Histomorphology of trauma in charred and decomposed remains

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Histomorphology of trauma in charred and decomposed remains

A Thesis Presented in Partial Fulfillment of the Requirements
for the Masters in Forensic Science
John Jay College of Criminal Justice
City University of New York

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Histomorphology of trauma in charred and decomposed remains

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This Thesis has been presented to and accepted by the Office of Graduate Studies, John Jay College of Criminal Justice in Partial Fulfillment of the Requirements for the Degree of Master of Science in Forensic Science.

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Abstract

Decomposition and burning of human remains are frequently encountered in forensic cases, and pose a challenge at determining an accurate cause of death. At autopsy, macroscopic techniques fail to reach beyond superficial layers of degraded tissue to identify wound morphology that characterizes blunt-force gunshot, and sharp-force trauma. The postmortem damage imparted on tissue with pre-existing injuries, obstructs the external features of trauma. Exploring beyond charred and decayed superficial tissue, histo-morphological patterns of injury are still identifiable as shown in this thesis. With detailed observation to the pattern of tissue disruption- an understanding of the type of trauma imposed can be found. Tissue injuries are exaggerated by thermal effects of burning and degraded by the taphonomic effects. This work aims to explore histomorphological findings of such challenging samples. Together with bone toolmark analysis, the information obtained from tissue trauma morphology can be applied to further develop models that can aid in the identification of trauma type and the tools used to inflict it.
Acknowledgement

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# Table of Contents

ACKNOWLEDGEMENT.......................................................................................................................... I  
TABLE OF CONTENTS ............................................................................................................................ II

INTRODUCTION........................................................................................................................................ 1

1. HISTOMORPHOLOGY OF TRAUMA BY TRAUMA TYPES, WEAPON USED AND TAPHONOMIC MODIFICATIONS ......................................................................................................................... 1  
   A. GUNSHOT WOUNDS ........................................................................................................................... 8  
   B. SHARP-FORCE TRAUMA .................................................................................................................. 12  
   C. BLUNT FORCE TRAUMA .................................................................................................................. 16  
   D. EFFECT OF DECOMPOSITION/CHRARING .................................................................................... 17  
   E. IMPACT ON FORENSIC SCIENCE ................................................................................................. 19  
   F. ETHICAL CONSIDERATIONS ........................................................................................................... 20

2. MATERIALS AND METHODS ............................................................................................................... 21  
   A. SAMPLES PREPARATION ................................................................................................................ 21  
   B. SAMPLES PROCESSING ....................................................................................................................... 21  
   C. SAMPLES HISTOLOGY PROCESSING .............................................................................................. 22  
   D. STAINING ........................................................................................................................................ 23

3. RESULTS .......................................................................................................................................... 24

4. DISCUSSION .................................................................................................................................. 38  
   A. TOOLMARK ON BONES .................................................................................................................. 38  
   B. HISTOMORPHOLOGY ..................................................................................................................... 39  
      i. Blunt-force Trauma ...................................................................................................................... 39  
      ii. Gunshot Trauma ......................................................................................................................... 41  
      iii. Sharp Trauma ........................................................................................................................... 43

5. LIMITATIONS .................................................................................................................................... 44

CONCLUSION/FUTURE DIRECTION ........................................................................................................ 46

SUPPLEMENTAL MATERIAL .................................................................................................................. 49

REFERENCES ......................................................................................................................................... 51
Introduction

This work aims to innovatively apply histological methods to severely damaged remains resulting from natural decomposition and effects of thermal destruction. Forensic analysis of human remains seeks to answer questions as to what was the cause of the death. The answer may not be obvious at first, especially when there is a lack of visible injury on human remains. At the onset of death, biological processes such as necrosis set in, such that in the presence of external factors, injured and non-injured tissue is degraded furthermore. In circumstances involving intentional burning or abandonment of remains, it becomes even more challenging to identify biological causes of death. Burning of human remains distorts external tissue and leaves remnants of charring and carbonization, which obliterates skin wounds. Especially, fatal physical trauma, which is documented as deep penetrating wounds on a victim’s body, can be further altered as a result of accelerated degradation. Taphonomic factors (unintentional) including rainfall, humidity, temperature and animal activity, all contribute to the natural rate of decomposition, leading to disintegration of human tissue.

1. Histomorphology of Trauma by Trauma Types, Weapon used and Taphonomic Modifications

Intentional damage to human remains, aimed to conceal identity and/or manner of death, result in even more extensive damage. Such destruction is seen in cases involving exposure to fire [with or without accelerant] that result in severe charring of external and underlying layers of the skin leading to nearly complete obliteration of a pre-existing
injury (Tümer et al., 2012). Charring carbonizes superficial and deeper layers resulting in disfiguration of internal and external injuries including connective and muscle tissue trauma. In a similar fashion, decomposition and entomologic activities further promote post-mortem degradation [that immediately begins at the point of death], producing advanced lesions, which mask the nature of pre-existing ones. Importantly, any secondary changes imposed on injured tissue pose a challenge at extrapolating a timeline of such traumatic events (McIntosh, Dadour, & Voss, 2017). Antemortem injuries are inflicted as the person is still alive, and perimortem injury is that which occurred at or near the time of death. Distinguishing these injuries from postmortem injuries, is the goal of the forensic pathologist, and serves to help infer which injury was fatal. Microscopically, these can be distinguished by noting signs of tissue repair surrounding the area of trauma. Antemortem and perimortem tissue injury typically shows signs of healing and repair, with elements of inflammation being markedly present (Dettmeyer, 2011). Repairing involves active recruitment of connective tissue and blood elements in patching the disrupted tissue structure. Postmortem injuries lack the presence of these elements because the body can no longer heal itself. Consequently, in the setting of post-mortem changes, the analysis of any pre-existing trauma marks is expected to be disfigured and should be interpreted with caution.

Post-mortem tampering of human remains is not uncommon in criminal investigations (Tümer et al., 2012). The intent to obliterate a body of evidence is to conceal evidence of a crime. For one, a perpetrator may hide trauma on the victims body in order to hide wounds which may be defensive in nature (Schmidt & Pollak, 2006). It is important to note that post-mortem tampering may not necessarily be criminal in nature:
these changes can take place as a result of catastrophic events such as plane crashes or unplanned natural decomposition (Obafunwa et al., 2015). Irrespective of the state of postmortem damage, determining the precise cause and manner of death must be done objectively and account for all possible postmortem modification, intentional or not. Medicolegal investigators need to have tools at their disposal to address the complexities of severely degraded human remains. The need to go beyond the outermost evidence of injury and evaluate tissue that has been irreversibly damaged, requires an understanding of tissue behavior under these conditions. Currently, routine autopsy is limited to the gross examination of the body and organs, and therefore does not address the challenges that may be encountered in putrefied and burnt remains (Madea & Saukko, 2007). On the other hand, histological examinations are routinely performed in clinical diagnostic settings, but its application to forensic studies is underutilized. In fact, trauma may not always be apparent during an autopsy to support a specific cause of death. In fact, one study points out that of the autopsy cases reviewed, 22% showed that traumatic lesions were better documented by histological findings than by gross examination alone (Lorin de la Grandmaison & Charlier, 2010), supporting that histological evaluation together with gross findings can help establish the precise cause of death. Presently, histomorphological assessments are not part of routine forensic analysis, which may be explained by the present lack of standardized criteria and peer-reviewed reports supporting its applicability to forensic investigations. Although histomorphology requires specialized training, its utility in aiding challenging cases can be shown in a specific study reported from an institution that requires use of routine [histological] hematoxylin
and eosin (H&E) staining as part of a systemic autopsy protocol: a retrospective analysis on 428 autopsies was undertaken by Lorin de la Grandmaison et al. (2010) and revealed:

“Mechanism of death not shown by gross anatomic findings was discovered by histology in about 40% of the cases. Cause of death was established by only histology in 8.4% of the cases. Microscopic findings affected the manner of death in 13% of the cases. Histology provided complimentary information about prior medical condition of the deceased in about 49% of the cases”. (p. 85)

This report sheds light on the impact of histology in death determination that may not necessarily be criminal in nature, yet still be impactful. If this is to be used as ancillary analysis to describe trauma wounds, an effort should be made to use histology to best identify the timing of injuries.

