercury and xenon lamps create huge amounts of energy, including the 'invisible' UV, which can destroy detectors in very short order. Keep in mind that the transmitted illumination for a microscope is fairly weak, and has virtually no UV emissions, which makes it safe for virtually all detectors.

In modern microscope design, the filters for doing epi-fluorescence illumination can be easily moved or exchanged. This presents the possibility of someone accidentally removing a protective filter, or not matching the exciter/emitter combination correctly. There is also a trend to use emission filter wheels in microscopy. This adds a great deal of flexibility to the system, but it also removes the emission filter from the beampath that goes to the eyes/oculars. This means that a great deal of the excitation light can reach the most important detector of all, your eyes. If this light happens to be either UV or NIR, it can destroy the retina without you actually 'seeing' it. It was thought for many years that the human eye was only damaged with UV light. We now know that violet and blue light may be just as harmful, and that green light, if strong enough, can also blind you. Red may be the safest in small doses, but near infra-red (nir) becomes dangerous again. (use bgg22 from Phila Optics Inc; or KG1/KG5 from Schott Glass).

Know the beampath of your microscope. Do not trust to chance or to others in the lab. If you have emission filter wheels, and therefore no emission filter in your cube/mount, ask that someone put UV-blocking optics in the oculars/eyepieces. There is usually room in the reticule shelf of the ocular. A 420 nm long-pass filter would block deep UV to 410 nm or so (L42 from Hoya Corp.; YG11 from Phila Optics Inc.; or gg420 from Schott). You still have to be concerned with very bright violet/blue/green light, but this light is 'visible' to you. Never look through the microscope while switching to a new/different cube. Move the filters/set, and then begin by looking several centimeters away from the oculars/eyepieces. Move your eyes closer to the focal plane of the oculars slowly. If you see a bright light, of any color, do not stare and make changes in the illumination system.

Dissolving Osmium Tetroxide the Easy Way

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Osmium crystals tend to cling to the glass ampoule walls making dissolving them difficult. Smashing the vials results in glass shards that may get into sample vials and can be dangerous, or carry into embedding media and damage diamond knives.

A simple trick to eliminate smashing osmium vials is to dip the sealed ampoule into liquid nitrogen. This releases the osmium crystals from the glass walls. Then simply break the vial using an ampoule cracker (available through EM supply houses) and pour the osmium crystals into a bottle containing the water for the OsO_4 solution. Wait a few minutes to let the ampoule warm-up, and it is easy to see if all of the crystals have been dumped out of the ampoule. Let the solution sit overnight at room temperature in a hood and the next day the crystals will be totally dissolved.

Another benefit is that if the solution is needed in a hurry, the bottle of water plus OsO_4 can be sonicated, and it is easily seen when the crystals are all dissolved. Just make sure the sonicator is in a hood and the bottle is well sealed.

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