Laser Ablation of Modern Human Cementum

The examination of trace element profiles

by

Lisa Lefever

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements of the degree of

MASTER OF ARTS

Department of Anthropology

University of Manitoba

Winnipeg, Manitoba

Abstract

This study used LA-ICP-MS on a documented sample of modern teeth to sample from a continuous line across the cementum increments thus creating a temporal line graph of the elemental composition against distance. The knowledge of cementum was extended through (1) a more complete elemental composition analysis and (2) the relation of element distribution to the ultrastructure structure throughout the life of a tooth. This study was exploratory and demonstrated that lead, zinc, mercury, and barium follow the same general line of changes, and most likely represent changes in health and exposure to these metals in the general environment. Copper, manganese and vanadium varied very little. Technological limitations prevented the examination of element levels in any one annulation.

Acknowledgements

I would like to thank my advisor, Rob Hoppa, who constantly encouraged me to finish, and managed to wade through my random writings and massive literature review. I greatly appreciate the chance to show I can eventually make my way through a thesis.

Secondly, I could not have accomplished any of this without the help from the rest of my external committee member, Dr Norman Halden, who graciously showed me the ways of laser ablation and trace elements, and granted me access to his cool toys. Also, listening to a Scottish accent makes everything that much more interesting.

Another big thanks goes out to my other committee member Dr Gregory Monks, who kept me on my cementum and grammatical toes.

Other thanks definitely goes out to Panseok Yang, who sat with me, explained, and entertained me while we lasered my teeth. Sorry for making you analyze so many teeth. I'd also like to thank the rest of the Geological Sciences department, mainly Dr Mostafa Fayek and Rong Liu, who let me see what the SIMS could do analysis- and explosion-wise.

A huge thanks goes to my friends and family back in Edmonton, who never stopped bugging me to finish this thing off. A special thanks to Megan Caldwell and Rebecca Kerby, who kept me 'sane' with coffee and Tim Hortons breaks, and advice on how to handle graduate life. I probably would have quit without your guys' support.

Laurie Shead also deserves a thank you for showing me the ways of BDIAL and the important software (and more than once). You also let me talk cementum with someone who actually understood what I was saying. Thanks to my dedicated Helper Monkey, Ben Collins, who sectioned and polished my numerous slides, a far cry from being allowed to play with lasers.

I also greatly appreciate all who attempted to read over my chapters (Kristjan Weigl, Katie Waterhouse, Nic Cawthorne, and my mom, whose brain I nearly broke with my extended lit review.

All of this research was made possible by the financial aid of the Canada Research Chairs Program, the Faculty of Geological Sciences, and the Anthropology Department of the University of Manitoba. I'd also like to thank Dr Curran from the Faculty of Dentistry, who collected all of the teeth.

Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

Since the 1950s, cementum increments have been used to estimate the age of mammals. Its application to humans as an age estimation method, however, was not undertaken until 1982 by Stott and colleagues. Since that time, there has not been an adequate explanation for the observed incremental structures within this tissue. These increments are being used as a method for age determination in humans with some degree of success (Stott et al., 1982; Naylor et al., 1985; Stein & Corcoran, 1994; Kvaal & Solheim, 1995; Rao & Rao, 1998; Sousa et al, 1999; Jankauskas et al., 2001; Wittwer-Backofen et al., 2004). There is the potential for a higher degree of accuracy, in regards to age estimation, if more is known about what was occurring within the incremental structures, leading to a better understanding of the causes and influences of cementum increments.

The main component of cementum, and all dental tissues, is hydroxyapatite, which is made up of calcium, phosphorous, oxygen, and hydrogen [$Ca_5(PO_4)_3OH$]. Other elements do exist within cementum, but only as trace amounts. While trace elements are unlikely the cause for the optical character of cementum increments, they may offer some potential of recording environmental changes within these structures. Ultimately though, this is subject to limitations of the analytical techniques.

Elemental analysis of cementum is possible using a variety of analytical approaches such as atomic absorption spectrophotometry (Tsuboi et al., 2000), x-ray microanalyser (Tsuboi et al., 2000; Alvarez-Perez 2005), electron microprobe (Hals & Selvig, 1977; Selvig & Hals, 1977; Atilla & Baylas, 1996; Tohda et al., 1996; Tsuboi et al., 2000; Alvarez-Perez, 2005; Rex et al., 2005), F electrode (Nakagaki et al., 1985; Murakami et al., 1987; Nakagaki et al., 1988), inductive coupled plasma-mass spectrometry (Arora et al., 2004), synchrotron x-ray fluorescence (Martin et al., 2004), and PIXE (Cohen et al., 1981; Charudhri, 1984).

Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) is a much newer form of analysis that offers an opportunity to analyze elements within calcified tissues with spatial resolutions on the order of a few microns and with sensitivities in the parts per billion range. The primary use of LA-ICP-MS in teeth/osteological research has been to determine nutrition, pollution, and migration through the examination of trace elements and isotopes in enamel and dentin. Only a handful of studies have examined cementum, but these have focused on animal species such as walruses and beluga whales, and only as a spot ablation methodology in several different locations (Evans et al., 1994; Evans et al., 1995; Outridge and Evans, 1995). Two studies have attempted linear ablation on teeth, Hewitt (2004) and Ghazi et al. (2000), though both of these studies were performed on dentin.

This study uses laser ablation on a documented sample of modern teeth to sample from a continuous line across the cementum increments thus creating a temporal line graph of the elemental composition against distance. Changes in the elemental profile most likely reflect changes in diet or nutrition over the course of the individual’s life from the time of tooth eruption.

Questions to be addressed include:

1. Is there any correlation between elemental changes and cementum increments?
2. Do increments that are slightly thicker (more pronounced than others) contain a different elemental profile than the other increments?

3. Is there an elemental difference between areas of faster growth (cellular cementum) versus slower growth (acellular cementum)?
4. Will an increment have the same elemental profile in different areas around the root?
5. What is the implication of different elements in teeth, particularly in cementum?
6. Do amalgam fillings affect the amount of mercury within cementum?

Expectations

Calcium, phosphorus and fluoride have been studied more extensively in cementum than other elements. It is expected that the Ca profile will roughly follow increments, which Hals & Selvig (1977) briefly mention in their electron probe study of cementum. This would follow the theory that the same amount of calcium is deposited in both light and dark increments, but that the space within which it is deposited is different, causing differences in concentration (Klevezal & Kleinenberg, 1969; Lieberman et al., 1990; Lieberman, 1994; Kagerer & Grupe, 2001; Wittwer-Backofen et al., 2004).

Although quantitatively different, other studies have shown that the profiles of Ca, P, and F are most likely related (Selvig & Hals, 1977). If Ca fluctuations match increments, then the profiles of P and F should also coincide. There is also the possibility that no element profile will match exactly with all increments. However, the more marked increments should cause some change in element profile, giving clues as to why they differ from the rest of the increments.

The elemental profile of one individual should differ from that of others, and teeth from the same individual should have the same profile once differing ages of eruption are taken into consideration since nutrition and dietary changes should affect all teeth. It is also expected that teeth with amalgam fillings will have a sudden increase in Hg.

Importance of this work

This study is exploratory and will increase knowledge of cementum through (1) a more complete elemental composition analysis and 2) the relation of element distribution to incremental structure throughout the life of a tooth. It is distinct from other chemical analyses, such as stable isotopes, which is a much more common form of dietary analysis, especially in archaeological human remains. This project is also distinct from physiological analyses of cementum, which has been one of the main focuses of cementum research. Unfortunately, since personal information beyond sex and age at extraction was not attained, interpretation of results will be speculative.

There are numerous potential applications to forensics, toxicology, and nutritional studies. Forensically, providing investigators with a more detailed analysis of unidentified human remains increases chances of identifying the decedent(s). Analysis of elements within cementum could be used to show migration patterns of individuals, giving investigators information on where in the world an individual has lived, or estimating age at time of exposure to elements such as lead. Strontium is already used to determine migration of individuals but is mainly restricted to pre-natal and childhood periods when enamel is still forming. Cementum is not restricted in the same fashion, and could provide migration information throughout the life of an individual, correlating the migration pattern with time through cementum increments. For this type of analysis, precise information is needed on strontium levels in different areas around the world.

Other identifying features of an individual may also be recorded within cementum. Since cementum is formed continuously (unlike enamel) throughout the life of the tooth once the root is formed, and its increments are formed roughly one pair per year, we would be able to link changes in elements to a particular time period of an individual's life. A sudden increase in mercury may be related to when an individual received an amalgam filling. This becomes a distinctive feature beyond the mere presence of the filling in cases where identification of human remains is an issue, although amalgam is no longer used. Other identifying information may include dietary information, such as the general age an individual became a vegetarian. Possible speculation on smoking habits is another area that may be determined through elemental analysis. Baranowska *et al.* (2004) found that zinc & lead levels were higher in the teeth of smokers in comparison to those of non-smokers. Although there are potentially numerous sources of the intake of zinc and lead from diet or environment, speculations can be made as to when an individual began smoking.

Cementum elemental analysis could feasibly be extended into toxicology analysis. Normally, toxicology investigators can use hair to examine past exposure to drugs and chemicals. Cementum records these exposures for a longer period of time than hair, especially for those individuals with short hair. This can provide investigators with more information.

Teeth are also great indicators of long term exposure to pollution and trace metals. Bone and dentin remodel and lose information. Enamel does not remodel, but has limited recording time (childhood). Cementum has very limited remodelling, if any, and is a record of almost the entire life-time exposure of an individual. Mammalian cementum, which is thicker, and is therefore easier to analyze, has been very useful in determining when individual mammals were exposed to trace metals (Evans *et al.*, 1994, 1995; Outridge & Evans, 1995). To date, no one has undertaken this type of study using human cementum, and my study will be able to show exposure to trace metals in individuals over their lifetime.

There is also the potential to expand upon the work of Kagerer and Grupe (2001) of investigating what is occurring within more marked increments. Their study linked these increments to pregnancies and certain diseases, such as renal disorder, but could not find any link to diabetes, liver or thyroid disorders, osteoporosis, or malnutrition. Conducting elemental analysis of cementum has the possibility to corroborate Kagerer and Grupe's theory by showing what is occurring within cementum during those periods of pregnancy and renal disease.

Elemental analysis of these more pronounced increments would show what else is occurring, and perhaps explain why osteoporosis lacks any influence on cementum increments, while other events involving calcium removal from body stores do affect increments, as was reported by Kagerer and Grupe (2001). Analysis could point to influences health has on the formation of increments.

There also exists the potential for aiding in estimating ages of individuals. If peaks and troughs of element profiles are correlated with increments, it may point out areas where increments are not as visible, further aiding in increment counts and age estimation from cementum.

Even if elemental peaks and drops do not coincide with cementum increments, linking certain episodes in an individual's life to elemental composition is still possible. By overlaying the linear profile onto the image of the cementum increments, one would only need to count the increments until the elemental change of interest to figure the rough age of the individual at the time of occurrence. The importance and implications of this study will be thoroughly discussed in the subsequent chapters.

Chapter 2: Dental Development

Introduction

Teeth are a great asset for age determination in physical anthropology. Cementum annulations are said to be reliable in adult estimation but assessment can be problematic. This chapter will review the dental tissue development, the biology of cementum annulation and cementum as an adult age estimator in humans, while the following chapter will review dental tissue chemistry.

Odontogenesis/Cementogenesis

Odontogenesis, the formation of teeth, can be broken down into six stages of development: initiation, bud, cap, bell, apposition, and maturation. The process of tooth development involves two of the three germ layers: the mesoderm and the ectoderm. The former produces the mesenchyme while the latter produces the epithelium. These two tissues, the mesenchyme and the epithelium, interact to induce in each other proliferation, differentiation, and formation, and eventually give rise to teeth. The first stage of tooth development, initiation, begins during the sixth prenatal week for deciduous teeth, while permanent teeth begin formation during the 4th prenatal month (Gray 1974; Ten Cate 1998; Avery & Steele 2002). The mesenchyme begins the development of teeth by influencing a cluster of ectodermal cells to give rise to the horseshoe-shaped oral epithelium. The neural crest cells then induce the mesenchyme to condense into the dental mesenchyme, which helps induce the oral epithelium to form the dental lamina layer. The development of the dental lamina begins in the midline of both dental arches and progresses posteriorly. A basement membrane, a thin layer of connective tissue, exists between the dental lamina and the mesenchyme. The permeability of the basement membrane allows communication between the two. This permeability is how the epithelial cells are able to induce the mesenchymal cells and vice versa.

The second stage of tooth development is the bud stage, which begins at the eighth prenatal week. At this point, the tooth germ begins its development. Penetration of the dental lamina into the mesenchyme forms the buds for which this stage is named. The mandibular arch will have 10 buds, which begin forming from the back of the arch progressing toward the front. The maxilla will also have 10 buds, but these buds begin forming in the front area where the deciduous incisors will form, moving toward the posterior of the arch. Each bud is the beginning of the enamel organ and corresponds to each future deciduous tooth.

The third stage of tooth development is the cap stage. At the ninth prenatal week, the condensed dental mesenchyme is acted upon by the dental lamina and becomes the dental papilla. The remaining mesenchymal cells surround the early enamel organ that has now become more cap-shaped. The mesenchymal cells have now formed the dental sac, which, together with the enamel organ and the dental papilla, form the tooth germ. Since the enamel organ has developed from the dental lamina, and the dental papilla from mesenchymal cells, the basement membrane still remains between the two tissues. This position is of importance because the basement membrane is the future dentinoenamel junction (DEJ).

The fourth stage of tooth development is the bell stage when the cells within the enamel organ differentiate into four different types of cells, which form into the following layers: outer enamel epithelium, stellate reticulum, stratum intermedium, and inner enamel epithelium. The outer enamel epithelium will protect the enamel organ during amelogenesis (the production and development of enamel). The stellate reticulum and stratum intermedium both help support the production of enamel. The inner enamel epithelium will differentiate into preameloblasts and, later, produce enamel. On the edge of the enamel organ, the outer and the inner enamel epithelium meet and form the cervical loop. This junction area becomes important in root formation. The enamel organ is growing at the same time as these layers are forming, and changes into a bell shape. As it grows, the enamel organ begins to envelop the dental papilla, which is also undergoing differentiation. The outer cells and central cells of the dental papilla are formed. These outer cells will differentiate into odontoblasts (the cells that form dentin) after being induced to do so by the preameloblasts. The central cells, on the other hand, will eventually form the pulp of the tooth. The dental papilla is also responsible for the shape of the crown and root of each tooth.

The fifth stage of tooth development, apposition involves the secretion of the dental tissues (dentin, enamel, and cementum). Though labelled as the fifth stage, the timing of this stage of development varies for each tooth, as teeth do not form and erupt all at the same time. The first event in this stage of development is the appearance of preameloblasts. Elongation of the inner enamel epithelium begins with differentiation into preameloblasts. The outer cells of the dental papilla are then induced by the preameloblasts to differentiate into odontoblasts, which line up along their side of the basement membrane that still exists between the two tissues. The odontoblasts deposit a layer of dentin matrix, which is also called predentin. Once mineralized and mature, predentin becomes dentin. Deposition of predentin begins at the centre of the incisal edge in anterior teeth or of the occlusal cusps in posterior teeth, and proceeds in an outward direction as does enamel matrix deposition. Once the first layer of predentin is deposited, the odontoblasts then induce preameloblasts to begin producing enamel matrix. At this time, the basement membrane begins to disintegrate, allowing predentin to come into contact with preameloblasts as they are formed, and to induce the preameloblasts to differentiate into ameloblasts. The basement membrane also mineralizes and produces the DEJ. Enamel production can begin once the DEJ has stabilized.

The sixth stage of tooth development corresponds to the completion of the tooth's crown and the initial formation of the root. The tooth begins its eruption from the dental crypt that has formed around it, and the inner and outer enamel epithelium extends away from the crown to encompass more of the dental papilla. This extension of the inner and outer enamel epithelium that makes up the cervical loop becomes Hertwig's epithelial root sheath (HERS). Between the dental papilla and the root sheath is another basement membrane. Within the root are still the outer and inner cells of the dental papilla. The outer cells are induced by HERS to differentiate into odontoblasts and produce predentin as a continuation of the "coronal" dentin. The inner cells become surrounded by the dentin and form the pulp chamber of the tooth. Hertwig's sheath does not induce the enamel epithelial cells to differentiate into preameloblasts; instead, once the root dentin formation is complete, HERS disintegrates along with the basement membrane, forming the future cementodentin junction (CDJ). The disintegration of HERS leaves behind cells that become epithelial rests of Malassez, the role of which is still unknown.

During this final stage of tooth development, the dental sac, which surrounded the enamel organ earlier during the cap stage, now comes into contact with the root dentin surface after the disintegration. This contact induces the cells of the dental sac to differentiate into cementoblasts (the cells that form cementum) that cover the entire root dentin area. After the cementum is deposited, the dental sac forms the periodontal ligament. At this time, the dental sac also migrates outward and begins forming the alveolar bone and the outline of the tooth crypt. In between cementum and dentin is an amorphous layer that has been described as both a hyaline layer and intermediate cementum whose origin has yet to be uncovered. It is devoid of both cells and collagen fibres (Selvig 1964).

Cementoblasts initially produce a substance called cementoid or precementum, which is the equivalent to predentin, in that it is the unmineralized version of the final tissue: cementum. A layer of cementoblast cells exists between the tooth surface and the periodontal ligament (Schroeder, 1991). The periodontal ligament may also deposit cementum, as fibroblast-like cells within the periodontal ligament may be capable of causing cells to differentiate into cementoblasts (Schroeder, 1991; Ten Cate, 1998). Cementoblasts insert cytoplasmic processes into cementum at right angles, essentially anchoring the collagen fibrils within it (Ten Cate, 1998). Cementoblasts continue to deposit cementoid and collagen that is continuous with the anchored fibrils (Ten Cate, 1998).

Cemuntum

Cementum can be categorized in three manners based on content of cellular material, presence or absence of collagen fibres, and their origin. When cementoblasts are producing cementoid and become trapped within this matrix, they become cementocytes. The presence or absence of these cementocytes determines if the cementum is cellular or acellular. Cellular cementum tends to be deposited at a faster rate than acellular cementum. This faster rate of deposition is the cause of cementoblasts getting caught within the cementoid matrix where they remain. Acellular cementum does not contain any cementocytes mainly due to the fact that it is deposited at a regular, more consistent rate, and cementoblasts are not overtaken by the matrix that they are producing.

With acellular cementum, the subsequent step is to determine whether or not collagen fibres are present within it. The role of cementum is to anchor the root within the alveolar socket by cementing periodontal ligaments to the dental root, as the other end of the PDL is attached to the alveolar bone. Cementum can also produce its own "intrinsic" collagen fibres. Afibrillar cementum does not contain collagen fibres, PDL or cementum in origin, whereas fibrillar cementum does.

With the presence of fibres established, one must determine the origin of the fibres to further categorize cementum. Cementum that contains periodontal ligament (PDL) fibres contain "extrinsic" fibres because the fibres within it are of an external origin. Once these extrinsic fibres enter into the cementum, they are sometimes referred to as Sharpey's fibres. They extend out from the PDL into the cementum and can become entwined with the intrinsic collagen fibres (Ten Cate, 1998). Sharpey's fibres are physically attached to it, which strengthens the bond between root and alveolar bone. Intrinsic fibres always run parallel to the surface of the root, but change directions by 90 degrees in successive layers to form a lattice-like pattern (Hillson, 1996).

Thus, there may be up to 5 types of cementum: acellular afibrillar cementum, acellular intrinsic-fibre cementum, acellular extrinsic-fibre cementum, cellular mixed-fibre/mixed stratified cementum, and cellular intrinsic-fibre cementum. In general, human acelllular cementum covers ½ to 2/3 of the cervical root, while cellular cementum is on the apical portion (Bosshardt & Selvig 1997; Ten Cate 1998; Avery & Steele 2002).

Incisors and canines do not generally contain cellular cementum (Bosshardt & Selvig 1997; Ten Cate 1998).

Cementum Annulations

The deposition of cementum forms a pattern of light and dark bands, the pairings of which are annual. There are several different words used in reference to these bands. Each light and dark band is a pair of 'increments', together forming an 'annulation'. Deposition of cementum throughout the year is not at a constant rate, with periods of increased and decreased activity. The thicker increments are seen as being representative of periods of growth (such as summer months), while the thinner ones are deposited during periods of rest or slower growth. Several different terms exist for both types of increments. Incremental growth or growth layers (Monks 1981), adhesion lines (Klevezal and Kleinenberg 1967), incremental growth lines (Grue & Jensen 1979), or simply summer lines, all refer to the thicker, light increments. The thinner increments are often referred to simply as winter lines.

Other descriptive terms such as translucent and opaque are dependent on the type of light being used to examine cementum. Since cementum is optically anisotropic, increments that are light and translucent when viewed with transmitted light will become dark when a polarizing lens or reflected light is used, and vice versa for the dark increments (Lieberman 1994).

Why the visual difference?

The reasons behind the optical occurrence of increments are as important as the reasons why annulations occur since one helps explain the other birefringent properties.

The two major components of cementum, collagen and hydroxyapatite crystals, give cementum birefringence. Both components follow analogous orientations throughout the tissue (most likely in relation to the intrinsic fibres). It is not entirely clear as to which type of collagen fibre is responsible for the main birefringence. Currently only speculation can be made on the reasons for the visual occurrence of annulations. Birefringence of the tissue implies orientation changes in fibres and hydroxyapatite crystals, while microradiographs imply a difference in density.

Fibre orientation differences

One common explanation for the visual presence of increments involves the differing orientation of collagen fibres and the corresponding hydroxyapatite crystals. This area of study is complicated by using such a general term for changes within three-dimensional space. Bone and turkey tendon studies are often referred to for fibre and crystal information since both tissues are larger and easier to examine than the intrinsic and extrinsic cementum fibres.

Cementum (intrinsic) fibres are being laid down parallel to the root surface while Sharpey's (extrinsic) fibres run perpendicular. Some researchers have theorized that changes in the orientation of Sharpey's fibres coincide with increments (Lieberman & Meadow 1992; Lieberman 1994; Bosshardt & Selvig 1997). This theory is problematic as cellular cementum does not always contain Sharpey's fibres, yet it still contains increments. If the changing direction of Sharpey's fibres were the true cause for the optical occurrence of increments, they should only exist wherever extrinsic fibres are present. Intrinsic fibre direction also cannot be deemed the increment-causing culprit as increments are found in areas that are purely extrinsic fibres (AEFC). If fibre orientation is responsible for incremental pattern, then both intrinsic & extrinsic fibres would have to be able to cause the pattern.

Hydroxyapatite crystallites have been noted to be precipitated along pre-existing collagen fibres in bone (Lowenstam & Weiner, 1989; Christoffersen & Landis 1991; Schmidt & Keil 1971). If the same applies to cementum, it is the changes in fibre orientation that needs to be explained.

Difficulties in determining causes for incrementation are further complicated by an alternate lamellar pattern that has been found within some annulations. Yamamoto and colleagues (1997, 1998, 2000) discuss a lamellar pattern of longitudinal and transverse intrinsic fibres within mature cellular cementum. These ‘layers’ are formed within the broader translucent increments. The appearance of the fibril direction is affected by the manner in which a specimen is prepared and sectioned. The research on alternate fibrillar pattern has not shown evidence for fibre orientation changes in a larger scale to correspond to increments.

Density

Mineralization differences between the light and dark cementum layers have been one of the longest standing theories behind the optics of cementum. One of the major difficulties with using the term mineralization is that it is seldom explained, nor the process well understood. Mineralization and calcification are often used interchangeably. Since the topic of mineralization and calcification is so complex, the use of the term 'density' will be used instead.

Annulations have been described as a set of one large band of poorly mineralized matrix (cellular cementum) and a thinner, more mineralized band (acellular cementum)

(Selvig 1965; Lieberman 1994; Bosshardt & Selvig 1997; Nanci & Ten Cate 2007). These terms are more common amongst animal studies since the difference in size between the two increments is much more obvious is animal teeth than in those of humans. Studies discussing ‘mineralization’ differences are actually referring to density differences. In terms of X-rays, a difference in density between some of the increments in acellular cementum has been demonstrated by Soni and colleagues (1960), Selvig (1977), Lieberman & Meadow (1992) and Tohda and colleagues (1996), though not all of the increments are visible on these microradiographs.

Cellular cementum is generally seen as less mineralized since its density is lower than that of acellular cementum. Its faster deposition rate and cell space gives the appearance of having a less concentrated matrix. The rate of deposition of matrix also differs between the two increments that make up an annulation, which is much more evident in cellular cementum, and even more so in animal cementum.

The fibres themselves are purportedly 'mineralized' to different levels. The Sharpey's fibres in acellular extrinsic fibre cementum (AEFC) have been found to be more mineralized than those found in cellular mixed stratified cementum (CMSC) (Dreyfuss & Frank 1964; Selvig 1965). Selvig (1965) observed that the outer layers of acellular cementum were rather homogenous in their microradiographic density, but did find that some of the increments were visible on the X-rays.

Other support for mineralization differences includes the use of hematoxylin. This stain is used in histology to stain for calcium salts. Theoretically, different amounts of calcium salts would be stained to differing degrees. Differences in the mineralization of successive layers of cementum, however, must include more than simply the amount of calcium salts. Differences in calcium salts do not explain the birefringent nature of cementum.

Renz and colleagues (1997) have been one of the few studies to show slight changes in Ca levels across cementum. When compared to the increments, however, the peaks and troughs could not be correlated. If mineralization of cementum is measured by Ca & Mg levels as reported by Selvig & Selvig (1962), then there is little possibility that the visualization of increments is caused simply by differences in mineralization.

Differences in tissue density, however, are not what give cemenetum its birefringence. There is little evidence to support the theory of mineralization differences, except that of density differences that appear in X-rays. Not only is there no elemental evidence for mineralization differences from past studies, the process of mineralization is complex and not well understood. This also leads to the question as to how mineral differences can be responsible for the birefringent nature/properties of cementum

It may not be a question of concentration as it is orientation of crystals and fibres. Recent researchers have suggested that it is not just mineral concentration, but crystal orientation that accounts for the appearance and nature of cementum increments (Wittwer-Backofen et al 2004).

Tohda and colleagues (1996) took microradiographs to examine relative mineralization differences in cementum. Although they found acellular cementum was fairly homogenous, the microradiographs showed some density differences. Their examination of exposed cementum showed a highly dense external surface. This, however, was not reflected in the Ca results of the electron probe analysis, but in those of the fluoride levels. Fluorine (F) shows where the tissue had become more mineralized from exposure. This does not mean that F levels would change with all mineralization changes since the increase in F occurs prior to caries, as a defence mechanism.

There are no clear explanations for the visual occurrence of cementum increments. We are unaware of what is physically occurring, making any theory mainly conjecture. Claims of mineralization differences have not been supported by elemental studies such as those of Cipriano (2002) and Kagerer & Grupe (2001). Cipriano (2002) correlated occurrences of more marked increments in great apes with particular cold winters, while Kagerer & Grupe (2001) correlated pregnancies, skeletal trauma, and one case of renal disorder to more marked increment for the particular year. The authors, however, could not correlate occurrences of diabetes, liver or thyroid disorders, osteoporosis, or malnutrition with marked increments. Neither study performed any chemical analysis to determine calcium levels in layers supposedly affected by a lack of calcium.

Age Estimation from Cementum Annulations

Animal cementum has been studied for over half a century. Its formation is the same as humans as it functions in the same manner, anchoring the tooth into its socket, allowing the animal to chew its food. Zoological studies (Sergeant & Pimlott 1959; Klevezal & Kleinenberg 1969; Grue & Jensen 1979) have clearly established the annual rate of cementum deposition and that the annulations in animals fluctuate in size seasonally. Within the zooarchaeological literature it was argued that the main reasoning for cementum increments was seasonal diets, where the nutrition and hardness of the food consumed varies greatly between summer and winter months. The difference in strain and nutrition were argued to affect the orientation of the collagen bands and the relative mineralization of the cementum in such a way that it caused visual layers in the deposition (Lieberman 1994). However, Burke and Castanet (1995) examined the teeth of domesticated animals fed a consistent diet year round and found that they still had clear changes between increments.

As a result of these earlier zooarchaeological studies, the use of cementum annulations in humans as a method of age estimation in adults was explored in the 1980s (Naylor et al 1985; Charles et al 1986; Condon et al 1986; Miller et al 1988). However, difficulties in validating the use of cementum annulations in age determination studies stem from the subjectivity of optically assessing these structures in human teeth. Unlike most mammals, humans have relatively small teeth and relatively long lifespans, making counting difficult.

Validation studies have varying success rates. Subjectivity of counting accounts for only a small amount of error. Inter-observer error accounted for five percent of variance , while only two percent of variance could be contributed to intra-observer error (Charles et al 1986: Condon et al 1986). Too many variables are not being controlled for in these validation studies, making comparisons between the studies difficult. The visualization of annulations can be influenced by methodology. That being said, the clarity of annulations in thin-sections does not necessarily lead to accurate age estimations (Wittwer-Backofen et al 2004; Renz & Radlinski 2005). There are other variables of differing degrees of importance that are not being controlled for in these validation studies; e.g.sample size, mean age, section thickness, decalcification, eruption tables, and direction of section (longitudinal versus cross sections). Even the manner in which they calculate the count differs between modal counts (Charles et al 1986; Condon et al 1986; Kagerer & Grupe 2001a; Blondiaux et al 2006), averaged counts (Lipsinic et al 1986; Miller et al 1988; Grobkopf 1990; Sousa et al 1999; Jankauskas et al 2001;

Wittwer-Backofen et al 2004), median counts (Renz & Radlanski 2005), and using the highest count (Kvaal & Solheim 1995; Wittwer-Backofen & Buba 2002). Occasionally, studies determine age by a single count of annulations (Stein & Corcoran 1994; Rao & Rao 1998).

The results of some studies are highly speculative. In the study by Renz and Radlinski (2005) one observer recorded a count of zero as well as 25 annulations on the same section in the same root quadrant. Other poor results/correlations may be attributed to counting methods. Lucas and Loh (1986) counted annulations from the microscope eye-piece, while most other studies use a photomicrograph.

A further complication at attempts to explain the occurrence of increments is the doubling phenomenon: the deposition of two sets of increments within one year. This phenomenon is widely reported and occurrences are documented in both humans and mammals alike. This phenomenon is not understood and has not been specifically studied. Wittwer-Backofen and colleagues (2004) hypothesize that the tendency of second premolars to bifurcate may cause incidences of "doubling". The authors found that when they examined thin sections of second premolars, many of the roots displayed a wavy pattern that suggested the roots were attempting to split into two. Wittwer-Backofen and colleagues (2004) felt that the confusion created by this wavy pattern may be responsible for double counts. Condon and colleagues (1986) had 4 cases of doubling in which all four were maxillary second premolars. Unfortunately, the doubling phenomenon is not limited to second premolars. Stein and Corcoran (1994) reported three cases of doubling in maxillary and mandibular central incisors, single rooted teeth. Regrettably, the sample was collected from 42 individuals and the authors fail to note if the teeth are from the same individuals. Thus, it is unknown how many different individuals had teeth affected by doubling represent, or if individuals had other teeth that did not display doubling.

Wittwer-Backofen and Buba (2002) have suggested a genetic cause for this phenomenon, however, the study conducted by Kagerer and Grupe (2001) involved an individual who demonstrated “doubling” in one tooth, but not another. A genetic origin would predispose all teeth to “doubling”. Stein & Corcoran (1994) noted that when teeth affected by doubling were viewed at a higher magnification, their increments were not as clear as in other teeth. It is possible that the doubling could be associated with the alternate lamellar pattern described by Yamamoto and colleagues (1997, 1998, 2000). The authors demonstrated multiple lines existing between increments (i.e, more than 2). Still, alternate lamellar pattern could be associated with the doubling phenomenon.

Summary

It is clear that the issue of understanding the formation and counting of cementum annulations for anthropological studies remains difficult. This study will examine the biochemistry of the cementum in order to ascertain whether changes in tooth biochemistry within the cementum can aid in resolving the annual structures themselves as well as more broadly contributing to our understanding of the formation of these structures and the role of life history analysis from dental tissues laid down throughout adulthood. As such, a review of the biochemistry of the dental tissues and methods for investigating their variation is warranted. These will be discussed in the following chapter.

Chapter 3: Tooth Chemistry

Introduction

This chapter examines of elements known to occur in dental tissues, their potential sources, and how they can be affected. Certain elements are essential for life; this includes calcium (Ca), magnesium (Mg), vanadium (V), manganese (Mn), copper (Cu), and zinc (Zn). The human body requires a certain amount per day, and if these requirements are not met, elemental deficiencies can result. The body takes steps to keep this from happening, such as loading the body with reserves to a certain extent. For example, most of the body's calcium is stored in the skeletal structure. When the body is not getting enough calcium to maintain proper function, calcium is removed from bones. The body's maintenance of these essential elements is ruled by homeostasis. Overdoses of elements may also occur, though the body tends to excrete the excess amounts of essential elements usually via intestine or kidneys to maintain homeostasis (Mertz 1981). Even with these safety features, consumption and absorption of excess essential elements can still happen.

Non-essential elements are not ruled by homeostasis, therefore they are more likely to be subject to variation. These elements are generally used to examine diet, environmental monitoring and heavy metal exposure. Essential elements have also been used for dietary purposes, and are discussed individually throughout this chapter.

Some elements are bone-seeking and are preferentially taken up into the hydroxyapatite structure of bone and most likely teeth as well. These include Pb, Zn, Mn, Ba, and Sr, all of which are metals of Group IIA on the periodic chart of elements.

Elements

Magnesium

Magnesium is an essential element that controls the mineralization of bone and most likely has a similar role in teeth (Serre et al 1998). Mg may affect Ca levels since the parathyroid hormone (PTH) requires Mg, and Ca needs PTH. Humans generally absorb Mg from foods such as nuts and vegetables which have very high and high levels, respectively. Meat, on the other hand, has low levels of Mg. This difference in Mg levels was the basis in some paleodietary studies interested in differentiating vegetarians from non-vegetarians (Blakely & Beck 1981; Francalacci 1989). There are inherent problems with this type of study. Firstly, Mg is an essential element and is therefore regulated within the body. Secondly, non-vegetarians generally still eat vegetables & nuts, and there is no simple accumulation of consumed (essential) elements.

Absorption of magnesium occurs within the small intestine. Factors that inhibit Mg absorption include dietary fibre, diseases or disorders affecting the small intestine or colon (renal disease), or alcoholism (WHO 2004; Gropper et al 2005).

Zinc

Zinc is part of several different systems, which include cell division and growth of bones. Although essential, there does not seem to be strict control over zinc. Severe deficiency can result in slower growth, affecting both sexual and skeletal maturity. Zinc mainly resides in muscle and bones, 30% of which is in the latter (WHO 1996). The amount of zinc in teeth has not been substantiated.

Factors that increase urine or sweat output, such as alcohol, exercise, and fevers, also increase the amount of zinc excreted from the body. Alcohol may also decrease zinc levels since zinc is involved in detoxifying alcohols in the liver. Stress, burns, surgery and weight loss all decrease zinc levels in the body, as well as pregnancy potentially which decreases zinc levels in plasma (Aggett & Favier 1993). Zinc levels in insulin-dependent diabetics are generally lower since zinc is involved in the production of insulin (Nakamura et al 1991). On the other end of the spectrum, increased levels of zinc have been correlated with smoking (Baranowska et al 2004).

Zinc is a vital part of the immune system. As a result, Zn levels in people suffering from immune disorders may vary greatly. Individuals afflicted with cancer tend to have higher levels of zinc (since it is a product of an overactive immune system) (Yoshinaga et al 1995). Immune-deficient individuals in all probability have lower levels of Zn within their system. Other non-nutritional complicating factors include soil levels (Haas 2006), tuberculosis (which has been linked to increased zinc levels in teeth) (Cruickshank 1949), and stress, burns, surgery, and weight loss all decrease zinc levels (Haas 2006).

Manganese

Though there is no known role in tooth development, Mn is used during skeletal development, with 50% of its body's stores in bones (Fraga 2005; Haas 2006). Diet is responsible for most of the Mn, coming from whole grains, nuts, tea, cloves, ginger, thyme, and bay leaves (Schroeder et al 1966). Plant sources contain higher levels of Mn than meat, which contain fairly low levels

Manganese is also present in drinking water and is used in industrial production of steel and iron alloys. It can also be found in cleaning products as the oxidant potassium permanganate, or in water treated with Mn greensands to make it more potable (WHO 2003). Absorption can also occur in the lungs if particles are inhaled, which mainly occurs in miners.

Homeostatic control over Mn may be greater than other essential elements as manganese does not accumulate within the body over time with normal exposure, nor does it decline with age (Schroeder et al 1966).

There are only a few factors that can complicate Mn absorption. Calcium can significantly decrease its absorption, while other elements, such as V, Fe, and Co merely compete with Mn (Schroeder et al 1966; Curzon & Cutress 1983; Davidsson et al 1991).

Copper

Copper is an essential element with several different roles in the human body. It is a part of complex proteins of connective tissues of the skeleton and blood vessels. Copper's role in the development of connective tissue also affects how bone forms since collagen is a major component of the tissue. Cu also affects the healing of tissues in the same manner. Copper deficiency can often lead to abnormal bone formation. The skeleton may become more fragile and develop osteoporosis, or scurvy-like changes, causing delayed maturation (WHO 1996; Abrams & Griffin 2004).

Dietary sources include organ meat, whole grains, nuts, seeds, oysters and dark chocolate, with animal sources containing a higher amount of copper (Klepinger 1984; Fraga 2005; Haas 2006). Copper pipes and cookware releases ions to the water and food in contact with them. Amalgam fillings may also give off minute amounts of copper.

Copper suffers from only a couple of complications, one of which is the competition for absorption between copper and zinc. Excess zinc will interfere with copper absorption; likewise manganese can also cause interference (Fraga 2005; Haas 2006). Copper serum levels can be affected by estrogen, causing increases during pregnancy or when taking birth control pills (Haas 2006). If the body does not excrete the excess copper during these periods, there is the potential to see when a female may have been pregnant.

Both deciduous and permanent teeth have been found to contain a range of levels in quantitative studies (Brown et al 2004; Derise & Ritchey 1974). However, individual needs vary, which would cause the quantitative amount within their teeth to differ

Lead

Lead is a non-essential element that is toxic at low levels. Being bone-seeking, the majority (95%) of Pb resides in the skeletal system, with a higher concentration in teeth (Strehlow & Kneip 1969; WHO 2001). Lead has been noted to vary in concentration in differing locations of bone and teeth. The general trend is an increased concentration within the surface of enamel, pulp, circumpulpal dentin and cortical bones (Brudevold et al 1977; Zoeger et al 2005; Bellis et al 2009). This pattern may be true of many of the extraneous elements incorporated into tissues. Carious teeth have been found to contain higher levels of Pb (and Hg) than healthy teeth (Boldevold et al 1977; Carvalho et al 1998; Tvinnereim et al 2000).

Pb in bone can be released back into the bloodstream during times of bone turnover or demineralization, both of which occur at a higher rate during pregnancy, lactation and menopause (Silbergeld et al 1988). This may cause increased Pb levels in cementum during these time periods.

As an element, lead can interfere with Ca metabolism and can even displace it, causing areas of weakness in bone (WHO 2003; Haas 2006). It also interferes with the absorption of Cu, and Zn (Haas 2006). By interfering with Zn absorption, Pb can suppress the immune system (Haas 2006).

Lead is more easily absorbed and more difficult to excrete in younger individuals (Calvery 1942; Keller & Roherty 1980; WHO 1996). Adults are capable of increased Pb absorption if on a liquid diet, when fasting, or when Pb is inhaled (Rabinowitz et al 1980; WHO 1996, 2001). Inhaled Pb is absorbed more than ingested lead (Kehoe 1961; WHO 2001). This increased absorption is evident in smokers who have been shown to have higher levels of Pb in their teeth (Baranowska et al 2004). Food is another major source of Pb. Lead is a naturally occurring metal present in soil and rocks, which is taken up by plants. Plants also acquire lead from Pb-containing insecticides or from fallout in industrialized areas (WHO 2001). 35% of an individual Pb intake comes from bread, cereals and drinks (WHO 1996).

Mercury

Like lead, mercury (Hg) is a non-essential, toxic element. Human intake of Hg is mainly from food unless occupationally exposed (WHO 2003). One of the main dietary sources is fish and sea food. They acquire Hg from the water they inhabit, accumulating it over time. Larger fish have higher levels of Hg because they have had longer to acquire Hg from the water. Mushrooms also accumulate Hg from the surrounding soils. Plants, however, do not accumulate Hg from soil, but may acquire it from pesticides and fungicides. Another source of Hg is amalgam fillings which are 40-50% Hg (Hahn et al 1989; Weiner & Nylander 1995; Carvalho et al 2002). Fillings both emit vapours and cause Hg ions to disperse through dental tissue (Hoffmann et al 2000; Carvalho et al 2002). There is a long standing debate as to how dangerous amalgam fillings actually are, especially when considering that 80% of inhaled Hg is absorbed (Weiner & Nylander 1995). Ingested Hg is not taken up to the same degree; less than 15% gets absorbed (Clarkson et al 1988). Skin can also draw in Hg when exposed to the element but at an insignificant amount (Hursh et al 1989)

Mercury was much more commonly used in the past before its toxicity was discovered. Past sources include make-up, medication, thermometers, teething powder, skin ointments. Even today, we have not completely eliminated its use in some batteries, fluorescent lighting, barometers, insecticides, rat poison, and electrical ‘gear’ (e.g. switches and rectifiers).

Vanadium

Although only a very small amount of vanadium is found in teeth (5-50 ppm from Chaudhri & Chaudhri 2006), it does play a role in bone and tooth formation. This enzyme-stimulating element is also involved in Ca metabolism (Haas 2006). Its salts have also been shown to stimulate the mineralization of bones & teeth (Rygh 1949).

Inhalation is the main point of absorption in humans since it is present in air pollution, and more concentrated in urban areas. Absorption through the skin is also possible. Soil and water contain varying amounts (WHO 2000). Foods have generally low quantities, though vanadium can be found in fats and vegetable oils. Once inside the body, V is mainly stored in bone and fat.

Strontium

Although not essential, strontium (Sr) is important to the skeletal system, increasing the strength of teeth and bones (Haas 2006). As a bone-seeking element, 99% of Sr in the human body resides in the skeleton (Haas 2006; Schroeder et al 1972). Structurally, Sr resembles Ca allowing it to replace some of the Ca ions in hydroxyapatite, where it is readily incorporated (LeGeros et al 1974). However, the body discriminates against Sr in favour of Ca. Because of this, Sr/Ca ratios have been used with (moderate) success in trophic studies.

Plant sources contain higher levels of Sr than meat, which contain fairly low levels. Because of this difference, Elias (1980) studied Sr levels in enamel in an attempt to discern different types of diets (vegetarian vs non-vegetarian). However, this study was very unsuccessful, having initially forgotten that non-vegetarians still consume large amounts of vegetables.

As with other trace element, large amounts of ingested Sr cause problems. When too much Sr is ingested, it can replace too many Ca ions in teeth and bones, disrupting their growth (Johnson et al 1966; Lenntech.com). This disruption causes animals to develop rickets (Follis 1955).

The majority of daily Sr intake comes from food, which vary greatly in their Sr content, while drinking water is responsible for 10% (WHO 2003). Sr levels in the air have been increasing because of human activity such as burning coal and oil combustion.

Pregnancy (and lactation) affects the absorption of Sr, increasing how efficiently it is absorbed by the body (Nielsen 1987). This may or may not be displayed in cementum.

Barium

Barium is a bone-seeking, non-essential element, 93% of which resides in bone and its connective tissues (Schroeder et al 1972). Paleodietary studies have examined Ba levels in archaeological human remains to determine whether they had a marine versus terrestrial animal diet, as marine animals have much higher Ba levels than terrestrial ones (Klepinger1984). Ba is also discriminated against in favour of Ca, but to an even higher degree than Sr. Unlike Sr, barium has not been found to increase with age (Schroeder et al 1972; Nielsen 1987).

Ba naturally occurs in water, as well as both igneous and sedimentary rock, and can accumulate within marine life (WHO 1996). Hard water generally contains more Ba than soft water (Curzon 1983). Areas of industrialization have increased levels of Ba in air, water and soil. Ba is used in several different industries, for fluorescent lamps, paint, bricks, tiles, glass, and rubber. The act of mining Ba releases it into the air. Individuals within these industries will be occupationally-exposed; otherwise humans primarily receive Ba from food (WHO 1996). Barium is generally not toxic unless occupationally exposed.

Absorption

Certain factors may influence the absorption levels of trace metals in teeth. Caries allow the infiltration of elements such as lead, zinc, and mercury (Tvinnereim et al 2000). Tvinnereim and colleagues (2000) found that tooth type, the presence of caries, and teeth with roots had differing effects on levels of Pb, Zn, and Hg in deciduous teeth from Norway. Selvig and Hals (1976) also noted that carious teeth had higher levels of zinc.

The incorporation of elements into the apatite structure does not occur equally. As mentioned earlier, bone-seeking elements (Pb, Sr, Zn, Mg, etc) are integrated much more easily and readily than others. Elements themselves are deposited uniformly around the root so levels within a particular increment will be consistent in different areas (Evans et al 1995; Stern et al 1999).

Diffusion

An important factor to consider with spatially sensitive analytical research is whether ions are able to move around within the tissue. It is of great concern that ions that are originally deposited into the crystallite structure stay there, otherwise temporal and spatial information is lost.

Extraneous elements present in the tissue fluid are incorporated into the hydroxyapatite during the initial crystallization (Bosshardt & Selvig, 1997). The concentration of these ions may change as more ions are adsorbed, or by substitution of other ions. Studies have also examined enamel and dentin to a certain extent; however agreement on the possibility of element diffusion within these tissues has not been ascertained. Several authors maintain that once an element is deposited in dental tissues, the element ion is unable to move around within the tissue (Curzon & Cutress, 1979; Losee et al, 1974; Curzon et al, 1974; Lee et al, 1999). However, limited diffusion through different dental tissues has been noted of certain elements, such as Cu, Zn, and Hg from amalgam fillings (Carvalho et al, 1998). These elements were noted to have the highest portion of the gradient right near the amalgam.

Homeostasis

The question of how much homeostatic control regulates essential elements is very important to this type of research. Teeth are extremely small reservoirs of elements in comparison to the rest of the body (skeleton, soft tissue, bodily fluids). As such, whether teeth are small enough to not be affected by homeostatic control is a vital issue. Several of the elements being investigated here are essential to life and theoretically are under strict regulation.

It is uncertain if teeth, especially cementum, are small enough to be able to record minute differences in homeostatically controlled elements. Teeth may record small changes in element levels, changes small enough to stay within homeostatic control. Changes in elemental patterns in cementum could reflect the body's changing need for essential elements. Non-essential elements have the most potential to vary since they are not under homeostatic control. Difficulties in this area stem from lack of knowledge as to what the trace element concentrations within teeth represent. Most anthropological research has concentrated on TE levels in bone in relation to diet or environmental exposure; however the repercussion that may occur within teeth when an individual experiences dietary deficiencies is unknown.

Cementum Studies

Cementum has never been a main interest of researchers, always being overshadowed by the more prominent dental tissues (enamel and dentin). This is partly due to the minute nature of cementum. Older technology required larger amounts of tissue in order to examine element concentrations. Separating cementum from dentin is a difficult task. Some studies have used the different densities of dental tissues to separate them once they have been pulverized. It was also difficult to obtain enough cementum to examine, especially when dealing with younger teeth that have not accumulated much tissue. With advancing technology, we are able to analyze smaller amounts of tissue with increasing accuracy, and in situ (discarding the tedious task of tissue separation).

The majority of past cementum studies examined fluorine (F) levels, in sound and exposed cementum, and its changes with age (Nakata et al 1972; Crawford et al 1983; Nakagaki et al 1985, 1988; Murakami et al 1987; Tohda et al 1996; Soyman et al 1997; Tsuboi et al 2000; Rex et al 2005, 2006). Fluorine, however, is not one of the elements that were chosen to be analyzed in this study. Other types of studies examine how diseased cementum differed from normal, sound cementum. Selvig & Selvig (1962) examined the changes in Ca, Mg and P with age and periodontal disease. Selvig & Hals (1977) chose to examine the elemental profiles of Ca, Mg, P, F, and S in order to see changes in exposed cementum which supposedly increases in mineral content. With the aid of the EPMA, they found that calcium levels increased, while Mg levels were unaffected by exposure. Hals & Selvig (1977) analyzed carious and non-carious cementum, finding that the highest levels of Ca and Mg were near the outer surface. Martin and colleagues (2004) used synchrotron X-ray fluorescence to take a general look at relative element intensities in cementum of two Peruvian mummies. They found that zinc was quite intense in cementum in comparison to dentin levels.

Most micro-analytical methods are capable of producing a very small beam to analyze a specimen; however, meaningful results may not be attained. A minute beam size is only capable of ablating minute amounts of tissue to analyze. So the question becomes how small the beam can be before the ‘machine’ is unable to detect a meaningful amount of trace elements. More tissue will be required to properly detect the trace elements in a sample than more major ones (such as Ca, P, F, etc.). For example, the electron microprobe (EPMA) is able to use a much smaller beam than the LA-ICP-MS to analyze a specimen, but it is unable to detect the lighter elements that is detected and analyzed by the LA-ICP-MS. The key to studying cementum is being able to (1) analyze microscopic amounts of tissue and (2) properly detect trace amounts of elements. The LA-ICP-MS has the potential to allow us to do both.

<u>LA-ICP-MS</u>

There are three general parts to the LA-ICP-MS. The laser, which uses photon radiation, ablates the sample material and forms an aerosol vapour of particulates within the sample cell. Argon gas is used to sweep these particulates to the inductively coupled plasma where the material is ionized and enters the mass spectrometer. Here, the ionized materials are separated by their mass and measured. The LA-ICP-MS system does not use an ion beam or charged particles to analyze a sample, and therefore does not need samples to be electronically conductive. This means that non-conductive materials can be analyzed without being coated by carbon or gold as in the cases of electron probe microanalysis (EPMA) or secondary ion mass spectrometry (SIMS), respectively.

LA-ICP-MS comes with several advantages over other methodologies, making it an attractive and somewhat ideal form of analysis for cementum. The LA-ICP-MS can perform in situ analysis of solids which allows spatial and temporal information to be assessed. The ablation process leaves a physical mark on the tooth surface which is microscopic and considered to be minimally destructive, aside from having to section the tooth to reveal the cementum. This microscopic line can be deemed beneficial as it allows a match-up of element profiles with the ablated area.

The efficiency of this analytical system is another benefit. It is capable of analyzing multiple elements simultaneously. The speed of analysis is also an advantage over other methods such as the SIMS - one line of spot analysis across cementum may take two days with SIMS, while a LA-ICP-MS line scan can be performed in a matter of minutes, calibration notwithstanding. The lack of complex sample preparation also adds to the time efficiency of this analysis, as well as reduces the possibility of contamination since samples are handled less and exposed to fewer substances.

LA-ICP-MS suffers from some disadvantages. The accuracy of the machine can drift over time if not constantly calibrated, but this is true of all instruments. LA-ICP-MS has several different areas of calibration and tuning, and has been noted to be quite complex by Cox and colleagues (1996). The plasma also has several different parameters that can be adjusted. Another fairly prominent issue is the need for matrix-matched reference material for proper calibration and to obtain quantitative results. Not all matrices ablate in the same manner, and may affect analysis. To limit this 'matrix effect', matrix-matched standard reference materials (SRM) are used for calibration and results in quantitative data. Cementum tissue does not have a matrix-matched SRM, which limits analysis to semi-quantitative results

There is also the issue of fractionation, where not all of the ablated material is sent to the ICP. When these larger particles fail to be swept away into the plasma of the ICP, they fall back down to the sample's surface. Here they form what is known as a 'halo effect'. In the case of spot analysis, ablation spots are located a certain distance apart to avoid re-ablating the extraneous material that is no longer in its proper spatial place. This avoidance can limit spatial analysis. Fractionation is not well understood, but can be reduced, although not eliminated all together (Chen 1999).

There are variations in relative instrumental sensitivity between elements, so not all elements can be measured to the same degree of certainty. Any element analysis has a minimum detection limit with which it is effective. Detection limits for LA-ICP-MS are reported to be approximately 0.5 to 5µg/g in apatite matrix (Chenery et al 1996). Theoretical detection limits for individual elements are as follows: 140 ppm (Ca), 10 ppm (Mg), 15ppm (Fe), 5 ppm (Mn and Cu), 2 ppm (Zn), 0.6 ppm (Sr and Pb), and 0.4 ppm (Ba) for a crater of 20µm wide and 10 µm deep (Chenery et al 1996). However, the limits of detection (LOD) can be calculated for individual elements in specific samples, using the peak intensity, the background levels, and the concentration of each element. The limits of detection are listed ppm (parts per million), which is the concentration of the elements; however, the data produced is generally given in intensity by counts per second (cps).

The homogeneity of cementum is not absolutely certain, though Hals and Selvig (1977) found that the calcium levels across cementum were relatively even. Heterogeneous materials are more difficult to analyse and quantify. If cementum proves to be more heterogeneous, performing line scans becomes even more suitable, as recommended by Sanborn & Telmer (2003).

There is a balance that must be struck between sensitivity and spatial resolution. The more material there is to analyze, the lower the limits of detection are, which increases the sensitivity but at the cost of spatial resolution. For example, a 5 µm beam will produce results with a much finer spatial resolution, but it cannot achieve the sensitivity to trace elements that a 30 µm beam has.

Standard Reference Material

When using LA-ICP-MS, reference material is needed to normalize or standardize the resulting element levels, putting the profiles into perspective and making them more quantitative. LA-ICP-MS on its own simply profiles the changes in an element's concentration within a substance. Using a material with known levels of elements set the baseline from which to calculate the quantitative levels of an element. Otherwise, the profile is qualitative: it shows how the element changes. True standard reference material does not yet exist for dental tissues. In order to achieve semi-quantitative results, an element that is relatively homogenous throughout the sample is used as an internal standard. All other elements are normalized against this one element's levels. In dental studies, calcium is generally used as an internal standard, and is used in this study as well.

Linear vs Spot

An advantage of performing a linear ablation rather than spot analysis is that there is no need to equalize the different areas of ablation in order to compare changes in element levels. It is also easier to see where changes are occurring since there is no real loss of information from having to leave fallout space between spots of analysis.

The laser output needs to remain constant so that the amount of tissue being ablated is the same as the laser travels (Sanborn & Telmer 2003). Ablation of sample is much more constant across homogenous material than heterogenous material (Sanborn & Telmer 2003). Linear ablation is seldom performed on dental tissues, as most studies prefer spot ablation. Budd and colleagues (1998) however, examined enamel & dentin with linear ablation in order to produce a 'line scan' of lead levels. More information is extracted from such a procedure as differing levels of elements are more easily seen when compared to intermittent spot ablation. The authors double checked their results by performing linear ablation in the opposite direction. This ensures that peaks and troughs in the line scan are reflective of element concentration and not a false reading or a "memory effect".

Spot analysis causes a 'halo effect' where ablated material that is not transported by the argon gas to the icp-ms is scattered around the pit made by the laser. The distance between spots must be further apart to keep from ablating material from the previous spot. With a line scan, the laser is ablating material as it crosses the specimen surface, leaving little time for deposition of extraneous material.

With linear ablation, more material is being ablated than with spot analysis. The more material being analyzed, the lower the detection limits (Chenery et al 1996). This will allow low levels of trace elements more likely to be determined. Line scans are also much faster and therefore more cost effective than performing spot analysis.

Summary

I have examined the possible implications different elements have in cementum analysis, as well as the use of laser ablation to look at TE in cementum. The next chapter will detail the sample to be examined, followed by my methodology

Chapter 4: Materials and Methods

Materials

Tooth Sample

Twenty-five permanent teeth from 13 individuals of documented age and sex collected from the Faculty of Dentistry at the University of Manitoba were analysed for this study. Although severely pathological teeth were not collected, several teeth displayed evidence of caries. The present sample is limited to single rooted teeth to avoid complications that can occur in areas of bifurcation. Also, single rooted teeth have larger roots to work with. The details of the sample are presented in Table 1. The sample distribution by sex and age are presented in Figures 1 and 2.

Table 1. Sample information

Individual	Tooth #	Tooth type	Age/Sex	Slide # analyzed	Times analyzed	Condition
1	2	max I2	57/M	3	1	carious
1	4	max I1		4	1	
4	3	mand I1	46/M	4	1	carious
4	4	mand I2		2	1	carious
4	5	mand I2		4	1	carious
13	2	max I1	32/F	4	1	carious
16	2	max C	23/F	4	1	carious
16	5	max I1		6	1	carious
19	2	mand I2	48/M	2	1	carious
19	3	mand I1		2	1	carious
22	2	max PM2	41/F	5	3	carious
22	3	max PM2		4	2	carious, amalgam filling
22	6	max I1		4	2	carious
22	9	mand PM1		5,6	2,2	amalgam filling
24	2	max I1	41/F	4	3	
24	3	mand C		5	3	
27	2	mand I2	57/F	4	3	PDL covering
28	2	mand I1	43/F	3	1	filling
28	4	mand I2		3	1	filling, alveolar bone
35	3	max C	33/M	6	2	
35	5	mand I1		4,6	1,2	
37	4	max C	78/M	4	1	carious
44	2	max I1	36/F	4	2	
48	1	max I	75/F	5	1	carious, filling
48	2	max I2		4	1	carious, filling

Figure 1 Count of tooth type by sex

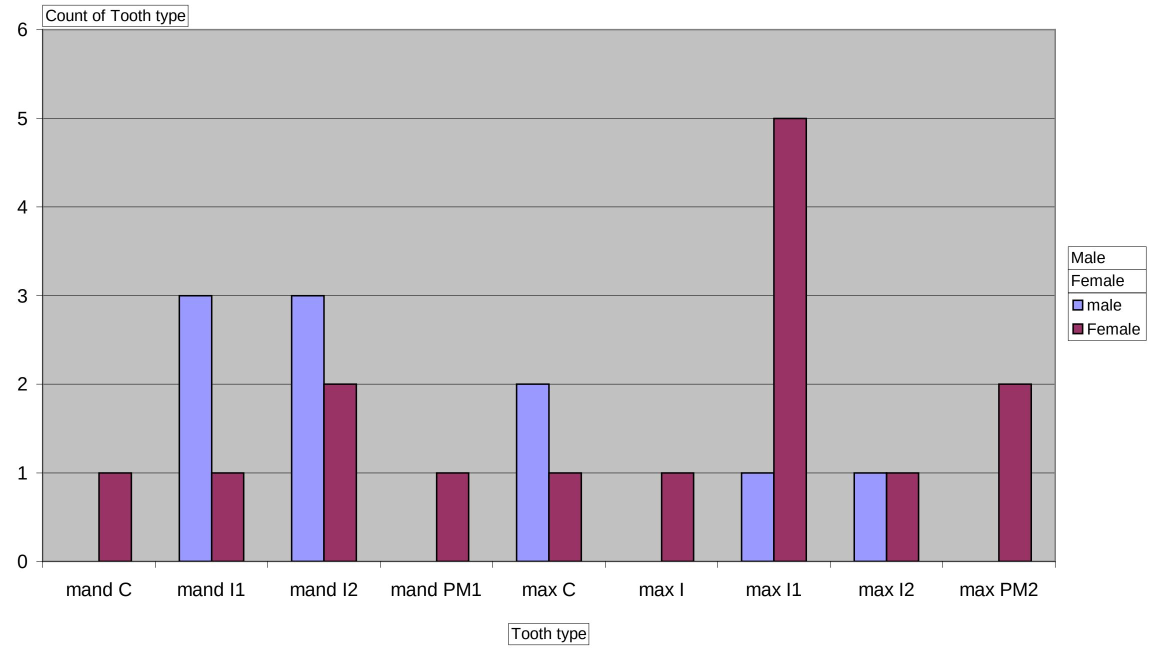
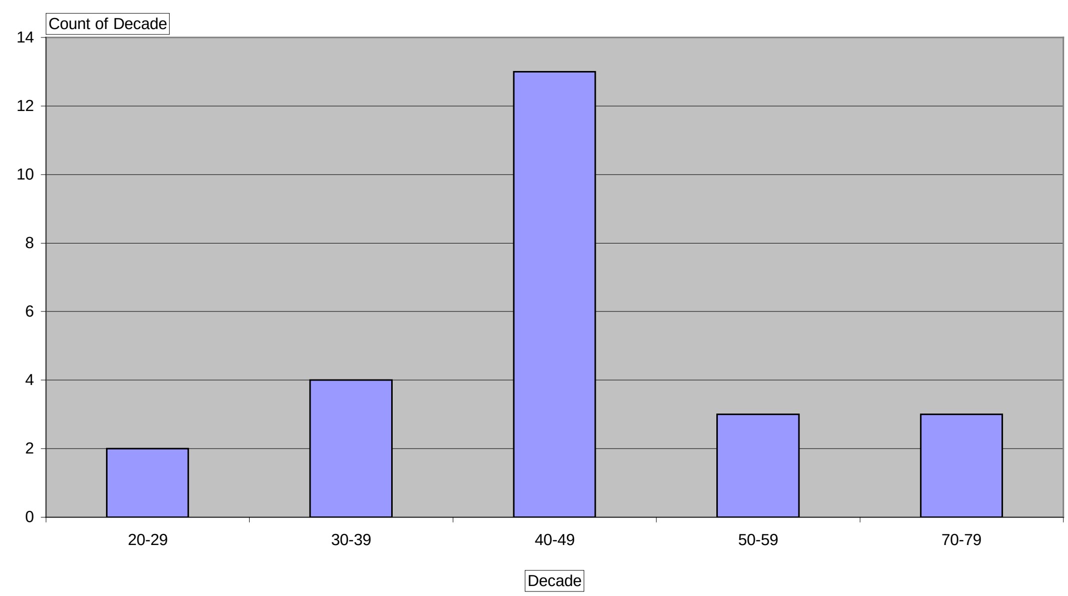


Figure 2 Count of teeth by decade



Methods

Sample Preparation

The teeth were first stored in 10% formalin and subsequently stored individually in distilled and deionised water, changed on a regular basis. As a further sanitary measure, the teeth were bathed for 1 minute in 70% ETOH (ethyl alcohol) as many of the roots still had soft tissue adhered. After being photographed and documented according to Buikstra and Ubelaker (1994), the teeth were left to dry naturally prior to being embedded. Blue Buehler specimen cups (SamplKup®) were used with a release agent applied to the interior so that the epoxy could be easily removed from the mould once hardened. In unused disposable cups, 3 parts resin (Buehler Epoxythin) and 1 part hardener were measured out, mixing them together for one minute with a wooden applicator stick. Buffers were first poured and allowed to cure in the SamplKup® so that the tooth was not lying directly on the bottom. Another batch of epoxy was mixed and the tooth placed on top of the buffer then covered with the epoxy. These were then placed within a vacuum chamber and pressurized to roughly 29 in/Hg to ensure few air bubbles remained. Specimens remained in vacuum chamber overnight, after which the chamber was slowly depressurized.

