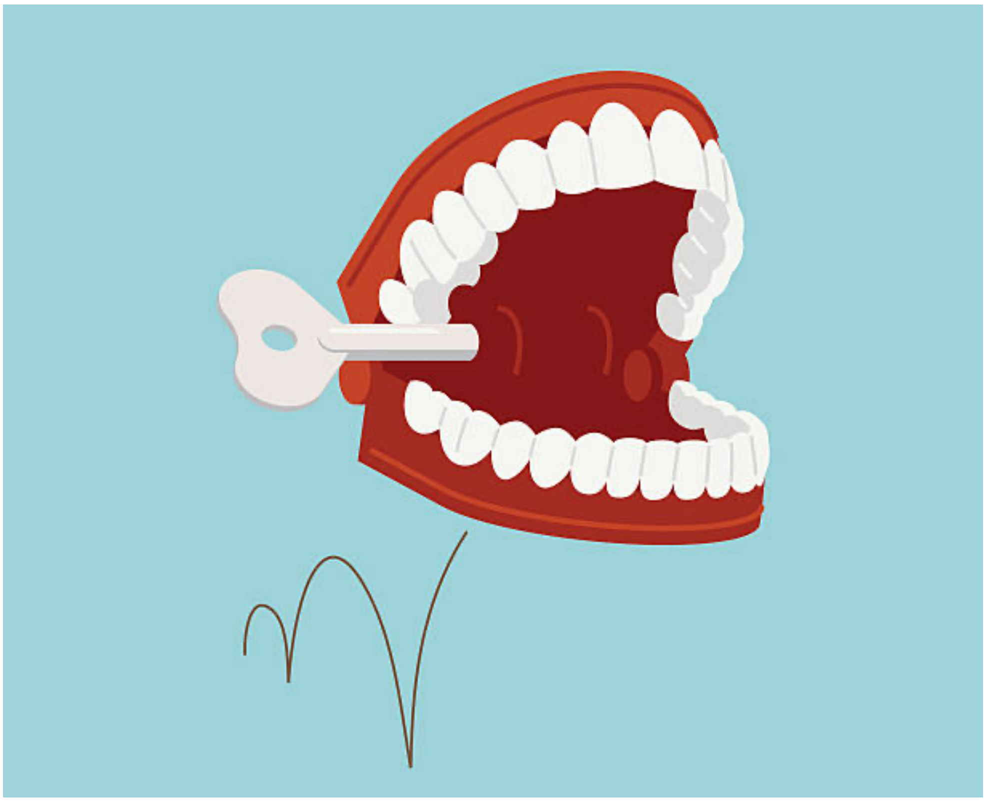
PULLING TEETH:
Application of Cementochronology to an Archeological Known-Age-at-Death Collection.



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Pulling Teeth:

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By Jessica J. van Dam

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Table of Contents

Table of Figures

List of Tables

CHAPTER 1: INTRODUCTION

Cementochronology, or the study of the cementum layer of teeth in relation to age, has been used to estimate age-at-death in humans since work done by Stott et al. in 1981. Cementochronology follows similar principals to dendrochronology, that is, counting tree rings which develop annually thereby 'ageing' a tree (McGraw, 2003). With trees the process is fairly simple because the moment the tree sprouts the layers can be counted and the precise age of the tree determined. However, with teeth, it is not only the cementum layers (cementum being the layer which covers the tooth root and attached to the surrounding bone, anchoring the tooth in place), which must be counted but also the average age at which a tooth erupts; these two numbers are then added and an age can be estimated (Naylor et al., 1985). While this process sounds quite simple, this method is not yet common in osteoarchaeology or in forensic settings.

Cementochronology can be useful in many circumstances, especially archaeological and forensic contexts when an individual's age-at-death estimate is limited by poor preservation or incompleteness of the skeleton. Several previous cementochronology studies using known age–at-death individuals have shown more accurate age estimations are achieved with smaller age ranges than macroscopic morphological techniques (Broucker et al., 2015; Maat et al., 2006; Naji et al., 2014; Naylor et al., 1985; Wittwer-Backofen et al., 2004). However, some of these studies have used individuals whose age was estimated using other skeletal aging methods, so the accuracy cannot be determined (Bertrand et al., 2014). The current methods, most notably the cutting angle, (the angle the saw blade is relative to the tooth's axis when taking the thin slices to create the microscope slides), used by different research groups have led to inconclusive or non-reproducible results (Renz & Radlanski 2006). It remains clear that more known age-at-death studies are needed to clarify and perfect the methodology of cementochronology.

1.1 Research Aim

The aim of this research is to test the reliability of cementochronology by using only known-age-at-death individuals, thereby adding to previous research on this method. It is also a sample collection of completely Dutch individuals which may show different results compared to samples with different ancestry or greater diversity. As part of this reliability test, this thesis will illustrate the efficacy of cementochronology in the hands of a novice practitioner. In addition, this research will offer a brief summary of previous cementochronology research.

Precise age estimates on adult individuals is important in archaeology and osteoarchaeology because it offers insight and greater understanding of the lives and deaths of past individuals. With more precise aging techniques osteoarchaeologists and archaeologists can correlate age to living conditions, disease prevalence, health, daily activities, and social practices. Cementochronology has the potential to be one tool for achieving greater precision in age estimation. It is also relevant for more recent deaths in forensic cases when a precise age could lead to the positive identification of a victim (Dirkmaat et al., 2008).

This research will use, when possible, a methodology protocol, known as the certified ISO 9001 protocol, but which will be referred to in this research as "the standard protocol", which was established in 2013 at the Direction de l'Archéologie in Douai, France. This protocol is used to help increase methodological consistency between researchers within cementochronology. It is summarized in *Figure 1* below, but essentially offers the steps and tools needed to create the thin section slides for microscopic analysis and documentation (Colard et al. 2015, 4). However, due to limitations with the descriptions within the standard protocol and availability of materials, deviations from the standard protocol were made. These deviations will be discussed in full in Chapter 4: Methods, as well as the limitations of the standard protocol in Chapter 6: Discussion.

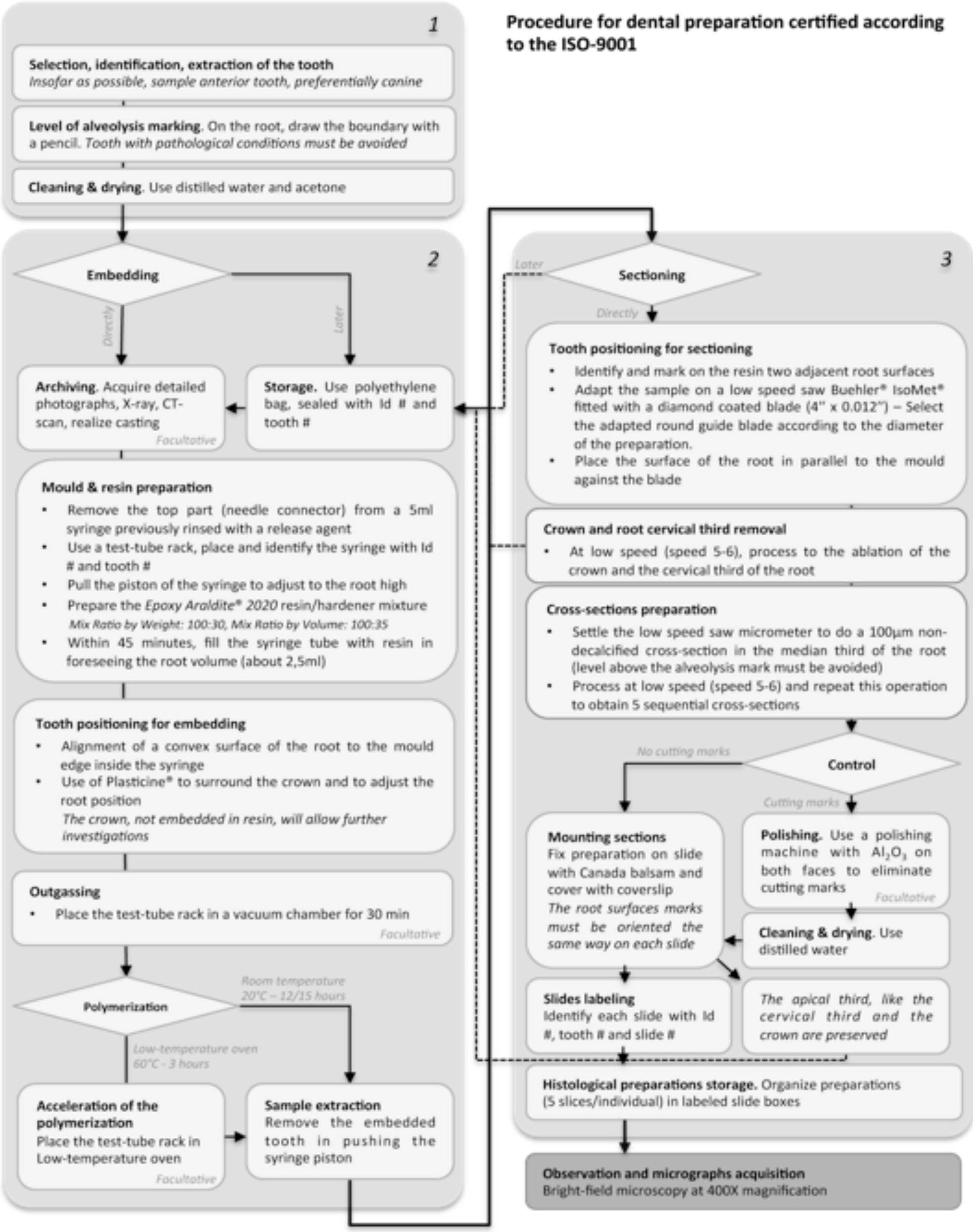

Figure 1. Flow chart designed by the cementochronology research program showing a methodology created from previous successful research. This is also known as the certified ISO 9001 protocol (Colard et al. 2015, 4).

1.2 Research Questions

The objective of this thesis is to investigate cementochronology as a reliable age-at-death estimation technique. Specifically, it will look at the methods outlined by the Cementochronology Research Program (Colard et al. 2015) and assess the practicality of this method for a novice researcher. For this thesis, a known age-at-death sample of 20 individuals has been selected from the Middenbeemster archaeological collection at the University of Leiden. This is due to archival records from the cemetery from which the collection was excavated which gives the identities and age-at-death for those individuals. The 20 individual sample was determined for a couple of reasons. First being that 20 is a sufficient number to run statistical tests and obtain meaningful results (according to the authors initial academic supervisor). Secondly, the method is both labour intensive and destructive. To process (extract, clean, cut samples, make slide and capture images of the slides) all 20 teeth took over three weeks, each tooth taking about 28 hours (though due to epoxy dry times some of this time was overlapped with other teeth). Therefore 20 was suggested by the authors initial academic supervisor who was familiar with the skeletal collection and deemed that there were 20 individuals who would fit the needs of this study and that 20 would be significant enough to notice a trend. This analysis will be run as a blind test. Specific research aims are as follows:

1.) Does the Cementochronology Research Program (CRP) protocol accurately and effectively estimate age-at-death in a Dutch post-medieval skeletal collection

Sub-questions:

i. Are the methods specified by the Cementochronology Research Program (CRP) practical and sufficient for the average or novice researcher?
ii. When methods are not specified for a particular step, does that compromise the outcome?

1.3 Thesis Structure

This thesis will be comprised of seven chapters. This chapter (Chapter 1) has covered an introduction and provides the aims of this thesis. Chapter 2 gives detailed literature review of the methodology used in age-at-death estimations. It also provides an introduction to cementochronology and an overview of previous cementochronology research. Chapter 3 provides the background and history of the study samples included in this research. Chapter 4 discusses the methods used for this research. Chapter 5 presents the results of this research. Chapter 6 discusses the research questions posed in Chapter 1 relative to the results. Chapter 7 concludes this thesis and is followed by the bibliography in Chapter 8.

CHAPTER 2: BACKGROUND

2.1 Age Estimation Techniques

In human osteoarchaeology there are several ways to estimate the age-at-death of an individual. In non-adults the growth and development of the skeleton is marked by somewhat regular changes which can help estimate age with relative accuracy, providing small age ranges (Schaefer et al., 2009). However, as people age and bone growth and development is completed, the skeleton begins a slow rate of deterioration which varies between individuals and between population groups (White et al., 2011). This can be further complicated by age, sex, ancestry, occupation and pathologies, which can have varied and different effects on bone structure, development and deterioration. In addition to this, some methods can be difficult to follow if the researcher is inexperienced, and the resultant large age brackets (which can be more than +/-20 years) all contribute to difficulties in accurately and precisely aging adult individuals. Even so, adult cranial and post-cranial aging techniques have been improved over the years (Ubelaker, 2008). Initially these methods were limited in that they were developed using specific populations (such as only white males), making their application less accurate when used on other populations (Ubelaker, 2008). However, recent research into the application of these methods on multiple and varied populations has improved their reliability and accuracy (Ubelaker, 2008; White et al., 2011). Despite these improvements, cranial and post-cranial aging methods remain limited in their precision, leaving researchers with large age ranges which can be problematic if there is legal relevance as in forensic cases (White et al., 2011).

The most popular of these age-at-death estimation methods are: the morphology of the symphysis of the os pubis; the morphology of the auricular surface of the ilium, cranial suture closure; and the morphology of the fourth sternal rib end (Garvin & Passalacqua 2012). Each of these methods will be summarised in the following sections and their limitations highlighted.

2.1.1 Pubic Symphysis

The pubic symphysis is where the two pubis bones meet on the anterior of the pelvis. The pubic symphysis morphology method has gained popularity with the work of Suchey and fellow researchers in the 1980s and 1990s (Katz & Suchey 1986; Brooks & Suchey 1990). Suchey and her colleagues (1990) used a collection of pubic symphyses gathered from autopsy, with mostly known age-at-death and known sex individuals. This study moved forward from the original research done by Todd in 1920 which had laid out a six-phase series of age groups of exclusively white males (Todd, 1920). Suchey and Brooks expanded this research to include both male and female samples, with six phases for both sexes (1990). This newer method looks at the overall morphological surface of the pubic symphysis by observing the way the pubic symphysis surface changes with increasing age. The earliest phase (1) shows a billowy surface with no defined edge while the later stages show a decrease or elimination of the billows which are replaced by a more even surface with a defined raised edge (*Figure 2*). While fairly reliable for individuals under 40 years old, Brooks and Suchey found it was a poor aging technique for older individuals at 40 years or older.

