Paraffin sectioning in the modern laboratory and the problem of static electricity: a review

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EDITORIAL

Paraffin sectioning in the modern laboratory and the problem of static electricity: a review

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ABSTRACT

Build-up of static electricity frequently hampers proper production and handling of serial paraffin sections produced on a microtome, a problem that is exacerbated in modern, fully climatized laboratories. In this context, we review a number of suggested remedies for static electricity, concerning increase of humidity, in one way or the other, and various kinds of anti-static devices. An excellent solution, the shockless one-point ionizing bar that we have implemented in our laboratory, albeit not new, is presented here in detail.

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Introduction

In several branches of zoology, microscopic examination of serial histological sections of organisms embedded in paraffin is of paramount importance. The production of the long ribbons with serial sections that are required for such zoological studies creates particular technical problems that the botanist rarely encounters as in this field generally only very short ribbons, or even single sections, suffice. In the present review we focus on the problem of static electricity.

The production and subsequent staining of histological sections for light microscopy involves techniques dating back to the mid-nineteenth century that have been reviewed and summarized in modern textbooks on general microscopic techniques for zoological applications, such as Gabe [1], Romeis [2], and Presnell and Schreibman [3]. Such histological sections are made on a microtome, at intervals varying between 2 and 8 µm, from tissues embedded in paraffin wax blocks, albeit these days traditional paraffin wax is generally replaced by standardised synthetic plastic-polymer paraffin waxes that have the same melting point, i.e. around 56°C.

Notably, the production of proper serial sections, i.e. ribbons with sections attached to each other, can be compromised by a considerable number of problems, for which potential remedies are presented in several texts [4]. One of these problems concerns the build-up of static electricity, which is formed as a result of the friction between the microtome knife blade and the paraffin block containing the embedded tissue. Static electricity is an imbalance of electric charges within or on the surface of a material and concerns electricity not flowing as a current.

Static electricity hampers the production of serial sections in three ways. First, it may be exceedingly difficult to start a proper ribbon when the first section bends either backwards over the edge of the knife or sticks firmly to the edge, so that upon the upstroke it attaches to the wax block and thus is lifted from the knife. Evidently, the frequently occurring recurvature of the very first section over the knife's edge one may counter by straightening it with the help of an artist's paint brush. However, generally this does not prevent the section attaching to the paraffin block upon its upstroke.

Second, the short ribbon with sections may stick to the knife because of the static electricity, so that the following series of sections are pressed against it, thus resulting in a compressed series of sections that are difficult to lift from the knife.

Third, even when ribboning of sections is achieved, the fully charged ribbon may be very difficult to handle, as it will try to stick to other metal parts of the microtome or to the hands of the operator. This situation has been aptly described by Lee [5]: 'It sometimes happens that the ribbon becomes *electrified* during the cutting, and twists and curls about in the air in a most fantastic and undesirable manner.'

Although some general texts suggest potential remedies for the build-up of static electricity, we feel that the subject has not received sufficient attention, particularly in view of

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the fact that several of the proposed countermeasures are difficult to implement in modern laboratories. Below we review the suggested remedies and discuss in some detail a very good solution, albeit not new, that we recently successfully implemented in our laboratory.

Countermeasures

Humidity

Build-up of static electricity during sectioning is particularly pronounced in dry weather. Therefore, a frequently suggested remedy consists of raising the humidity, in one way or the other. A simple way of doing this is postponing sectioning until the weather is more humid. In his old laboratory, the first author waited for a quiet and rainy day and then opened a window, thus effectively increasing the humidity in the room. Unfortunately, this simple countermeasure against static electricity is no longer available to researchers in current fully climatized laboratories.

Artificially increasing the humidity within the laboratory may be achieved by boiling water in an open pan, a remedy often suggested in the older literature [6], but which is rather impracticable. In case the water bath technique is used for mounting ribbons with histological sections onto glass slides [4], such a floatation bath may locally increase the humidity. In case one prefers the hotplate technique for mounting sections onto glass slides [4], use of an electric humidifier is a good alternative. We have rather successfully used humidifiers in rooms that were relatively small. However, in a large, modern, and fully climatized laboratory with many air-vents positioned in the ceiling, it is practically impossible to increase the humidity to such a level that it will sufficiently counteract the static electricity generated during the sectioning process. This situation forced us to construct a separate small chamber within the laboratory in which we placed the microtome and other necessary equipment, including a humidifier. This small chamber has a ceiling of transparent plastic and a door that can be closed. Operation of the humidifier in this closed-off compartment within the laboratory sufficiently increases the humidity. Although somewhat exaggerated, we sometimes refer to our microtomist's cubicle as the little sauna.

