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A Method of Mesa Trimming with Glass Knives for Obtaining Large Series of Ultrathin Sections

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Abstract—Ultrastructural analysis of tissue based on 3D reconstruction from serial ultrathin sections is one of the most adequate methods in studies of spatial organization of bio-objects. The sample preparation technique for 3D reconstruction includes the two most technically difficult procedures: an obtaining of stable ribbon of serial sections and mounting of this ribbon onto a slot grid coated with a support film. To mount the ribbon, special approaches and technical tools have been proposed and well evaluated. Much attention has also been paid to obtaining a large and stable ribbon, but this attention deals mainly with the choice of epoxy embedding media. The critical condition of obtaining the straight and stable ribbon is the precise parallelism of trailing and leading edges of mesa falling onto the knife cutting edge. The mesa trimming with dry diamond knife for cryoultratomy allows this condition to be maintained. In the present communication, the way of obtaining parallel sides of the mesa has been proposed with the aid of two forms of glass knives.

Keywords: electron microscopy, 3D reconstruction, ultrathin serial sections, diamond knife, glass knife, mesa trimming

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INTRODUCTION

Analysis of tissue structure with use of a "volume disector" based on serial sections (Sorra et al., 1998; Fiala, Harris, 2001) has been rightfully considered one of the most effective methods for analysis of the spatial organization of biological structures, while the subsequent quantitative analysis based on three-dimensional (3D) reconstruction is considered the "gold standard" in morphometry (von Bartheld, 2001).

The first 3D reconstructions at the ultrastructural level, specifically, those of synapses of the central nervous system, were performed in the 1950s–1960s on profiles of studied structures dissected from wax plates (Sjöstrand, 1958; Westrum, Blackstad, 1962). At present, methods of 3D reconstruction remain a priority not only in brain studies, but also are continuing to be published with refinement and facilitation of the technique of preparation of serial sections (Harris et al., 2006). In spite of the achievements of high-resolution electron tomography (Frank, 2006) and advances in the development of serial block-face scanning electron microscopy (SBFSEM) (Denk, Horstmann, 2004; Gatan, Inc., United States) and focused ion-beam scanning electron microscopy (FIB-SEM) (Knott et al., 2008; Bushby et al., 2011; FEI Co., United States), studies on serial sections so far have remained an adequate method of analysis of the ultrastructure of a large tissue volume (Hoffpauir et al., 2007).

Using serial sections, we were the first to show the presence of single taste receptor cells in the mammalian gustatory epithelium (Lindemann, 2001). Recently, in combination with analysis of a large series of semithin sections, we obtained the first data on the ultrastructure of dendritic synapses of identified dark and light pyramidal neurons in deep layers of the hippocampal neuropil (Klimenko, Rogachevskii, 2012).

The use of 3D reconstruction on ultrathin sections has been traditionally considered to be restricted by the necessity for surgical precision when working with sections, labor-intensity of the method, and significant time and finance expenditures. Studies carried out in our laboratory for many years with the use of 3D reconstructions have allowed us to develop well-reproducible methods of obtaining series of sections with minimal expenditures. The present communication provides us a possibility of publishing this experience.

The process of preparation for analysis of the structure of bio-objects with the use of 3D reconstruction on serial ultrathin sections requires adherence to several key rules. They consist in the necessity of (1) qualitative chemical tissue fixation, (2) adequately chosen epoxy resins embedding medium, (3) the presence of qualitative knives, (4) a specially shaped pyramid at the top of a tissue block, (5) obtaining of a sufficiently long ribbon of up to 100–200 serial sections, (6) accurate mounting of this series onto a slot grid coated with