After removal from the SamplKups, the embedded teeth were sectioned using an Isomet precision cutter (using distilled, deionised water). Although the tooth is advanced 1mm after each cut, the sections are less than that due to the width of the blade itself. Teeth with thicker roots were sectioned longitudinally, allowing a view of the cementum along the entire length of the root. Thinner roots were cross-sectioned to increase the chance of having good, usable sections. A small amount of epoxy was used to mount the sections onto petrographic slides.

Cementum annulations are difficult to see clearly on thicker sections, mainly due to superimposition, so they are ground down after being mounted. Ablation, however, cannot take place on sections that are thinned for visualizing annulations since the laser may ablate deeper than the amount of tissue that is left on the slide. For each tooth, one slide (occasionally two) was chosen for elemental analysis, while adjacent sections were ground down to a more appropriate optical thickness for viewing. Once this thickness was reached, the sections were polished using a polishing slurry (Buehler's Aluminum Oxide final polishing suspensions).

Sections selected for laser ablation were lightly ground and polished to remove any epoxy on the surface, and to erase saw marks. They were then put in an ultrasonic bath to clean them. Teeth with amalgam fillings were sectioned from the root end to crown, and the slides were not ground nor bathed to prevent amalgam particles from contaminating the rest of the section.

Cementum Imaging and Counting

Slides were examined using an Olympus BX51 microscope and photographed using a Roper Scientific Coolsnap fx digital microscopy camera and Image-Pro Plus software from Media Cybernetics. Images were further enhanced using Adobe Photoshop Elements (2.0). After enhancement, each dark increment of an annulation was marked for counting ease.

Laser Ablation Protocol

Each tooth was analysed with LA-ICP-MS using a UP-213 Laser Ablation System and a ThermoFinnigan Element 2 ICP-MS machine in the Department of Geological Sciences at the University of Manitoba.

Slides were put in the sample cell of the LA-ICP-MS. The sample cell was then sealed and the air purged. Background levels are checked to make sure they are at a reasonable level, and to calibrate a baseline. The standard reference material (NIST 610) is ablated just prior to sample analysis to further calibrate the machine. After a set of sample analyses, the SRM is again ablated to check that the machine's calibrations have not drifted in that time.

The area of interest was assessed on each slide and a line across the cementum, from the dentin out to the resin, was set for the laser to follow. Prior to ablating, the beam size, speed, and power output were selected. Once set, the computer/system recorded 60 to 100 seconds of background levels both prior to and following ablating the sample. These levels were later subtracted from the sample's element profile to show the 'actual' raw data. Between sample ablations background levels were left to level out prior to ablating a new slide.

The mass spectrometer collected raw counts per second (cps) of each analyzed element. Raw calcium levels were graphed to define the area of relatively stable levels. The initial blast of the laser ablates a higher amount of material causing an initial higher count of elements. Were the entire line to be normalized, the reduction in the initial hump would falsely depress element levels. The same is true for drops in calcium levels that occur as the laser exits the cementum into resin. The raw counts per second for each element was then calibrated against calcium, giving a standardized counts per second. Along with standardization, element levels are also corrected for background levels.

These adjusted profiles were then made into a line graph and laid over images of their respective laser line.

Electron Probe Micro-Analysis (EPMA)

To check the homogeneity of calcium throughout cementum, one slide was analyzed using the Cameca SX100 electron microprobe. After ensuring the surface of the slide is polished, the slide was carbon coated for 10-15 minutes since teeth are not electronically conductive, a requirement for analysis. Two standards of natural minerals were used for calibration before the cementum was analyzed. Spot analysis was performed from within the dentin out through the entire cementum into the resin. Each spot had a diameter of 10μm, making it a third of the size of the laser beam, but still large enough to potentially cover more than one annulation.

Analysis

The analysis of the data comprises two components: 1) the visualization and counting of the cementum annulations and 2) the collection of trace element data through the dental tissue. Visual assessments of profile patterns across the tissue are made in an attempt to correspond changes in element levels with cementum structure, including the cementum-dentin junction (CDJ). The use of a 30μm diameter beam, peaks and troughs cannot be assigned to any one particular annulation, but can be attributed to a specific time period. Patterns may emerge that can be linked to an individual's lifestyle or habitation. Evaluations of profiles from different teeth of the same individual will show any potential intra-individual variation that may exist. Intra-tooth variation is also examined through comparisons of profiles taken from different planes of the same tooth. The results of these analyses are presented in detail in the next chapter.

Chapter 5: Results

The results the electron microprobe and the cementum annulation demarcation are presented, as well as the elemental analysis for each individual.

Homogeneity of Ca from EPMA

The analysis of calcium levels was performed on one tooth using the electron microprobe (Figure 3). The EPMA shows relatively consistent Ca levels except for a drop at the cementum dentin junction (CDJ) and again later in the cementum. Upon examination of the second spot, there is a discolouration that appears directly underneath the analytical spot, which hindered the examination, as the same decrease occurs in oxygen and phosphorus levels. The final drop in element levels initially appears to occur before the resin; however, examination of the SEM photos of the edge of the cementum show that there is a separation between cementum and resin (Figure 4).

The LA-ICP-MS analysis of 22-3 also showed raw Ca levels to be homogeneous (Figure 5). The EPMA results support the laser ablation analysis, but do not definitively prove that Ca levels are homogeneous. It would be of interest to examine Ca levels of a tooth with variable levels as shown by LA-ICP-MS.

Cementum Annulations

Since this is not a validation study, the annulation counts were not performed as a blind test. The demarcation was in the interest to show the progress of structures versus the progress of the element profiles. Unfortunately, the annulations often were not visible

Figure 3 EPMA by weight for tooth 22-3

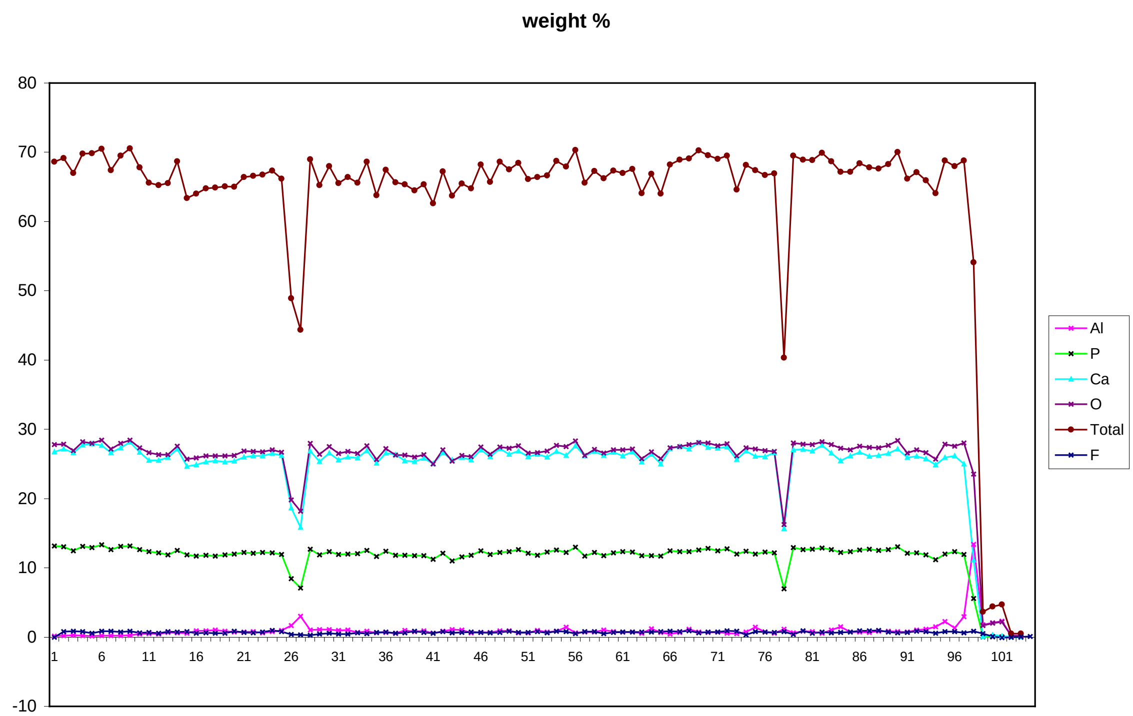


Figure 4 SEM photo of ablation line

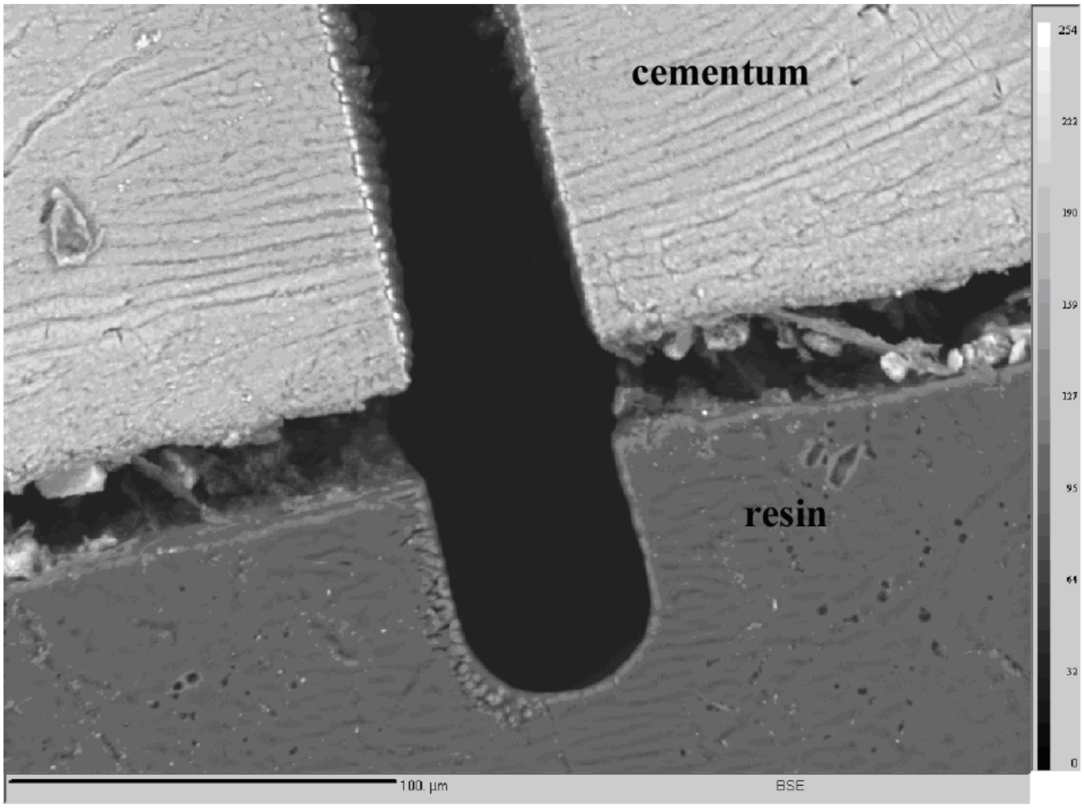


Figure 5 Raw laser ablation levels of Ca for tooth 22-3

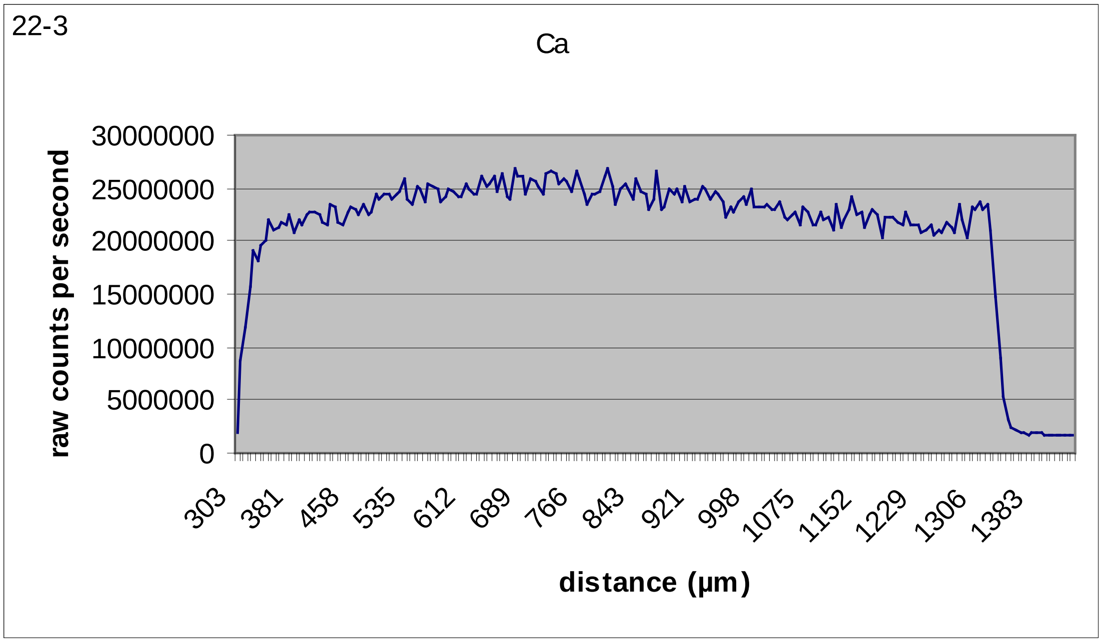
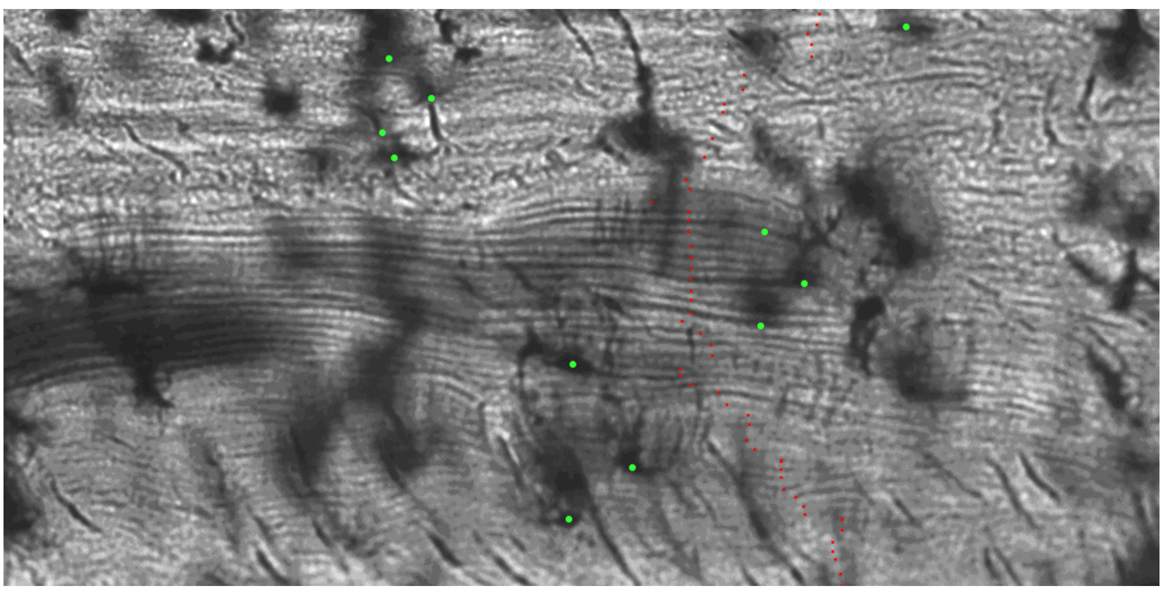


Figure 6 Tooth 22-3 (Green dots denote increments, while red dots show all visible lines)



in the area of the laser ablation lines. Many of the individuals in this study suffered from hypercementosis, an excessive deposition of cementum. This increase in tissue tended to correlate with an increase in apparent annulations at first examination. These extra 'lines' were not always wavy or fuzzy as are reported in cases of doubling (Wittwer-Backofen et al 2004). Teeth 22-3 (**F**igure 6) and 24-3 (**F**igure 7) may be good examples of possible alternate fibril pattern described by Yamamoto and colleagues (1997, 1998, 2000)

<u>Element Analysis</u>

1

Individual 1 was represented by 2 teeth (1-2 and 1-4). Each tooth was analysed once. The raw calcium levels of 1-2 (**F**igure 8) were much steadier than those of 1-4 (**F**igure 9); however, the background levels prior to analysis on tooth 1-2 showed much lower levels of calcium than 1-4, which may be related to this difference in Ca profiles.

Both teeth contained high levels of mercury (**F**igure 10 and Figure 11). With background mercury levels being low, the high content of Hg in the teeth can be seen as real; however, there is no information as to the presence or absence of amalgam fillings for this individual. Tooth 1-2 was carious and 1-4 has exposed dentin, both circumstances can allow mercury to be absorbed by the teeth. The mercury levels increased once the laser entered the cementum, and continued to increase with age.

Lead levels were high in both teeth, but had different patterns in cementum. A drastic increase in lead occurs at or just after the CDJ. Tooth 1-2 shows 3 peaks in lead levels (**F**igure 10), whereas tooth 1-4 (Figure 11) contains one peak lasting almost the

Figure 7 Tooth 24-3 (green dots mark the increments)

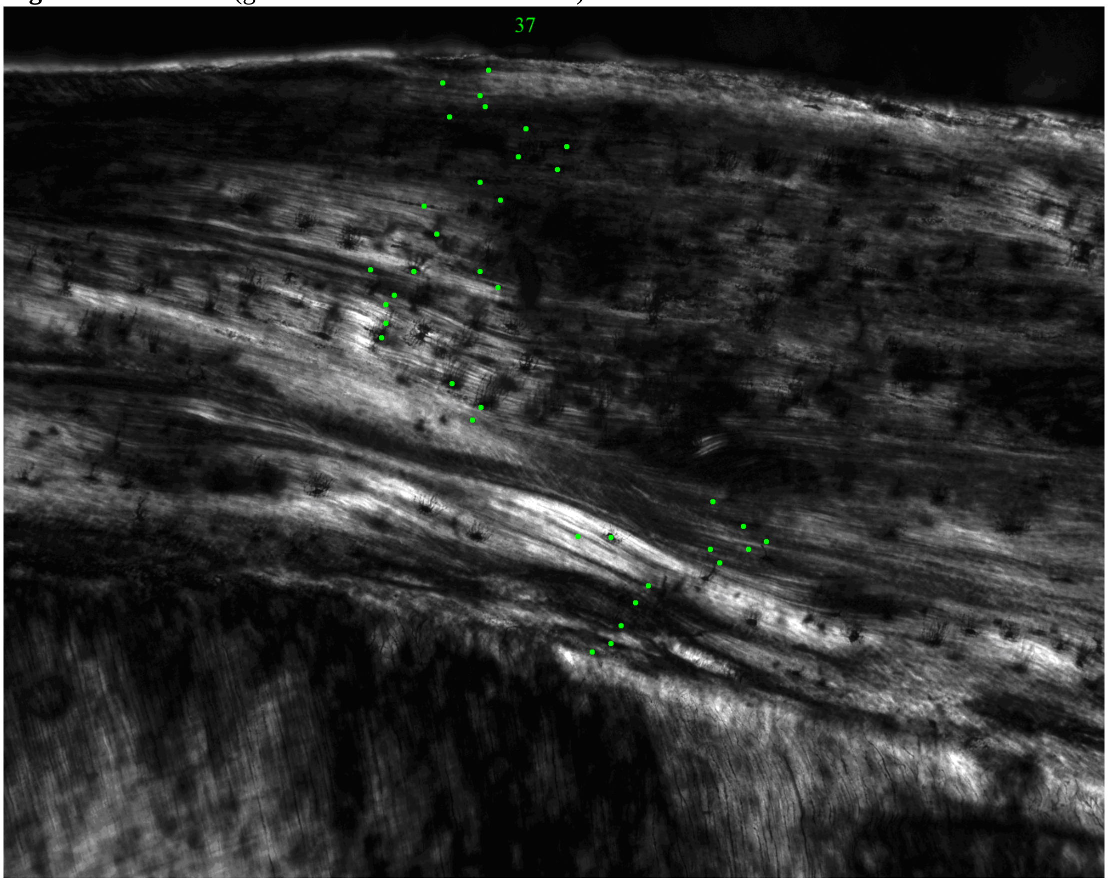


Figure 8 Raw calcium profile of tooth 1-2. X-axis is a proxy for age of tooth completion until documented age at extraction

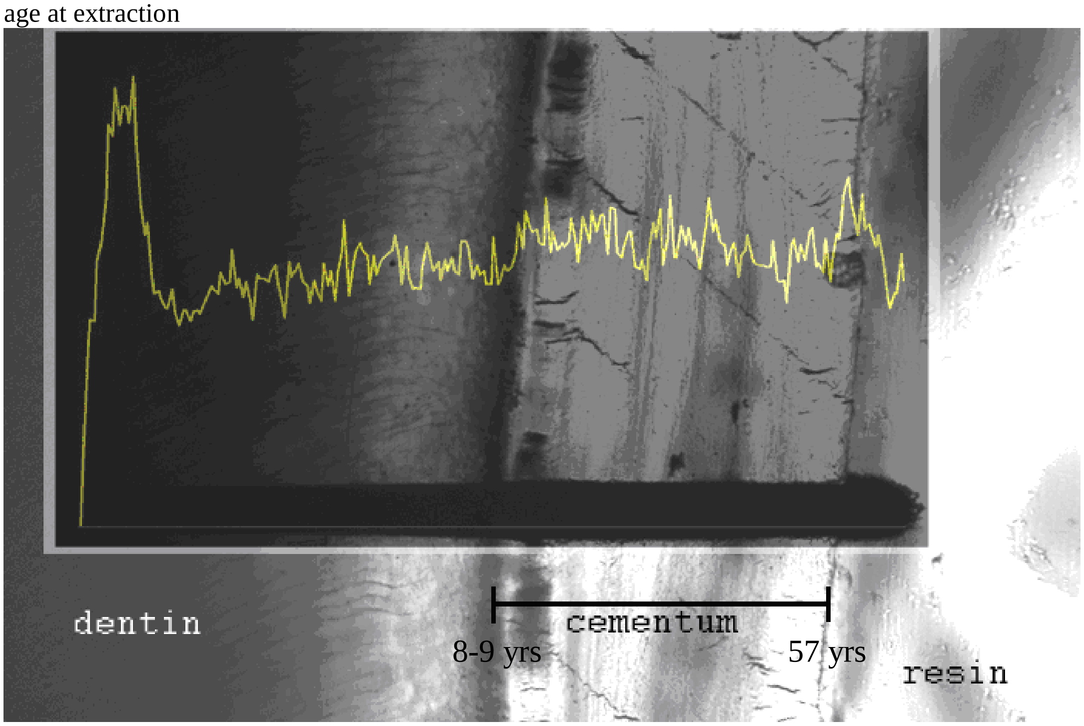


Figure 9 Raw Ca profile of tooth 1-4. X-axis is a proxy for age of tooth completion until documented age at extraction

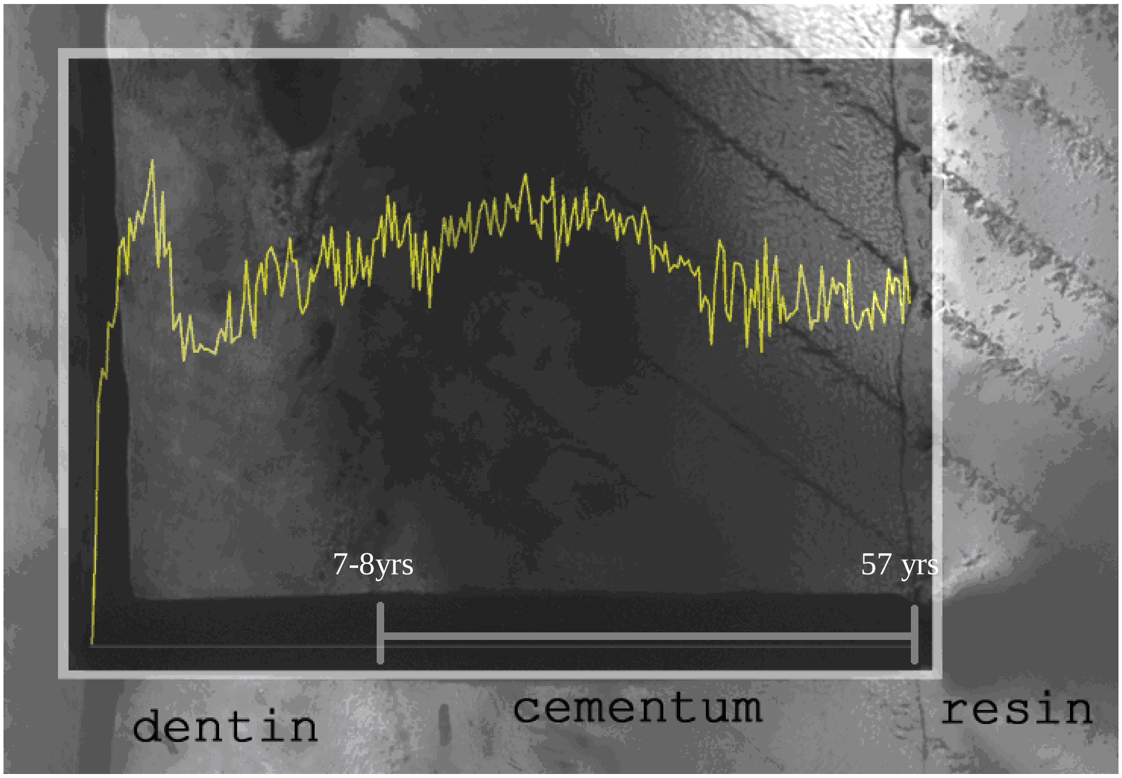
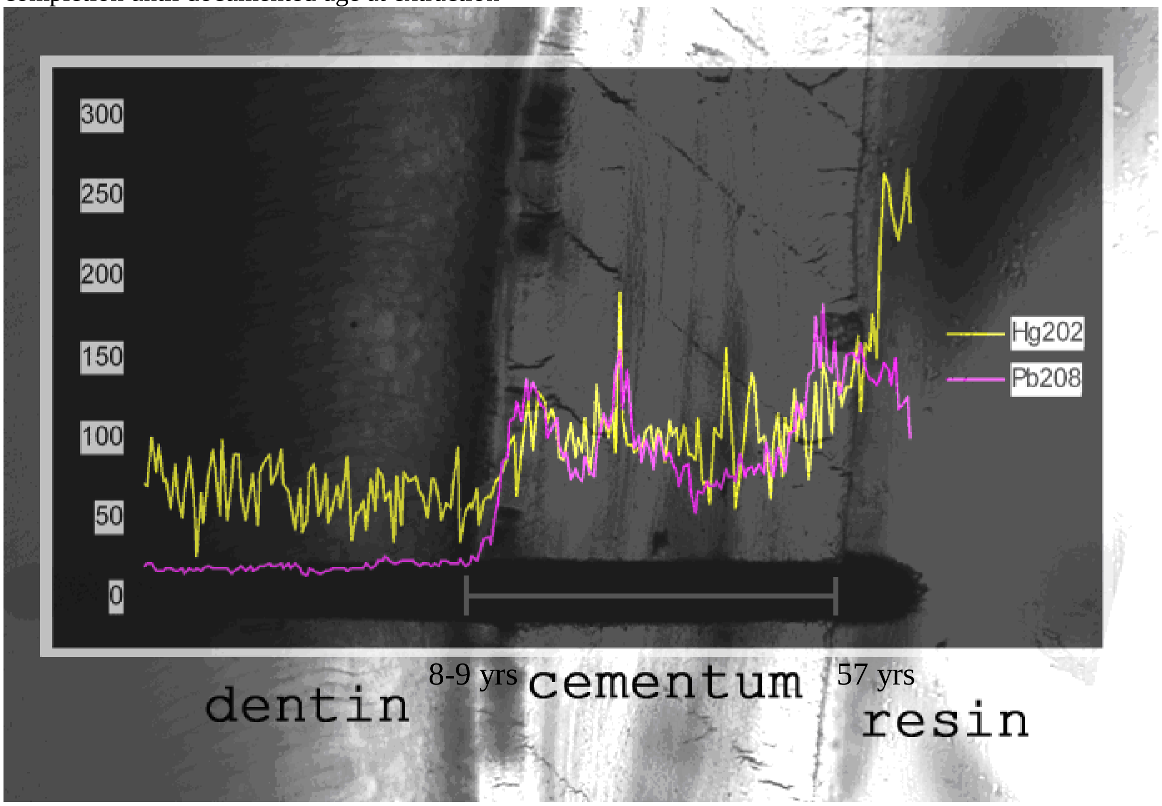


Figure 10 Standardized mercury and lead profiles of tooth 1-2. X-axis is a proxy for age of tooth completion until documented age at extraction



entire length of the cementum. Other elements were also found to be higher in cementum than dentin. These include Zn, Ba, and Hg.

Lead and zinc follow the same pattern until nearing the edge of cementum, where zinc greatly increases and lead levels fall. There is also a substantial peak in barium, copper, and manganese in the same area, though barium begins increasing just prior to the other elements. Barium and mercury follow the same profile pattern as zinc.

4

Individual 4 was represented by 3 teeth (4-3, 4-4, and 4-5). Each tooth was analyzed once. Analysis of tooth 4-4 and 4-5 were performed across acellular cementum, while tooth 4-3 was analysed across cellular cementum. Analysis of acellular cementum caused a compression of elemental profiles since deposition of elements is done in a much smaller area over the same period of time as cellular cementum. Elemental profiles for 4-4 and 4-5 were similar, however, tooth 4-3 showed more variation in elements. Tooth 4-3 also contained reparative cementum. Unfortunately other areas of the same tooth were not analysed, so comparison of patterns between regular cellular cementum and that of the reparative cementum could not be performed. The original CDJ could still be discerned from the reparative cementum that occurs within the dentin portion of the root. Lead, zinc, mercury (Figure 12) and raw calcium (Figure 13) levels showed an increase as the laser passed from the dentin into the reparative cementum, then again after the original CDJ. Magnesium (Figure 14) was also found to increase in concentration from dentin to reparative cementum, but not between reparative and regular cementum. The increase in magnesium is also seen in tooth 4-4 and 4-5.

Figure 11 Standardized mercury and lead profiles of tooth 1-4. X-axis is a proxy for age of tooth completion until documented age at extraction

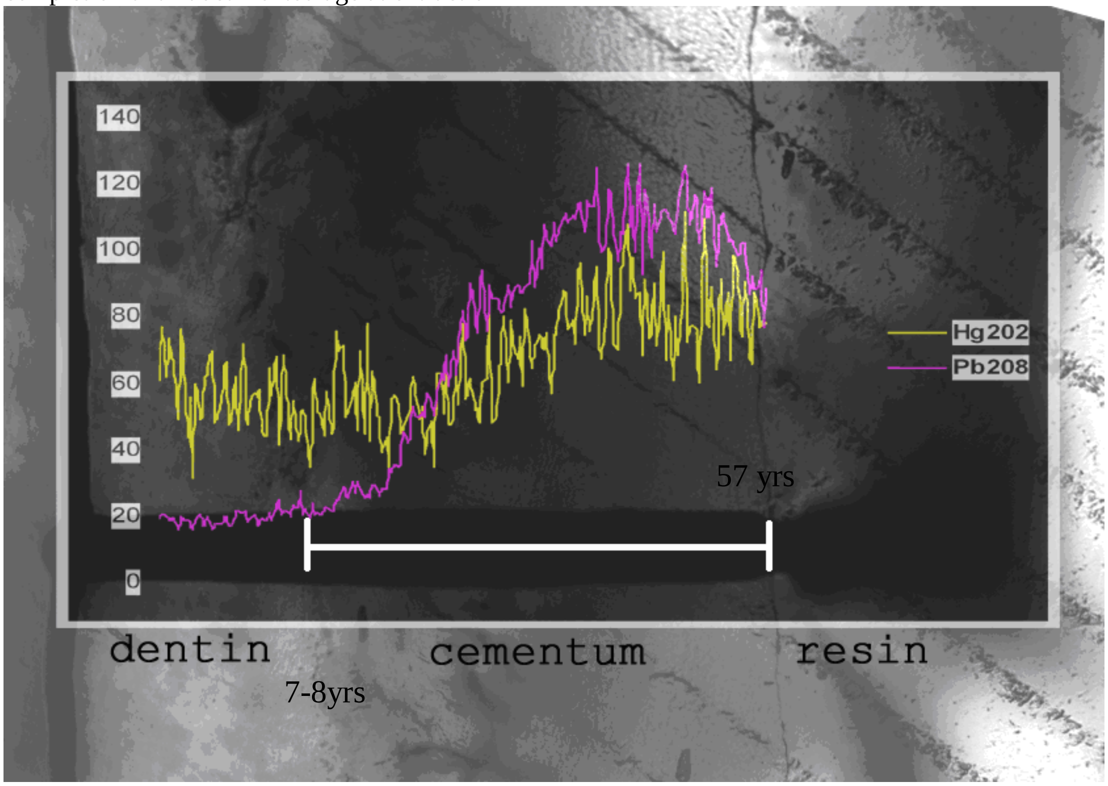


Figure 12 Standardized mercury, lead and zinc profiles of tooth 4-3. Y-axis on right-hand side is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

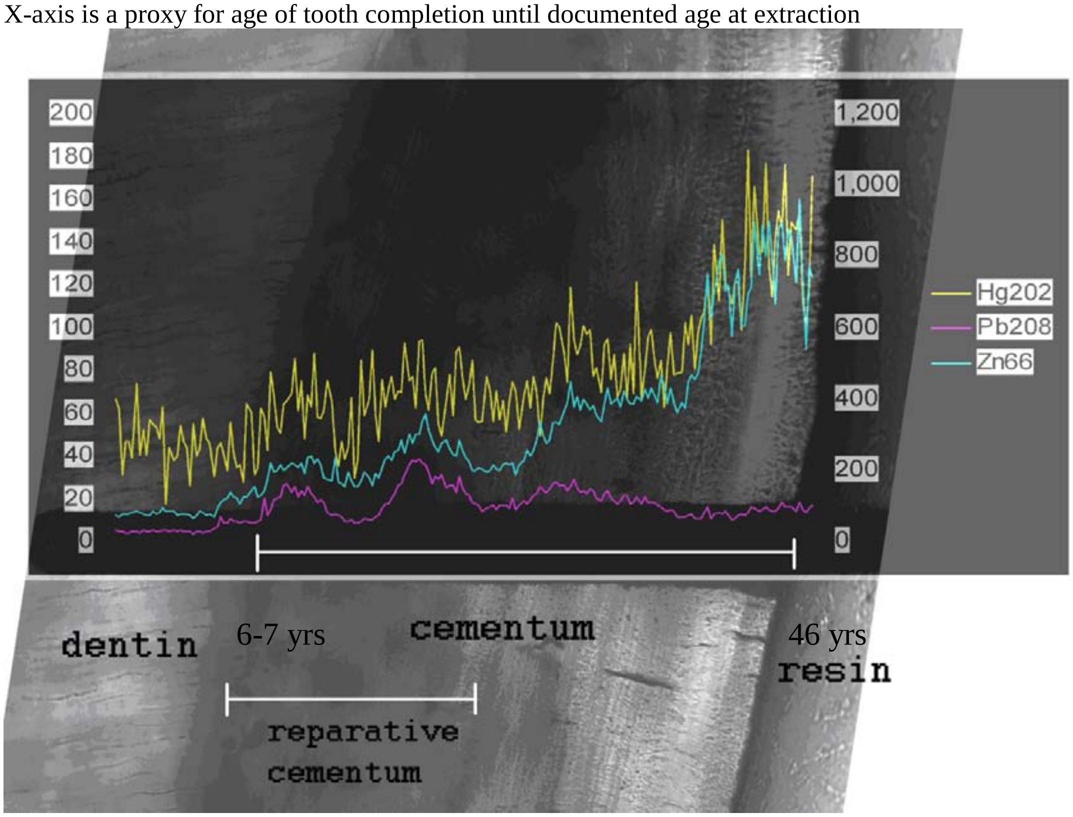


Figure 13 Raw calcium profile of tooth 4-3. X-axis is a proxy for age of tooth completion until documented age at extraction

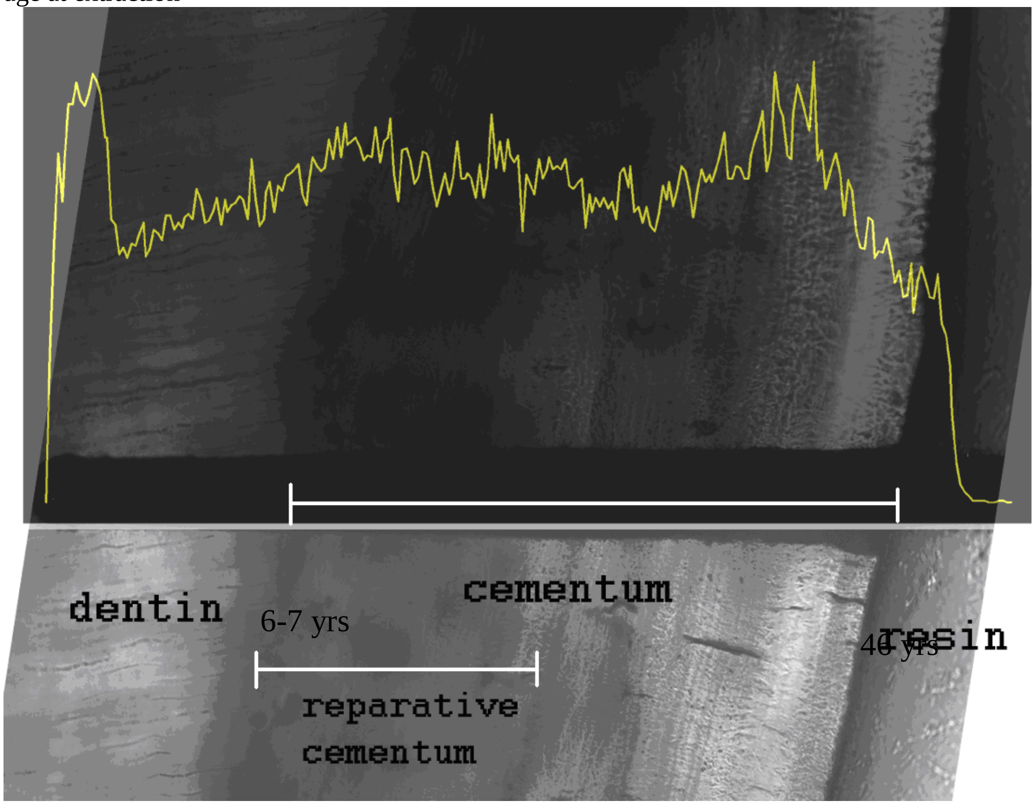
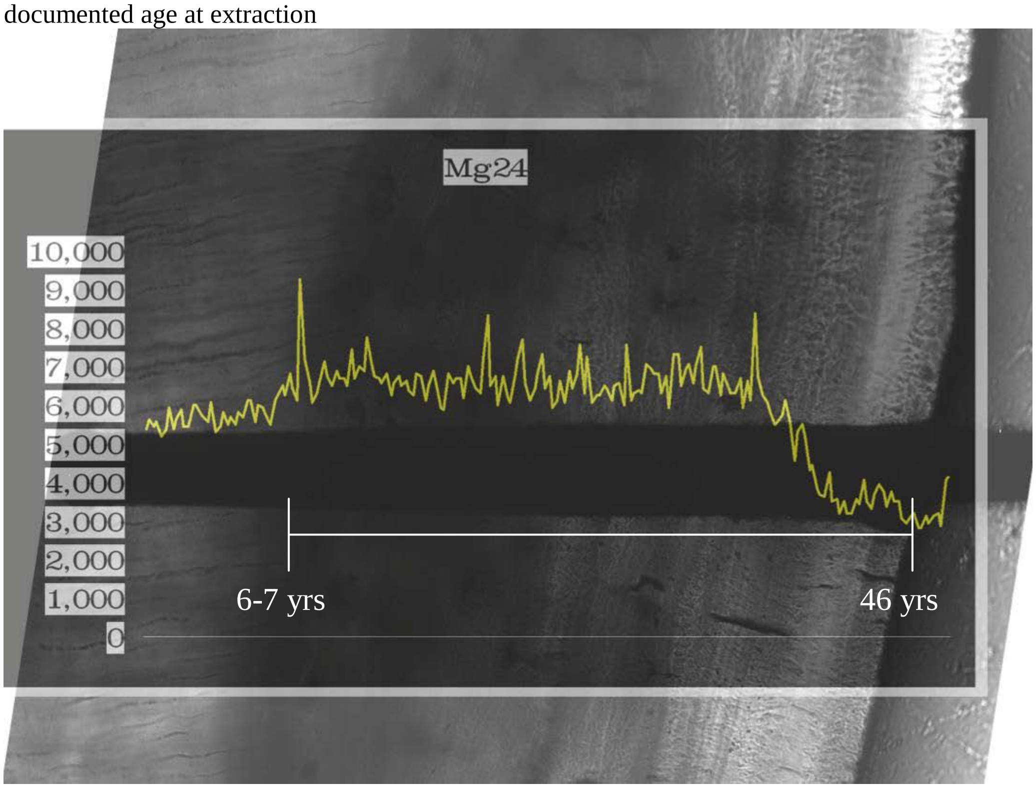


Figure 14 Standardized magnesium profile of tooth 4-3. X-axis is a proxy for age of tooth completion until documented age at extraction



All three teeth had high levels of mercury (Figure 12, Figure 15, and Figure 16 respectively), and moderately high lead levels. Tooth 4-3 has a large carie, exposing the dentin, while tooth 4-4 only displayed a small carie. There is no information as to the possibility of any amalgam fillings for this individual.

The profiles for lead, zinc and mercury (Figure 15 andFigure 16) all follow the same pattern in tooth 4-3. The elemental profiles for tooth 4-4 and 4-5 are very short, and it is difficult to determine if there is much difference between these three elements. Barium (Figure 17Figure 18, andFigure 19) did begin to increase just prior to Mn, Cu, Zn, and Hg as the laser neared the outer edge of the cementum.

13

Individual 13 only had one tooth analyzed (13-2). The tooth was carious and most of the enamel was missing. Mercury levels (Figure 20) were high, and could have been easily absorbed by the tooth through the exposed dentin. Zinc (Figure 20) and mercury share the same pattern. Lead (Figure 20), however, has only one broad peak and no other defining features, making it difficult to link to zinc’s profile.

Raw calcium levels (Figure 21) were not homogenous and peaks at the outer edge of the cementum, along with the usual zinc, mercury, and barium peaks. This peak in calcium may be the result of an increase in material being ablated, or an actual increase in calcium concentration in the tissue.

16

Individual 16 is represented by 2 teeth (16-2 and 16-5), both of which were analysed once. Lead in tooth 16-2 follows the same pattern as zinc, mercury and barium

Figure 15 Standardized mercury, lead and zinc profiles of tooth 4-4. Y-axis on right-hand side is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

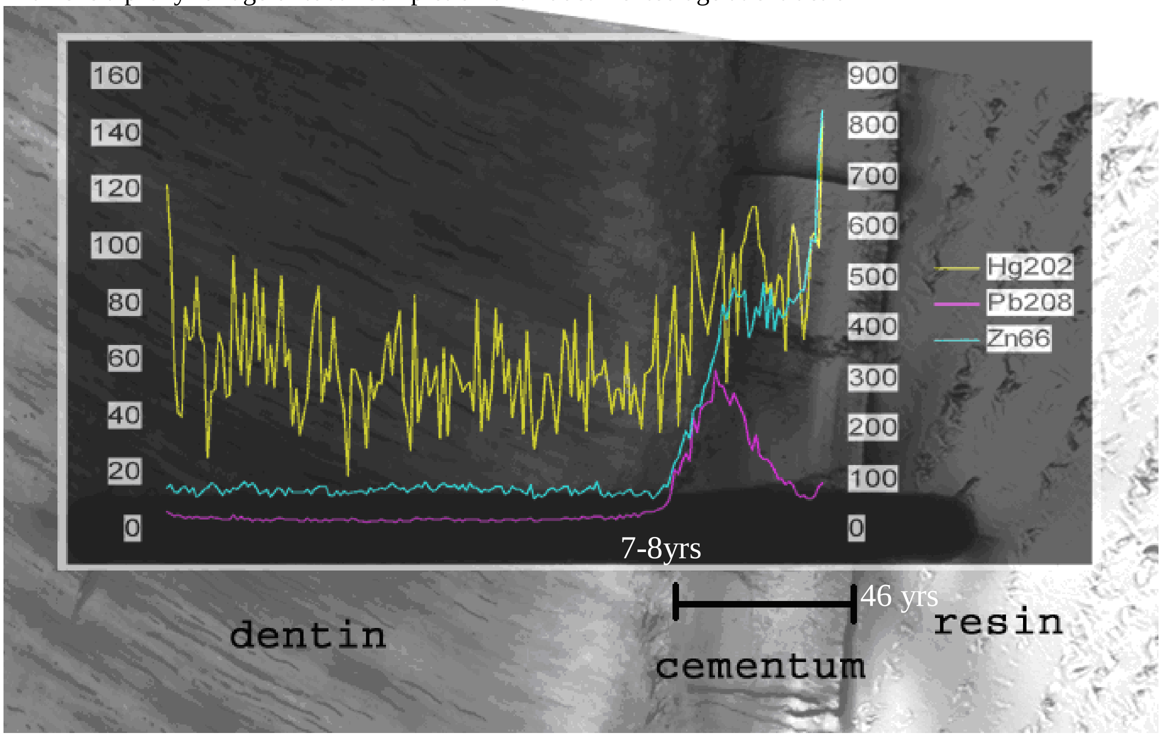


Figure 16 Standardized mercury, lead and zinc profiles of tooth 4-5. Y-axis on right-hand side is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

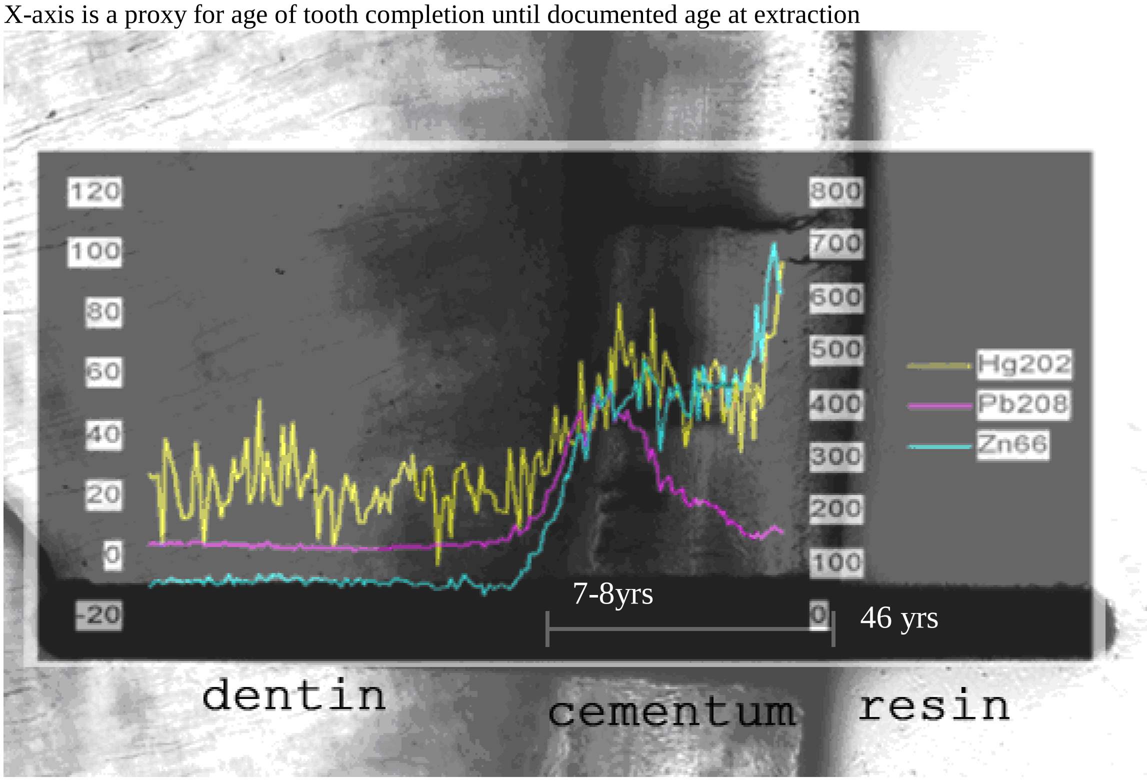


Figure 17 Standardized manganese, barium and copper profiles of tooth 4-3. Secondary y-axis (right-hand side) is for copper. X-axis is a proxy for age of tooth completion until documented age at extraction

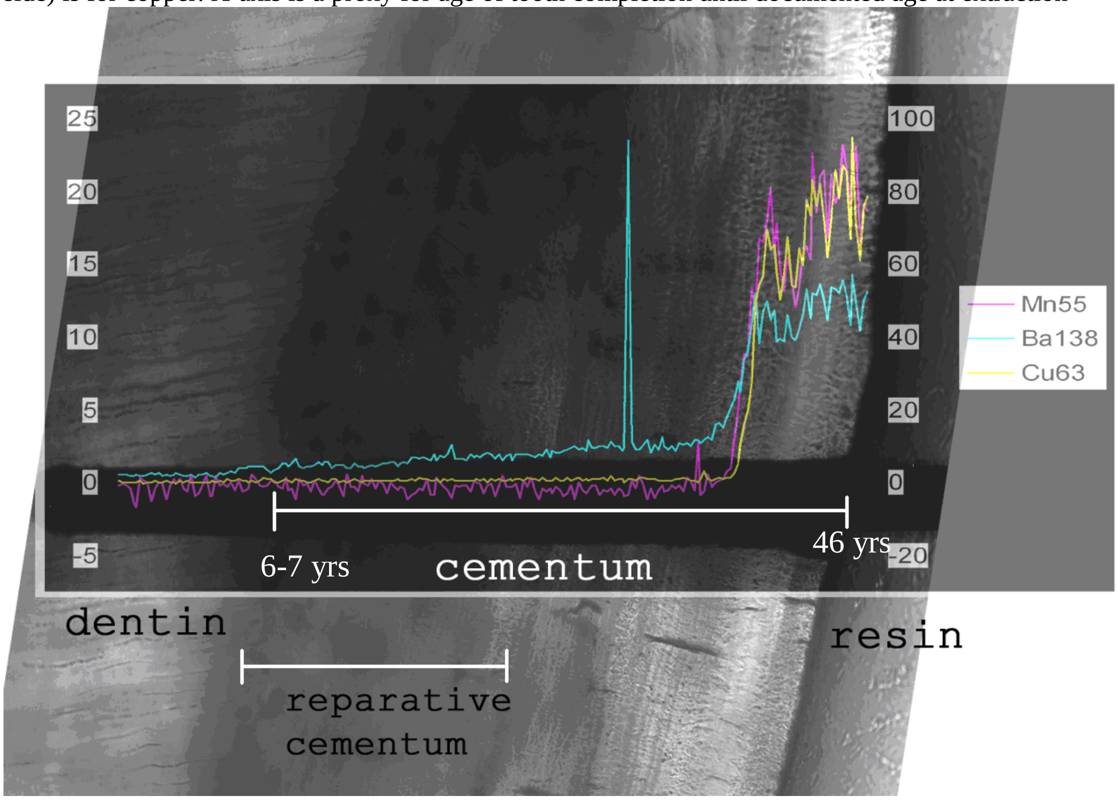


Figure 18 Standardized manganese, copper and barium profiles of tooth 4-4 (without outer peak) . X-axis is a proxy for age of tooth completion until documented age at extraction

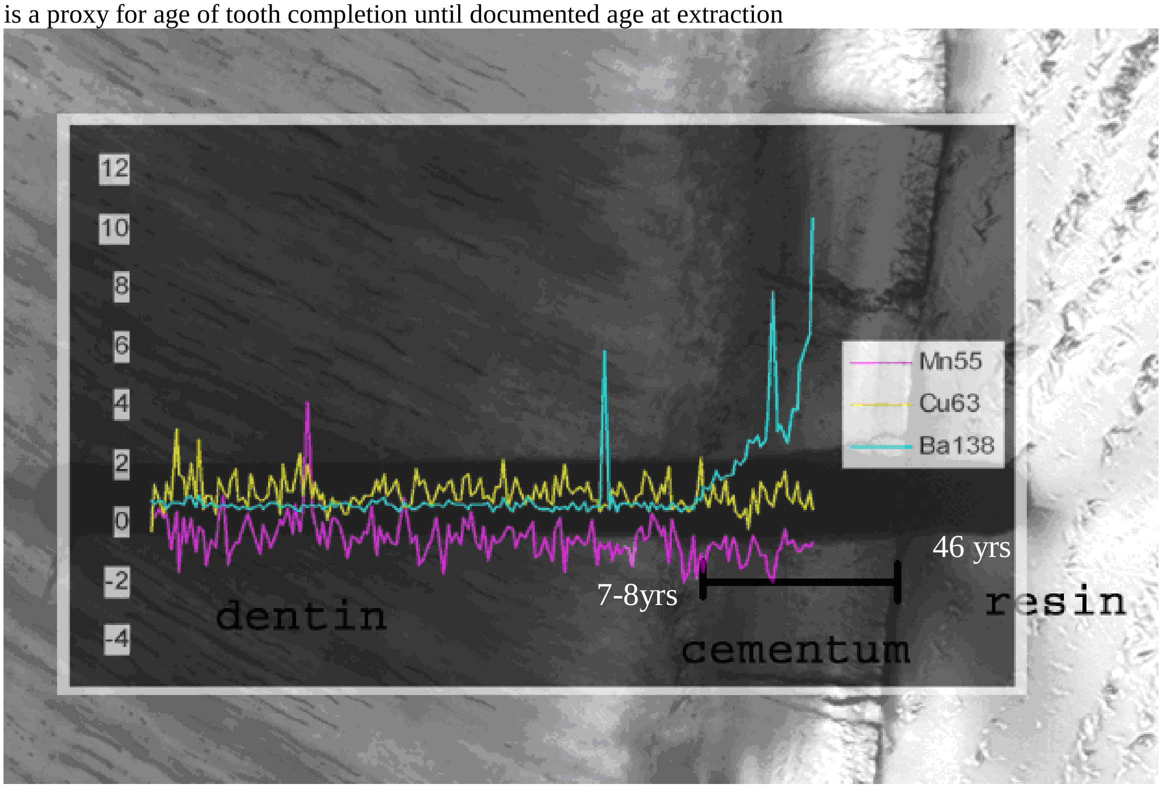


Figure 19 Standardized manganese, copper and barium profiles of tooth 4-5. Secondary y-axis (right-hand side) is for barium. X-axis is a proxy for age of tooth completion until documented age at extraction

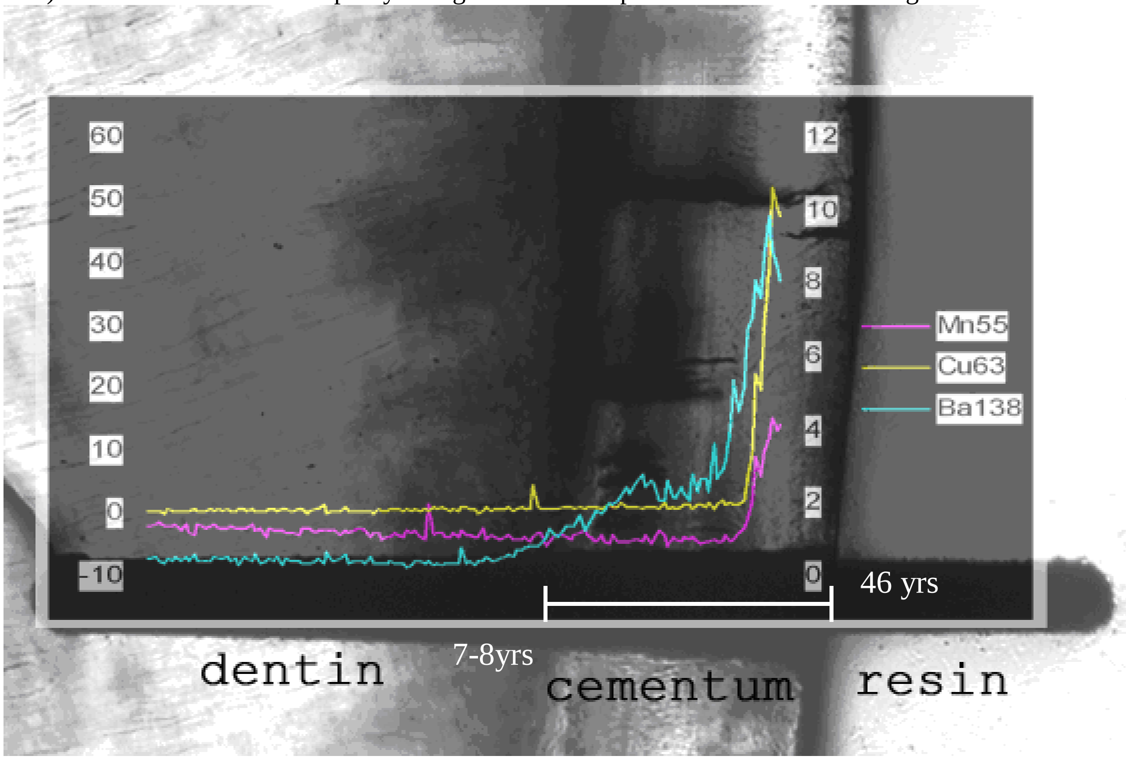


Figure 20 Standardized mercury, lead and zinc profiles of tooth 13-2. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

