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Using Cementochronology to Estimate Age-at-Death in Individuals with Pathological Dentition

by

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List of Abbreviations

AAC	Acellular Afibrillar Cementum
AEFC	Acellular Extrinsic Fiber Cementum
CDJ	Cementodentinal Junction
CEJ	Cementoenamel Junction
CIFC	Cellular Intrinsic Fiber Cementum
CMSC	Cellular Mixed Stratified Cementum
HERS	Hertwig's Epithelial Root Sheath
LC	Lower Canine
LI1	Lower First/Central Incisor
LI2	Lower Second/Lateral Incisor
LP4	Lower Fourth Premolar
PD	Periodontal Disease
PDL	Periodontal Ligament
$R^1/_2$	Root Half Length
R1	Round 1 of Counting TCAs and Calculating Age Estimates
R2	Round 2 of Counting TCAs and Calculating Age Estimates
RA	Round 1 and 2 Average
RCT	Root Canal Therapy
TCA	Tooth Cementum Annulations
UC	Upper Canine
UI1	Upper First/Central Incisor
UI2	Upper Second/Lateral Incisor
UP4	Upper Fourth Premolar

USING CEMENTOCHRONOLOGY TO ESTIMATE AGE-AT-DEATH IN INDIVIDUALS WITH PATHOLOGICAL DENTITION

Thesis Abstract--Idaho State University (2020)

Human teeth are made of the most durable material in the skeleton, which means they can be the only source of information to estimate age-at-death in forensic and archaeological investigations. Relatively predictable dental formation and eruption patterns allow investigators to provide reasonably reliable age estimates for infants, children, and young adults. However, once the adult dentition is fully formed, age estimation relies primarily on dental wear patterns, which are highly variable among individuals and populations.

The purpose of this research is to test the accuracy of *cementochronology* conducted on pathological dentition from modern, known-age individuals. Cementochronology is an age estimation method based on the examination of *cementum* – the material that covers the root surface of teeth and continues developing throughout life in uniform layers called *tooth cementum annulations (TCA).* This study assesses whether a novice observer can consistently obtain TCA counts, if different diseased teeth within a single individual provide consistent age estimates, and, ultimately, if this method offers sufficiently accurate and precise estimates to be applicable in forensic contexts.

KEYWORDS: cementum, cementochronology, age estimation, biological profile, dental anthropology, dental pathology, periodontal disease

Chapter 1 – Introduction

Estimating age-at-death from skeletonized individuals is an important step for both forensic anthropologists and bioarchaeologists. For forensic anthropologists, it is an essential component of the biological profile that can be used to support a presumptive identification or to narrow down searches of missing person records. In bioarchaeological contexts, knowing the approximate ages-at-death of the individuals at a site or in a collection is a critical first step for investigations of the health and demography of past civilizations. Additionally, it can reveal a great deal of information about population growth and density, fertility, mortality, and population regulation (Hassan, 1978), as well as increasing our understanding of how civilizations cared for their young and elderly populations.

Age estimation of juvenile remains is relatively straight-forward as the applied methods often rely upon the observation of somewhat predictable developmental processes that typically reach completion by early adulthood and are well-documented, such as tooth development (Moorrees, Fanning, & Hunt, 1963) and eruption (Ubelaker, 1978), or bone epiphyseal fusion (Cunningham, Scheuer, & Black, 2016; Cardoso, 2008). In contrast to juveniles, estimating age-at-death for adults can be a difficult task. Developmental processes that reach completion in early adulthood are not useful for adult age estimation, particularly for adults in the upper half of the lifespan (i.e., 50 years of age or older). Some methods have gained widespread acceptance for the analysis of adult remains, such as the observance of bone degeneration and metamorphosis of the pubic symphysis (Brooks & Suchey, 1990; Todd, 1920), iliac auricular surface (Lovejoy & Meindl, 1985; Buckberry & Chamberlain, 2002), or a combination of skeletal markers (Boldsen, Milner, Konigsberg, & Wood, 2002). However, these methods decrease in precision with increasing age due to the high variability of the observed physical markers, resulting in broad age categories or terminal age categories (e.g., 50+ years) that do not provide useful information.

An alternative approach to adult age estimation utilizes the tissue that forms on the outside surface of the tooth root, called *cementum*. This material is unique when compared to every other substance in the human skeleton because it continues to develop throughout life in uniform layers called *tooth cementum annulations* (TCA). Each annulation consists of one dark and one light band and is theoretically representative of one year of skeletal growth (Lieberman, 1994). By counting the annulations and adding that number to the age of likely tooth eruption, one can arrive at an age estimation of the individual using a method known as *cementochronology.* Because TCAs theoretically develop and remain unaltered throughout life, they are promising for estimating age in older individuals who cannot be accurately or precisely aged using traditional macroscopic methods. Research has shown that the margin of error when employing this method may be as little as ±2.5 years, even when applied to individuals in their 80s (Wittwer-Backofen, Gampe, & Vauper, 2004).

Despite numerous studies utilizing TCA for age estimation in humans that confirm the efficacy of the method, there is still no consensus on the applicability of the method on pathologically affected teeth. In fact, many researchers have explicitly stated that this method cannot or should not be applied to diseased teeth because of the likelihood that the disease will have altered the structure of the annulations (Pilloud, 2004; Alghonamy, Gaballah, & Labah, 2015). The exclusion of pathological teeth is problematic when considering the potential for cementochronology to fill a void in estimating the ages of older individuals in forensic settings who are likely to be affected by oral pathology. Nearly 20% of American people over the age of 65 have untreated dental decay (Dye, Thornton-Evans, Xianfen, & Iafolla, 2015), which is a significant factor in developing periodontal disease. The most severe manifestation of periodontal disease, periodontitis, affects between 35% and 47% of adults in the U.S., and 64% of those over 65 years old (Albandar, Brunelle, & Kingman, 1999; Eke, Dye, Thornton-Evans, & Genco, 2012). This is particularly troubling, because periodontitis is considered to be the most problematic pathology to affect the results of cementochronology studies due to its frequent and destructive presence in the specific area under analysis.

The purpose of this research is to test whether the application of cementochronology to pathological teeth by a relatively inexperienced observer produces accurate and precise age estimates for modern individuals, particularly for those over 50 years of age. Additionally, this study aims to assess the amount of variation in TCA analysis between tooth types from the same individual in to better understand how the accuracy of estimates can be affected by the selection or availability of certain teeth. Both objectives could supply useful information to forensic anthropologists and bioarchaeologists about the appropriate circumstances in which the method can be reliably applied.

Chapter 2 contains a brief introduction of cementum biology and deposition as well as the history of cementochronology as it pertains to the fields of zoology, zooarchaeology, forensics, and archaeology. It also introduces information about the nature and frequency of the dental pathology that may introduce error into cementochronology research. Chapter 3 describes the samples evaluated, as well as the materials and methods used to complete sample preparation and analysis. Chapter 4 presents the results of several analyses, including the accuracy of the estimates produced. Chapter 5 presents a discussion regarding the significance of the results from all applied analyses. Chapter 6 consists of conclusions about the applicability of this method on pathological teeth and suggestions for future researchers.

Chapter 2 – Literature Review

Cementum Biology

Cementum is one of several elements that make up the periodontium – the tissues that surround and support the teeth. The remaining elements of the periodontium are the periodontal ligament (PDL), alveolar bone, and gingival tissue in contact with the tooth. Cementum directly interacts with the PDL to form a joint between the alveolar bone and the tooth, called a gomphosis (Nanci, 2012). Cementum is avascular, categorized as cellular or acellular and fibrous or afibrillar depending on function and location along the length of the root, and experiences little to no remodeling after its deposition (Saygin, Giannobile, & Somerman, 2000).

The process by which cementum develops is the subject of ongoing study and there is currently no consensus among researchers as to the exact series of events that take place. A key point of uncertainty is the identity of the cells that are the precursors of cementoblasts. What follows is a summary of the most popular theory about the formation of cementum among researchers at the time of writing. Shortly after the crown completes formation, the root formation initiates, and the tooth begins the process of eruption that continues until it is in occlusion. The proliferation of epithelial cells residing at the cervical loop of the enamel organ known as the Hertwig's epithelial root sheath (HERS) signals ectomesenchymal pulp cells to differentiate into the odontoblasts that lay down a predentin matrix which, once mineralized, becomes the root dentin. The odontoblasts continue laying down the predentin matrix appositionally until the root reaches appropriate thickness (Nanci, 2012).

At this time, experts disagree on whether HERS cells begin to differentiate into cementoblasts, or ectomesenchymal cells of the dental follicle are signaled by the novel interaction with predentin to differentiate into cementoblasts (Nanci, 2012; Yagyuu et al., 2010). As the growing root begins to advance apically, the cementoblasts align themselves along the developing outer predentin margin and insert cellular processes into the unmineralized matrix to deposit collagen fibrils. Cementum and dentin fibrils intermingle with one another and, once calcified, form the cementodentinal junction (CDJ) once calcified. The mineralization process initiates at the inner margin of the predentin and spreads outward toward the cementum, allowing the fibrils enough time to blend together before mineralization occurs and anchors them to one another (Nanci, 2012).

The process by which cementum adheres to the periodontal ligament is very similar to its union with dentin. Cementoblasts on the root surface continue to secrete collagen fibers as they move further outward to the developing PDL. Concurrently, they also deposit non-collagenous matrix proteins that fill in empty spaces between the co-forming collagen fiber bundles. This growth continues appositionally until the cemental layer is approximately 15-20 μm in thickness, at which time it reaches the mineralized fibrous extensions of the PDL known as Sharpey's fibers (Grzesik, 2002; Nanci, 2012). The fibers from both tissues "stitch" themselves together, and the cementoblasts secrete only the non-collagenous matrix to encase the collagenous Sharpey's fibers before mineralization occurs (Nanci, 2012, p. 212).

Acellular Extrinsic Fiber Cementum

Cementum continues to develop in this manner and apically along with the developing root edge until it reaches approximately 60-90% its full length in single-rooted teeth (*Figure 1*), and 50-67% in multi-rooted teeth (*Figure 2*), with its extension increasing from posterior to anterior dentition (Bosshardt & Selvig, 1997; Yamamoto, Hasegawa, Yamamoto, Hongo, & Amizuka, 2016). The resulting material is known as acellular extrinsic fiber cementum (AEFC) or primary cementum and has the key function of providing secure attachment for the tooth through its connection to the periodontal ligament (Grzesik, 2002; Nanci, 2012; Colard et al., 2016).

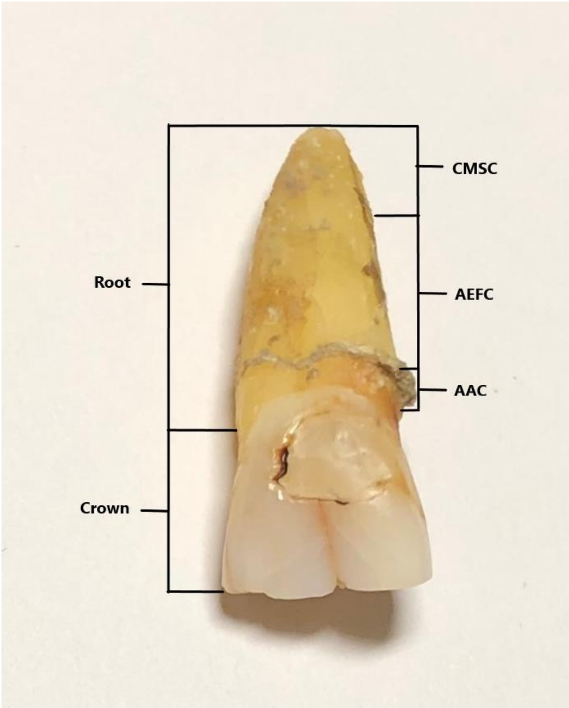

Figure 1. Anatomy & cementum distribution of a single-rooted tooth

The biochemical composition of AEFC is similar to bone, with some researchers estimating a mineral content of approximately 45-50% hydroxyapatite and organic content of 50-55% collagen and non-collagen protein matrix (Saygin et al., 2000; Nanci, 2012), while others maintain the proportions are closer to 60-65% and 35-40%, respectively (Colard et al., 2016). Once the initial layer of AEFC is deposited, right around the initiation of tooth eruption, it begins to develop appositionally and continues to do so throughout life. It is through this process the banding pattern of TCA is developed, which is the area of focus in cementochronology (*Figure 3*). Although it is still generally not understood what causes the alternating dark and then light bands of TCA, Colard and colleagues (2016) determined through Raman microscopy that the latter have higher mineral contents than the former.

Cellular Mixed Stratified Cementum

The cemental material covering the remaining apical portion of the root, consisting of 10-40% in single-rooted teeth (*Figure 1*) and 33-50% in multi-rooted teeth (*Figure* 2) is designated cellular mixed stratified cementum (CMSC) or secondary cementum (Bosshardt & Schroeder, 1996; Bosshardt & Selvig, 1997; Yamamoto et al., 2016). In humans, this type is found in smaller proportions on incisors and canines,

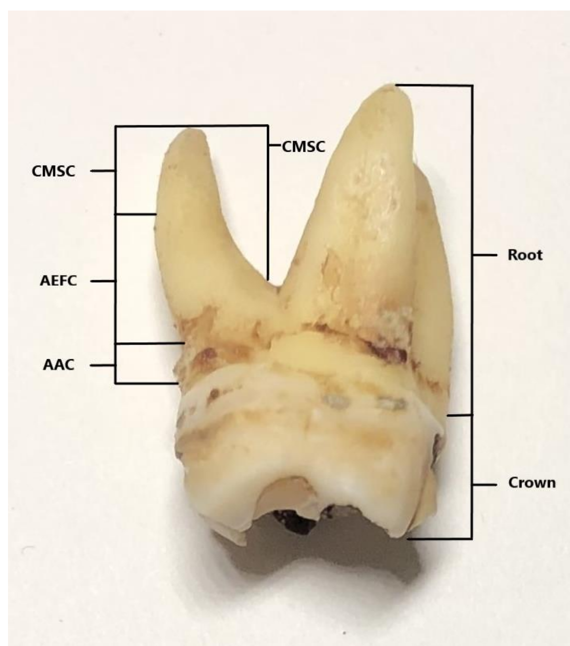

Figure 2. Anatomy & cementum distribution of a multi-rooted tooth

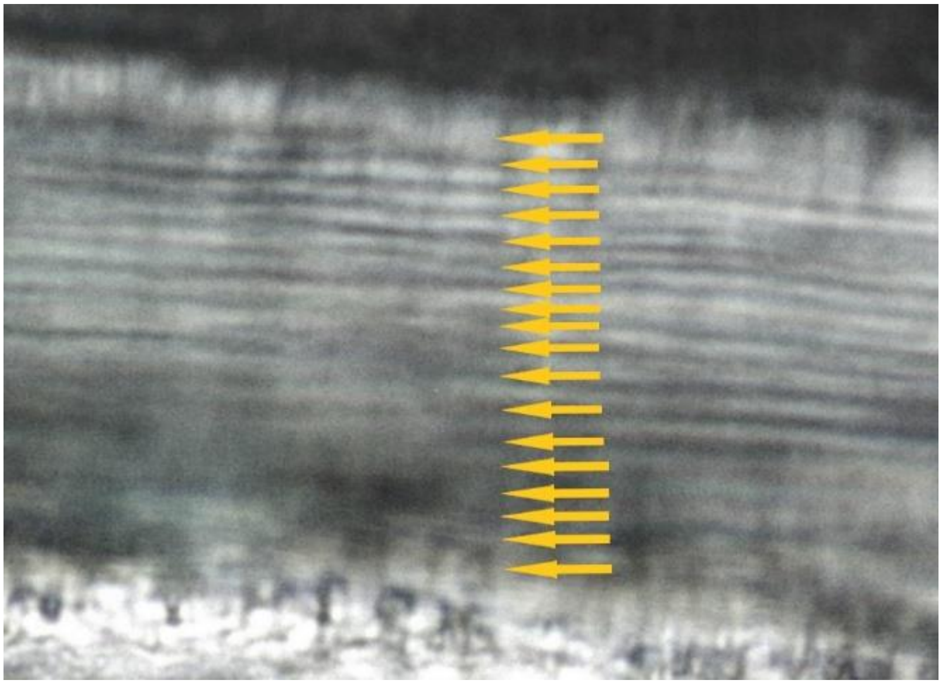

Figure 3. Image of TCAs found in AEFC

while its presence in molars and multirooted premolars is also seen between the roots, or what is known as the interradicular region (Bosshardt & Selvig, 1997; Nanci, 2012; Yamamoto et al., 2016).

While AEFC functions as the anchor for the fibers of the PDL, CMSC is an adaptive material whose main purpose is to compensate for occlusal wear throughout life. As the enamel on the occlusal surface of the tooth is worn down through constant grinding during mastication, this type of cementum is deposited at the root apex in order to move the tooth back into proper occlusion (Nanci, 20120).

CMSC is typically composed of stratified cellular intrinsic fiber cementum (CIFC), although intermittent layers of AEFC may also be present (Yamamoto et al., 2016). CIFC develops in virtually the same manner as AEFC though not as uniformly and at a more rapid pace (Bosshardt & Schroeder, 1996; Nanci, 2012). As suggested by its name, this type of cementum is characterized by the presence of mature cementocyte cells within its mineralized matrix that reside in lacunae, much like osteocytes found in bone. Unlike osteocytes, cementocytes in the CIFC are relatively disorganized and lack adequate communication with one another through canaliculi, especially nearer to the surface. Researchers believe that the PDL provides nourishment to the encapsulated cementocytes via diffusion, though lack of sufficient channels throughout means that cells in the inner layers are not often vital (Saygin et al., 2000; Nanci, 2012).

The biochemical composition of CIFC is approximately 45-50% mineralized and 50-55% organic materials (Saygin et al., 2000). The role of this adaptive cementum material is three-fold: it serves as a shock absorber during normal masticatory functions, it repairs or builds upon the root surface in instances of resorption or tooth movement, and it develops at the root apex to compensate for occlusal wear of the crown (Bosshardt & Schroeder, 1996; Bosshardt & Selvig, 1997; Grzesik, 2002; Nanci, 2012; Bertrand, Oliveira-Santos, & Cunha, 2019) Its near absence in certain tooth types is an indication that it plays a much smaller role in tooth support than AEFC (Nanci, 2012).

Acellular Afibrillar Cementum

The final type of cementum is called acellular afibrillar cementum and is located closest to the cementoenamel junction (*Figure 1*). It is an extension of the AEFC that slightly overlaps the enamel margin in approximately 60% of individuals, while it is slightly separated from or abuts the enamel in the remainder (Nanci, 2012). As its name suggests, this type is characterized by a lack of collagen fibers - an indication that it plays no role whatsoever in tooth attachment. It is easily discernible from the other types of cementum because its mineralized matrix lacks cementocytes and collagen fibers and is deposited in a relatively disorganized manner (Bosshardt & Selvig, 1997; Nanci, 2012).

Significance of Cementum Types in Cementochronology

All types of cementum mentioned above exhibit periodic growth increments, however, certain features of AAC and CMSC have caused researchers to deem them inappropriate for use in cementochronology studies. The thickness of cementum increases from approximately 50 μm at its cervical margin to 200 μm at its apical extension (Nanci, 2012). The irregular deposition patterns and relatively thin nature of AAC when compared to AEFC and CMSC means the annulations are compressed and, thus, difficult to distinguish from one another (Bosshardt & Selvig, 1997).

The adaptive nature of CMSC, particularly at the root apex, results in much thicker material than AEFC (Bosshardt & Schroeder, 1996; Bosshardt & Selvig, 1997; Nanci, 2012). One could presume that thicker cementum layers would result in more expanded and easily discernible increments, however, the irregular and unpredictable deposition of this material along with the sporadic inclusion of Sharpey's fibers and lacunae result in dramatic interruptions in the annulations (Bosshardt & Schroeder, 1996; Bosshardt & Selvig, 1997; Bertrand et al., 2019).

As the only cemental material that grows in regular increments and does not undergo remodeling after being laid down, AEFC is the type that is recommended for use in cementochronology studies (Colard, Bertrand, Naji, Delannoy, & Bécart, 2015; de Broucker, Colard, Penel, & Blondiaux, 2016; Bertrand et al., 2019).