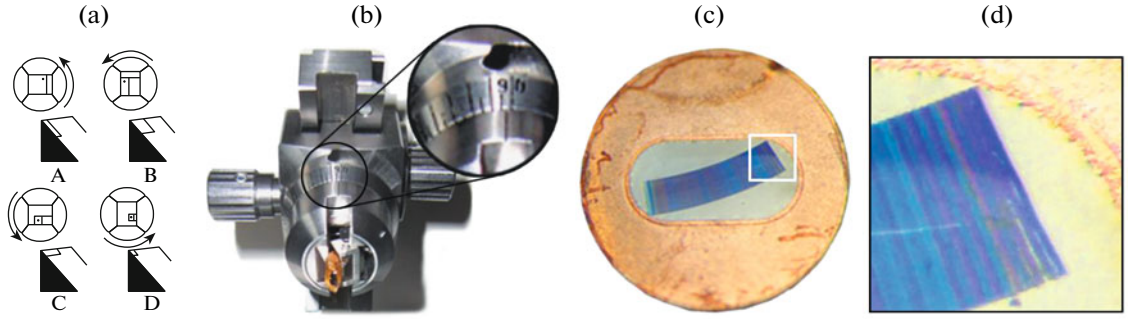

Fig. 1. Illustration of the method of mesa trimming with the use of specimen holder rotation and the resulting series of sections. (a) Means of mesa trimming with consecutive (A–D) turns of a block by 90° (by Mironov et al., 1994, with changes); (b) external view of a specimen holder of the Leica EM UC6 ultratome with a scale for specimen rotation to a desired angle; (c) a curved ribbon of 90 sections obtained with the Diatome Ultra 45° diamond knife and mounted onto a support film, mesa sides close to parallel obtained with a turn of the specimen holder by 180° and the deviation angle of the mesa sides from parallel amounting to less than 0.5°; and (d) magnified area of the same ribbon of sections, uneven section thickness and separation of sections from each other are obvious (on the right below).

a support film, and (7) staining of sections to rule out their contamination.

Regardless of the type of studied tissue (object), method of fixing it, properties of the embedding medium, or use of a diamond or glass knife, stability of the ribbon promotes retention of uniform section thickness and makes it easier to transfer the series from the fluid surface on the trough of the knife onto the coated grid without loss of sections, preserving thereby series' integrity.

In the present communication, which is addressed to researchers already experienced in ultramicrotomy, the key point in sample preparation for obtaining large series of ultrathin sections is considered, specifically the way to produce a trapezoid-shaped eminence at the tip of the tissue block for obtaining a stable section ribbon without the necessity of using an expensive diamond knife. This method is therefore available to any microscopic laboratory, and the presence of a knifemaker is not obligatory.

CONDITIONS FOR OBTAINING STABLE SERIES OF SECTIONS AND METHODS OF REALIZING THEM

Stability of a series can be achieved in several ways—an adequate choice of embedding medium or the use of adhesives applied to the lateral sides of the cutting eminence (Harris et al., 2006; Ruthensteiner, 2008). However, the key factor is parallelism of trailing and leading edges of the pyramid falling upon the knife cutting edge. Whereas, in routine ultratomy, it is enough to use a razor blade to obtain a manually truncated pyramid, the qualitative trapezoid for serial sections can be prepared only with ultramicrotome using a glass or diamond knife as a cutter or mill of trimmer. However, the use of trimmers, such as Reichert/Leica Ultratrim, Leica EM TRIM, or Leica EM TRIM2 (Austria/United States), is ruled out for this purpose. Since any heterogeneity of trimmed surfaces is a cause of fast dulling of the knife cutting edge, to preserve cutting properties of the knife in the course of obtaining a long series, the lateral sides of the pyramid should be as smooth as possible. This can be achieved only by trimming with ultratome using a microfeed in an automatic mode (Galey, 1963). The surfaces of the pyramid obtained with the help of a trimmer are more heterogeneous.

Mironov et al. (1994) described a way to obtain two parallel sides of a pyramid with a glass knife. For this, the knife holder is turned clockwise by 30° in the horizontal plane and one of the sides is trimmed; then, the knife holder is turned counterclockwise by 30° and the procedure is repeated by obtaining two parallel sides. The disadvantage of such an approach are the sloped sides of the truncated pyramid, which leads to an increase of the section area in the process of elongation of the series and, hence, to a change of cutting conditions. For homogeneity of cutting conditions at the beginning and end of the series, the eminence at the block face needs to have lateral sides at a right angle to its face surface. Obtaining such a "mesa" (resembling a mountainous relief with a flat top and steep slopes, widespread in the Southwest United States and Mexico), usually with the shape of a rectangular parallelepiped protruding over the tissue block surface, is called "the mesa-trimming technique" (Bozzola, Russell, 1999; Mironov at al., 1994).

Steep mesa sides can be obtained by trimming the tissue block with a sharp edge of a glass knife (Galey, 1963; de Bruijn, Mcgee-Russell, 1966; Mironov et al., 1994) by turning the specimen by an angle of 90° or 180° (Fig. 1a). Modern ultramicrotomes are equipped with specimen holders with a scale that allows turning the specimen by a certain angle (Fig. 1b). However, in practice, an attempt to turn the specimen exactly by the desired angle suffers failure. Even if the thereby-prepared mesa sides turn out to be close to parallel, the

difference even at 0.5° will produce a bend of the obtained ribbon of 100 sections by an angle of about 40°–50° (Fig. 1c). Deviation of the sides from parallel also is one of causes of unequal section thickness and rupture of the ribbon even before it is mounted onto the coated grid (Fig. 1d). Moreover, it is quite difficult to handle with the curved ribbon of sections on the trough, and even more so to mount it in the center of the support film. The curved ribbon will produce additional complications during selection and photographing of the region of interest in consecutive sections and subsequent alignment of serial images for 3D reconstruction.

