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A new chamber method for mounting tissue sections

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Abstract

We describe a method for mounting tissue sections onto slides that is faster and especially useful for those sections too thin or too large to be mounted easily by conventional methods. We have devised a chamber system for mounting tissue sections onto slides under buffer solution. Because of the buoyancy of sections in the buffer solution and the elimination of fluid surface tension, it is easy to move, turn, unfold, and spread even quite large or thin tissue sections. In-solution-mounting in this chamber also greatly reduces the force of the brush tip used to maneuver sections onto the slide, thus resulting in less damage to the tissue sections. This chamber greatly facilitates mounting multiple tissue sections onto a single slide. The new method is applicable to protocols that stain tissue sections either before (e.g. cytochrome oxidase) or after (e.g. cresyl violet) section mounting.
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1. Introduction

Tissue sectioning and mounting are among the most basic techniques in experimental neuroanatomy, and are routinely combined with other techniques such as electrophysiology (Baker et al., 1998; Xiao et al., 1999), or optical imaging (Nelson and Katz, 1995; White et al., 1999). Of these two fundamental techniques, tissue sectioning has received rather more attention than section mounting: There are a variety of studies on microtomy, covering almost every aspect: cryosectioning frozen tissue (Rijntjes et al., 1979; McElroy et al., 1993), improvement of the cryostat for large tissue sections (Waddell and Marlowe, 1969), the effect of embedding medium on the quantity of sections (Barthel and Raymond, 1990), variability of section thickness associated with the cutting device (Anthony et al., 1984), sharpening microtome knives (Pearse, 1973), or the relationship between the forces acting upon the tissue sections and the angle or speed of the blade (Allison and Vincent, 1990). One study even described a method to prepare histology sections without using a microtome (Troyer et al., 2002). In contrast, reports on techniques to facilitate mounting sections onto slides and minimize tissue damage and distortions are few and essentially share a common concept: attach a film (Bush, 1955), paper strip (Collewijn and Noorduin, 1969), or adhesive tape (Ornstein, 1986) to the surface of the frozen tissue before cutting, and then cut the tissue together with the support substrate. This method of mounting sections directly from the microtome blade, while minimizing distortions, has several disadvantages. Most importantly, this method does not permit sections to be transferred to an aqueous solution for further histological processing as some protocols require, or to cryoprotectant solution for long-term storage. In summary, microtomes and cryostats have been made easier to use, and can cut thinner sections more reliably. However, there has been less innovation in the method of mounting the sections onto glass slides, a time-consuming technique requiring meticulous care.

The traditional method is to use a fine brush to transfer a tissue section onto a glass slide. The thin sections are often twisted or folded because of the tissue's weight and the solution's surface tension. This is particularly true of brain sections with many surface irregularities (e.g. cerebellum), which tend to twist at the narrowest regions. It can be difficult to unfold or spread the folded tissue sections by the fine

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brush, and thinner sections may be damaged. It can also be tedious to put multiple sections side by side in a desired orientation on a single glass slide because mounting a second section next to a previously mounted section may disturb the mounted one and make it fold or even float away. Here, we present a novel method of mounting tissue sections onto the glass slides using a chamber we have devised for this purpose. This chamber simplifies the manipulation of tissue sections, regardless of their size or number.

2. Materials and methods

2.1. Tissue sectioning and histology

Ferrets (*Mustela putorius furo*) and naked mole rats (*Heterocephalus glaber*) were euthanized with i.p. injection of sodium pentobarbital (100 mg/kg), and intracardially perfused with a saline rinse solution, followed by 4% paraformaldehyde in 0.02 M phosphate buffered saline (PBS; pH 7.4). After a 30 min period of initial fixation, the brains were removed and sunk in 4% paraformaldehyde containing 30% sucrose. Tissue blocks were embedded in Frozen Section Medium (Richard-Allan Scientific) and cut on a cryostat (Microm, HM560) into 10 or 40 μm thick sections, which were collected directly into 0.02 M phosphate buffer (PB). Some sections were mounted on gelatin-subbed glass slides with our mounting chamber before being stained with cresyl violet; others were stained for cytochrome oxidase (CO) using a modified method of Wong-Riley (1979) before being mounted.