Beyond understanding the undisturbed normal human tissue and trauma-affected tissue, a few studies have aimed to document microscopic changes or histomorphological patterns of trauma in bodies that underwent postmortem charring and heavy decomposition. A study aiming to identify gunshot lesions on severely decayed remains determined that while a macroscopic examination can be used to identify gunshot wounds on skin that showed traces of decomposition, gunshot wounds from a 15-week decomposed body, were too degraded to provide any information (Gibelli et al., 2010). Severely putrefied samples pose a challenge because the extent of tissue degradation is so great, that remains become homogenized and indiscernible as a result of necrotic processes. Further studies focused on attempts to document decomposed tissue in a way that allows digital processing and manipulation of pictures and as such, identified stereo-optical microscopy to be the optimal method for documenting trauma wounds in
decomposing remains (Stanley, Hainsworth, & Rutty, 2017). For this reason, a firm understanding of undisturbed tissue and pre-existing trauma morphology is necessary to serve as a point of reference where degradation impedes trauma analysis.

Preliminary findings from studies on non-degraded trauma tissue showed that tools of the same class (i.e. serrated and non-serrated knives) produce similar wound pattern morphologies (Petraco, Petraco, & Pizzola, 2005; Pounder, Bhatt, Cormack, & Hunt, 2011). For example, since gunshot wounds result from high-velocity projectiles that perforate outer layers of the skin- circular entry and exit holes with flaps of torn skin tissue facing inward and outward, respectively have been observed (Karger, 2014). Penetrating injury from sharp stab wounds result in irregular but smooth edges at the point of impact, and vary in the depths of trauma (Crowder, Rainwater, & Fridie, 2013). Having the understanding that a particular wound is consistent with that of a sharp blade or a projectile, additional value can be extrapolated from tissue sampled along the path of injury and aid in determining the tool that was used. If the exposed tissue can be located, the internal corridor created by the tool can be analyzed for artifacts. Such findings may be foreign to normal human tissue, but successfully identified- it can provide insight as to the source of the object that transferred it (Delabarde et al., 2017). Environmental particles like dust, fibers, glass and combinations of such micro trace get deposited into tissue when a tool makes contact with it. If such exogenous material is detected, an effort to characterize it should be made to identify its composition. Although histological micro-trace analysis has not been explored in depth, few approaches have aimed to apply chemical and physical methods to identify sand particles, wood fragments, paint chips and metal fragments from tools such as a saw, wooden bat, and a knife in certain types of
wounds (Delabarde et al., 2017). Evidence such as this is capable of adding uniqueness to the study of a forensically relevant wound. The ability to approach histological cases from different perspectives such as with micro trace analysis adds more value in describing the unique signature of an injury.

Difficulties in trauma analysis are further compounded by taphonomic alterations (Thali et al., 2003). External actions of insect activity, temperature and humidity accelerate tissue degradation by promoting autolysis (Cockle & Bell, 2015). Since a penetrating injury leaks deeper layers of tissue, the extent of alteration to the open wounds may be affected by these factors. Smith (2014) found that “by adding trauma, a larger, more nutrient-rich orifice for insects to utilize” (p. 326) was observed. Macroscopic findings documenting these changes suggest that similar observations may exist on a microscopic level. Burning creates irreversible changes since outermost layers of skin are readily carbonized once exposed to extreme heat. Shriveling of tissue exposed to the face of fire has been reported in trauma inflicted remains, showing that wound morphology is changed in charred samples when compared to un-charred samples in Sus scrofa (Poppa et al., 2011). External layers of the skin get denatured and warped due to shrinkage of the dermis and epidermis (Gruenthal, Moffatt, & Simmons, 2012). Fatty tissue burns readily as it is composed mainly of carbohydrates, whereas bone tissue tends to persist and cut marks from knife wounds become less visible following burning (Kooi & Fairgrieve, 2013). Nevertheless, it is still possible to obtain viable evidence from burnt samples. In support of this, one study showed that a complete DNA profile can be obtained from bloodstains exposed to extreme conditions in which bloodstained objects were exposed to temperatures reaching as high as 700°C (Klein et al., 2017). With this
evidence, it is curious as to what extent does pre-existing trauma remain preserved in the postmortem burning process. Studies have shown that perimortem bone trauma can still be recognized after thermal exposure (Macoveciuc, Márquez-Grant, Horsfall, & Zioupos, 2017). Similarly, burnt soft tissue trauma has also been described using patterns of damage found in different tissue types (epidermis, fatty layers, muscles and organs) where sharp force and blunt force injuries were marked by specific identifiable morphologies (Symes, Dirkmaat, Ousley, Chapman, & Cabo, 2012).

At the onset of death, a body will invariably undergo natural postmortem decomposition. Natural decomposition involves multiple factors but is primarily driven by temperature. It has been shown that a warmer, wetter climate is the main driver of decomposition and variably accelerates it in Sus scrofa (Cross & Simmons, 2010). Left alone, taphonomic effects of the soil, insects, and scavenger activity speed up the rate of postmortem decomposition. Therefore, unintentional changes will invariably affect the state of remains. While only a limited number of studies have been performed on trauma-inflicted decomposed remains, the understanding is that pre-existing trauma affects the pattern of decomposition but not the rate of decomposition (Smith, 2014). Studying the effect of decomposition on an array of trauma wounds can elucidate a marker of decomposition, and a postmortem signature can be ascribed. A histological evaluation can reveal the nature of trauma lesions once a comparative analysis is performed with lesions that underwent decomposition. Preliminary findings from the current study can serve to fill the gap of understanding tissue injury and its response to postmortem changes in the setting of gunshot, sharp force and blunt force mechanisms. Trauma wounds resulting from these tools can be characterized by the pattern of disrupted tissue.
Then, these observations would be compared to the trauma tissue that has been burned or decomposed. Notable differences between the two types of observations include specific lesions being exaggerated as a result of thermal warping and shrinkage. Similarly, decomposed tissue is expected to show exogenous particles consisting of soil and artifacts of putrefaction. In the context of these studies, an intriguing and yet unanswered question remains: how does tissue as a whole, respond to trauma and to what extent can different types of trauma remain identifiable? This dissertation addresses the need for histomorphological investigations of charred or heavily decomposed bodies. The hypothesis driving our study is that if gunshot, stab and blunt force wounds are present in a heavily decomposed or charred body. This type of trauma can be characterized and can lead to identification of the type of tool/weapon used despite taphonomic changes. The goal of this work is to present a unique observation of the behavior of different types of lesions and how they react to thermal and other taphonomic factors.

a. Gunshot wounds

Penetrating trauma caused from high-speed projectiles is often documented by the presence of gunshot residue (GSR). Making up GSR are barium antimony and lead. As a projectile makes contact with soft tissue, bullet wipe is exchanged with surrounding tissue, leaving deposits of GSR around the entry/exit hole and along the internal pathway of travel in the body. A commonly used method of testing for GSR on surfaces is using energy dispersive X-ray spectrometry (SEM/EDS) that focuses on detecting these three metal particles. In gunshot wounds, a projectile enters the tissue with rapid force, and pierces through the epidermis, dermis, and connective tissue as it makes its way through
the organ and bone. On impact, a projectile deposits trace particles and carries them forward along the direction of travel until it comes to a complete stop [within the body or when it exits]. If the projectile passes through bone, entrance and exit holes can be distinguished by the beveling of the edges of bone. Grob-Perdekamp et al. (2011) showed that bullet wipe can distribute along the path of travel, radially in relation to the entry hole of a projectile, suggesting that sampling of tissue along a projectile path, is expected to reveal traces of GSR particles (Grob-Perdekamp et al., 2011). Visually, gunshot entry/exit wounds can be identified by observing a dark ring of hemorrhaging and tissue burning with dark soot particles (ecchymatic ring) surrounding the point of impact (Amadasi, Brandone, Rizzi, Mazzarelli, & Cattaneo, 2012). In instances where visible soot is identified, microscopic histological examination sheds light unto the fact that remnants of soot and bullet wipe can also be detected along the projectile path throughout and leading up to the exit hole. The ecchymatic ring is found to be more concentrated around the point of entry—at closer ranges and becomes less concentrated as the muzzle-to-target distance increases. However, positive identification of firearm soot as a marker of firing distance and or entry wound cannot be discernible from the bullet wipe embedded in the tissue (Perez & Molina, 2012). Of note: a lack of visible soot does not rule out a gunshot wound. Gibelli et al. (2010) showed pigskins that underwent gunshot trauma and then were subjected to various decomposition cycles after which, the entry gunshot wounds were sampled for histological evaluation. The severely decomposed tissue-testing for the presence of GSR was done by chemical tests prior to subjecting the sample for charring, because the charring effects have obliterated the morphology of the wounds (Gibelli et al., 2010). They concluded that thermal consequences produce a
distortion of pre-existing wound morphology and can therefore mask a gunshot wound as either an entrance or exit hole. Consequently in order to rule in the presence of GSR on charred tissue, a confirmatory test is needed to positively confirm the presence of GSR on carbonized tissue. Sensitive methods such as micro-computed tomography not only detect GSR gradient concentrations but are also able to quantify GSR within completely indiscernible remains (Fais et al., 2013). Nevertheless, utilizing non-destructive methods is the ideal option for situations where a sample exists in limited quantity- and a less destructive technique is necessary. One such example is inductively coupled plasma optical emission spectrometry (ICP-OES) which is used to see if GSR was detectable on charred bodies, with results stating that burning occluded only the surface of the wound morphology while the underlying tissue and the trauma reaching the dermis- persisted (Amadasi et al., 2012). In remains in which presence of entry/exit holes may be masked by tissue carbonization from fire, soot patterning was also shown to appear indiscernible among skin layer- further supporting the need to establish techniques aim at identifying soot from GSR in obliterated burnt remains (Collini et al., 2015).

