

Guide to Diamond Knife Use & Care

This guide has been a fixture in TEM labs for over 20 years and is used in most microscopy curricula as instructional material for knife care. We hope this information enhances your experience with diamond knives. In general, the guidelines you are about to read hold true for diamond knives of any age and made by any manufacturer.

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General Handling

A diamond knife is, at the same time, one of the most durable and fragile tools you will own. Diamond is among the strongest known materials, but this is only to forces applied in a compressive direction. Considering the wide range of materials that are cut successfully, from soft to hard, the diamond edge is quite durable.

The slightest pressure at right angles to the cutting edge, however, can chip it. Never allow the edge to come in contact with any object. Accidentally touching the edge with fingers, tools or clothing will nick the edge. Even so, with proper care and handling, your DDK diamond knife will provide years of useful service.

Receiving a Knife

Only knives that pass our rigorous inspection are released for shipping. There are several things you can do to insure that the same quality reaches you safely.

1. To avoid damage in-route, instruct your receiving department to not open the box.
2. When you receive the knife, inspect the red label on the front of the plastic knife box. If it is missing, torn or cut, notify DDK immediately.
3. Carefully remove the knife from its box, place it in the stage and begin sectioning.

During our final inspection the edge is thoroughly cleaned so no initial cleaning is required by you. Use blocks typical for your lab for your inspection. DDK diamond knives cut empty and tissue-filled areas equally well.

Diamond knives should only be used by people with experience in ultramicrotomy. When more than one person does ultramicrotome work, charge each individual with the care and use of their own diamond knife.

Section Thickness Card

Interference colors, created by the difference in phase of light reflected from the top and bottom surfaces of the section, are used to estimate the thickness of ultrathin sections between 40 and 250 nm thick. These phase differences occur as the speed of light changes when passing from air to the section and back. The color seen is dependent, therefore, on the index of refraction of the section as well as the section thickness.

Tips to Improve Knife Longevity

Diamond is, of course, the hardest material known. While the edge on your diamond knife may wear when cutting extremely hard or brittle materials, it is hard to accept that much wear occurs when cutting epoxy or acrylate embedments. How long will your new diamond knife last? It will last until the accumulation of damage to the edge, much of it accidental, renders no part suitable for cutting ultrathin sections.

You can extend the life of your new knife by careful handling to prevent severe accidental damage and relegating operations that cause damage to areas that are already damaged. Careful handling is an obvious

warning but we all could handle our knives a little smarter. When the knife is not in the ultramicrotome, keep it in the box. Use a small piece of tape to keep the box closed. Don't ever touch the edge of the knife with anything unintentionally and keep your knife out of situations where this could happen.

Whenever you are cutting the first block from a new batch, first use a used area of the knife edge. If you have a sample type that you've never cut before or someone gives you a block of theirs to cut, first use a used area of the knife edge. When you need to cut thick sections to rapidly get deeper into your specimen, use a used area of the knife edge. If the faintest shadow of doubt crosses your mind regarding how the knife edge will react to what you are about to do, use a used area of the knife edge.

Start cutting at one end of the knife edge. As that area becomes less able to cut publication quality sections, move to the next area. That used area becomes valuable for trying new things, taking ultrathin survey sections, lots of uses. In fact, the majority of your work will be done there with the area next to it used only when you are ready to take sections for micrography.

Frequently Asked Questions

1. What kind of diamonds are used?

"We use only the finest gem-quality diamond ..." "Have you seen the brown diamonds in those tennis bracelets ...?" In the diamond business today, gem-quality refers more to size than any other measure of quality. If it can be faceted, it is gem-quality and so we often compete with gem cutters for raw material. DDK looks beyond size to the overall shape, presence of inclusions, level of strain, and color when selecting raw material. We use large (one carat and up) stones that are clean (no visible inclusions at 10X) and pale yellow or better for our diamond knives. Perfect or nearly perfect octohedrons, known as crystals, are used for the perfection of structure they offer.

2. How is the edge put on?

After cleaving slabs from the diamond crystals, facets are ground and polished leading to the formation of an edge. Cleaving itself will not form a good edge because of the step-like formations found on cleaved surfaces. Diamond grows in plate-like fashion. When cleaved, the fracture plane will often jump between these growth lamellae. If two cleaved planes intersected, a sawtooth edge would be produced.

