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*Review*

# The Role of Histology in Forensic Investigations

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**Abstract:** histology, the processing and staining of cells, tissues and organs to highlight contrast, visualize and analyze their microstructure under microscopy, encompasses a vast array of techniques used to confirm, refine, or refute the macroscopic findings in forensic investigations, for example in the case of autopsies. This review presents several applications of histological protocols, staining methods and techniques to case studies, with the aim of illustrating the established, current and potential applications of histological investigations to forensic sciences. The list of disciplines include anthropology, cytology, dendrochronology, diatomology, entomology, fiber analysis, palynology,human and veterinary pathology, taphonomy and thanatology.

**Keywords;** autopsy; death; entomology; legal; medicine; postmortem

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## 1. Introduction

Histology provides the characterization of the microanatomy of healthy cells and tissues, while histopathology is the specialty of choice to observe pathological alterations. In both cases, tissue samples are collected from living or dead organisms, fixed in formaldehyde (usually 10% neutral buffered formalin), dissected, processed in a series of graded alcohols (70%-100%), embedded in paraffin wax, cut in thin sections, mounted onto glass slides and stained, with the aim of achieving a degree of cells and tissues differentiation observable by microscopy (O'Dowd *et al.*, 2020). In forensic sciences, histological and histopathological investigations are defined as ancillary, and can contribute to confirm, refine, or refute the macroanatomical_findings detected at the clinical examination, the death scene or the autopsy (Byard *et al.*, 2012; Dettmeyer, **2018;** Lau *et al.*, 2008). The application of conventional (e.g., Haematoxylin-eosin stain) and special histological techniques (e.g., immunohistochemistry) to forensic investigations is vast and diversified, including, but not limited to, the nature of the sample (e.g., cells, fibers, diatoms and insects), the age and the vitality of lesions (e.g., acute, subacute or chronic, antemortem, perimortem or postmortem) and the cause of death (natural vs. non-natural) (Dettmeyer, **2018**). As a widespread, relatively fast and cost-effective set of techniques, the advantages of histology have been recognized as essential to forensic investigations(De La Grandmaison *et al.*, 2010; Dettmeyer, **2018;** Lau *et al.*, 2008). This article aims to illustrate the established, current and potential applications of histology to forensic investigations through a brief introduction of selected techniques followed by exemplary case studies.

## 2. Applications of Histology in Forensic Sciences

### *2.1. Anthropology*

The identification of deceased individuals is crucial for legal and humanitarian reasons (Saukko *et al.*, 2016). In the case of decomposed remains, forensic anthropologists analyze the remaining hard tissues, such as bones and teeth, to determine the biological profile of any unidentified individual (Boyd *et al.*, **2018;** Hillier *et al.*, 2007b). Species, sex, age and population affinity are the fundamentals of general indentification, while a presumptive or positive identification can be achieved by adding personal features to the investigation, such as remnants of tattoos, prostethic implants and skeletal

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lesions or antemortem pathology in various stages of healing and/or development (Kaur *et al.*, 2015b). In the case of fragments that are too few, small or degraded to allow genetic analysis, histological methods can assist the diagnosis of species, human vs. non-human, relying on the presence of plexiform bone (Hillier *et al.*, 2007a), while age can be estimated by assessing the abundance and distribution of osteon fragments, Haversian canals and secondary osteons in cortical bone (Lynnerup *et al.*, 2006), or cementum incremental lines in teeth (Crowder *et al.*, 2018; Kaur *et al.*, 2015a).

#### 2.1.1. Case Study

In 2011, in the course of construction works in the immediate outskirts of a medium-sized city in the north of Italy, several bones were accidentally uncovered by excavators in a large shallow pit. Unfortunately, they were also heavily damaged and fragmented in the recovery process. The immediate macro-morphological appearance suggested a late juvenile or early adult non-human species, which was soon confirmed by histology. In fact, the abundance of plexiform bone and the absence of secondary osteons indicated the species as porcine (*Sus domesticus*) (Brits *et al.*, 2013; Caccia *et al.*, 2016; Mori *et al.*, 2005), and the bones resulted from the illegal dumping of cuts and partial carcasses after being consumed as food by itinerant communities intermittently camping in a close by designed area. Brits 2014, Caccia 2016 Mori 2005

### *2.2. Cytology*

In cases of sexual assault, the primary evidentiary material can be detected in intimate swabs, where spermatozoa and vaginal epithelial cells can be mixed. The application of genetic techniques for the personal identification of the perpetrator/s requires the separation of spermatozoa from mixed cell samples (Chapman *et al.*, 2020). Unfortunately, conventional methods may offer no assurance of effective cell separation (Hutchinson *et al.*, 2019; Puri *et al.*, 2019), so immunomagnetic bead separation techniques can be tested, based on the binding of an antibody, Hyaluronidase PH-20 (SPAM1), which targets the spermatozoa head. The fluorescent tagging of the antibody leads to the visualization of the binding by microscopy (Hutchinson *et al.*, 2019).