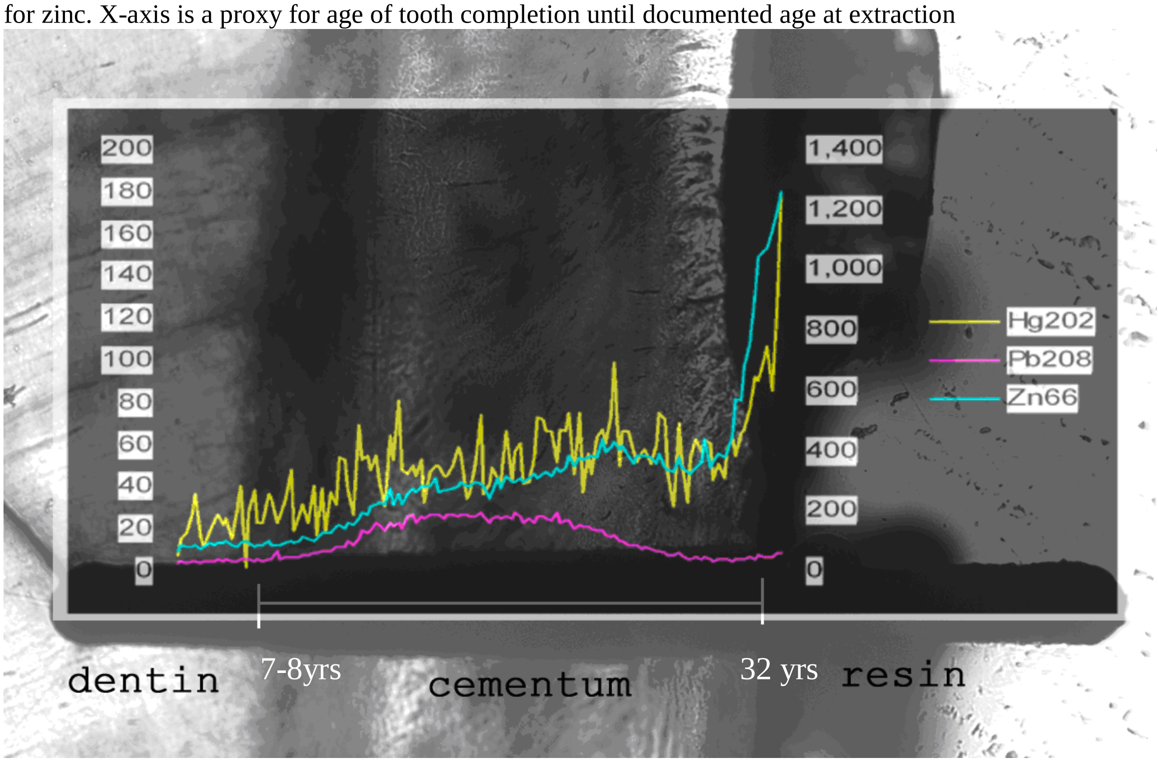


Figure 21 Raw calcium profile of tooth 13-2. X-axis is a proxy for age of tooth completion until documented age at extraction

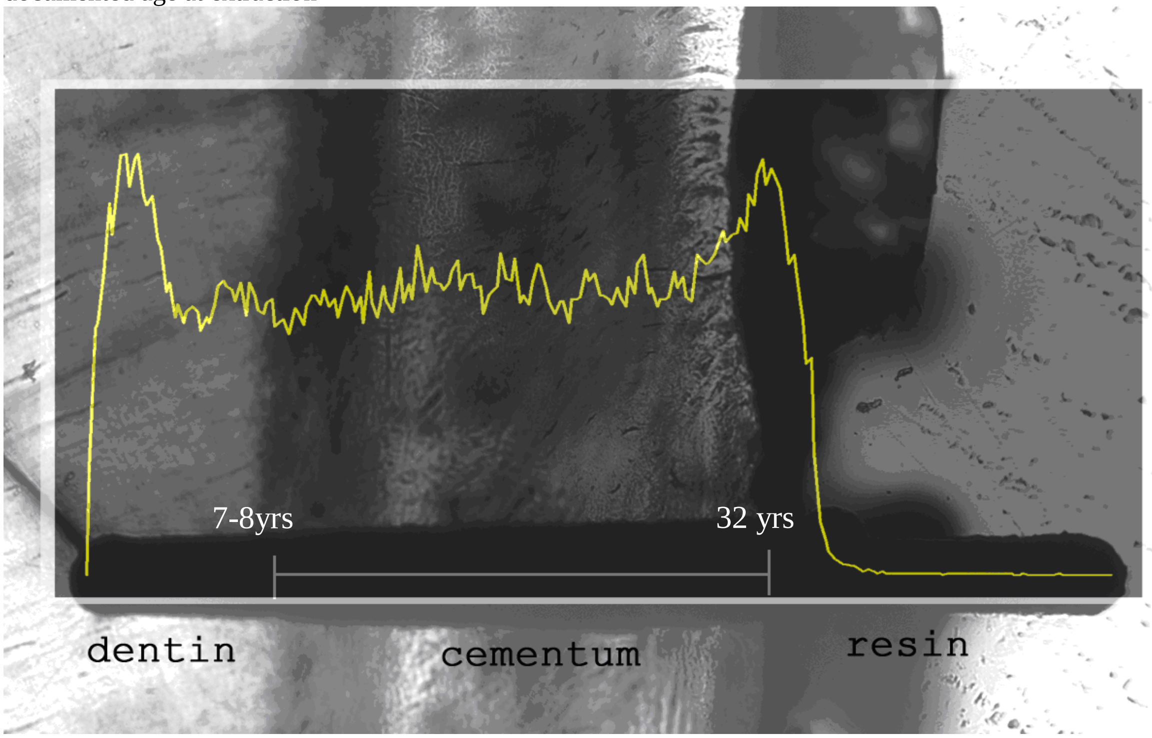
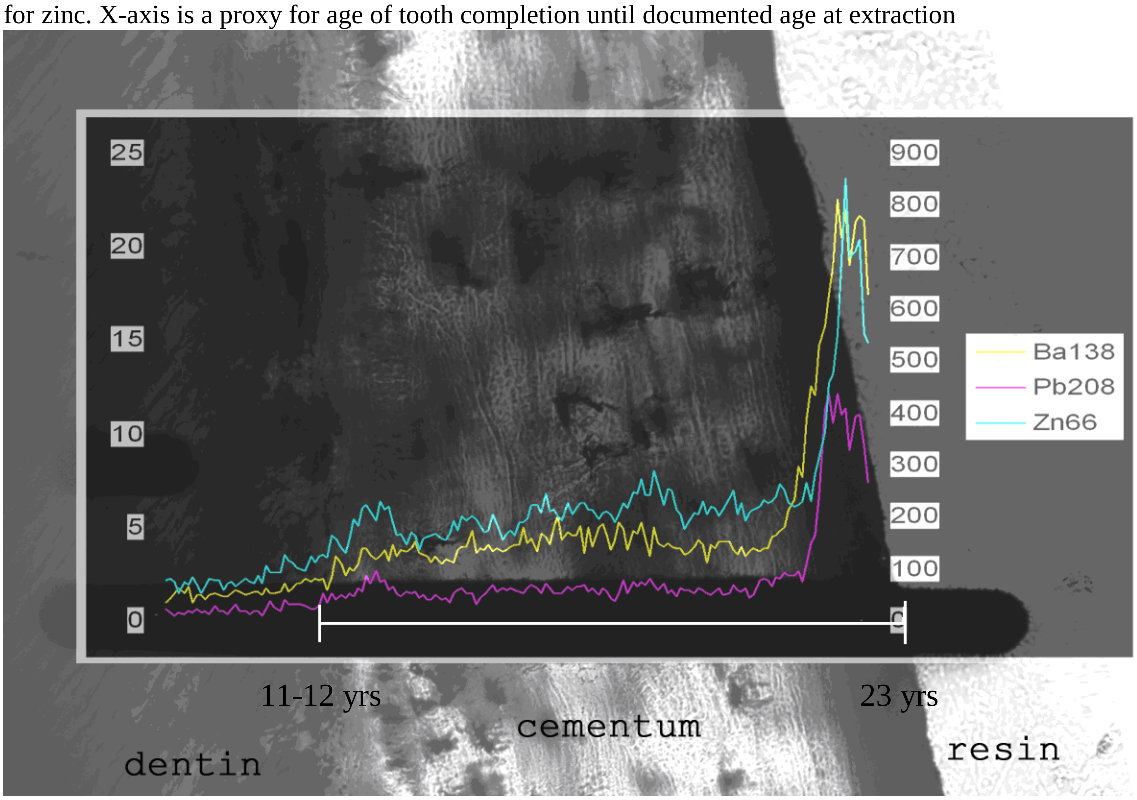


Figure 22 Standardized lead , zinc and barium profiles of tooth 16-2. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction



(Figure 22 and Figure 23), including the peak at the cemental surface, which is unusual. This could be from contamination though it depends if the other elements that peak at the surface are from contamination or not, but the peak also appears in 16-5, though not as large. The peaks occur at the start of the dark edge of the cementum.

Magnesium (Figure 24 and Figure 25) declines in both teeth as is its usual pattern. Lead, zinc and mercury slowly increased after the CDJ (**Error! Reference source not found.** and **Error! Reference source not found.**). The mercury levels in both teeth are low, as is the background levels. 16-2 contains two large caries, while 16-5 has no caries, and only a very small amount of dentin is exposed.

19

Individual 19 had two teeth analysed (19-2 and 19-3). Both teeth were cross-sectioned. Magnesium slightly increases prior to the CDJ, and does its usual decrease (Figure 28,and Figure 29 for tooth 19-3). Lead, zinc, barium, and mercury also increased (Figure 30 and Figure 31). Copper and mercury levels were erratic through the cementum (Figure 32). Both mercury and lead levels in both teeth were high.

The calcium levels in 19-3 take a sudden drop, then rise again, before the cementum edge (Figure 33). There is no apparent structural reason for this drop. The tooth did crack during analysis, which likely caused this drop; however, examining the underside of the slide is difficult. This drop in Ca levels is also apparent in the raw levels of the trace elements. With Ca being normalized, it levelled the drop in the calculated levels of the trace elements.

Figure 23 Standardized mercury profile of tooth 16-2. X-axis is a proxy for age of tooth completion until documented age at extraction

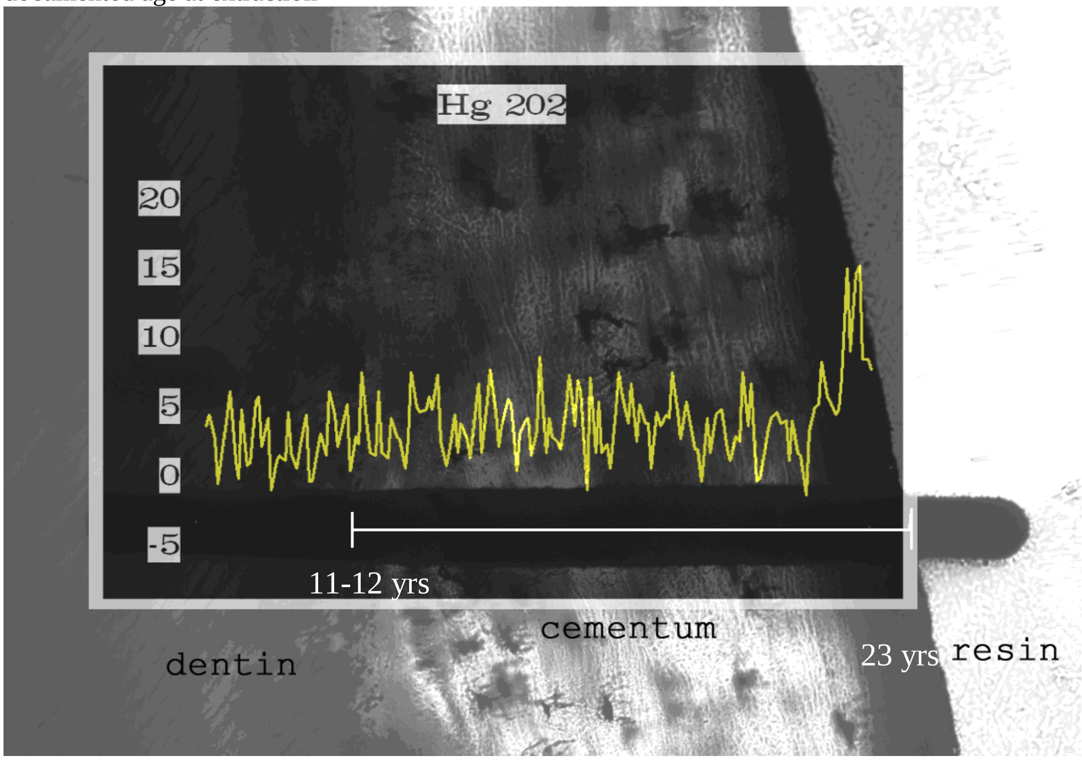


Figure 24 Standardized magnesium profile of tooth 16-2. X-axis is a proxy for age of tooth completion until documented age at extraction

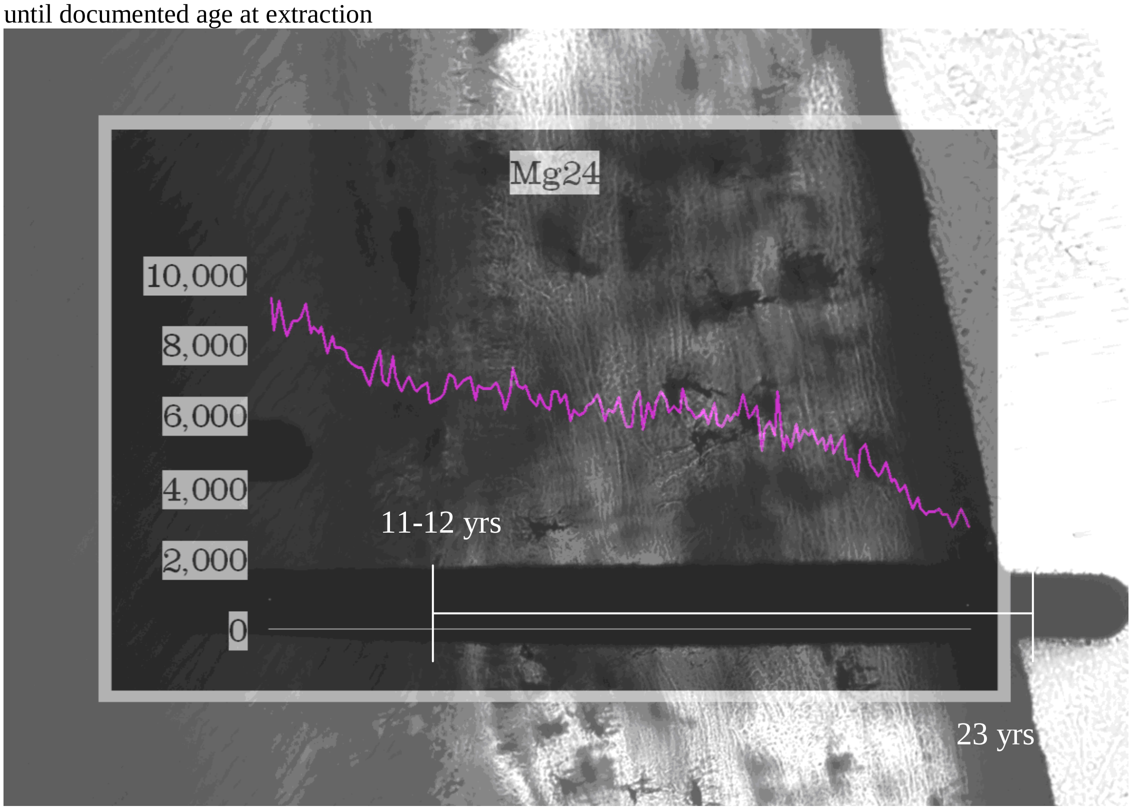


Figure 25 Standardized magnesium profile of tooth 16-5. X-axis is a proxy for age of tooth completion until documented age at extraction

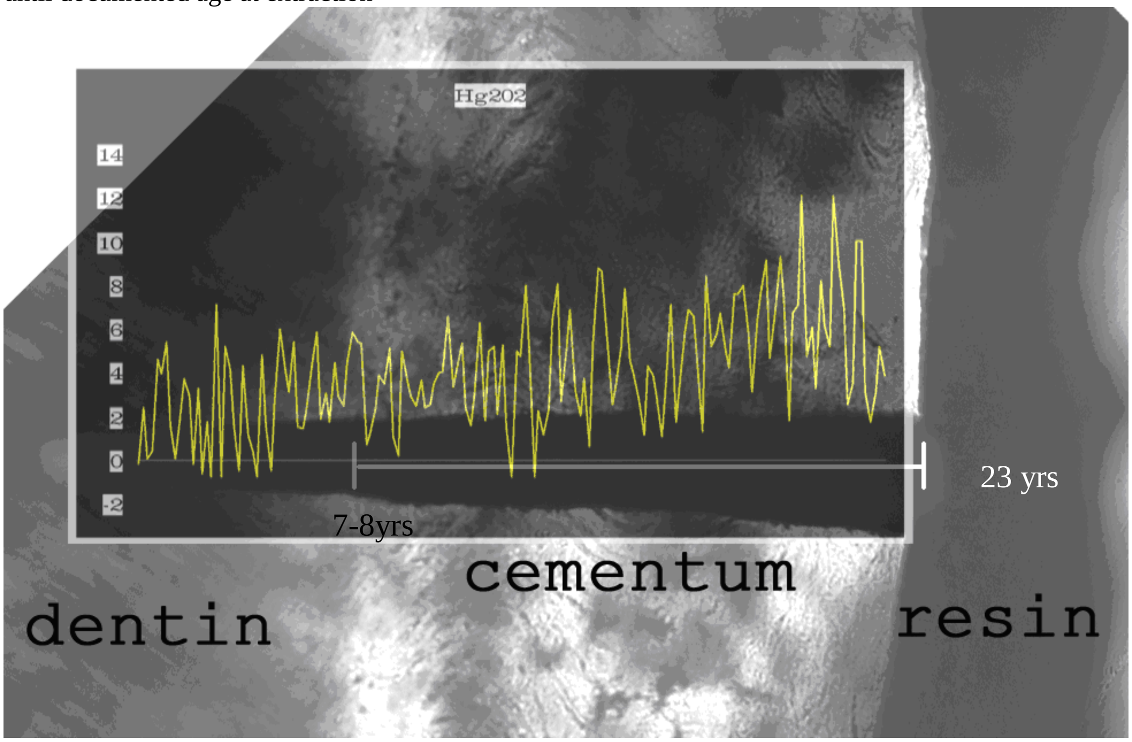


Figure 26 Standardized lead and zinc profiles of tooth 16-5. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

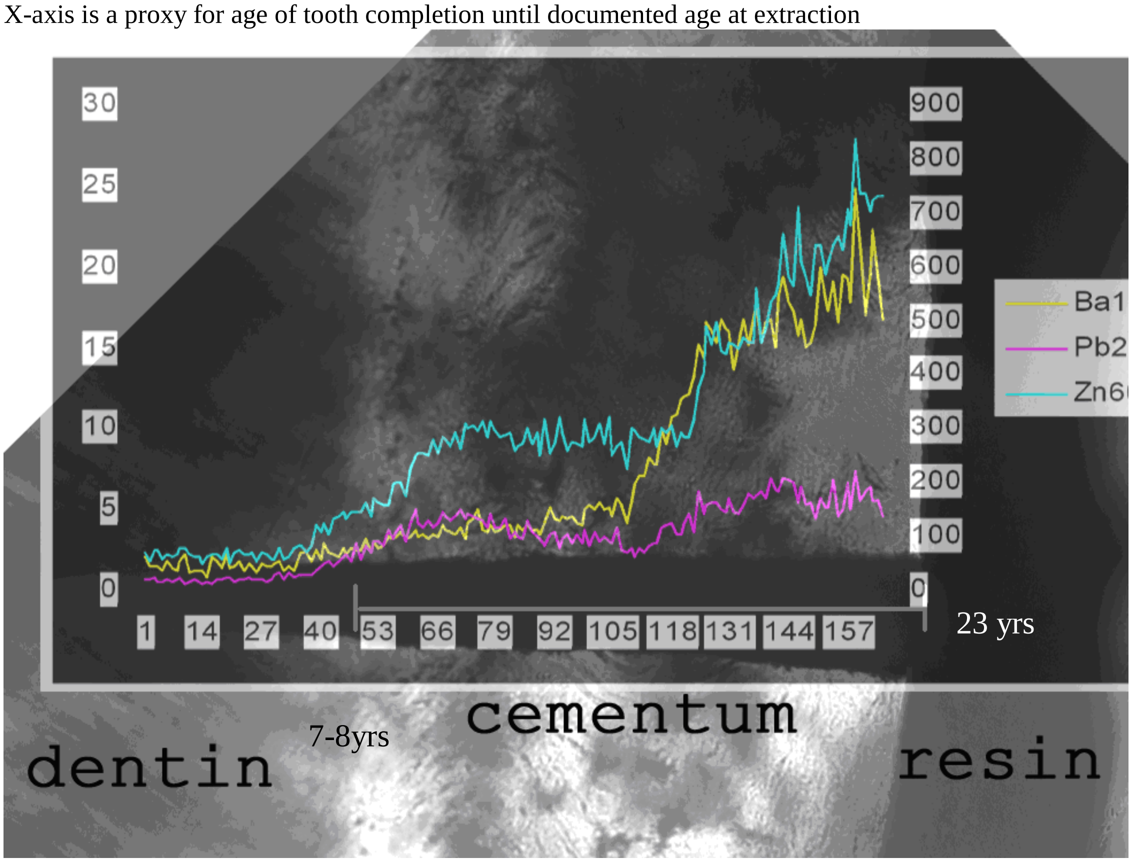


Figure 27 Standardized mercury profile of tooth 16-5. X-axis is a proxy for age of tooth completion until documented age at extraction

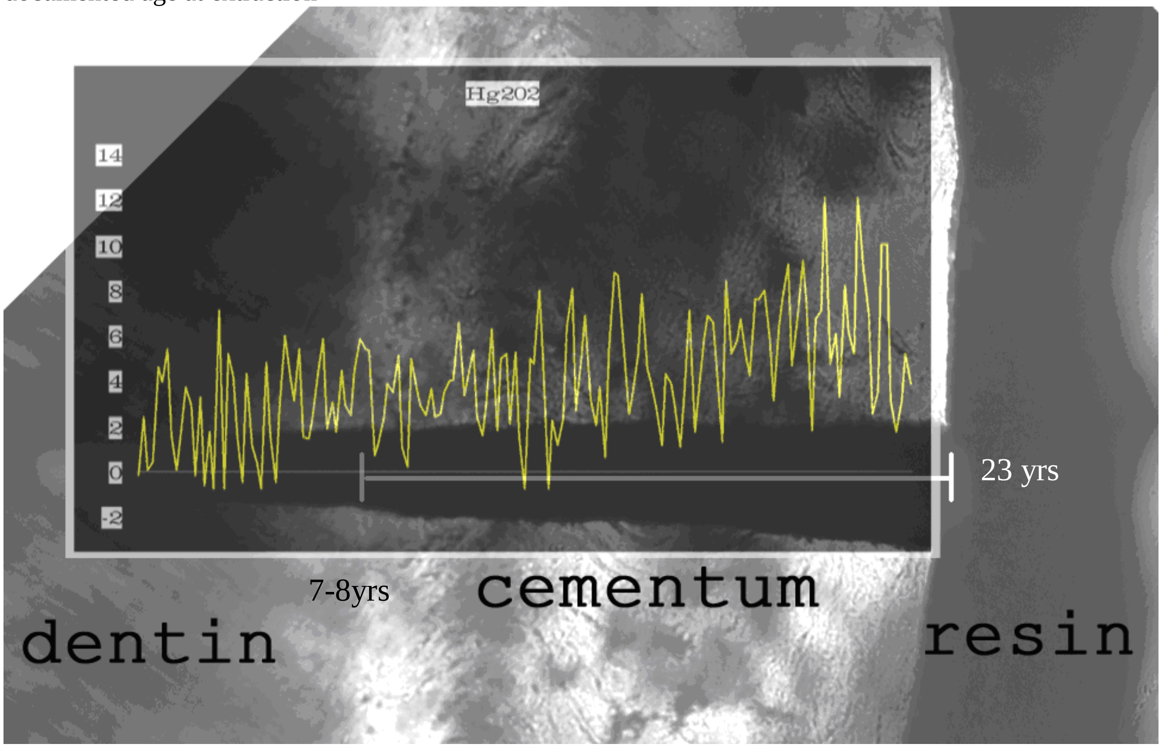


Figure 28 Standardized magnesium profile of tooth 19-2. X-axis is a proxy for age of tooth completion until documented age at extraction

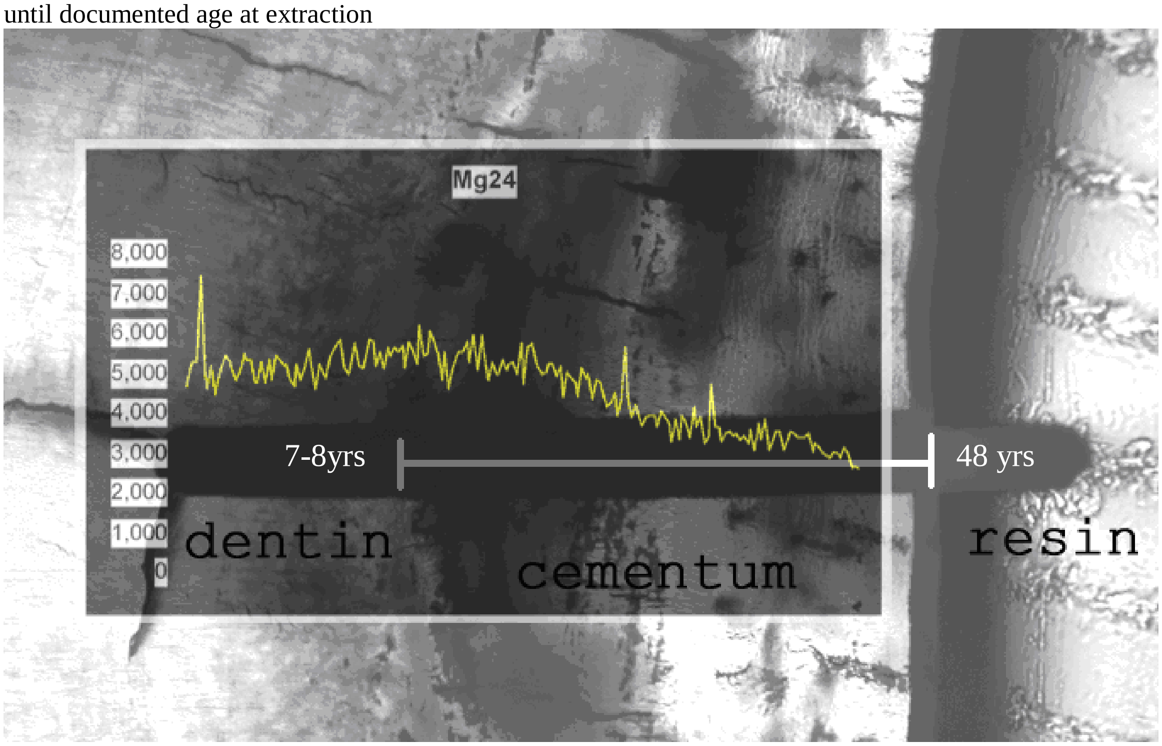


Figure 29 Standardized magnesium profile of tooth 19-3. X-axis is a proxy for age of tooth completion until documented age at extraction

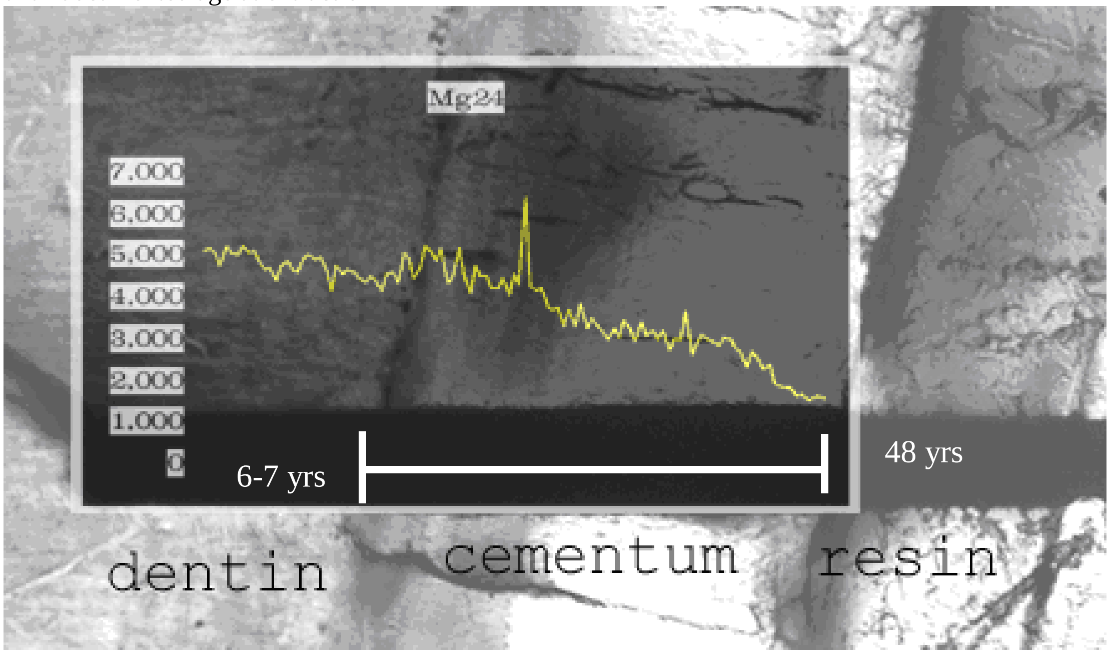


Figure 30 Standardized lead, zinc and barium profiles of tooth 19-2. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

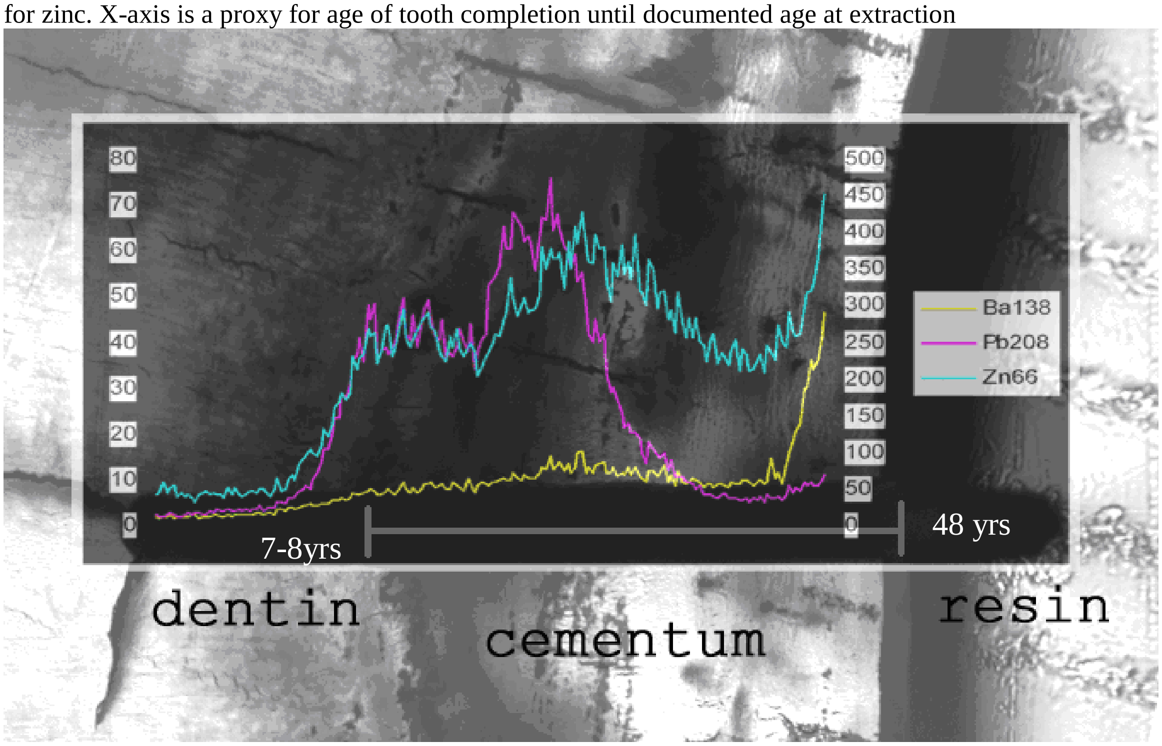


Figure 31 Standardized mercury profile of tooth 19-2. X-axis is a proxy for age of tooth completion until documented age at extraction

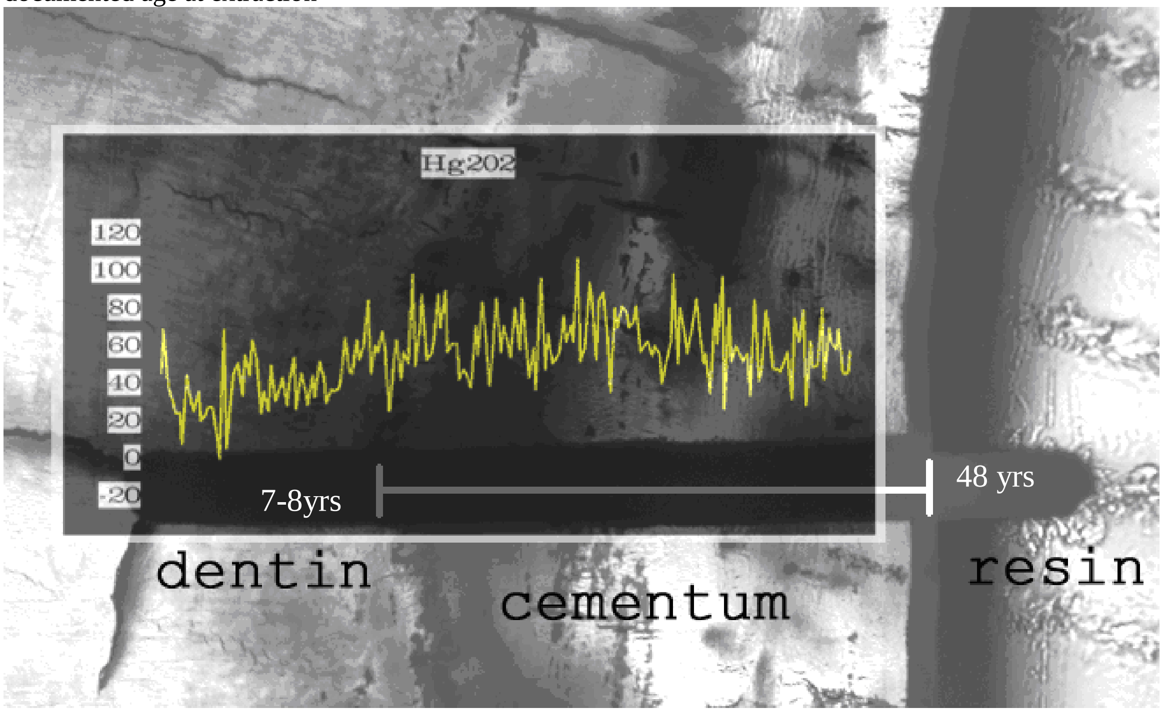
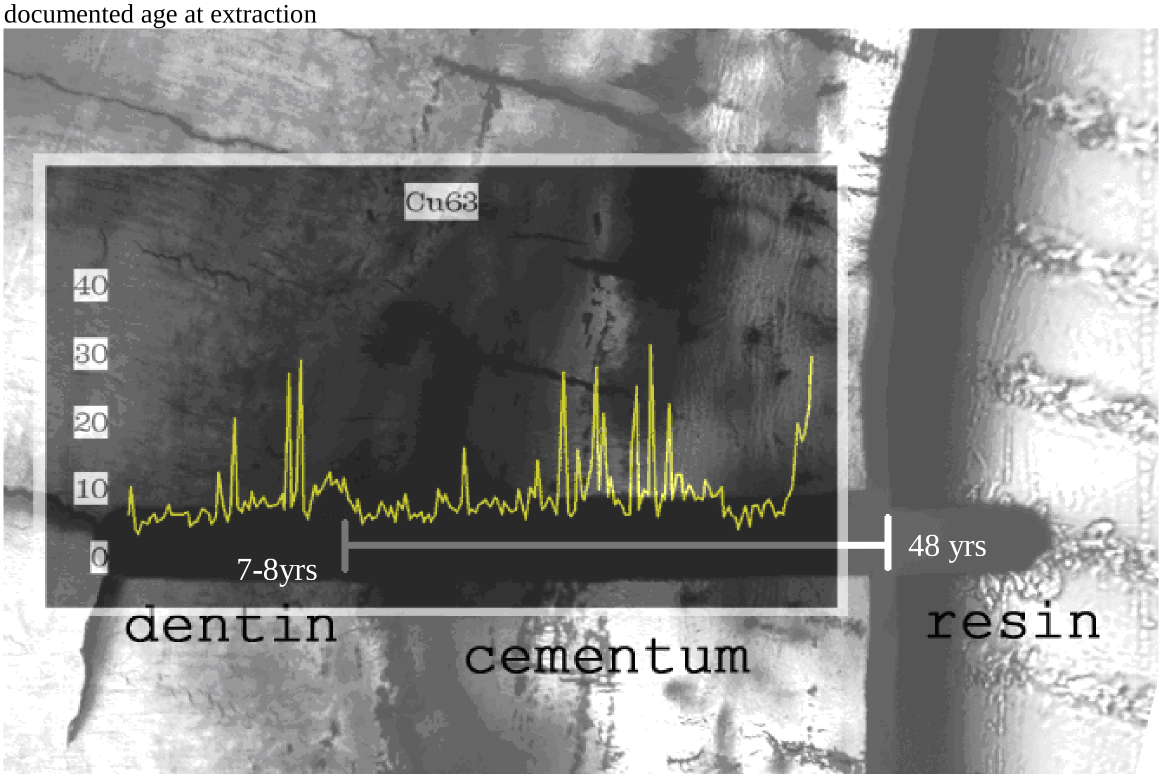


Figure 32 Standardized copper profile of tooth 19-2. X-axis is a proxy for age of tooth completion until documented age at extraction



22

The analysis for individual 22 is much more extensive than for any other individual. 4 teeth were analysed (22-2, 22-3, 22-6, 22-9). Each tooth was analysed several times.

22-2

In tooth 22-2, all three analyses were performed on the same side of the root since the other side was thin and mainly acellular. Line 1 has a double peak in the raw levels of the trace elements near the surface (Figure 35). The first peak occurs just as the laser beam would have reached the surface of the cementum. In other cases, the ‘surface’ peak lasts for as long as the surface is being ablated. This dip in levels before the second ‘surface’ peak is unusual. Raw calcium levels are not homogeneous through the cementum and peaks in the same area as the first surface peak seen in the raw trace elements profile (Figure 36).

Line 1 occurs closer to the apex of the root, and traverses more cellular cementum than the other 2 lines of analyses. The increased cellularity could be what is affecting the calcium levels. The darker areas of the cementum coincide somewhat with the drops in the calcium levels. The laser line appears slightly larger than either of the other two lines; however, there is a crack emanating from the one end of the laser line which allows the lasered area to split and appear larger. The other lines of analyses are not cracked and appear to be of similar size.

Line 2 (22-22) shows an increase in calcium levels in cementum in relation to dentin. There are some small changes in levels throughout cementum, but none as extreme as seen in line 1 (Figure 37). The darker cementum in line 2 does not coincide

Figure 33 Raw calcium profile of tooth 19-3. X-axis is a proxy for age of tooth completion until documented age at extraction

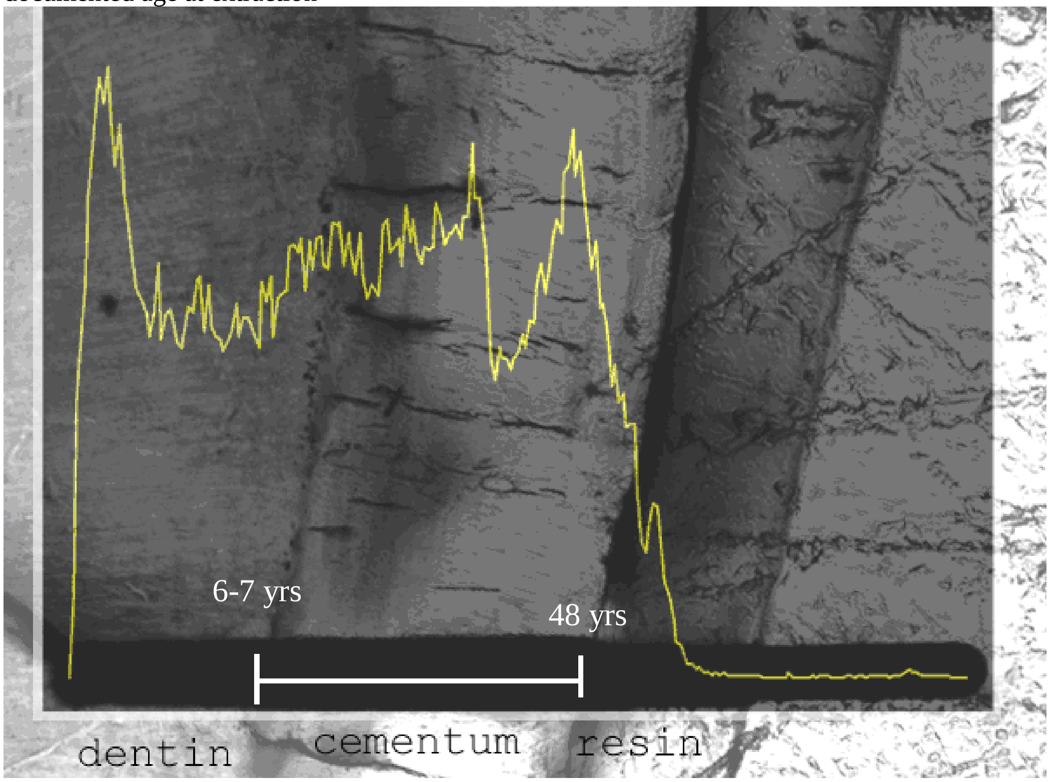


Figure 34 Raw calcium profile of tooth 19-2. X-axis is a proxy for age of tooth completion until documented age at extraction

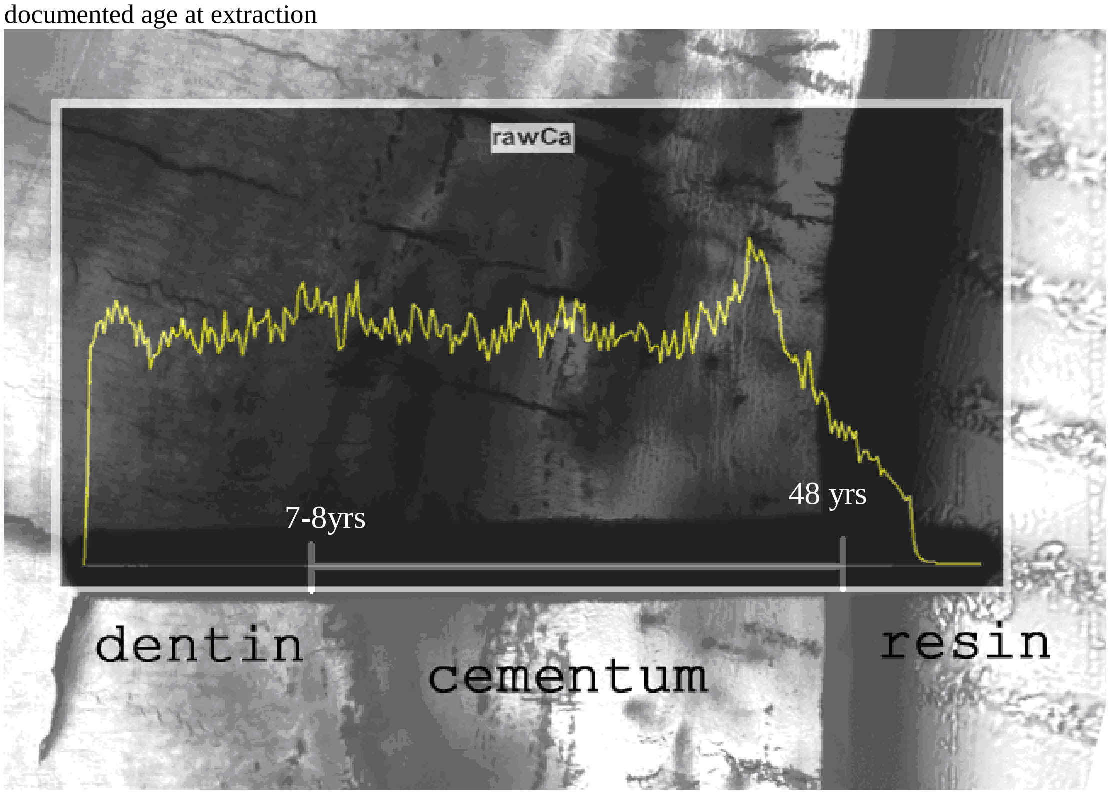


Figure 35 Raw profile of trace elements of tooth 22-2 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

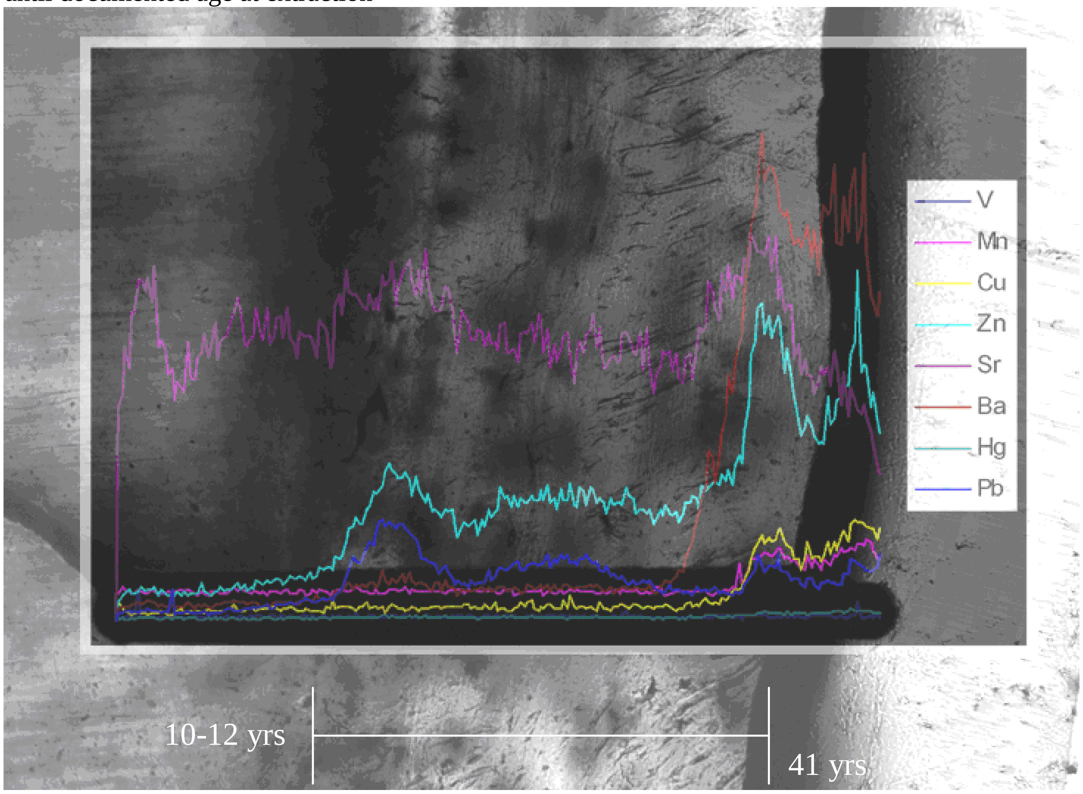
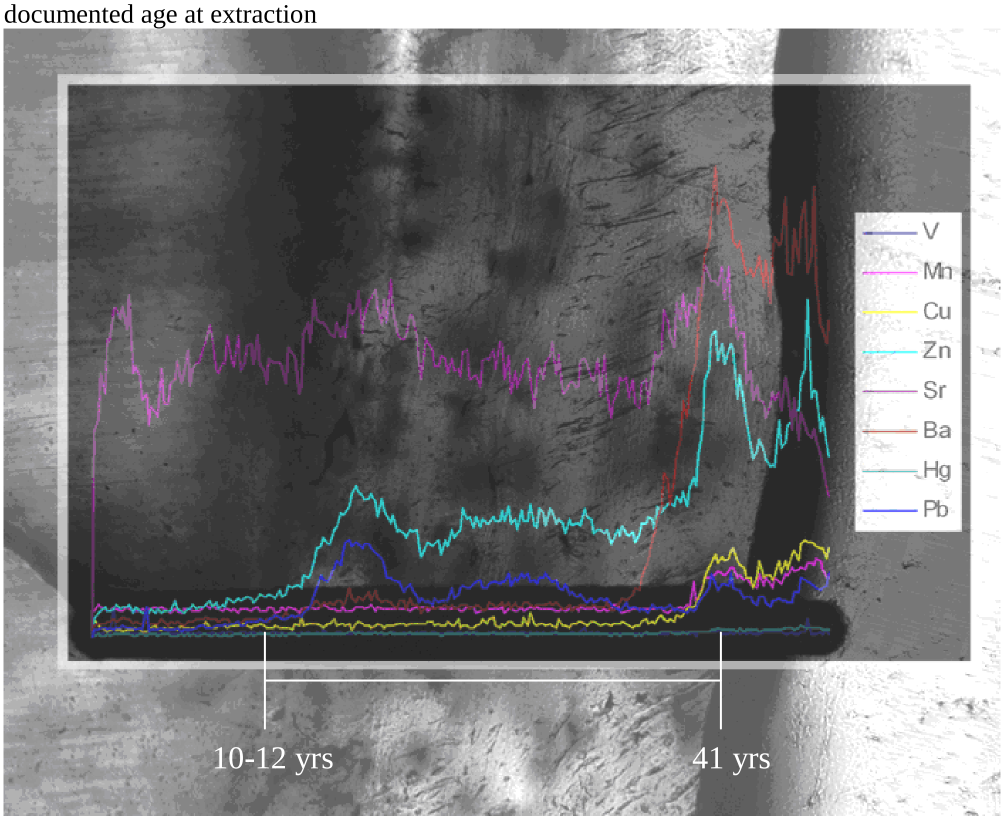


Figure 36 Raw calcium profile of tooth 22-2 line1. X-axis is a proxy for age of tooth completion until documented age at extraction



with a great change in calcium levels (Figure 38). Line 3 occurs in a less cellular area than the other two lines, and appears to be the most stable in calcium levels.

All 3 lines in tooth 22-2 are fairly close to each other and analyses are roughly the same; lead, zinc, mercury, and barium to a certain degree all follow the same profile pattern as each other, and in the different lines of analysis (Figure 39). Strontium (Figure 40,Figure 41, and Figure 42), however shows irregularities in lines 1 and 3 (22-2 and 22-23, respectably). Line 2, which lies between lines 1 and 3, was performed at 3µm/s, whereas lines 1 and 3 were at 2 µm/s. This slight difference in speed of the laser most likely is the cause of appeared variation.

There is also a difference in mercury levels (Figure 43, Figure 44, Figure 45). Line 1 has high levels of Hg and low background Hg levels (Figure 46). Lines 2 and 3 have low background and calculated Hg levels (Figure 46, Figure 47, Figure 48). I am unsure why this difference would occur.

22-3

Tooth 22-3 was analyzed twice with the LA-ICP-MS, and once with the EPMA. This is one of the two teeth with an amalgam filling. Both laser lines and the EPMA analysis were performed in the same area.

Line 1 (22-3) has inconsistent Zn, Ca, V, Ba, and Mg background levels (Figure 49). The background levels of Hg are high and fairly consistent (Figure 49). The high levels cause the calculated Hg levels to be low. The background levels are so high because the amalgam filling emits Hg vapours while in the ablation chamber, prior to ablation. Line 2 (22-32) also has high and steady Hg background levels (Figure 50). The ablated Hg levels were higher than those in line 1, and continued to increase throughout the ablation.