2.2. Mounting system

The mounting chamber is made of plexiglass (Fig. 1A). This setup consists of a mounting compartment (1), a buffer drawer (2), a buffer switch knob (3), which controls a buffer switch cylinder (4), and the mounting compartment base (5). The switch knob and switch cylinder are made from stainless steel; the cylinder penetrates the mounting compartment base. The switch allows buffer in the mounting compartment to flow down to the buffer drawer (Fig. 1B) via a fine hole through the cylinder and mounting compartment base. Turning the switch to the open state controls the rate of change of the buffer surface level in the mounting compartment. Traditionally, fine brushes are used to transfer and mount tissue sections. However, as tissue sections often adhere to the brush hairs, we use glass probes when needed to separate the tissue section and the tip of the brush. We make these probes by flame-heating one end of a glass pipette to bend and seal it to make a smooth blunt tip (Fig. 1C).

2.3. Using the chamber to mount large tissue sections

We use a slightly different procedure to mount large sections from that of medium or small sections. For mounting large sections, a glass slide (Fig. 1D) is stuck horizontally to four clay blocks (oil-based modeling clay) in the mounting compartment. We use oil-based clay because it does not dry for a very long time, can be reused, and still holds a slide underwater as it does in the air since it is water repellent. Before starting, the buffer switch is put in the closed state and the mounting compartment is filled with 0.02 M PB. The slide is kept at least 8 mm beneath the surface of the buffer solution. A fine brush is used to transfer a tissue section into the chamber buffer above the glass slide where the section will be mounted. During the transferring and mounting, we use glass probes to separate the tissue section from the brush if the tissue section sticks to the tip of the brush.

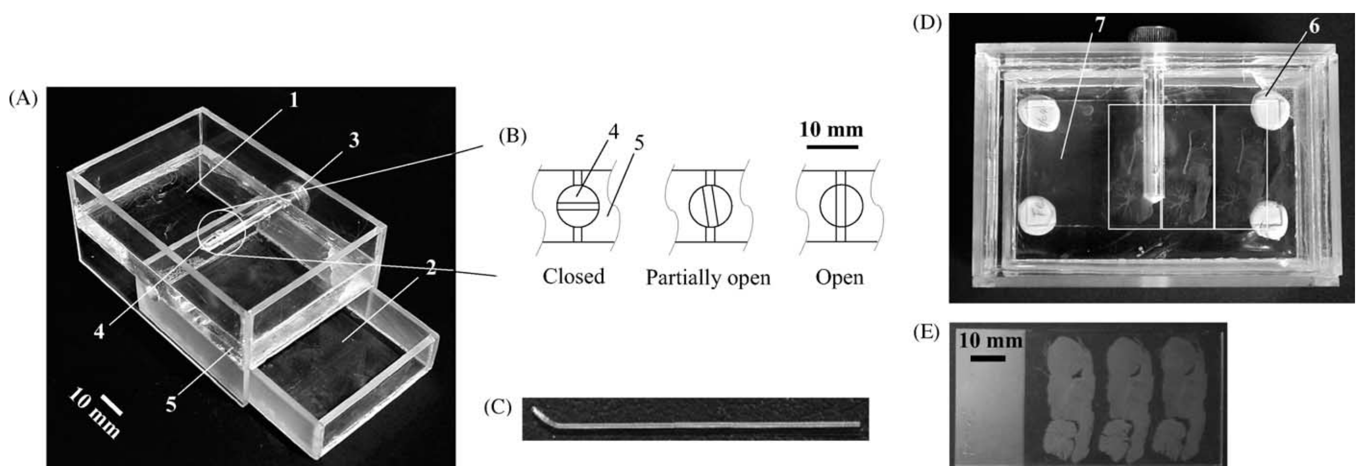

Fig. 1. (A) Design of the mounting chamber. (1) Mounting compartment; (2) buffer drawer; (3) buffer switch knob; (4) buffer switch cylinder; (5) mounting compartment base. (B) Cross sectional view of the buffer switch cylinder that penetrates the mounting compartment base. The buffer switch can be in the closed, partially open, or open state to regulate buffer flow rate from the mounting compartment to the drawer. The hole through the cylinder is roughly 1.5 mm in diameter. (C) Glass probe for handling tissue sections. (D) Tissue sections are mounted while immersed in the buffer solution. In this case, three 10 μm thick parasagittal sections of ferret brain (white boxes) have been mounted on one slide. (6) Clay blocks; (7) glass slide. (E) Once dried, the tissue sections adhere tightly to the glass slide and can be processed further.

