DIATOME® DIAMOND KNIVES HANDLING AND USE

INTRODUCTION

DiATOME® is the leading manufacturer of diamond knives for ultramicrotomy in biological and materials research. The expertise obtained in collaboration with our customers enables us to offer you the highest quality knives available in the market today. This technical note was written in order to allow easy handling and use of DiATOME® knives. Please contact us if you need any assistance, or if you have any special requirements in your ultramicrotomy applications.

HANDLING

A diamond knife is not as fragile as you might think. Taking into account the vast array of soft and hard specimens it cuts successfully, the diamond knife cutting edge is quite durable. With proper care and handling, a DiATOME® knife can withstand a great deal of use. However, in order to avoid any unfortunate mishaps, the following precautions should be taken when receiving the knife:

- Ask your receiving department not to open the box.
- Inspect the two securing stickers on the knife box and inform us if they have been cut or damaged.
- When unpacking the knife, care should be taken not to touch the cutting edge.
- Once the knife is unpacked, it is ready for use and does not need initial cleaning.

PREPARATION BEFORE SECTIONING

A perfect section ribbon can only be obtained from a well-trimmed block. The upper and the lower side of the block must be parallel to the knife edge. The block should not be too wide because this would substantially increase the cutting pressure. This may result in “chatter”.

We have found that the best trimming results of biological and materials research samples, at room and cryo temperatures, are obtained with our diamond trim blades:

- trim 45 (Item #123-TT-45)
- trim 20 (Item #123-TT-20)
- trim 90 (Item #123-TT-90)

CAUTION: If trimming is done with a razor blade, always use a fresh, degreased blade of good quality. If the razor blade is old and damaged, it has tendency to leave steel particles on the sample block. Upon sectioning, these particles will cause damage to the diamond knife’s cutting edge. If a sample block is precut with a glass knife, use only a clean new portion of the cutting edge. This will reduce the risk of glass particles sticking on the sample block. Avoid during the entire preparation of the sample blocks the embedding of hard particles from pipettes, razor blades, glass knives, etc.

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SECTIONING AT ROOM TEMPERATURE

The quality of sections will be determined by a number of factors. The following points should be checked before commencing sectioning:

- Set the clearance angle and the cutting speed as indicated on the guarantee card.
- Tighten all of the screws in the sample block holder, the knife holder, etc.
- Align the block (with the backlight on) parallel to the cutting direction (see *Figure 1a*).
- Align the block (with the backlight on) parallel to the cutting edge (see *Figure 1b*).
- Align the lower side of the block (with the backlight on) parallel to the cutting edge (see *Figure 1c*).

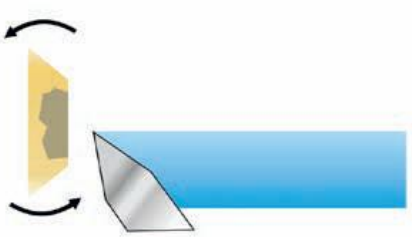

Figure 1a

Figure 1b

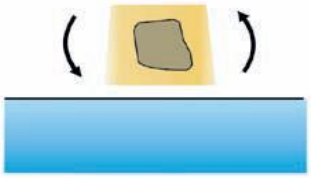

Figure 1c

The water in the boat should be level with the cutting edge and give a good reflection. *Exception*: for the sectioning of hydrophilic embedding materials, we recommend lowering the water level to a concave shape.

To avoid difficulties during sectioning and extend the life of a diamond knife, the following should be kept in mind:

- The sample block should be fully polymerized.
- Do not add solvents (i.e. acetone) to the distilled water in the boat. They may dissolve the sealing material between the knife and the boat.
- Avoid cutting thicker sections than the limits given for each type. See the limits below:
  - Ultra: 150nm
  - Cryo: 500nm
  - Histo: 2µm
- Exercise care when picking up sections. Do not touch the cutting edge with any solid objects (grids, loops, tweezers, etc.).
- Do not allow the sections to dry on the cutting edge.