Cementochronology in Other Mammalian Species

Zoology

The periodicity of mammal dental tissues was first recognized in the mid-twentieth century by researchers in the field of zoology (Scheffer, 1950; Laws, 1952). In each of the initial studies, researchers relied upon the examination of permanent canines taken from different species of seal, all of known age. The incremental deposition of the secondary dentin found on the exterior of the massive tusks used in both samples was visible to the naked eye, although they seemed to be less apparent on other tooth types (Scheffer, 1950). By counting the layers of this material, both observers reported strong correlations between estimates and known ages, with one finding that they were on average within one month of the animals' actual age (Laws, 1952) and the other claiming "accurate" results up to the age of four (Scheffer, 1950, p. 310). In addition, Laws found that the applicability of this method was sexually dimorphic as it applied to males of the species up to 20 years of age, but for females, it was only applicable up to 13 years (1952). Both researchers attributed the periodicity of the secondary dentin to annual dietary changes including fasting periods during molting and mating seasons (Laws, 1952), and amphibious lifestyles that result in aquatic and terrestrial prey at different times of year (Scheffer, 1950). Scheffer stated in his research that these incremental dental landmarks were limited to aquatic mammals, causing all research of its kind to be conducted on similar animals for the next decade (Laws, 1953; Nishiwaki, Hibiya, & Ohsumi, 1958; Laws, 1958).

The first study using dental increments to estimate age that was conducted on a solely landbound mammal was performed on a sample of forty wild mule deer and Columbian black-tailed deer, all of known age (Low & Cowan, 1963). Researchers in this study used only first incisors from deer that had been lifelong inhabitants of the Wildlife Research Unit at The University of British Columbia because they were less complicated than molars, and they were the first to erupt, giving them the potential to provide the most information. Aside from the fact that the method was proven to apply to land mammals, the essential takeaway from this study is that when the annual increments from both dentin and cementum were observed, it was discovered that those from cementum were more easily discernible and resulted in more consistent estimates.

Despite the apparent successes of cementochronology in zoology, the method was hard to conduct confirmatory studies in the wild. Without the ability to track animals from birth or near to it, determining the accuracy of the estimates was seemingly impossible. Spinage (1973, p. 184) determined that the methodology could and should be implemented in the wild through the use of complementary aging methods like dental wear patterns established through observation of animals in captivity because he believed it to be the "most promising method of age determination." Later studies have utilized the supplemental approach that Spinage had envisioned to estimate age in such wild animals as crabeater seals and Iriomote cats with reasonable levels of success (Laws, Baird, & Bryden, 2002; Nakanishi, Ichinose, Higa, & Izawa, 2009).

Zooarchaeology

Early on in the use of cementum for the determination of age, researchers hypothesized that the alternating bands were the result of more or less rapid growth caused by the relative availability of nutrition during different seasons (McEwan, 1963). In theory, the dark bands represent less nutritious fall and winter periods and the lighter bands represent the more nutritionally-rich spring and summer seasons (Lieberman D. E., 1993). This information is valuable, as it can provide researchers the ability to not only estimate the age at which an animal died, but also the season by examining the nature of the last visible annulation deposited before death (Burke & Castanet, 1995). While this theory had some relevance to zoological studies, it has been studied more in the context of zooarchaeological faunal assemblages.

The first studies to use cementochronology to learn information about human health and behavior were still accomplished through incremental analysis of animal dentition. After recognizing the success of the method applied to modern animal populations, researchers performed the same technique to an assemblage of faunal remains discovered at the archaeological shell midden site of Turner Farm in Maine (Spiess, 1976; Bourque & Morris, 1978). The area of interest was occupied by prehistoric populations at different periods around 5,000 BP and was chosen for both studies because of the relatively large collection of mammal remains associated with the occupation (Bourque & Morris, 1978). While the results of both experiments were not made explicit at the time of their respective publications, both researchers recognized that the ability to interpret season of death in such assemblages had the potential to produce a vast amount of information about the seasonal mobility, hunting patterns, and behaviors of past populations. The idea that the analysis of TCAs could contribute to such investigation grew in popularity in the following years. However, Bourque and Morris (1978) warned others that the periodicity of dental material was not well-established in other fauna and, thus, future researchers of cementochronology would have to consider that fact.

Nearing the end of the twentieth century, Liebermann aimed to standardize the methods by which cementochronology would be applied to animal remains in the archaeological context (1994). By standardizing the technique, he hoped that others would not be discouraged by the seemingly tedious and challenging task of producing the thin sections necessary for this approach. Liebermann strongly believed that the strength of this method pertained to the idea that teeth in these archaeological animal assemblages were often found still intact in the maxillae and mandibles, meaning the material to be observed was not influenced by human behavior or preservation. Additionally, he analyzed various sources for explanations of the incremental patterns and, in doing so, recognized that it likely resulted from a combination of the most credited factors: nutrition, biomechanical responses to stress and strain of seasonally available harder and softer foods, and hormonal cycles (Lieberman D. E., 1994).

The following year, research was conducted on the applicability of cementochronology to determine the season of death on two separate samples. The first sample was comprised of 16 horse teeth from contemporary animals with known seasons of death, and the second of 104 horse teeth from various archaeological sites in France dated from between 18,000 and 14,000 BP (Burke & Castanet, 1995). The main objective of this investigation was to assess the ability of researchers to apply this method to better understand its potential to produce accurate season of death estimates. By performing the analysis first on the modern, documented horse teeth, they were able to determine how TCA was deposited differently throughout the year based on their knowledge of the time of death for each animal. Building upon the analysis of the modern sample, they were then able to estimate better the season of death for the animals in the archaeological sample and, ultimately, which of the various sites in southern France were occupied by prehistoric peoples at different times of the year. An essential component of this study is the researchers' recognition that, although the environment seemed to influence the incremental deposition of cementum, there were also likely external influences such as genetics, which could have additional or possibly even more influence on its growth.

More recently, researchers who have applied cementochronology to various terrestrial mammals at archaeological sites have made additional discoveries about its usefulness in prehistoric contexts by implementing complementary approaches first utilized in zoology. Through a combination of TCA season-of-death analysis and previously studied tooth eruption and wear patterns of Norwegian reindeer, Takken Beijersbergen (2017) recognized changes in hunting patterns during several historic periods. The first and second periods of interest (275-350 CE and 1000-1100 CE, respectively) were represented by predominantly male reindeer remains that were hunted down at the prime of their adulthoods, while the final period (1200-1300 CE) produced remains from both sexes and at all stages of life (Takken Beijersbergen, 2017). These results indicated that hunters in the latest period were far less selective about their prey, which could very well have been associated with a scarcity of other food resources in the area at that time. Additionally, she compared the estimated ages produced by cementochronological analysis to those based on wear and eruption patterns and found that the resulting age category estimates were the same in 81% of the tested samples (Takken Beijersbergen, 2017).

In 2019, paleoanthropologists applied a similar complementary approach to ungulate remains found in Iberian sites associated with Neanderthal occupations. By using a combination of cementochronology and wear patterns, researchers in this study found the seasonality of death in various ungulates represented by a sample of 225 individual teeth (Sanchez-Hernandez et al., 2019). This study is notable because, for the first time, cementochronology was used to study the paleodemography and paleoenvironment of other hominins beside *H. sapiens*, as well as the dietary makeup of their prey via analysis of dental macro- and microwear patterns.

Cementochronology in Human Subjects

Forensic Applications

The first study to provide evidence that human dental material exhibited periodicity was published the same year as the original zoological study mentioned above (Scheffer, 1950; Gustafson, 1950). In this study, Gustafson (1950) scored multiple features of teeth taken from individuals of known age: attrition, secondary dentin, periodontosis, cementum, root resorption, and root translucency. Although the analysis of cementum was only reliant on its relative thickness when compared to the dentin, this study still established a periodicity in human dental material that had previously been unknown.

Despite this foundational research, the first study to implement cementochronology to human remains was not performed until several decades after the periodicity of cementum was first established (Stott, Sis, & Levy, 1982). Until this study, researchers had been reluctant to apply the age estimation technique to human remains because of the uncertainty of what caused cementum to develop in such a pattern. Stott and colleagues recognized that periodicity in cementum annulations had been established in all mammals that had been previously studied and hypothesized that human cementum would not be an exception. Their pilot study was performed using multiple teeth from three cadavers of known age, and the estimates it produced all fell within five years of the individuals' actual ages (Stott, Sis, & Levy, 1982). Because of this study's success, the authors acknowledged the profound potential of cementochronology in the fields of anthropology, forensic dentistry, and forensic medicine.

Following the success of the pilot study, other researchers began to apply the methodology to more extensive samples of modern human dentition for application in biological anthropology. In a series of studies from the same group of authors, researchers used cementochronology to estimate ages on a sample of 42 teeth taken from cadavers of known age. The first of these articles focused on the accuracy of the method with particular emphasis placed on variation between mandibular canines and premolars, as well as inter-observer error. They found that the rate of inter-observer error and intra-observer error were, on average, 5% and 2%, respectively (Charles, Condon, Cheverud, & Buikstra, 1986). Based on their results, they made multiple statements about the method's applicability and application. First, they claimed that it had better repeatability than more popular age estimation techniques that relied upon the examination of the pubic symphysis and iliac auricular surface. Second, they stated that demineralized thin sections produced the best results despite other practitioners' claims that the demineralization process was unnecessary (Stott, Sis, & Levy, 1982). Finally, they advised future researchers that the best results could be obtained by counting annulations in multiple areas from the same thin section and by using as many observers as possible (Charles et al., 1986).

The second study, written by the same group of authors, focused on the accuracy of age estimations for the same sample of known-age individuals from the first article. They found that their age estimates were reasonably accurate, with an average rate of error of 6 ±2.6 years (Condon, Charles, Cheverud, & Buikstra, 1986). While their results were similar to those found in past studies on other mammal species, they also found that females produced better results (possibly an indication of sexual dimorphism in TCA deposition) and that cementogenesis likely decreased with age.

Despite the finding that sex and increased age altered or decelerated the deposition of TCA and prior declarations that "determining the chronologic age of humans from cemental annulations in teeth is not possible" (Miller, Dove, & Cottone, 1988, p. 142), an extensive validation study conducted on a sample of 433 teeth from modern, known-age individuals indicated that factors such as age, sex, pathology, and tooth type did not affect their results. In addition, the researchers found that the method produced much more accurate estimates than all previous studies, with an average error of ±2.5 years (Wittwer-Backofen, Gampe, & Vauper, 2004). However, researchers involved in another study with a relatively large sample size of 116 teeth from 65 individuals of known-age discovered that those taken from young adults produced reasonably accurate results, while those from individuals over the age of forty produced estimates much younger than the actual age (Obertova & Francken, 2009).

According to the available research, cementochronology is applicable to teeth from all individuals, regardless of their biological sex, age, or state of dental health. There is, however, a possibility that teeth from older individuals produce estimates below their documented age and that women produce more accurate results than men. Research also indicates that the best way to reduce intra-observer error is to repeat the estimation process multiple times on the same tooth by multiple observers, when possible. With few exceptions, research suggests this method can be successfully applied to any forensic case in which the remains consist of human dentition.

Archaeological Applications

Due to the arguable success of cementochronology in human age estimation, the method has also been applied to human remains in archaeological contexts with variable accuracy. Obtaining age-at-death estimates for individuals from past people can inform archaeologists and bioarchaeologists about various aspects of population health, such as paleoepidemiology. One such archaeological study, consisting of teeth from 115 Mesolithic and Neolithic human remains, found age estimates based on cementum increments to be comparative to macroscopic osteological methods, although the authors warned that vastly different counts could be acquired from the same tooth when looking at different parts of the root (Roksandic, Vlak, Schillaci, & Voicu, 2009). This remained true even as observers obtained consistent results when they repeated the process. Another study conducted on 18 individuals from the Indian Mesolithic site of Damdama found that, although the age estimates produced were similar to those from osteological methods, cementochronology allowed them to place some of the individuals in their sample into more specific age categories (Schug, Brandt, & Lukacs, 2012).

Much like the zoological and zooarchaeological studies that preceded them, there has also been interest in finding the season of death in archaeological human remains. One study conducted on the remains of 18 individuals unearthed by flooding from a historical grave in Missouri found that TCA analysis produced results in all but one person that strongly correlated with estimates based on dental eruption patterns (Wedel & Wescott, 2015).

Technical Note: Variability of Elements Used for Human Studies

Despite attempts to develop a standardized protocol for performing cementochronology on human dentition, researchers continue to disagree about important variables of the technique (Colard et al., 2015; Wittwer-Backofen, 2012; Naji et al., 2014). For instance, most studies claim to have added the number of TCAs in their sample to the age of eruption for each tooth without defining which stage of eruption they are referring to (Stott et al., 1982; Wittwer-Backofen et al., 2004; Obertova & Francken, 2009; Gocha & Schutkowski, 2013; Naji, et al., 2014; Blondiaux, Naji, Audureau, & Colard, 2015). This is important because eruption is not a single event in development but, rather a process that is initiated when the tooth begins to move and is completed once it is in occlusion (Nanci, 2012). Without specification as to what eruption actually means in the context of cementochronology, inexperienced researchers have to decide for themselves which stage they want to use such as initiation, alveolar eruption, and mucosal eruption, and everything in between. It seems plausible that this lack of specification is a source for variation in accuracy between studies. Alternatively, some researchers choose not to concentrate on tooth eruption but, instead, add the number of annulations to average ages of other stages of development like root completion (Colard et al., 2015) or root mineralization (Oliveira-Santos, Gouveia, Cunha, & Gonçalves, 2016).

Another area of uncertainty among practitioners of cementochronology is the importance of sex-specific eruption ages. While many have chosen to use sex-specific ages of dental development (Wittwer-Backofen et al., 2004; Obertova & Francken, 2009; Gocha & Schutkowski, 2013; Oliveira-Santos et al., 2016), others have used general combined-sex development information in their research (Stott et al., 1982; Naji, et al., 2014; Colard et al., 2015). Those advocating the implementation of sex-specific eruption ages refer to the documented age differences in development stages between sexes ranging from 0-0.1 years in first molars, to 1.7 years in canines (Haavikko, 1970). However, the reference materials used in sex-specific TCA studies are relatively outdated and were established based on rather small samples of European populations (Adler, 1967; Haavikko, 1970; Schumacher, Schmidt, Böring, & Richter, 1990). In TCA studies that have not used sex-specific eruption ages, the most commonly used reference is based on a much larger sample size of 704 individuals also of European descent known as the London Atlas of Tooth Development and Eruption (Alqahtani, Hector, & Liversidge, 2010).

Cementochronology & Pathology

Periodontal Disease

Despite an increasing number of successful experiments implementing the technique, many researchers continue to question its reliability when applied to pathological dentition. Although 'periodontal disease' (PD) is technically an umbrella term encompassing any of the "Inherited or acquired disorder of the tissues [of the periodontium]," it is most often used to refer to the most common of these disorders – gingivitis and periodontitis (Pihlstrom, Michalowicz, & Johnson, 2005, p. 1809). Both oral diseases are the result of prolonged exposure of the gingiva to the microflora found in dental biofilm or plaque. With chronic exposure to the hundreds of species of bacteria found in oral microflora, the immune system inflammatory response results in both the bacteria and the affected individual to produce proteolytic enzymes that ultimately destroy connective tissues of the periodontium. The results of this process range in severity from gingival inflammation and bleeding, to gingival pockets formation, to loosening and then subsequent loss of dentition (Pihlstrom et al., 2005).

The inflammatory response of the gingiva that characterizes gingivitis often results in bleeding during flossing and brushing but is usually reversible, causing it to be considered the mildest form of PD. Despite its lack of severity, this form of periodontal disease affects between 50-90% of the world population, with variation by geographic location (Pihlstrom et al., 2005). Periodontitis is the progression of the immune-inflammatory response further into the alveoli, resulting in progressively deeper pockets between the teeth the gingiva as more of the periodontium is destroyed. In extreme representations of this disease, there is the destruction of alveolar bone, which increases tooth mobility and often leads to its loss. Unlike symptoms of gingivitis, once the biofilm bacteria invade these pockets, periodontitis is typically irreversible. The high mineral content of enamel means it is more difficult for the bacteria and enzymes to penetrate. However, breakdown of the periodontium leaves the less mineralized cementum on the root surface increasingly exposed to bacteria that can penetrate it as well as the underlying dentin, creating root caries or decay at the CEJ (Nanci, 2012; Gavriilidou & Belibasakis, 2019) (*Figure 4*).

Figure 4. Root caries at the CEJ of a tooth

In living people, the presence of periodontitis is diagnosed and monitored by probing the gingival pockets to determine the amount of periodontium destruction and detachment. In archaeological and forensic anthropological contexts, the presence of periodontitis on skeletonized remains is indicated by horizontal destruction of alveolar bone, resulting in even further root exposure (Nagaoka et al., 2009).

Frequency of Periodontitis

According to researchers, the frequency of periodontitis in United States residents seems to be increasing over time. Based on the results of one study conducted from 1988 to 1994 on 9,689 individuals, it was estimated that at least 35% of the U.S. population between 30 and 90 years of age had periodontitis

(Albandar et al., 1999). This study took into account the severity of the disease through periodontal probing, with approximately 22% exhibiting mild periodontitis (≤ 3 mm depth) and 13% having severe periodontitis (≥ 3 mm depth) on one or more teeth.

In a similar study based on data from the 2009-2010 National Health and Nutrition Examination Survey (NHANES) on 3,742 individuals, it was projected that 47% of the U.S. population had periodontitis (Eke et al., 2012). More specific categories for the severity of symptoms were presented as 8.7% being mild (2 or more interproximal sites with ≥ 4 mm depth or one site with ≥ 5 mm depth), 30% being moderate (2 or more interproximal sites with ≥ 5 mm depth), and 8.5% being severe (2 or more interproximal sites with ≥ 6mm depth) (*Table 1*).

Table 1. Frequency of periodontitis in the U.S. according to Albandar et al., 1999 and Eke et al., 2012

Study Time Period & Authors	**1988 – 1994 (Albandar et al., 1999)**	**2009 – 2010 (Eke et al., 2012)**
Overall Frequency (% of Population)	35	47
Mild Symptoms (% of Population)	22	8.7
Moderate Symptoms (% of Population)	-	30
Severe Symptoms (% of Population)	13	8.5

Some illnesses can increase individuals' susceptibility to contracting PD or can increase the severity of its expression, including HIV/AIDS, osteoporosis, diabetes, and several immune system disorders. Genetics can also play a role in PD susceptibility and development, as well as tobacco and alcohol use, elevated levels of stress, and poor nutrition (Pihlstrom et al., 2005). Studies have consistently shown that the Mexican-American and black demographics have higher rates of periodontitis than

American whites, and men are more likely to be affected by it than women (Albandar et al., 1999; Eke et al., 2012). Additionally, it has been shown that periodontal disease, along with caries, is one of the most common oral health problems among older people with one report claiming a frequency of periodontitis among people over 65 years old being 64% (Gavriilidou & Belibasakis, 2019; Eke et al., 2012).

While there are therapeutic and surgical treatments available to slow the progression of periodontal disease or reduce its symptoms, extreme alveolar bone loss and lack of finances can result in extraction. In a survey of 165 dental offices involving extractions performed on 6,134 patients, researchers determined that periodontal disease was the most common reason for the procedure at 35.9% (Murray, Clarke, Locker, & Kay, 1997). Through breaking down the reason for dental extraction by tooth type, the study revealed that PD was the most common cause for the removal of canines (43% from maxillae and 47.8% from mandibles) and incisors (49.3% and 68.9%) and was similar to removal due to caries in premolars (34.2% and 32.2%) and molars (44.6% and 35%).

It is essential to understand the frequency of periodontitis in the U.S. population in the context of this study because there is a strong possibility that randomly acquired teeth were extracted due to complications of periodontal diseased and, consequently, age estimates may be affected. It is particularly important to recognize the increased frequency of PD among older people, as one of the main concerns of this study is the applicability of cementochronology on teeth among such individuals.

PD in Cementochronology Studies

Cementochronology has gained some popularity in the twenty-first century, but practitioners disagree as to its applicability to pathological teeth with differing concerns over the effect that oral diseases have on the cemental structure. Because of its effect on cementum as well as other tissues of the periodontium, the presence of periodontal disease in general and periodontitis specifically concern researchers.

One study, conducted in Lithuania, tested the efficacy of the method using two separate samples — 51 extracted teeth from 49 contemporary dental patients and 43 canine teeth extracted from the "Stalin era" individuals interred in a mass grave (Jankauskas, Barakauskas, & Bojarun, 2001, p. 62). After conducting analyses on both samples, the researchers concluded that "minor pathologies" in teeth did not cause significant problems with their results; however, they did not clarify what should be considered minor or major pathologies (Jankauskas et al., p. 69).