Figure 37 Raw profile of trace elements of tooth 22-2 line2. X-axis is a proxy for age of tooth completion until documented age at extraction

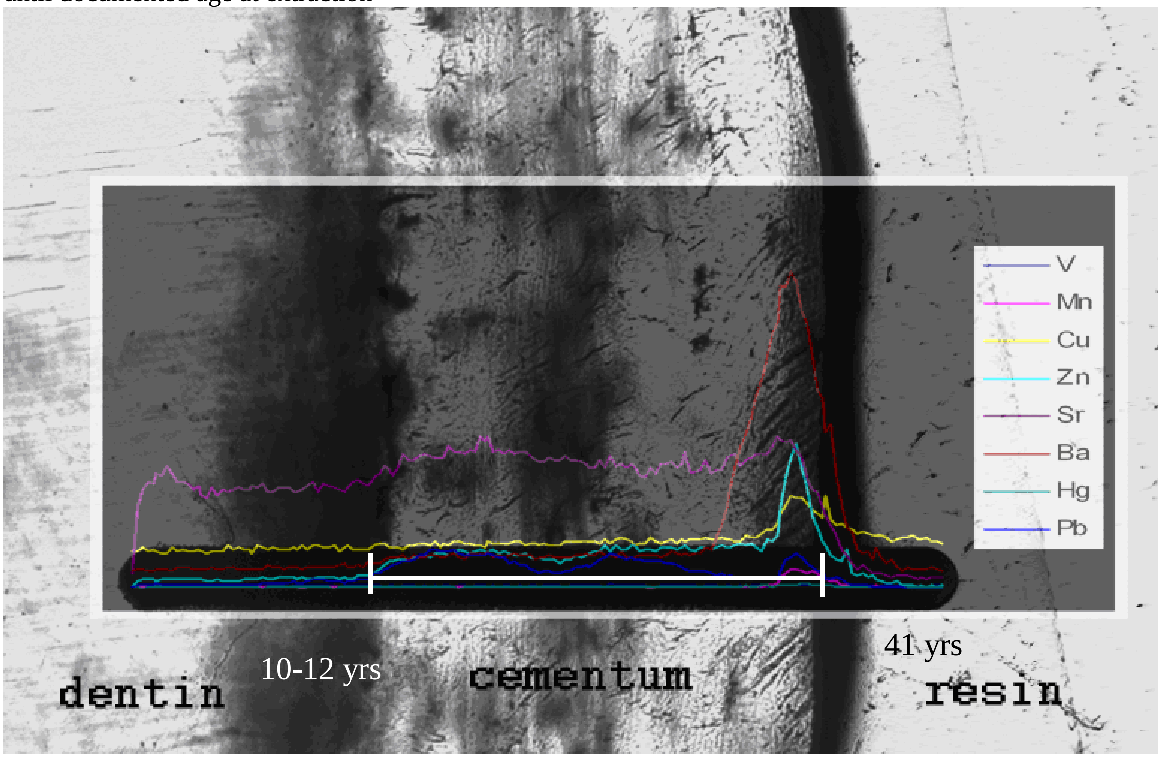


Figure 38 Raw calcium profile of tooth 22-2 line2. X-axis is a proxy for age of tooth completion until documented age at extraction

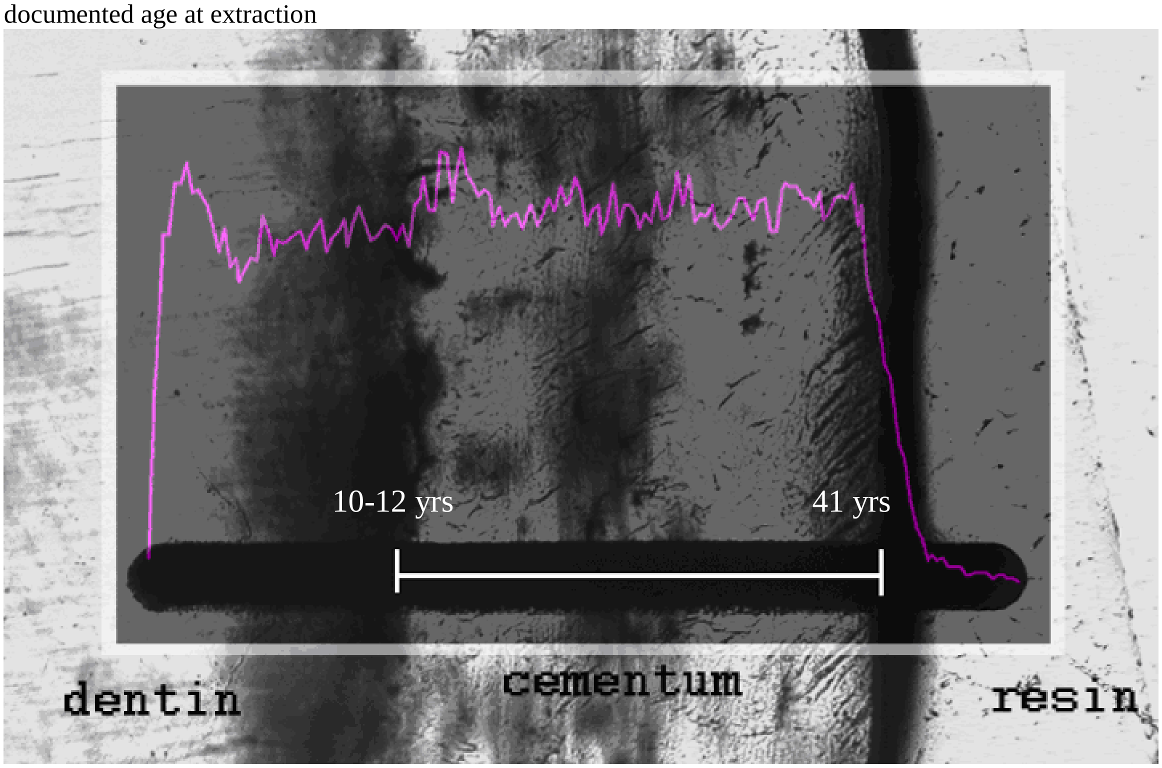


Figure 39 Raw profile of trace elements of tooth 22-2 line3. X-axis is a proxy for age of tooth completion until documented age at extraction

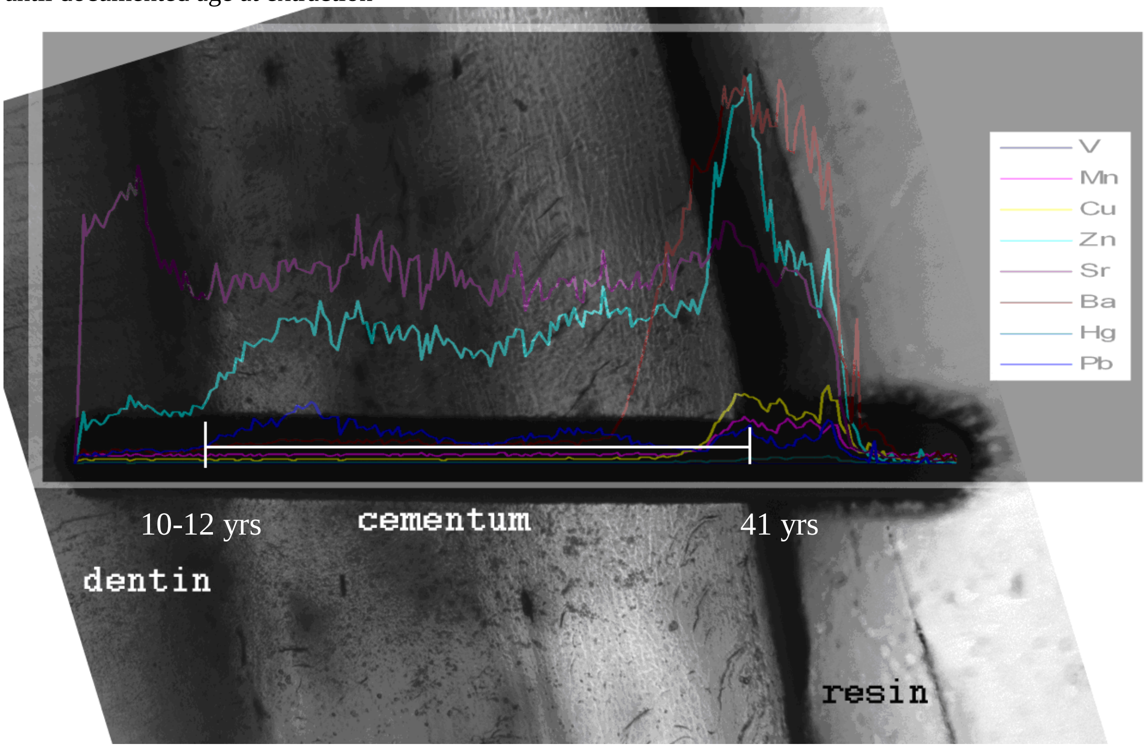


Figure 40 Standardized strontium profile of tooth 22-2 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

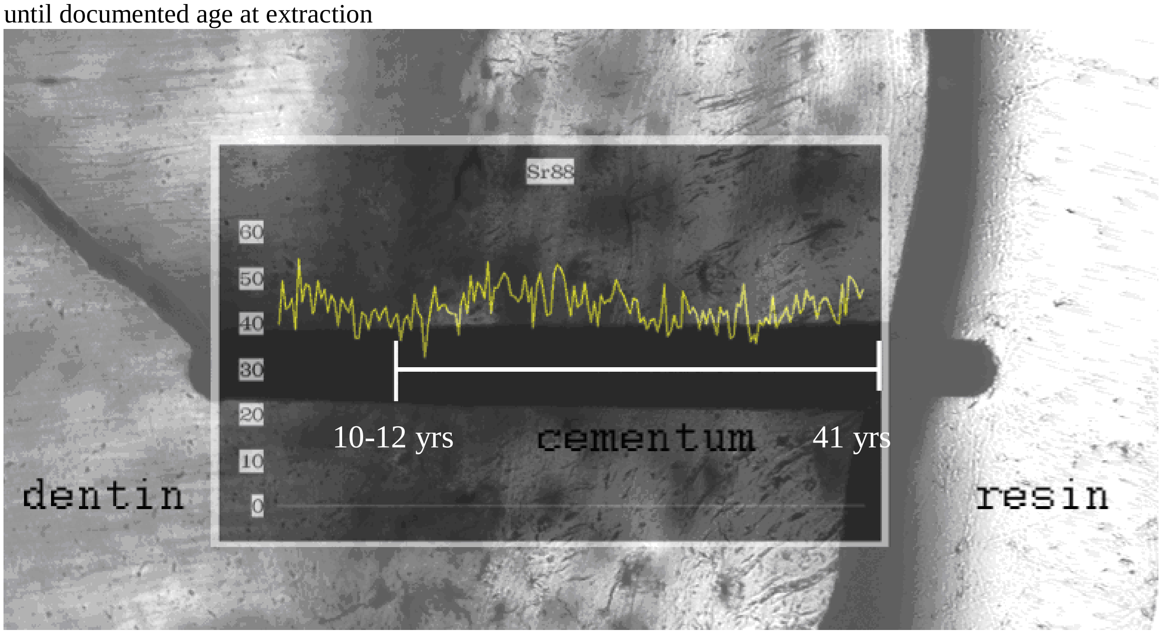


Figure 41 Standardized strontium profile of tooth 22-2 line2. X-axis is a proxy for age of tooth completion until documented age at extraction

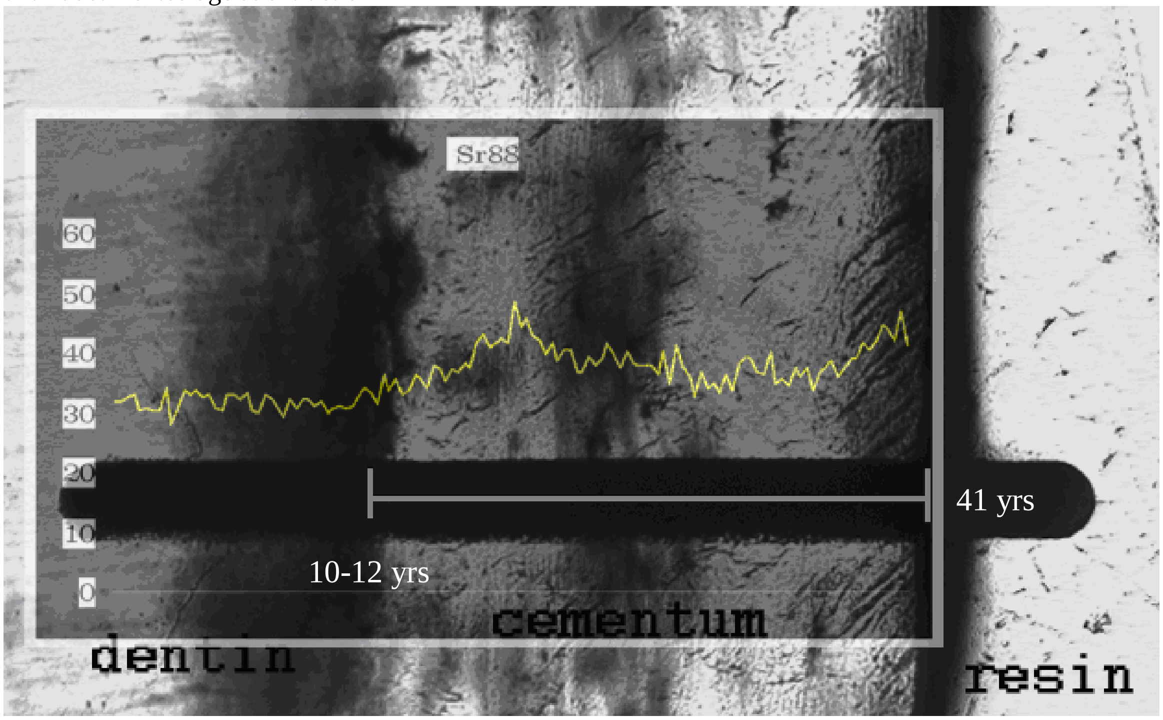


Figure 42 Standardized strontium profile of tooth 22-2 line3. X-axis is a proxy for age of tooth completion until documented age at extraction

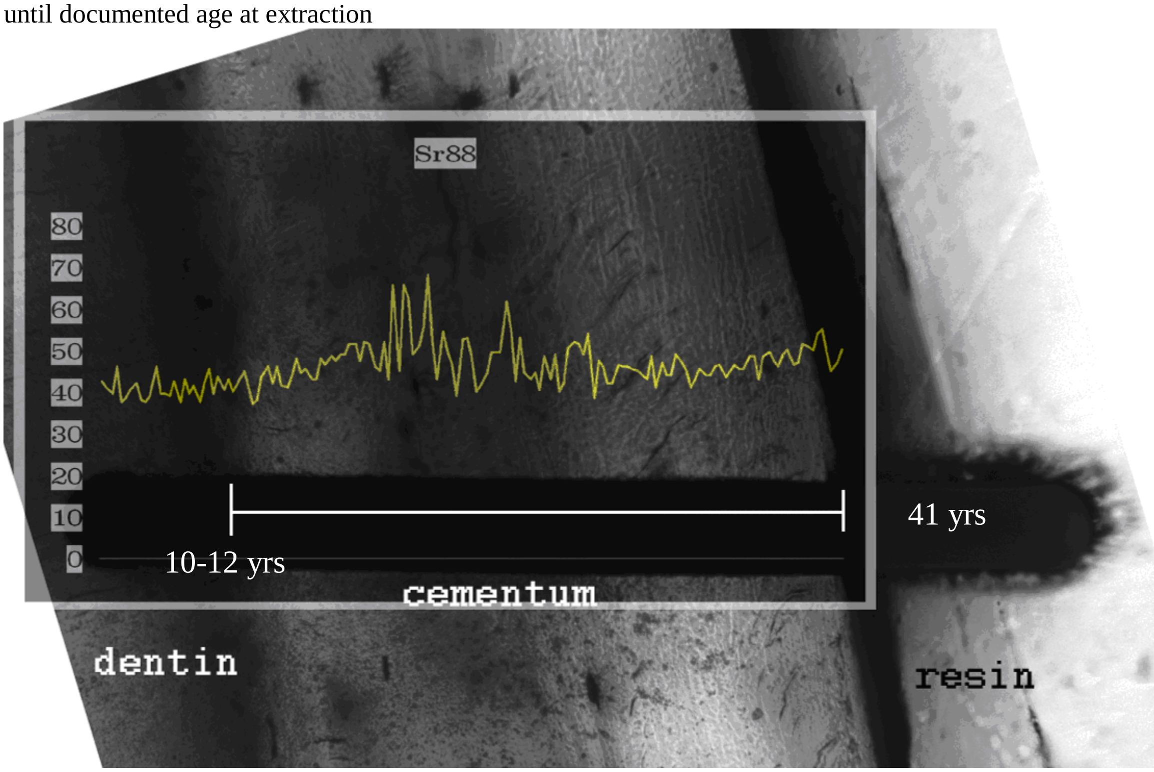


Figure 43 Standardized mercury profile of tooth 22-2 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

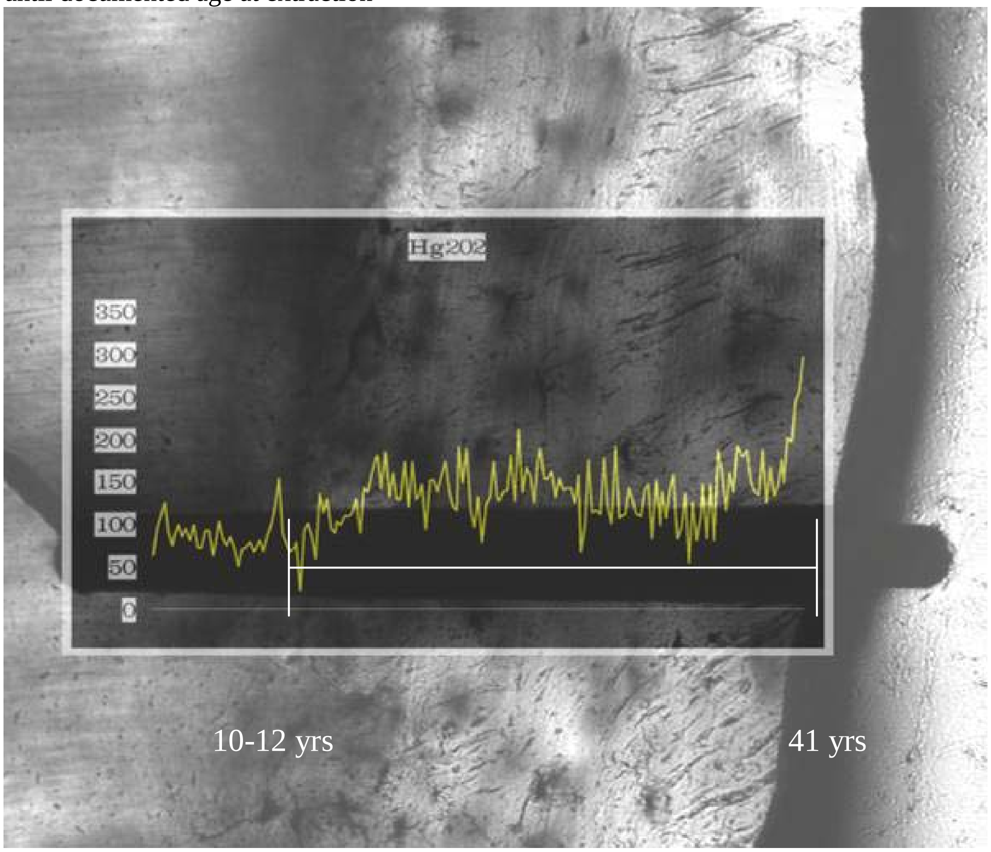


Figure 44 Standardized mercury profile of tooth 22-2 line2. X-axis is a proxy for age of tooth completion until documented age at extraction

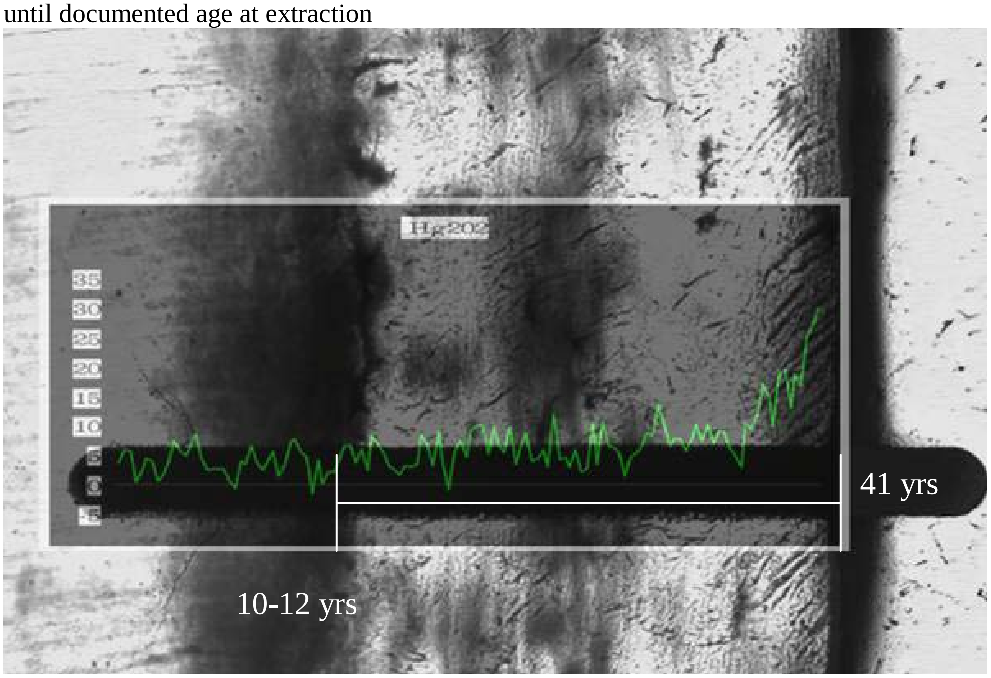


Figure 45 Standardized mercury profile of tooth 22-2 line3. X-axis is a proxy for age of tooth completion until documented age at extraction

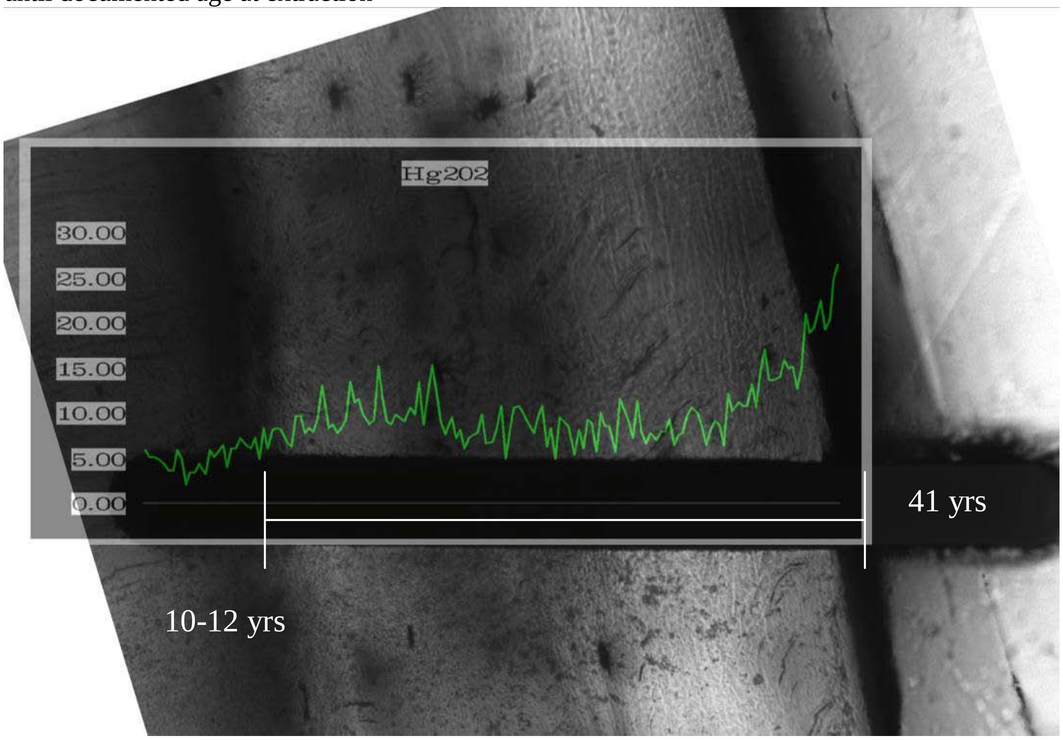


Figure 46 Background levels prior to ablation of tooth 22-2 line1

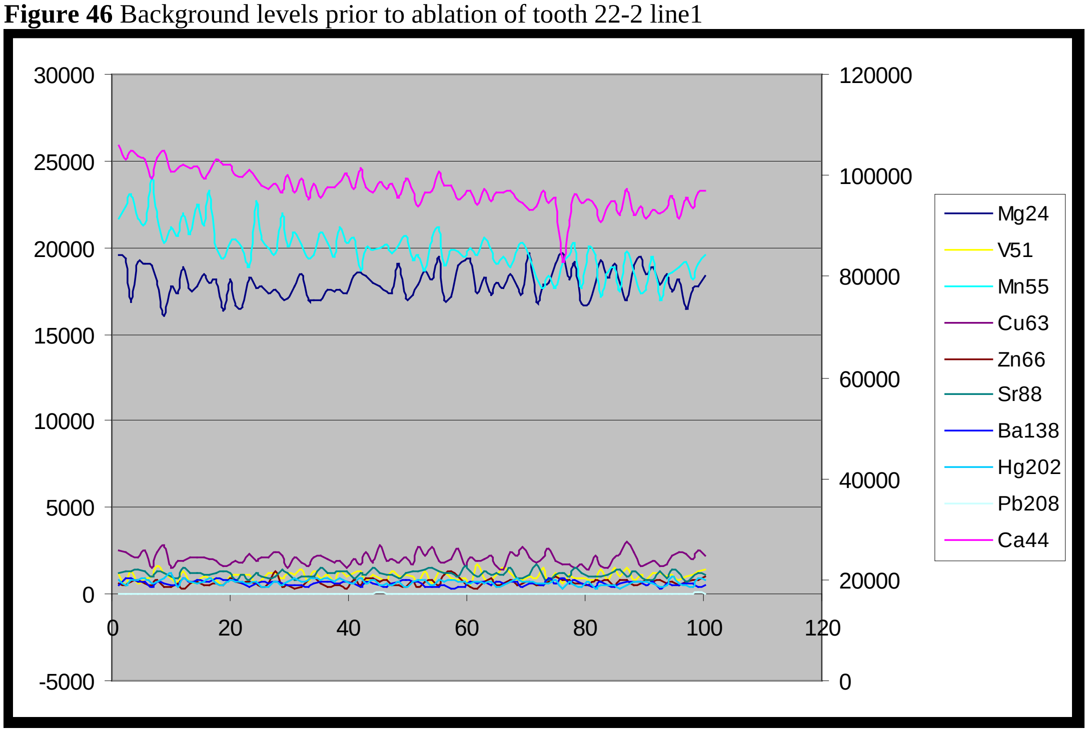


Figure 47 Background levels prior to ablation of tooth 22-2 line2

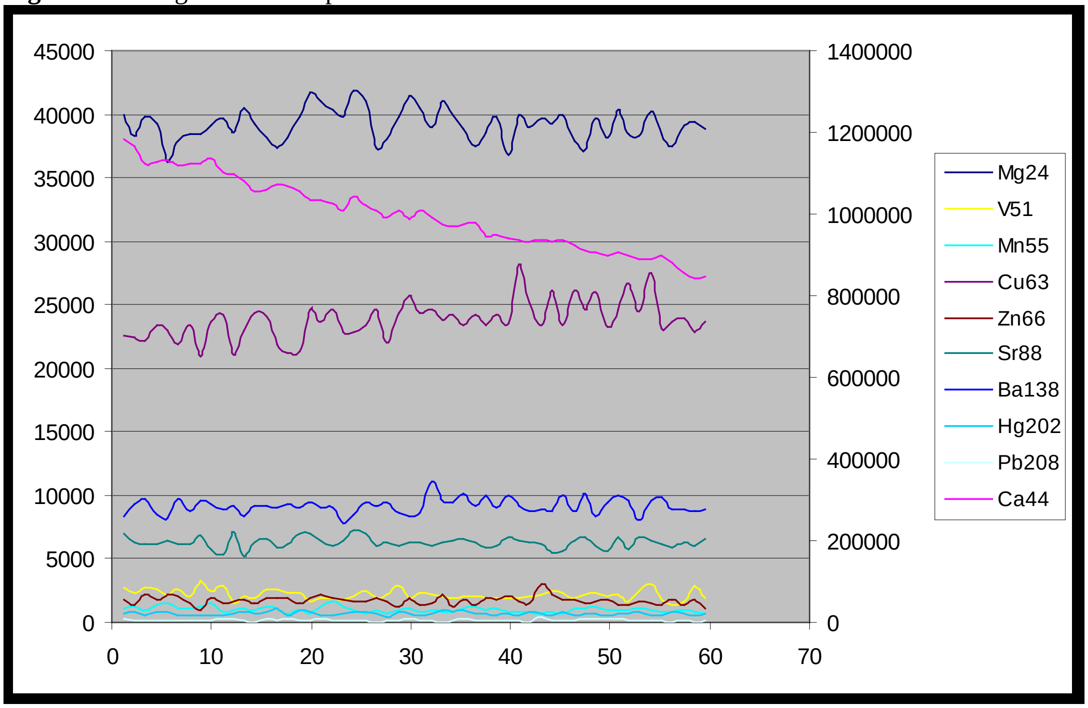


Figure 48 Background levels prior to ablation of tooth 22-2 line3

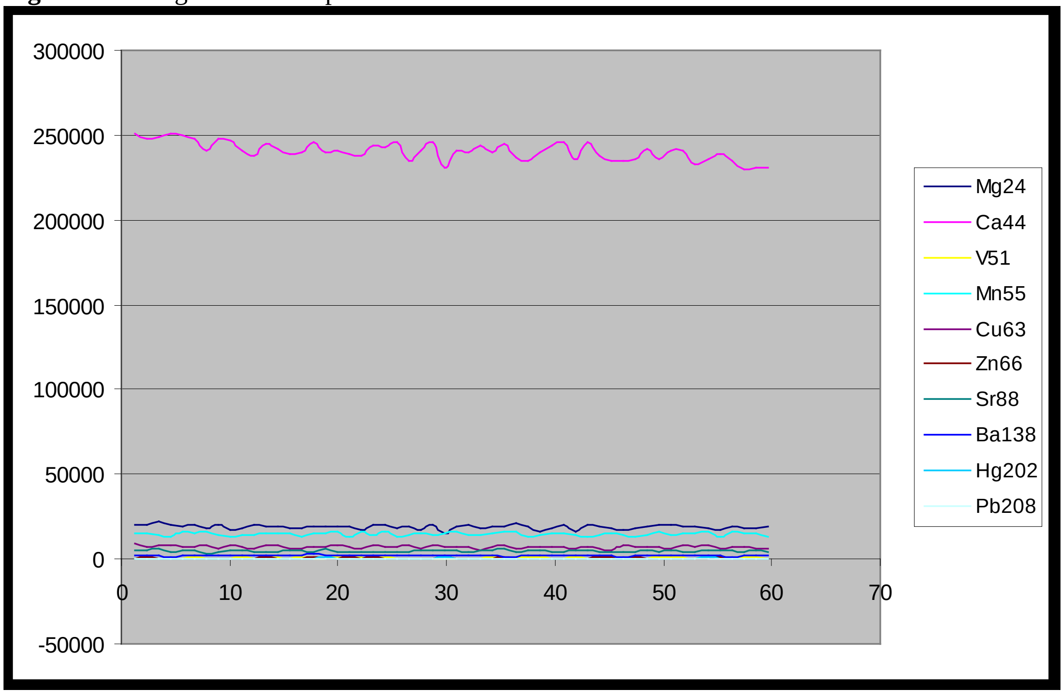


Figure 49 Background levels prior to ablation of tooth 22-3 line1

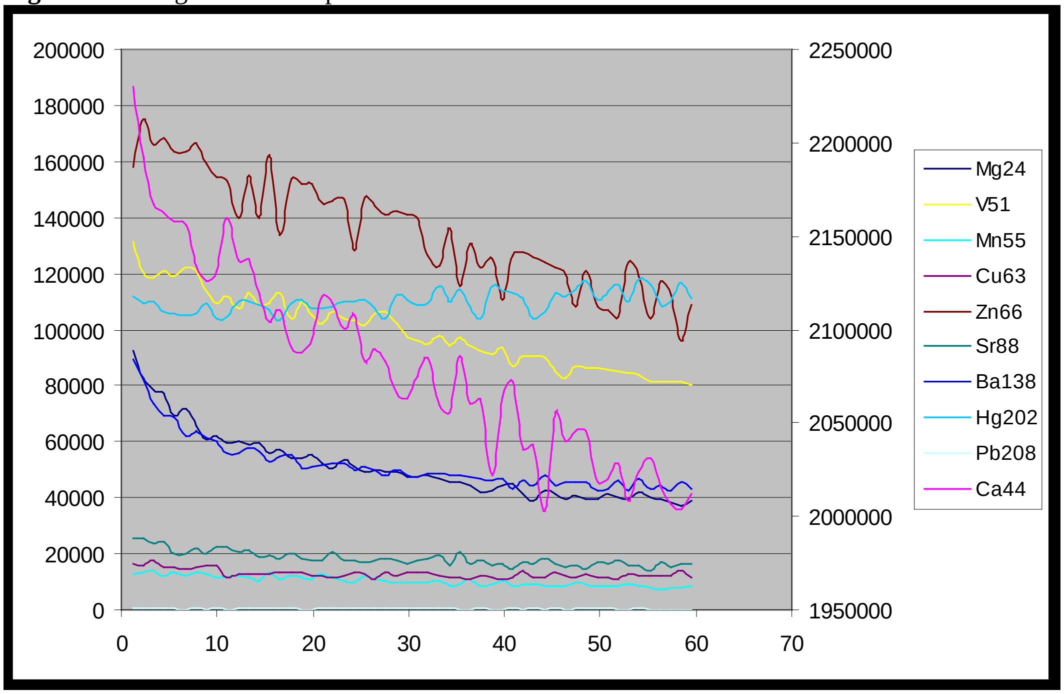
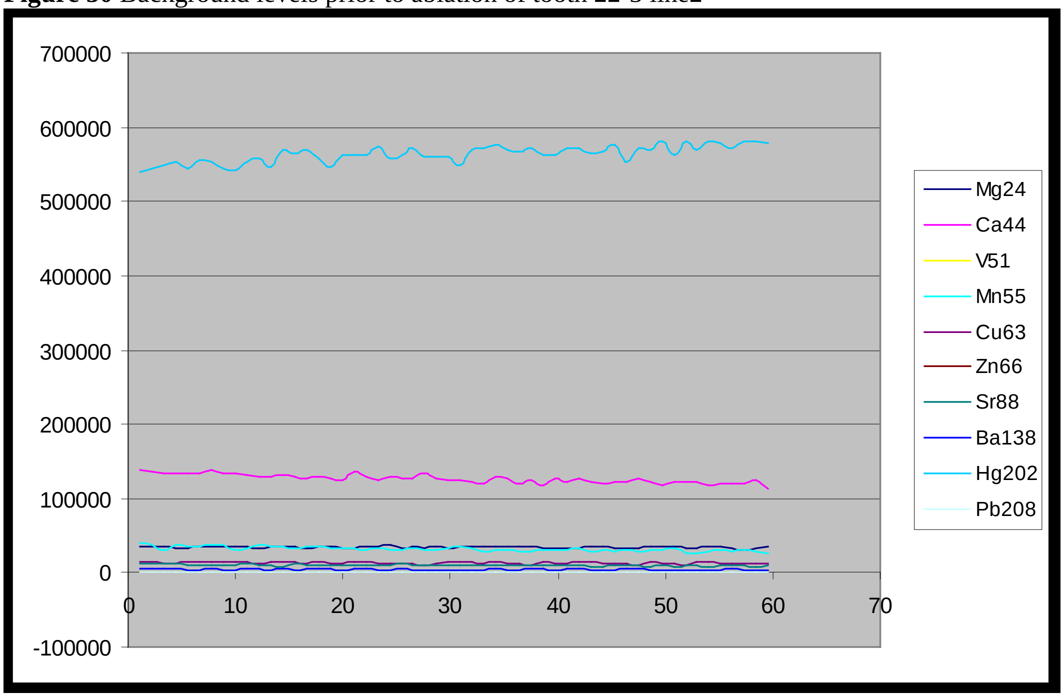


Figure 50 Background levels prior to ablation of tooth 22-3 line2



Tooth 22-3 has an excessive amount of cementum, which may be why Pb and Zn have such a more extensive profile, since there is more cementum to analyze (Figure 51). Patterns may be separated more than in other teeth. The last drop in levels of Pb (and 2^{nd} last of Zn) may correspond to an area of darker cementum that is observed in the same area on one of the subsequent thin sections for the tooth.

Line 1shows a crack emanating from the line into the dentin, which has allowed the tooth to separate a bit, making the ablation seem wider than it should. The laser mark in the resin is of similar size as that of line 2. Images taken with the SEM attached to the EPMA shows the separation of the cementum below the laser line.

The lead levels in line 2 are variable (Figure 52). The middle dip in levels corresponds to a dark area that runs through the cementum, parallel to the surface. It corresponds but may not be the cause of the dip. It is difficult to determine the cause of the darker cementum. Zinc also dips in the same area (Figure 52). The second large drop in zinc levels to an area with a narrow artefact or small crack on the slide. Other dark areas in cementum do not have the same effect. The size of the laser beam makes it difficult to determine how large, or small, a change would have to be to affect the levels detected.

The SEM photos also show that there is a space between the tooth and the resin. This space could have trapped material between the two, and may account for some of the large peaks that occur at the cementum edge, although many of the increases in elemental levels occur before the laser reaches that area.

22-6

Figure 51 Standardized lead and zinc profiles of tooth 22-3 line1. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

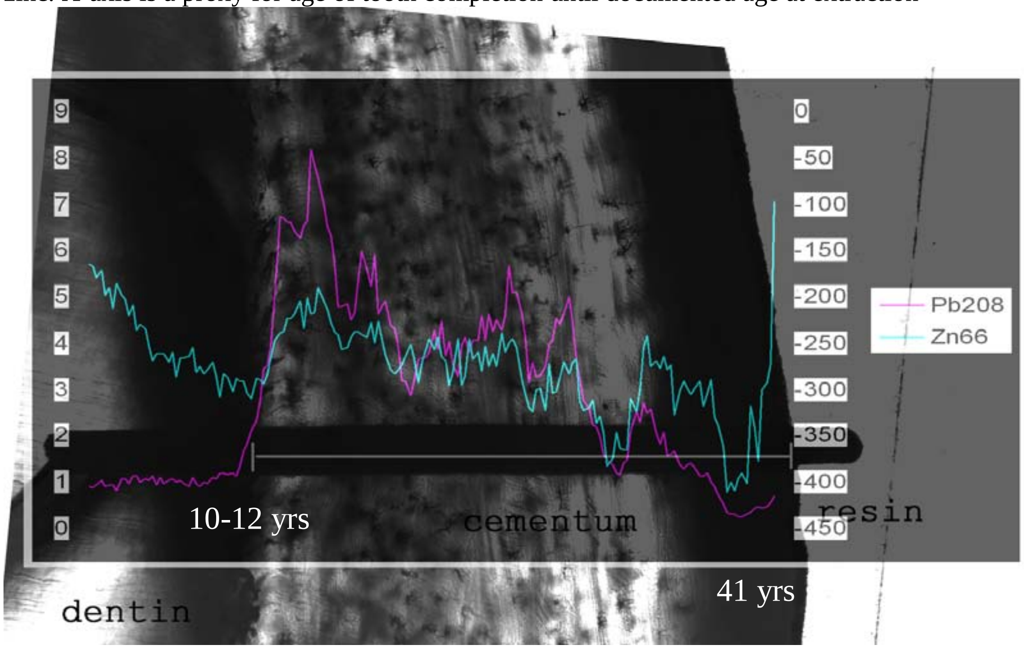
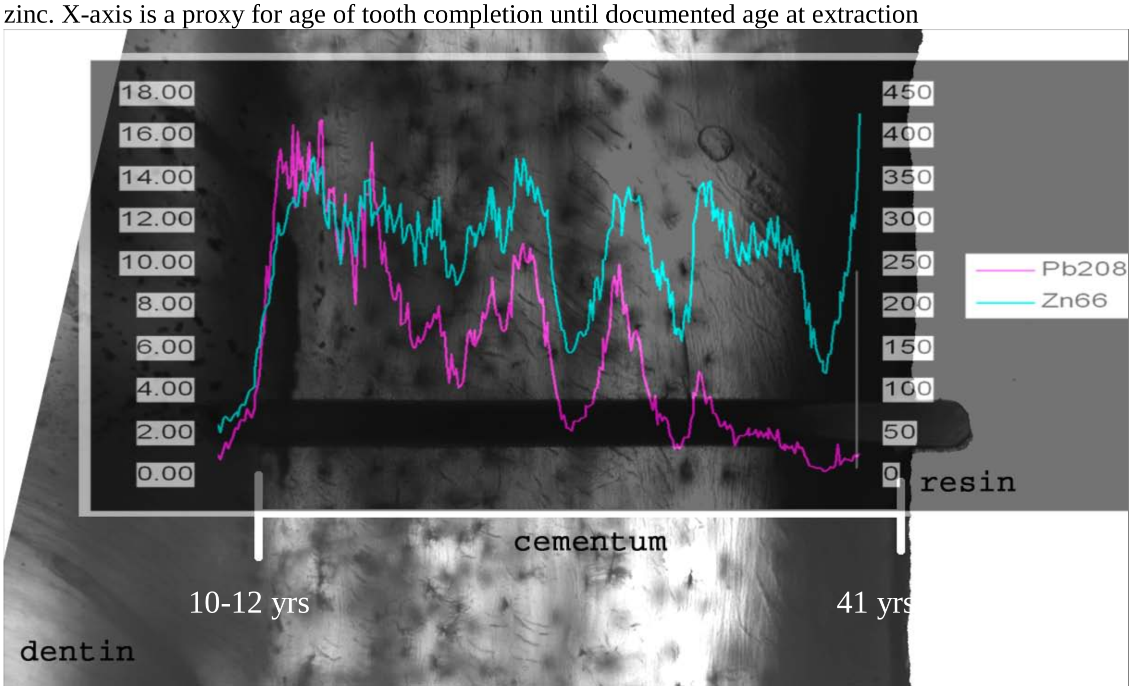


Figure 52 Standardized lead and zinc profiles of tooth 22-3 line2. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction



Tooth 22-6 was analysed three times; lines 1 and 3 (22-6 and 22-63, respectively) are close to each other, while line 2 (22-62) is further away. Line 1 appears larger; however there is a crack at the one end, allowing the cementum to separate a bit. None of the lines are particularly long since this tooth was cross-sectioned and the cementum was mainly thin and acellular. Lines 1 and 3 are in one of the more cellular areas.

Line 1 did not contain much dentin, so differences in tissue intensities cannot be determined. Lead, zinc and mercury follow the same pattern (Figure 54 andFigure 55). Barium's profile is too low to determine how closely it follows the three elements. The background levels of Ca, Mn, and Mg are unsteady at first, decreasing in intensity, before becoming more stable (Figure 56). The calculated profile of magnesium shows it decreasing fairly suddenly as the laser nearly reaches the outer part of cementum (Figure 57). The Mg levels through the rest of the cementum appear fairly stable. Mg levels in line 3 are also unusual. Levels drop suddenly before displaying a peak in levels when ablating the outer surface of the cementum and the surrounding resin. The other elements do not show the same drop prior to peaking. The drop, however, is similar to the outer decrease in Mg levels that occurs in line 1. The outer peak in Mg levels in line 3 could be from contamination since it is such an unusual occurrence.

Line 2 was analysed at 3µm/s which smoothes out some of the usual irregularities in analysis, and brings out the actual profile patterns of the elements. Hg is very variable, but does not have a discernable 'starting point' to determine when the amalgam fillings were implanted into the teeth of this individual (Figure 58). Levels are also low, in both the background and calculated levels (Figure 59). Copper levels in line 2 are more elevated than in either line 1 or 3 (Figure 60, Figure 61, andFigure 62).

Figure 53 SEM photo of tooth 22-3 showing line1 (on left) and line2 (right)

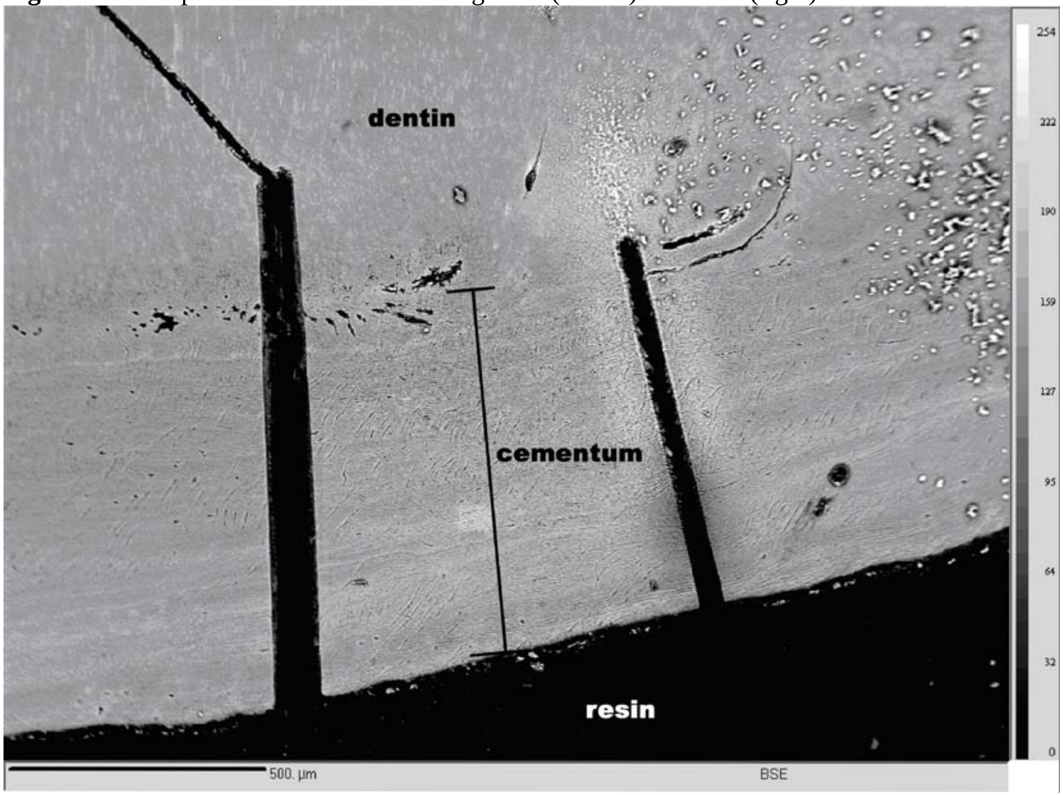


Figure 54 Standardized lead and zinc profiles of tooth 22-6 line1. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

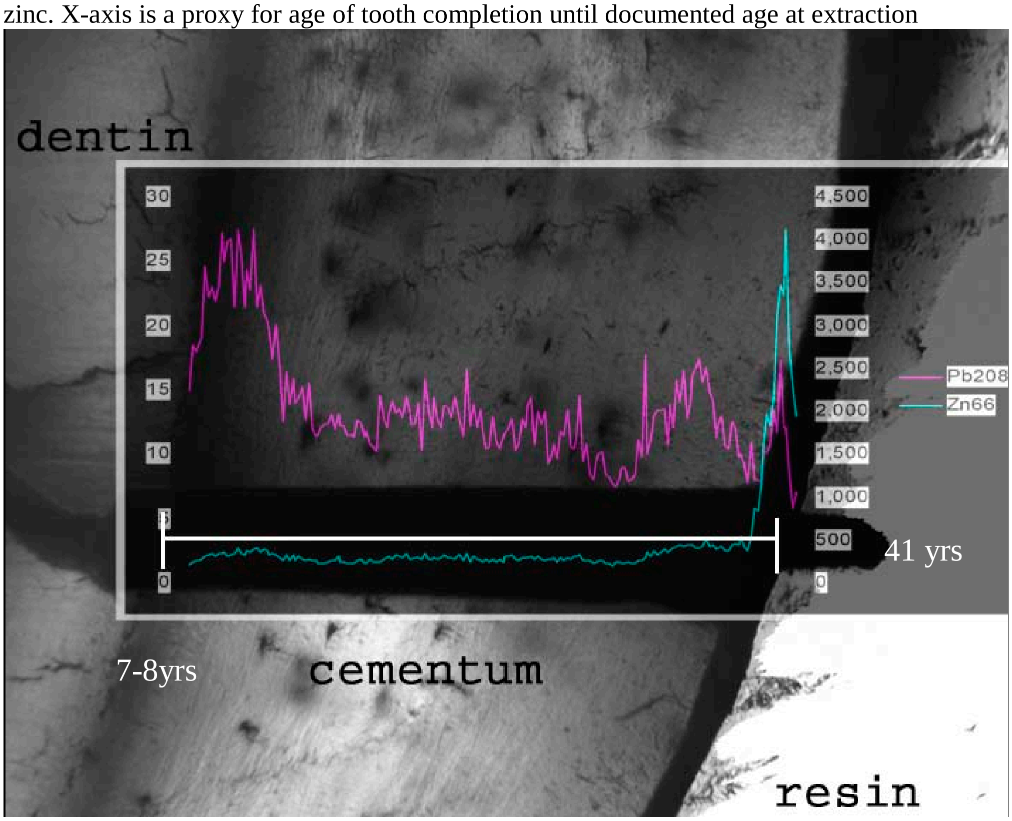


Figure 55 Standardized mercury profile of tooth 22-6 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

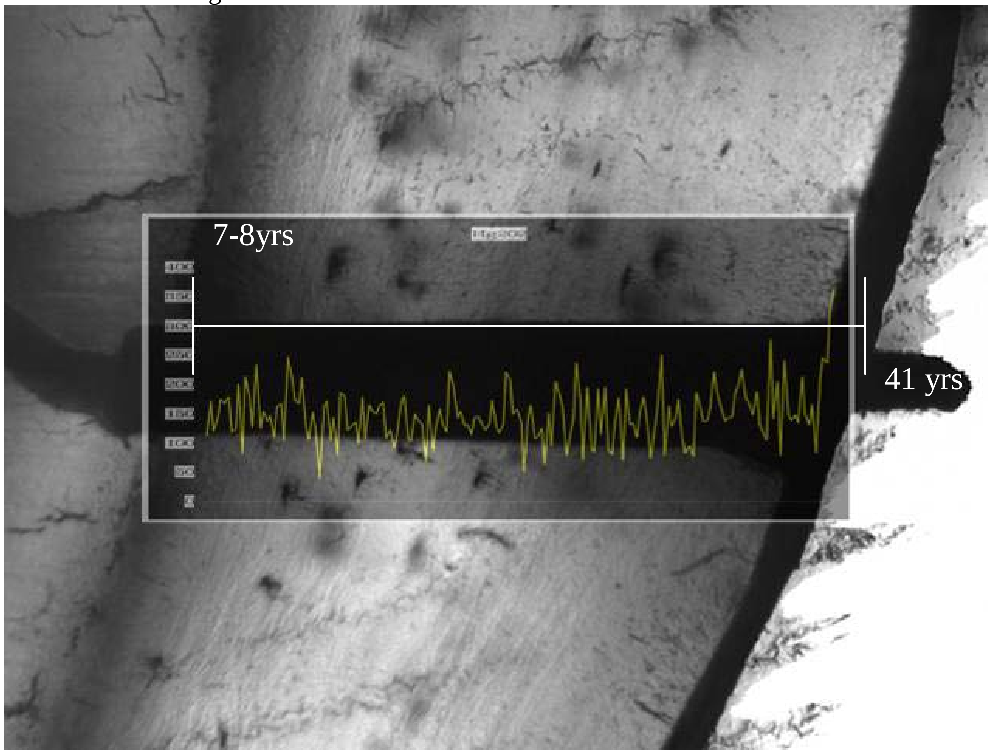


Figure 56 Background levels prior to ablation of tooth 22-6 line1

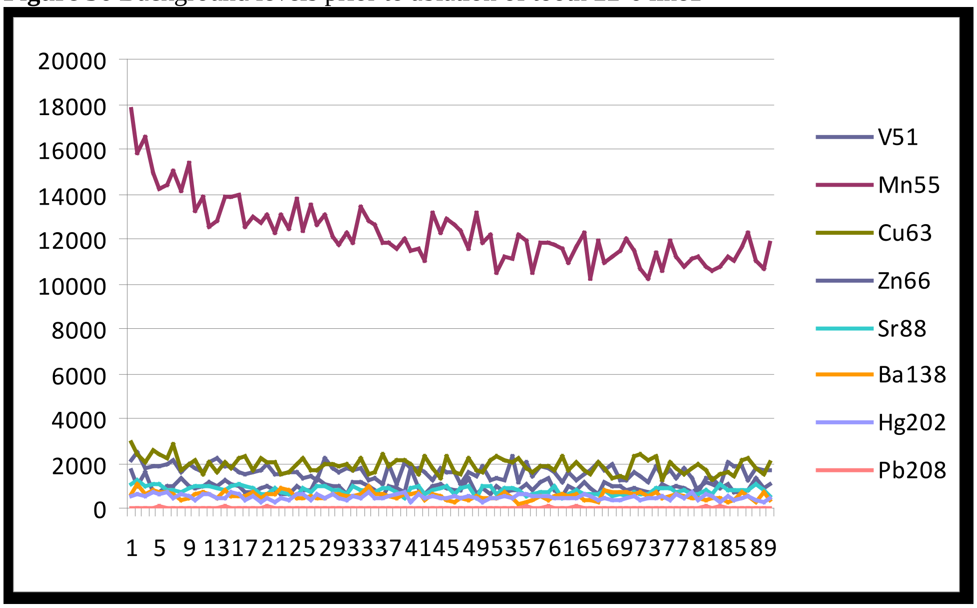


Figure 57 Standardized magnesium profile of tooth 22-6 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

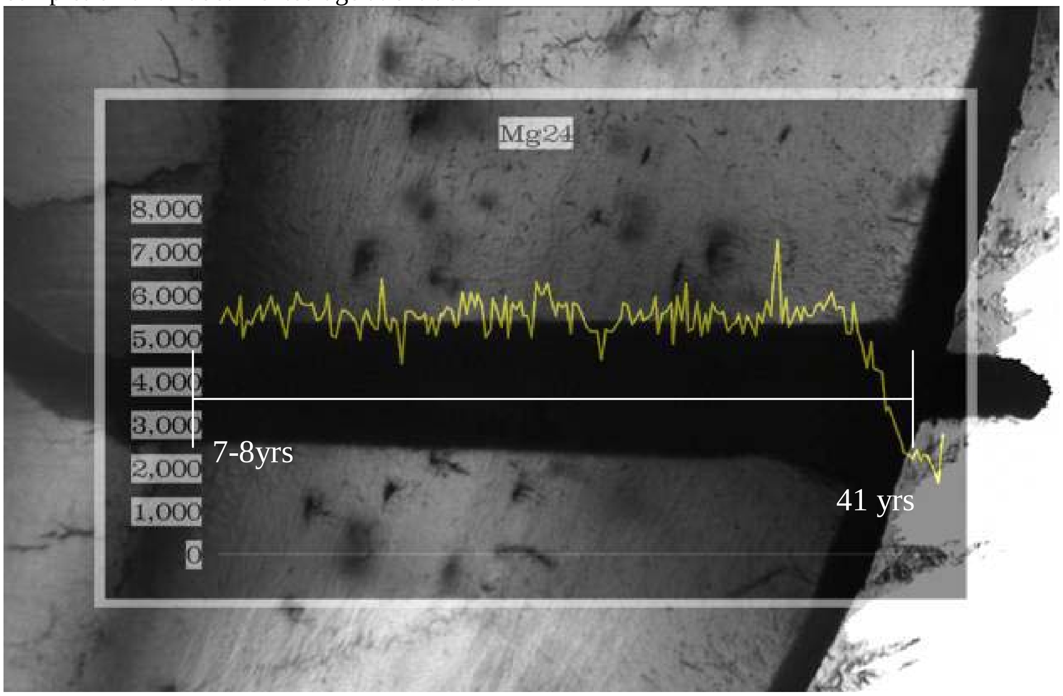


Figure 58 Standardized mercury profile of tooth 22-6 line2. X-axis is a proxy for age of tooth completion until documented age at extraction

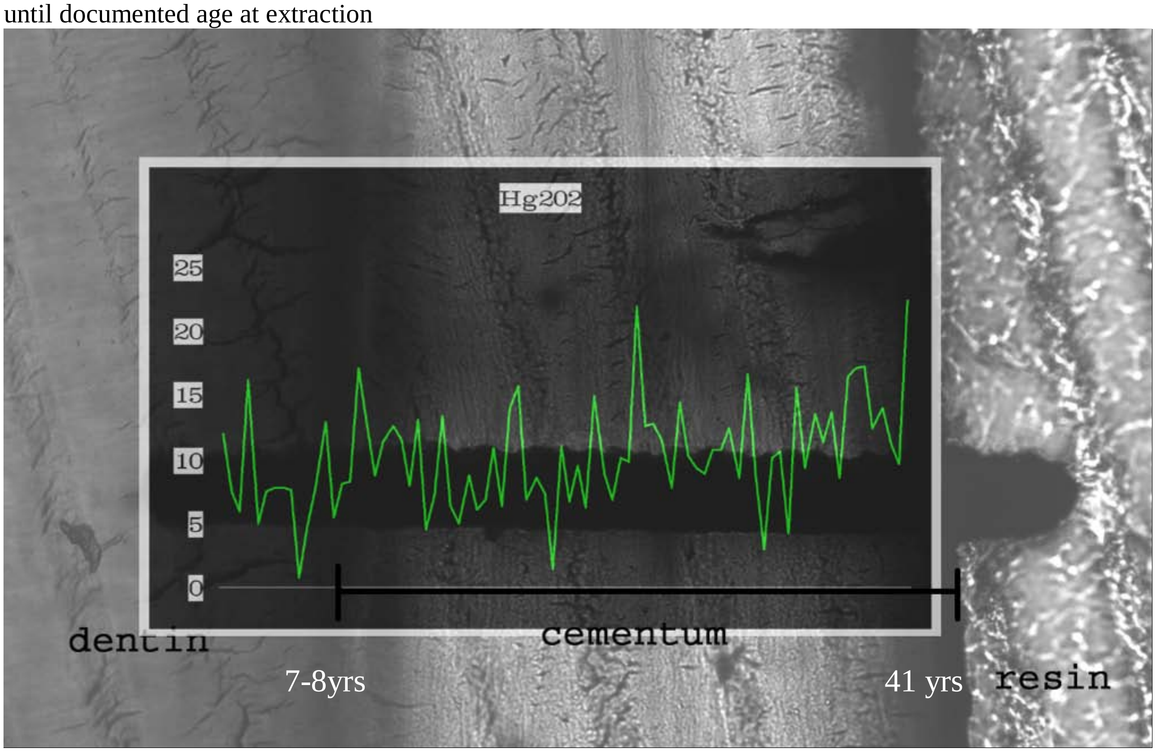


Figure 59 Background levels prior to ablation of tooth 22-6 line2

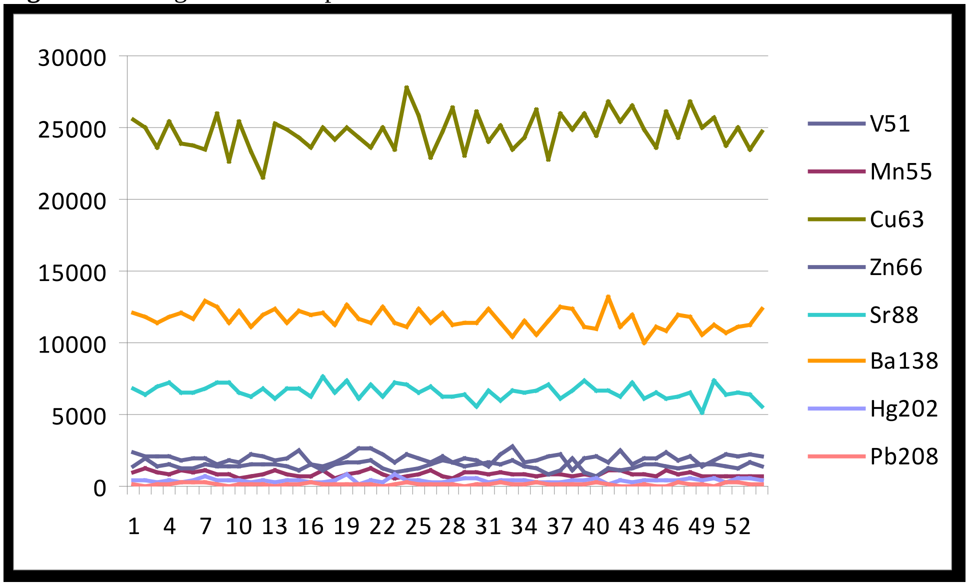


Figure 60 Standardized copper profile of tooth 22-6 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

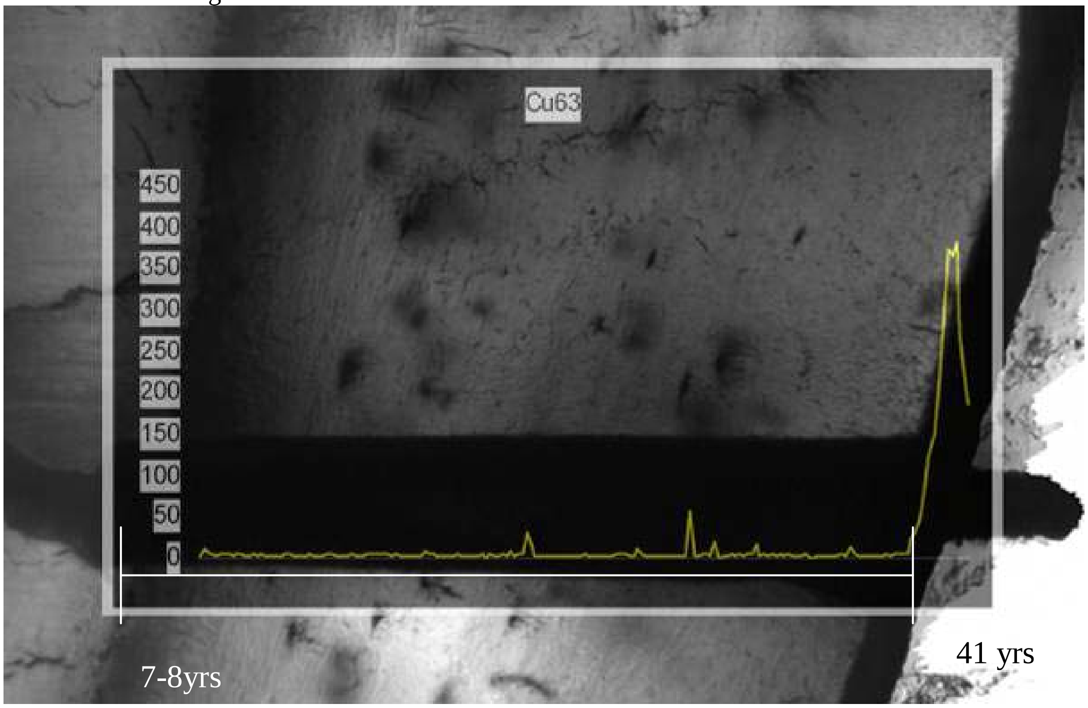


Figure 61 Standardized copper profile of tooth 22-6 line2. X-axis is a proxy for age of tooth completion until documented age at extraction

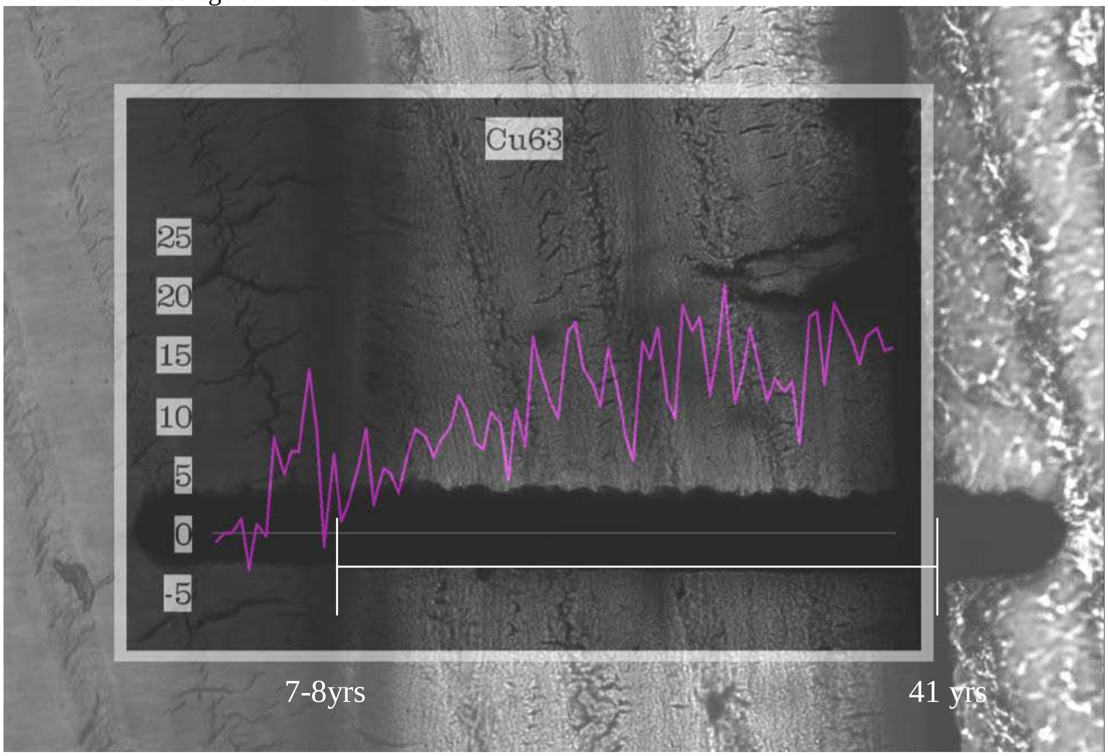
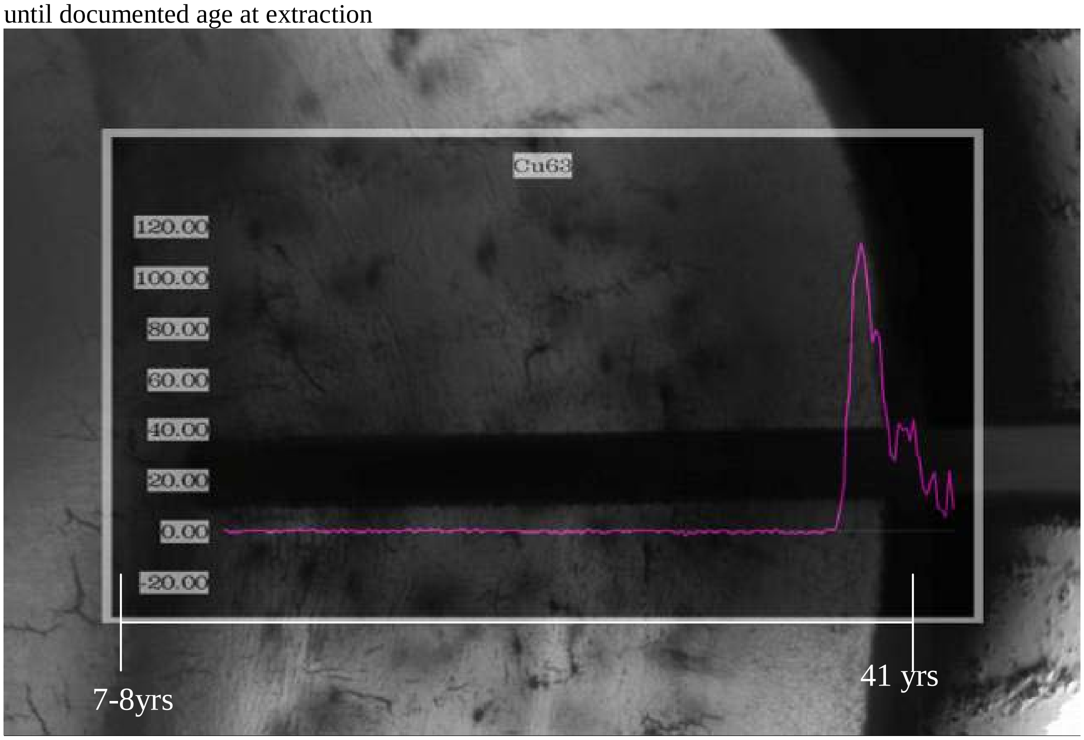


Figure 62 Standardized copper profile of tooth 22-6 line3. X-axis is a proxy for age of tooth completion until documented age at extraction



Copper levels steadily increase throughout the cementum. Line 2 was analysed on a day where analysis often showed high intensities of copper, mainly in background levels. This was due to contamination in the tubing from analysis performed the previous day of a material that contained a high amount of copper. Because of this, copper analysis of line 2 may not be true to the cementum levels. Lines 1 and 3 display constantly low levels of Cu until its usual ‘surface’ peak, suggesting the levels in line 2 are perhaps not real.