2.2.1. Data from 765 case studies analysed at the Section of Forensic Biology at Oslo University Hospital in the period 2013–2015 can be found in Fonneløp et al. (2019). In the study, positive findings of sperm cells could be detected in samples collected up to 72 h after deposition, and was less frequently detected in oral swabs, up to 12 h.

### *2.3. Dendrochronology*

Dendrochronology, the practice of dating tree rings, is a valuable tool in forensic investigations by assisting the estimation of the minimum PMI(Gärtner *et al.*, 2015; Pokines, 2018). Dating can be measured both in the trunk or in the roots, and the precision of this method relies on accurate sampling techniques coupled with histological staining, which can reveal rings that are not discernable through macroscopic observation. To achieve detailed image analysis, the process involves stabilising increment core samples taken from trees using a microtome, and covering them with a Non-Newtonian fluid, such as corn starch. This particular method enhances the structural stability by filling the open cells of the sample, thereby facilitating the cutting of thin sections with precision(Gärtner *et al.*, 2015). To visualise the cell contents, samples undergo fixation with Nawashin solution to stop all degradation processes within the cell and are washed with water before staining. Safranin-Astra blue and Picric-Aniline blue stains are then specifically applied to microsections of increment cores. These stains serve a dual purpose: to differentiate lignified and non-lignified structures and to enhance contrast for image analysis (Gärtner *et al.*, 2015; Piermattei *et al.*, 2015; Pokines, 2018).

#### 2.3.1. Case Study

In 2012 human perinatal remains were found in a shallow burial site after being disturbed by an excavator. A sealed plastic bag was located 0.25 m below the surface, containing hospital artifacts, such as a towel and an umbilical clip, that indicated a stillbirth. The location was known for the clandestine disposal of remains by families who could not afford other types of burial. A tree root from a pitch pine tree (*Pinus rigida*) was found growing across the remains. The root was sectioned, and the section flat surfaces sanded with progressively finer grit to obtain a polished appearance. Examined with a binocular microscope in bright-field technique under low power, 27 annual rings were counted, providing a likely minimum PMI of 27 years (1985). This case highlights the importance of analyzing smaller roots by microscopy, because they might not yield high ring counts at the macroscopic observation (Pokines, 2018).

### *2.4. Diatomology*

Diatoms are unicellular micro-algae, with sizes ranging from 5 to 500µm, primarily associated with aquatic ecosystems, although they can also be encountered in soil environments (Siver *et al.*, 1994). Their presence in soil may be influenced by factors such as past water flow, environmental conditions and specific ecological interactions. Diatoms exhibit a distinctive morphology, comprising a single cell enclosed by a silica frustule, a protective shell composed of two distinct parts with species-specific patterns, pores, and spines (P. A. Magni *et al.*, 2020b; P. A. Magni *et al.*, 2015). The resilient silica composition and highly specific morphology of different diatom species make them ideal trace evidence.

In forensic investigations the analysis of diatoms involves identifying species, assessing their assemblage and determining their abundance in soil, water, clothing or biological tissues. This information is then compared with the environments where the criminal activity is suspected to have occurred, usually bodies of water (Siver *et al.*, 1994). A typical application of diatom analysis in medico-legal and forensic pathology investigations is supporting mechanical asphyxia by drowning as the cause of death (Piegari *et al.*, 2019). During a drowning event, victims may inhale or ingest water containing diatoms, which can subsequently be found in various tissues. Moreover, diatom analysis can be used to infer crime scene locations by comparing diatoms recovered at the crime scene to reference samples, and can assist establishing the time of death by considering the diatom assemblage in relation to the season/s. Additionally, diatoms play a role in environmental tracing, linking materials or evidence to specific locations, such as the clothing of a suspect (P. A. Magni *et al.*, 2020a).

