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Section Cutting in Histopathology: An Update

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Abstract

Histopathology is the microscopic examination of tissues to diagnose and study diseases, more significantly the diagnosis of cancer. It plays a pivotal role in modern medicine, serving as the gold standard for definitive diagnosis in a wide range of conditions. Through meticulous analysis of tissue morphology (structure) and cellular characteristics, histopathology provides crucial information for disease classification, staging, and guiding patient management. In the world of histopathology, where microscopic details hold the key to diagnosing diseases, section cutting plays an unassuming yet critical role. Imagine a pathologist trying to decipher a complex puzzle from a bulky, opaque object. Section cutting transforms that object into a series of transparent, ultra-thin slices, revealing the intricate cellular architecture and hidden abnormalities within the tissue. This seemingly simple process is vital because it allows for optimal visualization. Sections with a precise thickness (typically 3–10 µm) enable light to penetrate the tissue effectively, revealing cellular and structural features under a microscope. Thicker sections would obscure vital information. Additionally, sectioning ensures a representative sampling of the entire tissue, capturing any uneven disease distribution. This is crucial as some areas may harbor the key to diagnosis. Furthermore, thin sections facilitate staining, which highlights different cellular components for clear and interpretable images. Finally, high-quality sections can be preserved for advanced techniques like immunohistochemistry. In essence, section cutting acts as the gateway to unlocking the secrets within a tissue sample. Without it, pathologists would be left with a limited view, potentially leading to missed diagnoses or inaccurate interpretations. Therefore, section cutting remains an irreplaceable cornerstone of histopathological examination.

Keywords: Microtome, analysis, diagnosis, cellular, sections

INTRODUCTION

Tissue Preparation for Sectioning

Histopathology relies on two main methods to prepare tissues for microscopic examination: paraffin embedding, and cryopreservation with OCT (Optimal Cutting Temperature) compound. Each caters to specific needs. Paraffin embedding, the workhorse of routine procedures, excels in durability and versatility. Tissues are dehydrated, cleared, and infiltrated with paraffin wax, creating a paraffin block for sectioning and long-term storage. This method is ideal for archiving and allows for a wide range of staining techniques. Cryopreservation with OCT offers a faster alternative, particularly for delicate tissues. The tissue is embedded in OCT, a protective compound, and rapidly frozen at ultra-low temperatures. This near-native state preservation makes it suitable for studying fragile structures or when speed is crucial. However, OCT-embedded tissues have shorter storage times and may not be compatible with all stains. Ultimately, the choice between paraffin embedding and cryopreservation depends on the specific needs of the tissue analysis. Paraffin remains the go-to for routine work due to its versatility and long-term storage, while cryopreservation shines for speed and delicate tissue preservation [1–5].

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Transforming a raw tissue sample into a diagnostic tool in histopathology requires meticulous processing. Two primary methods dominate this stage: paraffin embedding and cryopreservation with OCT (Optimal Cutting Temperature) compound. Each method utilizes distinct steps, impacting tissue integrity in different ways [6].

Paraffin Embedding: A Delicate Balancing Act [7–10]

1. ***Dehydration****:* The tissue undergoes a series of graded alcohol baths, progressively removing water. This dehydration is crucial as water can cause tissue distortion and hinder subsequent steps. However, excessive dehydration can lead to shrinkage and loss of cellular detail.
2. ***Clearing:*** Alcohols are replaced with solvents like xylene, making the tissue permeable to paraffin wax. While xylene efficiently clears the tissue, it can also cause some degree of cellular swelling and potentially extract certain lipids.
3. ***Wax Infiltration:*** Molten paraffin wax is introduced, saturating the tissue. This process provides stability and allows for thin sectioning. However, prolonged exposure to hot wax can damage heat-sensitive biomolecules.
4. ***Embedding and Blocking:*** The infiltrated tissue is placed in a mold and solidified into a paraffin block. This block can be stored for long periods, but prolonged storage may lead to gradual tissue degradation.

Paraffin embedding offers excellent durability and versatility, but each step requires careful monitoring to minimize potential damage to the tissue integrity and preserve the crucial details needed for diagnosis.

Cryopreservation with OCT: Speed and Near-Native Preservation

1. ***OCT Embedding:*** The tissue is directly embedded in OCT, a cryoprotectant compound that minimizes ice crystal formation during freezing. OCT minimizes tissue manipulation, preserving the natural architecture.
2. ***Rapid Freezing:*** The embedded tissue is rapidly frozen at ultra-low temperatures, typically using liquid nitrogen. This rapid freezing minimizes ice crystal formation, which can disrupt cellular structures. However, improper freezing techniques can still lead to some ice crystal formation and potential tissue damage.
3. ***Sectioning****:* Frozen sections can be prepared quickly, significantly reducing turnaround time for diagnosis. However, frozen sections are more delicate and require specialized handling compared to paraffin sections.