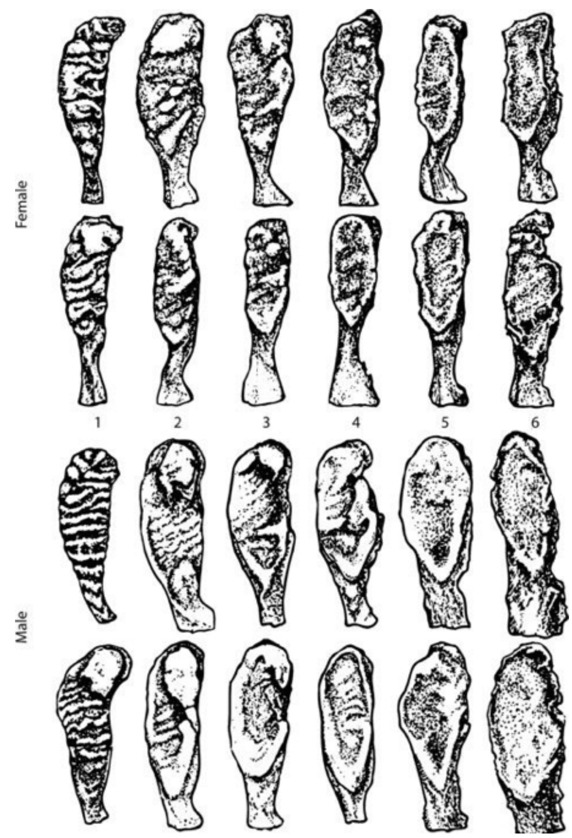

Figure 2. The 6 phases of pubic symphysis aging in both females (top two rows) and males (bottom two row). Left to right: youngest to oldest. (Suchey & Brooks, 1990 in white et al., 2011, 398).

2.1.2 The Auricular Surface

The auricular surface is the area on the ilium (a part of the hip bone) where it meets the sacrum (the bone at base of the spine the which connects to the hips). The method for determining age by examining the morphology of the auricular surface of the ilium was not developed until the mid1980s by Lovejoy and colleagues. Buckberry and Chamberlain improved upon Lovejoy's method in 2002 because the original methodology was difficult to replicate. They determined eight phases of aging, which are described using the terms transverse organization, surface texture, microporosity, macroporosity, and apical changes (Buckberry et al. 2002). The revision made the ilium method for aging much more accessible and increased the precision of the age estimates in older adults between 50 and 69 without decreasing its applicability to younger adults (White et al., 2011). However, the overall effectiveness of this method is questionable considering that the estimated age ranges can be very large as seen in table 1, below, showing the data from Buckberry and Chamberlain (2002).

Table 1. Table from buckberry and chamberlain's work on auricular surface of the ilium aging technique (2002). Composite score refers to the five descriptive terms: transverse organization, surface texture, microporosity, macroporosity and apical change

Composite Score	Stage	Mean Age and Standard Deviation	Median Age	Age Range
5 or 6	1	17.33±1.53 years	17 years	16-19 years
7 or 8	2	29.33±6.71 years	27 years	21-38 years
9 or 10	3	37.86±13.08 years	37 years	16-65 years
11 or 12	4	51.41±14.47 years	52 years	29-81 years
13 or 14	5	59.94±12.95 years	62 years	29-88 years
15 or 16	6	66.71±11.88 years	66 years	39-91 years
17, 18 or 19	7	72.25±12.73 years	73 years	53-92 years

2.1.3 Cranial Sutures Closure

The use of cranial suture closure as an age estimation technique was first developed Todd and Lyon in the mid 1920s. In their 1925 study they discuss the average ages of cranial suture closure on several sites of the cranium. Later in 1985, Meindl and Lovejoy adapted Todd and Lyon's 1925 research into a standardized method which identifies ten specific locations along the different sutures (Figure 3). This method rates the amount of closure on a scale from 0 to 3 with 0 being no ectocranial closure and 3 being full closure of the suture (Meindl & Lovejoy 1985, p.60). These sets of locations are broken into seven 'vault' locations and five 'lateral-anterior' locations, which includes two sites found in the vault section. The closure ratings at each site is added together and the composite scores are correlated with an age range and standard deviation. This data can be seen in Table 2 (Meindl & Lovejoy 1985; White et al. 2011). In 1994, Buikstra and Ubelaker updated this method to include 17 sites including several palatal sites originally laid out by Mann et al. in 1991, but they failed to include the other sites within the composite score system laid out by Meindl and Lovejoy (Mann et al., 1991; White et al., 2011). Years of research on this method have shown that cranial sutures close at varied rates, although the sphenooccipital synchondrosis is useful in establishing a minimum age as it is usually completely closed by 20-29 years (Krogman & Işcan, 1986). What is useful about the cranial sutures closure method is that if a skull is fragmentary but some of the sutures and the precise locations can be identified, they can still be used to estimate age.

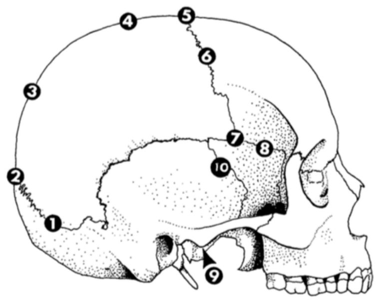

Figure 3. The original ten suture locations as laid out by Meindl and Lovejoy 1985.

Table 2. Table which shows the composite scores from cranial suture sites with the corresponding estimated ages as laid out by Meindl and Lovejoy 1985.

'Vault' Sutural Ages			'Lateral-anterior' Sutural Ages		
Composite Score	Mean Age	Standard Deviation	Composite Score	Mean Age	Standard Deviation
0	-	-	0	-	-
1-2	30.5	9.6	1	32.0	8.3
3-6	34.7	7.8	2	36.2	6.2
7-11	39.4	9.1	3-5	41.1	10.0
12-15	45.2	12.6	6	43.4	10.7
16-18	48.8	10.5	7-8	45.5	8.9
19-20	51.5	12.6	9-10	51.9	12.5
21	-	-	11-14	56.2	8.5
			15	-	-

2.1.4 Fourth Sternal Rib

The aging method using the fourth rib's sternal end is a technique which relies on three features of the rib: the pit depth, pit shape, and rim and wall configurations(Işcan et al. 1984). İşcan and colleagues determined six phases with good accuracy. However, their descriptions of morphological features are confusing which makes the method hard to replicate by less experienced researchers (White et al., 2011). Another problem with this method is that the identification of the fourth rib is often difficult, especially in archaeological cases. Preservation is a problem in archaeological settings and ribs are often damaged by taphonomic processes or poor excavation. Taphonomic processes, which are the external environmental factors which can effect the bone, weaken the already fragile sternal rib ends which are composed of a thin layer of bone compared to more robust bones such as the femora or humeri. This method also depends on known sex and ancestry which limits the use of this method for unknown individuals. However, given that this method is often combined with the others mentioned above, a good age estimate can be determined. Table 3 shows İşcan and colleague's results from their 1984 study, which illustrates the age ranges determined from the different features and stages of the aging fourth rib. They have broken the data down into each stage then added them up for a composite component score (İşcan et al. 1984). For younger individuals the age ranges stay small, with the smallest composite range 0 in the first stage where the composite score is 0 and the estimated age is 17. However, past age 21 the age ranges increase to 15 years and increase drastically from there.

Table 3. Data from İşcan and colleagues (1984) showing the estimated age ranges found using the fourth rib in

Stage or score	N	Mean age	SD	SE	95% Confidence interval of mean	Age range
I—Pit depth						
1	9	20.3	3.32	1.11	17.8–22.9	17–25
2	29	30.7	12.40	2.30	26.0–35.4	18–64
3	31	40.9	13.72	2.46	35.8–46.0	21–67
4	9	55.0	15.39	5.13	43.2–66.8	32–76
5	4	57.5	12.92	6.46	36.9–78.1	44–70
Total	82	37.9	16.15	1.78	34.8–40.9	17–85
II—Pit shape						
1	4	17.3	0.50	0.25	16.5–18.0	17–18
2	15	22.8	3.28	0.85	21.0–24.6	18–30
3	28	30.5	9.61	1.82	26.8–34.3	19–66
4	22	47.1	11.61	2.48	41.9–52.2	26–67
5	15	61.6	12.94	3.34	54.4–68.8	44–85
Total	84	38.4	17.26	1.88	34.7–42.2	17–85
III—Rim and wall configurations						
1	5	17.8	1.30	0.58	16.2–19.4	17–20
2	25	24.1	3.55	0.71	22.7–25.6	18–31
3	20	34.3	11.62	2.60	28.9–39.7	21–66
4	16	49.5	11.21	2.80	43.5–55.5	32–71
5	16	58.2	11.53	2.88	52.0–64.3	43–76
Total	82	37.8	16.67	1.84	34.2–41.5	17–76
Total component scores						
3	3	17.0	0.00	0.00	17.0–17.0	17–17
4	2	19.0	1.41	1.00	17.0–31.7	18–20
5	4	22.5	3.32	1.66	17.2–27.8	18–25
6	7	23.1	4.06	1.53	19.4–26.9	18–30
7	12	24.9	3.63	1.05	22.6–27.2	19–31
8	9	27.0	4.90	1.63	23.2–30.8	21–36
9	10	37.8	13.21	4.18	28.3–47.3	24–66
10	8	47.1	12.03	4.25	37.1–57.2	30–64
11	6	48.5	9.89	4.03	38.1–58.8	41–67
12	7	47.6	11.75	4.43	36.7–58.4	32–67
13	5	56.0	10.32	4.61	43.2–68.8	44–71
14	4	63.5	12.26	6.13	44.0–83.0	52–76
15	4	57.5	12.92	6.46	36.9–78.1	44–70
Total	84	37.3	16.81	1.83	33.8–41.0	17–76

2.1.5 Macroscopic Aging Techniques in Context

All of these methods (the pubic symphysis, auricular surface, cranial sutures, and fourth sternal rib ends) are often used together along with several others including cementochronology when estimating the age of an individual (Garvin, 2012). It is recommended by osteological professionals to average out the estimated ages gained from each method to reach a more accurate age range (Ubelaker, 2008; Garvin & Passalacqua 2012). However, each technique presents familiar problems. Preservation of the human skeleton is an issue which any study of archaeological material face. The biological nature of remains inherently leads to eventual decay and degradation meaning there is often very little left to study. This poor preservation is often compounded by damage inflicted during excavation, which can lead to missing small bones or further damage to delicate surface structures such as sternal rib ends or the pubis, which can be hit first by an unwary excavator with a shovel. Additionally, the macroscopic techniques present their own problems: interpretation of samples is highly dependent on the observations of the researcher and their experience in identifying the macroscopic features. This can lead to misidentification of age groups. Most macroscopic methods were developed using specific demographics which have limited populations so they must be applied with reservation to individuals who may fall outside the original biological profile. However, researchers often test these methods on different populations which increases their relevance (Gocha et al., 2015). In 2012, Garvin and Passalacqua conducted a study with 145 forensic anthropologists to determine which age-at-death estimation technique was most popular (Garvin & Passalacqua 2012). They found that most were most comfortable with and relied upon pubic symphysis, sternal rib ends, auricular surface, and cranial sutures or a combination of these. Cementum annuli (cementum lines i.e. cementochronology) was reported to be less widely used. Most who participated in the study stated that experience and time in the field were important when determining an age bracket which was often more narrow than the methods originally offered. While these methods can offer a possible large age brackets for an individual, cementochronology can decrease the size of the age bracket and is not limited by sex or ancestry (White et al., 2011).

2.2 Cementochronology

Cementochronology is an aging method that uses histological structures of the acellular cementum layer of teeth to estimate age-at-death. Acellular cementum is the outer layer around teeth roots, which from the time of eruption, adds a new layer annually (Naji et al. 2014; White et al. 2011). It differs from cellular cementum which also surrounds teeth roots but develops irregularly and often focuses on areas of high stress like the apex of the root tip (Naji et al., 2014). The cementum layer (both cellular and acellular) surrounds the dentin of teeth roots and helps to anchor the teeth within their sockets (Naji et al., 2014). These structures are illustrated in *Figure 4*.

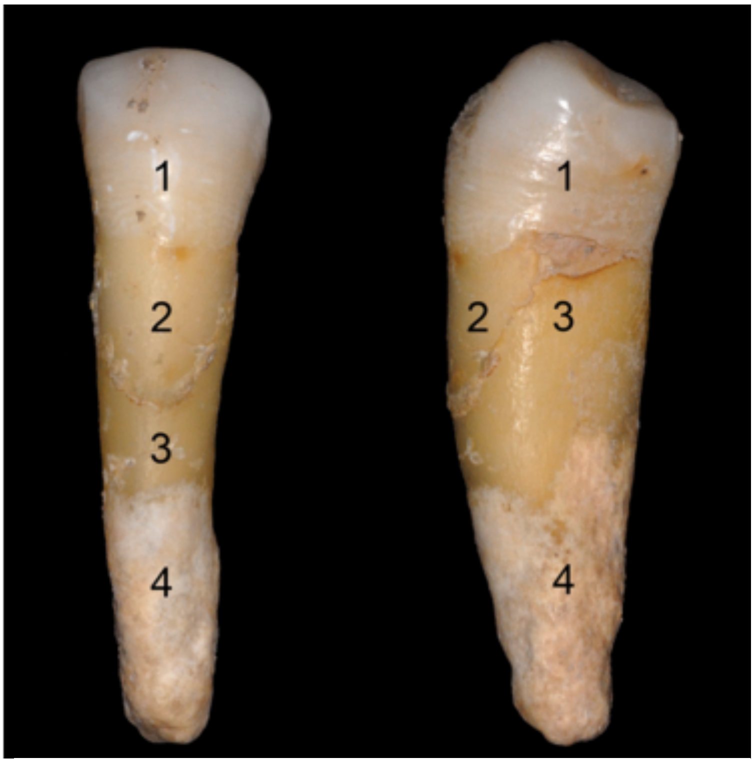

Figure 4. Premolars from Naji et al., 2014 study showing 1. Enamel, 2. Root dentin not covered by cementum, 3. Acellular cementum and 4. Cellular cementum.

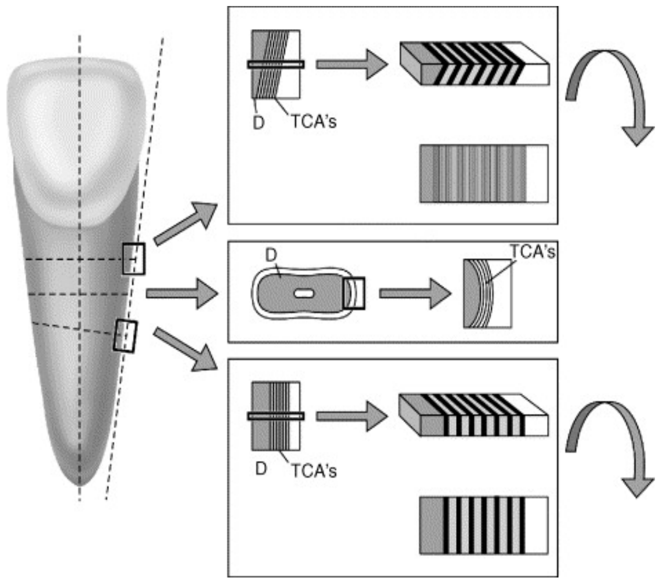

Figure 5. Shows a simplified illustration of the light and dark lines of the cementum layer and cutting angle used by Maat et al. 2006. TCA stands for and D is indicating the dentin of the tooth.

Acellular cementum layers are composed of light and dark lines when viewed under a microscope as illustrated in Figures 5 and 6, with each pair of lines indicating one year of growth (Bertrand et al., 2014; Maat et al., 2006).