To transfer sections from the trough of the knife and mount the series onto grids and the support film, well-recommended special technical tools and approaches were proposed long ago, such as the "perfect loop," method of Gay and Anderson (1954), micromanipulator of Barnes and Chambers (1961), device of Westfall and Healy (Healy, 1961; Westfall, Healy, 1962), Rostgaard's "third hand" (Behnke, Rostgaard, 1964), or the graceful method of Galey and Nilsson (1966) modified by Wegner (1971) and later by Mironov et al. (2008). Much attention has been paid to the large, and at the same time stable, series of sections, but this mainly concerns the choice of epoxy embedding media (Hayat, 2000).

In the foreign practice of serial ultratomy, for instance, in Prof. Christen Harris' laboratory, which is a lead one in this area (Harris et al., 2006; www.synapses.clm.utexas.edu), for these purposes, it is recommended to trim mesa sides with a dry diamond knife for cryoultratomy (e.g., CryoTrim 45°, Diatome US, Inc., United States). Such a knife has cutting edges on both the left and right sides (Fig. 2a). When mesa parallel sides are trimmed with a CryoTrim 45° knife, the specimen position remains unchanged, with only the knife position altering. The cutting face of the specimen block is first cut away on the left side, and then on the right, so two parallel edges are obtained (Fig. 2b, stages 2 and 3). The specimen is then turned by 90° along the long axis of the specimen holder arm, and the procedure is repeated (Fig. 2b, stages 4 and 5). The new CryoTrim 45° is rather expensive (from 60 thousand to 120 thousand rubles), but an older knife of this type, which is in any case unusable for cryoultratomy, cannot be found in every laboratory. If the study is designed for analysis of microbiological specimens with cells that have mineralized cell walls, even the new diamond knife will rapidly become unusable. Therefore, to prepare the mesa, the use of glass knives with a price is three orders lower than that of the diamond ones has doubtlessly remains actual. To obtain parallel sides of the mesa, Hanssen et al. (2010) used a glass knife with two sharp corners. To obtain such a knife, the fracture line of the glass square was shifted from the square center, which required a change of the knifemaker adjustments.

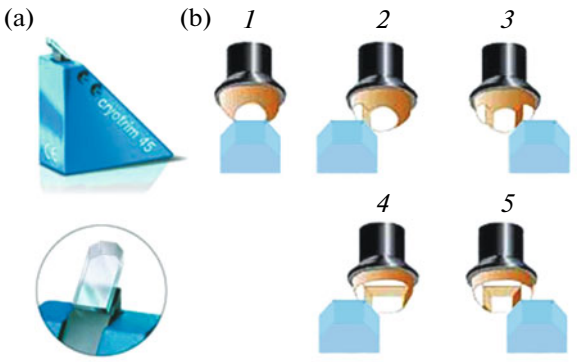

Fig. 2. External view of a dry CryoTrim 45° knife (a) and a procedure of block trimming using it resulting in parallel sides of the mesa (b).

(a) External view of the knife and profile of its diamond cutting edge (*round insertion*); (b) stages of mesa preparation: (*1*) trimming the specimen block front surface, (*2*) obtaining the left mesa side, (*3*) obtaining the right mesa side parallel to the left one, (*4*) object is turned by 90° to obtain the upper side of the mesa, and (*5*) obtaining the parallel lower side of the mesa (from www.diatomeknives.com with changes).

A simple method is presented below for obtaining two glass knives with "mirrored" profiles for trimming the mesa with parallel sides (patent application no. 2012157496 of December 27, 2012). The proposed method does not require any change of knifemaker settings. It is based only on a change of position of the glass square before a break into two knives; therefore, it may be carried out even in the absence of a knifemaker.

ESSENCE OF THE METHOD

Preparation of knives with LKB 7800 series knifemakers. The procedure of preparation of knives with an angle 45° according to the instruction for the LKB 7800 series knifemaker (Sweden; an analog of 7800 series knifemakers was produced in the Soviet Union by the Zhdanov medical-instrument-making plant under the trademark CCH-1) consists in that the square cut out from the glass strip is turned counterclockwise by 45°, is scored with a glass cutter, and is broken diagonally into two knives (Fig. 3, procedures A_1 and A_2). Such knives will have an even fractured sharp corner on the left (Fig. 3, *arrow* on upper *round insertion* (A_2); Fig. 4a), while the cutting edge will be formed by plane *d* (dirty) produced upon the manufacturer's breaking of the glass into strips. We used a knife of such a profile to trim the right side of the mesa (Fig. 4c). The knife cutting edge on the right is ended on the uneven surface of the *d* plane that has a trace of a fishscalelike fracture and is unacceptable for trimming the mesa side on the left (Fig. 3, *arrowhead on round insertion* above (A_2)).