Cryopreservation with OCT prioritizes speed and preservation of delicate structures. The rapid freezing minimizes tissue distortion, but proper handling is crucial to maintain tissue integrity during sectioning and analysis.

Microtomy and Cryostat Techniques [11–13]

In the realm of histopathology, after tissues are meticulously prepared through paraffin embedding or cryopreservation, the next hurdle lies in creating incredibly thin slices for microscopic examination. This crucial step is accomplished with two specialized instruments: microtomes and cryostats.

- ***Microtomes:*** Fig 1 These precision machines act as the workhorses of sectioning (Figure 1). They hold a very sharp blade and a mechanism for precisely advancing the tissue block towards it. With each pass, the microtome shaves off an exceptionally thin section in the form of Ribbon formation, typically ranging from 5 to 10 µm in thickness. Microtomes come in various configurations, but all share the ability to generate high-quality sections from paraffin-embedded tissues.
- ***Cryostats:*** Fig 2 While microtomes excel with paraffin, cryostats cater specifically to frozen tissues. These specialized freezers maintain ultra-low temperatures, typically around –70°C. The tissue block, embedded in OCT compound, is secured onto a specimen stage within the cryostat chamber. Similar to a microtome, a blade is used for sectioning. However, unlike the room

temperature setting of a microtome, the cryostat's freezing chamber ensures the tissue remains frozen during sectioning, allowing for creation of thin sections from cryopreserved samples.

- **Microtomes and cryostats: -** They are the unsung heroes of histopathology, transforming prepared tissues into microscopic marvels. Each instrument operates on distinct principles to achieve the same goal: generating ultrathin sections for detailed examination.

Microtomes: Precision Slicing [14–16]

The key to a microtome is its ability to move with incredible precision and control. This allows it to create incredibly thin slices. Here is a breakdown of its operation:

- The paraffin-embedded tissue block is securely mounted on a stage that can be meticulously adjusted in three dimensions (X, Y, and Z) for perfect alignment before sectioning.
- A very sharp blade, often made of steel or diamond, sits at the ready. This blade is crucial for creating clean and even sections.
- The micrometer is the key to the microtome's precision. It allows for incredibly fine adjustments in the distance, the tissue block is advanced towards the blade with each pass. This controls the final section thickness, typically ranging from 5 to 10 µm.
- As the handle is turned, a gear mechanism precisely advances the tissue block towards the stationary blade. With each pass, the blade shaves off a thin section, which is then collected for further analysis.

Cryostats: Masters of the Cold [17]

Imagine a special freezer that lets doctors slice tissues paper-thin, even when they are frozen super solid (Figure 2). That is basically what a cryostat is.

Figure 1. Rotatory Microtome.

Figure 2. Image of freezer.

Cryostat [18]

- Unlike the room temperature environment of a microtome, a cryostat boasts a freezing chamber maintained at ultra-low temperatures, typically around –70°C. This extreme cold ensures the OCT-embedded tissue block remains frozen solid during sectioning.
- Similar to a microtome, the frozen tissue block is secured onto a specimen stage within the cryostat chamber. This stage also allows for precise adjustments for optimal positioning.
- A sharp blade, similar to that of a microtome, is used for sectioning. However, the freezing chamber ensures the tissue remains rigid and easier to cut into thin sections.
- As with a microtome, a mechanism advances the tissue block towards the blade with each turn of a handle. The frozen tissue can be sectioned at similar thicknesses (5–10 μm) as paraffin-embedded blocks.

The core principle of a cryostat lies in maintaining a freezing environment to preserve the integrity of frozen tissues and enable creation of thin sections for microscopic analysis.

In the world of histopathology, achieving high-quality sections hinges on a delicate interplay between several factors.

Three key elements significantly influence the final product: blade angle, cutting speed, and tissue temperature.