3. What is the importance of diamond crystallographic orientation?

Crystal orientation is important when making diamond tooling since it determines the quality of edge possible and its durability. Good cleaved slabs have crystal orientations well within 1° of perfect, naturally. The orientation of edge that DDK uses has been used successfully since 1961, when diamond knives were first produced for sale. This orientation allows us to finish parallel to the edge providing a perfect, nick-free edge. We are able to use more of the diamond leaving more root for a more secure grip on the diamond and greater resharpener reserve.

4. What is this card that came with my knife?

Whether you are resharpener a knife or buying a new one, DDK guarantees that you will receive a continuous edge suitable for cutting ultrathin sections equal to or longer than the amount you have ordered. This card shows how much edge on the end(s) of your knife, over and above the amount you ordered, did not meet our quality standards. Our customers appreciate this "extra" edge as it is

very useful for trimming and/or survey sectioning.

5. The epoxy used to seal my old knife came off. What now?

First, be aware that the loss of this epoxy in no way affects the stability of the diamond in the boat; it only acts as a dam to keep water in the reservoir. In a pinch, you can apply some Duco cement with a sharpened applicator stick between the ends of the diamond and the boat. Otherwise, send the knife back to DDK and we will replace the epoxy, usually within a week.

6. How many times can my knife be resharpened?

Because of the extra long diamond slabs we use and the efforts we make to minimize the diamond removed, DDK is able to guarantee unlimited resharpenings for your diamond knife. Practically speaking, we can only remove material until there is no longer sufficient metal to hold the diamond securely. We find that with the longevity of our edge and expertise of our craftsmen, our diamond knives can last a lifetime in skilled hands.

7. Why do people use acetone in their boat water?

Some users find that acetone can help their cutting results in two ways: diamond wetting and section stretching. Diamond is naturally hydrophobic and some acetone in the water can help with bringing water to the edge. Acetone also promotes stretching of the sections by decreasing the water's surface tension and softening the plastic. All materials used in DDK's diamond knives are impervious to acetone and a wide range of other solvents. Check with your knife's manufacturer before trying this.

8. How long will my diamond knife last?

It is impossible to predict how long a diamond knife will last in your hands. Longevity is a function of what you are cutting, how often the knife will be used, and the amount of abuse it is subjected to. In clinical applications where diamond knives are used almost daily, knives may require resharpening every two years. There are DuPont knives over 20 years old still seeing frequent use in research laboratories. At every exhibit we attend, satisfied customers tell us they wish they could send business our way but their knife is still working well years after purchase. Clearly DDK diamond knives can provide years of quality performance depending upon the way in which they are used.

Presectioning Suggestions

To obtain a perfect section ribbon, the top and bottom of the block face must be parallel to each other and to the knife edge. If trimming with a razor blade, use a fresh degreased blade and change to a new blade before the final few cuts of your block are made. Avoid taking too thick a cut to avoid damaging the block face.

Do not allow particles from glass pipets, brushes, razor blades or glass knives to become embedded in the block during processing or trimming. Old razor blades and thick cuts of the block can leave particles behind. When sectioning, these particles damage the diamond's edge. For the same reason, use a clean new portion of a glass knife's edge to complete trimming the block face.

Excessive block width, section thickness, cutting speed or clearance angle increases the potential for chatter and compression because of the increasing cutting force required. Trim block faces as narrow as practical to control the cutting force.

Sectioning - Room Temperature

Good sectioning practice involves incorporating this list of simple rules into your everyday work habits.