A simple technique to raise very locally the humidity is by breathing out gently over the paraffin section and the knife. When the knife is still a bit cool one can see the moisture condensate on it and, thus, discharge the static electricity. This method, which was already suggested in the older literature [7] but also more recently [4,8], is only effective when the static electricity is not too strong. It should be noted that one should only breathe out very gently on the section and the knife during the ribboning as more forcefully blowing or huffing may cause the ribbon to fly about. When the knife becomes too warm, so that the moisture hardly condensates, one may cool it by spraying it with a freezing aerosol, although we do not specifically encourage the use of such aerosols. It is our opinion that this method of breathing should become engrained in the histological technique of the microtomist.

Another method for locally increasing the humidity is by using dry ice [9]. Chips of dry ice are placed in a tea-strainer or similar device, which is suspended immediately above the knife of the microtome and as closely as possible to the point where the sections spread on the knife blade. Moisture condensation due to the heavy cold air descending on the knife and the paraffin block may considerably reduce static electricity [4]. With respect to the container for the dry ice, we found a tea-strainer attached to a retort stand quite useful. However, others have devised custom-made dry ice containers that are fixed to the microtome [10,11].

Anti-static devices

In our experience, the use of an anti-static ribbon transporter belt greatly facilitates the work of the microtomist. Although it does not have any effect on the static charge building up on the knife and the paraffin block, such a conveyor belt [4] (Figure 1) makes the production and handling of long ribbons much easier, while it minimizes the static electricity of the ribbon that otherwise may wrap itself around parts of the microtome or

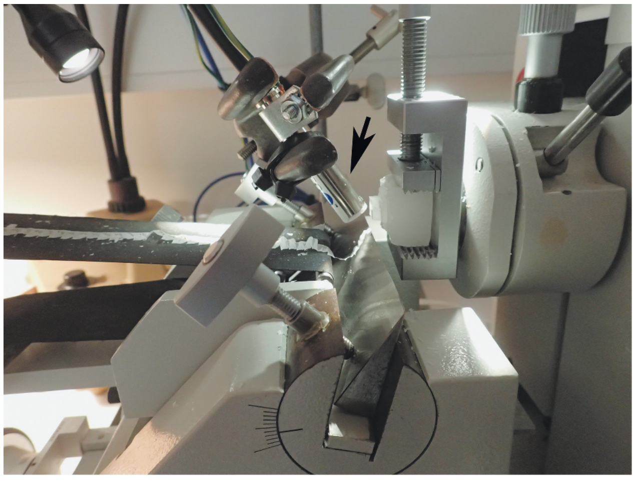

Figure 1. Close-up of the one-point bar (arrow) in position during sectioning. Visible are also paraffin wax block, resharpenable microtome knife blade, and part of the conveyor belt, carrying a ribbon with serial sections.

the operator. We have never dared to moisten the conveyor belt during the sectioning process, as suggested by Church and Kroeger [12]. In our opinion, such a conveyor belt is much more practical than a trough with water that catches the ribbon [13].

Earthing, or grounding, the microtome by means of a copper wire to water pipes or even to the earth electrode of the electricity grid is easily achieved and may theoretically be effective in discharging static electricity. On our microtome, it is even feasible to connect the copper wire directly with the knife by plugging it into the hole with screw thread on one side of the knife (this hole holds the handle during the sharpening of the knife through hand stropping, a technique that these days is almost obsolete, according to Presnell and Schreibman [3]). Although earthing or grounding the microtome is standard procedure followed by the present authors, its effect is difficult to assess and also has been questioned [4,14]. Further, in our practice, we refrained from earthing the operator, a remedy suggested by Bryan and Hughes [14], as this does not affect the build-up of static electricity due to the friction of the knife when it crosses the paraffin block.

Another kind of anti-static devices concern radioactive deionizers. In the 1950–60s, the instrument 'Reco Neutra-Stat' was advertised as an effective discharger of static electricity [1,15–17]. The device consisted of a small strip plated with polonium, which is placed closely to the knife and the specimen block. Polonium emits alpha particles that ionize the air and discharge the static electricity. A somewhat different device but based also on an ionizing unit consisting of a polonium element was described by Haskins and Nesbitt [18]. At the time, it was considered that alpha particles were harmless and formed no radiation hazard. However, these days polonium is treated much more circumspectly, a wise policy substantiated by the fact that it forms a component of tobacco and has been used to eliminate undercover agents and political opponents. The safety measures required during the use of polonium, combined with the fact that histologists are not trained radiological workers, in practice rule out the use of this device in the histological laboratory [19,20].