Applying histomorphological assessment to gunshot trauma is likely to reveal characteristic patterns. Studies reported that macroscopic examination of the presence of gunshot residue around entry and exit holes is directly related to the subsequent histological finding of GSR in 69 non-charred gunshot wounds. Additionally, the presence of soot was identified by routine H&E staining as black granular material consistent with GSR morphology (Cecchetto et al., 2012). The amount of GSR detected is consistent with the wound being either a gunshot entry or exit hole. The amount of GSR particles has been shown to be identified in entry wounds, whereas in exit wounds,
levels of GSR have been present in low levels particularly in the wound path leading up to the exit hole (Perez & Molina, 2012). Although this study was conducted on non-trauma inflicted samples, the utility of histological analysis in detecting GSR soot is very powerful especially in cases where gunshot trauma is obscured. In fact, Harada et al. (2012) report that a severely decomposed man, having a postmortem interval estimated to be 1-2 months, was found to have hemorrhaging signs of cranial damage during autopsy. On further investigation, histological staining of brain matter revealed tracks of potential soot material that motivated a re-sectioning from the hemorrhagic source wound to reveal discrete polygonal particles that were identified as soot deposition- resulting in a confirmed diagnosis of gunshot injury to the head (Harada, Kuroda, Nakajima, Takizawa, & Yoshida, 2012). Such an example describes microscopic features of soot particles furthermore supporting the value of these findings in autopsy cases.

Histomorphological findings in gunshot trauma can also provide supplemental information that help lead to accurate reconstruction of the manner of death. In a histological study reviewing data gathered from 28 bullet entry and exit holes, the authors found that entry holes showed evidence of increased hemorrhage surrounding the entry of the dermis. Traumatic affects from projectile were described: “charring, scorching with soot deposition" being prominent and consistent with entry wounds. Furthermore, pattern findings from exit holes were consistent with a “larger diameter, irregular edges of the wound and adipose tissue”. The findings concluded that although both entry and exit holes shared common morphological features, it is not always possible to distinguish entry vs. exit hole based on morphology alone (Baptista, D’Avila, & D’Avila, 2014).
summary, if a gunshot hole cannot be readily identified, supplementary testing may be
called upon to rule in/out the presence of GSR.

Confirmatory analysis should be done with the use of special staining that
specifically binds to GSR. Counterstaining with sodium rhodizonate (NaR) is a reliable
chemical test for gunshot trauma to be performed on gunshot wounds. NaR reaction is
specific to particles of lead which when bound, produce a dark staining red color.
Positive identification of gunshot wounds from decomposed pig samples has been shown
with using NaR testing together with neutron activation analysis (specific for identify
lead and antimony: components that make up the GSR). Having successfully stained for
GSR in decomposed samples, the study concluded that NaR analysis can be successfully
applied to decayed samples reliably up to the 9th week of decomposition [in soil] (Gibelli
et al., 2010). Recent studies reported that NaR can be reliably used to confirm gunshot
wounds irrespective of environmental pollution, showing that samples that did not have
any gunshot wounds were negative for NaR staining (Boracchi et al., 2017). NaR as a
supplementary test can therefore be used in conjunction with histological assessment of
gunshot trauma, without cross reactivity with soil contaminants.

b. Sharp-force trauma

An injury that results from a sharp force is typically caused by knife or saw,
having serrated and non-serrated blades. Class characteristics in bladed-tool and sharp-
edge weapons result from striations left behind in the impacted tissue, specifically the
kerf characteristics within the overall morphology of the wound pattern. These features
are used to describe class characteristics of tools such as knives, saw’s and complex
power tools and when used to inflict injury. In bone, striations are easier to identify than in soft tissue, because soft tissue is challenging due to its elastic nature (Kaliszan, Karnecki, Akçan, & Jankowski, 2011). The repeated sawing-like action of bladed tools that cut soft tissue and bone-leave behind unique markings that are complementary to the blade of origin, in which a striation pattern may be identified (Symes et al., 2012). Striation patterns have been reported in soft tissue as a result of serrated-type blades; however the absence of visible striations in soft tissue does not exclude a serrated-blade (Jacques, Kogon, & Shkrum, 2014). Even so, class characteristics have been observed on bone even after exposure to fire (Robbins, Fairgrieve, & Oost, 2015). A study using SEM detection show that toolmark features decrease by 40% in burned tissue when compared to the same features observed on pre-burned tissue (Kooi & Fairgrieve, 2013). On the other hand, under fluctuating thermal affects, toolmarks were observed showing in varying patterns, depending upon the type of tool used (Marciniak, 2009). A decline was seen in the positive identification of sharp force injuries from samples of charred Sus scrofa. A later study confirmed that stab marks and serration marks were still detectable after burning (Stanley et al., 2017). Overall, sharp tool trauma can be described microscopically, but if subjected to a charring cycle then the trauma pattern should be interpreted with caution.

Histologically, sharp-force trauma can be discerned by noting straight-line incisions and lacerations to superficial layers of the dermis. In wounds resulting from a penetrating blade, sharp corners flaking the previously intact tissue can be observed resulting from the slicing action of the blade (de Siqueira, Cuevas, Salvagni, & Maiorka, 2016). Kerf markings due to the impact with the blade floor can be detected in bone and
associated tissue (Crowder et al., 2013; Delabarde et al., 2017; Schmidt & Pollak, 2006). Unlike the traces of GSR seen in gunshot wounds, sharp force trauma may not reveal significant trace material. Bladed tools don’t normally shed particles, however Locard’s principle dictates that trace particles are transferred between interactions, but the lack of observable particles in trauma tissue, should not exclude sharp force impact. Presence of foreign material lodged in the tissue can be seen, particularly if the object impacting leaves trace of steel fragments, glass and soil particles. Chemical analysis has shown that in sharp force trauma inflicted on bone, 58% of analyzed cases, revealed that particle composition was concordant with the sharp object, suggesting that the source object can be identified by the particles found in the walls of the lesion (Pechníková et al., 2012). Sharp force lesions have been reported to be similar, described using histomorphology (Poppa et al., 2011). Determining information about the type of tool used is challenging since the nature of soft tissue is such that it has a tendency to re-form itself upon impact of a tool, due to its plastic nature. Attempts have been made to characterize toolmarks in soft mediums and harder mediums: replicating tissue and bone (Baiker, Pieterman, & Zoon, 2015; Crowder et al., 2013; Petraco et al., 2005). The nature of tissue is such that its elasticity reacts to recover from imposing forces. Toolmarks are not permanently cast in the tissue and such as trauma from blunt impact may be misinterpreted, having the tendency to recover from an impact. Sharp force trauma on the other hand, tends to leave permanent damage in the form of breaks in the continuity of connective tissue perpendicular to the direction of the incised wound (de Siqueira et al., 2016). These lesion patterns were documented in a veterinary study to show that sharp force trauma patterns as having linear or angular edges, whereas blunt force trauma tend to show
irregular edges A study that was submitted to the National Institute of Justice focused on the inter-observer assessment when looking at cut marks from bladed tools, attempting to document error rates. The study reported less than 50% observer agreement in assessing edge bevel marks in bone, whereas in casting wax and pig cartilage, there was a 96% agreement (Crowder et al., 2013). Metric assessments have suggested that lesion profiles vary depending on the type of tool and the variability of force applied using the tool. Symmetrical-bladed tools leave a smooth and uniform incision in bone marks, whereas asymmetrical blades leave a trauma incision path that is variable in length (V. DePrimo, personal communication, December 2, 2017). In cross-sectional knife mark profiles, width marks were wider in angled impacts when compared with 90-degree impacts (Cerutti, Magli, Porta, Gibelli, & Cattaneo, 2014). Bolliger et al. (2014) reported that toolmark depth can be informative and manual measurements can be acquired with similar sensitivity to digital computed tomography (CT). Wounds were inflicted into piglets, using a knife and a screwdriver, and then injected with contrast medium for CT imaging. At the same time, a dull tip probe was used to manually measure the depth of each wound. Measurements from CT-obtained data were compared to those recorded using a dull-tip probe. The results obtained from both methods, showed depth values to be within 11% deviation of the true values. Knife mark measurements were found to be uncomplicated when manually measured since the depth walls were smooth and accessible for the probe, whereas screwdriver marks were more difficult to probe due to the uneven depth walls and crushed channels produced from the tip of the screwdrivers (Bolliger et al., 2014). This study serves to support a practical method of measuring toolmark depths by hand, with an expected error rate of 11%.
c. Blunt force trauma