Similarly, Pilloud (2004) found that not only did periodontal disease alter the appearance of TCA to such a degree that accurate estimations could not be reached, but that on average pathological teeth would yield estimations 17.5 years above the actual age of an individual. These results, based on a sample size of 58 teeth, led Pilloud to recommend excluding pathological teeth from future studies. In contrast, the validation study performed by Wittwer-Backofen and colleagues (2004) found that the pathological dentition in their sample of 433 teeth – particularly those with periodontal disease – had no statistically significant influence on the accuracy of the researchers' estimations. They claimed that TCA age estimation was independent of periodontal disease and, consequently, could be effectively applied to forensic and archaeological remains with severe dental disease.

Using scanning electron microscopy (SEM), researchers examined 92 teeth extracted from 29 individuals due to PD to study how the disease affected cementum thickness (Bilgin, Gurgan, Nejat Arpak, Bostanci, & Guven, 2004). The authors determined that periodontal disease resulted in a significant loss of cemental tissue thickness on the affected sections of the root when compared to healthy sections. Despite this, researchers have continued to apply the age estimation method to periodontally affected teeth successfully.

Bertrand and colleagues (2014) evaluated the usefulness of cementochronology as a supplementary approach to age estimation for remains that are profoundly altered by osteological pathologies that can affect estimation by other, more commonly used methods. Using a sample of archaeological remains from four individuals, they determined that periodontal disease and other oral infections did not affect the applicability of the method. The following year, Alghonamy and colleagues (2015) published their results from an experiment in which they divided their sample of 60 teeth into four groups, separating them based on the sex of the individual and the presence or absence of pathology. Their research indicated that periodontally affected teeth produced results with "…highly significant differences…" between the known and estimated ages (Alghonamy, Gaballah, & Labah, 2015, p. 6). Because of the significantly erroneous estimates the diseased teeth produced, the researchers explicitly stated that they should not be used in future TCA analyses.

Research conducted on 49 teeth from 18 individuals who had foregone treatment for periodontal disease revealed that cementum seemed to continue to develop even as the alveolar bone was destroyed (de Broucker et al., 2016). TCA counts were significantly impacted on the apical $^1/_3$ of the root, although those from the cervical $^1/_3$ produced relatively accurate results, and the middle $^1/_3$ appeared to be unaffected. The authors concluded the study by proclaiming cementochronology to be the most reliable age estimation method available. Given the variability of results from cementochronology research conducted on pathological dentition over the last twenty years, it is not unexpected that many still question the technique's applicability in actual forensic and archaeological contexts where the sample is limited and one cannot simply disregard diseased teeth because they are not optimal subjects.

Summary and Conclusions

The periodicity of dental material is well-established, and there is a long history of the successful implementation of cementochronology in modern and archaeological remains from both humans and animals. Although some researchers have proclaimed the method to be ineffective in general and several have recommended avoiding its implementation in pathological dentition, studies with large sample sizes and supplementary analytical methods have claimed both to be untrue or, at least, exaggerated.

According to the sources provided in this section, by performing the ensuing analysis on the middle third of the tooth roots and avoiding the apical and cervical $^{1}/_{3}$, the expectation is that the counts will not be significantly affected by the presence of periodontal disease and reasonably accurate age estimates will be the result.

Chapter 3 – Materials & Methods

Study Materials

For this study, most of the teeth were acquired from dental offices in the Pocatello/Chubbuck, Idaho area, while the remainder came from two anatomical donors in the Idaho State University biology department. The anatomical donors had been deceased for approximately two years before the extraction of teeth selected for the purpose of this study. The teeth extracted at dental offices were recommended for extraction due to unknown clinical reasons, although oral pathology is the presumed underlying cause since dental professionals are unlikely to extract healthy dentition, with the exception of the third molars (Murray et al., 1997). All teeth were extracted between January and October 2019. Individuals were randomly assigned Participant IDs according to the dental office or lab of origin and the order in which they volunteered (e.g., AD-08 was the eighth person to volunteer to donate their teeth from Aspen Dental office). Each participant's sex was self-reported and no names or other pieces of identifying information were provided along with the randomly assigned identification numbers.

Cementochronology was performed on two separate samples. The first sample consists of sets of teeth taken from five individuals (3 teeth x 5 individuals; N=15). This sample was used to investigate the amount of variation in estimates from the same individual resulting simply from the evaluation of different teeth. All individuals in this "intraindividual sample" belong to separate age categories and, thus, this portion of the experiment has the potential to reveal information about tooth type variation changes with age and to supply information about the accuracy of the results in the remainder of the study (*Table 2*). The second sample, hereafter called the "interparticipant sample," also consists of 15 teeth total, but with only one tooth evaluated from each person. The purpose of this sample is to study the method's usefulness for pathological teeth and individuals of all ages, with particular attention paid to the estimates generated for those over 50 years of age (*Table 3*). All of the teeth used in this study are single-rooted and consist of first and second incisors (UI1, UI2, LI1, LI2), canines (UC, LC), and fourth premolars (UP4, LP4) from both the maxilla (U) and mandible (L). Although lower third premolars (LP3) are also single-rooted, none were donated for the use of this study.

Table 2. Intraindividual sample information

Participant ID	Age	Sex	Tooth Types	Tooth ID Numbers
AD-08	28	Female	LC, UI1, & UI2	8, 9, & 10
CC-03	64	Male	UP4, UI1, & LI1	24, 25, & 28
ISU-3622	80	Male	UI1, UP4, & LI2	31, 32, & 34
ISU-3702	75	Female	UI2, LI1, & LI2	35, 36, & 37
CC-04	33	Female	UI1, LI2, & LC	42, 48, & 49

Research Methods

Throughout the ensuing decades since the original human pilot study (Stott et al., 1982), several researchers have attempted to standardize the technique for cementochronology (Colard et al., 2015; Wittwer-Backofen, 2012; Naji et al., 2014). However, those attempts were not necessarily successful as the is still no standard procedure for sample preparation or TCA evaluation. Due to that fact, this study uses a combination of techniques from different publications depending on the availability and cost of supplies, and the amount of training and experience recommended to perform the them correctly.

Applied Methods

The teeth were assigned random identification numbers based on the order they were received, as all of them were collected over a period of several months. The teeth were decalcified by soaking them in a 10% neutral buffered formalin solution overnight and then allowing them to air dry for several hours.

Table 3. Interparticipant sample information

Tooth ID Number	Age	Sex	Tooth Type	Visible Pathology
2	63	Female	UI2	Caries/decay
4	75	Male	UI1	Extensive decay, crown absent
5	68	Male	LP4	Caries/decay, heavy occlusal wear
7	67	Female	UI2	Caries/decay, root apex resorption
10	28	Female	UI2	Extensive decay, ½ of crown absent
11	72	Male	UI1	Caries/decay, periodontal calculus
14	62	Female	LP4	Caries/decay, part of crown absent, pulp exposure
23	38	Female	UP4	Caries/decay
28	64	Male	LI1	Caries/decay, periodontal calculus
31	80	Male	UI1	Extensive decay, periodontal calculus
37	75	Female	LI2	Caries/decay, periodontal calculus, alveolar bone attached
48	33	Female	LI2	Caries/decay, periodontal calculus
52	69	Female	UC	Caries/decay on root surface, PFM crown, RCT, alveolar bone attached
53	69	Male	UI1	Caries/decay
54	65	Female	UI2	Caries/decay, PFM crown

The purpose of this first step was to remove the inorganic material in the tooth while preserving the organic material so that it was less likely to crack or splinter when cut (Cook & Ezra-Cohen, 1962). Next, they were individually photographed, and notes were taken on any visible pathology, prior restorations, or idiosyncrasies such as the presence of attached alveolar bone or the absence of the crown. Each tooth was then marked to outline the middle 1/3 of the root before being embedded in Buehler EpoThin® dual component epoxy resin. The middle $^{1}/_{3}$ was chosen for examination because it has been found to be less affected by the presence of PD than the other regions of the root, and it is the portion in which the AEFC is deposited (Nanci, 2012; de Broucker et al., 2016). Using a Buehler Isomet® 1000 saw, two 1mm-thick sections were cut from the root of each tooth. Then, employing a modified version of the Frost method (1958), they were hand-ground on both sides using aluminum oxide (Al_2O_3) solution and sandpaper in increasingly finer grits until they were approximately 0.5mm thick. Between each grit, the samples were sonicated in deionized water for approximately five (5) minutes. Once at the appropriate thickness, the surfaces were polished using a fine diamond paste and a microfiber cloth and sonicated in deionized water again for approximately five (5) minutes. Next, they were mounted on glass slides, and the grinding process was repeated until the samples were as thin as possible to reduce the presence of blurred annulations, at which time the slides were completed by adding a coverslip.

Digital images of the cementum from each sample were captured using a Leica® DM2500 polarizing light microscope (*Figure 5a*). The annulations were made more apparent and easier to count by altering the contrast and brightness of each photo using Paint 3D® photo editing software (*Figure 5b*). The annulations were counted (1 dark band + 1 light band = one annulation) using the drawing capabilities of the software to mark and keep track (*Figure 5c*). After counting the number of annulations from both cross-sections, the mean count number was added to the average age at which the tooth root reaches $^{1}/_{2}$ its full length ($R^{1}/_{2}$) . The age estimations are presented as ranges, and were obtained from the London Atlas by recording the minimum and maximum ages at which each tooth type was found to be at $R^{1}/_{2}$

(Alqahtani et al., 2010) (*Table 4*). This stage was chosen because it coincides with the approximate time that the middle $^{1}/_{3}$ of the root finalizes deposition of the intial layer of AEFC and the appositional increments begin to form (Nanci, 2012).

Two weeks after initially performing this analysis, new images were obtained from each tooth cross section and the counting process was repeated, producing a second set of age estimates. The photographs for the second round were taken independently from the first round, sometimes resulting in distinctly different cementum images from the same thin section. The results from both were compared to one another to test intra-observer consistency.

Table 4. Age ranges at which each tooth root reaches R$^{1}/_{2}$ (Alqahtani et al., 2010)

Maxilla		**Mandible**	
Tooth Type	Age at R$^{1}/_{2}$ (years)	Tooth Type	Age at R$^{1}/_{2}$ (years)
UI1	6.5 – 8.5	LI1	5.5 – 6.5
UI2	6.5 – 9.5	LI2	6.5 – 8.5
UC	7.5 – 11.5	LC	8.5 – 9.5
UP4	8.5 – 13.5	LP4	9.5 – 12.5

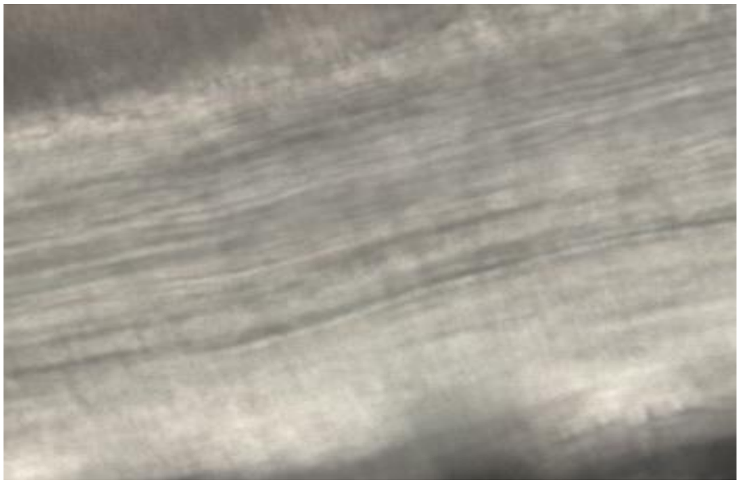

Figure 5a

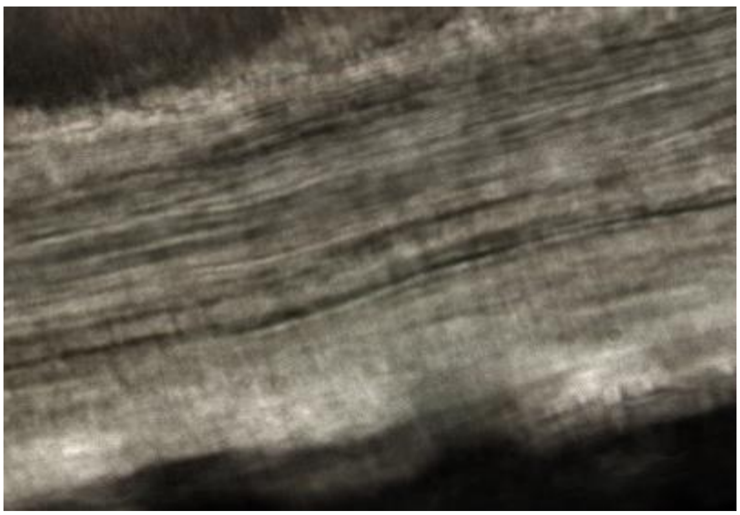

Figure 5b

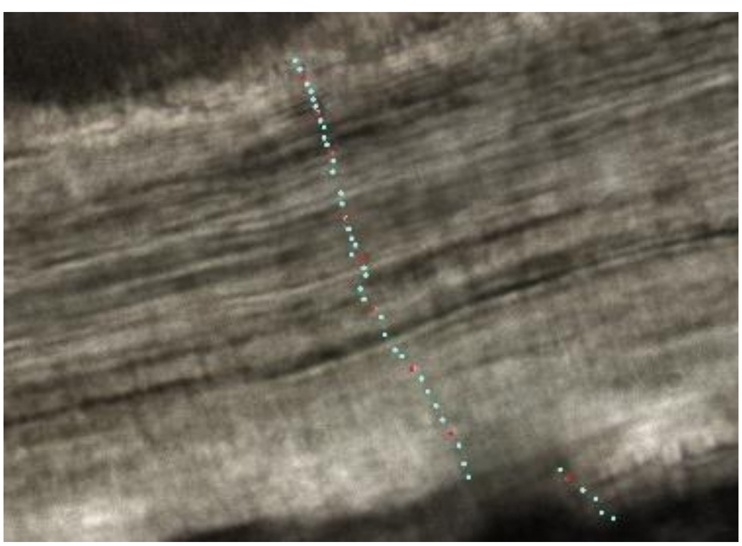

Figure 5c

Figure 5. (a) Initial image of TCAs from tooth #7 captured via microscope. (b) Same image with altered brightness and contrast settings to clarify annulations. (c) Same image with markers indicating positions of the TCAs (red markers are on every fifth TCA).

Chapter 4 – Results

In addition to the two sets of age estimates described in Chapter 3, an average of the two is also displayed in the results for both sample sets used in this study. The intention is to: A) test whether it is beneficial, per Charles et al. (1986), to count annulations in multiple areas of the same thin section and use the averages to produce estimates; and B) provide insight about the level of intra-observer error in studies conducted by inexperienced researchers observing pathological dentition. The apparent benefits or disadvantages of including the averages will be discussed in Chapter 5.

Intraindividual Sample

The main purpose of the intraindividual sample is to study the amount of variation in age estimations produced simply by using different teeth from a single individual. It is also crucial to look for patterns of intraindividual variation according to age and tooth type, to provide information about optimal research material selection in future research when there are multiple teeth available.

Intraindividual Sample Results by Participant

To study the amount of variation in TCAs found within the mouth of a single individual, the main area of focus is the measurement of error between the known age and the age estimate. This was determined by calculating the numerical ranges between these two values from the first (R1) and second (R2) rounds of examination as well as the average from both rounds (RA). Error rates below the known age are represented as negative values and those above the known age are represented as positive values (*Table 5*).

Table 5. Intraindividual sample results for R1, R2 & RA

Participant ID	Age	Tooth Type	TCA Count (R1)	Age Estimate (Round 1)	Distance from Known Age	TCA Count (R2)	Age Estimate (Round 2)	Distance from Known Age	TCA Count (Ave)	Age Estimate (Average)	Distance from Known Age
AD-08	28	LC	33	41.5 – 42.5	13.5 – 14.5	19	27.5 – 28.5	-0.5 – 0.5	26	34.5 – 35.5	6.5 – 7.5
		UI1	61	67.5 – 69.5	39.5 – 41.5	22	28.5 – 30.5	0.5 – 2.5	41.5	48 – 50	20 – 22
		UI2	32	38.5 – 41.5	10.5 – 13.5	36	42.5 – 45.5	14.5 – 17.5	34	40.5 – 43.5	12.5 – 15.5
CC-03	64	UP4	45	53.5 – 58.5	-5.5 – -10.5	45.5	54 – 59	5 – 20	45.25	53.75 – 58.75	-5.25 – -10.25
		UI1	34	40.5 – 42.5	-21.5 – -23.5	49	55.5 – 57.5	6.5 – 8.5	41.5	48 – 50	-14 – -16
		LI1	57.5	63 – 64	-1 – 0	49	54.5 – 55.5	8.5 – 9.5	53.25	58.75 – 59.75	-4.25 – -5.25
3622	80	UI1	60.5	67 – 69	-11 – -13	62.5	69 – 71	-9 – -11	61.5	68 – 70	-10 – -12
		UP4	82	90.5 – 95.5	10.5 – 15.5	71	79.5 – 84.5	-0.5 – 4.5	76.5	85 – 90	5 – 10
		LI2	56.5	63 – 65	-15 – -17	69.5	76 – 78	-2 – -4	63	69.5 – 71.5	-8.5 – -10.5
3702	75	UI2	49	55.5 – 58.5	-16.5 – -19.5	62.5	69 – 72	-3 – -6	55.75	62.25 – 65.25	-9.75 – -12.75
		LI1	49.5	55 – 56	-19 – -20	47	52.5 – 53.5	-21.5 – -22.5	48.25	53.75 – 54.75	-20.25 – -21.25
		LI2	50	56.5 – 58.5	-16.5 – -18.5	46	52.5 – 54.5	-20.5 – -22.5	48	54.5 – 56.5	-18.5 – -20.5
CC-04	33	UI1	29.5	36 – 38	3 – 5	30.5	37 – 39	4 – 6	30	6.5 – 38.5	3.5 – 5.5
		LI2	27.5	34 – 36	1 – 3	25.5	32 – 34	-1 – 1	26.5	33 – 35	0 – 2
		LC	27.5	36 – 37	3 – 4	29.5	38 – 39	5 – 6	28.5	37 – 38	4 – 5

For participant AD-08, a 28-year-old female, the error range of all three teeth (UI1, UI2, LC) and rounds of examination goes from a minimum of -0.5 to a maximum of 41.5, encompassing a total of 42 years. The estimates from R1 alone produced an error of 13.5 - 41.5, ranging 28 years, while R2 produced an error of -0.5 to 17.5 – a total range of 18 years. When viewing the estimates created by taking the average counts from R1 and R2, the error is 6.5 to 22 with a range of 15.5 years (*Figure 6*).

Of all the teeth from participant AD-08, UI2 shows the smallest amount of variation between all examination rounds at 10.5 to 17.5 years away from the known age, with a range of 7. LC has the next smallest error rates with a minimum of -0.5, a maximum of 14.5 years, and a total range of 15. UI1 has the highest estimate error rate of all teeth from this participant with a total range of 41 being between 0.5 and 41.5 years away from the known age. The only estimate for participant AD-08 that includes the known age is from the second round of examining the LC tooth. The resulting error for this tooth is -0.5

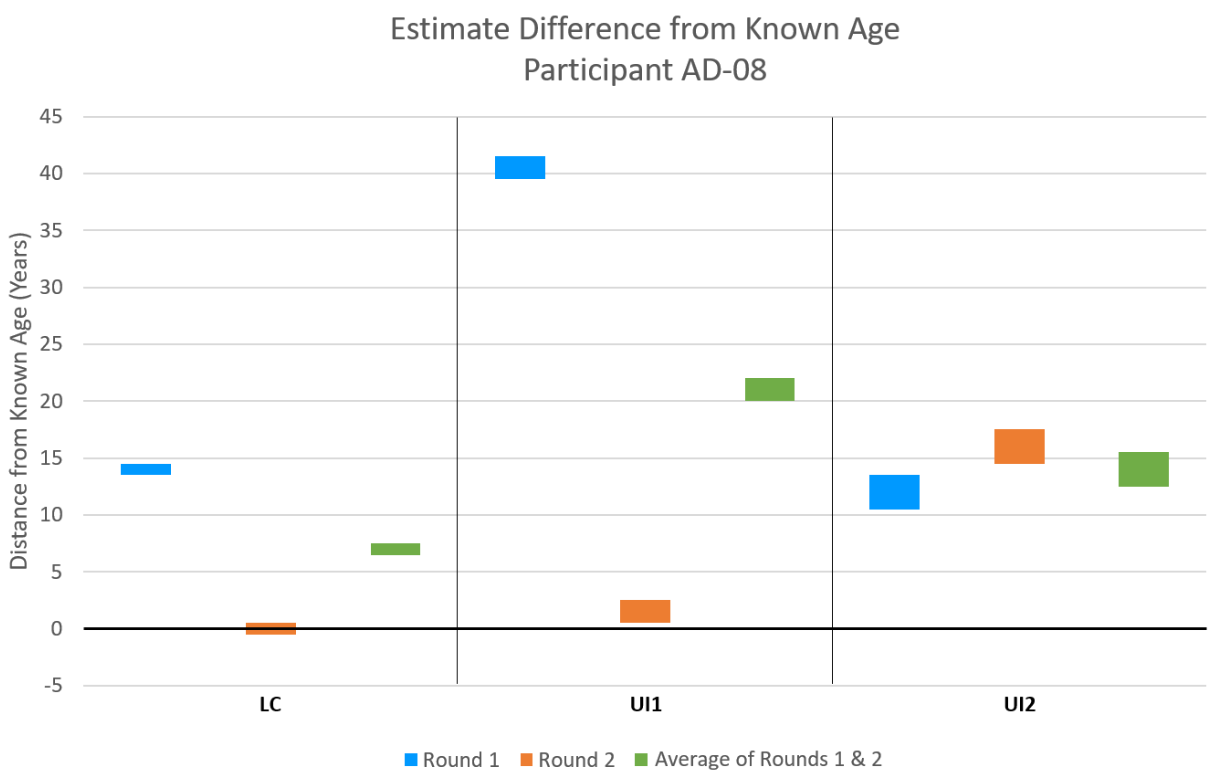

Figure 6. Graph showing the error or distance of estimates from the known age of participant AD-08

to 0.5 years, a range of only 1. R2 also produced the next closest estimate for tooth UI1, which has an error of 0.5 to 2.5 years (a total range of 2), while all other estimates land more than five years above the known age.

