

## Care and Use of Diamond Knives

The diamond knives supplied by TAAB are manufactured from natural gemstones and should therefore be treated with the respect related to their cost and value. A natural gemstone diamond is specially cleaved, ground and polished to provide a perfect cutting edge and then mounted and glued with an epoxy or methacrylate resin into a holder suitable for the ultramicrotome. Since the cutting edge of any diamond knife is extremely thin and fragile, great care must be taken to prevent any damage. The slightest pressure at right angles to the cutting edge could result in it becoming chipped. Diamond knives should preferably be used by people who are experienced in ultramicrotomy techniques and new users should obtain expert advice before their first sectioning sessions. We also strongly suggest that one person be charged with the care and use of each diamond knife. This is not to increase the sales of our knives (!), but to ensure the person who is going to use it takes full responsibility for the knife. This will lengthen the usable life, reduce costs by less frequent resharpening and reduce friction in the laboratory!

For best results with diamond knives these general recommendations should be followed although there are some procedures which are specific to each manufacturer.

### 1. Handling

Always take extreme care when handling a diamond knife and ensure the edge never accidentally comes into contact with any other object such as clothing or fingers

### 2. Clearance Angle

Diatome recommend 6° and this should normally be considered an upper limit when using knives from other manufacturers. A clearance angle that is too large can cause "bunching" or "compression" of the sections and increased pressure on the cutting edge. Reducing the clearance angle should eliminate this problem. Improper embedding can also be a cause of compression but diamond knives, unlike glass, are extremely tolerant of poor embedding and may disguise other problems.

### 3. Cutting Techniques

Diamond is amongst the strongest known material as long as the applied force is in the compressive direction. Stresses applied in any other direction on the delicate edge can cause nicks that can only be removed by resharpening. Our experience indicates that close adherence to the following recommendations will minimise danger to the edge and extend the life between resharpenings.

- Do not stop the microtome travel part way through a section or withdraw the diamond from a partially cut section. Always cut completely through the block as withdrawal may result in nicks in the edge, loosening of the diamond, or worse, tearing of the diamond out of its mount.
- Try to avoid cutting badly or partially cured blocks. Such blocks are the primary cause of epoxy debris being deposited on the knife edge that in turn is a major cause of knife marks in the sections. This epoxy debris can often only be removed by resharpening as it resists normal cleaning methods.
- Do not routinely cut sections thicker than 200nm (or 100nm for very hard blocks) at least until you become more experienced. Thick sections subject the diamond to excessive stress and may result in damage to the stone. Never cut sections without a flotation liquid in the bath.
- Do not allow particles from razor blades, glass knives or any other hard object to become embedded in the specimen block while trimming. These will certainly cause nicks in the knife edge. If you are required to cut specimens supplied from another laboratory make some exploratory cuts with a glass knife to ensure no hard particles are contained within, or have been embedded with the sample. We know of silica gel drying agent that was spun down with a cell suspension and caused havoc with all knives until detected.
- Do not allow sections to dry on the knife edge. They cause knife marks in the sections and can only successfully be removed by resharpening.

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#### 4. Cleaning

Cleaning before each microtomy session will give the best results but cleaning after each session is the best preventative against future problems.

- Before Sectioning - Detergent Soaking

The diamond knife can be soaked by placing it in a polystyrene foam coffee cup or similar (a glass or rigid container risks accidental edge damage) and adding a non-ionic detergent such as 1% Triton X-100 in distilled water. Leave for 1 - 8hrs depending on the stubbornness of the contamination. After soaking the knife should be rinsed well with distilled water from a water jet bottle. This should remove many residues and also improve the wettability of the knife.

Other common laboratory detergents containing caustic such as Alconox, 7-X or Photo-flo may remove the anodised finish from the boat and should be avoided.

- After Sectioning

On completion of sectioning care should be exercised to make sure that no sections or section debris remains attached to the cutting edge. Contact between the diamond knife and anything other than the specimen block must be kept to a minimum. With water still in the boat and by using an eyelash or similar flexible fibre, such contamination should be carefully teased away from the edge. After this dump the water from the boat and rinse with a jet of distilled water.

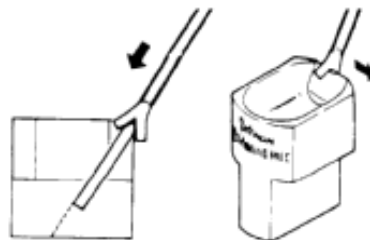
Do not attempt to blot the edge of the knife with a tissue but allow the knife to air dry or use a jet duster and store the knife in its box when not in use.