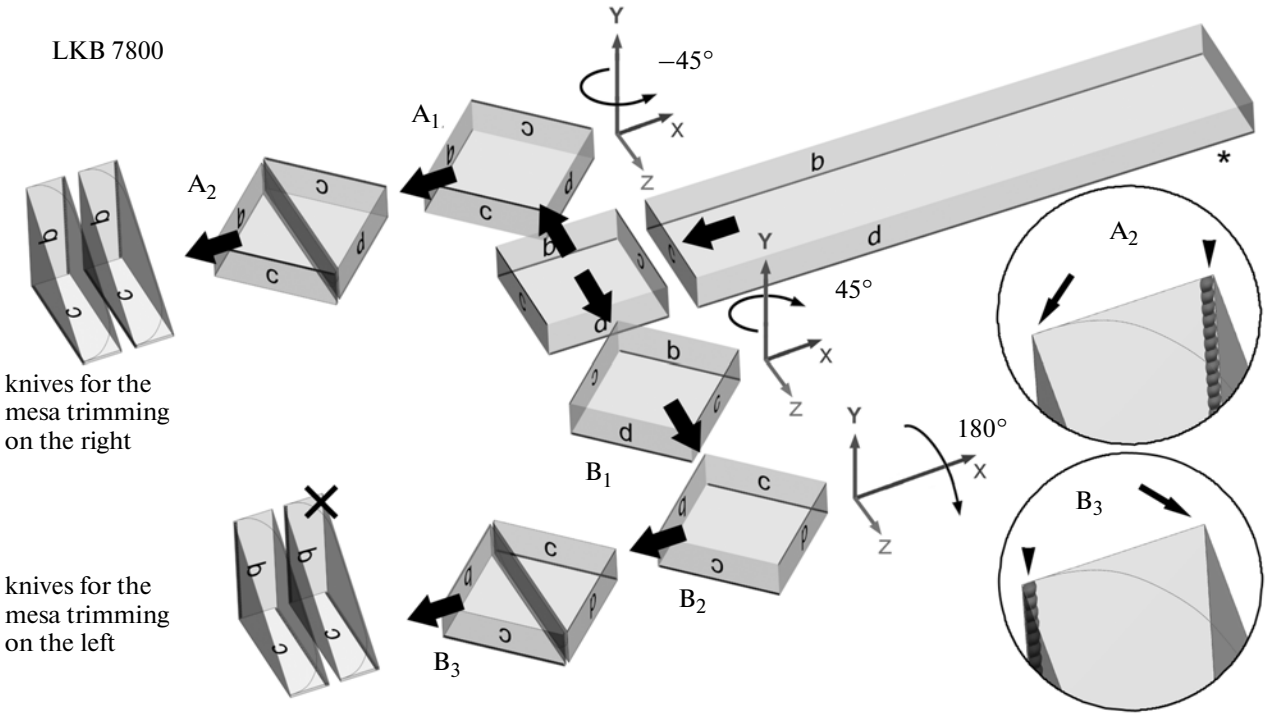

Fig. 3. Scheme of obtaining two types of glass knives with the LKB 7801 knifemaker.
(A_1 and A_2) Standard procedures of preparation of knives in accordance with the instructions for the knifemaker; (B1–B3) procedures of preparation of a knife with mirrored profile, one of the two knives as a rule having a broken right corner and being unacceptable for mesa trimming (*crossed out* at stage B_3). For clarity, planes of squares and knives are marked by letters *d* (dirty surface) and *c* (clean surface obtained with knifemaker); the position of the letters at each stage is changed according to the change of the square position. The glass strip on the knifemaker is initially disposed in such a way that a trace of a fishscalelike fracture resulting from the manufacturer's breaking of glass into strips is located below (marked by *asterisk*). Other explanations are in the text. Color illustrations with a high resolution are available as supplementary material at www.synapsis.ru.

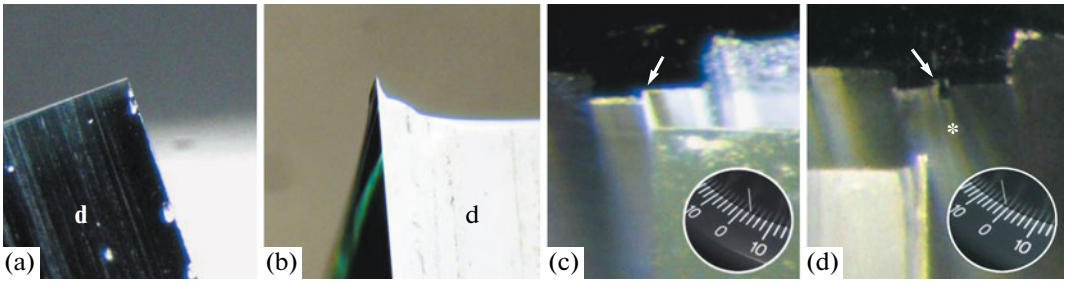

Fig. 4. Photograph of an ordinary knife (a) and a knife with a mirrored profile (b); a cutting edge is formed on the *dirty surface*.
(a) Sharp left knife corner for mesa side trimming on the right (rear view); (b) sharp right knife corner for mesa side trimming on the left (rear view); (c) mesa side trimming on the right (*arrow*), with trimming to be performed with the knife hardly rotated counterclockwise (*round insertion*); and (d) mesa side trimming on the left (*arrow*), with trimming to be performed with the knife hardly rotated clockwise (*round insertion*). Since the knife corner on panel *b* has a sharpened peak, the knife clearance angle upon mesa trimming on the left is to be reduced up to 2–1.5°. The obtained mesa with parallel sides is marked with an *asterisk*.