22-9

Tooth 22-9 was analysed 4 times, twice on section 5 and twice on section 6 of the tooth. This tooth is the other tooth in the study with an amalgam filling. Raw calcium levels for all 4 analyses were fairly consistent, except for the outer 1/3 of line 1 on section 6 (22-96). Calcium levels drop twice.

Lines 1 and 2 on section 5 (22-95 and 22-92, respectively) are on different sides of the tooth, both in cellular areas. Pb, Zn and Ba follow the similar patterns, though the lead and zinc levels are more ‘variable’ in line 1 than they are in line 2 (Figure 63,Figure 64, and Figure 65). The area of line 1 contains more cementum, though the analysis was performed at 4µm/s, while line 2 was 2µm/s. Faster laser speed usually causes profiles to be less erratic, not more detailed. One cemental difference which could possibly explain the changes that occur in line one and not line 2, could be the difference in cellularity. The decrease in lead and zinc levels in line 1 corresponds to an area of darker, more cellular cementum that does not appear in the area of line 2. The cementocytes may be lower the amount of elements being deposited, lessening the intensity. Levels are not necessarily higher in acellular areas, but may reflect element levels more accurately.

Figure 63 Standardized lead and zinc profiles of tooth 22-9 section5 line1. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

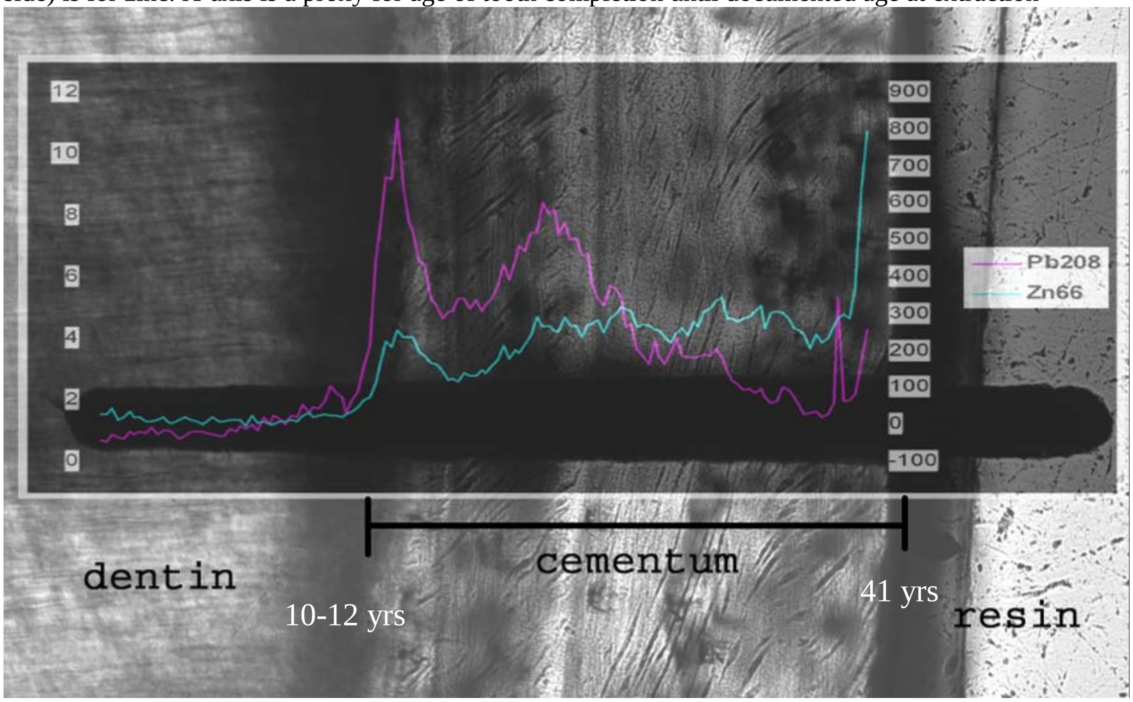


Figure 64 Standardized barium profile of tooth 22-9 section5 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

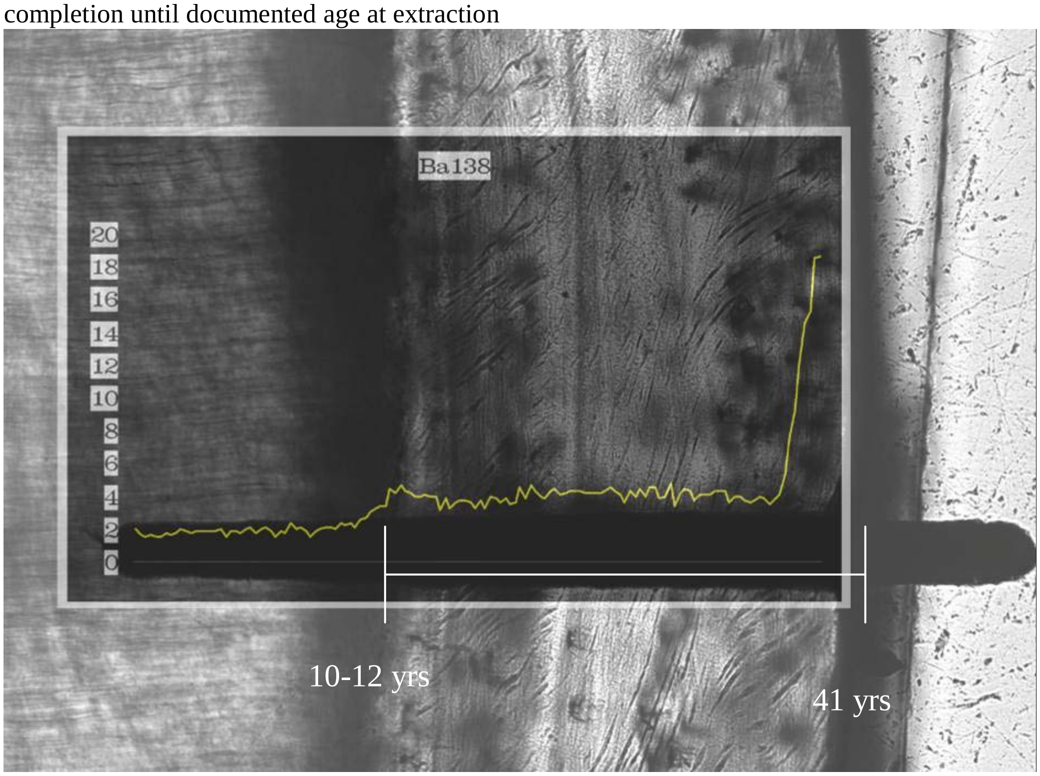


Figure 65 Standardized lead, zinc and barium profiles profile of tooth 22-9 section5 line2. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

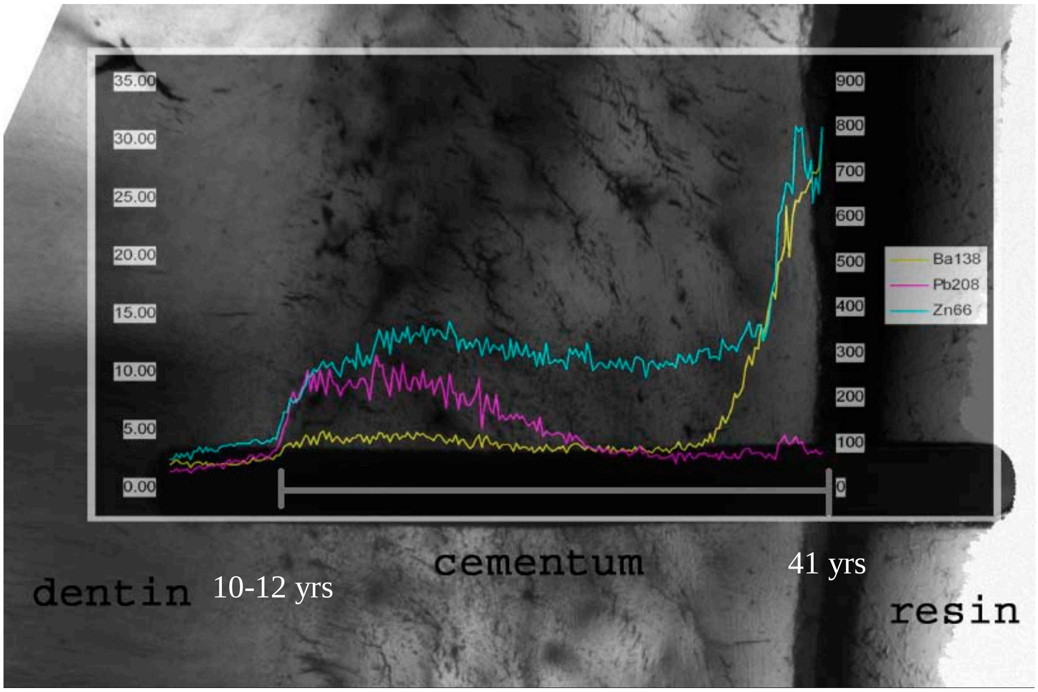
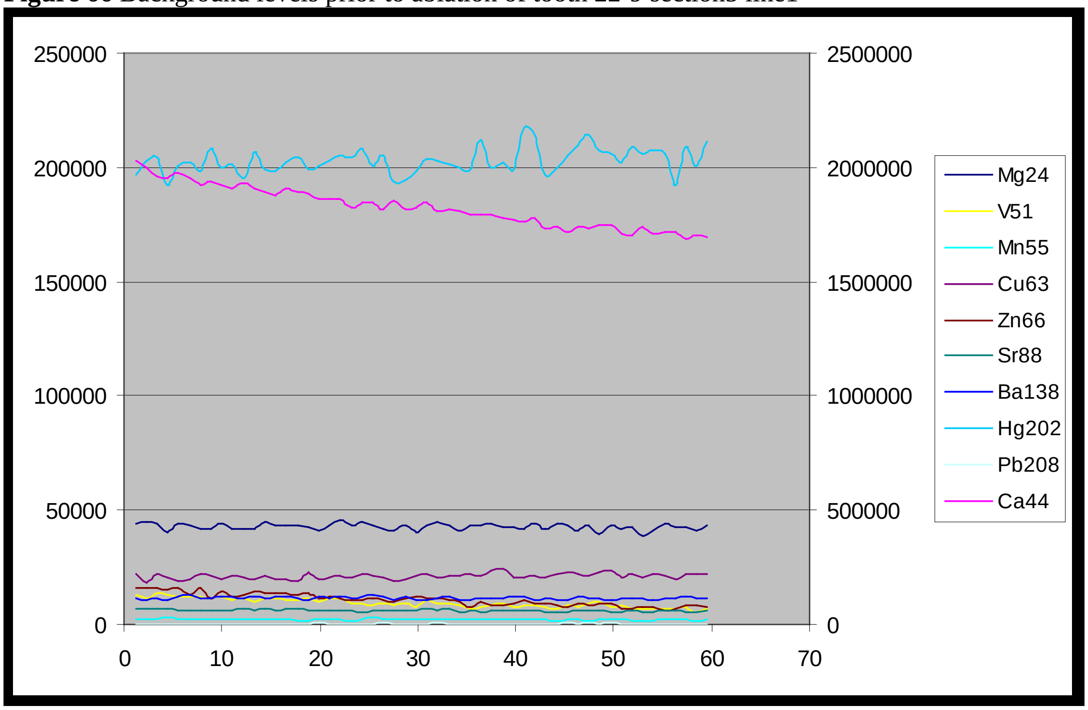


Figure 66 Background levels prior to ablation of tooth 22-9 section5 line1



The background mercury levels were very high due to the vapours being emitted from the section itself (Figure 66 and Figure 67). The Hg levels detected in the tooth were also high (Figure 68). The Hg profile in line 2 is unlike that of line 1 (Figure 69). There is a constant increase across cementum, but relatively constant levels in dentin. This pattern could indicate an infusion of Hg through cementum, from the exterior towards the CDJ. Raw levels show the same consistent increase towards the exterior of the cementum, and then a slow decrease as the laser exited the cementum into the resin.

Lines 1 (22-96) and 2 (22-93) on section 6 were ablated on the same side of the root, the same side as line 1 on section 5. Pb, Zn and Ba run along similar pattern and reflect the same profile as those in 22-95 (Figure 69). The profile of line 2 does appear slightly different, with these three elements peaking before the CDJ shown on the surface (Figure 70). The CDJ is not as defined as it is in the area of line 1, and there is a darker area in the corresponding dentin. The CDJ may angle in under the dentin and becomes ablated, or there is a pocket of reparative cementum under the dentin. Either way, line 2 shows the same profile pattern as line 1. The decrease in levels for Pb, Zn and Ba occurs in an area of higher cellularity in line 1. The area of line 2 does not show the same pattern in density of cementocytes.

Mercury levels were again high, in background and calculated levels. The two lines showed fairly opposite patterns. Line 1 showed a decrease in levels, while line 2 showed a consistent rise in levels after the CDJ, with a sudden large peak at the outer edge of cementum. This large edge peak could be contamination. It has already been shown that the amalgam filling gives off Hg vapours. The distilled water the tooth was stored in could have been contaminated with Hg from the filling, which the root could

Figure 67 Background levels prior to ablation of tooth 22-9 section5 line2

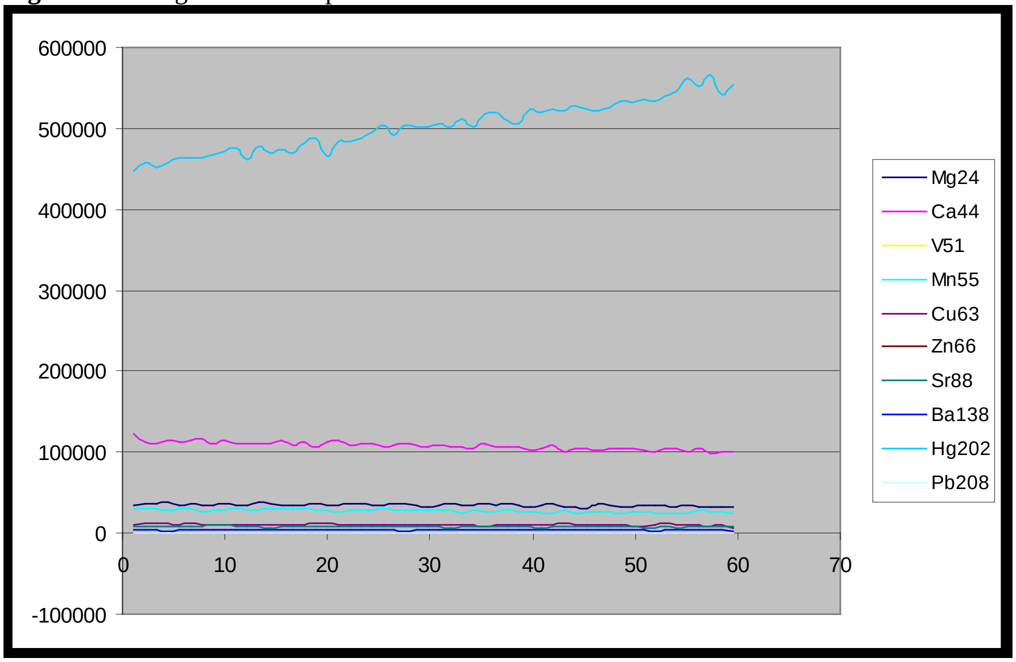


Figure 68 Standardized mercury profile of tooth 22-9 section5 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

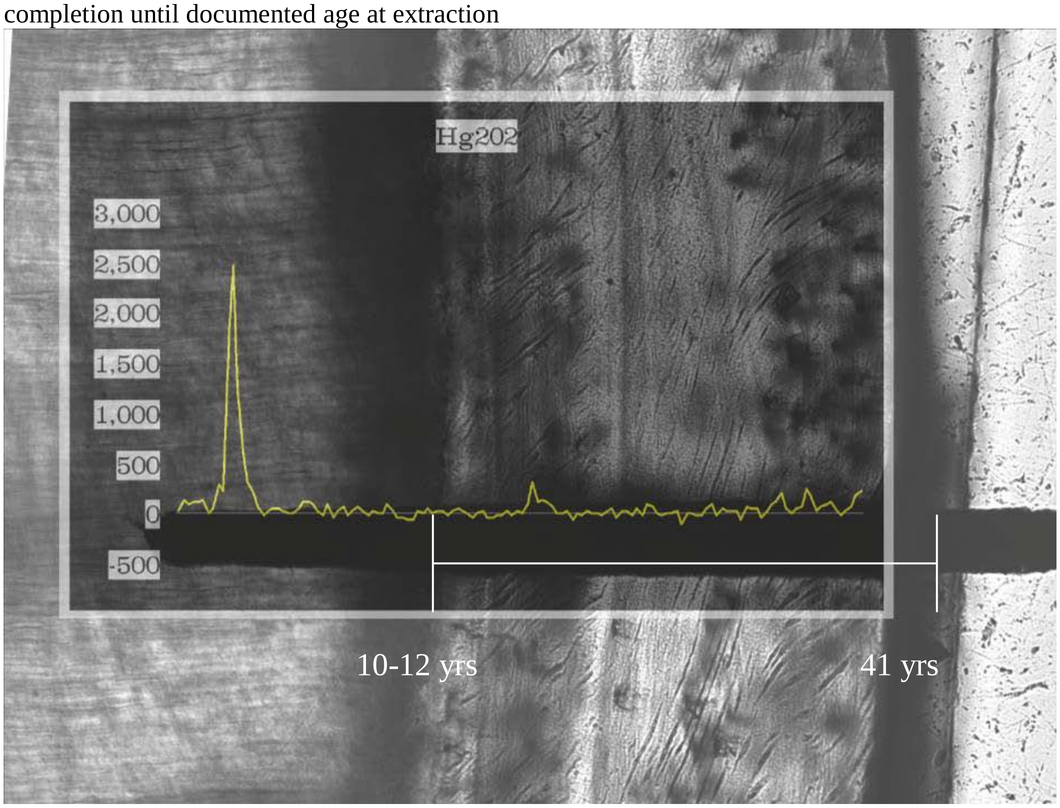


Figure 69 Standardized mercury profile of tooth 22-9 section5 line2. X-axis is a proxy for age of tooth completion until documented age at extraction

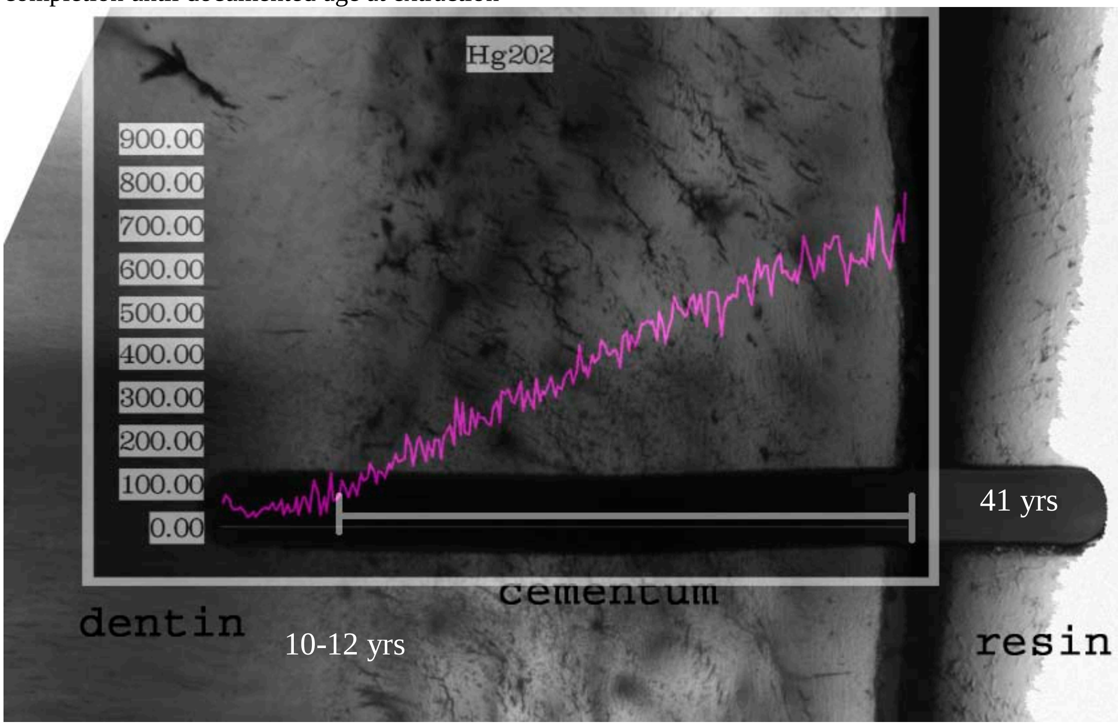
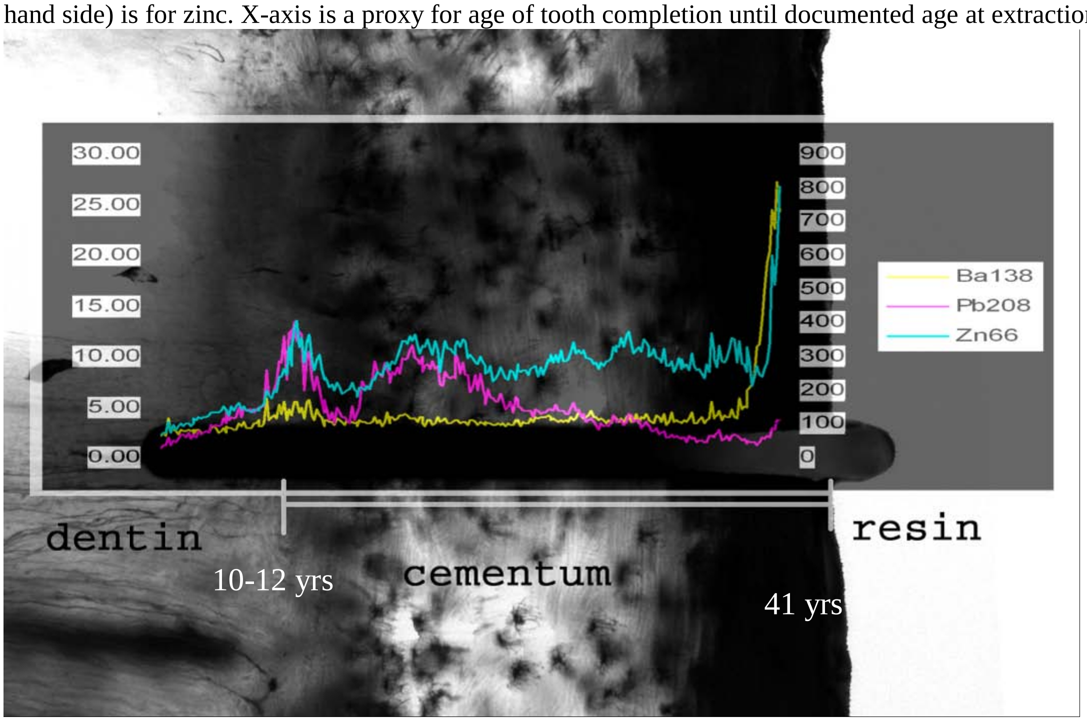


Figure 70 Standardized lead, zinc and barium profiles of tooth 22-9 section6 line2. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction



have taken up on the very exterior of the cementum. The sudden peak in line 1 can be seen in the raw levels.

Copper profiles are different between lines 1 and 2 (Figure 71 andFigure 72). In line 2, the copper follows the same general profile that is found in other teeth, extremely low levels through most of the cementum, with a sudden outer edge peak. Line 1 shows increasing copper levels throughout cementum. This pattern was somehow created during the normalization of calcium and the taking into consideration the background levels. The raw copper profile is very flat until the usual end peak.

Calcium levels through the first line drops twice in the outer third (Figure 73). The first drop coincides with the sudden lack of cementocytes. This is most likely a coincident. The second drop occurs near the outer edge, which is difficult to pin point due to a large dark outer edge.

24

Individual 24 is represented by 2 teeth (24-2 and 24-3). Tooth 24-2 was analysed 3 times, lines 1 and 3 are on the same side. The tooth was sectioned labial-lingually, and shows enough cellular cementum on either side of the root to be lasered. Tooth 24-3 was also analysed 3 times, again with lines 1 and 3 occurring on the same side. This tooth, however did not have much cementum on the side of line 2 (tooth was sectioned mesial-distally).

24-2

Line 1 (24-2) showed some irregularities in raw calcium levels (Figure 75). Levels increase, stabilize somewhat, then peaks slightly near the edge. The area of line 1

Figure 71 Standardized copper profile of tooth 22-9 section6 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

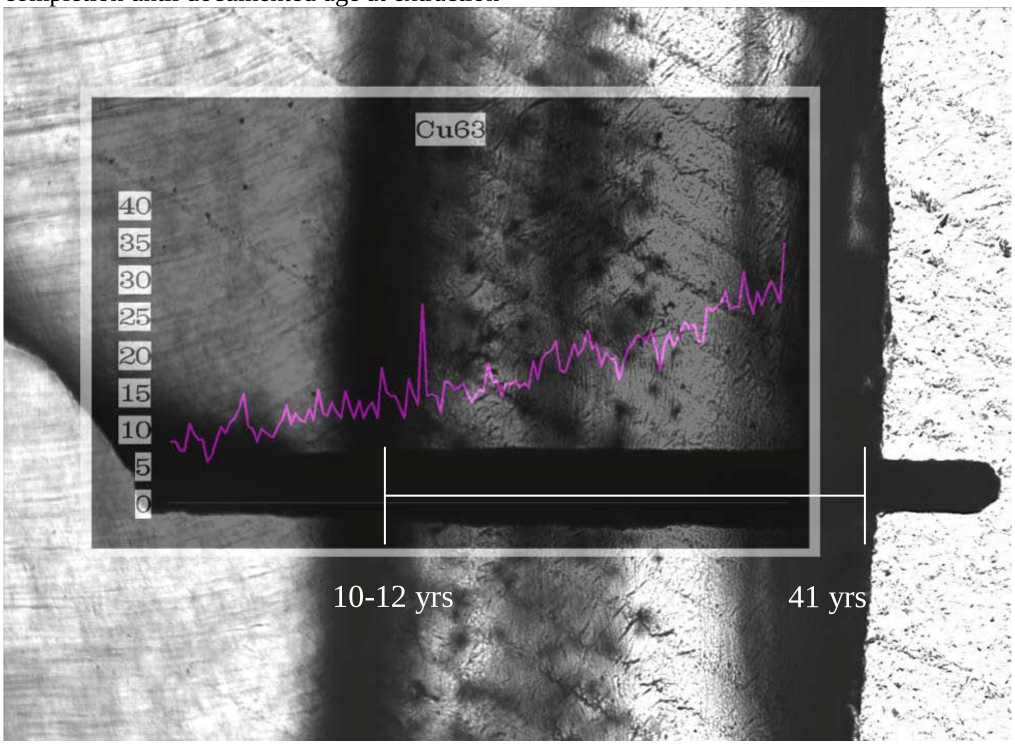


Figure 72 Standardized copper profile of tooth 22-9 section6 line2. X-axis is a proxy for age of tooth completion until documented age at extraction

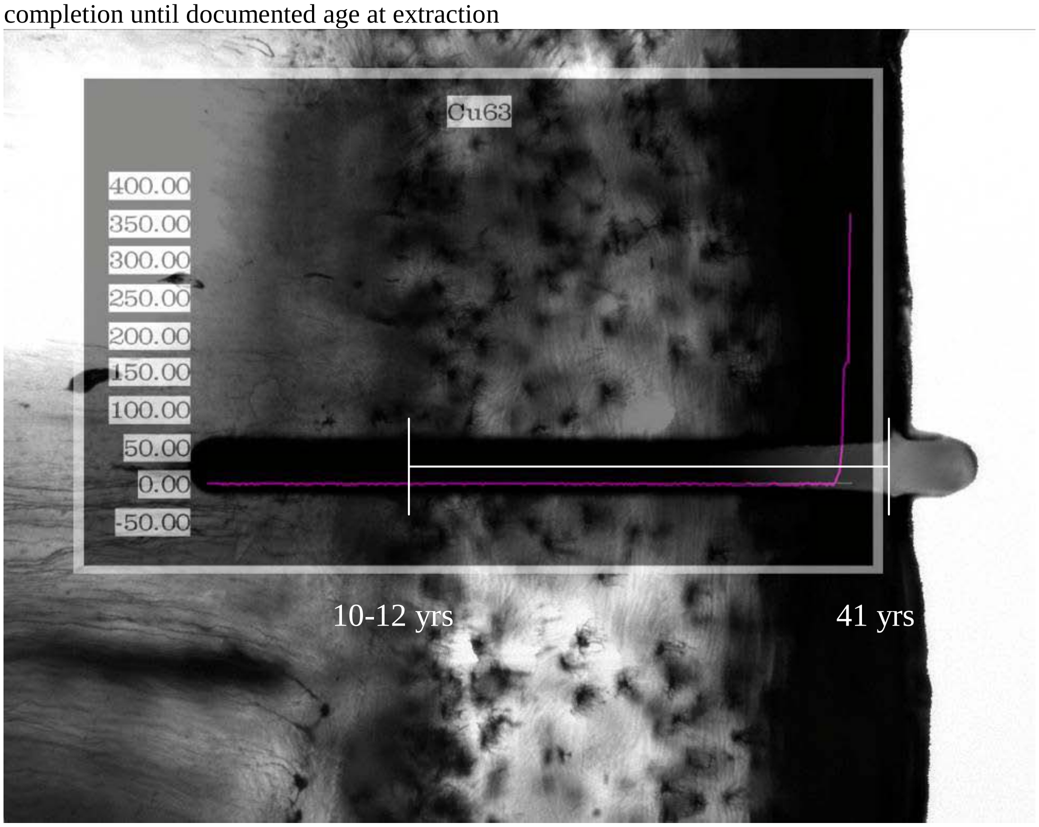


Figure 73 Raw calcium profile of tooth 22-9 section6 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

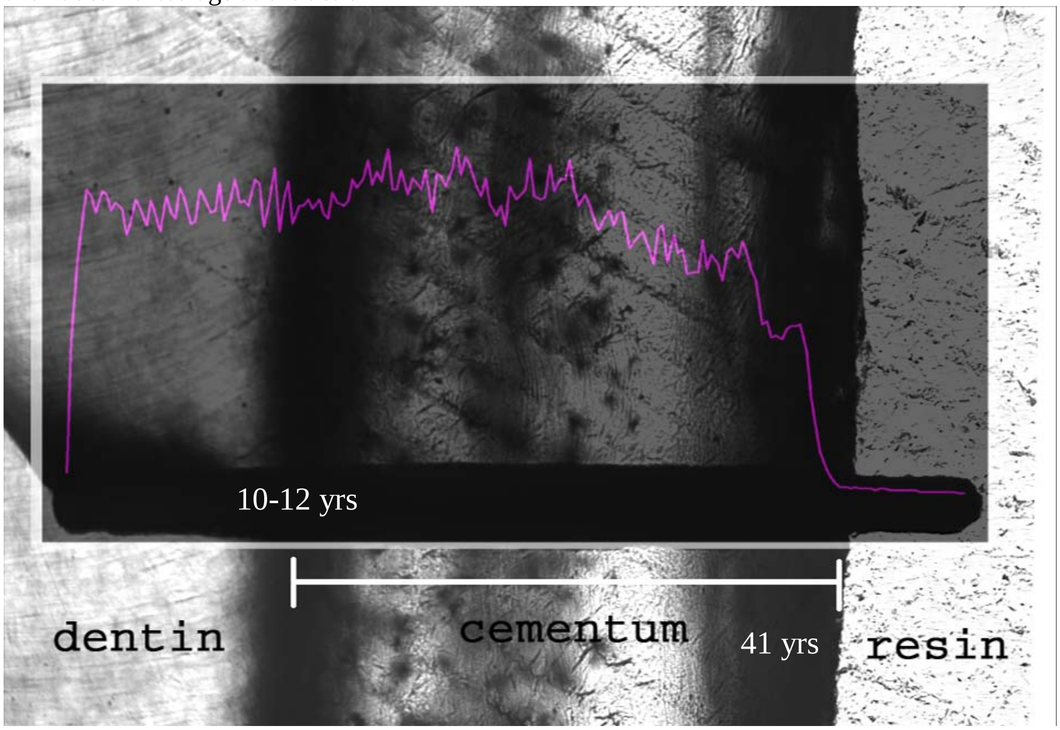


Figure 74 Raw calcium profile of tooth 22-9 section6 line2. X-axis is a proxy for age of tooth completion until documented age at extraction

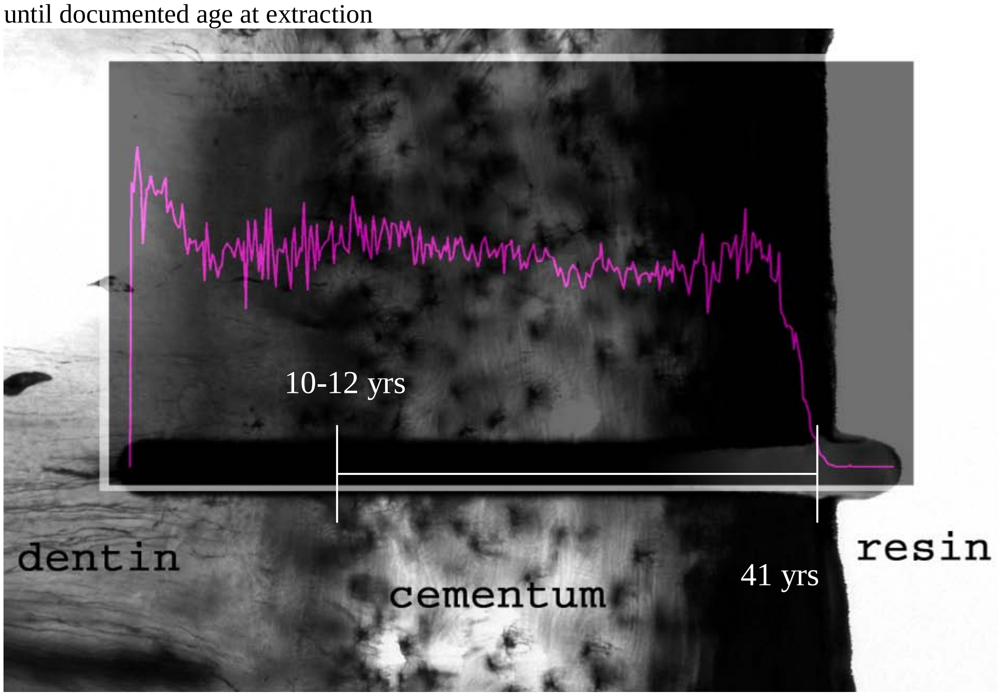


Figure 75 Raw calcium profile for tooth 24-2line1. X-axis is a proxy for age of tooth completion until documented age at extraction

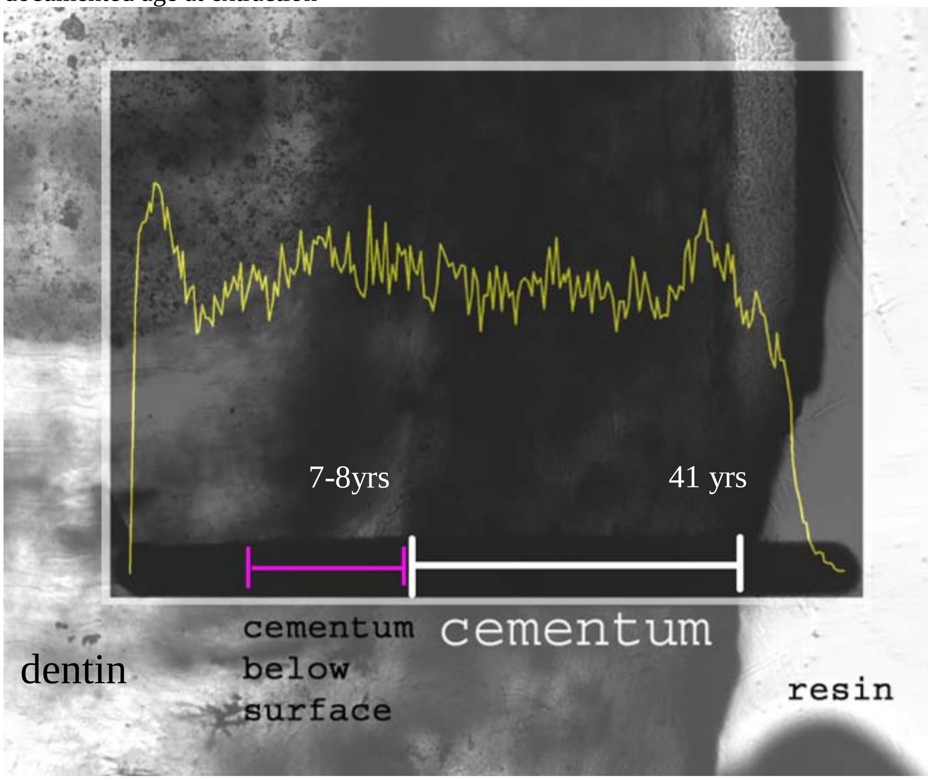
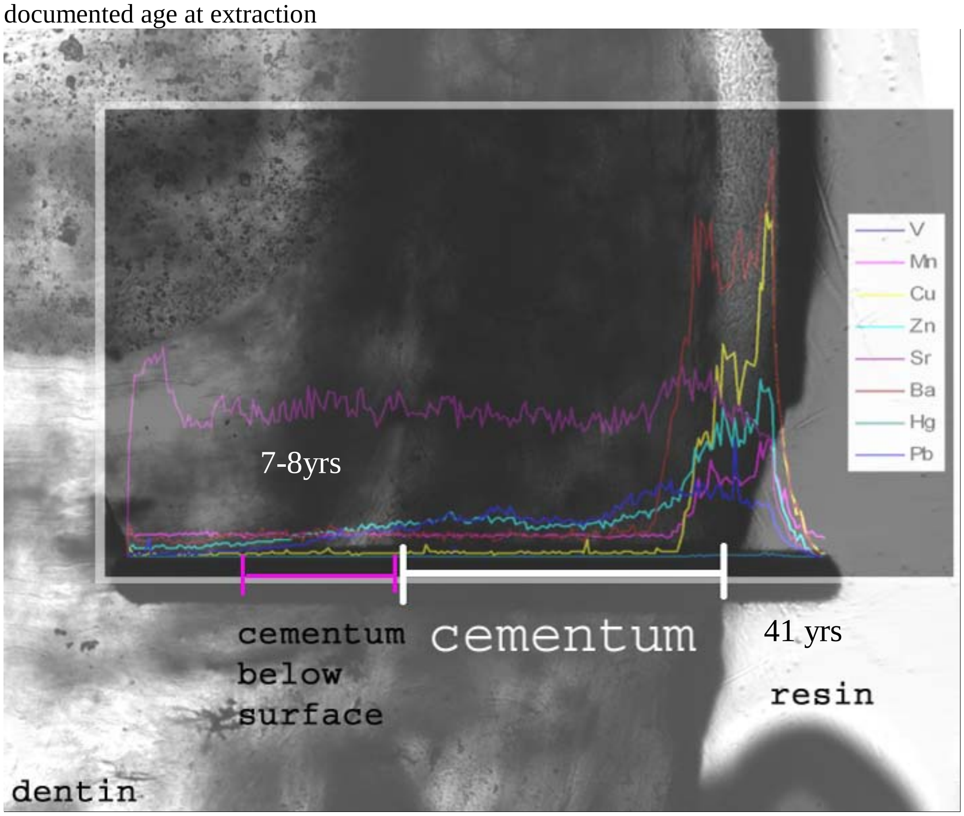


Figure 76 Raw trace element profile for tooth 24-2line1. X-axis is a proxy for age of tooth completion until documented age at extraction



is itself irregular. Upon close inspection, the CDJ appears stepped below the laser line, or the dentin is a bit unusual. Trace element levels gradually increase, making it difficult to denote exactly where cementum begins. The section could be cut on a sharp angle.

The raw levels of the trace elements show a prolonged increased, with no obvious reason why (Figure 76). As usual, Pb, Zn, and Ba follow the same pattern, with a gradual increase from dentin to cementum. Mg levels are stable, slightly increased in cementum but decreases drastically prior to the edge (Figure 77). The Hg levels in the cementum are high, while the background levels were low, implying the high levels are real. However, lines 2 and 3 have low background and calculated Hg levels.

The CDJ for line 2 is more straightforward, and is displayed in the lead and zinc profiles, which show a larger initial peak than in line 1 (Figure 78). Copper profile contains a pattern, but the background levels were high and falling due to copper being in the system from the previous analysis already mentioned.

Pb appears somewhat different from Zn (Figure 78), though this difference mainly involves the lack of increase near the outer edge. There is a small decrease in both lead and zinc that occurs in the same spot. The laser path is too large to see what would have caused this small decrease. The pattern of Pb and Zn are from line 2 differ from those in line 1. The Ba of lines 1 and 2 are more similar (Figure 78 and Figure 79).

Line 3 is near to line 1. From farther out, the CDJ appears fairly obvious, but upon close-up examination, it becomes more difficult to pinpoint. The lead levels (Figure 79) increase in the area that appears to be the CDJ (from farther out). There is a dark line in the cementum, parallel to the root surface, which may be a crack. There is a decrease in Pb, Zn, and Hg (Figure 79) that coincides with this line, which could be sheer coincidence. This decrease does not show up in raw calcium levels. Calcium does

Figure 77 Standardized magnesium profile for tooth 24-2line1. X-axis is a proxy for age of tooth completion until documented age at extraction

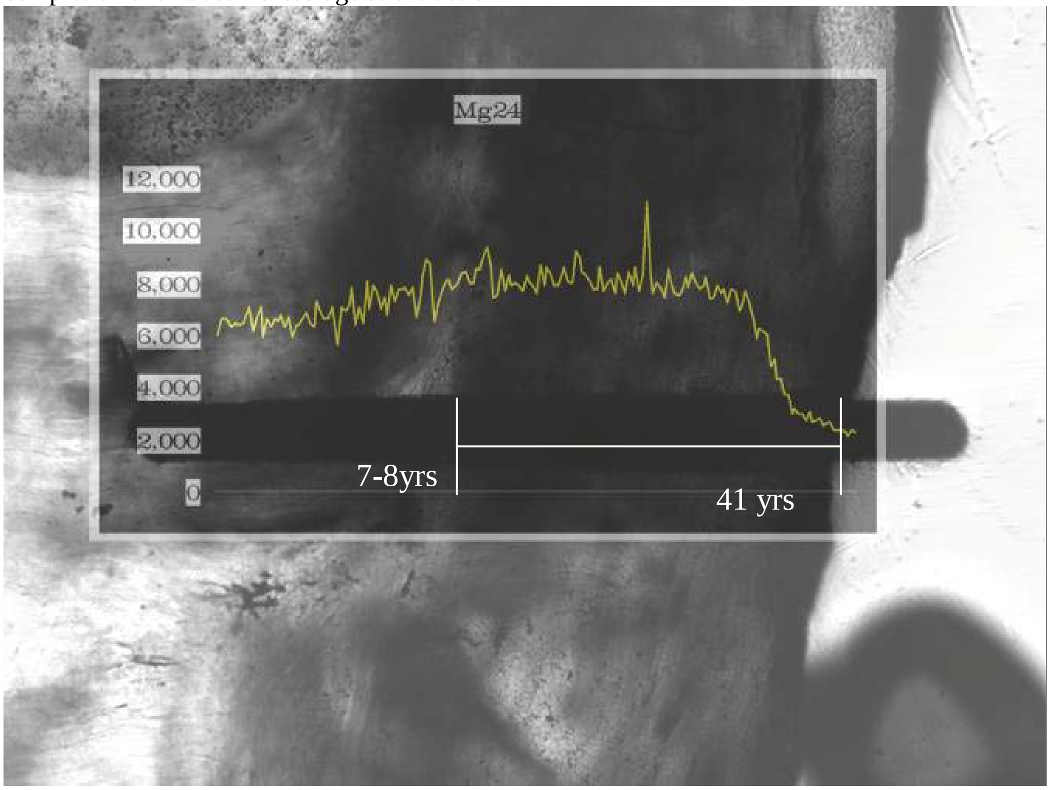


Figure 78 Standardized lead and zinc profiles for 24-2 line 1. X-axis is a proxy for age of tooth completion until documented age at extraction

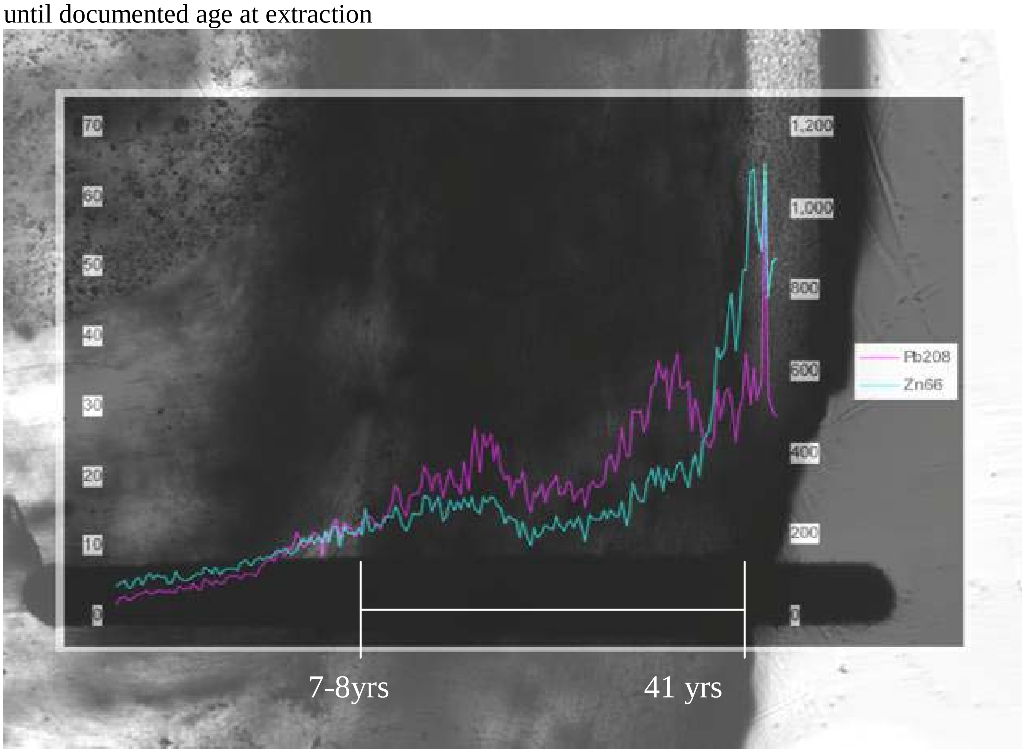


Figure 79 Standardized lead, zinc and barium profiles for tooth 24-2line2. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

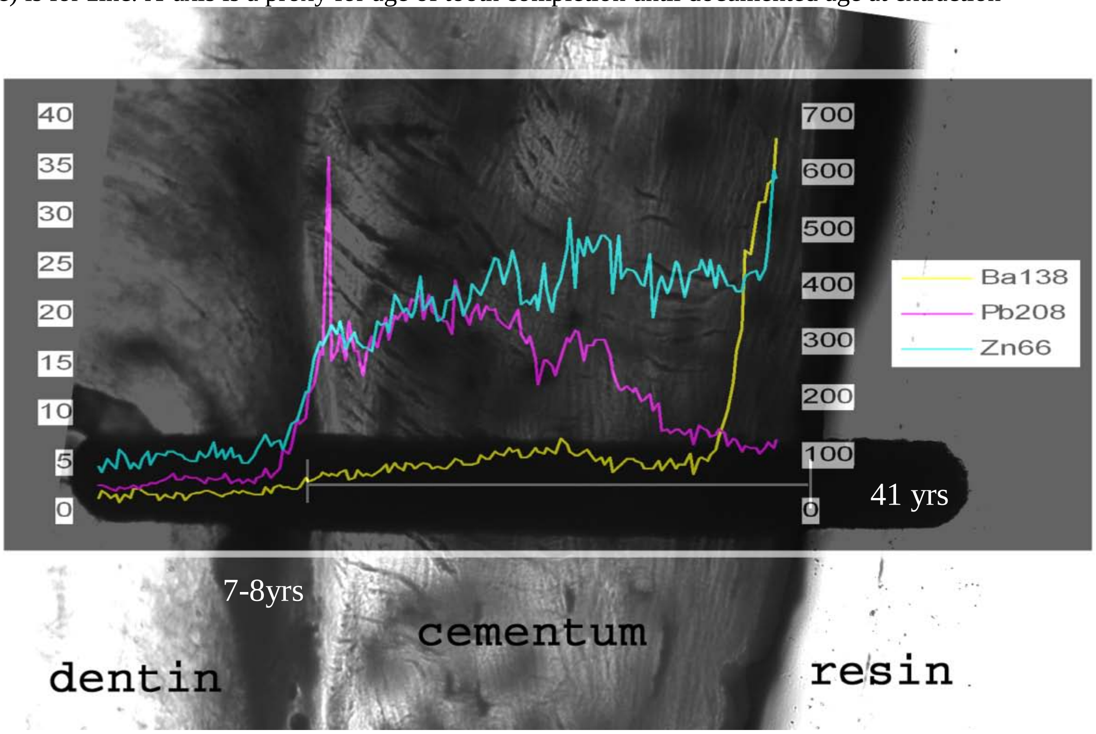
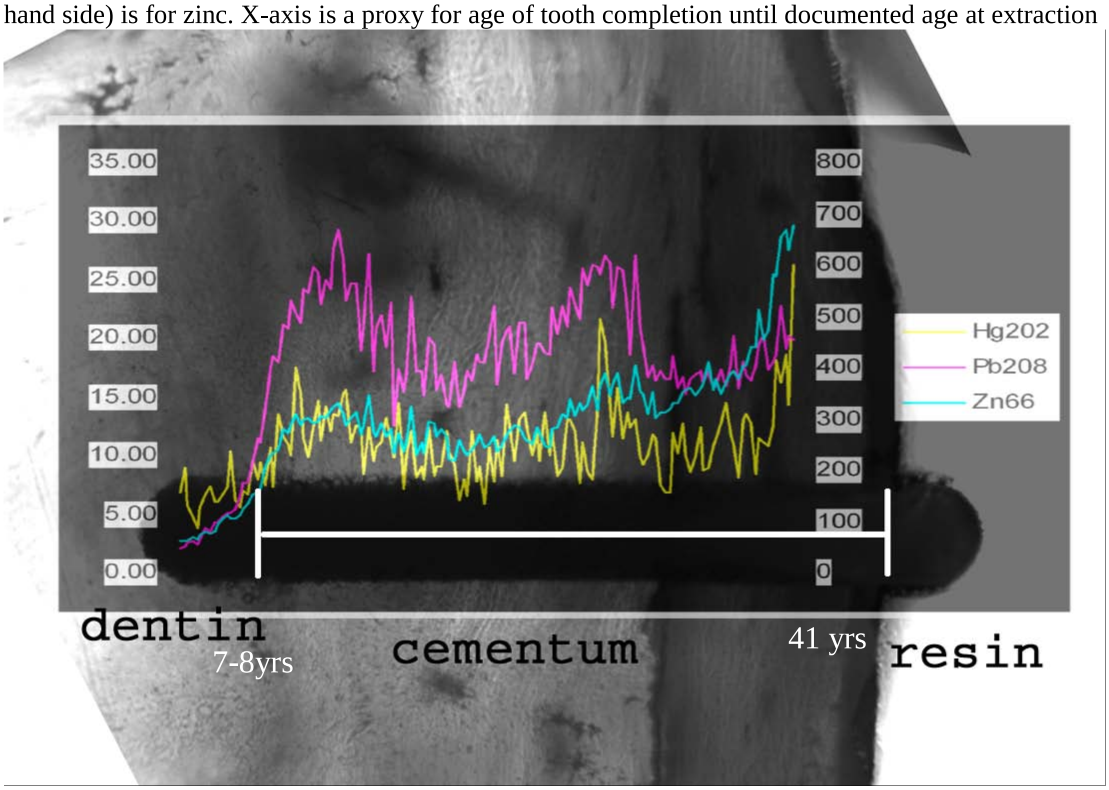


Figure 80 Standardized lead, zinc and mercury profiles profile for tooth 24-2line3. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction



increase over an area of slightly darker cementum (Figure 80). This could also be coincidence.

The profiles of Pb and Zn in line 3 follow the same pattern as each other. They are also similar to those of line 1, which is not surprising since the lines are not that far apart. Since Hg levels are not as high as they are in line 1, more of a pattern emerges, matching those of Pb and Zn.

24-3

Line 1 shows a large increase right after the CDJ in Pb, Zn and Hg (Figure 81 and Figure 82). Ba also shows an increase in the same area. Hg levels are high and erratic, making it difficult to determine how closely it follows Pb and Zn. Pb and Zn decreases and stabilizes over an area of darker cementum. Many other dark areas of darker cementum coincide with an increase in cementocyte concentration; however there is no visible increase in cementocytes on the surface.

Calcium levels are not homogenous (Figure 83). The first decrease occurs at the CDJ, while the next two may be correlated to the darker cemental area. The increase after the CDJ occurs in a lighter, most likely more acellular area. If the darker area of cementum is more cellular, the percentage of inorganic matter could be lower which would cause the calcium to be lower as well.

