How To Stick Loosely Adherent Cells To Glass Slides

Martin Spitaler
Imperial College, London, UK
m.spitaler@imperial.ac.uk

A simple and usually effective solution is to prewash the slides with 1M HCl, which increases the positive charge of the glass and thus sticks the cells to it. Simply leave it on the slides for 10 minutes, then wash 5 times with lots of phosphate-buffered saline and finally with water (to avoid salt crystals when drying).

The good thing: It's quick and effective independently of cell types, surface proteins, cell state (*e.g.* apoptotic cells), and it won't interfere with surface receptors, unlike glass coatings with *e.g.* polylysine.

The bad thing: The cells really *do* stick, *i.e.* they can't come off anymore. So don't use it for any experiments where cell migration or shape is important. I also would be careful with long-term experiments, it might interfere with cell division.

An alternative method is to cover the glass with polylysine (0.02%, 1 hour) before seeding the cells. Paraformaldehyde fixation will directly link the surface proteins to the polylysine.

One last note regarding polylysine: It can block lateral diffusion of surface proteins and thus interfere with cell signaling. To get around this problem, start fixation while the cells are in suspension or on untreated glass (5 minutes), then transfer the cells onto the polylysine-coated coverslips and spin them down with the fixative for another 5 minutes. This will give nicely physiological cells fixed onto the slides.

Just Say NO to Microtoming Silicon!

Ron Anderson
Microscopy Today, Largo, FL
randerson20@tampabay.rr.com

A recent discussion on the Microscopy Listserver on the subject of microtoming Si reminded many of us of this decades-old issue. Silicon is one of the easiest materials to prepare for TEM analysis with dozens of protocols available including chemical etching, electropolishing, mechanical polishing with and without ion milling, microcleaving, and the use of a focused ion beam instrument. The time involved in Si preparation can range from minutes to a few hours. Things become more complex if it is desired to prepare a Si semiconductor device with high specimen preparation spatial resolution *i.e.* prepping a prespecified, very small, structure.

Now and then a technologist, often times in the biological sciences, is asked to prepare a Si TEM specimen and wonders if Si can be microtomed. The results are almost always a pile of Si dust and frequently a damaged diamond knife.

So, no, you can't "cut" Si with a diamond knife. Si cleaves very easily. When the knife touches the Si, the Si cleaves along one of its preferred {111} crystallographic cleaving planes. That might be fine if you orient the Si such that a Si <111> crystallographic direction is perpendicular to the block face. Then if you are very lucky, you might get a cleaved piece of Si oriented with {111} faces (at the risk of your knife). Any article that I've seen that claims to have microtomed Si has shown lots of pictures of tiny {111} Si sections. Assuming that you are successful, so what! There is no, or very little interest, in anything in the semiconductor world or in potentially oriented thin films deposited on Si, that is best displayed on top of a Si cross section with a {111} block face orientation. The angles are wrong. Si wafers in semiconductor processing are almost all with (or very close to) a Si <001> direction perpendicular to the Si wafer surface. Devices fabricated in, or patterns deposited on, these wafers are not randomly oriented. They are almost always aligned with {011} planes perpendicular to the Si surface, *i.e.* the *x, y* orientations of the devices and patterns are parallel to <011> directions in the Si surface. Cross sections of interest for TEM or SEM analysis are oriented such that the microscope looks down on a {011} "block face." If you orient the specimen's block face so as to attempt to cut {011} cross sections, the diamond knife will touch the Si and immediately propagate a cleave down a {111} plane despite the orientation of the block. As the specimen in the block is constrained by the epoxy holding it, the result is a flake of Si dust from just the surface. When the knife comes around again you get more dust. An additional complication is that there are four different {111} planes in Si. If a cleave starts on one Si (111), the cleave might change direction and follow a different (111) plane as the thin section tries to bend up the knife angle as the knife advances. More dust.