Earlier, an even more radioactive element was suggested as countermeasure to static electricity generated during the production of paraffin ribbons, viz., radium [21,22]. A rod coated with radium bromide or a sealed tube containing this substance [23,24] is positioned close to that part of the microtome knife where the histological sections move down on the knife. The radiation emitted by radium ionizes the air and, thus, discharges the static electricity. Evidently, increased knowledge and awareness about the health hazards of radium rule out its application in the histological laboratory.

Non-radioactive antistatic devices were developed, if not improvised, on the basis of (a) a high-frequency apparatus connected to a custom-made electrode (about 20 cm long) made of wire-cored Christmas-tree tinsel [25], and (b) with the help of an induction coil from a Ford T-TT automobile connected, among others, with two brass or copper strips and two pieces of tinfoil of which one was cut to present 15–20 pointed projections [26]. In the last-mentioned device, the power supply was formed by a toy train transformer yielding 5–12 V or 4–6 Amp; the two strips were set close to and parallel to the knife of the microtome. A drawback of these custom-made devices is that they are not easily copied and neither are they commercially available.

The anti-static pistol forms another device that has been suggested for the removal of static electricity during histological sectioning with the microtome [27]. The pistol houses a high energy piezo-electric cell that generates a very high voltage that is fed to a discharge needle in its barrel. The voltage ionizes the air around the needle, with positive ions being formed when the trigger is squeezed and negative ions when it is released. During serial sectioning, the pistol is aimed, more or less, at the portion of the knife where the ribbon is formed. Although the device appeared to be effective, it has an obvious practical disadvantage. During the operation of a manual rotary microtome, one needs both hands during the production of serial sections, one hand for turning the flywheel and the other for handling the ribbon. Furthermore, constantly squeezing and releasing the trigger of the anti-static pistol must be rather fatiguing.

By far the most effective and harmless static eliminators concern the electrostatic ionizing devices. In industry, so-called ionizing bars are used to control the static charge, with relatively short bars being used in mini-environments, laminar flow hoods, and workstations, while much longer bars are used in the packaging, plastic, printing, and textile industries. Ionizing bars are connected with a high-voltage power unit. The voltage potential induces a strong electrostatic field that ionizes the surrounding air and thus removes the static charge.

Mattheij and Dignum [20] successfully used a short (10 cm) ionizing bar with seven point-electrodes in paraffin sectioning on a rotary microtome. In contrast to these workers, we found it difficult and cumbersome to install such an ionizing bar close to that part of the knife on the microtome where the paraffin sections are cut. In addition, and surprisingly, our preliminary tests with this device did not suggest that the build-up of static electricity was sufficiently countered. Therefore, we tried another kind of ionizing bar, viz., a One-Point Bar, which turned out to be highly effective.

We obtained this shockless One-Point Bar from the same company in The Netherlands that produced the bar described by Mattheij and Dignum [20], while the North American branch supplied Scadding [28] with a One-Point Bar. Such a bar is a small single point static bar that fits into tight spaces and effectuates spot neutralization of the static charge. Our bar measures only 1.05 cm in diameter and has a length of 5.70 cm. Its small size makes that it can be positioned very close to the knife and the paraffin block by using an old retort stand and a clamp, as well as a few tie wraps (Figure 1). Mounted in this way, we can easily rotate the clamp and thus bring the static bar close to the knife or turn it away from the microtome when this is required. Scadding [28] used a gooseneck arm for this purpose. The power unit (type A-Unit – A2A5S 230 V 50 Hz – 5kV) is a small box (length/width/height: 21 × 11 × 9 cm) that in our set-up is positioned beside the microtome, but the available cables allow the unit to be placed at some distance from it, when such is necessary and/or possible.

In this context, it is important to point out that medical histopathology generally has high specimen through-put. The latter necessitates the use of integrated specimen handling, processing, embedding, sectioning, staining, mounting, and block and slide storage systems that are often automated. In contrast to this procedure, taxonomic histology undertaken in natural history museums and in related departments at universities is generally on a small scale of only 1–2 specimens at a time, and the histological methods employed are invariably undertaken manually using simple traditional techniques. For example, Figure 1 shows a microtome with a block holder in which the jaws directly hold the trimmed paraffin blocks, or paraffin blocks mounted on wooden or metal block holders. These clamps contrast with block holders such as those made by large vendors of histology equipment and that are used in histopathology laboratories, and are specifically designed to hold and rapidly change the integrated paraffin wax-plastic cassette blocks. Furthermore, in Figure 1 a resharpenable microtome knife is shown. Although most laboratories now use disposable microtome blades, several companies still offer a resharpening service for traditional steel knives. Lastly, it is stressed that in taxonomic histology it is essential that during sectioning *all* sections should be retained (serial sectioning), and in their appropriate order, otherwise information on the anatomy of certain parts of the organism may be missed.