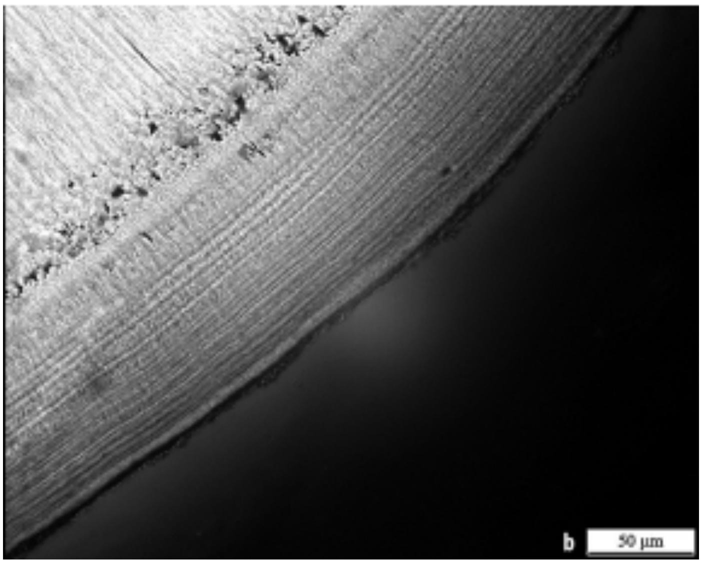

Figure 6. Shows a microscopic view of the light and dark lines of the cementum layer taken by Naji et al. 2014 (5).

It is unclear what causes these lines to appear with such regularity but they appear in all mammals, even those without clear seasonal changes to diet or environment (Naylor et al., 1985). Unlike bone which reabsorbs itself as it creates new bone, acellular cementum deposits new layers of tissue cumulatively (Naji et al., 2014). The formation of annual rings makes it an ideal method for estimating age-at-death because unlike macroscopic traits, these lines can be counted and be added to age of eruption to estimate age (Naylor et al., 1985). It is not as simple as dendrochronology, which counts tree rings to estimate age, because the lines in acellular cementum can be hard to visualize or differentiate. Methodologies for this technique are still being developed and will be discussed in section 2.3. Teeth are often the best preserved tissues in archaeological skeletal remains, which makes any reliable age estimation method using teeth highly desirable (Renz & Radlanski 2006). The following section provides a detailed literature review of various methodologies and techniques that use cementochronology.

2.3 Previous Research in Cementochronology

The idea for cementochronology initially began with research in animals (Stott et al., 1981). It was initially unclear whether this method could be used on non-hibernating animals such as humans (Naji et al., 2014). In the 1980s cementochronology was applied to humans with fairly good success resulting in several studies over the next four decades with varied outcomes (Stott et al. 1981;

Naylor et al. 1985; Wittwer-Backofen et al. 2004; Maat et al. 2006; Renz & Radlanski 2006; Aggarwal et al. 2008; Roksandic et al. 2009; Kasetty et al. 2010; Gocha & Schutkowski 2013; Gauthier & Schutkowski 2013; Bertrand et al. 2014; Gupta 2014; Naji et al. 2014; Broucker et al. 2015; Colard et al. 2015). This remains a seldom-used method due to inconsistent methodologies and the time-consuming and destructive nature of the process. Some researchers such as Renz and Radlanski have questioned if this method is worth the time and effort it demands and have identified its variable success in the hands of inexperienced researchers (Renz & Radlanski 2006). Another concern is that this method is destructive which, unlike macroscopic methods, poses ethical problems for important archaeological specimens (Naji et al., 2014). In 2012, scientists formed the Cementochronology Research Program (CRP) in the hope of solidifying a clear and logical protocol to make this method more streamlined for future researchers (Cementochronology.com, n.d.; Naji et al., 2014; Colard et al. 2015). There is also more recent research which uses radiographic technology to view cementum lines, eliminating the destructive nature of the technique and increasing accuracy in counting the lines by using custom-made software (Newham et al. 2021).

Since the 1980s there has been one group of French researchers who have taken a particularly active role in promoting cementochronology as a reliable aging method (Bertrand et al., 2014; Colard et al., 2018; Naji et al., 2014; Naylor et al., 1985; Stott et al., 1981). Naji and colleagues (2014) whose seminal work 'Cementochronology, to cut or not to cut?' offers an overview of the history of the method while introducing readers to their advances in organizing a research protocol. The paper also clarifies and provides solutions for the shortcomings voiced by previous researchers (Naji et al., 2014). The CRP was established in 2010 by Naji and colleagues (2014) in France and reached international researchers in 2012 at the American Association of Physical Anthropology annual meeting. Through this program, a protocol has been established laying out the recommended methods which have been shown to be the most effective when using cementochronology. This protocol has been made into a flow chart and can be found in Chapter 1.1, Figure 1.

Colard et al. specified in their article –*toward the adoption of cementochronology in forensic context*– that while any tooth type can be used for cementochronology, there is preference to the use of anterior and single rooted teeth (Colard et al., 2018). Upper canines have long roots, increasing the likelihood of their preservation *in situ* in archaeological remains making them ideal candidates for cementochronology (White et al. 2011; Renz & Radlanski 2006). Because of the likelihood of preservation within the skull, these teeth also often have well persevered cementum layer. Colard et al. (2015) suggest outlining the acellular cementum, which can sometimes break off the surface of the root unevenly, with a graphite pencil. The teeth are then washed in water, dried, and rinsed in acetone which removes any further moisture and evaporates quickly. In their article, they stipulate that the tooth should be imbedded in an epoxy resin such as Araldite 2020 with the root surface parallel to the mould edge so that the cut can easily be made at a 90^{o} angle to the root surface (Colard et al. 2015). These cuts are made with a diamond edge saw; ideally, five sequential slices are taken. The slices can then be mounted on glass slides, labelled, and viewed at 400x magnification under a microscope (Colard et al. 2015). For repeatability, digital photographs of the magnified slides are taken. This allows the images to be enhanced by increasing contrast and clarity. This method has since been applied to individuals with pathological conditions and on teeth with periodontal disease (Bertrand et al., 2014; Broucker et al., 2015). Both studies present good results finding good correlation between estimated and actual age.

Maat and colleagues (2006) made a significant contribution to cementochronology research by specifying the ideal cutting angle when preparing the thin sections. They point out that single rooted teeth are cone shaped and so the annular layers of cementum would be added as a cone. If that cone is cut perpendicular to its axis, the layers would overlap if seen through a microscope (Maat et al. 2006). However, if the cone was to be cut perpendicular to its exterior surface, the layers would line up when viewed under a microscope. This is dissimilar to tree ring annulations or dendrochronology which many compare to cementochronology (Renz & Radlanski 2006). Maat et al. (2006) include a clear and useful illustration shown in Figure 5. They concluded that the cutting angle should be perpendicular to the exterior surface of the root, a guideline which was adopted by the CPR's Protocol in 2010.

In 2004, Wittwer-Backofen and colleagues (2014) conducted a validation study of cementochronology. Unlike later researchers they focused less on methods but more on the results of their study. Their sample was very large compared to previous and later studies with 363 single rooted teeth deemed suitable for the research (Wittwer-Backofen et al., 2004). These teeth were extracted from living patients so their age-at-extraction, sex, and ancestry were known to the researchers. Because the researchers had such a large sample size, they were able to test whether sex, dental pathology, and/or age played any part in the reliability of cementochronology. The ages-at-extraction ranged from 12 to 96 years, with a 1.64:1 ratio of males to females. Tooth type varied but all were single rooted (e.g. incisors or canines). These teeth were extracted and then grouped by five causes: dental caries, periodontal disease, orthodontic care, odonto-prosthetics, and multiple pathologies. They found that in both males and females there was a positive correlation between cementochronology estimated age and actual age with a correlation coefficient of r=0.970 in males and r=0.978 in females regardless of reason for extraction and tooth type (Wittwer-Backofen et al., 2004). For correlation coefficients the results can rang between -1 to +1, negative correlation being -1, no correlation being 0 and +1 being a positive correlation. In this case there r value was nearly +1 showing a very high positive correlation. A number of teeth were excluded after cutting and preparation due to poor image quality of completely uncountable cementum lines due to irregularity such as a "wave pattern" which is often seen in fourth premolars. (Wittwer-Backofen et al. 2004). It should also be noted that fourth premolars, though often regarded as single rooted teeth, often bifurcate and are therefore generally less suitable for cementochronology.

A common problem with most traditional aging methods is that in older adults there is continuous change to the aging landmarks, making the features used for macroscopic aging difficult to isolate and group. This results in an age group for older adults beginning around 65 years with no upper limit. Similar expectations have surrounded cementochronology. However, Wittwer-Backofen et al. (2004)

found no decrease in reliability in older age individuals. Below in Figures 7 and 8, several graphs show the linear relationship between estimated age and actual age in males and females (Figure 7) and the relationship between estimated age and actual age in different tooth types with males and females marked as (O) and (X) respectively (Figure 8).

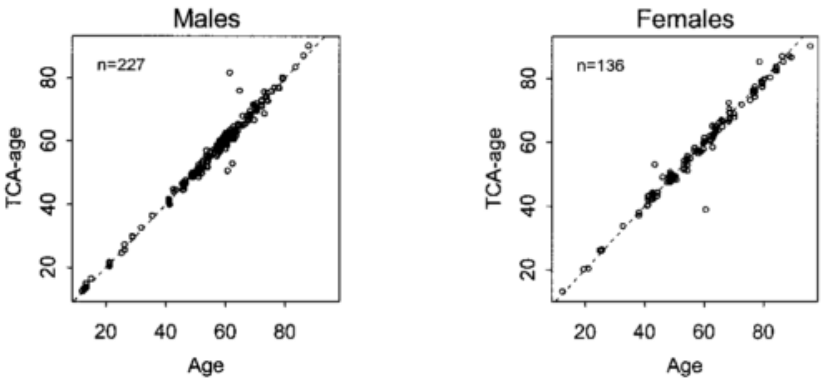

Figure 8. Sex differences in linear relationships between estimated age and actual age (Wittwer-Backofen et al., 2004, 123)

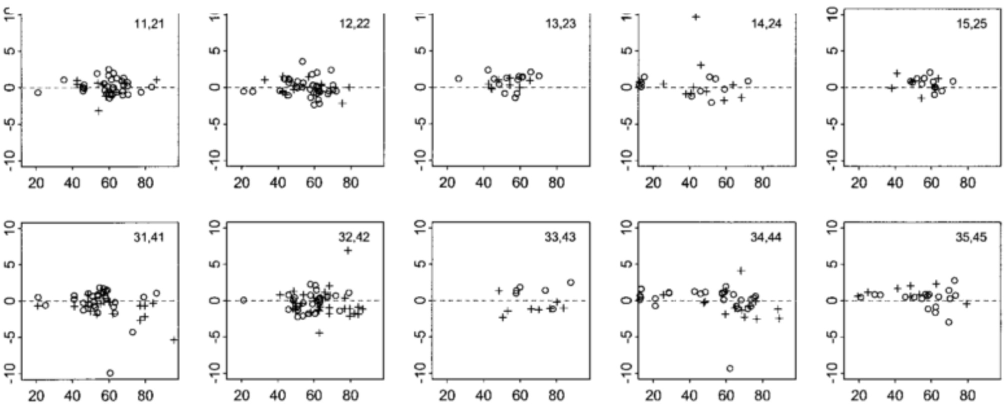

Figure 8. Relationship between estimated age and actual age shown with different tooth type. Maxillary on top and mandibular below (from left to right: medial incisor, lateral incisor, canine, 3rd premolar and 4th premolar).(Wittwer-Backofen et al. 2004, 123)

On occasions when macroscopic aging methods are hindered by damage or disease, cementochronology has been shown to still be useful. Individuals suffering from different types of bone dysplasia (achondroplasia, residual rickets, and osteogenesis imperfecta) as well as an individual diagnosed with leprosy were used in a case study by Bertrand et al. in 2014. Here the researchers found that the normal macroscopic methods proved inconclusive for aging since these diseases have unknown effects on the usual aging markers and landmarks (Bertrand et al., 2014). However, researchers saw no noticeable difference in cementum development in these individuals, suggesting the applicability of cementochronology on pathological individuals when other aging techniques are impaired (Bertrand et al., 2014).

A concern which is present for both archaeological and forensic cases is heat alteration. Teeth often survive fire well given the mostly inorganic composition of enamel and the protection of surrounding bone and soft tissues, especially the roots which are protected by the alveolus for most if not all of the burning process (Schmidt, 2015). This means they are an ideal means of aging in cases where the other aging landmarks are damaged (Schmidt, 2015). Gocha and Schutkowski (2013) investigated the effects of heat alteration to teeth and whether cementochronology could still be used on heat altered teeth roots. They found that while the cementum layer survived extreme temperatures (>600ºC), only 67% of the total number of sections were possible to count. Figure 9 is their graph

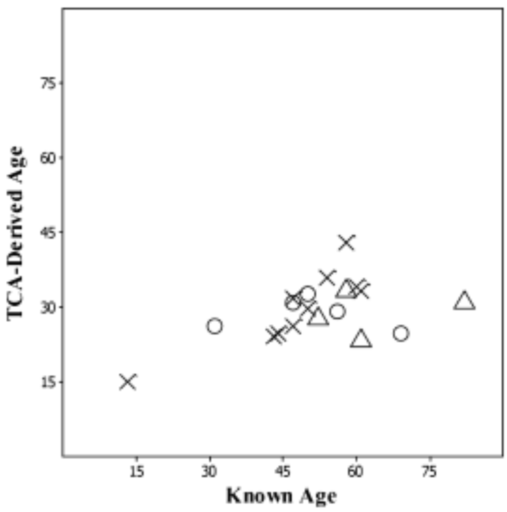

Figure 9. Estimated age vs. Known age with relation to heat alteration (Gocha and Schutkowski 2013, s153)

of the relationship between temperature (600ºC=X, 800ºC=△ and 1000ºC=O) , estimated age. and actual age for 17 individuals. Only the 600º group produced useful results.

There are three studies from 2008, 2010 and 2015 which claim to be about cementum annulation counts but in fact deal with cementum thickness (Aggarwal et al., 2008; Gupta, 2014; Kasetty et al., 2010). While this has no bearing on the current research, it is included to show the range of research being conducted on the cementum layer. In 2008, Aggarwal and colleagues found similar results to Wittwer-Backofen et al. (2004) in a much smaller study of 30 teeth. Specifically, they tested sex, tooth-type, and pathological differences in cementochronology reliability, finding little to no difference in any of these categories. It is important to note that their study used longitudinal sections of tooth roots which differs greatly from previous and later research which favours the middle third of tooth roots and a cutting angle of 90º to the exterior root surface. They also counted the lines with help from computer software by measuring the distance between two lines (y) and the width of the cementum layer (x) and calculating the approximate number of lines (n) based on (n)= x/y. Both differences in procedure make this study less helpful in determining the applicability of cementochronology as an aging technique in archaeological and forensic situations. What sets this research apart is the means of counting, which relied more on the width of cementum rather than an actual count. While this may minimize counting errors, it places greater emphasis on data relating to cementum thickness and age association rather than cementum annulations as is seen in Zander and Hürzeler's research of 1958. Aggarwal et al. concluded that cementochronology was a reliable method when using the methods they had devised.