Participant CC-03, a biological male, was 64 years old at the time that teeth UP4, UI1, and LI1 were extracted. The total error range for all teeth and all rounds of estimates is 23.5, being -23.5 to 0 years from the known age. The maximum and minimum margins of error are both found in the estimates from R1 as it exhibits the same range of 23.5 spanning from -23.5 to 0. RA represents the next most extensive error range for this participant being 11.75 from -16 to -4.25 years, while R2 shows the least with a range of 5 from -10 to -5 years outside the known age (*Figure 7*).

UP4 shows the smallest amount of error in estimates for this participant between R1, R2, and RA, with a range of 5.5 years from -5 to -10.5. LI1 falls in the middle of all three teeth from this participant in terms of the amount of error between rounds, as its estimates go from a maximum of 0 to a minimum of

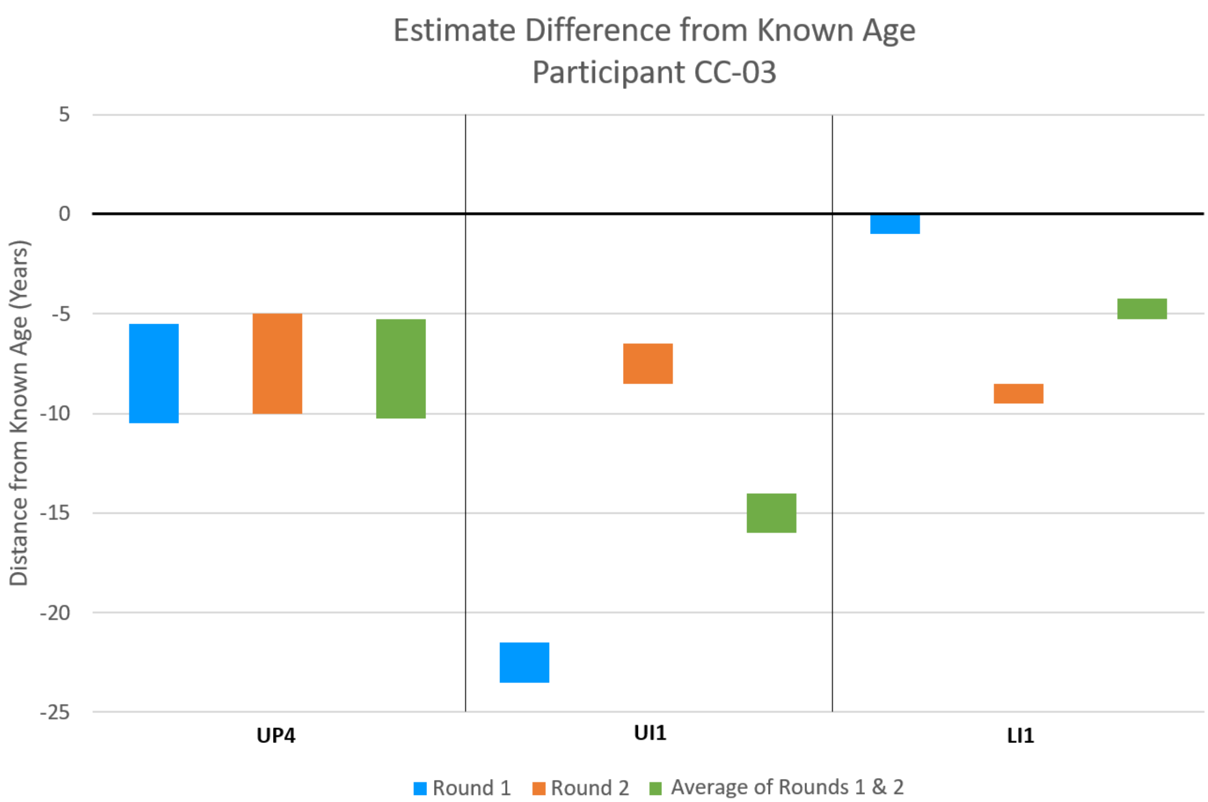

Figure 7. Graph showing the error or distance of estimates from the known age of participant CC-03

-9.5 years away from the known age, spanning a total range of 9.5. As with the previous participant, UI1 is shown to have the largest difference between rounds of examination, being from -6.5 to -23.5 with a range of 17 years. The only tooth that produced an estimate that encompasses the actual age is LI1 in R1, with a range from 0 to -1 years. All other estimates for CC-03 fall 4.25 years or more from the known age.

Participant 3622, a biological male, was 80 years old at the time of his death. The teeth extracted from this donor consist of a UI1, UP4 and LI2. The error range of estimates from all teeth and all rounds for this individual is 32.5, with a minimum of -17 and a maximum of 15.5 years. The estimates from R1 produced an error of -17 - 15.5, ranging 32.5 years, while R2 produced an error of -11 to 4.5 – a total range of 15.5 years. When viewing the estimates given by taking the average counts from R1 and R2, the error is -12 to 10 with a range of 22 years (*Figure 8*).

Of all the teeth from participant 3622, UI1 shows the smallest amount of error between all examination rounds at -9 to -13 years away from the known age, with a range of 4. UP4 and LI2 has similar

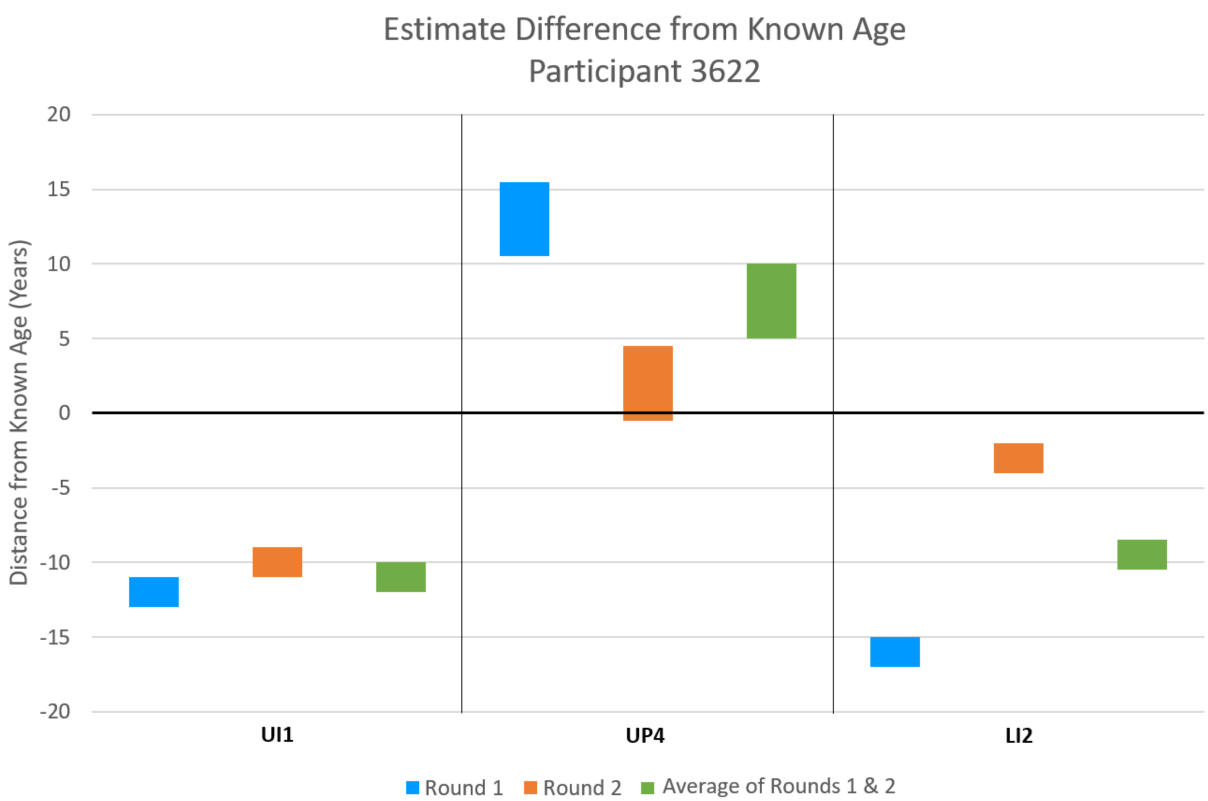

Figure 8. Graph showing the error or distance of estimates from the known age of participant 3622

error rates with the former showing a minimum of -0.5, a maximum of 15.5 years, and a total range of 16. LI2 has an error rate of -2 to -17 years away from the known age, with a total range of 15. The only estimate that includes the known ages from the second round of examining the UP4 tooth. The resulting error for this tooth is -0.5 to 4.5 years, a range of 5. R2 also produced the next closest estimate for tooth LI2, which has an error of -2 to -4 years (a total range of 2), while all other estimates land more than five years above the known age.

A biological female, participant 3702 was 75 years old at the time of her death, after which teeth UI2, LI1, and LI2 were extracted. The total error range for all teeth and all rounds of estimates is 19.5, being -22.5 to -3 years from the known age. The maximum and minimum margins of error are both found in the estimates from R2 as it exhibits the same range of 19.5 spreading from -22.5 to -3. RA represents the next most extensive error range for this participant being 11.5 from -9.75 to -21.25 years, while R1 shows the least with a range of 3.5 from -16.5 to -20 years outside the known age (*Figure 9*).

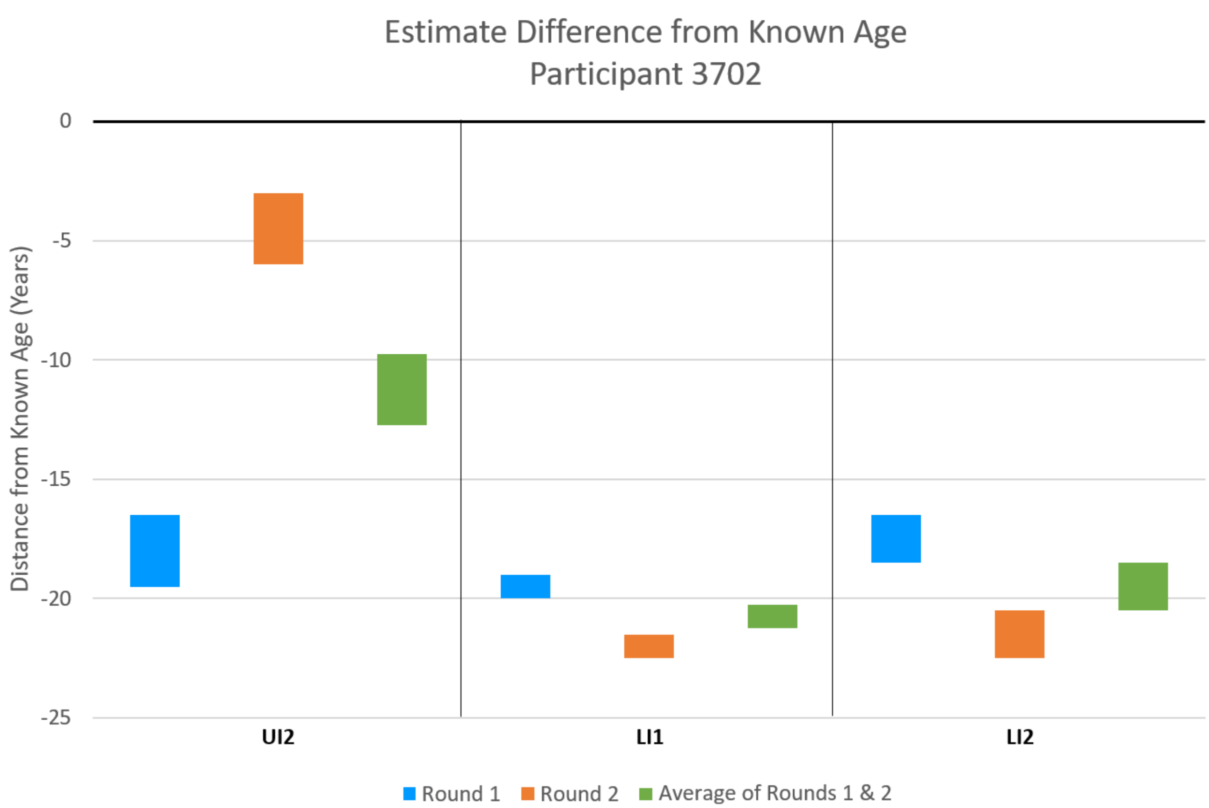

Figure 9. Graph showing the error or distance of estimates from the known age of participant 3702

LI1 shows the smallest amount of error range in estimates for this individual between all examination rounds, with a range of 3.5 years from -19 to -22.5. LI2 falls in the middle of all three teeth from this participant, as its estimates range from a minimum of -16.5 to a maximum of -22.5 years away from the known age, spanning a total range of 6. UI2 is shown to have the largest difference between rounds of examination, being from -3 to -19.5 with a range of 16.5 years. None of the teeth from this participant produced age estimates that match the known age. The estimate closest to the known age comes from the second round of analysis for UI2, which falls -3 years from the known age.

The final participant in the intraindividual sample, CC-04, is a biological female who was 33 years old at the time that teeth UI1, LI2, and LC were extracted. Between R1, R2, and RA, as well as all teeth, the overall error range is just 7, spanning from -1 to 6 years away from the known age. R1 provides the smallest error range of 4 (1 to 5), while that of R2 is the largest at just 7 (-1 to 6). RA falls in the middle, with a range of 5.5 years from 0 to 5.5 (*Figure 10*).

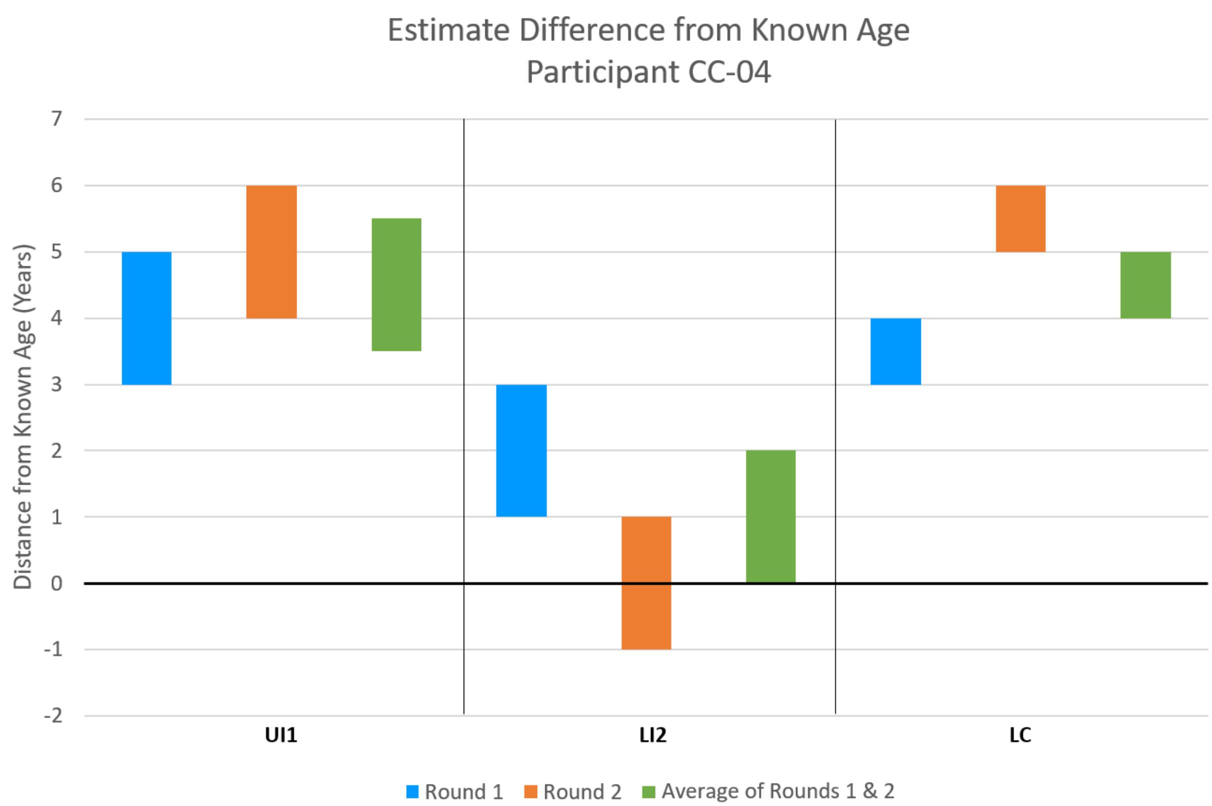

Figure 10. Graph showing the error or distance of estimates from the known age of participant CC-04

Both the UI1 and LC from participant CC-04 have relatively small error ranges in all rounds when compared to LI2. Both estimates are between 3 and 6 years from the known age for a total range of 3. Although LI2 displays the largest range of the three teeth, it is still rather small at just 4, with a minimum of -1 and a maximum of 3. Despite having the highest range and, thus, the most amount of variation between rounds of examination, examination of the LI2 provided the two closest estimates to the reported age with those from R1 (-1 – 1) and RA (0 – 2) both encompassing the known age.

Intraindividual Sample Results by Tooth Type

By analyzing estimation error rates according to tooth type, the results can reveal information about the accuracy of cementochronology according to what type of tooth is used as well as the variability of TCA display in a tooth type according to age. Again, to study the amount of variation in TCAs among different types of teeth, the measurement of error was determined by calculating the numerical ranges

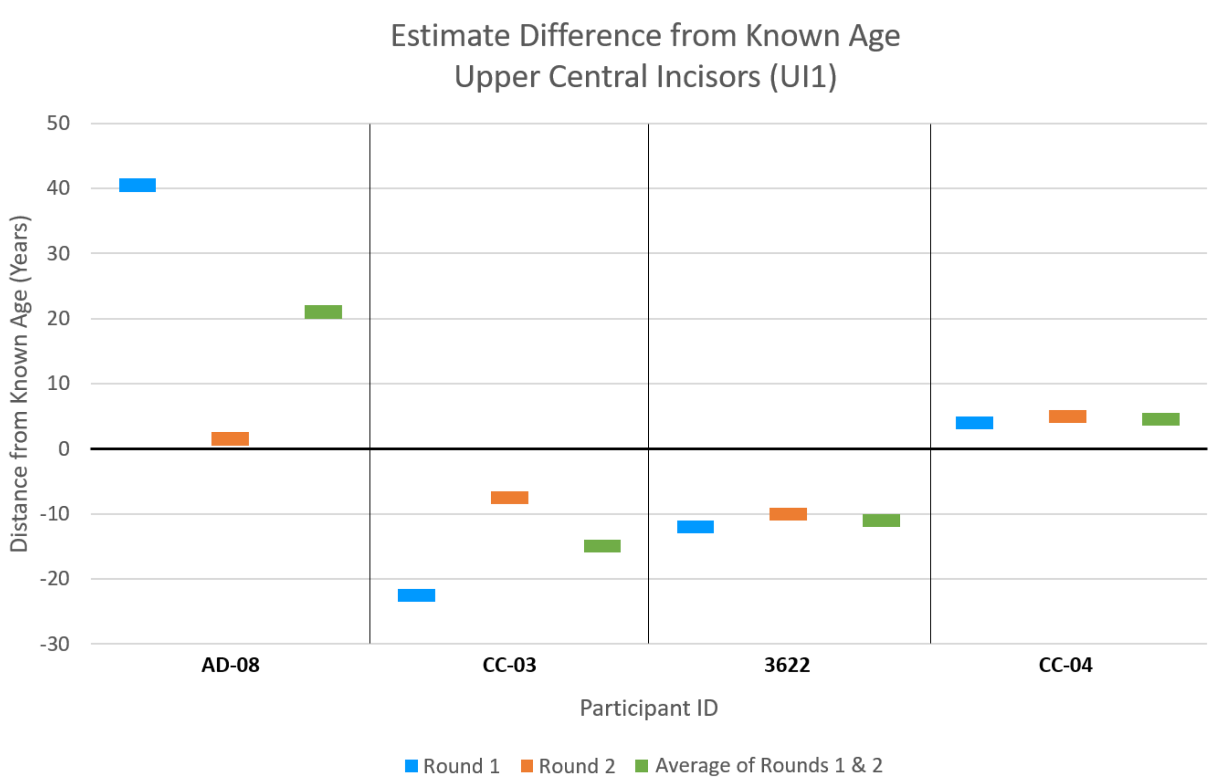

Figure 11. Graph showing the error or distance of estimates from known age for upper central incisors (UI1)

between the known age and the estimated age range from the first (R1) and second (R2) rounds of examination, as well as the average of the two (RA) (*Table 5*).

