Electron Microscopy Technique and Ultrastructural Assignment

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 Assignment

Ultrathin sectioning can also be performed with a glass knife. The preparation of a glass knife starts by selecting good glass rods from which they are made. The glass should be tough and preferably without consistency differences in the glass (Pittsburgh glass). The best way to make knives is according to Tokuyasu’s balanced break method using a Leica knife maker, a perfect knife is made by breaking a glass rod in two equal parts, next each part is broken in two equal parts etc., till squares of 2.5 x 2.5 cm are produced. This balanced breaking method yields squares, with freshly broken surfaces perpendicular to the upper and lower surface of the square because equal forces have been applied to the glass during the breaking. However, in practice it is virtually impossible to produce squares using this method due to imperfections in glass dimensions and tolerances in the knife maker. Therefore, the method has been adapted by Griffiths. He first makes double squares in a row from a glass rod. The freshly broken surfaces will not be perfect, but when a double block is broken into two squares the forces applied to each side will be identical thus ensuring at least one side of the square has a surface perpendicular to the upper surface. Two glass rods are broken into double blocks using the short scoring line and placed on a shelf in the same order as they were made. Next the twin squares are broken into squares. All right-side and left-side squares are collected separately in the order in which they are made. First all the right-hand squares are broken into triangles using the same length of the scoring device. The sharpest knives will be obtained when the breaking occurs exactly on the diagonal of the square. Furthermore, the crack should develop slowly using moderate forces and should be perpendicular to the upper surface of the square. To meet all these requirements, the midpoints of the upper pins of the knife maker must necessarily be positioned at equal distances from the scoring line. The support pins at the lower surface of the glass should be positioned exactly beneath the scoring line. These are factory settings, and should be checked if you suspect the pins to be ill positioned. (This can be checked by breaking several twin blocks. If the breaking plane shows the same aberration repeatedly, the positioning of the pins is wrong). To be able to fracture the squares along the diagonal the position of the square is carefully examined after each fracture and if necessary adjusted. If the counter piece is hardly visible and the sharp edge is made out of the freshly broken plane, the knife is probably quite good. Since positioning of the square may need some trials, first use all the right-hand squares (which are more identical to each other than mixture of right and left). When that is finished the same procedure is followed for the series of left-hand squares. Knives with a convex edge or with elevated stress lines should not be used.



Preparation of glass knives

**2. Knife Maker**

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