Gupta and colleagues (2014) followed essentially the same method as Aggarwal et al. (2008) for their research. Gupta et al. (2014) used longitudinal cross sections of 100 teeth and the distance between lines and the cementum thickness to estimate line count. They then measured dentin thickness to see if there was a correlation between cementum annulations count (thickness), dentin thickness, and age. They agreed with Aggarwal et al. (2010) that cementochronology was a reliable method when using the thickness of the cementum layer divided by the average distance between lines .

Kasetty et al. (2010) had a large sample of 200 single rooted teeth cut into two 100μm sections lengthwise. One section from each tooth was viewed on a slide using polarized light microscopy which increases the contrast between lines making them more easily countable. The other section was stained with Alizarin Red, to increase contrast between the lines and an image was captured with stereomicroscope. The cementum lines were then counted in the middle of the tooth root and the width of the cementum recorded from the apical end of the root. They found a positive correlation coefficient of r=0.42 which is considerably lower than others studies which for Wittwer-Backofen et al. who found age ranges no greater than 2.5 years (2004). For cementum thickness Kasetty et al. found a greater positive correlation coefficient of r=0.76. However, even at r=0.76, cementum thickness would not be reliable enough as an aging technique. They had several problems with the cementum lines and visibility, one of which was that some lines seemed to shift from one field of focus to another creating a doubling effect. This problem is explained by the study of Maat et al. (2006) who showed that cutting at a 90º angle to the axis of the root causes line overlapping and makes discerning separate lines difficult. It is possible that because the cuts were longitudinal instead of horizontal, the cementum lines were not aligned in the same way as they would be following the cutting angle of Maat et al. (2006). This misalignment could have caused blurring of the lines so they would be difficult to count consistently, which is the same problem when the tooth is cut horizontally along the axis instead of perpendicularly to the exterior surface. Overall, these researchers claimed that their research did not support the reliability of cementochronology.

In 2005, Renz and Radlanski conducted a validation study on the reliability of cementochronology and found it unreliable. They began with a sample of eight premolars, both third and fourth and upper and lower. The teeth were taken from a clinical dental setting with known age at extraction and teeth with noticeable pathologies were excluded. The team mostly followed the guidelines laid out by Stott et al. (1982). They proceeded to remove five 100μm sections from the middle third of the tooth perpendicular to the axis of the tooth root using a diamond coated saw without embedding the teeth in resin. They further ground these sections down to approximately 80μm and mounted them on glass slides after cleaning and dehydrating. They took digital images of each magnified slide at the buccal, lingual, distal, and mesial side resulting in about 20 images for each of the eight teeth. From these images, the researchers could count the cementum lines. However, they found that many of the thin sections broke and were not usable in the study, so while six of the teeth had a complete series of five thin sections the rest were interrupted by broken sections, so the series was incomplete. This inconsistency is similar to the 'wave lines' described by Wittwer-Backofen et al. (2004). Both researchers counted the lines in each quadrant on each slide for each tooth multiple times, finding it difficult to attain the same or similar count. Much to their surprise, they found that overall the cementum lines in each quadrant were not uniform and could therefore not be relied upon to count and estimate age.

It is important to note a few things about Renz and Radlianski's methodology. The angle at which they cut the roots differed considerably from what has now been established as the more effective angle (Maat et al., 2006). However, Renz and Radlanski performed their research before the publication of Maat et al. in 2006 and the protocol in 2010 so it is understandable that they were unaware of the potential problems regarding cutting angles. By treating teeth exactly like trees they likely misaligned the cementum lines under the microscope by cutting at a 90^{o} angle to the root axis. They also used teeth (premolars) which have been shown to have non-uniform line annulations, which as discussed by Wittwer-Backofen et al. (2004) is in part due to premolars sometimes having bifurcated roots despite being classified as 'single rooted'. The authors also had trouble with the thin sections breaking which may have been because the roots were not imbedded in resin. All these limitations make it unlikely that their results truly demonstrated that cementochronology was as unreliable as they stated.

In 2009, Roksandic and colleagues (2009) applied cementochronology to an archaeological sample from the Mesolithic/Neolithic period and experienced many difficulties. Beginning with 116 individuals with at least one single-rooted tooth, they extracted one tooth from each individual. They removed the crown and upper third of the root from each tooth, embedding the remaining root tip in resin. Next, they cut three 70-80μm sections from the middle third of each root. They then mounted the sections on slides and took digital images of the magnified cementum layer. Out of the original 116 teeth, countable cementum lines only appeared in 40, in at least one slide out of the series of three sections. The rest lacked a cementum layer or had compromised cellular structure. They employed three observers to count the visible lines. However, each attained different results and had age estimates with a +/- 25-year range which is comparable if not worse than some macroscopic methods. It is debatable, though, whether their angle of cutting may have accounted for some of the unclear cementum lines since the researchers failed to mention at what angle they cut their sections. They speculated that taphonomic processes had affected the cellular structure of the cementum layer leaving it unsuitable for cementochronology (Roksandic et al. 2019).

In conclusion, the discussed literature demonstrates that cementochronology is a generally reliable method for estimating age-at-death. It has been shown to give accurate and precise age results when tested on known-age-at-death individuals. Limitations and differences between methodologies could explain the differences between those researcher who found good results and those who did not. Unlike other macroscopic aging methods, cementum is not influenced by sex or ancestry. Teeth are usually the best preserved in archaeological contexts, surviving even heat alteration. Although there has been limited cementochronology research on individuals over the age of 55 years, it is nonetheless possible that this technique could offer a more precise aging method for older individuals, which is lacking in other aging methods.

Chapter 3: Materials

The teeth for this study were obtained from the Middenbeemster collection housed at the Laboratory for Human Osteoarchaeology at Leiden University.

The Middenbeemster collection is a collection of the skeletal remains excavated from the cemetery next to the church of Middenbeemster Netherlands. The excavation was conducted by *Hollandia Archeologen* and Leiden University in 2011. The cemetery was used from 1613 to 1866 AD by the inhabitants of Middenbeemster (Palmer et al., 2016). The collections consists of over 450 individuals from the Middenbeemster community, about a quarter of which are documented in archival records from 1829 onward (Inskip et al. 2018). This archival evidence makes these later burials ideal for study as their ages-at-death and burial locations are known.

The teeth were taken from only known-age-at death individuals with a total sample of 20 teeth taken from individuals with an age range from between 24 to 78. Nine of those individuals are over 55. Sex does not change tooth morphology at the cementum layer so sex was not taken into consideration when determining the sample (White et al., 2011). Only maxillary canines were chosen as they are large single rooted teeth which is an ideal criterion for cementochronology (Colard et al. 2015). Preservation was a consideration, however, due to the length of the canine roots, they often remain *in situ* within the maxilla which preserves the teeth quite well. Only teeth which remained within the maxilla were chosen as these were known to belong to the individual whereas teeth found as part of the skeletal assemblage but not *in situ* within the maxilla could have been from another individual. It is assumed there was no to little comingling of graves in this cemetery but as the author did not have access to the excavation records this was not assumed, hence the *in situ* tooth choice.

CHAPTER 4: METHODS

For this research, the protocol laid out by the Cementochronology Research Program (CRP) will be followed when possible using the materials and instruments available at Leiden University. The CRP protocol's purpose is to increase the use of cementochronology in different areas of research. Applying this protocol on a known-age-at-death sample, this current research can further validate the method. This method is explained concisely in a flow chart shown in Figure 10 (and earlier in Figure 1).

This research was conducted as a blind test, only the author's initial supervisor was aware of the age of the individuals. The information was provided by the supervisor once the cementum line counting was complete. All laboratory work was conducted at the Faculty of Archaeology at Leiden University. The osteological material was documented in the osteology laboratory and the slide preparations were completed in the chemical laboratory.

There were six steps utilised in the process:

1. Selection and Documentation (includes extraction)
2. Cleaning and Drying
3. Embedding
4. Sectioning
5. Slide production (mounting sections, slide labelling, etc...)
6. Analysis (observation and micrograph acquisition)

The CRP protocol summarises these six steps into three main procedures which covers more individual steps which can be seen below in *Figure 10*. For the sake of organization the author has decided to discuss the six steps as laid out above because the three step process laid out by the CPR was not always a linear process which could lead to confusion.

Overall, the entire process (steps 1 - 5) for a single tooth took 28 hours, including drying times. Due to the multiple observations needed for each photograph step 6 was a fairly short process in total but was spread over several months to account for intra-observer error. This will be discussed further in Chapters 5 and 6.

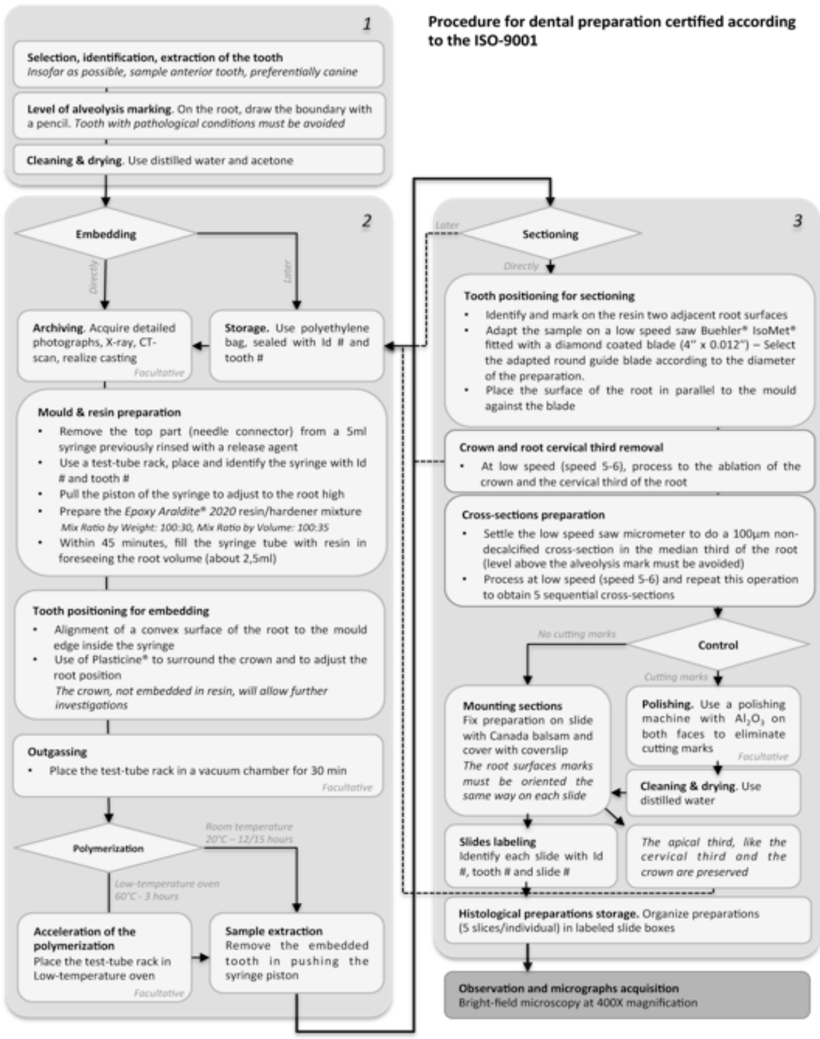

Figure 10. Flow chart designed by the cementochronology research program showing a methodology created from previous successful research. This is also known as the certified iso-9001 protocol (colard et al. 2015, 4).

4.1 Step 1: Selection and Documentation

The canines for this study were extracted from twenty individuals, yielding sixteen upper left canines, one upper right canine, two lower left canines and one lower right canine. Upper canines were preferred but do to the nature of archaeological material some upper canines were not present so lower canines were chosen. There should be no difference between right and left canines so the choice was based solely on which were present.

All teeth were placed in separate plastic bags which were labelled with their original site and find number, also known as their identifier number. Due to the destructive nature of this method, each tooth was measured using sliding callipers, dental pathological conditions were noted and photographs were taken. The CRP suggest CT scans and radiographs for complete documentation, however due to limited time and resources these documentation techniques could not be utilised.

4.2 Step 2: Cleaning and Drying

Each tooth was cleaned in distilled water using a sonicator, with the water changed every ten minutes until the water was clear which typically took 30 minutes, though some teeth were more soiled than others so cleaning could take as much as an hour. The teeth were then left to air dry in a slightly open petri dish for at least 24 hours until they could be imbedded. Before embedding the teeth were dipped in ethanol to remove any remaining contaminates and water as either could interfere with the drying of the resin. Here the CRP suggest acetone instead of ethanol and do not mention the use of a sonicator, though the level of soiling on teeth can vary. Teeth from the recently deceased may require a different cleaning method compared to archaeological specimens.

4.3 Step 3: Embedding

Next each tooth was imbedded in resin EpoThin Epoxy with the tooth laid horizontally in the mould and left to set for at least 24 hours in a vacuum chamber (Colard et al. 2015). The moulds used here were square or rectangular plastic embedding moulds. The CRP used cylindrical syringe moulds, the CRP also suggests leaving the samples in the vacuum chamber for 30 minutes. The remaining drying process is to be finished at room temperature or in a low heat oven.

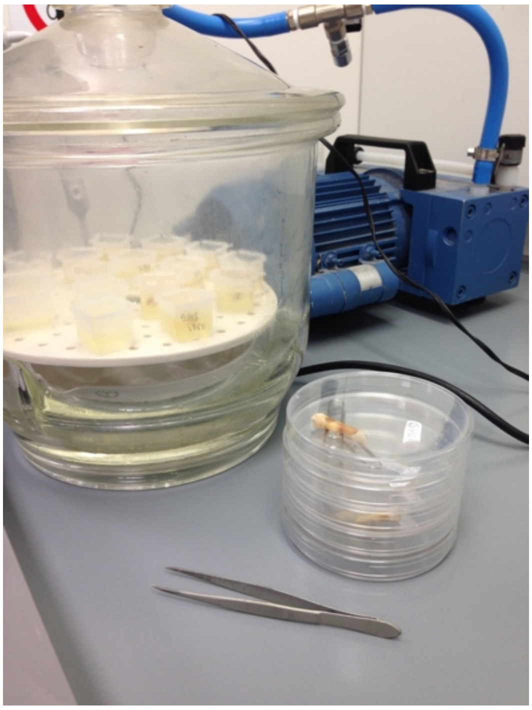

Figure 11. Photograph depicting the embedding process. In the large glass container (vacuum chamber) to the left are teeth already imbedded in epoxy, which are off-gassing within the vacuum chamber. In the plastic dishes to the right are teeth which will be embed

4.4 Step 4: Sectioning

Each tooth was removed from the moulds and a line was drawn on the epoxy which followed the exterior surface of the root (as was suggested by the CRP). Then another line was drawn at a 90º angle to the exterior surface, an example is shown in *Figure 12*.