To prepare a knife with a "mirrored" profile with a sharp corner on the right for trimming the block on the left side, it is possible to change positions of the holding forks fixing the glass square before the fracture (Fig. 5b). However, as in the case of the method of Hanssen et al. (2010) (Fig. 5c), this procedure requires turning back the forks' positions and then laborious long turning of the knifemaker along with checking of the quality of the obtained knives and with significant use of glass strips. This is why, after thorough turning of the holding forks' positions has been carried out, knifemaker settings are not changed for years.

In preparation of a knife with a "mirrored" profile without changes of the knifemaker settings, the square

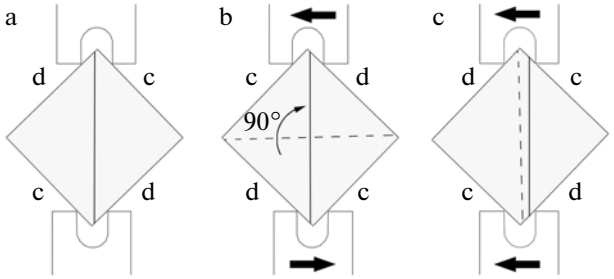

Fig. 5. Scheme of setting changes of an LKB series 7800 knifemaker to obtain knives with a mirrored profile.

(a) The first knife type can be prepared according to instruction to knifemaker; (b) to prepare the second type of knives with the "mirrored" profile, it is necessary to turn the glass square by 90° and change the positions of the holding forks (the direction is shown with *arrows*); and (c) obtaining a glass knife by the method of Hanssen et al. (2010). Vertical uninterrupted diagonal lines show planes of square break into two knives. On panels *b* and *c*, the positions of square break planes corresponding to the break plane on panel *a* are marked with a *broken line*; *dirty* and *clean* are the planes of manufacturer's break and the break of glass strip into squares, respectively.

cut out from the glass strip is to be turned clockwise by 45° and along the longest knifemaker axis by 180° (Fig. 3, *procedures* B_1–B_3). Upon a diagonal fracture of the square, one out of two knives will have a sharp corner on the right (Fig. 3, *arrow* on lower *round insertion* (B_3); Fig. 4b). Unlike the first type of knife, the sharp corner has the shape of a sharpened peak. We used this knife to trim the left side of the mesa (Fig. 4d). The left side of the cutting edge of this knife is restricted by the uneven surface of plane *d* that has a trace of a fishscalelike fracture and is unacceptable for trimming the block on the right side (Fig. 3, *arrowhead* on round *insertion below*) (B_3)).

In preparation of a knife with a "mirrored" profile, to prevent excessive actions, procedure B_2 (Fig. 3) may have been omitted and the square may have been broken into two knives after the clockwise turn (Fig. 3, B_1). However, in this case, as in Hanssen's method, the cutting edge will appear on surface *c* (clean). Meanwhile, creation of a sharp corner by surface *c* even in the case of the slow balanced break does not always provide satisfactory result (Fig. 8 below and explanation in the text).

Mesa trimming with two knives with "mirrored" profiles. The process of the mesa trimming is similar to the above-described method with use of a dry CryoTrim 45° diamond knife. In the process of trimming, the position of the tissue block also remains unchanged, which allows parallel sides to be obtained.

The optimal form of a mesa surface for picking up a large series of ultrathin sections onto one grid is an elongated parallelogram. Thus, with a width of the mesa between long parallel sides of about 20 μ, up to 100 sections can be placed per grid with a slot size of 1 × 2 mm. The degree of mesa deformation upon falling onto the knife edge depends on the distance between the long parallel sides. Whereas the mesa width between them amounts only to 20 μm, to decrease the mesa deformation in the course of ultratomy, it is worth trimming down by no deeper than 20 μm (Figs. 4c, 4d). Mesa trimming with Leica EM UC6 ultratome (Austria) at a microfeed of 20–50 nm and an falling rate of an object onto the knife of about 80–100 mm/s provides satisfactory results (Fig. 6a). Under the indicated conditions, with two glass knives