Blunt force tissue trauma results from a low-velocity impact and is generally characterized by deformation of tissue infrastructure, hemorrhage, and signs of healing such as resorption, clotting, and reorganization of tissue elements (Dettmeyer, 2011). Observing distortion in connective tissue fibers without a break in the continuity of the orderly tissue suggests some sort of impact. In a living organism, tissue elements respond to blunt-force deformation by reorganizing capillaries and cartilage to assist healing and for vascularization to take place. Therefore, signs of healing can be witnessed if the impact occurred prior to death (Byard, Gehl, & Tsokos, 2005). Conversely, post-mortem trauma tissue will lack signs of healing although deformities and disruptions in the tissue pattern are still observed. Blunt force trauma may also be penetrating: breaking outer layers of the epidermal tissue and piercing the underlying connective tissue. The extent of tissue damage has been described in relation to the amount of force applied by a blunt tip object. Newton’s law states that force is a product of mass times velocity, hence the extent of tissue deformation can be correlated with the amount of force of the blunt object (Shkrum, 2007). In damaged tissue, the extent of damage can be qualified by the severity of entrapped blood vessels and crushed fat cells surrounding the impaction area. If the force applied to the body is stronger than the tensile strength of the soft tissue such that the tool penetrates it, tearing and rupture of blood vessels and tissue network occurs (Wanek & Mayberry, 2004). Having said this, soft tissue trauma from a blunt force object has been described, but charring and decomposed affects have not been extensively studied. More studies to further document this in the context of the blunt force injury are needed.
d. Effect of Decomposition/Charring

The need for elucidating characteristics of trauma-inflicted tissue in response to extreme heat and decomposition has been acknowledged in recent work (Tümer et al., 2012). In extreme cases of burning, only the skeleton remains after the soft tissue is completely carbonized, leaving forensic evaluations to be assessed solely on bone (Bohnert, Rost, Faller-Marquardt, Ropohl, & Pollak, 1997). It is also evident that thermal subjection does not completely destroy signs of trauma (Poppa et al., 2011). Depending on the exposure to the fire, bone expands resulting in the formation of small heat-related micro-fractures that are longitudinal and transverse to the length of the bone (Symes et al., 2012). These heat-related changes produce a distinct burn signature, which can be used to discriminate against original pre-existing toolmarks, as Symes et al. (2012) reported that original toolmarks are protected from thermal changes and can still be distinguished from micro-fractures.

Identification of heat-induced micro-fractures

In the setting of pre-existing fractures that have been exposed to fire, it has been documented that circumferential lamellae have been shown to flake off around larger, pre or peri-mortem fractures (Schmidt & Uhlig, 2012). Thermal forces act to desiccate exposed tissue and in turn produce weaknesses in already fractured bone. Noting micro-fractures that were produced from thermal damage is important to document as it indicates that bone was exposed to burning. Bohnert et al. looked at the isolated affects of
burning applied to post-mortem bone. They noted that after exposing 20 intact skulls to incinerating temperatures of 600-820°C for up to 45 minutes, there were no heat-induced fractures observed, suggesting that it is possible to discriminate heat-induced fractures from trauma induced fractures (Bohnert et al., 1997). For one, the type of bone may affect the fracture formations in that long bone, flat bone and irregular type of bones may have a different susceptibility to fracture formation. Further work should be done, however, to better document the behavior of charred pre-existing fracture patterns and better characterize artifacts introduced from burning.

Histology of Charred Remains

Histologically, thermal injuries have been documented in the outer layers of the skin to show warped connective tissue fibers and elongated epidermal layers as a consequence of dehydration (Takamiya, Saigusa, Nakayashiki, & Aoki, 2001). Charring of the epidermis has been observed by flaking of the uppermost tissue as well as shrinkage and separation of different tissue layers (Imaizumi, Taniguchi, & Ogawa, 2014). Burning disintegrates tissue beginning with the outer skin, and reaching beyond while leaving a track of carbonized deposits. Symes et al. (2012) documented the sequence of degradation of human remains beginning with external modification of the skin which is described as blistering and splitting of epidermal layers; flexure of limbs which is seen as contraction of muscle tissue and retraction of muscle from the bone; loss of moisture leads to warpage of soft tissue and fractures of the bone are observed.

Histology of Decomposed Remains
There are inherent difficulties with analyzing decomposed remains, which may help explain the limited availability of studies performed on such samples. Due to the dissociative nature of the tissue that has undergone decomposition, sample handling becomes difficult since skin slippage disintegration of organs and structures are associated with putrefaction (Byard, Farrell, & Simpson, 2008). The extent of tissue degradation can produce severe liquefaction as a result of cell autolysis and identifying specific anatomical features can be obscured. Decomposition in the setting of trauma has been studied in single case reports (Gibelli et al., 2010; Harada et al., 2012). In all cases, gunshot wounds have been affected by decomposition yet histological characteristics of gunshot injury have been successfully identified. The influence of decomposition on pre-existing trauma has been shown to affect the pattern of decomposition on remains. With this in mind, the presence of penetrating wounds has been suggested to specifically influence this pattern by providing a greater access for entomologic activity (Bates & Wescott, 2016). Since humidity, temperature and necrotic lysis impart changes unto available surface tissue, the possibility of penetrating wounds being exposed to these factors immediately cannot be ruled out.

**e. Impact on Forensic Science**

The goal of this project is to identify and characterize the histomorphology of trauma in decomposed and charred tissue. The destructive nature of decomposition and fire on human remains presents a setback in forensic analysis. This study aims to describe the effects of heat and decay introduced unto existing trauma. Applying histological techniques to these compromised samples should help show the limitations of degraded
remains at the same time as bring to light the utility of histomorphology in trauma tissue. Moreover, it is also worth noting that the information presented in this thesis can serve as a guide to future studies that may go further in researching the behavior of trauma in decomposed and obliterated remains. The findings presented here serve to aid practitioners in the field on what features to anticipate in badly burned and severely decomposed remains. Furthermore, the conclusions reported from histomorphological evaluations intend to compliment current methods used in forensic investigations and contribute ancillary information in which current methods become limited. Finally, routine histological techniques can be applied to criminal cases in which the body of evidence has been altered and trauma identification remains unfound. Methods described in this work have been found to be economical and suitable to be applied in routine casework. Trauma histology has been explored at the gross (macroscopic) levels (see below), but the intent of this project is to go beyond the macroscopic features of trauma and explore the possibilities that histological investigations can reveal in identification of trauma in heavily decomposed or charred bodies.

f. Ethical Considerations

This work relied on the unbiased observation of the results obtained. For this reason, all work was performed with equal and unbiased handling to avoid variation from external variables. All samples were stored in a safe and secure containment to avoid disturbances of the samples. No animals were hurt in this process.
2. Materials and Methods

a. Samples preparation

The tissue samples in this study were obtained from *Sus scrofa* (domestic pig) at a local butcher. Three sets of “on-the-bone” ribs were obtained for knife and screwdriver trauma and two pieces of shoulder [including the bone] for gunshot trauma. The first set of ribs remained unaffected to be studied as control sample. The second rib set was stabbed with a household butcher knife at varying angles including 45-degree angle, 90-degree angle of impact. The third set of ribs was punctured with a set of screwdrivers with the same varying angles as the knife trauma. The first shoulder piece remained unaffected by trauma to be studied as a control sample. The second shoulder piece was shot at using a Ruger .22 long rifle. Consistent users produced all trauma wounds in order to avoid inter-user variability and all wounds were inflicted in triplicates.