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TRIMMING AT LOW TEMPERATURES

For successful cryo-sectioning of biological and materials research specimens, trimming is imperative. Our diamond trim blades (trim 45, 20, and 90) allow you to quickly and easily fulfill your trimming requirements.

For trimming, we recommend the same temperatures as set for the sectioning. It is advisable to use an ionizer, as it eliminates section debris sticking to the specimen and on the trim blade.

SECTIONING AT LOW TEMPERATURES

At low temperatures, the handling of the diamond knives is the same as for room temperature. Be careful when picking up the dry cryo-sections from the diamond surface. **The cutting edge may not be touched with any solid objects (i.e. loops, tweezers, or grids).** Please contact us for more information.

Dry Cryo Sectioning

The following temperatures are recommended for the cryo chamber, knife, and specimen:

- Sucrose infiltrated biological samples: Semi-thin sections -80°C, ultra-thin sections -110°C to -120°C.
- Frozen hydrated biological samples: -150°C.
- Polymers, rubber: -120°C (or below the glass transition temperature).

Dry Cryo-Ultramicrotomy Sectioning

The use of an ionizer is recommended, as it allows the cutting of perfect section ribbons and easy collection.

Cryo-Sectioning with a Boat Liquid (i.e. DMSO/water 50/50%)

Set the following temperatures:

- Cryochamber: -120°C.
- Specimen: -120°C.
- Diamond Knife: -30°C.

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CLEANING

Method 1

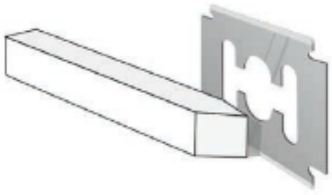

Figure 2a

Immediately after picking up the sections, remove all unused sections with a hair curl or an eyelash. Then clean the knife edge as follows:

- Empty the boat, dry carefully with filter paper (**without touching the knife edge**) and remount it in the ultramicrotome knife stage.
- Take one of our polystyrene sticks and bevel it to an angle of approx. 60° using an oil-free razor blade (see *Figure 2a*).
- Dip the rod into 70-80% ethanol and shake off the excess.
- Pass the rod over the cutting edge without applying pressure (see *Figure 2b*).

Figure 2b

Mechanical cleaning, as mentioned above, is absolutely necessary after cutting poorly polymerized blocks (i.e. Lowicryl® polymerized in a freeze substitution apparatus).

Method 2

If you do not feel comfortable using Method 1, there is an alternative.

Immediately after picking up the sections, proceed as follows:

- Remove all unused sections with a hair curl or an eyelash.
- Rinse the knife thoroughly with distilled water.
- Take a can of clean, pressurized air and blow the water off of the cutting edge.

Method 3

If sections or debris dry on the knife edge, we recommend the following cleaning procedure:

- Place the knife in distilled water, adding one or two drops of mild liquid dishwashing detergent.
- Let the knife sit overnight.
- Remove the knife and rinse with distilled water.
- Proceed to clean the knife using Method 1.

Cleaning of the Cryo Diamond Knives

- Remove the knife from the cryo chamber (**before heating the chamber up**).
- Rinse the knife under tap water to warm it up.
- Dip the polystyrene rod in 50% ethanol.
- Pass the rod over the cutting edge without applying pressure (see *Figure 2c*).
- Dry the knife with clean canned air.

Figure 2c

CAUTION: We do not recommend sonification for the cleaning of any of our diamond knives.
Solvents and acids should never be used under any circumstances.

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RE-SHARPENING

DiATOME® diamond knives can be re-sharpened an unlimited number of times due to special attention given to minimizing the amount of grinding during the sharpening procedure. The cutting edge length is not affected.

Our sophisticated manufacturing and re-sharpening methods are unique and allow us to guarantee that your re-sharpened DiATOME® knife will be of the same superior quality as your new DiATOME® knife.

GUARANTEE

Before delivery, each knife (new or re-sharpened), is subject to extensive testing. We only ship knives that perform to the highest standards.

In the unlikely event that you experience difficulties, or for any reason you are unhappy with the performance of your knife, please contact us immediately. A short description will allow us to solve the problem to your full satisfaction.

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