- ***Blade Angle****:* Imagine a dull butter knife versus a razor-sharp chef's knife. The angle of the blade on a microtome or cryostat has a similar impact. A perfectly angled blade creates clean, even sections, minimizing tissue distortion and crushing artefacts. Conversely, a dull or improperly angled blade can tear and compress the tissue, compromising the quality of the section and potentially obscuring crucial diagnostic details.
- ***Cutting Speed****:* While haste often makes waste, the same is not necessarily true for sectioning. There is an optimal cutting speed for achieving high-quality sections. A slow cutting speed can lead to excessive manipulation of the tissue, potentially causing compression and tearing. Conversely, excessively fast cutting speed can generate uneven sections with chattered edges. Finding the sweet spot between these extremes ensures smooth, even sections for optimal analysis.
- ***Tissue Temperature (Paraffin Embedding):*** For paraffin-embedded tissues, maintaining the correct tissue temperature during sectioning is crucial. If the tissue block is too cold, it becomes brittle and prone to cracking during sectioning. Conversely, if the block is too warm, the paraffin wax can become soft and smear, obscuring cellular details within the section. Microtomes often have features to control the temperature of the tissue block, ensuring it remains at the optimal consistency for smooth and informative sectioning.

These factors, along with proper blade maintenance and appropriate section thickness, all contribute to achieving high-quality sections. A skilled technician understands the delicate interplay of these elements and can create sections that are not only aesthetically pleasing but also provide a clear window into the microscopic world of the tissue, ultimately aiding in accurate diagnoses.

EMERGING TECHNIQUES IN SECTION CUTTING

While microtomes and cryostats reign supreme in histopathology sectioning, innovative technologies are emerging on the horizon. Vibratomes, for instance, offer an alternative for sectioning very fragile tissues like brain slices. These instruments utilize a vibrating blade, minimizing pressure on the tissue and potentially reducing compression artifacts. Additionally, ultrasound-assisted microtomy (UAM) is gaining traction. UAM employs low-frequency ultrasound waves during sectioning, which can improve section quality for challenging tissues or those requiring ultra-thin sections for advanced analysis techniques. These advancements, while still evolving, hold promise for expanding the capabilities of histopathological sectioning and potentially unlocking new avenues for tissue analysis. While

microtomes and cryostats have long served as the workhorses of histopathological sectioning, novel technologies are pushing the boundaries. Two such advancements generating interest are vibratomes and ultrasound-assisted microtomy (UAM). Let us explore their potential advantages and limitations compared to traditional methods.

Vibratomes: A Gentle Touch for Fragile Tissues

Vibratomes offer a distinct advantage for sectioning exceptionally delicate tissues, such as brain slices. Unlike traditional microtomes that utilize a static blade, vibratomes employ a vibrating blade. This vibration minimizes the pressure exerted on the tissue during sectioning, potentially reducing compression artifacts that can distort cellular structures and hinder diagnosis. This gentle approach makes vibratomes particularly valuable for studying tissues prone to tearing or compression during conventional sectioning. However, vibratomes also come with limitations. Compared to microtomes, they may offer less precise control over section thickness, potentially impacting downstream analyses. Additionally, vibratomes often require specialized training for optimal utilization, adding a layer of complexity to the workflow.

Ultrasound-Assisted Microtomy (UAM): Sharper Sections for Demanding Needs

UAM presents a unique approach to sectioning, incorporating low-frequency ultrasound waves during the process. These ultrasound waves are thought to weaken intercellular bonds, facilitating smoother cutting and potentially improving section quality for challenging tissues. UAM also holds promise for generating ultra-thin sections, which can be advantageous for certain advanced analysis techniques. Despite its potential benefits, UAM is still in its early stages of development. The long-term impact of ultrasound on tissue integrity requires further investigation. Additionally, UAM instrumentation can be more expensive compared to traditional microtomes, potentially limiting its widespread adoption.

The Evolving Landscape of Sectioning Techniques

Vibratomes and UAM represent exciting advancements in histopathological sectioning. While each offers unique advantages, limitations exist. Vibratomes excel with delicate tissues but may compromise on precision. UAM tackles challenging tissues and potentially enables ultra-thin sections, but its long-term impact and cost require further exploration. Ultimately, the choice of technique depends on the specific needs of the tissue and the desired analysis. As these technologies mature and integrate with traditional methods, the future of histopathological sectioning promises to be even more precise and informative, leading to even more accurate diagnoses.

CONCLUSION

In conclusion, section cutting stands as a fundamental yet often underappreciated cornerstone of histopathology. Thin, high-quality sections act as a gateway, unlocking the microscopic secrets harbored within a tissue sample. The meticulous processes of paraffin embedding or cryopreservation, followed by precise sectioning with microtomes or cryostats, ensure optimal visualization of cellular and structural features. This detailed information empowers pathologists to make accurate diagnoses, ultimately impacting patient care and treatment decisions. The field of sectioning technology is constantly evolving. Recent advancements like vibratomes and ultrasound-assisted microtomy (UAM) offer exciting possibilities. Vibratomes provide a gentle touch for fragile tissues, while UAM tackles challenging samples and potentially enables ultra-thin sections for advanced analysis. As these technologies mature and integrate with traditional methods, the future promises even more precise and informative sectioning. Beyond these specific advancements, ongoing research explores laser microtomy, which utilizes laser beams for sectioning, potentially offering advantages in speed and reduced heat impact on tissues. Additionally, automation is making inroads into sectioning, promising increased efficiency and consistency in histological workflows. Looking ahead, the future of section cutting lies in a synergistic approach. Traditional methods like microtomes and cryostats will likely remain the workhorses, while novel techniques like vibratomes and UAM will find their niche