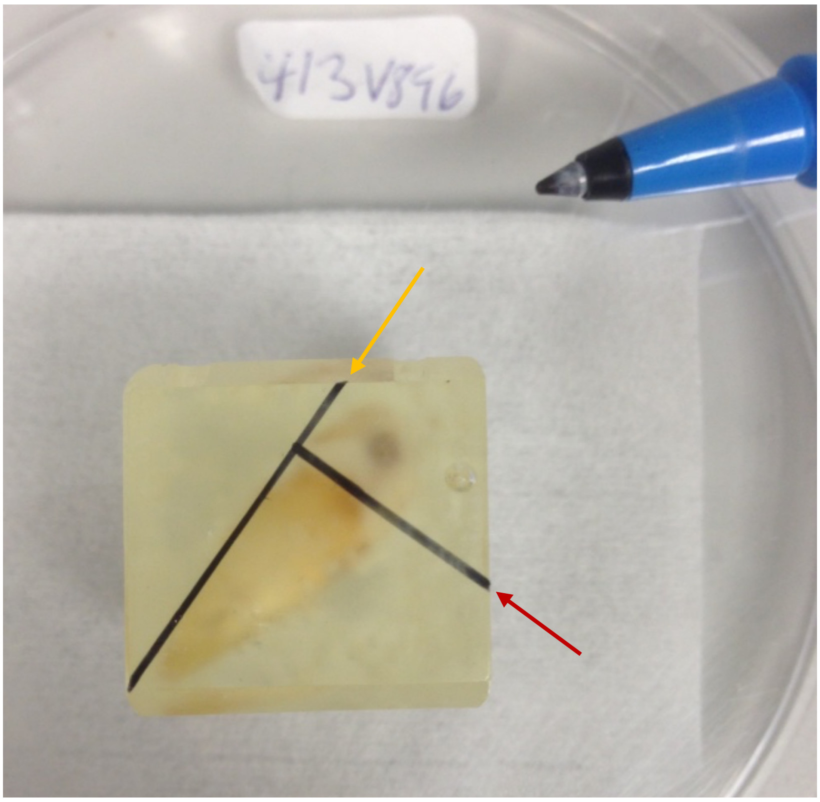

Figure 12. Photograph showing a tooth imbedded in epoxy where the exterior surface of the root has been marked, indicated by a yellow arrow. Then a line drawn perpendicular (90º) to indicate the ideal cutting angle, indicated by a red arrow.

Approximately five thin sections were taken from each tooth root at the middle third of the root at a 90º angle to the exterior surface of the root using a *Diamond Edge ISOMet® 1000 Precision Saw*. Each section was around 400µm thick. These thin-sections were further thinned by hand polishing using two different grades of sandpaper on both sides of the thin-section. The CRP suggests using a polisher if there are saw marks, but specified polishing is not required if no saw marks are visible.

4.5 Step 5: Slide Production

The freshly polished thin-section was adhered to a glass slide using Bison Colle Seconde Lijm, an ethyl-cyanoacrylate 'super glue'. A glass coverslip was adhered over the sample, sandwiching the sample between glass and adhesive, creating a histological slide. Here the CRP recommends Canada balsam to adhere the sample to the slide and coverslip.

Figure 13. Photograph showing the sanding machine in the background, with the metal mount for the slide to be used in the sanding machine, to the left and marked in green. The ethyl cyanoacrylate used for mounting the samples to the slide to the right and marked

4.6 Step 6: Analysis

Finally, each slide was viewed through a Leica DM 1000 at 400x magnification. One to four photographs were taken of each slide, guaranteeing that the lines would be countable in at least one of the pictures. The best photograph was chosen to make the official counts. The 'best' photograph was chosen based upon which had the most area in focus with good contrast between the lines in the majority of the photo. If the photograph had low line contrast, Pixlr, a web browser based photograph editor, was used to increase the contrast and light levels.

Each tooth (photograph) was counted twice and documented in separate excel sheets, with a month (approximately 30 days) in-between each count to account for intra-observer error. Counting was done using a laptop screen and mouse arrow. No marks were made on the photographs. Occasionally, some sections of the photograph were not in focus, likely due to an uneven surface or adhesive, so the counting was made where lines were the most clear. If a section of clear lines only lasted part of the photograph, a major line was followed to a different section of the photograph to where the lines became clearer. This is illustrated below in Figure 14, where the blue arrows indicate the trajectory of sight used to count the lines. The more vertical lines show where the lines could be counted where the horizontal lines show where a major line was followed to reach a more clear section.

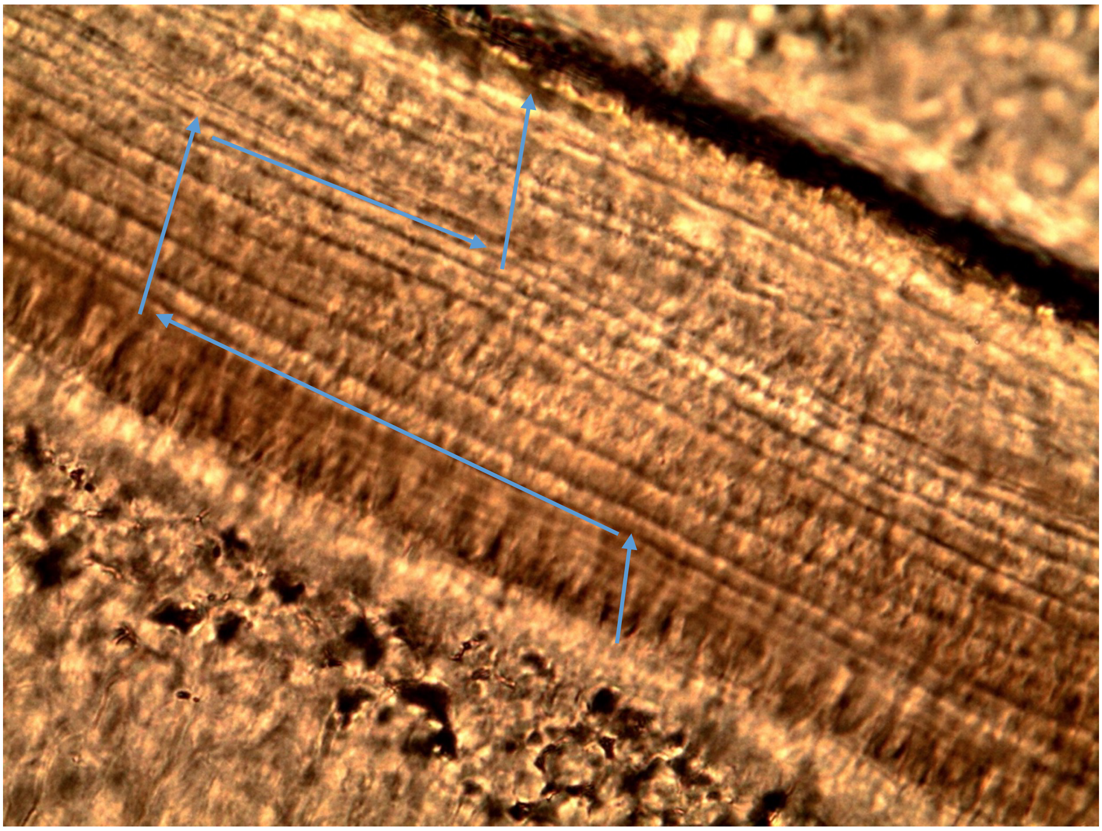

Figure 14. Photograph of tooth s59v133 at 400x magnification. Edited on Pixlr. The blue arrows indicate direction of counting progress.

An average of these two counts were taken and used to estimate age. This was done by taking the average count added to the approximate age of eruption. For this research tooth development data was taken from *Juvenile osteology: A laboratory and field manual* by Schaefer, Scheuer and Black (Schaefer et al. 2009). Since it is unclear when cementum lines begin to form on teeth, an upper and lower limit for age of eruption was decided based on growth charts illustrated by Ubelaker (1979) referenced in Schaefer et al. 2009 (Schaefer et al. 2009, 95). Because the root formation of canines begins before full eruption, the lower (younger) age estimates are 9 years for lower canines and 10 years for upper canines and the upper (older) estimates are 12 years for lower canines and 15 years for upper canines. These estimates do include a margine of error of +/-24-30 months. In the data, which will be presented below, an upper and lower age estimate will be given along with the actual age.

Step 6.1: Statistics

Pearson's correlation coefficiant will be determined for both age estimates. Along with Pearson's correlation coeffient, Intraclass Correlation Coefficient (ICC) will be determined for the authors count consistency. The data were broken into smaller age groups to determine if age, and at what age, reliability might be effected. These age groups are essentially arbitrary as there is no clear agreement on what is "old age." Therefore, several age groups will be isolated, analysed for correlation and discussed. These groups are 'Younger Set' and 'Older Set' where the data was split in half when the actual ages were listed chronologically, meaning the younger set ranges from age 24-45 and the older set 54-78. Three smaller groups will also be considered. These being, 'Set 1', 'Set 2', and 'Set 3', where in a similar way to the first groups, the first 7 individuals will make up set 1, the next 7 set and the last 6 set 3. This means that set 1 covers ages 24-39, set 2 ages 42-56 and set 3 ages 59-78. This analysis will be calculated using Microsoft ® Excel 2022.

The data gathered and results from these statistical analyses will be explained next in Chapter 4 and discussed further in Chapters 5.

CHAPTER 5: RESULTS

Much of this research was focused on the methods surrounding cementochronology, and by extension illustrate the reliability of the method in the hands of a novice researcher. There will be a summary of the results, followed by the data being divided into different groups which should help to illustrate how accurate the method was on different age groups.

Table 4 provides a summary of the data collected. As discussed previously in Chapter 3, the estimated age is determined by adding the average age of tooth eruption to the average cementum line count which produces the estimated age. For this study the average age of tooth eruption is taken from *Juvenile osteology: A laboratory and field manual* by Schaefer, Scheuer and Black (Schaefer et al. 2009).

Table 4. Table showing the gathered and calculated data for this research..

Tooth ID#	Tooth Type	Count 1	Count 2	Count Avg.	Low Estimate	High Estimate	Average Estimated Age	Actual Ages	Difference
S40V64	ULC	30	35	32.5	42.5	47.5	45	24	21
S338V721	ULC	17	15	16	26	31	28.5	33	4.5
S482V1048	ULC	17	22	19.5	29.5	34.5	32	36	4
S59V133	ULC	27	32	29.5	39.5	44.5	42	38	4
S524V1120	ULC	41	35	38	48	53	50.5	39	11.5
S413V896	ULC	23	28	25.5	35.5	40.5	38	39	1
S101V0131	ULC	36	43	39.5	49.5	54.5	52	39	13
S473V1003	ULC	22	32	27	37	42	39.5	42	2.5
S466V1010	ULC	33	36	34.5	44.5	49.5	47	43	4
S435V929	ULC	44	45	44.5	54.5	59.5	57	45	12
S155V1509	LRC	52	45	48.5	57.5	60.5	59	54	5
S383V880	ULC	16	17	16.5	26.5	31.5	29	55	26
S53V290?	LLC	18	19	18.5	27.5	30.5	29	55	26
S347V741	ULC	43	40	41.5	51.5	56.5	54	56	2
S92V124	LLC	47	46	46.5	55.5	58.5	57	59	2
S386V848	ULC	38	47	42.5	52.5	57.5	55	61	6
S436V991	ULC	26	26	26	36	41	38.5	64	25.5
S486V1088	ULC	29	38	33.5	43.5	48.5	46	68	22
S390V831	ULC	27	37	32	42	47	44.5	71	26.5
S356V864	ULC	24	21	22.5	32.5	37.5	35	78	43

As the table demonstrates, there is variation between the tooth's actual age and its estimated age. Of the 20 samples used in this study, only two individuals had a relatively small difference of 2.5 years while another two individuals had a difference of over 20 years. Seven teeth had a difference of less than five years while the remaining nine samples were over the age of 55 and only two of these were estimated within five years of actual age.

The following graph depicts the reliability of the first and second counts (Figure 15), of the author which illustrates that there was little Intra Observer Error in this research. This graph shows the reliability coefficient, r, in the top right corner and was determined using Pearson's Coefficient where r shows correlation between two sets of numbers. Results of 0 to -1 show a negative correlation while results between 0 and +1 show a positive correlation. In this case r=+0.8799 illustrating a positive correlation. By running the Intraclass Correlation Coefficient (ICC) test, which assesses the consistency between quantitative data, a deeper understanding of reliability can be determined. ICC can range from 0 to 1 where 0 is not reliable and 1 is perfectly reliable. Between counts 1 and 2 ICC=0.86 showing good reliability. Both the positive correlation and the ICC results indicate that the author was fairy reliable in her counts.

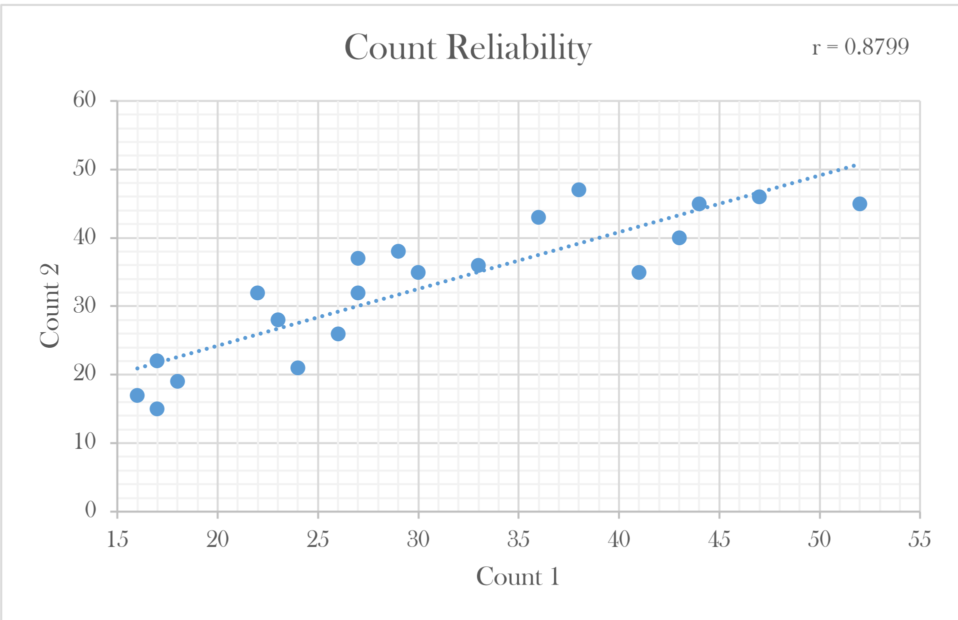

Figure 15. First and second counts. Graph showing the reliability between the first count and the second count, illustrating the reliability of the author to count the same number of lines each time. r=+0.8799.

While most statistical results show R (r^2), this by default gives a positive number as a negative multiplied with a negative equals a positive. Because corelation was important for these results the author decided to report the data in the r form which indicates if the correlation is positive or negative. While this will vary from other work on the subject which reports the R value instead of r, the author hopes the presence of negative and positive correlation will help clarify the data for the reader.