Diatoms are obtained from the evidence of interest via the diatom test, that is a series of processing steps to collect and concentrate the diatoms associated with the evidence. The concentrated material is then mounted onto microscope slides using a medium that preserves the diatom structures, such as Naphrax, Hoyer's solution, or glycerin, before undergoing thorough microscopic examination at high magnification. The identified diatoms are then compared to reference databases or known samples, enabling forensic experts to draw valuable insights and establish potential connections between the evidence and specific environments, locations or time. Histological staining techniques commonly used for tissues are not typically applied to diatoms due to their unique composition and characteristics. However, some specific staining techniques may be employed for diatoms to enhance selected features or facilitate microscopy observation, such as Haematoxylin- Eosin (H&E), methylene blue and rhodamine dyes (Badu *et al.*, 2015; Cristóbal *et al.*, **2020;** Kucki *et al.*, 2012).

#### 2.4.1. Case Study

The discovery of a deceased 34-year-old male in a gutter line in a rural area was followed by a forensic investigation. Unfortunately, the lack of available forensic expertise led to the burial of the body. The body was exhumed six months later to determine the cause of death. Despite the advanced stage of decomposition, a fractured hyoid bone, dislocated maxilla and mandible and an intact skeleton were revealed. Chemical analysis of the left clavicle and right femur was conducted to detect diatoms and traces of poison. Simultaneously, water from the death site was collected for comparative analysis. This comprehensive approach aimed to unravel the circumstances surrounding the individual's demise and shed light on potential forensic indicators, such as diatoms

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and toxic substances (P. A. Magni *et al.*, 2020a). Despite finding diatoms in the bones, the water sample taken from the drowning site tested negative for diatoms. This discrepancy raises potential explanations, including improper preservation of water samples from the gutter line, ante-mortem penetration of diatoms into the bloodstream through the intestinal or respiratory tract, or the possibility that the suspected drowning site was not the primary crime scene. Notably, the absence of details on the methodology for the diatom test, particularly whether staining was applied to the samples, introduces a critical element. The lack of staining may have contributed to a false negative result in the water sample analysis. Ensuring clarity in the testing methodology and incorporating supplementary tests to validate the results is imperative for achieving a more precise and reliable interpretation of the findings in forensic investigations (Khurshid *et al.*, 2021).

### *2.5. Entomology*

Forensic entomology is a specialized branch of entomology that focuses on the study of insects and other arthropods in relation to forensic investigations. In criminal cases, insects colonizing cadavers can be used to estimate the minimum time-since-death (or postmortme interval – PMI). Additionally, forensic entomology can assist in other aspects relevant to criminal investigations, such as determining the timing of neglect on humans or animals, identifying the primary location of the crime, and detecting the presence of toxicological substances in the remains (Byrd, & Tomberlin, 2020). Information is obtained through the identification and aging of the insect evidence, correlated with the environmental situation (Bambaradeniya *et al.*, 2023). The identification and aging of insects are typically conducted by assessing specific morphological characteristics, and histological techniques can enhance this process. Moreover, histology can be instrumental in obtaining more precise results when insect samples are inadequately preserved due to incorrect collection and storage practices or as part of a cold case investigation (P. A. Magni *et al.*, 2012).

#### 2.5.1. Case Studies

##### Blowfly Immature Aging

When a cadaver is displaced in a terrestrial and temperate environment without major physical of chemical impediments to the arrival of insects, blow flies (Diptera: *Calliphoridae spp*.) are generally the first to colonize. Similarly, blow flies can also colonize orifices, wounds, and skin of living animals and individuals that are unable to care for themselves. The vast majority of blow fly species are oviparous, and they lay translucent eggs that allow for the observation of embryogenesis occurring inside. Assessing the development of the first instar larva inside the egg provides valuable information regarding the time since oviposition, supporting the estimation of the minimum PMI or time of negligence. Standard histology is a simple and practical approach to evaluate the age of the egg (Pais *et al.*, 2018) and has also offered positive results for the age estimation of blowfly pupae. The pupal stage comprises >40% of the immature component of the blowfly lifecycle, during which vast internal and external morphological development occurs (Voss *et al.*, 2016). Stained pupal section revealed differences in brain and thoracic muscle development throughout the pupal development (Brown *et al.*, 2015).

In the forensic investigation of a double homicide, the examination of fly eggs on a male victim aimed to pinpoint the time of death. Using conventional light microscopy, scanning electron microscopy (SEM), and micro-computed tomography, the analysis identified the carrion fly species and documented their developmental stages. This comprehensive approach enabled an accurate estimation of the time of death. The presence of carrion fly eggs, when considered alongside other investigative data, strongly implicated the suspect perpetrator's presence at the crime scene during the critical timeframe, significantly contributing to the timeline reconstruction of the crime (Bozó, 2023).