The tissue section is then gently spread and unfolded with the fine brush in the solution above the slide. Once the tissue section is untwisted and in the desired orientation, and positioned appropriately over the glass slide, the entire tissue section is positioned onto the glass slide fully and evenly by applying light pressure with the tip of the brush. The section then adheres to the slide surface because of its weight, water pressure, and the gelatin on the subbed slide surface. Next, a second tissue section is transferred into the mounting chamber. If this tissue section is upside down or in the wrong orientation, in order not to disturb the previous section, the next one should be floated away from the first tissue section, and then turned over and floated gently back to the side of the first tissue section. It can then be positioned onto the glass

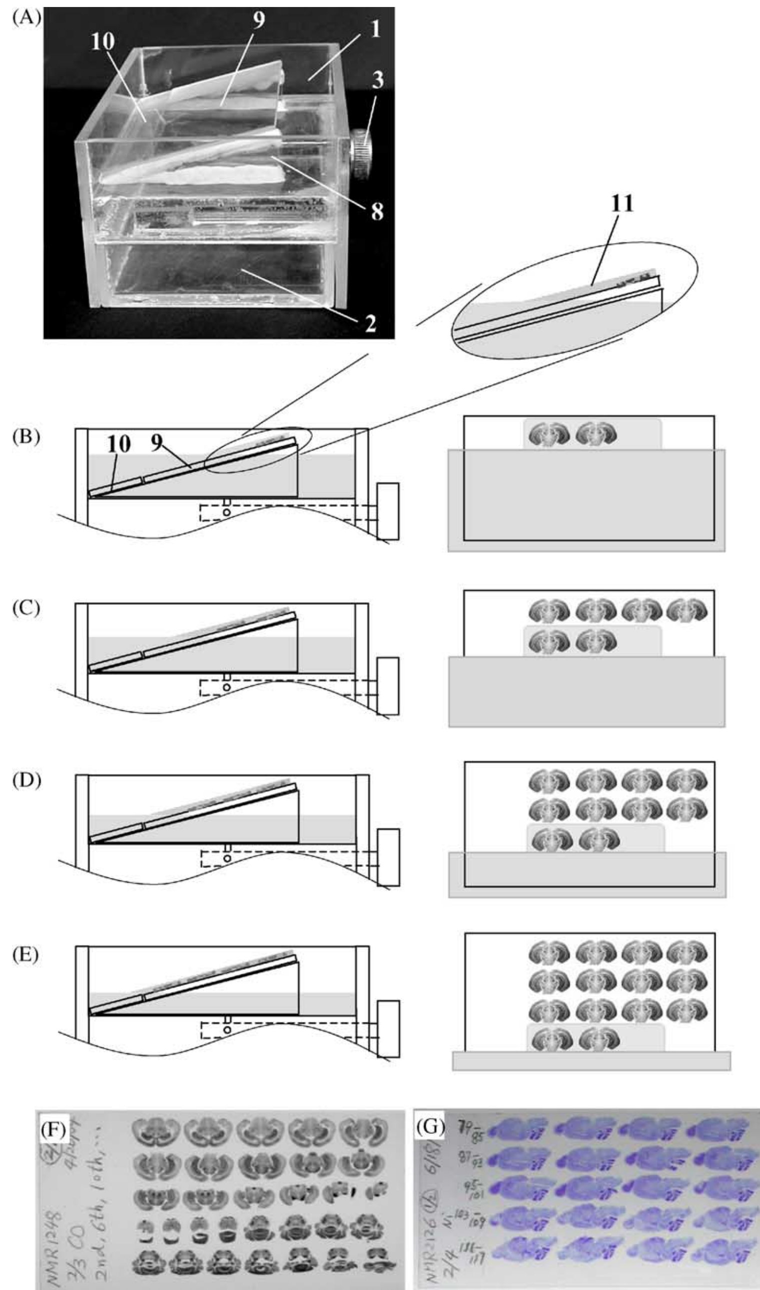

Fig. 2. Mounting multiple rows of tissue sections on a slide. (A) Side view of the mounting chamber and the slide. The slide is angled roughly 13–15° from the horizontal plane and not submerged completely in buffer. (1) Mounting compartment; (2) buffer drawer; (3) buffer switch knob; (8) slide holder; (9) glass slide; (10) a strip of glass slide. The two slide holders (8) are made from plexiglass and stuck to the mounting compartment base (5) by clay. The strip of glass slide (10) is cut from the identical glass slide as the ones used for mounting tissue sections. This strip (10) and the slide (9) are stuck by clay side by side to the slide holders. One edge of the strip (10) touches the mounting compartment base (5). (B–E) The buffer level is allowed to fall for mounting successive rows of sections. Left panels are side views of the mounting compartment and the slide; right panels are top views of the slide. Shaded regions indicate buffer. (F) Cytochrome oxidase stained coronal sections of naked mole rat brain. (G) Cresyl violet stained parasagittal sections of naked mole rat brain.