Figure 16 and 17, illustrates the estimated and actual age data in two types of graphs. The first (Figure 16) shows the average age estimate (blue) and the actual age (orange) as a bar graph where the discrepancies can more clearly be seen. The second (Figure 17) shows the same data but as a scatter plot where the correlation can be seen both as a linear trend line and as the correlation coefficient, which is noted in the upper right corner.

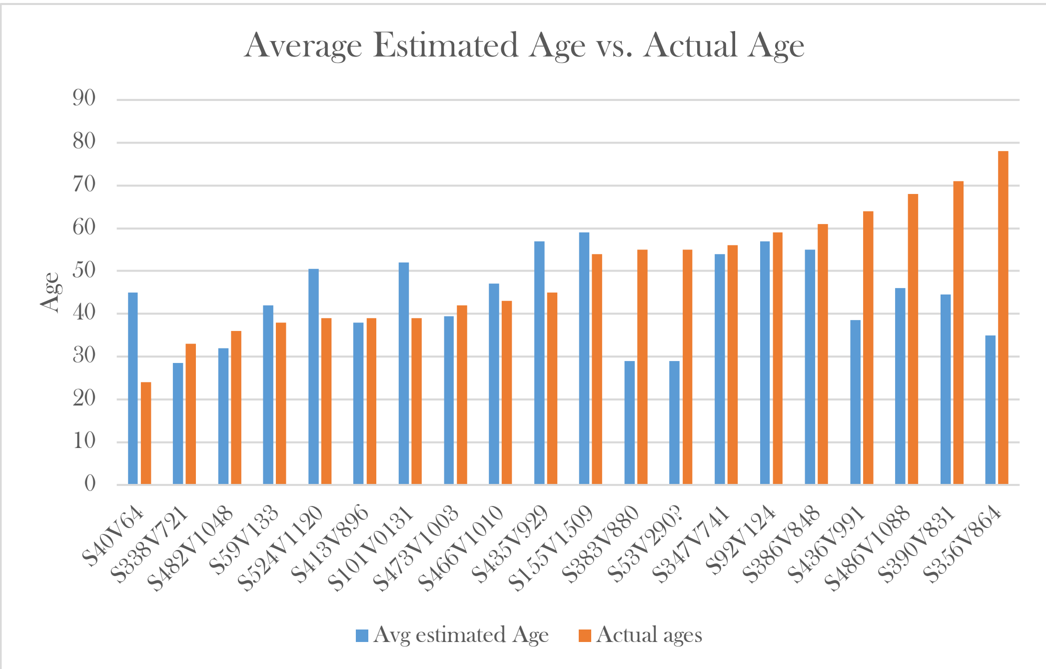

Figure 16. Actual and estimated age. Graph showing the actual age in orange and the average estimated age in blue, which illustrates the discrepancies between the estimated age and actual age. Note that the actual ages were ordered chronologically from youngest (left) to oldest (right).

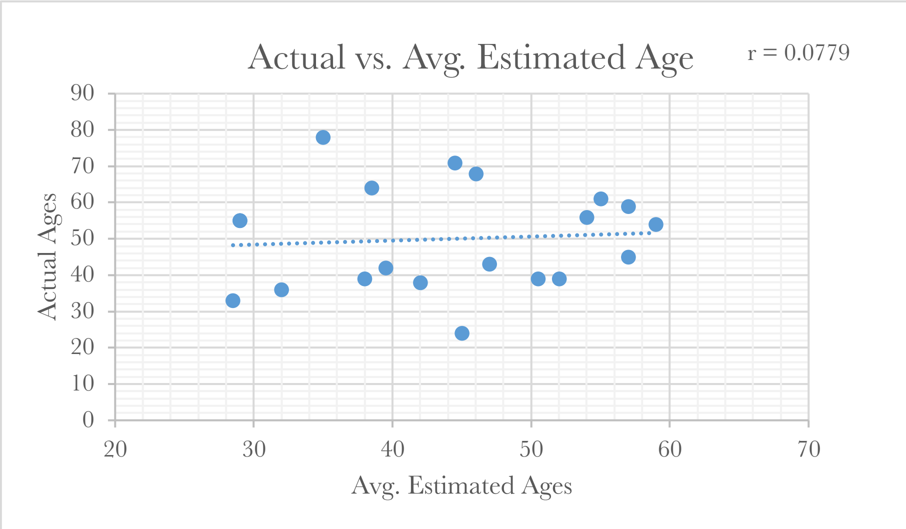

Figure 17. Actual and estimated age. Graph showing the actual age and the average estimated age in a scatter plot where the correlation coefficient (r) is noted in the top right corner. r=0.0779 is positively correlated, however as it is nearly zero there is almost no correlation.

Of further interest was whether the low or high age estimates showed a closer correlation to actual age, especially as compared to the average age estimate which was used for the majority of this testing. *Figure 18* shows the low estimated age and the actual age with r noted in the top right corner. *Figure 19* shows the high estimated age and the actual age with r noted in the top right corner.

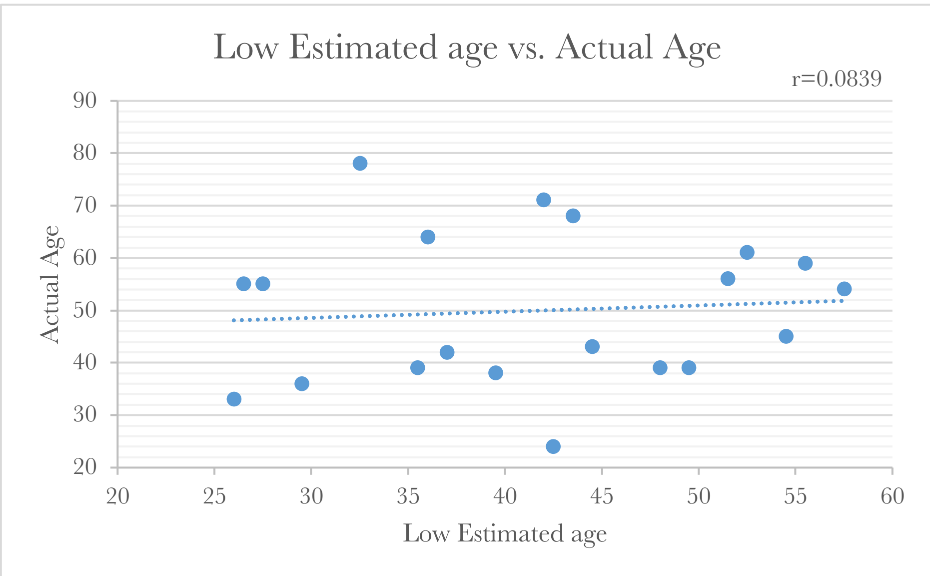

Figure 18. . Actual and low estimated age. Graph showing the actual age and the average estimated age in a scatter plot where the correlation coefficient (r) is noted in the top right corner. r=0.0839 is positively correlated, however as it is nearly zero there is almost no correlation.

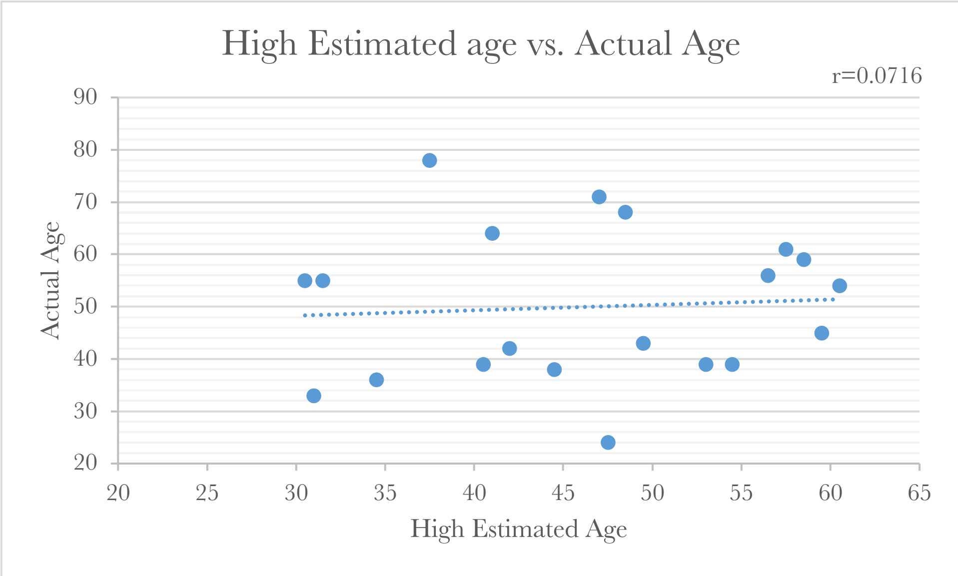

Figure 19. Actual and high estimated age. Graph showing the actual age and the average estimated age in a scatter plot where the correlation coefficient (r) is noted in the top right corner. r=0.0716 is positively correlated, however as it is nearly zero there is almost no correlation.

Figure 20 shows the Younger Set of individuals, ages 24-45 years old and *Figure 21* shows the Older Set, ages 54-79 years old. These two groups were determined by

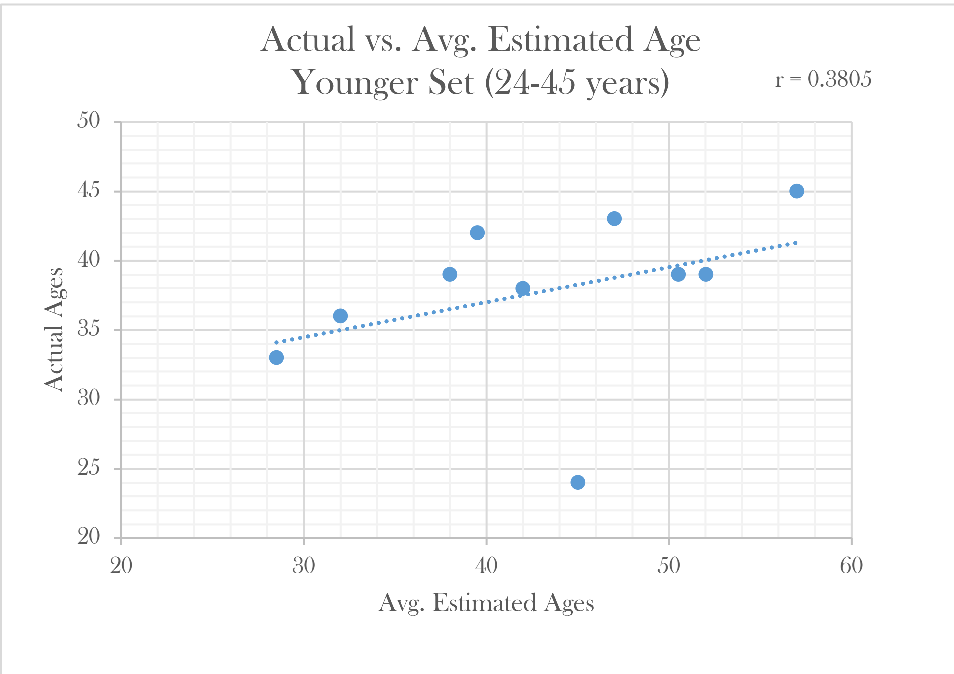

Figure 20. Younger set. Graph showing the younger half (24-45 years) of the estimated and actual age reliability. r=+0.489.

arranging the data chronologically according to actual age and breaking the set of 20 into two equal groups.

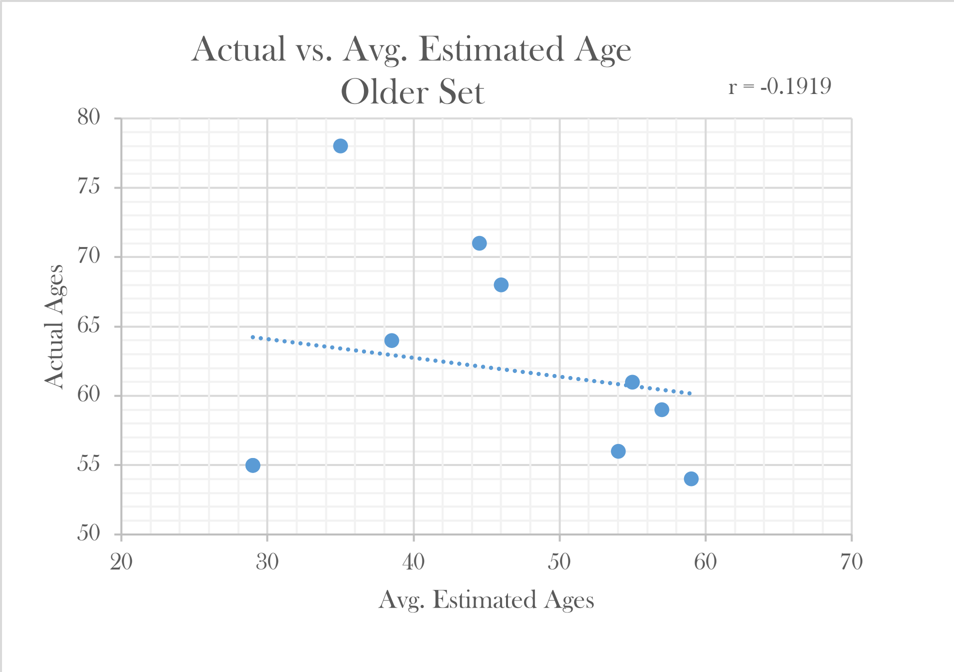

Figure 21. Older age group. Graph showing the older half (55-79 years) of the actual and estimated age data. r=-0.063

The data was further broken into three different age groups, Set 1 is 7 individuals, ages 24-40 years old, shown in *Figure 22*, Set 2 is 7 individuals, ages 40- 56 years old, shown in *Figure 23* and Set 3 is 6 individuals, ages 59-78 years old and shown in *Figure 24*. These sets hope to illustrate the reliability of the method within more isolated age groups. Because of the small sample size (20 individuals), the groups were determined by keeping each set as a similarly sized group (7, 7 and 6). There has also been disagreement about how to isolate different age groups within adults as age groups are often defined by the society not by skeletal markers. Therefore these groups were chosen arbitrarily based solely on the sample size divided by three. While this may not agree with other data subgroups within similar research the author hopes it will keep the age groups unbiased. The correlation coefficient (r) is indicated in the top right corner for each set's graph.

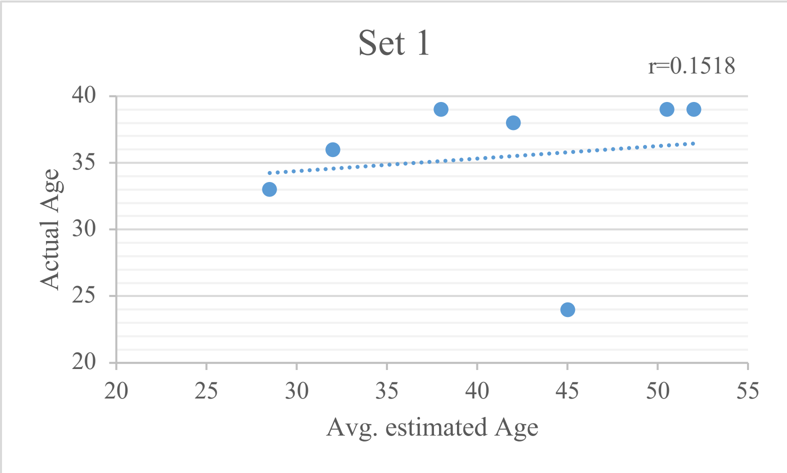

Figure 22. Set 1. Graph showing the actual and estimated ages of set one (24-40 years). r=0.1518.