##### Insect Species Identification

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Histology is an important step required for a correct identification blow fly species, especially for immature stages such as eggs, larvae, full and empty puparia. For the eggs, species identification via SEM has been used, however an easier and less expensive technique is using light microscopy following potassium permanganate staining technique (Bunchu *et al.*, 2012; K. Sukontason *et al.*, 2004). The eggs can be permanently mounted on slides features like hatching pleats, width and morphology of the plastron, morphology of the plastron area surrounding the micropyle and chorionic sculpturing can be considerend alongside the size for identification purposes (Sanit *et al.*, 2013). A recently proposed reliable alternative uses a slightly modified standard laboratory processing run with 1 h fixation in 10% formalin, 2 h softening in Molliflex and H&E staining (Pais *et al.*, 2018).

Similarly, SEM is utilized to examine the ultrastructure of all other blowfly immature life stages, offering the advantage of appreciating critical characteristics to differentiate closely related species (Sanit *et al.*, 2017). However, light microscopy is more commonly preferred, employing the hydroxide clearing method for larvae (Bunchu *et al.*, 2012) and the potassium hydroxide clearing technique for pupae (K. L. Sukontason *et al.*, 2007). These techniques facilitate the observation and the measurement of blowfly immatures species-specific characteristics, such as the cephalopharyngeal skeleton, anterior and posterior spiracles, papillae, and spines.

The investigation of highly decomposed human remains recovered from a well required the intervention of a forensic entomologist 20 months after the initial inquiry. The body was found dismembered and, initially, the entomological evidence was overlooked and lost. In the course of a second autopsy, insects were discovered in bone cavities, but were poorly preserved. The delayed discovery necessitated the application of multiple microscopic techniques to highlight the species-specific features leading to the taxonomical identification (Paola A. Magni *et al.*, 2013).

### *2.6. Fibers*

In forensic investigations, trace evidence is routinely gathered also in the form of fibers. In Western Australia, a database was established for a cold case investigation that spanned 24 years, where fibers emerged as crucial evidence in a sexual assault and two homicide cases (Aljannahi *et al.*, 2022; Powell *et al.*, 2021). Fibers, spanning synthetic, plant-based, fur or recycled, prove instrumental in connecting victims to suspects and specific locations (Carr, **2017;** Marková, **2019**). Forensic laboratories often employ light microscopy, alongside commercial products like Shirlastain®, to distinguish between various fiber types. Polarising light microscopes, SEM and fluoresence microscopy are commonly utilised for fiber examination (Aljannahi *et al.*, 2022; Marková, **2019**).

#### 2.6.1. Case Study

A small thread was obtained from the coat of a suspect in a sexual assault case, where the victim's pink shirt was damaged. Dye analysis indicated the presence of Reactive Red 195, while diode array detection (DAD) indicated yellow and blue dyes. The cotton fibres present in the thread from the suspect matched the colour and fluorescence of the cotton fibres present in the victim's damaged shirt. The results of the dye analysis and fluorescence microscopy strongly suggest that the small thread discovered on the suspect's coat originated from the victim's shirt (Schotman *et al.*, 2017).

### *2.7. Palynology*

Palynology is the study of palynomorphs, e.g., fungal spores, plant spores and pollen. In the last 25 years, palynology has proven effective as trace evidence for geolocation in forensic investigations (Wiltshire, 2016*)*. Various extraction procedures exist for preparing pollen samples for analysis, e.g., centrifugation and dissolution of the background matrix. Some laboratory methods may degrade or destroy the pollen, with the extent depending on the specific pollen taxa and their location. Acetolysis, a standard procedure, is employed to eliminate undesirable lipids and debris, however, this approach can damage some palynomorphs (Laurence et al., 2019*)*. No specific staining is required, although the choice of mounting media is crucial to a correct visualisation using light

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microscopy, transmission electron microscopy (TEM) and SEM. Glycerine jelly is commonly used for mounting slides in forensic palynology (Bryant, **2013**). The disadvantages of these methods can be summarised in high costs and the need of time, access to expensive equipment and expertise in the interpretation of the results (Bryant, **2013;** Wiltshire, 2016).