slide adjacent to the previous section without disturbing it. The fluid disturbances caused by gently moving the next sections are too small to fully dislodge the previously mounted sections. If there is still space on the slide, the same method may be used to position additional tissue sections onto the slide. When one glass slide has been completed (Fig. 1D), the switch is turned to the fully open position. The buffer then flows down to the buffer drawer through a hole in the center of the mounting compartment base (Fig. 1B) at the rate of about 1 drop/s. When the buffer surface level has decreased to about 3 mm above the glass slide, the switch is turned to decrease the buffer flow rate so as not to disturb the mounted sections. We find that rates not exceeding roughly 1 drop every 2 s do not disturb the mounted sections. The buffer solution is allowed to gently flow down to the lower buffer drawer until the surface of the buffer solution is beneath the glass slide. The tissue sections will then adhere firmly to the glass slide, and the glass slide can be removed from the chamber for further processing (Fig. 1E). The next glass slide is then positioned in the chamber. The buffer drawer is pulled out, and the buffer poured back into the mounting compartment for use with the next slide.

2.4. *Using the chamber to mount smaller tissue sections*

For mounting smaller sections, a glass slide is stuck to clay blocks in the mounting compartment tilted in such a way that the long edge of the glass slide is parallel to the long edge of the chamber while the short edge of the mounting slide is tilted roughly 12–15° (12° for thinner sections like 10 μm, 15° for thicker sections like 40 μm) to the bottom of the mounting compartment (Fig. 2A).

When mounting larger tissue sections, the glass slide is completely submerged in the buffer solution. By contrast, when multiple rows of smaller sections are to be mounted onto one slide, the level of buffer solution should reach only to the bottom of the first row of the sections that will be mounted (Fig. 2B). The fine brush is used to first apply a thin layer (Fig. 2B: number 11) of the buffer solution onto the glass slide where the first row of sections will be. Then the brush is used to transfer a tissue section into the buffer solution in the mounting compartment. The tissue section is then gently spread and unfolded with the fine brush in the solution. Once the tissue section is untwisted and in the desired orientation, unlike mounting large sections the section is made to glide up on a thin layer of buffer. The lubrication created by the thin buffer layer makes this possible, and prevents tissue damage. After the first row of sections is done, the buffer switch is opened until the buffer level decreases to about the bottom of the second row of the sections that will be mounted (Fig. 2C). The same method is used to mount the second row of sections. This procedure is repeated until all rows are done (Fig. 2D and E). A strip of glass slide (Fig. 2B: number 10) is placed by the edge of the slide (Fig. 2B: number 9) to make sure that the last row of tissue sections can glide smoothly onto the slide like previous rows. Fig. 2F and G show slides with multiple brain sections mounted using this chamber, stained for either cytochrome oxidase or Nissl substance. The regularity of the multiple sections mounted on each slide is readily apparent.

3. Results and discussion

We used our method to mount ferret brain tissue sections, which are typically about 25–36 mm long and 13–14 mm wide (10–40 μm thick). The sections mounted by the new method lie flat and even and are in good condition, as well as having the desired orientation and sequential order on the slide. We use a slightly different procedure to mount smaller tissue sections from that used with larger sections. Because we mounted only one row of the large sections on each slide (Fig. 1E) and the forces from the buffer flowing out of the mounting compartment approximately counteract those from opposite sides on each section (Fig. 3A), the sections drifted little when the buffer flowed out of the mounting chamber. However, when we mounted the smaller sections using the same method, the two rows of the sections near the edge drifted away when the buffer flowed down to the buffer drawer. This is because the forces from buffer flow are unbalanced on the edge (Fig. 3B) compared to the middle section of the slide. Furthermore, the greater area of the large tissue sections compared to the smaller ones (300–500 mm^2 versus 50–60 mm^2) makes them adhere better and thus less likely to float away during the mounting process. When mounting smaller sections, we unfold and untwist a section in the buffer and make use of a thin layer of buffer to glide the section to its desired position. The lubrication of the thin buffer layer facilitates moving the section, significantly decreasing the forces exerted by the fine brush. We decreased the surface level of