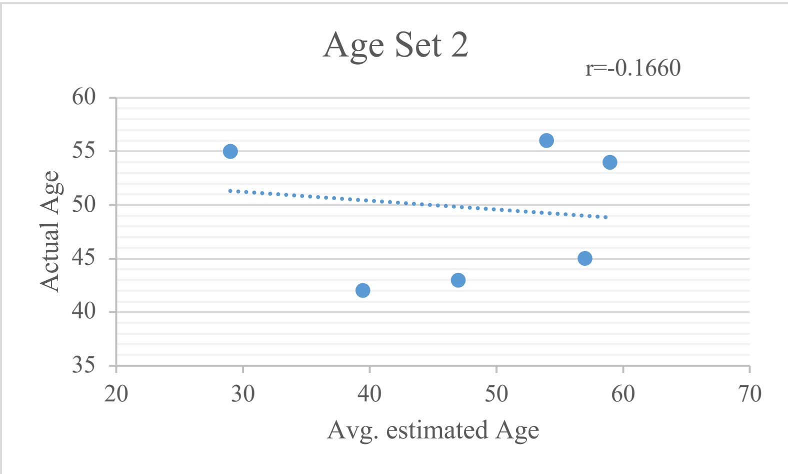

Figure 22. Set 2. Graph showing the actual and estimated ages of set 2 (40-56 years). r=-0.1660.

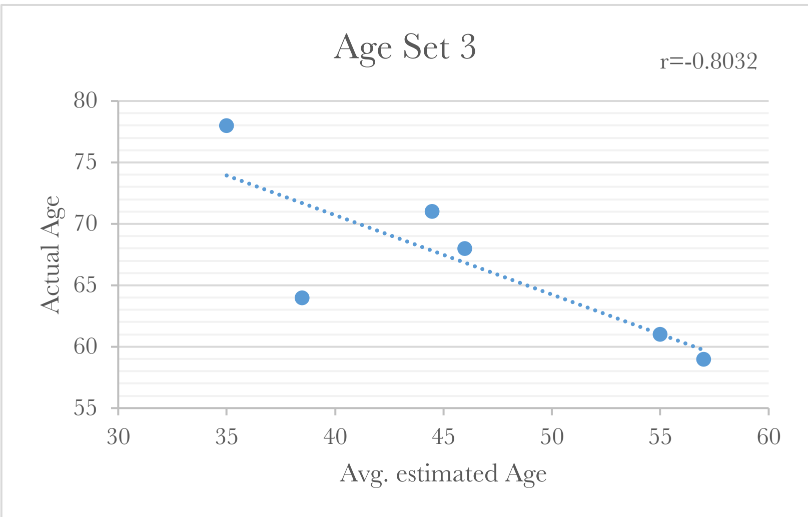

Figure 22. Set 3. Graph showing the actual and estimated ages of set 3 (59-78 years). r=-0.8032.

For these three sets, since there was a slightly higher correlation for the younger age estimate than the higher, correlation based on the lower age estimate was also tested. For Set 1 using the lower age estimate r=0.1518, Set 2 r=-0.1475 and Set 3 r=-0.805.

In summary, the author showed low intra observer error (was reliable counting), in as much as can be assessed from a sample of 20 counted twice, indicated by ICC=0.86 which shows good reliability. The method was less reliable than was expected at estimating age with only a small age range. The only possible exception is Set (24-40) with a correlation coefficient r=0.1518, where n =10. Although not highly reliable it nevertheless shows some correlation between actual and estimated age. The sample size of 20 in this study may be somewhat small to draw definitive conclusions, especially when broken down into even smaller groups. The meaning and interpretation of this data will be discussed in Chapter 6.

CHAPTER 6: DISCUSSION

This research assed 20 canines from the Middenbeemster collection held at the University of Leiden Osteology laboratory. The aim of the study was to use cementochronology to estimate the age-at-death of these individuals. Specifically the author was interested in the reliability of cementochronology using a recognized method (ISO 1009 Protocol). As part of this the author recognizes that she is a novice to cementochronology so the effectiveness in novice hands was an element of interest. Overall this research has addressed the set aims and the main findings are interpreted below. Alongside this, limitations of the study and future research avenues are identified in this chapter.

6.1 A Review of the Results

As was previously shown in Chapter 4, the results for cementochronology estimating the age of 20 known-age-at-death individuals was only moderately reliable. Depending on the age-at-eruption used to calculate estimated age the correlation coefficient (r) ranged from +0.0716 to +0.0839. While these are positive correlation results, they are still *very low* when considering that absolute correlation is +1.0. It is interesting to note that the lower estimate is the slightly more correlated result at r=+0.0839. The average age estimate used for much of this study had r=+0.0779, which is lower than the lower age estimate but higher than the higher age estimate. This may indicate that cementum may begin to develop clear lines at a younger age, possibly before the tooth root has finished forming.

It is also very clear from the bar graph shown in *Figure 16*, that the estimated ages are drastically lower than the actual age in older individuals. This is supported by the reliability of Set 3 (ages 59-78) where r=-0.8032, which shows a very high negative correlation. For these older individuals it would be interesting to consider if a certain number of years could be added to the estimated age to bring the estimate closer to the actual age. To do this for a skeleton with no known-age-at-death, it would need to occur in tandem with macroscopic aging techniques to determine that the skeleton is at least a certain age but this would come with complications. it would be difficult to decide what minimum age would need extra years added to the estimated age. Say the minimum age was 50, at which point 20 years would be added to the estimate determined from cementochronology. For this data this would bring 5 individuals (S383V880, S53V290?, S436V991, S486V1088 and S390V831) into an age range within about 6 years of the actual age. However, it would increase the estimated age for four individual to more than a 25 year difference (S155V1509, S347V741, S92V124 and S386V848) because they were already within a 6 year difference of the original estimation. This also ignores the eldest individual in the sample whose actual age was 78 and estimated age was 35, a 43 year difference, so a 20 year addition would help but not bring it nearly close enough to the actual age. Therefore, with this data sample alone it seems impossible to the determine whether some kind of formula could be determined to increase the accuracy of the estimate.

Set 2 (ages 40-56) is also negatively correlated where r=-0.166, though this is closer to 0 and therefore less negatively correlated. Even Set 1 (ages 24-40) which is positively correlated is still low as r=0.1518. These were assessed using the average age estimate, however using the lower age estimate which was slightly more positively correlated only made a large difference to Set 2. Using the lower age estimate for Set 2 r=-0.1475 compared to with the average age estimate where r=-0.1660. While these are still negatively correlated the lower age estimate brought the r value closer to 0 and therefore closer to a positive correlation.

Looking at the data outside of the different age sets, it seems almost random whether an individual was estimated with under or over a 5 year difference. While the younger individuals were more likely to be estimated closer to their actual ages, several individuals in the older age group were estimated with only a 2 year difference to their actual ages (S347V741 and S92V124). Three individuals in the younger age groups who were all 39 when they died, only one was estimated under a 5 year difference (S413V896) whose estimated age was 38. The other two individuals (S101V0131 and S524V1120) were estimated to be 52 and 50.5 respectively, a 13 and 11.5 year difference. While some of these could be considered outliers, there is very little consistency based on age. Based on the r values for all groups it is clear there is little to no correlation between the estimated and actual ages even between the age groups no matter how they are divided. It is unclear why there is such inconsistency, if the younger individuals had all been within a 5 year estimate and the older individuals all with over a 5 year estimate it would be clear that accuracy decreases with age. However, these results show so much inconstancy that it is nearly impossible to draw any conclusion between the actual age of the individual, the age group they could be placed in and the subsequent estimated age. While r values could likely be increased by eliminating the seeming outliers, this further highlights that with cementochronology, specifically done by a novice, the results cannot be trusted to give an age estimate any closer than those estimated using non-destructive macroscopic techniques.

Despite the inconsistent results, the author was reliable in her ability to count the cementum lines twice with a correlation coefficient r=+0.8799 and ICC=+0.86. These results show a high positive correlation and high rate of reliability as both are close to +1, showing low intra observer error.

6.2 Limitations of the Study

Given the results summarised both in Chapter 4 and above in Chapter 5 section 5.1, it is unfortunately clear that the method offered unreliable results. Even ignoring the unreliable results, there are more limitations to this study which will be discussed in this section.

A fundamental problem was the small sample size. While a sample size of 20 is enough to notice trends, it is not sufficient if the data is grouped into smaller groups. Sets 1-3 had 6-7 individuals in each set which only gives a small glimpse of reliability but is certainly not sufficient to draw any large conclusions. If the original sample had included 60 individuals and each smaller group 20 (if divided evenly by three) then the trends and reliability could be evaluated with more confidence.

The sample is also from an archaeological sample. Though fairly young in terms of an archaeological record (less than 200 years), the burial environment could still have led to taphonomic changes to the cementum layer (Pokines & Symes, 2014). Even though teeth roots can be somewhat protected while in-situ in the bone it is unknown if the burial environment could affect the cementum. Taphonomic agents have been shown to cause degradation to the skeleton it has also been shown that teeth often survive best. However, it is unknown whether the cementum layer is in anyway effected. Studies done to test the effect of fire and heat on the cementum layer show that while heat and fire does lower the reliability those researchers still had successful results (Gocha and Schutkowski, 2013).

What is certainly true is the lack of data on the development of the cementum layer and its post-mortem and post-depositional decay process and the effects of different taphonomic agents on the layers. If for example the erosion of the external layers of the cementum caused a loss of layers, the counts would come out significantly less than expected. However, given the data here, which shows both over and under counts, this may not be the case. Also, considering the low positive correlation it is difficult to draw any conclusions especially considering that the 2/3rds of the data (sets 2 and 3) show a negative correlation.

6.2.1 Application of the Standard Protocol

Of high interest to the author was the feasibility of using the standard protocol created by Colard et al. (2015, 4). Ultimately the author hoped that by using the standard protocol, she would be adding to the research and provide an indication of how effective the method is to an average researcher. Obviously the hope was that the method would be clear, straight forward and offer reliable results, similar to previous researchers. The protocol itself looks fairly straight forward, but it does not explain how and where different tools or materials will make a difference to the reliability of the results. There is also a steep learning curve when creating the thin sections. Unfortunately, this was poorly addressed both by the initial supervisor and the author. Lab access and instruction were difficult to gain and ultimately the creation of the slides was rushed due to time constraints. This highlights well that the protocol, which looks fairly simple, is in fact very labour intensive and difficult to master in a short time. For those unfamiliar with creating thin sections of samples imbedded in epoxy for use as histological samples, adequate instruction, supervision and practice is highly recommended. Time estimates for creating the thin sections should also not be underestimated. This was certainly a failure on behalf of the author who failed to anticipate the time needed and the problems which might arise with this study.

Despite the shortcomings of the author in creating the thin sections, photographs of all the thin sections were taken meaning that the sample of 20 could be studied. However, given how poor the reliability of the results achieved in this research it is clear that there are certainly limitations of the method. These limitations will be discussed in the next sections.

6.2.2 Limitations of the Standard Protocol

As mentioned several times in this paper, it was hoped that the standard protocol would be source of information and offer clear guidance in the execution of this research. However, some deficiencies became apparent as this research was conducted. In broad terms the protocol was clear and well organized, but it spoke in specifics where I think to make it accessible to the general researching public more broad examples or options should be mentioned. Specifical examples of this will be discussed in terms of each step.

The first step in the protocol includes; selection, identification, extraction of the tooth, level of alveolysis marking, cleaning and drying. The author subdivided this one step into two, 1. Selection and Documentation and 2. Cleaning and Drying. Selection and Documentation was fairly straight forward and was based on the availability of the samples and the information provided on those samples. 'level of alveolysis marking' was less clear and not clearly defined. This step was skipped for most of the samples as the epoxy obscured the graphite lines. Cleaning and drying were also straight forward in theory, however the protocol does not say how to clean with distilled water and acetone, only that they should be cleaned with these. For this research the teeth were cleaned using a sonicator, i.e. the teeth were submerged in distilled water and sonic vibrations through the water loosened and removed surface dirt and debris. To ensure the teeth were fully dry and any surface oil or grime was removed, they were dipped in acetone. This was done especially because the epoxy may fail to adhere properly to the teeth if there were any impurities left on the surface. In several cases while cutting the thin sections, the area of tooth separated from the epoxy. This could be due to too small a surface area so the epoxy lost adhesion, alternately the surface of the teeth were not cleaned sufficiently which also caused adhesion failure.

The next stages defined by the protocol were; embedding, archiving and storage, mould and resin preparation, tooth positioning, outgassing, polymerization with notes on time required, and sample extraction. The author summarised this section as one step, Embedding. In the protocol they were specific in how to embed the teeth in syringes. As may be considered common knowledge, there are many types of moulds available for use with epoxy. Syringes of the proper size and quantity were unavailable therefore square and rectangular plastic moulds were used. The choice for each tooth depended on the size of the tooth as some of the canines needed the extra length of the rectangular moulds. These moulds made outgassing the epoxy convenient as many samples fit in the vacuum chamber simultaneously. Presumably it should be more important that the teeth are imbedded than by what method they are imbedded. However, later they clarify that by using a syringe it allows for the crown to remain free of the epoxy, which would minimize the destructiveness of the method. So rather than only mentioning the syringe method an 'or' section would have been helpful, especially if consideration such as destructiveness are noted earlier in the protocol. Of concern too is the type of epoxy used. In the protocol they suggest *Araldite 2020*, however this type of epoxy resin was unavailable at the time. Instead *EpoThin* Epoxy was used. However the author believes this epoxy was insufficient for its purpose. The epoxy is meant to form a mass around the tooth at or near a similar hardness, however this epoxy was considerably softer than the tooth. This could be due to inaccurate measurements or ratio of the two components which are mixed to create the epoxy. It could also be due to contamination of the epoxy which may have inhibited the proper curing. In this case the author agrees with the protocol that the type of epoxy may be important in creating the most stable entity to be sampled. Of consideration is that Araldite 2020 is a very common epoxy used in many applications so the access to this epoxy should be easy for most researches with little financial strain. The author also wondered if outgassing was strictly necessary. Since not every lab has access to a vacuum chamber it would be interesting to test whether there are alternative methods for ensuring there are no bubbles around the tooth.