The total error range of estimates for all upper central incisors (UI1) from R1, R2 and RA extends from -23.5 to 41.5 – a span of 65 years. Those from participants 3622 and CC-04 show small amounts of variation, with the former being between -9 and -13 away from the known age – a range of 4 – and the latter being between 3 and 5 – a range of 2. The tooth from participant CC-03 shows a great deal more variation with an error range of 17 from estimates -6.5 to -23.5 years away from the known age. The highest amount of variation is found in the tooth from the 28-year-old participant AD-08, which has a range of 41 years from 0.5 to 41.5 (*Figure 11*).

Although no UI1 produced an estimate range that contains an individual's actual age, the closest estimate was obtained during R2 evaluation of the tooth from AD-08. That estimation range falls 0.5 to 2.5 years above the known age while the next closest are all three teeth from CC-04, with that of R1 being 3 to 5, RA being 3.5 – 5.5, and R2 being 4 – 6. Two of the UI1, from AD-08 and CC-04, consistently produced estimates above the actual age while the other two consistently produced estimates below.

For the combination of all six estimates produced from the two UI2 teeth in the sample, the range of error extends from -19.5 to 17.5, or 37 years total. Those from AD-08 are less varied between each other with a range of only 7 (10. – 17.5), while those from 3702 are more varied with a range of 16.5 (-3 – -19). The UI2 from AD-08 consistently produced estimates above the known age and those from 3702 produced estimates below it. No estimates fall directly on the known age and the closest is from R2 for 3702 (-3 – -6) (*Figure 12*).

The final maxillary tooth type, UP4, produced an overall error in estimates of -5.5 to -10.5, for a total range of 25 years. The estimate for participant CC-03 is less varied between rounds of examination and counting than the one from 3622. The first shows an error range of 5.5 with estimates falling

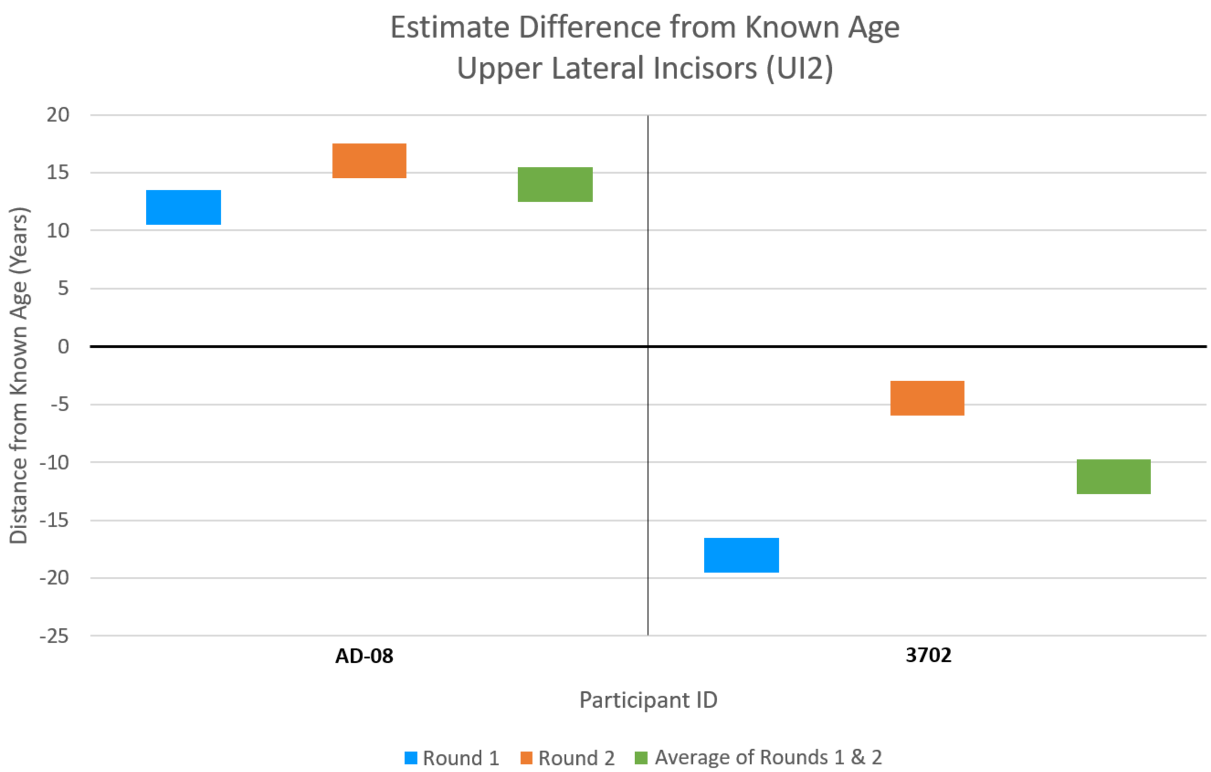

Figure 12. Graph showing the error or distance of estimates from known age for upper lateral incisors (UI2)

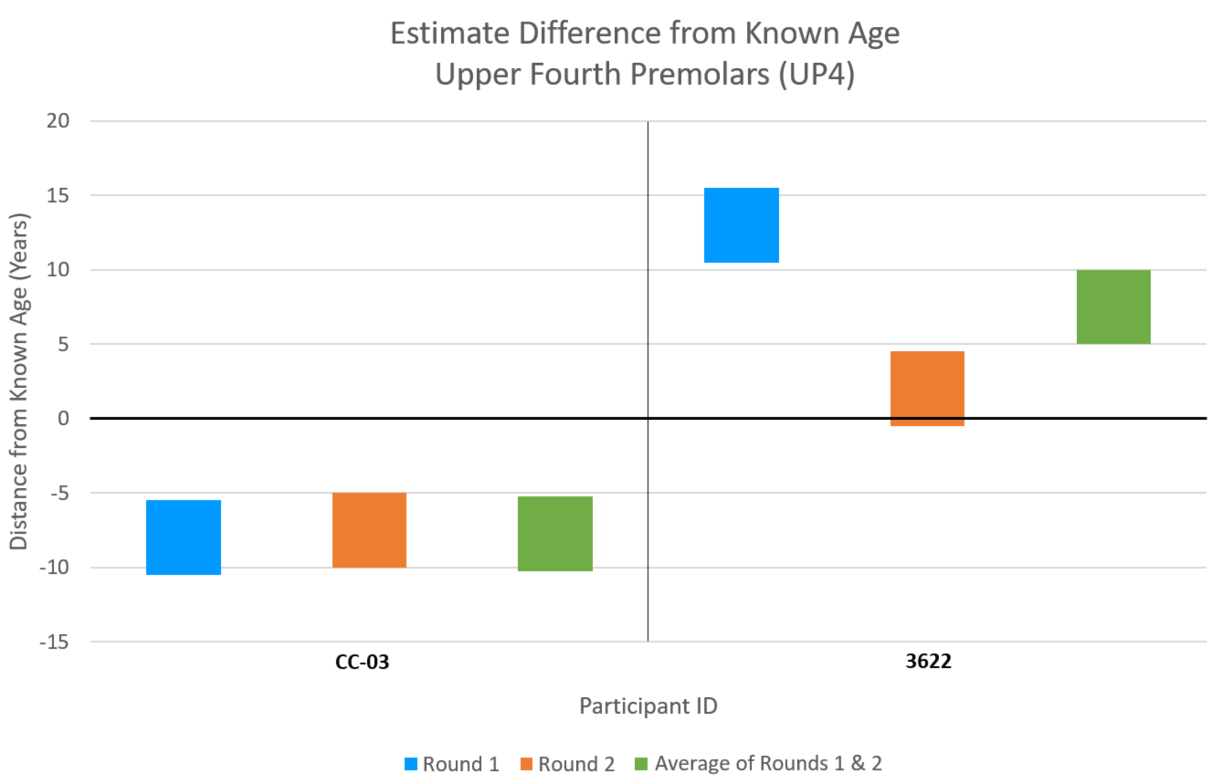

Figure 13. Graph showing the error or distance of estimates from known age for upper fourth premolars (UP4)

between -5.5 and -10.5, and the second shows a range of 16 with estimates falling between -0.5 and 15.5 away from the known age. The R2 estimate of the tooth from 3622 falls within the known age, with an error range -0.5 to 4.5, while the results from R1 and RA both fall above it. All the estimates from participant CC-03 fall below the known age (*Figure 13*).

The total error range of estimates for all lower central incisors (LI1) from R1, R2, and RA is 22.5, extending from a minimum of -22.5 to a maximum of 0 years outside of the participants' known ages. Those from participant 3702 show a small amount of variation, being between -19 and -22.5 away from the known age – a range of 3.5. The tooth from participant CC-03 shows a slightly more variation with an error range of 9.5 from estimates -9.5 to 0 years away from the known age. The only estimate from this tooth type to incorporate the known age of the individual is from the R1 examination of CC-03, with an error range of -1 to 0 (*Figure 14*).

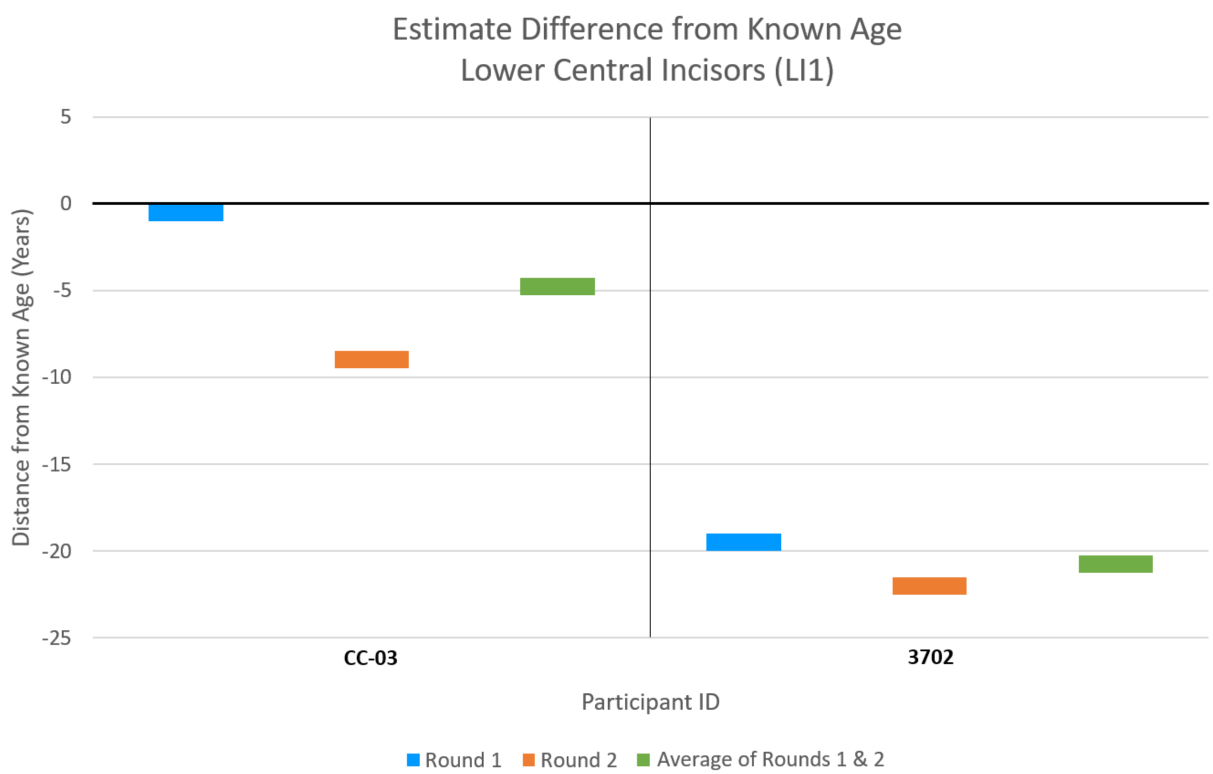

Figure 14. Graph showing the error or distance of estimates from known age for lower central incisors (LI1)

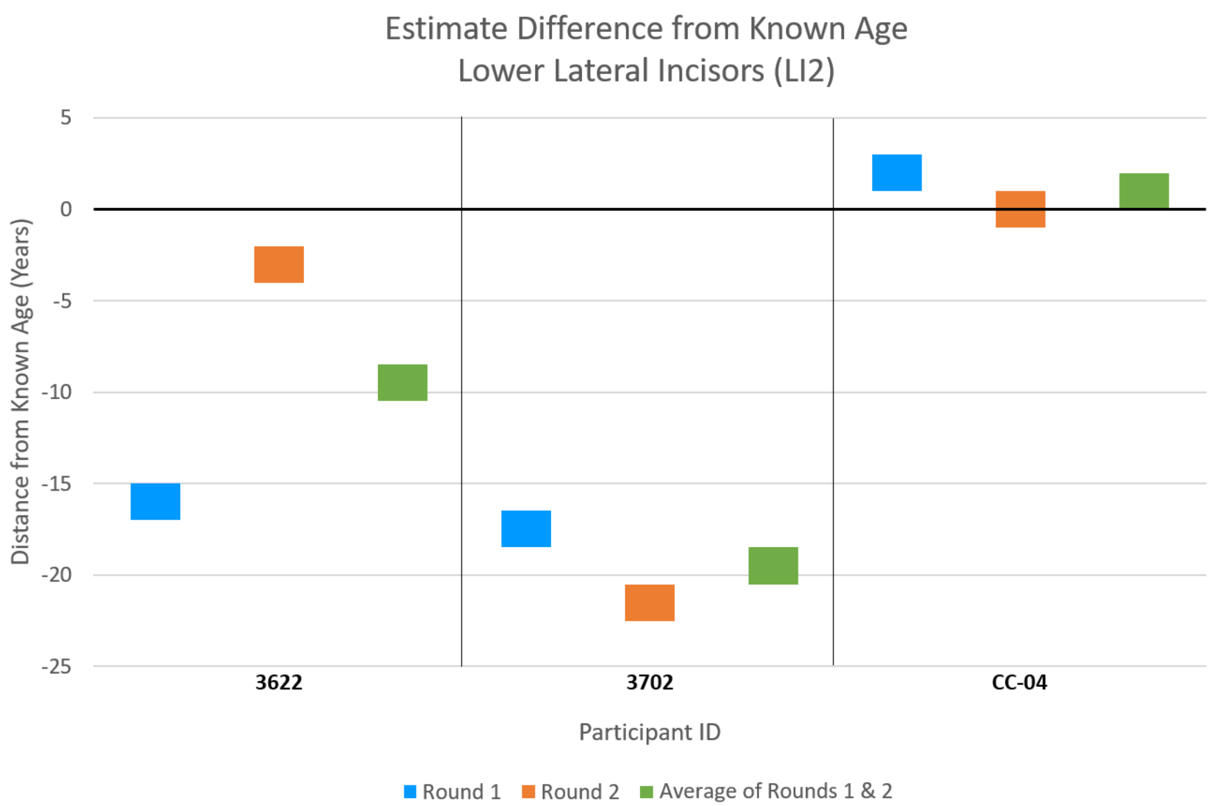

Figure 15. Graph showing the error or distance of estimates from known age for lower lateral incisors (LI2)

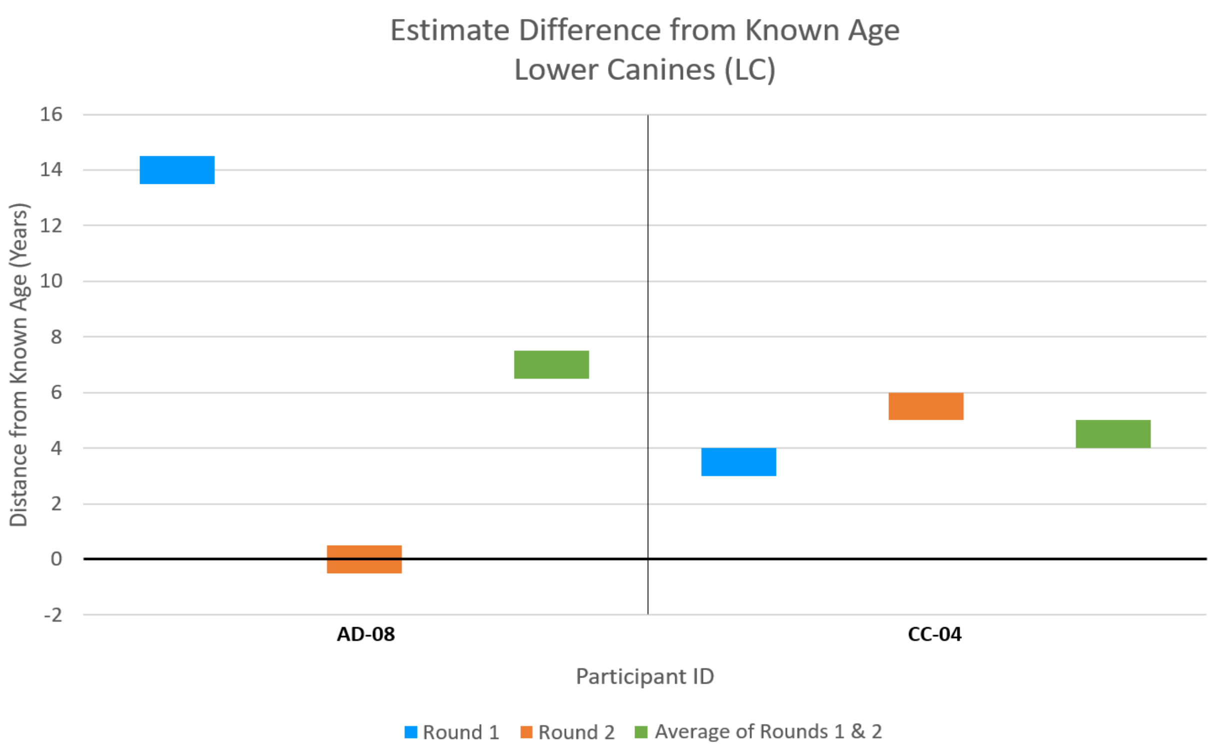

Figure 16. Graph showing the error or distance of estimates from known age for lower canines (LC)

The lower later incisors (LI2) produced similar estimates errors to those of the LI1 teeth, with an overall range of 25.5 going from -22.5 to 3. With a total of three teeth of this type, the one taken from participant CC-04 produced estimates with the least amount of variation. Additionally, the tooth from CC-04 shows the results closest to the known age, with two of the estimates (R2 and RA) surrounding the known age and the final estimate only being one year off. R1, R2, and RA estimates for this tooth have an error range of 2 being between -1 and -3 years from the known age. The tooth from 3702 has an error range of 6 (-16.5 to -22.5), and the one from 3622 has the most amount of variation with an error range of 15 (-2 to -17) (*Figure 15*).