Line 2 occurs in a more acellular location. Dentin was hardly analysed, and the elemental profiles are not as interesting as those in line 1. Pb, Zn and Hg follow similar patterns (Figure 84), but may be slightly different from those of line 1. The section was cut on an angle, allowing the outer cementum surface below the resin to angle out, causing the broad elevated levels in most of the elements. Raw calcium is much more

Figure 81 Raw calcium profile of tooth 24-2 line3. X-axis is a proxy for age of tooth completion until documented age at extraction

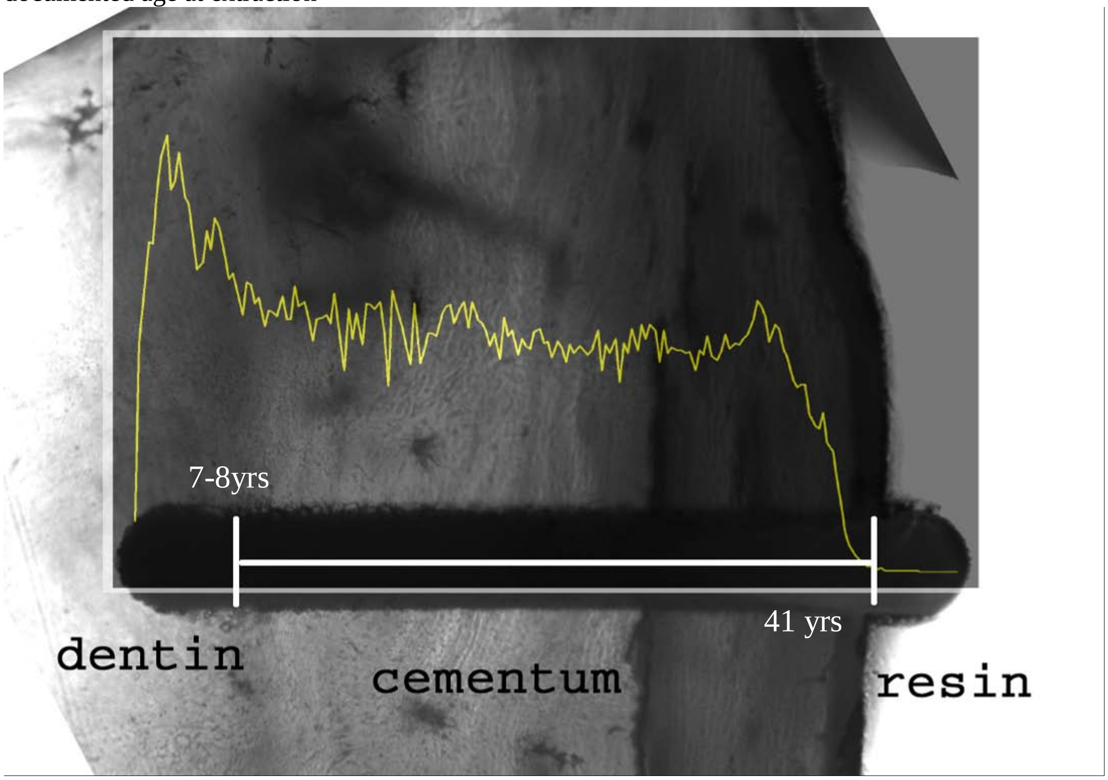


Figure 82 Standardized lead and zinc profiles of tooth 24-3 line1. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

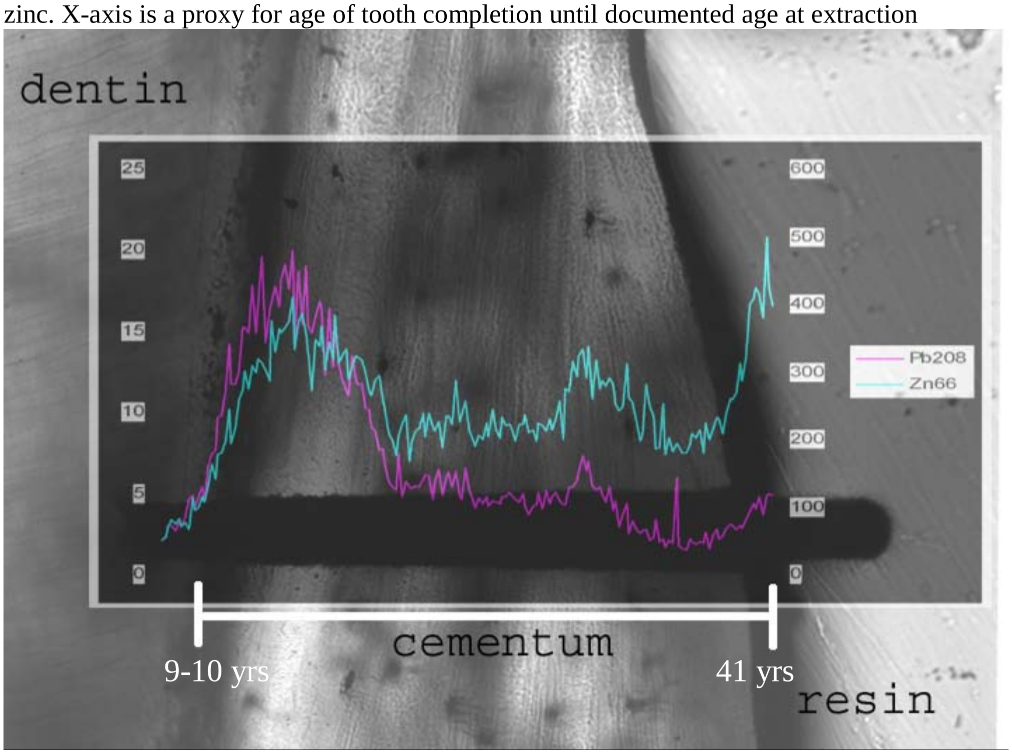


Figure 83 Standardized mercury profile of tooth 24-3 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

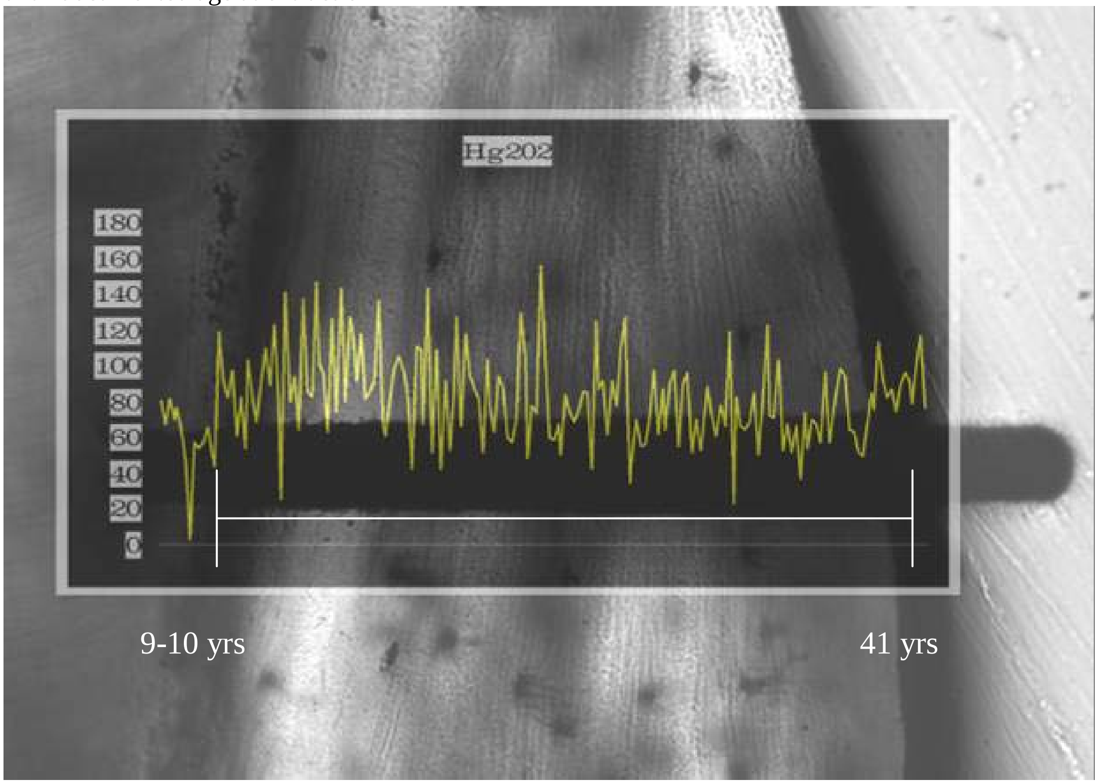
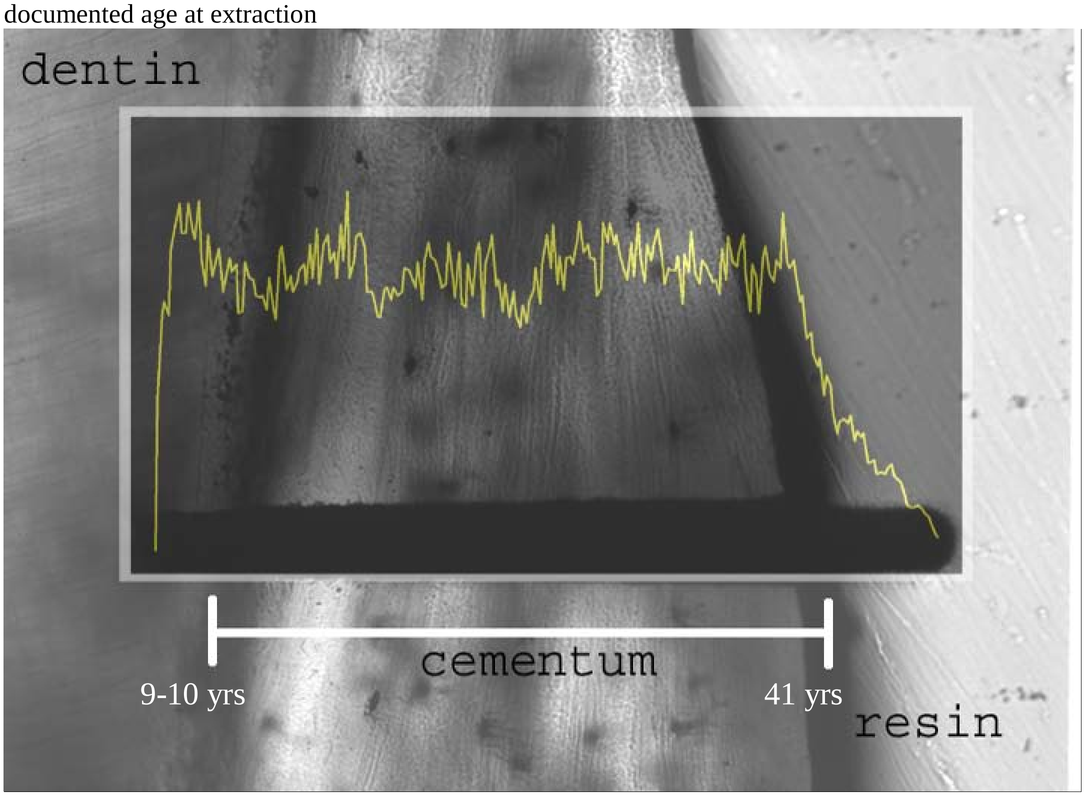


Figure 84 Raw calcium profile of tooth 24-3 line1. X-axis is a proxy for age of tooth completion until documented age at extraction



stable, with a slight increase in the outer third, and a decrease in levels when the exterior of the cementum below the resin is ablated (Figure 85).

Line 3, which is near line 1, appears very similar in Pb, Zn, Hg, and Ba levels (Figure 86 and Figure 87), although there appears to be an extra small peak in the levels in line 3. Raw calcium levels are not homogenous (Figure 88), but do not follow the same pattern as that in line 1. The levels decrease throughout the tissue until near the outer edge where it increases.

Cellularity of the cementum may be a complication for line 3. The highest levels of lead occur over the most acellular area of the cementum. The levels afterwards are quite low in comparison, especially in lead. The rest of the cementum is dark and fairly full of cementocytes.

27

Individual 27 is represented by one tooth (27-2) which was analysed only once. There is a great increase in lead after the CDJ (Figure 89), occurring in a dark cellular area of cementum. This does not follow the hypothesis that cellularity may negatively influence elemental levels. Zn, Hg and Ba follow the same pattern as each other (Figure 90), which increases to differing degrees across the cementum, and coincide with the increasing acellularity of the cementum. It is difficult to tell if lead and zinc are actually different, or if they are similar and that the end segment is different as it usually is. Cementum is too narrow for a more possibly extensive pattern.

The surface peaks occur prior to the laser reaching the edge of the cementum. There appears to be an increase in the width of the laser path, which may be the reason for this earlier peak. The raw calcium levels also peak in the same area (Figure 91). The

Figure 85 Standardized lead, zinc and mercury profiles profile of tooth 24-3 line2. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

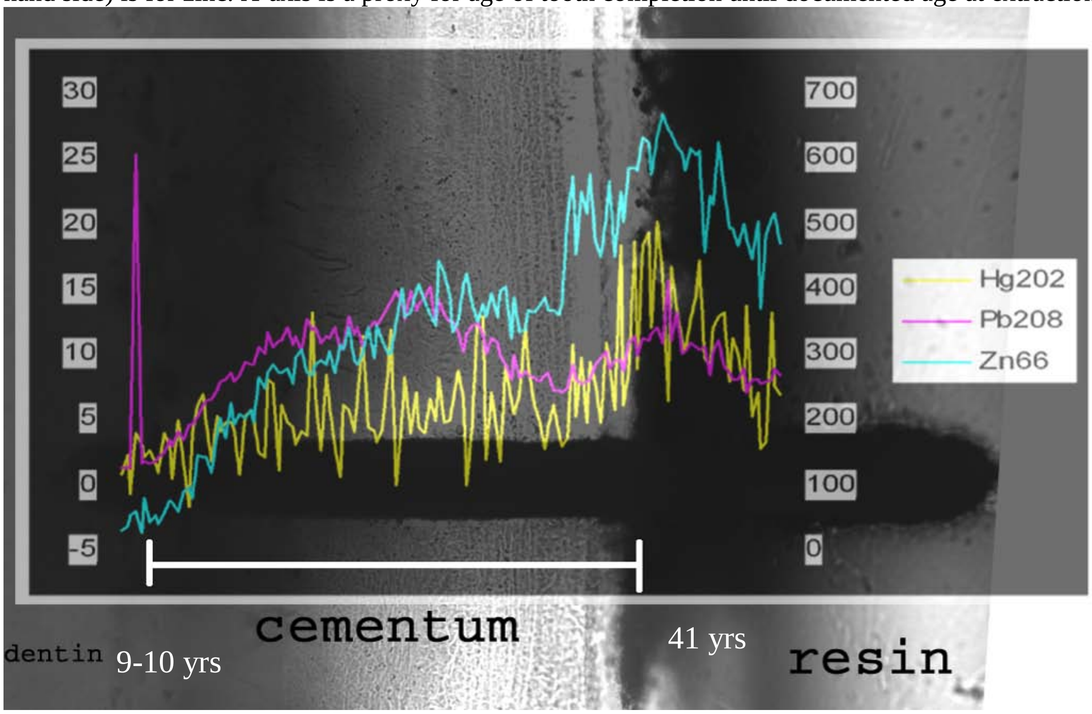


Figure 86 Raw calcium profile of tooth 24-3 line2. X-axis is a proxy for age of tooth completion until documented age at extraction

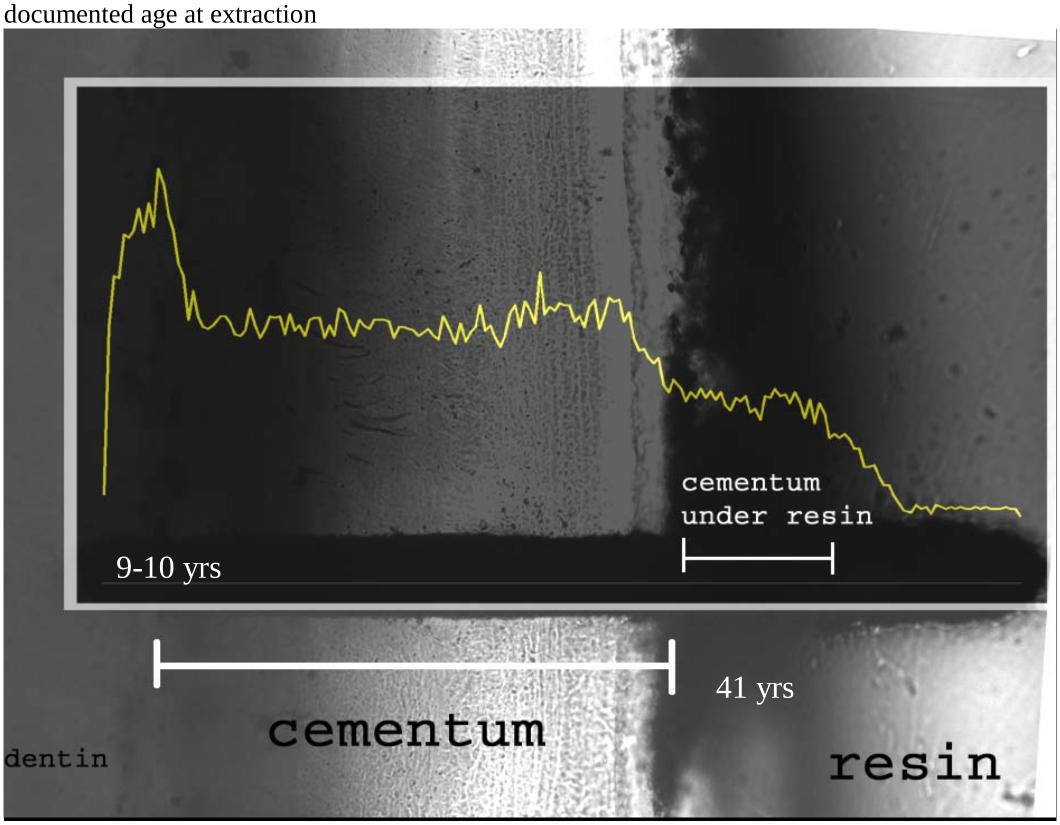


Figure 87 Standardized lead, zinc and mercury profiles profile of tooth 24-3 line3. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

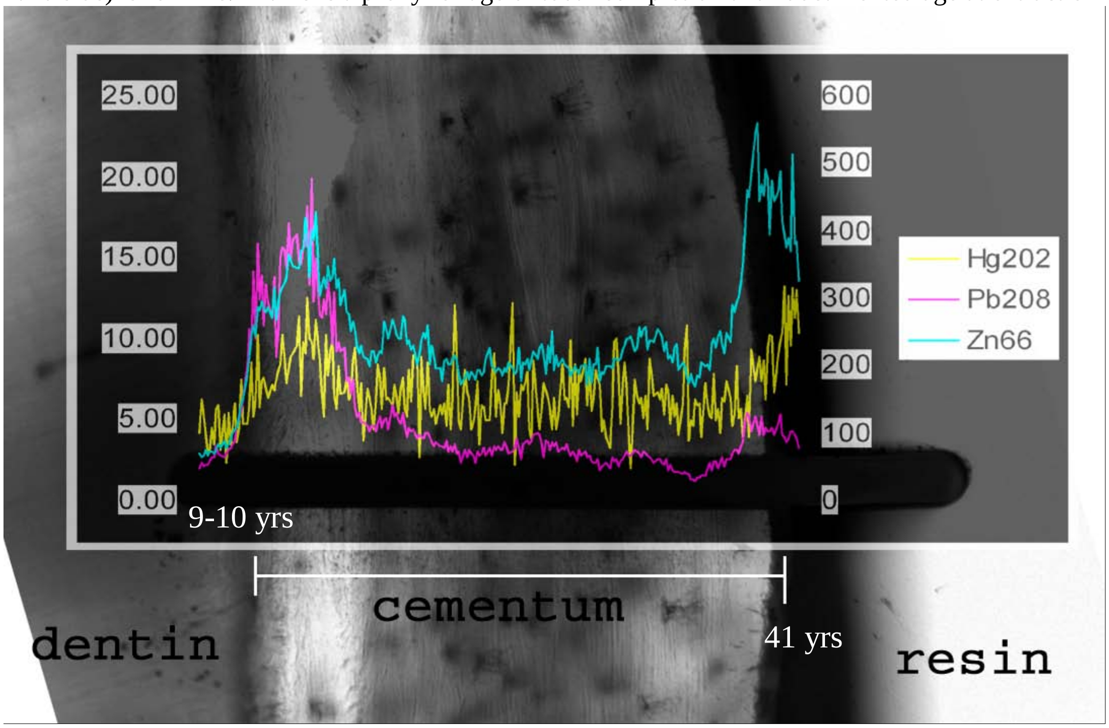


Figure 88 Standardized barium profile of tooth 24-3 line3. X-axis is a proxy for age of tooth completion until documented age at extraction

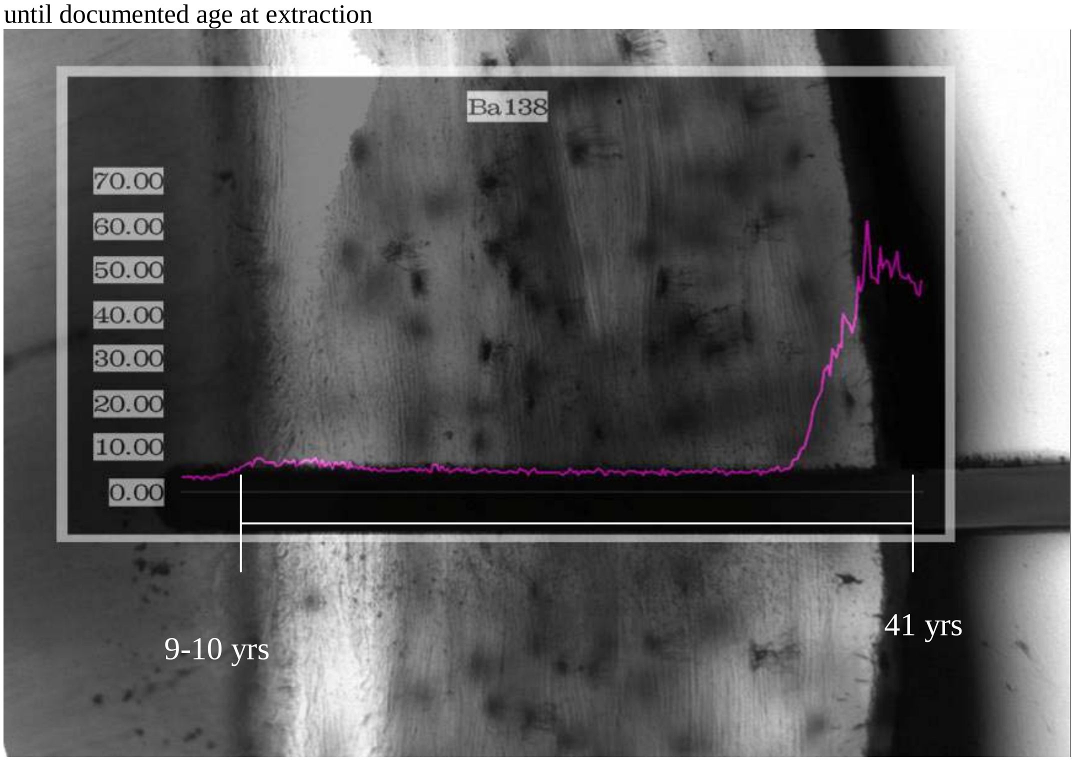


Figure 89 Raw calcium profile of tooth 24-3 line3. X-axis is a proxy for age of tooth completion until documented age at extraction

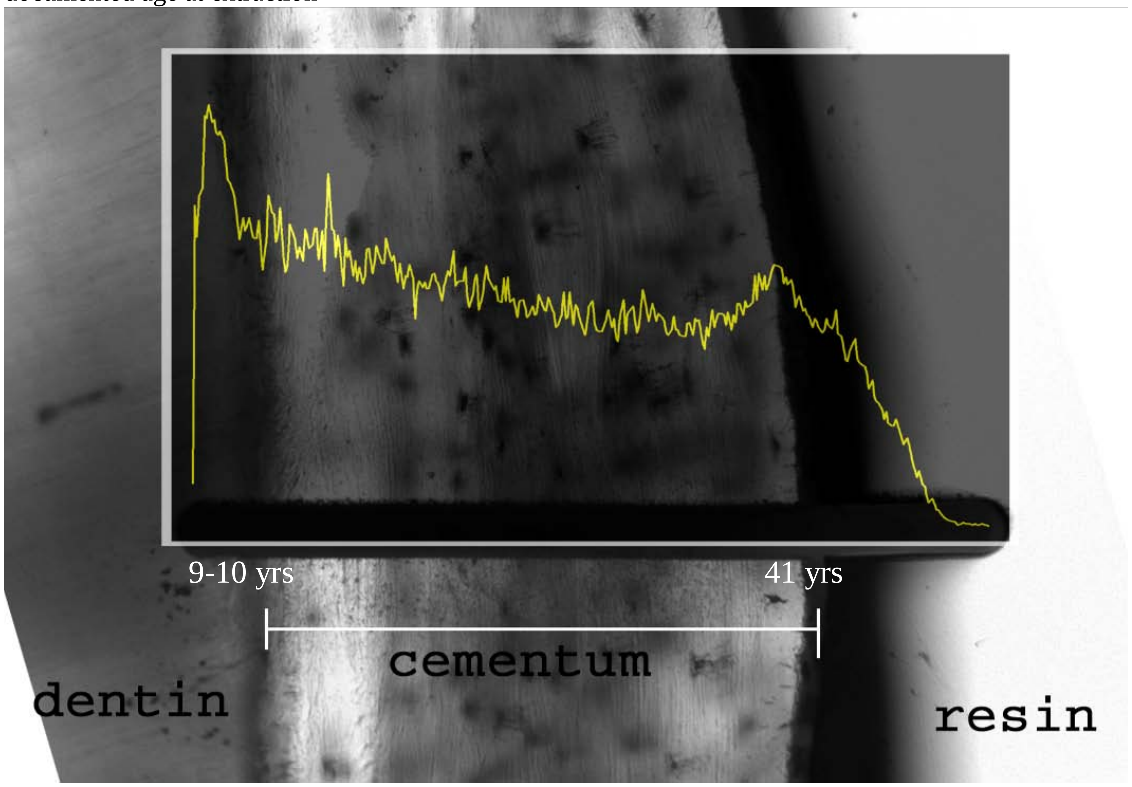
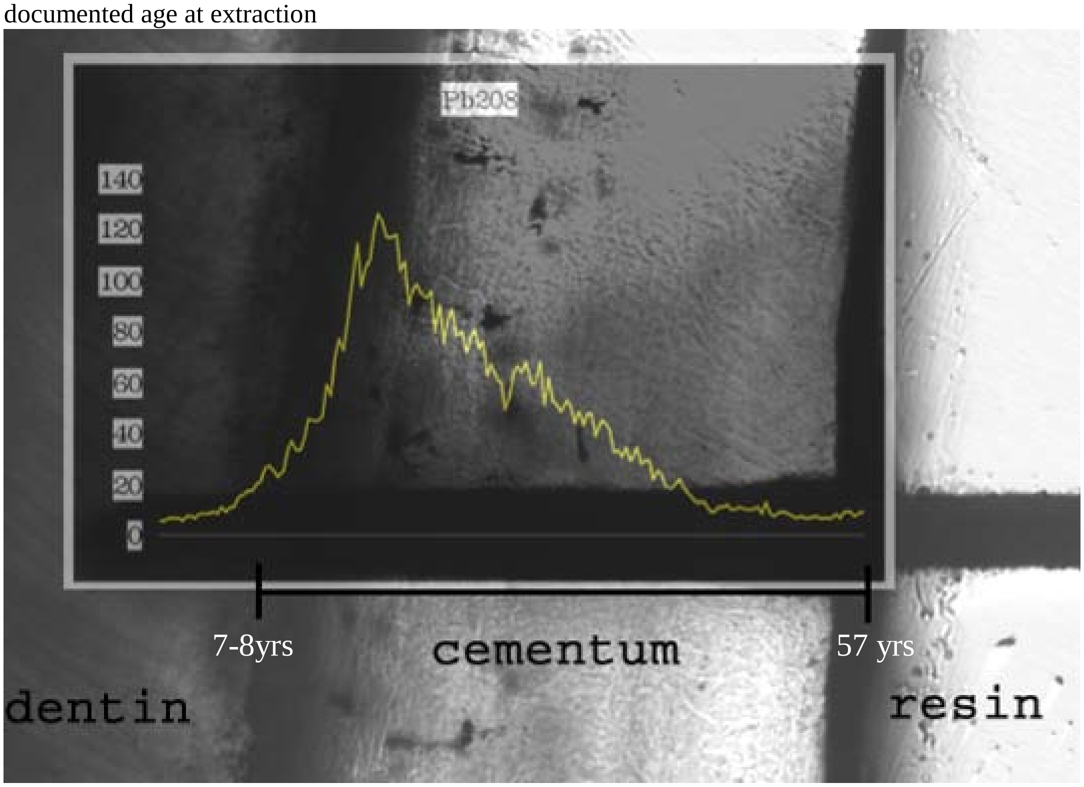


Figure 90 Standardized lead profile of tooth 27-2. X-axis is a proxy for age of tooth completion until documented age at extraction



levels in the trace elements were elevated enough not to be erased during the normalization of calcium levels. The raw calcium profile also shows a lull in levels prior to the end increase, with no ultrastructural reason.

There is a double peak in surface levels in some of the trace elements, just like in 22-2. Double peak occurs in the raw levels, so it is not an anomaly from Ca normalization.

Levels of lead and mercury are high. The tooth did not show any signs of caries upon inspection. The deposition of these higher levels could be from high exposure during the life of the individual, and not through absorption through exposed dentin.

28

Two teeth represent individual 28 (28-2 and 28-4). Each tooth was analyzed once. Both teeth have low levels of Hg, and both have composite fillings. The element profiles of both teeth are fairly boring, with only one lead peak. 28-2 was sectioned longitudinally, while 28-4 was cross sectioned, and lasered in the area of the most cementum.

Tooth 28-2 shows Pb and Zn (Figure 93) increasing at the same point, but changing after that. Zn increases greatly near the edge, as is its custom, and creates an apparent difference between it and lead. Zn, Ba, and Hg (Figure 93and Figure 94), to some extent, follow similar patterns. There is a streak of darker cementum that potentially accompanies the slight increase in strontium. Raw Ca levels are stable across cementum, though more so over the lighter, more acellular area. Raw calcium levels for tooth 28-4 are almost as homogenous as in tooth 28-2 (Figure 95 and Figure 96). The calcium does show an increase in levels at the outer edge.

Figure 91 Standardized zinc, barium and mercury profiles profile of tooth 27-2. Secondary y-axis (right-hand side) is for barium. X-axis is a proxy for age of tooth completion until documented age at extraction

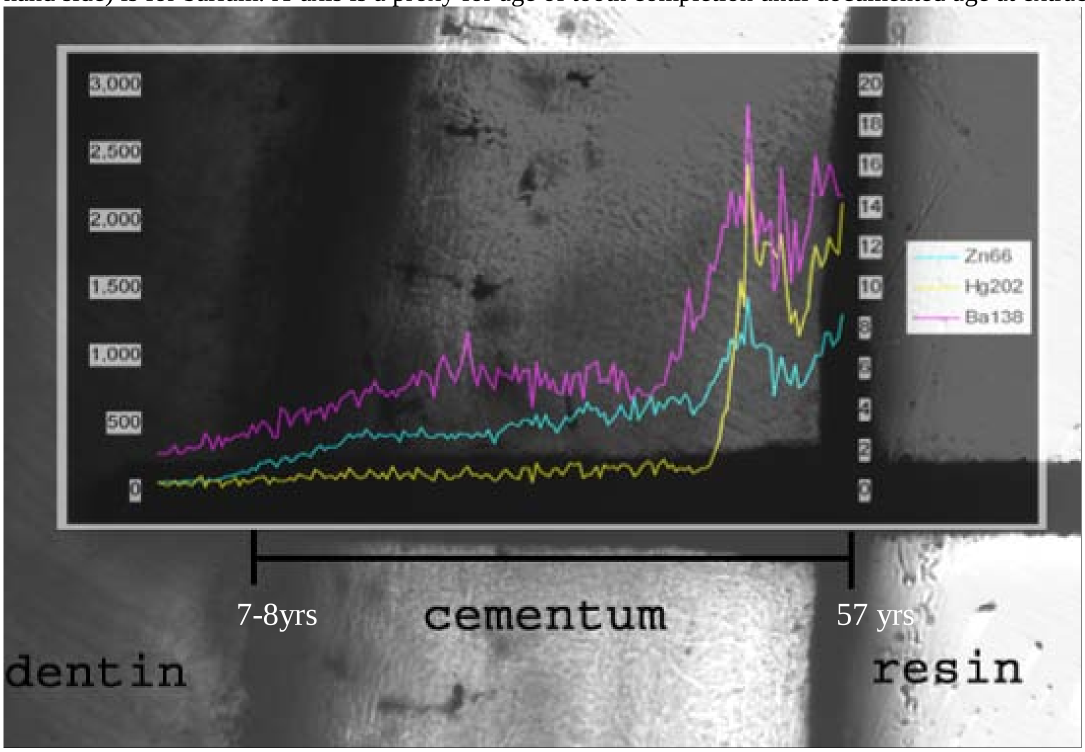


Figure 92 Raw calcium profile of tooth 27-2. X-axis is a proxy for age of tooth completion until documented age at extraction

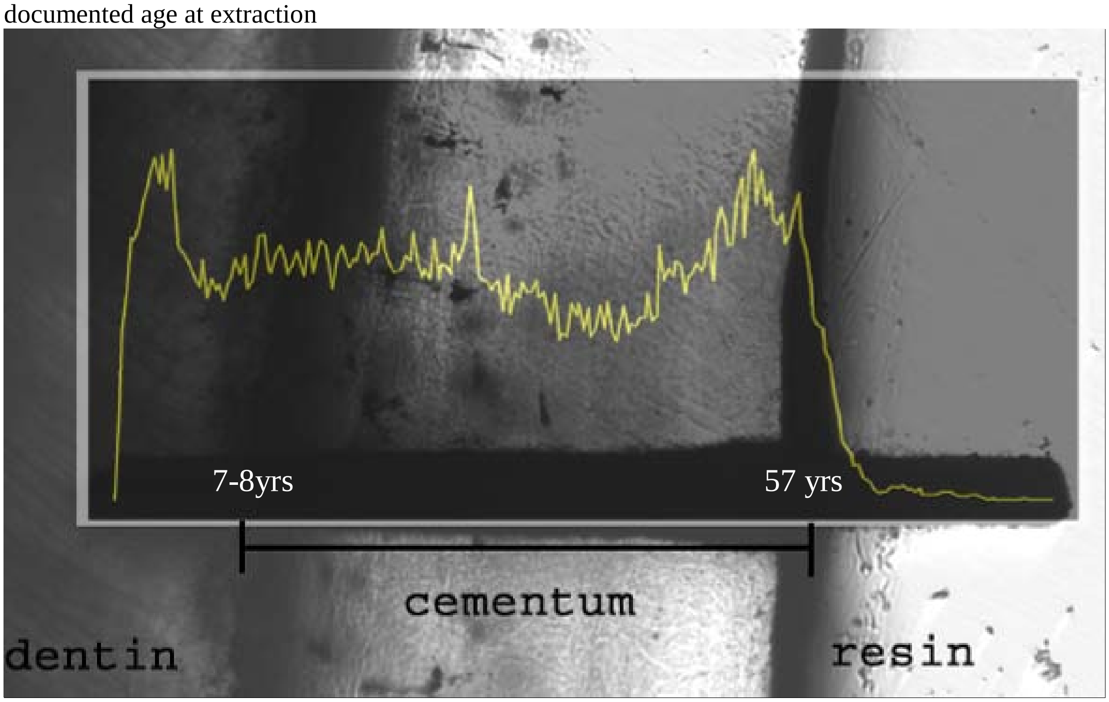


Figure 93 Standardized lead and zinc profiles of tooth 28-2. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

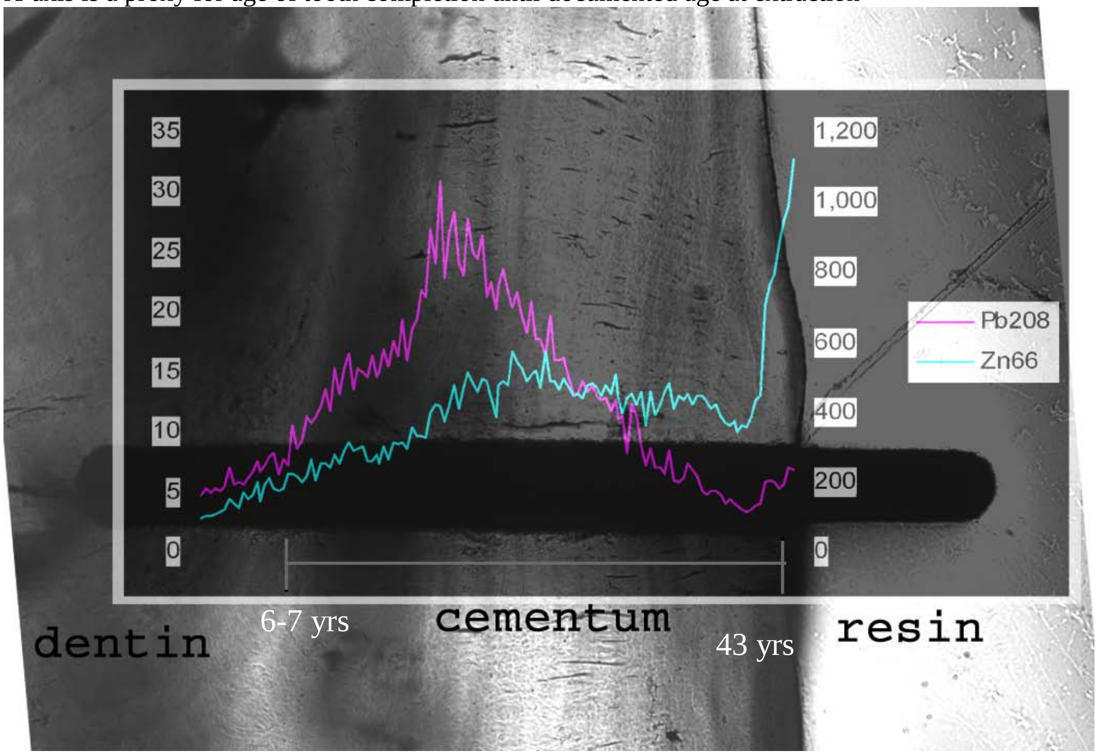


Figure 94 Standardized mercury and barium profiles of tooth 28-2. X-axis is a proxy for age of tooth completion until documented age at extraction

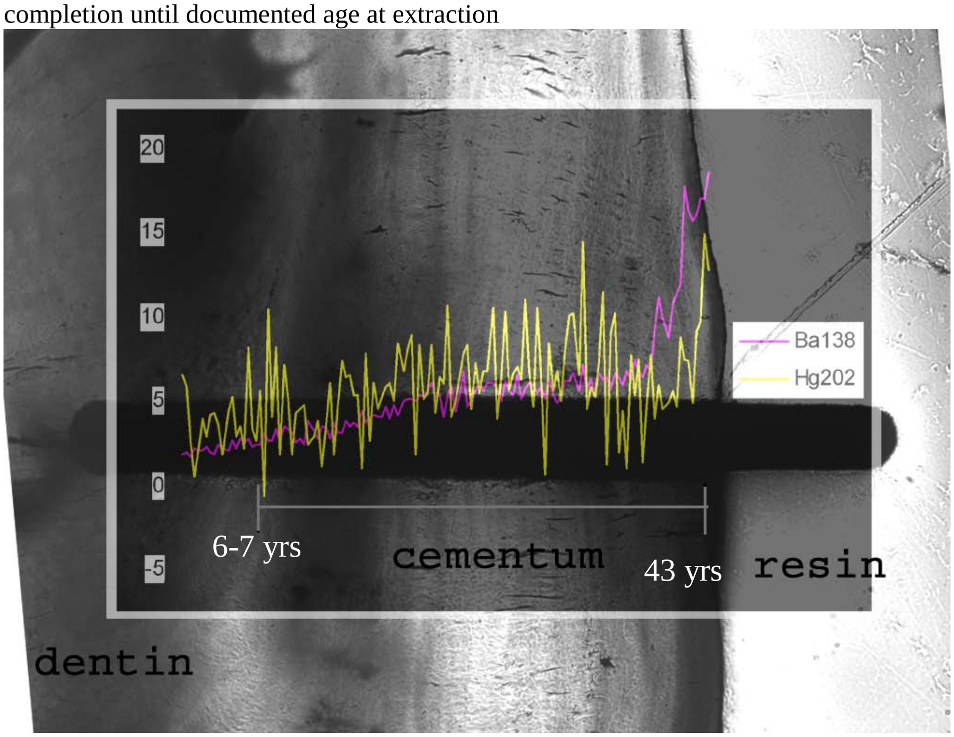


Figure 95 Raw calcium levels in tooth 28-2. X-axis is a proxy for age of tooth completion until documented age at extraction

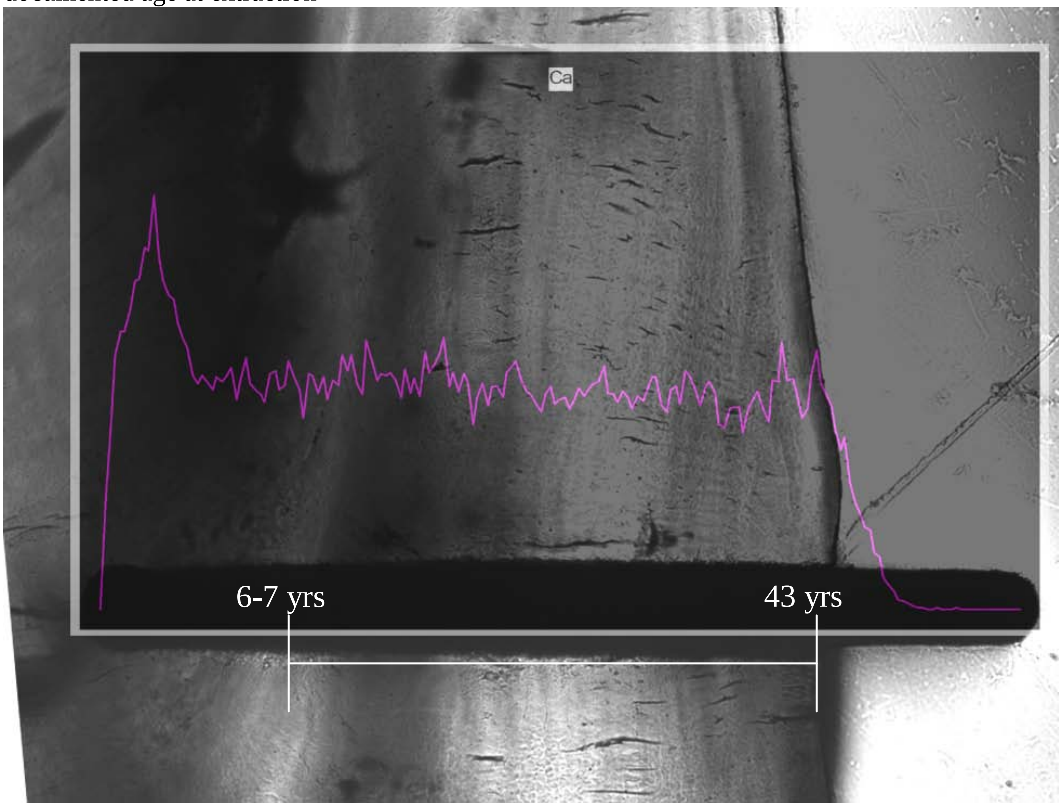
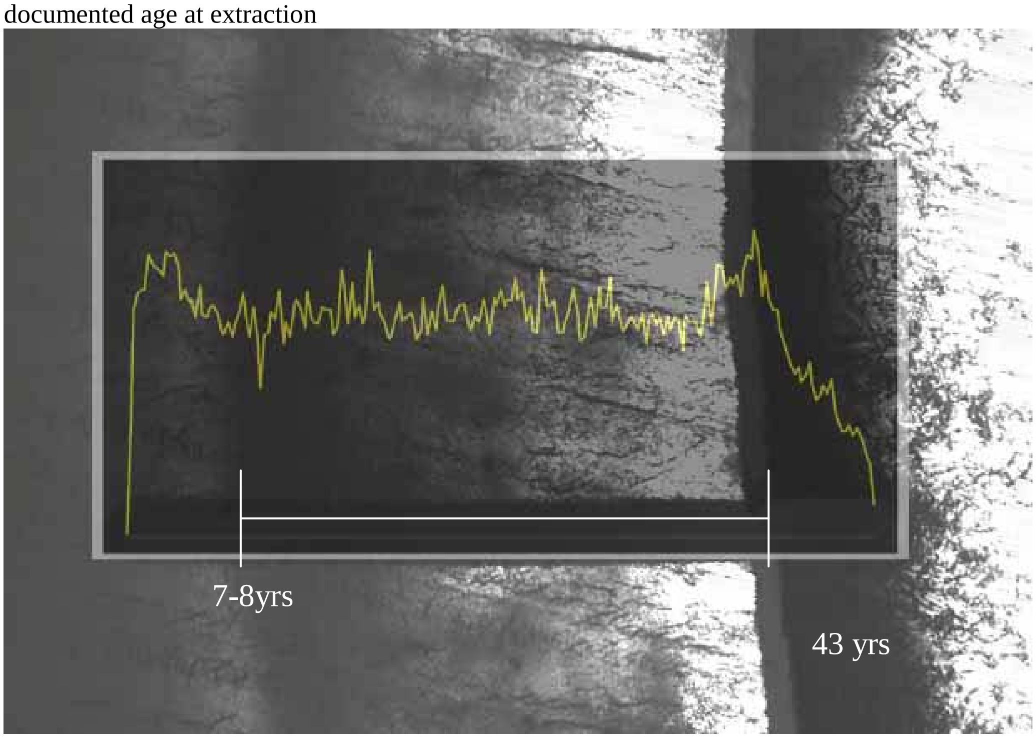


Figure 96 Raw calcium levels of tooth 28-4. X-axis is a proxy for age of tooth completion until documented age at extraction



Tooth 28-4 shows similar elemental profiles as tooth 28-2, but at a later time in the individual's life. The increase in lead does not appear straight after CDJ (Figure 97). Tooth 28-2 erupted 1-2 years ahead of 28-4. This difference in time goes against the spatial difference in profile patterns.

35

Individual 35 is represented by two teeth (35-3 and 35-5).

35-3

Line 1 (35-3) was accidentally ground off, but marked on the image from previous photos of the line. This shows that the laser did not penetrate the entire width of the tooth section. Pb, Zn, Hg, and Ba (Figure 98 and Figure 99) all follow similar profile patterns, though Ba does not seem quite as patterned. Mg drops before CDJ, then increases in cementum (Figure 100).

Lasering did cause the tooth to crack. Calcium levels (Figure 101) do not suddenly drop at any point, suggesting the crack occurred after analysis, and should not be considered as having any effect on the elemental profiles. Calcium does decrease slightly throughout the cementum.

Pb and Zn of line 2 (35-33) follow a similar pattern (Figure 102), which also resembles those in line 1. One main difference is the end peak seen in lead levels, preceded by sharp decrease. This decrease is also found in Zn. Barium levels are unusually high in line 2 (Figure 102), and background levels cannot explain this difference from line 1. The severe and sharp rise in barium is also seen in manganese.

There is a drop in raw calcium levels with no obvious reason (Figure 103). There is a crack emanating from the start of the ablation line which may travel the length of the

Figure 97 Standardized lead levels of tooth 28-4. X-axis is a proxy for age of tooth completion until documented age at extraction

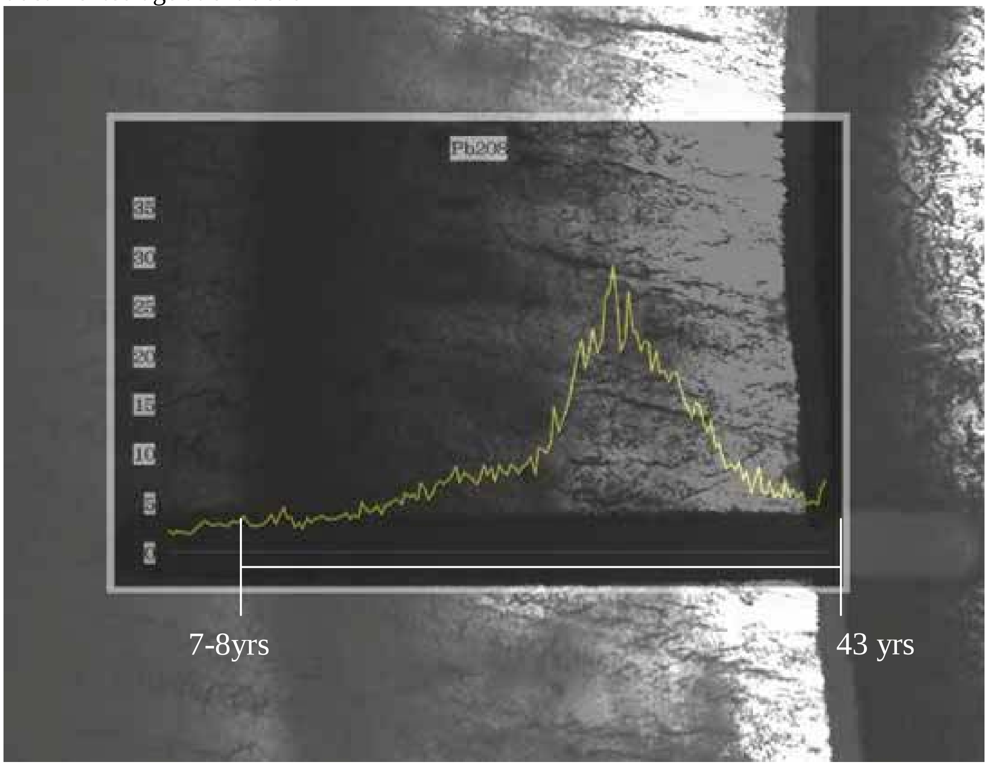


Figure 98 Standardized lead, zinc and barium profiles of tooth 35-3 line1. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

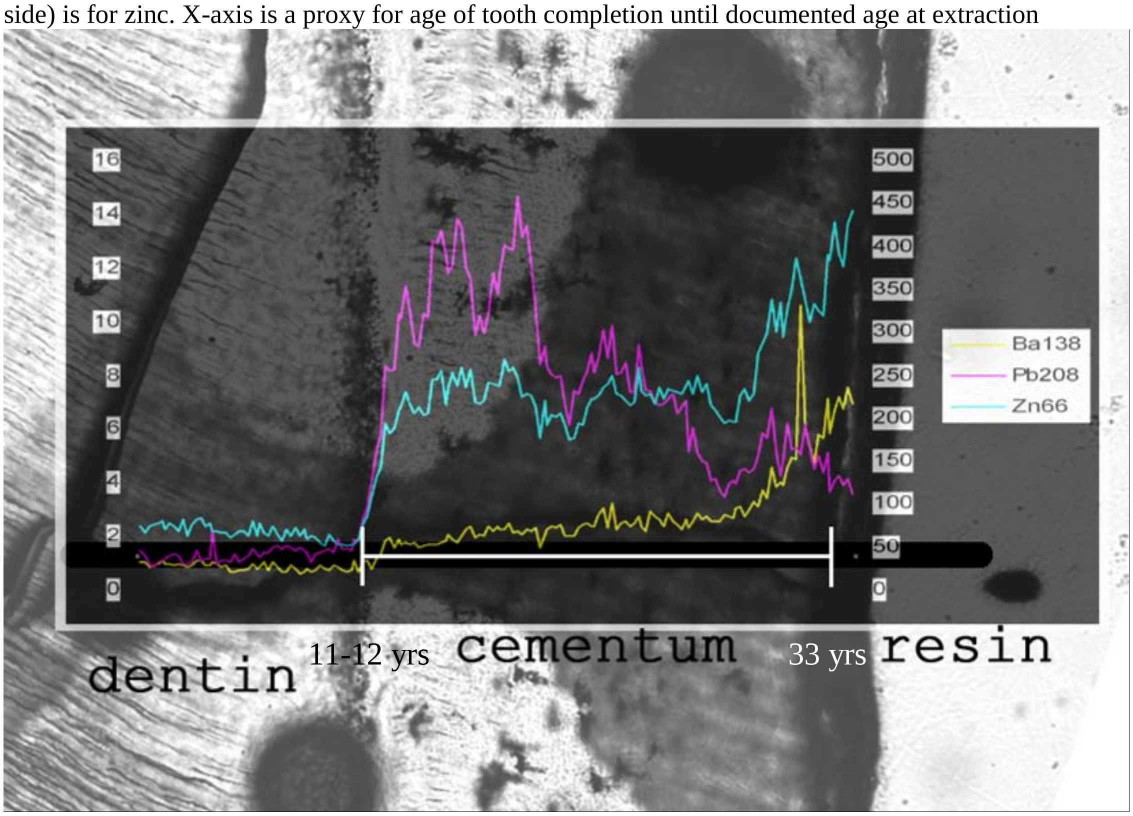


Figure 99 Standardized mercury profile of tooth 35-3 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

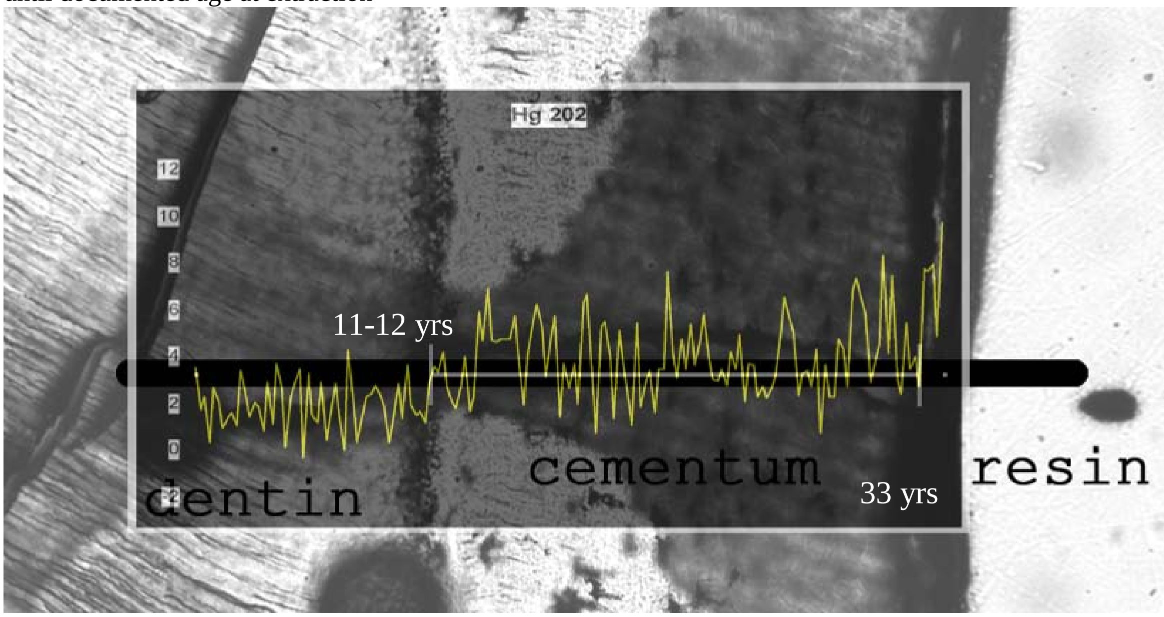


Figure 100 Standardized magnesium profile of tooth 35-3 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

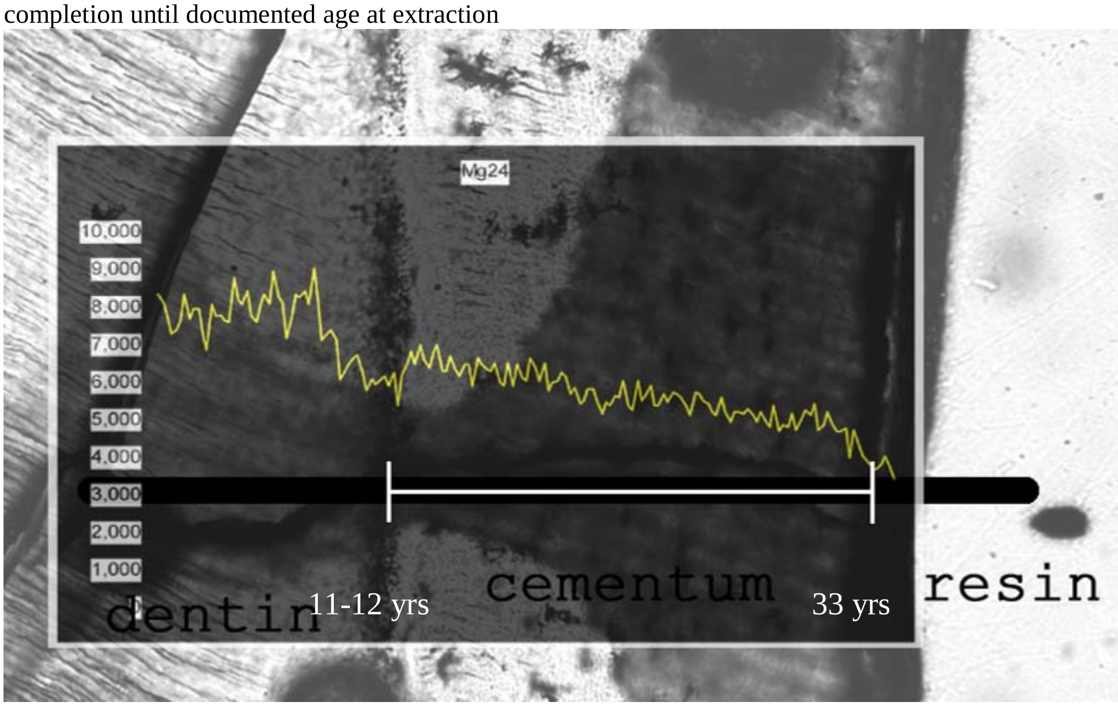


Figure 101 Raw calcium profile of tooth 35-3 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

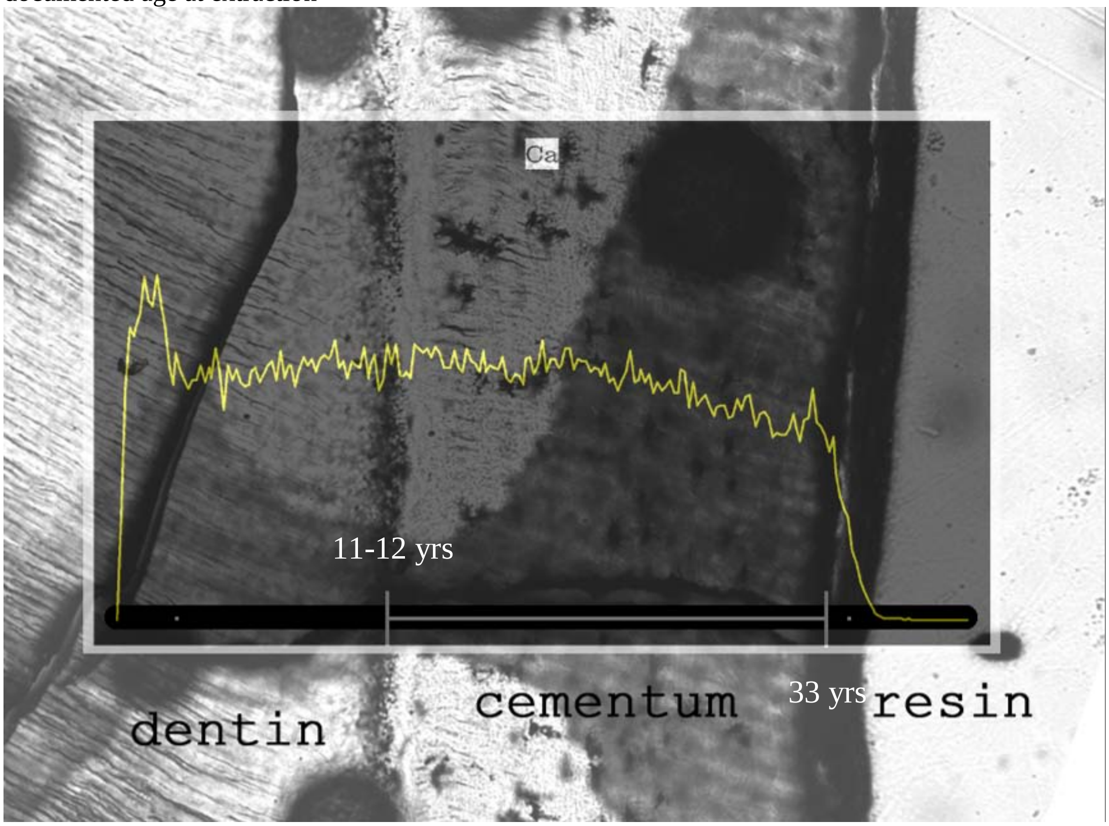


Figure 102 Standardized lead, zinc and barium profiles profile of tooth 35-3 line2. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

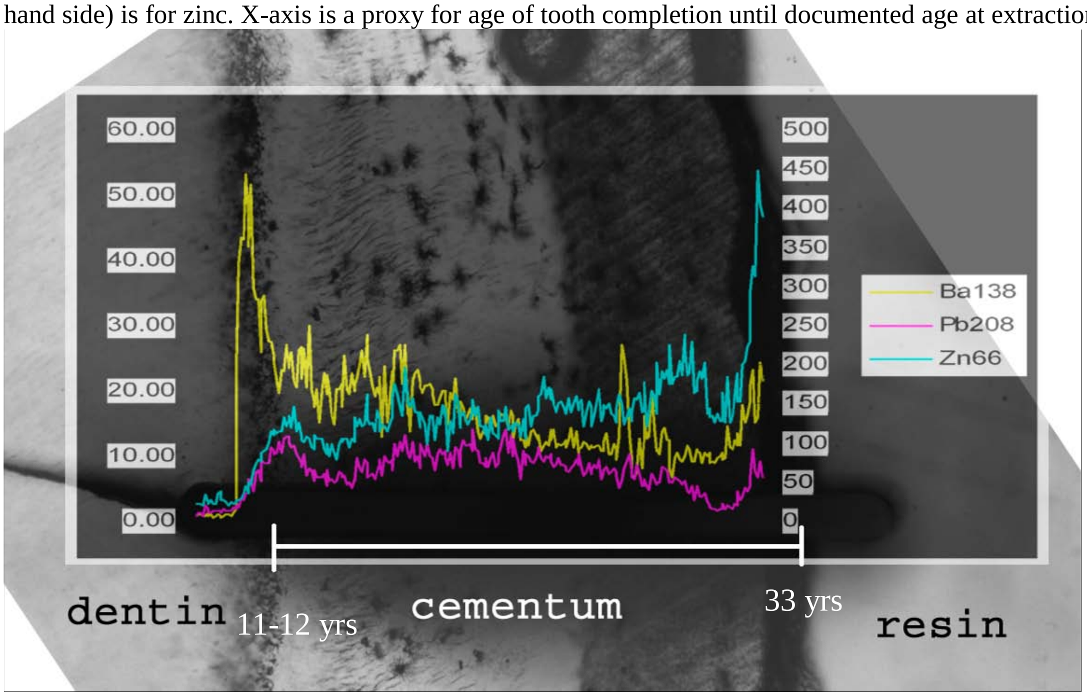
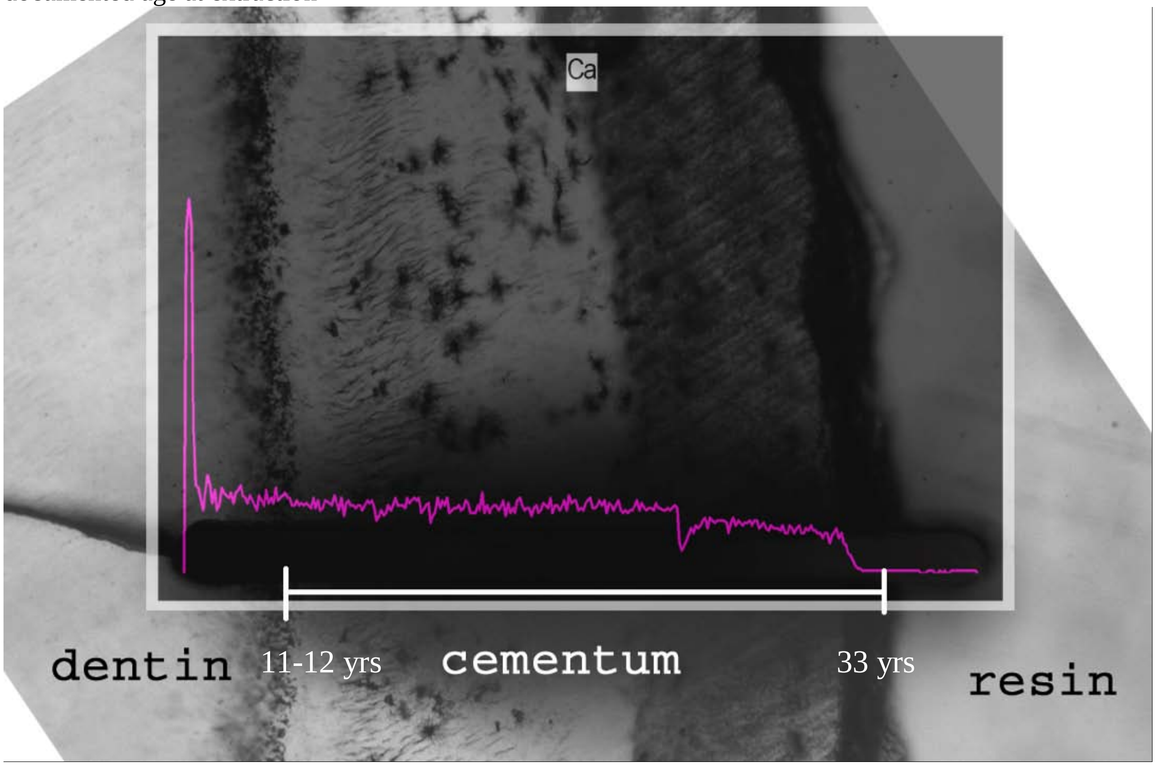


Figure 103 Raw calcium profile of tooth 35-3 line2. X-axis is a proxy for age of tooth completion until documented age at extraction



line and could have caused the drop in levels. This drop can be seen in the raw levels of the trace elements. With the normalization of calcium, this drop in levels is equalized and not seen in the calculated profiles. The elemental profiles of line 1 are less jagged than those of line 2. This is due to the slightly faster speed of the laser (3µm/s versus the usual 2µm/s that was performed for line 2).