The next step specified by the protocol is sectioning which they further divide into; tooth positioning, crown and root removal, cross-section preparations, separation of a control group and non-control group for removing and keeping saw marks, cleaning and drying and mounting. They suggest a *Buehler IsoMet* saw fitted with a 4"x 0.012" diamond coated blade. This is a very specific piece of equipment and the author suggests that any similar saw fitted with a diamond coated blade should be an option and mentioned in the protocol. If the specific brand and size is crucial to the protocol then it should be explained why this is the case. For this research the saw available saw was a *Diamond Edge ISOMet® 1000 Precision Saw,* but the size of the blade was unknown to the author. The thickness of the blade made it difficult to fully know the exact thickness of the sample which was being cut. The saw is designed in such a way that the thickness of the section can be chosen, however, even though each section should have been 0.4 mm the difference in actual thickness seemed to vary greatly. This became most obvious when the saw blade was too thick to allow too thin of a section to be cut and the blade would jump from its initial incision plane to the outside edge. In this same vein there were no instruments to gain an accurate thickness measurement of these slices. In an attempt to make the slices even, hand sanding and polishing was employed, but of course this was also inexact. Presumably if a more exact measuring system had been available, the author could have been more consistent in the thickness of the slices made, thereby discerning if slide thickness played into the readability of the cementum lines. While digital callipers were available, multiple measurements taken of the same sample in the same location showed different results indicating the precision of the callipers were not exact enough to be useful. Also problematic was the varying sizes of teeth which meant they sometimes only fit into the moulds in a certain position, unlike in the protocol which advises a specific alignment to allow for the correct cutting angle later. As it was, the author did her best to ensure that the cutting angle was 90º to the exterior surface but, this was sometimes an approximation, especially as more slices were made and the curve of the tooth exterior surface meant that angles were changing and no longer 90º. This was unfortunately unavoidable as the natural curve of the tooth could not easily be accounted for when taking the thin sections. In total, only one or two thin sections were taken successfully from each tooth though the desire was to get four to six sections each. Often the epoxy would come of while in the process of cutting which could lead to the tooth section breaking. Sometimes the section was so thin it would bend and break or be lost. Eventually it was determined taking a slightly thicker section was safer, though this also meant that the section would need to be sanded more to achieve the perceived proper thickness.

In the protocol the authors suggest a mechanical sander. While a mechanical sander was available, the process of adhering the sample to a slide so it could be sanded, then removing it often destroyed the sample. Also the mechanical sander, which should have sanded the surface evenly, typically wore only certain areas away or the sample would bounce over the surface. Therefore the samples were hand sanded by using only water to adhere the sample to a fingertip and smoothed across different grades of sandpaper until the sample was considered thin and smooth enough. However, this did not mean that all the saw marks were removed and that the samples where a uniform thickness. This meant that once mounted on the glass slides the field of focus was often different across the sample, meaning that while one area was in focus another would be out of focus.

This challenge when viewing the samples under microscope could be due both to an uneven surface but also could be due to the adhesive which was used. The recommended adhesive in the protocol was *Canada Balsam*, a mounting medium which has a similar transparency to glass when dry. In this study, an *ethyl cyanoacrylate,* aka. 'superglue' was used. When viewed under the microscope it seemed as if the adhesive refracted the light in a strange way, creating small rainbows, obscuring a lot of detail and sometimes seeming to double lines or features. This is shown below in *Figure 25* where in an unedited image of sample S347V41 is shown in colour. By looking at the central section of the cementum lines the lines seem to look slightly rainbow with red and green coming through as prominent colours. While this could have been a trick of the microscope or the camera attached to the microscope it was never the less a hinderance when viewing and attempting to count the lines. Since no other mounting medium was tested it is unclear what caused the strange light refraction or if there was another factor for this. When looking at other researchers images they appeared much clearer so it would be interesting to see how their equipment and materials differed both from this study and the protocol. In *Figure 26* is an example of the cementum photos Colard et al. obtained in their research. Of note is that that changed their image to black and white, which is something that is not mentioned in the protocol.

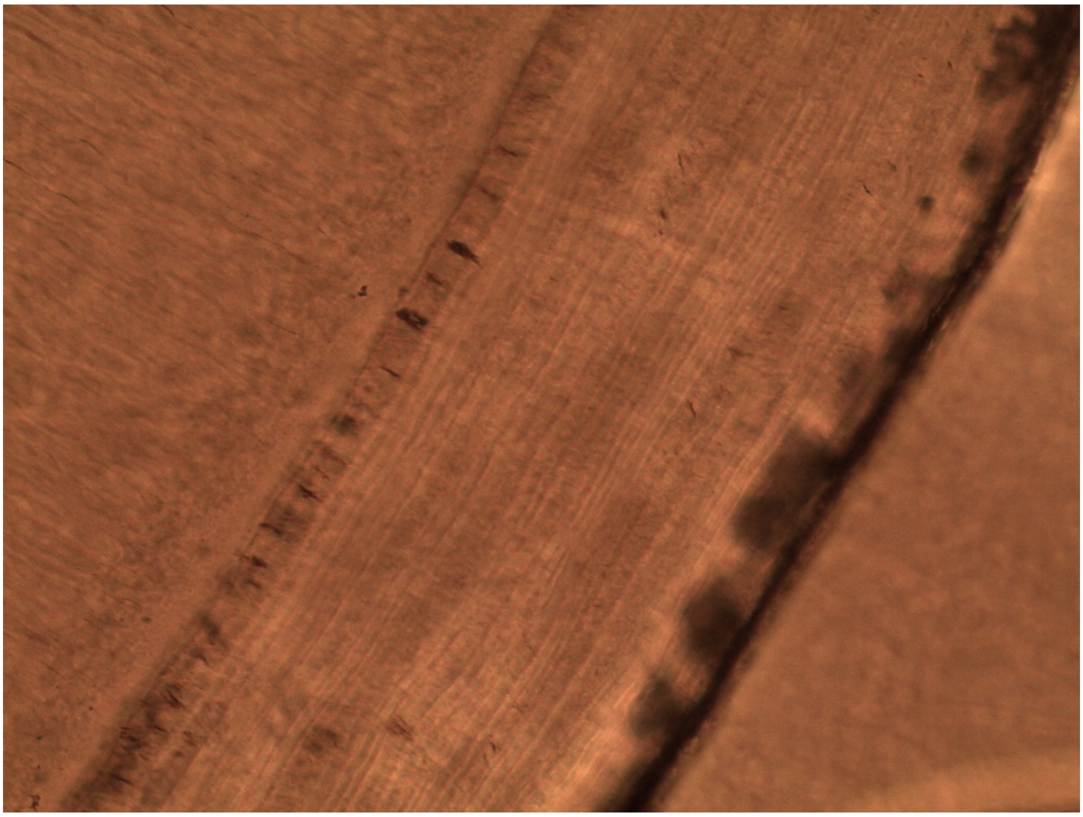

Figure 23. Unedited image of sample s347v741. While the cementum layer is a clear section running diagonally through the image, the lines are somewhat obscured. This is could be due to the microscope, the microscope camera, the mounting medium or an uneven surface of the sample.

Finally, possibly the area with the least information within the protocol refers to analysis and counting under the microscope. while the authors specify they samples should be viewed and photographed at 400x magnification there is no other information provided. They give no explanation to if they edited their photographs to improve clarity or how exactly they counted the lines. As shown above in Figure _ the image is in black and white, is switch colour photographs to black and white the standard? In Chapter 3 the author reported on the somewhat 'zig-zag' nature of the counting process, the goal being to count the clearest lines possible even if that meant counting across the images in a varied pattern rather than in a straight line. If for example the protocol said to draw a straight line through the image perpendicular to the cementum lines and to only count the lines which intersect with that line, I would imagine I would have significantly different results than those I produced. This is because sections of my photographs were out of focus or blurred. Because of this I employed a randomized 'zig-zag' pattern to follow the clearest lines so as to count the maximum possible. So if instead I had followed one line I would likely have missed many lines due to poor visibility. This difference should be part of the protocol so as to keep the method as uniform as possible. This type of clarification would also improve the reproducibility of counting. If for example, if a line was drawn perpendicular to the cementum lines then that line could be kept in place for each count, isolating the location of counting to a small area instead of the entire photograph.

6.3 Recommendations for Future Work

6.3.1 Cementum Development and Decay

There are several areas which the author believes should be explored further in a vein to improve and develop cementochronology. The first pertains to the general knowledge surrounding cementum. Specifically looking at the development of cementum and ascertaining when new lines begin to form relative to age of eruption. Then understanding how cementum forms and develops over time, specifically what may cause line divergence as mentioned in __. They document that some areas of cementum can have lines which appear to separate and re-join later creating uneven numbers of lines. Then finally understanding more specifically how taphonomy and burial environments can affect the cementum. This study used archaeological known-age-at-death samples, but studying samples which have not been buried may help provide a base-line for reliability.

6.3.2 Diverse Sample Studies

It is assumed that sex and ancestry has little effect on tooth development in terms of cementum. However, there are clear age differences for age at eruption between males and females which could certainly effect the reliability of age estimates using cementochronology. As has been noted throughout the physical anthropological world, many standard samples used to create age estimation techniques use non-diverse samples, often using Caucasian populations which are not representative of a diverse population (Garvin et al. 2015). It is therefore recommended that known-age-at-death samples from a diverse group of individuals is used to conduct future studies in cementochronology. This may also mean assessing tooth eruption ages in diverse groups. This research is likely easily attainable within the dental industry as regular radiographs would show tooth development in children. However this assumes regular dental appointments for children which would provide a biased sample since dental care is not accessible in all countries and to all children. While many studies exist for tooth development in children and generalisations have been made it would be important to understand if ancestry or sex in different regions of the world play any part in the reliability of these studies.

6.3.2.Better guidelines in counting

As mentioned in section 5.2.2 the protocol glosses over the photograph acquisition, editing and counting process. More detail on this process should be included in the protocol so that every area of the protocol is specific and replicable. Alternately a discussion by the authors of the protocol should asses the multiple ways photographs could be edited and the lines counted. Of specific interest would be using a sample of photographs, where there is a control with other copies made with different edits (e.g. black and white), then different counting methods used. This research could provide a glimpse into how editing the photographs and counting procedures would affect the reliability of the method. Of recent interest to the author is the use of cell phone cameras to take pictures using a microscope. The camera lenses on many microscopes are difficult to focus even if the sample is in focus through the eye piece(s) of the microscope. By hovering a cell phone camera over the eye piece, a photo can be taken which represents more accurately the focus of the eye piece compared to the camera built into the microscope. Because the author was unaware of this possibility at the time of the photography (2016) and the improved phone camera quality since then (year of publication 2022), it would be interesting to attempt to photograph the same samples using a phone camera and see if the clarity is any better.

6.3.3 Suitability for a Novice Researcher

As may have been evident from both Chapter 3 (methods) and earlier in this Chapter, the cementochronology process has a steep learning curve and is not easily mastered. The development of the protocol was intended to streamline the process and make it replicable to a variety of researchers in different fields. However, considering the challenges, limitations and results of this study the author believes those wishing to use cementochronology reliably and regularly should seek professional instruction and guidance. The author felt generally unsupervised for the instruction on how to create and analyse the cementum samples. While this may or may not have affected the results it is important to note that simply having access to materials and equipment may not be sufficient to attain reliable results.

A further limitation was the sample size and the origin of the sample. Because the archaeological collection had only a limited quantity of known-age-at-death individuals the sample size was kept small at 20. This was also because of the limited time allowed to complete the research for master's students, which was compounded by limited lab access and instruction on the equipment needed. It is worth considering whether the unknown quality of archaeological teeth samples effected the results and whether a larger sample or a more diverse sample would have altered the results in anyway. In addition it would have been helpful to have more time available in the lab to practice creating the thin sections so that the research could have been completed more efficiently.

CHAPTER 7: CONCLUSION

The aims of this research was to provide further research on cementochronology using the Cementochronology Research Program's Protocol. This was done using a sample of known-age-at-death individuals from an archaeological context. Of further interest was how successful this method could be in the hands of a novice researcher.

As was summarised in Chapter 2, multiple age estimation techniques exist which can be used on skeletal remains. Unfortunately, due to the nature of aging skeletons which behave differently between each individual these methods have different levels of reliability. This is to say that many of the age ranges which can be determined from these methods can be very large (+/-20 years in some cases). While this type of estimated age range is typically acceptable in archaeological contexts, forensic contexts often need narrow age ranges as this can mean the difference between having an unidentified skeleton and an identified individual. However, more precision when aging any skeletal remains is valuable as this can tell us much about the lives and deaths of past individuals.

Cementochronology has been shown to offer more accurate and precise age estimates for some researchers (Broucker et al., 2015; Maat et al., 2006; Naji et al., 2014; Naylor et al., 1985; Wittwer-Backofen et al., 2004). However, for others they found difficulties with the method, inconclusive results or poor results comparable to macroscopic aging methods (Bertrand et al. 2014; Renz &Radlinski, 2006). It is a destructive technique so researchers are understandably cautious in applying it to skeletal remains as the accuracy of the results must outweigh the loss of material. Therefore it is valuable to test the method thoroughly to prove its reliability or lack thereof.

This research was conducted to add to the previous data on cementochronology and highlight its efficacy in the hands of a novice research. While it was shown that there was fairly little intra observer error on the part of the author, the accuracy of the age estimations were far from ideal. While when tested in groups, the youngest group showed the highest positive correlation between actual age and estimated age, two individuals in their 50's were estimated within 2 years of their actual age. While another two in their 50's were estimated within 26 years of their actual age. And compared to the youngest individual whose actual age was 24 was estimated as 45, a 21 year difference. This shows that the consistency between the age groups is not nearly so simple as first appears from the correlation tests run on the data. This seemingly random inconstancy highlights the unreliability of cementochronology, at least in the hands of a novice researcher.

While this unreliability could be solely due to the inexperience of the author, it is also possible that some elements of the method used caused certain problems. Most notably, is the quality of the photographs of the cementum lines. This poor quality is possibly due to the thickness of the thin sections and/or by the limitations of the camera attached to the microscope. it would be interesting to see if improvements of the image quality could improve the results in any quantifiable way. As the slides are still held with the original skeletal material, a different image capturing method could be tested. This of course would require further research time and possibly more or different equipment which was not possible within the scope of this thesis. This is one of several future research avenues related to cementochronology. One other notable avenue is the prospect of computer programmes which could be created to count the cementum lines within a photograph. This has already been used in recent research conducted by Newham et al., who used radiographs (X-Rays) to image the cementum lines in rhesus macaque lower first molars (2021). By using radiographs they removed the destructive element of the method as the teeth did not need to be cut. Then they applied a computer software programme to count the lines in the radiograph image. They found that both the radiograph and software combination yielded good and accurate results, and noted that it compensated for several of the pitfalls noted in previous cementochronology research.

The possible benefits and supposed accuracy of cementochronology does not ignore some of the problems inherent with this age estimation technique. Steps have been made to implement an international protocol for the method but adoption is not yet universal. Even with a standard protocol, the method is labour intensive, especially for large samples. As researchers have pointed out, lack of experience with counting the cementum lines can easily lead to miscounts and errors. It is also a destructive technique which is not well suited for rare, irreplaceable archaeological samples. However, despite these drawbacks it is important to continue research into the method as it may be the most precise and accurate age estimation method. Ideally this thesis will provide insight to future researchers in the fields of osteology and cementochronology.

CHAPTER 8: BIBLIOGRAPHY

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