The final tooth type used in this sample is the lower canine (LC), which has an overall estimate error between -0.5 to 14.5 for a total range of 15. The minimum and maximum points of that overall range are both produced by the first tooth in the sample, taken from AD-08. The tooth from CC-04 resulted in an estimate with a much smaller error range of 3, being between 3 and 6 years above the known age. While only the R2 analysis of the AD-08 tooth produced an estimate that includes the known age, all others produced estimates above the actual ages of the participants (*Figure 16*).

<u>Interparticipant Sample</u>

The main objective in analyzing the interparticipant sample is to test the overall accuracy and reliability of cementochronology on pathological dentition. It is also a goal of this study to observe any visible patterns of decreased accuracy in age estimates with increasing age, as that is a problem many have noticed with the technique. The raw data from this portion of the study can be found in *Table 6.*

The first round of estimates has a relatively weak correlation with the known ages of the participants at 0.67 (*Figure 17)*. In this round, only 6.67% of the teeth (n = 1) produced estimates that include the known age. As for the remainder of the estimates, 20% (n = 3) fall within the ±2.5-year range, another 20% fall within the ±2.5-5-year range, 6.67% (n = 1) fall within the ±5-7.5-year range, 13.33% (n =

2) fall within the ±7.5 – 10-year range, 20% (n = 3) fall within the ±10 – 15-year range, and the final 13.33% (n = 2) fall outside of the ±15-year range (*Figures 18 & 19*).

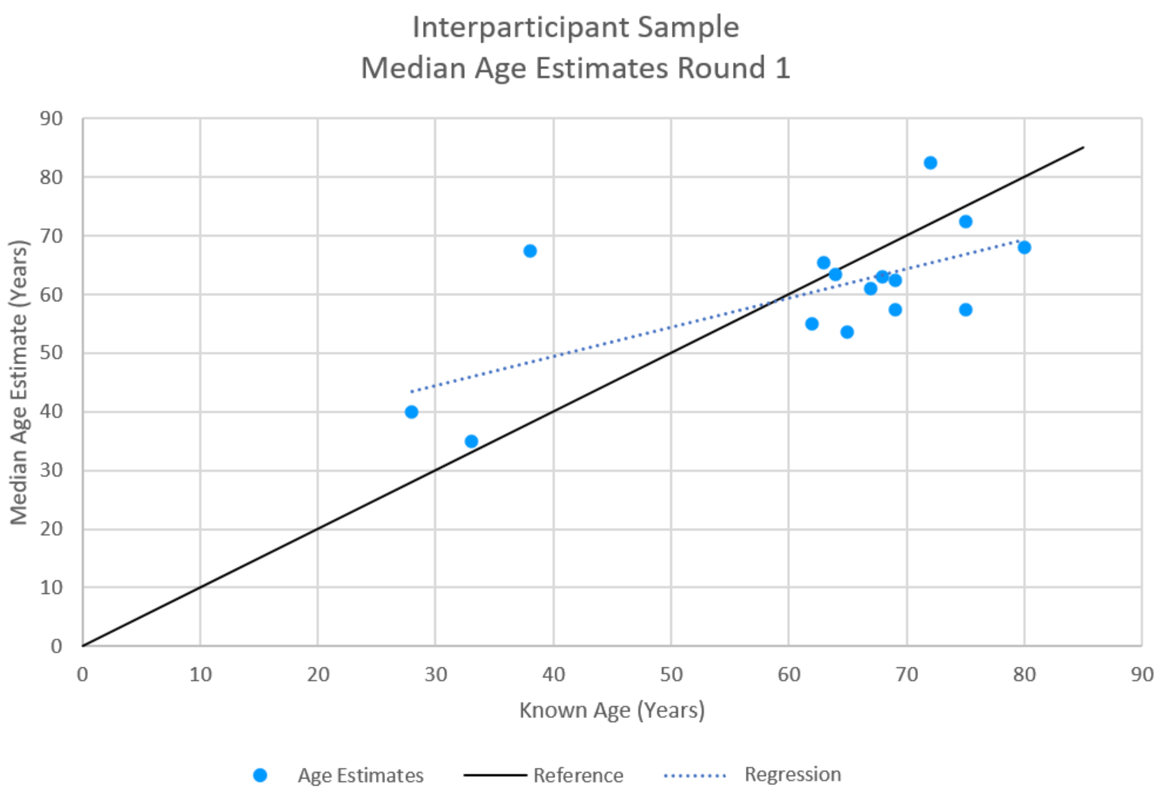

Figure 17. Scatter plot showing the median age estimates compared to the known ages for R1 of the interparticipant sample

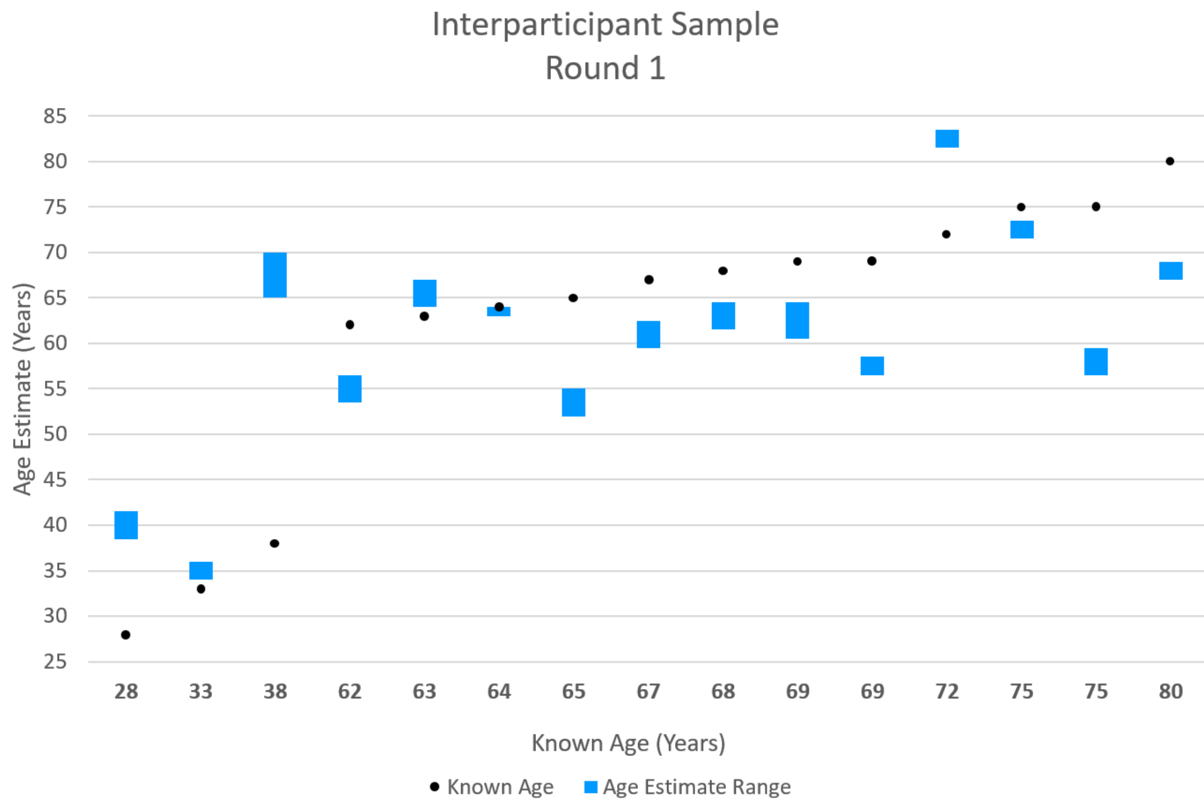

Figure 18. Graph showing the results for Round 1 of the interparticipant sample

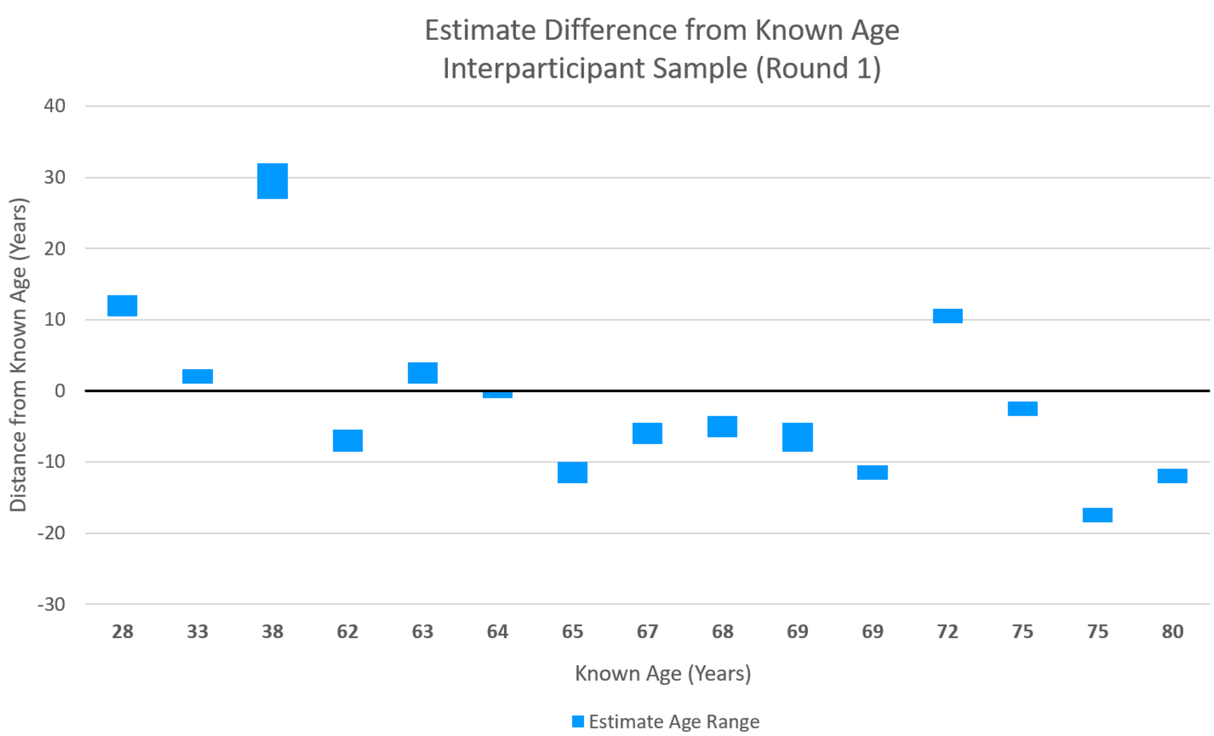

Figure 19. Graph showing the error or distance of estimates from known age for R1 of the interparticipant sample

Table 6. Interparticipant sample results for R1, R2, & RA

Participant ID	Age	Tooth Type	TCA Count (R1)	Age Estimate (Round 1)	Distance from Known Age	TCA Count (R2)	Age Estimate (Round 2)	Distance from Known Age	TCA Count (Ave)	Age Estimate (Average)	Distance from Known Age
2	63	UI2	57.5	64 – 67	1 – 4	52.5	59 – 62	-1 – -4	55	61.5 – 64.5	-1.5 – 1.5
4	75	UI1	65	71.5 – 73.5	-1.5 – -3.5	74.5	81 – 83	6 – 8	69.75	76.25 – 78.25	1.25 – 3.25
5	68	LP4	52	61.5 – 64.5	-3.5 – -6.5	50	59.5 – 62.5	-5.5 – -8.5	51	60.5 – 63.5	-4.5 – -7.5
7	67	UI2	53	59.5 – 62.5	-4.5 – -7.5	54	60.5 – 63.5	-3.5 – -6.5	53.5	60 – 63	-4 – -7
10	28	UI2	32	38.5 – 41.5	10.5 – 13.5	36	42.5 – 45.5	14.5 – 17.5	34	40.5 – 43.5	12.5 – 15.5
11	72	UI1	75	81.5 – 83.5	9.5 – 11.5	49	55.5 – 57.5	-14.5 – -16.5	62	68.5 – 70.5	-1.5 – -3.5
14	62	LP4	44	53.5 – 56.5	-5.5 – -8.5	50	59.5 – 62.5	-2.5 – 0.5	47	56.5 – 59.5	-2.5 – -5.5
23	38	UP4	56.5	65 – 70	27 – 32	40.5	49 – 54	11 – 16	48.5	57 – 62	19 – 24
28	64	LI1	57.5	63 – 64	-1 – 0	49	54.5 – 55.5	-8.5 – -9.5	53.25	58.75 – 59.75	-4.25 – -5.25
31	80	UI1	60.5	67 – 69	-11 – -13	62.5	69 – 71	-9 – -11	61.5	68 – 70	-10 – -12
37	75	LI2	50	56.5 – 58.5	-16.5 – -18.5	46	52.5 – 54.5	-20.5 – -22.5	48	54.5 – 56.5	-18.5 – -20.5
48	33	LI2	27.5	34 – 36	1 – 3	25.5	32 – 34	-1 – 1	26.5	33 – 35	0 – 2
52	69	UC	53	60.5 – 64.5	-4.5 – -8.5	53.5	61 – 65	-4 – -8	53.25	60.75 – 64.75	-4.25 – -8.25
53	69	UI1	50	56.6 – 58.5	-10.5 – -12.5	51	57.5 – 59.5	-9.5 – -11.5	50.5	57 – 59	-10 – -12
54	65	UI2	45.5	52 – 55	-10 – -13	44	50.5 – 53.5	-11.5 – -14.5	44.75	51.25 – 54.25	-10.75 – -13.75

The tooth with the furthest estimate from the actual age comes from the 38-year-old and shows an error range of 27 – 32 years. Only one of the estimates from R1 lands directly on the known age – the tooth from the 64-year-old participant. It has an error range of -1 – 0, while those from the 33-year-old and 63-year-old are each only 1 to 3 years away from the correct age. For the younger individuals included in this sample, all the estimates from R1 fall above the actual age. For the remaining participants, all of which were over 60-years old at the time of tooth extraction, all but two estimates land on or below the actual age.

The second round of estimates has a stronger correlation than the first at 0.76 (*Figure 20*). In R2, the number of estimates including the documented age increases to 13.33% (n = 3). 6.67% (n = 1) are within ±2.5 years of the known age, 13.33% are within ±2.5-5 years (n = 2), another 13.33% (n = 2) are within ±5-7.5 years, 20% (n = 3) are within ±7.5-10 years, 26.67% (n = 4) are within ±10-15 years, and the final 26.7% are outside of ±15 years (*Figures 21 & 22*).

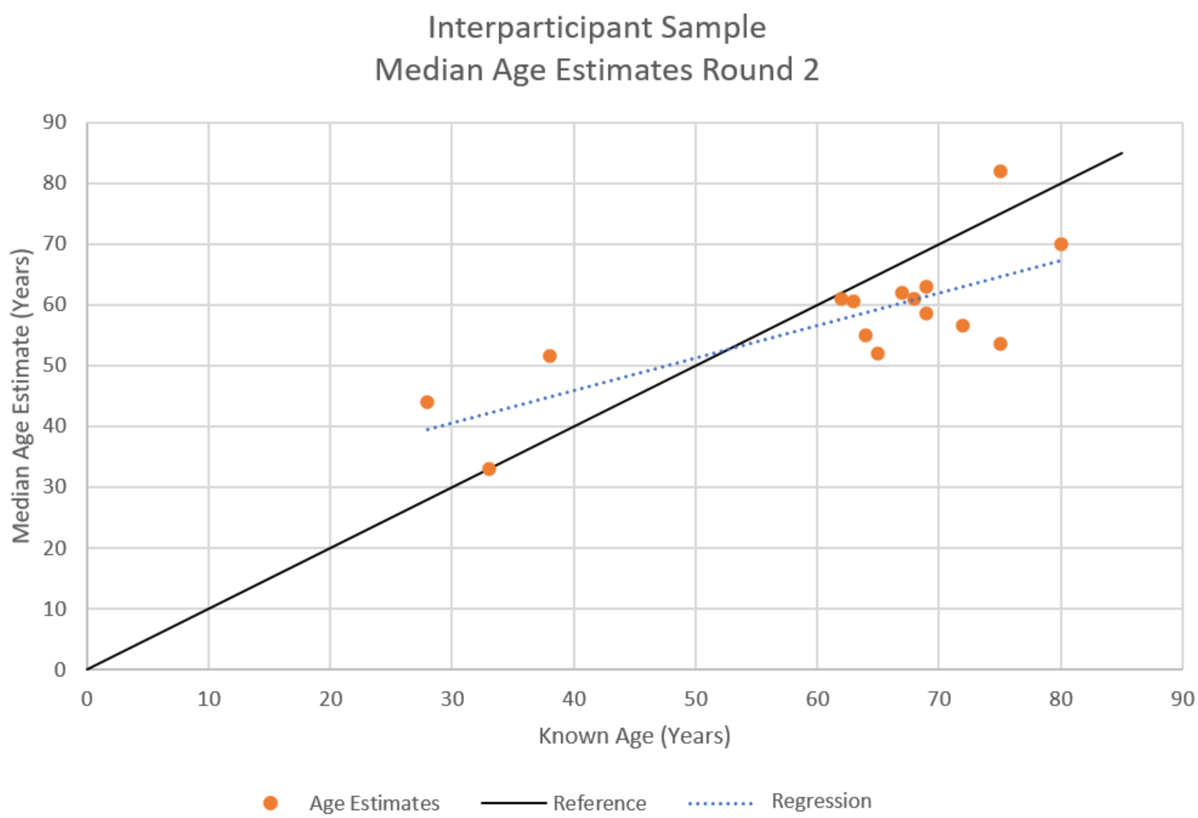

Figure 20. Scatter plot showing the median age estimates compared to the known ages for R2 of the interparticipant sample

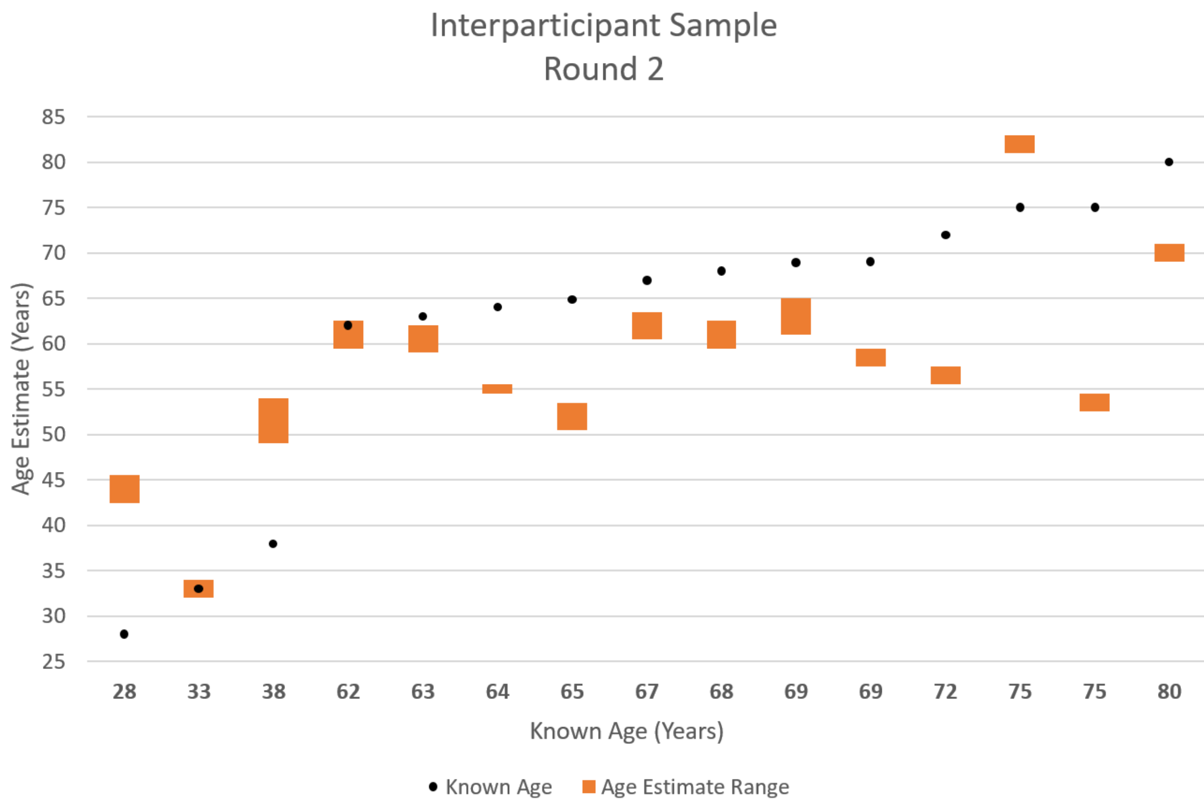

Figure 21. Graph showing the results for Round 2 of the interparticipant sample

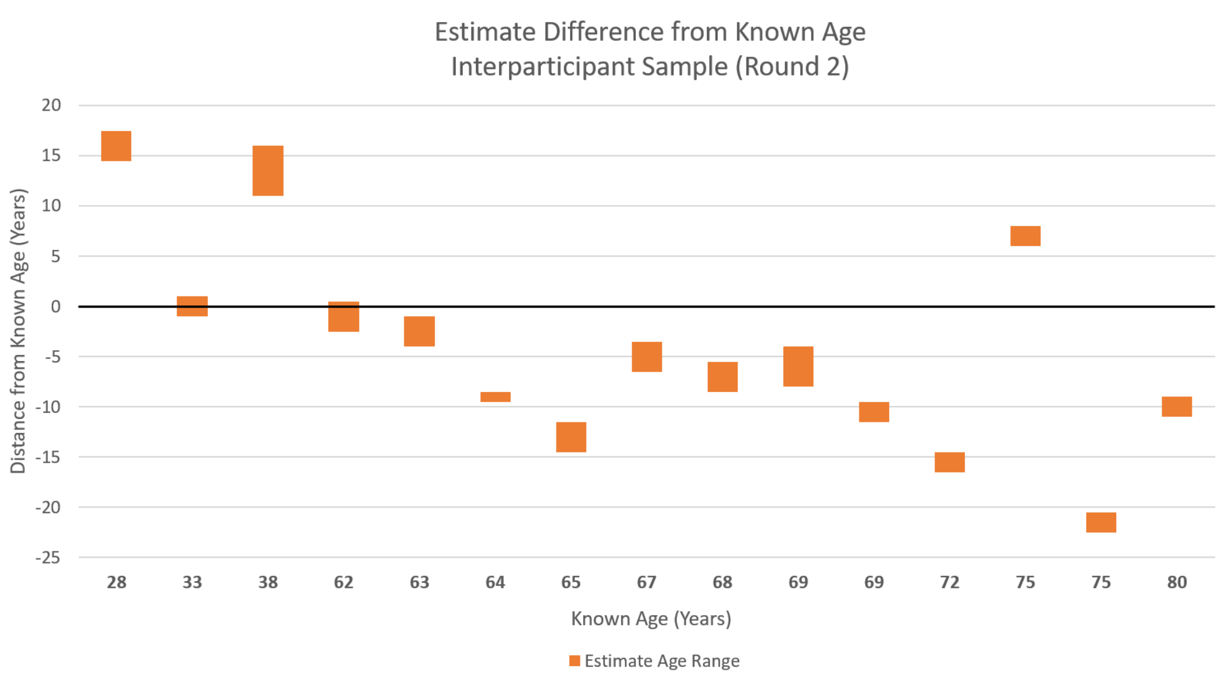

Figure 22. Graph showing the error or distance of the interparticipant sample of estimates from known age for R2