- Edge Cleaning Devices

In the event that difficulties with sectioning become evident and epoxy debris on the diamond edge is thought to be the cause, cleaning of the edge itself may be tried. The use of edge cleaning devices should be minimised. If you follow the basic precautions of cutting well-cured blocks and removing section fragments from the edge after each sectioning session it should not be necessary to use tools more than once or twice a week. They have the greatest potential for producing fine nicks in the knife-edge and should only be used by people experience in handling diamond knives.

A polystyrene or pith rod is the safest tool for edge cleaning. It should be sharpened to a chisel edge of approximately 45° using a razor blade cleaned with a solvent to remove any traces of oil or grease. Before using, dip the rod into distilled water, blot on filter paper and it is ready for use. **DO NOT USE DRY.**

A cleaning stick of soft wood such as a toothpick or applicator may also be used for cleaning after proper preparation to remove the natural resins present. This is accomplished by soaking the sticks for 24hrs in alcohol and then allowing to thoroughly air dry before use. The stick must be spatulated (i.e. sharpened to a thin chisel edge with a clean razor blade before use). Dip the stick into distilled water, blot on filter paper and it is ready for use. An alternative for the experienced user is to spatulate the stick, chew the end in the mouth gently for 30 seconds or so and use directly on the edge. Our experience indicates that the softer end is safer for the edge and it appears to be cleaned better. Whichever method you choose, on no account use edge cleaning devices dry.



Whilst viewing the knife edge under the dissecting microscope the flat end of the stick should be gently pressed against the cutting edge of the diamond knife as though to split the stick. The stick should always be wiped parallel to the cutting edge, never obliquely or at an angle, and should always travel the entire length of the diamond. Start at one end of the edge but not on the resin holding the diamond in the mount as this could drag debris down onto the edge.

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Water, detergent or even vinegar can be used to help remove clinging debris that may cause streaks in cutting although in most cases distilled water is the recommended cleaning fluid. 1% Triton solution will generally remove any remaining debris if necessary. White vinegar as it comes from the bottle is an effective cleaning solution in truly stubborn cases. After exposure to these agents the knife should be rinsed promptly and thoroughly with distilled water, dried and put away securely in its box if it is not going to be used again immediately.

- Ultrasonic Cleaning

Sonication is not recommended

### **General Cautions**

Never clean the knife with organic solvents. While the knife itself is impervious to cleaning agents, the epoxy/methacrylate compounds and boat are not. Strong detergents, bleaches and bases may remove the anodised finish of the boat.

Keep mechanical cleaning techniques to a minimum

Never subject the knife to bending stress or exert force in a direction perpendicular to the edge.

The ability to produce good clean sections on a continuous basis requires that the utmost attention be given to maintaining a clean sectioning environment. The use of uncontaminated boat fluid for supporting cut sections and the use of reagent grade solutions for cleaning will help to ensure that your diamond knife achieves maximum results over a long period of time.

### **5. Storage**

The diamond knife should be kept in the storage box furnished with it in a clean, out-of-the-way place to prevent accidental damage or dropping it when not in use.

### **6. Resharpener**

Properly cared for all the diamond knives supplied by TAAB should last many years of cutting operations without problems. The onset of compression and an increase in knife marks may indicate that resharpener is needed. Although not essential, we think it is preferable that the original manufacturer carries out resharpener. A diamond knife can be resharpened many times and will cost 50 - 60% of the cost of a new knife.

### **7. Selecting a knife**

The manufacturers represented by TAAB provide knives of the highest quality. Choosing a supplier or knife may be made on the grounds of speed of supply, personal preference, third party reference or price.

What size of knife should I buy?

Your budget may have more influence on this decision than any other factor. However, if this is not a major problem then there are some other criteria to consider. What is the largest block size you will be required to cut? No point in having a knife smaller than this. Some users employ the maxim of buying the largest knife their budget allows on the grounds it will take a long time to use the entire edge. They move along the edge a bit at a time as it becomes scored or damaged. Others say that a large edge has more chance of becoming damaged, particularly as the 'unused' part must be cleaned after use every time as well as the 'used' part. It might therefore be better to buy two smaller knives keeping one in reserve for when the first goes for resharpener. Our personal recommendation is to go for two knives of your maximum anticipated section size if funds allow, or get as close to a 3mm edge length as you can afford. Whatever rationale you adopt, we guarantee that once you have used diamond knives you will feel lost without them.

### **8. Conclusions**

Despite the foregoing warnings and precautions, there is no doubt that a diamond knife is one of the finest investments a TEM laboratory can make. The time saved in obtaining results is considerable and purchase can be justified on this benefit alone. They can also reduce frustration, as they are extremely tolerant of poor processing, can cope remarkably well with uneven block hardness and can cut thinner and larger sections than is possible with glass. They can sometimes cut blocks that are impossible with glass thus saving expensive and embarrassing experimental or clinical re-runs.