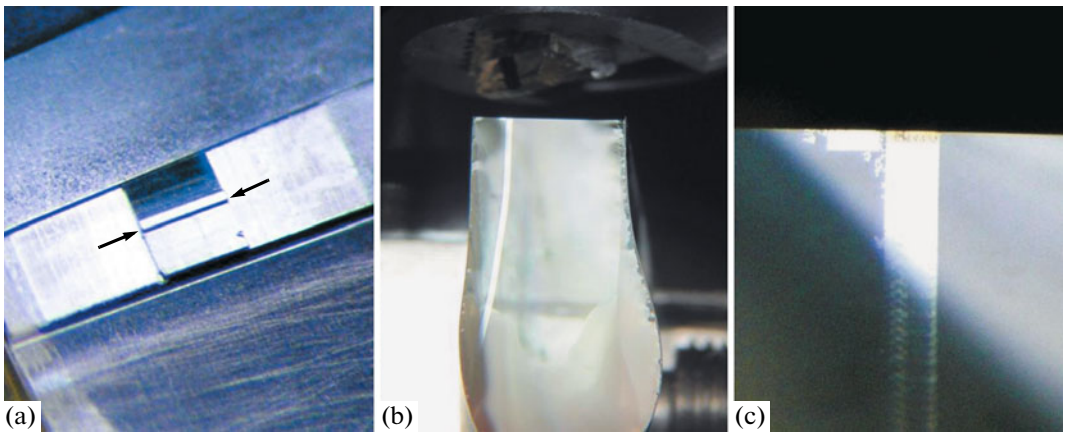

Fig. 6. Example of mesa and stable series of section obtained with the help of glass knives.

(a) Photograph of mesa with parallel sides prepared with two glass knives of mirrored profile, area ≈250 × 45 μm (marked with *arrows*); (b) series of sections obtained with a glass knife from the mesa presented on panel *a*; and (c) the same ribbon of sections near the knife edge at the higher magnification. The ribbon on the water surface more than 9 mm long consists of more than 200 sections. The instruments and parameters of the presented series are object, osmificated hippocampus; embedding medium, EMbed812/NMA/DDSA (ratio of mixtures A : B = 3 : 7 in accordance with Luft (1961)); ultramicrotome, Leica EM UC6; glass knife, 45° (width 6 mm); knifemaker, LKB 7801; knife clearance angle, 6°; trough with distilled water; section thickness, 60 nm; and cutting speed, 1.6 mm/s.