1. Align the block face so that it is planar to the cutting arc and the bottom is parallel to the cutting edge.
2. Check that all thumbscrews and clamps are tightened, i.e. the block holder, the knife holder, stage adjustments, etc.
3. Set the clearance angle and cutting speed as listed on the knife box and in the ultramicrotome manual.
4. Set the water level even with the cutting edge and to give a good reflection.
5. Solvents may be added to the boat in any concentration in order to control the surface tension, avoid extraction of specific sample components, etc.
6. Avoid cutting sections thicker than the limits given for each specific knife, since thick sections requiring excessive cutting force may damage the edge. With an ultramicrotome knife, do not cut thicker than 200 nm, 100 nm for very hard blocks. With a Histo-Knife, do not cut sections thicker than 5 microns.
7. Re-approach the knife edge each time the clearance angle, block orientation or knife lateral position is changed or the knife is replaced in the stage.
8. Sectioning partially-cured blocks is the primary source of epoxy debris on the diamond knife edge, a major cause of knife marks on sections. Each block must be fully cured.
9. Be especially careful when moving or collecting sections. Do not touch the cutting edge with any solid object (grids, loops, tweezers).
10. Do not allow sections to dry on the knife edge. Dried sections on the edge are a frequent cause of knife marks and compression.
11. Do not stop block travel or change the clearance angle during a cut or withdraw the diamond knife from a partially cut section. Withdrawal may result in gross edge damage or loosening of the diamond from its metal mount.

Sectioning - Cryo Temperature

Research shows that diamond knives can cut higher quality cryo sections than glass knives. The more durable diamond knife cuts thinner sections with less compression and greater uniformity.

Although the optimum temperature will depend on the specimen being cut, start with these rules of thumb:

- Trimming and semi-thin sections: -60• C
- Ultrathin sectioning of fixed tissue: -80• C to -110•C
- Ultrathin sectioning of unfixed tissue: -120• C

Good sections of fixed tissue are tinged blue or gold, unfixed tissue sections are cellophane-like. Temperatures too high give a soft block and compression, temperatures too low give a hard block and small ice chips. Vary the temperature to optimize your results.

Electrostatic charging in the cryo chamber causes cryo sections to stick to the knife edge, curl, bunch up, and fly away. The lower the temperature, the more pronounced these problems become. If these

problems make your cryo sectioning overly difficult, use our Statikiller ionizer together with our cryo diamond knife.

Cleaning Procedures

Cutting good clean sections requires maintaining a clean sectioning environment. Use clean boat fluids while sectioning and reagent grade solutions for cleaning.

Three Step Method

Good habits of knife care will give best results. Use these three steps when sectioning to avoid problems:

1. Before sectioning - Soak the diamond knife edge in a non-ionic detergent, such as Triton X-100, diluted to 0.1% in distilled water. This softens any debris on the edge and improves wettability of the diamond.
2. After collecting sections - Make sure that no sections, or section debris, remain attached to the cutting edge. Using an eyelash, or other flexible fiber, carefully tease section debris away from the edge.
3. After sectioning - Make sure no sections remain on the edge and dump the water out the back of the boat, not over the diamond edge. Rinse the knife with distilled water and carefully wipe the bowl with a tissue (but do not attempt to dry the wet edge of the knife).

Edge Cleaning Devices

Clean the diamond knife edge if epoxy debris is thought to be causing sectioning difficulties. Minimize the use of edge cleaning devices by cutting well-cured blocks and removing section fragments from the edge after sectioning.

A high-density polystyrene rod, available from DDK and many EM disposable suppliers, is the safest tool to use for diamond knife edge cleaning. Sharpen it to a chisel edge of 45° using a double-edge razor blade. **DO NOT USE DRY.** Dip the rod into your cleaning solution and blot on filter paper before use.

While viewing the knife edge under a dissecting microscope, press the chisel end of the stick against the edge of the diamond knife as though to split the stick. Wipe the stick parallel to the cutting edge, never obliquely or at a right angle, across the entire length of the diamond knife edge on each cleaning stroke.

Cleaning Solutions

Try distilled water or ethanol first. A 0.1% Triton solution will usually remove any remaining debris. White vinegar is good for truly stubborn cases. Cleaning with the stick or by soaking with these solutions is often sufficient. There are other, stronger solutions which can be used in unique situations. If the above solutions do not give the desired results, contact us to learn about cleaning fluids of last resort.

Always rinse with distilled water when finished.

Cryo-Knife Cleaning Tips

Before baking out the cryo chamber, remove the diamond knife. Hold the knife under a stream of distilled water or leave it in a safe place until it has warmed up. Any of the cleaning methods from above can then be used.

Sonication and Acids

Sonication is not recommended for the cleaning of any of our diamond knives and strong acids should NEVER be used.

Troubleshooting

Wetting Problems - not enough

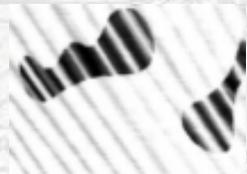
Causes - hydrophobic contamination

Cures - clean the edge with alcohol or saliva and a cleaning rod, overfill the boat with distilled water for a few minutes.

