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Improved Cutting of One-Micron Plastic Sections using Qwick Glass Knives

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One-micron plastic "thick" sections, usually stained with toluidine blue, are indispensable light microscopic adjuvants for the selection and evaluation of tissue blocks prior to electron microscopy. Plastic thick sections may also be the only practical alternative to routine histologic sections when tissue specimens are limited or too small for processing in paraffin. In all cases, however, plastic sections provide superior light microscopic resolution compared to paraffin sections, but lack the compatibility with the wide variety of special stains that make paraffin sections so versatile.

Plastic Sections and Glass Knives

The cutting of plastic sections has evolved over the years since Latta and Hartmann[1.] introduced knives made from plate glass in 1950 to the present use of knives made from commercially available glass strips and most recently the use of diamond-edged "histo knives." Whether hand-made or commercially obtained, these knives are similar in design: a sharp cutting edge with a trough or reservoir for the collection of sections on a liquid surface.

For those who prefer to make their own glass knives, the process of making troughs may be tedious and time consuming. Up to now there have been two generally accepted ways to make knives with troughs. Commercially available pre-made troughs, either metal or plastic, may be affixed to the cutting edge of the knife by means of melted paraffin or beeswax. Or, alternatively, troughs may be constructed using electrical or mylar tape wrapped around the cutting edge and made leakproof by sealing the heal of the trough to the glass using paraffin, beeswax, or finger-nail polish. While these methods do provide very serviceable knives and excellent sections, an easier and quicker method is described.

We found that glass knives with excellent trough-like qualities may be prepared in a quick, simple, and convenient manner. The following protocol describing the making of "qwick knives" was improvised out of necessity, having run out of knives with conventional troughs and a mountain of EM blocks to cut.

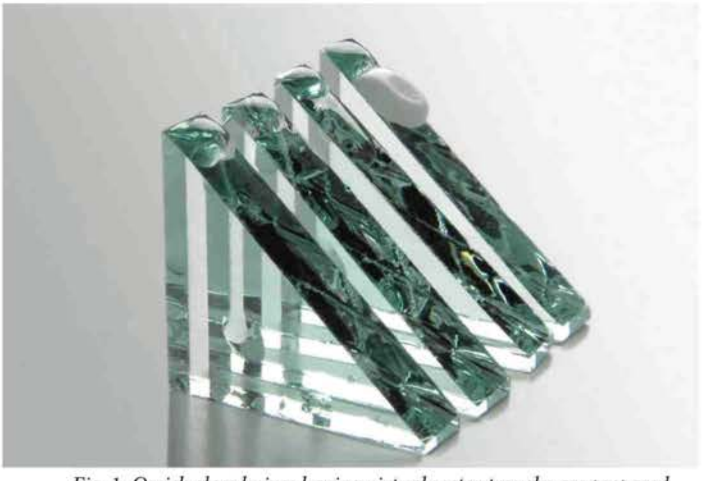

Fig. 1: Qwick glass knives having virtual water troughs are prepared by application of various hydrophobic substances including (left to right) pap pen, finger nail polish, histologic mounting medium, and paraffin. A water droplet added between the hydrophobic substance and the cutting edge of the knife provides a suitable water surface for the collection of sections cut from an epoxy-embedded tissue block.

Preparation of Qwick Knives

Initially, standard glass knives are broken from glass strips using an LKB knife breaker. Qwick knives are then formed by running a line of one of several wetting-resistant, hydrophobic substances (described below) from edge to edge across the hypotenuse surface of the triangular glass knife parallel to and approximately 5 mm below the cutting edge. With that done, qwick knives are complete and do not require any other fabrication or attachment. Qwick knives are easy to make and many can be made in a fraction of the time needed to prepare conventional knives with troughs, hence the name "qwick knives".

Individual qwick knives are placed into the knife holder of an ultramicrotome and the space between the hydrophobic substance and the cutting edge is filled with several water droplets administered by a 5 ml syringe fitted with a 24-gauge hypodermic needle. Enough water is added to form a liquid pool with a notably convex surface (fig. 1). The wetting-resistant hydrophobicity of the applied substance will contain the water as a small puddle and prevent it from running down the slope of the knife. Surface tension of the water is enough to prevent flow over the lateral edges and down the sides of the knife. If the line of hydrophobic substance is much lower than 5mm from the cutting edge it becomes difficult to keep the upper portion of knife wet as the water settles to the lower portion of the improvised trough.