Once the trauma was inflicted, each rib set and shoulder piece was equally divided so that one half was subjected to a charring cycle over a fire pit. The complementary set was placed into a wiring cage and buried in a shallow grave for decomposition. The charred samples were subjected to charring for 20 minutes and then packaged and delivered to the laboratory for processing. The decomposition interval was 5 weeks long while temperature was recorded daily. At the completion of the 5-week interval, the samples were recovered from the shallow grave and packaged to be delivered to the laboratory for processing.

b. Samples processing

At processing, samples were visually assessed for trauma marks and excised using a surgical kit. Tissue sections were excised to include from the area of the wound and were no bigger than a 5mm x 5mm by 5mm sections. Cuts were made longitudinally to
the depth of the wound, and cross-sections where prepared. At processing, each tissue sample was excised and labeled in cassettes. The associated bone was carefully separated and blanched by boiling for 2 hours to remove residual soft tissue. Then the bone was incubated in a 3% hydrogen peroxide solution overnight at room temperature to bleach the bony structure. Finally, the bone was rinsed with tap water and allowed to dry. The bones were evaluated by performing blunt-end probe measurements of the toolmark imparted on them. Triplicate measurements were obtained of the depth, breadth, length and width of all visible toolmarks and recorded. Bone mark measurements were processed in R studio (RStudio Team, 2015). Separately, the associated soft tissue samples were processed by routine histological methods.

c. Samples histology processing

The first step was to formalin fix the tissue. Cassettes housing the soft tissue samples were fixed using 10% neutral buffered formalin (NBF 10%, Sigma) by incubating them for 72 hours at 4°C. Afterwards, the cassettes were rinsed in 70% ethanol and stored at 4°C. Then, cassettes were processed for paraffin embedding. First, a dehydration step was initiated to remove water from the tissue: a series of ethanol baths to dehydrate the tissue using denatured alcohol: two baths of 95% denatured alcohol (Flex 95, Fisher) for 2 hours at 55°C while stirring, followed by one bath of 100% denatured alcohol (Flex 100, Fisher) for 2 hours at 55°C while stirring and finally incubating in 100% denatured alcohol overnight while stirring. Once the tissue were dehydrated, they were put through two series of clearing baths which facilitates removal of proteins and hardens the cell walls: this was achieved by incubating in a xylene substitute reagent (Clearing 1, Fisher) at 55°C for two hours while stirring. Once the
tissue was hardened, the samples were then put through an infiltration step where liquid paraffin enters the tissue to occupy the space: samples were put through two baths of liquid paraffin (Paraplast, VWR) 65°C for two hours while stirring. Finally, the samples were embedded into paraffin by emerging the samples into stainless steel molds filled with paraffin and allowed to cool. The formalin fixed paraffin-embedded (FFPE) tissue blocks was then cut using a microtome (Leica) into 3-5 \( \mu \)m thick sections on positively charged slides.

d. Staining

Staining was performed on prepared slides. Slides were first de-paraffinized in order to remove paraffin to allow stain reaction to take place. This took place by submerging the slides in three changes of a xylene substitute (Clear-Rite, Fisher) for 3 minutes each followed by three rounds of dehydration with denatured alcohol (100% Flex, Fisher) for 1 minute each, and 95% Flex for one minute. The slides were briefly rinsed with tap water and then rinsed with deionized water. Next, the slides were placed incubated in Hematoxylin stain solution (Hematoxylin 7211, Fisher) for 2.5 minutes allowing the stain to penetrate and stain the nuclei in blue. After rinsing with tap water, a clarifier (Clarifier 1, Fisher) was used to allow differentiation staining to take place of the different tissue components. The slides were then rinsed with tap water for 30 seconds, and placed into a bluing reagent (Bluing Reagent, Fisher) for 30 seconds to [allow a proper alkalinity to increase the resolution of the Hematoxylin]. The slides were first rinsed with tap water, and then rinsed in 95% Flex. Next, they were immersed in Eosin stain (Eosin-Y, Fisher) for 1 minute which stains cytoplasmic components in pink.
Finally, slides were placed into three changes of 100% Flex for 1 minute each and followed by three changes of Clear-Rite for 1 minute each and then dried.

A counterstain for the detection of GSR was performed using sodium rhodizonate (NaR) after H&E. One hour prior to H&E staining, a 0.3% NaR solution was prepared by diluting 0.3 g of sodium rhodizonate dibasic (Sigma, R1609) in 100 mL of distilled water. Also, a 1% solution of tartaric acid was prepared by diluting 1g of L (+) tartaric acid (Sigma, 251380) in 100mL of distilled water. Once the slides were stained with H&E, two drops of 0.3% NaR solution was placed on the tissue and one drop of 1% tartaric acid was added for 1 minute of reaction. Then the slides were rinsed off with distilled water and cover slipped. A biological microscope was used for histomorphological examination of the routine analysis and special stained samples. High-resolution images were acquired using digital imaging software CaseViewer2.8 (3DHistech Ltd., 2017).

3. Results

Bone samples were analyzed based on the measurements of the toolmarks. Each bone revealed a primary and a secondary appreciable toolmarks, imparted by knife and screwdriver and compiled into a dataset (Supplementary data, Table 3). Dimensions of length, width and depth were obtained in triplicates from 16 recovered bones. In knife (sharp force trauma) impacted bone, the average length of the primary toolmark was 14.48 mm; average width was 1.04 mm and average depth was 2.69 mm. Bones that were impacted at a 90-degree angle with a knife showed the average width of the mark to be 0.36 mm whereas marks impacted at other varying angles were 1.19 mm in width. In
screwdriver-impacted bone, the average length of the primary toolmark was 14.79 mm; average width was 3.12 mm and average depth was 1.8 mm. To explore the relationship between width, length and depth of different types of marks, the first approach was to utilize a scatterplot comparing pairs of the data. R-studio (RStudio Team, 2015) was used to process the dataset by looking for correlation between variables- demonstrated as a pairs plot in Figure 1.

**Figure 1. Scatter plot of toolmark measurements**

![Exploration of Toolmark measurements](image)

Since a strong relationship was not observed among the variables, a small clustering was noticed between width and depth of the primary mark. A subsequent pairwise analysis was performed on widths. On further evaluation, width-pairs were subjected for statistical interrogation using a t-test. Wilcoxon test was used to determine if there was a significant difference between screwdriver and knife: widths, lengths and depths of the trauma marks. After performing this test with the 3 variables, the findings revealed that there is a significant difference between the widths of knife and screwdriver marks at $\alpha = 0.1$ (p-value= 0.06), but no significant difference was found between lengths (p-value= 0.627) and depths (p-value= 0.368) of the marks (Figure 2).

**Figure 2. Screwdriver and knife marks on bone: Wilcoxon T-test**

Wilcoxon rank sum test with continuity correction generated in R studio showing kw1 (Knife width) and sw1 (screwdriver width) skewed distribution. P-value = 0.06139 at $\alpha=0.1$ Alternative hypothesis: The median widths of both samples are different.

A second exploratory approach was using principle component analysis (PCA). Since the measurements did not show an evident correlation, PCA was used to extrapolate variation among the dataset. In this method, the data was put through orthogonal transformation
into linearly correlated variables. The variables were plotted into the first dimension because 84% of the variance among the toolmarks was captured in the first principle component (Figure 3).