The tooth with the highest rate of error in R2 is from 75-year-old participant 3702, with a range of -20.5 to -22. The next most erroneous estimate comes from the 72-year-old individual, ranging from a minimum of 15.5 to a maximum of 18.5 years. Two of the teeth from younger participants show the next highest error ranges, with the estimate for the 28-year-old being 14.5 to 17.5 years and the estimate for the 38-year-old showing some improvement from R1 with an error range of 11 to 16 years difference. The closest estimates to the known ages from this round come from the 33-year-old and the 62-year-old. Both estimates include the known age, with the former being 32 to 34 and the latter being 59.5 to 62.5. With the exception of the estimate from the 33-year old participant falling evenly on either side of the correct age, all the younger participants are estimated to be much older than their actual ages in this round as with R1. Again, the majority of the remaining older participants are estimated to be younger than their known age, with the exception of the tooth from 75-year-old that produced an estimate the includes the reported age. A comparison of the results from R1 and R1 can be found in *Figure 23.*

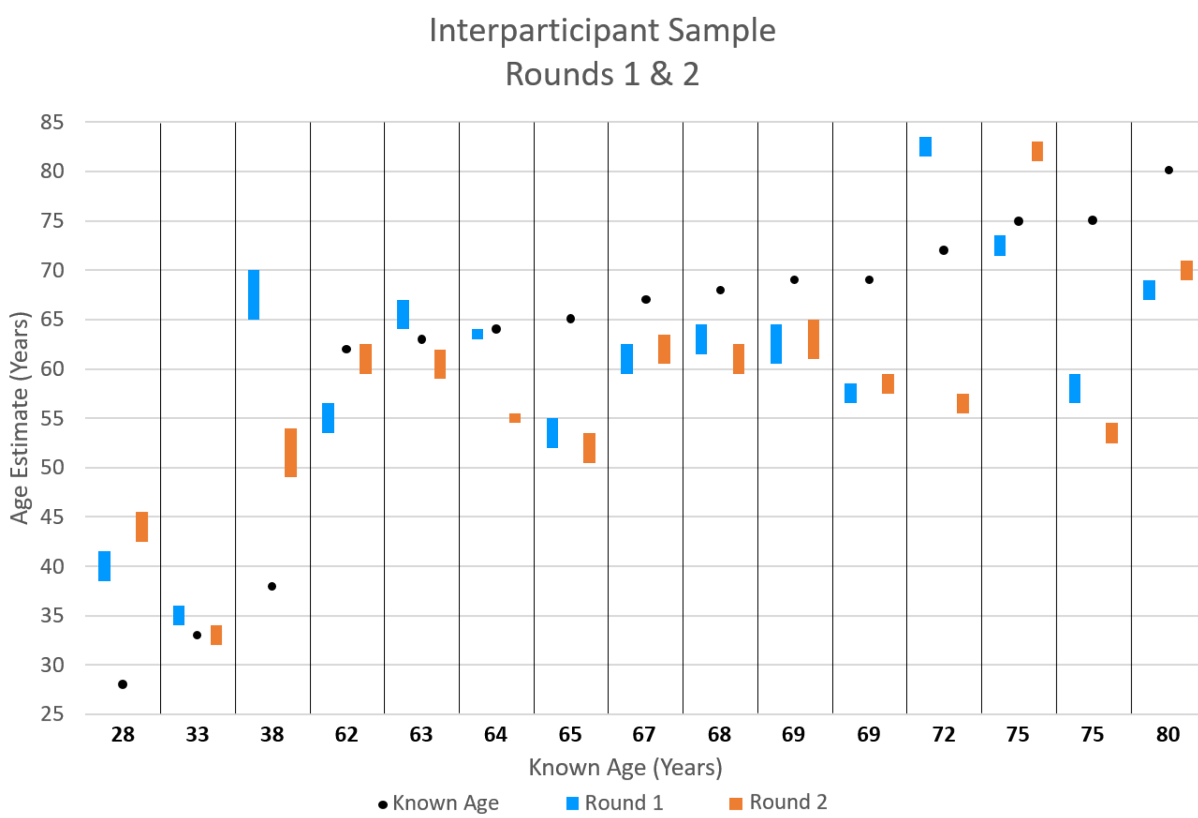

Figure 23. Graph showing the results for Rounds 1 & 2 of the interparticipant sample

When averaging the counts between R1 and R2 to produce new estimates (RA), the correlation between estimated and known age is stronger than either of them individually at 0.77 (*Figure 24*). The number of estimates that incorporate the known age is the same as R2 at 13.33% (n = 3), and the number of estimates within ±2.5 years of the known age is the same as R1 at 20% (n = 3). The percentage of estimates falling within ±2.5-5 years is the highest in this round at 26.67% (n = 4), and, for the first time, no estimates fall within ±5-7.5 years of the known age. The percentages for the final three categories of ±7.5-10 years, ±10-15 years, and upwards of ±15 years are equally distributed in this round at 13.33% (n = 2) each (*Figures 25 & 26*).

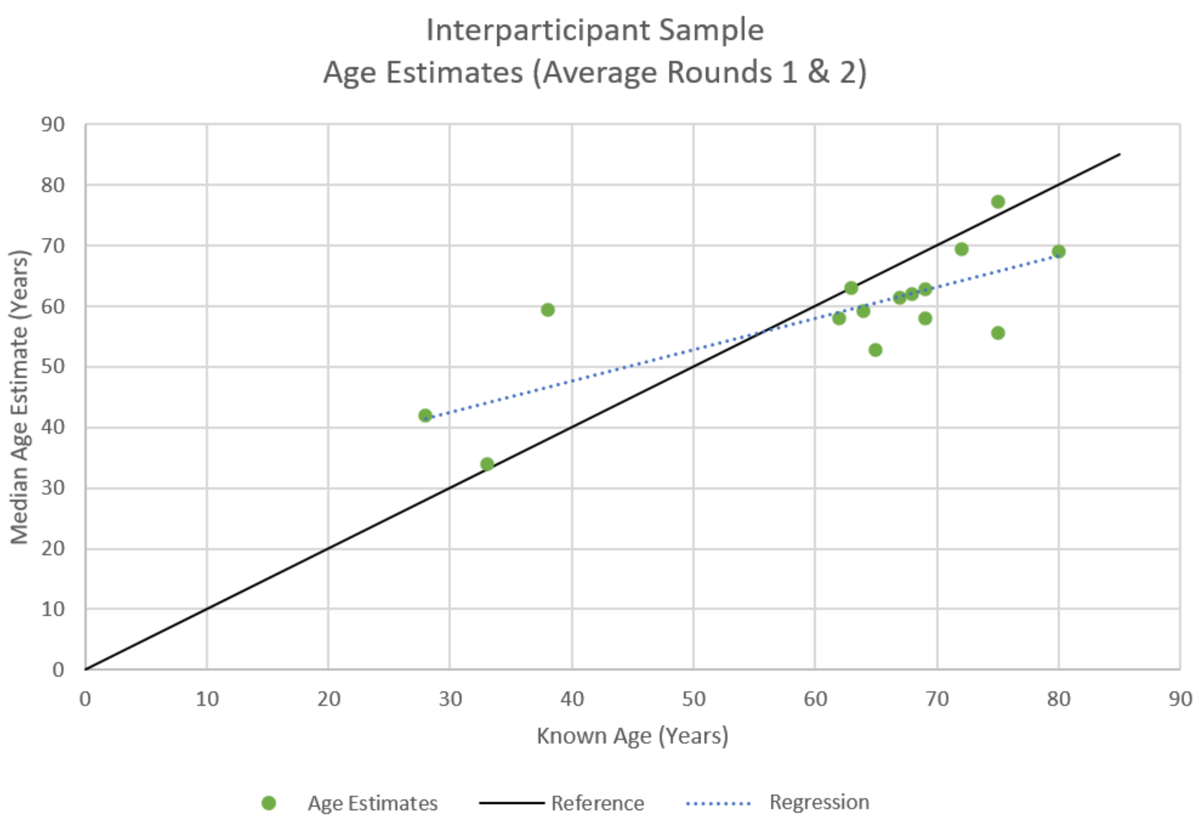

Figure 24. Scatter plot showing the median age estimates compared to the known ages for R1 &R2 of the interparticipant sample

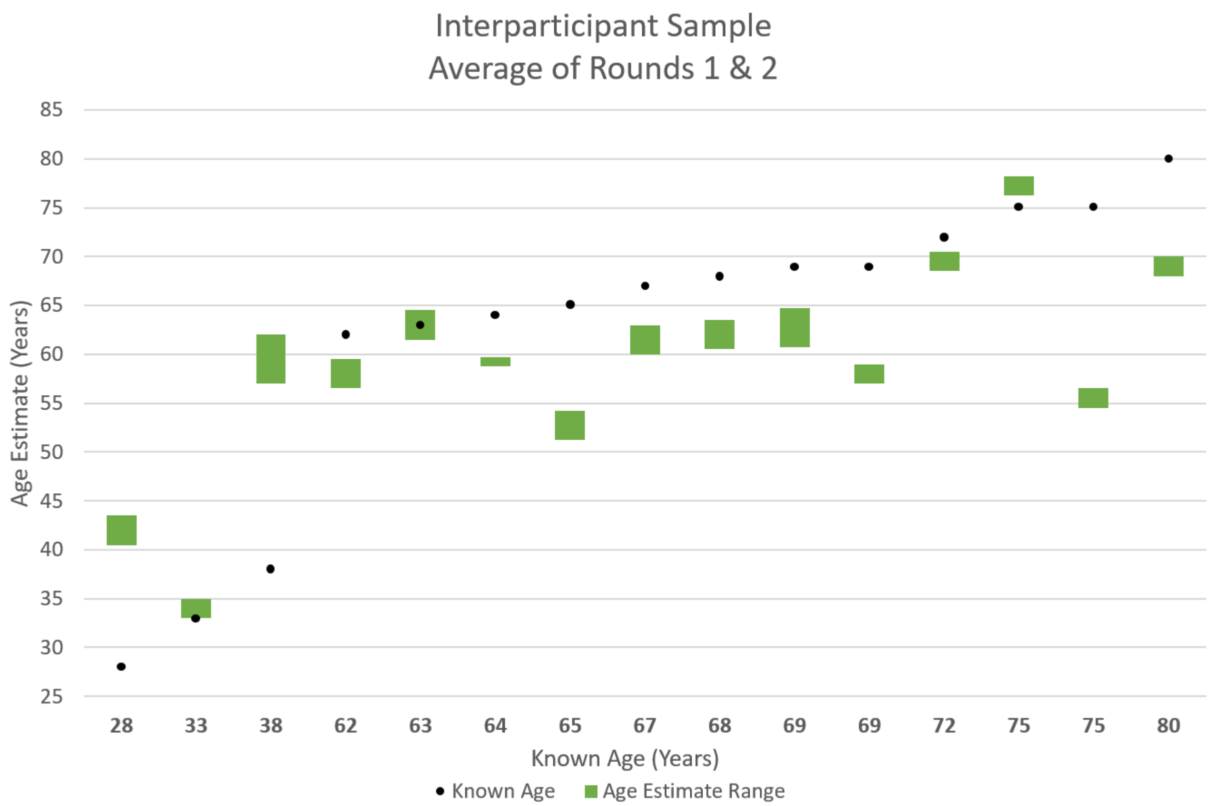

Figure 25. Graph showing the results for R1 & R2 of the interparticipant sample

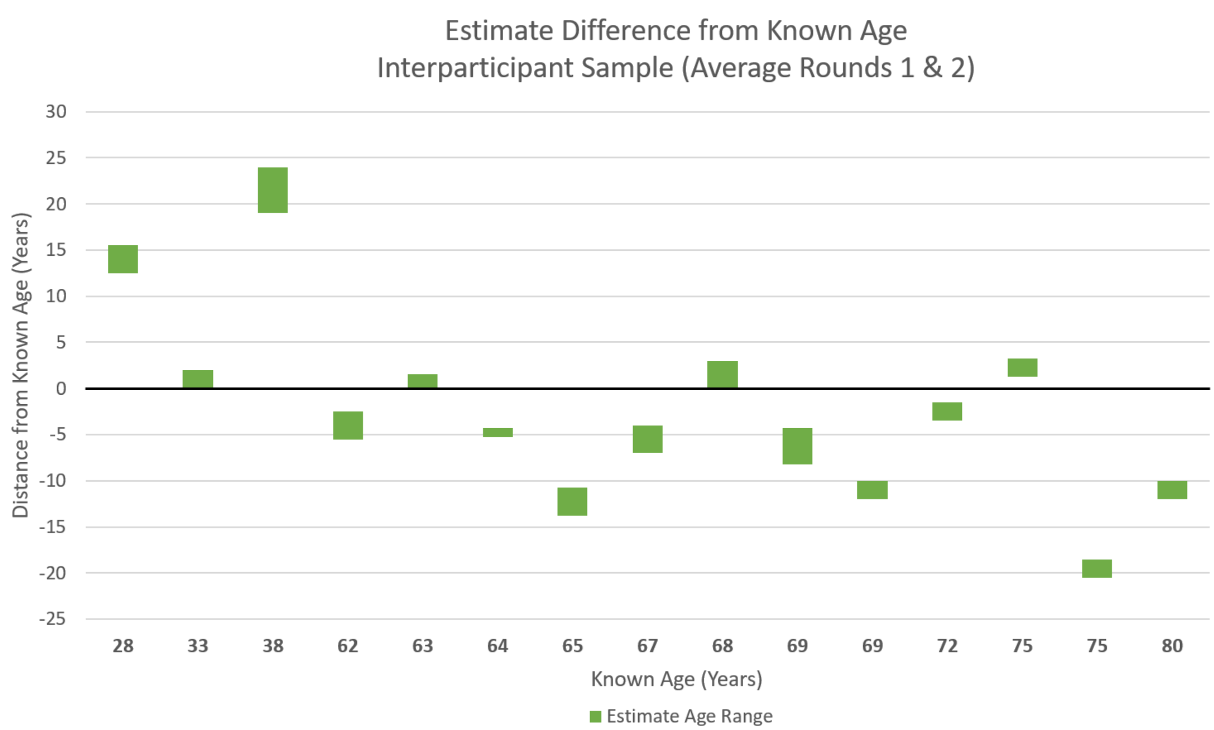

Figure 26. Graph showing the error or distance of estimates from known age for the averages of R1 & R2 of the interparticipant sample

The estimate that falls furthest from the known age comes from the 38-year-old, at 57 – 62, or an error rate of 19 to 24. Again, the tooth from participant 3702 has one of the highest estimate error rates at -18.5 to -20.5. The tooth from the 33-year-old participant once again produced an estimate that includes the documented age, along with the tooth from the 63-year-old participant. The same pattern found in R1 and R1 persists in RA since the teeth from younger participants consistently provided estimates at or above the correct age while all but one of the remaining teeth from older participants produced estimates below the correct age.

Chapter 5 – Discussion

What follows is a discussion of the results for both samples, with additional information concerning the amount of intra-observer error for both. To interpret the results of each sample more clearly, it is crucial to recognize how intra-observer error – the variance is estimates produced by the same observer on the same sample, but at a different time – has influenced them both. Following the discussion on intra-observer error, the results from the intraindividual and interparticipant samples individually.

Intra-Observer Error

Intraindividual Sample

Assessing the amount of intra-observer error in this sample is accomplished by comparing the range in age estimates and error ranges between R1 (Round 1 Age), R2 (Round 2 Age), and RA (Rounds 1 & 2 Average) for each participant, as well as the amount of error and error range between all rounds for each tooth type. When looking at the total range of age estimates for each participant, the second round of estimates (R2) shows less variation than the first round (R1) in three individuals (60%). This seems to indicate that estimates improve when they are repeated, which is likely due to the observer having more experience.

When comparing the age range of estimates for the average round (RA) to the round with the least variation between R1 and R2, there was less variation in only one individual (20%). There is a similar pattern when observing the amount of error between rounds of examination for each participant. For three of the participants (60%), the error ranges from R2 are closer to the known age than those from R1. In comparing the error for each participant between RA and the round with the closest age estimates to known age between R1 and R2, the former's is consistently further from the known age in all participants observed. This indicates that taking the average between separate counts to produce a new estimate does not necessarily decrease the amount of error, especially in studies such as this one that have small sample sizes and only two rounds of examination.

Age estimates are closer to the known age in R2 than in R1 for three of the tooth types (50%), while they are equal in two of the others (33.33%). When comparing the accuracy of estimates in RA to the round with the estimates nearest to the known age between R1 and R2, the average does not produce better results for any of the tooth types. The ranges between minimum and maximum error measurements for each tooth type are consistently smaller in R2 than in R1, while those from RA are smaller in two of the tooth types (33.33%) than those from R2. R2 shows an improvement in age estimate accuracy and precision than R1 for all participants and tooth types. While the results from RA were closer to the known age for certain participants or types of teeth, overall, it did not present any substantial improvement compared to the individual rounds.

Interparticipant Sample

Analysis of intra-observer error in the interparticipant sample is accomplished solely by observing the difference in age estimate accuracy for each tooth between R1, R2, and RA. There is no need to include error or age estimate ranges in this particular analysis because, when determined for only one tooth, both ranges will always be equal to the amount of variation of $R^{1}/_{2}$ for each tooth type.

For this sample, R1 and R2 show the same amount of error, as estimates were closer to the known age in 50% of teeth in each round. For tooth number 2, the results were equally as close to the documented age in both rounds, though the average of the two (RA) was closer to the known age than either one. In two other teeth, the results from RA were closer to the known age than either R1 or R2. This means that the RA estimates were closer to the reported age for three individuals, or 20% of the total sample.