According to Nicholson [29], anti-static pistols and ionizing bars would be inadequate for use with ultrathin sectioning and may even increase the static charge. The problem could be removed by closing off the open end of the point bar by a grounded grid. In other words, a grounded grid is interposed between the discharge needle(s) and the static-bearing surface of the knife and the paraffin block [29]. The end of our point bar, as detailed above, is open but, nevertheless, the device is highly effective in removing static electricity in standard paraffin sectioning, albeit we did not test its effectiveness in ultrathin sectioning.

Electrostatic ionizing bars produce ozone near the bar. However, the concentration is very low, so that Mattheij and Dignum [20] actually failed to detect generated ozone with an ozone measuring tube near their ionizing bar with seven point-electrodes. Another test was performed on 1 November 2012 by SIMCO-ION company on their 1500 MEB ionizing bar, which has an effective length of 97 cm, with power being supplied by an A-Unit (A2A7S); ozone was measured by means of a HIVUS ozone meter (in-house document of SIMCO-ION, The Netherlands, R. Achterkamp *in litt.*). Concentration of ozone stayed well below 0.025 ppm.

It should be noted that the MEB ionizing bar is much larger than the one-point bar, contains multiple point-electrodes, and is powered by a higher voltage. Therefore, the concentration of ozone produced by a single one-point bar must be even lower, corroborating also the negative result obtained by Mattheij and Dignum [20].

The Netherlands Food and Consumer Product Safety Authority (NFPA) has specified an official directive regarding a health warning label that should be provided with apparatuses that generate ozone [30]. For larger rooms, it is 3 mg ozone per hour. A standard laboratory is about 135 m^3, with the air being refreshed six times per hour. This implies that an ozone producing device should not emit more than 0.062 ppm per hour in order to stay under the NFPA-limit. The ozone production of the one-point bar stays well below this value. Nevertheless, as the human nose is very sensitive, it may be possible to smell ozone in close proximity of the ionizing bar. But, this low production of ozone can be neglected during the operation of the ionizing one-point bar. In case, a histologist feels unconvinced by this data and risk assessment, it is always possible to use a mask with an activated carbon cartridge for protection against ozone.

Table 1. Cost analysis of suggested remedies for static electricity generated during the process of histological paraffin-sectioning. The column safety refers to safety in relation to the microtomist.

Remedy	Costs (US $)	Safety	Availability	Disadvantage
Electric humidifier	295	Very low risk	Commercially available	May not sufficiently increase humidity in modern laboratory
Microtomist's cubicle	1825	Very low risk	Custom-made	Smaller workspace; it can get rather warm inside
Freezing aerosol	18	Low risk	Commercially available	Aerosolized gases
Dry ice machine + CO_2 cylinder	1800 + 180	Very low risk	Commercially available	Relatively high costs
Tea-strainer	12	Very low risk	Commercially available	None
Anti-static conveyor belt	variable	Very low risk	Not commercially available	Only available 2nd hand for older types of microtomes
Grounding of microtome with 2 m insulated copper wire	3.5	Very low risk	Commercially available	Requires some handiness
Radioactive deionizers	not applicable	High risk	No longer commercially available	Health hazards
Custom-made non-radioactive antistatic devices	negligible	Very low risk	Not commercially available	Requires some handiness
Anti-static pistol	235	Very low risk	Commercially available	Impractical
One-point ionizing bar (including power unit)	825	Very low risk	Commercially available	Very low production of ozone

Conclusion

The various solutions that have been proposed for increasing the humidity in the laboratory – in itself a good countermeasure against static electricity – are either difficult to implement, or become less effective, or even ineffective, in a modern, fully climatized laboratory with its mechanically generated supply air and exhaust air, changing the air at least six times per hour. Antistatic devices, such as earthing or grounding of the microtome and radioactive deionizers, are either hardly effective or no longer permitted under current health and safety regulations. The non-radioactive antistatic devices described in the literature were custom-made and are not easily copied and neither are these commercially available. From the electrostatic ionizing devices, the commercially available shockless One-Point Bar is the perfect and safe device to overcome the problem of static electricity generated during the production of serial paraffin sections on a microtome stationed in a modern, fully climatized laboratory. A general cost analysis (in US $) of the various suggested remedies is presented in Table 1.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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