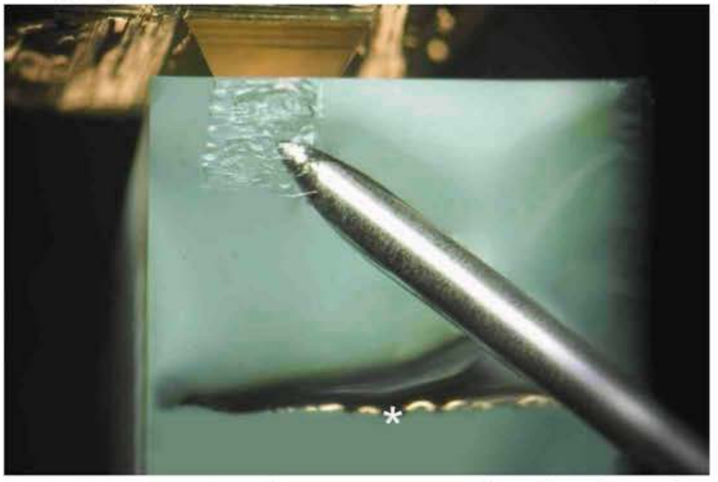

Fig. 2: A one-micron plastic section is cut onto the surface of a virtual water trough formed by application of a hydrophobic butadiene rubber barrier (*) using a pap pen. A hypodermic needle (or other implement) is slid underneath the section for transfer to a glass slide.

Use of Qwick Knives

Initially, qwick knives may be used in a dry state to polish off the face of a pre-trimmed block. Once the face is polished, water may be added and sections taken with the next cycle of the microtome. The cutting of sections proceeds as usual, but while using qwick knives the microtomist experiences greater efficiency with the microtome and the boredom of sectioning is relieved. With each cycle of the microtome, sections extend from the knife-edge across the convex surface of the water allowing the tip of a hypodermic needle or other implement to be inserted under the section (fig. 2). The section is then lifted from the water surface and transferred to a water droplet located on a glass slide. With successive cutting cycles, the transfer of each section can be timed to match the rhythm

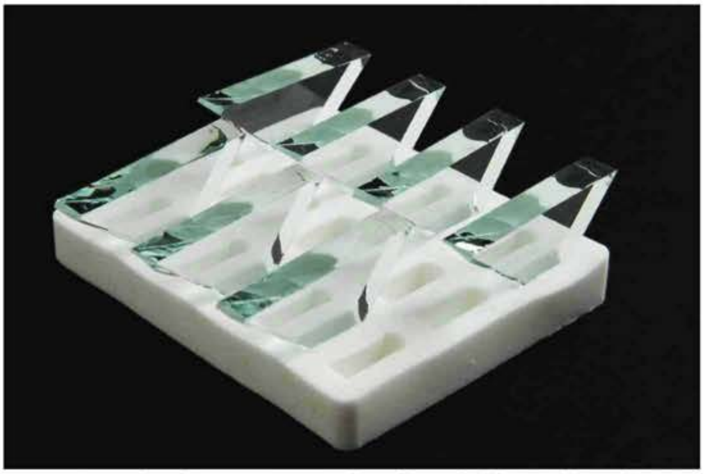

Fig. 3: Glass knives are supported by a flat embedding mold in order to prevent running of histologic mounting media down the slope of the knife. Knives are dried overnight at room temperature or in a 70ºC oven for at least an hour before use.

of the microtome so that five or six sections can be accumulated quickly on the glass slide. The glass slide is then placed on a hot plate or held over a gentle flame. With moderate heating, the water is evaporated to dryness and the section is allowed to attach to the slide. Sections are then stained in a conventional manner using 0.73% toluidine blue and 0.27% basic fuchsin in a 30% methanolic solution with a few drops of saturated sodium borate over a hot plate or low flame.

Choice of Hydrophobic Substances

We tried several different hydrophobic substances while making qwick knives including histologic mounting medium (acrylic resin), finger nail polish (acrylate copolymers), paraffin wax, pap pen (butadiene rubber), and Vaseline (petroleum jelly).