Summary and Conclusions of Intra-Observer Error

This shows that the intra-observer error in cementochronology analysis for age estimation can be reduced by counting the TCAs from a single tooth multiple times in different areas, because more experience increases an observer's ability to identify them with repetition. By taking the average of the counts from multiple rounds, the improvement that is made over time is often no longer observable and, thus, it does not seem to be beneficial. For the teeth that showed improved accuracy for age estimates in RA, it seems to be the result of estimates from R1 or R2 falling above the known age while the other fell below, causing the average to fall somewhere in the middle. However, for those that have both estimates from R1 and R2 falling either above or below the known age, the RA can only be better when compared to the one with the most error.

Due to the insignificant results of using count averages to produce estimates in this study, it appears that it should not be used in research with similarly small sample sizes. It is also possible that increasing the number of rounds of examination could improve the average estimate, since experience is shown to decrease error in counts. As the applicability of using average TCA counts has been called into question in this study, only the results from R1 and R2 will be analyzed in the remainder of the discussion.

Intraindividual Sample

Intraindividual Sample by Participant

The estimates from this sample do not show any obvious patterns in terms of the amount of TCA display variation between multiple teeth from a single individual. Although the variation in age estimates from all individuals were consistently smaller in R2 than in R1, some are still relatively large. Three of the participants (28, 75, and 80 years of age) have combined age estimate ranges from all three teeth that span more than 15 years. The remaining two participants have much smaller amounts of variation between teeth with age estimation ranges of 5 and 7. The relatively small amount of variation in two individuals whose ages fall in the middle of all participants suggests that there is no relationship between the age of the individual and the amount variation in their teeth.

Intraindividual Sample by Tooth Type

When analyzing this sample by tooth type, between both rounds of examination the UI1 teeth have the highest estimate error range of 65 years, while the LC teeth have the lowest range of 15. The UI2 teeth have a range of 37, while the remaining UP4, LI1 and LI2 teeth have similar ranges, falling between 22.5 and 25.5. These results suggest that pathological lower canines have the least amount of variation in the display or visibility of TCAs, making them the optimal choice for the application of cementochronology when there are multiple diseased teeth available to choose from. In addition, due to the high degree of variation in TCA display in upper central incisors, it is possible that they should be not be the first choice for researchers. However, three of the UI1 teeth used in this study produced estimates that were relatively close to the known age, suggesting the high amount of variation does not significantly affect the resulting estimates.

All the teeth from the two youngest participants (28 and 33 years of age) produced estimate ranges above or including the correct age in both rounds of examination. The majority of teeth from the remaining participants (64 to 80 years old), produced estimate ranges below or including the correct age in both rounds, with the exception of the UP4 from the 80-year-old which falls slightly above the correct age in the first round. It seems as if the visibility of TCA's for all types of pathological dentition are consistently overestimated in younger individuals and underestimated in older individuals.

Interparticipant Sample

As with the intraindividual sample, the results of the interparticipant sample indicate that TCAs are mostly overestimated in younger individuals and underestimated in older individuals. The teeth from the youngest individuals all produced estimates that fall at or above their known ages while all the teeth from the older individuals produced estimates that fall at or below their known ages, except for those from the 63 and 72-year-olds in R1 and one of the 75-year-olds in R2. However, the relatively small sample size and absence of representation of median age groups means this pattern cannot be confidently be applied to the general population.

There does not seem to be any relationship between the age of the individual and the error of estimates their teeth produce. The only teeth to consistently produce estimates in both rounds that include or fall within one year of the known age come from the 33 and 63-year-old participants. The teeth from the 38-year-old and one of the 75-year-old individuals produced estimates with the highest degrees of error in both rounds. This means that the amount of error in estimates from this sample is more likely to be due to the relative type or severity of pathology present, and not increased age.

Possible Sources of Error in Results

One of the main sources of error in age estimates produced during the course of this study is likely to be observer inexperience. This is confirmed by the fact that error consistently decreased in the second round of estimates compared to the first, as experience provided less confusion about the distinction between annulations. Additionally, more experience in the second round of observations led to better images that depicted that annulations more clearly (*Figures 27 & 28*). Therefore, the inexperience of the researcher in both using the microscopic equipment as well as observing the TCAs themselves can result in erroneous age estimates.

As discussed in Chapter 2, several researchers have noted the irregularity of TCAs on periodontally diseased teeth. Although the reasons for extraction are unknown for the teeth used in the current study, it is likely that the they were removed due to a pathological condition such as periodontitis and, thus, their TCAs have been altered or damaged. Such damage interrupts and blurs the annulations, making

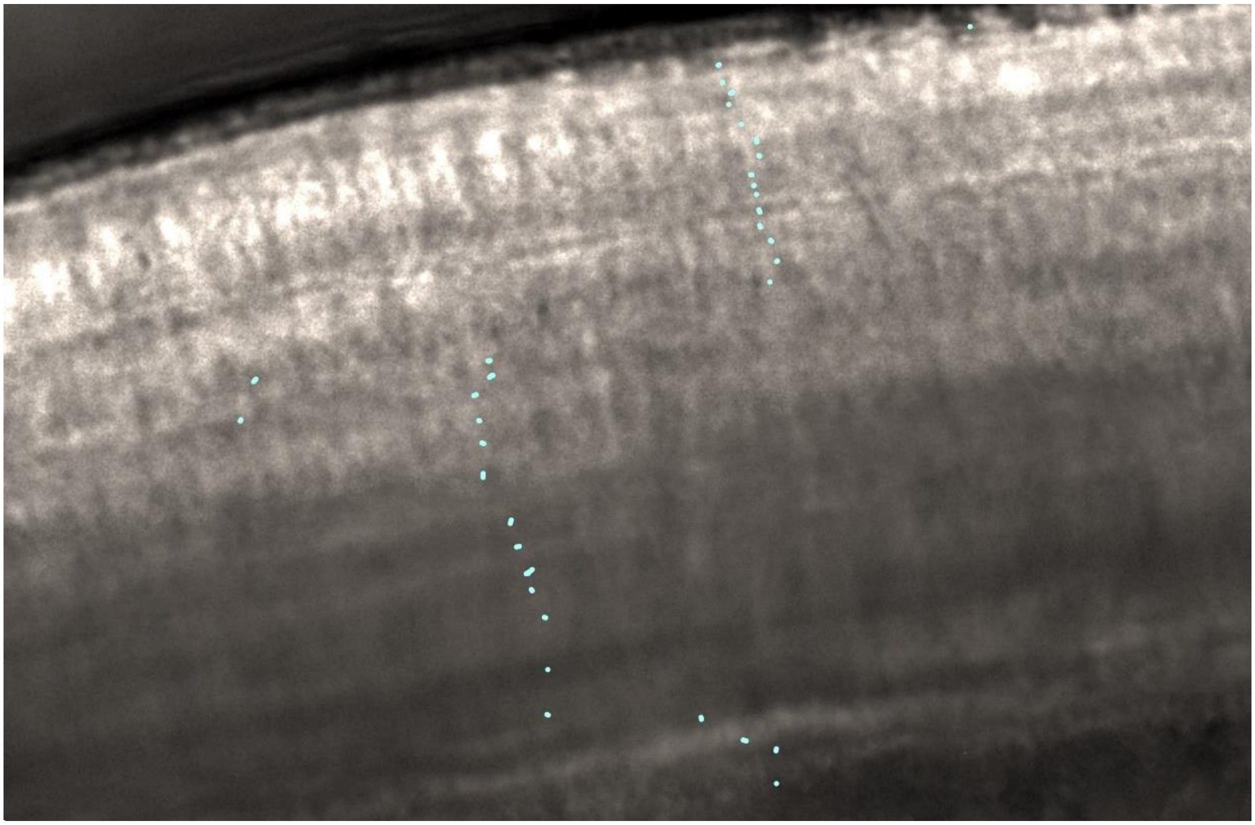

Figure 27. Image of LC tooth taken from participant AD-08. This image is from R1 and the light blue dots mark TCAs

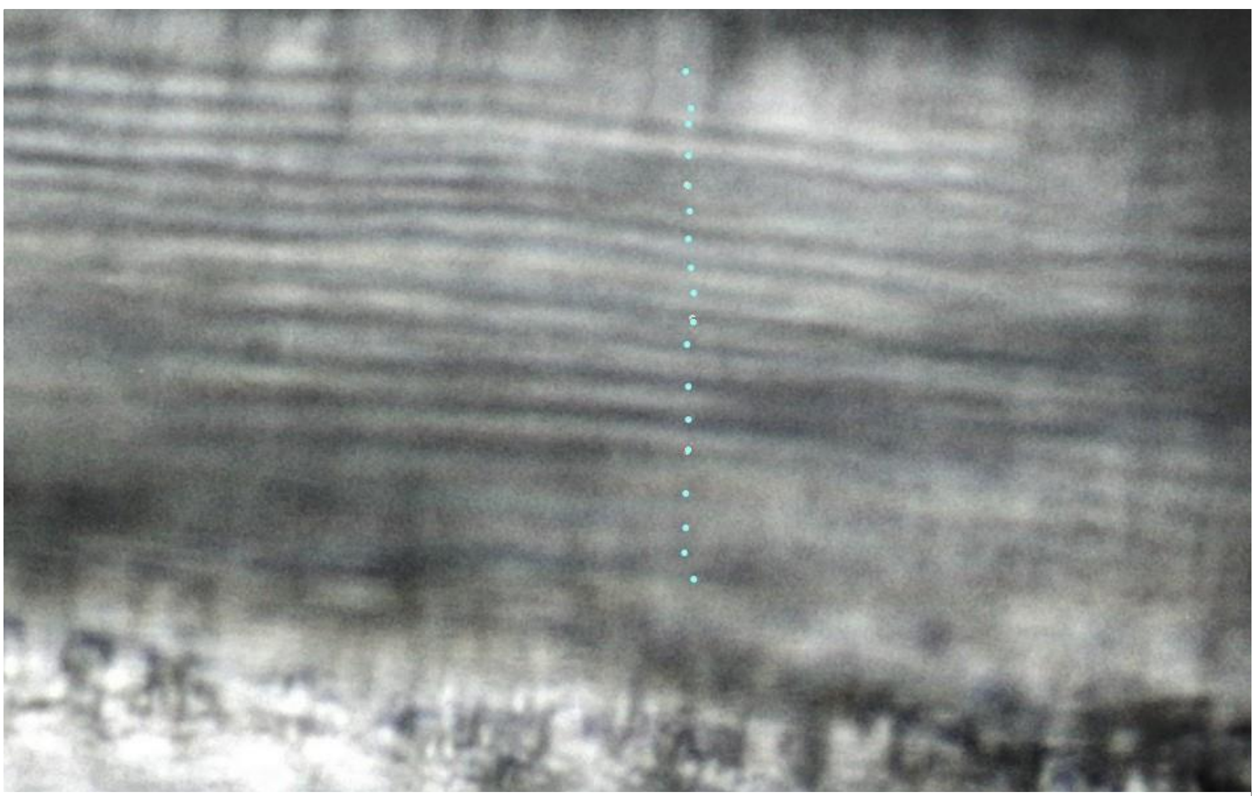

Figure 28. Image of LC tooth taken from participant AD-08. This image is from R2, showing significantly clearer TCAs than the image from R1 (Figure 27)

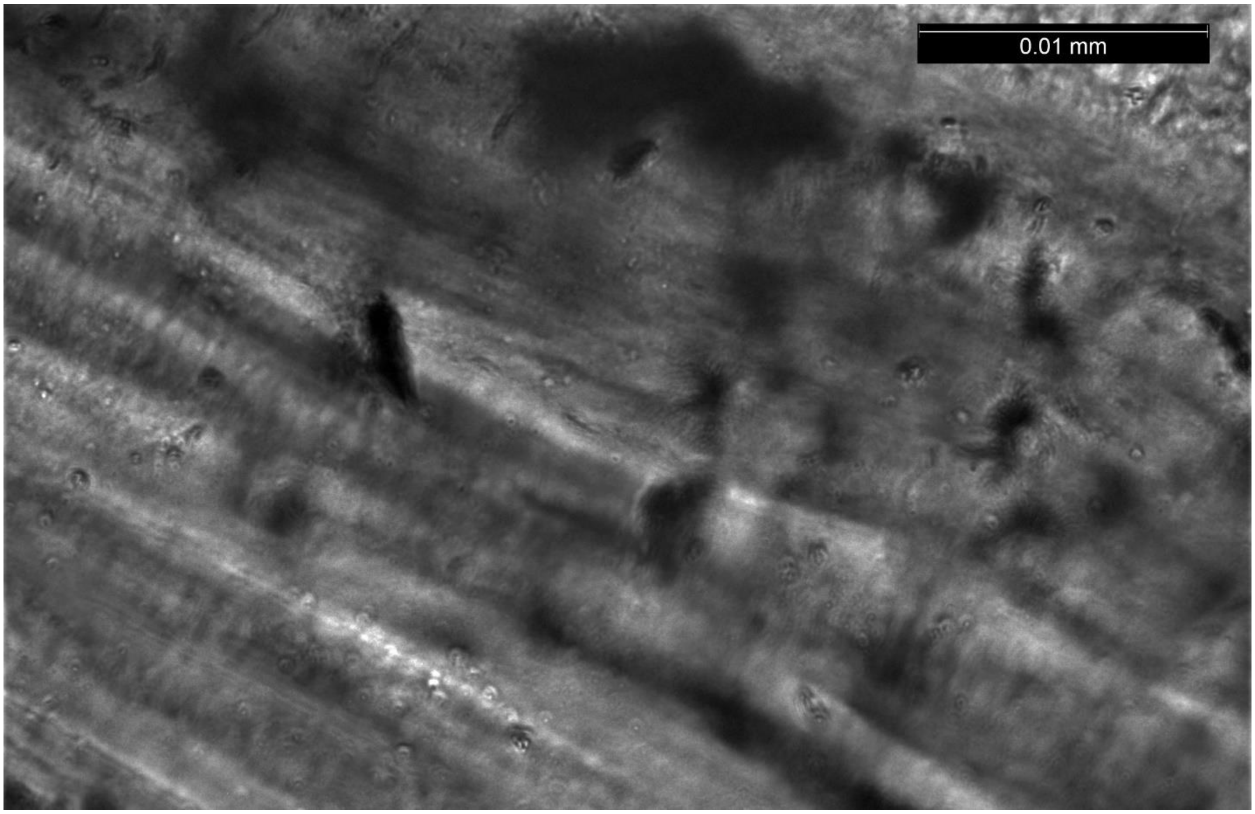

Figure 29. Image of tooth 42 taken during R1 (large dark areas interrupting TCAs are pathological)

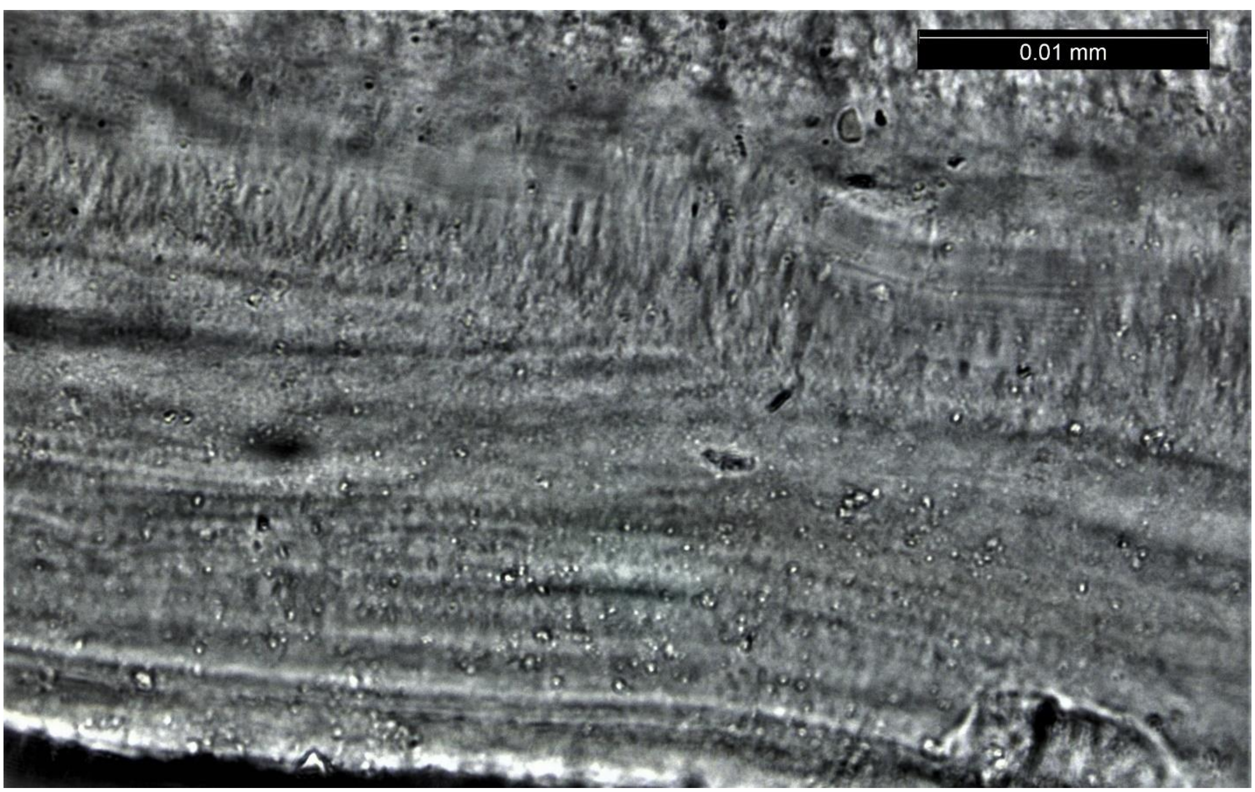

Figure 30. Image of tooth 42 taken during R2, still showing pathological disruptions in TCAs, though with much less severity than shown above in Figure 29

them difficult to distinguish from on another (*Figure 31*). During the two rounds of investigation, the images were sometimes taken from different areas of the circumference of the thin section, chosen solely for the visibility of the annulations. This means that the relative error rates in a single tooth between R1 and R2 or between different teeth may be attributed to the presence and severity of pathology in the specific area observed (*Figures 29 & 30*).

Chapter 6 – Conclusions

Cementochronology has been established as a robust method for estimating age in skeletonized individuals through the publication of numerous studies over several decades. The reliability of this method has been proven in multiple studies using human and other mammalian dentition. Despite confirmation of its reliability by many researchers, others hesitate to implement its use in their own work, partly due to the uncertainty of its applicability to pathological dentition.

Despite producing results far outside of the previously reported error range of ±2.5 years (Wittwer-Backofen et al., 2004), the method still has great potential to provide useful age estimates when applied to pathological teeth. The main sources of error in this study are most likely observer inexperience and irregularity of TCAs due to the presence of pathology. Pathology such as periodontitis damages the cementum and disrupts or blurs the annulations, making them difficult to distinguish from one another. Error from both sources can be diminished by repeating the technique and gaining experience in the process. Estimates were closer to the known age in the second round of observations compared to the first round, as experience provided better ability to both observe or recognize distinct annulations and to select areas of the thin section for observation. Additionally, the presence of pathological landmarks can be better recognized and the ability to acquire counts can be improved despite their presence through added experience.

Intra-observer error in cementochronology can be improved by increasing the number of times each tooth is examined, as experience reduces the amount of error in estimates. Taking averages of the TCA counts to produce new estimates is not applicable in studies such as this one with relatively small sample sizes and few rounds of observation. It is possible that the averages could be more effective for a more experienced researcher, by either increasing the amount of observations made on each tooth, or both.

The results of the research presented here indicate that there can be a high degree of variation of TCA display between diseased teeth from a single individual. There also seems to be a high degree of variation in the error of estimates produced from upper central incisors and a relatively low degree of variation in lower canines. Both of these observations do not seem to be influenced by age however, one factor that does seem to be influenced by age, is the presence of estimates above or below the known age. Older individuals consistently produced estimates that are at or below their age while younger individuals have the opposite effect. Even though few estimate ranges incorporate the known age of the subject, 66.67% fall within ten years, despite different levels of variation of TCA display based on tooth type or age. It appears that, since the observed variation does not seem to be directly influenced by the respective age of the individual, that it is mostly due to the type or severity of present pathology.

Suggestions for Future Research

It is recommended that future researchers of cementochronology in pathological teeth use larger sample sizes that include individuals in the median age ranges that were not used here (i.e., between 40 and 60-years-old). By doing this, they may be able to obtain a more complete understanding of estimate error distribution patterns for pathological teeth according to age in both interparticipant and intraindividual studies. It is recommended that future researchers use lower canines when multiple diseased teeth are available, as they appear to have the smallest amount of variation in estimate error of any tooth type. Finally, inexperienced researchers should observe and count TCAs on the same tooth as many times as possible since more experience is shown to improve estimations and reduce error over time.

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