I had a response back on my Si microtoming tome on the listserver from a biologist who wrote: "People like me read this and go 'OooKay ... what's a {111} plane and how would I know how I have such a thing aligned for anything, much less microtomy?' " Answer: Any Si semiconductor wafer you find will most likely have a <001> direction perpendicular to its surface, as stated previously. If it is a whole wafer there is probably a flat and a notch ground into the wafer's edge at right angles to each other. The families of crystal {011} planes are oriented at 45 and 90 degrees with respect to each other. The flat is parallel to one of the {011} planes in the family. The notch is perpendicular to a second {011} plane at 90 degrees with respect to the flat. If it is a processed Si chip, look at the *x, y* coordinates defined by the structures on the surface of the chip. They are parallel to {011} planes. {111} planes are oriented at 35 and 90 degrees with respect to {011} planes. Try cleaving the wafer at 45 or 90 degrees with respect to the flat or notch. When you get a piece with a 35 degree slope with respect to the surface, the slope part will be a {111} face. OR, just hit the Si wafer with a hammer and look for a piece with shiny 35 degree sloped edges. But, my biological friends, why are you making your heads hurt by reading this? My point is to explain why you won't want to damage the edge of your diamond knife creating Si dust. Now you know that the dust particles will have 35 degree sloped sides! Using a microtome to make Si cross sections is like using a screw driver to drive a nail. Instead of fighting the reality that Si cleaves, why not exploit this fact and prepare really great thin specimens by the microcleaving technique.[1].

Reference

[1] Walck, S.D. and McCaffrey, J.P., MRS Proceedings #480, p. 149, 1997. Or Google micro cleaving plus Walck or McCaffrey.

Fortress™ FIB Holder with CastleGuard™ Protection

Fortress™ FIB Holders are reusable holders that secure FIB samples that are held in a specific orientation without the use of adhesives, adhesive pads, or conductive paint. Fortress™ FIB Holders can be used to position a thin, whole or cut TEM grid/disk in an orientation such that either an in-situ or an ex-situ FIB lift-out technique can be used to attach a FIB-prepared sample. Physical protection of the mounted sample on the grid is provided with the CastleGuard™ protection design. CastleGuard™ protection provides a rigid support structure that shields the sample while allowing required access for processing. Fortress™ FIB Holders are designed to fit into the SS200 SampleSaver™ storage container for storage and transport in an inert environment. Up to 30 Fortress™ FIB holders can be stored in a single SS200 SampleSaver™ storage container.

Special Features:

- Unique clamping mechanism secures sample without over tightening.
- Standard 1/8" pin mount fits most FIBs and SEMs.
- Peripheral support structure protects specimens during handling and transport.
- Economical design allows user to prepare and store samples on the same holder.

SampleSaver™ Storage Container

The SampleSaver™ Portable Storage Container system is a specialized container that enables a user to replace the reactive atmospheric air typically present in the container with an inert gas species. Once a sample is stored inside the inert atmosphere, the sample does not undergo reactions such as oxidation during storage and/or transportation. It is ideally suited to the preservation of microscopy samples that may undergo changes during the delay between sample preparation and analysis.

Special Features:

- Suitable for SEM, TEM, SPM, EBSD, AFM and FIB samples as well as Liquid Chromatography Vials
- Prevents oxidation of samples and coatings
- Sample racks for holding SEM stubs, customized TEM grid boxes, AFM discs, Microscope Slides, LC Vials, FIB Holders and Metallographic mounts
- Samples remain secure during transport
- Preserves chromium coated SEM samples
- Simple leak check feature to verify integrity
- Sample racks are interchangeable and color coded

SBT

SOUTH BAY TECHNOLOGY, INC.

1120 Via Callejon | San Clemente, CA 92673 | U.S.A. | 949-492-2600 | Fax: 949-492-1499
Toll Free: 800-728-2233 | email: info@southbaytech.com | www.southbaytech.com