**Figure 3. Bone toolmark data shown in 3D PCA**

Toolmark data is clustered in the first principle component suggesting that the variance of the data is best explained by PC1. “Control”, “Decay” and “Char” represent the postmortem condition of the sample. Primary (1), secondary (2) and tertiary (3) sampling was done on the Knife (K), and screwdriver (S) trauma. Samples are sub-numbered to represent the samples obtained in triplicates.

To further investigate the data, a hierarchical clustering was done (Figure 4). A dendrogram showed two main clusters, not including the two outlier clusters screwdriver-
decay (Decay S7) and knife-charred (Char K2.1); control-knife (Control K1.2) and
decay-knife (Decay K2) trauma samples. These two groups are fused at a much higher
height then the rest of the samples, suggesting that they are most dissimilar from one
another. The height (distance) between all samples was 0-13, suggesting that there is little
difference in height between clusters. The gunshot entry and exit holes were observed to
be in an exclusive cluster apart from knife and screwdriver marks, suggesting that
gunshot holes are dissimilar from other markings on bone.

Figure 4. Dendrogram of toolmark measurements on bone

Hierarchical clustering of toolmark measurements showing gunshot holes subdivided from
knife (blue) and screwdriver marks (yellow). Little dissimilarity is seen among all marks.
The morphological assessment of blunt-force trauma using a screwdriver with a blunt tip end and beveled sides was performed on un-charred and fresh specimen (controls). Gross observation revealed a rectangular shape indentation on the epidermal layers of the skin corresponding to the negative impression of the dull-tip of the screwdriver as it impacted the sample. Below the superficial layers of the dermis, the path of impact revealed a rectangular-shaped corridor. In some samples the impact of the blunt tip tool was severe enough to puncture the specimen and produce macroscopic destruction of tissue that flapped away from the remaining body. At the site of impact, trauma was noted in the form of tissue destruction of localized muscle fibers and breakage of the dermal layers. The external layers of the skin showed torn and ripped edges, particularly the epidermis. Adipose tissue, which is typically observed as a honeycomb network composed of adipocytes, was disrupted revealing a tearing of this network. Damage was also observed as abrupt discontinuation of previously intact muscle fibers: which appeared broken and terminated in uneven edges (Figure 5a).

Samples that were subjected to thermal damage, presented with a noticeable dark-staining peripheral layers of the samples. Dermis of the samples was observed to be dark staining with shriveling of various skin layers in the form of flakes. A clearly visible darkly stained epidermis was observed (Figure 5b). These layers showed to be disintegrating and shriveling was noted in severely damaged areas. Of note, cracks were observed at the epidermis-dermis boundaries. Some charred samples revealed the utmost outer layer to be disconnected from remaining layer and flaked off. Adipose tissue was absent surrounding the area of burning. Blunt force trauma in charred samples was
identified as deformation of the muscle layers. Since thermal damage resulted in warping and shriveling of the external layers, breakage at these sites was observed as a larger opening cutting into muscle tissue. No signs of thermal changes were observed at the deep level of blunt force impact. The changes were observed for the most part on the surface of the samples.

The blunt force trauma samples that were retrieved after decomposition presented with gross putrefaction noted all over the specimens’ peripheral layers. Tissue liquefaction reaching deep into the muscle layer was noteworthy on macroscopic view. As anticipated, the superficial skin layer was absent and darker staining tissue was more prominent where the dermis would have been (Figure 5c). In samples that had retained parts of the epidermis, those layers appeared to be sloughed off and were detached from the rest of the tissue. Tissue deformation was observed in the outer layers of the muscle that was also exposed to decomposition. The site of injury showed invasive decomposition that was marked by dark-stained muscle tissue. The decayed samples showed a greater degree of dark staining involving underlying tissue beyond the tissue that was exposed to the taphonomic elements. Dark staining tissue was also observed to be surrounding foreign particles, detected at the surface of the skin, consistent with soil matter.
Figure 5. Histomorphology of Screwdriver Trauma

(a) 2x
Control sample showing deformation of muscle tissue with a sharp force component present (torn muscle fibers)

(b) 3.5x
Charred sample Arrow points to charring external layers (dark staining). Blunt force deformation seen as a wave-like depression.

(c) 2x
Decomposition. Arrow points to shredded edges of superficial dermis layers. Dark staining purple color is consistent with necrosis at 5 weeks.
Macroscopic evaluation of gunshot-afflicted injury to the shoulder sample revealed visible entrance wounds on the surface of the skin, and also several still visible exit wounds confirming that the projectile passed through the sample. The entry wound had a faint soot deposit where the projectile impacted it (ecchymatic ring). Entrance holes on the scapula showed smooth round edges with bony splinters facing inward towards the direction of travel from the projectile. Whereas exit holes show protruding bony edges that face outward facing the direction of the travelling projectile. Exit holes reveal jagged edges with splintering of the bony tissue. Microscopically, observing the large amount of tissue damage surrounding the impact readily recognized the entrance wound. The dermis appeared shattered and fragmented with jagged edges of dermal layer and connective tissue protruding in random directions. Adipose tissue was noted to have shattered edges in the area of impact as well as fiber breakage noted in Figure 6a. Consistently, debris was detected as small dark staining particles embedded within muscle tissue. Notably, translucent amorphous material was detected in crevices of tissue that resembled cellulose nitrate from primer residue. Charred samples showed a similar pattern of tissue damage when compared to the control sample. The projectiles impactful force left tears in the connective tissue as observed by jagged and uneven edges of the dermis. Adipose tissue was absent in charred samples, a finding that was previously reported in similar gunshot samples by Baptista et al. (2014). Dark staining superficial dermis layers were identified at the edges of the samples (Figure 6b). Nitrocellulose was identified as amorphous deposits of translucent particles. The outermost dermis layers appeared to be detached from the remaining tissue samples. Decayed gunshot samples were
distinguished in that the dermis layer was absent for the most part. Some dermal layers if present stained dark purple. Putrefaction was so extensive that outer layers of the sample were severely degraded and dermal layers were homogenized. This was recognized by the lack of dermal tissue (Figure 6c). Nitrocellulose was not observed in decayed samples. However, small dark staining aggregates of foreign particles were seldom observed wedged in the muscle tissue as well as on the periphery.

**Figure 6. Histomorphology of Gunshot Trauma**

(a) 2x  
Control (exit) wound. Arrow points to damage to adipose tissue due to exiting projectile.

(b) 2x  
Charred entry hole. Arrow points to nitrocellulose particles retained in the tissue from entering projectile.
Sharp force trauma samples showed signs of surface penetration when screened macroscopically. Sharp impact holes were identified in by neat and clear incisions with
varying depths. Microscopically, signs of tissue injury were evident by severed fibers in the connective and muscle tissue. Breakage of these fibers revealed sharp edged terminals (Figure 7a). Muscle fibers were observed in most samples to be sheared and appeared to resemble short threaded fibers versus long and orderly bundles. Charred samples showed dark staining peripheral dermal layers which appeared dislocated from the rest of the tissue sample having shredded ends that were severed from a stabbing motion inflicted by the blade (Figure 7b). Charring was noted by identifying a thin dermal layer consisting of only a few layers. Impact wounds were challenging to identify in charred samples, let alone to presume the source of the trauma. Decayed knife trauma samples presented with dark staining tissue in the outer areas that was also devoid of the dermis layers [as previously observed with other trauma samples]. The putrefaction degraded the samples greatly so that the outer layers were not present revealing prominent muscle tissue that was dark red staining. Sharp force trauma was not readily identifiable in most samples (Figure 7c), however general tissue destruction was noted by the presence of abruptly broken epidermal fibers and shredded muscle fibers. Shredded epidermal layers have been observed in some samples, but it is unclear of the source of trauma. Decomposition affected the external dermis layers resulting in muscle tissue to be stained dark purple indicating exposure to the decomposition.
Figure 7. Histomorphology of Knife trauma

(a) 5x
Control sample showing cross-section of knife wound. Arrow points to muscle fiber discontinuation due to penetrating blade.

(b) 5x
Charred sample showing carbonized external layers of the knife entry wound. Arrow points to severely shredded tissue.