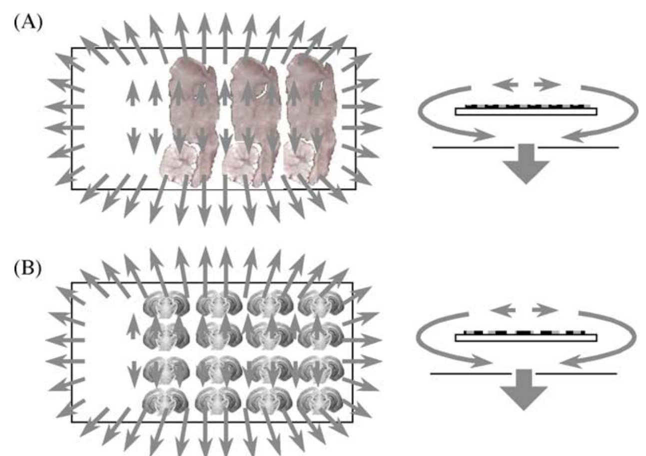

Fig. 3. Analysis of forces from decreasing the buffer level. (A) The forces from buffer draining approximately counteract on one row large sections on a horizontally placed slide. (B) The forces from buffer draining differ between central and peripheral rows of middle size sections on a horizontally placed slide. Left panels are top views of the slide; right panels are side views of the slide.

the buffer one row at one time. In this way, buffer flow does not disturb previously mounted sections (Fig. 2B–E). On the other hand, this method is not optimal for mounting large thin sections as these could be damaged by this process of using the brush to glide sections up to their positions on the slide.

Cutting and mounting tissue sections is a basic histological technique widely used in a variety of disciplines. There are two common ways to produce brain sections: the brain may be sunk in a sugar solution and cut when frozen, or the brain may be embedded in a medium and cut at room temperature. Frozen sectioning is commonly used in neuroanatomical studies because the sections can be cut rapidly, and there is less shrinkage than is commonly associated with harsher procedures employing embedding media. However, mounting tissue sections is time-consuming, and each frozen section may be distorted in a variable, uncontrollable way by the traditional mounting methods (Swanson, 1992). These disadvantages limit the use of frozen cutting. To overcome these disadvantages, we introduced a new chamber method for efficiently mounting tissue sections, especially those sections too thin or large to be mounted easily by conventional methods.

The traditional mounting method is to put the tissue section onto the glass slide and spread it out using a fine brush. Because of the liquid surface tension, when transferring the tissue section with the brush onto the slide, tissue sections are prone to twist and fold around the brush tip. It is very easy to distort or damage tissue sections by the brush when spreading folded sections in this way, especially when they are thin or delicate. Using the traditional method, after a tissue section is done, excess buffer around the section is blotted off. This method makes it tedious to put multiple sections close together on the glass slide because the thin layer of buffer around later-mounted sections can cause already-mounted tissue sections to float, ripple or fold again. Another mounting method is to stick tissue sections to the glass slide directly from the knife of the microtome or cryostat. This method also has limitations. Many histochemical staining protocols require that the sections be stained free-floating before being mounted. Furthermore, mounting sections directly from the microtome blade means one cannot easily position multiple sections close to each other in the desired orientation or order.

Our new mounting chamber has several advantages. First, because there is buffer buoyancy force supporting the thin sections and there is no liquid surface tension on them, it is easy to move, unfold, turn, and spread the thin tissue sections. This is particularly important for mounting thin ($<20\,\mu$m thick) sections. Second, the buoyancy force and lack of surface tension also reduces the force of the brush tip required, resulting in less damage to the tissue sections. This is also true of some classic mounting procedures. However, as we have described, our chamber facilitates the manipulation of delicate tissue sections, permitting the user to control the flow of buffer away from the slide smoothly without disturbing mounted sections. Third, while the precise time required to mount sections depends on the researcher's skill and experience, this chamber can reduce the time involved. We find that using the chamber reduced the time needed to mount sections like those in Fig. 2F and G by 30–60 min. Finally, because the chamber makes it possible to mount all sections in one row or all sections on a slide together at one time and then decrease the buffer level, it is easy to put sections side by side close together in the desired orientation on the glass slide (Figs. 1E and 2F, G). The value of this goes beyond appearance; more sections on each slide means fewer slides are required. Our experience suggests we use roughly one third fewer slides. This saves the time and labor of slide subbing, permits one to stain more sections at one time, and reduces the space needed to store tissue slides.

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