2.7.1. Case Study

In 2015, a sealed trash bag containing the partly decomposed remains of a female child floated to the shore of Deer Island, in Boston Harbor (MA, USA). The child was given the moniker 'Baby Doe', as she could not be positively identified despite her clothing and a blanket. Clothing and hair samples were sent to the U.S. Customs and Border Protection Laboratories and Scientific Services Directorate lab in Houston, Texas. 200-300 pollen grains were recovered, however, the investigation identified only two species of Cedar pollen (*Cedrus spp.)* as relevant, since that particular Cedar pollen is found in arboretums. Safranin-O, a common stain for plant material, was applied to all samples. Further investigations led to the arrest of the child's mother and her boyfriend, who lived 4 km away from the Arnold Arboretum of Harvard University (Laurence *et al.*, 2019).

*2.8. Human Pathology*

The microscopic examination of human tissues is a fundamental in the diagnostics of both clinical and forensic pathology (Dettmeyer, **2018;** O'Dowd *et al.*, 2020).The microstructures composing tissues can be differentiated by conventional stains, such as Haematoxylin-Eosin, Congo red (amyloid) and Van Gieson (increased connective tissue in fibrosis), immunohistochemistry, based on the binding between antigen and antibody to highlight proteins, or special techniques, such as the TUNEL assay to detect nuclear fragments in apoptotic cells, in situ hybridization to detect DNA/RNA in tissue, cells or chromosomes, and techniques for Electron microscopy (Dettmeyer, **2018;** O'Dowd *et al.*, 2020). The application to forensic investigations is vast and diversified, including but not limited to clinical malpractice, the confirmation of natural death (e.g., myocardial infarction) and the dating of wounds (Li *et al.*, 2020; van de Goot *et al.*, 2014; Vignali *et al.*, 2023).

2.8.1. Case Study

A few recent studies discuss the role of tadalafil, a human cyclic guanosine monophosphate–specific phosphodiesterase, type 5 inhibitor (PDE-5) primarily administered in erectile dysfunction (ED), as a contributing cause of death in subjects with pre-existing cardiac risk factors or disease (Nagasawa *et al.*, 2021; Rust *et al.*, 2012; Schirripa *et al.*, 2023). In a recently described forensic case, the histopathological finding of ischemic cardiopathy (myocardial sclerosis, mild coronary and severe aortic arteriosclerosis) in an apparently healthy older male supported cardiogenic shock as the cause of death in a tadalafil user.

*2.9. Veterinary and Wildlife Forensic Pathology*

Animal abuse and welfare, biodiversity, identification, non-human evidence at crime scenes and wildlife crime are only a few of the many modalities that fall under the broad category of veterinary forensics. Associative evidence, such as hairs, bite marks or plant-based materials originating from pets and animals can be utilised to direct investigations in a variety of cases, including but not limited to trauma analysis, wound ballistics and poisoning (Byrd, Norris, *et al.*, **2020;** Linacre *et al.*, **2013;** Merck, **2013**). The greatest difference between veterinary/wildlife and human forensic histopathology is the wide range of different taxa involved. However, it has proved crucial to determine the cause and the manner of death in cases of drowning, neglect, sexual assault and stillbirths. The most impactful limitation is the decomposition of non-human cadavers, usually more advanced than those reported for most human bodies object of forensc investigations (Delgado *et al.*, 2021).

2.9.1. Case Study

An adult male domestic cat was found with arrows piercing through the skull and the neck. After conducting a radiograph of the skull, the cat was humanely euthanised and a whole-body postmortem computed tomography (PMCT) identified the cause of death as non-natural, by a penetrating trauma to the head. After the autopsy, histopathology indicated interstitial nephritis in the absence of any other serious disease *(Watson et al., 2020)*. This would have contradicted any potential excuse for attempting to kill the cat, for example the need to cease their suffering caused by a terminal condition *(Watson et al., 2020)*.

### *2.10. Histotaphonomy*

When postmortem modifications involve hard tissues, such as bone and teeth, their study at the microscopical level is known as histotaphonomy (Fernandez Jalvo *et al.*, 2016). Microscopic alterations in bone microstructure and reconstruction of post-mortem processes have been studied for over a century, and hold relevant applications within forensic medicine (Brönnimann *et al.*, 2018). For example, it has been established that the macroscopic appearance and the microscopic preservation of bone in the archaeological and fossil record can appear inconsistent (Kontopoulos et al., 2016), with the degree of surface preservation not necessarily reflecting the degradation of the organic component of bone (90% type-I collagen). The degradation of collagen reflects the protein content of bone (Lebon *et al.*, 2016; Madden *et al.*, 2018), and can be be utilised to estimate short PMIs and postmortem submersion intervals (PMSI). A protein naturally insoluble in water due to spatial structure and high molecular weight, collagen can be degraded by chemical or enzymatic hydrolysis, that is the attack of microorganisms producing protease enzymes. Chemical and enzymatic hydrolysis are known to start a few months postmortem (Boaks *et al.*, 2014; Jellinghaus *et al.*, 2019), to continue over time (Hoke *et al.*, 2013) and to constitute an essential step in the process of bone decay (Edda E. Guareschi *et al.*, 2021; Pfretzschner, 2006; Trueman *et al.*, 2002).