Wetting Problems - too much

Causes - electrostatic charging, hydrophilic embedding resins

Cures - increase the room humidity, dry the block face with filter paper, use an antistatic gun, lower the water level to a concave shape



Chatter

Causes - external vibrations, faulty microtome, loose stage clamps, cutting pressure is too high, clearance angle too small

Cures - change the location, have the microtome checked, tighten holder clamps, reduce the block width, increase the clearance angle, decrease cutting speed



Compression

Causes - soft block, included angle too high, dull knife, clearance angle too high, speed too high

Cures - make harder blocks, switch to a low angle knife, send your knife for evaluation, reduce the clearance angle, reduce the cutting speed

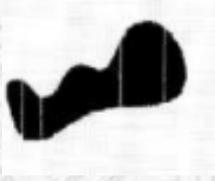


Streaks

Causes - touching of edge with fingers or tools, hard particles in block or

specimen, worn diamond knife

Cures - keep objects away from the edge, follow trimming recommendations, check your processing protocols for use of glass and other hard materials, use different knives for your hard vs. soft specimen



Striations

Causes - fine edge imperfections, poor block polymerization, inhomogeneous blocks.

Cures - try a new part of the edge, make sure your blocks are fully polymerized, try a new block

Resharpener

Properly cared for, a DDK diamond knife will last for many years of cutting. The inability to cut "clean" thin sections, the onset of compression, or an increase in knife marks may indicate that resharpener is needed.

The goal of resharpener any diamond knife is to restore its original length and cutting ability. Our unique methods of manufacturing and resharpener diamond knives allow us to guarantee that your resharpener DDK knife will be of the same superior quality as your new DDK knife.

DDK strongly advises that your DuPont or DDK knife only be resharpener by DDK. The large quantities of knives, resharpener by other companies, that we receive for evaluation and correction confirm the uniqueness of our manufacturing and quality assurance processes.

A DuPont or DDK diamond knife can be resharpener many times, limited only by the amount of diamond remaining within the metal shank and the amount of diamond removed from the edge. Delaware Diamond Knives now guarantees unlimited resharpener on its diamond knives due to the long diamonds used and the attention given to minimizing the diamond removed.

Custom Services

At DDK, we hope to make ultramicrotomy as easy as possible for our customers. Our popular custom testing program can add a degree of confidence to your work.

Send us a sample if you are having problems with a specimen, are unsure which type of knife to use in your particular application, or just to insure that your new knife will cut your embeddings. We will determine which type of diamond knife works best and supply grids to demonstrate those results. There is no charge for this service.

In addition, our craftsmen have experience in making and repairing diamond knives and tools of a wide variety. We would like to be a part of helping you develop tools to perform the special tasks your research requires.

Share your design with us or call with your ideas. You will find us willing to turn your ideas into reality and help evaluate their usefulness. Normally, there is no charge other than that for the product manufactured.

Warranties & Guarantees

At Delaware Diamond Knives, a large part of the cost of a diamond knife is the extensive testing each one is subjected to prior to shipment. One hundred percent of the entire edge of every knife is tested by cutting sections and examining them with the electron microscope. New knives, sharpened knives, ultrathin knives, even Histo Knives must pass these rigorous sectioning standards before shipment to you.

As a result, we confidently guarantee that our diamond knives, whether new or resharpened, are free from defects in material or workmanship. A diamond knife thought to be defective for any reason should be returned to Delaware Diamond Knives, Inc. We, in turn, will correct defects by repair or replacement, at our option. This guarantee is void if, in our opinion, the diamond knife has been damaged due to negligence or misuse.

Closing

We hope that the information in this booklet has been of assistance, either in educating or reinforcing your knowledge of diamond knives' use in ultramicrotomy. The work in our laboratories, however, is not the only source for the information contained here. Thousands of satisfied customers through the decades we have been involved with diamond knives have contributed to the contents of this manual. We welcome your contributions regarding applications, techniques, and care of diamond knives, too.

We take special pride in the results you get with our products. If ever we can assist in improving your results, please use our toll-free number to call and be sure to look for additional DDK products in the future.