35-5

Tooth 35-5 was analyzed 3 times on two different sections. Section 4 was analysed once (35-54). Pb, Zn, Hg and Ba are all similar (Figure 104 andFigure 105). The edge levels of Zn and Ba peak, drop, then peak again. The second end peak corresponds to the outer surface of cementum under the resin. The drop in levels is puzzling. The initial increase in Pb and Zn is large. Hg also increases noticeably after the CDJ, but not to the same extent. There is a general lull in levels that occurs over the area of darker cementum. Pb decreases as the cellularity of the cementum increases. The most acellular cementum, which is the most exterior portion, does not create much of a peak in Pb levels, though Zn does, which could just be part of its usual end peak. Magnesium (Figure 106) drops in intensity at or slightly before the CDJ, matching that seen in tooth 35-3. The levels through the cementum barely decrease, which is also seen in the other tooth.

Line 2 (35-56) was performed on section 6 of tooth 35-5. Pb, Zn, Hg and Ba all show similar profiles (Figure 107 and Figure 108), which are somewhat similar to those in line 1. The same dark cementum occurs in the same lull area.

The initial increase after the CDJ of line 2 shows a step before reaching its highest levels in Pb and Zn. Neither of the other two lines of analysis shows this. The three lines

Figure 104 Standardized lead, zinc and barium profiles of tooth 35-5 line1. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

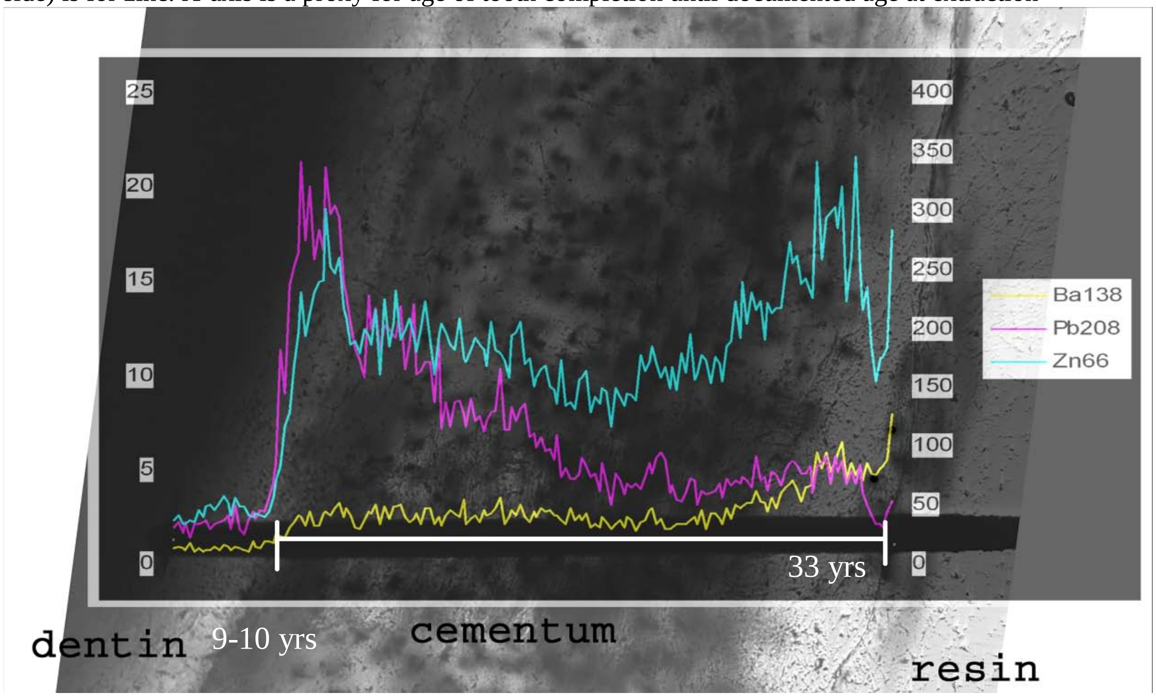


Figure 105 Standardized mercury and barium profiles of tooth 35-5 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

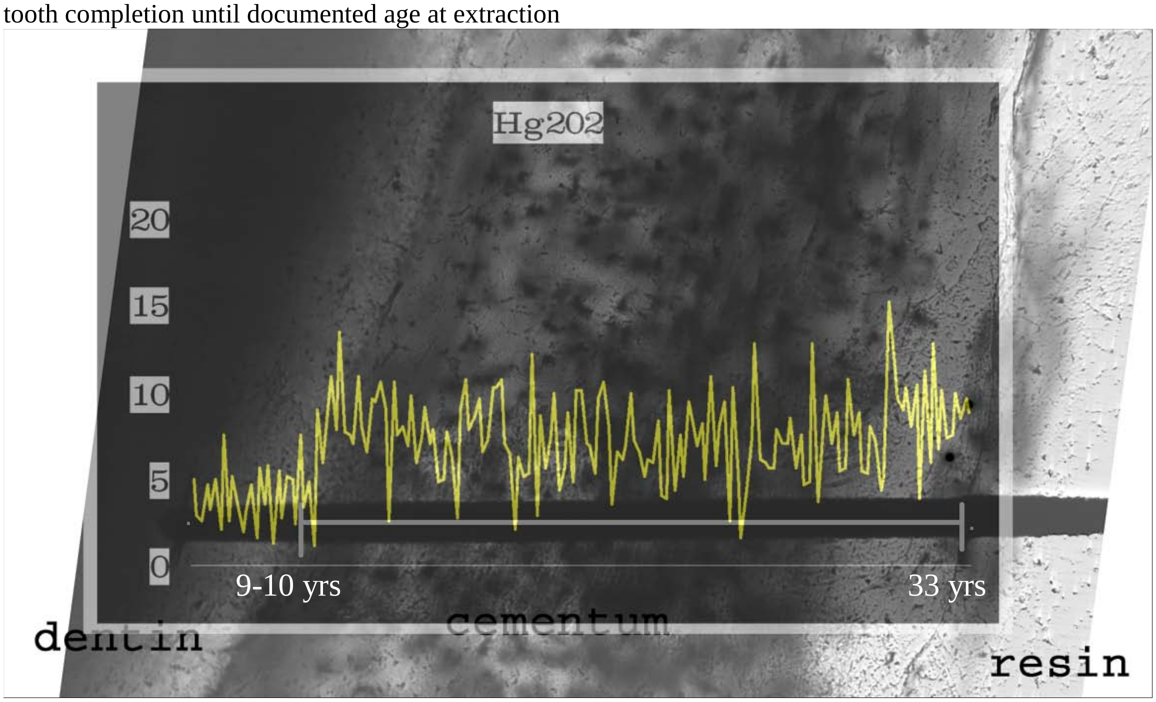


Figure 106 Standardized magnesium profile of tooth 35-5 line1. X-axis is a proxy for age of tooth completion until documented age at extraction

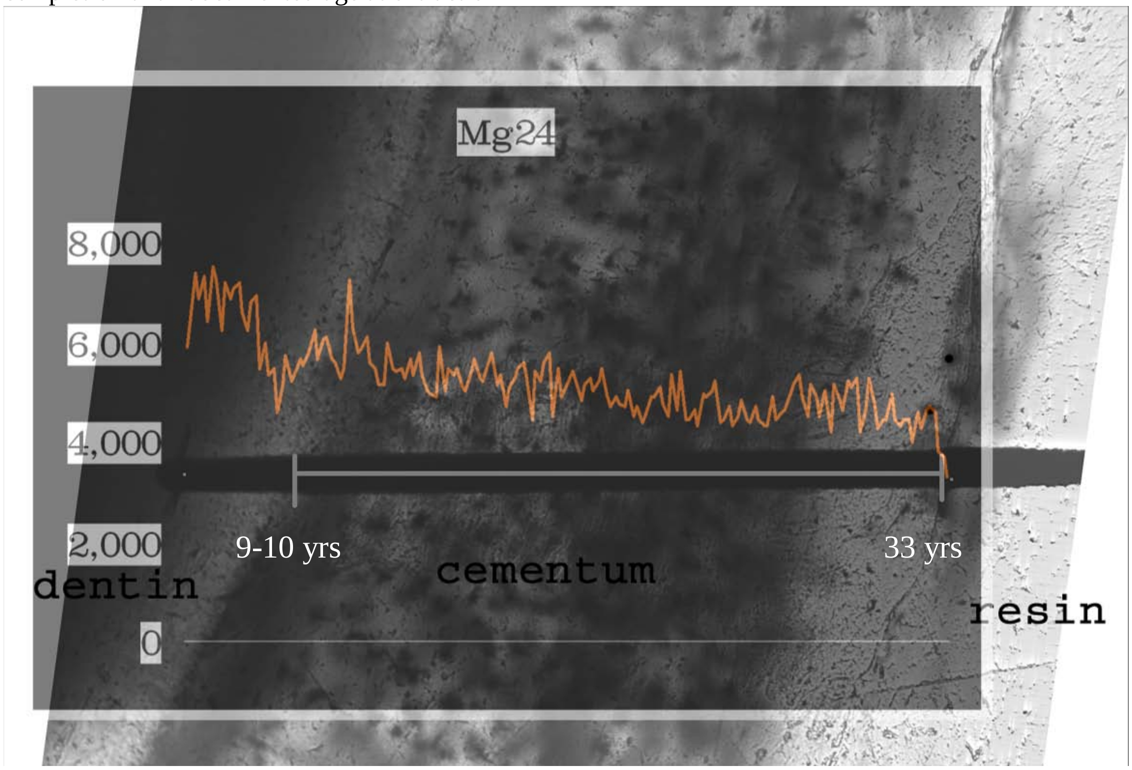


Figure 107 Standardized lead, zinc and barium profiles of tooth 35-5 line2. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

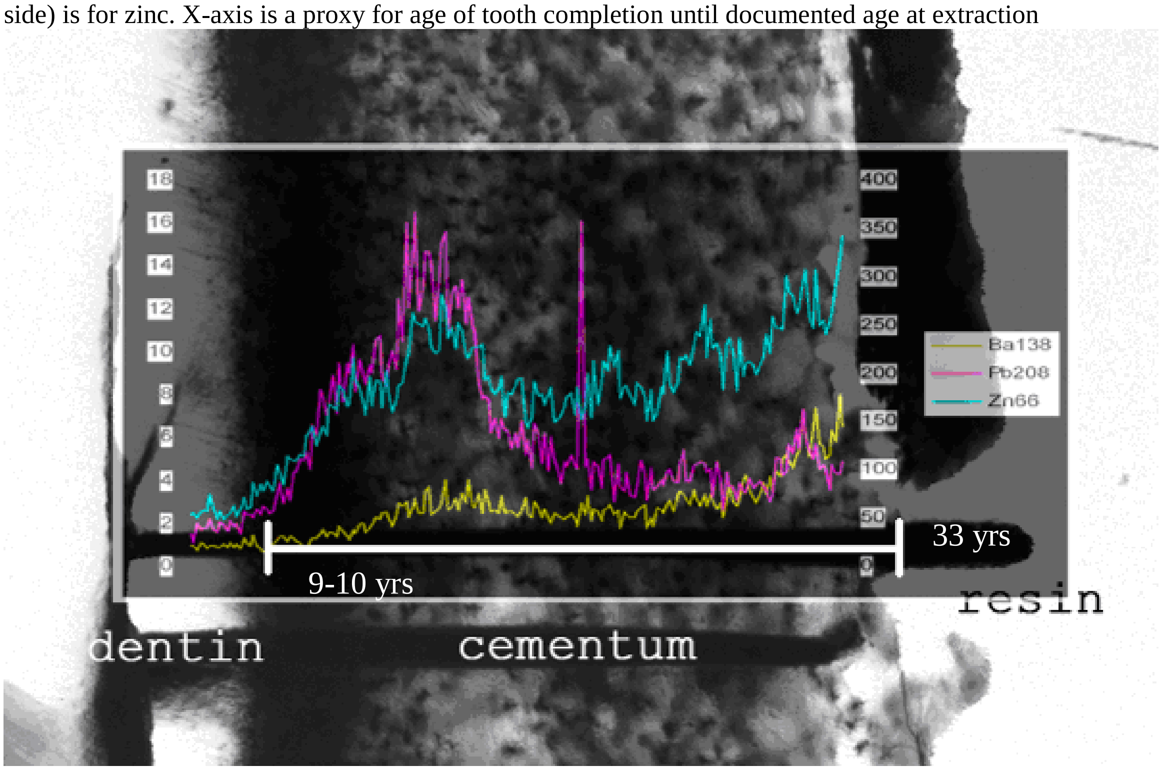


Figure 108 Standardized mercury profile of tooth 35-5 line2. X-axis is a proxy for age of tooth completion until documented age at extraction

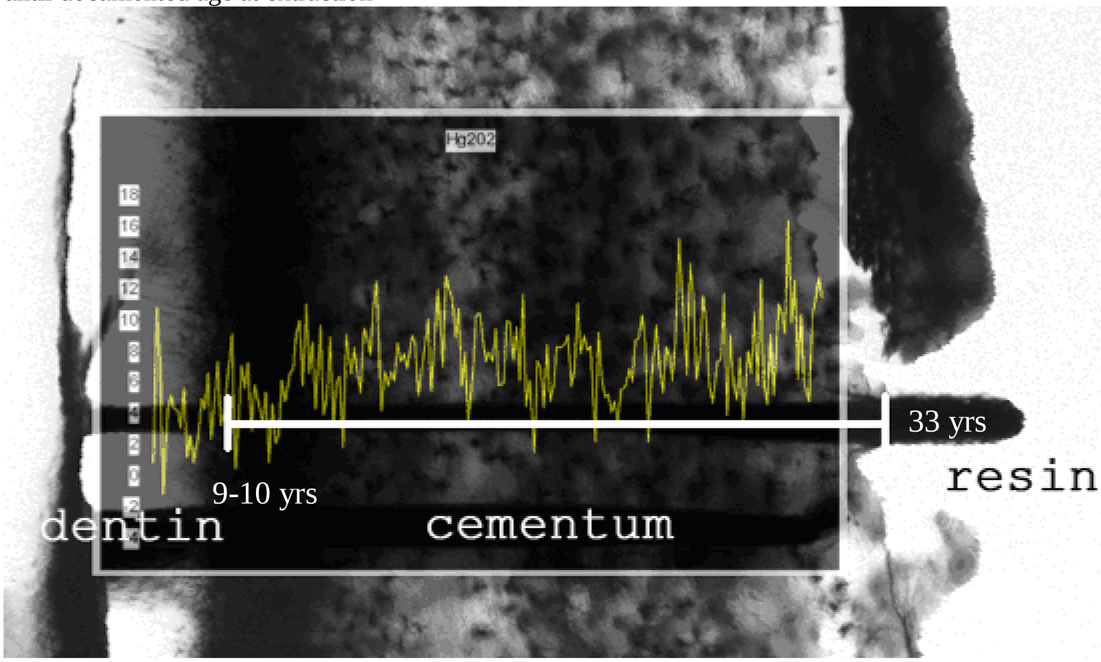
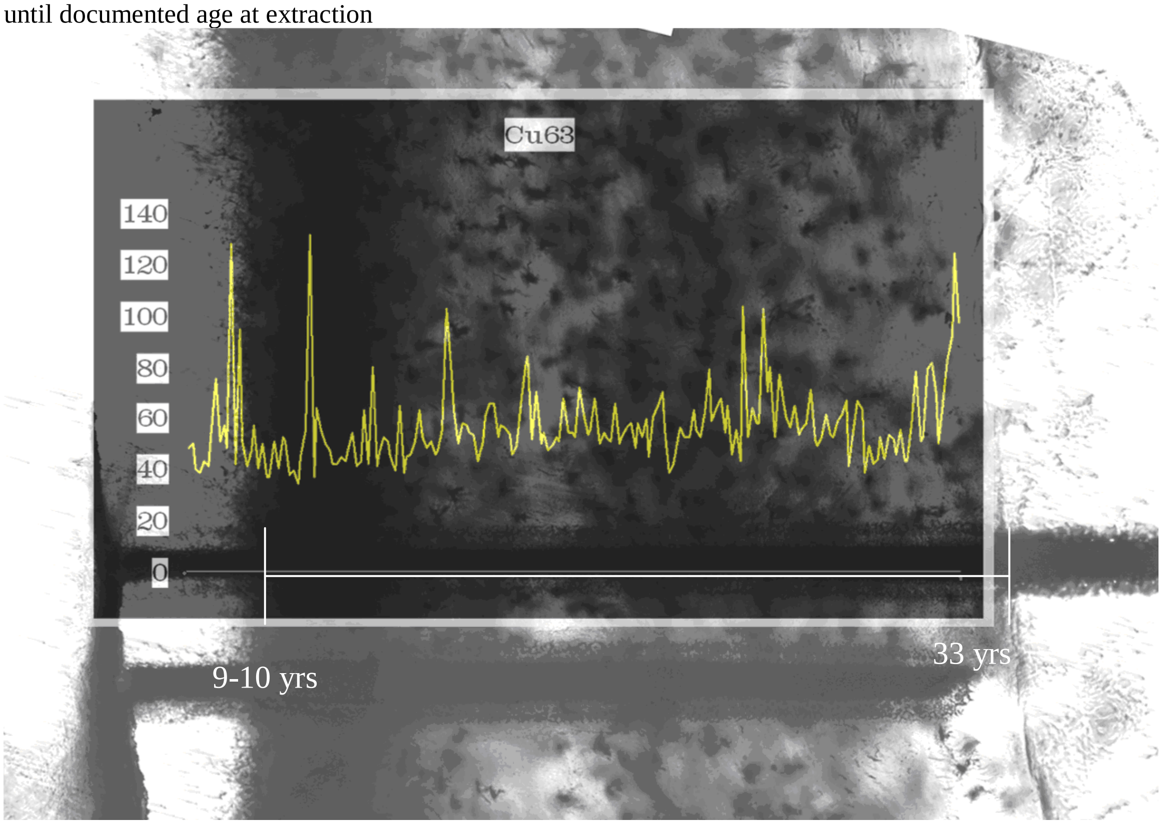


Figure 109 Standardized copper profile of tooth 35-5 line2. X-axis is a proxy for age of tooth completion until documented age at extraction



were performed at different speeds; lines 1, 2 and 3 were performed at 4, 3 and 2μm/s, respectively. The cementum width of line 2 is larger than that of line 3, which may be the reason for the step not being noticeable in line 3 even though more time is taken to analyse it.

Line 2 also has higher copper levels (Figure 109), which are not present in either of the other two lines. Vanadium also shows elevated levels at the edge of cementum, which again is not seen in lines 1 and 3.

Line 3 shows a dip in raw Ca levels near the edge of the cementum (Figure 110). This dip is in the same area as many of the increases in the trace elements. The increases are seen in the raw levels as well so they are not a product of Ca normalization. The image is too dark to tell where exactly the cementum ends or if there is a structural reason for this increase/dip in levels. The peak in elements (Pb, Zn, Hg, Ba) (Figure 111 and Figure 112) does appear to occur prior to the edge and therefore earlier than normal. Aside from the slower increase in Pb, Zn, and Ba levels, the elemental profiles cannot be correlated to the changes in cellularity of the cementum.

Mercury is low in all three lines; tooth 35-3 also showed relatively low Hg levels. There are no caries in this tooth or the other (35-3). It is unknown if there are any amalgam fillings in the mouth.

Elemental levels for the tooth show some slight differences. Line 1 in tooth 35-3 matches the patterns in line 1 of tooth 35-5. Line 2 of tooth 35-3 shows lulls in Pb and Zn levels that do not appear in any of the other lines of analysis.

37

Figure 110 Raw calcium profile of tooth 35-5 line3. X-axis is a proxy for age of tooth completion until documented age at extraction

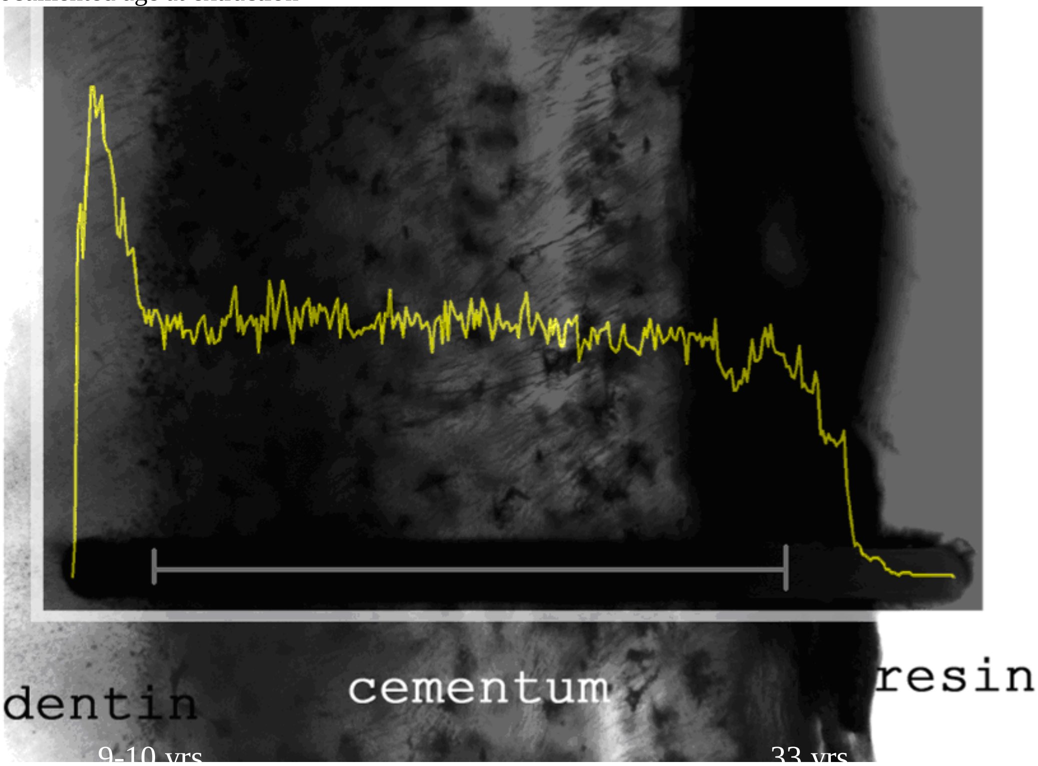


Figure 111 Standardized lead, zinc and barium profiles of tooth 35-5 line3. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

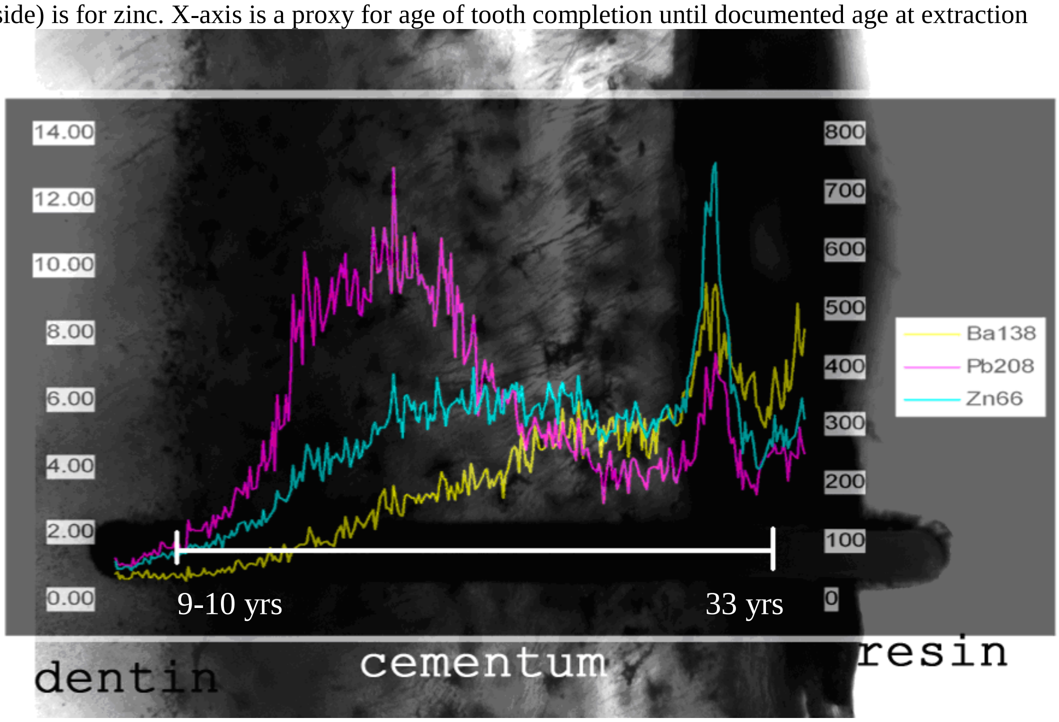


Figure 112 Standardized mercury profile of tooth 35-5 line3. X-axis is a proxy for age of tooth completion until documented age at extraction

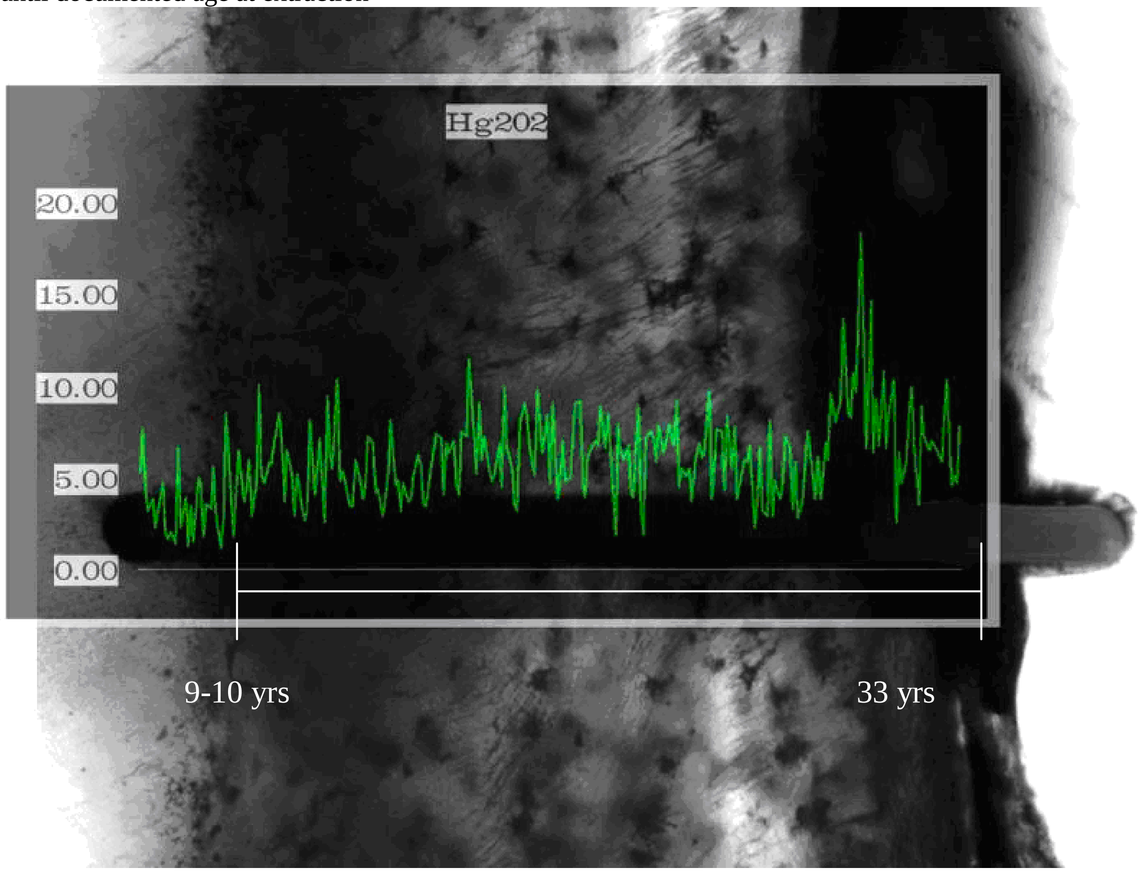
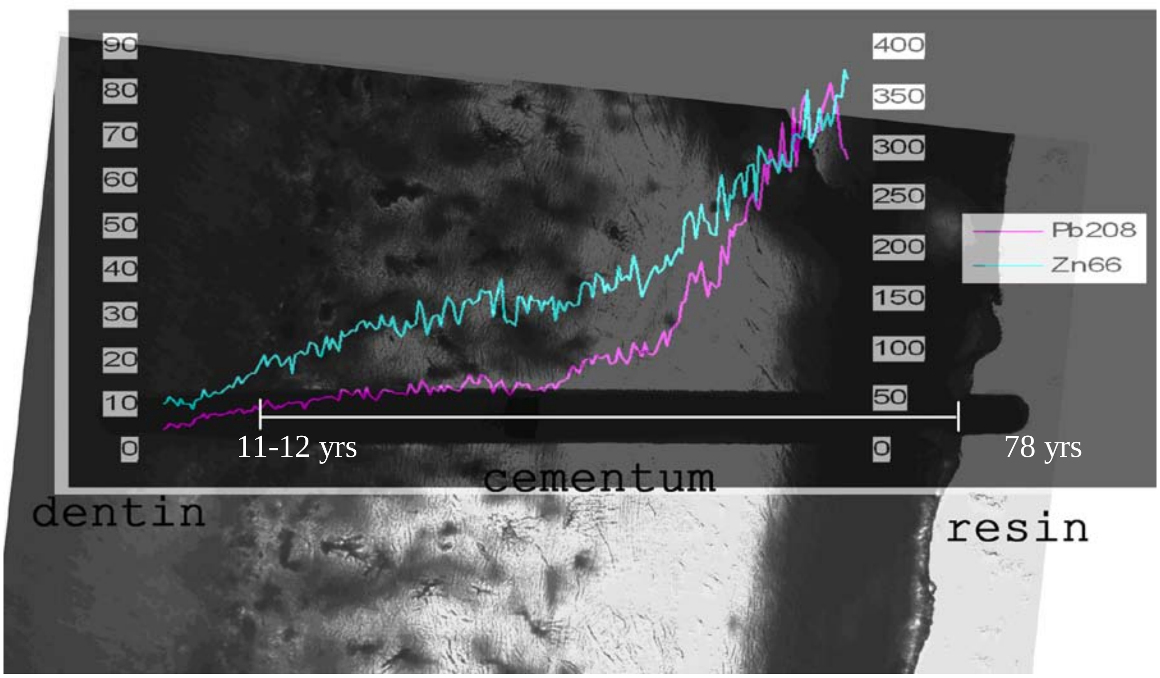


Figure 113 Standardized lead and zinc profiles of tooth 37-4 line1. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction



Individual 37 is represented in this study by one tooth (37-4) which was analysed twice. Line 1 (37-4) and line 2 (37-43) were performed next to each other, making it unlikely that were would be any differences in elemental profiles. The difference that does exist between the two lines is due to the speed of the laser; line 1 was performed at 3µm/s while line 2 was completed at 2µm/s. The slight differences in the appearance of the profiles should be a result of the speed differences. Line 2 shows more specific peaks and drops in lead and zinc levels.

Lead, zinc and barium all follow similar patterns in line 1 (Figure 113) as they do in line 2 (Figure 114). There is an unusual rise in lead in the outer 1/3 of cementum. The sharper rise does correspond to where the cementum becomes more acellular. Unfortunately, since lines 1 and 2 were performed so close together, and there was no explicit analysis performed in an area of absolute acellular cementum, the increase in lead cannot specifically be associated with the occurrence of acellular cementum. Lead levels fall as the usual elements peak. Zinc shows the same increase through the acellular area. The increase in lead and zinc after the CDJ is fairly small, though slightly more noticeable in zinc.

The tooth is carious and contains very high peak lead levels, which have been linked. Mercury levels are low implying that there are no amalgam fillings in the rest of the mouth.

44

Individual 44 is represented by a single tooth (44-2) which was analysed twice. Both lines were performed on the same side of the root, fairly close together. Pb peaks before CDJ, then again right after (Figure 115). Second peak is much larger. Zn does not

Figure 114 Standardized lead and zinc profiles of tooth 37-4 line2. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

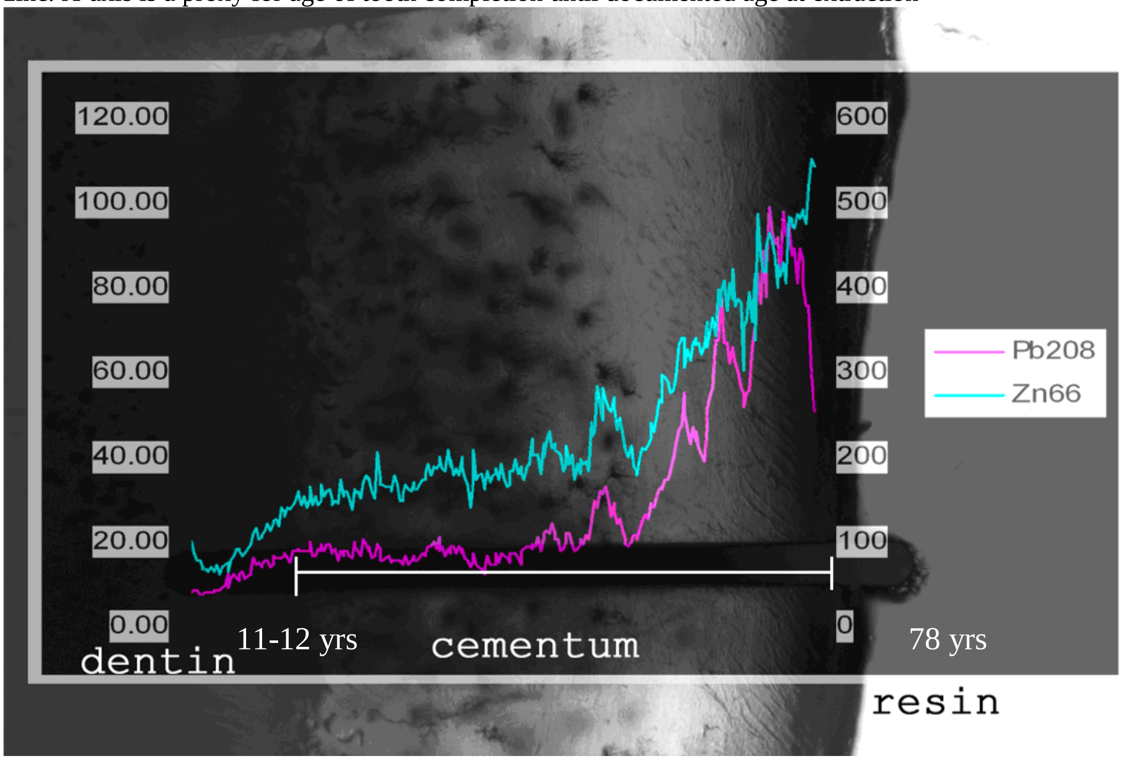
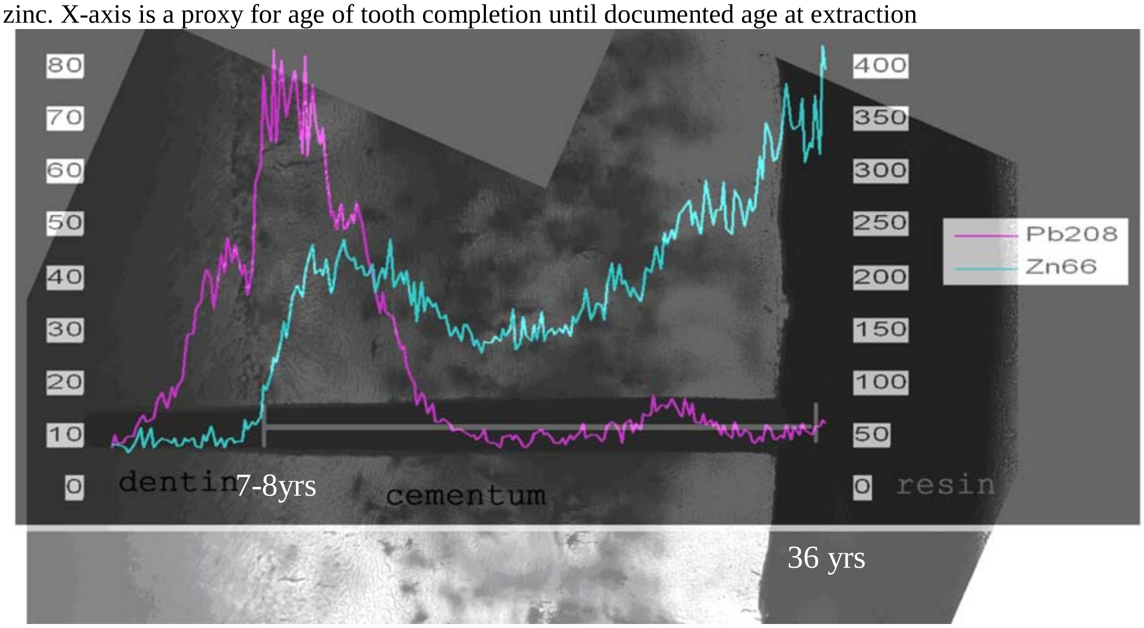


Figure 115 Standardized lead and zinc profiles of tooth 44-2 line1. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction



share the peak prior to the CDJ (Figure 115). This dentin peak is seen in the analysis of line 1 but not in line 2 (Figure 116), though the rise in Pb levels does start to occur prior to the CDJ (or maybe just as the laser begins to ablate the beginning of the CDJ). Upon examination, there is no structural explanation for the lead found in the dentin. There is no visible possibility of the CDJ curving in below the surface, and Zn does not follow the same pattern. The shadow that extends past the surface edge suggests that the cementum under the resin angles out which would suggest that the CDJ would also angle out (CDJ below surface would occur after the surface CDJ, from left to right).

As the cementum becomes more cellular, Pb and Zn levels fall. Zn levels begin to rise before cementocytes thin out. As mentioned previously, the cellularity of the cementum may not be correlated to elemental levels, but merely coincidental. Zn increases sharply at the CDJ with line 1 being slightly steeper than line 2. The end of lines 1 and 2 appear different at edge of cementum, however the calculated portion of Zn in line 1 does not go as far as line 2 and is cut short of the decrease seen in line 2. Looking at the raw levels of Zn, one can see the decrease.

The tooth does not appear to have any caries. The high lead levels which occur at the beginning of the cementum cannot be correlated to absorption through carious tissue. Mercury levels are low, which may imply that there are no amalgam fillings in the rest of the mouth.

The ablated line in 44-2 is not even throughout its run. The density of material has an effect on the amount of material that gets ablated. Dentin is denser than cementum, allowing a larger amount of cementum tissue to be ablated. This is not seen in all specimens (this cementum may be less dense than others). The size difference of laser lines between tissues is not seen in line 2. One other possibility involves the crack that

Figure 116 Standardized lead and zinc profiles of tooth 44-2 line2. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction

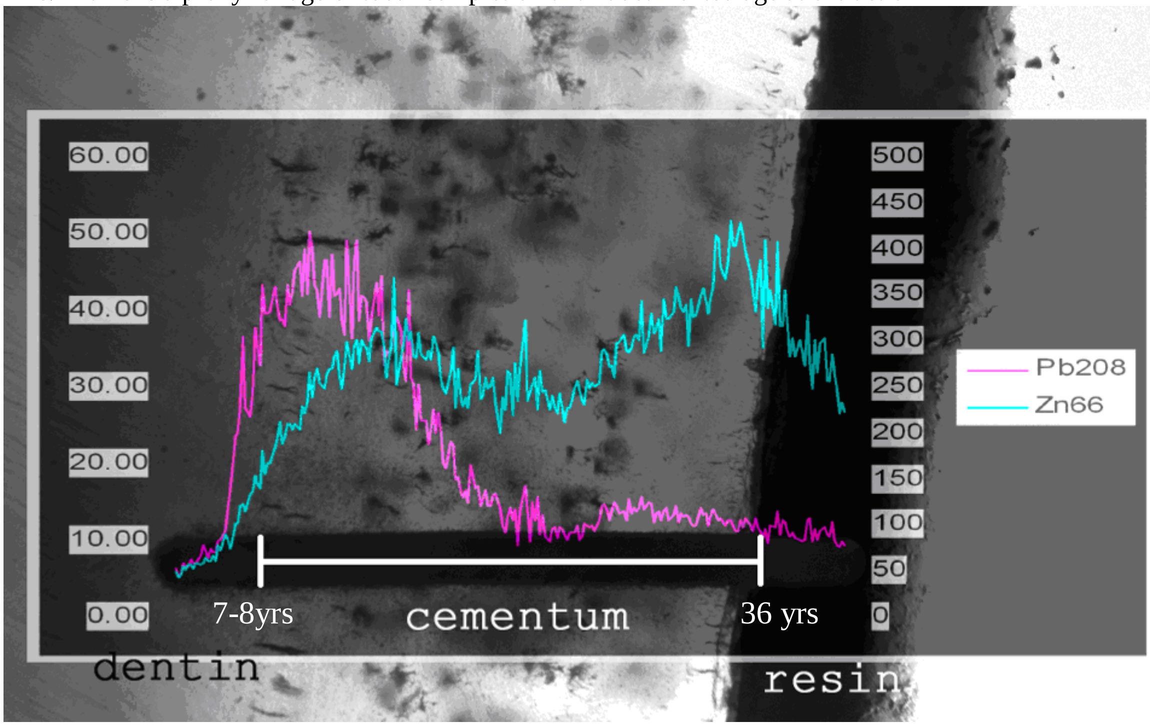
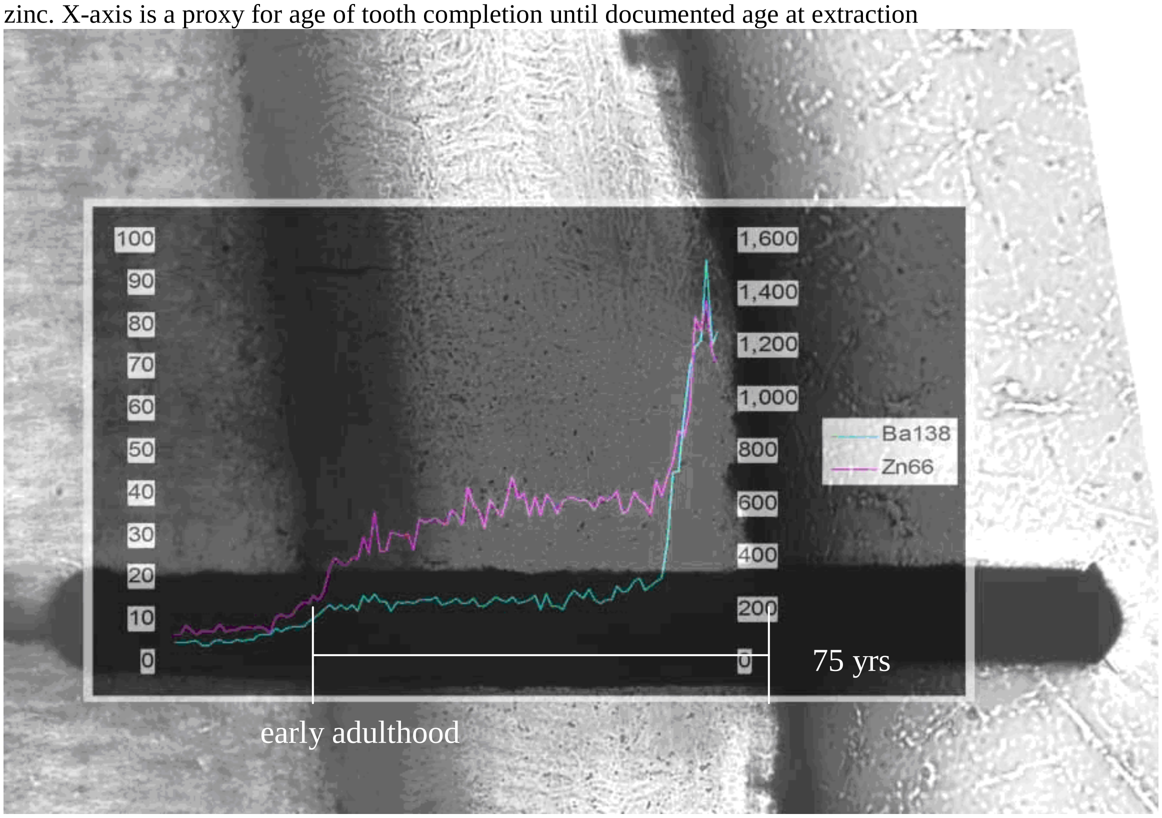


Figure 117 Standardized zinc and barium levels in tooth 48-1. Secondary y-axis (right-hand side) is for zinc. X-axis is a proxy for age of tooth completion until documented age at extraction



emanates from the start end of the laser, which would allow the cementum to pull away and ‘widen’ the appearance of the line.

48

Individual 48 was represented by 2 teeth (48-1 and 48-2), both of which were cross-sectioned and analysed once each.

48-1

The cementum analysed in tooth 48-1 was thin and acellular. It is much more difficult to locate any cellular cementum when teeth are cross-sectioned. It is difficult to determine much from such a narrow/short analysis. This individual was 75 years old when the tooth was removed, so there are many years of information packed into a small space.

With such narrow cementum, it is difficult to determine how close or different some of the element profiles are. Zinc and barium do appear similar (Figure 116), especially since both have a double end peak seen in the raw levels. Copper also has multiple edge peaks (Figure 117), while strontium only has one (Figure 118). Calcium stays moderately homogeneous throughout the cementum. Lead levels (Figure 119) are quite high and the tooth is carious, which may be related.

The size of the laser beam in comparison to the cementum thickness means the beam reaches the edge, and where the most interesting patterns (element-wise) are at about 1/3 of the way through.

48-2

Figure 118 Standardized copper levels of tooth 48-1. X-axis is a proxy for age of tooth completion until documented age at extraction

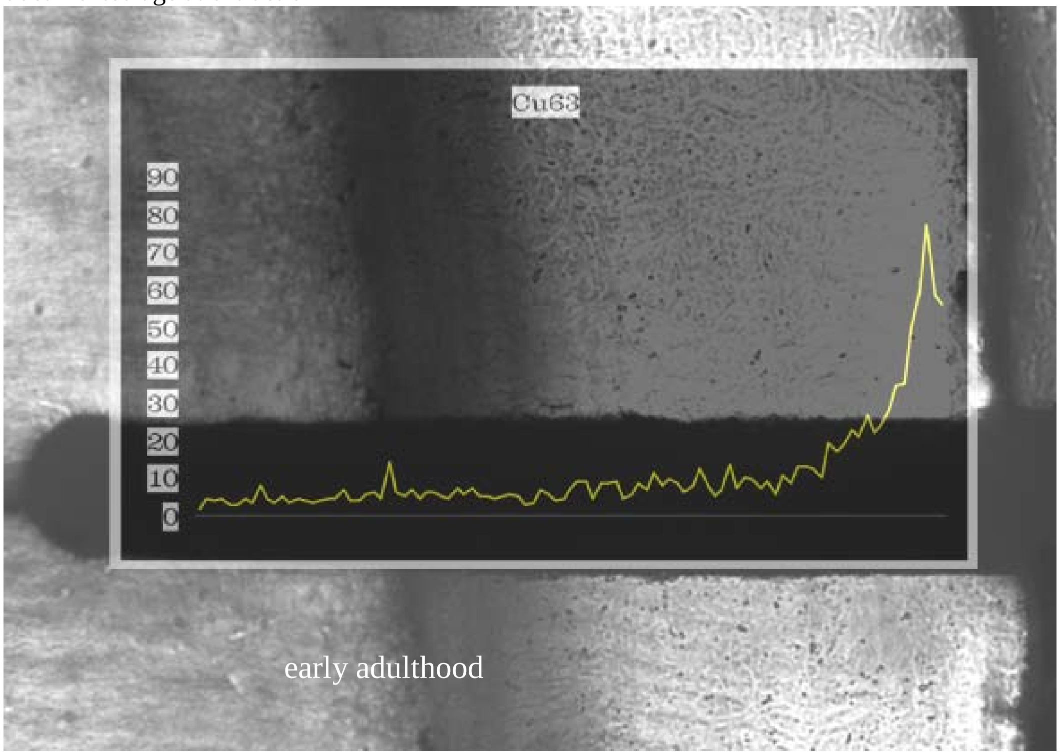


Figure 119 Standardized strontium levels of tooth 48-1. X-axis is a proxy for age of tooth completion until documented age at extraction

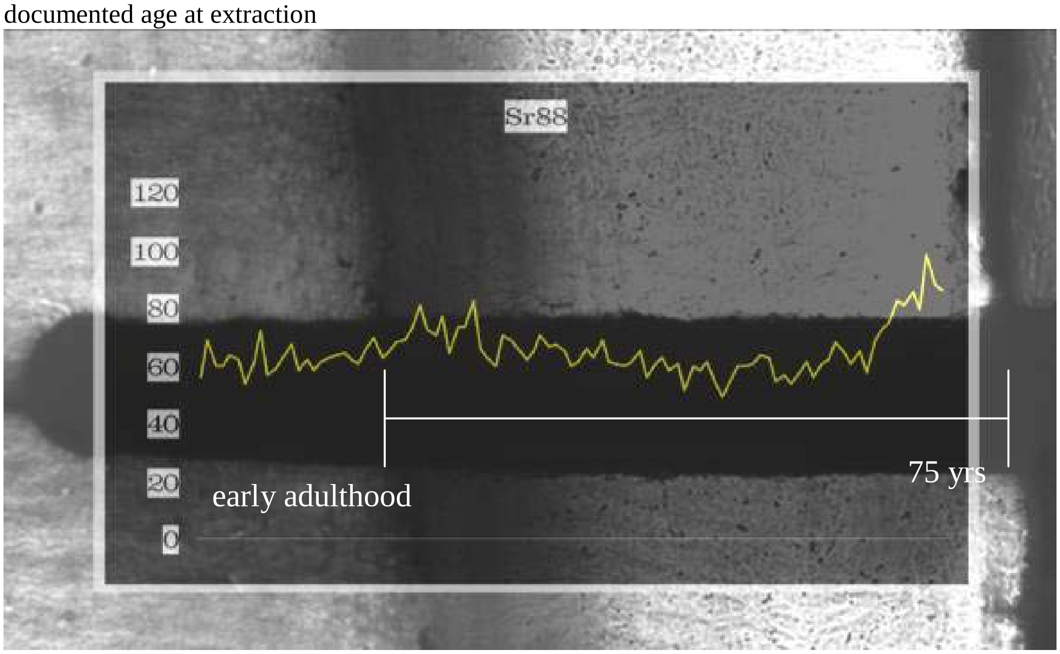


Figure 120 Standardized lead levels of tooth 48-1. X-axis is a proxy for age of tooth completion until documented age at extraction

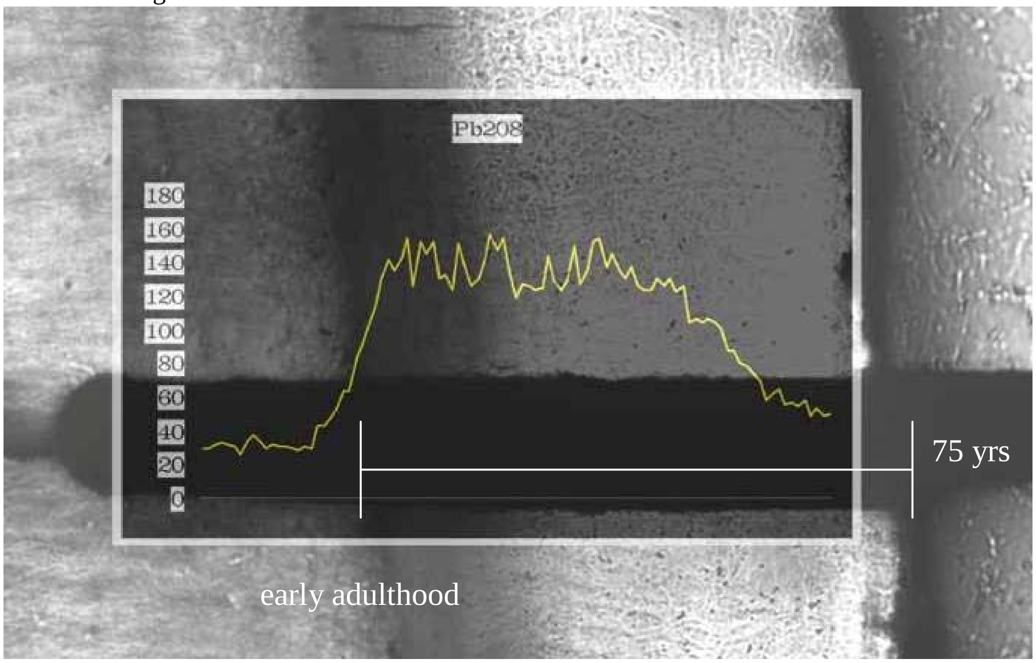
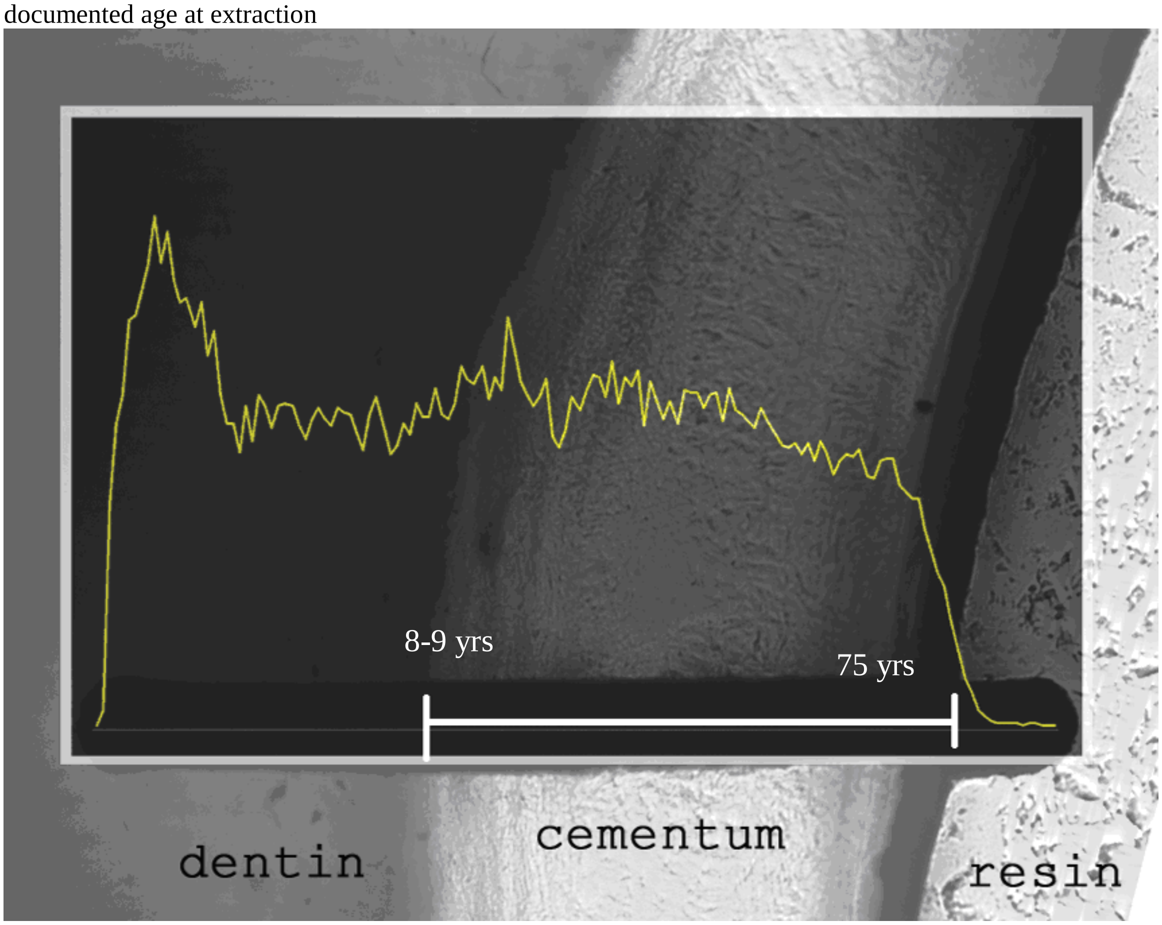


Figure 121 Raw calcium profile of tooth 48-2. X-axis is a proxy for age of tooth completion until documented age at extraction



Tooth 48-2 does not contain much cementum. Ca is variable (Figure 120), with changes occurring during and after the CDJ. The rest of the cementum slowly decreases in Ca levels. The large drop occurs as the laser reaches the outer edge. The cementum after the CDJ appears less uniform than the majority of the cementum. Only the cementum near the outer edge appears to change as well; however these two areas do not share the same variation in Ca levels.