2.10.1. Case Study

A recent study by the authors describes one of the very few taphonomic experiments involving bone submerged in natural saltwater and freshwater, compared to air-exposed bone and to non-exposed controls. The macroscopical and microscopical characterization of terrestrial mammalian bone submerged for up to 24 months includes algae colonization and adipocere formation on the submerged samples, desiccation of the analogous fragmentary soft tissues exposed to the air, persistence of bone marrow within the medullary cavity throughout the experiment and preservation of bone microstructure, with clearly identifiable and regularly distributed vascular channels, lamellae and osteocyte lacunae. Collagen, stained with picrosirius red (Junqueira *et al.*, 1979; Lattouf *et al.*, 2014)and quantified by QuPath® (Bankhead *et al.*, 2017) appears to degrade faster underwater than in subaerial exposure, whereas bacterial bioerosion has only been observed in the samples exposed to the air, possibly due to the different and more abundant species of microbioeroders. The results indicate that some parameters of bone taphonomy and diagenesis can, with due caution, predict the depositional environment in PMIs and PMSIs of at least 12 months (E.E. Guareschi *et al.*, 2024, under review).

### *2.11. Histothanatology*

Decomposition processes, such as autolysis, adipocere formation and putrefaction, can limit forensic diagnostics after death by modifying the anatomical structure of organs, tissues and cells (Dettmeyer, **2018;** Magni *et al.*, 2020). The knowledge of the appearance of decomposition processes, especially in their early stages, when still macroscopically imperceptible, can assist in the detection and differentiation of both pathological findings, e.g. thrombosis vs. postmortal blood clot, and clues to estimate the PMI, e.g., fungal colonization, which will become particularly defined in the intermediate and later stages, when histology can disclose crucial details and timelines of death, for example through the analysis of the gastric content or the lung tissue.

2.11.1. Case Study

In a recent study, the microscopical analysis of the soft tissues and skeletal remains of 21 human analogues *(Sus domesticus)* left to decompose in three distinct types of cemetery disposal, inhumation, traditional entombment and aerated entombment, confirmed that the process of decomposition is promoted by the presence of drainage, microfauna and oxygen in the burial environment, and is hindered by waterlogged and anoxic conditions, which favour the formation of adipocere. This involves critical consequences in the urban cemetery management of densely populated countries in temperate climates, with related environmental and public health issues. Specifically, partially skeletonized bodies need a second disposal after exhumation, with additional cost, family distress, labour and space (Edda Emanuela Guareschi et al., 2022).

## 3. Discussion

The debate about the value of systematic histological examination in medico-legal and forensic autopsies (De La Grandmaison *et al.*, 2010; Lau *et al.*, 2008) appears to be pacifying, because the crucial role of histology has been gradually confirmed over the last decade (Chen *et al.*, 2021; Fronczek *et al.*, 2014). This review, although not systematic and not exhaustive due to the massive volume of literature on the same, or closely-related, topic, shows that the relevance of this role is not limited to the human body, but involves many other fields of forensic investigations, with the ultimate aim of closing cases and ensure justice. While accepted as highly valuable, the access to histology by law enforcement and forensic practitioners is characterized by some meaningful challenges, such as the need of a dedicated wet laboratory, with inherent cost (Molina *et al.*, 2007) and accreditation requirements (e.g., International Standard ISO 15189:2022), the specific expertise in the interpretation of samples, and the admissibility of the materials and the reports presented as court evidence (Cirielli *et al.*, 2021). Therefore, securing an agreement with a public or private histology laboratory, usually associated to a hospital, is essential to ensure access to specialized expertise and equipment. In conclusion, histology serves as an indispensable tool in forensic investigations, offering detailed microscopic examination of materials, usually of organic nature, to complete macroscopic examinations. The interdisciplinary approach, combining anatomical, pathological, and molecular techniques, underscores its critical contribution to modern forensic sciences.

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