(c) 10x
Decayed tissue. Arrow points to a cross-sectional view of a stab wound with dark-staining margins surrounding the elliptical shape incision of the wound.

Temperature was recorded throughout the length of decomposition (Figure 8). The impact of higher temperatures (unseasonably warm for March: 80F.) on the rate of decomposition may have accelerated the extent of decay. A concerning point in this study is that warmer weather could potentially accelerated decay farther than intended, such that most tissue injury could have been obliterated.
Figure 8. Temperature records for Waymart, PA: Site of decomposition

Maximum and minimum temperatures were recorded during the 5-week decomposition interval. Maximum temperature was 91°F, and minimum temperature was 31°F. High temperatures accelerate the rate of decomposition.
4. Discussion

a. Toolmark on bones

Toolmark length, depth and width measurements of the 35 bone markings revealed gunshot holes to be dissimilar from screwdriver and knife toolmarks. The average depth of knife marks (2.69 mm) was greater than the average depth in screwdriver marks (1.8 mm), which may be attributed to the nature of injury that is intended from sharp-force and blunt-force, respectively. In this experiment, knife trauma was sharp and penetrating, while screwdriver trauma represented blunt-force impacts. With this distinction, the blunt tip of the tool is anticipated to deform the tissue rather than penetrate it since the tip of the object is dull rather than sharp. In cases where the blunt tip may cause severe damage and break through tissue, the depth of the wound is expected to be smaller than other sharp-tip objects. The width of knife marks (1.19 mm) measured from wide-angle impacts was greater than the width of knife marks from 90-degree impacts (0.36 mm). The Wilcoxon T-test proved a statistically significant difference between the width measurements of the knife and screwdriver marks. These results corroborates the conclusions obtained by Cerrutti et al., (2014) who showed that there is a strong association between asymmetrical blades and the width of the lesion. From these findings, the marks of the asymmetrical blade of the knife and the symmetrical tip of the screwdriver can be discriminated by metric assessments of lesions widths. From these observations, one can suggest development of a machine-learning model that can process widths of toolmarks to generate a likely association of the source tool.
Exploration of toolmark data suggested that there was little variation among all measurements (PCA) yet hierarchical clustering revealed two major clusters, of which gunshot holes showed an isolated aggregate. Among the clusters, toolmark measurements encompassed samples from charring and decomposition, as well as the control samples showing a spread of these measurements without a particular pattern. These data qualify toolmark measurements as being relevant to document. Overall, there is little dissimilarity among all toolmarks in the setting of charred and decomposed samples. Further work should explore the specific relationship among the different types of samples.

b. Histomorphology

i. Blunt-force Trauma

Blunt-force impact unto soft tissue can be simply observed as deformations in muscle and connective tissue. Histologic analysis of soft tissue impacted with a blunt tip tool (screwdriver) revealed wound marks of various depths including penetrating ones reaching past the dermis into the muscle tissue and bone. The resilient structure serves to protect the body from physical injury. This point is important to emphasize because in cases of low-velocity impact, muscle tissue exhibits a plasticity that moves to contract to oppose the impacting trauma (Pette & Staron, 2000). The results obtained here, illustrate this nature by observing deformations in fibers around the impact site. Penetrating injuries from screwdriver showed a sharp-force component from forceful penetration breaking through superficial layers and resulting in bone formation of bone marks. Localized tissue damage was noted in all samples impacted with a screwdriver. All samples presented evidence of deformation as crushed layers of dermal and connective tissue leading up to the wound track. Also, adipose tissue showed signs of compression
by evidence of depressed adipocyte layers mixing in with disrupted muscle fibers. The disrupted muscle layers were observed as severed and isolated fragments. Recently, similar observations have been reported, documenting a disrupted appearance of myofibers and lacerated muscle tissue in blunt-force trauma (Barington & Jensen, 2017). Samples that were subjected to burning presented with a characteristic trauma signature: dark staining superficial layers that were exposed to fire appeared dark blue-black in color and the tissue immediately beneath it showed homogenized striations. Blunt force characteristics of depressed tissue was noted as an indentation of longitudinal fibers throughout the path of injury. Moreover, the injury channel stained dark blue indicating change in tissue necrosis in the path of injury. The channel was identified in burnt samples irrespective of the charred appearance of the external layers. The decayed samples presented severe fragmentation consistent with putrefaction. The fragmented segments of external tissue were observed as sheared edges at the cut margin and irregular borders segmented from the main body of remains. Necrotic tissue stained darker with increased blue staining suggesting tissue lysis and decay. A notable channel of dark staining associated to the screwdrivers’ track of impact was observed in the muscle layer. Wound tracks were identified according to the purple staining channel, although muscle deformation cannot be characterized due to it being homogenized in appearance. Trauma is identified in the staining of the internal track of the object. In the setting of postmortem changes, internal blunt force trauma tissue was identified in the control sample and the burnt sample. In the decomposed sample, a trauma pattern was identified as dark staining of the wound channel, but a characteristic mechanism cannot be ascertained in extensive putrefaction.
ii. Gunshot Trauma

Gunshot trauma samples showed a greater area of tissue disturbance when compared to the blunt force trauma samples. This is an expected finding since the impacting force is due to a high-velocity projectile, whereas blunt force trauma results from a lower-velocity impact. Upon entry of the projectile, the track of the projectile results in a temporary cavity as a result of expanding kinetic energy radially from the direction of travel. This temporary expansion returns to a smaller size because of tissue elasticity. Muscle tissue was generally intact except where muscle fibers appeared shredded as a result of injury and object impact. Projectile entry holes showed beveling of tissue flaps facing internally (as a result of external force) and exit holes having beveling of tissue externally (as a result of internal force). Severe adipose tissue destruction was observed and deformation of adipose tissue was depressed into the deeper layers of muscle tissue. Cracking of outer dermis layer was observed for all types of trauma samples that were subjected to charring. Epidermal cracking is typical of dehydration and shrinking cells. Foreign particles were identified in all tissue types as small deposits of black clumping particles. They were prominently observed at the entry wounds and sporadically identified within the tissue. These particles were deposited from the fast traveling projectile and may be originating from the firearm and the environment. Foreign material was detected in decayed samples as small dark staining aggregates. This is an expected finding since the tissue samples were exposed to the soil when buried in the shallow grave as well as, being lodged within the tissue as a result of an exposed opening from the screwdriver impact. Decomposition imparted severe necrotic effects
that degraded external layers and involved muscle tissue. As a result, wound injuries were identified with difficulty in severely liquefied remains. Injuries were observed as dark a staining area that was previously red-pink in color. The projectiles exposed internal opening of the tissue, which served to allow decomposition to affect this tissue immediately, which is seen as areas of purple necrosis in the margins of the projectile track.

Nitrocellulose particles [from primer residue] were observed in generous deposits in entry hole samples and identified less frequently in samples obtained from along the projectiles path. Such observations noted that residue was ovoid, clear, translucent particles that were wedged into crevices of the tissue that was sampled along the track of the projectile. Characterization of primer residue in this experiment is supported by similar results that describe nitrocellulose as ovoid and translucent in appearance (Dolinak, Wise, & Jones, 2008). Presence of primer residue was confirmed with counterstaining with NaR, which resulted in dark staining residue along the damaged tissue, suggesting that the path of the projectile is marked with bullet trace. Figure 6d shows an exit hole with prominent deposits of nitrocellulose. Confirmatory chemical testing using NaR (Figure 6e) shows positive staining of lead (scarlet red color change from the normal pink and blue tissue) at the edges of the exit wound. Sodium rhodizonate confirmed the presence of primer residue in charred and decomposed tissue as a positive test for gunshot trauma.

Wound injury in gunshot-affected samples was qualified in this experiment as patterns of multi-level destruction of fibers, and exaggeration of the size of the tissue openings from these injuries. Patterns of necrosis were identified as purple staining tissue
surrounding the margins of the projectile path. Gunshot injury can be identified in charred tissue, but limited in decomposed tissue. This may be dependent on the extent of putrefaction. Severe putrefaction results in degradation of tissue layers, therefore the presence of primer residue may be a helpful confirmatory test to confirm this type of trauma.