applications. By embracing these advancements and fostering continued research, histopathology can unlock even greater diagnostic accuracy, propelling personalized medicine forward.

REFERENCES

1. Singh AP, Saxena RA, Saxena SU. A study on the working of blood bank. J Med Health Res. 2022 Jan 10; 7(1): 1–5.
2. Singh Ajit Pal. Importance of papanicolaou staining in gynecologic cytology. EPRA Int J Multidiscip Res (IJMR). 2022; 8(9): 216–219.
3. Litjens G, Sánchez CI, Timofeeva N, Hermsen M, Nagtegaal I, Kovacs I, Hulsbergen-Van De Kaa C, Bult P, Van Ginneken B, Van Der Laak J. Deep learning as a tool for increased accuracy and efficiency of histopathological diagnosis. Sci Rep. 2016 May 23; 6(1): 26286.
4. Ismail SM, Colclough AB, Dinnen JS, Eakins D, Evans DM, Gradwell E, O'Sullivan JP, Summerell JM, Newcombe RG. Observer variation in histopathological diagnosis and grading of cervical intraepithelial neoplasia. Br Med J. 1989 Mar 18; 298(6675): 707–10.
5. Van den Bent MJ. Interobserver variation of the histopathological diagnosis in clinical trials on glioma: a clinician's perspective. Acta Neuropathol. 2010 Sep; 120(3): 297–304.
6. Singh AP, Saxena R, Saxena S. Plasma apheresis procedure. EPRA Int J Multidiscip Res (IJMR). 2022 Jul 20; 8(7): 205–18.
7. Singh AP, Mouton RJ, Sharma MK, Ihotu-Owoicho AI. When will this pandemic end? A review. J Basic Appl Res Int. 2021 Dec 29; 27(10): 42–45.
8. Singh AP, Batra J, Saxena R, Saxena S, Kumar C. Alarming rise in professional Blood donors and its repercussions. Cardiometry. 2022 Dec; 1(25): 1394–6.
9. Batra J, Singh AP, Saxena R, Saxena S, Goyal K. Blood components and its usage: a clinical insight from diagnostic lens. J Pharm Negat Results. 2022 Oct 12; 13(6): 1747–50.
10. Obiajulu CV, Ochanya OE, Singh AP. Adverse reaction after blood donation in blood bank. Int J Pure Med Res. 2022 Oct 1; 7(10):.
11. Singh AP, Batra J, Khan SS, Saxena R, Saxena S. Diagnosis and treatment of Wilson disease: An update. Cardiometry. 2022 Dec; 1(25): 1397–400.
12. Singh AP, Saxena R, Saxena S, Batra J. An Update on Emergency Contraceptives. International Journal of Food and Nutritional Sciences (IJFANS). 2012; 11(2): 65–70.
13. Singh Ajit, Heldaus Jerome, Msaki Andrew. Hemodialysis Complications: A Clinical Insight. Int J All Res Educ Sci Methods. 2023; 11(3): 640–647. 10.56025/IJARESM.2023.11323640.
14. Singh Ajit, Saxena Rahul, Saxena Suyash. An elaborated study on bio-medical waste and its successful administration. Int J Recent Sci Res. 2022; 13(8): 2121–2126. 10.24327/ijrsr.2022.1308.0436.
15. Singh Ajit, Batra Jyoti, Saxena Rahul, Saxena Suyash. Susceptibility to Cervical Cancer: An Overview. Int J Food Sci Nutr. 2022; 11(2): 731–739.
16. Singh AP, Saxena RA, Saxena SU. Hemoglobin estimation by using copper sulphate method. Asian J Curr Res. 2022 Jul 13; 7(1): 13–5.
17. Singh AP, Saxena RA, Saxena SU. Protocols for blood collection in a blood bank. J Med Health Res. 2022 Sep; 7(2): 16–21.
18. Singh AP, Saxena R, Saxena S. Cytopathology: An Important Aspect of Medical Diagnosis. Res Rev: J Oncol Hematol. 2023; 12(3): 13–18.