We found that histologic mounting medium made excellent knives, but needed overnight drying at room temperature or at least an hour in a 70ºC oven prior to use. The knives also needed to have the mounting medium applied and dried with the sloped portion of the knife in a horizontal position in order to prevent running of mounting medium. We achieved this by supporting the knives in a flat embedding mold prior to application and drying of the mounting medium (fig. 3). Once dried, the histologic mounting medium was ready to hold a moderate reservoir of water next to the cutting edge of the knife appropriate for collection of sections.

Finger nail polish dried rapidly and the knife was ready to use soon after fabrication. Only a single application of nail polish was needed to produce a knife with a water surface suitable for collection of sections. The size of the water droplet could be varied from one having slight surface curvature to one that was quite rounded. A surprising amount of water could be added before the nail polish barrier was overwhelmed.

Warm liquid paraffin was readily obtained from our histology laboratory. About 1 ml of liquid paraffin was drawn up into a standard Pasteur pipette. So that paraffin would not rapidly cool and harden inside the pipette, the pipette was preheated over a gentle flame or warming plate prior to being dipped into the paraffin. With gentle pressure on the pipette bulb, a thin line of liquid paraffin could be drawn across the sloped portion of the knife as described above. The paraffin hardened quickly on the cool glass surface forming a sizable water dam. Knives made with paraffin could be made quickly as needed at the time of sectioning and did not require extended drying as did knives made with mounting medium. Since the paraffin was not only hydrophobic but also more bulky than the other substances, knives made with paraffin could hold more water than any of the others. However, knives made with paraffin had some disadvantages. They would occasionally slowly leak if the paraffin hardened too quickly with poor adhesion to the glass. We found that, in order to avoid interruption of sectioning due to a slow leak, water could be added by means of the syringe/hypodermic at intervals to compensate for the water loss. Another disadvantage was that, with the making of numerous knives, the preparation area became spotted with many hardened droplets of paraffin. Despite the disadvantages, qwick knives prepared with paraffin performed well.

We were astonished at the performance of knives made with the pap pen (Liquid Blocker; Cat. No. 6505, Newcomer Supply, Middleton, WI 53562). Pap pens are used typically for segregation of liquids on a glass slide to avoid mixing of reagents. Our immunology laboratory uses pap pens to encircle different antibody solutions on a single slide used in the immunologic labeling of histologic sections. For our purposes, a single swipe of the pen across a glass knife gave a precisely positioned, near invisible barrier that held an enormous amount of water at the knife's edge. Knives made with a pap pen were the quickest and easiest to prepare and required very little drying time. They could be made in a matter of minutes prior to sectioning and provided a virtual water trough that was perfect for collection, lifting, and transfer of sections. Knives made with a pap pen were clearly our favorite.

Vaseline was applied to glass knives and sculpted by use of a flat toothpick. As water was added, the water and petroleum jelly slid down the slope of the knife rendering it useless.

Summary

Qwick knives are ideal for the busy electron microscopy laboratory. And in institutions where electron microscopy is being squeezed, qwick knives save both time and money. They are more economical than the commercially available "histo knife" and easier and quicker to make compared to knives made with plastic or metal troughs or knives made with tape. All the qwick knives except those made with Vaseline performed well. Those prepared with the help of a pap pen were easiest to make and the felt tip of the pen allowed precise placement of the hydrophobic barrier. The pap pen, nail polish, and paraffin permitted knives to be prepared almost immediately before sectioning, thus guaranteeing that the glass cutting edge would be at its sharpest. Knives made with histologic mounting medium equaled the performance of the others but needed to be made at least an hour in advance of sectioning.

Qwick knives allow the successful preparation of 1-micron "thick" sections from plastic blocks of all sizes including those with large faces. Qwick knives may also be useful in cutting thin sections from blocks having relatively small faces. Qwick knives may even be useful to the novices first honing their sectioning skills in electron microscopy, but not yet ready to graduate to a diamond knife.

Acknowledgment

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Reference

1. Latta, H. and Hartmann, J. F. (1950). The use of a glass edge in sectioning for electron microscopy. Proc. Soc. Exptl. Biol. Med., 74, 436.