**iii. Sharp Trauma**

Sharp force trauma samples revealed that sharp-force injury in the form of torn fibers involving multiple tissue types. Connective and muscle tissue showed discontinuity in previously intact fibers. Cut edges were seen along the cross-sectional view of the incision. Although, a unique characteristic of knife soft tissue trauma is that sharp severed fibers were noted, a blunt-force component may also be observed. In the samples assessed, shredded tissue fibers were seen penetrating external, connective and muscle tissue. Neat and smooth margins of incisions have been observed in cross-sections of the incision. Torn layers were not deformed, which can be used to discriminate against blunt-force impact. However, deformations in the deepest tissue (e.g. bone) were observed. Knife trauma was observed in burnt tissue. Signs of thermal injury were noted as dark staining layers with flaking of outer segments. Thermal effects resulted in tissue shrinkage and exaggeration of tissue cracks imposed by incision from the knife. The channels of sharp injury were v-shaped and identified in charred and decomposed samples. Decomposition was identified around the margins of the incision of wound channels as purple staining tissue invading radially outward away from the exposed tissue. Dark staining tissue that was exposed to decomposition was identified on external layers. Extensive fragmentation was also noted as shredded pieces and separated clumps
of tissue budding off the main body. It is worthwhile to point out that foreign particles
were not observed in these samples. However, the presence of foreign matter cannot be
ruled out. In the context of thermal and taphonomic changes, sharp force incisions were
described irrespective of the external patterns imposed by charring and decomposition.

Different types of trauma were identified based on patterns of histological
staining. In charred remains, different types of injuries were identified by patterns of
overstressed disruption. Depression of multiple tissue layers in a wave-like pattern was
identified in blunt force trauma. Discontinuation of fibers in the connective and muscle
tissue and sheared fibers- lacking evidence of concussive depression of muscle tissue was
identified in sharp force trauma. In addition, pre-existing injury was further amplified, by
expansion of injury tracks from shrinkage and delamination. Shrinkages affected the size
of the exposed trauma. In decomposed trauma samples, injured tissue was identified by
dark-staining areas invading deep layers of muscle. In sharp-force trauma, tissue that has
been exposed showed radial color changes emanating from the source of the wound.
Putrefaction was exaggerated by increased temperatures and showed severe decay of the
external layers evident by fragmentation. Gunshot trauma was more challenging to
identify in decomposed tissue as a result of extensive putrefaction and the size of the
gunshot hole. Non-specific damage was identified in the tissue, yet NaR provided
positive confirmation for gunshot trauma.

5. Limitations

Studying the effects of decomposition and burning on soft tissue and bone
presents with inherent challenges. The primary limitation in this study was the limited
amount of specimen available. Ideally, a complete specimen would be prepared for such an experiment having a complete skeleton and tissue as to replicate a real life model. It is challenging to study behavior of trauma in a limited specimen having incomplete limbs and missing tissue. Therefore, future studies should consider obtaining an intact piglet for trauma studies. As so, given the post-mortem state of the samples studied, typical features of tissue healing were not emphasized due to the post-mortem state of the specimen at the time of affliction. Hence, it is important to keep in mind that observing microscopic signs of healing may indicate signs of perimortem trauma. Our dataset consisted of bone mark measurements for knife and screwdriver trauma. Gunshot trauma was inflicted on the shoulder part of the specimen, piercing two of the scapula bones that were recovered. Since gunshot marks on bone consisted of only a sample set of two, they were not considered as part of the total dataset since comparison of scapula gunshot wounds is not comparable to comparison of rib bone with toolmarks.

Secondly, climate effects on the rate of decomposition must be considered. Although the temperature during this study was generally mild, drastic drops in temperature were documented during the duration of the experiment, which may slow the rate of putrefaction. With this said, extensive putrefaction can be expected with warmer temperatures, which in turn may appear over-putrefied, and trauma dissection becomes more challenging due to the homogenization of remaining tissue. These factors must be considered in order to avoid over-interpretation of the results.

In this study, sample preparation and histological processing was performed manually employing steps that are usually facilitated by using automation. In large medical facilities, routine histological processing is done using automated tissue
processors that produce batches of ready stained slides in a matter of hours, whereas in this study, all tissue processing was performed manually over a period of 2-3 days. Hence, this method is subjected to a human error component and may lack consistency in the quality of the stained slides.

Conclusion/Future Direction

Collectively these results demonstrate that soft tissue trauma, having undergone postmortem thermal damage and decomposition, retains histological evidence of injuries. Using a comparative approach, patterns of injury can be studied along with intact tissue to document markers of different types of trauma. It remains possible to identify specific tool lesions in blunt, gunshot, and sharp force trauma injuries. First, trauma that has been exposed to a charring cycle can be identified by the presence of dark staining dermis layers that came into contact with the tool. This was noted in blunt force, gunshot and sharp-force trauma. Moreover, as a result of shrinkage, tissue delamination and cracking of exposed tissue is an expected finding that suggests evidence of such thermal damage. Second, decomposition provided a histomorphological signature in the samples studied, lacking the outer most layers and involving deeper muscle layers. If the sample shows extensive dark staining, fragmented regions beyond the dermal layers, and lack of connective tissue, putrefaction may be the cause. It is important to keep in mind that tissue damage should be correlated to the interval of decomposition, where a shorter interval may result in milder decay when compared to the extensive decay that was observed in samples obtained at 5 weeks.
As with soft tissue, toolmark measurements obtained from trauma-associated bones revealed distinct patterns. Statistically, screwdriver, gunshot and knife marks showed to be dissimilar from one another, suggesting that further research should focus on sub-classifying these differences. Gunshot holes have been shown to be distinctly different from screwdriver and knife marks most probably due to the round shape of gunshot holes when compared to elongated and irregular shape of screwdriver and knife marks. However in the setting of postmortem changes, toolmark measurements revealed no pattern of distinction, suggesting that postmortem changes may not affect the length, width and depth of bone marks. Also, screwdriver and knife marks can be further differentiated based on the width of measurements. A statistically significant difference was identified within these marks. This suggests that with additional interrogation, it may be possible to develop a statistical model that can utilize these values so that it can be used to generate potential tool analysis provided that a metric of width is submitted for calculation. Metric assessment of trauma-inflicted bone together with histological findings supports a conclusion that trauma patterns are unique among different types of injuries.

Finally, histomorphological features that are representative of blunt-force, gunshot and sharp-force trauma were identified in charred tissue and to an extent in decomposed tissue. Blunt-force and sharp-force impacts produced depression of multiple layers and penetrating incisions, respectively. To identify either one, a closer look at individual tissue types can reveal a pattern of tissue disruption. Concussive depressions are associated with blunt-force impact, whereas sheared edges and discontinued fibers are consistent with sharp-force impact. In gunshot trauma, the amount of force being carried
with the projectile shows to be related to the extent of tissue damage resulting in disordered cut margins and shredded layers of adipose, connective and muscle layers. To further elucidate the behavior of tissue injury under different types of traumatic force, supplementary work on this topic should be performed to quantify and relate the amount of force imparted on an object and compare with the extent of tissue destruction.

The aim of this study was to show the utility of routine histological methods in addressing the challenges of obliterated trauma tissue. Based on the findings obtained in this work, microscopic evaluation showed evidence of tissue damage located beneath the charred and decomposed layers. We therefore conclude that histological techniques can be utilized to examine microscopic evidence beneath the layers of damage introduced from postmortem changes. This serves to aid practitioners by looking beyond severely putrefied and charred remains, and determine patterns consistent with different types of injury.

Determining the cause of death lies at the root of forensic pathology. In cases where wounds and trauma are unaltered, a cause of death analysis is simple. But it is common for trauma to be obscured by postmortem criminal actions. Perpetrators may be motivated to cover up traces of their crime by burying the victim in a shallow grave or burn the remains and escape identification. In either case, the effects of charring or decomposition upon pre-existing trauma are indeed destructive and hard to identify in regular autopsy proceedings. In this study, we have shown that adding histomorphological investigations can successfully identify specific types of trauma where gross examination has failed to do so.
Supplemental Material

Table 1. Label identification

1. Screwdriver “S”
2. Knife “K”
3. Gunshot “G”
Numeric designations refer to the different areas of trauma sections
Numeric sub-divisions refer to the cut of a sample out of a series (e.g. triplicate)

Table 2. Sample Identification

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Table 3. Dataset of toolmark measurements

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