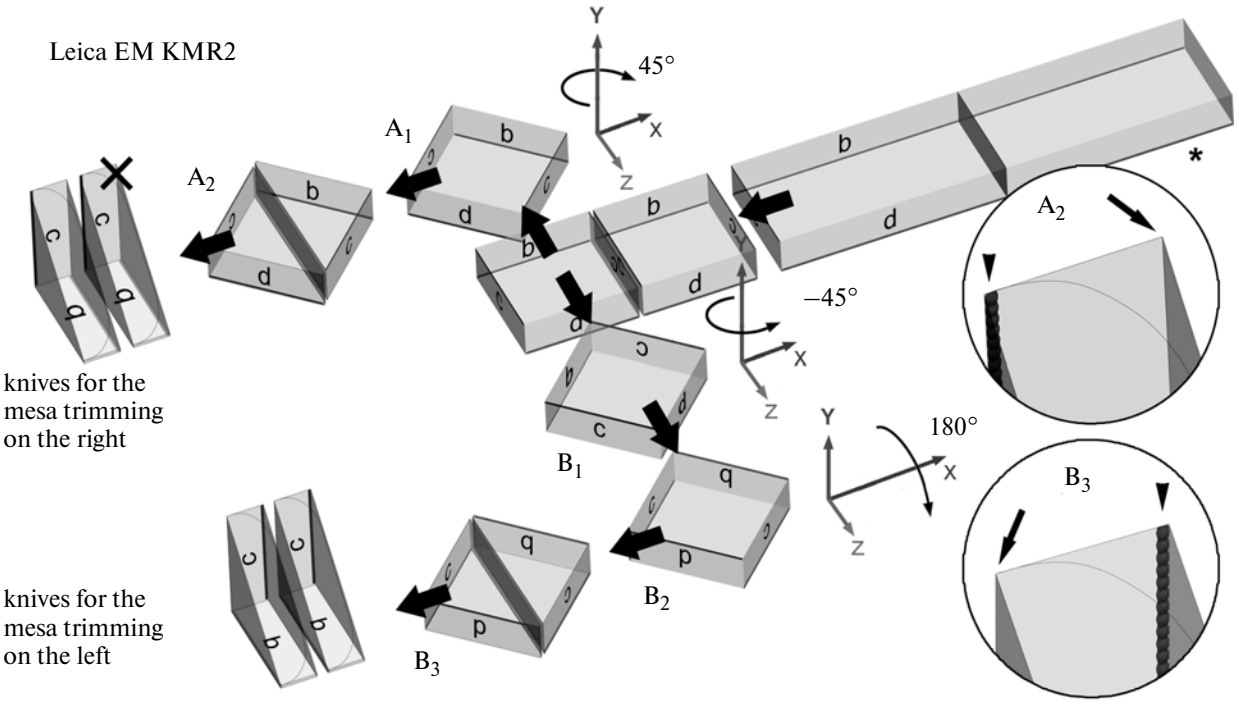

Fig. 7. Scheme of obtaining two types of glass knives with the Leica EM KMR2 knifemaker.
(A_1 and A_2) Standard procedure of making knives, one of them as a rule having a broken right corner and being unacceptable for mesa trimming (*crossed out* at the stage of A_2). (B_1–B_3) Procedures of preparation of a knife with a "mirrored" profile. Designations are the same as in Fig. 3.

with "mirrored" profiles, it is possible to trim up to ten or more Epon mesas of high hardness; the ratio of mixtures A : B = 3 : 7 by Luft (1961). With a mesa prepared in the described way and with observance of the general rules of ultratomy, it is not complicated to obtain a stable ribbon of 100–200 sections with either a diamond or glass knife (Figs. 6b, 6c). At the initial stage of obtaining the series, just after the first sections involving the entire cutting face of the mesa have been cut, it is useful to create conditions that rule out temperature and air fluctuations near the ultratome. For this purpose, in foreign practice, a transparent box is mounted onto an antivibration table enclosing the ultratome (Harris et al., 2006). In the absence of such a box, quite enough to leave the ultratome behind and get back to it only when the ribbon will have reached the desired length.

Practical use. We studied the three-dimensional structure of synapses on apical dendrites of dark (hyperchromic) and light neurons in *Str. radiatum* of rat CA1 hippocampal neuropil at a depth of about 150 μm from neuronal bodies where the vast majority of excitatory synapses are formed by hippocampal trisynaptic loop projections (Klimenko, Rogachevskii, 2012). The morphology of dark neurons is characterized by a reduction in optical density when apical dendrites protrude into the neuropil, which makes the dendritic profiles undistinguishable from dendritic profiles of light neurons. To determine if dendrites belong to dark or light neurons, we used a series of 200–300 semithin (thickness of 1 μm) sections with a large area of hippocampal CA1 field in the plane parallel to the neuronal layer (perpendicular to apical dendrites). At a depth of 150 μm, the dendritic diameters are about 2 μm, which in aligned sections allows to trace the dendrite from the neuronal body to the last section in the series.

For structural analysis of synapses located on this dendrite, in the corresponding area of the tissue block face, a mesa was prepared to obtain a series of ultrathin sections. Since the direction of the apical dendrite course is initially unknown, so that the dendritic profile would not go outside the boundaries of images, it was necessary to obtain images captured from a large area of semithin sections. This eventually affected the time of their processing. In each case, the entire process from obtaining the series of semithin section up to determination of the position of the dendrite of the identified neuron on the block face took no less than 1 month. Therefore, from the areas of block faces determined in the way described above, it was necessary to obtain ribbons of serial sections the stability of which would have been completely ruled out a probability of section loss in the process of ultratomy and rupture of the series during its transfer and mounting onto grids.

In the above-described work of Klimenko and Rogachevskii (2012), we did not use diamond knives of the CryoTrim 45° type. To prepare a mesa with an area of 15/30 × 100/250 μm, we used two types of glass knives with "mirrored" profiles prepared with an LKB 7801 knifemaker (Sweden) in accordance with the method proposed in the present work.

Preparation of two types of knives with new-generation knifemakers. The preparation procedure of glass knives with more modern models of knifemakers is different. The method of knife preparation with the Reichert-Jung type 705202 (Reichert-Jung, Austria), the Leica EM KMR2 (Austria), and the most constructionally refined Leica EM KMR3 (Leica Microsystems, United States) and GKM-2 (RMC, Boeckeler Instruments, Inc., United States) is based on the principle of the balanced break in accordance with Tokuyasu and Okamura (1959) and Griffiths et al. (1983) for obtaining glass knives for cryoultratomy. The process of knife preparation begins from breaks of the glass strip into fragments of equal weight, up to obtaining glass squares (Fig. 7). The method is also distinguished by the fact that, in the process of breaking squares into two knives, the cutting edge is formed by plane *c*. For this purpose, the square obtained from the glass strip is turned clockwise by 45°, scored, and broken into two knives (Fig. 7, *procedures* A_1–A_2). One of the two knives will have a sharp corner on the right via which the left side of the block can be trimmed. The left side of the cutting edge of this knife is restricted by the uneven surface of plane *c* that has a trace of a fishscalelike fracture and is unacceptable for trimming the mesa side on the right (Fig. 7, *arrowhead* on *round insertion* above (A_2)). The second knife, as a rule, has a broken right corner and is unacceptable for mesa trimming. To obtain a knife with a "mirrored" profile without a change in the knifemaker settings, to trim the block on the right, it is possible to use a rule similar to that proposed above for preparation of two types of knives with LKB 7800 series knifemakers. In particular, prior to the break into two knives, the glass square needs to be turned counterclockwise by 45° and by 180° along the long knifemaker axis (Fig. 7, *procedures* B_1–B_3). The prepared knife will have a sharp corner on the left (Fig. 7, *round insertion* below (B_3)); this allows making the side parallel to the first one by trimming the mesa on the right side.