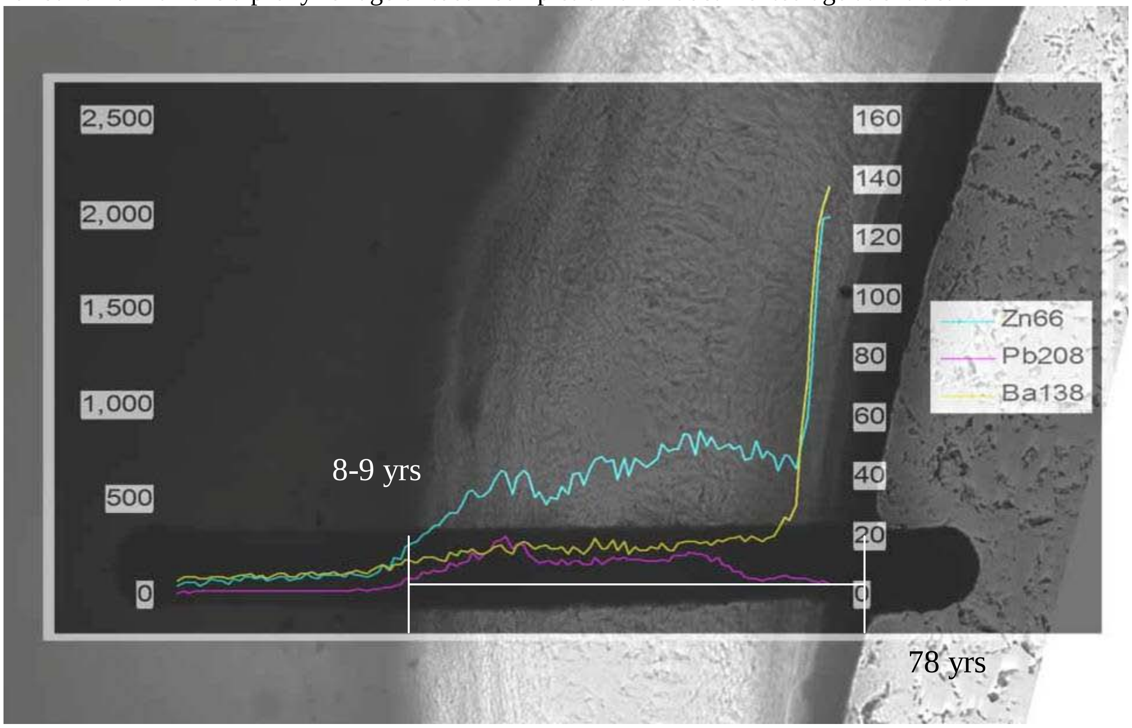
With the shortness of the analysis, it becomes difficult to see how closely elements change with each other. Zinc and barium follow similar profiles, but it is difficult to determine if lead follows along a similar path (Figure 121). The end portions of lead profiles have differed from those of zinc, mercury, and barium in the rest of the teeth in the study.

The tooth was carious with very high lead levels, higher than those in tooth 48-1. Tooth 48-2 had both composite fillings and exposed caries, but low mercury levels, implying no amalgam fillings in the rest of the mouth.

Summary

Each individual was discussed as per trends seen in their elemental analysis. The following chapter will examine the broader trends seen for each element.

Figure 122 Standardized lead, zinc and barium levels of tooth 48-2. Secondary y-axis (right-hand side) is for barium. X-axis is a proxy for age of tooth completion until documented age at extraction



Chapter 6: Discussion and Conclusions

Introduction

Having examined the element profiles for each individual in the previous chapter, I will explore the analysis of each element and the general trends found, as well as answer the initial questions posed.

Lead

This study shows differences in intensities of lead. Low levels are in the range of less than 20 parts per million, while high levels are 75ppm and over. Previous studies have observed that carious teeth have higher levels of Pb than healthy teeth (Buldevold et al 1977; Carvalho et al 1998; Tvinnereim et al 2000). The results of this study do not appear to correspond to these previous findings, though the analysis here is relative. High levels do not solely correspond to the presence of caries, nor does the presence of caries always correspond to high Pb levels. Individual 16 shows low levels of lead with the presence of caries. Individual 22 also shows the same occurrence of low lead intensities with carious teeth. The background lead levels were not elevated, and therefore could not have falsely lowered the calculated levels for either individual. Individuals 27 and 44 show high intensities of lead without the presence of a carie. Unfortunately, it is not known if other teeth of the individuals contain caries or fillings. There did not appear to be any differences in levels between the sexes.

Individual 22 shows an immense variation in lead exposure throughout their life, the source of which also contains zinc, barium, and most likely mercury. Individuals 1 and 4 also show multiple peaks of lead. The other individuals display one or two larger peaks of lead. This could indicate a difference in lifestyle. Those with less variable lead intensities are not changing their exposure to whatever source is providing the lead, zinc, barium and mercury. This could imply they are living in the same area.

Individual 37 experienced high exposure to lead later in life, which has not been the case in the other individuals of the study. Five teeth from four individuals show high levels of lead at the exterior portion of cementum. The lead profile for individual 37, who is 78 years old, shows a more variable, longer exposure to higher levels of this element. Zinc, barium and mercury follow the same pattern.

Zinc

Zinc is an essential element that should be under homeostatic control. The large variations in zinc intensities imply that if there is bodily control, there must be a large variation within which it works. The rest of the essential elements (Ca, Mg, Mn, Cu, and V) remain much more consistent throughout the cementum.

The outer peaks in intensities show that zinc is most likely a part of the collagen fibres of the periodontal ligament that have yet to be made a part of the cementum, and mineralized. It is not clear if collagen fibres within the cementum can affect the zinc levels, but it is unlikely since it is well linked to lead levels, and lead has a high affinity for inorganic matter.

The large changes in zinc levels leaves the question open as to whether or not zinc can be used to spot disease or ailments that have been linked to changes in serum zinc levels. If the predominant source for zinc in cementum is from cigarette smoke or drinking water, then occurrences that affect zinc and not the other elements that normally follow zinc could demonstrate incidences of disease, ailment, or a period of major healing and recovery.

Magnesium

Magnesium demonstrates two general patterns, either a constant level across cementum, or the more common slow decrease, as reported by other studies (Selvig & Hals 1977; Tsuboi et al 2000). This lack of variation supports the inability to use Mg to assess dietary differences between individuals.

Magnesium is reported to be lower in cementum than in dentin (Neiders et al 1972; Hals & Selvig 1977). Several of the teeth did show higher levels after the CDJ (4-3, 4-4, 4-5, 13-2, 19-2, 22-6, 24-2, 35-5, 48-2), however, not all lines of analysis for each tooth showed this increase. It is unclear why this occurs or what it indicates.

Most of the Mg analyses show a decrease near the outer aspect of cementum. This pattern of decrease in levels is not an age-dependent occurrence. The youngest individual of the study was 23 yrs old (individual 16), with 11-12 years of cementum on 16-2 (Figure 24) and 7-8 yrs on 16-5 (Figure 25). The decrease in Mg occurs a quarter of the way through the cementum. The oldest individual of the study (individual 37) is 78 years old and has a constant rate of magnesium deposited throughout the cementum (Figure 122). If the decrease were age-dependent, it would occur at the same age for all of the individuals. The onset of this decrease occurs in the same general area of cementum. Since age is not a factor in this decrease, then it is also not an indication of the body's inability to absorb magnesium later in life, or the body's lessening need for it. This decrease could be an indication that the exterior of cementum is not as mineralized as the rest of the cementum. Magnesium is known to play a role in controlling mineralization in

Figure 123 Standardized magnesium profile of tooth 37-43 (78 yrs old) . X-axis is a proxy for age of tooth completion until documented age at extraction

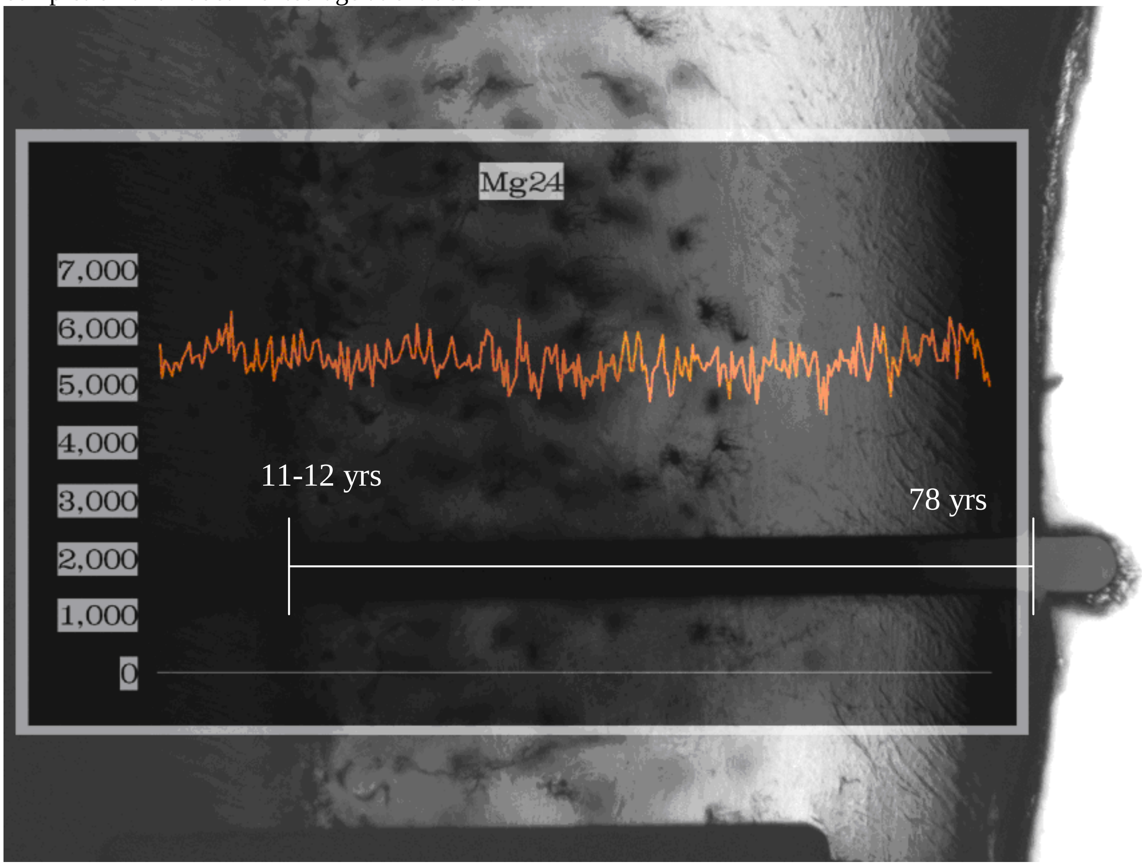
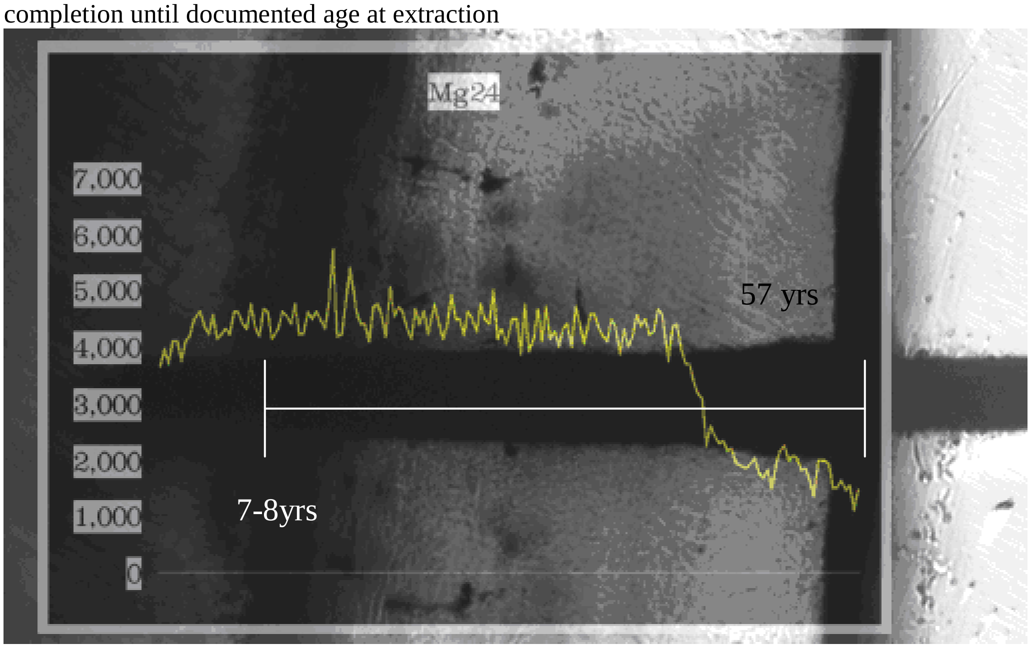


Figure 124 Standardized magnesium profile of tooth 27-2 (57 yrs old) . X-axis is a proxy for age of tooth completion until documented age at extraction



bone, which may also be true for the mineralization of teeth (Alvarez-Perez et al 2005; Serre et al 1998). The increase of mineralization of the outer portion of cementum suggests that Mg particles are able to be move, and be incorporated into previously deposited cementum. If this is true, then use of Mg to examine an individual's life may be futile. Also, none of the individuals in this study demonstrated periods of low Mg intensities prior to the expected decrease, so it cannot be determined whether Mg deficiencies are recorded within the cementum. It is also not known if any of the individuals suffered from any deficiencies.

Another interesting point in the Mg profiles is that the rate of decrease differs between individuals. Tooth 4-3 (Figure 14), 24-2 (Figure 77) and 27-2 (Figure 123) decreases more suddenly than others. Since these decreases are steeper than the other teeth of the study, it could be an indication of disease or a disorder such as renal disease or alcoholism (Berdanier 1998). Diseased cementum has been reported to have lower Mg levels than non-diseased (Hals & Selvig 1977). However, without ancillary information regarding the health of these individuals (which is not available) it is impossible to say if disease played a role for the decrease of magnesium in these teeth.

Strontium

Like magnesium, strontium does not have a variable profile like zinc or lead. It is also the only element to be noticeably affected by the calcium normalization since it follows calcium fairly closely. Two general patterns occur in the Sr profiles of this study. The profiles either show relatively constant Sr intensities, or they show a lull in the latter third of the cementum, followed by an increase. These two patterns may be correlated with sex. All 5 males show a relatively consistent level, with only individuals 1 (Figure

124) and 35 (Figure 125) showing an increase near the edge of the cementum. Five of the eight females demonstrate the lulled pattern, while three do not. Individual 24 remains relatively flat and individual 28 does not show much of a lull. Individual 27 does show a decrease in Sr levels after an increase that occurs during the first half of the cementum, but there is no increase near the edge of cementum to denote a simple lull in levels.

The lull in Sr intensity occurs for an extended period of time, covering the time period when pregnancy may occur. Mothers transfer both strontium and calcium to their offspring during pregnancy and lactation (Humphrey et al 2007). However Sr levels have been shown to be higher in women during their reproductive years most likely in preparation for pregnancy (Blakely 1989). In either case, it would be helpful to know if and when any of these individuals were pregnant to see if this Sr lull can be correlated to the stress of pregnancy and lactation.

Barium

Barium is bone-seeking but has also been noted to be in connective tissue (Schroeder et al 1972). This suggests that its presence is in both organic and inorganic portions of bone, and theoretically teeth. The drastic increase near the outer cemental edge shows the increase in organic material, most likely in the form of unmineralized PDL fibres. This also suggests that there may be a higher concentration in organic matter than inorganic. The barium profile follows the same pattern as lead, zinc and mercury, implying all of these elements are coming from the same source.

Mercury

Analysis of mercury shows that it occurs in relation to lead, zinc and barium.

Figure 125 Standardized strontium profile of tooth 1-2. X-axis is a proxy for age of tooth completion until documented age at extraction

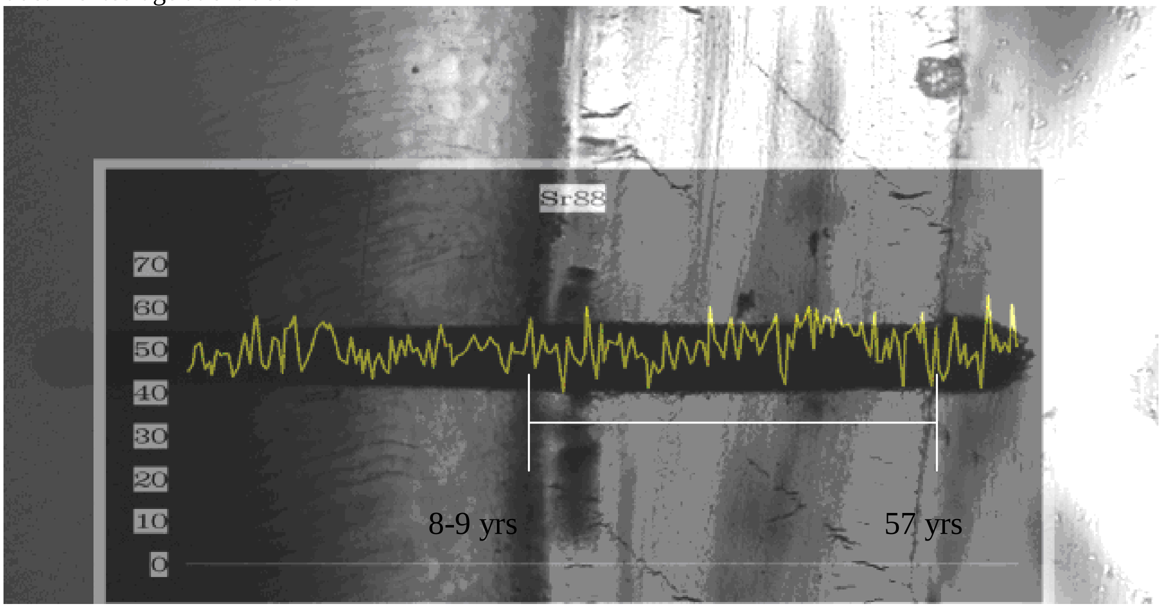
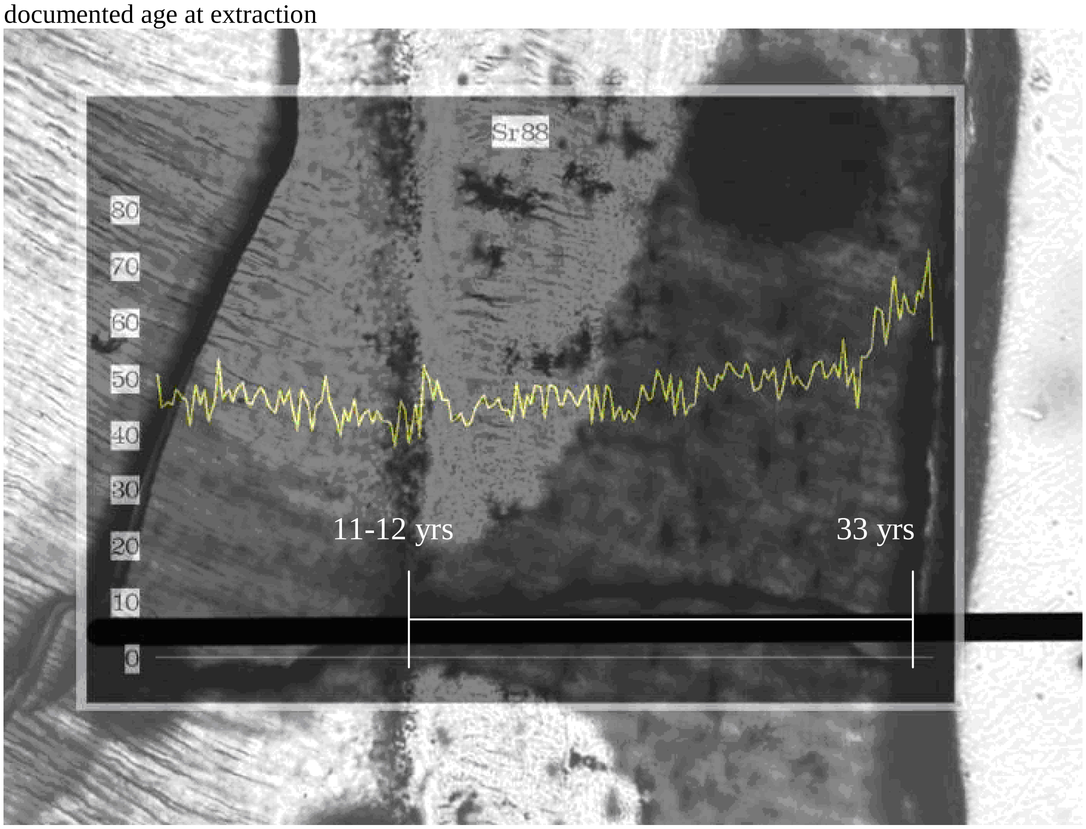


Figure 126 Standardized strontium profile of tooth 35-3. X-axis is a proxy for age of tooth completion until documented age at extraction



It is thought that the source of mercury in teeth is from amalgam (Carvalho et al 2002). The general composition of amalgam is 40% mercury, 20% silver, 15% tin, 15% copper and 10% zinc (Carvalho et al 2002). Lead is not a part of amalgam fillings, yet it shares a similar profile to mercury, which suggests that the mercury in the cementum of these individuals comes from a source other than amalgam.

Minute amounts of mercury have been found to diffuse from amalgam fillings through enamel, dentin and into the root canal (Carvalho et al 2002; Hoffman et al 2000). The results of this study show that there is not a diffusion gradient from dentin to cementum, since the cementum has the higher levels. There is also no simple increase from the CDJ to cemental surface to imply any diffusion of mercury from the exterior inwards, though initially 22-32 and 22-92 appear to have a consistent increase from CDJ to cementum surface. However, once the background levels of Hg are consulted, it can be seen that the consistent increase is most likely a continuation of the increasing background levels of Hg which is caused by Hg vapours emitted from the amalgam. This accumulation of vapours can be further exacerbated by mercury's ability to stay within the ICP-MS, causing a 'memory effect' (Harrington et al 2004). Another line of analysis performed near the initial laser line should have been performed to examine what, if any, variations could be attested to be valid.

The mercury profiles of the other teeth in my study do not exhibit migration patterns or a consistent increase which implies that the mercury levels are from the time of deposition of the tissue.

Copper, Manganese and Vanadium

Copper did not vary much in its profile between individuals, most likely because its concentration in teeth is so minute. Since copper intensity in cementum is not that high, it becomes difficult to say much about the possible sources of copper, and if pregnancy or use of birth control pills could be detected by having higher levels over certain periods of a female's life. The high levels of Cu near the exterior of the cementum are most likely due to its role in connective tissues.

Although manganese is mainly associated with bone, some is linked with organic material (Fore & Morton 1952). A recent study compared Mn levels in different organs and tissues of the human body, finding that hair and nails contain high levels manganese, showing that this element does have an affinity for collagen (Yoo et al 2002). The manganese profile presents basically one general trend, which is the increase in levels once the laser begins ablating the organic material present on the cemental surface. The same increase is observed in zinc, barium, copper, and mercury, and is most likely correlated to the increase of unmineralized collagen present in the cementum tissue. The background levels of manganese were fairly high, greatly diminishing any other patterns that may occur within cementum. The lack of variation in Mn levels does not make the element worthwhile to analyse in cementum.

Vanadium levels varied even less than manganese, also showing only minute amounts in cementum. This lack of variation and concentration does not make vanadium a worthwhile meaningful element to analyse.

<u>Trends</u>

With this analysis, it is of use to examine the correspondence of different elements to aid in determining possible sources for these elements. Conjoining profiles can also lead to a better understanding of what may be occurring without the benefit of a personal history of these individuals.

Lead and Zinc

One of the most distinct relationships observed in the cementum was the correlation of lead and zinc patterns. Other studies have also noted this correlation (Strehlow & Kneip 1969; Lee et al 1999). All individuals in this study except individual 27 showed a corresponding pattern between lead and zinc. Mercury and often barium also follow a similar pattern. This correlation of element patterns points to a shared source. Brass fixtures are known to leach both lead and zinc into tap water (Grosvenor et al 2005), however, this would not account for the barium and mercury observed. Another possible source is cigarette smoke. Smokers, as well as those exposed to second-hand smoke, have been found to have increased levels of both lead and zinc (Baranowska et al 2004; Malara et al 2006). Cigarettes also contain barium and mercury (Iskander et al 1986; Chiba & Masironi 1992). Many individuals in this study have fairly large increases in element levels at or just after the CDJ. This would imply exposure to cigarette smoke at a very young age and raises some serious issues regarding health. However, a closer examination of personal and medical information would be required to accurately assess the possible source(s) of these elements.

Occasionally the relative sizes of the lead and zinc peaks did not correspond. This occurred in individual 22 and individual 35 (35-3). The first peak of lead may be larger than the second peak, whereas the first peak of zinc is smaller than the second peak (e.g. 22-2, 22-96, 35-3). This difference could be because children are more susceptible to lead absorption since they absorb 4-5 times more and excrete less than adults (WHO

1996). If the lead and zinc are coming from the same source, as is suggested by the similar profiles in all individuals in the study, individuals may be absorbing a higher percentage of lead versus zinc at the younger age.

Individual 27 shows the outer two thirds of Pb and Zn do not match up; however, zinc and barium stay very similar throughout the cementum. The change in similarity between lead and zinc levels occurs earlier than in the rest of the teeth, and points to a change in element sources, where lead and zinc are most likely coming from different sources. Another possibility, although unlikely, is that the body has changed its ability to absorb lead, zinc and barium. Lead is a bone-seeking element and difficult to limit its absorption. As for increases in zinc and barium absorption, there are many diseases and ailments that can cause an increase in zinc levels, but there are none reported to cause barium to follow suit.

Outer Edge

The outer portions of zinc and lead profiles diverge from each other. This is also the area where Ba, Hg, Cu and Mn peak. The occurrence of these outer peaks is not age dependent, but likely occurs because of an increase in organic matter at the outer edge of cementum in the form of collagen fibres. All of these elements are known to occur in the organic fraction of skeletal tissue (Bratter et al 1977; Schroeder et al 1972). In their examination of cementum with an electron microprobe linescan, Selvig and Hals (1977) noted an organic layer on the exterior of the root. The authors used sulphur levels to help determine the general degree of organic material throughout the cementum, finding that there was an increase in sulphur from the CDJ to the root surface.

The occurrence of these outer peaks initially appears to differ as to when they occur. However, the tooth sections were not cut completely perpendicular to the outer surface causing the edges to either angle out or in. When the edge is angled out, the peaks are extended as the laser is able to penetrate the resin and ablate some of the outer cementum surface below. When the edges are angled in, the laser ablates the outer cementum below the surface before the laser reached the cementum edge that appears on the surface of the section. This causes the end peaks to occur sooner.

Cellularity and Ultrastructure

The cellularity of the cementum may influence the elemental levels prior to the cemental surface. Some of the analyses passed over dark areas of cementum, which may or may not be more cellular. If the darkness is caused by a mass of cementocytes, the area could be more cellular; however, it is extremely difficult to determine what is occurring. The darker areas of cementum can be seen in many of the sections (at least 11 analyzed sections). In some of these sections, changes in some element levels coincide with the darkness. In 22-3, I noted a decrease in lead and zinc in lines 1 and 2, but not in correspondence to all of the dark areas present. Tooth 22-9 shows a dark area that appears to coincide with a drop in lead and zinc in line 1 (22-95). The area of line 2 (22-92) does not show a dark area, nor do Pb and Zn change. 22-96 shows the same drop in Pb and Zn over the dark area which has shifted more towards the centre of the cementum. The Pb and Zn profiles in the other teeth of individual 22 are similar, but not highly correlated to those in 22-9, allowing the possibility that these dark areas of cementum can influence element levels.

Some darker areas correspond with difference reactions from element profiles. 27-2 shows a great increase in Pb over its dark cementum. Tooth 35-5 shows an increase of Pb and Zn over a dark area in the 35-54 analysis line, near the CDJ. The two other lines of analysis (35-56 and 35-53) take place over a similar, but wider dark area right after the CDJ. Lead and zinc do increase, but only partially over the dark area. There is a second dark area visible in 35-54 and 35-56, but not 35-53. Lead and zinc levels over this second area decrease in all three lines of analysis. The results from this tooth may indicate that the darkening of cementum could occur for different reasons, and that changes in levels could simply be a coincidence. Further investigation of dark areas of cementum and what they are is needed to determine if this is actually an ultrastructural change in cementum, and how it could be influencing element levels.

Differences between cellular and acellular cementum are difficult to determine. There appears to be some dissimilarity. Tooth 24-3 had 3 different laser analyses from the same slide. Line 2 (24-32) was performed in a more acellular area, while lines 1 and 3 were fairly close together and in cellular cementum. The initial peak found in lead, zinc, and mercury is not present in the analysis of line 2. Either there are some differences in element profiles between the two types of cementum, or the element peaks are a local phenomenon. Acellular cementum is problematic to analyse due to its minute width. Comparing it to cellular cementum becomes more complicated because element profiles are compressed and many years of information become ablated at any given time, more so than what occurs in wider, cellular areas.

Diffusion

The issue of the possible diffusion of elements through dental tissues is an important consideration. The element profiles do not suggest any diffusion is occurring in this study. Lead, zinc, mercury, and barium all increase in cementum from dentin. If these elements were diffusing into cementum, dentin would have the higher element levels. Mercury also does not appear to increase from the exterior cementum surface inward, suggesting that any mercury present in the mouth from amalgam fillings are not diffusing inwards from the cementum surface. The peaks in elements at the surface are very likely associated with organic material present on the surface; however, if they are evidence of contamination, their diffusion inwards is extremely limited. All of this suggests that the element levels reflect the overall levels at the time of cementum deposition.

Limitations

This study suffered from four general limitations; informational limitations, technological limitations, theoretical limitations and the physical limitations of cementum. The restriction of personal data of the individuals who donated their teeth meant I was unable to confirm or dismiss potential elemental information.

The inability to get much from the profiles of copper, manganese, and vanadium is a mixture of technological, physical and theoretical limitations. On the technological end, the LA-ICP-MS is a great way to analyse cementum, however the detection limits of trace elements restricted my analysis to a laser beam that is larger than any one annulation. The technology is not at the point yet to assess the small changes in their intensities. There is also the possibility that the profiles of these elements will not display any changes in the body's exposure or use due to homeostatic control. These three elements are considered essential for life which means the body will try to protect itself from deficiencies, as well as over-exposure. Zinc appears to be an exception since it is one of the most varied elements examined in this study. It is obviously not as strongly inhibited as other essential elements.

The use of an internal standard restricted my results to only semi-quantitative element levels. External standards are required to properly calibrate raw levels into quantitative ones. The external standards are matrix-matched to the sample to severly limit any differences in ablation. There has yet to be a standard reference material to match the matrix of cementum.

Physically, we are limited by the form of the tooth root. When a root is sectioned, the overlapping structures of cementum are slightly offset due to the conical nature of the root and our inability to section a tooth so that the cementum and structures below the surface are exactly at a 90 degree angle. The laser ablates a certain amount of tissue below the surface. With the offset of structure, the farther below the surface that the laser ablates, the more admixture of different structures there will be. The speed of the laser will also affect the amount of tissue ablated. A slower speed will ablate further below the surface, but at least in this study, more information was attained than with a slightly faster speed.

Other limitations include my analysis of mercury and its correlation to amalgam fillings, which was restricted due to Hg fumes being emitted by the filling. I would strongly recommend removing the crown from the root prior to embedding so that the amalgam is left untouched.

Future Directions

The main issue is the access to personal information which will be needed to take this type of research further. The ability to analyse with lower limits of detection along with a smaller laser beam will also increase the possibilities. Element profiles would become much more defined with a smaller laser. The ultrastructure of cementum may be better associated with any possible elemental changes with more minute sampling. We could test the theory of whether or not there is a change in calcium levels in association with more marked increments. Other changes in element levels in connection with marked increments could point to diseases such as tuberculosis, or stressful events like pregnancy and skeletal trauma.

Further exploration of possible sex differences in strontium profiles is warranted. The knowledge of it and when pregnancies occurred with respect to the decreases in strontium would also aid in determining the possibilities of this element.

The possibility that the mercury from amalgam fillings can be deposited at the time of installation and onward should be reassessed with the removal of the crown and filling prior to sectioning. The removal will eliminate the increased ambient mercury within the sample chamber, and allow for a more precise measurement of mercury's intensity in cementum.

Conclusions

It is obvious that cementum does contain an enormous amount of information which is beginning to be accessed by linear ablation. There is less of an averaging effect due to either mass tissue analysis, or time, as is the case of elemental analysis of bone, which assesses exposure of the past 8 -10 years of an individual's life. With linear ablation of cementum, we can see changes in exposure to metals such as lead over the majority of an individual's life, from the time of tooth eruption onwards.

Returning to questions originally asked, the elemental profile for any one increment could not be determined with the present level of technology and detection limits, which also precluded analysis of the more pronounced increments. This analysis did provide general agreement of elemental profiles in different areas of the root, implying that an increment should have similar levels in any location on the root. The comparison of cellular to acellular cementum proved difficult because of the amount of cementum the laser beam was able to ablate at any given time, and the narrowness of acellular cementum. The spatial resolution versus time represented by acellular cementum was fairly vague since there was much averaging of tissue levels. In essence, the larger the space of the annulations, the narrower the time span the laser averages. The mercury levels in most of the teeth are most likely not from amalgam fillings, and the teeth that did contain amalgam fillings proved difficult to analyze due to the very presence of the fillings and the mercury vapours they produced.

Other findings include the following:

- It is important to compare raw and background levels of elemental counts to their calculated levels. Comparisons to raw levels will show what changes the normalization of Ca may have done. Examination of background levels will show if the intensities of the elements have been diminished by excessively high levels present within the sample chamber
- Trends are easier to detect when multiple elements are compared to each other

- Analysis of cellular cementum is much easier and provides a more accurate profile, especially with a larger laser
- Lead and zinc are frequently associated with each other, as well as mercury and barium
- Diffusion of ions is very limited

This study has demonstrated the benefits of using LA-ICP-MS on a tissue that records information throughout life. There is much more information to be attained through linear ablation.

References

Abrams, S. A. and I. J. Griffin

2004 Microminerals and bone health. In *Nutrition and Bone Health*, edited by M. F. Holick and B. Dawson-Hughes, pp. 377-390. Humana Press Inc, Totowa, NJ.

Aggett, P. and A. Favier

1993 Zinc. *International journal for vitamin and nutrition research* 63(4):301-307.

Alvarez-Perez, M. A., O. Alvarez-Fregoso, J. Ortiz-Lopez and H. Arzate

2005 X-ray microanalysis of human cementum. *Microscopy and Microanalysis* 11(4):313-318.

Arora, M., S. W. Y. Chan, B. J. Kennedy, A. Sharma, D. Crisante and D. M. Walker

2004 Spatial distribution of lead in the roots of human primary teeth. *Journal of Trace Elements in Medicine and Biology* 18(2):135-139.

Atilla, G. and H. Baylas

1996 Electron probe analysis of cementum surfaces. *J Marmara Univ Dent Fac* 2(2-3):510-4.

Avery, J. K. and P. F. Steele

2002 *Oral development and histology*. Thieme, New York.

Baranowska, I., L. Barchanski, M. Bak, B. Smolec and Z. Mzyk

2004 X-ray fluorescence spectrometry in multielemental analysis of hair and teeth. *Polish Journal of Environmental Studies* 13(6):639-646.

Bellis, D. J., P. J. Parsons, J. Jones and D. Amarasiriwardena

2009 Evaluation of Laser Ablation Inductively Coupled Plasma Mass Spectrometry for the Quantitative Determination of Lead in Different Parts of Archeological Human Teeth. *Spectroscopy Letters* 42(8):491-496.

Berdanier, C.

1998 *Advanced Nutrition*. CRC Press, Boca Raton, Fla.

Blakely, R. L.

1989 Bone Strontium in Pregnant and Lactating Females from Archaeological Samples. *American Journal of Physical Anthropology* 80(2):173-185.

Blakely, R. L. and L. A. Beck

1981 Trace-elements, nutritional-status, and social-stratification at Etowah, Georgia. *Annals of the New York Academy of Sciences* 376(DEC):417-431.

Bosshardt, D. D. and K. A. Selvig

1997 Dental cementum: The dynamic tissue covering of the root. *Periodontology 2000* 13:41-75.

Bratter, P., D. Gawlik, J. Lausch and U. Rosick

1977 Distribution of trace-elements in human skeletons. *Journal of Radioanalytical Chemistry* 37(1):393-403.

Brown, C. J., S. R. N. Chenery, B. Smith, C. Mason, A. Tomkins, G. J. Roberts, L. Sserunjogi and J. V. Tiberindwa

2004 Environmental influences on the trace element content of teeth - implications for disease and nutritional status. *Archives of Oral Biology* 49(9):705-717.

Brudevold, F., R. Aasenden, B. N. Srinivasian and Y. Bakhos

1977 Lead in enamel and saliva, dental caries and the use of enamel biopsies for measuring past exposure to lead. *J Dent Res* 56(10):1165-71.

Budd, P., J. Montgomery, A. Cox, P. Krause, B. Barreiro and R. G. Thomas

1998 The distribution of lead within ancient and modern human teeth: Implications for long-term and historical exposure monitoring. *Science of the Total Environment* 220(2-3):121-136.

Buikstra, J. E. and D. Ubelaker

1994 *Standars: for data collection from human skeletal remains*. Arkansas Archeological Survey, Fayetteville, Arkansas.

Burke, A. and J. Castanet

1995 Histological Observations of Cementum Growth in Horse Teeth and their Application to Archaeology. *Journal of Archaeological Science* 22(4):479-493.

Calvery, H. O.

1942 Trace elements in foods *Journal of Food Science* 7(4):313-331.

Carvalho, M. L., J. P. Marques, J. Brito, C. Casaca and A. S. Cunha

2002 Hg, Bi, Cu and Zn distribution in human teeth treated by dental amalgam measured by synchrotron microprobe. *Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions with Materials and Atoms* 196(1-2):148-154.

Carvalho, M. L., T. Pinheiro, M. A. Barreiros, C. Casaca, A. S. Cunha and P. Chevallier

1998 Amalgam components drift in teeth-toxicity risks: A preliminary approach. *Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions with Materials and Atoms* 137:913-918.

Charles, D. K., K. Condon, J. M. Cheverud and J. E. Buikstra

1986 Cementum Annulation and Age-Determination in Homo-Sapiens .1. Tooth Variability and Observer Error. *American Journal of Physical Anthropology* 71(3):311-320.

Chaudhri, M. A.

1984 PIXE analysis of human dental cementum. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 3(1-3):643-647.

Chen, Z. X.

1999 Inter-element fractionation and correction in laser ablation inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry* 14(12):1823-1828.

Chenery, S., T. Williams, T. A. Elliott, P. L. Forey and L. Werdelin

1996 Determination of rare earth elements in biological and mineral apatite by EPMA and LAMP-ICP-MS. *Mikrochimica acta*:259-269.

Chiba, M. and R. Masironi

1992 Toxic and trace-elements in tobacco and tobacco-smoke. *Bulletin of the World Health Organization* 70(2):269-275.

Christoffersen, J. and W. J. Landis

1991 A contribution with review to the description of mineralization of bone and other calcified tissues invivo. *Anatomical Record* 230(4):435-450.

Cipriano, A.

2002 Cold stress in captive great apes recorded in incremental lines of dental cementum. In *Folia primatologica*, pp. 21. vol. 73.

Clarkson, T. W., L. Friberg, G. F. Nordberg and P. R. Sager

1988 *Biological monitoring of toxic metals*.

Cohen, D. D., E. Clayton and T. Ainsworth

1981 Preliminary investigations of trace element concentrations in human teeth. *Nuclear Instruments and Methods* 188(1):203-209.

Condon, K., D.K. Charles, J.M.Cheverud, and J.E. Buikstra.

1986 Cementum annulation and age determination in Homo sapiens. II. Estimates and accuracy. In *American-Journal-of-Physical-Anthropology*, pp. 321. vol. 71.

Cox, A., F. Keenan, M. Cooke and J. Appleton

1996 Trace element profiling of dental tissues using laser ablation inductively coupled plasma mass spectrometry. *Fresenius Journal of Analytical Chemistry* 354(2):254-258.

Crawford, A. W., W. J. Sampson and H. J. Debruin

1983 Shallow Fluorine Depth Profiles of Cementum in Periodontal-Disease - a Pilot-Study. *Journal of dental research* 62(7):806-810.

Cruickshank, D. B.

1949 The frequency distribution of the zinc concentrations in the dental tissues of the normal population. *Biochemical Journal* 44(3):299-302.

Curzon, M. E. J. and T. W. Cutress

1983 *Trace elements and dental disease*. Wright/PSG, Boston.

Curzon, M. E. J., F. L. Losee, R. Brown and H. E. Taylor

1974 Vanadium in whole human enamel and its relationship to dental caries. *Archives of Oral Biology* 19(12):1161-1165.

Davidsson, L., A. Cederblad, B. Lonnerdal and B. Sandstrom

1991 The effect of individual dietary-componenets on manganese absorption in humans. *American Journal of Clinical Nutrition* 54(6):1065-1070.

Derise, N. L. and S. J. Ritchey

1974 Mineral-composition of normal human enamel and dentin and relation of composition to dental-caries. 2. Microminerals. *Journal of Dental Research* 53(4):853-858.

Dreyfuss, F. and R. Frank

1964 Microradiographie et microscopie electronique du cement humain. *Bull Group Int Rech Sci Stomatol Odontol* 7:167-181.

Elias, M.

1980 The Feasibility of Dental Strontium Analysis for Diet-Assessment of Human-Populations. *American Journal of Physical Anthropology* 53(1):1-4.

Evans, R. D., P. M. Outridge and P. Richner

1994 Applications of Laser-Ablation Inductively-Coupled Plasma-Mass Spectrometry to the Determination of Environmental Contaminants in Calcified Biological Structures. *Journal of Analytical Atomic Spectrometry* 9(9):985-989.

Evans, R. D., P. Richner and P. M. Outridge

1995 Micro-Spatial Variations of Heavy-Metals in the Teeth of Walrus as Determined by Laser-Ablation Icp-Ms - the Potential for Reconstructing a History of Metal Exposure. *Archives of Environmental Contamination and Toxicology* 28(1):55-60.

Follis, R. H., J. A. Bush, G. E. Cartwright and M. M. Wintrobe

1955 Studies on copper metabolism. 18. Skeletal changes associated with copper deficiency in swine *Bulletin of the Johns Hopkins Hospital* 97(6):405-413.

Fore, H. and R. A. Morton

1952 The manganese in bone. *Biochemical Journal* 51(5):598-600.

Fraga, C. G.

2005 Relevance, essentiality and toxicity of trace elements in human health. *Mol Aspects Med* 26(4-5):235-44.

Francalacci, P.

1989 Dietary Reconstruction at Arene Candide Cave (Liguria, Italy) by Means of Trace-Element Analysis. *Journal of Archaeological Science* 16(2):109-124.

Ghazi, A. M., S. Shuttleworth, S. J. Angulo and D. H. Pashley

2000 New applications for laser ablation high resolution ICPMS (LA-HR-ICP-MS): Quantitative measurements of gallium diffusion across human root dentin. *Journal of Analytical Atomic Spectrometry* 15(10):1335-1341.

Gray, H.

1974 *Gray's Anatomy: the unabridged running press edition of the american classic*. Running Press.

Gropper, S. S., J. L. Smith and J. L. Groff

2005 Magnesium. In *Advanced Nutrition and Human Metabolism*, edited by J. L. Groff and S. S. Gropper. Thomson & Wadsworth, Belmont, CA.

Grosskopf, B.

1990 Age-Determination by Counting Incremental Lines in Dental Cementum of Burial Human Teeth. *Zeitschrift Fur Rechtsmedizin-Journal of Legal Medicine* 103(5):351-359.

Grosvenor, A. P., R. R. H. Martin, M. Giuliacci and M. C. Biesinger

2005 Evaluation of multiple analytical techniques in the study of leaching from brass fixtures. *Canadian Journal of Analytical Sciences and Spectroscopy* 50(2):97-107.

Grue, H. and B. Jensen

1979 Review of the formation of incremental lines in tooth cementum of terrestrial mammals. *Danish Review Game Biology* 11:1-48.

Haas, H. and B. Levin

2006 *Staying Healthy with Nutrition: the complete guide to diet and nutritional medicine*. Celestial Arts, Berkeley.

Hahn, L. J., R. Kloiber, M. J. Vimy, Y. Takahashi and F. L. Lorscheider

1989 Dental silver tooth fillins - a source of mercury exposure revealed by whole-body image scan and tissue-analysis. *Faseb Journal* 3(14):2641-2646.

Hals, E.. K. A.Selvig

1977 Correlated electron probe microanalysis and microradiography of carious and normal dental cementum. In *Caries research*, pp. 62. vol. 11.

Harrington, C., S. Merson and T. D'Silva

2004 Method to reduce the memory effect of mercury in the analysis of fish tissue using inductively coupled plasma mass spectrometry. *Analytica Chimica Acta* 505:247-254.

Hewitt, B. R.

2004 *Health and mortality at Fidler Mounds*, University of Manitoba.

Hillson, S.

1996 *Dental Anthropology*. Cambridge University Press, Cambridge.

Hoffmann, E., H. Stephanowitz, E. Ullrich, J. Skole, C. Ludke and B. Hoffmann

2000 Investigation of mercury migration in human teeth using spatially resolved analysis by laser ablation-ICP-MS. *Journal of Analytical Atomic Spectrometry* 15(6):663-667.

Humphrey, L., M. Dean and T. Jeffries

2007 An evaluation of changes in strontium/calcium ratios across the neonatal line in human deciduous teeth. In *Dental perspectives on human evolution: state of the art research in dental paleoanthropology*, edited by S. Bailey and J. Hublin, pp. 303-320. Springer, 2007.

Hursh, J. B., T. W. Clarkson, M. G. Cherian, J. J. Vostal and R. Vandermallie

1976 Clearance of mercury (Hg-197, Hg-203) vapor inhaled by human subjects *Archives of Environmental Health* 31(6):302-309.

Iskander, F. Y., T. L. Bauer and D. E. Klein

1986 Determination of 28 elements in american cigarette tobacco by neutron-activation analysis *Analyst* 111(1):107-109.

Jankauskas, R., S. Barakauskas and R. Bojarun

2001 Incremental lines of dental cementum in biological age estimation. *Homo-Journal of Comparative Human Biology* 52(1):59-71.

Johnson, A. R., Armstron.Wd and L. Singer

1966 Strontium incorporation into dental enamel *Science* 153(3742):1396-&.

Kagerer, P. and G. Grupe

2001 Age-at-death diagnosis and determination of life-history parameters by incremental lines in human dental cementum as an identification aid. *Forensic science international* 118(1):75-82.

Kehoe, R. A.

1961 Metabolism of lead in man in health and disease *Archives of Environmental Health* 2(4):418-&.

Keller, C. A. and R. A. Doherty

1980 Effect of dose on lead retention and distribution in suckling and adult female mice *Toxicology and Applied Pharmacology* 52(2):285-293.

Klepinger, L. L.

1984 Nutritional Assessment from Bone. *Annual Review of Anthropology* 13:75-96.

Klevezal, G. A. and S. E. Kleinenberg

1969 *Age determination of mammals from annual layers in teeth and bones*. Program for Scientific Translations, Jerusalem, Israel.

Kvaal, S. I. and T. Solheim

1995 Incremental Lines in Human Dental Cementum in Relation to Age. *European journal of oral sciences* 103(4):225-230.

Lee, K. M., J Appleton, M. Cooke, F. Keenan, and K. Sawicka-Kapusta

1999 Use of laser ablation inductively coupled plasma mass spectrometry to provide element versus time profiles in teeth. In *Analytica chimica acta*. vol. 395.

Legeros, R. Z., W. P. Shirra, M. A. Miravite and J. P. Legeros

1974 Biological and synthetic amorphous calcium phosphates. *Journal of dental research* 53(FEB):117-117.

Lieberman

1990 Computer image-enhancement and analysis of cementum increments as applied to teeth of gazella-gazella In *Journal of archaeological science*, pp. 519. vol. 17.

Lieberman, D. E.

1994 The Biological Basis for Seasonal Increments in Dental Cementum and their Application to Archaeological Research. *Journal of Archaeological Science* 21(4):525-539.

Lieberman, D. E. and R. H. Meadow

1992 The biology of cementum increments (with an archaeological application). *Mammal Review* 22(2):57-77.

Losee, F. L., T. W. Cutress and R. Brown

1974 Natural elements of periodic table in human dental enamel *Caries Research* 8(2):123-134.

Lowenstam, H. A. and S. Weiner

1989 *On Biomineralization*. Oxford University Press, New York.

Lucas, P. W. and H. S. Loh

1986 Are the incremental lines in human cementum laid down annually? *Ann Acad Med Singapore* 15(3):384-6.

Malara, P., J. Kwapulinski and B. Malara

2006 Do the levels of selected metals differ significantly between the roots of carious and non-carious teeth? *Science of the Total Environment* 369(1-3):59-68.

Martin, R. R., S. J. Naftel, A. J. Nelson, A. B. Feilen and A. Narvaez

2004 Synchrotron X-ray fluorescence and trace metals in the cementum rings of human teeth. *Journal of environmental monitoring : JEM* 6(10):783-786.

Mertz, W.

1981 The Essential Trace-Elements. *Science* 213(4514):1332-1338.

Miller, C. S., S. B. Dove and J. A. Cottone

1988 Failure of use of Cemental Annulations in Teeth to Determine the Age of Humans - Response. *Journal of forensic sciences* 33(6):1305-1306.

Monks, G. G.

1981 Seasonality Studies. *Advances in Archaeological Method and Theory* 4:177-240.

Murakami, T., H. Nakagaki, Y. Sakakibara, J. A. Weatherell and C. Robinson

1987 The Distribution Pattern of Fluoride Concentrations in Human Cementum. *Archives of Oral Biology* 32(8):567-571.

Nakagaki, H., K. Kawai, T. Murakami, Y. Sakakibara, N. Ohno, J. A. Weatherell and C. Robinson

1988 Fluoride Distribution and Histological Structure of Human Cementum. *Archives of Oral Biology* 33(4):257-264.

Nakagaki, H., J. A. Weatherell, M. Strong and C. Robinson

1985 Distribution of fluoride in human cementum. *Archives of Oral Biology* 30(2):101-104.

Nakamura, T., A. Higashi, S. Nishiyama, S. Fujimoto and I. Matsuda

1991 Kinetics of zinc status in children with IDDM. *Diabetes Care* 14(7):553-7.

Nakata, T. M., R. J. Stepnick and I. Zipkin

1972 Chemistry of human dental cementum - effect on concentration of ash, fluoride, calcium, phosphorus and magnesium. *Journal of Periodontology* 43(2):155-&.

Naylor, J. W., W. G. Miller, G. N. Stokes and G. G. Stott

1985 Cemental Annulation Enhancement - a Technique for Age-Determination in Man. *American Journal of Physical Anthropology* 68(2):197-200.

Neiders, M. E., J. W. Leitner, W. A. Miller and J. D. Eick

1972 Electron-probe microanalysis of cementum and underlying dentin in young permanent teeth. *Journal of dental research* 51(1):122-&.

Nielsen, F.

1987 Other Elements: Sb, Ba, B, Br, Cs, Ge, Pb, Ag, Sr, Sn, Ti, Zr, Be, Bi, Ga, Au, In, Nb, Sc, Te, Tl, W. In *Trace element in human and animal nutrition*, edited by W. Mertz, pp. 415-463. Academic Press, Inc, San Diego.

Outridge, P. M. and R. D. Evans

1995 Effect of Laser Parameters and Tooth Type on the Ablation of Trace-Metals from Mammalian Teeth. *Journal of Analytical Atomic Spectrometry* 10(9):595-600.

Rabinowitz, M. B., J. D. Kopple and G. W. Wetherill

1980 Effect of food-intake and fasting on gastrointestinal lead absorption in humans *American Journal of Clinical Nutrition* 33(8):1784-1788.

Rao, N. G., and N.N. Rao

1998 CCTV study of cemental annulations in determining the age from single tooth. In *Indian journal of dental research*, pp. 41. vol. 9.

Renz, H. and R. J. Radlanski

2006 Incremental lines in root cementum of human teeth--a reliable age marker? *Homo* 57(1):29-50.

Renz, H., V.Schaefer, H. Duschner, and R.J. Radlanski

1997 Incremental lines in root cementum of human teeth: an approach to their ultrastructural nature by microscopy. In *Advances in dental research*, pp. 472. vol. 11.

Rex, T., O. P. Kharbanda, P. Petocz and M. A. Darendeliler

2005 Physical properties of root cementum: Part 4. Quantitative analysis of the mineral composition of human premolar cementum. *American Journal of Orthodontics and Dentofacial Orthopedics* 127(2):177-185.

2006 Physical properties of root cementum: Part 6. A comparative quantitative analysis of the mineral composition of human premolar cementum after the application of orthodontic forces. *American Journal of Orthodontics and Dentofacial Orthopedics* 129(3):358-367.

Rygh, O.

1949 Recherches sur les oligo-elements. 3. sur l'importance du strontium, du vanadium, du baryum, du thallium et du zinc dans le scorbut *Bulletin De La Societe De Chimie Biologique* 31(9-10):1408-1412.

Sanborn, M. and K. Telmer

2003 The spatial resolution of LA-ICP-MS line scans across heterogeneous materials such as fish otoliths and zoned minerals. *Journal of Analytical Atomic Spectrometry* 18(10):1231-1237.

Schmidt, W. J. and A. Keil

1971 *Polarizing microscopy of dental tissues: theory, methods, and results from the structural analysis of normal and diseased hard, dental tissues and tissues associated with them in man and other vertebrates*. Pergamon Press.

Schroede.Ha, I. H. Tipton and A. P. Nason

1972 Trace metals in man - strontium and barium *Journal of Chronic Diseases* 25(9):491-&.

Schroeder, H. A., J. J. Balassa and I. H. Tipton

1966 Essential trace metals in man: manganese. A study in homeostasis. *J Chronic Dis* 19(5):545-71.

Selvig, K. A.

1965 The fine structure of human cementum. *Acta Odontol Scand* 23(4):423-41.

Selvig, K. A. and E. Hals

1976 Periodontally diseased cementum studied by correlated microradiography, electron-probe microanalysis and electron-microscopy. *Journal of Dental Research* 55:B165-B165.

1977 Periodontaly diseased cementum studied by correlated microradiography, electron-probe analysis and electron-microscopy. *Journal of Periodontal Research* 12(6):419-429.

Selvig, K. A., and S.K.. Selvig

1962 Mineral content of human and seal cementum. In *Journal of dental research*, pp. 624. vol. 41.

Sergeant, D. E. and D. H. Pimlott

1959 Age determination in moose from sectioned incisor teeth. *Journal of Wildlife Management* 23(3):315-321.

Silbergeld, E. K., J. Schwartz and K. Mahaffey

1988 Lead and osteoporosis - mobilization of lead from bone in menopausal women. *Environmental Research* 47(1):79-94.

Soni, N. N., G. van Huysen and H. M. Swenson

1962 A Microradiographic and X-ray Densitometric Study of Cementum. *Journal of Periodontology* 33:372-378.

Sousa, E. M. D., G. G. Stott and J. B. Alves

1999 Determination of age from cemental incremental lines for forensic dentistry. *Biotechnic & Histochemistry* 74(4):185-193.

Soyman, M., H. Ulukapi, S. Oztezcan, F. Gurdol and Y. Guven

1997 Fluoride concentration and profile in different cementum surfaces. *J Marmara Univ Dent Fac* 2(4):582-7.

Stein, T. J. and J. F. Corcoran

1994 Pararadicular Cementum Deposition as a Criterion for Age Estimation in Human-Beings. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* 77(3):266-270.

Stern, R. A., P. M. Outridge, W. J. Davis and R. E. A. Stewart

1999 Reconstructing lead isotope exposure histories preserved in the growth layers of walrus teeth using the SHRIMP II ion microprobe. *Environmental science & technology* 33(10):1771-1775.

Stott, G. G., R. F. Sis and B. M. Levy

1982 Cemental Annulation as an Age Criterion in Forensic Dentistry. *Journal of dental research* 61(6):814-817.

Strehlow, C. D. and T. J. Kneip

1969 Distribution of lead and zinc in human skeleton. *American Industrial Hygiene Association Journal* 30(4):372-&.

Ten Cate, A. R.

1998 *Oral histology: development, structure, and function*. 5th ed. Mosby, St Louis.

Tohda, H., O. Fejerskov and T. Yanagisawa

1996 Transmission electron microscopy of cementum crystals correlated with Ca and F distribution in normal and carious human root surfaces. *Journal of Dental Research* 75(3):949-954.

Tsuboi, S., H. Nakagaki, Y. Takami, H. Eba, J. Kirkham and C. Robinson

2000 Magnesium and fluoride distribution in human cementum with age. *Calcified tissue international* 67(6):466-471.

Tvinnereim, H. M., R. Eide and T. Riise

2000 Heavy metals in human primary teeth: some factors influencing the metal concentrations. *Science of the Total Environment* 255(1-3):21-27.

Weiner, J. A. and M. Nylander

1995 An estimation of the uptake of mercury from amalgam fillings based on urinary-excretion of mercury in swedish subjects *Science of the Total Environment* 168(3):255-265.

WHO

1996 *Trace elements in human nutrition and health*. World Health Organization, Geneva.

2004 *Vitamin and mineral requirements in human nutrition*. World Heath Organization and Food and Agriculture Organization of the United Nations, Geneva.

Wittwer-Backofen, U. and H. Buba

2002 Age estimation by tooth cementum annulations: perspectives of a new validation study. In *Paleodemography: age distribution from skeletal samples*, edited by R. Hoppa and J. W. Vaupel, pp. 107-128. Cambridge University Press, Cambridge.

Wittwer-Backofen, U., J. Gampe and J. W. Vaupel

2004 Tooth cementum annulation for age estimation: Results from a large known-age validation study. *American Journal of Physical Anthropology* 123(2):119-129.

Yamamoto, T., T. Domon, S. Takahashi, N.M.D. Islam, and R. Suzuki

2000 Twisted plywood structure of an alternating lamellar pattern in cellular cementum of human teeth. In *Anatomy and embryology*, pp. 25. vol. 202.

Yamamoto, T., T. Domon, S. Takahashi, N.M.D. Islam, R. Suzuki, and M. Wakita

1998 The regulation of fiber arrangement in advanced cellular cementogenesis of human teeth. In *Journal of periodontal research*, pp. 83. vol. 33.

Yamamoto, T., T. Domon, S. Takahashi, and M. Wakita

1997 Formation of an alternate lamellar pattern in the advanced cellular cementogenesis in human teeth. In *Anatomy and embryology*, pp. 115. vol. 196.

Yoo, Y.C., S.K. Lee, J.Y. Yang, S.W. In, K.W. Kim, K.H. Chung, M.G. Chung and S.Y. Choung

2002 Organ distribution of heavy metals in autopsy material from normal Korean. *Journal of Health Science* 48(2):186-194.

Yoshinaga, J., T. Suzuki, M. Morita and M. Hayakawa

1995 Trace-elements in ribs of elderly people and elemental variation in the presence of chronic diseases. *Science of the Total Environment* 162(2-3):239-252.

Zoeger, N., P. Wobrauschek, C. Streli, G. Pepponi, P. Roschger, G. Falkenberg and W. Osterode

2005 Distribution of Pb and Zn in slices of human bone by synchrotron mu-XRF. *X-Ray Spectrometry* 34(2):140-143.