We had no possibility to test the method with the newest models of knifemakers, but the functionality of the method and identity of the results have been checked and confirmed on two previous models: the Reichert-Jung type 705202 and the Leica EM KMR2. The construction of the GKM-2 knifemaker differs in the position of the scoring wheel (it is located underneath a glass strip), but the orientation of glass strips and squares during preparation of knives with KMR2, KMR3, and GKM-2 knifemakers is identical.

Choice of plane forming the cutting edge. During preparation of two types of glass knives to obtain a cutting edge, we used surface *d*. Our choice is dependent on the profile of the rear knife surface near the sharp corner (Figs. 3, 7, *arrow* on round insertions). The closer this surface to the plane (Fig. 8a), the higher the quality of the trimmed mesa. The profile of this plane can depend both on the knifemaker settings (positional relationship of balance pins and braking pins) and on the quality of manufacturer-produced glass strips. The high quality of the cutting edge formed on surface *c* and the least curvature of this surface are achieved using the slow balanced break (Hagler, 2007). However, even with the slow balanced break of the glass strip into squares, the lowest part of newly formed surface *c* (opposite to scored line) does not always form a right angle with the bottom surface of the square (Fig. 8b). If surface *c* near the sharp corner obtained by the method of Tokuyasu and Okamura (1959) and Griffiths et al. (1983) (Fig. 7) is far from the plane, making knives with "mirrored" profiles with modern knifemakers for mesa trimming can be done with the rule of Fig. 3. In this case, the cutting edge and corner will be formed by surface *d*.

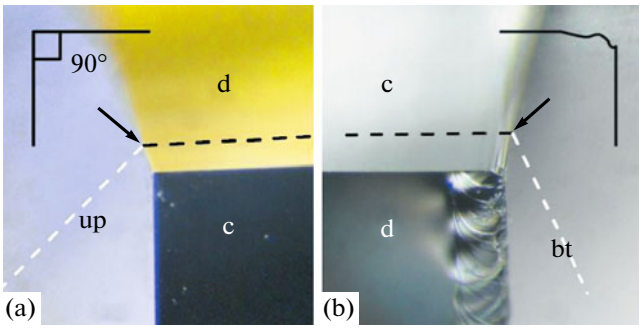

Fig. 8. Choice of planes of glass squares *dirty* and *clean* for obtaining a knife cutting edge.

(a) The square is obtained by the standard procedure with the LKB 7801 knifemaker. The profile of the plane *dirty forms* a right angle (*arrow*) on the edge. The cutting edge (*black strokes*) obtained on the plane *dirty* forms a sharp corner suitable for trimming the mesa side, (up) upper square plane; (b) the square is obtained by the slow balanced break (3 min) with a Reichert-Jung series 705202 knifemaker. The profile of the surface *clean* does not form a right angle on the edge (*arrow*). The cutting edge (*black strokes*) obtained on the plane *clean* will be unacceptable for trimming the mesa side; (bt) bottom square plane. The upper parts of the figure are supplemented by schematic drawings of transverse sections of square edges.

CONCLUSIONS

This paper describes the means of trimming parallel mesa sides to obtain a stable series of ultrathin sections with two glass knives having "mirrored" profiles. The method has been checked using several knifemakers—LKB 7800 (Institute of Biochemistry and Physiology of Microorganisms of the Russian Academy of Sciences, Pushchino), LKB 7801 (Institute of Cell Biophysics of the Russian Academy of Sciences,

Pushchino), Reichert-Jung series 705202 (a branch of the Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Pushchino), and Leica EM KMR2 (Central Research Laboratory of Research Institute of Applied Fundamental Medicine of Nizhni Novgorod State Medical Academy)—and can be implemented with the use of new-generation knifemakers—Leica EM KMR3 and RMC GKM-2—without changing their settings.

The two knives made in the above-described way, being three orders of magnitude less expensive than diamond ones, allow preparing up to ten or more mesas on blocks with soft tissue embedded in a mixture of epoxy resins. The proposed method is simple, based only on a change of glass-square orientation upon a break into two knives, and may be implemented with the use of virtually any currently available knifemaker and even during manual making of glass knives.

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